ICHA 2023

20th International Conference on Harmful Algae

HAB Science and Human Well-being

ABSTRACT BOOK

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Conference logo and front cover were designed by Dr. Shigeru Itakura and Dr. Yoko Cho, respectively.

Welcome message from ICHA 2023 chair

As the global outbreak of the novel coronavirus (COVID -19) has finally calmed down, we are pleased to announce the hosting of ICHA 2023 in Hiroshima, bringing together more than 500 participants from 40 countries around the world for the first time in five years. The Local Organizing Committee (LOC) and the Scientific Committee (SC) of ICHA 2023 would like to express our sincere gratitude to the ISSHA executives and participants for their various forms of cooperation.

During the preparation of the ICHA 2023, the LOC has been facing some difficulties on the COVID19 pandemic and rising prices in the world. In this exceptional situation, we sincerely appreciate that ISSHA members could understand giving up online and in person hybrid meetings, that was initially announced by the LOC, and holding in-person meetings, exclusively.

The people of Japan have a long history of intimate ties with the coastal environment which is defined by the concept of "Sato-Umi" whereby biological productivity and biodiversity have been nourished through the intimate interactions of humans with their coastal ecosystems. The theme of the conference will be "HAB Science and Human Well-being". The goal is to exchange relevant scientific information towards a greater understanding of HAB mechanisms, better and timely predictions of HAB occurrences and mitigating their negative impacts.

In addition to the Opening Lecture and 9 Plenary Lectures, there will be 216 oral presentations, 199 poster presentations, and 45 ignite talks on 16 different topics (Ecology, Biology and biogeography, Community/Species interactions, Taxonomy, Microbiomes and omics, Prediction and modeling, Monitoring and mitigation, Ciguatera and benthic HABs, Ichthyotoxic HABs, Cyanobacterial HABs, Toxins, Biosynthesis and detection methods, Toxicology, Surveillance and management, HABs in a changing world, Socio-economic impacts, Emerging issues).

We ensure that the ICHA 2023 will make a significant contribution to the scientific knowledge on harmful algae, and we sincerely hope that all participants will thoroughly enjoy their experiences in the International City of Peace, Hiroshima, along with Japanese cuisines and Sake.

Finally, we extend our deep appreciation to Dr. Wayne Litaker, our ISSHA president, for the tremendous support provided over the past year in the preparation for ICHA 2023.

ICHA 2023
November 5 - 10 HIROSHIMA JAPAN

The 20th International Conference on Harmful Algae

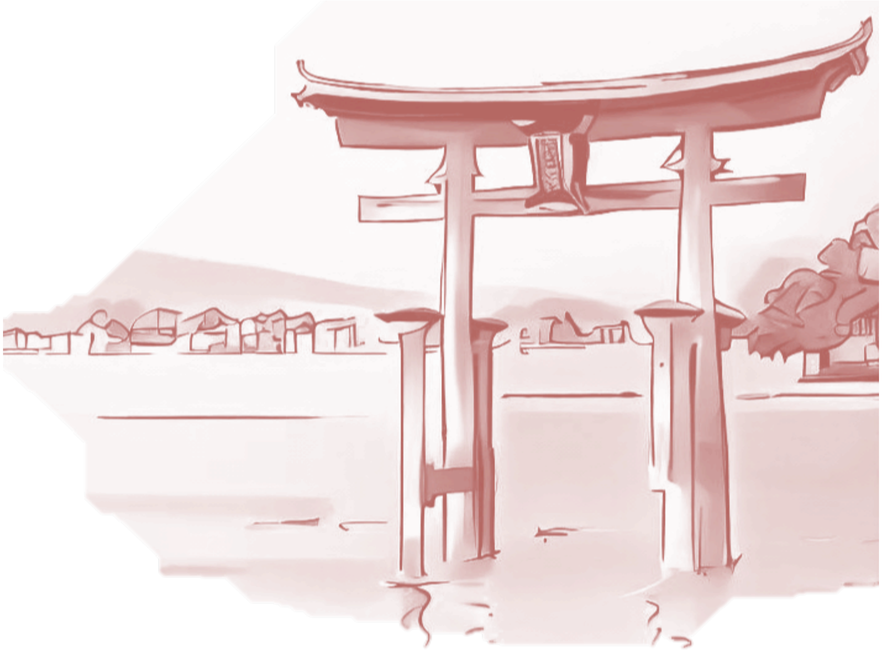
Ichiro Imai

Ichiro Imai

Chair of ICHA 2023

ICHA Proceedings Guidelines

Presenters of all contributed talks and posters will have the opportunity to submit their papers for inclusion in the Proceedings of the International Society for the Study of Harmful Algae**. Your paper must be submitted no later than 1st March 2024.** Paper submission instructions will be emailed to you after the conference. The Proceedings of ISSHA Conferences are published under the auspices of the International Society for the Study of Harmful Algae (ISSHA). The complete proceedings will be published on the ISSHA website no later than 12 months after the conference, but reviewed, revised and accepted submissions will be uploaded prior to that as they are completed. Editors of the proceedings are selected jointly by the ISSHA Conference Committee, the Local Organizing Committee, the Science Committee, and may be from within and from outside the three committees as required to have an editorial team and an editor-in-chief with the required scientific background and editorial experience.



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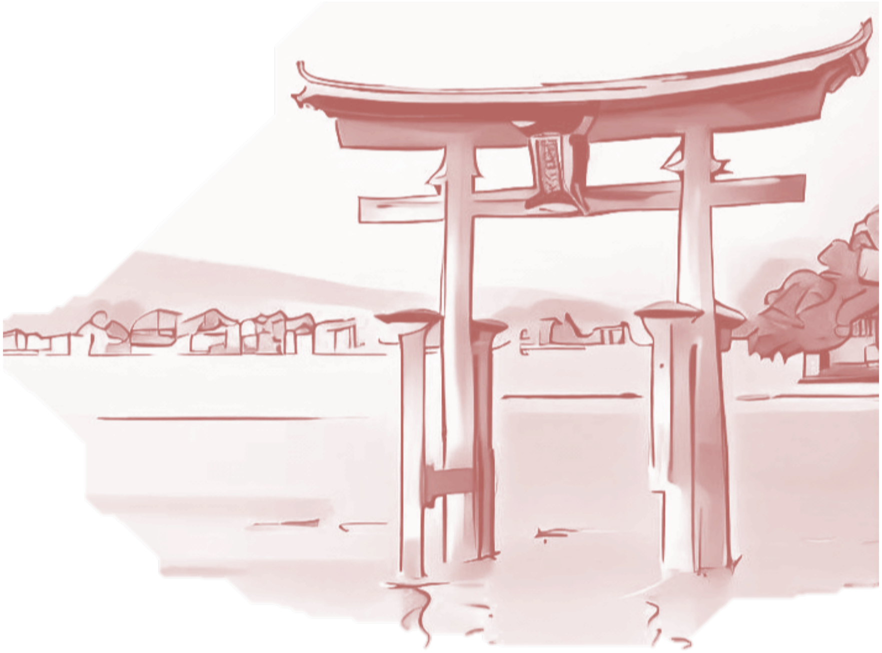

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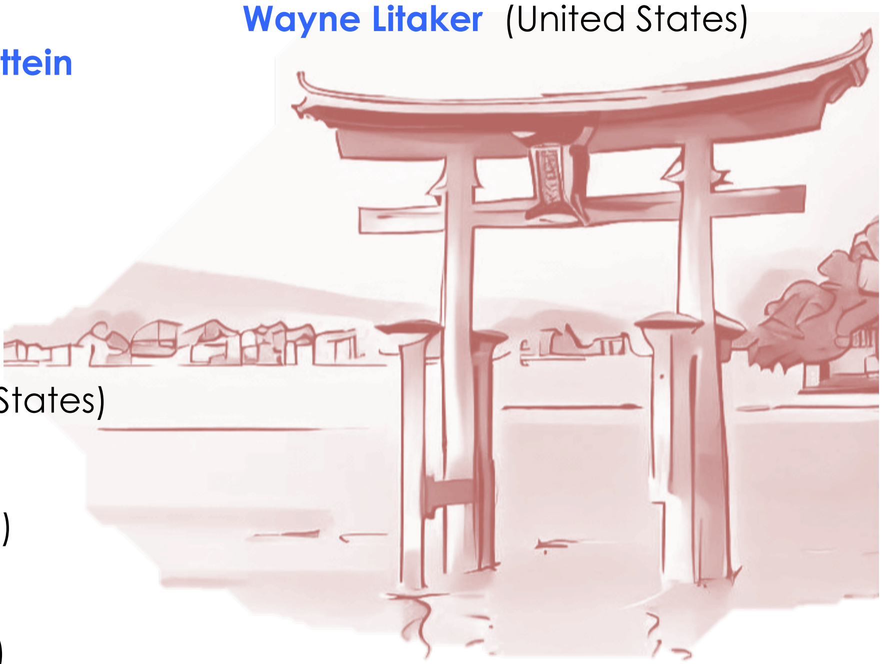

Opening Lecture

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Opening Lecture

OL

What conditions caused naughty algal blooms to turn into harmful ones?

Yasuwo Fukuyo[1,2]

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We have various old documents reporting algal blooms in Japan. Some report fish mass mortality, some report harmful influences on coastal society, and others report high fish catch. After rapid development of aquaculture of shellfish and fish, report on negative impact increases, and scientific research was promoted. Nutrient level in coastal waters have close relation to bloom occurrences, especially those causing oxygen depletion and fish mortality. To decrease the blooms, reduction of nutrients has been successfully implemented. But it has also negative side effects to aquaculture and coastal fisheries. In northern Japan, there is an old saying, “Do not eat shellfish when snowmelt water runs into coastal seas.” It was only when shellfish aquaculture developed and transportation of live shellfish to far areas was enabled that strict regulation of market closure with toxin monitoring became necessary. It is also considered as one of the results of forgetting the old saying. In order to mitigate negative impacts on society and solve problems caused by algal blooms, proper scientific understanding on algal blooms is not enough. With sound understanding of social and cultural background at each locality, application of the scientific findings to the society is essential.

Plenary Lectures

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Plenary Lecture
PL01

HABs in Aotearoa: climate change, high-tech solutions and kaitiakitanga

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Climate change is causing the range expansion of harmful algal bloom (HAB) species into new coastal regions. Marine heatwaves now occur regularly across the Pacific Ocean and Aotearoa New Zealand's coastal waters have been warming at nearly four times the global average. These changes are impacting HABs and causing the expansion of tropical/sub-tropical species into Aotearoa New Zealand's historically temperate region. For example, *Gambierdiscus* and *Fukuyoa* are now established in the northern regions. Understanding the risk of emerging seafood poisoning syndromes, such as ciguatera, is difficult due to a lack of knowledge of the biogeography of causative species, variation in toxin production even at strain level, limited understanding of the flux of toxins in the food-web, and difficulties in analysing environmental and seafood samples. Our recent research in Aotearoa New Zealand and across the Pacific uses novel molecular and analytical chemistry technologies that are highly sensitive and specific for HAB species and the toxins they produce. This allows full characterisation of HAB species over wide geographic latitudes and can help determine how toxins accumulate through the marine food web. We are ultimately working towards fit-for-purpose monitoring techniques that are rapid, cheap, and easy to use for the communities that need them. For example, we are collaborating with indigenous communities in Aotearoa New Zealand to establish rapid-test kits for kaitiakitanga (guardianship)-based monitoring programmes, and for surveillance of mātaitai (food gathering areas) and kaimoana (seafood), to ensure safe seafood harvests and a reduction in the often-debilitating foodborne illnesses caused by HABs.

Plenary Lecture
PL02

Harmful algal blooms (HABs) in the tropics: What have we learned in Malaysia for the past two decades?

Po Teen Lim[1], Kieng Soon Hii[1], Sing Tung Teng[2], and Chui Pin Leaw[1]

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Malaysia has been constantly affected by Harmful Algal Blooms (HABs). In this talk, I'll review what we have learned about PSP, ASP, CFP and BHABs and Ichthyotoxic algae. PSP was no longer restricted to Sabah or *Pyrodinium bahamense* but eventually involved more toxin producers in wider area. Region-wide distribution of *Alexandrium tamiyavanichii* has been confirmed by qPCR and metabarcoding. What we learned from the prolonged blooms of *A. minutum* and underlying bloom mechanism will be shared. Although no human amnesic shellfish poisoning (ASP), high species diversity of *Pseudo-nitzschia*, (>30 species), was discovered and confirmed morphologically and genetically, including nine novel species. Toxigenic diatom *Nitszchia navis-varingica*, despite its low density, was widely distributed in coastal water. The contamination of domoic acid in shellfish mollusks and stranded marine mammals indicated the risk of ASP in this region. The unprecedented blooms of *Karlodinium australe* in 2014-2015 have resulted in massive mortality of wild and cultured fishes in Johor strait, where the economic losses have reached record level. This situation was exacerbated further by the frequent fish-killing algal blooms along the waters. The fish killing mechanisms of some harmful species were clarified. The use of NGS and development of HAB database for metabarcoding should be adopted as a remedy to unpredictable emerging event. Several Ciguatera poisoning events were confirmed since 2017. Our research on benthic harmful algal communities at different microhabitats pointed to the scenario that degradation of coral reefs would provide favorable microhabitats for the proliferation of *Gambierdiscus* and *Ostreopsis* spp. Understanding of HABs under the influence of climatic and non-climatic drivers is crucial for better management and mitigation of the impacts.

Plenary Lecture
PL03

Are the members of the genus *Gambierdiscus* responsible for ciguatera poisoning in coastal Japan?

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Occasional and sporadic outbreaks of ciguatera poisoning (CP) have been reported in the subtropical coastal regions of Okinawa and the temperate regions of mainland Japan, respectively. We have attempted to clarify ciguatoxin producers in Japan. First, we analyzed the phylogeography and intraperitoneal toxicity of *Gambierdiscus* spp. in mice, which revealed three toxic species and one nontoxic species of the genus distributed in shallow waters of Japan. Among these, the toxic species *G. scabrosus* and nontoxic *G. jejuensis* are predominant in subtropical and temperate Japanese waters, respectively. The results suggested that *G. scabrosus* may be involved in CP in Okinawa. We found *G. silvae* in deep waters in Japanese coastal waters. This species showed acute oral toxicity and *i.p.* toxicity in mice, suggesting its involvement in CP. However, toxin analysis using LC-MS/MS revealed no ciguatoxins (CTXs) in any of the five *Gambierdiscus* species mentioned above, including *G. silvae*. Recently, three novel phylotypes of *Gambierdiscus* were discovered by metabarcoding. However, the strains of the novel phylotypes have not yet been established, and their CTX productivity has not been clarified. Considering all these results, three possibilities regarding ciguatoxin producers in Japan arise: 1) *G. silvae* may produce "CTX-related" compounds that cause CP in Japan; 2) the three novel phylotypes, discovered by metabarcoding, may produce ciguatoxins; and 3) unidentified organisms, including those not belonging to *Gambierdiscus,* may be involved in CP. Extensive research is underway to identify the source of CTXs in Japan that result in CP.

Plenary Lecture
PL04

The significance of parasitic interactions in the ecology of harmful microalgae

Esther Garcés[1]

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The idea that the diversity of a community can influence pathogen transmission and dynamics can be traced back over 50 years, when C.S. Elton observed that "*outbreaks (of infectious diseases) most often happen on cultivate or planted land… that is, in habitats and communities very much simplified…*". Based on our current understanding of the ecological framework of host-parasite interactions, the structure of the host community can indeed lead to diverse effects in terms of parasite emergence and transmission. By applying the concept of "*simplified biological communities*" in the marine ecosystem, we focused on high-biomass dinoflagellate blooms, which represents a transient state of low diversity in microplankton communities locally dominated by single species. Using this perspective, we closely examined marine interactions involving blooming dinoflagellates and their parasites, namely specialists and generalist zoosporic parasitoids in the group of Perkinsozoa (Alveolata) and Chytrids (Fungi). Our data revealed the conspicuous presence of these parasitoids in these high-density mono-specific blooms of the species *Alexandrium minutum*, *A. taylori*, *Dinophysis sacculus*, *Barrufeta bravensis*, *Levanderina fissa*, *Gymnodinium litoralis*, *Ostreopsis* sp. and *Durinskia baltica*. Numerous parasitoid species were recurrently detected, sometimes coexisting in the same host species, and clearly some of them representing new diversity yet to be described. These parasitoids appear to have adapted to their blooming hosts, thereby facilitating the maintenance, spread and recurrence of the parasite population. By providing new insights into the diversity, physiology, and ecology of marine parasites, particularly zoosporic parasites infecting phytoplankton, we can develop more comprehensive approaches to the dynamics of harmful algal blooms.

Plenary Lecture
PL05

Can plastic pollution amplify the spread of toxins from benthic microalgae through the food web?

Luiz L. Mafra Jr.[1], Estela Pires[1], Talita V. V. Batista[1], Isabel do P. Leite[1], Maria Carolina S. Kutz[1], Jennifer C. G. da Silva[1], Mariana Matiazzi da Silva[1], Ana Julia R. Soteras[1], and André Menegotto[2]

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Marine plastic debris provides a significant surface area for potential colonization by harmful microalgae and for the adsorption of their toxins. Furthermore, floating plastics are expected to substantially expand the substrate area available for benthic microalgae in the ocean, intensifying the transfer of potent toxins through pelagic food webs. In a series of laboratory trials using different analytical approaches, we demonstrated that the colonization of benthic microalgal cells and the adsorption of lipophilic toxins depend on the exposure time and different characteristics of the plastics, including polymer composition and the particle size, shape and aging degree. We also measured the toxin uptake and potential harmful effects of microplastic (MP) particles, coated or not with okadaic acid (from *Prorocentrum lima*) or ovatoxins (from *Ostreopsis* cf. *ovata*), to brine shrimps, mussels and microalgal cells. In parallel, using secondary data, we quantified the available surface area of MP and macroplastic debris of different sizes and shapes in multiple oceanic regions to evaluate the potential role of floating plastics as vectors for the transfer of toxins from three widespread benthic dinoflagellates: *Gambierdiscus* spp., *O.* cf. *ovata* and *P. lima* complex. This was finally assessed in a field survey, in which the presence of harmful microalgae and toxins was examined in dead-stranded sea turtles containing varying amounts of plastic debris in their digestive tracts. Altogether, our findings indicate that plastic pollution may increasingly exacerbate the negative impacts of benthic harmful algal blooms to marine organisms and human health in certain coastal areas.

Plenary Lecture
PL06

Development of HAB mitigation using modified clays in China

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The impacts of harmful algal blooms (HABs) on economies, public health, ecosystems, and aquaculture across the globe have all increased in recent decades, and this has been acutely the case in China. Flocculation is an essential approach to control HABs in the ocean, comparing with other means. However, the application of traditional flocculants in marine environment is limited, as the insufficient aggregation and slow settlement of them to HAB organisms caused by hydrodynamic influence, wave disturbance, etc. Such limitations can be overcome by Modified Clay (MC) technology. Using natural clay particle as a carrier, the surface of which is physically modified as to enhance the electrical neutralization, to strengthen the function of absorption bridging and sweep netting, and to improve the algicidal ability. As a result, the HAB organisms can be aggregated effectively and settled down to the bottom rapidly. The technology was first used in the field in 2005, and has been developed about twenty years in China. Up to now, it is the most accepted method and widely used technology in China. A MC based comprehensive technology system for HAB control has been established, including development of functional MC products and supporting equipment, eco- and environmental evaluations of MC, standards and protocols for on-site applications, etc. Within this presentation, the research, development, and application of MC technology in China would be generally introduced, focusing on the principle and theory, safety assessments, and application cases. This also provides a reference for HAB mitigation in other countries of the world.

Plenary Lecture

PL07

Chemical studies on biosynthetic and metabolic pathways of marine toxins

Mari Yotsu-Yamashita[1], Yuta Kudo[1,2], Shigeki Tsuchiya[1], Satoshi Numano[1,3], Yuko Cho[1], Keiichi Konoki[1], Yasukatsu Oshima[4], and Kazuo Nagasawa[5]

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The pufferfish toxin, tetrodotoxin (TTX), and the paralytic shellfish toxin (PST), saxitoxin (STX), are the major marine neurotoxins that are regulated due to concern of lethal food poisonings. These toxins are potent and specific blockers of $\mathrm{Na_v}$. TTX is detected both in marine animals and terrestrial amphibia. Biosynthetic genes of TTX have not been discovered yet, even though it is drawing high attention. We have been approaching TTX biosynthesis by finding many natural analogues and related compounds mainly from pufferfish and newts. Comparison of the chemical structures of TTX related compounds between pufferfish and newts suggested different biosynthetic pathways towards TTX between marine and terrestrial environments. The stepwise and multiple oxidation reactions were suggested in the marine environments, while monoterpene origin was suggested in the terrestrial environments. On the other hand, the putative genes of STX biosynthesis have been reported from the toxin producing cyanobacteria and dinoflagellates. The several STX biosynthetic enzymes have been heterologously expressed and their possible functions have been identified. However, the process of formation of the tricyclic skeleton of STX and order of the oxidation reactions in the ring and side chain are remained unclear. We chemically synthesized putative biosynthetic intermediates of STX to identify them in the toxin producing dinoflagellates and cyanobacterium, as well as to perform the incorporation experiments using the stable isotope labeled compounds. In this symposium, we will present our recent results for biosynthetic and metabolic pathways of TTX and PSTs.

Plenary Lecture
PL08

Global warming and harmful cyanobacterial blooms: from scientific insights to societal impacts

Dedmer B. Van de Waal[1,2]

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[2] Institute of Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands.

Global warming is expected to increase the occurrence, magnitude, and duration of harmful cyanobacterial blooms worldwide. At the same time, societal demands for clean surface water are increasing with hotter and drier summers. Yet, we currently lack a global view on the impacts of warming on cyanobacterial blooms, and the way these are perceived by society. Moreover, intensified use of surface waters will call for optimized monitoring that assesses human health risk, while avoiding unnecessary closure of surface waters. In this talk, I will present a global analysis where we assess the impact of warming on public awareness of harmful cyanobacterial blooms. Moreover, I will explore how near real-time measurements can help make short-term forecasts of cyanobacterial blooms. Together, these may guide the development of tailored monitoring programs and early warning signals for harmful cyanobacterial blooms.

Plenary Lecture
PL09

Lessons from *Pseudo-nitzschia* around the world

Vera L. Trainer[1], William P. Cochlan[2], and Charles G. Trick[3]

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Cells of the diatom genus *Pseudo-nitzschia* are cosmopolitan, found throughout the World's oceans. However, the expression of these cells as toxic *Pseudo-nitzschia* blooms is more sporadic in space and time. *Pseudo-nitzschia* blooms are most frequently found in upwelling zones, causing economic, social, and cultural damage to communities through the transfer of the taxon-specific neurotoxin, domoic acid, to shellfish, planktivorous fish, and mammals. For these blooms to have such impacts, they must achieve either very high cell densities or be dominated by cells with elevated domoic acid levels. Anticipating toxic events caused by *Pseudo-nitzschia* has proven difficult because no single driver triggers the production of domoic acid. However, research on *Pseudo-nitzschia* events and culture experiments worldwide has provided insights into the conditions promoting increased toxigenicity and rapid, specific growth rates in this genus. Elevated toxin production by some species of *Pseudo-nitzschia* is linked to both macro- and micro-nutrient availability. In contrast, the ability of *Pseudo-nitzschia* cells to proliferate quickly is linked to their aptness to acquire macronutrients with greater efficiency than other phytoplankton species, including many other diatoms. Upwelling HAB species are usually adapted for both low or pulsed nitrate supply, readily enabling *Pseudo-nitzschia* to acquire nitrate at both elevated and low ambient concentrations. This, together with the taxon's distinctive response to low iron conditions, and a competitive iron affinity system permit *Pseudo-nitzschia* to persist and then to grow rapidly when exposed to iron-replete waters. These strategies, along with others, contribute to the resilience and worldwide success of *Pseudo-nitzschia*.

Oral Communications

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Taxonomy

O-001

New records of three raphidophytes in Philippines: Taxonomic identification and phylogeographic insights

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Marine raphidophytes are notoriously known to cause devastating harmful algal blooms in many countries worldwide. However, of the 7 genera, only one species, *Chattonella subsalsa* was so far reported from Philippine waters. In this study, three previously undocumented species of raphidophytes were detected in Zambales, Central Luzon and their unialgal cultures were successfully established. Cellular morphology was examined using light microscopy and molecular phylogeny was inferred using partial LSU rDNA and ITS sequences. Cells of *Chattonella marina* were teardrop- to ovoid-shaped, measuring 30.6 μm long, and chloroplasts were yellowish-brown and oval or irregularly shaped. Cells of *Fibrocapsa japonica* were typical, which was oval-shaped, 20.15 μm long, and possessed prominent striations at the posterior end. Cells of *Heterosigma akashiwo* were small (12.19 μm long) and asymmetrical in shape. Phylogenetic analyses showed that *C. marina* from the Philippines was positioned in a well-supported clade composed of *C. marina* from Korea, Japan, Brazil and Hong Kong; *F. japonica* clustered with *F. japonica* from the USA, Brazil and Germany; and *H. akashiwo* was positioned in the clade with *H. akashiwo* from Norway, Japan and USA. Furthermore, the brine shrimp lethality assay revealed that the highest experimental cell concentration (7.27×10^5 cells L^{-1}) of *F. japonica* exhibited 10% *Artemia* mortality after 48 h exposure while *H. akashiwo* showed 40% *Artemia* mortality at maximal cell concentration (1.11×10^6 cells L^{-1}) after 48 h of exposure. This is the first report of *C. marina*, *F. japonica* and *H. akashiwo* in the Philippines. Their occurrences in this region suggest potential risks to aquaculture farming and further investigation is warranted.

Taxonomy
O-002

Genetic differentiation between red and green types of *Noctiluca scintillans* in Jakarta Bay, Indonesia

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Noctiluca scintillans is a globally distributed harmful algal bloom (HAB) species that causes fish mortality and damage to fisheries. It exhibits two distinct appearances, i.e., red and green, with red *N. scintillans* (RNS) being widely distributed and green *N. scintillans* (GNS) being restricted to tropical waters. The taxonomic relationship between these two types, same species or not, remains unclear. In this study, therefore, we investigated the genetic sequences and morphological characteristics of *Noctiluca* in coastal Indonesia, where both red and green types exist. The cell surface of Indonesian specimens (RNS) exhibited rough surfaces, whereas that of the Japanese specimens collected for comparison had smooth surfaces. Genetic analysis revealed considerable divergence in the sequences of RNS from Indonesia compared to those from other known sequences registered in GenBank, suggesting the presence of a distinct Indonesian RNS species. Furthermore, genetic analysis revealed that Indonesian GNS formed a separate clade from the Indonesian RNS population, suggesting that the two types are distinct species. These findings provide the first evidence of coexisting RNS and GNS types in the same location and highlight their distinct genetic compositions. Consequently, this study provides a new perspective on the current taxonomic classification of the *Noctiluca* genus, which currently recognizes only one species. These results highlight the importance of revisiting the taxonomy of *Noctiluca* and considering the genetic and morphological differentiation within this globally distributed HAB species.

Taxonomy
O-003

Phylogenomic analysis of the genus *Brasilonema* (Nostocales, Cyanobacteria) with the descriptions of two novel species: *B. kendallianum* sp. nov., *B. urbanum* sp. nov.

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Terrestrial or subaerophytic environments are potentially inhabited by diverse cyanobacterial lineages that are relatively less studied than aquatic and benthic counterparts. *Brasilonema* is a terrestrial genus of cyanobacteria that grows in a broad range of subaerophytic habitats, from subtropical to tropical areas. To further explore the diversity of *Brasilonema*, cyanobacterial material was collected from an urban environment, isolated, and identified using morphology, 16S rRNA phylogenies, 16S-23S rRNA ITS sequence dissimilarities, and phylogenomics. Three species of *Brasilonema* were identified, two of which are new to science: *B. kendallianum* sp. nov. and *B. urbanum* sp. nov. Our phylogenomic analyses also corrected the classification of two established species, *B. octagenarum* and *B. tolantongensis*. ELISA and LC-MS data indicated that microcystin production was found for the first time in five *Brasilonema* species: *B. santannae* (BLCC-T43), *B. tolantongensis* (BLCC-T49, BLCC-T61, BLCC-T120), *B. fioreae* (BLCC-T72), *B. octagenarum* (BLCC-T74), and *B. urbanum* sp. nov. (BLCC-T112). Investigation into the influence of nitrogen form (nitrate, ammonium, urea) and concentration (BG_{11}, BG_{11}-halved nitrogen, BG_{11o}) on toxin production revealed that ammonium and urea did not induce the production of microcystin, while BG_{11}-halved nitrogen was observed to cause high levels of microcystin. The application of this work provides new insights into the phylogenomic diversity and the toxin production of these terrestrial cyanobacteria.

Taxonomy
O-004

Influx of toxin-producing *Pseudo-nitzschia* from the Northeast continental shelf to an east coast US estuary

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Pseudo-nitzschia, a cosmopolitan neurotoxin-producing diatom genus, has been observed for decades in Narragansett Bay (NB), Rhode Island, USA but led to toxin-related shellfish harvest closures for the first time in 2016-17. Species of this genus vary in their ability to produce domoic acid, so taxonomic identification is critical for understanding the ecological drivers of these harmful algal blooms. Since many *Pseudo-nitzschia* species are morphologically indistinguishable under light microscopy, we used molecular barcoding of the internal transcribed spacer region 1 (ITS1) and high throughput sequencing to characterize species composition. From over a decade of monthly samples, we found that *P. australis*, a high domoic acid-producing species, was not present in NB until just prior to the 2017 closure, meaning it was recently introduced to NB and likely played a role in the toxic event. The appearance of *P. australis* has motivated further research investigating recurring or episodic influxes of species into NB from the Northeast continental shelf (NES). In collaboration with the NES LTER project, we sequenced samples from nine biannual cruises along a transect originating about 40 km from the mouth of NB and extending to the NES break. To better understand the ecological connectivity of these two systems, we compared NES species composition to that of NB during the four weeks prior and following each cruise. High similarity of winter NB and NES *Pseudo-nitzschia* assemblages suggests greater ecosystem connectivity than in summer months, which may lead to increased frequency of species influx to NB during the winter.

Taxonomy

O-005

Key biosynthesis processes of proteins containing neurotoxin *β*-*N*-methylamino-*L*-alanine in the endoplasmic reticulum of marine diatoms

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The neurotoxin *β*-*N*-methylamino-*L*-alanine (BMAA) has received widespread attention for damaging marine ecosystems and threatening human health. Here, stress from environmental factors can be a stimulus for the synthesis of BMAA-containing proteins in diatoms, and differential centrifugation was used to determine the distribution of BMAA in organelles. The production of BMAA-containing proteins was significantly stimulated and was universal, the BMAA yield of three diatoms (*Thalassiosira andamanica*, *Thalassiosira allenii* and *Thalassiosira minima*) increased by 2.2-fold, 2.5-fold and 7.7-fold, respectively, when diatom growth was limited by iron. The changes of transcriptome pathway under iron limitation combined with the results of co-culture of diatoms and cyanobacteria, the pathways possibly related to BMAA-containing proteins biosynthesis were valine, leucine and isoleucine degradation; arginine biosynthesis; alanine, aspartate and glutamate metabolism; protein processing in endoplasmic reticulum; SNARE interactions in vesicular transport and proteasome. Deeply, the differential centrifugation determined that BMAA-containing proteins was mainly distributed in the endoplasmic reticulum and the Golgi apparatus. Based on the findings in both studies, we speculate that the synthesis of BMAA-containing proteins may arise from a simple catalytic reaction. Cysteine embedded in the peptide chain was used as a substrate, which was catalysed by the cysK enzyme to form BMAA-containing proteins together with methylamine. Moreover, the reduced ubiquitination-mediated proteolysis and vesicle trafficking precision through the COPII system will also aggravate the accumulation of BMAA-containing proteins in diatom.

Taxonomy
O-006

Rapid classification of harmful algae using holographic microscopy

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Holographic microscopy has a greater depth of field than conventional microscopy imaging and can rapidly image all algae in a water body with a certain thickness. However, the complex holographic image processing process takes longer time, which weakens the advantages of holographic imaging. Therefore, this study proposes a method to detect the raw hologram using neural network, which greatly reduces the detection time based on achieving the accurate identification of harmful algae. In this study, a coaxial 28-fold magnification holographic microscopic imaging system based on the Mach-Zendel interferometer structure was established to image a 200 μm thickness of water. Eight algae species of harmful algae, *Alexandrium tamarense* (AT), *Chattonella marina* (CM), *Karlodinium veneficum* (KV), *Heterosigma akashiwo* (HA), *Karenia mikimotoi* (KM), *Prorocentrum micans* (PM), *Prorocentrum donghaiense* (PD), and *Prorocentrum lima* (PL), were selected as the target species. The detection of the raw hologram is performed by training a neural network, which saves the time of running the algorithm while ensuring the accuracy. We believe that the proposed method represents an effective approach for the real-time algae monitoring.

Community/Species Interactions
O-007

Free fatty acids and reactive oxygen species as potential allelopathic substances affecting *Gymnodinium catenatum* (Gymnodiniales: Gymnodiniaceae)

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Allelopathy refers to a biochemical interaction among competing microalgae in which donor species produce specialized metabolites that can cause inhibitory effects on the growth of susceptible species. This phenomenon can participate in the regulation of harmful algal blooms. The dinoflagellate *Gymnodinium catenatum* is negatively affected by allelopathic interactions with the raphidophyte *Chattonella marina* var. *marina*. This study explored the effect of free fatty acids (FFA) and reactive oxygen species (ROS), which are known to be produced by *C. marina* and were previously suggested as potential allelochemicals affecting *G. catenatum*. An analysis of *C. marina* detected 16:0 (12.5 ± 0.01%), 18:4ω-3 (15.4 ± 0.37%) and 20:5ω-3 (35.4 ± 0.70%) as the most abundant fatty acids during the exponential phase. These fatty acids and hydrogen peroxide (H_2O_2) were used individually in dose-response bioassays with cultures of *G. catenatum* in exponential phase. Results show that these metabolites affect chain-forming qualities and morphology, including loss of motility and signs of chlorosis in *G. catenatum*. The fastest mortality upon fatty acid exposure was observed with the polyunsaturated fatty acid 18:4ω-3 (LD_{50} = 1.7 ± 0.19 mg L^{-1} at 24 h). Exposure to H_2O_2 caused mortality mainly after 12 h (LD_{50} = 112.8 ± 7.51 μM). In addition, FFA and H_2O_2 increased superoxide dismutase (an antioxidant) activity in *G. catenatum*. These results suggest that FFA and ROS are, at least partially, involved in allelopathic interactions affecting *G. catenatum* and contribute to elucidate their potential role as allelochemicals in marine environments.

Community/Species Interactions
O-008

Allelopathic effect on competition between the epibenthic dinoflagellates *Amphidinium thermaeum* and *Coolia malayensis* in laboratory conditions

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The genera *Amphidinium* and *Coolia* are typical co-existing species in marine benthic ecosystems. They produce a wide range of secondary metabolites within variable amounts of mucilage that may cover their substrate; however, the control mechanism of colonization and competition is unclear. We propose that it might be through allelopathic interactions. In the current study, the allelopathy between *Amphidinium thermaeum* and *Coolia malayensis*, both isolated from Bahía de La Paz, Gulf of California, were analyzed. Three experiments were performed using 300 mL allelopathy flasks: i) mixed cultures, ii) both species cultured without cell contact (separated by a membrane of 5 μm), and iii) cultures with the addition of 50 and 100% of cell-free filtrates. For all bioassays, cells of each species were harvested during the exponential growth phase. All experiments were inoculated with initial densities considering cell biovolume ratios of 1:1 and 1:2. The results showed the dominance of *A. thermaeum* in mixed cultures, as well as the effects on growth and morphological changes in *C. malayensis* (loss of motility, chlorosis, ecdysis, and cell lysis). Similar effects were observed in cultures exposed to cell filtrates without dilution. In cultures without cell contact, no allelopathic effects were observed in any of the species. These species appear to compete for space through allelopathy, mainly through contact, which is reflected in a decrease in cell density. Mucilage plays a direct and possibly active role in the transport of allelochemicals by increasing the surface of contact.

Community/Species Interactions
O-010

Unraveling energy allocation and predator interactions in toxic diatoms: Insights into *Pseudo-nitzschia* ecological dynamics

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Pseudo-nitzschia is a toxic diatom species that pose a significant risk to marine ecosystems. The impact on the marine food web is assessed continuously. Recent research has uncovered a fascinating interaction between *Pseudo-nitzschia* and its predators, copepods. Copepods release a specific amide compound called copepodamide when they are present. This compound serves as a signal to induce se toxin production in *Pseudo-nitzschia* diatoms. *Pseudo-nitzschia* produces toxins as an efficient defense mechanism against predation. To build upon this direct observation study, this study aims to investigate how *Pseudo-nitzschia* allocates resources when exposed to copepodamides under limited light conditions, assessing the trade-offs between growth and defense. We analyzed silicate content, toxin levels, growth rates, and carbon, nitrogen, and phosphorous allocation in *Pseudo-nitzschia seriata* to track energy usage under multiple stressors. Toxin-producing *Pseudo-nitzschia* can sustain approximately 50% of the growth rate exhibited by non-toxic algae, indicating a substantial energetic cost associated with toxin production. Understanding the energy allocations in *Pseudo-nitzschia* under multiple stressors provides valuable insights into the ecological dynamics of toxic diatoms and their interactions with predators. Moreover, it sheds light on the potential impacts of copepod-induced stress on population dynamics and toxin production.

Community/Species Interactions
O-011

Cluster analysis reveals different HAB-related species assemblages in the northern Adriatic

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The Northern Adriatic is a shallow, semi – enclosed coastal sea with steep, spatio-temporal gradients of ecological factors. This ecosystem is characterised by a dynamic and rich phytoplankton community, mostly dominated by diatoms in terms of total abundances. Phytoplankton dynamics is under the influence of freshwater nutrient input by Po river. Proximity of coast enabled monthly sampling and good spatial resolution in the period from 2020 – 2023. Several potentially harmful blooms of diatoms *Pseudo-nitzschia* spp. and domination of dinoflagellates like *Gymnodinium* spp. and *Azadinium* spp. were observed. In order to perform detailed analysis of phytoplankton succession, clustering method was used instead of seasonal grouping of samples which often tends to be used in phytoplankton analysis. Clustering revealed the succession of species assemblages in which different indicator species were found. Some of these indicator species were either responsible for blooms or were dominant in certain timespans over the year. Analysis showed that clusters differed in abundances, diversity and seasonality in succession. Ordination showed that *Pseudo-nitzschia delicatissima* complex is positively correlated to temperature or salinity, while surprisingly, small dinoflagellates are not. A shift from bimodal to unimodal succession in the northern Adriatic could be connected with dinoflagellate domination in spring.

Community/Species Interactions
O-012

Growth and morphological responses of *Dolichospermum flos-aquae* to grazing by protists

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Lake ecosystems around the world are suffering from harmful cyanobacterial blooms as a result of global warming and eutrophication. Interspecies interaction is among the major factors affecting cyanobacterial bloom development. Nitrogen-fixing cyanobacteria like *Dolichospermum* can achieve nutrient compensation through heterocyst differentiation for rapid population growth. Recently, we found that the protist *Paramecium multimicronucleatum* is able to graze on the filamentous *D. flos-aquae*, partially suppressing the population growth of cyanobacteria. Protozoan grazing reduced the number of short filaments remarkably, resulting in the increased average length of filaments in cyanobacterial population. In addition, heterocyst differentiation was induced by protozoan grazing. The higher the grazing intensity faced by cyanobacteria was, the earlier the heterocyst was formed, together with the higher frequency of heterocyst detected. Transcriptome and proteome analysis revealed that grazing by *Paramecium* induced changes of multiple pathways in *Dolichospermum*, including carbon fixation, biofilm formation, nitrogen metabolism, pentose phosphate pathway, oxidative phosphorylation, photosynthesis, ABC transporters and others. In particular, the genes encoding nitrate/nitrite transport system substrate binding protein and nitrogenase molybdenum ferritin chain in nitrogen metabolism were significantly up-regulated, indicating the enhanced nitrogen fixation. The pathways associated with phosphorylation and carbon fixation were also upregulated with predicted increased energy production. The findings highlight the possible ecological function of heterocyst differentiation in mediating protozoan and nitrogen-fixing cyanobacteria interactions.

Ciguatera and Benthic HABs
O-013

Discovering bioactive compounds produced by toxic *Gambierdiscus* species

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The genus *Gambierdiscus* produces a complex array of bioactive secondary metabolites, which range in toxicity and mode of action. These include ciguatoxins (CTXs), maitotoxins (MTXs), gambierones, gambieric acids, gambierol and gambieroxide. While CTXs are known to be the causative agents of ciguatera poisoning (CP), the contribution of the other secondary metabolites is not yet understood. Adding to the complexity of CP, only *G. polynesiensis* has been definitively shown to produce Pacific CTXs using liquid chromatography mass spectrometry (LC-MS), yet the known distribution of this species does not align with global intoxication events. In addition, most species display some bioactivity when using *in vitro* and *in vivo* assay techniques, demonstrating the presence of unknown compounds. Discovering the bioactive compounds from these additional *Gambierdiscus* species is, therefore, paramount to determining their role in CP. Two recently described species, *G. cheloniae* and *G. honu*, were the subject of a compound discovery project where MTX-6, MTX-7 and several gambierone analogues were identified. With the inclusion of these new toxins, the secondary metabolite profiles of *Gambierdiscus* and co-occurring benthic dinoflagellate species was mapped using targeted LC-MS analysis. Representative isolates from thirteen *Gambierdiscus*, three *Fukuyoa* and five *Coolia* species were quantitatively analysed for CTXs, MTXs and gambierones, and qualitatively analysed for gambieric acids, gambierol and gambieroxide. The results, when coupled with toxicity data, have highlighted several species that are good candidates for future compound discovery projects. This is the most comprehensive study performed on the secondary metabolite profile of the *Gambierdiscus* genus to date.

Ciguatera and Benthic HABs
O-014

Effects of temperature, salinity, and their interactions on growth and ciguatoxin production, of the benthic dinoflagellate *Gambierdiscus polynesiensis*

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Gambierdiscus polynesiensis produces ciguatoxins (CTXs), causative agents of ciguatera poisoning (CP). This species is found in the tropical/subtropical Pacific regions, including French Polynesia, the Cook Islands, and Rangitāhua/Kermadec Islands (a territory of Aotearoa/New Zealand). Warming seawater and changes to salinity levels along New Zealand's coastal waters are forecasted in the near future, and this may increase the risk of *G. polynesiensis* proliferating in the region. It is, therefore, important to evaluate the effects of temperature and salinity on the growth and production of CTXs by this species. This study evaluated the growth characteristics of *G. polynesiensis* strain CAWD267, isolated from Rarotonga (Cook Islands), based on a growth experiment at five temperatures (17.5, 20, 25, 27.5, and 30 °C) in combination with four salinities (25, 30, 35, and 40). Production of CTXs was evaluated using cell pellets harvested at the stationary phase, for each combination above, using a liquid chromatography-tandem mass spectrometer. The strain grew at 20–27.5 °C and at all salinity levels tested. The maximum values of growth rate and cell density were 0.33 divisions/day and 785 cells/mL, respectively, at the conditions 25 °C and salinity 35. On the other hand, the maximum values of CTX cell quota, CTX concentration, and CTX production rate were 29.9 pg/cell, 18.2 ng/mL, and 1.01 ng/mL/day, respectively, at the conditions 27.5 °C and salinity 35. This result suggests a condition of 27.5 °C coupled with salinity 35 may be a critical factor in the risk assessment of CP occurrence associated with *G. polynesiensis*.

Ciguatera and Benthic HABs
O-015

Mapping the predicted distribution of the benthic dinoflagellate *Gambierdiscus polynesiensis* after forecasted global warming in New Zealand

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The effects of a warming ocean are anticipated to have significant implications for seafood safety. *Gambierdiscus polynesiensis*, a dinoflagellate species known to produce ciguatoxins (CTXs) responsible for ciguatera poisoning (CP), is found in the tropical/subtropical Pacific regions, including the Cook Islands and Kermadec Islands, which are part of New Zealand's territory. With the increasing concern of range expansion of this species due to associated climate change, understanding the potential risks of CP in New Zealand waters is critical. This study mapped the predicted distribution of *G. polynesiensis* strain CAWD267 in response to forecasted changes in ocean temperature and salinity. Specifically, temperature-salinity relationships of CAWD267 were revealed through laboratory growth experiments and toxin analyses and combined with multiple global climate model (GCM) scenarios. Statistical modeling techniques were used to develop a spatially explicit risk assessment of ciguatera HABs and associated toxins in a changing ocean. Preliminary results indicate a notable expansion of suitable habitat for *G. polynesiensis* in the future, driven by the projected changes in ocean temperature and salinity. The model outputs reveal potential hotspots of *G. polynesiensis* growth along the coasts of New Zealand, highlighting areas with elevated risk of CP outbreaks. Further research is necessary to fully understand the complex interactions between environmental variables and the growth and toxin production of such CP-related species. However, the outcomes of this research will provide valuable insights for coastal management and public health strategies, to proactively mitigate and anticipate the associated risks on seafood consumers and human health.

Ciguatera and Benthic HABs
O-016

A multi-disciplinary mid-term evaluation of the impact of climate change on the occurrence of ciguatera in the Balearic Islands Archipelago

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The Mediterranean Sea is exposed to climate change and prediction exists of increased seawater temperature up to +3,5°C by 2100. *Gambierdiscus* spp. was first described in Crete in 2004, and *Fukuyoa paulensis* in the Balearic Islands (BI) in 2016. A study on the occurrence of *Gambierdiscus* spp. in the BI started in 2016 and is currently ongoing. The first occurrence of *Gambierdiscus* in the BI is set to 2016. Originally *G. australes* was considered the only species of the genus in the archipelago. Genetic analyses through Sanger sequencing, probe-based biosensors, and metabarcoding shows that *G. excentricus* and *G. carolinianus* are also present. *Gambierdiscus* spp. are not restricted to the warmest months and were detected in winter (December and January) when seawater temperatures may decrease below 13°C. *Gambierdiscus* spp. reach values above 1000 cells/g wet weight of macroalga in the months of August, September and October in red (*Digenea simplex*, *Jania virgata*, and *Ellisolandia elongata*) or brown macroalgae (*Cladostephus spongiosus*, *Cystoseira sensu lato*, and *Dictyota dichotoma*). The spatial distribution of *Gambierdiscus* spp. includes the main 4 islands (Formentera, Ibiza, Mallorca and Menorca) with interannual persistence in all of them from 2017-2022. Although evidence of CTX-like toxicity in fish needs to be confirmed, we describe CTX-like toxicity of *G. australes* from the BI and presence of and MTX5, 44-methylgambierone, Gambierone, Gambieric acid A, B, C, and D through LC-MS/MS. We advise that ciguatera may become a major issue in the Mediterranean Sea in the future.

Ciguatera and Benthic HABs
O-017

Toxicokinetic mechanisms and food web dynamics of Caribbean ciguatoxins

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The prediction and management of ciguatera in seafood vectors in the Caribbean region remains a challenge due to the lack of integrated fisheries and cross regional data on the environmental and physiological factors that contribute to fish toxicity. Recently, we have been able to gain an improved understanding of the causative algal toxin sources, characterize new Caribbean CTXs and their metabolites in fish, and gained insight on the in vivo kinetics of bioaccumulation and depuration in marine species. However, CTXs are present in trace amounts in contaminated fish matrices creating a continuing analytical challenge. In this study, we examined and tracked a variety of CTXs from stationary primary producers through to higher predatory fish from the US Virgin Islands and other regions in the Caribbean. Composite toxicity was quantified, and species-specific toxin profiles (including multiple novel variants) were identified by LC-MS/MS and LC-HRMS methods. Both toxicity and toxin profiles were compared to size, age, sex, location, trophic position, and related stable isotope ratios as a proxy for diet. While relationships between size, age, and sex did not fully explain toxicity or toxin diversity in fish tissues, differences were identified between species and in offshore vs nearshore collected fish that were further evaluated using compound specific stable isotope analyses. Given the extensive impacts that ciguatera has in affected communities and the global reach of this public health issue, the synthesis of multiple data streams is long overdue and essential to effective risk management.

Ciguatera and Benthic HABs
O-018

Benthic harmful algal blooms in the Mediterranean Sea and the tropics: drawing from similarities and differences

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Under the combined pressures of climate change and existing human activities, the oceans and seas are experiencing habitat loss and community shifts. In the tropicalization of the Mediterranean Sea, nearly warming twice as fast as the global average, there has been significant increases in harmful algal bloom (HAB) events over the past three decades, with the toxin-producing microalgae of the genera *Ostreopsis* and *Gambierdiscus* of particular concern in coastal benthic ecosystems. These benthic harmful algae pose threats to human and environmental health and food safety, thereby affecting the livelihoods of coastal populations, and the sustainable use of the marine environment in blue economy sectors. While the occurrence and health impacts of *Ostreopsis* blooms are well-documented in the Mediterranean, the characterization of *Gambierdiscus*-related events primarily exists in tropical areas. This presentation aims to adopt a 'One Health' approach, utilizing field and laboratory studies, large dataset analyses, and literature reviews to explore the similarities and differences in adverse events related to *Ostreopsis* and *Gambierdiscus* in both tropical areas and the Mediterranean Sea. By bridging these knowledge gaps, we aim to gain valuable insights to enhance monitoring and prediction efforts for sustainable development, aligning with the objectives of the Ocean decade. Keywords: harmful algal blooms, benthic algae, *Ostreopsis*, *Gambierdiscus*, Mediterranean Sea, tropical regions, 'One Health' approach, food safety, coastal communities.

Taxonomy
O-019

Morphological and molecular taxonomy of epibenthic dinoflagellates from the Gulf of California

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Epibenthic dinoflagellates live associated to substrates on the seafloor. Some species can produce toxins and form benthic harmful algal blooms. However, taxonomic information about epibenthic dinoflagellates from Mexico is scarce, complicating the understanding of their diversity, biogeography, ecology, and toxicity. The objective of this study was the morphological and molecular identification of epibenthic dinoflagellates from the southern Gulf of California. Samples were collected in Bahía Concepción, Bahía de La Paz and in Ensenada de La Paz from 2015 and 2022. Individual cells were isolated mainly from macroalgae collected at depths of <10 m. Horizontal tows of the water column in shallow zones (<2 m) were also carried out. Thirty-four strains were identified by light and scanning electron microscopy, and partial sequences of 5.8S, 28S and ITS of rDNA were analyzed. The combination of these tools allowed the identification of 13 species of epibenthic dinoflagellates, belonging to the genera *Amphidinium*, *Coolia*, *Gambierdiscus*, *Ostreopsis*, *Prorocentrum*, *Symbiodinium* and *Vulcanodinium*. Characteristics were described to differentiate three complexes: *Prorocentrum cassubicum*/*P. norrisianum*; *P. micans*/*P. koreanum*; and *P. triestinum*/*P. redfieldii*. The taxonomic controversy between *P. mexicanum* and *P. rhathymum* could not be clarified. This study includes the first sequences of *Ostreopsis lenticularis*, *Gambierdiscus carpenteri*, *Prorocentrum concavum*, and for the first time *Prorocentrum norrisianum* was identified in the Gulf of California. Additionally, this study contributes to the knowledge of eight species of epibenthic dinoflagellates in which the production of toxigenic compounds has been reported (e.g., *Vulcanodinium rugosum*).

Taxonomy
O-020

Plastid molecular phylogeny of the unarmored dinoflagellate family Kareniaceae suggests multi-parallel haptophyte symbioses

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Establishment of permanent plastids through haptophyte endosymbiosis is a key evolutionary event that enabled unarmored dinoflagellates in the family Kareniaceae to form fish-killing blooms by photosynthesis. It has been generally believed that the haptophyte plastid was permanently established at a common kareniacean ancestor and inherited by modern species. However, several pieces of evidence conflicting this view have been recently found, making it questionable how many times haptophyte symbiosis occurred in this group. Here we show that kareniacean plastids are derived from multiple symbioses of haptophytes, rather than from a single symbiosis. By sequencing five plastid-encoded genes from 18 species in the Kareniaceae (*Karenia* 5 spp., *Karlodinium* 6 spp., *Takayama* 4 spp., and unknown affiliation 3 spp.), we resolved their molecular phylogeny. Compared with the host nuclear-encoded rDNA phylogeny, the topology of plastid phylogeny in *Karenia* was almost identical to that of host phylogeny, suggesting that plastids are inherited from the common ancestor of the genus. However, the topology of plastid phylogeny in *Karlodinium* and *Takayama* were mostly inconsistent with that of host phylogeny. Notably, *Karlodinium ballantinum* plastid was more than 99% genetically identical to a haptophyte *Emiliania huxleyi*, indicating the very recent plastid origin. Plastids of unknown affiliation 3 spp. were in haptophyte origins but inconsistent with their positions of host kareniacean phylogeny in any of them. From these results, we concluded that the Kareniaceae started from an ancestral species with transient haptophyte symbionts, and subsequently the 13 derivative lineages independently established the permanent plastids.

Taxonomy
O-021

A possible new dinoflagellates species in the family Ceratoperidiniaceae causing brick-red discoloration of the cultured yesso scallops in northern Japan

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Discoloration of bivalves is known to occur due to the accumulation of specific photosynthetic pigments in gland tissues from phytoplankton blooms. Discolored oysters were reported to cause economic damage to culture farmers. In 2018 and 2019, we observed brick-red discolorations of the cultured yesso scallops from Funka Bay and Sanriku Coast in northern Japan. To understand the causative plankton species and pigments, microscopic observations of seawater samples and LC/MS and H-NMR analyses of the colored midgut gland tissues of the scallops were conducted. Concerning the results, peridinin; which was dinoflagellate-specific and water-soluble carotenoid, was detected from the colored gland tissues, and predominance of a gymnodinioid photosynthetic dinoflagellate was observed from the same depths in Funka Bay as the discoloration of the cultured scallops was found. Thus, this unarmored dinoflagellate was concluded as a causative species, but the species could not be morphologically identified. We attempted further morphological and molecular phylogenic analyses from the naturally isolated cells. The cells possessed a circular acrobase around the apex connecting with the sulcus extension, honeycomb amphiesmal vesicles, and a hyaline membrane, which are common characteristics of the family Ceratoperidiniaceae. Additionally, some species-specific morphological characteristics were observed. In the phylogenic tree by LSU rDNA genes, the sequences of the cells were grouped under the genus *Kirithra* in the family Ceratoperidiniaceae but separated as a distinct taxon. These morphological and phylogenic features strongly suggest that the isolated cells are a new species.

Taxonomy
O-022

Morphology, biology and taxonomy of goniodomin-producing *Alexandrium* species: what we know and what we don´t

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Species of the dinophyte genus *Alexandrium* are widely distributed and are notorious bloom formers and producers of various potent phycotoxins. Among the 32 taxonomically accepted species there are six identified producers of goniodomins (GD), i.e. polyketide macrolides which may cause liver and thymus damage in mice, are cytotoxic, and have been associated with mortality in aquatic invertebrates. The species are *A. monilatum*, *A. hiranoi*, *A. pseudogonyaulax*, *A. taylorii*, and two newly described species *A. limii* and *A. ogatae*. Among those, *A. monilatum* is known to cause large fish kills in estuaries of the US east coast, *A. taylorii* is forming recurrent blooms in the Mediterranean, and *A. pseudogonyaulax* is of current concern in Europe, where new occurrences and high biomasses are being increasingly reported from northern temperate waters. All GD-producing species share a metasert or exsert first apical plate (not rhomboidal/insert) constantly disconnected from the pore plate, which is the trait used by Balech to define the subgenus *Gessnerium*. However, while all GD producing species form a highly supported monophyletic group in rRNA based phylogenetic analyses, several species of *Gessnerium* sensu Balech, for instance, *A. insuetum*, *A. margalefii*, and *A. pohangense* were grouped with other species in the subgenus *Alexandrium*. In this presentation we will review the current state of knowledge on GD producing species, including challenges in morphological differentiation, peculiarities in their behavior and life cycles, as well as species and strain-specific differences in toxin profiles.

Taxonomy
O-023

Exploring the diversity of small *Heterocapsa* species (Dinophyceae) in the NW Mediterranean

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The *Heterocapsa* genus includes cosmopolitan species prone to forming blooms in coastal and estuarine environments. Among them, *H. circularisquama* and *H. bohaiensis* represent toxic species responsible for shellfish mass mortality, and prawns and larvae of crabs mortalities, respectively. Although *Heterocapsa* blooms have been reported in various regions of the Mediterranean Sea, comprehensive investigations of their diversity in the NW Mediterranean are lacking. In this study, we cultured 14 *Heterocapsa* strains obtained along the Catalan Coast in the NW Mediterranean Sea, over a one-year period. Thought molecular characterization using LSU and SSU rDNA sequences, we were able to identify 7 distinct *Heterocapsa* species, including *Heterocapsa minima* and *Heterocapsa niei*. The remaining strains represent 5 new species proposed herein, based on phylogenetic and morphological analyses. The features of these newly discovered species were examined using various microscopy techniques. Additionally, haemolytic tests were conducted to assess their toxicity. To better understand their distribution in the study area, we analysed their presence in available metabarcoding datasets. Our findings reveal that some of the species have a wide distribution along the Catalan coast and presented high relative abundances. This study expands our knowledge of the diversity of small *Heterocapsa* species in the NW Mediterranean, and indicates that the *Heterocapsa* genus is far from being fully complete. The characterization of their morphology, toxicity, and distribution contributes to a comprehensive understanding of *Heterocapsa* blooms in the region, ultimately enhancing our ability to assess their ecological impacts and potential risks to human activities.

Taxonomy
O-024

New toxins and toxic species - and what about their names? – News from the IOC- taxonomic reference list group

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The number of toxic species are steadily growing, and thus is the IOC-taxonomic reference list of harmful species. New species are e.g. found in *Prorocentrum*, *Alexandrium*, *Centrodinium*, *Gonyaulax*, *Ostreopsis*, *Azadinium*, *Heterocapsa*, *Chattonella* and *Pseudo-nitzschia*. Species change names, but efforts trying to conserve some of those that are commonly used, like an ongoing effort to conserve the name *Alexandrium*. The list is continuously being updated. Work is ongoing on improving the taxonomic reference list e.g. by inclusion of verified DNA sequences and compiling a list of harmful but non-toxic species.

Community/Species Interactions
O-025

Two consequential changes in eukaryotic plankton diversity pertinent to occurrence and absence of HABs

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Species diversity and structure of plankton community reflect the quality and health of water, and thus indicate the influence of environmental factors and processes. Given that microalgal HABs are caused by rapid proliferation of one or a few toxic or deleterious species, it is reasonable to anticipate a shift in eukaryotic diversity between non-HABs and HABs periods. Here we report two consequential changes pertinent to HABs in eukaryotic plankton diversity. The first case is the effect of a natural dinoflagellate (*Prorocentrum donghaiense*) bloom on the structure and function of eukaryotic plankton community. The results showed significant differences in plankton community structure between blooming and non-blooming periods. The species richness was positively correlated to resource use efficiency (RUE), and more importantly, the cell density of *P. donghaiense* exhibited significant negative correlation with RUE. Our results demonstrated HABs reduce RUE via reducing species richness (corresponding to a less occupancy of the trophic niches), which supports the previously documented notion that niche partitioning enhances RUE. The second case is the effect of massive cultivation of commercially-viable seaweed *Gracilaria lemaneiformis* on the diversity of eukaryotic plankton community. We found that the biodiversity of eukaryotic plankton community was significantly higher in the seaweed cultivation area than that in the nearby control area as reflected in OTU richness, evenness and dominance for total plankton community. These results explicitly demonstrated *G. lemaneiformis* cultivation could enhance biodiversity of plankton community via allelopathy, which prevents one or several plankton species from blooming and consequently maintains a relatively higher biodiversity.

Community/Species Interactions

O-026

Characteristics and patterns of HABs in China coastal waters during the last 20 years

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A new focus is needed in HAB research to build understanding of how the HAB species may change with climate and how to setup models to predict and precaution HABs. Analysis of long term data is helpful to understand the patterns of change of HABs. The data of nearly 20 years indicate that the characteristics of HABs in China are year-round, whole sea-area, multiple species and high risk. They displayed distinct spatial-temporal characteristics. The large scale HABs mostly concentrated in the Bohai Sea and south-east China Sea, the primary organisms were dinoflagellate which lead to huge economic loss of mariculture and damage to ecosystem. The review will help better understand the rule of HAB in coastal waters, predict and early warning of HABs in China.

Community/Species Interactions
O-027

Allelopathy in marine dinoflagellates. What have we learned?

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Biotic factors such as allelopathy have been recognized to play a significant role in regulating marine microalgae communities. To understand the complex and subtle allelopathic interactions among microalgal species, extensive research is still needed. The dinoflagellate *Gymnodinium catenatum* is a paralytic toxin-producing species known to form harmful algal blooms (HABs) in several regions. The allelopathic response of *G. catenatum* to cells and cell-free media of co-existing species was evaluated. Cells and cell-free media of the raphidophyte *Chattonella marina*, and dinoflagellates *Margalefidinium polykrikoides* and *Gymnodinium impudicum* have allelopathic effects towards *G. catenatum*. Several triggering effects have been detected: growth inhibition, changes in cell morphology, and response to cellular stress, all of which lead to cell lysis; also, an increase in the activity of caspase-3 suggests an induction of programmed cell death. When transferring cells to media without allelochemicals, a low number of cells can survive, in which toxin content per cell increases; chain-forming cells have a higher growth rate than single cells, and temporary cysts can be formed, suggesting that the survival strategy of *G. catenatum* is migration through the chemical cloud, encystment, and an increased toxicity. Allelopathic studies in epibenthic dinoflagellates are scarce, due to the complexity of cultivating these species; however, similar responses, such as growth inhibition and alterations in cell morphology that lead to cell lysis are observed in *Coolia malayensis* when exposed to *Amphidinium* species. Information on biotic interactions allows decision-makers to foresee the consequences of human and climate-driven impacts on the fragile marine ecosystems.

Community/Species Interactions
O-028

Characterization of marine bacteria that support growth of *Heterosigma akashiwo* under phosphate-limiting conditions

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Heterosigma akashiwo occasionally forms dense bloom, resulting in its predomination in the local population. While eutrophication is linked to the bloom formation, the precise mechanism of the process is still obscure. Nitrogen and phosphorous (P) are macronutrients for the alga and are regarded to be limiting factors for bloom formation. The total concentrations as well as the chemical forms are important for the bioavailability of these components to the alga. For example, inorganic phosphate is generally regarded as the most bioavailable form of the element, while its concentration is much smaller than that of the dissolved organic phosphate in the marine environment. Marine phosphorous cycling driven by environmental microorganisms presumably plays a crucial role in the determination of bioavailability of the element to phytoplankton, thus the propagation of the organisms. Here, we attempted to identify marine bacteria that support the growth of *H. akashiwo* under the P-limiting condition. We isolated bacterial strains from marine sediments obtained from the areas where the bloom of the species is frequently observed. From the several isolates obtained from aerobic and anaerobic conditions, we identified a few bacterial strains that promote the growth of the alga under P-limiting conditions. We observed that *H. akashiwo* phagocytoses various bacterial strains, and some specific species supports the propagation of the alga, presumably utilized then as a P-source. Our study reveals the novel nutritional uptake pathway that may contribute to the propagation of the alga, or to the bloom formation.

Community/Species Interactions
O-029

The interactions among marine fungal chytrids and blooming dinoflagellates

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The function and role of aquatic fungi, including basal clades such as Chytridiomycota (chytrids), have been largely overlooked in marine environments. However, recent research has emphasized that marine fungi, as osmo-heterotrophs, play a significant role in biogeochemical cycles. In this study, we show that fungi are an important component of communities during coastal dinoflagellate blooms. Fungal groups, primarily Ascomycota and Basidiomycota, undergo changes over time as the bloom progresses, and chytrids start to develop when dinoflagellates reach peak abundances. Chytrids encompass both saprotrophic and parasitic fungi that influence the growth and development of dinoflagellate blooms. They are not only found in the water column but also in sediments and in the epiphytic community, where they reach higher abundances. Chytrid outbreaks during dinoflagellate blooms are usually dominated by a few species, highly influenced by the dominant hosts present in the community and with observed prevalence < 5%. The diversity of marine chytrids remains largely unknown, leading to the detection of many unidentified species. Overall, this research highlights the importance of understanding the role of aquatic fungi, particularly chytrids, in marine ecosystems and their interactions with dinoflagellate blooms. By shedding light on their diversity and ecological functions, we can gain a better understanding of the intricate dynamics within these ecosystems and their impact on harmful algal blooms.

Community/Species Interactions

O-030

Bacterial effect on red tide formation caused by *Karenia selliformis* on the coast of Hokkaido, Japan

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In the fall of 2021, a large-scale red tide bloom occurred along the east coast of Hokkaido, Japan, killing a wide range of marine organisms such as salmon and sea urchins. This red tide was primarily composed of a dinoflagellate, *Karenia selliformis*, which originated from the coast of the Kamchatka Peninsula. It was the first report of *K. selliformis* red tide in Japan. Although the direct reasons for inducing the growth of *K. selliformis* remain unknown, previous simulation-based studies suggest that some potential physical factors, such as relatively high water temperature and warm currents, may affect bloom formation. In this study, axenic strains of *K. selliformis* and 40 coexisting bacterial strains were established to evaluate the bacterial effect on *K. selliformis* growth. Among these bacterial strains, *Sulfitobacter* strain #17 significantly induced the growth of *K. selliformis*. Cell extract of strain #17 could also induce the growth of *K. selliformis* in a concentration-dependent manner. In contrast, the bacterial cell extract did not induce the growth of other dinoflagellate strains, *Alexandrium catenella*, *Takayama* sp., *T. acrotrocha*, and *Heterocapsa minima*. Strain #17 was mesophilic, and thus the high water temperature in the warm currents can promote the bacterial growth. These results suggest that this kind of bacteria may be the driving force for both red tide formation and species succession.

Ciguatera and Benthic HABs
O-031

Dereplication of *Gambierdiscus balechii* extract for toxin screening

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Natural marine toxins have a significant impact on seafood resources and human health. Several species from the genus *Gambierdiscus* have been documented to produce a suite of natural marine products that are known to be biologically active, including ciguatoxins (CTXs) and maitotoxins (MTXs). Dereplication is a screening process used for microbial extract profiling to identify biologically active substances and known compounds responsible for the observed activity. In this study, a multidisciplinary approach based on the combination of cell based (Neuro-2a) cytotoxicity bioassay-guided micro-fractionation screening process coupled with liquid chromatography-high resolution mass spectrometry, which was crucial in revealing, for the first time, the presence of a putative C/I-CTX analogue in a *Gambierdiscus balechii* (VG0920) extract. Through cytotoxicity assays, three biologically active fractions exhibiting CTX-like and MTX-like toxicity were identified. MTX-like compounds were purported but not confirmed in this study. Using a commercially available standard, the presence of 44-methylgambierone was also confirmed at a concentration of 8.6 pg cell^{-1}. In the absence of a C-CTX standard, a naturally incurred fish extract of *Sphyraena barracuda* was used to confirm the presence of the putative I/C-CTX analogue from the *G. balechii* extract. To-date no commercially available C-CTX standard exists and the identification of a benthic harmful algal species that can be cultured and is capable of producing I/C-CTXs can alleviate an analytical gap that requires naturally incurred animal material.

Ciguatera and Benthic HABs
O-032

Stereochemical diversity of Caribbean ciguatoxin analogues

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Ciguatera poisoning (CP) is endemic in subtropical and tropical regions from the consumption of ciguatoxin (CTX) contaminated fish. The recent discovery of Caribbean CTXs (C-CTXs) in *Gambierdiscus* spp. resulted in identification of a precursor analogue, C-CTX5, that can be chemically and enzymatically reduced into C-CTX1/2 or further reduced to C-CTX3/4. The two reducible sites on C-CTX5 are the ketone at C-3 and the hemiketal at C-56. Initial chemical reductions of C-CTX5 into C-CTX3/4 resulted in 2 LC–HRMS peaks with a ratio that differed from that observed in fish samples. In theory, this reduction could have produced 4 stereoisomers of C-CTX3/4 and existing literature reports the reduction of C-CTX1/2 into C-CTX3/4 with a similar ratio of products to that observed in the fish. The observed difference in peak intensities from the reduction of C-CTX5 suggests that C-3 epimers were also produced, prompting a more detailed study of the products of this reduction. LC–HRMS with a slow gradient was used to show the four stereoisomers of C-CTX3/4. This methodology was applied to naturally incurred fish tissues to determine the distribution of these CTX analogues. The results showed that C-CTX1/2 is a mixture of C-3 epimers, and that C-CTX3/4 are a mixture of 4 epimers. This may also be the case for many of the uncharacterized C-CTXs in fish that are derived from C-CTX5, revealing an unexpected complexity in C-CTX analogue profiles. These data indicate that there is variability in the enzymatic reduction of C-CTXs in fish, leading to varying ratios of the C-3 and C-56 stereoisomers in the fish tissues. Further work is necessary to determine the toxicological effects of these stereoisomers relating to CP.

Ciguatera and Benthic HABs
O-033

Rapid and sensitive detection of ciguatoxins with a single-step immunosensing strategy

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Ciguatera is a seafood-borne disease caused by the ingestion of fish contaminated with ciguatoxins (CTXs). Previously considered to be geographically limited to tropical areas, ciguatera is now being reported outside these locations. This serious threat to the public health makes clear the need to develop and validate efficient and rapid methods for the detection of CTXs. In our group, we have developed several biosensors, all based on antibodies, for the detection of CTXs in both fish and microalgae. Magnetic beads and electrodes coated with multi-walled carbon nanotubes have been used as capture antibody immobilisation supports, and electrochemical measurements from detector antibody have been recorded with a conventional but also a portable potentiostat connected to a smartphone. These immunosensors have been proved to detect CTX1B in fish at the levels proposed by the US FDA. They have also been able to provide CTX1B and CTX3C equivalent contents in *Gambierdiscus* and *Fukuyoa* cultures and in seawater. Latest efforts have been focused on the simplification of the protocols. In this direction, a single-step assay, only requiring a subsequent washing step and the corresponding signal measurement, has been developed and applied to the analysis of CTX-containing fish. Thanks to the simplicity and rapidity of the protocol, together with the storage stability of the reagents, this bioanalytical tool can certainly facilitate CTXs analysis and ciguatera risk assessment, and contribute to research and monitoring activities.

Ciguatera and Benthic HABs
O-034

One decade of efforts toward addressing the risk of Ciguatera in the Caribbean: the Cuban experience

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Ciguatera poisoning is considered the most prevalent non-bacterial seafood intoxication globally. In Cuba, although ciguatera constitutes the primary cause of algal-derived seafood intoxication, very little information was available ten years year ago on the causative dinoflagellate species and the occurrence of ciguatoxins in the marine food web. With the support of the International Atomic Energy Agency, since 2009 capacities have been gradually established in several countries of the Caribbean to help address this problem in the region. As a result several protocols have been implemented in Cuba focus on cell-based and toxin monitoring. Natural and artificial substrates are used for sampling benthic microalgae in order to enumerate cells and to establish cultures. Several clones of *Gambierdiscus* and other benthic species have been successfully isolated and maintained. Information on species identity has been obtained through international collaboration, however, the establishment of a molecular biology laboratory for this purpose is underway. The Receptor Binding Assay has been implemented for detecting ciguatoxins in fish tissues and several alternatives are currently being evaluated to maintain its operability. However, despite all the achievements, there are still many challenges to face. Access to information on ciguatera events collected by public health authorities has been very limited. Due to logistical issues sampling has been limited to areas located the central region of Cuba. On the other hand, with the new fishing law the collection of regulated species has also been up to now impossible. Much remains to be done in terms of strengthening national inter-institutional collaboration and effective communication in order to continue advancing on this topic.

Ciguatera and Benthic HABs
O-035

Ostreopsis cf. *ovata* trend along the Conero Riviera (northern Adriatic Sea) over two decades

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Harmful blooms of the toxic dinoflagellate *Ostreopsis* cf. *ovata* have been a recurrent phenomenon along the Mediterranean rocky coasts in the last decades. Since their first records, there has been a widespread belief that the extension of this dinoflagellate well-known in tropical areas to higher latitudes was due to the global warming and the general rise of seawater temperature. Blooms of *Ostreopsis* cf. *ovata* along the Conero Riviera (northern Adriatic Sea) occur between the end of the summer and the beginning of the autumn since 2006. *Ostreopsis* cf. *ovata* abundance data collected from its first record to nowadays were analyzed to better define the interannual trend of this phenomenon and its possible linking to certain climate change predictors. A significant increasing trend in the magnitude of *Ostreopsis* phenomenon was observed up to year 2012, then a stabilization at relatively low values was observed. This trend does not follow the incessant increase in water temperature observed during the last three decades in Adriatic Sea, but rather recalls that of an invasive species, although the provenience of *O.* cf. *ovata* in the Mediterranean Sea is still unresolved. Even if the *Ostreopsis* bloom in this area seems to slightly lessen in the last decade, *Ostreopsis* abundances still reach values up to 10^3 cells cm^{-2} which could be harmful to human health.

Ciguatera and Benthic HABs
O-036

The role of habitat dynamics in the facilitation of *Ostreopsis* spp. blooms

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In recent decades, recurrent *Ostreopsis* spp. blooms have been recorded throughout the globe, causing public health issues and mass mortalities of invertebrates. *Ostreopsis* species are benthic and develop in shallow waters in close relation with a substrate, but possible substrate preferences are still ambiguous. A literature review summarized information on *Ostreopsis* spp. blooms related to the habitat at different spatial scales, the micro- (substrate), meso- (community) and macrohabitat (ecosystem). The sampled substrate and the ecosystem where *Ostreopsis* spp. were collected were generally reported and described in the studies, while the description of the mesohabitat was rarely reported. *Ostreopsis* spp. were generally described as attached to biotic substrates and in particular macroalgae, even in studies conducted in coral reefs, where macroalgae are generally not dominant (but they can be in case of coral reef degradation). In both temperate and tropical areas, *Ostreopsis* spp. were mostly sampled on algal species usually forming medium or low complexity communities (erect or turf-forming algae), often characteristic from post-regime shift scenarios, and rarely on complex communities (such as kelp forests). Experiments in the field on both the northern (France) and southern Hemisphere (New Zealand) suggested similar results, but a high variability of Ostreopsis abundances was recorded, especially in especially in the Mediterranean Sea, where seaweeds are generally smaller and their distribution vary at a small scale. The presented work highlights the need of collecting more information about the mesohabitat (i.e. macroalgal communities) where important *Ostreopsis* spp. blooms develop, as much as of the underlying mechanisms driving eventual differences on *Ostreopsis* spp. abundances. This knowledge would allow a better risk assessment of *Ostreopsis* spp. blooms, identifying areas at high risk on the base of the benthic habitats present. More research is in progress in the framework of different projects (Bentox, University Côte d’Azur and FORESCUE, Biodiversa +, European Community) in order to asses the presence of a link between the recent increase in frequency and magnitude of blooms and the loss of complex macroalgal communities (i.e. marine forests).

Biology and Biogeography
O-037

A review of *Alexandrium* genus around the Mexican Pacific Coasts with an approach to its molecular taxonomy and toxicology in Mazatlán Bay, Sinaloa

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Around the world, the distribution of *Alexandrium* genus is well known, some species produce different toxins that could affect the ecosystem or indeed, affects human for the consumption of seafood contaminated by marine toxins. Nowadays, the genus *Alexandrium* has been questioned in their taxonomy. In Mexico, the distribution of *Alexandrium* is uncertain, some authors have made efforts to establish the distribution around the Gulf of Mexico and in the Mexican Pacific Ocean (MPO), however, the complexity of the identification of the species by light microscopy and the lack of taxonomists specialized in the genus, could derivate in wrong identifications. Here we summarize the records of *Alexandrium* genus from coasts of the MPO, also from some culture samples from Mazatlán Bay, Sinaloa (subtropical MPO), cell morphologies are presented by LM. Production of PSP toxins and its compositions were examined by HPLC and molecular phylogeny was inferred from LSU, SSU and ITS rDNA. As results, 18 species of the *Alexandrium* genus are reported in the MPO, in which 9 produce PSP toxins. Also, 3 more species produce another type of toxins. In Mazatlán Bay, from the culture samples, *A. compressum, A. cohorticula*/*A. tamiyavanichii, A. fraterculus* and *A. monilatum* are presented with LM. In addition, LSU, SSU and ITS matches with the species, except for *A. compressum* whose sequences are not yet available. 10 cultures of Mazatlán Bay could produce PSP toxins in the range of 1-10 pmol PSP toxins eq. cél^{-1} which are characterized by carbamoil-toxin group.

Biology and Biogeography

O-038

Speciation and evolution of cryptic species of *Alexandrium*, *Centrodinium* and PST production

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Despite their fundamental importance in global marine and climate systems, the processes of evolution and speciation in marine planktonic dinoflagellates remains a puzzle. Dinoflagellates have asexual and/or intermittent sexual reproduction with diploid cyst stages, large population sizes, with theoretically high dispersal, and were once considered ubiquitous with shallow population structuring. Surprisingly, dinoflagellate populations have often shown large, highly structured genetic diversity that persists over time, and cryptic species have been found in many previously identified species. In the cryptic species of the former *Alexandrium tamarense* species complex, which cause many or most of the PST uptake into commercial shellfish worldwide, several species are found occurring sympatrically in some areas of the world. Differences in PST production may be indicative of differences in copy number in *sxt* genes, or the distinct transcriptional processes that regulate their expression. Selection has played a role in the evolution of a paralogous copy of *sxtA* across *Alexandrium* spp., indicating the potential role of ecological processes in their diversification. Here, we investigated speciation, and the evolutionary trajectories and rates in *Alexandrium* species, focusing on the former *A. tamarense* complex and the related genus *Centrodinium*. We generated transcriptomes of 17 dinoflagellate strains and conducted phylogenetic analyses based on large multi-gene alignments of proteins and nucleotides. Evolutionary hypotheses will be presented, with specific reference to the diversification of dinoflagellates vis-à-vis ecology of PST production and HABs.

Biology and Biogeography

O-039

Metabarcoding revealed a high diversity of Amphidomataceae (Dinophyceae) and the seasonal distribution of their toxigenic species in the Taiwan Strait

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The dinophyte family Amphidomataceae includes the genera *Azadinium* and *Amphidoma*. Four of these species are known to produce azaspiracids, which are lipophilic phycotoxins accumulating in shellfish. The diversity and biogeography of Amphidomataceae is far from yet resolved. Here we performed a time series sampling of both water and sediments in the Taiwan Strait and surrounding waters. Metabarcoding was performed to unveil the diversity of Amphidomataceae targeting internal transcribed spacer (ITS1) region, followed by quantitative PCR (qPCR) with modified primers for *Az. poporum* and *Az. spinosum* ribotypes. The diversity of Amphidomataceae was revealed from the water samples with the aid of ITS1 based molecular phylogeny, showing eight new *Azadinium* clades and several ZOTUs (zero-radius operational taxonomic units) grouping together with *Am. languida*. Moreover, eleven known *Azadinium* species including three ribotypes of *Az. poporum* and *Az. spinosum*, were detected. The seasonal occurrence and sources of these toxic *Azadinium* species in the Taiwan Strait will be discussed. Our results highlight the rich diversity of Amphidomataceae and risk potential of azaspiracids in the Taiwan Strait and surrounding waters.

Biology and Biogeography

O-040

The effect of light intensity for the growth, vertical distribution and photosynthesis of *Karenia mikimotoi*

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A dinoflagellate species, *Karenia mikimotoi*, has been known to form heavy red tides and severely impact marine fish culture. *K. mikimotoi* has the characteristic of being located in mid-deeper layer on sunny days. In this study, we incubated *K. mikimotoi* in under 6 steps light condition (PFD = 47, 81, 232, 269, 452, 664 μmol photons^{-2} m^{-1}) and a columnar aquarium (7.4 cm ϕ × 200 cm high) which are illuminated from above due to examine the effect of light intensity for the growth, vertical distribution and photosynthetic parameters obtained by PAM fluorometry. Growth rate of *K. mikimotoi* was higher with light intensity stronger, however, the growth rate was saturated at PFD of 232 μmol photons^{-2} m^{-1} and the *Fv/Fm* value declined significantly with stronger PFD. Because the decline of *Fv/Fm* indicates the inhibition of PSII, it was suggested that this strong irradiance was harmful for their PSII center. On the other hand, ETR maintained high value at PDF of 232 μmol photons^{-2} m^{-1}, suggesting that PSII of *K. mikimotoi* was suffered photoinhibition, however, they increased electron turnover and avoided to decline of functional PSII centers. In the column experiment, *K. mikimotoi* population accumulated to the surface of water column before lighting, while, after lighting, they descended to sub-surface layer immediately. Both *Fv/Fm* and ETR showed highest values at the layer, and *K. mikimotoi* would migrate themselves from the surface to the sub-surface to avoid inhibition of PSII and get the optimal light irradiance.

Biology and Biogeography
O-041

On the morphological and phylogenetic diversity of cryptophytes of the *Teleaulax-Plagioselmis-Geminigera* clade and the species identity of kleptoplastids from *Dinophysis*

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The origin of the kleptoplastids in *Dinophysis* spp. has been identified using plastidial molecular markers. This has pointed to cryptophytes of the *Teleaulax/Plagioselmis/Geminigera* clade as the main source of these kleptoplastids worldwide, while more recently, plastids from the *Rhodomonas/Storeatula* clade were also reported in southern Chile. Despite their ecological relevance, the diversity and taxonomy of these cryptophytes has been poorly addressed. Their small size makes them candidates to harbour morphologically (pseudo-)cryptic diversity and, additionally, dimorphism has been shown in a few cases. Here we show results from the analysis of more than 50 cryptophyte strains that belong mainly to the *Teleaulax/Plagioselmis/Geminigera* clade from different origins. Sequences of partial plastidial 23S rDNA, psbA and rbcL genes were obtained. Selected strains were observed under the SEM and TEM (ultrathin sections) for their morphological characterization and to uncover dimorphic clades. Phylogenetic analyses showed 11 phylogroups within the *Teleaulax/Plagioselmis/Geminigera* clade, most of them representing distinct morphospecies: eight from marine environments, two from freshwater and one from continental brackish waters. Five of the clades were confirmed to be dimorphic (i.e. contained *Teleaulax*- and *Plagioselmis*-morphotype strains), including the one most frequently represented by sequences from *Dinophysis*. The *Teleaulax*-morphotype strains from the latter are consistent with Butcher's description of *Cryptomonas acuta*, the basionym of the type species of *Teleaulax*, but not with *Rhodomonas amphioxeia* Conrad (basionym of *T. amphioxeia*). Finally, sequences reported from Chilean *Dinophysis* were found to be identical to those of two *Rhinomonas* cf. *fulva* strains. Funded by the Basque Government project GIC21/82.

Biology and Biogeography
O-042

Discover novel resting cyst-producers in dinoflagellates with multiple approaches

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While resting cysts have been well demonstrated to play vital roles in inoculating and initiating harmful algal blooms (HABs) of dinoflagellates, only about 10% of the currently accepted species of dinoflagellates have been proven as cyst producers. On the other hand, the number of cyst-producing species has been gradually increasing, e. g. most of recently described new species were confirmed as cyst producers. We will present our demonstrations of many resting cysts that are novel either internationally or nationally. Firstly, via laboratory culture-based observation of life history, we demonstrated sexual mating, formation of planozygotes and diploid cysts, and germination of cysts in *Karenia mikimotoi*, *Karlodinium veneficum*, *Prorocentrum donghaiense*, *Akashiwo sanguinea*, and then we detected the presence and distribution of these species of cysts in the sediments of Chinese seas using an approach combining fluorescence in situ hybridization-light microscopy-single cyst sequencing for an ~1400 bp LSU rRNA gene (FISH-LM-SCS) and qPCR. Secondly, we identified dinoflagellate resting cysts via applying an approach combining direct SCS and metabarcoding high throughput sequencing to hundreds of sediment samples from four seas of China. This approach discovered many species of cysts that have never been reported either in the literature or from China or the investigated regions. The approach also detected many cysts of toxic or HABs-forming species and many more cysts that could not able to be convincingly annotated to any described species. Our pursuits not only found many resting cysts of vital ecological importance, but also pinpointed a fundamental feature of the life cycle of dinoflagellates.

Ichthyotoxic HABs
O-043

Global socio-economic impacts from fish-killing algal blooms: A roadmap towards more effective management and mitigation

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Finfish aquaculture mortalities by HABs have caused massive socio-economic impacts worldwide, exceeding the impact from shellfish biotoxins. The most potent fish-killers include members of the widely distributed, unrelated dinoflagellate genera *Alexandrium, Karenia, Karlodinium* and *Margalefidinium*, raphidophytes *Chattonella* and *Heterosigma*, dictyochophytes *Pseudochattonella* and *Vicicitus*, and haptophytes *Chrysochromulina* and *Prymnesium*. These species have in common their propensity to produce lytic compounds that irreparably damage gill tissues of fish which ultimately die from suffocation. Except for advances with *Prymnesium* (prymnesins), *Karlodinium* (karlotoxins) and *Karenia brevisulcata* (brevisulcenals), the mechanisms of how such microalgae kill finfish (which may include ROS and FFA as co-factors) remain poorly understood. Broad scale ecosystem impacts are lesser from raphidophytes and dictyochophytes that require cellular contact for harmful effects, compared to *Karenia* and *Prymnesium* where intracellular or excreted toxins are responsible. Hurdles that limit progress in our understanding of ichthyotoxins include: HABs at fish farms are not a research priority until a major bloom occurs; data sharing between industry and scientists is very limited; and lack of standardized methods to detect ichthyotoxins in low concentrations dissolved in seawater. Fish killing HAB events are disasters and should be treated as such, and we actively need to pursue broad-scale strategies to control blooms e.g. by clay flocculation of algal biomass and/or targeted mopping up of ichthyotoxins. This GlobalHAB "white paper" provides a roadmap for scientists, aquaculturists and insurance brokers to improve management of fish-killing algal blooms that put pressure on seafood security for an ever increasing human population.

Ichthyotoxic HABs
O-044

The dual-face impact of marine heatwaves on the blooms of harmful dinoflagellate *Margalefidinium* (=*Cochlodinium*) *polykrikoides* in Korean coastal waters

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In 2018, the bloom of harmful dinoflagellate *Margalefidinium* (=*Cochlodinium*) *polykrikoides* occurred under abnormally high water temperature (WT) conditions caused by marine heatwaves (MHWs) in Korean coastal water (KCW). To understand the impact of MHWs on *M. polykrikoides* bloom in 2018, we conducted field surveys (horizontal and vertical investigation) and laboratory experiments (physiological, genetic, and behavioral analysis). The 2018 strain was able to grow above 28°C, despite being an East Asian ribotype, and the growth rate was relatively low at 30°C. Under unusual early stratification conditions caused by MHWs ($p < 0.01$, Kruskal–Wallis test), the blooms occurred more earlier than the average outbreak in the last 17 years in KCW. However, the bloom duration in 2018 was exceptionally short, which was associated with high WTs ($R^2 = 0.52$, $p < 0.01$). In field, most of the *M. polykrikoides* population was at a depth of 3–6 m during the day, where the WT was significantly lower ($p < 0.01$; Kruskal–Wallis test) than in the surface layer (0 m), suggesting the usage of DVM to avoid high temperature stress. In a laboratory experiment to verity this thermotaxis, we found that a trend of greater DVM velocity when moving from "unfavorable" water temperature (30°C hot and 12°C cold) to "favorable" water temperature for growth (optimal 24°C) of *M. polykrikoides*. Our findings indicated that in relation to MHWs, early stratification condition plays a positive role in developing bloom, but maintaining bloom under high WT conditions are negatively affected, such as the reduced growth rate and limited DVM behavior.

Ichthyotoxic HABs
O-045

Interplay between ichthyotoxic dinoflagellate *Karenia mikimotoi* and marine bacteria isolated from blooming water and cultivable phycosphere

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Karenia mikimotoi is one of the most toxic and deadly harmful algal bloom (HAB) causative species. The blooms of this species always lead to massive mortality of fish and shellfish. In 2016, outbreaks of blooms of this species killed more than 200 tons of fish in various fish-farming zones of Hong Kong. Recently, the interaction between HAB species and marine bacteria has attracted considerable research attention. Marine bacteria is speculated to play important role in HAB. Some bacterial strains have shown to bear algicidal activity on HAB species and several reports also demonstrated that the toxicity of HAB species can be affected by certain bacteria associated with the HAB species. However, study on associations between *K. mikimotoi* and marine bacteria is greatly limited and our understanding in this area is very poor. In this study, we aimed to investigate the interplay between *K. mikimotoi* and marine bacteria in terms of algicidal effect and ichthyotoxicity modulations of the bacteria on *K. mikimotoi*. There are three main parts in this study: (1) establishment of axenic culture for *K. mikimotoi*; (2) determination on algicidal effects of a bacterial strain (P4) isolated from the aforementioned algal bloom in 2016 on *K. mikimotoi*; and (3) investigation on how ichthyotoxicity of *K. mikimotoi* is being affected by its associated bacteria co-existing in the algal culture. We developed an in-house methodology for the establishment of axenic culture for dinoflagellates. This methodology combined density gradient centrifugation, antibiotic treatments, and serial dilution techniques. The axenic status of the *K. mikimotoi* culture has been maintained more than 60 generations without the use of antibiotics which indicated that our method is a highly promising approach for the generation of axenic dinoflagellate cultures. Based on the results obtained from the algicidal experiments, P4 exhibited significant algicidal effect on the *K. mikimotoi* cells and was bacterial dose and growth phase dependent. At 25% v/v dose, 100% of algicidal efficiency was achieved in 48 hours. The highest algicidal efficiency was recorded when both P4 and the algal cells were at stationary growth phase. Interestingly, more prominent algicidal effect was observed when P4 was co-culturing with xenic *K. mikimotoi* cells. We compared the ichthyotoxicity between xenic and axenic *K. mikimotoi* cultures using *in vitro* bioassay with fish gill cell line. Surprisingly, significant increase of ichthyotoxicity was observed in axenic *K. mikimotoi* cultures. Further, the algal ichthyotoxicity was not as expected to return back to its original level as observed in the xenic culture when the associated bacteria were added back to the axenic culture. We also found that different bacterial isolates could have different specific modulatory effects on the algal ichthyotoxicity. Proteomic analysis revealed that proteins in cellular metabolism and chloroplast were affected significantly in *K. mikimotoi* when the algal cells were exposed to P4 / its associated bacteria.

Ichthyotoxic HABs
O-047

Regulation of photosynthetic and hemolytic activity of *Phaeocystis globosa* under different light spectra

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Phaeocystis globosa frequently proliferates in eutrophic waters and forms ichthyotoxic algal blooms that cause massive fish mortalities in marine ecosystems. One of the ichthyotoxic metabolites was identified as the glycolipid-like hemolytic toxin, reported to be initiated bunder light conditions. However, the association between hemolytic activity (HA) and photosynthesis of *P. globosa* remained unclear. Light spectra (blue, red, green and white) and 3-(3,4-dichlorophenyl)-1, 1-dimethylurea (DCMU) were selected as the stressors to stimulate the hemolytic response of *P. globosa* in relation to the light and dark photosynthesis reaction. Hemolytic activity in *P. globosa* was sensitive to the light spectrum as it decreased from 93% to nearly undetectable (1.6%) within 10 min of transfer from red (630 nm) to green light (520 nm). This indicates that the vertical transformation of *P. globosa* from deep to surface waters (dominated by green light and all light spectra, respectively) may drive the hemolytic response in coastal waters. However, regulation of photosynthetic electron transfer in the light reaction of *P. globosa* was excluded by the evidence of inconsistent response of HA to photosynthetic activity. The biosynthesis of HA may interfere with the pathway of photopigments diadinoxanthin or fucoxanthin, and the metabolism of three and five carbon sugars (GAP and Ru5P, respectively), which ultimately lead to changes in the alga’s hemolytic carbohydrate metabolism.

Ichthyotoxic HABs
O-048

Massive Fish Killing in the River Oder in Poland/Germany in Summer 2022 – deciphering the disaster and insights into toxicity

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In summer 2022, a massive and unprecedented toxic algal bloom killed more than 400 t of fish and mollusks in the Oder River, along a 200 km stretch in Poland and Germany. We compiled relevant climatic and hydrological evidence, satellite remote sensing, records of monitoring stations, cell counts, water and toxin chemistry, as well as genomic data. Our analyses suggest that the coincidence and combination of multiple factors were preconditional: steady effluents of brines into the headwaters elevated salinity, and together with replete nutrients, high water temperatures and low river discharge levels during the 2022 summer heat wave. This enabled the mass development of the toxic haptophyte *Prymnesium parvum* and the release of the toxins (prymnesins). Furthermore, we investigated whether organic micropollutants may lead to mixture effects and add to the toxicity of the algal toxins. *In vivo* assays towards other algae, daphnia and zebrafish embryos were conducted to estimate the hazard for nontarget aquatic organisms which were only moderately increased over what is typically observed in rivers and wastewater treatment plant effluents. However, the extracts caused remarkable neurotoxic effects *in vitro* in a human neuronal cell line, where neurite growth was impaired. Mixture toxicity modeling demonstrated that the neurotoxic effects were mainly caused by the detected prymnesins with some contributions by other organic micropollutants. Under climate change scenarios society needs to brace against future compound extreme event because of the diverse and wide-ranging adverse impact in addition to the fish kill.

HABs in a Changing World
O-049

Long term *Alexandrium* cyst and *seda*DNA record in a 9000-year sediment core from east coast Tasmania, Australia

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Toxic *Alexandrium catenella* dinoflagellate blooms have unexpectedly impacted the seafood industry along the Tasmanian east coast since 2012 (up to 150 mg STX eq/ kg in mussels). To investigate the history of these blooms, we applied palynological microscopic cyst analyses (enhanced by primulin staining) combined with sedimentary ancient DNA analyses (*seda*DNA) in sediment cores collected inshore and offshore of Maria Island, Tasmania, Australia. Inshore, highest abundances of *Alexandrium* cysts (364 cysts per g^{-1} dry sediment) occurred in surface (0-1 cm) sediments but cysts were detected throughout the 35cm long core (deposited over the last ~144 years). Offshore, low concentrations of morphologically similar cysts were observed throughout a 268 cm-long core (deposited over the last ~9,000 years). All inshore *Alexandrium* cysts responded to primulin staining, but very few offshore cysts stained, presumably due to bilayer cyst wall degradation. Long term preservation of *Alexandrium* cysts is widely disputed, hence in addition we used *seda*DNA (metagenomic shotgun sequencing combined with hybridisation capture) to target the genus *Alexandrium*. Our *seda*DNA results show that *Alexandrium* has recently (from ~15 years ago) become more abundant in inshore waters (up to 854 reads per sample) but has been present offshore in low abundance (< 20 reads per sample and increased damage with depth, indicating authentic *seda*DNA) throughout the last ~9,000 years. These results point to a previously cryptic *Alexandrium* population recently stimulated by changing environmental conditions.

HABs in a Changing World

O-050

Photo-physiology and mixotrophic grazing by dinoflagellate *Karenia brevis* on *Synechococcus* with different quality

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In 2005, it was reported that *Karenia brevis*, which forms red-tide almost annually in Florida's Gulf coasts, was capable of facultative mixotrophy, and that it could graze on small cells such as *Synechococcus*. However, since then, only limited research (< 5 studies) has been conducted on mixotrophy of this species. Here, we examined if quality of prey could affect grazing responses by *K. brevis*. Prey cultures, nitrogen (N) and phosphate (P) replete, N-limited, and P-limited *Synechococcus,* were inoculated into *K. brevis* with varying ratios. Photo-physiology of prey as well as grazer were also monitored. A total of 4 experiments were conducted, including 1 using *K. brevis* from batch culture (exponential growth) and 3 using chemostat cultures at 60% max growth rate. In most of the experiments, densities of N, P-replete, N-limited and P-limited *Synechococcus* controls increased significantly (2.1 to 4.1-fold) while those with grazers increased less (0.9 to 1.5-fold), resulting in ingestion rates that ranged from 24.4 to 92.0 *Synechococcus* *K. brevis*$^{-1}$ d^{-1}. No consistent patterns were observed in ingestion rates on different quality of *Synechococcus* but ingestion rates were a function of prey-to-grazer ratios across all treatments (R^2=0.76). The relative electron transport rate, $rETR_{max}$, increased with time in prey controls but, in prey with grazers, $rETR_{max}$ dropped significantly after 24-72h after inoculation. No significant changes in densities and photo-physiology were found in the grazers with prey. This demonstrates that presence of *K. brevis* can not only directly affect prey by grazing on them but can also indirectly affect prey.

HABs in a Changing World
O-051

Evaluating the potential future impacts of *Ostreopsis* cf. *ovata* via controlled experimental exposure of naïve shellfish from Great Britain

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The genus *Ostreopsis* is known from several locations globally with *Ostreopsis* cf. *ovata* being a species of concern, having been shown to produce harmful effects at multiple trophic levels These include cases of direct poisonings of humans, via marine aerosols and skin contact, evidence of toxin accumulation in seafood and both lethal and sublethal effects on marine species, including bivalve molluscs. The compounds responsible for these impacts, certainly in humans, belong to a family of toxins known as palytoxins, with ovatoxins being a common component of *O.* cf. *ovata* toxin profiles. A recent assessment suggested that, although *O.* cf. *ovata* is not currently present in Great Britain (GB), a risk of future occurrence exists, as climate change warms temperate waters. With the species now present on the French Atlantic coast it is close to GB. To pre-emptively assess the impacts an emergence of this species could have in the Southwest of GB a series of tank studies were undertaken. These exposed naïve *Mytilus sp.* from GB to two strains of *O.* cf. *ovata,* one producing palytoxins from North-western Adriatic Sea (Italy), and one not. During the study the ovatoxin containing strain caused a cessation of feeding for the exposed mussels and resulted in rapid mortalities. Only limited toxin uptake was found in mussels exposed to the toxic strain, indicating a serious potential for environmental damage and a commensurate food security risk if ovatoxin producing strains of *O.* cf. *ovata* were to successfully establish in GB waters in the future.

HABs in a Changing World
O-052

Investigating the molecular and physiological responses of temperature-adapted toxic *Pseudo-nitzschia* to ocean warming

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Changes in environmental conditions can induce toxic species of the diatom *Pseudo-nitzschia* to dominate algal blooms, causing widespread damage to marine ecosystems. In recent years, an increase in bloom frequency has been linked to climate change. *Pseudo-nitzschia multiseries* responds favorably to warm temperatures, leading to the possibility that this highly toxic species could dominate coastal blooms in a warmer ocean. In our project, we aim to understand the physiological and molecular changes involved in this adaptation. To predict how *P. multiseries* will respond to ocean warming, we have experimentally grown three strains of toxic *P. multiseries* to over 500 generations to either ambient cold temperatures (14°C) or anomalously warm temperatures (24°C). These strains were monitored for their responses to warm temperature, including growth rate, biomass, nitrogen uptake kinetics and domoic acid (DA) production. We compared the genomes and transcriptomes of these temperature-evolved strains to discover intra-strain genetic variations and differential gene expression in the key pathways involved in DA biosynthesis, stress response, growth, reproduction, and nitrogen metabolism. The final goal of this project is to link the physiological manifestations of these molecular changes to our observations of phenotypic changes to obtain a holistic picture of future HAB events in a changing ocean.

HABs in a Changing World

O-053

Study of bloom-causing species in Acapulco Bay over a 20-year period (2000-2023)

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The objective of this study was to document the species responsible for blooms in Acapulco Bay over a 20-year period (2000-2023) and analyze their relationship with El Niño-Southern Oscillation (ENSO) and local currents. A total of 57 collections were conducted from 2000 to 2023, using Van Dorn bottles to obtain 560 samples, which were quantified using the Utermöhl method. The association between harmful algal blooms (HABs) and ENSO was examined using standardized values of the Multivariate ENSO Index, and significance was evaluated through the Olmstead-Tukey quadrant sums method. Regional currents were analyzed using the Google Earth Engine. Based on cell density and high relative abundance (>60%), a total of 55 blooms were recorded, mainly occurring during the rainy season (June-October) and the dry-cold season (November-March). Other authors reported an additional 37 blooms, resulting in a total of 102 blooms. These blooms consisted of 44 taxa, including 21 diatoms, 21 dinoflagellates, and 2 from other groups, with the former being predominantly harmless. Out of the identified species, 67 blooms were associated with noxious species, including 11 known to commonly produce toxic HABs, such as four taxa of *Pseudo-nitzschia* spp. and various dinoflagellates. Abundance analyses of *Pyrodinium bahamense* var. *compressum* and *Gymnodinium catenatum* revealed a tendency to produce HABs during La Niña conditions. Furthermore, 52 additional taxa found in Acapulco were considered potential HABs due to their low densities. Most dinoflagellate blooms occurred under the influence of tropical waters from the Mexican Current, while diatoms were associated with the rainy season.

HABs in a Changing World
O-054

Analysis of harmful algal blooms in the Mexican Tropical Pacific: A remote sensing approach

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The Mexican Tropical Pacific is influenced by three main ocean currents: the Equatorial Current, the North Equatorial Current, and the California Current. When these currents converge, they cause the upwelling of deep sediments and the occurrence of numerous upwelling phenomena. The objective of this study was to analyze Harmful Algal Blooms (HABs) in the Mexican Tropical Pacific with areas exceeding 1 km^2 using remote sensing during the period from 2019 to 2023. MODIS Aqua satellite images were utilized via Google Engine, utilizing the sensor bands of 743-753 nm, 673-683 nm, and 662-672 nm with monthly significance. Additionally, post-processing was conducted using SNAP and QGIS software. Three zones with high HAB occurrences were identified: Banderas Bay (BB) in Nayarit-Jalisco; Acapulco Bay (AB) in Guerrero; and Salina Cruz (SC) in Oaxaca. In the case of BB, HAB dynamics were persistent and associated with significant anthropogenic influence, runoff, and organic waste from hotels. AB experienced HAB dynamics predominantly during two periods throughout the year, linked to upwellings caused by the convergence of warm and cold currents. Salina Cruz showed inferred connections to the Pacific Refinery and the geological formation as the endpoint in the Gulf of Tehuantepec. Furthermore, the recorded data revealed a higher occurrence of HABs compared to the current literature reports.

Biology and Biogeography

O-055

Light microscopy and metabarcoding of 16S and 18S reveals the distribution of harmful algae in a salinity gradient from the Baltic Sea to the Kattegat-Skagerrak, NE Atlantic

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Harmful algal blooms are recurrent phenomena in the Baltic Sea and the Kattegat-Skagerrak. The diversity, geographical and seasonal distribution of eukaryotic taxa were investigated using light microscopy (Utermöhl method) and by metabarcoding of partial 16S and 18S rDNA gene sequences. The Protist Ribosomal Reference Database 2 was used as reference list for barcoding sequences. It is not without problems to use such short sequences for identifying HAB taxa to species. This will be discussed. Environmental parameters were analyzed as well. In total >230 samples were collected at 17 locations over 14 months. At least 36 species from the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae were observed in the combined dataset. The distribution of HAB taxa was strongly seasonal and dependent on salinity. The number of HAB taxa in the Kattegat-Skagerrak (near surface salinity ~15-30) was higher than in the brackish water Baltic Sea (salinity ~3-7). Fish killing species observed include *Prymnesium polylepis, Pseudochattonella verrucophora, Akashiwo sanguinea, Chrysochromulina leadbeateri* and *Karenia mikimotoi*. Surprisingly the 18S data show that also *K. brevis* was present. The 18S results show that *Margalefidinium polykrikoides* and *Margalefidinium fulvescens* were present, previously unreported from this sea area. The distribution of DST producers *Dinophysis* spp. was strongly salinity dependent. Several taxa of the PST-producing genus *Alexandrium* were present. *Pseudo-nitzschia* species include *P. pseudodelicatissima*, *P. pungens* and *P. seriata*. Metabarcoding of 18S rDNA revealed some surprising results and the advantages and disadvantages of the method will be discussed together with 16S results not ready when preparing abstract.

Biology and Biogeography
O-056

Species-specific seasonal distribution of *Skeletonema* resting cells in coastal sediments

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The cosmopolitan bloom-forming diatom *Skeletonema*, often observed in coastal waters, largely underpins coastal ecosystems and fisheries. Although *Skeletonema* does not produce toxins, their blooms lead to hypoxia in summer and surface oligotrophy in winter. They consequently severely damage bivalve fisheries and seaweed aquaculture. However, it has not been well-investigated which species forms the blooms after the phylogenetic revisions of *Skeletonema* with multiple pseudocryptic species. We thus developed a novel species-specific qPCR technique, demonstrating the *in situ* seasonality of each species in Japanese coastal water of Ariake Sound. They form resting cells during their unfavourable seasons, although water-sediment coupling of diatoms little investigated. Here, we quantified species-specific distributions of resting cells of *Skeletonema* in sediments from 7 coastal stations by conducting serial-dilution incubation of the sediments in enriched seawater to estimate the resting cell abundance with species-specific primers (namely MPN-PCR technique), targeting the estival *S. costatum* and brumal *S. dohrnii* and *S. japonicum*. *S. costatum* showed the highest resting cell abundance in August, particularly near the river mouth, during their bloom season. Although *S. dohrnii* dynamically varied each season, whereas *S. japonicum* had highest abundance in the river mouth during their bloom season. In the non-bloom seasons of each species, the highest abundance was observed in the river mouth, indicating physical transport by estuary circulation. Our study suggests that the distributions of *Skeletonema* resting cells were biologically controlled in the bloom seasons, whereas coastal basal currents could also convey the resting cells.

Biology and Biogeography
O-057

Comparative genome and transcriptome analyses in the cosmopolitan diatom genus, *Skeletonema*

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In this study, a comparative genome analysis was carried out to get deep insights into gene functions in planktonic diatoms showing the rapid growth and forming of extensive blooms. We sequenced the genome of nine species (12 strains) in the genus *Skeletonema*, using a hybrid assembly pipeline in combination with Illumina short-reads and Nanopore long-reads. The estimated genome sizes were 40.3-69.3 (53.7 ± 8.6, average ± SD) Mbp in *S. ardens* (SA), *S. costatum* (SC), *S. dohrnii* (SD), *S. grevillei* (SG), *S. japonicum* (SJ), *S. menzelii* (SM), *S. pseudocostatum* (SP), *S. subsalsum* (SS), and *S. tropicum* (ST), respectively, and it was much larger in SC. After scaffolding, the numbers of contigs were 94-348 (186.5 ± 93.1, average ± SD), and it was the smallest in SM and the largest in SC. Lengths of N50 were 0.35-1.09 Mbp (0.69 ± 0.22). The predicted gene numbers were 15,275-21,376 (16,994 ± 1,620), and it was the smallest in SP and the largest in SM. The presence of repetitive elements was also checked. The percentages were 11.0-41.1% (26.8 ± 10.6). BUSCO completeness using eukaryotic genes were 90-98 (94.6 ± 2.8) %. The complete plastid and mitochondria genomes sizes were 126,904-127,378 (127,153 ± 142) bp, and 36,119-41,359 (38,553 ± 1,825) bp, respectively. Gene organization of the plastid genome was similar and conservative among species, but those in the mitochondria genome were variable. We will present more detailed information at the conference.

Prediction and Modeling
O-058

Probabilistic weekly risk forecasts for harmful algal blooms and associated biotoxins using ensembles of machine learning and multi-level Bayesian models

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Predicting harmful algal blooms (HABs) and their associated toxins is essential to minimizing their impact on human and farmed fish health as well as facilitating cost-effective industry responses. To aid in aquaculture management, the website https://www.HABreports.org publishes weekly risk forecasts for Scotland based on expert interpretation of historical trends and recent monitoring of HAB-forming taxa, biotoxin concentrations, and environmental conditions with an emphasis on shellfish aquaculture. Here, we present a quantitative complement to these forecasts with ensembles of probabilistic models drawing on publicly available datasets beginning in 2015. For six HAB taxa and three biotoxins, six candidate models (random forests, multi-layer perceptron neural networks, gradient boosted trees, ridge-regularized generalized linear models, multivariate adaptive regression splines, and multi-level Bayesian models) were parameterised using environmental and autocorrelative predictors, including past conditions, 5-day environmental forecasts, and local and regional trends in HAB taxa densities and biotoxin concentrations. Data for two years were withheld for out-of-sample evaluation. Ensemble predictions were calculated for each observation, weighting models by 10-fold cross-validated area under the precision-recall curve. Ensemble predictions for the two out-of-sample test years showed substantial improvement over the null model across several evaluation metrics, with best performance for toxins produced by *Dinophysis*. While no single candidate model emerged as universally superior, ensemble predictions consistently matched or outperformed the best model across metrics, producing a stable optimal meta-model. These weekly operational predictions provide a rigorous model-based supplement to help inform risk forecasts.

Prediction and Modeling
O-059

Projecting climate change influences on *Alexandrium catenella* in the Gulf of Maine

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The Gulf of Maine is a region with recurrent harmful algal blooms of the dinoflagellate *Alexandrium catenella* that cause closures of shellfish beds in coastal and offshore waters. To quantitatively investigate potential influences of climate change on bloom distribution and intensity, we coupled a dynamically downscaled climate model with an established regional model of circulation and *A. catenella* dynamics. *A. catenella* cell concentrations from the 3-d model are converted to toxicity in terms of saxitoxin equivalents (STX) based on first-order uptake and depuration kinetics. Simulations are run for complementary 25-year periods: a recent hindcast (1994-2018) and a climate projection of conditions at the end of the century (2073-2097). For the hindcast we compare the modeled STX at the coast with toxicity observations from shellfish monitoring stations over the simulation period. We find that the model provides skill at seasonal and interannual time scales, including the distribution of toxicity along the coastline. In the climate model simulations, physical conditions in the Gulf of Maine are altered from the hindcast including increased temperature, decreased salinity, increased stratification, and changes in circulation patterns. The climate simulations show overall increases in concentrations of *A. catenella* and STX at the coast. STX concentrations exceed regulatory limits for shellfish bed closure starting earlier in the year and for longer periods than in the hindcast period. We diagnose the factors contributing to the projected increase in *A. catenella* with climate change.

Prediction and Modeling
O-060

Accumulations of flagellates caused by interactions between vertical swimming and physical processes in the upper ocean

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Flagellates occasionally form blooms with higher rates of cell density increase than the rates observed in laboratory experiments. We hypothesize that the higher rates of the increases are related to accumulations of flagellates caused by interactions between their vertical swimming and physical processes (fluid motion) in the upper ocean. To examine the interactions, we conducted numerical experiments using a Lagrangian particle-tracking model coupled with a hydrodynamic model (Large-eddy simulation model). Particles simulating three flagellates with same diel vertical migration (DVM) rhythm (upward: 0200–1600, downward: 1600–0200) and with different swimming speeds (*Chattonella* spp.: 0.8 m h^{-1}, *Karenia mikimotoi*: 2.2 m h^{-1}, and *Cochlodinium polykrikoides* (=*Margalefidinium polykrikoides*): 4.0 m h^{-1}) were released in the stratified ocean forced by surface winds and surface waves. Results of the experiments show that depth of the maximum particle-density layers changed due to DVMs. In the afternoon, particles accumulated in the vertical direction up to several times compared to nighttime. Higher wind speeds induced stronger turbulence, making surface accumulations decreased and particle trajectories different than trajectories of only DVMs especially in slow swimming flagellate *Chattonella*. In the case of moderate wind speed and *C. polykrikoides*, horizontal accumulations with striped structure in the surface convergent areas, which were caused by the balance between upward swimming speed and downwelling velocity of Langmuir circulations, increased particle-density in one or two order of magnitude. For monitoring of harmful algal blooms, we need to consider these processes occurring in relatively short timescale compared with their growth.

Ichthyotoxic HABs
O-062

Ichthyotoxicity of the harmful dinoflagellate *Alexandrium pseudogonyaulax* and predator-prey interactions with the copepod *Acartia tonsa*

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The dinoflagellate *Alexandrium pseudogonyaulax* has recently proliferated in Northern European waters, thereby seemingly replacing previously dominating *Alexandrium* species. *A. pseudogonyaulax* produces the phycotoxins goniodomins (GDs) and not yet characterized lytic substances, however the ecological effects of both are mostly unknown. *A. pseudogonyaulax* supernatants and GDs exhibited a cytotoxic and lytic effect on the RT-gill W1 rainbow trout cell line, while GDs showed no lytic activity in a complementary *Rhodomonas salina* bioassay. Therein, cytotoxicity was determined by a cell viability assay and lytic effects by measuring the excretion of lactate dehydrogenase, a proxy to assess tissue damage. These findings suggest an ichthyotoxic effect of *A. pseudogonyaulax*, however the responsible molecule(s) and the mechanism of action remains unclear. Furthermore, predator-prey interaction experiments between *A. pseudogonyaulax* and the copepod *Acartia tonsa* (investigated life-stages: N_4, C_4, adult copepods) revealed a grazer-induced stimulation of GD production in *A. pseudogonyaulax*. In addition, *A. tonsa* feeding on *A. pseudogonyaulax* exhibited decelerated morphological development and enhanced mortality rates. Moreover, the hatching rate of *A. tonsa* eggs subjected to the supernatant of *A. pseudogonyaulax* was drastically reduced. Altogether, these findings extend the reported harmful effects of *A. pseudogonyaulax* on other marine species of ecologically important trophic levels and highlight the potential danger towards the economical exploitation of marine resources such as shell- and finfish.

Ichthyotoxic HABs
O-063

Morphology, molecular phylogeny, and ichthyotoxicity of naked dinophytes *Karlodinium* species from Malaysia

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Genus *Karlodinium* is one of the naked dinophytes that caused fish kill events worldwide. In 2014-2015, blooms of *K. australe* have been reported to cause massive fish mortality in the Johor Strait, severely impacting the aquaculture industries of Malaysia and Singapore. These events have risen the concern to further investigate the species diversity and ichthyotoxicity of *Karlodinium* in Malaysian waters. Clonal cultures of *Karlodinium* were established and identified based on detailed morphological observations and molecular analysis. A total of eight species of *Karlodinium* were identified: *K. armiger, K. australe, K. azanzae, K. ballantinum, K. decipiens, K. gentienii, K. veneficum,* and *K. zhouanum*. The species identity of *Karlodinium* was further supported by the molecular phylogenetic inferences of internal transcribed spacer (ITS) and large-subunit (LSU) ribosomal DNA markers. Results of *Artemia* bioassay showed that *K. australe, K. veneficum*, and *K. ballantinum* caused mortality of *Artemia*. While fish assay showed that *K. australe* is highly ichthyotoxic. This study demonstrates, for the first time, a high diversity of *Karlodinium* species from Malaysia.

Toxicology

O-064

Allelopathy of *Skeletonema costatum* on *Karenia mikimotoi* in laboratory cultures: Physiological parameters and transcriptome profiling analysis

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The roles of allelopathy on succession of marine phytoplankton communities remain controversial, especially for the development of blooms. Physiological parameters measurement (Fv/Fm value, MDA content, SOD activity, ATP activity, cell size, chlorophyll content, apoptosis and cell cycle) and whole transcriptome profiling analysis were used to investigate allelopathy effect of *Skeletonema costatum* on *Karenia mikimotoi*. Allelopathic effects were dose-dependent for filtrate cultures and crude extract cultures and high-concentration allelochemicals had the ability to destroy algal cells rapidly. Several metabolic processes related to ribosome and RNA transport, glycolysis/gluconeogenesis, photosynthesis, cell membrane maintenance and osmoregulation, which responded to oxidative stress, cell cycle arrest and apoptotic caused by allelochemicals. Photosynthetic system disfunction and abnormal energy metabolism led to ROS accumulation and ions exchange limitation. Due to *K. mikimotoi* scavenged excessive ROS easily (activity levels of SOD enzymes were increased), so oxidative damage was not the main cause of the growth inhibition. The accumulation of inorganic ions resulted in changing of osmotic pressure and increasing of cell volume, making cells more fragile to environmental stress and also prone to rupture. The influence of allelochemicals on the development of competition algae indicated that allelopathic effects had a more important role in community succession.

Toxicology
O-065

Toxicity of *Dinophysis* spp.: Conventional toxin productions and negative impacts on bivalves from monoclonal cultures and feeding experiments

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Species of the genus *Dinophysis* are cosmopolitan dinoflagellates associated with the production of diarrheic shellfish toxins (DSTs) responsible for the contamination of commercial shellfish with potent toxins and subsequent transfer to human following consumption and poisoning with the Diarrhetic Shellfish Poisoning (DSP). The toxin types and production kinetics have remained intractable due to difficulties in establishing mixotrophic *Dinophysis* cultures, until very recently. In addition, *Dinophysis* spp. have been found to negatively affect bivalve species, during feeding experiments that were intended to understand the kinetics of toxin intakes-toxin depurations for aquaculture and fisheries management (Basti et al. 2014). In this presentation, we demonstrate and discuss the growth and toxin productions of several species of *Dinophysis* from established monoclonal cultures. In addition, we present our results on effects of three species of cosmopolitan *Dinophysis* on early-life stages, juveniles, and adult commercial bivalve species, i.e. Pacific oyster (*Magallana gigas*, former *Crassostrea gigas*), Japanese pearl oyster (*Pinctada fucata martensii*), Noble scallop (*Mimachlamys nobilis*) and Japanese scallop (*Mizuhopecten yessoensis*). Our results show that toxin productions in *Dinophysis* spp. are species- and strain-specific, indirectly impacted by temperature that mainly affects growth, and that the toxicity to bivalve species is also species-specific and life-stage species (both the *Dinophysis* species and the shellfish species) and that it is unrelated to conventional DSTs.

Toxicology
O-066

Investigating the *in-vitro* toxicity mechanisms of prymnesins from *Prymnesium parvum*

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Prymnesins, produced by *Prymnesium parvum*, are believed to be responsible for fish kills when this species blooms. A-type prymnesins have been shown to disrupt membrane structure and increase conductivity across membranes. Their exact mode of action, however, remains unclear. Current hypotheses include interactions with membrane sterols and the involvement of ions. Sterol interactions were tested in hemolysis and cell viability assays after co-incubation of prymnesins with sterols. The impact of prymnesins on RTgill-W1 cells with altered membrane cholesterol content was evaluated. Selected ion-free media were prepared for cytotoxicity testing and cellular changes were imaged using confocal microscopy. Co-incubation with cholesterol and epi-cholesterol pre-hemolysis reduced the hemolytic potential by about 50% for prymnesins, irrespective of sterol concentration. This effect could not be observed in RTgill-W1 cells, however in HCEC-1CT cells a slight decrease in cytotoxicity could be measured. Exposure to prymnesins in calcium-free medium seemed to accelerate cytotoxic effects. The resulting nuclear area was significantly higher compared to exposure to prymnesins in standard medium. In chloride-free medium, cell structures remained virtually intact, with nuclear area and circularity remaining stable compared to the solvent. The study suggests that membrane sterols are involved in the toxicity of these ichthyotoxins.

HABs in a Changing World

O-067

A spring toxic red tide of *Alexandrium minutum* in the Rías Baixas (NW Iberian Peninsula, Spain): is this the new normal?

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On April 25th, a massive red tide spread in the inner Ría de Pontevedra (NW Iberian Peninsula), an important seabed shellfish area. The authorities determined harvesting closures during several weeks because of PST values above regulatory limits. Microscopical and genetic analyses confirmed the toxic dinoflagellate *Alexandrium minutum* as responsible species. The affected area, close to a river mouth, registered salinities of 23 on that date. Seawater discolorations lasted up to May 10th in the northern margin of the ría, particularly in the port of Campelo. The passage of low pressures systems across the western Iberian Peninsula brought southerly winds and persistent rain episodes in the area. The situation changed in May with high pressures and winds turning north. Remote sensing of red waters was hampered by cloud cover except on 27th April, where the bloom was visible in a high-resolution image of Sentinel-2. Abundance of *A. minutum* ranged ~$30\text{-}120 \cdot 10^6$ cells L^{-1} during samplings carried out in the port of Campelo, Chl *a* values reaching up to 694 mg m^{-3}. eDNA metabarcoding provided further insights into the composition of the dinoflagellate assemblage, vastly dominated by *A. minutum,* and cytometric analysis (CytoSense) supplied morphometric characterization of the bloom. Toxins profile in the water column showed the dominance of GTX1,4, and minor amounts of GTX2,3. Additional analyses were also carried out both in marine invertebrates and *A. minutum* isolates from Campelo. This is the second red tide by *A. minutum* ever recorded in the region after a previous episode in 2018. The implications of these PSP episodes in the near future are discussed.

HABs in a Changing World
O-068

Toxic blooms of *Alexandrium catenella* in the warming Alaskan Arctic: a synthesis

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The Arctic Ocean is changing due to climate-driven warming, leading to an increased risk of *Alexandrium catenella* blooms and paralytic shellfish toxins. While recognized as a concern in southeastern Alaska, the distribution, dynamics and impacts of blooms north of the Bering Strait are largely unknown. This talk synthesizes six years of observations and analyses, highlighting the growing threat to Alaskan Arctic ecosystems and the coastal communities dependent on marine resources. Key findings include: 1) an exceptionally large and dense *A. catenella* cyst bed in the Chukchi Sea with concentrations among the highest reported globally; and 2) evidence that climate-driven warming of surface and bottom waters can now support complete life cycle progression near the cyst bed in some years. Two mechanisms for blooms and toxicity in the Alaskan Arctic are proposed: 1) transport of blooms from the Northern Bering Sea through the Bering Strait, as originally proposed by Natsuike and co-workers; and 2) self-initiating and self-seeding blooms near the cyst bed. The large Arctic cyst accumulations are hypothesized to arise from repeated cyst deposition from transported blooms with minimal losses because of suppressed germination during colder years. This was observed in 2022 when a massive bloom transported from the south was observed in Bering Strait, while cold bottom temperatures limited germination from the Arctic cyst bed, resulting in minimal losses and no self-initiating bloom. These findings highlight a significant and ecosystem-wide threat to marine resources which, in the Alaskan Arctic, is a significant threat to human health and food security.

HABs in a Changing World
O-069

Predicting future shellfish toxin dynamics in the rapidly changing climate of Southeast Alaska

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Paralytic shellfish poisoning (PSP) caused by toxins produced by the dinoflagellate *Alexandrium* are a threat to human health and subsistence shellfish harvesters in Southeast Alaska. There have been more than 150 cases of PSP in Alaska since 1970 and concerns over the safe harvest of shellfish has led many subsistence users to reduce or stop harvesting important diet and cultural resources. Since 2016 the Southeast Alaska Tribal Ocean Research (SEATOR) network has been monitoring shellfish toxin concentrations in more than a dozen communities in an effort to encourage recreational and subsistence harvesters to make informed decisions about shellfish safety. Recent models and traditional ecological knowledge have identified important environmental variables such as sea surface temperature (SST) and air temperature to predict harmful algal bloom dynamics on short time scales. Here we incorporate future climate projections for the region into random forest models to predict changes to the potential *Alexandrium* bloom window in coming decades (2031-2060). Models were developed using observed concentrations of PSP toxins in blue mussels (*Mytilus trossulus*) collected by SEATOR from 2016-2023 and used to predict toxin concentrations using gridded downscaled climate projections for Southeast Alaska. We describe changes to the traditional safe harvest season coinciding with warming SSTs and spring air temperatures. Maintaining access to safe shellfish is a top priority for Alaska Native tribes in the region, and a critical issue for establishing and fostering food security in a changing climate.

HABs in a Changing World

O-070

Domoic acid in a warmer California Current: Emergence of *Pseudo-nitzschia multiseries* as a climate "winner"

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Harmful Algal Blooms in the California Current have been dominated by two toxigenic species of *Pseudo-nitzschia*, *P. australis* and *P. multiseries.* For the past several decades *P. australis* has been dominant while *P. multiseries* has remained in the background. In the largest toxic blooms, such as the 2014-15 warm event, *P. australis* has emerged as the "winner" from a more diverse species assemblage. *P. australis* generally does not produce toxin above about 20° C based on laboratory-based culture studies and field data, leading to suppression of toxin in warm waters of the California Current and the suggestion that toxicity will gradually move poleward with climate change. New results with isolates of *P. multiseries* from the Pacific Northwest and Monterey Bay show that *P. multiseries* behaves fundamentally differently, with maximum toxin production at temperatures >20°C and a shift towards very high levels of dissolved domoic acid production with increasing temperature. Forced evolution experiments at elevated temperatures also suggest that toxicity will increase in these strains. These results suggest that domoic acid may be enhanced along the entire California Current as *P. australis* moves poleward and *P. multiseries* replaces it in warmer southern waters. The consequences of very high levels of dissolved domoic acid are poorly understood but could lead to enhanced chronic exposure for marine organisms, breakthrough in water filtration systems, and potential accumulation in marine organisms. Taken together, these results suggest that past conditions are a poor predictor of a future warm ocean.

HABs in a Changing World
O-071

Effects of ocean acidification on the growth and domoic acid production of the diatom *Pseudo-nitzschia multiseries* from the California Current System

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Toxic blooms of the diatom genus *Pseudo-nitzschia* are common in the coastal waters of eastern boundary upwelling systems (EBUS), including those of the California Current System (CCS) off the west coast of the United States where ocean acidification (OA) is unequivocally observed. However, the relationship between toxic blooms of elevated domoic acid (DA) concentrations and regions of decreased pH levels is still uncertain and variable. Our laboratory study used unialgal cultures of acclimated *P. multiseries* – a toxigenic species isolated from Monterey Bay, a well-known DA hotspot, to examine this relationship and determine whether ocean acidification promotes cellular growth and/or DA production in response to declining pH under both macronutrient-sufficient and -deficient conditions expected in episodic upwelling regions such as the CCS which exhibits silicate limitation of diatom growth in some areas. Unlike earlier studies with other strains and species of *Pseudo-nitzschia,* OA does not affect the specific growth rate achieved by *P. multiseries*. Although cellular DA levels greatly increase with silicate deficiency, there is no significant relationship with pH, and resultant rates of DA production do not increase at reduced pH levels of 7.95 and 7.80 relative to 8.10 (control). However, at the lower pH treatments tested, cells decrease their affinity for silicate and cannot acquire this fundamental nutrient required for cell division at these lower 'threshold' concentrations for silicate uptake. Our results demonstrate the importance of using ecologically relevant species and suggest that the impacts of OA differ greatly among toxigenic species and their degree of macronutrient sufficiency.

HABs in a Changing World
O-072

Biologically Enhanced Biochar - A sustainable and natural method to eliminate cyanotoxin from drinking water

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The occurrence of Cyanobacterial blooms in natural water sources are problematic due to their potential to produce highly toxic compounds such as microcystins. Carcinogenesis and severe liver damage are observed after microcystin ingestion, resulting in fatality in severe cases. The severe toxicity of microcystins, specifically Microcystin-LR, has resulted in the implementation of strict drinking water limits of 1 µg/L by the World Health Organisation (WHO). Cyanobacterial blooms are becoming more common in natural water supplies, due to increasing global temperatures and eutrophication. Current water treatment systems for the removal of toxic materials are expensive and often inefficient in microcystin removal, therefore, new technologies are urgently required. Here we demonstrate the potential of an effective low-cost sustainable solution for microcystin removal from contaminated water, utilizing naturally occurring microorganisms combined with biochar to create biologically enhanced biochar (BEB). The biochar provides a low-cost porous support for the immobilization of naturally occurring microbial communities. Highly toxic microcystin-LR (MC-LR) was used to influence the microbial colonization of the biochar by the natural lake water microbiome. These BEBs were then shown to rapidly and effectively remove microcystins from contaminated water sources with long lasting efficacy. This system was also shown to effectively remove microcystins in the presence of live cyanobacteria. This proof-of-concept study demonstrates a sustainable, scalable & safe nature-based water treatment solution.

Prediction and Modeling
O-073

Detection and monitoring of harmful algal blooms in operational fjord systems

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Phytoplankton modeling in coastal waters and inland lakes has primarily focused on abundant groups like diatoms and flagellates, relying on remote chlorophyll-a (chl-a) measurements for validation. However, predicting and tracking the occurrence of harmful algal species remains challenging. The Norwegian University of Science and Technology (NTNU) has developed an observational pyramid comprising sensor platforms operating at different scales, including small satellites with hyperspectral imagers, UAVs with remote sensors, and autonomous surface and underwater vehicles with instrumentation for thermodynamic and biologic observations. These platforms jointly measure environmental variables to monitor the ocean's state. This paper investigates the integration of predictive model outputs from a computational model domain encompassing the Trondheim Fjord in Norway into the operational assets of the NTNU observational pyramid. It explores how estimated states from a predictive ocean model can inform the system about potential harmful algal events. Although the model cannot directly predict harmful algae, it can forecast phytoplankton distributions in terms of the abundance of diatoms and flagellates. Using these predictions, the local operational assets can be directed to sample targeted areas and determine the presence of harmful species. This integrated approach enhances operational monitoring of phytoplankton and improves harmful algal bloom identification and monitoring. Integrating predictive models with the NTNU observational pyramid enables targeted data collection and informed decision-making in aquaculture management. It enhances understanding and management of harmful algal blooms, supporting sustainable practices and ecosystem health.

Prediction and Modeling

O-074

Predicting exposure of saxitoxins to Pacific Walruses using toxin trophic-transfer models

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Climate change is causing drastic alterations to the Alaskan Arctic ecosystem including ocean warming resulting in unprecedented rates of decline in sea ice extent and delayed sea ice formation. These altering environmental conditions are a gateway to unprecedented ecological changes in the Alaskan Arctic marine ecosystem, including the rise of frequent and toxic harmful algal blooms (HABs) consisting of the dinoflagellate *Alexandrium catenella*. It is unknown how the suite of neurotoxins, paralytic shellfish toxins (PSTs) including saxitoxin (STX), produced by *Alexandrium* move throughout Alaskan marine food webs. Additionally, levels of PSTs detrimental to the health of the Pacific walrus (*Odobenus rosmarus divergens*), an important resource to coastal communities in western Alaska, are unknown. The objectives of this study were to construct trophic-transfer models of STXs throughout a critical marine food chain consisting of phytoplankton (*Alexandrium* vegetative cells and cysts), benthic invertebrates (clams, worms, and snails), and walruses using opportunistic samples collected on various cruises throughout Arctic and Subarctic Alaskan waters during 2019 – 2022. Preliminary results found trophic transfers models accurately predicted movement of STXs from phytoplankton to worms and snails, while further improvement to model parameters are required for predicting STXs in clams. Using observed toxin values in walrus prey from 2022, models found walruses were exposed to similar oral doses of STXs as in 2019, an anomalously warm year. While preliminary, these models will help management identify dangerous bloom conditions that may threaten the health of walruses as well as the food security of coastal communities throughout western Alaska.

Prediction and Modeling
O-075

Machine and deep learning approaches to automated classification of Philippine HABs species

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Red tide occurrences pose an ever-present threat to coastal communities in the Philippines as they can lead to shellfish poisoning and massive fish kills. Unfortunately, most efforts in monitoring algal blooms in the country are still based on manual sampling and collection, which commonly equates to a delayed government response. In this paper, the feasibility of establishing an automated HABs monitoring pipeline utilizing the Imaging FlowCytobot (IFCB) is investigated by training and evaluating a series of tree-based and convolutional neural network models in the identification and quantification of local HABs species. A composite dataset containing IFCB images of local species spanning 19 classes across 153,187 images was established using existing open source annotated IFCB image databases. For the tree-based models, a standard feature extraction process was first accomplished, followed by feature selection, model training, and hyperparameter tuning. For the deep learning approach, data preprocessing followed by data augmentation and transfer learning were implemented. In both approaches, oversampling was done to compensate for class imbalance. Furthermore, feature ranking techniques, such as tree-based impurity and SHapley Additive exPlanations (SHAP) for the tree-based models and Gradient-weighted Class Activation Mapping (Grad-CAM) for the deep learning approaches, were also implemented. A 5-fold cross validation shows a high degree of predictive power, with some models reaching 99.11% accuracy and 97.66% F1-score. These results show promising opportunities for automated near real-time HABs monitoring in the Philippines.

Prediction and Modeling
O-076

Development of a forecast model for harmful algal bloom (HAB) over the long term in Sabah's coastal waters

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The coastal waters of Sabah experience harmful algal blooms (HAB) frequently, which negatively impact the local economy. This research aimed to develop machine learning models that could predict the occurrence of two specific HAB species, *Pyrodinium bahamense* and *Margalefidinium polykrikoides*, based on sea surface temperature, sea surface salinity, wind velocity, solar irradiance, and chlorophyll *a*. Cell abundance data from 2004 to 2018 were obtained from the Department of Fisheries Sabah's HAB monitoring program, while environmental data were obtained from the Copernicus Marine Services and NASA POWER databases. The dataset was used as input for the models, with 75% allocated as training data and 25% as test data. The XGBoost machine learning algorithm was employed to create the predictive models, and its hyperparameters were optimized using 10-fold cross-validation. Several adjustments were made to improve the model's performance, such as excluding zero-value data from Sabah's east coast, incorporating 1-week mean values of environmental variables, and modifying the training-test split ratio. After fine-tuning the hyperparameters, the model for *P. bahamense* did not accurately fit the data, showing relatively low prediction accuracy ($R2 = -0.298$, $MAE = 0.626$, and $RMSE = 0.825$). Conversely, the model for *M. polykrikoides* performed well, demonstrating a reasonable level of prediction accuracy ($R2 = 0.597$, $MAE = 0.581$, and $RMSE = 0.682$). This model can be utilized for future predictions to assess the risk of harmful algal blooms in Sabah's coastal waters.

Prediction and Modeling
O-077

Forecasting harmful algal blooms for the aquaculture industry in Mediterranean coastal waters

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Marine aquaculture in the Mediterranean has been threatened by harmful algal blooms (HABs) in events mainly associated to species that produce saxitoxins, okadaic acid and dinophysistoxins. Furthermore, but to a less extent, by species that produce domoic acid, yessotoxins and ichthyotoxins. Monitoring programs have produced robust long term time series on the abundance of different taxa and physico-chemical parameters at aquaculture sites. These time series allow us now to produce forecast models which can be fueled with the results of ongoing monitoring programs to improve early warning systems advancing the information about the level of HABs risk expected in the following weeks. Forecast models based on neural networks, built on phytoplankton times series from Alfacs Bay (Western Mediterranean), result in very low errors of classification: 0% for *Dinophysis caudata*, <3% for *Dinophysis sacculus*, <4% for *Dinophysis*, <1 % for *Pseudo-nitzschia* and 6% for *Alexandrium minutum*. The length of observations required to forecast phytoplankton abundance varies among taxa. The sensitivity analysis showed that 5 years of observations for *Karlodinium* provided optimal results, but a longer series was needed for *Pseudo-nitzschia*. Abrupt changes in trends may diminish forecast capabilities and therefore a continuous improvement of the models is needed.

Prediction and Modeling
O-078

Understanding shellfish biotoxin contamination and predicting DSP events: The Portuguese case study

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Closures to shellfish harvesting due to temporary contamination with marine toxins are a major bottleneck for the effective growth of shellfish production in Europe, and particularly in Portugal. The project "MATISSE - A Machine Learning-Based Forecasting System for Shellfish Safety" aims to overcome the negative impacts of such decision-upon-event processes by developing an early warning system for shellfish safety. Historical data generated by IPMA on the routine analysis for toxins quantification in shellfish, and phytoplankton from shellfish producing areas were used, complemented by meteorological, abiotic, and metabolic data, and also by satellite data from the Copernicus program. Seasonal patterns across the Portuguese coast and variables time-lagged correlated with biotoxin concentration in shellfish were disclosed. Moreover, their predictive role was assessed in autoregressive and artificial neural network models applied to multivariate time-series data for forecasting mussel contamination beyond the safety limits 1 to 4 weeks ahead. The ability to forecast interdictions to shellfish harvesting and production represents an invaluable contribution to decision-making, in particular, in the definition of proper actions to be taken upon closures regarding production management, stock distribution and storage, thus reducing waste and economic losses.

Toxicology

O-079

An update on the need to review toxicological data for marine toxins

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Marine toxins have been regulated in Europe according to an unusual criterion: compounds lethal to a mice are toxins to humans. This has created an odd legislation that is being corrected very slowly. The criteria to consider a toxin based on mice lethality has been proven to be biased. In addition to this, current legislation that sets a monopoly on a unique technology, mass spectrometry, has evidenced the total opposed pathways followed by analytical chemistry and toxicology. European legislation requires a solid analytical technology that needs to be implemented based on weak toxicological evidence (toxicity equivalency factors). This contradiction is becoming evident as recent works show that what has been accepted until now, even the mechanism of action of the toxin groups, needs to be revisited. This presentation will review the state of the art on the toxicity of toxin groups, toxicity equivalency factors, and how all this is being conditioned by the accelerating change in the toxin profile worldwide, but especially in Europe, due to global warming. The examples of NSP, DSP, TTX, PLTx and CTX will be used as a point in case.

Toxicology
O-080

Brevetoxins emergent toxins in France: Evaluation of BTX-3 mouse acute toxicity by oral gavage

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Brevetoxins (BTXs) constitute a family of lipophilic marine phycotoxins produced by the dinoflagellate *Karenia brevis*, but other dinoflagellates are likewise assumed synthesizing BTX-like analogs. BTXs can accumulate in shellfish, fish and diverse marine organisms. Exposure to BTXs in humans may occur by seafood ingestion, inhalation through aerosolized marine water, or by skin contact. BTXs produce the so-called neurotoxic shellfish poisoning (NSP), characterized by neurological, gastrointestinal and/or cardiovascular symptoms. In 2018, BTXs were detected in mussels from a Mediterranean lagoon in Corsica Island (France), and afterwards repeatedly during the cold season, representing an emerging risk, not only in France, but also in Europe. To prevent health risks associated to the consumption of contaminated shellfish in France, ANSES recommended a guidance level of 180 µg BTX-3 eq./kg shellfish meat for the occurrence of BTXs in shellfish. In addition, it recommended assessing the effects of BTXs by an acute oral toxicity study in rodents. The CEA team conducted this study. BTX-3 was used as the reference compound, employing various functional tests and male and female Swiss mice. Results that will be presented at the 20th International conference on Harmful Algae (ICHA) will provide points of departure that can be used to derive an acute oral reference dose (ARfD).

Toxicology
O-081

Effects of dinophysistoxin-1 on the growth, photosynthesis, antioxidant responses, and cell cycle of *Isochrysis galbana*

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The phycotoxin dinophysistoxins are widely distributed in the global marine environments and potentially threaten marine organisms and human health. In this study, effects of the dissolved dinophysistoxin-1 (DTX1) on the growth, photosynthetic efficiency, pigment contents, oxidative stress response and cell cycle of the marine microalga *Isochrysis galbana* were investigated. Growth of microalgae was significantly inhibited by DTX1 with 0.6 ~ 1.5 μmol L^{-1} in a 96-h batch culture, corresponding the 96 h-EC50 of DTX1 at 0.835 μmol L^{-1}. The maximum quantum yield of PSII (F_v/F_m), the maximum relative electron transport rate ($rETR_{max}$), light utilization efficiency (α) and half-saturated light irradiance (I_k) were obviously reduced by DTX1 at 1.5 μmol L^{-1} during 96-h exposure. Contents of most of pigments were generally reduced by DTX1 with a dose-depend pattern in microalgal cells except for diatoxanthin. The ROS levels were increased by DTX1 with 0.6 ~ 1.5 μmol L^{-1} after 72-h exposure, while the contents or activities of MDA, GSH, SOD and CAT were significantly increased by DTX1 at 1.5 μmol L^{-1} at 96 h. Cell cycle analysis showed that the I. galbana cell cycle was arrested by DTX1 at G2/M phase, and the cell size and intracellular complexity were also increased in the 24-h batch culture. This study enhances the understanding of the chemical ecology effects of DTX1 on marine microalgae.

Toxicology
O-082

Algal toxin exposure risks to Pacific walruses and Bowhead whales in the Alaskan Arctic during the anomalously warm ocean conditions of 2019

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Climate change-related ocean warming and ongoing reduction in Arctic sea ice extent, duration and thickness increase the risk of harmful algal blooms in the Alaskan Arctic. Two toxigenic species of concern include the dinoflagellate *Alexandrium catenella* and some diatoms in the genus *Pseudo-nitzschia*. These harmful algae produce the potent neurotoxins, saxitoxin and domoic acid, that impact marine wildlife health and cause the human illnesses known as paralytic shellfish poisoning (PSP) and amnesic shellfish poisoning (ASP), respectively. This talk presents data on algal toxin concentrations quantified in Arctic food web samples of phytoplankton, zooplankton, benthic clams, benthic worms, and pelagic fish collected during the summer 2019 from the Beaufort, Chukchi, and northern Bering seas. The northern Bering Sea average sea surface temperature from May to September in 2019 was the warmest on record since recording began in 1900. Ecologically relevant toxin doses of saxitoxin and domoic acid to walruses feeding on clams in the northern Bering Sea as well as bowhead whales feeding on krill in the Alaskan Beaufort Sea will be discussed in terms of exposure risks and potential health impacts.

Toxicology
O-083

Characterisation and toxicological activity of three different *Pseudo-nitzschia* species from the northern Adriatic Sea (Croatia)

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Diatoms of the genus *Pseudo-nitzschia* are cosmopolitans spread in seas and oceans worldwide, with more than 50 described species, dozens of which have been confirmed to produce domoic acid (DA). Here, we characterized and investigated the toxicological activity of secondary metabolites excreted into the growth media of different *Pseudo-nitzschia* species sampled at various locations in the northern Adriatic Sea (Croatia) using human blood cells under in vitro conditions. The results revealed that three investigated species of the genus *Pseudo-nitzschia* were capable of producing DA indicating their toxic potential. Moreover, toxicological data suggested all three *Pseudo-nitzschia* species can excrete toxic secondary metabolites into the surrounding media in addition to the intracellular pools of DA, raising concerns regarding their toxicity and environmental impact. In addition, all three *Pseudo-nitzschia* species triggered oxidative stress, one of the mechanisms of action likely responsible for the DNA damage observed in human blood cells. In line with the above stated, our results are of great interest to environmental toxicologists, the public and policy makers, especially in light of today’ s climate change, which favours harmful algal blooms and the growth of DA producers with a presumed negative impact on the public health of coastal residents.

Toxicology
O-084

Effect of *Microcystis* on *Daphnia* telomere length is dependent on clonal variation

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Cyanobacterial blooms, more frequent with rising temperatures, often lead to the availability of poor-quality food for keystone grazer *Daphnia*. There is growing evidence that understanding clonal variation in *Daphnia* fitness in response to cyanobacteria is crucial for predicting evolutionary responses to harmful algal blooms. Telomere length (TL) has emerged as a promising molecular tool to assess individual fitness in wild animals, but its relationship with food quality and telomere dynamics in aquatic invertebrates remains poorly understood. In this study, we examined how TL and individual fitness were influenced when *Daphnia* faced toxic cyanobacterial stress. A transplant garden experiment was conducted, rearing four clones of *Daphnia magna* in the presence and absence of low-quality food (toxic Microcystis). Results revealed that toxic *Microcystis* reduced somatic growth rate, reproduction, and population growth. Clonal responses varied, with tolerant clones exhibiting higher fitness than sensitive clones. However, tolerant clones experienced significant telomere attrition when grazing on *Microcystis* compared to the control group. Surprisingly, sensitive clones displayed longer telomeres in the grazing on *Microcystis* group than the control group. This dynamic negative relationship highlights the interplay between telomere erosion, individual growth rate, and reproductive output. These findings shed light on the complex interactions between cyanobacterial pressure and telomere dynamics in *Daphnia* clones, providing insights into the potential impacts on individual fitness.

HABs in a Changing World
O-085

Sharp contrast in the host preference of two epiphytic dinoflagellate genera (*Gambierdiscus* and *Ostreopsis*) in Jeju coasts, Korea

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We compared *Gambierdiscus* spp. (GAMBI) and *Ostreopsis* spp. (OSTRE) in host preference by quantitatively analyzing data from 520 macro-algal samples collected six times (bimonthly) from six stations in Jeju coastal waters. GAMBI and OSTRE showed significant contrasts in commonality and dominance (cells per gram wet weight, CGWT) among all five epiphytic dinoflagellate (EPD) genera. Of total of 1108 abundance data for each EPD genera on 81 species of substrate macroalgae, GAMBI (95, 8.6%) and OSTRE (206, 32.0%) were at the two extreme ends. Of the 81 macro-algal samples top 7 species with more than 30 EPD abundance data (EADat) were all red algae, with *Amphiroa* sp., *Gelidium amansii*, *Plocamium telfairiae* taking the lead with 113, 129, and 100 EADat, respectively. In *Amphiroa* sp. GAMBI and OSTRE accounted for 9.7 and 28.3 % of the 113 EADat, respectively, representing EADat numbers at the two extremes. In the other two macroalgae, *G. amansii* and *P. telfairiae*, GAMBI and OSTRE again represented the two extreme numbers of EADat among the 5 EPD genera examined. Sample mean CGWT for GAMBI (1357) and OSTRE (175) in *Amphiroa* sp. were at the extremes whereas those of OSTRE (175) and GAMBI (1839) were at the top among the 5 EPD genera in *G. amansii* and *P. telfairiae*, respectively. Present example of the sharp contrast in host preference between GAMBI and OSTRE currently requires further long-term in situ monitoring of the two potentially toxic EPD species and preferred host macroalgal species in Jeju coastal waters.

HABs in a Changing World
O-086

Multi-decadal trends in *Karenia mikimotoi* blooms in Japan

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The harmful dinoflagellate *Karenia mikimotoi* frequently kills fishes and mollusks in mariculture in Japan. For identifying multi-decadal trends in blooms of this species, we analyzed the 30-year data (1991–2020) from the annual reports, "Red Tides in the Seto Inland Sea" and "Red Tides in the Kyushu Waters." For analyses, we used three spatial scales; the smallest was water divisions (e.g., bays), the medium was "Seto Inland Sea" and "Kyushu waters," and the largest was western Japan. For each spatial scale and year, we organized the number of blooms and damage cases, amount of damage, maximum cell density, period, and onset dates of the first and last blooms from the annual reports. Since the definition of *K. mikimotoi* bloom varies among the data reporting prefectures, we also created and used a filtered dataset based on the maximum cell density. We performed the non-parametric Mann-Kendall test for data without autocorrelation and the modified Mann-Kendall test for autocorrelated data. For both non-filtered and filtered datasets, the maximum value of maximum cell density and period of bloom showed statistically significant increasing trends in the scale of western Japan. In the same spatial scale and both datasets, the results indicated the earlier occurrence of the first bloom of the year but no significant trend in the timing of the last bloom. In the water division scales, increasing and/or decreasing trends were found for multiple data categories. Our results imply the multi-decadal intensification of *Karenia mikimotoi* blooms in Japan.

HABs in a Changing World
O-087

The impact of marine heatwaves on phytoplankton community changes in the subarctic coastal region of southeastern Hokkaido, Japan

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We conducted monthly field campaigns to investigate the seasonal and annual changes in the phytoplankton assemblages in the subarctic coastal region of Akkeshi Bay, open to the western subarctic Pacific, for 4 years running from January 2019. During our observations, red tides primarily consisting of the dinoflagellate *Karenia selliformis* occurred in the autumn of 2021. The pigment concentration of gyroxanthin-diester, a proxy for the abundance of *Karenia* spp., measured by Ultra-high-performance liquid chromatography (UHPLC) and the cell counting of *Karenia* spp. by light microscopy showed the bloom lasted from September to December 2021 in Akkeshi Bay. Before the *Karenia* spp. bloom in Akkeshi Bay, we found the highest cell concentration of *Synechococcus* measured with flow cytometry in August 2021 after the record-breaking marine heatwave. The *Synechococcus* abundance in August 2021 was 4-fold higher than those in August 2019, 2020, and 2022. An excitation-emission matrix and parallel factor analysis (EEM-PARAFAC) distinguished 6 different fractions of dissolved organic matter containing humic- and protein-like components. We found the higher fluorescence intensity of protein-like (tyrosine- and tryptophan-like) components in the samples with higher cell densities of *Karenia* spp. (> 500 cells/ml), suggesting that protein-like components were likely derived from *in situ* biological production and supported the *Karenia* spp. bloom. We conclude that the biological interactions among *Synechococcus*, *Karenia* spp., and bacteria communities would contribute to the onset and persistence of the *Karenia* spp. bloom in the Pacific coast of Hokkaido in autumn 2021.

HABs in a Changing World
O-088

Evolution of harmful algal blooms in the coastal waters of the Bohai Sea and the Yellow Sea, China

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Harmful algal blooms (HABs) have severe impacts and raise great public attention. The HABs vary at regional and local scale under the joint impacts of multiple environmental stresses, such as climate change, eutrophication, mariculture development and so on. In this study, statistical and GIS tools were used to analyze the evolution features of HABs in the coastal waters of the Bohai Sea and the Yellow Sea based on datasets of HAB events and environmental variables. Our analysis shows that HABs in the Bohai Sea increase their frequency over the period studied, although the increase has plateaued in the last decade. The seasonal distribution of HAB events has clearly expanded, and the main hotspot moved from Bohai Bay to coastal waters of Qinhuangdao over the three periods. A unique feature of HAB evolution is the rapid shift of typical HAB-forming microalgae from dinoflagellates to haptophytes and pelagophytes over the three periods, with a trend toward diversification, noxiousness and miniaturization. In the Yellow Sea, there is an increasing dominance of HABs formed by dinoflagellates in terms of frequency, scale, seasonality, spatial distribution and species. The HABs in the northern Yellow Sea and southern Yellow Sea exhibit different evolution features. The recurrent large-scale green tides in the southern Yellow Sea from 2007 may lead to the decrease of interannual and seasonal frequency of HABs formed by microalgae.

HABs in a Changing World
O-089

Summer *Karenia brevis* blooms in eastern Gulf of Mexico: Climate change, importance of mixotrophy and relation to increasing hypoxia events

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Blooms of the toxic dinoflagellate *Karenia brevis* occur almost annually in the eastern Gulf of Mexico, typically initiating in early fall and terminating before summer. However, several blooms that have occurred in recent years have over-summered and were sustained above the temperature optima for growth. Hypoxia is more likely to occur in parallel with a red tide bloom in warmer ocean temperatures and notable hypoxic events occurred concurrently with seasonally prolonged *K. brevis* events in 2005, 2014 and 2018. This emerging hypoxia on the traditionally nutrient-poor continental shelf is hypothesized to result from mechanisms that differ from eutrophication-induced hypoxia. Increased fluxes of nutrients and fresh water to the Gulf of Mexico after hurricanes may fuel both *K. brevis* events and hypoxia as such extreme precipitation events discharge large pulses of nutrient and organic materials onto the continental shelves. *Karenia* can also supplement its nutrition via mixotrophy. Warming reduces oxygen solubility, increases stratification and reduces nutrient supply to surface waters, creating conditions conducive to mixotrophic species such as *K. brevis*. Based on recent measurements of light-dependent respiration by *K. brevis* using ^{18}O isotopes and analysis by membrane inlet mass spectrometry, we show that a substantial fraction of gross primary productivity can be respired in organic rich waters. Future climate conditions, including warmer waters and extreme precipitation events, may lead to larger and more prolonged *K. brevis* blooms, increased heterotrophy and seasonal hypoxia in the eastern Gulf of Mexico.

HABs in a Changing World

O-090

The effect of hurricane disturbance on benthic microalgal communities in the Northern Gulf of Mexico

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The 2021 Atlantic hurricane season was the third most active on record with 21 named storms and seven hurricanes forming, driven by La Niña and increased sea surface temperatures among other climate factors. After a prior year of significant hurricane activity in the northern Gulf of Mexico we aimed to evaluate the effect of these disturbance events on benthic microalgal (BMA) communities on petroleum structures (rigs) that form interconnected artificial reef ecosystems. Artificial tiles (n=18) were deployed for 28-42 days at rig platforms approximately 10 km southeast (nearshore) and 40 km south (offshore) of Dauphin Island, Alabama. Once retrieved, six tiles were pooled to form three replicates at each depth and site per deployment. Data loggers were maintained throughout all deployments to record continuous light, temperature, and salinity. During the second deployment (August 29-31, 2021) major Hurricane Ida moved through the field sites. Tiles were retrieved following Ida and a third deployment was conducted to capture post-hurricane BMA settlement. The epiphytic community harvested from the tiles were sieved to collect the 20-200 µm BMA fraction and partitioned for culture establishment, microscopy, and metabarcoding. Alpha diversity was comparable across deployments, locations, and depths. Community structure, however, differed between nearshore and offshore locations and pre- and post-hurricane. Interestingly, the ciguatoxin producer *Gambierdiscus* sp. was not observed before Ida at any site, but several species were identified post hurricane. These data highlight the potential of hurricane disturbance events to modify BMA communities and may allow harmful species to establish into new areas.

Ecology
O-091

Objectif Plancton: a citizen science program to assess small-scale phytoplankton variability in a macrotidal environment (Bay of Brest, France)

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Synoptic observations of small-scale (<1 km) variability in phytoplankton community composition based on in situ sampling are rare. Thanks to a citizen science program called "Objectif Plancton", such sampling of phytoplankton communities has been performed in a macrotidal environment (the bay of Brest, Brittany, France) since 2014. Using a predefined protocol, and a simplified plankton sampling kit, 17 volunteer boaters sample, simultaneously, distinct geolocated sites. Sampling is conducted three times per year, in April, June, and September. Collected samples are brought back to the laboratory for subsequent analysis of phytoplankton community composition and other environmental variables. Recent results are presented, including measurements of abiotic parameters and different methods used to characterize phytoplankton communities, i.e.: light microscopy counts, chemotaxonomy (HPLC), and flow cytometry. Despite strong tidal currents in the bay of Brest, the data reveal a non-uniform spatial structuring of phytoplankton communities at small scales, with distinct patches (clusters) dominated by different taxa. Multivariate analyses, and an assessment of hydrodynamic connectivity between stations obtained from a lagrangian flow network, suggest that the observed small-scale spatial structuring of phytoplankton communities can be explained by the combined influences of environmental forcings; themselves spatialized due to the relative influence of the rivers flowing into the bay compared to that of the adjacent Iroise Sea.

Ecology
O-092

Time series analysis of the cyst production of toxic dinoflagellates in the Cape Blanc upwelling region, NW Africa between 2003 and 2020

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The pressure of the recent climate change on marine ecosystems affects the bloom dynamics of toxic dinoflagellates. Cysts of toxic bloom-forming dinoflagellates can form a seed bank in the sediments. To gain a better knowledge of how climate change might affect the cyst production of these species, it is essential to have a long and precise record of their cyst production over time. Here, we present an 18 years-record of dinoflagellate cysts export flux in the upwelling region off Cape Blanc, Mauritania collected by a moored sediment trap. In this region that belongs to the world's most productive marine ecosystems, 73 dinocysts taxa were identified of which 3 were potentially produced by toxic species. Cyst production of these species; *Gymnodinium* spp., *Lingulodinium polyedra*, and *Protoceratium reticulatum* occurred relatively continuous throughout the time series. By applying a time series analysis, we gained insight into the periodicity and variation of their export fluxes in the studied time interval that is characterized by rapid global warming. By comparing the cyst export flux with atmospheric and hydrographic changes in the research area, we could obtain insight into the driving factors of cyst production. For instance; irregular peaks of *Gymnodinium* spp. export flux were observed in 2004 and 2007, which coincided with the coldest sea surface summer temperatures of our time series.

Ecology
O-093

Biophysical trapping and phased mating during a *Dinophysis acuminata* bloom in Nauset Marsh

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Dinophysis are specialist mixotrophic dinoflagellates that can cause diarrhetic shellfish poisoning. They must feed on *Mesodinium* ciliates to grow yet these species are seldom observed together *in situ*. How then do *Dinophysis* blooms develop? In this study, the mechanisms driving development and decline of blooms occurring in the Nauset Marsh (Cape Cod National Seashore, MA, USA) were investigated. Changes in cell abundance, morphology, and behavior were observed by an Imaging FlowCytobot (IFCB) that was operated alongside a profiling sonde that recorded changing physico-chemical conditions within one of the Nauset's terminal kettle ponds. *Mesodinium* were rare preceding and during the 2-3 weeks of *Dinophysis* bloom development in 2015 and 2021. Cell concentrations reached peaks of over 10^6 cells L^{-1} but did not proliferate through division. Instead, they accumulated through advective import and selective retention caused by their formation of dense, subsurface thin layers that persisted for up to six weeks. These features were tightly associated with oxyclines that occurred below the pond's pycnocline and shallow outlet channel, preventing dissipation of blooms through tidal and wind-driven mixing. *Dinophysis* also underwent repeated cycles of phased mating within these features during predawn hours and over several successive days. Sex during extended prey scarcity may enable these cells to benefit from genetic repair or to reallocate kleptoplasts and other cellular resources. Persistence of the Nauset populations may have also been promoted by aggregation in a relatively suboxic zone that improves access to nutrients or limits grazer activity.

Ecology
O-094

Interactions between *Dinophysis acuminata* and zooplankton grazers during harmful algal blooms

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Blooms of *Dinophysis acuminata* are annual events in estuaries around the globe, yet zooplankton impacts on these HABs has been poorly studied. To better understand these interactions, an Imaging FlowCytobot (IFCB) was used to assess plankton dynamics during blooms of *Dinophysis* in NY in 2019, 2020, and 2021. “Fullness” of *Dinophysis* (as quantified by the IFCB) paralleled the presence of their prey *Mesodinium* and enhanced fullness continued through the bloom period. Field experiments were performed with the copepod, *Acartia tonsa*, and indigenous zooplankton communities to assess how changes in zooplankton grazing affected *Dinophysis*. During bloom initiation, there was minimal grazing on *Dinophysis* by protozooplankton, while during the peak bloom period, there was significant grazing demonstrating their role in allowance and control over the course of these HABs. Experimental additions of juvenile *A. tonsa* caused increases in *Dinophysis* densities likely via trophic cascades. In culture, rates at which *A. tonsa* grazed *Dinophysis* were not different than rates when fed a non-HAB in both monoculture and mixed algal diet experiments. Prolonged grazing (> two weeks) on *Dinophysis*, however, caused a significant ($p<0.05$) reduction in copepod survival compared to a non-harmful algal diet suggesting that as blooms intensify and progress, reduced survival of copepods may release protozooplankton from grazing pressure promoting protozooplankton grazing on *Dinophysis* and contributing to bloom demise. Collectively, this study demonstrates that zooplankton grazing can play a key role in both allowing and preventing HABs caused by *Dinophysis*.

Ecology
O-095

Looking into the past: Photophysiological perfomance in ´revived´ 50 year old toxic *Nodularia spumigena* from Baltic Sea sediment suggest adaptation to climate warming

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In the Baltic Sea, toxic bloom-forming cyanobacteria have experienced different phases of climate change in their history, nevertheless, little is known about their adaptive traits to cope with climate warming. After a bloom, resting spores are deposited to the sediment where they can survive for decades and represent natural archives of adaptation. We compared photophysiological performance as a temperature dependent process in historic and recent *Nodularia spumigena* strains resurrected from a dated sediment core taken from the Eastern Gotland Basin. Laminated layers down to 51cm depths were selected for germination experiments at favourable conditions. *N. spumigena* strains only germinated from the sediment subsurface (~2018) and 12 cm depth (~1972). The strains were isolated and identified based on morphology and 16S RNA. Recent and 50-year-old strains were then used to characterize oxygen production and respiration under increasing light and temperature conditions up to 40°C. Furthermore, photosynthetic efficiency (Y(II)) was recorded over the growth period at different temperatures. The results show that light compensation (I_c) and saturation point (I_k) of *N. spumigena* has not changed significantly over the last 50 years even if I_c of recent strains was twice as high on average as in the old strains. I_K differed in mean value by 50 µmol photons m^{-2} s^{-1}. In recent strains the highest oxygen production was between 20-25°C, whereas the 50-year-old strains exhibited their optimum between 10-20°C. Respiration optimum was at around 35°C for all strains. The results indicate that photophysiological adaptation to warming has taken place in Baltic *Nodularia spumigena*.

Ecology
O-096

High resolution biological and physical profiling of the demise of domoic acid during the relaxation of upwelling

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Conventional marine field studies such as weekly pier sampling or ship-based expeditions have traditionally been limited due to the inability to sample continuously over space and time. This study couples traditional ship-based sampling with state-of-the-art long-range autonomous underwater vehicles (LR-AUV) and constant vertical profiling Wirewalkers sampling to create a multi-omics dataset of microbial community composition, metatranscriptomes, total metabolomes and metadata to characterize the chemical-microbe network in Monterey Bay, CA. In this region whose reputation is characterized by charismatic toxic *Pseudo-nitzschia australis* diatom blooms, a baseline background *Pseudo-nitzschia* population is studied during this non-bloom Spring, demonstrating that the same mechanisms that drive dynamics of a naturally occurring baseline process apply when exacerbated. These data reveal physical dynamics that led to upwelling relaxation and the demise of domoic acid production, preventing this seed population from sparking a wide-reaching toxic bloom. Furthermore, biosynthetic intermediate molecules to domoic acid N-geranyl-L-glutamic acid (L-NGG), 7'- hydroxy-L-NGG, and 7'-carboxy-L-NGG, are reported to be detected in the ocean for the first time using solid phase extraction coupled with untargeted LC-MS/MS techniques.

Monitoring and Mitigation
O-097

RPA and nanopore sequencing for rapid field deployable identification of *Alexandrium*

Robert George Hatfield[1]

[1] Cefas

The dinoflagellate genera *Alexandrium* includes multiple toxin producing species, producing a suite of compounds including potent neurotoxins. As some species are not toxin produces, the ability to elucidate them when enumerating for routine monitoring is of great value. Unfortunately this is very challenging using light microscopy meaning that cell counts are often not well correlated with toxin bioaccumulation. Although numerous molecular assays have been developed for the detection of this genera, they tend to only work on a specific species. This means that multiple assays would be needed if there is more than one potential threat. These assays also tend to use PCR to amplify DNA which requires lengthy and expensive DNA extraction to prevent inhibition. The assay presented avoids these drawbacks by using recombinase polymerase amplification, an isothermal amplification technology that is notably resilient to inhibition. Nanopore sequencing is then performed on the amplicons to provide real time phylogenetic identification. The process is rapid, can be multiplexed and in-silico analysis indicates inclusivity of all known saxitoxin producing variants. A single laboratory validation indicated the ability to identify a single cell in 50mL and a field study indicated good correlation between the number of reads attributed to toxic variants and toxins detected in shellfish flesh as well as total reads correlated with cell counts.

Monitoring and Mitigation
O-098

Exploring omics technologies for effective HABs monitoring in Southern Chile

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Harmful algal blooms (HABs) monitoring programs require substantial logistical and financial resources to effectively detect, provide early warnings and mitigate its consequences, and a limited number of institutions worldwide have achieved this level of effort. In Chile, the Fisheries Development Institute has conducting the largest-scale monitoring program for over 15 years. This program encompasses more than 300 sampling stations monthly, spanning a vast geographical range from 36°S to 53°S covering diverse oceanographic features, including fjords/channels and the exposed Pacific Ocean. Molecular detection methodologies, such as metabarcoding and metagenomics, have been integrated as complementary approaches to enhance the monitoring. Utilizing metabarcoding, we sequenced a fragment of 18S ribosomal gene in 34 sites, enabling us to assess the diversity and community composition of microphytoplankton. The analysis revealed 6,400 Amplicon Sequence Variants (ASVs) assigned to principal classes, exhibiting variations in richness and dominance between the studied areas. The observed diversity patterns indicated spatial separation between the fjord/channel and the exposed Pacific Ocean, likely influenced by characteristic environmental parameters such as temperature, salinity, and nutrient levels. Additionally, metagenomic analysis identified 4,670 genes associated with temperature response, light intensity, nutrient assimilation, and toxin production, providing insights into the key metabolic pathways underlying physiological and metabolic changes in the phytoplankton community. This data offers valuable information for future management strategies aimed at predicting and mitigating HABs events in Chile. This research contributes to a deeper understanding of the complex dynamics of HABs and supports efforts to protect both human health and the marine environment.

Monitoring and Mitigation
O-099

Prediction of *Chattonella* proliferation based on photosynthetic activity evaluation with a pulse amplitude-modulation fluorometry

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In recent years, a pulse amplitude-modulation fluorometry (PAM fluorometry) has become a commonly used technique for assessing states of plant photosynthesis. This method involves illuminating plants with modulated light pulses and simultaneously measuring *in vivo* chlorophyll-*a* fluorescence, allowing estimation of the extent of electron transfer in photosystem II. In particular, *Fv/Fm,* a maximum quantum yield under dark condition, is known as an indicator of nutrient status and temperature stress in plants. In this study, we investigated whether the proliferation and physiological state of *Chattonella*, a notorious fish-killer in Japan, could be assessed using *Fv/Fm* measurements with the PAM fluorometry. The results of culture experiments showed that, during the exponential growth phase, *Fv/Fm* exhibited high values around 0.7, which gradually declined after reaching a steady state mostly due to phosphorus limitation. Such *Fv/Fm* declines well coincided with declines of daily cell divisions and with remaining phosphorus concentrations in the culture media, and phosphorus spikes to the media immediately increased *Fv/Fm* values followed by growth increases. In conclusion, *Fv/Fm* measurements in *Chattonella* monitoring well depicted the growth status and the nutrient deficiencies, especially phosphorus limitation, and was considered to be a useful indicator for assessing its proliferation during red-tide events.

Monitoring and Mitigation
O-100

Development of a novel fluorometer to detect harmful algal bloom species and applications to a HABs monitoring

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Improving technology for harmful algal blooms monitoring is an important issue in fisheries industries. We have investigated the feasibility of monitoring harmful algal species using specificity of chlorophyll fluorescence. We concentrated on the chlorophyll fluorescence spectrum peaks of *Karenia mikimotoi* (Km) and *Chattonella marina* (Cm) and noticed they were slightly shifted towards longer wavelengths compared to others. We also confirmed that the Fluorescence spectral Shift Index (FSI), which is defined as the ratio of fluorescence intensities at two different wavelengths (670, 690 nm), can represent the degree of fluorescence spectrum shifts. Based on them, we have developed an *in-situ* Harmful Algal Indication sensor (HAI sensor), which measures FSI to detect the existence of harmful algal species. As a further application to understand more detailed field status, we have developed an estimation method of harmful algal abundance using FSI, and improved HAI sensor to output the estimated abundance as HAD (Harmful Algal Density). The performance evaluation of HAI sensor measurements for cultured samples showed that FSI and HAD detected and estimated harmful algae and its abundances, respectively. In the field evaluations, we compared FSI and HAD against the microscopic observation results, and HAI sensor successfully detected vertical distributions and monitored developmental dynamics of Km by vertical profile observations and long–term mooring, respectively. Furthermore, FSI of *Karenia brevis* and *Karenia selliformis* also showed high value comparable to Km. Thus, we expect that HAI sensor is also useful in regions where those HABs occur, such as Gulf of Mexico and Hokkaido coast.

Monitoring and Mitigation
O-101

PhytO-ARM, an open source/open design toolkit for deployment of adaptive HAB monitoring systems

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An open-source, open-design toolkit for automated HAB monitoring and response has been developed to support HABON-NE, a region-scale network of advanced HAB sensors that serves the northeast U.S. Called PhytO-ARM, the kit can be configured to serve a wide range of sensor deployments and needs. It has been built from our experience creating platforms that programmatically and adaptively reposition Imaging FlowCytobot (IFCB) and other sensors in the water column. Most components have been migrated to commercial-off-the-shelf ones that are easier to replace, maintain, and share with new users. Software components are coded as containerized ROS nodes, making them easy to deploy across one or several devices as required to address wide-ranging needs. Through collaboration with McLane Research Laboratories, we've also updated the IFCB sensor so that it powers a connected CTD sonde and can classify its images onboard using convolutional neural network models. Systems are currently configured and deployed from trailerable barges, ships of opportunity, and autonomous surface vehicles. We have also developed systems specifically for aquaculture that respond to the presence of HABs by sending alerts and taking automated actions at shellfish nurseries. Ongoing development is expanding PhytO-ARM capabilities to integrate new nutrient and carbonate system sensors with the aim of better understanding how increasing stratification, eutrophication, and acidification of coastal oceans may promote the expansion and intensification of HABs. These tools are freely available and adaptable for new ocean sensing applications.

Monitoring and Mitigation
O-102

In-situ segmentation and post-processing of phytoplankton image data captured onboard an autonomous surface vehicle

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The utilization of autonomous surface vehicles (ASVs) for in-situ identification of phytoplankton can considerably expedite species identification of a bloom, surpassing the time required by conventional methods involving sample collection and analysis. Onboard imaging systems are used in-situ to capture images of the underlying species, and the resulting data sets can either be transmitted back to a taxonomist or be fed into a machine learning model for inference. Should the vehicle be in a remote location with limited connectivity, unnecessary data should be removed before transmitting, and prior to inference, the data must be segmented and processed. Methods for post-processing and segmenting microscopic images of phytoplankton captured by an autonomous surface vehicle are presented. Lightweight image manipulation methods using open-source software such as *ImageJ* and *FIJI* are used to remove stuck particles from the image data, as well as normalize the background and perform thresholding to generate binary masks well suited for inference in a machine learning model. The study will also investigate whether pigment-specific analyses can be carried out to target characteristics unique to certain species of phytoplankton, using the additional information given by a hyperspectral imager (HSI). The proof of concept is demonstrated and evaluated by feeding processed image data of phytoplankton into a neural network for unsupervised classification.

Emerging Issues
O-103

The Amnesic Shellfish Poisoning toxin, domoic acid: the tattoo of the King scallop *Pecten maximus*?

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Domoic acid (DA) is a neurotoxin produced by diatoms of the genus *Pseudo-nitzschia* and is responsible for Amnesic Shellfish Poisoning (ASP) in humans. The king scallop *Pecten maximus* is a high valuable resource frequently exposed to *Pseudo-nitzschia* blooms, which is capable of accumulating high amounts of DA compared to other bivalves, retaining the toxin for months or even a few years. This work aimed to decipher what are the main physiological mechanisms involved in the kinetics of slow depuration of DA in *P. maximus* in comparison to fast-depurator species. The development of an anti-DA immunohistochemical technique allowed to visualize that a part of the DA-signal was trapped within autophagosomes distributed within the cytoplasm of the scallops digestive cells. The subcellular time-tracking of DA revealed a strong relationship between autophagy and slow DA-depuration ($r = 0.8$, $P < 0.05$). Furthermore, an *in vitro* exposure of digestive gland slices of scallops *P. maximus* and mussels *Mytilus edulis* to dissolved DA revealed a strong upregulation of autophagy-related transcripts in the scallops, and an overexpression of glutamate receptors (GR) and membrane solute-carriers (SLC) genes in mussels, which could support differences in recognition and absorption/excretion of DA between slow and fast depurators. This work provides new evidence about the physiological mechanisms involved in the long retention of DA in *P. maximus* and represents a baseline to explore procedures to accelerate decontamination in this species.

Emerging Issues
O-104

Enhanced adsorption of highly toxic microcystin analogues onto virgin and aged six microplastic types

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Water quality is a global environmental concern, and microplastics are contributing to the issue. It is becoming increasingly evident that microplastics can act as a vector for micropollutants, including cyanotoxins like microcystins, when co-existing in the environment. This study evaluated the interaction of eight microcystins (MC-RR, -YR, -LR, -WR, -LA, -LY, -LW, and -LF, 5 μg/mL each) in contact with polypropylene and polyethylene terephthalate (10 g/L). Additionally, a mixture of MC-LR, -LW, and -LF (1 μg/mL each) was placed in contact with virgin and artificially aged polypropylene, polyethylene, polyethylene terephthalate, polyamide, polystyrene, and polyvinyl chloride microparticles (median size < 45 μm). The microparticles were acquired commercially and artificially aged. A detailed characterization of the microplastics proved to be crucial for accurate data interpretation; the analytical findings uncovered inconsistencies in purity and size. The type, weathering of the microplastics, and microcystin hydrophobicity were found to be key factors of adsorption. Polypropylene showed the greatest adsorption of microcystin, adsorbing greater amounts of all analogues in the mixture containing eight (83-100% adsorption) and three (80-100% adsorption) analogues. The microplastic weathering also impacted the adsorption of microcystin. All aged microplastics adsorbed microcystins, while no adsorption was observed by virgin polyamide. Moreover, the more hydrophobic and more toxic analogues (MC-LW, -LF) showed greater adsorption onto virgin and aged particles compared to hydrophilic analogues (MC-RR, -LR). The findings indicate that highly toxic hydrophobic microcystin analogues have a higher potential to be transported into the food chain by microplastics, especially after the plastic has undergone environmental weathering.

Emerging Issues
O-105

Microcystin inhalation can trigger immune responses in lung and gut microbiome dysbiosis via gut-lung axis: An acute mouse model study

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While many studies reported on health impacts via ingestion pathways of microcystin (MC), health effects from inhalation pathway is unclear. We investigated the impacts of MC inhalation on the lung immune responses by examining cell counts of immunocytes in bronchoalveolar lavage (BAL) fluids and gut microbiome by determining bacterial community structures and predicting functions. For this, C57BL/6J female mice were exposed to MC via inhalation once a day with two dose groups (Group 1, 25 µg/kg; Group 2, 50 µg/kg) and compared against a control group. The exposure duration was 3 days, and 24 hours after the last inhalation, all mice were sacrificed. Fecal samples were collected before the first inhalation and before the sacrifice, and DNA was extracted and sequenced for gut microbiome analysis. The results show that neutrophils and monocytes in BAL fluid significantly increased in the MC group ($p < 0.1$). The MC groups (Group 1 and 2) showed decreasing trends of alpha diversity, whereas the control group showed increasing trends. At the phylum level, beta diversity of gut microbiome from the MC inhalation group (Group 2) showed significantly less bacterial community structure shift compared to the control ($p = 0.01$). The bacterial genera in which abundance altered positively correlated with functional shift (harmful or unhealthy mouse gut), whereas negatively correlated bacteria were depicted as beneficial or commensal. Bacterial functional prediction using PICRUSt2 result shows that pathways involved in heme biosynthesis and peptidoglycan synthesis were significantly increased in the MC exposure groups. This pilot study found that MC inhalation could affect the gut through systemic crosstalk of immunological signals, which could affect the gut microbial community via the gut-lung axis.

Emerging Issues
O-106

Accumulation of ciguatoxins in the New Zealand greenshell mussel and changes in their transcriptomes

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Ciguatera poisoning (CP) is a non-bacterial seafood poisoning mostly caused by fish and shellfish consumption and is estimated at up to 500,000 cases yearly. CP is caused by the potent neurotoxins ciguatoxins (CTXs) produced by benthic microalgae from the genus *Gambierdiscus*. The vectors of CP are diverse, and many are high-valued commercial seafood species. CTX transfer in the marine food web is complex, preventing the development of effective monitoring programmes and risk assessments. It is predicated that CP will become a risk in aquaculture species with climate change. In this project, the commercially and culturally important green lipped mussel *Perna canaliculus* was exposed to toxic *G. polynesiensis* strains in individual tanks. The bioaccumulation of CTXs using the neuroblastoma cell-based assay (CBA-N2a) and liquid chromatography tandem mass spectrometry was assessed in different tissues. *G. polynesiensis* strains with different toxin profiles (high and low CTX production) were used in this study and their effects on the organisms will be compared. In addition to the lipophilic CTXs, *G. polynesiensis* produces hydrophilic gambierone analogues and these were concurrently monitored. This is the first time that bioaccumulation has been assessed for this toxin class. The effects of the toxins, and different ecologically relevant concentrations of the toxins, on the organisms will also be studied using transcriptomics. These results will allow identification of genes that are differentially expressed in the organisms exposed to ciguatera toxins and determine the impacts of these toxins on survival rates and stress response of exposed animals (e.g., heat shock proteins).

Emerging Issues

O-107

How to integrate the qPCR method into HABs monitoring? First results of an international workshop promoted by GlobalHAB

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The international science program on HABs, GlobalHab, has recently identified the need to integrate the analysis of environmental DNA by molecular methods to improve HAB monitoring and to develop early risk alert systems. Given the need for species specific identification and abundance evaluation, the qPCR/dPCR methodologies have been suggested as the most suitable for HAB species molecular monitoring. To foster the integration and the application of these approaches, GlobalHAB has endorsed and supported the workshop in Hiroshima (Japan) on the 3-5, November 2023 (prior to the ICHA conference). International experts in the molecular field from different countries and continents have been invited to participate in person to the workshop, which comes after 4 online preparatory meetings. The aims of this workshop are: 1) compare and contrast qPCR/dPCR methods in the context of all eDNA approaches, 2) establish how and to what extent qPCR/dPCR could be used in monitoring of HABs considering the limits of the methods, 3) select target species to be monitored with qPCR at a global scale, 4) agree on common protocols adapted to HAB monitoring systems, and 5) identify gaps and expand on existing international guidelines. The outcomes of the workshop will be shared with the international community. At the ICHA conference in Hiroshima, the first results of the workshop will be presented and discussed in order to gain consensus for the initiative.

Emerging Issues

O-108

Severe acute dermatitis in Senegalese fishermen associated with *Vulcanodinium rugosum* and its cytotoxic metabolite portimine A

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In November 2020, and to a lesser extent also in 2021, a "mysterious illness" has struck Senegalese fishermen, with severe acute dermatitis reported in over one thousand artisanal fishermen, following exposure through drift-net fishing activity in an off-shore area South of Dakar (Senegal; West Africa). Infectious character of the illness was rapidly excluded, and analysis of raw seawater suggested microalgal contamination. Samples of raw water, GFF-filtered plankton, and biomass collected off nets in a fishing canoe operating in the area were examined (depending on sample type) by light microscopy, biomolecular analysis (qPCR), targeted (LC-MS/MS) and non-targeted chemical analysis (LC-HRMS), as well cellular models of human skin (keratinocytes and primary skin cells). Microscopy revealed the presence of *Vulcanodinium rugosum*, which was subsequently confirmed by qPCR. Targeted analysis confirmed the presence of pinnatoxin H, portimine A, and traces of analogs in both GFF filters and biomass (maximum of portimine A = 0.2 mg g^{-1} biomass). A comparison of the biomass from Senegal with a culture of a *V. rugosum* strain from the South of France (IFR-VR-001) confirmed that 372 molecular features (48%) were identical in the biomass from Senegal and the culture of the French isolate of *V. rugosum*, again suggesting that *V. rugosum* was a major constituent of the biomass fishermen had been exposed to. Cellular assays on keratinocytes confirmed the high cytotoxicity of portimine at sub-nanomolar levels. The high concentration of portimine A in the biomass collected in a barge combined with its cytotoxicity in dermal cells indicates that portimine A is a plausible cause of the dermatitis reported by Senegalese fishermen.

Ecology

O-109

Are *Heterosigma akashiwo* and its virus HaV fashionistas? -Clonal but Variable-

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Heterosigma akashiwo is a notorious red tide-causing raphidophyte distributed in the tropical~subarctic regions. At the final stage of its bloom, increase in viral titer was observed (Nagasaki et al. 1994), and therefore viral infection is thought to affect red tide dynamics and disintegration (Tarutani et al. 2000). Among DNA and RNA viruses infecting this alga, Heterosigma akashiwo virus (HaV) has been most intensively studied for their intraspecific infection specificities (Nagasaki and Yamaguchi 1998; Tarutani et al. 2000; Tomaru et al. 2004). *H. akashiwo* and HaV strains have highly diverse intraspecific reactivities, however, the mechanism determining their infection specificity remains unknown. We performed frequent samplings at the Uranouchi Inlet in Japan, established a culture collection of *H. akashiwo* and HaV, selected the representative host and viral strains, and then we conducted cross-reactivity test; consequently, they were respectively divided into five and four groups based on their reactivities. Also, most probable number assay revealed the concentration of infectious units were significantly variable when distinct host strain was used as host. Hence, we hypothesized that a clonal viral lysate comprises virions differing in host specificity. Furthermore, it is striking that the restoration of cell growth or emergence of viral-resistant cells occurred in some host-virus combinations. Both *H. akashiwo* and HaV may be diverse in infection specificity even within a clonal culture. We are currently investigating the mechanism supporting these phenomena.

Ecology
O-110

Phaeocystis: A global enigma

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The genus *Phaeocystis* is distributed throughout the ocean, with blooms commonly occurring on continental shelves. This phytoplankter is unusual in that it has two major morphologies: solitary cells and cells embedded in a gelatinous matrix. Only colonies form blooms. The colonies commonly grow to 2 mm in diameter, but some regions support colonies that reach 3 cm. It has been suggested that the large size and mucilaginous envelope allow the colonies to escape predation, but the data are inconsistent as to whether colonies are grazed. Cultured *Phaeocystis* can also inhibit the growth of co-occurring phytoplankton or the feeding of potential grazers. Colonies and solitary cells use nitrate as a nitrogen source, although solitary cells also can grow on ammonium. Given the size of colonies, it has been suggested that *Phaeocystis* might be a major contributor to carbon flux to depth, but in most cases, colonies are rapidly remineralized in the upper 300 m and are not a quantitatively significant source of organic matter to the deep sea. The occurrence of large *Phaeocystis* blooms is often associated with a low and highly variable light and nitrate-rich environment, with *Phaeocystis antarctica* blooms being linked additionally to high iron availability. Emerging results indicate that different clones of *Phaeocystis* have substantial genetic plasticity, which may explain its appearance in a variety of environments including those previously unrecognized in warmer subtropical systems. This variability also makes extrapolation from culture studies to the ocean challenging. Given the recent documentation of *Phaeocystis* appearing in new systems, this trend likely will continue in the future, given that *Phaeocystis* has a marked ability to acclimate to a variety of oceanographic conditions, such as variable nutrient concentrations, temperature and irradiance levels.

Ecology
O-111

Long-lasting extraordinary spring bloom of Amphidomataceae (Dinophyceae) and AZA-2 in the Argentine Sea

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During two research expeditions along the Patagonian shelf in the SW South Atlantic, a mixed, high abundance bloom of Amphidomataceans was sampled in the mid shelf (45.5-46°S, 62-61°W) in spring 2021. A reddish water discoloration was observed in the bloom area. The bloom was first sampled on 16th November with up to 32 million cells/L. Twelve days later, the bloom was still persistent: 14 million cells/L, at a station ~50 km distant from the first sampled location. The magnitude of this bloom is a global record for these dinoflagellates so far reported in the literature. Amphidomatacean species represented 98-99% of the total phytoplankton abundance. *Azadinium spinosum* (60%) and *A. dalianense* (36%) dominated, while other taxa were *A. dexteroporum*, *Amphidoma parvula* and *A. languida*. The few accompanying protists were other naked dinoflagellates and ciliates. In both stages of the bloom, AZA-2 was detected in 20µm net tows and in bottle samples, with values up to 2122 pg/L. This toxin was previously found in an argentine ribotype B strain of *A. spinosum*, which is characterized by a particularly slender cell shape. Such cells were identified in the present bloom with low abundance (0.1 to 0.4 % of total Amphidomataceae), which would correspond to AZA-2 cell quotas of 17-42 fg/cell. The bloom was associated with strong water column stratification, deep mixed layer depth and high chlorophyll concentration. Retention mechanisms may favor the occurrence and persistence of the bloom in the mid shelf where frontal systems develop in spring.

Ecology

O-112

Temperature effects on cell abundances of four *Alexandrium* species revealed by long-term monitoring using qPCR

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Paralytic shellfish poisoning (PSP) has occurred every year in Jinhae Bay, Korea for the last four decades. Despite the long history of PSP events, the organism responsible for PSP is not clear and little is known about seasonal variations of *Alexandrium* species in Jinhae Bay. To address this issue, 30 *Alexandrium* cultures were isolated in Jinhae Bay and the LSU rDNA of the cultures were sequenced. Four *Alexandrium* species were identified; *A. catenella* (Group Ⅰ), *A. pacificum* (Group Ⅳ), *A. affine* and *A. fraterculus*. Based on the sequence information, quantitative real-time PCR (qPCR) using TaqMan was developed for enumeration of the four *Alexandrium* species. Assay specificity and sensitivity of the qPCR were confirmed by testing against related organisms and comparing qPCR results with microscopy. And then the qPCR was used to investigate temporal changes in abundances of the four *Alexandrium* species in Jinhae Bay from 2011 to 2022. The field survey showed that *A. catenella* mostly appeared in spring (March to May), *A. pacificum* appeared in every month (higher abundances in May to July), and *A. affine*/*A. fraterculus* appeared in summer to autumn (July to November). Strong PSP events occurred when *A. catenella* appeared at higher cell densities in April ($P<0.05$), whereas PSP occurred longer periods when *A. pacificum* appeared at higher cell densities in June ($P<0.01$). Also, strong and long PSP events occurred when surface water temperature was lower than average ($P<0.01$), whereas concentrations of dissolved inorganic nitrogen were not associated with the *A. catenella* cell densities and PSP events in Jinhae Bay.

Ecology
O-113

Effects of abiotic parameters on growth and toxin production of three strains of *Alexandrium pseudogonyaulax* (Dinophyceae) from the Danish Limfjord

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The marine dinoflagellate *Alexandrium pseudogonyaulax* is a widely distributed Harmful Algal Bloom (HAB) species that was first detected in the Mediterranean Sea. More recently, *A. pseudogonyaulax* occurred in much colder Northern European waters and for the last 1.5 decades it appears to replace the formerly more common species *A. catenella* and *A. ostenfeldii* in the highly eutrophic Limfjord (Denmark). From there it apparently spreads against a salinity gradient into the Baltic Sea. *A. pseudogonyaulax* produces the macrocyclic polyketide toxin Goniodomin A (GDA), that has been shown to lead to invertebrate mortality in laboratory exposure experiments, and the spreading could thus be of concern for the environment and human health. In this study, we measured growth and toxin production of three strains of *A. pseudogonyaulax*, isolated from the Limfjord, in response to different bottom-up factors including temperature, salinity, nitrogen availability and CO_2-concentration. Results show a broad salinity and temperature tolerance, resulting in growth of all strains between temperatures of 12 and 27 °C and between salinities of 10 and 40, with positive growth rates ranging from 0.06 to 0.33 d^{-1}. The highest cell quotas of GDA were measured at low temperatures and correlate with cell volumes. For two strains, GDA amounts were almost unaffected at all salinities, while the cell quota of the third strain decreased at salinities ≥ 30 about 20-fold compared to lower salinities. In summary, these findings show a high ecological tolerance of the Limfjord population of *A. pseudogonyaulax*, together with distinct intraspecific physiological differences within the population.

Ecology
O-114

Insights into the green seawater discoloration of *Lepidodinium chlorophorum*: from cellular ecophysiology to bloom development and ecosystem impact

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Blooms of the green-lineage dinoflagellate *Lepidodinium chlorophorum* (Green Seawater Discolorations, GSD), have been associated in southern Brittany (NE Atlantic, Vilaine Bay, France) with mass mortalities of cultivated bivalves. Combining *in situ* biological, genetics, satellite, and biogeochemical data with ecophysiological experiments on dinoflagellate cultures and oysters, we propose the first ecological conceptual model that allows understanding bloom phenology and its harmful effect on the ecosystem. In non-blooming period, rare *Lepidodinium* eDNA was detected in the sediments and any benthic cyst was detected, suggesting the existence of pelagic resting forms from which bloom can initiate. Increase in abundance in the field were correlated to ammonium inputs after sediment resuspension. Freshwater inputs and thermal stratification favored the establishment of GSD. During the advanced bloom phase, remote sensing showed the extension of the GDS and highlighted the importance of tidal currents in bloom spatial dynamics. Citizen science data showed that blooms can last more than one month. *Lepidodinium chlorophorum* produces high amounts of Transparent Exo-polymeric Particles (TEP), and in culture the presence of bacteria induced an overproduction of these compounds. *In situ*, bacteria are supposed to remineralize TEP to ammonium, permitting the bloom duration. However, TEP may have a negative effect on filtering bivalves. Experiments on the ecophysiological recovery of the oyster *Crassostrea gigas* exposed to dinoflagellate GSD concentrations showed the production of acid glycoconjugates by digestive tubules, and impaired filtration, and a reduced food assimilation in animals. This conceptual model can favor further studies in other ecosystems impacted by blooms of this species.

Monitoring and Mitigation
O-115

Automated optical approaches for *in vivo* multiscale monitoring of phytoplankton communities and HABs in the English Channel and North Sea

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Automated optical observations of phytoplankton communities are currently implemented in eastern English Channel and southern North Sea on different platforms (fixed autonomous stations, moorings, dedicated cruises and measurements/ships of opportunity) as a complement of long term monitoring networks. These systems are characterized by significant connectivity to adjacent seas, strong hydrodynamics and low to high riverine inputs, influencing biogeochemical and biological processes like high productivity and recurrent phytoplankton blooms, including Harmful Algal Blooms-HABs of potential impact on marine food webs, as well as on human health and economy. Because of Global Change, they are also facing unprecedented changes (including extreme events, heat waves) that need to be considered into the dynamics, ecological assessment and budget. Integrated phytoplankton observations are carried out combining reference methods with innovative automated *in vivo* imaging inflow/*in situ*/benchtop devices, pulse shape-recording flow cytometers, as well as *in vivo* multispectral fluorometers. These measurements provide information on the distribution and dynamics of phytoplankton functional groups (flow cytometry) and main taxa (imaging) at high spatial and temporal resolution assigning community changes to different bloom situations (including HABs) and pelagic habitats state, complementing physical, biogeochemical and biological variables. Interoperability in automated observations as well as in data treatment tools are being consolidated. Moreover, harmonisation in data pipelines conform to the FAIR principle and synthesis based on conventional statistics as well as on IA/ML approaches (used to cluster environmental states and forecast events) makes it possible to address scientific, societal and economic challenges through a new perspective, facing global and local changes.

Monitoring and Mitigation
O-116

The California HAB Early Warning system: a prototype system for a national HAB Observing System in the U.S.

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In the last decade, the ocean science community has developed several novel sensors and methods for monitoring and predicting a diversity of HAB events. These include the Imaging FlowCytobot (IFCB), the Environmental Sample Processor (ESP), and various biophysical modeling systems optimized for HAB prediction. IFCB and ESP instruments are commercially available, have demonstrated efficacy for a range of HAB detection and study challenges, have a growing user base around the world, and are thus at Technology Readiness Level (TRL) 8-9. I will describe our efforts to develop a national capacity to accelerate research to operations for HAB technologies and monitoring efforts so they can be better integrated into management tools, monitoring, and predictive frameworks, while also providing standardization of data collection, processing, interpretation, and archive. This will be accomplished through regional and national coordination, establishment of a HAB Data Assembly Center (HABDAC), and an effort to harmonize and mature existing technologies towards higher readiness levels in collaboration with partner U.S. IOOS Regional Associations (RAs). Specifically, the HABDAC will provide a centralized location for existing HAB data products, provide cyberinfrastructure for processing near real-time imagery and sampling feeds, host a common code repository for machine learning models and training data sets, and provide data science and data management support to research teams producing information products. The prototype focuses on CA given the long-running Harmful Algal Bloom Monitoring and Alert Program (HABMAP), the California Harmful Algae Risk Mapping (C-HARM) predictive system, and 12 operational IFCBs in California.

Monitoring and Mitigation
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The California Imaging FlowCytobot Network: automated image classification and data integration for state-wide HAB monitoring

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The California Imaging FlowCytobot (IFCB) Network consists of 9 IFCB units deployed along the California coast, with the goal of providing real-time data for Harmful Algal Bloom (HAB) monitoring. Image data products, including raw images as well as morphological descriptors of the imaged organisms, are available in real-time through the IFCB Dashboard (https://ifcb.caloos.org). The California IFCB Network has developed a comprehensive image database that serves as the foundation for training and validating of the automated image classification algorithms. We leverage taxonomic expertise to ensure the high-quality annotations of plankton images. The CA IFCB Image Database currently spans three monitoring sites in Southern California: Scripps Pier, Del Mar and Newport Beach. We apply a Convolutional Neural Network (Inception v3) to classify images collected by the IFCBs into 127 taxonomic categories. Here, we illustrate high-resolution, classifier-derived abundance time series for selected HAB taxa recorded at the three Southern California sites, focusing on *Lingulodinium* and *Pseudo-nitzschia* spp. that were responsible for major HAB events in the region in the recent years. Classification-derived abundance data are integrated into the Data Access and Control (DAC; https://habdac.portal.axds.co/) system for effective HAB management. The HAB DAC data portal serves as a centralized hub for HAB monitoring, gathering pertinent information from existing HAB data products and making it more accessible it to stakeholders and decision makers. The ongoing development of automated classification algorithms for underwater plankton images and data integration and visualization workflow within the IFCB Network represents a significant advancement in HAB monitoring along the California coast.

Monitoring and Mitigation
O-118

Yessotoxin, a shellfish-killing toxin, impact the health and harvestability of many shellfish species in the Salish Sea, Washington, USA

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The Salish Sea, the inland waterway of Washington State, USA, is home to numerous shellfish species harvested for commercial, recreational, subsistence, and cultural uses. Washington State is the largest producer of farmed shellfish in the United States, supporting more than 3,200 jobs and an estimated annual economic contribution of $270 million. Summer bivalve shellfish mortalities have been observed in the Salish Sea for nearly a century and attempts to understand and mitigate these losses have been only partially successful. In 2016-2017, during a massive summer mortality event, a maximum concentration of 2.20 mg/kg total yessotoxin was measured in Salish Sea bivalve shellfish, a value known to cause shellfish mortalities in other parts of the world. In the present study, 13 species of shellfish were collected from ten locations in the Salish Sea, focusing on shellfish that are routinely harvested for subsistence by Coast Salish Indigenous communities. During May-October 2021, the concentration of yessotoxins ranged from undetectable to 0.14 mg/kg in the North Salish Sea, and from 0.03 – 2.36 mg/kg in the South Salish Sea. Furthermore, 100% of the sampled bivalves from the South Salish Sea had quantifiable yessotoxin. The summer shellfish die-offs that impact both commercial and subsistence harvests may be more tightly linked to yessotoxin exposure than previously thought due to their ubiquitous presence. Looking to the future, climate change, urban runoff, and drought will increase the likelihood that shellfish contain biotoxins, highlighting the food sovereignty and security issues experienced by people who rely on shellfish as important food.

Monitoring and Mitigation
O-119

Screening of lipophilic toxins in marine invertebrates from NW Iberian Peninsula (Spain). Are mussels good toxicity indicators?

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The presence of lipophilic toxins has been well studied in bivalve mollusks, but not in other marine invertebrates. However, in order to assess the human health risk, it is necessary to characterize the toxins present in a given production area and their potential impact on commercial species. The aim of this study is: 1) to screen the prevalence and concentrations of lipophilic toxins in 9 groups of marine invertebrates from NW Iberian Peninsula; 2) to evaluate the validity of wild mussels as sentinel organisms. 1131 specimens (733 bivalves, 188 gastropods, 83 crustaceans, 56 cnidarians, 48 echinoderms, 9 polychaetes, 8 cephalopods, 4 sea squirts and 2 poriferous) were sampled between April 2021 and December 2022 and analyzed by LC-MS/MS for 14 lipophilic toxins. 13-desm SPXC and PnTXG had the highest prevalence in most of the invertebrate groups, with special mention to OA in polychaetes and sea squirts. Maximum concentrations were recorded for OA in polychaetes and crustaceans, and for DTX2 in polychaetes, even with levels above the legal limit. The toxins concentrations were, in general, higher in mussels than in non-bivalve invertebrates from the same sampling point. The only exceptions were: for OA and DTX2, one polychaete sample and three mean values for gastropods and for 13-desm SPX C, one echinoderm sample. Significant positive correlations in mean concentrations were obtained between mussels and four groups (echinoderms, crustaceans, gastropods and cnidarians). Mussels can be considered good biological indicators for lipophilic toxins. Polychaetes could be an exception, but further surveys would be needed to draw definitive conclusions.

Monitoring and Mitigation

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Screening of paralytic shellfish toxins in non-traditional vectors in NW Iberian Peninsula (Spain)

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Paralytic shellfish toxins (PSTs) are potent marine neurotoxins with worldwide distribution, mainly associated with dinoflagellates. In NW Iberian Peninsula the main species responsible for paralytic shellfish poisoning are *Alexandrium minutum* and *Gymnodinium catenatum*. Our aim is to evaluate the presence of PSTs in marine invertebrates and fish, non-traditional PSTs vectors. A total of 828 samples were collected between April 2021-2023. Eight groups of invertebrates (e.g. echinoderms, gastropods, cnidarians, crustaceans, cephalopods, polychaetes, ascidians and bivalves) and fish were studied. Wild mussels were collected whenever possible on the same sampling point and date to compare PSTs results with the other groups. Samples were analysed using i) HPLC with fluorescence detection and pre-column oxidation and ii) LC-MS/MS. PSTs were detected in 253 samples (including gastropods, cnidarians, echinoderm, polychaetes, porifera, crustaceans and bivalves), of which 229 samples showed quantifiable levels. The typical toxic profile included STX, GTX2, GTX3, GTX1, GTX4. In addition, dcSTX, NEO, C1, C2, GTX5 and C3, C4 were also found in certain samples. Our results suggest the existence of other potential vectors than bivalves, including edible organisms of great economic value. These findings stress the need of further surveys on non-traditional vectors for a better public health protection. Correlation statistical study between mussels, crustaceans and gastropods suggest the validity of the bivalve as sentinel for these organisms. Further extensive surveys would be needed to draw definitive conclusions for cnidarians and the other groups studied.

Toxins, Biosynthesis and Detection Methods
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Colchicine induced dynamic changes in saxitoxin biosynthesis and metabolism in the dinoflagellate *Alexandrium pacificum* (Group IV)

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Intracellular levels of saxitoxin and its analogues (STXs) in paralytic shellfish toxin-producing dinoflagellates are controlled by a balance between degradation and biosynthesis in response to marine environmental fluctuations and stresses. This is the first study to show the usefulness of combining *in vivo* labeling with stress treatment. A toxic strain of the dinoflagellate *Alexandrium pacificum* (Group IV) was cultured in colchicine-containing ^{15}N-labeled sodium nitrate-medium. The intracellular content of all isotopomers of precursor amino acids, biosynthetic intermediates, and major STXs were determined over time by the column switching high resolution liquid chromatography-mass spectrometry, quantitatively. Based on the results of statistical analysis, we proposed the hypothesis that STXs are biosynthesized by *de novo* and salvage pathways. In colchicine-treated cultures, the per-cell content of total STX analogues did not change significantly over time; however, the production rate of each pathway differed greatly. Those for *de novo* biosynthesis of all compounds were decreased by colchicine until Day 3, while those for salvage pathway were not changed. It suggested that colchicine inhibited nitrate assimilation upstream of glutamate biosynthesis in *de novo* pathway. Subsequently, enhancement of biosynthesis by both pathways was observed. Dynamic analysis provides new insights into the complex mechanisms regulating STX biosynthesis and metabolism in dinoflagellates.

Toxins, Biosynthesis and Detection Methods
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Targeted analysis of phycotoxins in combination with Neuro2A bioassay screening as a comprehensive approach for toxin monitoring of shellfish

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In the Netherlands shellfish from production area are routinely monitored for the presence of regulated shellfish toxins by LC-MS/MS analysis. However, the emergence of marine and freshwater toxins in geographical areas where they have never been reported before is a concern of considerable impact on (sea)food contamination, and consequently, on public health. A strategy has been devised in order to gain insight into the presence of emerging and unknown toxins whereby samples are screened with a Neuro2A effect assay. The advantage of this approach is that unexpected or yet unknow toxins, causing an effect in the bioassay, are detected. A previous study with shellfish samples from Chile, showed a good correlation between the bioassay outcomes, that of the *in vivo* mouse bioassay and MS analysis. The Neuro2A method has recently been optimized and validated for both hydrophilic and lipophilic phycotoxins. Up to now 3 years of monitoring samples (n =~1,200) have been used for the screening of lipophilic phycotoxins with use of the Neuro2A effect assay. MS and bioassays outcomes were nicely in line with each other. Four hundred samples (one year of monitoring) have been used for the screening of hydrophilic phycotoxins. When suspected samples occur that cannot be directly confirmed by LC-MS/MS analysis, further measurement will be carried out to using fractionation and high resolution MS to (semi)identify the possible phycotoxin. During the presentation the approach and results of the application will be addressed.

Toxins, Biosynthesis and Detection Methods
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Distribution of phycotoxins and associated harmful algae in the fjords and channels of the Tierra del Fuego Archipelago, South America

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During a research expedition in the fjords and channels of Tierra del Fuego (53 - 60°S, 66 - 72°W), South America, phycotoxin distribution was assessed in the 4-week duration of the cruise by vertical net hauls in January/February 2022. Domoic acid was more abundant in open oceanic waters such as in the Cockburn and Ballenero Channels and the Drake Passage. In contrast, most of the phycotoxins produced by dinoflagellates (SPX, DTX, PTX, YTX, PST) were more abundant in inner waters of the Tierra del Fuego Archipelago. Generally, the abundances of phycotoxins correlated well with abundances of potential producers. Whereas PST toxin profiles of field samples were conserved throughout the entire cruise transect, distinct YTX profiles were found between stations belonging to Magellan Strait (MS) system on one side and Beagle Channel (BC) on the other. The distinct YTX profiles are indicative of the presence of different producing species, i.e. *Protoceratium reticulatum* in MS and *Lingulodinium polyedra* in BC. These results are consistent with cyst abundances of both species in MS and BC. On a temporal scale, an onset of a massive bloom of *Alexandrium catenella* was observed in the central BC and Garibaldi Fjord between end of January and mid February 2022 with PST levels exceeding 700 µg/net haul.

Toxins, Biosynthesis and Detection Methods
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Acute and chronic *in vivo* effect of pinnatoxins in rodent. An attempt to decipher how this emergent class of marine toxins crosses the blood-brain and placental barriers

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Pinnatoxins A and G (PnTx-A, PnTx-G), produced by the dinoflagellate *Vulcanodinium rugosum,* exhibit high acute oral toxicity in rodents. This effect is related to the exceptional ability of these toxins to interact with various subtypes of nicotinic acetylcholine receptors (nAChRs), in particular the muscle and neuronal $\alpha 7$ subtypes. Digital autoradiographic analyses of tritiated PnTx-G after acute and chronic oral administration to mice revealed similar biodistribution profiles with accumulation in the gastrointestinal tract and rapid elimination, mostly by feces. To further assess the *in vivo* PnTx-G biodistribution, ^{3}H-PnTx-G was administered orally to adult rats, revealing a small percentage of the injected dose in peripheral organs (liver, spleen, skeletal muscles) as well as in various brain regions (hippocampus, hypothalamus…), highlighting the ability of the toxin to cross the intestinal barrier and the blood-brain barrier (BBB). After intravenous ^{3}H-PnTx-G injection to pregnant rats, the toxin crossed the placental barrier and was recovered in various central and peripheral embryo tissues. *In vitro* models of a cell-based rat BBB model and an *ex vivo* human cotyledon model were further used to identify how PnTx-G crosses the BBB and the placental barrier, respectively. The first results suggest a passive transport of ^{3}H-PnTx-G across the BBB, while a facilitated toxin transfer through the materno-fetal barrier seems to occur *via* a specific transporter yet to be identified. Altogether these findings reinforce the interest to further study the potential risk to human health associated with long-term consumption of contaminated shellfish.

Toxins, Biosynthesis and Detection Methods
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Challenges in phycotoxin analysis in marine mammal biological matrices

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Marine mammals (MMs) have great potential as sentinel species to assess the effect of harmful algal blooms (HABs) on the marine ecosystem and public health. Despite this, phycotoxin exposure of MMs remains largely unknown worldwide. Moreover, working with MM tissues and fluids is often challenging due to their complex matrices, the limited sample availability, and the lack of standardized protocol for phycotoxin analysis of MM samples by liquid chromatography coupled to post-column derivatization and fluorescence detection (HPLC-FLD) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Currently, most of the scarce studies published do not accurately assess the matrix effects (ME) and their role in ion suppression or enhancement in LC-MS/MS. Considering all the above, the present study aimed to select suitable extraction methods to minimize MEs. Different clean-up steps were tested in various MM matrices (feces, stomach content, and liver, among others) including hexane washing, protein precipitation, and lipid freezing. Most of the latest work has focused on solid phase extraction (SPE), carrying out different types of SPE (normal, reverse and ion exchange phases) and various cartridge sorbents. So far, results show that the ME is highly dependent on the analyzed tissue. Also, the tested techniques have proven to be inefficient in significantly reducing the ME and achieving good recoveries at the same time for the assessed phycotoxin classes. For this reason, mixed-mode SPE phases and matrix-matched calibration curves for each type of tissue or fluid will be explored to compensate for ME.

Toxins, Biosynthesis and Detection Methods
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Highly variable toxin profiles in *Dinophysis norvegica* strains from different geographical locations

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Dinophysis spp. have long been known to produce the okadaic acid (OA) and pectenotoxin (PTX) families of toxins. Recent advances in LC–HRMS analysis and culturing techniques enabled deep untargeted analysis of the toxin profiles of cultured strains of *Dinophysis norvegica* isolated from the Northeast and Northwest coasts of the USA, and from Norway. *D. norvegica* strains from the Northwestern USA produced PTX2, while the Northeastern strains produced dihydrodinophysistoxin-1 (dihydroDTX1) and putative PTX12. The newly isolated Norwegian strain from the Oslofjord produced a novel OA variant and a tentatively identified novel PTX variant, but no PTX2, PTX12 or dihydroDTX1. Interestingly, a water sample from the Oslofjord contained PTX2, PTX12 (identical to putative PTX12 from the Northeastern US *D. norvegica*), OA, DTX1, and DTX2, as well as the novel OA- and PTX-variants identified in the Norwegian *D. norvegica* strain, but not dihydroDTX1. The novel OA variant from the Norwegian strain was detected in a reference material that included Norwegian mussels, suggesting that it may accumulate in shellfish in the same way as other OA/DTXs. LC–HRMS revealed that OA-group toxins were sometimes completely esterified in *D. norvegica* cultures, potentially leading to underdetection of these toxins unless a hydrolysis step is included, but novel diol ester forms were identified in the cultures using LC–HRMS, as were glucosides of the OA/DTX variants. The results suggest that the toxin profiles of *Dinophysis* spp. may be richer than previously thought, varying dramatically with location even within a single species.

Ecology
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HAB response to agriculturally derived nutrient loading

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Monterey Bay, California (USA) is an open embayment, the dynamics of which are shaped by complex interactions between oceanographic and anthropogenic influences. These processes act synergistically to facilitate harmful algal bloom (HAB) events that threaten the rich resources in one of the world's most diverse marine ecosystems. The Bay is surrounded by intense year-round agriculture characterized by repeated fertilizer applications. Run-off is collected into tile drains which discharge into sloughs that feed two main river systems (Salinas River and Pajaro River) and deliver nutrients into Monterey Bay. Continuous nutrient loadings from agricultural run-off and natural processes act in concert to provide nutrients along the shoreline that can fuel HAB events. To examine the ability of nutrients derived from agricultural run-off to promote growth of HAB species, we used mesocosms to measure cell health, growth and toxicity responses of *Alexandrium* sp. and *Pseudo-nitzschia multiseries*. "Raw" agricultural runoff and agricultural runoff treated in woodchip bioreactors were used as nutrient sources, and differences in nutrient source quality on promoting algal growth was examined through daily sampling for particulate and dissolved nutrient fractions. HAB growth response measurements and nutrient fractions were also taken from a year long campaign of monthly cruise transects along the Pajaro River mouth, during which we opportunistically sampled from separate *Margalefidinium* and *Pseudo-nitzschia* blooms. These nearshore field results are being put into the context of overall nutrient loadings from this river stem, including recent reductions from improved agricultural practices. This approach is guiding assessment and improvements of nutrient reduction systems.

Ecology
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Intra-specific genomic and metabolic diversity of bloom-forming cyanobacteria at different spatial scales

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Cyanobacteria exhibit remarkable genomic diversity at the intra-species level, thought to play a major role in Adaptation of marine Synechococcales to spatial environmental variations. However, this aspect is underestimated in the study of bloom development and Ecology of harmful cyanobacteria. Coupling genomics and metabolomics, here we describe the diversity of three cyanobacterial species from distinct Orders, one responsible for perennial highly dense populations, *Limnospira fusiformis* (Oscillatoriales), and two cyanoHAB, *Aphanizomenon gracile* (Nostocales) and *Microcystis aeruginosa* (Chroococcales). At *L. fusiformis* species level, our results showed that the strains were distributed on a phylogenomic tree according to their sampling sites, correlating with highly divergent genetic contents and metabolisms, clearly distinct at the level of specific fatty acids and sugars production. But beyond biogeographical divergences, large genotypic diversity was also characterized within studied populations for all species. In each of them, a large pangenome associated with a very extensive accessory gene set was found despite similar genome size and high similarity indices among genomes. These variations were mainly associated with mobile genetic elements and biosynthetic gene clusters. For some of the latter, metabolomic analysis confirmed the production of related secondary metabolites, such as cyanotoxins (*e.g.*, saxitoxins and microcystins) and carotenoids, which are thought to play a fundamental role in the cyanobacterial fitness. Altogether, these results suggested that large genotypic and metabolic diversity is a common feature largely shared into the Cyanobacteria phylum at high and low spatial scales, and raised questions about the impact of intra-population diversity in bloom development and regulation.

Ecology
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The differential ability of wastewater- and fertilizer-derived nutrients to alter the intensity, diversity, and toxicity of harmful cyanobacterial blooms in North American lakes

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Harmful algal blooms are promoted by excessive nutrient loading and while fertilizers and sewage are the most prevalent nutrient sources in most watersheds, the differential effects of these nutrient sources on HABs are unknown. Here, we tracked cyanobacterial HABs (CHABs) and performed experiments in five North American lakes including Lake Erie where fertilizers, membrane (0.2 µm)-filtered sewage (dominated by reduced forms of nitrogen) or sand- and membrane-filtered sewage (dominated by nitrate) were offered as nutrient sources to CHABs at N-equivalent and environmentally realistic concentrations. Phytoplankton communities were evaluated fluorometrically, microscopically, and via high throughput gene sequencing, and levels of microcystin and the $\delta^{15}N$ content of particulate organic nitrogen (δPON^{15}) were quantified. Fertilizer and both wastewater sources significantly increased the abundance of cyanobacteria in all experiments ($p<0.01$ for all) with sand-filtered sewage having a less potent stimulatory effect compared to membrane-filtered sewage and effects of all nutrient sources on eukaryotic phytoplankton generally being less intense and consistent. Fertilizer significantly increased microcystin levels and decreased the δPON^{15} whereas wastewater significantly increased δPON^{15} ($p<0.05$). *Microcystis* was the genus most consistently promoted by nutrient sources (all experiments), followed by *Cyanobium* (50% of experiments) with increases in *Microcystis* biomass most consistently elicited by membrane, but not sand filtered, wastewater. Collectively, results demonstrate that wastewater from differing types of sewage discharge and fertilizers can promote CHAB intensity and toxicity, while concurrently altering CHAB community composition and δPON^{15} with sewage discharged through sand muting bloom intensity and raw sewage favoring *Microcystis* blooms.

Ecology
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Eight years of weekly eDNA monitoring in the north-western Pacific: an overview of the HAB species detected

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Detection of marine organisms based on environmental DNA (eDNA) has expanded the knowledge of species richness, distribution, and appearance patterns. eDNA data from a single location sampled weekly over eight years was used to detect as many species as possible, including harmful algal bloom (HAB) species. Their appearance patterns and associations with biotic and abiotic factors were also investigated. In total, > 2500 operational taxonomic units (OTUs) associated with unique species were detected, and 52 of those were associated with HAB species. Their detection was aided by three different molecular markers, including a marker targeting dinoflagellates. Each marker helped to detect unique species that were not shared with other markers. The highest number of unique HAB species was detected based on 18S ribosomal RNA gene (14 OTUs). The general appearance patterns associated with the HAB species as well as their associations with environmental parameters and other species in the community were also characterized. The study shows that the long-term eDNA-based monitoring approach provides comprehensive knowledge of the species present as well as their associations with biotic and abiotic factors. This is especially important for local environmental agencies, aquaculture as well as for the general public to avoid or mitigate the negative effects associated with the outbreaks of HABs.

Ecology
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Understanding the factors influencing harmful algal blooms in Patagonian fjords and channel system: insights from historical data and recent studies

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Harmful algal blooms (HABs) have been a persistent phenomenon in Patagonian fjords and channel system for more than 50 years. The occurrence of marine toxins produced by microalgae in Chile was officially recognized in 1972. Recent natural and anthropogenic threats to the marine system in Chilean Patagonia have led to changes in terrestrial and aquatic ecosystems. The unprecedented retreat of ice fields in the southernmost regions has resulted in increased freshwater runoff into adjacent glacier and fjord system. In this study, we present a comprehensive review of historical data and recent experiments, including the results from the 2019 PROFAN cruise. We develop a hypothetical and conceptual model to illustrate climate-hydrologic-ocean interactions with implications for the estuarine environment of the Magellan region. We include two scenarios that consider initiation processes, phytoplanktonic succession associated with the progression of HAB events in the southernmost fjords. These processes include haline stratification, temperature, seasonal availability of light and nutrients availability, with effects on plankton community structure along a longitudinal gradient. The results also highlight the impact of HABs on the aquaculture industry and the need for mitigation and adaptation strategies and provide new insights into HABs in Patagonia`s inland seas of and suggest the prevailing environmental conditions that would favor the appearance of new HAB species.

Ecology
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Toxic ecological relationships: Evaluation of toxic compounds exuded from *Karenia selliformis* on marine microalgae

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The dinoflagellate *Karenia selliformis* produces toxic compounds that can harm other phytoplankton species, impact the food chain, and be detrimental to birds, mammals, and fish. It has been suggested as a hypothesis that the primary ecological purpose of producing and releasing these toxic compounds is to inhibit the growth of other species of microalgae that may be considered competitors (allelopathy). Due to the high toxicity exhibited by this species in Chile, our main objective was to evaluate the effect of exudates from the Chilean strain of *K. selliformis*, CREAN_KS02, isolated from the Aysén Region (43°S), on other phytoplankton species. The bioassays considered the exposure of cell-free exudates of *K. selliformis* on different target microalgae taxa, including *Phaeodactylum tricornatum, Thalassiosira pseudonana, Rhodomonas salina, Dunaliella tertiolecta*, and two species of *Heterosigma akashiwo,* CCMP302 and HA-01, from different origins. Effects on cell density, photosynthetic efficiency, production of reactive oxygen species (ROS), and morphology of the exposed cells were evaluated. After 12 hours of exposure, the abundance of *T. pseudonana, R. salina*, and *H. akashiwo* CCMP302 significantly decreased, reaching 0 cells/mL, while no significant effect was observed on *D. tertiolecta* compared to the control. Regarding photosynthetic efficiency (Fv/Fm), only diatoms *T. pseudonana* and *P. tricornatum* showed a decrease. On the other hand, significant differences in ROS production were observed in *T. pseudonana, D. tertiolecta*, and *H. akashiwo* CCMP302. Our results suggest that the exudates from *K. selliformis* produce species-specific interaction effects and do not affect all coexisting microalgal species in the same manner.

Monitoring and Mitigation
O-133

Investigating the accumulation of dissolved domoic acid in mussels from Monterey Bay, California

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Domoic acid is of particular concern for eastern boundary current regions, such as the central California coast of the United States. Populations of *Pseudo-nitzschia australis* and *Pseudo-nitzschia multiseries* cause near annual blooms related to upwelling-favorable winds in the spring and summer, leading to ecological and economic losses. Due to its chemical stability, domoic acid has been shown to persist in marine environments for months after causative blooms dissipate. In addition, sampling for dissolved domoic acid via direct water analysis and solid phase adsorption toxin tracking (SPATT) from the Santa Cruz Municipal Wharf suggest bloom de-coupled toxin events and show rising concentrations of dissolved domoic acid in recent years. Human and animal health risks associated with long-term, low-dose exposure to domoic acid are still being determined, and it is not clear to what extent traditional regulatory methods track dissolved domoic acid. The present study uses experiments with dissolved domoic acid and California mussels to elucidate dissolved toxin accumulation in mussels. Here we show that domoic acid is accumulated through the gills and digestive system, though concentrations are lower than domoic acid accumulated by feeding on toxic cells under the same experimental conditions. The mussel digestive gland had the greatest domoic acid affinity and retained domoic acid longer than other tissues. Results show that mussels are not an adequate sentinel for dissolved domoic acid, and alternate monitoring methods such as SPATT are more advantageous for understanding toxin circulation in the absence of an active harmful algae bloom.

Monitoring and Mitigation
O-134

Real-time HAB alerts using in-situ sampling and AI image classification

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For the aquaculture the industry in Scotland early warnings of HABs are key in planning preventative action, site management and mitigating against economic loss. Automated in-situ devices can provide massive amounts of data in near-real time about phytoplankton communities in key locations but there are still challenges with analysing and disseminating this information in a way that is useful to the aquaculture industry. Working together with Scottish Sea Farms, SAMS and UHI Shetland have developed stakeholder focused tools which builds on AI image classification and utilizes data captured by the Imaging Flow CytoBot (IFCB). Together these tools provide near real-time alerts for key HAB forming species, allowing the aquaculture industry to dynamically respond to emerging threats.

Monitoring and Mitigation
O-135

Investigation of limit of detection and quantification for digital PCR assays: the case study of *Azadinium spinosum* (Dinophyceae) in Irish waters

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Azaspiracids (AZAs) are a group of lipophilic toxins associated with Azaspiracid Poisoning (AZP) in humans due to the consumption of contaminated shellfish. AZA toxins are produced by some species of marine nanodinoflagellates from the cosmopolitan family Amphidomataceae. Ireland is one of the most affected countries globally by AZA, where blooms of toxigenic *Azadinium spinosum* have been leading to prolonged closures of shellfish farms. DNA-based techniques such as quantitative PCR (qPCR) and digital PCR (dPCR) are extremely sensitive methods that have shown great potential for routine screening of environmental samples, with the former being successfully applied in the detection and relative quantification of some species of Amphidomataceae in Irish waters. In contrast, dPCR has been used to a lesser extend despite its ability to enable absolute quantification of low-copy targets and potential to mitigate enzymatic inhibitory effects. Thus, the present study tested the accuracy of two dPCR systems (StandardBioTools' BiomarkHD and QIAGEN's QIAcuity) using a probe-based assay targeting the LSU-rDNA gene in samples of known target concentration (i.e. nine points 5-fold standard serial dilution of *A. spinosum* batch culture). Limit Of Detection (LOD) and Limit Of Quantification (LOQ) thresholds were estimated to infer transferability, repeatability and reproducibility of the assay across the different systems. Results will be presented on the use of *A. spinosum* toxigenic ribotype as a model organism with the aim of detecting AZA-producers in environmental samples. The final goal of this study is to develop reliable multi-species assays to be implemented in the Irish National Monitoring Program of harmful phytoplankton.

Monitoring and Mitigation
O-136

The mitigation potential of harmful algal blooms (*Alexandrium catenella*, *Pseudo-nitzschia* spp., *Dinophysis acuminata*, *Margalefidinium polykrikoides*) by cultivable seaweeds

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While many macroalgae have been shown to inhibit the growth of HAB phytoplankton, there has been a lack of data on the macroalgae/HAB pairs most relevant for the global aquaculture industry. Here, we present results demonstrating mitigating effects from relevant macroalgae/HAB pairs – predominantly, the effects of *Ulva* spp. on warmer-water HAB species including *Margalefidinium polykrikoides* and *Dinophysis acuminata*, and the effects of sugar kelp (*Saccharina latissima*) on cool-water HABs including *Alexandrium catenella* and *Pseudo-nitzschia* spp. Algicidal activity was present for all pairs; in bloom water incubations, *Ulva* significantly reduced (92% reduction) bloom populations of *M. polykrikoides* compared to control ($p < 0.05$), *S. latissima* significantly reduced bloom populations of *A. catenella* (73-95% reduction, $p < 0.005$) and bloom populations of *Pseudo-nitzschia* spp. (69-75% reduction, $p < 0.05$). Mitigation potential is also demonstrated in many of these pairs using multiple strains of each species in culture. Co-incubation of forage fish (*Menidia* spp., *Cyprinodon variegatus*), *M. polykrikoides*, and *Ulva* spp. demonstrated that =live *Ulva* thalli significantly extended fish survival upon exposure to the ichthyotoxic *M. polykrikoides* in every case, with *Ulva* completely mitigating fish mortality in some cases. The presence of sugar kelp in shellfish enclosures significantly decreased toxin accumulation in shellfish fed toxic HAB phytoplankton; kelp significantly lessened saxitoxin accumulation ($p < 0.05$) from *A. catenella* in *Mytilus edilus* and domoic acid accumulation ($p < 0.05$) from *Pseudo-nitzschia multiseries* in razor clams (*Ensis* spp.) demonstrating the efficacy of seaweeds in inhibiting both HABs and the trophic transfer of HAB toxins.

Monitoring and Mitigation

O-137

A tale of caution: the effect of media nutrients on light-driven cyanobacteria removal studies

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Climate change and anthropogenic pollution give rise to more frequent and severe harmful cyanobacterial blooms which challenge potable water treatment worldwide. Light-driven treatment such as photolysis is a proposed alternative for *in-situ* control of cyanobacteria. A common way of verifying the effects of light-driven treatments on cyanobacteria is by performing lab-scale experiments where cyanobacteria are cultured in growth media (e.g., BG-11). In the current study, six *Microcystis aeruginosa* strains (SCIENTO, NIES 1099, B2666, PCC 7820, 7813 and 7806) in BG-11 media were exposed for seven days to UV-A (365 nm) irradiation, which we have previously shown to be effective for targeted cyanobacterial and toxin removal. Photosynthetic activity significantly decreased after 24 hours of irradiation with samples showing no photosynthetic activity by the end of the experiment. Intra- and extracellular microcystin (MC) concentrations were markedly decreased in UV-A treated samples with a combined microcystin removal of 86%. It was observed, however, that the nutrients present in the BG-11 medium used in the current experiments (e.g., nitrate and iron) enhanced the UV-A photolytic effects on *M. aeruginosa* photosynthetic activity and microcystins concentration. MC-LR dissolved in BG-11, pure water and artificial fresh water was exposed to UV-A irradiation to confirm the effect of nutrients in light-driven treatments for cyanobacteria control and a MC-LR removal of 56%, 22% and 6%, respectively, was observed. It is important to consider the media composition for lab-scale experiments focused on cyanobacterial removal in order to effectively evaluate light-driven strategies for cyanobacteria and toxin removal.

Monitoring and Mitigation

O-138

A modified stratification index method to assess reservoir water quality trends

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Harmful algal blooms (HABs) have been a harm to reservoir health for decades and it is believed that as climate changes and temperatures rise, frequency and severity of HABs are likely to increase as well. Understanding relationships between physical factors in a reservoir and bloom trends is key for keeping rising blooms at bay. A modified stability index based on temperature dependent water density at multiple depths was adapted into a code to process high frequency reservoir monitoring data. Metrics and visual tools were developed to use this stability index to objectively analyze how stratification, – including intensity, start date, and turnover point – algal concentrations, and other water quality characteristics are changing, and how they are likely to change in the coming decades. The stability index time series was compared to time series of water temperature, water depth, chlorophyll-a, and blue-green algae observations at several United States Army Corps of Engineers (USACE) reservoirs. A percentage for each site was generated, indicating if blooms were occurring each year near onset of stratification, maximum stratification, or turnover. Bettering our understanding of when stratification is occurring within reservoirs, as well as when blooms are occurring, will lead to more informed management decisions and better HAB control. The ultimate goal will be to applying this method to a high volume of USACE reservoirs, in order to analyze regional trends, proactively combat HABs, and improve reservoir water quality.

Toxins, Biosynthesis and Detection Methods

O-139

Environmental detection of *Pseudo-nitzschia* spp. and domoic acid biosynthesis in the Santa Barbara Channel, 2019-2022

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The proliferation of well-known harmful algal bloom genus *Pseudo-nitzschia* (PN) poses a significant threat to fisheries, marine mammal survival, and public health via the production of neurotoxin domoic acid. Genes encoding DA biosynthesis were recently discovered and can now shed light on how and why this neurotoxin is produced in nearshore environments. Located in the Southern California Bight, the Santa Barbara Channel is a hotspot for such toxigenic PN blooms that led to a marine mammal stranding crisis in August-September 2022. This study investigated *Pseudo-nitzschia* spp. and domoic acid biosynthesis in the Santa Barbara Channel using a combination of short- and long-read amplicon sequencing, metatranscriptomics, and metabolomics. Long-read Nanopore sequencing of the region connecting the eukaryotic ribosomal DNA small (18S) and large subunit (28S) allowed for high species-level resolution of *Pseudo-nitzschia* produced by short-read 18Sv4, 18Sv9, and ITS2 amplicon sequencing. The frequency of monthly sample collections on the Plumes and Blooms Cruises (NOAA, UCSB) between 2019-2022 highlighted distinct seasonal assemblages and gene expression patterns of *Pseudo-nitzschia* spp. Results shed light on oceanographic conditions associated with species-specific expression of domoic acid biosynthetic genes, in connection with elevated particulate domoic acid concentrations up to 22,720 ng/L.

Surveillance and Management
O-140

Assessing the risks for the novel azaspiracid shellfish toxin AZA-59 in the Pacific Northwest Region of the United States

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In the Pacific Northwest Region of the United States, the first illnesses due to lipophilic shellfish toxins, specifically diarrhetic shellfish poisoning from dinophysistoxin-1, occurred in 2011. To assess the potential presence and relative risks from additional lipophilic shellfish toxins such as azaspiracid in the region, solid-phase adsorption toxin tracking (SPATT) sampling and a targeted effort to culture *Azadinium* was implemented in 2014-2015. Several *Azadinium* species were isolated and one species, *A. poporum*, was found to produce the novel azaspiracid AZA-59 exclusively. This compound was further detected in trace quantities on SPATT with no AZA-1, 2, or 3 detected. Mass culturing of *A. poporum* produced sufficient toxin to confirm its identity as 3-hydroxy, 7–8 dihydro AZA-1, with a 24 h LD_{50} in mice of 122 micrograms/kg, after intraperitoneal injection. A provisional TEF of 0.8 was established based on the corresponding LD_{50} for reference AZA-1 of 92 micrograms/kg. Specific qPCR analysis for *A. poporum* in combination with additional SPATT sampling from 2016-2018 found *A. poporum* and AZA-59 to be widely distributed in low abundance in Puget Sound and surrounding coastal waters. Screening of shellfish for AZA-1, 2, 3, and 59 by targeted LC-MS/MS during this same period detected AZA-59 (only) in 3% of samples with a maximum concentration of 2.6 micrograms/kg. At this time, AZA-59 appears to be a relatively low risk to shellfish consumers in the region, but management strategies developed as part of this project can be used to mitigate risks if *Azadinium* and/or AZA increase in the future.

Toxins, Biosynthesis and Detection Methods
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Evaluation of the cytotoxic activity of methanolic extracts from the dinoflagellate *Amphidinium* against human breast and lung cancer cell lines

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Dinoflagellates produce an extensive array of secondary metabolites with potential biotechnological and biomedical applications. In recent years, these compounds have attracted attention because of their structural diversity, which confers a variety of biological activities. Members of the genus *Amphidinium* produce compounds with known cytotoxic activities. This research aimed to evaluate the cytotoxic activity of three *Amphidinium* strains against two panels of human cancer cell lines. The strains AeSQ172, AeSQ177, and AeSQ181, isolated from San Quintin, Baja California, Mexico, were described morphologically and identified as *A. eliatiensis* based on sequences of the ITS1-5.8S rRNA-ITS2 and 28S large-subunit (LSU) rRNA gene operon. Crude methanolic extracts were obtained from cultured biomass and the solvent was removed. Dry extracts (MeOH-free) were mixed with 0.1% DMSO and evaluated for cytotoxic activity against seven human cancer cell lines: four lung cancer (carcinoma A549 and H661; adenocarcinoma H1437 and H1563) and three breast cancer (carcinoma T-47D; adenocarcinoma MCF-7 and MDA-MB-231). Our results showed that the application of methanolic extract at 100 µg mL^{-1} significantly decreases the cell viability of all tested cell lines. In breast cancer cell lines, the percentage of inhibition was 19% for MCF-7 and T47-D. In lung cancer cell lines, the percentage of inhibition was 16% for A549, 16% for H661, and 21% for H1467. MDA-MB-231 (breast cancer) and H1567 (lung cancer) cell lines showed the highest cytotoxic response, with 29% and 27%, respectively. These promising results prompted further fractionation to obtain purified compounds with higher cytotoxic activity.

Toxins, Biosynthesis and Detection Methods

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Non-target analysis of marine algal toxins using passive samplers with liquid chromatography–high-resolution mass spectrometry

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Liquid chromatography–high-resolution-resolution mass spectrometry (LC–HRMS) offers the potential for non-target analysis (NTA) of marine algal toxins. In this study, passive samplers deployed at various sites in Puget Sound, Washington State (USA), between 2016 and 2018, were analysed by LC–HRMS with data-dependent acquisition (DDA) using metabolomics software for data handling. Method and data workflows were modified from those previously established for assessment of algal biotoxin reference materials. Methanolic extracts of the Diaion HP20 resin samplers were analysed by reversed-phase LC with DDA MS/MS analysis in both positive and negative ionization modes. Through a combination of an exclusion list for background peaks, an inclusion list for known toxins, and software optimization for detection of related compounds, a comprehensive profile of algal toxins in the study location was established. Toxin classes observed over the three years were consistent, but with variations between sites and in trends over time. Dinophysistoxin-1, pectenotoxin-2 and yessotoxin were prevalent, with the software detecting many class-related analogs not typically monitored using targeted methods. The recently reported azaspiracid-59 was not automatically detected by NTA, but was found at trace levels through manual extraction of MS data. Classes of cyclic imines including gymnodimines and spirolides were also found. This work demonstrates the power and applicability of LC-HRMS and NTA in algal toxin screening, while the information generated on spatial and temporal toxin occurrence in Puget Sound is valuable for future research on harmful algae and toxins in the region.

Toxins, Biosynthesis and Detection Methods
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Paralytic shellfish toxin screening using bioelectronic tongue

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Phytotoxins produced by marine microalgae, such as paralytic shellfish toxins (PSTs), can accumulate in bivalve molluscs, representing a human health concern due to the life-threatening symptoms they cause. Development of rapid tools such as sensors for PST detection is challenging since this toxin group comprises more than 50 compounds with toxin profiles varying among different PST producing species. Several assays and biosensors proposed for detection of PSTs are mostly based on antibodies to saxitoxin (STX), which is the most common PSTs worldwide. As PST profile detected in bivalves from Portuguese coast during *Gymnodinium catenatum* blooms differs due to the predominance of N-sulfocarbamoyl and decarbamoyl toxins, usefulness of these biosensors is quite limited. The present work aimed at the development of bioelectronic tongue comprising potentiometric chemical sensors and enzymatic biosensors as a rapid tool for detection of N-sulfocarbamoyl and decarbamoyl PSTs. Analytical characteristics of 8 potentiometric chemical sensors with PVC plasticized membranes were determined in the solutions of 6 major PSTs (dcSTX, GTX5, dcGTX2+3, C1+2, dcNEO, GTX6). Sensors displayed cross-sensitivity to all studied toxins except GTX6, to which low sensitivity and low selectivity were observed. For the detection of GTX6 enzymatic biosensor based on carbamoylase enzyme and potentiometric sensor sensitive to dcNEO was developed. Sensor detection limits were in the range from 0.03 to 0.7 μmolL^{-1} allowing PST detection at the concentration levels corresponding to the legal limits. Selection of the sensors for the bioelectronic tongue for simultaneous quantification of major PSTs in bivalve extracts was carried out using genetic algorithm.

Toxins, Biosynthesis and Detection Methods
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Exploration of *Vulcanodinium rugosum* toxins and their metabolism products in mussels from the Ingril lagoon hotspot in France

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Vulcanodinium rugosum is the producer of pinnatoxins, pteriatoxins, portimines and kabirimine, considered as emerging marine biotoxins. Over 2018, we assessed the toxin contamination of shellfish collected monthly in Ingril lagoon, a site known as a hotspot for *V. rugosum* growth. This short time-series study gave us an overview of the presence and seasonal variability in shellfish of all toxins associated to this dinoflagellate. Complementary strategies were implemented, involving LC-MS/HRMS acquisitions and LC-MS/MS targeted analysis. Pinnatoxin-G was quantified throughout the year, with the highest value observed in June (467.5 µg/kg), whereas Pinnatoxin-A was systematically quantified at trace levels. Portimine-A was detected between May and September. One of the main findings of this study is the identification of a series of fatty acid esters of PnTX-G. Apart from the conjugation with palmitic acid, the other fatty acid esters of PnTX-G identified had been never reported in European shellfish, so far. Unknown metabolites were also detected using various approaches, including molecular networking. We identified a series of unknown fatty acid esters of portimine-A. Fractionation experiments and semi-synthesis of several analogs were performed to confirm their identity. Some other compounds detected were putatively attributed to known *V. rugosum* metabolites or biotransformation products since reference materials were not available to ensure their identification. Finally, another compound was considered an unknown analog of pinnatoxins; acquisition of more comprehensive structural information is needed to characterize it more precisely. Findings of our study will contribute to a better characterization of the hazard that cyclic imines, produced by *V. rugosum* or resulting from their biotransformation, pose.

Ecology
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Patterns and drivers of toxic *Pseudo-nitzschia* spp. abundance identified with an Imaging FlowCytobot in the Northern California Current System

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Harmful algal blooms caused by the diatom genus *Pseudo-nitzschia* are increasing along the California Current System. Many *Pseudo-nitzschia* spp. (PN) can produce the neurotoxin domoic acid, which is transferred through marine food webs by ingestion of PN cells. Through marine heatwaves and ocean acidification (OA), the consequences of climate change are already being experienced in the California Current System, so there is a growing need to understand how these stressors will affect toxic PN blooms. There are examples of the linkage between warm anomalies and increased PN abundance and toxicity, notably the massive toxic bloom of *P. australis* in 2015 that closed major fisheries throughout the U.S. West Coast. However, the effects of OA on PN blooms are inconclusive, as most of what we know comes from laboratory experiments, where only one or two variables were changed. Those studies suggest that the effects of OA depend on the full range of in situ dynamics, including PN strain diversity, interactions with other environmental variables, and competition with other phytoplankton. Here, we explore the abiotic and biotic drivers of PN abundance and toxicity in the NCCS using observations from a NOAA fishery survey during the summers of 2019 (heatwave) and 2021 ("normal"). An Imaging FlowCytobot was installed on the survey to collect continuous phytoplankton data, which was paired with observations of temperature, salinity, pCO2, nutrients, and domoic acid. The realized ecological niches occupied by small and large (generally more toxic) size PN in each of the study years are compared to assess drivers of toxicity.

Ecology

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The occurrence pattern of the generalist parasitoid *Parvilucifera* (Alveolata, Perkinsozoa) of dinoflagellates revealed by a high-frequency time series data

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Current knowledge about the diversity and ecophysiological characteristics of the generalist parasitoid *Parvilucifera* (Aleveolata, Perkinsozoa) has mostly come from occasional samplings during dinoflagellate host blooms and laboratory experiments. However, which factors are relevant to the occurrence pattern of the parasitoid in the field has not relatively received much attention. To address this issue, we conducted a high-frequency protist monitoring with focus on the occurrence, prevalence, and impact of parasitoid *Parvilucifera* (Aleveolata, Perkinsozoa) with the dynamics of dinoflagellate hosts at a fixed station in Jinhae Bay, Korea during 411 days from April 2020 to May 2021. The study site was characterized by several bloom events, which were caused by several alternate dinoflagellate hosts throughout the daily time-series monitoring period, irrespective of the season. Despite the presence of a variety of potential hosts throughout the sampling period, the infections by *Parvilucifera* spp. in surface water were detected in only five dinoflagellates species and even occurred only during the abundance of dinoflagellate *Akashiwo sanguinea* reaching up to 10^3 cells L^{-1}. The result from the high-frequency time-series data suggests that the occurrence of *Parvilucifera* is highly associated with a density threshold of the preferred host species (*A. sanguinea*). Moreover, the generalist parasitoid *Parvilucifera* showed a pattern of alternative host use such as parasite spillover mostly in the presence of the preferred host. The results obtained from this study would provide an insight to understanding how the parasitoid interacts with its preferred host and even potential hosts in the field.

Ecology
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Diversity and niche differentiation of the *Amoebophrya* species complex infecting harmful dinoflagellates in Korean coastal waters

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Marine dinoflagellates are important primary producers in ecosystems, and some bloom-forming dinoflagellates are known to produce toxins that can cause disease in human health. In this study, the dynamics of *Amoebophrya* (Syndiniales), a group of parasites that are fatal to a wide range of marine dinoflagellates were daily monitored between April 2020 and May 2021 in Jinhae Bay, located on the southern coast of Korea. During the high-frequency monitoring period, a total of five dinoflagellate genera (*Alexandrium*, *Dinophysis*, *Heterocapsa*, *Prorocentrum*, and *Scrippsiella*) were infected by *Amoebophrya* species complex, and the timing of occurrence and prevalence of the parasitoid complex were different for each host. Additionally, using the sequences of *Amoebophrya* obtained by the single-cell PCR and DNA metabarcoding, the genetic diversity and haplotype networks of the *Amoebophrya* species complex were analyzed. The species-specific resources and regulators defining the realized niches of the *Amoebophrya* species complex and their hosts were identified using the Outlying Mean Index (OMI) analysis. The results would contribute to a better understanding of the dynamics of the bloom-forming dinoflagellates that may be affected by parasitoids with different niches.

Ecology
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Genus *Pseudo-nitzschia*: The physiology of success in a highly structures and dynamic phytoplankton ecosystem

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The northern Adriatic is a very convenient ecosystem to observe phytoplankton under a variety of different conditions. Steep gradients of nutrient concentration/availability in this shallow basin allow the observation of the very same species under changing nutrient regimes on small temporal and spatial scales. The genus *Pseudo-nitzschia* is among the most abundant and most frequent diatoms in the northern Adriatic phytoplankton community over the last four decades. After studying *in situ* behaviour of this genus along the aforementioned gradients, we set out and isolated key species for in vitro experiments, where we analysed their reactions to contrasting nutrient regimes. In earlier studies, we found that alkaline phosphatase extracellular activity as well as membrane lipid remodelling are major reactions of phytoplankton to phosphate limitations. However, each species is using those functions in its own way to ensure survival and success within the phytoplankton community. Having identified phosphate limitation as a major shaping factor, we here present how harmful algae species of the genera *Pseudo-nitzschia* ensure successful competition within the phytoplankton community. Here we report data on species-specific growth rates under different nutrient regimes, phosphate uptake rates, alkaline phosphatase activity, localization and activation patterns and characteristics of alkaline phosphatase activity in four *Pseudo-nitzschia* species regularly occurring in the basin. Our results demonstrate a high interspecific variation in metabolic responses to phosphate limitation in sympatric congeneric species. The ecological significance and function appears hence to be defined on species level and appears far from homogeneous within genera.

Ecology
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Dangerous hitchhikers: attachment, transport, and survival of HABs-causing species in floating plastics debris

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Mismanaged plastics that end up in the ocean provide different microbiota with new substrates to colonize and form a distinct microenvironment now known as the 'plastisphere'. Among the microorganisms attaching to the plastic surface however are known toxic dinoflagellates, which may be transported with the drifting plastics. Many aspects of the HABs-plastics interaction as well as the mechanisms involved in the attachment however remain little understood. Here, we combined amplicon and shotgun metagenomics coupled with microscopy to determine the presence and diversity of HABs species naturally occurring in floating plastics. Results showed the high diversity of HABs in floating plastics collected from the different environments in the Philippines, shown by both sequences and microscopy. Within the plastics-associated community, HABs cells were embedded within the biofilm matrix and might be benefitting from the myriad of interactions with other microbiota such as nutrient recycling. We then tested the ability of *Alexandrium* cf. *pacificum* to survive various conditions akin to oceanic gradients while attached to LDPE sheets. Significantly higher attachment of *Alexandrium* cells was observed on plastics with mature biofilms especially when nutrients in the surrounding environment were limited, indicating the role of the biofilm in ensuring the viability of the cells. Succession studies were also conducted to understand mechanisms of recruitment of HABs to the plastisphere community explored by 18S rRNA gene sequencing. Results of this study have several implications in understanding the role of plastics as a potential new dispersal mechanism for HABs species.

Ecology
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Saxitoxin dynamics underlying bloom development and persistence in the bioluminescent HAB species *Pyrodinium bahamense*

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Pyrodinium bahamense is a saxitoxin (STX)-producing bioluminescent dinoflagellate that blooms in the Indian River Lagoon (IRL), Florida, the bioluminescent bays in Puerto Rico, and the Indo-Pacific. STX is a potent neurotoxin that binds with high affinity to the voltage-gated sodium channel in humans and causes Paralytic Shellfish Poisoning. Its role in the ecology of *P. bahamense* remains unknown. While *P. bahamense* previously existed in Florida with no know record of toxicity, toxic blooms have emerged over the past 20 years. The core STX biosynthetic pathway genes have been identified. *SxtA4* is essential for toxin synthesis and so serves as a molecular proxy for toxicity. Our lab developed a single-cell multiplex PCR assay targeting the 18S rRNA and sxtA4 genes in *P. bahamense* and found that some cells within the same sub-populations (both IRL and Puerto Rico) lack *sxtA4*. This assay is now being used to assess genotype frequency and toxin dynamics among *P. bahamense* blooms from multiple sites in the IRL. Our data show the *sxtA4+* genotype frequency to be ca 100% among multiple dates and sites in "early" blooms (March and April) or pre-bloom sub-populations, but that as the season progressed, the *sxtA4-* genotype emerged. A general trend among sites showed the toxin quota per cell decreased as cell abundance increased. *SxtA4* transcript abundance is being quantified to identify whether the underlying molecular mechanism of toxicity decrease is due to solely to genetics (i.e. increase in *sxtA4-* cells) or if repression of *sxtA4* by *sxtA4+* cells is also occurring.

Monitoring and Mitigation
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Possibility of suppressing harmful algal blooms (HABs) using artificial reefs (water stirring blocks) installed on the coastal seabed

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This study introduces a possibility of environmentally friendly mitigation method to suppress harmful algal blooms (HABs) by utilizing artificial reefs (AT-V). In recent years, efforts have been made to artificially resuspend coastal bottom sediments using fishing boats and other means to facilitate the germination of diatom resting stage cells and promote the growth of resulting diatom vegetative cells. These efforts aim to suppress blooms caused by harmful flagellates. However, these methods are relatively expensive as they require energy source like fuel oil, which limits the trial times they can be implemented in the field. The AT-V reef is designed as a regular hexagonal pyramid-shaped concrete block (with dimensions of W = 6.86m, D = 5.94m, H = 2.5m, and a weight of 9.80 tons). It is expected to resuspend bottom sediments and create water movement according to the currents from all directions (360°). The AT-V reef operates without external power and is expected to facilitate the continuous resuspension and diffusion of coastal bottom sediments over an extended period, utilizing natural currents such as tidal currents, wind-driven currents, and density currents. If sediment resuspension and water stirring capability of this product are proven to be sufficiently effective for diatom germination and proliferation in natural environments, the proper installation of AT-V reefs (considering the flow characteristics of each coastal area) can contribute to the successful implementation of “Sato-Umi” practices, which have the potential to suppress HABs and enhance the productivity of coastal areas by harnessing the inherent power of nature.

Monitoring and Mitigation

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A review on environmentally friendly strategies against harmful algal blooms

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Since there are currently no practical countermeasures for harmful algal blooms (HABs) other than passive measures such as feeding stop and cage moving, there is an urgent need of effective and environmentally friendly strategies against HABs. Biological control strategies are considered to be more environmentally friendly than physicochemical methods. We proposed that the developments of seaweed beds and seagrass beds are effective against the occurrences of HABs. Seaweed farmings or combined farmings of fishes and seaweeds would be promising. Their effectiveness is due to the function of algicidal bacteria that attach to the surface of seaweeds and seagrasses with enormous densities (usually > million/g). HABs are scarce in the waters of Akkeshi-ko Estuary and some part of Puget Sound in US with big scale *Zostera* beds. As another strategy against HABs, we proposed lifting diatom resting stage cells (requiring light for germination) from bottom sediments into euphotic layer with bottom tillage to proliferate vegetative cells after germination. Nutrients will be quickly consumed by growing diatoms, and HABs of flagellates would be prevented. *Chattonella* red tides were prevented by sea bottom tillage in Tomonoura. Toxic *Alexandrium* blooms have been suppressed by bottom tillages in 2020-2023 in Osaka Bay. The minimum requirement in HAB strategies is that "The ecosystem must not be adversely affected by implementation of control measures". The concept for sustainable fisheries "Sato-Umi" is "High productivity and biodiversity in the coastal sea area with human interaction". Environmentally friendly strategies against HABs should be included in this concept.

Monitoring and Mitigation
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Is Peroxymonosulfate (PMS) a better alternative to hydrogen peroxide treatment for the *in situ* mitigation of cyanobacteria harmful blooms (cyano-HABs)?

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Peroxymonosulfate (PMS), is used in the water treatment industry (especially pool and spa) as it can do targeted oxidation for the removal of emerging contaminants and disinfection. PMS was also used in bench scale experiments for the removal of cyanotoxins from drinking water. Despite that, there is limited information in the literature regarding its application on mitigating toxic cyanobacteria *in situ*. Other peroxide compounds such as H_2O_2 have been extensively used on cyanobacteria contaminated sites with varying efficiencies. Though efficient, the requirement for high H_2O_2 oxidant to restore contaminated sites can negatively affect non-targeted species (phytoplankton and zooplankton), which ultimately impose limitations on H2O2 in-situ application. Herein, PMS was investigated, as an alternative peroxide compound for its algicidal properties on two cyanobacteria species (*Microcystis* sp. and *Aphanizomenon sp.*) and its toxicity on non-targeted zooplankton species (*Echinogammarus veneris* sp.). Surface water collected from Kouris Reservoir (Cyprus), was spiked with pure cultures and PMS doses of 1-5 mg/L (H_2O_2 equivalents) were added to monitor the destruction of cyanobacterial cells and identify the lethal concentration for both the cells and Gammarus species. The photosynthetic activity of cyanobacteria was monitored for up to 48 hours by capturing the instantaneous fluorescence and the quantum yield of the Photosystem II, while the zooplankton mortality was recorded at each time interval. Treatment experiments showed that both species required as low as 3 mg/L PMS, while toxicity studies on zooplankton showed that species are more sensitive to multiple than single PMS doses which is opposite to liquid H_2O_2.

Monitoring and Mitigation
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CyanoSTUN™ (Cyanobacteria Suppression Through Ultraviolet-C Neutralization): Results from field trials with a UV-enabled boat for mobile suppression of CyanoHABs

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Cyanobacteria are the most common cause of harmful algal blooms (HABs) in freshwater systems in the United States. Cyanobacteria HABs (cyanoHABs) negatively affect ecosystems and water resources through the production of hazardous cyanotoxins. Current methods for mitigating cyanoHABs are generally resource-intensive and have adverse effects on off-target species and the surrounding ecosystem. In this study, we engineered the Cyanobacteria Suppression Through Ultraviolet-C Neutralization (CyanoSTUN™) vessel, an agile pontoon boat equipped with a wastewater-grade UV-C reactor that irradiates raw HAB-contaminated surface water with UV-C germicidal light (254nm), and we tested its ability to suppress both the growth of biomass and the production of cyanotoxins in cyanoHAB-affected lakes in the Northeastern and Midwestern United States during the 2023 bloom season. During treatment, no chemicals were added to the water, there was no pretreatment, and no surface water was removed from the waterbody. This presentation will report findings from the field trials including treatment efficacy, rate, scalability, and impact on other algal species. The objective of CyanoSTUN™ is to give US Army Corps of Engineers water managers confronted with a cyanoHAB the ability to rapidly and safely stun a bloom for an extended period or destroy it entirely without causing off-target ecological effects.

Monitoring and Mitigation

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Diospyros mespiliformis, a bioflocculant agent, removal and lysis of cyanobacteria cells: Characterization, adsorption, thermodynamics, and kinetic study

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Harmful algal blooms are increasing worldwide. We report on a study of *Diospyros mespiliformis* (DM), for the removal of cyanobacteria under laboratory conditions. The coagulants were: DM with no shells (M2), methanolic extracts with shells (M4), methanolic extracts without shells (M5), and aluminum sulphate (M6). The before FTIR characterization of DM revealed similar carboxyl, hydroxyl and carbonyl groups with bands at 1760-1690 cm^{-1}, 3300-3600 cm^{-1}, and 1900 cm^{-1} and 1600 cm^{-1}. The after FTIR bioflocculation showed a shift to 2922 cm^{-1} and 1028 cm^{-1} which suggested that the functional groups -C-H, -P-H and -C=O were present. The optimization process was investigated and the optimum parameters were particle size, $75 \leq x \leq 125$ μm, pH (6), bioflocculant mass (0.1 g), an initial concentration (30 mg L^{-1}), contact time (80 min) and room temperature (25°C). The maximum removal efficiency by these optimum parameters was 95%. The adsorbent materials followed a pseudo-second-order kinetic model and the Freundlich isotherm model. The negative $\Delta G°$ values may suggest the spontaneous adsorption of material onto cyanobacteria. The positive values of $\Delta H°$ may suggest the reaction was endothermic. The positive values of $\Delta S°$ may suggest a strong affinity for cyanobacteria *Microcystis aeruginosa* was the dominant cyanobacteria species. Sixteen microcystin variants were identified by their mass/charge ratio (m/z). Eight unidentified peaks may be attributed to the DM material and/or could be new cyanotoxins. The field samples from aquaculture farms showed a percentage removal was variable, ranging from 10 to 90% with M4 and M5 emerging as potential candidates.

Monitoring and Mitigation
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Shifting the paradigm: In-reservoir UV-LED TiO_2 photocatalysis for the selective removal of cyanobacteria – a mesocosm study

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Potentially harmful cyanobacteria challenge potable water treatment with high biomass events and dissolved toxic and nuisance metabolites globally. Retrofitting existing water treatment infrastructure is often impractical, if not impossible, and often prohibitively expensive. In a paradigm-shifting move we propose in-reservoir pre-treatment of cyanobacteria-contaminated raw waters to ease the burden on existing water treatment infrastructure. In an iterative design approach over three years, treatment modules have been designed, refined, and optimised in bench and pilot-scale studies for in-reservoir deployment. TiO_2-coated beads made from recycled glass are employed in conjunction with UV-light emitting diodes (LEDs) to create highly reactive hydroxyl radicals that preferably remove cyanobacteria and subsequently released cyanotoxins from raw water. In a mesocosm study in a drinking water reservoir in Brazil water quality parameters were markedly improved within 72h of deployment and cyanobacterial presence was decreased by over 90% without affecting other phytoplankton communities. The treatment system is virtually plastic-free, low cost, utilises recycled materials and could ultimately be powered by renewable energies, thus providing a true green treatment option. We have conclusively demonstrated that a paradigm-shift towards in-reservoir treatment is not only possible but feasible and can provide a valuable addition to conventional water treatment methods. The modular nature of the treatment system does lend itself to use in other settings such as wastewater treatment and other at source pollutant-removal scenarios.

Toxins, Biosynthesis and Detection Methods
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Structural and functional characterization of a novel cyclic imine toxin

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The present contribution describes the discovery of a novel cyclic imine toxin purified from a dinoflagellate strain. This compound was purified to homogeneity and its molecular mass and formula were determined by HRMS. The structure of this novel compound was solved by NMR spectrometry (800 MHz). The compound bares a six membered spirocyclic imine embedded in a carbon macrocycle in a similar, but not identical, way to other members of the cyclic imine toxins family. At nanomolar concentrations, the novel compound behaves as a potent antagonist of human $\alpha 7$ nicotinic acetylcholine receptor, while at micromolar concentrations, it displays an agonistic activity towards the same receptor subtype. Further, the novel compound also possesses potent cytotoxic and apoptotic activities against human cancer cell lines. Altogether, its physico-chemical features and functional activities singularize this novel compound from the other members of cyclic imine toxin family of dinoflagellate origin composed of ~43 congeners.

Toxins, Biosynthesis and Detection Methods
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Field validation of the qualitative Neogen™ lateral flow immunoassay for paralytic shellfish toxin monitoring in Southern Rock Lobster hepatopancreas tissues

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Recurrent blooms of paralytic shellfish toxin (PST) producing *Alexandrium catenella* on the east coast of Tasmania, Australia, have repeatedly impacted the wild Southern Rock Lobster fishery (valued at >51 Million US$/annum). To effectively manage this risk, commercially available PST test kits are attractive, as they offer rapid results at substantially reduced costs (~US$60/sample). We here report on a successful field validation of the commercial Neogen rapid test to substitute currently used LC-MS/MS analysis (~US$340/sample). The test kit was challenged against 128 samples containing various toxin profiles and concentrations representing those detected during past Tasmanian biotoxin seasons (2016-2019). The Neogen test kit provided a high confidence in detecting PST (probability of detection of 1) in high-risk samples (PST concentrations ≥0.4 mg STX.2HCl equiv./kg). The probability of detection of PST decreased with PST concentration in samples containing <0.4 mg STX.2HCl equiv./kg. The results suggest that the Neogen test kit could be best employed as a screening assay at the start and end of the biotoxin season, when samples are most likely to contain PST below this level. At the height of the bloom, when PST levels >0.4 mg STX.2HCl equiv./kg are expected, the Neogen test is not likely to be cost-effective, as positive results would have to be followed up with chemical analysis to confirm PST concentrations and inform management decisions. Opportunities for reducing analytical costs and increasing sample turnaround time by including the Neogen test or similar qualitative screening assays in the Tasmanian Southern Rock Lobster PST monitoring program are discussed.

Toxins, Biosynthesis and Detection Methods

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The genus *Amphidinium* in Mexico: Morphological and molecular characterization, amphidinol identification, and new analogs

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Defining the classification and species boundaries of dinoflagellates of the genus *Amphidinium* has been challenging due to high morphological variability, the absence of visible large rigid thecal plates, and related methodological difficulties. Recent attention has focused on the genus because of the production of polyketide metabolites, some of which have demonstrated significant bioactivity. These compounds could play an important role in species interactions within benthic microhabitats and may even contribute to the ciguatera syndrome symptoms. Amphidinols (AMs) are of particular interest, since they have been circumstantially associated with fish kills caused by *Amphidinium* massive blooms of planktonic *Amphidinium*. In this study, seven *Amphidinium* isolates from the Mexican Pacific, the Gulf of California, and the Gulf of Mexico were identified to species by morphological analysis by light- and scanning electron microscopy, and by by sequencing the variable D1 and D6 LSU domains and ITS1 and ITS2 regions of the rRNA gene. We identified the previously recorded *A. carterae* and *A. operculatum* and confirmed the first records of *A. eilatiensis, A. massartii*, and *A. theodorei* in Mexican waters. The LC-MS/MS analysis of seven strains corresponding to *A. massartii, A. operculatum*, and *A. eilatiensis* corroborated the production of 17 AMs: ten previously known (AM02, -04, -05, -06, -07, -09, -11, -14, -15, and -17), four recently reported (N7, N8/N9, N12, and N13), and three new variants (U1, U2, and U3). This study underscores the importance of investigating both the morphological and molecular richness of species and the bioactive metabolites of the genus *Amphidinium*.

Toxins, Biosynthesis and Detection Methods
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Experimental studies of the aerosolization of toxins produced by *Ostreopsis* cf. *ovata*

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We will present a summary of the experiments conducted to characterize the aerosolization process of toxins produced by *Ostreopsis* cf. *ovata*. A total of 26 experiments were conducted by incubating in a bubble-bursting aerosol generation chamber, natural microplankton and microbenthic communities obtained during *O.* cf. *ovata* blooms in 2019, 2020 and 2021. The concentration of different PLTX analogues in the seawater and in the aerosols collected on PM_1 and PM_{10} impactors was estimated. Ovatoxin analogues *a* to *g* and isobaric PLTX (iso-PLTX) were present in the seawater samples, while in the experimentally generated aerosols all the analogues were also detected except OVTX-f and iso-PLTX. Considering the whole experimental data set, toxin aerosolization occurred mainly during the first three-four hours of the incubation and decreased during the subsequent hours. This trend was observed in experiments with *Ostreopsis* cells collected under different physiological phases of the natural blooms, and with different cell concentrations incubated in the chamber. This pattern suggests, on one side, that toxins could be released as a response to the overall experimental manipulation, from collection to incubation. In addition, it could indicate that in nature, toxins would not be continuously produced but synthesized and released only under certain conditions. In this sense, the different physiologic states of benthic and planktonic cells during the blooms could be involved in the variability of the total toxin concentration in the aerosols collected on the impactors, while the contribution of dissolved toxins, not measured in this study, should be investigated in future research.

Toxins, Biosynthesis and Detection Methods

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The magical mystery tour that is dinoflagellate sterolysin biosynthesis some assembly (correction) required

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We assembled a single large transcript (>14Kb) with three consecutive ketosynthase (KS) modules, that are conserved across all species of dinoflagellates. *Karlodinium veneficum* and *Amphidinium carterae* known producers of sterolysin, and *Akashiwo sanguinea*, which does not produce a sterolysins. When comparing the three species, the acyl transferase (AT) subunit in the triple module KS was present only in the non-toxic species and missing in the toxin producing species. Using ^{14}C labeled acetate and liquid chromatography mass spectrometry (LC/MS), we found that cerulenin inhibition resulted in the cessation of both fatty acid and sterolysin production. The KS domain must be activated by the enzyme, phosphopantetheinyl transferase (PPTase). The PPTase activates the thiolation domain by transferring the phosphopantetheinate group from Coenzyme A to the KS thiolation domain creating a free thiol group upon which the natural products are synthesized. *Amphidinium carterae* appears to have three PPTases, two of which PPTase 1 and 2, are very similar except that PPTase 2 does not appear to have a stop codon and was never observed as a full size protein. The remaining two PPTases (PPTase 1 and 3) had alternating expression patterns that did not directly correlate to the thiolation domain required in lipid biosynthesis. This lack of pathway segregation by PPTases is a completely novel way of synthesizing natural products compared to bacteria and fungi, likely due to the acquisition of both photosynthesis and natural product/lipid synthesis during dinoflagellate evolution that were not present in the common ancestor.

Toxins, Biosynthesis and Detection Methods
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Insights into the chemistry of microalgal toxins involved in recent northern European fish killing events

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In recent years, two major harmful algal bloom events (HABs) have taken place in northern parts of Europe, which had considerable economic and ecological consequences. Understanding the underlying mechanisms and identifying the specific toxins responsible for the observed fish killings are crucial for effective monitoring and mitigation. In 2022, the haptophyte *Prymnesium parvum* grew to high densities in the Oder River, running through both Poland and Germany. Having recently described that strains of *P. parvum* can be grouped into either A-, B- or C-chemotypes, depending on the type of prymnesins they produce, we set out to isolate, cultivate and characterize the toxin profiles of 10 individual strains from the Oder HAB. All strains had practically identical B-type prymnesin profiles. Intriguingly, HPLC coupled with ion-mobility QTOF mass spectrometry, showed that the structural diversity of the prymnesins was even larger than previously reported. In May–June 2019, *Chrysochromulina leadbeateri* caused massive fish kills in several fjords of northern Norway. Since the chemistry of this species has not been characterized, we are currently using RTgill-W1 gill cells in bioassay-guided isolation towards the structural elucidation of the related fish-killing toxins. Our initial studies have demonstrated that two recent and an older strain of *C. leadbeateri* have very similar natural product profiles, and that some highly toxic, likely polyketide derived compounds are only present in one fraction from the bioassay-guided separation procedure. Altogether, this presentation will give an update on our current investigations and new insights into the chemistry of these important fish-killing microalgae.

Ecology
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Composition and spatial-temporal dynamics of phytoplankton community shaped by environmental selection and interactions in the Jiaozhou Bay

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The Jiaozhou Bay as a model marine ecosystem in China has been intensively investigated over the last 90 years. However, detailed phytoplankton community composition, spatial-temporal dynamics, and its assembly mechanism were still unclear. To address these, we systematically examined the composition and spatial-temporal dynamics of phytoplankton in the Jiaozhou Bay through high-throughput sequencing of 18S rDNA V4. Analysis of 468 samples from 12 sampling sites over one full year revealed much higher phytoplankton diversity than previous reports, and strong seasonal succession patterns. Some phytoplankton also showed spatial variations, although the phytoplankton community didn't show significant distance-decay pattern. Environmental factors (especially temperature), species-species interrelationships and unique resting stages were uncovered to be the main contributors instead of stochastic process in shaping the phytoplankton community assembly. The overwhelming positive correlations between phytoplankton and other protists suggested that coevolution might be critical in this marine ecosystem. Complementary distributions of different amplicon sequence variants (ASVs) of same genera, such as *Skeletonema marinoi* (ASV_2) and *Skeletonema tropicum* (ASV_263) of the genus *Skeletonema*, suggested that phytoplankton have evolved differentially to exploit a wide range of ecological niches. This study laid a solid foundation for ascertaining phytoplankton composition and spatial-temporal dynamics in temperate seawaters and mechanisms underlying phytoplankton community assembly, allowing in-depth studies of marine ecology.

Ecology
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Spatial distribution of benthic cysts of *Heterosigma akashiwo* (Raphidophyceae) in sediments of Puget Sound, Washington, USA

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Heterosigma akashiwo (Raphidophyceae) blooms have caused large economic losses to aquacultured fish for several decades worldwide. However, the knowledge of the initiation of blooms of *H. akashiwo* is still limited by the lack of information about the cysts of *H. akashiwo* in sediments. In this study, the distribution of *H. akashiwo* cysts was investigated using the Most Probable Number (MPN) method to characterize their abundance in sediments collected throughout Puget Sound, Washington, USA. The densities of cysts in the sediments collected from 12 - 31 January 2013 ranged from the lower limit of detection (< 200 MPN g^{-1} wet sediment) to a maximum of 59,000 MPN g^{-1} wet sediment, which is the highest density of *H. akashiwo* cysts recorded to date in the world. The regions of high cyst densities coincided with areas where vegetative cells were earlier observed during the bloom season of the same year. The sites showing elevated cyst abundance in sediment function as “seed beds” for *H. akashiwo* blooms occurring in the spring and summer, providing probable clues for forecasting “hotspots” of its bloom.

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Distribution and diversity of dinoflagellate cysts in surface sediments from the Meishan Bay, Zhejiang

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Dinoflagellate cysts can serve as seed banks for algal blooms and are also used to indicate water eutrophication. Most previous research on dinoflagellate cyst distribution has focused on open waters and natural bays, with fewer studies in semi–enclosed artificial bays. Meishan Bay was once an open bay with access from the north and south. However, after the construction of northern and southern dams in 2012 and 2017 respectively, it became a semi–enclosed artificial bay. In this study, six surface sediment samples were collected from the inner and outer waters of Meishan Bay. A total 36 species of dinoflagellate cysts were identified, with an abundance ranging from 187 to 1456 cysts/g. The average cyst abundance was higher in the inner water than in the outer water due to an increase in harmful algae blooms, a decrease in suspended matter content, hydrodynamics and sedimentation rate, an increase in water transparency, and the water eutrophication inside the bay. The Shannon–Wiener diversity index (H') ranged between 1.62–2.40, and Pielou's evenness index (J') ranged between 0.58–0.81. Both were significantly higher outside than inside the bay, reflecting lower ecosystem stability and a higher possibility of red tides inside the bay. Both the percentage of abundance and species number of toxic dinoflagellate cysts were significantly higher inside the bay than outside. A total of 16 red tide–causing species and 8 toxic species of dinoflagellate cysts were identified, among which the yessotoxins–producer *Protoceratium reticulatum* was dominant in stations S3 to S6. The construction of the dam not only enhanced the difference in distribution of dinoflagellate cysts in surface sediments between inside and outside the bay but also increased the risk of toxic and harmful red tides inside the bay. Therefore, more attention should be paid to monitoring toxic and harmful dinoflagellate inside the bay.

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Probing “active” and “inactive” genes of particular significance in the resting cysts of dinoflagellates

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The facts that >75% of marine HABs are caused by dinoflagellates and that ~40% of HABs-causing microalgae are dinoflagellates manifest the vital importance of investigating all aspects of the biology and ecology of dinoflagellates in the international community of HABs research. The life history and its relevant molecular biology are among those of fundamental significance because the switch between vegetative growth and resting stage (cysts) plays a vital role in the HABs dynamics and, more generally, the remarkable adaptive strategies of dinoflagellates. Unfortunately, the molecular processes and functional genes underpinning the dormancy and revival of dinoflagellate cysts had remained to be a completely black box until very recently. Therefore, during the last 8 years we conducted a series of investigations aimed at revealing the genes responding to the life history shift of dinoflagellates, particularly those actively expressed in the resting cysts. Via an approach combining comparative transcriptomics, physiology, and UHPLC-MS/MS and ELISA survey of the resting cysts and vegetative cells of the cosmopolitan HABs-causing species *Scrippsiella acuminata*, we observed that TCA cycle was still a crucial pathway of energetic metabolism for resting cysts under aerobic condition, especially at the early stage of dormancy, and its expression elevated at higher temperature and light irradiation. The energy consumption of resting cysts reaches a low but somehow stable level within a short time period and is lower at low temperature, darkness, and anoxia than that at ambient condition. We also confirmed a classical plant hormone, abscisic acid (ABA), may play a vital role in regulating encystment and maintenance of dormancy, akin to its function in seed dormancy of higher plants. Our findings together are believed to considerably enhance current understanding of the molecular processes in resting cysts of dinoflagellates.

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Nutrient level changes in coastal areas of Japan from a view point of aquatic palynomorph assemblage analysis

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In general, nutrient load increased form land brings enhance primary production and then marine recourses in water column becomes rich. This is good for eutrophication. However, oversupply of nutrients forms red-tides. This breaks the usual food chain and surplus organic matter deposited on the bottom. In the bottom layer increase of oxygen consumption bring regeneration of nutrients. These nutrients are recycled to water column. For the reconstruction of past marine environments, palynomorphs consisting of dinoflagellate cysts, ciliate lorica and cyst, foraminiferal linings, and turbellarian egg capsules have been employed for long time. Among these palynomorphs, dinoflagellate cysts and crustacean resting eggs reflect environmental changes of water column and foraminiferal linings suggest them of bottom layers. Studies using aquatic palynomorph assemblage analysis carried out on several sediment core samples of Japan. Around 1890s eutrophication started as suggested increase of dinoflagellate cysts (Oslo Fjord Signal) and crustacean resting eggs. Such eutrophication brought increase of marine resources. Around the mid of 1940s, eutrophication might once stop and this change was reflected in decrease of dinoflagellate cysts and crustacean resting eggs. This situation might be due to less anthropogenic activities during World War II. Around 1960s, eutrophication re-started remarkably and serious red-tides frequently occurred. This was suggested by rapid increase of heterotrophic dinoflagellate cysts (Heterotroph Signal) and crustacean resting eggs. Nutrient load much increased form land due to vigorous anthropogenic activities. Foraminiferal linings remarkably decreased around 1960's. This situation induced a development of hypoxic water and re-generated nutrients were promoted.

Monitoring and Mitigation
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Most treatments to control algal blooms are not effective: meta-analysis of field experiments

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Harmful algal blooms negatively impact freshwater, estuarine, and marine systems worldwide, including those used for drinking water, recreation, and aquaculture, through the production of toxic and non-toxic secondary metabolites as well as hypoxic events when algal blooms degrade. Consequently, water resource managers often utilize chemical, bacterial, physical, and/or plant-based treatments to control algal blooms. However, awareness of available treatments may be limited, and there is ambiguity among the effects of algal bloom treatment across studies. Such variation within the literature and lack of knowledge of other tested treatments leave uncertainty for water resource managers when deciding what treatments are best to control algal blooms. Our primary objective was to synthesize data from 39 published and unpublished studies that used one of 28 chemical, bacterial, physical, and/or plant-based algal bloom treatments in field experiments on various water quality measurements, including phytoplankton pigments and cell density, microcystin, and off-flavors (i.e., taste and odor compounds). Across all studies and treatment types (227 effect sizes), algal bloom control was observed when measured at the time of greatest decline following treatment or at the end of the experiment. However, these findings were primarily mediated by only four chemicals, namely copper sulfate, hydrogen peroxide, peracetic acid, and simazine. None of the bacterial, physical, or plant-based treatments were shown to significantly improve water quality by themselves. Results from this synthesis quantitatively showed that most treatments fail to improve water quality in the field and highlight the need for more research on alternative treatments.

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Application of lake restoration: an ecofriendly approach using photocatalyst technology for improvement of biodiversity in recreational lake

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Various sizes of freshwater are undergoing eutrophication due to feeding extra nutrient in freshwater stream and lakes. Recently several physiochemical approaches have been applied in lakes to restore water quality or maintain current conditions. However, the attempted technologies are causing adverse effects on the environment due to breaking natural bioremediation cycle either by blocking nutrients or using algaecide material. In addition, eutrophication is accelerating due to continuous inflow of polluted water, which is a need to find a new solution on emerging technology to remediate extra nutrients with sufficient speed to avoid more accumulation of pollutants. In this study, a photocatalyst material with some organic material applied to the water body and it shows the restoration and improving biodiversity compared to inflow water from rivers This method is an eco-friendly restoration by photocatalysis and was applied to Seokchon Lake in Seoul city, South Korea. The results verified the physical, chemical, and biological effects. Since Seokchon Lake is maintained by flowing in water from the Han River, almost 10% of the lake water monthly feed to the lake. As a result of observation, it was found that most of the cyanobacteria and microalgae were introduced from the Han River. Green algae were 4.13 x 10^3 Cells ml^{-1} in Seokchon Lake and 7.07 x 10^3 Cells ml^{-1} in influent water, and diatoms were 7.73 x 10^3 Cells ml^{-1} and 10.17 x 10^3 Cells ml^{-1} respectively. For blue-green algae, Seokchon Lake was 3.8 x 10^3 Cells ml^{-1}, while influent was 4.3 x 10^3 Cells ml^{-1}. The dominant species of green algae are *Tetraedron minimum* and *Scenedesmus* sp., and diatoms were *Fragilaria crotonensis* and *Tabellaria* sp., respectively. The blue-green algae are *Microcystis* sp. and *Dictyosphaerium* sp. Another data were pH 8.8, TN 0.5 mg/L, TP 0.02 mg/L, BOD 4.5 mg/L, COD 9.2 mg/L, SS 15 mg/L, DO 8.25 mg/L, indicating that the water quality grade was 2nd stage. No rapid physicochemical changes were observed during the experiment period, and no effect on aquatic organisms such as fish, plankton and plant were observed. This study can be an alternative to the method of improving the quality of polluted natural lake water.

Monitoring and Mitigation
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A novel immobilized algicidal bacteria (DinoSHIELD) for controlling harmful dinoflagellates: A mesocosm study in the Broadkill River, DE, USA

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Dinoflagellates, notorious for causing toxic HABs, may be effectively controlled by algicidal bacteria, including *Shewanella* sp. IRI-160. Previous research developed a bio-control technology (DinoSHIELDs) using alginate hydrogel to immobilize *Shewanella* sp. IRI-160. DinoSHIELDs continuously release bacteria-derived algicides targeting dinoflagellates while limiting the dispersion of bacteria. Although effective in laboratory experiments, the application of DinoSHIELDs in the field requires exploration. Here, we conducted a 6-day field mesocosm experiment to evaluate the effects of DinoSHIELDs on water quality and natural microbial communities. Compared to controls, there was a significant decrease in dinoflagellate abundance by Day 2 in mesocosms treated with DinoSHIELDs, with no negative effects on overall photosynthetic biomass. Moreover, qPCR analysis of *Shewanella* released from DinoSHIELDs showed a peak (870 cells/mL) on Day 1, but densities of released *Shewanella* declined to 16 cells/mL by Day 6. These counts were significantly lower than native total bacterial levels in the study area, which typically exceed 10^7 cells/mL during summer. Furthermore, minor decreases in pH (< 0.2 unit decrease) and dissolved oxygen levels (< 1 mg/L decrease with all concentrations exceeding the hypoxia level) were observed; this suggests increased heterotrophic activity in the DinoSHIELDs treatment compared to controls. Concentrations of inorganic nutrients (nitrate/nitrite, ammonium, and phosphate) were not significantly different from controls. Additional work includes 18S rRNA sequencing analysis to understand effects of DinoSHIELDs on microbial community composition. Overall, the results indicated that DinoSHIELDs may be an effective biocontrol strategy for dinoflagellate bloom management and do not negatively impact the natural microbial community.

Monitoring and Mitigation
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A novel immobilized algicidal bacteria (DinoSHIELD) for controlling harmful dinoflagellates: Transitioning research to the field

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The toxic dinoflagellate, *Karenia brevis*, presents a major human health and economic threat to coastal communities in the Gulf of Mexico. Previous research investigated a bacterium, *Shewanella* sp. IRI-160, which secretes a group of water-soluble algicidal compounds that control the growth of dinoflagellates, including *K. brevis*, without the requirement of direct bacteria-algae contact. Further studies showed no evidence of negative impacts on other phytoplankton, invertebrates or juvenile finfish. However, repeated field application of high concentrations of bacteria or algicidal filtrate may raise concerns about biosafety. Recent research demonstrated the algicidal activity of immobilized *Shewanella* sp. IRI-160 and the algicidal filtrate in surface water-deployable and -retrievable alginate hydrogels (DinoSHIELDs), suggesting an effective alternative to direct application of the bacteria or algicidal compounds. Furthermore, at concentrations required to kill *K. brevis*, DinoSHIELDs were recently demonstrated in *in situ* mesocosms (2900 L) to have a negligible effect on the microbial community with respect to non-target species. Current research is focused on scaling-up production to conduct a field demonstration treating up to 4000 m^2 on the west coast of Florida, USA. The area under study will be confined using barriers (i.e., shoreline, turbidity curtains, and/or bubble curtains) to limit diffusion out of the study plot. The overall goal of the field study is to optimize delivery of the algicide from DinoSHIELDs containing either the immobilized *Shewanella* sp. IRI-160 or cell-free algicidal products and demonstrate the potential utility of this technology for continuous red-tide management in the Gulf.

Monitoring and Mitigation
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Control of *Karenia brevis* blooms through various flocculation and sedimentation methods

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Near-annual blooms of *Karenia brevis* along the SW Florida coast and the worldwide proliferation of other HABs in aquatic systems result in environmental, economic, and human health effects. These impacts emphasize the need for effective strategies that reduce the effects of HABs on coastal resources and communities, while causing minimal impact on the environment. Strategies for flocculation and sedimentation of cells are at the forefront of current HAB control technologies, with regular application in marine and freshwater systems in some parts of the world. Flocculation and sedimentation can be achieved using combinations of clays, polymers, coagulants and other materials, sometimes with other chemicals acting as algaecides or contributing to toxin breakdown. Through multiple projects with research partners, we have examined the efficacy of several compounds, including mineral clays, polyaluminum chloride (PAC), activated carbon, calcium peroxide (CaO_2) and polyphenolic compounds, individually and in combination, for the destruction and removal of *K. brevis* cells and toxins. PAC-modified kaolinite clay rapidly removed cells from the water column (>90% in ≤6hrs at clay loadings ≥0.3g/L), although cells remained viable in floc for >48hrs and toxin removal was ≤50%. The addition of 5% curcumin with clay resulted in 100% cell death within 6hrs. PAC without clay but with 150mg/L CaO_2 resulted in 100% cell death within 2hrs. 0.5g L^{-1} activated carbon with luteolin resulted in ~90% cell removal at 24hrs and >97% removal within 72hrs. This presentation will overview these studies, summarize results and consider the feasibility of applying these approaches as bloom control strategies.

Monitoring and Mitigation
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Mitigation of *Prymnesium parvum* blooms in experimental conditions and its consequences

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In summer 2022 a huge bloom of *Prymnesium parvum* occurred in the Oder River in Poland and Germany, which spread for hundreds of kilometers. It had dramatic consequences, including extensive fish and clams kill. It is considered the largest such disaster ever noted in inland waters. Studies on the environmental factors supporting the bloom, and research on possible mitigation measures have begun. Hydrogen peroxide is one of the chemicals proposed to limit *Prymnesium* blooms. We conducted laboratory experiments using hydrogen peroxide with the final concentration of 40, 80 and 120 mg/l of 35% H_2O_2. We studied its influence on phytoplankton dominated by *P. parvum* and the consequences of such treatment for newly inoculated phytoplankton. *P. parvum* disappeared from water already 16 hours after application. Its presence did not resume for two weeks. Prymnesins dropped initially by half and were mostly in water fraction. After the addition of new inoculum dominated by *P. parvum* to all tanks, we noted an increase in the abundance of *Prymnesium* and its dominance as well as an increase in the concertation of prymnesins in the cells. The highest number of *Prymnesium* and prymnesins were noted in the tank with the lowest initial concentration of H_2O_2, while the lowest was in the control tank and the tank with 120 mg/l of hydrogen peroxide. At the end of the experiment, *P. parvum* dominated overwhelmingly in all tanks accounting for 85-99% of the biomass. We discuss the reasons for the dominance of *Prymnesium* after the disappearance of H_2O_2.

Cyanobacterial HABs
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Morphology and molecular characterization of bloom-forming *Microcystis aeruginosa* and the diversity of cyanobacteria in Sarawak, Malaysia Borneo

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In 2020, two massive blooms of *Microcystis* were recorded in man-made lakes in Sibu and Kuching, Sarawak, Malaysia Borneo. Water samples were collected and preserved for cell enumeration, and species identity was confirmed by morphological and molecular characterizations. The toxicity potential of the species was further assessed by molecular detection on the presence of mcy genes, *mcyA* and *mcyE*. The bloom densities of *Microcystis* in the two events yielded 1.72 and 1.94×10^6 colonies/L. The results of microscopic observation showed that the bloom samples were dominated by coccoid cells containing gas vesicles, with the cell diameter ranging from 3.67 to 5.87 µm. Cells formed irregular aggregated colonies in both bloom samples and cultured strains. Based on the 16S rRNA gene phylogenetic analysis, three newly obtained sequences of *Microcystis* strains from Kuching were cladded with *M. aeruginosa* VN314 from Vietnam. One newly obtained sequence of *Microcystis* from Sibu grouped together with VN515. The 16S molecular distances revealed a high similarity (99.18-99.85%) to *M. aeruginosa*. The mcy genes, *mcyA* and *mcyE*, were detected in the strains. This study served as the first report of toxic *M. aeruginosa* blooms in freshwater lakes of Malaysia. Field surveys in Sarawak also revealed a high new diversity of taxa, including *Aerosakkonema funiforme*, *Ammassolinea attenuate*, *Cephalothrix komarekiana*, *Raphidiopsis curvata* and some are undescribed taxa. Comparisons of the morphology and molecular phylogenetic relationship of these undescribed taxa will be discussed.

Cyanobacterial HABs
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Detection of filamentous cyanobacteria blooms using imaging and pulse shape flow cytometry, and optical sensors

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Cyanobacteria form harmful blooms that can have adverse effects on recreational use, health, and ecosystem functioning. These blooms have been heavily studied for decades, but some questions related to species dynamics and bloom development are still unresolved. Traditional monitoring methods, such as light microscopy samples, provide only sparse information not enough to improve the understanding of bloom formation, development and dispersion. Optical phycocyanin, Chl *a* and turbidity sensors for high-frequency observations have been utilized widely, but they lack species level information and reference to biovolume/biomass information with similar timescale. Emerging high-frequency imaging flow and pulse shape cytometry applications, such as Imaging FlowCytobot (IFCB) and Cytosense (CS), could provide community composition with filament specific biovolume estimates. To get better understanding how these different methods describe the filamentous cyanobacteria blooms we compared multi-year summer biomass data collected with an IFCB with data sets collected with CS, phycocyanin and Chl *a* fluorescence, turbidity and light microscopy from Utö Atmospheric and Marine Research station, Baltic Sea between 2018 and 2022. Chl *a* fluorescence may provide a proxy to describe the overall phytoplankton abundance, however, it ignores cyanobacteria with low Chl *a* fluorescence. Phycobilin pigments fluorescence have been used to target cyanobacteria biomass more specifically. Cyanobacteria biomass derived from IFCB compared better with phycocyanin fluorescence than Chl *a*. Turbidity also followed a similar pattern with the IFCB timeseries. IFCB and CS described the bloom in similar manner and the IFCB results were comparable with light microscopy, demonstrating that such technologies can also support cyanobacteria monitoring.

Cyanobacterial HABs
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How different Dissolved Organic Matter (DOM) can influence phytoplankton community structure

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Dissolved organic matter (DOM) plays a major role in abiotic and biotic processes within aquatic ecosystems. Studies comparing the effects of different DOM at varying concentrations on the structure of phytoplankton communities, especially in regards to the cyanobacteria, are currently limited. The present study aimed to determine how different types of DOM affect the structure of phytoplankton communities. Water samples containing natural phytoplankton communities were collected from 3 sites at Lake Hume, Australia and exposed to three DOM treatments (Control (no added DOM), and 2 mg-C L^{-1} of a highly humic DOM (AT) and 2 mg-C L^{-1} of a more autochthonous DOM (MI)) for fourteen days. The type of DOM was shown to influence the abundance of the potentially toxic cyanobacteria *Chrysosporum ovalisporum*. Significantly lower cell counts were recorded in presence of the Appletree Creek DOM (AT) which is characterized as: humic rich; higher C/N ratio; highly aromatic; high molecular weight; higher allochthonous molecules; and lower protein-like substances. In contrast, a significant increase in the number of cells of the same cyanobacteria was observed in the presence of the Minnamurra River DOM (MI) which is characterized as: fulvic-rich; lower C/N ratio; lower aromaticity; lower molecular weight; high autochthonous molecules; and higher in protein-like substances. Community composition also differed significantly between the two DOM treatments and compared with their respective controls. This suggests that DOM may play an important role in either stimulating or inhibiting growth of harmful algae and has the ability to influence the phytoplankton community structure.

Cyanobacterial HABs

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Mitigating cyanobacterial blooms (cHABs): An introduction of powdered-diatomaceous earth (pDE) in contaminated freshwater set-ups

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Diatomaceous earth (DE) is a naturally occurring material and is predominantly composed of light elements and other non-metallic substances. It has excellent physical properties – low density, permeable structure, and high resistance to chemicals. On the other hand, cyanobacterial blooms (cHABs) pose a hazard to the environment and human health. Ingestion through contaminated drinking water may result in gastrointestinal problems. To solve this issue, powdered-diatomaceous earth (pDE) was introduced to contaminated water. The main objective of this study is to mitigate cHABs through flocculation techniques. The Philippine DE was utilized and underwent crushing and pulverizing at ≈63 μm. Scanning electron microscopy (SEM) was employed to evaluate the morphology of pDE. A 1000 mL of contaminated water (CW) from a freshwater pond was prepared. One hundred grams (100 g) of pDE was directly poured into the CW and left for several hours to complete the reaction. Results showed a build-up of flocculated sediment at the bottom of the container. Several species of *Aphanothece* sp., *Choococcus* sp., *Nodularia* sp., and *Pseudanabaena* sp. were among the dominant cHABs identified as revealed in the optical light microscope. Experiments show that pDE is an excellent flocculating agent of cHABs in contaminated water since it has permeable structures from skeletons of diatoms as revealed in SEM.

Cyanobacterial HABs

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Cyanotoxin-encoding genes as markers to predict cyanobacterial harmful algal blooms and cyanotoxin production

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Cyanobacterial harmful algal blooms (CyanoHABs) produce excessive amounts of cyanotoxins, mainly microcystins (MCs) and anatoxins (ATXs), significantly threatening aquatic ecosystems and public health. Accurately predicting CyanoHABs is essential to develop effective CyanoHAB prevention strategies, but current CyanoHAB prediction approaches are limited, complicated, and difficult to use. To address this need, we recently reported using cyanotoxin encoding genes as targets for a novel early warning system that can predict cyanotoxin production in various lakes in United States. Results obtained from studies at an inland and a west coastal freshwater lake with typical reoccurring MC- and ATX-producing CyanoHABs, respectively, revealed that *Microcystis* as the major MC producers in June and July in the inland lake, while, *Dolichospermum* as the major ATX producers proliferated in May and June in the coastal lake, leading to a MC- or ATX-dominated HAB event. The abundance of MC or ATX encoding genes and their transcript levels also significantly correlated with total cyanotoxin concentrations detected, when expressed in logistic equations, which accurately forecasted cyanotoxin production. Furthermore, an association between the quantity of cyanotoxin producing genes and expressions of critical nutrient metabolisms associated genes were also observed revealing intense cyanotoxin production co-occurred with pronounced cyanobacterial nutrient metabolic activities during a CyanoHAB event. Overall, these studies elucidated the potential drivers during the CyanoHAB event. More importantly, we have shown the feasibility of using our early warning system in forecasting a CyanoHAB event and as a useful tool to guide CyanoHAB prevention and mitigation practices in inland and coastal freshwater lakes.

Cyanobacterial HABs

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Comparative genomics of novel cyanobacteria *Sphaerothrix gracilis* isolated from microplastics reveal insights to plastic utilization and bloom potential

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Microplastics are prevalent contaminants across many ecosystems. These particles are present for long periods in marine ecosystems and form a suitable habitat for the colonisation of organisms. A new cyanobacterial genus and species, *Sphaerothrix gracilis*, was isolated from the surface of a foam microplastic particle from tropical coastal regions. Draft genome sequencing and average nucleotide identity (ANI) strongly suggests that *S. gracilis* is a new genus and species within the Leptolyngbyaceae. Comparative genomics of *S. gracilis* and closely-related species of *Leptolyngbya* and *Geitlerinema* showed that *S. gracilis* had a distinct genome structure and metabolic profile. A total of 700 core genes in *S. gracilis* were found to be involved in pathways related to cyanobacteria harmful algal blooms, as curated in the pathway database CyanoPATH. These genes were mainly involved in nitrogen fixation (22%), assimilation of vitamins and trace metals (14%) and toxin production (13%). *S. gracilis* revealed the presence of terpenes, polyketides and non-ribosomal peptide synthetases and also demonstrated the genetic capacity to produce toxic secondary metabolites such as leukotoxin, anachelin, and puwainaphycin. Functional annotations also showed that *S. gracilis* contained genes associated with the transformation of nylon and polylactic acid. Antibiotic resistance genes were also found in *S. gracilis*, with probable resistance to glycopeptides, fluoroquinolone and tetracycline. This study highlighted the presence of novel species of cyanobacteria of bloom potential originating from the plastisphere, with capacity for future pharmaceutical and industrial applications.

Microbiomes and Omics

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Microbiomes of Australian *Gambierdiscus* species and comparative genomics of bacterial associates

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Understanding bacterial microbiomes of phytoplankton can provide vital insights into their growth, physiology and toxicity. Our study focused on four *Gambierdiscus* species: *G. carpenteri, G. lewisii, G. lapillus,* and a second *G. lapillus*-related genotype. We combined traditional culture-based methods with high throughput 16S rRNA amplicon sequencing (Oxford Nanopore) to analyse microbiome composition. While our culturing approach recovered 14 bacterial genera from 145 isolates, our sequencing method detected over 200 genera. This underscores the depth of microbiome diversity not captured by culturing alone. Across all *Gambierdiscus* species, the dominant bacterial classes were Gammaproteobacteria, Alphaproteobacteria, Flavobacteriia, Cytophagia, and Sphingobacteriia. Bacteria attached to cells differed from those in the culture medium, with attached microbiomes showing a higher proportion of Gammaproteobacteria and Alphaproteobacteria. Despite overarching similarities, we identified differences in microbiomes among the *Gambierdiscus* species at the genus and species levels, indicating that *Gambierdiscus* species have a unique set of bacterial interactions and dependencies. Several core microbiome members, notably *Magnetospira* sp. (QH_2) and *Neptuniibacter caesariensis* (attached) and *Sedimenticola thiotaurini*, *Magnetospira* sp. (QH_2) and *Spongiibacter marinus* (unattached), showed a prevalence >80% across all samples. Furthermore, we sequenced complete genomes of microbiome bacteria, including several *Alcanivorax*, a genus known to degrade hydrocarbons and promote phytoplankton growth. We will discuss our comparative genome analysis and highlight the unique features of these *Gambierdiscus*-associated bacterial genomes. Our study reveals the remarkable diversity and species-specific variations among bacterial microbiomes of *Gambierdiscus* species. Our findings provide the basis for further exploring the functional role of these microbial communities play in the dynamics of harmful algal blooms.

Microbiomes and Omics
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Exploration of the *Microcystis* phycosphere: comparative analyses of nitrogen assimilation and bacterial communities in North American lakes

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The cyanobacterium *Microcystis* is a non-diazotrophic species which naturally occurs in large colonies shown to contain a distinct microbiome of epiphytic and embedded bacteria that differ from bulk water column bacteria. Preliminary examination of community composition of these bacteria indicated that *Microcystis* colonies may be enriched with taxa involved in nitrogen (N) cycling relative to whole and free-living bacteria. This study focuses on nitrogen assimilation by *Microcystis* colonies by quantifying assimilation of ^{15}N labeled nutrients and N_2 gas with high throughput sequencing. We conducted weekly experiments from six lake systems with *Microcystis* blooms from June to November 2020-2022 including Lake Erie. Sample water was separated into three distinct fractions: *Microcystis* associated bacteria, free-living bacteria, and the whole water community. Aliquots from each fraction were used to measure ^{15}N isotopic assimilation of nitrate, ammonium, urea, and nitrogen gas, nitrogen fixation was assessed by acetylene reduction, and DNA samples were used for high throughput sequencing of 16s rRNA and *nifH* genes. *Microcystis* colonies had a preferential assimilation of urea, on average, >50% of total N uptake, and significantly higher fixation of N_2 compared to free-living bacteria, with rates up to five-fold higher. Principle coordinate analysis of beta-diversity revealed significant divergences of *Microcystis* colonies and free-living bacteria across all lakes. Sequencing of the *nifH* gene revealed both heterotrophic bacteria and cyanobacteria comprised diazotrophic communities within *Microcystis* colonies. Collectively, this study demonstrates that N assimilation rates and pathways of *Microcystis* colonies differ significantly from free-living plankton communities.

Microbiomes and Omics
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Diversity of HABs from the Texas coast (Gulf of Mexico) with a focus on extreme events: a full length 18S rDNA metabarcoding approach

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More than half of the 153 known HAB species are found in the Gulf of Mexico and most of these HAB species have been observed on the Texas coast. Using a metabarcoding approach, samples from the Texas Observatory for Algal Succession Time series (TOAST) were examined for the presence of HAB species. Metabarcoding is a rapid and effective means to investigate microplankton community composition and species diversity. In this study we focused on harmful algal bloom species. Our objective was to examine the seasonal variation in HAB diversity over the three-year period 2019-2022 in comparison with results from the short 6-week period following the extreme flooding event after Hurricane Harvey in 2017 and Hurricane Nicholas in 2021. Results suggest the diversity of HABs increases after an extreme event. Given the anticipated increase in the number and intensity of hurricanes with climate change, it is important to determine how this will impact the diversity and potential for blooms of harmful algae in the Gulf of Mexico as well as other parts of the world.

Microbiomes and Omics
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Different strains induce different threatens - How *Microcystis* genotype distinctions may influence the ecotoxiciological effects of blooms on fish

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One of the most prevalent and notorious bloom-forming freshwater cyanobacteria is *Microcystis*, whom toxicological impairs yet remain incompletely investigated. Based on our previous studies, we hypothesize that some emerging *Microcystis* metabolites (in addition to microcystins (MCs)) are of (eco)toxicological concern and should be further investigated. To this end, we explore the ecotoxicological potential of different *Microcystis* genotypes producing various bio-active metabolite cocktails, including cyanopeptolins, microginins, aeruginosins, or microcyclamides, on Medaka fish model. We focused on the early molecular response of the adult fish to a simulated realistically intense 4-days bloom with 4 distinct *Microcystis* genotypes, comprising 2 MC-producing (PMC 728 and 807) and 2 non-MC-producing (PMC 810 and 826) strains. We handle a microcosm experiment to study the dynamics of the gut microbiota composition using 16S rDNA sequencing and of the gut, liver, and muscle metabolomes as a functional proxy using LC-MS metabolomics, and use strains, water and faeces as controls. Basically, this study highlights the high complementarity of multi-omics approaches to unravel microbiota and holobiont metabolic responses that help us to address the ecotoxicological response at the organism scale. Interestingly, it also reveals how different can be the fish responsiveness to the different strains. Indeed, while fishes exposed to PMC 728 exhibit microbiota dysbiosis signature, the PMC 826 strain remarkably perturbates the fish digestion process inducing even more pronounced metabolic alterations, when the 2 others provoke more limited perturbations. Moreover, the present results highlight the importance of considering cyanobacterial diversity at the genotype scale to assess the realistic environmental risk of cyanobacteria on fish. This finding subtly depicts the complexity of natural fish exposure to cyanobacterial bio-active and toxic compounds and may explain why, so far, research fail to observe simple linear relation between cyanobacterial cyanotoxin production during bloom events and toxin contents within the different fish organs.

Microbiomes and Omics
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High-resolution multi-omics analyses provide insights into a toxic algal bloom and cellular processes of the ichtyotoxic and mixotroph *Chrysochromulina leadbeateri*

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The formation of harmful algal blooms (HABs) and the ecological factors driving them remain poorly understood. Strategies of the bloom forming species to avoid top-down control mechanisms, such as grazing and competition, are crucial in formation of HABs and understanding bloom dynamics. In 2019, the mixotrophic haptophyte *Chrysochromulina leadbeateri* caused a fish-killing HAB event in Northern Norway. Studying the emergence of blooms and factors stimulating toxin production is challenging due to the difficulty or impossibility of inducing blooming phenotypes in culture for many species, limiting descriptive and traditional analyses. However, meta-omic approaches enable the study of HAB events in their natural environment, connecting environmental observations with biological processes to identify potential drivers and impacts. In this study, we observed differences in the metabolome and gene expression of *C. leadbeateri* between a location affected by a bloom and an unaffected location. We identified cellular processes associated with metabolic processes and bloom formation. Bioassays confirmed toxicity in the bloom area, although the specific toxin could not be identified. We found several polyketide synthase (PKS) genes, identified by peptide homologies, associated with toxicity and their expression correlated with inorganic nitrogen and phosphate availability. Metabolomic and metatranscriptomic analyses revealed increased expression of processes like endocytosis, autophagy, and lysozyme, indicating the use of mixotrophy as a nutritional strategy during bloom formation. Our findings suggest that *C. leadbeateri* exploits its toxicity to dominate the community and increase its prey availability for nutritional supplementation. This study demonstrates the utility of metatranscriptomics and metabolomics aligned with ecological metadata in understanding eco-evolutionary processes in natural environments. Identifying stimuli and metabolic and cellular processes driving HABs could aid in predicting and preventing future blooms.

Microbiomes and Omics

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Biology and ecology of the invasive diatom *Cymbella janischii* – what's happening in the mat?

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Cymbella janischii is a freshwater benthic diatom that was previously considered endemic to the United States. However, since its first detection in Japan in 2006, the species has been dispersing, and it is now known to be widespread in Japanese rivers and streams. The species attaches to stone surfaces using mucilage stalks, and occasionally forms massive blooms, resulting in a thick mat that covers riverbeds, giving a detrimental effect on the habitat of fish and insects, as well as negatively impacting river landscapes. Despite the urgent need for a better understanding of the biology and ecology of *C. janischii* to prevent further expansion of its distribution, our knowledge about this species is currently limited. In this study, our aim is to gain basic insights into *C. janischii* using DNA metabarcoding and metagenome analyses, as well as various microscopic techniques for observations of the mats. To date, we have detected several bacterial and fungal species that may be correlated with the mat formation. The bacteria in the mat possess genes involved in the biosynthesis of the vitamin B family, which could benefit the growth of *C. janischii*. The presence of genes encoding diphosphatases in the genome of *C. janischii* indicates the possibility that the species catalyzes the hydrolysis of diphosphates. Furthermore, a 3D reconstruction of the mat using microscopies and nano-focus X-ray CT supports the idea that the interior of the mat is filled with a dense soup of organic substances, which could be utilized by *C. janischii* and co-existing organisms.

Toxins, Biosynthesis and Detection Methods
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Development of an LC-MS/MS method for the detection of microcystins in marine and estuarine shellfish

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In recent years, increased attention has focused on the risks from exposure to cyanotoxins, such as microcystins (MCs), through consumption of freshwater seafood. Several reports have shown that these primarily freshwater toxins can also be found at varying concentrations in marine and estuarine shellfish. To better assess the concentrations found in these vectors, and the risks they pose to consumers, a validated analytical method is required. To date, several protocols have been published, but the recovery of the targeted MCs have varied. In addition, many methods based on mass spectrometry suffer from varying degrees of matrix interference. The major difficulty for extraction and accurate detection of MCs is their polarity with the Kow between -1 to ~4. Herein, an extraction and detection protocol were developed for MCs (i.e., MC-LA, -LF, -LR, -LW, -LY, -RR, -YR, and nodularin-R) from Eastern oysters (*Crassostrea virginica*), blue mussels (*Mytilus edulis*), hard clams (*Mercenaria mercenaria*) and softshell clams (*Mya arenaria*). Samples were extracted using 20 mL of 80/19.9/0.1 Methanol/water/formic acid. MCs were analyzed using a Waters Acquity UPLC system with Sciex QTrap mass spectrometer operated in negative ESI mode. The evaluation of the method was based on spike recovery, matrix effects, precision, specificity, and ruggedness. Overall, the recovery ranged from 85 to 117%. Matrix effects were between 90 and 120%. The extraction protocol reduced matrix interference from other compounds and provided cleaner extracts which will benefit the monitoring efforts.

Surveillance and Management
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Performance of high-throughput microfluidic real-time qPCR technology for the simultaneous detection of six *Alexandrium* species in environmental samples

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Several species of the genus *Alexandrium* are associated with the production of Paralytic Shellfish Toxins (PSTs) causing the human illness known as Paralytic Shellfish Poisoning (PSP) through the consumption of contaminated filter-feeding molluscs. The threat to public health and the severe economic impact on shellfish industries that PST events constitute urge the development of new, efficient, and reliable monitoring tools. High-throughput PCR systems such as the Standard BioTools' Biomark HD, which uses microfluidics technology to process samples at nanolitre-scale volumes, have the potential to perform up to 9.216 reactions simultaneously, providing significant cost and time advantages. This study investigated the transferability of six previously published qPCR markers targeting *Alexandrium minutum, A. tamarense, A. catenella, A. mediterraneum, A. tamutum,* and *A. ostenfeldii* to the Biomark HD real-time qPCR System. The efficiencies, Limits of Detection (LOD), and Limits of Quantification (LOQ) of each assay were first assessed on traditional qPCR using double-stranded synthetic DNA fragments (dsDNA). As expected, the efficiencies of amplification, slopes, and r^2 values confirmed the robustness of the assays. The LODs, calculated using curve - fitting methods, ranged between 2.30 and 14.23 copies per reaction. Calibration curves with dsDNA and specificity tests on clonal strains of different *Alexandrium* species were run on the Biomark HD, where seventy-two samples (controls and field) were tested simultaneously for all the assays. The Biomark HD results were compared to conventional qPCR, to investigate the reliability of microfluidics technologies in identifying and quantifying simultaneously six *Alexandrium* targets in environmental samples.

Surveillance and Management
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It takes a village: Meeting the complex challenges presented by HABs in Alaska through the Alaska harmful algal bloom network

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Oceans play an integral part in the social, cultural and economic well-being of Alaska. Marine living resources are a primary source of food throughout the largest state in the USA, and harmful algal blooms (HABs) are a growing but unpredictable risk to many stakeholders and communities. Subsistence shellfish harvesting, commercial aquaculture operations, and wildlife are all potentially impacted by the toxins produced by HABs. The size, remoteness and geographic diversity of Alaska's coastlines and oceans add to this complexity – communities throughout Alaska have different levels of knowledge, history, and relationship to HABs, as well as varying levels of infrastructure to address the threat. As climate change continues to impact the physical characteristics of the oceans, HABs are responding in unpredictable ways and there is a growing need to better understand the factors that lead to HAB events in different regions throughout Alaska and how their effects can be mitigated. Due to these complexities, there are many researchers, environmental coordinators, regulators, state and federal agencies, and community members that have come together to become the Alaska Harmful Agal Bloom Network. The network provides a statewide approach to the awareness, research, monitoring, and response to HABs with the goal of reducing the risk to humans and wildlife. This presentation will describe the current state of knowledge on HABs in Alaska, present ongoing research and monitoring activities, and discuss the importance of this work to protect the well-being of Alaskan communities.

Surveillance and Management

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Dogs, drones, and genes: Developing a holistic monitoring framework for HABs

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The state of Utah, USA has 53 waterbodies (and counting) affected by harmful algal blooms (HABs) spread across 220,000 km^2. Monitoring these waterbodies for cyanotoxins is critical as they serve as both popular recreational destinations and key drinking water supplies. In recent years, Utah has combined a variety of innovative tools to develop a holistic monitoring framework that protects the public, facilitates HAB mitigation and prevention, and still fits within practical budget, lab, and personnel constraints. Core components of this framework, highlighted in this presentation, include: (1) A broad-scale community outreach program. Utah partners with recreation and environmental organizations, the regional poison control center, and healthcare providers. Monitoring is further aided by a volunteer-powered “HAB Squad”. (2) Vigorous research collaboration. Utah applies cutting-edge science. New in 2023, is the application of two qPCR methods (one a quick-screen tool developed in house) to yield rapid and predictive results on HAB development and toxicity. (3) Broadscale remote sensing. Utah uses drones, satellite imagery, and other remote sensing tools to expand the application of more traditional point-sampling techniques. This monitoring framework has the potential to be applied at state and regional scales around the globe. This presentation will highlight the benefits and structure of our holistic approach, in addition to sharing key findings on the drivers and trends behind HABs in the western USA.

Cyanobacterial HABs
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Expansive alien cyanobacteria in Central-East Europe - Do they pose a serious threat to aquatic ecosystems?

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During last decades, expansion of potentially toxic cyanobacteria have been commonly observed. The reason of the expansion is still not fully explained but several hypotheses related to climate changes, occurrence of different ecotypes and accelerated eutrophication of reservoirs are the most frequently considered. Increasing abundance of expansive cyanobacteria is potentially associated with serious threats they may pose to invaded ecosystems by producing toxic metabolites and outcompeting native species. We have studied distribution pattern and environmental factors enhancing expansion of invasive cyanobacteria, *Raphidiopsis raciborskii*, *Raphidiopsis mediterranea*, *Sphaerospermopsis aphanizomenoides* and *Chrysoporum bergii*, in Central East Europe during the last two decades. The study showed increasing occurrence of *R. raciborskii* and *S. aphanizomenoides* while *R. mediterranea* and *C. bergii* were less common. *R. raciborskii* has increased its contribution to total phytoplankton biomass since it was first reported in Polish lakes, but so far it has never been found as a dominant species. Genetic and LC-MS/MS analysis showed that any of the isolated strains was capable of producing well known cyanotoxins. *R. raciborskii* strains isolated from Polish lake showed, however, ability to produce unknown metabolites evoking neurotoxic effects. Moreover, we noted that expansive cyanobacteria caused significant changes in phytoplankton communities. Mean air temperature, conductivity and TP concentrations were found to be the most important factors driving the occurrence of the expansive cyanobacteria. They occurred more frequently in turbid and shallower lakes. Eutrophication and climate changes may therefore stimulate further expansion of alien cyanobacteria in temperate zone, with unpredictable consequences for the ecosystem.

Cyanobacterial HABs
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Susceptibility and toxicity of cyanobacteria exposed to environmental antibiotics concentrations

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The diffusion of antibiotics and antibiotic resistance in the environment poses several questions. Non target organisms as cyanobacteria or natural bacteria can be affected by antibiotic released in the environment, as well as develop/acquire antibiotic resistance under the selective pressure of sub lethal, very low concentrations. The susceptibility of different species of cyanobacteria has been determined for various classes of antibiotics and an extremely large variability has been observed. Some studies have shown a possible role for cyanobacteria in the spread of antibacterial resistance, however very few data are available on the effects of antibiotics on cyanobacterial toxicity. Since one suggested role of cyanotoxins is to protect the cells from oxidative stress, we asked whether toxic cells were more resistant than non toxic strains. We exposed several monoclonal strains to a panel of 7 antibiotics, chosen within the most diffuse and the most relevant classes of antibiotics, with different mode of action and observed a highly variable susceptibility. We subsequently tested the effects of two possibly naturally occurring concentrations of erythromycin on natural populations of *Planktothrix rubescens*, freshly collected from Albano Lake (Rome, Italy). Both concentrations of erythromycin inhibited the natural population more than the monoclonal strains. The percentage of toxic cells (*mcyE* carrying cells) did not change over time, but a variation in microcystins cell quota has been observed, suggesting that antibiotics in the environment not only could stimulate the selection of resistant cyanobacteria, but could affect the production of toxins, thus changing the toxicity of the bloom.

Cyanobacterial HABs
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Multiyear analysis of bacterial community dynamics reveals key bacterial players in the regulation of *Microcystis* blooms in Daechung Reservoir

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Although the relationship between *Microcystis* and bacterial communities has been extensively investigated, most studies focused on their interaction for one year rather than for multiple years, leaving our understanding of the contribution of bacterial communities to the development of *Microcystis* blooms unsatisfactory. To determine key bacteria that are crucial for the formation of *Microcystis* blooms, we collected samples from three sites (Chuso, Hoenam, and Janggye) in the Daechung Reservoir for three years (2017, 2019, and 2020). Our results showed that the major modules in co-occurrence networks were related to the formation, maintenance, and decomposition of *Microcystis* blooms, suggesting that bacterial communities play a significant role in the regulation of *Microcystis* blooms. *Microcystis* blooms have been found to alter the diversity, assembly processes, and functional characteristics of bacterial communities. As environmental parameters and *Microcystis* bloom intensity were similar between stations rather than between years, the bacterial community associated with *Microcystis* blooms was more variable across years than between stations. Although bacterial communities vary greatly from year to year, they shared core ASVs, including *Pseudanabaena*, *Microscillaceae*, *Sutterellaceae*, *Flavobacterium*, *Candidatus*, *Aquiluna*, and *Bryobacter*. These core ASVs were also identified as key indicators of bloom stage, suggesting that they are the fundamental biological elements for the development of *Microcystis* blooms in Daechung Reservoir. Overall, our study highlighted that to gain a more comprehensive understanding of how microbial community responds to *Microcystis* blooms, a one-year survey is not sufficient, but a multiyear analysis is essential with diverse stations and weather events.

Cyanobacterial HABs
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Threshold for flow management to mitigate harmful cyanobacterial blooms in the large river with consecutive dam-scale weirs

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Nakdong River is the second largest river in South Korea and used as a major source of drinking, industrial and agricultural water for a large catchment area. For drought mitigation and flood control, eight large dam-scale weirs were simultaneously constructed along the approximately 200km middle-to-lower reach. These large weirs can regulate the water flow through epilimnetic or hypolimnetic discharges. Harmful cyanobacterial blooms (cyanoHAB) have been becoming serious concerns in the Nakdong River, especially after construction of eight weirs. Typical bloom-forming cyanobacterial genera, *Microcystis* and *Aphanizomenon* have been developing to massive blooms in mainly four downstream weirs area every summer. Flow management is one of the direct, short-term measures which can suppress the formation of cyanobacterial blooms or flush the developed blooms in the eutrophic river. In this study, we evaluated the applicability of flow management strategies for mitigation of cyanoHABs in the Nakdong River. The threshold of natural flushing events like heavy rain in mitigating cyanoHABs was assessed. The weekly average value of the ratio of discharge to ordinary discharge in the four downstream weirs must be at least 250% for *Microcystis* biomass to drop below 50,000 cells mL^{-1}. In the laboratory semi-continuous culture, hydraulic retention time (HRT) shorter than 4 days could suppress *Microcystis* growth. To reduce the pre-developed *Microcystis* or *Aphanizomenon* blooms (>100,000 cells mL^{-1}) to less than 10,000 cells mL^{-1}, which is the criteria of harmful cyanobacteria alert system in Korea, within one week, HRT should be maintained for 2 days or shorter.

Cyanobacterial HABs
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Effects of water level management on algal blooms in the large river system

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Harmful Algal Blooms (CyanoHABs) have been occurring annually in Korea causing severe water quality problems as consequence of the construction of large weirs in the river system. In regulated river, the magnitude of discharge rate as well as water level can be manipulated to disrupt the development of cyanobacterial blooms. The present study was performed to evaluate the effects of hydrological operation in the consecutive weir area to reduce residence time and dilute cyanobacteria abundance. HABs were decreased at the nine weirs which were fully or partially operated in summer (Jun. to Sep.) from 2018 to 2021. Sejong, Gongju and Bakje weirs located in the Geum River which were fully operated, cyanobacterial abundance were significantly decreased as 85% than the usual periods of 2013 to 2017. In particular, the hydraulic residence time (HRT) showed less than one day during the summer in 2021. In the case of partially operated weirs as Seungchon and Juksan located in Yeongsan River, harmful cyanobacteria cell density in 2021 were decreased to 75% and 37% than the previous years from 2013 to 2017 respectively. However, the result of Nakdong rivers with long stretches of slow flow condition was not clear during the study periods. Thereby, The effect of the water level fluctuations to manage the cyanobacterial blooms depends on the local conditions including watershed properties, meteorological factors (rainfall patterns and prolonged droughts) as well as inflow water quality.

Cyanobacterial HABs
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Cyanosphere interactions as an important elements in controlling harmful algal blooms

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Bacteria co-occurring with cyanobacteria are one of the biotic factors influencing bloom development, knowledge of their ecological interactions is essential. The aims of our studies were: 1) to describe bacterioplankton dynamics, diversity and functional potential of different cyanobacterial blooms from Polish lakes; 2) to obtain culturable bacteria with algicidal properties. Blooms dominated by filamentous cyanobacteria (*Planktothrix*, *Limnothrix*) were accompanied by potential cyanobacterial parasites (Verrucomicrobia). Blooms with *Microcystis* domination were associated to opportunistic bacteria (representatives of *Roseomonas, Flavobacterium*) and nitrogen-transforming bacteria (Burkholderaceae). Considering the seasonal changes, the nitrogen-transforming proteobacteria dominated the bacterioplankton assemblages in early summer. Later, a mixed bloom of *Snowella, Aphanizomenon* and *Microcystis* appeared, bacterial diversity decreased, and potential cyanobacterial parasites/predators increased. The post-summer was significantly dominated by toxigenic *Microcystis* with potentially predatory taxa (Vampirovibrionales), and bacteria that may indicate the sanitary status of water (Sutterellaceae). Potential algicidal bacteria were observed with low abundances (*Rheinheimera*, ≥3%). Despite this, six algicidal candidates representing Firmicutes, Gammaproteobacteria and Bacteroidetes were selected for laboratory co-cultures. Cyanobacterial strains (filamentous and coccoid morphotypes) had different sensitivity against algicidal strains, with almost no effect against green algae. Two bacterial strains (*Morganella, Bacillus*) showed the highest algicidal effect against cyanobacteria. Changes in the expression of the cyanobacterial genes *psb*A (photosystem II repair) and *rec*A (DNA repair) indicated the disruption of physiological processes during the first 30h. This study showed that the filamentous cyanobacterium *Aphanizomenon gracile* (first time tested in detail) was more sensitive to algicidal bacteria than the commonly studied *M. aeruginosa*. Research funded NSC 2019/33/B/NZ8/02093 ALGICYDY.

Microbiomes and Omics
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Detecting harmful algal taxa on the West Coast of the United States and Canada using DNA metabarcoding

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Alexandrium, *Dinophysis*, *Heterosigma*, and *Pseudo-nitzschia* are harmful phytoplankton genera that have been found along the West Coast of the USA and Canada. Although they can form blooms that dominate the phytoplankton community, they are often a minor, but still concerning, component of the community. DNA metabarcoding is a powerful genetic tool used to characterize phytoplankton communities. DNA metabarcoding can provide a site-to-site comparison to identify areas with high or low abundances of harmful taxa relative to the entire community. We applied 18S rRNA metabarcoding to investigate phytoplankton communities from samples collected in the spring of 2016 on the NOAA West Coast Ocean Acidification research cruise from San Francisco, California, USA to Haida Gwaii, British Columbia, Canada, where associated microbial, chemical, and physical oceanographic data were collected concurrently. Analysis of the metabarcoding data revealed high variability in harmful taxa along the West Coast. High relative abundances of *Alexandrium* and *Pseudo-nitzschia* were observed at known harmful algal bloom initiation sites, such as Heceta Bank in Oregon and the Juan de Fuca Eddy off Washington State and Vancouver Island, Canada, as well as in the coastal and inland waters of British Columbia. Furthermore, we detected harmful taxa along depth profiles, up to 227 meters. Ongoing analyses are aimed at assessing the potential relationship of microbial community structures and physicochemical parameters (e.g., carbon dioxide measurements, nutrient concentrations) to the presence or absence of harmful taxa. These efforts will bring nucleic acid metabarcoding into the suite of tools currently used for harmful algae studies.

Microbiomes and Omics
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Exploring the diversity and community assemblages of benthic harmful dinophytes by eDNA metabarcoding coupled with the artificial substrate sampling method

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Harmful algal blooms in the benthic system (BHAB) are a major environmental problem that has increased worldwide in the context of global climate change. The harmful effects of BHAB on human health due to ciguatera poisoning (CP) make it one of the research priorities in HAB science today. From an ecological monitoring perspective, the following key research questions often arise: what are the distribution patterns of BHAB species in areas of CP vulnerability? And what are the possible relationships in benthic community composition? In this presentation, we investigated the molecular diversity of benthic dinophyte community assemblages on a tropical fringing reef of Perhentian Islands, Malaysia by applying DNA metabarcoding approach using the artificial substrate sampling method (AS). We assess the sensitivity of metabarcoding for BHAB species detection, evaluate the potential application in the quantitative representation of species, and compare the effectiveness of AS and natural substrate sampling methods for eDNA analysis. Our findings showed that the method is sensitive in detecting BHAB species with a higher taxonomic resolution, particularly of the taxonomically difficult species of *Gambierdiscus* and *Ostreopsis*; the results also discovered several new taxonomic records of benthic dinophytes in the region. Furthermore, the genetic diversity of toxic ribotypes of *Ostreopsis ovata* can be revealed by this approach. Finally, we discuss the feasibility of this molecular approach coupled with AS sampling method as a fundamental basis of consideration in BHAB monitoring strategies in potential CFP-prone areas.

Microbiomes and Omics

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Characterizing Cyanobacterial HABs Microbiomes: Beyond *Microcystis*

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Cyanobacterial harmful algal blooms (cyanoHABs) are a global phenomenon, degrading water quality and aquatic ecosystems. Several genera of cyanobacteria are known to form cyanoHABs (e.g., *Dolichospermum*, *Microcystis*, *Raphidiopsis*). Though dominated by cyanobacteria, these cyanoHABS are a diverse collection of microbes including archaea, bacteria, fungi, and protists including eukaryotic phytoplankton, known as the microbiome. However, these communities are more than the sum of their taxa. These organisms act in concert, relying on each other for nutrient recycling and/or auxotrophy to fill in missing genomic gaps and thus benefitting the community and potentially increasing bloom proliferation and intensity. While this concept of the interactome is not new, its role in cyanoHABs dynamics has been limited to the common bloom-forming genus *Microcystis*. Here we explore metagenomic data from *Dolichospermum*, *Microcystis*, and *Raphidiopsis* dominated blooms within Lake Okeechobee to evaluate their taxonomic composition as well as their interactome. Results indicate that there are distinct associated microbiomes between these three bloom-forming taxa. Furthermore, the functional roles of these taxa are explored to identify which traits are unique and shared among bloom communities.

Microbiomes and Omics
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In-situ assessment of bloom microbiomes associated with the HAB dinoflagellates *Gymnodinium catenatum* and *Tripos furca*

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Phytoplankton cells have complex inter-relationships with their microbiomes, bacteria that interact within a cell-associated micro-zone (the phycosphere) around "host" cells where metabolites, growth promoters/inhibitors and cell-signalling molecules can be exchanged at effective concentration. Microbiomes differ from more abundant "background" bacterial communities, modify host physiology in species-specific ways, are essential for ocean primary production and the growth of many HAB species. However, the challenge of distinguishing and sampling the functionally relevant cell-associated microbiome from a complex microbial background means we know little about these microbiomes in natural systems. We addressed the sampling problem using multi-spectral sorting flow cytometry to identify and sort individual phytoplankton species directly from blooms and characterised the cell microbiomes using both amplicon sequencing (16S, V3-V4, Illumina) and cultivation-based approaches. In-situ bloom microbiomes of HAB dinoflagellates *Gymnodinium catenatum* and *Tripos furca* were sampled multiple times from the Derwent Estuary (Tasmania, Australia) during 2021-2022. Microbiome composition varied over time, and differed significantly from background (unsorted) bacterial communities and also between the two species. Both cultivation and amplicon sequencing recovered microbiome community structures dominated by a narrow diversity of gamma- and/or alpha-proteobacterial families Vibrionaceae, Shewanellaceae, or Pseudoalteromonadaceae; substantially different from alpha-proteobacteria-dominated microbiomes recovered from bulk bloom water sampling and crude size-fractionation. In-situ microbiomes also differ from uni-algal lab cultures which are likely modified by algal media and cultivation-related selection processes, highlighting limitations of culture-based models for understanding microbiome-HAB species dynamics in natural systems. Instead our data reinforce the need develop and apply approaches to assess bloom microbiome diversity, dynamics and functional relationships in-situ and at scales relevant to phycosphere microenvironments.

Microbiomes and Omics
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Comparative gene expressions of *Pseudo-nitzschia pungens* co-cultured with different bacterial phylotypes

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The microbiome of *Pseudo-nitzschia* is known to influence the physiology of their diatom hosts but the molecular underpinnings of these influences are particularly understudied. Here we compared the enriched molecular pathways and different differentially expressed genes (DEGs) of *Pseudo-nitzschia pungens* when co-cultured with various phylotypes of associated bacteria. We specifically looked for DEG's involved in nutrient utilization, photosynthetic activity, pre-DA biosynthetic pathway, stress responses, growth responses and secondary metabolite production to compare and differentiate the specific molecular effects elicited by each bacterium on *Pseudo-nitzschia* transcriptome. We found that those bacteria that commonly co-occur with *Pseudo-nitzschia* have showed more significant influence on the gene expressions of the diatom host than the ones that are rarely present during *Pseudo-nitzschia* blooms. We will further discuss the implications of this study on the evolutionary relationship of *Pseudo-nitzschia* and its microbiome associations.

Microbiomes and Omics
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Genomics and eco-active organic matter in oceans: the roles of harmful and other algae, with emphasis on physical effects

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Dissolved organic matter (DOM) in the ocean represents about 662 billion tons of C, 200 times more than the living biomass. It is produced mainly by microbial primary production. The largest fraction of this DOM is old (>weeks to months) and both chemically and biologically recalcitrant. The remaining fraction is young (seconds to weeks), more labile and surface active, and powerfully changes the rheological properties in the bulk water and at interfaces including the sea surface microlayer (SML). Correlation has been observed between *in situ* chlorophyll concentrations and rheological thickening, while the greatest viscosity increases have been measured in blooms and cultures of the harmful algae, European *Karenia mikimotoi*, *Phaeocystis globosa* and *Noctiluca scintillans*. Surface foams and scums, as well as "viscous water" also occur in blooms of other microalgae and cyanobacteria. Microbes controlled by their genes produce the main DOM molecules, largely polymeric complexes of sugars, amino acids, fatty acids and nucleic acids. The properties of ocean water and its interfaces and its biogeochemical fluxes may thus be engineered by plankton genes. These fluxes influence ocean and atmospheric climate. Viral infection may furthermore modify prokaryotic and eukaryotic genes and their expression. Therefore, these ocean plankton genomes and the fluxes and climates they influence may be subject to Darwinian-type selection. Research programs need to integrate ocean ecology, rheology, biogeochemistry and genomics, to find the associations among them. High-biomass HABs may be notable eco-engineers, where the effects of the eco-engineering, exopolymers and causative DNA are spatially associated and easy to research.

Surveillance and Management

O-205

Development of rapid techniques for the *in-situ* detection of *sxtA4* and *sxtG* involved in saxitoxin biosynthesis

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Some species of marine dinoflagellates belonging to the genera *Alexandrium, Gymnodinium catenatum* and *Pyrodinium bahamense* can produce the neurotoxin saxitoxin (STX) and its analogues, which can accumulate in shellfish without visible effects on the hosting organism. Their presence is hazardous for human health, and can led to socio-economic impacts for fisheries and aquaculture sectors. Early detection of either toxins or toxin producing species is therefore of paramount importance. Several methods have been developed to achieve this objective, most of which are laboratory based, reliant on highly specific skills, expensive and time consuming and are not able to identify all the species simultaneously. In this work, a duplex PCR assay that simultaneously targets the *sxtA4* and *sxtG*, genes, which are involved in the synthesis of STX, is presented. Additionally, an enzyme linked oligonucleotide assay (ELONA) is developed for the colorimetric detection of PCR amplicons. The specificity of the approach was verified using genomic DNA from non-toxin producing congeneric species and with other microalgae genera. This approach focuses on the genes responsible for toxin production as an alternative to focusing on the species. It will provide better early monitoring strategies, and future studies will explore the potential of this strategy for on-site analysis of possibly contaminated shellfish.

Surveillance and Management

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Harmful algal blooms dynamics and toxic outbreaks in coastal waters of Central-Southern Pacific Ocean Chile (36° - 44°S)

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Harmful Algal Blooms (HABs) occur frequently in the fjords and channels of Southern Chile, causing important public health effects, economic losses and social impacts. This study evaluated the phytoplankton community and the occurrence of HABs species related to coastal environments from 2016 to 2023 in Central-Southern Pacific Ocean Chile (CSPO; 36° - 44°S). Hydrographically we have identified 3 latitudinal transition areas in CSPO: Biobío (BB), La Araucanía-Los Ríos (AR) and South of the Chacao channel (LL). HABs occurred with a high abundance of dinoflagellates during the 2016 autumn and 2018 summer when the favorable atmospheric–oceanographic conditions allowed the proliferation of *A. catenella* (5,000 and 375 cells mL^{-1}, respectively) with a high concentration of PST (6.6 x 10^3 and 1.5 x 10^3 µg STX eq/100g, respectively) in LL. In addition, during 2018 summer a massive bloom of *Kareniaceae* (390 cells mL^{-1}) killed millions of pelagic and benthic marine animals affecting the coastal areas between LL and AR. During 2019 summer a *Dinophysis acuminata* (317 cells mL^{-1}) with a pectenotoxins (PTX-2) production (54 µg/kg) was recorder in BB. The diatom *Pseudo-nitzschia* cf. *australis* was registered during 2022 summer and autumn (2,000 and 200 cells mL^{-1} in LL and BB, respectively), these events promoted the accumulation of domoic acid (DA) in shellfish (140 and 23.3 µg DA kg^{-1}, respectively), with shellfish harvesting closures in both areas. This study provides valuable baseline data that could sustain monitoring activities and management decisions in CSPO and project future actions, especially in a climate change scenario.

Surveillance and Management
O-207

Official control protocol in the Canary Islands (Spain) for detecting CTXs in commercial fish from first sale points

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In recent years climate change has led to different outbreaks of ciguatera poisoning in the Canary Islands. The first case was detected in 2004, in which two fishermen caught a 26 kg amberjack (medregal negro; *Seriola Rivoliana*), that they consumed, poisoning five family members. From then until now, more than 120 people have been poisoned in the Canary Islands for this cause. The Government of the Canary Islands has involved by implementing an official control program since 2011. The experience acquired in recent years through this program has made it possible to establish risk species in relation to minimum weights for all fish that are marketed, from which a specimen must be analyzed and obtain a negative result, before being able to go on sale for human consumption. The current list contains the following species with weights equal to or greater than those indicated: island grouper (*Mycteroperca fusca*, 7 kg); amberjack (*Seriola* spp, 13 kg); dusky grouper (*Epinephelus marginatus*, 12 kg); bluefish (*Pomatomus saltatrix*, 9 kg) and wahoo (*Acanthocybium solandri*, 35 kg). The result of this program is very safe and all fish with a negative result are released to the market for consumption. No person has been poisoned during these years, and more than 11,000 fish have been evaluated. It must be highlighted that the prevalence of CTX-positive fish detected in the Canary Islands has risen from 9.9% in 2017 to 16.7% in 2022. The causes of the new outbreaks of ciguatera poisoning detected are discussed in this work.

Socio-economic Impacts

O-208

Transition of social perception on shellfish toxin and poisoning: Through a text analysis of newspapers in Japan

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Shellfish toxin causes various negative impact to the society, which includes self-imposed restriction of shipping shellfish to markets, self-imposed restriction of opening clam picking parks, and shellfish poisoning. This paper aims at clarifying transition of social perception of the shellfish toxin and shellfish poisoning in line with development of scientific knowledge on the shellfish toxin. Three major newspaper companies, namely Asahi, Mainichi, and Yomiuri were targeted in analyzing the article on shellfish toxin. The article of the three newspaper from 1872 to 2023 were searched using the word "shellfish toxin". As a result, 850 articles of Asahi, 542 articles of Mainichi, and 932 articles of Yomiuri, in total, 2324 articles were found which include the word "shellfish toxin". Text analysis of those articles was conducted by using a free software named KH Coder. Majority of the articles refers to self-imposed restriction of shellfish shipping to markets and deregulation of the self-restriction. Another focus of the articles is the impact on clam picking called SHIOHIGARI. Articles on shellfish poisoning cases decreased in line with the regulation by the government imposed. Relationships between the government regulation, understanding and scientific knowledge on shellfish toxin, and tones of the articles are also discussed.

Socio-economic Impacts
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Social drivers of knowledge, beliefs and practices on harmful algal blooms: Lessons from fishing communities in Biliran and Leyte regularly exposed to HABs

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In Eastern Visayas, shellfish poisoning cases have caused deaths and economic losses despite management efforts, making harmful algal blooms (HABs) a significant social concern. This study examined the social dimensions of HABs in Eastern Visayas to elucidate factors affecting community awareness and actions relating to HABs. By looking at the social systems of HABs, this study can influence policy formulations to better HABs monitoring strategies of local governments. Knowledge, Beliefs and Practices (KBP) survey was employed to 334 small scale artisanal fishers who regularly consume green mussels, *Perna viridis* in Carigara, Leyte and Caibiran, Biliran that have regular red-tide bans. Data shows that at least 50% of the population is knowledgeable of basic scientific concepts of HABs including its causes, occurrence, algal toxins, exposure levels, and socio-economic impacts, however *t-Test* shows that knowledge level in fishing communities of Leyte is significantly higher than knowledge levels of Biliran (*p-value*<0.001) which might be an outcome of the high number of shellfish poisoning in Leyte. *Factor analysis* of belief variables revealed three major factors that influence variance in community perception including (a) government and individual action (25.4% variance), (b) lifestyle and social setting (15.9% variance) and (c) environmental impacts (7.6% variance). *Spearman rho correlation* shows moderate positive relationship between knowledge levels and government and individual action (*correlation coefficient* = 0.45, *p-value*<0.001) which calls for improvement in government monitoring systems and translation of these knowledge to good fishing practices.

Socio-economic Impacts

O-210

Warning And Technology to Combat Hazards of Harmful Algal Blooms in Region VIII (WATCH HAB R8) and other efforts on HAB mitigation in E. Visayas, Philippines

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Region VIII the political region of Eastern Visayas, is the most affected region in terms of Paralytic Shellfish Poisoning in the Philippines. This is primarily caused by *Pyrodinium bahamense* blooms. Starting 2015, harmful algal blooms (HABs) and shellfish bans have occurred almost monthly, shifting from one bay to the other, in the waters and bays of the different islands in the region. The Warning And Technology to Combat Hazards of Harmful Algal Blooms in Region VIII (WATCH HAB R8) Program funded by the Department of Science and Technology of the Philippines is comprised of three projects to find solutions and mitigate the impact of HABs in the region. These are (1) Establishment of Database on Seasonal Variability of phytoplankton community in Five HAB affected bays in Region VIII (2) Understanding the Social Dimension of Red Tide Phenomenon and, (3) Design and Development of Diatomite Clay to Mitigate HABs. The Philippine HAB monitoring agency focusses only on *P. bahamense* monitoring. Now, there is a phytoplankton database that has two years of monthly phytoplankton and physicochemical data; and cyst maps, for five bays in the region. Monitoring is now extended to two more years in select islands of the region. Knowledge, beliefs and practices of the local communities in Samar island have been documented; and is now extended to two other islands, Biliran, and Leyte. Diatomaceous earth proves to be effective in clearing out HABs in the laboratory setting. Additionally, efforts to alleviate the economic losses of mussel farmers are explored.

Cyanobacterial HABs
O-211

Taxonomy and phylogeny of geosmin-producing *Dolichospermum* species

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Although many species names have been reported for geosmin-producing cyanobacterial species of the genus *Dolichospermum*, and the geosmin-producing gene, *geoA*, has been reported in Genbank using many species names, our re-analysis using morphology and phylogeny showed that many species were misidentified and that the number of species that actually produce geosmin is limited. *D. hangangense*, in particular, was misidentified to many species. This may have been due to the use of immature acinate for identification. Similarly, *D. minisporum*, which has recently been noticed as a source of mold odor in the Lake Biwa system, has also been misidentified to many species and should be noted. In both species, it was found that some strains belonging to the same clade phylogenetically have *geoA* genes and others do not. As genetic analysis progresses, the errors of traditional identification by morphology are becoming clearer. To avoid confusion, re-identification by morphology and phylogeny, including those of the past, should be continued. On the other hand, many reports of mold odor and toxin genes including *geoA* did not register the barcode genes (*rbcL*, *rpoC*, PC) necessary for re-identification. In the future, it is recommended that the barcode sequences should be registered together to confirm the identification.

Cyanobacterial HABs
O-212

Microcystins and metabolites in European noble crayfish *Astacus astacus* in Lake Steinsfjorden, Norway

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Lake Steinsfjorden, an important Norwegian location for noble crayfish (*Astacus astacus*), is often affected by cyanobacterial blooms of *Planktothrix* spp. These blooms may cause microcystin contamination of the crayfish. Investigation of crayfish samples by ELISA showed presence of microcystins (MCs) in crayfish from June to September in 2015 and in October 2016. PCR analysis confirmed presence of *Planktothrix* biomass in their stomach. MCs were also found in the lake water. LC–HRMS analysis on filters from water samples and on a selection of the crayfish tissue extracts from the original study, revealing the presence of known and previously unreported MCs. The MCs in the crayfish in spring and early summer appeared to originate from the *Planktothrix*, whereas the MCs in the crayfish in late summer and autumn appear to be novel metabolites from an unknown source not present in the water column. The results indicate that the concentration of MCs in the water column is not a good indicator of potential MC contamination of crayfish in Lake Steinsfjorden.

Cyanobacterial HABs
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Spatiotemporal diversity of marine benthic CyanoHABs and their potential as reservoirs for harmful eukaryotic algae

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Benthic cyanobacterial mat (BCMs) proliferations, or benthic cyanoHABs, in marine environments are occurring frequently, partly driven by nutrient loading and climate change. Reports of BCMs occurring on both Florida coasts have increased, from mangroves to seagrass beds and corals, demonstrating a need to understand the diversity involved in BCM formation. As part of a continued monitoring of this environmental health threat, benthic proliferations were sampled on the Florida coasts between 2021 and 2022. Cyanobacterial mats were collected, eDNA was extracted, and the 16S rRNA of cyanobacteria were amplified, as well as the 18S rRNA of accompanying eukaryotes. In addition to amplicon sequencing, isolation, characterization, and genome sequencing of select strains were also performed. Results indicate the homocytous filamentous *Dapis*, *Okeania*, and *Sirenicapillaria* dominated BCMs across the Gulf Coast of Florida. LC-MS/MS analyses revealed presence of anatoxin-a, dihydroanatoxin-a, malyngamide C, and cylindrospermopsin, within either the water column or mat material. Several known bloom forming dinoflagellates including *Amphidinium* and *Alexandrium*, were also captured within the BCMs. Results from this study indicate a need to further assess the spatial and temporal aspects of these blooms, in addition to their cyanobacterial and eukaryotic community structure, influencing factors and the production of toxic and bioactive compounds.

Cyanobacterial HABs
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Detoxification and recycling of the harvested microalgae with organic food waste using salt-tolerant mushroom strains

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Cyanobacterial blooms in lakes, reservoirs and rivers have been environmental and social issues due to its toxicity, odor, etc. Among the cyanotoxins, microcystins exist mostly within the cyanobacterial cells and they are released from the cells. Therefore, an innovative technology is needed to detoxify and environment-friendly recycle the harvested microalgae. This study develops detoxification method of microcystins of the harvested microalgae through recycling harvested microalgae with food waste using salt-tolerant mushroom strains, natural ecosystem decomposer. In this study, the harvested microalgae is mixed with the food waste and then the mixed toxic organic waste is used as mushroom compost by adjusting water content of about 70% using cellulose. The mushroom compost is bottled, sterilized, and the salt-tolerant mushroom spawn is inoculated. The mushroom is then cultured and grows in the temperature, humidity and CO_2 controlled environment. During this eco-friendly toxic organic waste recycling process, diverse bacteria or various enzymes of the salt-tolerant mushroom strains decompose the microcystins as well as the organic waste, and produce nutritious and antibiotic organics. Using PHLC/Mass analysis, it was verified that 99.8% of the microcystins of the harvested microalgae was detoxified in the harvested mushroom as well as in the recycled mushroom biomass compost. Cultured biomass compost after mushroom harvest can be used for the organic fertilizer, functional bio-feed and RE-100 biomass renewable energy. In this eco-friendly organic waste recycling process, no toxic material, no wastewater nor sludge is generated with value added recycling biomass compost and thus sustainable ESG with zero-waste circular economy.

Cyanobacterial HABs
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Use of a novel photocatalyst to mitigate freshwater and marine eutrophication and forestalling the generation of harmful algal blooms (HABs)

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The intensified and expanded threat of eutrophication (often manifesting in harmful algal blooms (HABs)) to aquatic ecosystems, public health and the global economy, necessitates approaches that safely and effectively mitigate these occurrences. Here, we report on the use of a novel TiO_2-based photocatalyst capable of processing excess organic matter in the water column and sediment, fostering biodiversity and returning the ecosystem to a stable state that naturally forestalls dense and toxic blooms of cyanobacteria in North American lakes and the dinoflagellate, *Alexandrium catenella,* in marine settings. Using water from a NY lake during a summer, the application of the photocatalyst processed excess nutrition reducing levels of total nitrogen (TP) and phosphorus (TP) ($p<0.01$) thereby forestalling *Microcystis*-bloom density by more than 70% over a one-month period. Application of the photocatalyst to large volume mesocosms (300 L) filled with NY lake water in fall, yielded an even larger relative reduction of *Microcystis* bloom populations, significantly reducing biomass and microcystin levels by an order of magnitude, while also significantly reducing levels of TN, TP, respiration rates, and total suspended solids and significantly increasing water clarity and the relative abundance of diatoms. Experiments with water from a cyanobacterial HAB in Florida demonstrated the photocatalyst significantly reduced or completely forestalled cyanobacterial populations and cylindrospermopsin concentrations while significantly increasing the biomass of green algae and diatoms ($p<0.01$). Finally, the photocatalyst significantly forestalled expansion in cell densities and saxitoxin levels in cultures of North American strains of *A. catenella*. Given that the by-products of photocatalytic reactions are H_2O, O_2, and CO_2, our results suggest this novel photocatalyst has the potential to rehabilitate ecosystem biodiversity and thereby forestall the appearance of freshwater and marine HABs.

Cyanobacterial HABs
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Assessing the impact of cyanotoxins and water quality on aquatic life in reservoirs

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We assessed the extent of pollution in an essential public water supply reservoir (southeastern Brazil). An environmental monitoring study was performed at the Billings Reservoir (at the water catchment site) to assess the water quality in 2017, 2018, and 2019. Physicochemical parameters were analyzed, quantifying the total cyanobacteria and the cyanotoxins microcystins (MCs) and saxitoxins (SXTs), as well as their possible ecological risk to the aquatic environment. We also determined metals and metalloids (As, Ba, Cd, Pb, Cu, Cr, Fe, Mn, Ni, Zn, and Sb) and fecal bacteria (Escherichia coli). Monthly samplings were performed for 2017, 2018, and 2019 (totaling 36 sampling campaigns). Metals, metalloids, and E. coli values were below the maximum limit allowed by the Brazilian legislation. High concentrations of total cyanobacteria (3.07×10^4 – 3.23×10^5 cells/mL), microcystin variants MC-LR (0.67–23.63 μg/L), MC-LA (0.03–8.66 μg/L), MC-RR (0.56–7.92 μg/L), and MC-YR (0.04–1.24 μg/L), as well as the saxitoxins GTX2 (0.18–5.37 μg/L), GTX3 (0.13–4.40 μg/L), and STX (0.12–2.92 μg/L) were detected. From an ecotoxicological point of view, the estimated values for the risk quotient (RQ) for microcystins and saxitoxins were largely greater than 1, indicating a high risk to aquatic life. Therefore, further efforts need to be made to delay the eutrophication of the reservoir.

Poster Communications

(including posters also presented during an Ignite Talk Session)

ICHA2023
November 5 - 10 HIROSHIMA JAPAN

The 20th International Conference on Harmful Algae

Ecology
P-001

Life cycle dynamics of *Alexandrium catenella* during a large-scale Bering Strait bloom event

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Rapid warming and the recent discovery of a massive *Alexandrium catenella* cyst bed have raised concerns about the intensification of paralytic shellfish poisoning risk in the Pacific Arctic. This was the motivation for a HAB-dedicated cruise in summer 2022, studying waters from the northern Bering Sea to the western Beaufort Sea. An Imaging FlowCytobot (IFCB) was configured to sample from an underway seawater line, allowing for real-time identification of HAB cells along the cruise track. Using this method, a large-scale *A. catenella* bloom was first detected in the northern Bering Sea on July 25; over the course of six weeks, this bloom was advected through the Bering Strait and onto the Chukchi Shelf. At its peak, cell concentrations reached >150,000 cells liter^{-1}, and >80% of the phytoplankton biomass in surface waters was composed of *A. catenella*. Further analysis of IFCB imagery reveals the presence of vegetative cells, gametes, and planozygotes throughout the course of the bloom. Spatial and temporal patterns of these cell stages indicate that sexual fusion occurred after the bloom passed through the Bering Strait, resupplying the Chukchi Shelf cyst bed. Comparison of surface sediment samples collected north of the Bering Strait in summer and fall 2022 support this conclusion, with an average 6x increase in cyst abundance following the bloom. This event provides a clear case study of how advected blooms from the Bering Sea can feed the *A. catenella* population of the Chukchi Shelf, maintaining a source for future Arctic HABs as bottom temperatures rise.

Ecology
P-002

Effects of temperature and salinity on growth of *Karenia mikimotoi* and *Karenia papilionacea* cultures

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Karenia mikimotoi and *Karenia papilionacea* are harmful dinoflagellates widely found in coastal waters of temperate and subtropical regions. These species often form blooms in warm coastal waters where temperature and salinity which exhibit dramatic seasonal changes and occasionally pose a threat to aquacultures. To understand their bloom dynamics, we investigated and compared the effects of temperature and salinity on *K. mikimotoi* and *K. papilionacea* cultures. Clonal strains of *K. mikimotoi* and *K. papilionacea* were isolated from the Uranouchi Inlet, Japan. They were cultivated at five temperatures (10, 15, 20, 25, and 30 °C) in combination with four salinities (20, 25, 30, 35, and 40). Growth rates obtained under these conditions were statistically compared. Both cultures of *Karenia* species grew well at 15-30 °C and salinities of 20-40. To data, *K. mikimotoi* grew at a low temperature of 10 °C while *K. papilionacea* did not grow at the same temperature. This suggests that *K. mikimotoi* has a low temperature tolerance. Maximum growth rates of *K. mikimotoi* and *K. papilionacea* were obtained at 20－25°C and 25－30°C, respectively. Further, *K. papilionacea* grew rapidly at a high salinity of 35. These dinoflagellates represent different growth responses to temperature and salinity; compared to *K. mikimotoi*, *K. papilionacea* appears to prefer warmer waters and may have an ecological advantage in bloom forming in such warm waters of temperate and subtropical regions.

Ecology

P-004

Effects of intrusion and retreat of deep cold waters on the red tides species in the South Sea of Korea

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To explore the effects of eutrophic deep waters offshore on red-tide outbreaks, waters were collected from 0, 20, and 40 m depths at an offshore station in the South Sea of Korea, and the growth rate of each major red-tide dinoflagellate *Prorocentrum donghaiense*, *Tripos furca*, *Alexandrium fraterculus*, and *Margalefidinium polykrikoides* in the waters was measured. No species grew at dissolved inorganic nitrogen concentrations (DIN) of < 7.0–8.4 μM, but the growth rates of all four species rapidly increased and became saturated at 12–15 μM DIN. On July 31, 2020, the DIN was 4.1–4.6 μM in 0–10 m depth waters but 8.9–18.4 μM in 20–50 m depth waters. Under these circumstances, considering that calculated reachable depths of *P. donghaiense*, *T. furca*, *A. fraterculus*, and *M. polykrikoides* were 10, 15, 24, and 52 m, respectively, *A. fraterculus* and *M. polykrikoides* can reach the deep waters and grow, but *P. donghaiense* and *T. furca* cannot. However, if the deep waters intrude and ascend by 10–20 m relative to that on July 31, four species can reach deep waters and grow, whereas if the deep waters retreat and descend by 10–20 m, only *M. polykrikoides* can reach the deep waters and grow. Red-tide outbreaks by *M. polykrikoides* in the South Sea in 2014 occurred after the deep water retreat. Thus, the position of the eutrophic deep waters, affected by their intrusion and retreat and solar insolation, can affect outbreaks of harmful *M. polykrikoides* red tides.

Ecology
P-005

The largest recorded *Alexandrium pacificum* bloom in south-eastern Australia: toxicity and impacts

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Over the past two decades, the coastal region of south-eastern Australia has been experiencing blooms of *Alexandrium pacificum*, a Paralytic Shellfish Toxin (PST) -producing species. Since the commencement of biotoxin testing along this coastline in 2004, the first detection above the regulatory limit was reported to be sevenfold greater (7.2 mg/kg PST STX equivalent), in blue mussels (*Mytilus galloprovincialis*) during the spring of 2016. Subsequently, PSTs related to *A. pacificum* have recurred annually. Here we report on the largest PST event recorded in SE Australia to date. In 2022, a widespread bloom of *A. pacificum* spanned ~750 km of the SE Australian coastline, with PSTs reported above the regulatory limit in oysters (*Saccostrea glomerata*) at 9.4 mg/kg, mussels (*Mytilus galloprovincialis*) at 3.8 mg/kg, as well as lobsters at 1.1 mg/kg for the first time. Using single-cell isolation, light microscopy enumeration, molecular genetic tools and LCMS/MS for toxin determination, we describe this event, including the identity of the causative species, its abundance, toxicity and the environmental conditions at the time of this bloom. We discuss the presence and abundance of *A. pacificum* and PST production in relation to ecological factors and long-term changing ocean conditions in the region. Climate change and its associated effects on highly variable coastal environments are hypothesized to exacerbate harmful algal blooms. Therefore, it is crucial to conduct analyses of harmful algal communities in relation to environmental factors over adequate temporal and spatial scales to determine long-term trends and minimize the impacts of future PST outbreaks.

Ecology

P-006

Effect of harmful algae on the ephyrae of the moon jellyfish *Aurelia aurita*

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Although algae typically serve as a food source for jellyfish in marine food webs, this study aimed to investigate the potential opposite scenario, where harmful algae could have detrimental effects on jellyfish. Our research focused on the moon jellyfish *Aurelia aurita*, which has a global distribution and is known to cause significant damage to human health and aquaculture. Given the common occurrence of *A. aurita* and several algal species in coastal waters, understanding their interactions from an ecological perspective is crucial. We examined the potential noxious effect of six species of harmful algae on the ephyrae stage of *A. aurita*. The results showed that the rhythmic pulsation behavior of *A. aurita* could be significantly suppressed when exposed to high concentrations of various algal species. Particularly, *Heterosigma akashiwo* was observed to induce morphological damage to the *A. aurita* ephyrae. In general, this study provided a comprehensive understanding of the interaction between harmful algae and jellyfish ephyrae. Moreover, the findings suggest that harmful algal blooms have the potential to suppress the mass occurrence of Aurelia medusa.

Ecology
P-008

Bloom development of toxic dinoflagellate *Alexandrium catenella* (Group I) in Jinhae-Masan Bay, Korea: Germination strategy of resting cysts in relation to temperature and salinity

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To better understand the role of resting cysts in relation to the outbreak of paralytic shellfish poisoning and bloom dynamics in Jinhae-Masan Bay, Korea, this study investigated the germination features of ellipsoidal *Alexandrium* cysts, which are isolated from sediments collected in winter (January) and summer (August) in the Jinhae-Masan Bay, under different combinations of temperature and salinity. Morphology and phylogeny of germling cells revealed that the ellipsoidal *Alexandrium* cysts are assigned to only *Alexandrium catenella* (Group I). The cysts could germinate across a wide ranges of temperatures (5-25℃), with germination success within 5 days, although at 25℃ very low cumulative germination rates (≤13.3%) (winter samples) and no germination (summer samples) were observed. This result indicates that a continuous seeding for maintenance of vegetative cells in the water column may occur through a year without the endogenous clock to regulate germination timing. In addition, the cyst germination of *A. catenella* (Group I) was not controlled by salinity changes. Interestingly, *A. catenella* (Group I) cysts isolated from surface sediments in winter and summer exhibited different ranges in germination temperatures. This seems to reflect the differences in temperature ranges that resting cysts experience, indicating that *A. catenella* (Group I) may have a high adaptive potential to changes in seasonal temperature. Based on obtained results, this study provides the schematic scenario of bloom development of *A. catenella* (Group I) in Jinhae-Masan Bay, Korea.

Ecology

P-009

Diatom viruses isolated from coastal waters and sediments in western Japan

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Although the significances of diatoms in coastal environments have been recognized, the effects of diatom viruses as its decreasing factor are not fully understood. The first diatom virus was reported in 2004, a virus infecting *Rhizosolenia*. After the initial discovery, *Chaetoceros* viruses and pennate diatom viruses have been isolated and characterized. The report on diatom host-virus systems have been accumulated, so far. The diatom viruses are grouped into two types based on genomic features, single-stranded (ss) RNA and ssDNA. The ssRNA diatom viruses harbor ca. 9kb ssRNA genome with two open reading frames (ORFs) encoding putative replication-related proteins and capsid proteins. Phylogenetic analysis based on the deduced amino acid sequence of the RNA-dependent RNA polymerase domains supported the monophyly of these viruses. The genomes of ssDNA diatom viruses are composed of circular ssDNA (ca. 6kb) which includes at least two ORFs for replication-related proteins and capsid proteins. These diatom viruses are all lytic to their respective host diatoms; and their infection is strain-specific. These discoveries contribute for understanding the diatom ecology in coastal environments.

Ecology
P-010

Bloom formation of colony-forming harmful diatom *Thalassiosira diporocyclus* in the Kagoshima Bay and its significance as prey for some copepods

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The colony-forming diatom species, *Thalassiosira diporocyclus*, which forms large colonies up to several millimeters, often makes condense blooms in the coastal areas of Japan along with the Kuroshio Current. This species is recognized as a harmful alga since its gelatinous colonies become entangled in fishing nets, making trawling difficult. However, little is known about factor of bloom formation and their significance as food items for mesozooplankton. We investigated the abundance of *T. diporocyclus* in spring in Kagoshima Bay and conducted feeding experiments of the dominant copepods on *T. diporocyclus*. Abundance of *T. diporocyclus* was the highest at the bay mouth where upwelling events occurred due to intrusion of the warm water mass originated from the Kuroshio Current. This suggests *T. diporocyclus* makes bloom due to the nutrient supply accompanying upwelling in the front between the coastal areas and the Kuroshio Current. Colony size was 1.7±1.0 mm in average, reaching a maximum of 10.1 mm. At the feeding experiment, the ingestion rates of *Calanus sinicus*, *Eucalanus* spp. and *Oncaea* spp. on the colonies were quite low, but they actively ingested on the single cells. The single cells of *T. diporocyclus* after disintegration of the colonies would be important food items for the dominant copepods, while their colonies are not utilized.

Ecology
P-011

Different ecological niche adaptation of four harmful raphidophytes in Southeast Asia

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Chattonella is a harmful microalga that has caused fish kills in Australia, Brazil, China, India, Japan, Mexico, the United States, and Southeast Asia. Recently, the coexistence of four *Chattonella* species (*C. subsalsa*, *C. marina*, *C. tenuiplastida*, and *C. malayana*) in Southeast Asia has been clarified, suggesting possible adaptation of the species to different ecological niches. This study aimed to compare the effects of temperature and salinity on their growth to explore their niches. A representative strain of each species was investigated in ten temperatures (13.0–35.5°C) and five salinities (15–35). Maximum specific growth rates were highest for *C. subsalsa* (0.65±0.01 d^{-1}), followed by *C. malayana* (0.47±0.03 d^{-1}), *C. marina* (0.45±0.02 d^{-1}), and *C. tenuiplastida* (0.39±0.01 d^{-1}). All species grew > 10^4 cells mL^{-1}. *C. subsalsa* showed the widest growth temperature (20.5–35.5°C). Optimal temperatures were the lowest in *C. marina* (25.5°C), then *C. subsalsa* (28.0°C), *C. tenuiplastida* and *C. malayana* (30.5°C). The optimal salinities were overlapping, i.e., *C. subsalsa* and *C. malayana* (30), *C. marina* and *C. tenuiplastida* (25). The ecophysiology of *C. tenuiplastida* and *C. malayana* was first elucidated in this study and results suggested their limited distribution in warm tropical waters. Our *C. subsalsa* and *C. marina* were comparable to those in the temperate waters. This study highlighted the wide distribution of *C. marina* was attributed to its adaptation to a lower temperature, and high harmful bloom potential of *C. subsalsa* in tropical Asian waters given its high adaptability and coincidental distribution with historical fish kill locations.

Ecology

P-012

Interaction between a harmful alga *Microcystis* and marine phytoplankton

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Microcystis is one of the major freshwater microorganisms causing cyanobacterial harmful algal bloom that is known to secrete a toxin called microcystin. Although the toxins produced by *Microcystis* have been extensively studied, the interaction between *Microcystis* and marine phytoplankton has been little studied. Due to the geographical characteristics of Korea, there are many estuaries where the large scale of *Microcystis* blooms enters, so *Microcystis* and its toxins entering the marine environment cannot be ignored. Therefore, we conducted experiments on whether *Microcystis* affects diverse marine phytoplankton, which often occurs in the estuary. The marine phytoplankton includes diatoms, cryptophytes, raphidophytes, and dinoflagellates. At a high concentration of *Microcystis aeruginosa*, the growth rates of dinoflagellates and diatoms were inhibited, while the other groups of phytoplankton were not. The filtrates of *Microcystis* also showed a different effect depending on the phytoplankton. This study provides insight into the impact of *Microcystis* on the phytoplankton in estuarine ecosystems.

Ecology

P-013

Distribution and genetic diversity of *Pseudo-nitzschia* around Obama Bay, Japan

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Pseudo-nitzschia is known to produce domoic acid, which causes amnesic shellfish poisoning. In recent years, there have been reports of damage to fisheries due to bloom, mainly along the east coast of North America. Although there have been no cases of shellfish poisoning in Japan, we considered it important to evaluate the potential risk of domoic acid in Obama Bay, because oyster cultivation is very active in this area. In this study, we surveyed three sites in Obama Bay for one year from November 2021 to understand the occurrence of *Pseudo-nitzschia* and we also started sampling at seven sites in the coastal area from June 2023. At each site, *Pseudo-nitzschia* were counted by microscopy, and metabarcoding analysis was conducted to reveal the species composition of *Pseudo-nitzschia*. In addition, CTD observations and nutrient analyses were done to understand the relationship with seasonal changes in *Pseudo-nitzschia* abundance. Cell counts of the genus in the bay ranged from 0.24 to 8.4×10^4 cells/L during the year, with a significant increase in September at all sites and a further increase in October at two of the sites. The environmental factors that were highly relevant to cell counts differed from site to site. We plan to analyze these factors in more detail, referring to seasonal change of environmental factors. Metabarcoding first targeted the *rbc*L gene, but was unable to detect *Pseudo-nitzschia* successfully. Therefore, we are now using *Pseudo-nitzschia*-specific primers to amplify only the genus to accurately reveal species composition. The results of the re-analysis will be reported at the conference.

Ecology
P-014

Mechanism of massive spring diatom bloom in the Oyashio region

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Diatoms, well-adapted to turbulent and nutrient-rich condition, are making blooms in coastal and upwelling waters. In the pelagic Oyashio region, western North Pacific, massive diatom blooms, such as observed in coastal regions, occur extensively every spring. These blooms could support high biological productivity in this region, one of the world's richest fishing ground. For spring blooming of diatoms in this region, both processes; supply of sufficient nutrients (including N, P, Si and Fe) and seeding of active diatom cells have been recognized to be essential. The aim of this study is to elucidate mechanism of the spring diatom blooms in the Oyashio region by autoecological approach, focusing on seeding and growth processes. Conditions of spring diatom blooms and population dynamics of key diatom species in this area were examined by long-term monitoring data and metatranscriptome analysis of diatom communities in the Oyashio off the southeast coast of Hokkaido, Japan.

Ecology
P-015

A new sampling device to measure resuspension flux of dinoflagellate cysts from bottom sediments in coastal waters

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Dinoflagellate cysts resuspended from bottom sediments into the water column may be an important inoculum at temperatures, oxygen and light levels more optimal for cyst germination. However, little is known about the conditions for resuspension of cysts from the bottom sediments. We have developed a new device to measure the resuspension flux of cysts (cysts m^{-2} day^{-1}). The basic structure of the device is a sediment trap in which the opening at the top is covered with a lid and reverse U-shaped tubes are attached to the holes drilled in the lid. The devices were moored at 1 and 5 m above the bottom at a site (11 m deep) in Ago Bay, Japan, and successfully collected resuspended cysts from bottom sediments, particularly the cysts of the red tide-forming dinoflagellate, *Scrippsiella acuminata*. Microscopic observations on samples collected from March to July 2020 revealed resuspension fluxes of *S. acuminata* cysts of $1.36 – 3.21 \times 10^4$ and $0.10 – 0.77 \times 10^4$ cysts m^{-2} day^{-1} at 1 and 5 m above the bottom, respectively. The data indicate that this new device can detect temporal and spatial variations in the flux of resuspended dinoflagellate cysts from the bottom sediments. Furthermore, the device can also monitor the difference in resuspension flux of other microalgae that possess resting stages in various coastal waters, contributing to our understanding of the ecology of these organisms.

Ecology
P-016

Seasonal and horizontal distribution of harmful dinoflagellate *Karenia digitata* in the Seto Inland Sea, Japan

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Dinoflagellate *Karenia digitata* is a unicellular harmful alga, and its red tide causes damage to fisheries, such as killing fish, bivalves, and seaweeds (Nori). Ecological information of the species is insufficient, e.g., seasonality of occurrence and optimal growth condition, because of its irregular occurrence. In addition, difficulty in morphological identification make us the monitoring difficult. In this study, to clarify the seasonal and horizontal distribution of *K. digitata* in the Seto Inland Sea where this species was first reported in Japan, we investigated *K. digitata* using a quantitative PCR (qPCR) for the rDNA-targeted molecular detection and identification. From 2018 to 2022, two types of surveys were conducted: monthly monitoring in Bingo-Nada, where a red tide of *K. digitata* and fishery damages have been occasionally reported, and several wide-area surveys in the eastern part of the Seto Inland Sea including Bingo-Nada during the survey period. *Karenia digitata* were detected by qPCR in the Bingo-Nada from September to January (the season is autumn to winter in Japan) every year, although it was occasionally detected in the spring. The peaks of cell density converted from DNA copy number were detected in autumn. In the survey of the wide areas conducted in October or November 2020, *K. digitata* was detected widely from the center to the eastern part of the Seto Inland Sea. These results indicate that the occurrence of *K. digitata* has seasonality with the peaks in autumn, and its distribution is wide in the central and eastern Seto Inland Sea.

Ecology
P-017

Allelopathy effect of *Margalefidinium polykrikoides* on toxin production of *Pyrodinium bahamense*

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The co-occurrences of *Pyrodinium bahamense* and *Margalefidinium polykrikoides* had been reported in Sabah coastal water. To understand the factors contribute towards the occurrences of these Harmful Algal Blooms (HABs) species, an allelopathy study of *M. polykrikoides* on *P. bahamense* was carried out by exposing filtrates of *M. polykrikoides* to live cells of *P. bahamense*. Three different volumes of *M. polykrikoides* bloom and culture filtrates (10, 20 and 50 mL) were mixed with 150 mL of *P. bahamense*. Paralytic shellfish poisoning (PSP) produced by *P. bahamense* was analysed using an isocratic postcolumn derivation HPLC method with fluorescence detection. The inhibition of *P. bahamense* was found in the bloom of filtered *M. polykrikoides* with a high toxin content of 45.658 fmole STXequiv.cell^{-1}. *P. bahamense* produced decarbamoylsaxitoxin (dcSTX), saxitoxin (STX), gonyautoxin-4 (GTX4), gonyautoxin-1 (GTX1), gonyautoxin-3 (GTX3) and gonyautoxin-5 (GTX 5). This study shows that the co-occurrences of both species might affect the PSP concentration produced by *P. bahamense* during bloom. Therefore, the findings provide important information for the monitoring of HABs particularly in Sabah coastal waters for human health and food security.

Ecology
P-018

Salinity effects on growth, oxidative stress, and compatible solutes in *Alexandrium minutum*: Insights into osmoregulation mechanisms

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Microalgae in estuarine and brackish water face the challenge of coping with complex salinity fluctuations, where osmoregulation plays a vital role in their survival and growth. *Alexandrium minutum*, a toxic dinoflagellate, serves as an ideal model for studying osmoregulation in dinoflagellates due to its wide range of salt tolerance and ease of laboratory cultivation. In this study, domesticated strains of *A. minutum* were obtained by long-term passage cultivation under different salt concentrations (15, 20, 25, and 30). We examined their growth status, oxidative stress levels (SOD and CAT), and concentrations of compatible solutes that commonly found in eukaryotic algae (DMSP, glycine betaine, and proline). Significant differences in specific growth rate and oxidative stress levels were observed among the different salt conditions, highlighting the critical role of salinity in *A. minutum* growth. Notably, a salinity value of 25 was identified as the optimal growth condition for *A. minutum*. Furthermore, concentrations of glycine betaine and DMSP exhibited clear positive correlations with salinity, indicating the significant roles of these compatible solutes in *A. minutum*'s salt adaptation. This study addresses the research gap concerning the osmoregulation mechanism of *A. minutum*, providing theoretical support for further related studies.

Ecology
P-019

Mapping harmful microalgal species by eDNA monitoring: a large-scale survey across the southwestern South China Sea

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A large-scale sampling was undertaken during a research cruise across the South China Sea in August 2016, covering an area of about 100,000 km^2 to investigate the molecular diversity and distributions of micro-eukaryotic protists, with a focus on the potentially harmful microalgal (HAB) species along the east coast of Peninsular Malaysia. Environmental DNAs from 30 stations were extracted and DNA metabarcoding targeting the V4 and V9 markers in the 18S rDNA was performed. Many protistan molecular units, including previously unreported HAB taxa, were discovered. Our findings also revealed interesting spatial distribution patterns, with a marked signal of compositional turnover between latitudinal regimes of water masses, where dinophytes and diatom compositions were among the most strongly enhanced at the front. Our results further confirmed the widespread distribution of HAB species, such as the toxigenic *Alexandrium tamiyavanichii* and *Pseudo-nitzschia* species, and the fish-killing *Margalefidinium polykrikoides* and *Karlodinium veneficum*. The molecular information obtained from this study provides an updated HAB species inventory and a toolset that could facilitate existing HAB monitoring schemes in the region to better inform management decisions.

Ecology
P-020

Measurements of primary production and light-dependent respiration in harmful algae blooms

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The accumulation of biomass during Harmful Algal Blooms (HABs) can lead to hypoxia conditions as oxygen is consumed during respiration and aerobic decomposition in the senescent phase of the bloom. However, little is known about how different conditions might influence respiration rates. Traditionally, respiration rates have been assumed to be constant, and are typically measured under dark conditions, but these rates can, in fact, be light-dependent. This study investigated how light intensity affects the oxygen production and respiration of several HABs. Gross Primary Production (GPP), Net Primary Production (NPP), and Gross Respiration (GR, i.e., the sum of metabolic processes consuming O_2) were measured during blooms of different species. These measurements were performed using a new, rapid $H_2{}^{18}O$ enrichment technique with Membrane Inlet Mass Spectrometry (MIMS). Ten-point irradiance curves were used to assess the relationship between GPP, NPP, and GR. Our results showed that GPP and NPP curves exhibit the classic saturation response to light intensity for both *Microcystis* sp. and *Karenia brevis*. However, GR-irradiance curves were variable in magnitude and pattern among the species. *K. brevis* presented higher respiration rates in the higher lights, which represented a substantial fraction of the GPP, being even greater than NPP rates. These results indicate that different groups may have distinctive respiration responses to maintain redox balance and/or prevent light-induced damage. Determining how oxygen production and respiration rates vary among species under specific environmental conditions is essential to understand where and when hypoxia events might be developed in future climate conditions.

Ecology
P-021

Interactions between the red-tide dinoflagellate *Tripos furca* and common heterotrophic protists

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The mixotrophic dinoflagellate *Tripos furca* causes red tides in many countries. To understand its population dynamics, mortality due to predation and growth rate should be assessed. In prior studies, the heterotrophic dinoflagellates *Noctiluca scintillans*, *Polykrikos kofoidii*, *Protoperidinium steinii*, and mixotrophic dinoflagellate *Fragilidium subglobosum* were known to ingest *T. furca*. However, if other common protists are able to feed on *T. furca* has not been tested. We explored interactions between *T. furca* and nine heterotrophic dinoflagellates and one naked ciliate. Furthermore, we investigated *T. furca* abundance and common heterotrophic protists in coastal-offshore waters off Yeosu, southern Korea, on Jul 31, 2020, during its red tide. Among the tested protists, the heterotrophic dinoflagellates *Aduncodinium glandula*, *Luciella masanensis*, and *Pfiesteria piscicida* fed on *T. furca*. However, the heterotrophic dinoflagellates *Gyrodiniellum shiwhaense*, *Gyrodinium dominans*, *Gyrodinium jinhaense*, *Gyrodinium moestrupii*, *Oblea rotunda*, *Oxyrrhis marina*, and the naked ciliate *Rimostrombidium* sp. did not. However, *T. furca* did not support the growth of *A. glandula*, *L. masanensis*, or *P. piscicida*. From Jun 30 to Sep 5, 2020, *T. furca*-dominant red tides prevailed in the South Sea of Korea. The maximum abundance of heterotrophic dinoflagellates in Yeosu on Jul 31, 2020, was as low as 5.0 cells mL^{-1}, and *A. glandula*, *L. masanensis*, and *P. piscicida* were undetectable. Furthermore, the abundances of the known predators were very low or negligible. Therefore, no or low abundance of effective predators might be partially responsible for the long duration of *T. furca* red tides in the South Sea of Korea in 2020.

Ecology
P-022

Dynamics of *Alexandrium catenella* bloom and the influence on paralytic shellfish toxins in shellfish in the coastal waters of Qinhuangdao, China

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Since 2016, paralytic shellfish toxin (PSTs) poisoning incidents have occurred in Qinhuangdao, the Bohai Sea. *Alexandrium catenella* producing gonyautoxin 1-4 is suggest to cause for PSTs poisoning incidents; however, bloom dynamics and influence on PSTs in shellfish are still unknown. In this study, three investigations covering Qinhuangdao inshore were executed in summer and autumn 2020, and spring 2021; and sixteen investigations covering four sampling stations were executed from July 2020 to July 2021. The results showed *A. catenella* bloom occurred from the end of March to the middle of April when seawater temperature is 5-10℃. There was high abundance of *A. catenella* cysts in Qinhuangdao coastal surface sediment. Cyst abundance in summer was greater than in autumn and spring, and it was greater in north coastal waters than that in the south. Prior to *A. catenella* bloom in the end of March, spatial distribution of *A. catenella* cysts was consistent with vegetative cells, implying *A. catenella* cysts in local sediment may be cause for the blooms. During *A. catenella* bloom, PSTs exceeded the regulatory limit for PSTs (800 μg STXeq/kg tissue) in multiple kinds of shellfish, and they had different features of PSTs accumulation and elimination. Both mussel and oyster accumulated PST faster than scallops, and mussels accumulated more content of PST than oysters; however, scallop retained PSTs for a longer time. These results will provide a scientific understanding for the formation mechanism of *A. catenella* blooms and a basis for early warning of PSTs poisoning incidents in the Qinhuangdao.

Ecology

P-024

First HAB of the dinoflagellate *Pyrodinium bahamense* var. *bahamense* in the lagoon of Terminos, Campeche, Mexico

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Dinoflagellates are the most abundant and diverse group of toxin-producing microalgae with harmful potential according to UNESCO; when environmental conditions are not favorable for them, they begin an accelerated reproduction, causing Harmful Algal Blooms (HAB) that may or may not be toxic, depending on the organism that causes it. *Pyrodinium bahamense* var. *bahamense*, has been reported for the state of Campeche and in the Terminos lagoon, as well as in the Pom-Atasta lagoon complex, with high abundances; it is a species with a high toxic potential, generating saxitoxins and analogous compounds. The lagoon of Terminos is the second largest coastal lagoon in Mexico, considered an important natural protected area from an ecological, fishing and oil level point of view. This paper presents the first HAB report for the species *Pyrodinium bahamense* var. *bahamense* in the lagoon in the dry and rainy seasons of 2107. The cells of *Pyrodinium bahamense* var. *bahamense* presented an increase in their size that may be due to an ability to search and store nutrients present in the water column when the supply of new nutrients is limited or episodic; the HAB occurred when the nutrient concentrations of NH_4^+, NO_3^-, NO_2^-, PO_4^{3-}, SiO_2 were low, therefore the favoring the size increase of *Pyrodinium bahamense* var. *bahamense* due to the storage of nutrients to survive. With the help of remote sensing, the HAB was validated in the area where *Pyrodinium bahamense* var. *bahamense* was found.

Ecology
P-026

Diversity and temporal dynamics of the marine diatom genus *Chaetoceros* in two coastal areas of the NW Mediterranean Sea

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Chaetoceros Ehrenberg is one of the most cosmopolitan and diverse diatom genera in the oceans and plays an important role in food webs and biogeochemical cycles of carbon and silica. Some species can reach bloom abundances and others can be harmful to fish, causing physiological damage or even death. In this work, the diversity and temporal dynamics of *Chaetoceros* were explored, combining classical and molecular approaches, in surface waters of two environmentally different coastal zones of the Catalan coast (Barcelona and Blanes Bay), covering several years from 2004 to 2019. Metabarcoding of the V4 region of 18S rDNA obtained monthly during 8-years in Blanes recovered a total of 41 ASVs belonging to *Chaetoceros*, 27 of which correspond to well-known species. The most abundant and frequent ASVs corresponded to *C. tenuissimus*, *C. socialis*, *C. vixvisibilis* and *C. curvisetus*. Additionally, the abundance of twenty-four *Chaetoceros* species was followed monthly by light microscopy during two years in the two coastal areas. The obtained results confirmed *C. curvisetus*, *C. socialis* and *C. vixvisibilis* as the most abundant (maximum abundance > 10.000 cells L^{-1}) and frequent (> 30%) species in the two areas, together with species of the *C. lorenzianus* complex. During the studied period, *C. tenuissimus* was only detected in high abundances in Barcelona. These species presented similar temporal dynamics in both areas with maximum abundances at different times of the year responding to different ecological conditions. The diverse environmental parameters that control the different *Chaetoceros* species succession patterns are discussed.

Ecology
P-027

Comparison of photoprotective mechanisms between a raphidophyte *Chattonella marina* var. *antiqua* and a diatom *Skeletonema costatum*

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The past dominant diatom species, *Skeletonema* spp., have declined recently in the Seto Inland Sea of Japan, and this is considered to be attributed to oligotrophication of the sea. Meanwhile, harmful raphidophyte species, *Chattonella* spp., still occur densely in summer. We assume recent increase in solar insolation at the area might help *Chattonella* domination over *Skeletonema*, and their photoprotective mechanisms under high light exposure were investigated. Cultures of *Skeletonema costatum* s.s. and *Chattonella marina* var. *antiqua* were maintained in 1 L chemostat cultures (N/P = 16) for 24 or 30 days, respectively. The balanced-phased cells were exposed to a moderate and a high light (PFD = 150 and 800 μmol-photons m^{-2} sec^{-1}) for each 1 hour, and photosynthetic responses and their xanthophyl pigments involved in excess-light dissipation were measured. *S. costatum* exhibited higher non-photochemical quenching (NPQ) under high light, but quantum yield of photosystem II was lower than *C. antiqua*, insisting *S. costatum* could not protect PSII irrespective their active photoprotection. These trends of NPQ induction were supported by de-epoxidation status of xanthophylls, i.e., diadinoxanthin and violaxanthin cycles. While *S. costatum* exhibited diadinoxanthin cycle only, *C. antiqua* actively employed both diadinoxanthin and violaxanthin cycles, as indicated by higher accumulation of the de-epoxidated pigments, indicating *C. antiqua* could effectively handle high light. Lhcx proteins known to contribute NPQ coordinating with xanthophyll cycle were also identified in *S. costatum* and *C. antiqua*, and six or four Lhcx genes were detected. The gene expressions for Lhcx family will be presented in the poster.

Ecology

P-028

Effect of light emitting diode on the growth and biochemical composition of *Chlorella vulgaris*

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In the case of green algae *Chlorella vulgaris*, the efficiency of removing nutrients such as nitrogen and carbon varies depending on the light emitting diode (LED) wavelength. Therefore, in this study, the growth specificity and physiologically active substance changes of *C. vulgaris* (PKVL7422) according to LED wavelength and light amount were reviewed. In this study, *C. vulgaris* showed high growth rates at blue and red wavelengths. The carbohydrate content was the highest at the green wavelength of light intensity 10 μmol/m^2/s, which showed a low growth rate. Protein (334 μg/g) and lipid (716 μg/g) showed the highest content at the normal blue wavelength of 100 μmol/m^2/s light intensity. There was no clear difference in the change in the growth specificity of *C. vulgaris* according to the mixing wavelength. There was no significant difference in carbohydrate content ($P>0.05$), and protein was 1.5 times higher than blue single wavelength, and lipids showed similar content. The growth rate according to the light and dark cycle control was the highest at 12L:12D with a red wavelength of 0.63/day and a blue wavelength of 0.59/day, and there was no clear difference in carbohydrate, protein, and lipid content. There was no statistically significant difference in carbohydrates in the biochemical composition change according to the two-stage culture of each growth stage ($P>0.05$). Protein showed 1.5 times higher content than blue single wavelengths, and lipids showed similar content. Therefore, if the growth specificity and biochemical composition change results of microalgae by LED wavelength are used, it is expected that a light culture system that considers high efficiency and functionality using LEDs and microalgae will be established in the future.

Ecology
P-029

Generation and release of reactive oxygen species by symbiotic zooxanthellae under copper stress

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Generation of reactive oxygen species (ROS) by symbiotic zooxanthellae under external stress, i.e., heat or high light, have been proved to be the main driver of coral bleaching. High levels of copper would be one of the oxidative stresses for zooxanthellae, however, the generation and release mechanisms of ROS responsible for Cu stress have not been clearly elucidated. Therefore, the photosynthetic and ROS inhibition experiment was first conducted by the addition of Diphenyleneiodonium (an NADPH oxidase inhibitor), rotenone (a mitochondrial complex I inhibitor) and 3-(2,3-dichlorophenyl)-1,1-dimethylurea (a PSII inhibitor) as well as copper to *Symbiodinium* sp., with the result showing that approximately 50% of ROS were generated from plasma membrane NADPH oxidase when under high copper stress. Moreover, low copper, i.e., 3~6 μM, stimulated the production of ROS in long time exposure (96h), which was then scavenged by glutathione (GSH), and regulated by catalase (CAT) and peroxidase (POD). High copper, over 9 μM, stressed the cells since the first 24h. To mitigate the cellular oxidative damage, the extra ROS was first regulated by CAT, POD and superoxide dismutase (SOD), then actively released into the medium. The ROS releasing process may not through the cell membrane by the evidence of no significant variation of saturated fatty acids (SFA)/unsaturated fatty acids (UFA), but over 4 times of ROS releasing into the medium under high Cu stress would lead to a great threaten to the symbiotic 'zooxanthellae-coral' microsystem.

Ecology

P-030

The ecology of *Ostreopsis ovata* spreading along the rocky coast of the eastern Adriatic

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Species from the genus *Ostreopsis* can be observed around the Mediterranean. In recent years observations are reported from more and more locations. Along the north eastern Adriatic coast *Ostreopsis ovata* is reported from more and more places and observed abundances appear to increase with the years. Here we report on the ecology of this observed spread of *Ostreopsis ovata* and on the changes in benthic communities that are observed during this spread. On the base of spatio-temporally resolved biodiversity data and further ecological parameters, transport pathways along with climate change related changes in habitat ecology are discussed in an attempt to predict the near future development of Ostreopsis ovata along the north eastern Adriatic Sea.

Ecology

P-031

Harmful phytoplanktonic resting stage assemblages in the central Mediterranean Sea

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Several phytoplankton species, including HAB (harmful algal bloom) species, produce resting stages that deposit onto the sea bottom, forming seed banks or assemblages playing a crucial ecological role in maintaining microbial populations in future environments. The aim of this study, was to investigate the phytoplankton assemblage structure in relation to environmental drivers and human pressures. In particular, resting stages of diatoms and dinoflagellates, including HAB taxa, in the surface sediments of three Mediterranean regional areas were analysed. Abundance of target resting stages was determined by the application of different real time PCR assays. Using multivariate data analysis, we found that resting-stage abundance seemed related to depth. Moreover, regional differences in the resting-stage assemblages were evident and environmental drivers were correlated with those regional differences. Three main groups of samples were defined according to sea surface temperature (SST) and depth thresholds. Samples from the Adriatic Sea (average SST $<$ 18°C) showed the higher abundance and species richness, while deep samples from all other basins (depth $>$ 368 m) were poorer and less diverse than those from shallower sites (depth $\leq$ 368 m). Diatom and dinoflagellate resting stages were the most represented taxa and, among the dinoflagellate cyst taxa, the potentially harmful *Alexandrium minutum* and *Gymnodinium* spp. were found to be the most abundant species. The study of the resting-stage assemblages structure can be regarded as a time- and space-integrated response to environmental conditions which can favor or prevent sedimentation of resting stages giving also information on future phytoplankton assemblages.

Biology and Biogeography

P-032

Effects of silicate on growth, morphology and life-cycle switching in silicoflagellates

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Silicoflagellates are unicellular algae with a trademark basket-like external siliceous skeleton. They also have another naked stage in their life-cycle that is distinct from the skeleton-bearing stage. There have been some reports of fish mortalities coinciding with the bloom of silicoflagellates, especially when they proliferate as the naked stage. However, the factors that induce the transition between the skeleton-bearing and naked stage are unknown. Silicate, a material of siliceous skeletons, is a candidate environmental factor that affects their skeletogenesis. In this study, we examined the effects of silicate concentration on the growth, morphology and life-cycle switching of the silicoflagellates using the skeleton-bearing stage of cultured strains *Octactis octonaria* and *Dictyocha fibula*. We first observed that the specific growth rate μ (d^{-1}) did not differ ($n=3$, $p>0.05$) between a silicate-replete medium (10–190 μM) and a silicate-deplete medium (<0.6 μM) in both strains. In contrast, skeletal morphology of silicoflagellates changed with the silicate concentrations. For example in *O. octonaria*, the thickness of several parts of skeleton in silicate-deplete medium was significantly thinner ($n=30$, $p<0.05$). Most notably, upon silicate depletion, both strains grew with no skeletal structure. This loss of skeleton was reversible: when skeleton-less cells were transferred to a silicate-replete medium, skeleton regeneration occurred. It should be noted, however, the skeleton-less cells obtained under the silicate-deplete condition differed from cells in the naked stage in having rough cell surface and many tentacles. These results demonstrated that silicate critically affects the skeletogenesis of silicoflagellates, but not their growth or life-cycle.

Biology and Biogeography

P-033

Quorum sensing mediated activities of growth-inhibiting bacteria against the toxic dinoflagellate *Alexandrium catenella* (Group I)

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The developments of strategies for preventing and controlling harmful algae, including the PSP, are urgent global priority. We demonstrated that the biofilms of seagrass and seaweed beds serve as natural environments for the habitats of algicidal bacteria against HABs. We demonstrated abundant existence of growth-inhibiting bacteria against the PSP causing dinoflagellate *Alexandrium catenella* (Group I) from the seagrass beds in Akkeshi-ko Estuary and the coastal waters adjacent Akkeshi Bay. The attack mechanisms of the growth-inhibiting bacteria were investigated. When the bacteria were added to algal cultures as liquid culture where they were dispersed, at most no algicidal activities occurred. When algicidal bacteria were added to algae in the form of colonies, those bacteria exhibited a higher capacity of algicidal activity with the same cell density of bacterial addition. Based on these findings suggesting the involvement of quorum sensing in algicidal activities, we conducted culture experiments with the addition of β-cyclodextrin, which inhibits quorum sensing. As a result, the occurrence of growth inhibition was confirmed in the co-cultivation experimental conditions with algae and bacterial colonies. On the other hand, no growth inhibition was observed in the experiment in β-cyclodextrin-added conditions. In the future, it is suggested that experiments considering quorum sensing will make it possible for better understandings of the interactions between harmful algae and algicidal bacteria in natural environments.

Biology and Biogeography
P-034

Potential distribution of the genus *Alexandrium* in the Seto Inland Sea

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Genus *Alexandrium* includes many species that cause paralytic shellfish poisoning and fish mortality. Many of these species have ability to form dormant cysts and occurrence of the vegetative cells in water is transient. Therefore, it is inferred that the distribution that can be understood by monitoring of vegetative cells is limited. In this study, we investigated the potential distribution of *Alexandrium* species by metagenomic analysis targeting the dormant cysts in sediment in the Seto Inland Sea. In April 2021 and May 2022, sediment samplings were conducted at 40 stations in the eastern part (Kii Channel – Hiroshima Bay) and at 14 stations in the western part (Hiroshima Bay – Suo Nada) of the Seto Inland Sea, respectively. Metagenomic analysis was conducted based on DNA extracted from the sediment samples. As results, a total of 19 *Alexandrium* species (including four unknown species) were detected. Focusing on *A. tamarense* species complex (At complex), distribution of *A. catenella* (group I) was widely in the surface sediments, while that of *A. pacificum* (group IV) was limited. Nontoxic species of *A. tamarense* (group III), which has not been reported to occur in Japan, was detected in several samples of vertical distribution survey from the western Seto Inland Sea. The result suggests that other species of At complex other than *A. catenella* and *A. pacificum* distribute potentially along the coast of Japan. In this presentation, we will also report the distribution of other potential toxic and harmful *Alexandrium* species.

Biology and Biogeography
P-035

Expansion of HAB species in Hokkaido, northern Japan, after 2010s

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To reveal the recent situation of the expansion of HAB species around Hokkaido, northern Japan, we considered the past occurrences of HAB events since 1970s. Novel HAB species were detected after 2013 as well as the toxic dinoflagellates *Alexandrium catenella* (Group I) and *Dinophysis* spp., producing shellfish toxins. Notorious red tide species *Chattonella marina*, *Karenia mikimotoi* and *Margalefidinium polykrikoides*, and toxic species *Ostreopsis* sp. and *Prorocentrum lima* had been newly recorded in 2010s. These species are supposed to be introduced to the west coast of Hokkaido from western Japan via the Tsushima Warm Current system. In the autumn 2021, the harmful red tide due to *Karenia selliformis* occurred on the Pacific coast of eastern Hokkaido, and great amounts of fishery species (e.g., sea urchin *Strongylocentrotus intermedius*, chum salmon *Oncorhynchus keta* and sea snail *Neptunea* spp.) were killed by the HAB event. The harmful event is assumed to be caused by the introduction of *K. selliformis* from the offshore area of north Pacific via the Oyashio. It is important to elucidate geographical distribution of HAB species and to monitor further expansion of HAB species via ocean current system in subarctic regions.

Biology and Biogeography

P-036

Potential impact of the *sxtA4* gene diversity on the paralytic shellfish toxin production in the toxic dinoflagellate *Alexandrium pacificum*

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It is unclear how the genus *Alexandrium* exhibits high intraspecific physiological variability, resulting in variations in production of paralytic shellfish poisoning toxins (PSTs) depending on the strains. Thus, we investigated the intraspecific variation in PST production of the eight *A. pacificum* strains depending on the four different temperature conditions (15 °C, 20 °C, 25 °C, and 30 °C). As a results, PST content was clearly different depending on *A. pacificum* strains irrespective of temperature conditions, and the level of PST content in each strain was higher under unfavorable growth conditions. There was a clear difference in PST composition between the two strains (KM2 and KM5): PST components in strain KM5 were more diverse than those in strain KM2. To examine whether or not this difference might be derived from intraspecific genetic diversity between the strains, we investigated the sequences of the *sxtA4* gene which is a core gene for PST production using a cloning method. Interestingly, the *sxtA4* gene in strain KM5 (88 single nucleotide polymorphisms; SNPs/615 bp) had more SNPs than that in strain KM2 (54 SNPs/615 bp). Given these findings, the number of SNPs might be positively associated with the level of PST diversity in *A. pacificum*.

Biology and Biogeography
P-037

Mapping of four main harmful algal species in the East China Sea (Yangtze River estuary) and their possible respondence to main ecological status via a global vision

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The Yangtze River Estuary (YRE) is the most severely affected sea area for harmful algal blooms (HABs) in China. *Karenia mikimotoi*, *Margalefidinium polykrikoides*, *Prorocentrum donghaiense* and *Heterosigma akashiwo* are the main HABs species in East Asian Sea. The four HABs species were detected and quantified via quantitative real-time PCR (qPCR) in this study. Estimated maximum cell abundance of *K. mikimotoi*, *M. polykrikoides* (East Asian Ribotype, EAR), *P. donghaiense* and *H. akashiwo* were 1.6×10^5, 1.3×10^5, 1.6×10^5 and 1.2×10^7 cells·L^{-1}, respectively. HABs dominated by *H. akashiwo* and *P. donghaiense* occurred at station S27 on July 22, 2020. In the studied sea area, turbidity, pH and salinity may be the main factors affecting the blooms of *K. mikimotoi*, *M. polykrikoides* (EAR) and *P. donghaiense*, while temperature may be the main factor affecting the blooms of *H akashiwo*. The results reveal a distributive pattern of the four HABs species in the YRE under the different ecological status. It provides a practical case for the future construction of warning systems for the four HABs species and HABs.

Biology and Biogeography
P-038

Availability of iron to harmful algal species

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Iron is an essential trace metal and a limiting factor for microalgal growth, but dissolved iron concentrations in natural waters are extremely low. Dissolved iron fractions consist largely of colloidal hydrolysis species and most of those are bound by organic ligands in natural waters. The mechanism of iron uptake by eukaryotic microalgae is not yet fully understood because of the intricate iron chemistry of natural seawater and the difficulty in cultivation of axenic microalgae under uncontamination. To clarify utilization of the iron for the harmful algal species, we examined the growth experiments using a developed artificial synthetic medium in the presence of different dissolved organic iron concentrations. Axenic clonal cultures of harmful algal species were used in this study. These were 3 species of flagellates *Heterosigma akashiwo*, *Heterocapsa circularisquama*, *Karenia mikimotoi* and 4 species of diatoms *Asteroplanus karianus*, *Chaetoceros lorenzianus*, *Coscinodiscus wailesii*, *Skeletonema marinoi-dohrnii* complex. These minimum cell quotas of iron were calculated 0.0065, 0.058, 0.35, 0.11, 0.0013, 15, 0.14 pmol cell^{-1} respectively. The utilization of organic iron is thought to be useful for the growth of these algal species under inorganic iron-deficient environments. These results suggest that the iron speciation gives significant effects on controlling the harmful algal bloom formation in coastal areas.

Biology and Biogeography
P-039

Cultured dinoflagellates of different ages display varying characteristics

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In the lower region of Chesapeake Bay, the toxic dinoflagellate *Alexandrium monilatum* forms near-annual "red tides" in late-summers when the average water temperature exceeds 25°C. It is notorious for bioluminescence and the occasional co-occurrence of aquatic animal kill events. During an *A. monilatum* bloom peak, cells have a dark red pigmentation and form long cell chains up to 80 cells in length. As a bloom declines, pigmentation lightens to yellow, cell chains break up, and sexual stages including gametes and zygotes are observed. A clonal isolate of *A. monilatum* established from a 2007 bloom in the York River no longer displays some of these field-observed characters, including bioluminescence and formation of long chains, whereas newly established isolates from 2020 and 2022 maintain them. Secondly, during the exponential growth phase the per cell goniodomin-a toxin content is higher in the newer isolates, and the 2007 isolate has a faster growth rate overall. We hypothesize that these differences are a result of adaptation over time to the controlled environment of growth in laboratory culture. Consideration should be given to the fact that results from experiments with old and new isolate cultures might be different. Scientists should be mindful of the age of their isolates when using them for experimentation.

Community/Species Interactions

P-040

Development of a long-term preservation method for the parasitic dinoflagellate *Amoebophrya* sp. by infecting the genus *Alexandrium*

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The endoparasitic dinoflagellate *Amoebophrya* spp. has attracted attention as a biological control agent for harmful algal blooms. For practical marine applications of the parasite, developing a method for its stable culture and preservation is vital. Despite the great effort and high cost required for parasite maintenance, only a few reports exist on its preservation methods. The purpose of the present study was to determine the appropriate conditions for long-term preservation of the parasite. Preliminary experiments showed that this parasitic species cannot be cryopreserved; however, we found that *Amoebophrya* sp. within host cells could be preserved by suppressing the progress of the infection cycle at low temperatures. Parasitism of *A. catenella* (Group I) and *Amoebophrya* sp. was allowed to progress in a well of 12-well microplates in successive cultivation condition (14 °C), and then each plate was transferred to the following temperatures: 14 (control), 6, 4, and 3 °C. Subsamples were collected and tested monthly to determine whether the preserved *Amoebophrya* sp. was capable of reinfecting the host. The results showed that when preserved at 14 °C, *Amoebophrya* sp. perished within 2–3 weeks. When preserved at 6 and 3 °C, reinfection with *Amoebophrya* sp. was not confirmed one month after preservation. In contrast, when preserved at 4 °C, reinfection was confirmed one month after preservation. Our findings provide an important basis for establishing a method to use this parasite as a biological control agent for HABs.

Community/Species Interactions

P-041

Inhibition mechanism and dose effect of mangrove leaves on harmful algae in coastal South China

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Harmful algal blooms (HABs) have intensified worldwide during these decades, mainly due to eutrophication. Mangroves are usually located in eutrophic water bodies but rarely have harmful algal blooms. Therefore, whether the presence of mangroves can inhibit the growth of harmful algae has become a topic worthy of discussion. This study investigated the impact of the presence of mangrove plants on the phytoplankton community along the coast of South China and found that mangrove vegetation may affect water quality and shape phytoplankton composition. The impact of mangrove plants on harmful algae was then further evaluated under laboratory conditions. The results suggest that the effects of mangrove leaf extract on harmful algae are species-specific, both in mangroves and in harmful algae. *Kandelia obovata* has a general inhibitory effect on selected harmful algae. The inhibitory effect of *K. obovata* on HAB species *Karenia mikimotoi* and *Alexandrium tamarense* was enhanced with the increase of the dose, and the inhibitory effect of the low-dose repeated exposure was longer than that of the high-dose single exposure. Mangrove leaf extract induces excessive production of reactive oxygen species in algae, causing lipid peroxidation, reducing photosynthetic pigments, and reducing photosynthetic efficiency, thereby inhibiting their growth or even killing them. These results demonstrate mangrove plants, especially *K. obovata*, have huge potential to control HABs.

Community/Species Interactions
P-042

Photoacclimation of *Dinophysis acuminata*: touching the limits of kleptoplastidy?

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Several species of the dinoflagellate *Dinophysis,* responsible of diarrheic shellfish poisoning, are known to acquire photosynthetic capacities through sustained acquisition of kleptoplastids of cryptophyte origin. Initially, cryptophyte microalgae are the prey of the red tide forming ciliates, *Mesodinium* spp., that steal and use their nuclei, mitochondria and plastids, thereby becoming photosynthetic. These ciliates can in turn be victims of kleptoplasty by *Dinophysis* species. This study aims at understanding whether plastid photoacclimation capacities are altered in the cellular environment of the hosts. Using the triple culture system *Plagioselmis prolonga* - *Mesodinium rubrum* - *Dinophysis acuminata*, we set up an experiment in which the three organisms were shifted from high to low irradiances, in order to induce synthesis of photosynthetic complexes, such as phycoerythrin. Our results show that photoacclimation to lower irradiance is possible in the three organisms but that it is much constrained by the availability and the photoacclimation level of preys. Confocal microscopy measurements showed that it does not rely on an increased ingestion rate of plastids. Interestingly, green light utilization by photosystem II was optimal in the cryptophyte but always lower in ciliate and dinoflagellate stolen plastids, suggesting a modification of phycoerythrin light absorption efficiency. We hypothesize that phycoerythrin synthesis, which requires nuclear and plastidial genes, is altered in the dinoflagellate that lack the cryptophyte nucleus. Overall, our study suggests that light harvesting is less efficient in the translocated plastids and that competitiveness of the dinoflagellate for light is highly dependent on the photoacclimation level of the preys.

Community/Species Interactions

P-043

A study on the changes of dominant microalgae in Geum River near *Haematococcus pluvialis* culture facility

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South Korea has four seasons and the amount of precipitation and water temperature constantly change depending on the season, it has good conditions for conducting research on the cell cycle of cyanobacteria. *Microcystis aeruginosa*, a toxic cyanobacteria, is a major causative agent of water blooms, and many related damages have been reported. Therefore, studies on the effects of changes in the biological and physical environment of *Microcystis aeruginosa* on their development are ongoing. In this study, biological changes of surrounding organisms related to cyanobacteria were tracked in a tributary of the Geum River near the *Haematococcus pluvialis* culture facility. After sampling and environmental analysis with NGS over the past two years, studies have shown that water blooms when certain factors, such as water temperature and nitrate, are increased. In addition, it was found to be accompanied by changes in the composition of zooplankton and microbes before seals. According to the zooplankton, the microalgae community that previously flourished was removed to provide a space for other microalgae to grow, and at this time, it was confirmed that when the temperature rises, it leads to the growth of cyanobacteria. Therefore, it is expected that changes in the surrounding biocommunity will help predict the occurrence of cyanobacteria, which should be careful of contamination in microalgae culture facilities.

Community/Species Interactions
P-044

Allelopathic effects of diatoms on the growth of *Chattonella marina* var. *antiqua*

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The raphidophyte *Chattonella marina* var. *antiqua* (hereinafter called "*C. antiqua*") and diatoms often form alternating blooms, and it is thought to be causally related to interspecific competition between *C. antiqua* and diatoms via allelopathy and macronutrients. First, in this study, we examined the effects of filtrates prepared from seawater samples, including diatoms, on the growth of *C. antiqua*. Our results indicated that *C. antiqua* growth was inhibited by the filtrate prepared from seawater samples, which includes high cell densities of diatoms. Then, we investigated the allelopathic effects of eight strains of diatoms on the growth of *C. antiqua*. As a result, *C. antiqua* growth was promoted or inhibited depending on the kind of diatom species. Especially, the filtrate of *Thalassiosira nitzschioides* showed the strongest inhibitory effect of the eight strains on *C. antiqua*. Thus, growth interactions between *T. nitzschioides* and *C. antiqua* were examined in bi-algal cultures. When initial cell densities of *T. nitzschioides* and *C. antiqua* were both 10^2 cells mL^{-1}, the growth of *C. antiqua* was strongly suppressed by *T. nitzschioides*, but the growth of *T. nitzschioides* was virtually the same in both bi-algal and mono-algal cultures. Furthermore, the growth of *C. antiqua* was notably suppressed in bi-algal cultures when initial cell densities of *C. antiqua* and *T. nitzschioides* were 10^2 cells mL^{-1} and 10^4 cells mL^{-1}, respectively. Therefore, species-specific growth interactions between *C. antiqua* and diatoms may have a crucial role in the growth dynamics of *C. antiqua*.

Community/Species Interactions

P-045

Interactions between *Microcystis*, the cause of harmful algal blooms, and marine heterotrophic protozoa

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Microcystis is one of the freshwater cyanobacteria and causes harmful algae blooms in many countries. Large-scale outbreaks of *Microcystis* in lakes and rivers enter the estuary and coexist with many marine organisms. When the *Microcystis* cells are destroyed due to the high salinity, the microcystins in the cells are released, causing damage to the marine environment and marine organisms. Therefore, the interaction between *Microcystis* and marine protists should be studied. To find out their relationship, we coincubated *Oxyrrhis marina* and *Polykrikos kofoidii*, marine heterotrophic protozoa abundant in the marine environment, along with *Microcystis*. The results showed that *P. kofoidii* was unable to feed on *Microcystis*, resulting in a negative growth rate of -0.65 d^{-1}. On the other hand, *Oxyrrhis marina* was able to feed on *Microcystis* and showed a positive growth rate of up to 1.2 d^{-1} with increasing prey concentration. However, at specific high concentrations of *Microcystis*, the growth rate of *O. marina* decreased. The results indicate that *O. marina* has the potential to control *Microcystis* entering the marine environment but also that high concentrations of *Microcystis* can inhibit the growth rate of marine protozoa. It also suggests the need to study the interactions of *Microcystis* with other marine heterotrophic protozoa, and such studies will improve our understanding of the impact of *Microcystis* on marine ecosystems.

Community/Species Interactions

P-046

Harmful algal bloom proliferation promoted by allelopathically-induced trophic transfer of nitrogen

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Allelopathy is a biological mechanism that can promote by harmful algal blooms (HAB) via the inhibition of sympatric phytoplankton. To examine the impacts of allelopathically regenerated N on HABs, a series of experiments were performed using multiple allelopathic HAB species including the dinoflagellates *Alexandrium catenella* and *Margalefidinium polykrikoides*, and the pelagophyte, *Aureoumbra lagunensis*. These HABs were paired with the cosmopolitan dinoflagellate, *Akashiwo sanguinea*, that was labeled with $^{15}NO_3^{-}$- or $^{15}NH_4^+$, allowing the release and transfer of N to be traced as a time course during allelopathic interactions. During all experiments, the allelopathic inhibition of *Akashiwo* was accompanied by increases in cell densities, growth rates, and the $\delta^{15}N$ content of the HAB species, evidencing the transfer of N liberated from *Akashiwo*. The cellular transfer of ^{15}N and release of dissolved N was higher when *Akashiwo* was grown with $^{15}NO_3^-$ compared to $^{15}NH_4^+$ suggesting a differential subcellular-compartmentalization of N sources. Regardless of the type of N, HABs obtained 60–100% of their cellular N from lysed *Akashiwo* cells and there was an enrichment of the $\delta^{15}N$ content of the dissolved NH_4^+ pool post-lysis of *Akashiwo*. Collectively, the results demonstrate that beyond facilitating species succession, allelopathy can supply HABs with regenerated N and, therefore, is likely important for promoting and sustaining HABs. Given that allelopathy is known to be a dose-dependent process, allelopathy may induce a positive feedback loop, whereby competitors are lysed, N is regenerated, HABs are intensified and, in turn, become more strongly allelopathic.

Community/Species Interactions

P-047

Microbial community coexisting with *Pyrodinium bahamense* cyst

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Marine phytoplankton cells grow in close association with a complex microbial associate community. These microbial communities are known to affect the growth, behavior, and physiology of the algal host. However, knowledge regarding the microbial communities coexisting with phytoplankton cysts is limited. *Pyrodinium bahamense* is a dinoflagellate that produces saxitoxin and causes paralytic shellfish poisoning. When conditions are unfavorable for vegetative growth, *P. bahamense* produces resting cysts in the marine environment. In this study, we will investigate the composition of the microbial community coexisting with harmful algal cyst of *Pyrodinium bahamense* in Puerto Bay, Puerto Princesa City, Palawan. This bay is known for the frequent bloom of *P. bahamense* which affects the local fisheries. To conduct this study, two methods will be used. First is to isolate the microorganisms from the sediment and cyst and culture them in the laboratory in an anoxic environment. Marine agar will be used to isolate bacteria while potato dextrose agar will be used to isolate fungi. The other method is to subject the sediment and cyst samples in 16S rRNA gene amplicon sequencing for the bacterial community and ITS sequencing for the fungal community. The assessment of microbial communities associated with harmful algal bloom species like *P. bahamense* should be studied more extensively as more data is needed to ascertain the functions of associated microorganisms responsible for cyst production.

Community/Species Interactions
P-048

First report of *Amoebophrya* sp. infecting the harmful dinoflagellate *Karenia* species

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As a biological control method for harmful algal blooms (HABs), endoparasitic dinoflagellates, *Amoebophrya* spp., are attracting attention because they infect and kill over 70 species of their host dinoflagellates. However, studies on *Amoebophrya* species infecting the genus *Karenia,* one of the most important HAB species worldwide, have not yet been conducted. In 2020, for the first time, we successfully isolated *Amoebophrya* sp. that infected *K. mikimotoi* from Osaka Bay, Japan. The present study aims to clarify the life cycle, taxonomy, host range, and characteristics of the infection process of *Amoebophrya*. The isolated parasite strain exhibited the typical life cycle of *Amoebophrya*. Genetic analysis of the nuclear 18S and 28S rDNA revealed that the parasite was a novel strain unregistered in GenBank. *Amoebophrya* sp. isolated in this study could infect *K. mikimotoi* and *K. papilionacea* but not *K. selliformis* and the other 14 dinoflagellates, indicating high host specificity. A laboratory co-culture experiment of *K. mikimotoi* and *Amoebophrya* sp. was conducted under optimal conditions, and distinct suppression of host growth was observed with the addition of the parasite compared with that in the control. The prevalence increased rapidly from 36 h after the addition of the parasite and peaked at 60 h (98 %). Given its high infection ability and host specificity, the parasite *Amoebophrya* sp. can be used as an effective biological control agent for reducing *Karenia* blooms without detrimental effects on the marine environment.

Community/Species Interactions

P-049

DNA metabarcoding analysis of phytoplankton community composition in Tokyo Bay, Japan

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DNA metabarcoding has been widely applied to phytoplankton investigation as a rapid and high-resolution tool. Tokyo Bay is an epitome of eutrophic embayment in the world with high primary productivity, however, the information of phytoplankton community using DNA metabarcoding approach is still limited. In the present study, phytoplankton species composition was monitored by metabarcoding method simultaneously with light microscopy. DNA metabarcoding detected 1805 unique eukaryotic OTUs (operational taxonomic units), and 740 OTUs could be classified into eight phytoplankton phyla. Among the phytoplankton phyla, 409 and 148 OTUs were from dinoflagellates and diatoms, respectively. The third phytoplankton phylum was Chlorophyta (57 OTUs). When comparing the results at the genus level with light microscopy, more than twice as many genera were detected by the metabarcoding approach. In the case of diatoms, 23 and 37 genera were identified by light microscopy and metabarcoding, respectively. In contrast, the number of genera (39) in metabarcoding for dinoflagellates were significantly higher than that (12) in light microscopy, indicating hidden diversity. A possible explanation for this is parasitism. Parasitic dinoflagellates of *Amoebophrya* were frequently detected by metabarcoding, but not by light microscopy. We also detected parasitic protists of *Pirsonia* on diatoms and diatom-consuming amoeba. In conclusion, the DNA metabarcoding method revealed more algal species than light microscopy. Furthermore, we possibly analyze the interaction between phytoplankton and their consumers using the metabarcoding approach.

Community/Species Interactions
P-050

Microalgal species isolation and the establishment of monoclonal culture from samples collected from mangrove water and pneumatophores

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Mangroves are a unique ecosystem along tropical and subtropical coasts that provides ecological functions and services, including providing habitat for various microalgae species, including harmful algal bloom (HAB) causative species. In recent years, numerical studies found that various microalgae species were growing and attached on the surface of pneumatophores which are the aerial roots of some common mangrove species found in the coastal wetlands. In addition, highly abundance and diversity of microalgae species have been recorded in mangrove water. However, ecological roles of these microalgae found in mangrove and their possible associations with mangroves as well as HAB are still poorly understood and require more research. Isolate and establish monoclonal cultures for these microalgae species is the prerequisite for carrying out laboratory experiments. Most of the current algal isolation methods and established algal cultures were mainly focused on marine and freshwater samples and we found these methods work very well for the algal isolation from the mangrove water. Yet, algal isolation from pneumatophore samples is very challenging and report on systematic techniques or procedures for the isolation of microalgae from pneumatophores is not available in the literature. Hence, the present study aims to compare the differences in microalgae species isolation from pneumatophores and mangrove water and try to establish the protocol for pneumatophore algal isolation. Compared to mangrove water samples, the algal isolation from pneumatophore samples was much more challenging. We found that the algal concentration of the pneumatophore samples is usually low, and the algal species are firmly attached on the surface of the pneumatophore which make it very difficult for isolation. It takes additional treatments for detaching the algal species from the pneumatophore, but risk of algal death or lysis of cells may also increase due to such treatments and the long processing time. In this regard, we applied “enrichment culture method” together with optimized vortex condition for detaching the algal cells. Our results showed that these methods are effective for the algal isolation and culture establishment from pneumatophore samples. Currently, we have established around 16 monoclonal microalgae cultures from the mangrove samples and all these cultures are maintained in the algal culture collection facility at our laboratory – The Metropolitan Algal Repository and Supply (MARS).

Community/Species Interactions
P-051

Comparison of wild and mutant strains of an algicidal bacterium *Pseudoalteromonas* sp. strain A25

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Pseudoalteromonas sp. strain A25 is a yellow-pigmented colony forming marine bacterium with the ability to lyse the diatom *Skeletonema marinoi-dohrnii* complex NIES-324. In previous study, some of the proteins induced in the stationary phase of this bacterium might be involved in the algicidal process. We isolated a pigmentation mutant strain of strain A25 with white colony (strain A25W1), characterized by a reduction of its algicidal activity. When the same amount of periplasmic fraction extract of late logarithmic phase of strain A25 or strain A25W1 inoculated into strain NIES-324 culture, it was observed that the extract of wild strain lysed diatoms completely twice as fast as that of mutant strain. The proteolytic activity of periplasmic fraction extract of strain A25 was also surpassed that of the mutant strain. The genomic analysis of strain A25 revealed that the genome consists of two circular DNAs of approximately 3.9 Mbp and 0.85 Mbp. Within this two contigs, a total of 3,945 CDSs were identified. Comparative examination of the entire nucleotide sequences of strain A25 and mutant strain A25W1 exposed the existence of thirty mutations. One of the mutation sites had CDS annotated as OmpR family, while no CDS associated with protease genes were detected near the mutation sites. These findings suggest that A25W1 potentially exhibit diminished synthesis algicidal-related compounds at the transcriptional or regulatory level.

Community/Species Interactions

P-052

Effects of monsoons and locations to the phytoplankton communities from HAB-affected coastal areas in Palawan Island, Philippines

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Palawan is an elongated strip of islands that is bounded by major marine biogeographic regions, the West Philippines Sea and the Sulu Sea. In addition, communities near the coast are sustained by the abundant fishery resources of the island, especially the Puerto Princesa Bay (PPB) and Malampaya Sound (MS). However, these coastal areas are experiencing a recurring phenomenon of Harmful Algal Blooms (HABs), threatening the lives and livelihood of the Palaweńos. Also, PPB and MS have differences in terms of physical characteristics, and climatic and oceanographic conditions that could influence the blooms of harmful algae and dynamics of phytoplankton in general. Hence, understanding the variations of phytoplankton assemblages and density between these two areas would be vital in fisheries management specifically for fish and mussel farmers, and shellfish gleaners. We used a 20 μm conical plankton net with 30 cm mouth diameter and a vertical tow method of phytoplankton collections for qualitative and quantitative analyses. Furthermore, the identification and counting were done using the conventional microscopy and the use of Imaging Flow Cytobot (IFCB) technology. The results of this study will be beneficial for gathering more data on HABs species and its dynamics, for possible trend and prediction of blooms.

Community/Species Interactions

P-053

Distribution, diversity and infection of the parasitic dinoflagellate *Amoebophrya* in the coastal waters of China

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Amoebophrya is an obligate endoparasite infecting wide ranges of marine organisms in coastal and oceanic waters. 14 dinoflagellate taxa were found to be infected by *Amoebophrya* spp., and the prevalence ranged from 0.03 % to 2.50 % in the coastal waters of China. Sequencing of environmental genomic DNA revealed substantial diversity and significant regional heterogeneity of Amoebophryidae sequences derived from the coastal waters of China, while no significant correlation was observed among geographical locations. Phylogenetic analyses of 18S rDNA sequences derived from individual *Amoebophrya*-infected cells indicated the host divergence of the parasitoid and lend credence to the multiple species assumption. An *Amoebophrya* sp.-*Akashiwo sanguinea* co-culture was established from Chinese coastal waters. The lifespan of *Amoebophrya* sp. ex. *A. sanguinea* was approximately 58 h. The infective dinospores can survive up to 78 h in ambient waters, but gradually lose their infectivity. The parasitoid was unable to infect other dinoflagellate species, its infection rate reached as high as 91% when the ratio of dinospores to host cells was 20:1. Infected host cells died eventually and released approximately 370 dinospores/cell. The host nuclear structures were rapidly degraded by *Amoebophrya* infection, and the chloroplasts of parasitized host cells remained intact until the parasite filled the almost entire cell structure. Nevertheless, infected cells showed sustained but lower levels of photosynthetic performance (~ 64 % of control cultures), and the photosynthesis-related genes were significantly down-regulated. The results further revealed the broad host range, wide distribution, substantial diversity and parasite-host interactions of *Amoebophrya* in the coastal waters of China, that should not be neglected in future studies on the succession of HABs, as well as the ecological significance of this parasitoid in marine microbial food webs.

Taxonomy

P-054

Development, phylogeny, and host range of a novel *Phagomyxa*-like parasitoid infecting marine dinoflagellates

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Dinoflagellates serve as the host for a variety of parasitic organisms. During the intensive samplings in Korean coastal waters in 2017, *Phagomyxa*-like parasitoids of dinoflagellates have been detected in all over the Korean coastal waters and a strain of the parasitoid was successfully established in culture from the seawater samples of Jinhae Bay, Korea in 2023. The new parasitoid has similar life cycle to other parasitoids of dinoflagellate such as syndiniales *Amoebophrya*, perkinsea *Parvilucifera*, and chytrid *Dinomyces*, which all have infective zoospores and feeding and reproductive stages. The new parasitoid was most characterized by the conspicuously hyaline body with dark-brown residual body within host cell, which closely resembles the genus *Phagomyxa* (Cercozoa, Phytomyxea), parasites of diatom and filamentous brown algae. Phylogenetic analyses based on the SSU rDNA sequences also revealed that the new parasitoid clustered together with genus *Phagomyxa*. The result from host range experiment of the new parasitoid showed that infections were detected only in dinoflagellates with variable degrees of susceptibilities.

Taxonomy
P-055

Taxonomy and phylogeny of *Pseudo-nitzschia* in Iceland

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Recent changes in oceanographic conditions have led to drastic shifts in phytoplankton community compositions in the North Atlantic and the Polar waterfront, with a northward expansion of temperate phytoplankton. Icelandic waters are at the edge of two different ecosystems, the Arctic and the Atlantic. Generally, occurrences of HAPs, including *Pseudo-nitzschia* are predicted to increase in the Arctic, because of trends in decreasing sea ice, increased temperatures, and increased light penetration in the water column. Little is known about the diversity, occurrences, bloom dynamics or chemical mechanisms of *Pseudo-nitzschia* around Iceland. The monitoring of HAPs performed using LM indicates at least four different morphological types. Only one species, the toxigenic *P. seriata,* has been identified sufficiently. We collected samples at several coastal and oceanic sites around the island to study the diversity of *Pseudo-nitzschia*. Several cultures were established by micropipette. Cell morphologies were observed by LM, SEM and TEM. Phylogeny was inferred from the hypervariable 18S rRNA, LSU rDNA and ITS sequences obtained using the Sanger sequencer platform. The results of this survey will produce the first characterization of this genus in Iceland and provide a baseline for future assessment of *Pseudo-nitzschia* in Iceland.

Taxonomy
P-056

First report of *Coolia* sp., an epibenthic dinoflagellate from Cancabato Bay, Tacloban City Philippines

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We report the first record of a *Coolia* species, an epibenthic dinoflagellate, in Cancabato Bay, Tacloban City, Philippines. This genus is typically found in tropical and subtropical waters and often coexists with ciguatoxigenic dinoflagellates. A live specimen of *Coolia* sp. was collected from the coastal waters near seagrass beds at a depth of 1 meter and established into clonal cultures. Based on light microscopy, cells are globular with slightly asymmetrical theca and have an average cell length and cell width of 28.71 um ±3.87 and 25.87 um ±2.77 (n=35), respectively. Phylogenetic analysis based on the 28S LSU rDNA demonstrated the genetic uniqueness of the *Coolia* sp. from Cancabato Bay compared to known *Coolia* species, while the 18S rDNA phylogenetic tree placed it in a separate clade. These findings suggest the possibility of a new species. However, further phylogenetic analyses using different markers are required for confirmation. Morphological assessment using fluorescence microscopy and SEM will enhance the species' characterization to strengthen this claim. Ecological data, including physico-chemical parameters, environmental thresholds, toxicity profile, and pigment analysis will provide insights into its ecological niche.

Taxonomy
P-057

Classification and morphology of nano-flagellates collected from Jiaozhou Bay

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Over the past 90 years, extensive research has been conducted on the phytoplankton in Jiaozhou Bay. However, there remains a lack of understanding regarding the composition, diversity, and presence of toxic species among the flagellates with particle sizes ranging from 2 to 20 μm. Therefore, the purpose of this study is to employ morphological identification and high-throughput sequencing techniques to investigate the nano-flagellates in the waters surrounding Jiaozhou Bay. The results of morphological identification revealed the presence of 28 species, encompassing five phyla and eleven classes, within the collected samples. Further analysis indicated that Chlorophyta and Cryptophyta accounted for 21.42% and 28.57% of all identified species, respectively. Furthermore, this study identified 17 previously undocumented species in Jiaozhou Bay, which had not been identified through morphological identification methods in this area before. In addition to the newly discovered species, this research also revealed the presence of toxic and harmful microalgae, including *Heterocapsa pygmaea* and *Heterosigma akashiwo*. Furthermore, the study identified potentially toxic species such as *Karenia* sp. and *Olisthodiscus luteus*. These findings have significant implications for understanding the dynamics of the nano-phytoplankton community in the marine ecosystem. This study lays the foundation for future investigations into the dynamic properties of the nano-phytoplankton community in the marine ecosystem surrounding Jiaozhou Bay.

Taxonomy

P-058

Classification and eco-physiological study of new species of *Picochlorum* from Korean coastal waters

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The genus *Picochlorum* is a marine microalga belonging to the green algae family, which typically has a fast growth rate and contains large amounts of intracellular lipids, making it a key material for biofuels. Recently, a species similar to *Picochlorum* was established from the waters of Geoje, Korea. Based on morphological observations and phylogenetic analysis using 18s rRNA sequences, it was revealed that *Picochlorum* sp. (GJ210610) had a similar cell size and shape to the previously reported *Picochlorum* species and belonged to the *Picochlorum* clade. However, our strain of *Picochlorum* formed a distinct clade from other species. In addition, for the first time in the genus of *Picochlorum*, the secondary structure of the ITS2 sequence was revealed. To investigate the physiological characteristics, we examined the temperature-dependent growth rates and fatty acid composition of *Picochlorum* sp. The results showed that the cells died at 30℃ and only grew between 15 and 25℃. The cells grew to a maximum concentration of 13×10^6 cells ml^{-1} at 15℃ while a maximum specific growth rate was 1.04 d^{-1} at 25℃. Fatty acid analysis showed about 36% fatty acids per cell. Morphological and phylogenetic analyses revealed that *Picochlorum* sp. (GJ210610) is a new species belonging to the genus of *Picochlorum*. The results of this study may contribute to a better understanding of the taxonomy and ecology of the genus *Picochlorum*.

Taxonomy
P-059

Morphology and cytotoxicity potential of the marine thecate dinophyte *Heterocapsa* (Dinophyceae) from Malaysia

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Species of the small thecate dinophyte *Heterocapsa* have been previously associated with mass mortality of bivalve mollusks. To investigate the species diversity and toxicity potential of this genus, thirty-four strains of *Heterocapsa* were established from Malaysian waters. Three known species, *H. bohaiensis*, *H. huensis*, and *H. rotundata*, and three newly described species, *H. borneoensis*, *H. limii*, and *H. iwatakii* were examined microscopically based on their detailed morphologies. The species were differentiated by cell size, shape, displacement of the cingulum, shape and position of the nucleus, the number of pyrenoids and their position, and body scale ultrastructure. The species delimitation was robustly supported by the molecular data. A light microscopy-based key to species of *Heterocapsa* is established, separating two major groups of *Heterocapsa*, i.e., species with a single pyrenoid and multiple pyrenoids. Bioassays were conducted by exposing *Artemia* nauplii to *Heterocapsa* densities of 1–5 × 10^5 cells mL^{-1}, and treatments exposed to *H. borneoensis* showed naupliar mortality, while no naupliar death was observed in the treatments exposed to cells of *H. bohaiensis*, *H. huensis*, *H. limii*, and *H. iwatakii*. Naupliar death was observed during the initial 24 h for both tested *H. borneoensis* strains, and mortality rates increased up to 50% after 72-h exposure. This is the first report on the diversity and cytotoxicity potential of *Heterocapsa* species from Malaysian waters.

Taxonomy

P-060

Take our (almost) 25 year phytoplankton dataset

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The Algae Lab at the North Carolina Division of Water Resources (NC DWR) has a public record phytoplankton database which dates back to at least 2000. Algal bloom monitoring has been in place since 1984. Freshwater lakes throughout the state have been sampled every May-September in accordance with river basin sampling schedules since 2000. Three coastal rivers have been sampled monthly since 2000. Two freshwater lakes have been sampled monthly since 2011. Phytoplankton taxa are identified to at least genus level. Data include cell counts (cells/ml), biovolume (mm^3/m^3), cell size ranges (um), taxa list with photographs, and the lab's Standard Operating Procedures manual. Most phytoplankton samples have corresponding nutrient and water chemistry data with location coordinates. Please contact us at https://algae.nc.gov.

Taxonomy

P-061

Taxonomy and phylogeny of unarmored dinoflagellates of the family Kareniaceae isolated from Hokkaido, Japan

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In a devastating harmful algal bloom of *Karenia selliformis* occurred along the eastern coast of Hokkaido, Japan in September–November 2021, co-occurrences of other kareniacean dinoflagellates were observed. Since the occurrence information of kareniaceans is limited in Hokkaido, we established six species of kareniacean cultures in 2021 and 2022, and their morphology and phylogeny were examined by use of LM, SEM and molecular phylogeny based on LSU rDNA and ITS region. Molecular phylogeny showed the presence of *Karenia longicanalis*, *Kr.* cf. *longicanalis*, *Kr. mikimotoi*, *Karlodinium* cf. *digitatum*, *Takayama* cf. *acrotrocha*, and *Takayama* sp. Cells of *Kr. longicanalis* and *Kr.* cf. *longicanalis* had the long apical structure complex, striations on the epicone, and lobate chloroplasts. The former had a centrally located nucleus and 4–7 chloroplasts, while the latter had an anteriorly located nucleus and 18–52 chloroplasts. *Kr. mikimotoi* were found in both years and phylogenetically similar to previously reported strains. *Kl.* cf. *digitatum* were related to *Kl. digitatum*, but its cell length was 14.6 μm, which was smaller than the described *Kl. digitatum* (21.5 μm in length. *T.* cf. *acrotrocha* belonged to the clade of the species. *Takayama* sp. was phylogenetically related to *T. tasmanica* but its cells were 35.2–48.8 μm long in the field samples, which is apparently larger than *T. tasmanica* (16–27 μm long).

Taxonomy
P-062

Morpho-molecular characterization of a new non-toxigenic *Amphidoma* species from the Asian Pacific

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The marine thecate dinoflagellate *Amphidoma languida* is the only species known to produce azaspiracids (AZAs) in the genus *Amphidoma*, which belongs to Amphidomataceae with *Azadinium*. It has so far been reported mainly from the Atlantic, although the related DNA sequences have been detected also in the Pacific. In this study, morphology, phylogeny and AZAs production of nine *Amphidoma* strains isolated from Japan, Malaysia and Philippines were investigated. Phylogenetic trees inferred from rDNAs (SSU, ITS, and LSU rDNA) showed the monophyly of our nine strains, which was a sister to the clade of *A. languida* including toxigenic strains from the Atlantic. AZAs were not detected from any strains by LC-MS/MS. Cells were ovoid, and 8.7–16.7 µm in length and 7.5–14.0 µm in width. A large nucleus in the hyposome and a spherical pyrenoid above the nucleus were observed. Thecal tabulation was typical of *Amphidoma*, Po, X, 6', 6'', 6C, 5S, 6''', 2''''. A ventral pore was located on the anterior left of 1'. The presence of a ventral depression, on the anterior margin of the anterior sulcal plate, was different from *A. languida*. A large antapical pore containing about ten small pores was observed. We recognize the non-toxigenic *Amphidoma* is an undescribed species discernible from the most related toxigenic species *A. languida*. Its SSU rDNA (V4) sequences in the Tara Ocean environmental DNA database showed a wide distribution, not only in the Asian Pacific.

Taxonomy

P-063

Species diversity of kareniacean dinoflagellates in the Philippines, including the detection of an undescribed *Karlodinium* species

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Unarmored marine dinoflagellates belonging to the family Kareniaceae have been detected in an increasing number of coastal areas in the Philippines, but have not been identified based on recent taxonomic systems. For example, a harmful red tide of *Takayama* sp. in Bolinao Northwestern Philippines has linked with massive fish mortality events in 2016 and 2018. In an attempt to examine their diversity in Philippine archipelago, samples were collected from nine sites in southern Luzon and one site in Davao Gulf, Mindanao. Overall, a total of 18 strains of kareniacean dinoflagellates belonging to *Karenia* (*K. mikimotoi*, *K. papilionacea*), *Takayama* (*T. acrotrocha* and *T.* cf. *tasmanica*) and *Karlodinium* (*K. ballantinum, K. decipiens, K. gentienii, K. zhouanum*) were established in the laboratory and identified based on their morphology and molecular sequences. Presence of *K. gentienii* and *K. decipiens* in Davao Gulf reported in this study are first country reports. For the undescribed *Karlodinium* sp. (strain GBSUB5) from Subic Bay, Zambales, their cells measuring 10.9 µm long and 6.9 µm wide were the smallest in this genus. A round nucleus was predominantly positioned at the anterior part of the cells. Based on LSU rDNA marker, the GBSUB5 was closely related to *K. australe* and *K. azanzae*. However, the GBSUB5 can be distinguished from these two closely related species in smaller cell length, anterior nucleus position and round shape. Hence, GBSUB05 is a novel *Karlodinium* species to be described.

Taxonomy

P-064

Molecular phylogeny and morphological characterization of *Pyrodinium bahamense* from Dumanquillas Bay in Southern Philippines

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Toxic *Pyrodinium bahamense* blooms in the Philippine waters have been consistently reported since the early 1980s. To date, it remains one of the most prevalent HABs species, which has been linked to most of the PSP-related toxicity events in the country. Although widely distributed, understanding of the phylogenetic diversity and possible connectivity of the different strains in the country remains little understood. This includes *Pyrodinium* strains in Dumanquillas Bay in southern Philippines, which has exhibited high cell abundance with high toxicity levels being detected almost all year round. Therefore, this study aims to explore and investigate the molecular phylogeny and morphological structure of *P. bahamense* isolated from Dumanquillas Bay and determine its relationship with the other strains in other geographic regions of the country. Results of this study will provide better understanding of the diversity and distribution of *Pyrodinium* in the Philippines as well as their potential connectivity. These will also help improve the detection of occurrence and to determine suitable mitigation strategies to help safeguard public health and livelihood.

Taxonomy

P-065

Morphology and phylogeny of a novel *Scrippsiella* species from Central Luzon, Philippines

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Blooms of the marine armored dinoflagellate *Scrippsiella* have been reported worldwide. In May 2022, a small dinoflagellate with a morphological resemblance to *Scrippsiella* was isolated from fish farming sites at Obando, Bulacan, Central Luzon, Philippines. For further investigation in particular its species identification, cellular morphology and thecal plate tabulation of the established culture were examined using light and scanning electron microscopy. Its phylogenetic position was inferred from the sequences of LSU rDNA and ITS region. Plate formula of Po, x, 4', 3a, 7'', 6c, 5s, 5''', 2'''' was consistent with other *Scrippsiella* species. Unlike *S. masanensis*, the hypotheca was relatively shorter than the epitheca and the overall cell length was small measuring 20.9 µm long. Among the three intercalary plates which differ in size, 2a plate was asymmetrical and hexagonal in shape. Thecal pores were observed; pores on the plate boundaries of 1''' and 4''' plates. The longitudinally elongated nucleus was located at the center, occupying a part of the epitheca and hypotheca. Cysts were formed in the clonal culture and the smooth surface differs from spinous cysts of most *Scrippsiella* species. In the LSU rDNA and ITS phylogeny, this species did not position within any known *Scrippsiella* species. Brine shrimp lethality assay showed that the culture was non-toxic to *Artemia*. The unique morphological characteristics and phylogenetic placement of the isolate suggests that it is a novel species to be described.

Taxonomy
P-068

Characterization of *Alexandrium* and *Protoceratium* strains isolated from Greek coastal waters (NE Mediterranean Sea) and the use of LAMP method for their detection

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Marine armored dinoflagellates of the genera *Alexandrium* and *Protoceratium* include potentially toxic species and it is important to use all available tools for their accurate detection and unambiguous identification. Both morphotaxonomy (under epifluorescence and scanning electron microscopy) and phylogeny were investigated for *Alexandrium* and *Protoceratium* strains isolated from Greek coastal waters. Cells were isolated from samples collected within the framework of the Potentially Toxic Marine Microalgae Monitoring Program conducted by the Laboratory Unit for Harmful Marine Microalgae of the Aristotle University of Thessaloniki (LUHMM-AUTh). Cells from cultures were further used for the isolation of genetic material, the amplification of appropriate regions by Polymerase Chain Reaction and sequencing to search for their genetic identity. *A. fraterculus*, *A. taylorii* and *P. reticulatum* were morphologically identified and phylogenetically clustered distantly from similar and related taxa, such as *A. tamiyavanichii* and *Pentaplacodinium saltonense*. Furthermore, a detailed investigation into the optimization and validation of a LAMP method protocol specifically designed for the above mentioned genera was conducted. The sensitivity of LAMP method was tested by comparing its results with those of quantitative Real Time PCR that was used as the gold standard. According to the findings of our study, this is the first time that the presence of *Alexandrium fraterculus* and *Protoceratium reticulatum* is genetically confirmed in the Greek coastal areas, while a trustworthy and accessible tool for the early detection and monitoring of HABs is suggested.

Microbiomes and Omics

P-069

Temporal diversity and co-occurrence of dominant viruses and their potential phytoplankton hosts in coastal waters in Western Philippines

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Marine viruses could influence bloom dynamics of phytoplankton including that of harmful algal species. However, their diversity and ecology remain little understood, especially in tropical coastal environments like the Philippines. Here, we looked at the diversity of dominant viruses and their associated hosts in known HABs hotspot mariculture areas of Bolinao and Masinloc in northwestern Philippines using combined amplicon and shotgun high throughput sequencing. Results showed that the diversity of the viruses were largely influenced by the occurrence of their eukaryotic hosts, mainly driven by changes in monsoons. Specifically, the northeast monsoon, characterized by more stratified and colder surface waters, was dominated by *Prasinoviruses*, a period which also favored growth of picoeukaryotic hosts mainly Chlorophytes. On the other hand, samples from the southwest monsoon period were accompanied by increased abundance of fragments belonging to *Picornavirales* and coinciding with the high abundances of diatoms such as *Skeletonema* and *Chaetoceros*. This period was characterized by stronger winds leading to mixing but lower nutrients potentially due to high assimilation. Notably, bacteriophages were high in both periods, which may be associated with the sustained high organic matter production in both areas and seasons due to mariculture activity, favoring heterotrophic bacterial growth. This study is the first to profile marine viruses in the Philippines, which also significantly contributes to our understanding of viral communities in tropical coastal waters and their potential roles in HABs occurrences.

Microbiomes and Omics

P-071

Exploring global gene expression & metabolite production in Narragansett Bay *Pseudo-nitzschia* isolates under nutrient limiting conditions

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In 2016, Narragansett Bay, Rhode Island experienced its first shellfishery closure in response to a harmful algal bloom caused by diatoms in the *Pseudo-nitzschia* genus. *Pseudo-nitzschia* are known for producing the potent neurotoxin, domoic acid (DA). Human ingestion of DA can cause amnesic shellfish poisoning, resulting in short-term memory loss, brain damage, and in severe cases, death. Understanding the mechanisms that induce DA production are critical to public health and safety. Previous work from our group has shown that DA concentrations in Narragansett Bay are elevated when inorganic nitrogen and phosphorus are low, suggesting linkages between environmental nutrient stress and DA production. To better understand these environmental triggers of DA production, we have isolated multiple strains of DA producing *P. multiseries* and *P. pungens* from Narragansett Bay and confirmed elevated DA production in response to silicate, phosphate and nitrate limitation. An experiment design limiting phosphate, nitrate and nitrogen source switching (growth in urea) in culture paired with global gene expression profiling will illustrate the connection between nutrient metabolism and DA production in Narragansett Bay *Pseudo-nitzschia*. We are also investigating suites of metabolites produced in these physiological experiments and assessing if they are conserved across species, strains, and nutrient conditions. Although previous studies have identified specific compounds associated with *Pseudo-nitzschia* they suggest that there are still thousands of unidentified primary and secondary metabolites that may play a role in modulating DA production or the interactions between *Pseudo-nitzschia* and their associated microbiome which may modulate toxin production and profiles.

Microbiomes and Omics

P-072

Microcystis strain dynamics throughout seven years of Lake Erie harmful algal blooms

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Microcystis-dominated cyanobacterial harmful algal blooms (cHABs) occur annually in Lake Erie, the shallowest and most eutrophic of the Laurentian Great Lakes. There is considerable inter- and intra-annual variation in cHAB characteristics such as timing and duration, extent, and toxicity. These characteristics are largely driven by weather and nutrient loading, which in turn produce varying niches for different *Microcystis* strains. Using metagenomic data collected from the 2014-2021 Lake Erie cHABs, we tracked *Microcystis* strain dynamics and the presence and abundance of *Microcystis* genes. Diverse strains of *Microcystis* were observed to co-occur in Lake Erie cHABs, with differences in their gene content related to nutrient acquisition, carbon concentrating mechanisms, and production of secondary metabolites like microcystins and other cyanotoxins. The spatiotemporal relationship between strains, gene abundance, nutrient availability, and environmental conditions were evaluated to identify strains and genes associated with increased fitness under varying bloom conditions. Understanding these relationships can likely improve models and forecasts of cHAB severity and toxicity, and inform policy designed to reduce the socioeconomic and human health impacts of cHABs.

Microbiomes and Omics
P-073

Ecological diversity of harmful algae-attached bacteria in the Southern coastal ecosystem, South Korea

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The dynamics of particle-attached and free-living bacterial communities can be observed through changes in common harmful algal species. Therefore, we analyzed phytoplankton, bacterial communities, and inorganic nutrient factors from the Jangmok coastal ecosystem at weekly intervals for 210 d. The changes in the phytoplankton community were classified into six groups according to the predominant phytoplankton species. These were, harmful algal blooms of *Akashiwo sanguinea* (late autumn), *Cryptomonas* cf. sp. (early winter), *Skeletonema marinoi-dohrnii* complex (winter), *Pseudonitzschia delicatissima* (early spring), *Cryptomonas* cf. sp. (spring), and diatom complexes such as *Chaetoceros curvisetus* and *Leptocylindrus danicus* (early summer). The indicator species for particle-attached bacteria (PAB) and free-living bacteria (FLB) during each phytoplankton bloom period was based on the indicator value of 59 and 32 species, respectively. In addition, correlation analysis showed that PAB had a higher correlation with phytoplankton than FLB. The PABs, ASV#002 (*Kordiimonas lacus*), #050 (*Aurantibacter aestuarii*), #013 (*Polaribacter marinivivus*), and #075 (*Sedimenticola thiotaurini*) were strongly correlated with *A. sanguinea* blooms ($r > 0.90$). Additionally, PAB showed significant correlations with *P. delicatissima*. These results suggest that extracellular polymeric substances may be involved in the relationship between bacteria and phytoplankton. PAB showed higher alpha diversity than FLB and contained amplicon sequence variants corresponding to 96% of all bacteria. Moreover, potentially pathogenic bacteria were more prevalent in the PAB community. These results highlight the importance of substrate or habitat specificity in potentially pathogenic prokaryotes in the marine environment. It is unclear whether these findings are the result of selection by phytoplankton or bacteria; however, a strategic relationship is found among them. Further study is needed to determine whether this relationship is essential for survival between two organism communities.

Microbiomes and Omics
P-074

Co-variance between free-living bacteria and *Cochlodinium polykrikoides* (Dinophyta) harmful algal blooms, South Korea

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To understand the co-variance between common free-living bacteria and *Cochlodinium polykrikoides* harmful algal blooms (HABs) and their metabolic functions, we investigated 110 sampling sites in the Southern Sea of South Korea. These sampling sites were divided into three groups based on environmental factors and phytoplankton data with a similarity of 85% using non-metric multidimensional scaling. One group represented high-severity *C. polykrikoides* blooms, while the other two represented low-severity or no blooms. In high-severity HABs, inorganic phosphorous and dissolved organic carbon concentrations were strongly correlated with *C. polykrikoides* density ($p < 0.01$). This may reflect the changes in biochemical cycling due to inorganic and organic substrates released by HAB cells (or by cell destruction). Furthermore, 88 common bacterial operational taxonomic units (OTUs, with mean relative abundance > 1%) were identified. These included Gammaproteobacteria (36 OTUs), Flavobacteriia (24), Alphaproteobacteria (18), and other taxa (11). When *C. polykrikoides* blooms intensified, the relative abundances of Gammaproteobacteria also increased. OTU #030 (*Flavicella* sp., Flavobacteria, 96%) was positively correlated with *C. polykrikoides* abundance ($r = 0.77$, $p < 0.001$). Functional analysis based on the dominant bacterial OTUs revealed that chemoheterotrophy-related functions were more common in high-severity sites of HABs than in other groups. Therefore, the occurrence of HABs highlighted their interactions with bacteria and affected the bacterial community structure and metabolic functions.

Microbiomes and Omics

P-075

Annual succession of dinoflagellate community on temperate coast zone (the southern coast of South Korea)

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To identify the succession of dinoflagellates on the southern coast of South Korea, the total phytoplankton transition was analyzed through 18S rDNA Metabarcoding analysis at 3 day intensive (311 time series monitoring) from November 2018 to June 2020. In a total of 16,224 ASVs (amplicon sequencing variables), 43% of dinoflagellate taxa (total 217 ASVs) appeared, and 66% of dinoflagellates appeared in total relative abundance. The dinoflagellates community was classified into seasonal groups using Bray-Curtis dissimilarity. In common dinoflagellates taxa (with a mean relative abundance >1% in all samples) appearance was selected as 8 dominant taxa. The predominant taxa of *Heterocapsa rotundata* (All times), *Akashiwo sanguinea* (Oct, 2019, Mar-May, 2020), *Gymnodinium* sp.(May-Jun,2019), *Euduboscquella* sp. (Nov-Dec, 2018, Dec, 2019, Jan-Feb and May-Jun, 2020), *Amoebophrya* sp. (Jun-Jul, 2019, Jan, 2020), *Karlodinium veneficum* (Jan, Mar and Aug-Sep, 2019), *Katodinium glaucum* (Apr, Nov, 2019), *Dissodinium pseudolunula* (Feb and Aug, 2019, Feb-Mar, 2020). Overall, *Heterocapsa rotundata* was predominant on the coast of Geoje from 2018 to 2020. Also, harmful species such as *Akashiwo sanguinea* and *Gymnodinium* sp. and parasitic and dinoflagellate *Amoebophrya* sp. increased, whereas *Heterocapsa rotundata* rapidly decreased. This reduction suggests that growth inhibition is due to harmful substances in the dinoflagellates or intra-species community transitions due to host-parasite relationships. In further studies, we will investigate why these predominant species are affected by environmental factors through ecological analysis between the environment and major indicator species.

Microbiomes and Omics
P-076

Metabolome responses associated with chronological aging in the harmful dinoflagellate, *Karenia mikimotoi*, can predict future bloom demise

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This study examined the alterations of metabolic profiles over the growth curve in cultured dinoflagellate *Karenia mikimotoi* under nutrient (nitrogen (N) or phosphorus (P)) deprived conditions. A key criterion for the diagnosis of late stationary phase was further explored to identify when the dinoflagellate cells will enter bloom demise. The cultured dinoflagellate cells (initial density: 1.3 × 10^2 cells/mL) were incubated under N and P controlled conditions: 2 mM-N and 0.1 mM- P replete (NP-rep), N-depleted (N-dep, 0.2m M- N and 0.1mM-P), and P-depleted (P-dep, 2m M- N and 0.0033 mM -P) medium. The results demonstrated differential expression of metabolites for key process involved in energy production, photosynthesis, and carbon metabolism, which suggests that metabolic restructuring is required for coping with chronological aging or nutrient deprivation. Furthermore, we found hallmark of the late stationary phase: remarkable increase in the ratio of glucose to glycine, which represents an indicative of dinoflagellate cells entering into bloom demise. Further, this is also detected in the late stationary phase of the cultured diatom *Chaetoceros tenuissimus*, indicating that this may be the general criterion for other phytoplankton species. The present findings will provide insights regarding chronological aging and criterion for phytoplankton bloom demise prediction.

Microbiomes and Omics

P-077

Genomics and eco-active organic matter: the roles of harmful and other algae, with emphasis on physical effects

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In marine and freshwater systems, algae, other protists and bacteria (*sensu lato*) live both in suspension and associated with surfaces. These organisms secrete extracellular organic matter (OM) following the expression of their genes. This OM includes signaling, allelopathic and toxic molecules, and it physically affects processes including adsorption and the diffusion of nutrients, toxins and gases, as well as aerosol production at the sea surface and in heat-reflecting sea-foam. All these OM molecules may thus be classified as eco-active. Through large programs, huge databases of eDNA and eRNA sequences are being rapidly compiled. Matching of these sequences to their expressed and associated eco-active OM molecules, is required, because it will help to understand how genes in marine ecosystems are in producing OM with different physical-mechanical-rheological and surface properties that bio-engineer biogeochemical fluxes. HABs, particularly high-biomass ones, play a special role in this subject because here molecules are easier to detect, and processes are more intense and easier to measure. This is undoubtedly even truer in easily visible structures such as organic aggregates, foam and cyanobacterial scums.

Microbiomes and Omics
P-078

Domoic acid depuration after a *Pseudo-Nitzschia* bloom: can bacteria be used as a tool to accelerate the King scallop's depuration

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Harmful algal blooms occur globally and can have harmful consequences for marine organisms. These consequences extend beyond marine life, as certain toxins can accumulate and increase in concentration along the food chain. One such example is domoic acid (DA), a neurotoxin produced by microalgae belonging to the *Pseudo-nitzschia* genus. When levels of DA in commercially exploited species surpass the safety threshold, fishing activities may be legally halted for extended periods, sometimes spanning years. *Pseudo-Nitzschia* blooms can be particularly problematic for bivalve molluscs. Unlike species like the blue mussel *(Mytilus edulis*), which rapidly eliminate DA, King scallop *(Pecten maximus*) retain the toxin for extended periods. In 1998, Stewart et al. proposed that the differential ability to eliminate DA could be attributed, in part, to variations in gut microbiota composition among bivalve species. This study investigates the microbiota present in mussels and scallops and its potential role in DA elimination, with the long-term goal of developing probiotics to enhance DA depuration. DA-contaminated King scallops were exposed to either mussel digestive gland homogenate or mussel feces for a two-week period. Subsequently, changes in the bacterial communities within the scallops' digestive glands were examined using metabarcoding techniques to determine if the transfer of microbiota from mussels to scallops occurred. Analysis of the results showed a possible transfer of the Maribius genus from mussel feces to the digestive gland of scallops. Moreover, the presence of Chlamydia only in scallops could be a lead to understanding the long retention of DA in their digestive gland.

Microbiomes and Omics

P-079

High host-specificity of the *Roseobacter* clade to harmful dinoflagellate *Margalefidinium polykrikoides* blooms in fields

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Roseobacter clade and Flavobacteria are known to have species-specific interactions with phytoplankton blooms. In recent, it is suggested that *Roseobacter* may have more highly specific interactions with phytoplankton than Flavobacteria in laboratory experiment. However, there is no study for their interaction through field study. To investigate species-specific interactions in fields, bacterial communities were identified from four distinct *Margalefidinium polykrikoides* bloom events via next generation sequencing (NGS) assay and bloom associated bacterial OTUs were analyzed. As a result, the predominant shared free-living (FL) and algae/particle-associated (PA) bacterial communities were similar among the four distinct bloom events, but the FA and PA bacterial communities clearly differed. In FL bacterial communities, the Rhodobacteraceae and Flavobacteriaceae families were the predominant taxa, whereas Flavobacteriaceae and a few members of the Gammaproteobacteria were prevalent in PA bacterial communities. Thus, at higher taxonomic levels (the family level), these bacterial taxa were relevant and had species-specific interactions with *M. polykrikoides* blooms. In NMDS analysis, operational taxonomic unit (OTU) communities within the Flavobacteriaceae and Vibrionaceae were not well clustered and were dissimilar among bloom events. However, in the *Roseobacter* clade, OTU communities were not only well clustered by FL and PA bacteria, but also OTUs #17 and 842 were commonly predominant in distinct *M. polykrikoides* bloom events. These results newly show that the *Roseobacter* clade had more species-specific interactions with *M. polykrikoides* blooms than did Flavobacteriaceae in fields.

Microbiomes and Omics
P-080

Translational costs and regulation in *Amphidinium carterae*: Insights from synchronized cultures and novel quantification methods

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Dinoflagellates have the highest respiration rates compared to other algal species. We hypothesize that protein translation, a highly energy-dependent process, may be the reason behind this heightened respiration rate. Our prior findings suggest that translation regulation plays a crucial role in regulating gene expression in dinoflagellates, with transcript levels remaining constant. In this research, we successfully synchronized *A. carterae* cultures and quantified photosynthesis, respiration and translation throughout a diel cycle using novel techniques. We measured cellular oxygen production and utilization throughout repeated day-night cycles using a novel continuous culture monitor. Our observations showed a distinct diel cycle with notable oxygen increases during the light cycle and significant decreases during the dark phase, resulting in an oxygen deficit. To determine when protein translation occurred in the diel cycle, we utilized puromycin-incorporation (SUnSET method) as a measure of translation rates. Our results revealed maximum rates in translation during the day with decreases at night. We quantified changes in abundance of translation initiation factors (eIF4E) and compared their abundance directly to transcript levels over the cycle. We also quantified the energy sources for these mechanisms, specifically measuring ATP, ADP, and AMP levels. Collectively, these findings shed new light on the tight link between the light and dark cellular processes in dinoflagellates.

Microbiomes and Omics

P-081

Metabarcoding of dinoflagellates from red tide waters in Eastern Visayas, Philippines reveals presence of potential HABs species previously overlooked

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In the last 15 years, a total of 150 paralytic shellfish poisoning cases was recorded in Eastern Visayas with at least 6 deaths, and most of these cases are due to the consumption of green mussels *Perna viridis* harvested from HABs infested waters of Carigara, Leyte and Caibiran, Biliran. In June 2022, no red tide bans were issued in these waters indicating *Pyrodinium bahamense*-free month after 5 long years. Environmental water samples were collected from these previously *Pyrodinium bahamense* infested waters to profile phytoplankton community and identify toxic species awaiting bloom. Phytoplankton was counted using light compound microscope and targeted SSU rDNA and LSU rDNA was examined using Illumina MiSeq platform for molecular metabarcoding. A total of 24 genera was observed microscopically in all bays, dominated by the diatoms *Skeletonema, Thalassionema, Chaetoceros* and *Pseudo-nitzschia*. Dinoflagellates were observed but only comprises about 4% of the population and is dominated by HABs agents *Dinophysis* and *Prorocentrum*. Interestingly, *Pyrodinium bahamense*, the main culprit of HABs in the region was not observed microscopically, which backs the government's non-issuance of red tide bans. However, metabarcoding from same water reveal the presence of *Pyrodinium bahamense* in small quantities, indicating its presence and potential bloom recurrence. Given the global increase in HABs, it is suggested to use quick and precise tools such as molecular metabarcoding on top of morphological observations for ecological studies and future prediction.

Microbiomes and Omics
P-082

Functions of epiphytic microbiota on pneumatophores of *Avicennia marina* and possible host-microbe interactions through preliminary proteomic analysis

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Pneumatophores of mangrove plants are occasionally submerged in water and harbor diverse epiphytic microbial communities including bacteria and microalgae. Metagenomic analysis, which could unravel the taxonomic composition of microbiota and their potential functions based on the analysis of gene sequences, however, may not entirely reflect their actual functions. By using proteomic and metaproteomic approaches, we could possibly elucidate the actual roles of both hosts and microbes and look into what happens in the field. In this study, LC-MS/MS was used to obtain peptide sequences separately from pneumatophores and epiphytic microbiota. Due to highly limited protein reference databases for both samples, we constructed protein sequence databases from a published transcriptome of pneumatophores of *A. marina* and a metagenome of their epiphytic microbiota obtained by our team. A total of 384 and 377 unique proteins were identified via sample-specific database search in pneumatophores and epiphytic microbiota respectively. Within the metaproteome of epiphytic microbiota more than half of proteins were assigned to gammaproteobacteria, followed by bacteroidota and cyanobacteria. This matched exactly with the taxonomic distribution previously derived from metagenomic reads, supporting the feasibility of taxonomic classification using metaproteomics. Another blastp search was limited to only common phyla of microalgae and the top-hit species was an unclassified Synechococcaceae. All proteins from pneumatophores were assigned to eudicots, showing successful removal of microbes from the host. Interestingly great similarity of molecular functions was found between proteins from pneumatophores and those from epiphytic microbiota, of which most were involved in binding nucleotides, nucleoside phosphates and nucleic acids.

Microbiomes and Omics

P-083

Bacterial community associated with the toxic dinoflagellate *Akashiwo sanguinea* and potential HAB-causing diatom *Coscinodiscus* spp. in Puerto Princesa Bay, Philippines

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Bacteria associated with harmful algal bloom (HABs)-forming species play important roles in their bloom initiation/decline and toxin production. Many aspects of the ecology and mechanistic processes involved in this association however remain little understood. Here, we investigated the cultivable associated bacteria of a toxin-producing species, *Akashiwo sanguinea*, and the bloom-forming diatom *Coscinodiscus* spp. To do this, single algal cells of the target algae were manually isolated through micro-pipetting. The isolated algal cells were washed intensively with sterile filtered seawater to remove non-associated bacteria and then cultured in an F/10 medium. Successful algal isolates were used for bacterial isolation using agar plating. The phylogenetic identity of the isolates was determined via 16S rRNA gene sequencing. Two bacteria were successfully isolated from the diatom *Coscinodiscus* spp. and were coded as CC. These isolates were white with CC-1 having a circular shape while CC-2 with irregular shape. Both bacterial isolates were found to be Gram-positive. Phenotypic characterization showed their ability to produce catalase and gelatinase with salt tolerance ranging from 2% to 10%. Other assays were then conducted to further characterize their potential roles in the association. Results from this work will pave the way for a better understanding of the interaction between bacteria and phytoplankton that may affect changes in phytoplankton blooming events in the marine environment.

Microbiomes and Omics
P-084

Morphological and molecular characterization of *Akashiwo sanguinea* in Cancabato Bay: Insights into population genetic diversity and potential regional variations

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Akashiwo sanguinea (formerly known as *Gymnodinium sanguineum*) is a harmful alga that could have negative ecological impacts on marine ecosystems and human health. However, comprehensive molecular studies characterizing and understanding the genetic diversity within populations of A. *sanguinea* using different molecular markers in different regions are limited. This study presents morphological and molecular descriptions of clonal cultures of *A. sanguinea* from Cancabato Bay, with a focus on the partial D1-D3 region of the 28S LSU rRNA gene. Molecular analyses were conducted on DNA extracted from ten-day old *A. sanguinea* cultures isolated from a 20 µm plankton net samples. Preliminary findings showed the presence of potential regional polymorphisms variations between the isolates and those described from other localities. Morphological characterization using light and high-resolution microscopy however did not indicate significant variation, potentially indicating cryptic diversity despite identical morphology. These sequence variations may be influenced by environmental factors impacting genetic diversity and population differentiation. Understanding the genetic characteristics and variations of *A. sanguinea* is crucial for elucidating its biological and ecological dynamics and impacts. This analysis contributes to our knowledge of this harmful algal species. Further molecular studies however are needed to establish regional variations in *A. sanguinea* populations. Implications and potential applications of the research findings will be discussed. Continued research is necessary to further deepen knowledge and develop effective strategies to mitigate the effects of this harmful algal species.

Prediction and Modeling
P-085

Probabilistic models for harmful algae detection: Application to the Norwegian Coast

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Toxic marine algae pose significant risks to human health due to the accumulation of toxins in shellfish for consumption. We have developed probabilistic models to predict the likelihood of HAB presence and outbreaks along the Norwegian coast, which can help optimization of the national monitoring program and the planification of mitigation actions. We employ Support Vector Machines to calibrate probabilistic models for detecting the presence and increased harmful concentrations (HC) of eight toxic algae found along the Norwegian coast. The target taxa are *Alexandrium* spp., *Alexandrium tamarense*, *Dinophysis acuta*, *Dinophysis acuminata*, *Dinophysis norvegica*, *Pseudo-nitzschia* spp., *Protoceratium reticulatum*, and *Azadinium* spinosum. The predictors are some environmental drivers: sea surface temperature and photosynthetic active radiation obtained through remote sensing, and mixed layer depth and sea surface salinity from modeled reanalysis. The probabilistic models are trained with data from 2006 to 2013 and tested with data from 2014 to 2019. The presence models demonstrate good statistical performance across all taxa, with R (event frequency versus predicted probability) ranging from 0.69 to 0.98, RMSE from 0.84% to 7.62%, and ROC-AUC scores from 0.56 to 0.8. Predicting the probability of HC is more challenging and the HC models only reach skill with four taxa (*Alexandrium* spp., *A. tamarense*, *D. acuta*, and *A. spinosum*). There are significant differences in seasonal and geographical variability and sensitivity to the environmental drivers of the different taxon, which are presented and discussed. The models can predict geographical regions and periods with a higher risk of toxic species detection and optimize the regional monitoring program of harmful algae. The method can be easily extended to other regions as it relies only on free available remote sensing and model data and already running national programs for monitoring toxic algae.

Prediction and Modeling

P-086

Evaluation of the effects of *in-reservoir* UV-LED/TiO_2 photocatalysis on cyanobacteria and green algae cell integrity using a novel statistical approach

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Cyanobacterial blooms have negative impacts on the multiple uses of water, hamper potabilization, and increase the risks to human and aquatic ecosystem health. Previous studies have shown that advanced oxidation processes using titanium dioxide and UV illumination are an effective method for the treatment of natural water, mitigating the impacts of cyanobacterial blooms. In the current study, the effects of a UV-LED-driven TiO_2 photocatalytic treatment on trichome and cell membranes of cyanobacteria and green algae species/genera were evaluated. Organisms included *Raphidiopsis raciborskii, Dolichospermum* sp., *Geitlerinema* sp., *Microcystis* sp., *Pseudanabaena* sp., *Planktothrix agardhii*) and four genera of green algae (*Aulacoseira* sp., *Coelomorom* sp., *Fragilaria* sp., *Monoraphidium* sp.). The study was conducted at pilot scale inside a drinking water reservoir located in the tropical semi-arid region of Northeast Brazil which is historically dominated by cyanobacteria. Due to the large amount of data collected and its variability, expected for large-scale *in-situ* experiments, conventional statistical analyses (descriptive statistics) did not lead to clear conclusions about the experimental outcomes. Applying a novel set of statistical methods allowed us to conclude, with a known error margin, that UV/TiO_2 photocatalysis impacted the cell integrity of green algae and cyanobacterial species differently. Green algae generally were more resistant to the treatment than cyanobacteria. *Planktothrix agardhii* was the most resilient cyanobacterium evaluated with resistance comparable to green algae. Additionally, the chances of finding intact cells in both phytoplankton groups were greatly reduced after 336 h of treatment.

Prediction and Modeling

P-087

Modeling the distribution and abundance of *Alexandrium catenella* in Chilean fjords

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The fjords of southern Chile exhibit confirmed evidence of the presence of harmful microalgae and marine toxins since the seventies in the last century. Currently, there is a diverse range of microalgae and toxins that have been confirmed for the fjord system (41-55°S), being *Alexandrium catenella* and the paralytic toxins those that have caused the most effects to public health and productive activities. It is currently recognized that in Chile, *A. catenella* is distributed along our coastline between Coliumo (36°S) to Mariotti islets (55°S), being more abundant in the fjords. For this reason, in the last 15 years in the Chilean fjords, in addition to developing permanent monitoring actions to timely detect harmful microalgae and marine toxins, research actions have also been developed to have a hydrodynamic model that allow us to appreciate the processes that characterize the fjord system, and it has also been possible to incorporate particle tracking dispersion models, which are also applicable to the case of harmful blooms. In this context, this contribution presents the efforts to couple field sampling, laboratory experimentation and particle dispersion models for the understanding of algal blooms. Simulation results of the 2018 and 2022 blooms using inert particle scattering are presented. In addition, the advances of an individual-based model (IBM) that includes biological aspects of the particles for the simulation of the distribution and abundance of *A. catenella* in southern Chile are presented.

Prediction and Modeling

P-088

Factors driving cyanobacterial blooms in Lake Okeechobee (FL, USA) and development of predictive bloom duration and intensity models

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Lake Okeechobee is the eighth largest freshwater lake in the United States. It provides multiple environmental services including regional flood control, water supply for the local communities, agricultural interests and maintenance of the Everglades ecosystem, recreational activities, and critical wildlife habitat. The lake has become increasingly eutrophic over the past five decades and now experiences intense cyanobacterial blooms that degrade water quality and often produce potent cyanotoxins. Monitoring bloom development and distributions in real-time is challenging because blooms can develop rapidly, vary in magnitude, and shift location rapidly in response to wind-drive circulation patterns. Current models can describe wind driven circulation patterns, wind mixing, nutrient inputs, and other factors relevant to controlling bloom formation, but only partially capture the short-term (one to five day) and longer-term (two week to a month) changes in cyanobacterial biomass and distribution. The goal of this study was to examine ways to improve predictive Lake Okeechobee cyanobacterial biomass and distribution models. This involved a comprehensive review of both the physical and biological factors controlling cyanobacterial bloom formation and current modeling efforts. Resulting recommendations for improved cyanobacterial bloom forecasting will be presented and include: (1) improving short-term predictive models by coupling predicated 3-5 day windspeeds from the National Weather Service with wind-driven circulation models and lake-wide cyanobacterial biomass distributions obtained from satellite imagery and (2) longer-term projections based on historical data and estimated upcoming changes in runoff, temperature and wind associated turbidity/light limitation conditions over next month, which that are updated weekly based on satellite imagery.

Prediction and Modeling
P-089

Application of a quantitative molecular method to characterize abundance and distribution of *Alexandrium* cysts for NOAA's HAB Forecasting

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Alexandrium catenella is a paralytic shellfish poisoning-causing dinoflagellate that overwinters as a resting cyst and germinates into the water column in the spring and summer. Using winter cyst distribution, forecast tools in the Gulf of Maine (GOM) and an early warning system in Puget Sound, Washington have been developed to mitigate human health risks and economic effects of shellfish closures. The current protocol for cyst enumeration by fluorescent microscopy is time consuming and requires highly specific training for cyst identification. This MERHAB project, funded by NOAA, includes development of a new quantitative polymerase chain reaction (qPCR) methodology for *A. catenella* cysts that is being evaluated against the standard microscopy protocol with the goal of producing more rapid and accurate cyst abundance data. Assay development has shown the importance of using a qPCR standard curve based on cysts instead of a diluted amplicon, necessitating concentration of a large number of *A. catenella* cysts from the environment. Furthermore, interannual and regional variability in rDNA gene copy number requires development of a new standard curve for each cyst survey. qPCR standard curves were constructed and compared using cysts from GOM, Puget Sound and Kodiak, Alaska. Cyst maps from all three regions will be presented and comparison of microscopy-based and qPCR-based cyst abundances will be presented for the GOM. To assess temporal variability in rDNA gene copy number, seasonal comparison of qPCR standard curves was completed for Quartermaster Harbor, Puget Sound.

Prediction and Modeling
P-090

Comparative analysis of the applicability of prediction programs for the evaluation of microcystin toxicity

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There is a tendency to reduce the use of animals in scientific experiments and that is why in silico models that make predictions have gained relevance. A molecule can be evaluated using prediction models, which allow the comparison of its chemical structure with others used for its training. These models make it possible to relate the chemical structure of the molecule to biological activity. Simulation programs may have limitations related to their applicability domains. Microcystins, toxins produced by cyanobacteria, have not been extensively evaluated in prediction programs. Its use makes it possible to obtain information about the toxicity of microcystins and their variants. Purpose of this study: to compare six ADMET prediction simulators for microcystin toxicity analysis (Admet Predictor®, AdmetLab 2.0, AdmetStar 2.0, SwissADME, T.E.S.T., and Ecosar) to evaluate the variants MC-LR, MC-RR, MC-YR, and MC-HarHar. After carrying out the applicability domain evaluation of the six programs for microcystins, it was possible to verify that four of them can be used with the chosen microcystins: AdmetSar, Admet Predictor®, SwissADME, and T.E.S.T. Comparative results of variants on models of lipophilicity, permeability, intestinal absorption, transport proteins and environmental biodegradation were obtained. SwissADME, as it is a drug-trained program, presented the worst performance among the evaluated programs when its predictions were compared with experimental data in the literature. Admet Predictor®, on the other hand, was the program that presented the highest number of predictions about microcystin variants, being comparable with data obtained from in vivo experiments.

Prediction and Modeling

P-091

Environmental variable selection and synthetic sampling methods for improving the accuracy of algal alert level prediction model

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Currently in South Korea, algal alert systems are being implemented at 29 points in the Four Major Rivers. The algal alert levels are determined based on the cyanobacteria cell density measured at real-time monitoring points. These levels for section related to water supply activities are classified into normal, caution, warning, and blooming. The current purpose of the algal level early warning system is to focus on developing post-occurrence response measures for occurrence of harmful algal blooms. However, the prediction for algal alert levels in advance is possible to suppress the growth of harmful birds and secure safe and clean water resources. In this study, to predict algal alert levels in advance, machine learning model was constructed using comprehensive information from the national monitoring network, weather measurement data, and real-time algal alert level data. Furthermore, to improve the accuracy of the prediction model, variable selection techniques were utilized to select environmental variables that affect algal alert levels. Various sampling techniques were applied to improve the performance of the model, minimizing prediction errors that occur during the model training process due to data imbalance. When constructing the prediction model using the original data without considering variable selection and sampling techniques, the results showed prediction accuracies of 50% for the caution level (Level-1) and 62.5% for the warning level (Level-2). However, when applying non-linear variable selection techniques and the Synthetic Minority Over-sampling Technique-Edited Nearest Neighbor (SMOTE-ENN) sampling technique, the constructed model exhibited prediction accuracies of 85.7% for Level-1 and 75.0% for Level-2.

Prediction and Modeling
P-092

Prediction of harmful algal blooms for early warning using deep learning and machine learning models

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The harmful algal blooms (HABs) can degrade the water quality and cause problems in water utilization. To mitigate problems, the monitoring and prediction of HABs have been conducted by many countries. The prediction practice of the current algal alert system is implemented to assess the present status of water eutrophication. The aim of this study is to establish early warning systems for HABs, offering guidance for proactive management. In this study, Data-driven methods such as machine learning, and deep learning were utilized to predict HABs. One-dimensional convolutional neural network (1D-CNN), artificial neural network (ANN), and random forest (RF) were constructed to predict the alert levels (3 classes) of algal blooms in advance. The data used for the models consisted of nine years of water quality, hydrological, meteorological data collected in a reservoir that showed the frequent occurrence of algal blooms. The results showed that the overall accuracy of prediction performance in 1D-CNN, ANN, and RF models were 0.774, 0.994, 0.923 in the training step and 0.526, 0.563, 0.636 in the test step. The test performance indicated that the RF model achieved the highest performance of accuracy, while the 1D-CNN model achieved the lowest accuracy. With the small datasets, 1D-CNN showed low performance due to the characteristic of deep learning, which is optimized for the efficient processing of big datasets by the consideration of complexity. Therefore, relatively small datasets can be suitable for RF model, and if intended to use a deep learning model, larger datasets would be required in prediction.

Prediction and Modeling

P-093

The present and future of HAB monitoring and forecasting in Chesapeake Bay to support shellfish management

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Several phytoplankton species in Chesapeake Bay are known to produce toxins and negatively impact important shellfisheries. High frequency monitoring through satellite remote sensing is beneficial in protecting public and environmental health in US waterways. The success of these tools relies on unique optical signatures of monospecific, high biomass blooms. In Chesapeake Bay, a variety of dense algal blooms appear throughout the year in mixed assemblages. While dinoflagellate blooms can be detected from satellites, the species cannot be determined using satellite data alone. For example, blooms of the HAB species *Alexandrium monilatum* and *Margalefidinium polykrikoides* that occur in late summer in southern Chesapeake Bay have similar optical properties and are often co-located. Algorithms applied to the Sentinel-3 Ocean and Land Colour Imager (OLCI) have improved resolution, providing better detection and characterization of algal blooms. Higher spatial resolution imagery is now available, through Sentinel-2, in narrow coastal systems. Future efforts employing higher spatial, spectral, and temporal resolution (NASA's PACE, and GLIMR instruments, and the operational Geostationary Extended Orbit ocean color instrument (OCX)) will enhance HAB detection and forecasting in coastal and freshwater systems. Heuristic models constructed with information regarding ecological niche, combined with satellite data, can aid in species-specific identification in complex systems. Combined with NOAA's Phytoplankton Monitoring Network, an in-situ Citizen Science approach to measure the relative abundance of key HAB species, we hope to improve our ability to respond to HABs in Chesapeake Bay, to support resource managers and provide information and tools for onsite farm management of shellfisheries.

Monitoring and Mitigation

P-094

New strategies for marine and freshwater toxins mitigation

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The presence of contaminants in water represents a worldwide public health issue. Phycotoxins and cyanotoxins are the largest groups of compounds that most frequently appear in water since are produced by phytoplankton and cyanobacteria in aquatic environments during harmful algal blooms. Traditional water treatment procedures such as coagulation and filtration are unsuccessful at removing toxins. As a result, there is an urgent need to develop innovative techniques to reduce toxic contamination. Thus, mitigation strategies and detoxification methods must be developed to avoid human and animal intoxications. In this sense, nanotechnology has become a viable tool for the remediation of contaminated water in recent decades since nanostructures have a vast surface area and allow the combination of numerous reactive components. In this context, the aim of this work was to establish the potential of magnetic nanostructures for adsorbing cyanotoxins and phycotoxins from water solutions and algae cultures. Toxin concentration in solutions containing a mixture of marine or freshwater toxins was evaluated using ultra-performance liquid chromatography-tandem mass spectrometry (UHPLC-MSMS) before and after treatment with nanostructures. After incubation, nanostructures were magnetically separated from solutions and, subsequently adsorbed toxins were recovered by extraction with organic solvents by vortex mixing and sonication. Nanostructures show good results reducing up to 80% of phycotoxins, such as saxitoxins, spirolides, azaspiracids and okadaic acid and cyclic peptides cyanotoxins. This adsorption was also observed when nanostructures were used in harmful algal cultures of *Gymnodinium catenatum*, *Prorocentrum lima* and *Microcystis aeruginosa*. Therefore, the application of nanotechnology in water treatment could be a viable way to reduce the impact of natural toxins.

Monitoring and Mitigation

P-095

Harmful hitchhikers on marine plastics in Singapore

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Plastic waste has become a pervasive environmental issue, particularly in marine ecosystems. It provides additional habitats for marine phytoplankton, including potentially toxic bacteria and harmful algal species. It is known that a large proportion of ocean plastics end up beached or floating in coastal zones. Singapore is an island nation located at the end of the Malayan Peninsula and is one of the world's top shipping hubs. Therefore, plastics enter Singapore's coasts from land-based activities, maritime inputs and surrounding waters. Plastics originating from beyond coastal zones may introduce foreign harmful algal species that are not normally present in Singapore's marine phytoplankton community. Furthermore, environmental changes of coastal waters exacerbated by climate change and industrialisation may cause the proliferation and eventual bloom formation of the harmful algal species attached to these plastics. However, there are limited studies using Nanopore sequencing to detect potential harmful algal species in environmental samples in Southeast Asia. This study examined the preliminary detection of harmful algal species attached to plastics found on a beach. 16S and 28S rRNA sequences were obtained for barcode amplicon sequencing using the Nanopore MinION sequencing platform. New species of toxic cyanobacteria in the genus *Sphaerothrix* was found. Harmful genera such as *Karenia*, *Karlodinium* and *Levanderina* were also detected on the plastics. The presence of harmful algae on plastics may introduce new species into the local environment leading to fish kills. Therefore, to mitigate blooms, the rapid detection of harmful species in the field is critical for routine monitoring.

Monitoring and Mitigation
P-096

Life without chargaff's rules: Thymine dioxygenase as a proposed enzyme for the synthesis of 5-hydroxymethyl uracil in dinoflagellates

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The genomes of core dinoflagellates contain a fifth nucleotide base, 5-hydroxymethyl uracil (HOMeU). This fifth nucleotide complementary base pairs with adenine, replacing about forty percent of thymine in the genome. While the function of HOMeU is unknown, it is hypothesized that it either plays some role in the regulation of gene expression or is a vestigial structure from an ancestral genome. Thus, enzymes involved in the production of HOMeU are good candidates for harmful algal bloom (HAB) mitigation. We find that incorporation of HOMeU occurs during the polymerization of DNA rather than as a post-synthetic modification because i) HOMeU exists within the deoxyribose and ribose nucleotide pools and ii) the DNA polymerase is not able to distinguish thymine from HOMeU. It is likely that thymine is oxidized and hydrolyzed to form a HOMeU:adenine pair. We identified a hypothetical thymine dioxygenase (TD) transcript in *Amphidinium carterae* that is found in all dinoflagellates. The predicted activity of the enzyme is to produce 5-hydroxymethyl uracil, succinate, and CO_2 from thymine, 2-oxogluterate, and oxygen. *In vitro* studies with recombinant produced TD examined the substrate specificity and kinetics of this presumptive conserved protein. DMOG (Dimethyloxalylglycine), an antagonist of α-ketoglutarate cofactor, was examined as an inhibitor of the activity. Polyclonal antibodies then examined whether this enzyme is produced only in the core dinoflagellates that express HOMeU and not in other species of the broader dinoflagellata phyla. Our findings ultimately introduce new mechanisms for HAB mitigation should TD activity inhibition or protein knockdown prove lethal for dinoflagellates.

Monitoring and Mitigation

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Long-term harmful algae bloom (HAB) monitoring in Monterey Bay, California, USA

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The California Current System (CCS) is a highly productive system that promotes/supports marine organisms and important fisheries. CCS productivity is threatened by the ongoing issue of harmful algae blooms (HABs). California's statewide HAB network and forecasting system (CalHABMAP) is a network of nine stations along the California Coast that provides weekly monitoring data to improve our understanding of HAB event timing, distribution, and species composition. Efforts are made to streamline methodologies and analyses across stations in order to produce comparable data sets accessed by a variety of stakeholders (e.g., water quality/fisheries managers, marine mammal entities, public health departments). Monterey Bay contains two CalHABMAP stations (Santa Cruz Wharf in the north, Monterey Municipal Wharf II in the south) that provide over a decade of HAB species counts and other associated measurements. We know from these data sets and targeted studies that phytoplankton populations can differ between these two locations. A weekly public blog post (https://phytoblog.sites.ucsc.edu/) allows for easy access to qualitatively compare species presence and general weather/water conditions. Further in-depth analyses of HAB species (microscopy, molecular probes), toxins, nutrients, and chlorophyll are also performed and publicly accessible via NOAA's ERDDAP portal – a free, open-source data server where consistently formatted CalHABMAP station datasets are stored for easy download. Examples of annual trends in select HAB species within Monterey Bay are presented alongside representative parameters (e.g. temperature) to demonstrate the utility of weekly monitoring. New technologies are enhancing these data sets to provide deeper insights and inform HAB forecasting efforts.

Monitoring and Mitigation
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Modified clays remove *Karenia brevis* cells and decrease brevetoxin concentrations throughout the water column

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Florida red tide blooms caused by *Karenia brevis* are responsible for massive economic losses, ecological damage, and human health risks. Bloom mitigation technologies must be environmentally safe, economically feasible, applicable at large scale, and effective at eliminating cells and toxins. Clay flocculation is a promising technique applied successfully for mitigation of several HAB species, including pilot-scale (e.g., ~1,000 m^2) field studies for *K. brevis*. This study seeks to understand how clay flocculation alters *K. brevis* cell and brevetoxin concentrations over time and at various depths. 80-L, 120 cm-tall tanks containing approximately $8x10^5$ cells/L *K. brevis* were treated with MCIV or GC03.5 clay. Three hours after application, cell concentrations were reduced to $1.1x10^5$ and $4.1x10^4$ cells/liter by MCIV and GC03.5 respectively, while the control density increased to $9.5x10^6$ cells/liter. By T_{24}, control cell density increased to $1.2x10^7$ cells/L while both clay treatments exhibited complete cellular removal. In the control, total toxin concentrations increased 60%, 39%, 40% and 200% compared to T_0 at 3, 24, 48, and 72 h, respectively. GC03.5 reduced total toxins by 29%, 71%, 83% and 91%. With a total toxin decrease of 25% by T_{72}, the MCIV treatment maintained toxin levels similar to T_0, but converted toxins from parent congeners BTX-1 and BTX-2 to reduced (BTX-3) and oxidized (BTX-B5) forms. No differences were observed between surface, middle, and bottom depths, indicating that treatment was similarly active throughout the water column and did not concentrate toxins at depth. Ongoing studies are investigating potential bioavailability of toxins in settled floc.

Monitoring and Mitigation

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First report of *Noctiluca scintillans* blooms in Singapore waters

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Harmful Algal Blooms (HABs) of a large, green, bioluminescent dinoflagellate *Noctiluca scintillans*, have become more frequent in the Western Pacific Region in recent years, leading to enhanced interest in monitoring and detection of such blooms. In the year 2022, two *Noctiluca scintillans* blooms were observed in Singapore waters. The first was reported in Northern Singapore waters at Changi and Pasir Ris Beach in March 2022 while the second was reported in Southern Singapore waters at Raffles Lighthouse and St. John's Island in November 2022. Blooms generally form under favorable hydrobiological settings. Eutrophic conditions coupled with timely succession of phytoplankton and zooplankton communities and predator prey oscillation could be the driving force for sustained blooms of *Noctiluca scintillans* in the Western Pacific Region. No mass mortalities of marine life were observed in this report, however, *Noctiluca scintillans* blooms have previously been responsible for mass mortalities of coral, finfish and shellfish associated with oxygen depletion and ammonium toxicity which occurs during the decay of blooms. At times, massive economic losses were incurred. The prevalence of blooms exacerbated by increasing coastal urbanisation highlights the need for monitoring and detection of such blooms.

Monitoring and Mitigation
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Fish kill and harmful algal bloom events in Singapore waters

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Singapore coastal environments are increasingly affected by regular harmful algal blooms. In December 2009, a massive fish kill event was observed in northern coastal water due to HABs caused by toxic dinoflagellates: *Karlodinium australe* and *Karenia mikimotoi*. The increase in frequencies of HABs have led to enhanced interest in monitoring and detecting of such blooms. HABs monitoring started in Singapore from 2010 to 2023. During the period between 2014 and 2016, three massive HABS were observed due *Karlodinium* spp. and *K. mikimotoi*. Moreover, fish kill was caused by toxic *Karlodinium* spp. During early 2016, prolonged *K. mikimotoi* blooms of more than three months were observed. For the period between 2017 and 2019, mainly diatom blooms were observed. However, from 2020 to 2021, several *Karlodinium* spp. blooms were observed with no associated fish kills. On the other hand, during 2023, blooms caused by other dinoflagellates such as *Peridinium* were observed for a prolong period of several weeks. Blooms in Singapore were generally formed during the neap tide and under favorable physical settings. The eutrophic conditions found in our northern coastal water could be the driving force for bloom to be formed and sustained. Moreover, in recent years, due to the change in our environmental conditions, more new species, and new record of HAB species were identified. Therefore, it is critical to continue our HAB monitoring rapidly and cost-effectively to protect our marine resources which we depend on greatly as well as human health.

Monitoring and Mitigation
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Trends of diarrhetic shellfish toxins in Portuguese bivalves and environmental variables 2001-2022: impacts from drought and wildfires

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In mainland Portugal high levels of diarrhetic shellfish poisoning toxins (DSTs) originating from *Dinophysis* spp are accumulated in bivalves every year. The occurrence of DSTs in spring and early summer has been positively related to precipitation. Continental runoff supplies regularly nutrients to the marine food chain. Precipitation is highest on the northwest (NW) than on the southwest (SW) or south (S) coasts. A NW to S decreasing gradient in DSTs is commonly found, similar to the latitudinal decrease of macronutrients (N, P and Si). A decrease in average precipitation after 2003 led to a reduction in the interannual average concentration of DSTs after 2008 on the NW coast and the S coast. However, the accumulation of DSTs on the NW coast increased in the period 2020-2022. In the last two decades, extensive forest wildfires were recurrent, but the area burned reduced sharply after 2017 following tighter fire control measures. Low levels of DSTs or a low percentage of weeks with samples above the RL from the NW coast were related to high burned areas in the previous year. The recent increase in 2020-2022 of DSTs, a planktonic biomarker in bivalves, was also coincident with the similar temporal increase in either the recruitment or the biomass of *Sardina pilchardus*, a small pelagic planktivorous fish in decline after 2005-2006. Low sardine recruitment or low sardine biomass were coincident with low precipitation or high burned areas. Wildfires degrade coastal water quality, as seems reflected both in the bivalve DSTs and the sardine stock time series.

Monitoring and Mitigation

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Application of Imaging FlowCytobot in East China Sea -- Approaching of fast *in situ* growth and feeding behavior by *Mesodinium rubrum*

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High-resolution optical imaging system, Imaging FlowCytobot (IFCB), has become the fundamental tool in monitoring the plankton diversity in marine ecosystems. One of the beneficial uses of IFCB is to understand the *in situ* morphological, behavioral and ecological response in complex environmental condition. During spring bloom season (March to May 2022) of East China Sea, *Thalassiosira* and *Prorocentrum* are the two dominant genus, and proportion of diatom slightly decreased, while that of dinoflagellates increased a lot. Peak bloom appeared at middle of May, contributed mostly by dinoflagellate *P. donghaiense*. The mixotrophic *Mesodinium rubrum* was observed almost in all samples, ranging from 80 to 6,600 cells/L. The fastest *in situ* growth rate of *M. rubrum* reached 1.23/day within 3 days (March 7~9th). Thereafter, over 9.9% of cells were captured in feeding behavior, resulting to the increases of cell biomass from 2,378 to 5,228 cells/L within 24h. As the prey of *Dinophysis*, *M. rubrum* bloom would possibly lead to the succession of *Dinophysis*, driven to the serious diarrhetic shellfish poisoning outbreaks.

Monitoring and Mitigation
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The value of the SoundToxins partnership: an early warning system for HABs in Puget Sound, USA

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Washington State is a national leader in farmed bivalve shellfish, with an industry that employs more than 3,200 people in family wage jobs and contributes an estimated $270 million to the economy. The SoundToxins partnership was established in 2006 as a cost-effective monitoring program to provide an early warning of harmful algal bloom (HAB) events to aquaculturists and the Washington State Department of Health (WDOH) through weekly monitoring of phytoplankton and associated environmental conditions. This program was recently expanded to provide alerts of the presence shellfish-killing HABs to aquaculturists. SoundToxins has 92 participants, including commercial aquaculturists, tribal managers, State agencies, environmental learning centers, and private citizens, who monitor HABs at more than 35 sites around Puget Sound. The early warning provided by SoundToxins has averted human illnesses from toxic shellfish that would have negatively impacted market and harvester confidence. Here, the net benefits of SoundToxins to aquaculture producers and shellfish consumers was determined using a series of in-person interviews and a written survey completed by WDOH and shellfish producers. WDOH also helped to define the "counterfactual", important in determining the causal effect of an "intervention" such as SoundToxins.

Monitoring and Mitigation

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Efficacy of a virus-based biological control method against the early stages of the harmful dinoflagellate *Heterocapsa circularisquama* bloom

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In Japan, aquaculture of bivalves such as oysters and pearl oysters has been severely threatened by blooms of the harmful dinoflagellate, *H. circularisquama*. With the urgent need to develop preventive approaches, we have developed and applied a biological control method using an algal virus called HcRNAV contained in marine sediments. In past microcosm experiments, spraying sediment including HcRNAV on seawater containing high density of *H. circularisquama* resulted in accelerate its decline. In this study, a tank trial was conducted to test whether the spraying method would be effective in the early stages of the *H. circularisquama* blooming. The trials were conducted using seawater (Ex.1) and nutrient-enriched seawater (Ex. 2). In Ex1, in which natural seawater was inoculated with sediment including HcRNAV, the density of *H. circularisquama* decreased until it reached only ~1.7 % of the initial after three days (GLM, $p < 0.01$). In Ex 2, in the treatment spraying with sediment containing HcRNAV resulted in a significant decrease in the density until it reached only ~1.3 % of the initial after two days (GLM, $p < 0.01$). The nutrient-enriched seawater reduced *H. circularisquama* density faster. The results suggest that different nutrient concentrations might affect the density and composition of diatoms in ambient seawater, but have little effect on the decrease in density of *H. circularisquama* itself. Hence, spreading sediment containing HcRNAV in the early stages of *H. circularisquama* blooming was indicated that it is effective in suppressing proliferation of *H. circularisquama*.

Monitoring and Mitigation
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Dynamics in natural microbial communities treated with algicidal amines targeting *Karenia brevis*

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Efficacy and environmental safety are priorities when developing methods for control of Florida red tide (*Karenia brevis*, Dinophyceae). A combined dose of 0.9 ppm ammonium and 7.5 ppm putrescine (NP), demonstrated control of *K. brevis* in unialgal cultures. To evaluate NP's performance in more realistic conditions, two experiments were conducted with microbial communities collected from a site subject to annual red tides. In a 72-hour microcosm with red tide communities, NP reduced *K. brevis* cell density by over 75% within 24 hours and kept it significantly lower than controls. Total brevetoxin concentration decreased over time in both NP and control microcosms. While total brevetoxins were higher in NP, primary brevetoxins (BTX-1 and BTX-2) decreased. Diatoms initially declined in NP microcosms but recovered, becoming the dominant phytoplankton group. In a 7-day mesocosm conducted with non-red tide communities, dinoflagellates decreased, demonstrating specificity for dinoflagellates over other phytoplankton taxa. By Day 6, NP mesocosms had higher richness and diversity of heterotrophic eukaryotes than controls. Trends in ammonia, pH, dissolved oxygen, and turbidity in NP suggested high respiration and a bloom of heterotrophs. Oxygen initially decreased while turbidity increased before returning to initial levels. Conversely, an increase in ammonia and decrease in pH stabilized at altered values for the duration of the experiment. Putrescine had a half-life under 24 hours in both experiments. Overall, NP controlled dinoflagellates including *K. brevis* in mixed communities, with effects on water chemistry that necessitate further testing and consideration by managers.

Monitoring and Mitigation

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Morphological plasticity bestows ciliate *Euplotes charon* W413 mitigation capacity upon *Karenia mikimotoi*

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The utilization of predatory organisms for the biocontrol of harmful algal blooms (HABs) has been overlooked because of concerns regarding biological invision and the lack of efficient candidates. Addressing these challenges, we investigated the removal efficiency of nine *Euplotes* strains isolated from coastal waters of Fujian, China, against the noxious dinoflagellate *Karenia mikimotoi*. The results revealed that *Euplotes charon* W413 exhibits significant grazing capacity on *K. mikimotoi*, supported by its unique morphological plasticity enabling enhanced feeding through substantial increases in cellular size. Importantly, *K. mikimotoi* cells were found to be nutritionally adequate food sources for W413, facilitating its rapid proliferation. Laboratory experiments demonstrated that even at low initial densities (0.01 cells/mL), W413 effectively inhibited the development of simulated *K. mikimotoi* blooms. Moreover, higher W413 loads (100 cells/mL) resulted in the complete removal of *K. mikimotoi* blooms containing 30,000 cells/mL within four days. This study contributes to our understanding of the feeding selectivity of *Euplotes* species and identifies W413 as a promising candidate for the biological mitigation of *K. mikimotoi* blooms. However, before applying this method in field settings, several key issues need to be addressed. These include assessing the viability and efficiency of *Euplotes* in diverse natural environments, developing cost-effective bait and suitable facilities for industrial-scale cultivation, and evaluating the long-term ecological impact of introducing exogenous *Euplotes* to planktonic ecosystems. Additionally, exploring the genomic basis of W413 for its morphological plasticity presents an interesting avenue for future scientific investigations.

Monitoring and Mitigation

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HAB-causing phytoplankton species and the water quality in Jibatang River Estuary, Calbayog City

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Estuaries are productive aquatic ecosystems that have natural salinity gradients as well as fluctuations in nutrient concentrations from riverine discharge. The accumulation of nutrients in estuaries can reduce water quality, which leads to organic pollution. It can also influence biological communities like phytoplankton and cause harmful algal blooms (HAB). Phytoplankton are good bioindicators for assessing water quality. Hence, this study assessed the phytoplankton community, along with HAB-causing species, as water quality bioindicators in the Jibatang River Estuary, Calbayog City. The phytoplankton community structure, physicochemical parameters, and water quality were assessed in the estuary, as well as the relationship between phytoplankton structure and physicochemical parameters. A total of 13 HAB-causing phytoplankton species were observed in the estuary. The months of October and November were dominated by 62% *Cyclotella* sp. (2.2×10^4 cells/L) and 20% *Peridinium* sp. (7.5×10^3 cells/L), respectively. However, the months of December and January, which had the highest phytoplankton populations, were dominated by 21% *Skeletonema* sp. (2.0×10^4 cells/L) and 43% *Leptolyngbya* sp. (2.3×10^4 cells/L), respectively. The Integrated Phytoplankton Indices (IPI) were lower during the HAB-dominated months compared to non-HAB months. This may indicate lower water quality conditions in the estuary during these months.

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Screening of cycle imine toxins in *Mytilus galloprovincialis* in Neretva river and Bay of Mali Ston

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Cyclic imines (CIs) are a newly discovered group, a subclass of highly toxic organic substances, called marine biotoxins. CIs are macrocyclic compounds with imine and spiro-linked ether moieties, produced by harmful marine algal species, dinoflagellates. Although the levels of cyclic imines in shellfish are not regulated and these compounds have not yet been associated with poisoning in humans, they are characterised by their fast-acting neurotoxicity and lethality by respiratory arrest in mice. In our study, for the first time in Croatia, analogues of the three largest groups of CIs - spirolides (SPXs), gymnodimines (GYMs), and pinnatoxins (PnTX) - were screened in mussel samples of *Mytilus galloprovincialis* collected every two months from February 2021 to December 2022 in the Neretva River and Bay of Mali Ston. The bay is an important shellfish breeding area in the southern part of Croatia, as it is under the intermittent influence of freshwater from the delta of the Neretva River. Both studied areas have a great diversity of habitats, especially aquatic ones, and are considered extremely important for the protection of nature and biodiversity. High-resolution mass spectrometry (HRMS) based on a TOF analyzer is used for the detection and determination of GYM-A, GYM D, SPXC, 13-desmethyl-SPXC, Iso-13-desmethyl-SPXC, 13-desmethyl-SPXD, 13,19-didesmethyl-SPXC, 20-methyl-SPXG, PnTX-A, and PnTX-G. Fragments of the studied SPX and GYM analogues were detected by the multiple reaction monitoring (MRM) method at low levels in a smaller part of the samples collected mainly during the warm season.

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Reduction of the impact of exposure to harmful marine microalgal species via the use of a UV-LED g-C_3N_4-photocatalytic curtain

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A common impact of harmful marine microalgae is the contamination of shellfish with a range of natural toxins, which can ultimately impact human health if consumed. The management strategy for human health protection is to temporarily close shellfish production areas when they become contaminated until regulated toxin concentrations drop below established thresholds. These closures work well to prevent human illness when systems are well implemented but they can cause substantial socioeconomic damage to those communities where the shellfish production occurs. Reduction/elimination of toxin uptake into shellfish to prevent contamination would present an opportunity to maintain aquatic production, even during periods of harmful microalgal growth. A system utilising photocatalysis to treat freshwater contaminated with cyanobacteria and cyanotoxins showed promise in this regard. As such the system was modified for use in a marine environment. Following small scale studies which demonstrated the efficacy of the UV activated g-C_3N_4 catalyst against two microalgal species, *Alexandrium catenella* and *Prorocentrum lima,* one a paralytic and the other a diarrhetic shellfish toxin producer, a larger tank system was designed for the exposure of mussels with and without the mitigation device. The presence of a UV-LED g-C_3N_4-photocatalytic curtain within this system reduced the uptake of toxins into *Mytilus sp.* during a three-day exposure, when compared to controls. This highlights a proof of concept of a novel mitigation strategy in the marine environment at pilot scale.

Monitoring and Mitigation

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Yessotoxins in an aquaculture area off the Basque coast: their occurrence in mussels and in its potential producers

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Monitoring of phycotoxins and toxic phytoplankton in a bivalve aquaculture area off the Basque coast (south-eastern Bay of Biscay, Atlantic Europe) has revealed the occurrence of different phycotoxins poisonings. Here we address the poisoning by yessotoxins (YTXs), reporting on their occurrence and shedding light on the species responsible for their production. YTXs regulated in the EU were analysed in mussels by LC/MS (229 samples from April 2016 to December 2022). Yessotoxin (YTX) was the most frequent (54% of samples) and abundant analogue (its oxidation product 45-hydroxy-YTX could be quantified in 37% of the samples). Homo-YTX and its oxidation product 45-hydroxyhomo-YTX were always below their quantification limits. During most of the study period, YTXs concentration was below the regulatory limit (3.75 mg YTX eq. kg^{-1}). However, higher peaks were found from May to October 2021 (4-6 mg YTX eq. kg^{-1}). Observed potential YTXs producers were *Lingulodinium polyedra* and *Protoceratium reticulatum*. Low concentrations of *Gonyaulax spinifera* were also found throughout the year. In June 2022 we isolated seven strains of *P. reticulatum* and two of *L. polyedra*. Sequencing of ribosomal markers (partial LSU, ITS region) confirmed identifications and showed that *P. reticulatum* strains shared identical ITS sequences with Mediterranean strains in clade B (sensu Wang et al. 2019). Whereas *L. polyedra* strains did not produce detectable amounts of toxins, most *P. reticulatum* strains produced high amounts of YTX (32±26 pg/cell) but no Homo-YTX, pointing to clade B of *P. reticulatum* as the main source of YTXs in mussels in the Basque coast.

Monitoring and Mitigation
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Labour-saving HAB monitoring surveys using an automated water sampling microscopy system: Validation of performance on cell density estimation in laboratory experiments

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To reduce the damage of HABs, higher frequencies of HAB monitoring surveys is needed, however, the effort required is high. To address these issues, in recent years, an automated water sampling microscopy system (seaMS, manufactured by Nishimura Shokai) was developed. SeaMS is a device that delivers microscopic film in real-time and has the potential to mechanize HAB monitoring surveys. However, there are two problems associated with using seaMS, which are: (1) the system cannot estimate cell density; (2) species identification needs to be carried out visually. This study reports results of the experiments and analyses on problem (1) stated above. Laboratory experiments were carried out in the following order: sample incubation (*Chattonella antiqua*, *C. marina*, and *Karenia mikimotoi*), microscopy, seaMS filming, and data analysis. Regression analysis was then performed with cell density as the objective variable and the number of cells counted as the explanatory variable. The results showed that the relationship between cell density (y) and number of cells (x) of *C. antiqua*, *C. marina*, and *K. mikimotoi* were $y=386.08+1698.43x$, $y=868.58+2076.55x$, and $y=228.37+2653.49x$, respectively ($p < 0.001$). In the future, these results can be used for in-situ observations to validate the accuracy made by seaMS. If adequate observational accuracy is then achieved, labour-saving HAB monitoring surveys will be possible using seaMS.

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Biomarker development for *Pseudo-nitzschia* spp. and its implications to effective HABs surveillance in the Philippines

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Many *Pseudo-nitszchia* species can produce domoic acid (DA) that causes Amnesic Shellfish Poisoning (ASP). Although several species and their blooms have been reported in the different waters of the Philippines, effective strategies are lacking to monitor their occurrence and potential harm. For this purpose, molecular biomarkers could become useful for identifying and quantifying the causative organism and their potential toxicity. Here, biomarkers targeting the 18S and 28S rRNA genes and DA-synthesis related genes were developed for species detection and putative toxicity detection. Application of the biomarkers were developed mainly using PCR-based tools and sequencing as a low-cost, accessible, and affordable technology for developing countries. Environmental DNA samples from various sites of the Philippines were tested to detect various species of *Pseudo-nitzschia* where they have been reported to occur. Our results showed that *P. brasiliana* was predominantly the species that is seen in the Philippines. DA measured via HPLC and ELISA, indicated that the species can potentially be toxigenic, indicating the effectiveness of the biomarkers as monitoring tools for detecting the presence of the species and its potential toxicity. The developed biomarkers could be useful as a screening method in areas prone to *Pseudo-nitzschia* HABs occurrences. This technology will further help improve early warning systems and monitoring programs specifically for ASP outbreaks.

Monitoring and Mitigation
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Characteristics of phytoplankton assemblages by seasonal short-time scale monitoring using HPLC pigment analysis in semi-enclosed harbor

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Short-time scale monitoring is necessary to investigate the detailed scenario of phytoplankton succession. A chemotaxonomic analysis, a functionally efficient way, was performed every week for a full year in the semi-enclosed Jangmok Bay to determine the composition of major phytoplankton assemblages including small phytoplankton. The pigment analysis using HPLC exhibited that micro-sized phytoplankton largely dominated more than 80% of the assemblages during the survey period while picoplankton contributed up to 50% in spring. Unlike the common blooms in temperate waters, multiple blooms occurred; two were in summer and other in fall. The high abundance of diatoms in summer succeeded to an intensive bloom of cryptophytes with a one-week time lag, which was followed by a dinoflagellate bloom in fall. The additional microscope analysis revealed an ecological succession of major phytoplankton species: a bloom of fast-growing *Pseudo-nitzschia* sp. in summer was replaced by a bloom of *Cryptomonas* sp. a week later of the diatom bloom, and then further succeeded to an *Akashiwo sanguinea* bloom in fall. The dominance of relatively small-sized *Cryptomonas* sp. led to mismatching between microscope and chemotaxonomic analysis during the second bloom, indicating the size-dependence matters. In contrast, for the *Akashiwo* bloom, the microscope analysis was significantly underestimated by the chemotaxonomic analysis. The major three blooms were coincident with two main rainfall events in summer and fall. Particularly, after the heavy rainfall in the fall, a large discharge from the Nakdong River was likely to drive the abruptly high growth of *Akashiwo*.

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Toward field installation of a low-cost plankton imaging system for continuous monitoring and data acquisition of harmful algal blooms

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Autonomous plankton imaging systems are becoming increasingly important for the monitoring of harmful algae. Most of these systems are expensive, and therefore installation at multiple sites or depths is often not financially feasible. The PlanktoScope is an open-source hardware and software platform that enables quantitative plankton imaging, and one can be built for several hundred US dollars. In this poster, we introduce the progress of our ongoing project to develop a low-cost system that can continuously monitor and collect time-series data of plankton in the field, using the PlanktoScope. As a first step, we built the PlanktoScope v2.1 and shot images of cultured harmful algal species, such as *Karenia mikimotoi* and *Chattonella* spp. Some of these images allowed rough visual identification. The PlanktoScope pumps the water into the flow cell through silicon tubes for imaging. Continuous pumping of natural seawater is expected to result in biofouling inside the flow cell and tubes. Therefore, we are developing a system that keeps these parts sufficiently clean for at least a week. We are also planning to enhance the data storage by making changes to the hardware and software so that frequent imaging can be achieved. In conclusion, we are aiming to develop an inexpensive system that allows real-time detection and continuous data acquisition of harmful algal blooms in the field (e.g., mariculture sites).

Monitoring and Mitigation
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Using particle size distribution (PSD) to automate imaging flow cytobot (IFCB) data quality in coastal California, USA

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The use of imaging flow cytobots (IFCBs) for plankton research is increasing worldwide and coordinated IFCB networks are being developed to monitor harmful algal blooms (HABs) in several coastal regions. Monitoring programs with IFCBs designed to run continuously can generate up to 70 samples per day creating a wealth of image data. Ideally, data streams are monitored daily (real-time) to assess data quality assurance and quality control (QA/QC). However, front end data QA/QC can be cumbersome for personnel and is often left for a later date once thousands of data files have accumulated. Particle size distribution (PSD) is used to inform food web dynamics, calculate total community biomass, and calculate radiative transfer in ocean remote sensing. PSDs can be automatically generated from equivalent spherical diameter (ESD), a measure derived from IFCB image processing, and in previous work, anomalous PSDs from IFCB data identified bloom events in San Francisco Bay, CA. We propose that variations in PSDs also reveal "bad" data allowing for some automation in backend QA/QC procedures. As more and larger IFCB networks come online worldwide, the need for automated data QA/QC is prudent to increase the efficiency of working with these datasets. While full automation of IFCB data QA/QC is unlikely, using PSDs to automatically flag data allows users to focus their efforts on determining whether data are questionable or reflect shifts in community structure.

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Occurrence, causative microalgae, and bioaccumulation of lipophilic marine biotoxins (LMTs) in the South Sea Coast of Korea

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Aquaculture farms have been established along the South Sea Coast of Korea, supplying most of the country's domestically consumed seafood. However, annual harmful algal blooms pose a potential threat to seafood safety. This study aimed to determine the spatial and seasonal distributions of 12 lipophilic marine biotoxins (LMTs) in phytoplankton and mussels in the region in 2021. Solid-phase adsorption toxin tracking (SPATT) was used to monitor the cumulative compositions of LMTs in seawater. LMT concentrations were also analyzed in twelve commercially available species of domestic shellfish to evaluate the potential risks to human health. *Gonyaulax spinifera* and *Dinophysis acuminata*, causative microalgae of yessotoxins (YTXs) and pectenotoxins (PTXs), respectively, showed high densities in the region from May to July. This period corresponded to high LMT concentrations in phytoplankton and mussels. Phytoplankton mainly contained PTX-2 and homo-YTX, with a maximum concentration of 2300 ng g^{-1} wet weight (ww) in May. In contrast, mussels mainly contained homo-YTX and YTX, with a maximum concentration of 1300 ng g^{-1} ww in July. LMTs-producing microalgae showed low densities and concentrations after July, whereas mussels accumulated toxins until September. Okadaic acid, dinophysistoxin-1, and azaspiracid-2 were detected in SPATT but not in seawater, phytoplankton, or mussels. YTXs were detected in domestic seafood samples of mussels, red scallops, and pen shells, but the concentrations were below the European Food Safety Agency recommended standard of 3.75 mg YTX-EQ kg^{-1}. Moreover, the hazard quotient was less than 100 in all scenarios, indicating that the risk was not significant.

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Seasonal variability of phytoplankton diversity and abundance in Puerto Princesa Bay, Palawan, Philippines

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Harmful algal bloom (HABs) events have been recurring in Puerto Princesa Bay, northeastern Palawan since 2013. Current methods in monitoring however conducted by the relevant government agencies are haphazard and frequently experience delays due to the limitations of methods being used (i.e., manual count or mouse bioassay). Understanding the ecology of the blooming organisms, such as its variability with seasons, is important in potentially predicting occurrences of such events. This is to ensure timely warning and advisories to the public to prevent losses in life and livelihood. Here, we determined the diversity and abundance of phytoplankton with emphasis on HABs species in Puerto Princesa Bay. Bi-monthly sampling using the standardized vertical tow of 20 μm plankton net were collected in several sites within the bay, coupled with light microscopy to profile the communities. At the community level, significant shifts in phytoplankton communities were observed between southwest and northeast monsoon, associated with the changing conditions. Results also revealed a total of 40 phytoplankton genera composed primarily of *Chaetoceros*, *Thalassionema*, *Rhizosolenia*, *Coscinodiscus*, and *Ceratium* with abundance ranging from 100-552 cells. L^{-1}. Interestingly, seven HABs-causing species were identified: *Akashiwo*, *Amphora*, *Dinophysis*, *Gymnodinium*, *Nitzschia*, *Noctiluca*; and *Pseudo-nitzschia*. The toxic *P. bahamense* was mainly observed to increase during the southwest monsoon, when runoffs were also higher, consistent with the previous years. Implications to monitoring and potential prediction of occurrences are also discussed.

Monitoring and Mitigation

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Species-specific detection and quantification of harmful blooms of *Chrysochromulina leadbeateri* (Haptophyta) using quantitative PCR

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Ichthyotoxic haptophyte algae blooms are increasing in magnitude and frequency, with can have devastating impacts on ecosystems and human societies. These ichthyotoxic blooms are rising rather sporadic with unpredictable environmental conditions and therefore monitoring attempts are rather complicated and normally just performed by cell counts and microscopical observations. Most of these ichthyotoxic microalgae are rather small in size and belonging to the nanoplankton, particular the haptophytes like e.g., *Prymnesium parvum, P polylepis,* and *Chrysochromulina leadbeateri*. In 2019, a massive bloom never so far reported in these extent and intensity of *Chrysochromulina leadbeateri* in northern Norway was reported causing massive mortalities of farmed salmon. The differentiation of potential toxic *Chrysochromulina* species with their non-toxic congeners is hampered by their morphological similarity. Therefore, the application of molecular tools for monitoring are needed, such as quantitative real time PCR assays for a reliable detection, discrimination and quantification. In this study we introduce a new quantitative PCR assay dedicated to detect and discriminate *C. leadbeateri* in natural systems or environmental set ups using SYBR Green and Taq Man assays. Quantitative PCR results show the distribution of *C. leadbeateri* found in the polar region of northern Norway, Svalbard and Greenlandic waters. Results from qPCR are aligned with data based on metabarcoding data sets from these sampling campaigns. Our data show the polar expansion of *C. leadbeateri* and point out the potential risk for further blooms. This can be problematic in near future with expanding aquacultures to the north and the importance for fishery in these pristine areas and their societies. Climate change driven environmental changes within these geographic areas could lead to a higher risk of rising and recurring ichthyotoxic blooms due to natural or anthropogenic induced radical changes.

Monitoring and Mitigation

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High resolution underway observations of harmful algal bloom dynamics in the Baltic Sea and the Kattegat-Skagerrak using AI assisted automated imaging in-flow

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Early detection and warning of harmful algal blooms are desirable e.g., to protect aquaculture and tourism industries and also for protecting human health. We used state of the art image analysis and deep learning approaches to collect high resolution data on the distribution of harmful algae in the Baltic Sea and the Kattegat-Skagerrak. The Imaging FlowCytobot (IFCB) was deployed as part of a ferry box system on R/V Svea during cruises in the area collecting water samples underway approximately twice per hour resulting in a spatial resolution of ~ 11 km (~6 nautical miles). Organisms in the size range of ~10-150 µm were imaged. A training set of images annotated by phytoplankton identification experts was used for developing classifiers for automated identification of HAB taxa. The toxin producing cyanobacteria *Nodularia spumigena* was abundant in the Baltic proper in summer. The non-toxic cyanobacteria *Aphanizomenon flos-aquae* was also present in high biomass. Phycotoxin-producing dinoflagellates including *Dinophysis* spp. were also quantified. The diatom genus *Pseudo-nitzschia* was also identified. By combining results from the IFCB with data from bio-optical sensors such as fluorometers and satellite remote sensing of ocean colour, the development of cyanobacteria blooms could be followed.

Monitoring and Mitigation
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Baselining phytoplankton communities and potential HABs species in major ports of Palawan, Philippines

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The Philippines, being an archipelagic country, highly depends on seas and seaports for its domestic and international commerce and travel. However, seaports can serve as avenues for the introduction and spread of harmful algal blooms (HABs) such as via ballast water transfer. This is true for island provinces like Palawan, which had its first HABs report in 1998 in Malampaya Sound, Taytay. Since then, it has expanded to other areas such as Puerto Princesa Bay and Honda Bay, which are all important embayments both for fisheries and transport. These emphasize the importance of monitoring to understand dispersal pathways of HABs due to the high connectedness of seaports. To date, no baselining work has been done on phytoplankton communities in the different ports of Palawan. Here, we profiled the phytoplankton diversity in 11 major ports covering the entire province. To do this, an opportunistic 30-L bucket method and microscopic examination were used. Results showed a total of 44 genera of phytoplankton, with the highest density being in the port of Roxas (1493cells/L). A total of nine potential HABs species were identified but with varying abundances. These include *Ceratium* spp., *Protoperidinium* spp., *Gonyaulax* spp., *Noctiluca* spp., *Amphora* spp., *Prorocentrum* spp., *Pseudo-nitzschia* spp., *Ornithocercus* spp., and *Dinophysis* spp. The highest diversity of HABs were found in ports of San Vicente, El Nido and Buliluyan, all having four species. Communities were further elucidated using the Imaging Flow Cytobot. Implications to the mitigation of dispersal of HABs around the province will be discussed.

Monitoring and Mitigation

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Phytoplankton assemblages in surface sediments collected from Onagawa Bay and Matsushima Bay

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In order to investigate different species of phytoplankton and the suitability or unsuitability of their presence on shellfish aquaculture, phytoplankton assemblages were investigated by DNA analysis of surface sediments collected in Onagawa Bay and Matsushima Bay. Diatoms were dominant at many stations in both bays, although in some stations green algae were dominant. Weighted UniFrac analysis (which is quantitative and considers the dominant species) revealed no clear differences in the structure of the phytoplankton assemblages between these bays. Diatoms are ubiquitous in the northeastern coast of Honshu, Japan, so the differences between bays is not clear. In unweighted UniFrac analysis (which is qualitative and considers minor species), the structure of the phytoplankton assemblages tended to vary with the bay. The minor species (producing fewer detected sequences) found in Onagawa Bay and Matsushima Bay were different, suggesting that minor species were either endemic in the bays or adapted to the particular environment of each bay. In addition, among the minor species detected were the dinoflagellate *Dinophysis* sp. (which causes diarrheal shellfish poisoning), the raphidophyte *Heterosigma* sp. (a harmful alga) the haptophyte *Phaeocystis antarctica*, and the pelagophyte *Aureococcus anophagefferens*. Another two species, *Phaeocystis* spp. and *Aureococcus* sp., are also classified as harmful algae, although these species have less attention for no damage to aquaculture in Japan. It is concluded that DNA sequencing of sediments is a useful contribution to the data required for effective management of shellfish aquaculture.

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Near infrared hyperspectral imaging: a powerful tool for toxin-producing microalgae detection in seawater

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Harmful algal blooms (HABs) are a recurrent problem in coastal regions around the world. Currently, identification of toxin-producing microalgae species relies on microscopy and molecular techniques, which are expensive, time consuming and require specialized and highly qualified operators. The purpose of this work is the development of a rapid and cost-effective approach to detection and identification of harmful algae in seawater based on near infrared (NIR) hyperspectral imaging. Hyperspectral image (HSI) is a special case of a conventional digital image, where every pixel is represented by a spectrum instead of intensity of color channels. NIR spectroscopy is a sensitive technique for discrimination of microorganisms, and its combination with imaging was expected to further increase sensitivity enabling identification of toxic algal species in the presence of complex and diverse phytoplankton assemblages. In this work HIS was applied to seawater samples collected at Aveiro lagoon in Portugal at two sampling locations, from May 2020 to June 2021. Sampling was carried out twice a month or weekly during blooms. After filtration of lugol fixed samples, NIR images of filters were recorded. Classifiers were calculated for HAB-forming taxa common at the Portuguese coast, including *Pseudo-nitzschia* spp., *Gymnodinium* spp., and *Dinophysis* spp., using phytoplankton identification done by optical microscopy as a reference. Classification accuracy in validation varied from 87% for azaspiracid- producing species to 98% for *Pseudo-nitzschia* spp. and *Gymnodinium* spp. HSI was demonstrated to be a promising tool for rapid detection of toxic microalgae in seawater.

Monitoring and Mitigation

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Assessment of current practices to disseminate alerts and information for the effective communication of HABs warnings and events in selected sites in the Philippines

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Information dissemination is an integral part of HABs mitigation and management. Timely alerts or warnings would ensure public health and safety as well as reduce the potential economic losses caused by HABs. In the Philippines, the BFAR National Office has the sole authority to release Shellfish Bulletins for harvest and consumption bans based on periodic monitoring (centralized). However, some BFAR Regional Offices have also been granted permission to issue their own bulletins (decentralized) due to the frequency and severity of HABs occurrences in their locality. Given the need to establish an effective information dissemination and warning system, assessing these frameworks could provide insights on how to improve communication of HABs events to the public. Here, we determined the efficiency of a centralized vs. decentralized framework on information dissemination via interviews with relevant national agencies, local government units, and stakeholders. Results showed longer cascade time (48 hrs to 2 wks) for centralized systems due to the longer approval process before any information can be downloaded to the locality. This affects the ability of the agencies to deliver timely warnings and notices, posing risks to public safety. In contrast, in decentralized systems, information and alerts only take <24 hours as it effectively cuts dispatch time after testing the samples. Feedback from stakeholders confirmed the expediency of the decentralized process but also revealed the need for more education campaigns about HABs. Efficiency of the different mediums and channels used to communicate information and their implications to effective warning systems are also discussed.

Monitoring and Mitigation
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Different molecular approaches for identification and quantification of HAB species

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Harmful algal blooms involve several phytoplankton species and these phenomena have become common in worldwide coastal waters, causing negative impacts on human health, ecosystem quality and affecting economic and commercial activities of the interested coastal areas. Indeed, a multiplicity of toxins or bioactive compounds can be produced affecting many different marine resources. For the identification and quantification of potentially harmful microalgae, microscopy-based methods are commonly used, but they are time consuming and require the complex recognition of morphological features. Thus, methods for rapid, sensitive and specific identification and quantification of target harmful microalgae in marine environments have been developed for exploring phytoplankton assemblages' composition and diversity. Molecular methods based on PCR, real-time PCR, microarray systems and even on eDNA have been developed and applied. Genus, species- specific primers and probes were designed on ribosomal DNA sequences (i.e., SSU, LSU, 5.8S, ITS1 and ITS2 regions). These markers are suitable for studying the phylogeographic and phylogenetic relationships, population genetic structure and diversity in marine harmful microalgae. These molecular methods have been applied to different type of samples from cultured strains to field samples as surface seawater, macrophyte, sediment and aerosol samples in the Mediterranean Sea. These molecular tools proved to be successful for rapid, specific, and sensitive detection and enumeration of potentially harmful phytoplankton taxa. Moreover, these high throughput methods could be applied in water monitoring programs to explore the community biodiversity and dynamic also with the aim of the control and management of HABs in marine coastal waters.

Monitoring and Mitigation
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Decrease in the frequency of diarrhetic shellfish poisoning (DSP) events associated to shifts in phytoplankton populations and changes in analytical methods for biotoxin analysis

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Shellfish closures were frequent in Alfacs Bay between 1998 and 2013, lasted around 40 days per year on average and most of them were enforced after obtaining positive results in the mouse bioassay test for DSP. Among the different species of microalgae that produce okadaic acid, in Alfacs Bay it is *Dinophysis sacculus* the species that reaches the highest abundances. The maximum abundance since 1990 has been 19,110 cells/L in January 1998 followed by 15,600 cells/L in May 2003. Its abundance has decreased since then and has been very low until February 2022 when it reached 3,660 cells/L and okadaic acid was detected over regulatory level in mussels and razor clams with a maximum concentration of 410 and 215 micrograms of okadaic acid equivalents per kilogram in February 2022 and March 2023 respectively. The implementation of the extended LC-MS/MS method for lipophilic toxins in routine since the year 2015 has allow to detect the recurrent presence of pinnatoxins. Their presence together with the occasional presence of yessotoxins suggest that some of the previous closures would have been a result of false positives of the mouse bioassay test.

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The *sxt* gene and paralytic shellfish poisoning toxins as markers for the monitoring of HAB dinoflagellate blooms

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Paralytic shellfish poisoning (PSP) is a syndrome caused by the consumption of seafood contaminated with saxitoxin and its derivatives (STXs). These toxins are a family of neurotoxins produced also by eukaryotic dinoflagellates. The first two genes that start the biochemical synthesis of STX are *sxtA* (*polyketide synthase*) and *sxtG* (*amidinotransferase*). The accumulation of these compounds in seafood has a major economic impact on aquaculture industries. Due to the increased risks posed to human health by PSP toxins worldwide, it is crucial to investigate the potential correlation between STX-production and *sxt* gene content in environmental samples during toxic blooms. This study aimed to illustrate a potential scenario of STX producing harmful *Alexandrium minutum* and to investigate STX-related risk in the Mediterranean Sea. Field samples were collected during a bloom event. Field samples were processed for quantitative molecular qPCR and LC-HRMS^n analyses. The median *sxtA1* gene copy number/cell was 2.2 and no difference in *sxtA1* gene copy number was found among *A. minutum* populations. A qPCR assay was applied to quantify *sxtA1* gene copy number in *A. minutum* field samples. A positive correlation was found between abundance and *sxtA1* gene copies by qPCR in surface water. Further, *sxtA1* gene content was correlated with toxin presence in environmental samples to provide an indication of PSP risk during a bloom. Efficient monitoring strategies by combined molecular and chemical methodologies can play a fundamental role in preventing and managing health and economic risks related to STX-producing *Alexandrium* spp. blooms in coastal and marine farm areas.

Ciguatera and Benthic HABs

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Evaluation of differences in acid stability of ciguatoxin congeners from the Pacific by LC-MS/MS

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Ciguatoxins (CTXs) are the causative toxins of the worldwide food intoxication called ciguatera fish poisoning (CFP), affecting > 50,000 people annually, mainly in tropical and subtropical regions. CTXs are ladder-shaped polyether molecules produced by toxic dinoflagellates, which are translocated through the food chain to larger fish, converting into numerous congeners and increasing toxicity. It has been reported that the toxicity of one of the CTXs from the Pacific disappeared by heating in 1M-HCl. Despite the possibility that acids can abolish CTX toxicity, the acid stability of most CTX congeners has not been studied. Therefore, in this study, the stabilities of six calibrated-CTX congeners (CTX1B type: CTX4A, 52-epi-54-deoxyCTX1B, 54-deoxyCTX1B, and CTX1B; CTX3C type: CTX3C and 51-hydroxyCTX3C) in various acidic solutions were evaluated by LC-MS/MS. This study revealed that the CTX3C congeners are stable even in 5% (1.4 M) HCl, unlike the CTX1B congeners. Moreover, CTX1B decreased slightly overnight at ambient temperature in 0.1% (0.028 M) HCl, whose pH (about 1.6) is similar to gastric juice. Therefore, the stability in artificial gastric fluid (pH 1.6, 37°C) for up to 2 hours was evaluated. The six CTX congeners changed at distinctly different rates in artificial gastric fluid. Specifically, CTX4A and 52-epi-54-deoxyCTX1B epimerized immediately, CTX4A and CTX4B decreased rapidly, CTX1B decreased slowly, and 51-hydroxyCTX3C remained almost unchanged. This result suggests that CTXs may be absorbed after structural changes at different rates in the stomach, which is crucial for accurately assessing the risk of CFP among fish with different congener profiles.

Ciguatera and Benthic HABs

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Shedding light on Gambierdiscus: Diel patterns and their interspecies variability

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Gambierdiscus is a genus of large epibenthic dinoflagellate found in tropical and sub-tropical regions. Species in this genus are of interest because some produce potent neurotoxins known as ciguatoxins (CTXs). CTXs enter the food chain by grazing of these cells, and consumption of these contaminated seafoods often results in gastrointestinal, cardiovascular, or neurological symptoms collectively termed ciguatera poisoning (CP). This highlights the importance of accurate monitoring of *Gambierdiscus* environmental populations for early warning of a potential CP event. Pelagic dinoflagellates exhibit diel vertical migration, however the presence of a diel migration has not been established in benthic dinoflagellates which might affect important sampling and monitoring efforts of cells like *Gambierdiscus*, as well as toxin trophic transfer models if systematic shifts in cell locations occur. In this work, we demonstrate diel patterns in the movement of multiple species of *Gambierdiscus* in culture, and show marked and quantifiable differences in those patterns between species. We found that *G. belizianus* actively moves during the day period, while *G. carolinanus* does so at night while kept under the same light regime. *G. caribaeus* and the more toxic *G. excentricus* moved more around the transition from the light to dark period. This differentiation in movement timing, when viewed in the light of varying toxin levels between species, may have implications on toxin monitoring from environmental samples. Our findings underline the necessity of considering diel migration in the effective monitoring of benthic harmful algae.

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Accumulation of ciguatoxins in herbivorous fish and their diet in Japan

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Ciguatera poisoning is a foodborne illness caused by the consumption of marine organisms contaminated with ciguatoxins (CTXs), posing a significant food safety concern worldwide, including Japan. While herbivorous fish in the South Pacific have been found to accumulate CTXs through grazing on dinoflagellates attached to macroalgae, there is currently no information available on CTX accumulation in herbivorous fish or their dietary habits in Japan. This study aimed to investigate CTX accumulation in herbivorous fish (specifically surgeonfish) from Okinawa, Japan and to elucidate their dietary preferences using metabarcoding analysis. Three species of surgeonfish (*Acanthurus nigrofuscus*, *Acanthurus dussumieri*, and *Naso unicornis*) were captured in June and November 2022 at two sites in Okinawa. Flesh extracts from the fish were analyzed for CTX1B and CTX3C levels using fluorescent sandwich ELISA. The dietary composition of these species was determined through metabarcoding analysis targeting the 18S rDNA, as previously reported by Homma et al. (2022). Among the 43 surgeonfish specimens examined, 14 and 8 specimens were found to contain CTX1B and CTX3C, respectively, with the highest concentrations being 1.6 and 1.3 ng/kg. Analysis of gut contents revealed that surgeonfish primarily consumed Metazoa, Dinoflagellata, and Ochrophyta. Notably, no sequences from the genus *Gambierdiscus*, known as a CTX producer, were detected. This study represents the first report on CTX accumulation and dietary habits of surgeonfish analyzed using metabarcoding techniques in Japan.

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Depth distribution of benthic harmful dinoflagellate assemblages in Madeira Island: implication in view of climate changes

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Climate-driven alterations in coastal systems affect variations in frequency, intensity, and geographic expansion of Benthic Harmful Algal Blooms (BHABs). Warmer temperatures induce tropical species to migrate towards higher latitudes or deeper habitats to find more physiologically tolerable conditions. The aim of the present study was to understand how climate change could affect the distribution of BHAB species, investigating the influence of different environmental factors on their growth dynamics. The vertical distribution of five BHAB genera (*Ostreopsis, Gambierdiscus, Prorocentrum, Amphidinium* and *Coolia*) was investigated in the south coast of Madeira Island over the 2022 summer period using standardized artificial substrates deployed at 2 depth levels (4 and 13 m). Depth-related abiotic parameters, such as light, temperature, water motion and nutrient concentrations were examined as drivers of the algal distribution in the water column. Three vertical distribution patterns were observed: cell abundances of *Ostreopsis* resulted higher at 4 m, while *Gambierdiscus, Prorocentrum* and *Amphidinium* were more abundant at 13 m, and *Coolia* resulted to be ubiquitous. The recorded environmental parameters explained a high percentage of the observed algal distribution. In detail, light emerged as the most significant parameter, confirming laboratory results obtained with *O.* cf. *ovata* cultures, for which the best growth performance was observed at the highest light intensity and lowest temperature tested. The results highlighted the importance of considering depth when investigating the ecology of BHAB, to predict their future migrations and assess related human health risks, also in view of implementing future monitoring programs.

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The first occurrence of massive blooms, benthic dinoflagellate (*Ostreopsis* sp.) in seagrass areas of Eastern Gulf of Thailand

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The first occurrence of brown mucous covering seagrass leaves along the seagrass areas of Rayong province, the eastern coast of the Gulf of Thailand, occurred on February 2nd, 2023. Seawater was colorless, not smelled, and there was no film on the water's surface. Seagrass leaves with water samples were collected and sent to the lab for analysis. The results showed that brown mucous was composed of the potentially massive toxic benthic dinoflagellates. Morphological analysis revealed that *Ostreopsis* sp. is the dominant species with the highest density of 6,266 cells g^{-1} wet weight seagrass leaves in Nern Kor areas. For the adjacent seagrass area, Namdang Beach, the cell density was 424 cells g^{-1} seagrass leaves. The seawater qualities (temperature, salinity, pH, DO, and nutrients) of the Nern Kor area meet Thai marine water quality standards for recreation. But Namdang Beach showed that phosphorous and nitrate were higher than the standards. This is the first record of potentially toxic benthic dinoflagellates bloom in seagrass areas of Thai water. In the past, potentially toxic benthic dinoflagellates were less attention in Thailand, but this study might raise more awareness of Ciguatera Fish Poisoning in the future.

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The potential role of marine plastic debris as a dispersal vector for harmful epiphytic dinoflagellate *Fukuyoa koreansis*

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Marine plastic debris (MPD) can have a significant impact on marine ecosystems because it can potentially function as a dispersal vector for toxic and alien organisms. We performed laboratory experiments to assess the possible function of MPD (polyethylene film and polypropylene rope) as a dispersal vector for the harmful epiphytic dinoflagellate *Fukuyoa koreansis*. Under non-agitation conditions, there was no significant difference in growth between the experimental groups ($p > 0.05$, Chi-square = 0.228, Kruskal-Wallis test), implying that the presence of MPDs did not have a statistically significant impact on the growth of *F. koreansis*. After 15 days, growth without MPD was about 25-fold greater without agitation than with agitation (150 ± 42 cells mL^{-1} *vs.* 6 ± 1 cells mL^{-1}). When grown with both MPD types and agitation, there were 4-times more attached cells on MPD (78 ± 1 %) than without agitation. These findings imply that the presence of attachment substrates, such as MPD, plays a vital role for the growth of *F. koreansis* in an unstable water mass. Moreover, MPD may serve as a habitat or shelter for *F. koreansis*, potentially functioning as a dispersal vector for this harmful epiphytic dinoflagellate.

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Marine benthic dinoflagellates – their relevance for science and society

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The species composition of marine microalgae in benthic habitats is quite distinct from planktonic habitats. Our understanding of benthic dinoflagellate biodiversity, biogeography, toxicology and ecology has improved but is still rudimentary. Benthic harmful algal blooms have attracted increasing interest because of the impact of ciguatera poisoning, which appears to be increasing worldwide in recent years. This book is an updated summary of the taxonomy of currently described taxa and includes morphological and molecular genetic information for species identification. It contains the most comprehensive review of benthic dinoflagellate toxins published so far. Methods of study are also described and the health and economic impacts of benthic dinoflagellates for society are highlighted. The book will be a fundamental contribution to improving the monitoring of benthic dinoflagellates worldwide. 242 species in 63 genera are presented, illustrated with more than 240 color images, about 250 electron micrographs, and more than 330 drawings. Compared to the first edition, 64 new species, 20 new genera and 19 new combinations are described. All chapters have been revised to reflect the latest knowledge and more than 150 new publications have been integrated. The methods section has been expanded to include light microscopy, molecular genetic methods for detection and quantification, and toxicity tests and toxin analyses. The chapter *Relevance for science and society* has been added. In parallel to the new edition, matrix keys will be available online via the pages of the "Centre of Excellence for Dinophyte Taxonomy" (CEDiT https://www.dinophyta.org/identification-keys/).

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Benthic harmful microalgae and their impacts in South America

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Public awareness about Benthic Harmful Algal Blooms (BHABs) has increased substantially over the past few decades. Even so, reports of BHABs remain relatively scarce in South America (SA). This study provides a comprehensive overview of the current state of knowledge on BHABs in the continent, by integrating data from published articles, books, and technical reports. We recorded ~300 different occurrences of potentially toxic BHAB species over the Caribbean, Atlantic and Pacific coasts, mostly in marine (>95%) but also in estuarine areas located from 12°36’ N to 54°53’ S. The study listed 32 infrageneric taxa of potentially toxic benthic dinoflagellates and eight of estuarine cyanobacteria occurring in SA, with the greatest species diversity recorded in the equatorial-tropical zone, mainly in northeastern Brazil (Atlantic), Venezuela and Colombia (Caribbean), and Galapagos Islands, Ecuador (Pacific). Local strains of *Amphidinium*, *Gambierdiscus, Coolia* and *Prorocentrum* spp. produced toxic compounds of emerging concern. *Prorocentrum lima* species complex followed by *Ostreopsis* cf. *ovata* were associated with most BHAB events in SA. Whereas the former has caused the contamination of multiple marine organisms and cases of Diarrhetic Shellfish Poisoning in subtropical and temperate areas, the latter has been associated with faunal mortalities and is suspected of causing respiratory illness to beach users in tropical places. Ciguatera Poisoning has been reported in Colombia (~240 cases; no deaths) and Venezuela (60 cases; two deaths), and may be also a risk in other places where *Gambierdiscus* spp. and *Fukuyoa paulensis* have been reported, such as Galapagos Islands and the tropical Brazilian coast.

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Development of genetic markers related to ciguatoxin (CTX) production in *Gambierdiscus* species responsible for ciguatera poisoning

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Ciguatera poisoning (CP) is the most common seafood toxin-associated illness in the world. It is caused by consuming fish contaminated with ciguatoxins (CTX) which are primarily produced from dinoflagellates of the genera *Gambierdiscus* and *Fukuyoa*. *Gambierdiscus* species are endemic in tropical to warm-temperate waters and are increasingly expanding to new areas due to changes in sea surface temperatures, nutrient loading, eutrophication and habitat disturbance. These environmental triggers are assumed to regulate CTX production in *Gambierdiscus* species. There is a lack of knowledge regarding the genetic regulation of CTX in species such as *G. polynesiensis* and the recently discovered *G. holmesii*. In this work, the expression of genetic markers associated with CTX production in *Gambierdiscus* species under different environmental and growth conditions will be determined. Here, transcriptomic analyses will be used to compare genetic factors for the differential gene expression of CTX genes in *G. polynesiensis* and *G. holmesii*. This study will unveil the knowledge of CTX biosynthesis in *Gambierdiscus species* which aids in developing novel CP monitoring tools in the near future. Detection of specific genes and genetic markers responsible for CTX will help not only Research Institutes and Bioanalytical companies but also the fishing industries. Ultimately, the possibility to detect hotspots of ciguatera before an intoxication event will implement monitoring strategies and protect human health.

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Spatiotemporal distribution of ciguatoxins from long-term monitoring in the Florida Keys Marine Sanctuary

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Ciguatera poisoning (CP) is an emerging issue for Florida, with a long history of sporadic cases from the Florida Keys, South Florida, and from residents traveling to the Bahamas. Due to climate change causing an increase in seawater temperature, it is hypothesized that this could cause an increase in CP incidence and range. In this study we aimed to evaluate spatiotemporal trends and environmental drivers in ciguatoxin (CTX) levels in the epiphytic dinoflagellate community using samples collected from 6 distinct long-term monitoring sites in the Florida Keys National Marine Sanctuary that vary in depth and benthic cover. Replicate samples of dominant macroalgal genera were collected monthly over a six-year period, 2012 - 2018. A sodium channel specific mouse neuroblastoma assay was used to screen extracted algal samples for the presence of CTX-like and palytoxin (PLTX)-like activity, and overall neurotoxicity. Liquid chromatography-mass spectrometry was then employed to further investigate the toxin profiles in positive samples. Spatiotemporal data on algal abundance and toxicity identified site specific trends that were related to habitat type. These data will be explored to evaluate trends and to contribute to predictive models that further incorporate temperature, wind/wave, and other environmental parameters. These data not only provide a solid baseline in CTX and other benthic algal toxin trends but will further our understanding of changes over time and space to improve our predictive capacity for ciguatera in vector organisms.

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Benthic sand-dwelling dinoflagellates community in Pyoseon Beach of Jeju Island, Korea during spring and summer 2022

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Benthic dinoflagellates are important primary producers in benthic ecosystems, and some species are highly toxic. While they are mostly found in tropical and subtropical waters, global warming is expanding their habitat to temperate waters. Jeju Island, located at the southernmost tip of the Korean Peninsula, is one of the areas most affected by climate change and urgently needs monitoring of toxic species to prepare for ecological and health damages. This study investigated the distribution and species diversity of benthic dinoflagellates community in the sandy intertidal zone of Pyoseon Beach, Jeju Island, during spring and summer 2022. Presented study identified a total of 23 genera and 48 species of benthic dinoflagellates, including four potential toxic species, eight Korean unrecorded species. *Amphidinium, Prorocentrum, Heterocapsa, and Sinophysis* were the most commonly appearing genera. The potential toxic species were more prevalent in summer. The monthly average abundance of benthic dinoflagellates ranged from 4 to 10 cells/cm^3, with the highest abundance in June (23%). The number of species of each station ranged from 2 to 13, and Shannon's diversity index ranged from 0.25 to 2.31. The average diversity index of the lower intertidal zone was higher than the upper intertidal zone, except in August. This study provides valuable data on seasonal species diversity and the potential toxic species of benthic dinoflagellates in Jeju Island. Long-term monitoring will enable further research on the impact and change of benthic ecosystems due to climate change and the structure and function of benthic ecosystems.

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Toxicity Screening and toxin profile of *Gambierdiscus* spp. and fish from La Réunion Island (Indian Ocean)

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Several outbreaks have been recorded in the Island of La Réunion (SW Indian Ocean) since 1986. Toxicities and toxin profiles of *Gambierdiscus* and fish from the Indian Ocean are poorly documented. In this work a multidisciplinary study on the toxicities by neuro-2a cell-based assay, magnetic bead-based immunoassay and toxin profile by Liquid chromatography coupled to mass spectrometry (LC-MS/MS) and high-resolution mass spectrometry (LC-HRMS) were implemented for four *Gambierdiscus* species (*G. belizeanus, G. balechii, G. pacificus*, *G.* cf. *pacificus* and *G.* ribotype 2) and three fish species (*Carcharinus leucas, Lutjanus bohar* and *Variola louti*) from La Réunion island. *G. belizeanus* showed the highest toxicities with values from 0.76 to 0.14 pg equiv. CTX1B/cell. The two main compounds that were detected were gambierone (171.7 to 3499.4 ng equiv. gambierone/ml) and 44-methyl-gambierone (380.4 to 3745.2 ng equiv. 44-methyl-gambierone /ml). Toxicity of shark (*Carcharhinus leucas*) and fish (*Variola louti* and *Lutjanus bohar*) was also analyzed ranging from 0.013 to 1.27 ng equiv. CTX1B/g in flesh, 2.55 to 16.4 ng equiv. CTX1B/g in liver, and 0.5 to 3.12 ng equiv. CTX1B/g in viscera. Several potential CTX congeners were identified: the flesh of the *V. louti* after a Ciguatera incident confirmed the presence of CTX1B, 52-epi-54-doxyCTX1B and 54-deoxyCTX1B. In the second *V. louti* individual and *L. bohar*, CTX1B was identified in the three tissues. The *C. leucas* samples revealed the presence of a complex CTX contaminant profile showing congeners of CTX3C-group.

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First record of a *Coolia* sp. in the tropical waters of the Philippines

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Studies on benthic dinoflagellates in the Philippines have been significantly limited despite many of these species being shown to produce potent toxins, including *Gambierdiscus* and *Coolia* species. Interestingly, although *Gambierdiscus* has been reported to occur in many areas of the country, none of the known *Coolia* species have been reported in the Philippines so far. Here, cells with morphologies similar to *Coolia* sp. were obtained from the mariculture waters of Bolinao, Pangasinan in northwestern Philippines. Multiple strains were then successfully cultured. Cell measurements of strain BOLCO1 through light microscopy revealed cell lengths ranging 24-28 µm, widths ranging 26-29 µm, and dorso-ventral depths ranging 27-30 µm, mainly corresponding to some smaller species of *Coolia*. Sequence similarity search for the D1/D3 region of the 28S rRNA gene further revealed *C. malayensis* and *C. monotis* as the most similar matches. Phylogenetic analysis however clustered out the isolate to the *C. monotis* clade with 0.99 posterior probability value. Several lines of evidence indicate that our isolate was also a putative *C. monotis*, whose presence in the Philippines has not been previously recorded. It is thought to be limited to colder waters as its only currently available representative isolates were from North Atlantic and European waters. Information on the distribution of benthic dinoflagellates in the Philippines is limited, and the awareness on the presence of toxin-producing species is valuable to the fisheries-dependent country.

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Evaluation of Ciguatoxin-like toxicity in the trophic web in Sardina del Norte and Risco Verde, two hotspots of *Gambierdiscus* spp. in Gran Canaria Island

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Due to the incidence of cases of CP in the Canary Islands, a study of the population dynamics of *Gambierdiscus* spp., was performed in Sardina del Norte (SN) and Risco Verde (RV) every month from May 2021 to November 2022, as well as the CTX-like toxicity present in captured fish from both points. Fish collection was conducted during November 2021. For the purpose of toxicity assessment, fish species from the different trophic levels were collected (n = 60 in SN, n = 54 in RV) as well as sea urchin pools (n = 9 in SN, n = 10 in RV) and 9 octopuses (n = 7 in SN, n = 2 in RV). CTX-like toxicity was analysed using the Neuro-2a cell-based assay. In fish and octopus, flesh and liver/hepatopancreas of each individual were analyzed. Toxicity in carnivorous fish from SN was observed: *Sphyraena viridensis*, *Seriola* spp., *Muraena augusti*, and *Gymnothorax unicolor*. Some herbivorous/omnivorous fish species displayed toxicity: *Pagrus pagrus*, *Pseudocaranx dentex*, *P. auriga*, *Dentex gibbosus*, and *Sparisoma cretense*. In RV, toxicity was evidenced in sea urchins, *Octopus vulgaris* and carnivorous fish such as *Seriola* spp., *M. augusti, M. helena, G. unicolor*, and also in herbivorous/omnivorous *P. pagrus, P. dentex, Diplodus sargus cadenati, D. sargus cervinus*, and *S. cretense*. CTX activity was evidenced through marine food web in sampled areas, and for the first time CTX-like toxicity was displayed in sea urchin and octopus samples in the Canary Islands.

Ichthyotoxic HABs
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Using high-throughput techniques to understand the growth of the fish-killing haptophyte *Chrysochromulina leadbeateri* in Northern Norway

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Harmful algal blooms (HABs) have been a global cause of substantial mortality in fish farms. In 1991 and 2019, the haptophyte *Chrysochromulina leadbeateri* formed extensive toxic blooms in Northern Norway. The 2019 event was especially devastating, resulting in the death of more than 14,500 tons of farmed salmon (*Salmo salar*). Cultures were isolated from both blooms, now kept at the Norwegian Culture Collection of Algae (NORCCA). To gain a better understanding of the growth patterns of this bloom-forming alga, we conducted *in vitro* experiments using a high-throughput microplate-based approach. The experiment assessed growth with different temperatures, salinities, and irradiance in a 3-way factorial design. Our findings indicated that *C. leadbeateri* exhibits the highest growth rates at temperatures between 13–15°C and salinities from 28–30 under a constant irradiance of 50 µmol $m^{-2}s^{-1}$. Our experiments also showed increased growth rates with higher irradiance, ranging from 10–100 µmol $m^{-2}s^{-1}$. Data from the 2019 blooms showed the highest cell concentration of *C. leadbeateri* in Balsfjorden, where the temperature hovered around 7°C, and the salinity was between 32–33. The current study will use the growth data obtained from our experiments with information about environmental conditions and the geographic distribution of *C. leadbeateri* from metabarcoding samples in the metaPR2 database to better understand the bloom dynamics of this species. This will also be combined with a phylogenetic analysis of the *Chrysochromulina* genus.

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Analytical strategy based on extraction, bioassay and untargeted LC-MS analysis for the determination of neurotoxins in Raphidophyceae *Heterosigma akashiwo*

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Heterosigma akashiwo (Raphidophyceae) is described as one of the ichthyotoxic microalgae responsible for the formation of harmful algal blooms (HABs), which cause significant losses in the aquaculture industry due to their wide global distribution. Despite the efforts made, there is still no certainty regarding the ichthyotoxic mechanisms or the nature of the toxic compounds produced by *H. akashiwo*. Previous studies have demonstrated that the extract obtained from *H. akashiwo* CCMP302 biomass (New Zealand) has a neurotoxic effect on the Neuro-2a neuronal model. This effect corresponds to the inhibition of voltage-dependent Na^+ channels, and it also exhibits an anaesthetic efficacy like commercial products when evaluated in zebrafish. Furthermore, analytical methods have not yet detected compounds related to brevetoxins. To characterize the chemical nature of the compounds responsible for the neurotoxicity and anaesthetic effect of *H. akashiwo*, we developed an analytical methodology based on liquid-liquid fractionation (Liq-Liq) coupled with solid-phase extraction (SPE) guided by Neuro-2a cell bioassay, zebrafish model, and LC-MS. According to the Liq-Liq fractionation of the hydromethanolic extract, the compounds of interest are distributed in the studied organic fractions according to the Neuro-2a bioassay, exhibiting anaesthetic activity only in the most apolar fraction in the zebrafish model. Subsequently, this active fraction was further fractionated using SPE, and anaesthetic activity was observed only in the two intermediate hydromethanolic fractions. Finally, the characterization of the active fractions through untargeted LC-MS analysis can contribute to the identification of potential bioactive compounds and the study of ichthyotoxic mechanisms that cause mass fish mortality.

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mRNA-seq analysis on *Karenia brevis*-exposed larval stage Java medaka *Oryzias javanicus*

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Blooms of harmful dinoflagellate *Karenia brevis* poses a threat to marine life due to the brevetoxins they release. However, the mechanism of action of brevetoxin on immature fish larvae is still largely unexplored. This study investigated the effects of *K. brevis* exposure on the gene expression of *Oryzias javanicus* (henceforth Java medaka) larvae using mRNA-seq analysis. Java medaka larvae (<24h post-hatch; n = 5) were exposed to ~1.0 x 10^4 cells mL^{-1} *K. brevis* (CCMP 2281). The total RNA of each individual larvae was extracted using ISOGEN II. The read quality for the sequencing files were checked using the fastp and FastQC software and analyzed using STAR (ver.2.7.10), SAMTools (ver.1.16.1), and edgeR (ver.3.40.2) programs. Visualization and pathway analyses of the reads were then conducted using iDEP web application (ver.1.1). RNA-seq analyses revealed that 511 genes were differentially expressed through *K. brevis* exposure with 264 upregulated genes and 247 downregulated genes. Genes that promote adaptation and homeostasis under stress environments (*pacc1* and *INHBC*), especially for neuroprotection (*npas4a*), were differentially upregulated compared to the control. Simultaneously, genes relating to muscle functions (*kbtbd12*), reticulophagy regulation (*retreg1*), calcium homeostasis (*guca1a*), and organogenesis (*fkbp5*) were differentially downregulated. *K. brevis* exposure impaired the expression of these genes which are thought to have aligned with the known neurotoxic properties of brevetoxin. The effects of post-*K. brevis* exposure muscle and organogenetic impairment in fish may carry on to adulthood.

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Understanding fish-killing mechanisms of HABs from different aspects based on the observations of multiple species of dinoflagellates

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Harmful algal blooms (HABs) cause severe impacts on ecosystems, fisheries, aquaculture and even human health. While dinoflagellates are responsible for ~75% events of marine HABs, dinoflagellates are toxic to marine animals and human because they produce various toxins. However, most of the toxins have not been fully identified, particularly those causing fish kills, the so-called ichthyotoxins. Up to date, only a few types of compounds, such as the karlotoxins, have been proven to be true ichthyotoxins with complicated structures. Consequently, the fish-killing mechanisms for most dinoflagellates are largely unknown. Here we synthesize our recent investigations on the fish-killing toxicity of the dinoflagellates *Alexandrium leei*, *A. insuetum*, *Margalefidinium polykrikoides*, *M. fulvescens*, *Karlodinium veneficum*, and *K. australe* in order to gain novel understandings of fish-killing mechanisms of HABs from different aspects. Based on these works, we infer that: 1) At least for some "toxic" dinoflagellates, production of toxins may possibly a part of their trophic modes both in terms of the process and outcome; 2) fish-killing and mortality of other organisms may not be caused by toxins but rather by the phagotrophy or micro-predation for a number of HABs-causing species more than what previously thought; and 3) phagotrophy may play vital roles in the function of some dinoflagellates both in their top-down and bottom-up regulations of the population dynamics, including the formation of HABs for these species. We believe these perspectives from different aspects provide new insights into the ecological significance of ichthyotoxicity and phagotrophy exhibited in many HABs-causing microalgae.

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Influence of biotic and abiotic factors on prymnesin profiles in three strains of *Prymnesium parvum*

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Golden alga, *Prymnesium parvum*, poses a significant threat to aquatic ecosystems globally by producing prymnesins, which are potent polyether toxins that cause rapid decline in fish, shellfish and mollusk populations. Prymnesins are categorized into A-, B- and C-types based on variations in their carbon backbones and multiple analogues of each type have been detected that vary in the degree of glycosylation, chlorination, the number of rings and double bonds. Three *P. parvum* strains (UTEX 2797, K-0374 and PPSR01) were grown under various conditions to investigate the influence of growth phase, nitrogen availability, light intensity, and salinity on prymnesin profiles. While some growth conditions resulted in strain-specific changes in prymnesin profiles, all three produced increased proportions of prymnesins with higher degrees of glycosylation at later stages of growth. The impact of decreased salinity was significant in two strains, where the proportion of tri-chlorinated prymnesins represented more than 95% of the prymnesin profiles. By gaining an understanding of the effects of biotic and abiotic factors, culture conditions could be modified to promote specific prymnesin profiles to support the development of analytical reference materials. Furthermore, evaluating prymnesin profiles in combination with toxicity assays will provide insights into prymnesin biosynthesis, mode of action and toxicity.

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Toxicity effect of the algicidal supernatant from bacterium P4 and the *Karenia mikimotoi* cells after exposed to algicidal supernatant on fish gill cell line

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Algicidal bacteria have been researched for decades and revealed an efficient measure to control harmful algal bloom. Most of the algicidal bacteria exhibit an indirect algicidal effect which expressed the algicidal substance(s) to kill the target algae. An algicidal bacterium P4 was previously isolated from an ichthyotoxic algal bloom in 2016 and showed strong indirect algicidal activity to the causative agent, *Karenia mikimotoi* (KMHK). However, the toxicity effect of the algicidal substances and released algal toxin from the algal cells under algicidal event to other marine organisms was still unclear and was necessary to understand before the application. In this study, fish gill cell line RTgill-W1 was used to investigate the possible toxicity effect by determining the cell viability using AlamarBlue stain after the 120 min exposure to 25%v/v of the P4 bacterial supernatant of an algicidal bacterium P4 (spent bacterial medium only) and the 90 min exposure to the algicidal supernatant treated axenic algal cells. Based on our data, there were no significant drops ($p \le 0.05$) in gill cell viability after exposure to the P4 bacterial supernatant when compared to the controls (algal medium L1 control and bacterial medium $ST10^{-1}$ control), which had maintained nearly 100% cell viability. These results demonstrated that this algicidal supernatant was non-toxic to fish gill *per se*. Interestingly, when comparing to the positive control (i.e fish gill cell line treated with KMHK), a significant decline ($p \le 0.05$) of KMHK ichthyotoxicity was observed in both KMHK cells after exposure to the algicidal supernatant and the bacterial $ST10^{-1}$ medium. The attenuation of ichthyotoxicity of axenic KMHK after these treatments still required more research in the near future.

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Toxic microalgae in Norwegian waters (ToxANoWa) – Uncovering fish-killing mechanisms of phytoplankton from Scandinavian waters

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The ToxANoWa project is the result of a HAB event in Northern Norway in May-June 2019. The responsible species for the fish-kills was *Chrysochromulina leadbeateri*. Field samples collected at the time were used to establish cultures of *C. leadbeateri*. Little is known about why this species form blooms and may be toxic to fish. ToxANoWa aims to obtain better knowledge on growth preferences, toxicity and produced toxin(s). The project also aims to study species in the related genus *Prymnesium*, responsible for fish-killing HABs worldwide. *Prymnesium parvum* produces prymnesins that are highly toxic to fish, but the chemistry and toxicology of the prymnesins are still not well understood. ToxANoWa aims at producing selected prymnesins to make them available for research and monitoring purposes. We also work on the elucidation of the chemical structures of C-type prymnesins, a new variant of prymnesin, recently discovered by ToxANoWa-collaborators. In addition, the ToxANoWa team will raise prymnesin antibodies that may be used to develop research and monitoring tools to simplify selective extraction and detection of these toxins. Another *Prymnesium* species, *P. polylepis*, was responsible for a large fish toxic bloom in the Kattegat and Skagerrak in 1988. It is still unknown what the toxin compounds are that is harmful to fish and their mode of action, but application of novel technologies in ToxANoWa will hopefully provide additional clues. This project is a collaboration between the Norwegian Veterinary Institute, University of Oslo, the Technical University of Denmark and the National Research Council of Canada.

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Responses of medaka fish experimentally exposed to ichthyotoxic dinoflagellate *Karenia mikimotoi*

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The harmful algae species *Karenia mikimotoi* can be found in different locations all over the world. The algal bloom caused by this species can kill massive amount of aquatic organism due to their high ichthyotoxicity. During December 2015 to February 2016, massive algal blooms of *K. mikimotoi* were happened in Hong Kong. In such algal blooms, more than 200 tons of fish were being killed, which led to significant financial loss. We have successfully isolated the algal bloom causative species *K. mikimotoi* and established a monoclonal culture denoted as KMHK. In this study, we compared the ichthyotoxicity of KMHK with two other *K. mikimotoi* strains (NIES2411 from Japan and CAWD133 from New Zealand) using marine medaka fish bioassay. Within three hours, KMHK killed 50% of the fish, whereas the Japanese strain NIES2411 killed 100% of the fish. However, it was observed that no fish would be harmed by the New Zealand strain CAWD133 throughout 24 hours exposure. The result was aligned with experimental data obtained from an in vitro bioassay using fish gill cell line (RTgill-W1). Interesting, it was found that the ichthyotoxicity of KMHK has been attenuated when compared to a similar medaka fish bioassay that we had performed previously for this species in 2016. The experiment showed that KMHK could achieve 50 % mortality in around 30 minutes. We speculated the reduction of ichthyotoxicity of KMHK may be attributed to a series of antibiotic treatments introduced to the algal culture for the prevention of bacterial contamination. Such treatments might disrupt or change the bacteria composition associated with KMHK which may in turn alter the ichthyotoxicity of the algal cells. However, more research in this area is needed.

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Effects of *Karenia brevis* on behavior, mortality and reproductive success of the Florida stone crab, *Menippe mercenaria*

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Karenia brevis blooms cause fish kills, turbid water and hypoxic events, all of which can negatively impact local fisheries. These blooms are prevalent along Florida's gulf coast and have extended through the summer months in recent years. The stone crab, *Menippe mercenaria,* is a ~$25 million fishery that occurs primarily along Florida's gulf coast where these blooms develop. During summer, stone crabs are reproductive and release larvae that are transported offshore. Using laboratory-based exposures we determined lethal and sublethal effects of *K. brevis* on *M. mercenaria* adult and larval stages, as a co-stressor in adult crabs, and on reproductive success. Sub legal adult crabs exposed to "high" abundance of *K. brevis* ($> 1 \times 10^6$ cells L^{-1}) had 42% decrease in survivorship, 67% decrease in food consumption and decreased reflex response. Crabs exposed to *K. brevis* plus severe hypoxia exhibited a 43% decrease in survival and experienced a 62% increase in lethargy. Larval stages experienced 100% in high and 30% in medium (1×10^5 cells L^{-1}) of *K. brevis* and demonstrated reversal of swimming behavior. No differences in stone crab embryo development or hatching success were observed after exposure to medium *K. brevis* abundance, although female crabs showed significantly higher stress levels and prolonged hatching. These studies were the first to demonstrate negative effects of *K. brevis* on the Florida stone crab, presenting the critical need of further investigation to fully understand how red tide may impact sustainability of the fishery.

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Characteristics of *Prymnesium parvum* from the first harmful bloom in the Polish river

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Prymnesium parvum, the haptophyte, is one of the most harmful algae species. Its mass occurrence usually results in serious environmental and socio-economic losses. In Poland, the first *P. parvum* bloom occurred in summer 2022 and had dramatic consequences for the Odra River ecosystem. At the peak of the bloom in 2022, *P. parvum* density was more than 600 mln cells/L. The fish kills were recorded both in 2022 and 2023, when the population reached 20-30 mln cells/L. The aim of our study was to characterise *P. parvum* from the Odra River and to determine its specific genetic and chemical traits. Genetic analyses indicated the presence and expression of genes encoding the enzymes involved in prymnesis (PRMs) synthesis, while LC-MS/MS analysis confirmed the production of three type-B variants of these ichthyotoxins. Changes in the profile of PRMs were observed between different places and in different seasons, suggesting that the population is not clonal. The optimisation of analytical procedure and isolation of pure PRMs, allowed for quantitative analyses of the cell-bond and free form of the toxin during several months of *P. parvum* bloom. Toxicological *in vitro* studies were also conducted and differences in the activity of PRMs variants were explored. As permanent occurrence of *P. parvum* in Polish water bodies is expected, the new knowledge about the dynamics, diversity, and toxicity of the species can support the development of an effective strategy for harmful bloom management.

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Hemolytic toxicity of fish-killing dinoflagellates isolated from HABs-affected bays in Luzon, Philippines

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Several ichthyotoxic dinoflagellates have been reported to occur in the Philippines but the method of how they elicit mortality remains unknown. One suggested mode is via hemolytic toxin production, posing a significant risk for aquaculture and fisheries. In this study, the hemolytic toxicity of some frequently reported HABs-causing species from different bays in Luzon, Philippines was investigated. A total of 7 species including *Heterocapsa* (*H. philippinensis*, *H. borneoensis*, and *Heterocapsa* sp.), *Amphidinium carterae*, *Biecheleriopsis adriatica*, *Chattonella subsalsa*, and *Scrippsiella mosanensis* were isolated. Results showed varying growth rates at 25°C with optimal light conditions with *H. borneoensis* exhibiting the highest maximum specific growth rate (0.51 ± 0.04 d^{-1}) and *H. philippinensis* being the slowest (0.13 ± 0.03 d^{-1}). Using the same growth conditions, hemolytic toxicity of the cultures was quantified using an Erythrocytes Lysis Assay, employing rabbit erythrocytes. Among the four experimental samples (culture supernatant, ultrasonic ruptured cells, supernatant of ultrasonic ruptured cells, and dark incubated samples), the ultrasonic ruptured cells were hemolytic in all species except that of the supernatant of *S. mosanensis* and *C. subsalsa* which was much higher than the lysate. However, no hemolytic activity was detected on the supernatant of ultrasonic ruptured cells in *B. adriatica* and *A. carterae*. Surprisingly, *H. borneoensis* and *H. philippinensis* showed hemolytic activity under dark conditions. These findings will shed light on the potential impacts of these ichthyotoxic species on aquaculture and the risk of massive fish mortality in the country when it blooms.

Cyanobacterial HABs

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Combined effects of CO_2 and nitrogen on the ecophysiology of a toxic cyanobacterium

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Eutrophication has led to the proliferation of harmful cyanobacterial blooms, which are expected to be further promoted by climate change stressors like elevated pCO_2. These environmental changes may enhance the density and toxicity of blooms by affecting the eco-physiology of cyanobacteria. Changes in the eco-physiology of cyanobacteria manifest as changes in a range of their traits like growth, nutrient uptake, and toxin production. As such, we can use a trait-based approach to mechanistically understand the underlying cellular processes that lead to the success of toxic cyanobacterial species. A trait-based ecological approach relates cellular and functional traits of species to population and community dynamics. In this study, we assessed how changes in CO_2 levels combined with low and high nitrogen (N) levels affected growth, nitrogen uptake, cellular elemental stoichiometry, amino acid content, toxin production, and the underlying regulation of genes in three toxic strains of *Microcystis aeruginosa* (HUB524, PCC7820, NIES1088). Our findings showed interactive effects between CO_2 and N on growth, stoichiometry, and toxin production consistently across strains. For example, with increasing CO_2, cellular C:N ratios increased in low N but decreased in high N treatments. Regarding nitrogen uptake kinetics, the three strains responded differently to varying CO_2 levels. Currently, amino acid and gene regulation data are being analyzed to determine the underlying regulatory processes that led to these observations. So far, our findings show stoichiometrically predictable responses in key traits, which will support our understanding of the future impacts of CO_2 and nitrogen variation on cyanobacterial growth and bloom toxicity.

Cyanobacterial HABs

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Taxonomy, function, and molecular mechanisms of the algicidal bacterium, *Pseudomonas* sp. Go58, derived from water-plant biofilm

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The mitigation of nuisance cyanobacterial blooms has increasingly emphasized environmentally friendly approaches, with particular attention given to the utilization of algicidal bacteria exhibiting potent anti-microalgal activity. In this study, we isolated a bacterial strain, Go58, from the biofilm of the water plant, *Trapa* sp., which exhibited significant algicidal activity against the bloom-forming cyanobacterium *Microcystis aeruginosa*. Whole genome sequencing analysis of the strain Go58 revealed its potential classification as a novel species closely related to *Pseudomonas protegens*. Further investigation of the strain's culture broth identified the presence of pyoluteorin and its novel analogue, pyoluteorin B, which demonstrated remarkable toxicity against cultured cyanobacterial species, including *M. aeruginosa* and *Anabaena cylindrica*, while displaying reduced toxicity towards eukaryotic microalgae and other aquatic organisms. Notably, the production of pyoluteorin was found to be enhanced in the presence of the target cyanobacterium. To assess the effectiveness of the strain Go58 and pyoluteorin in a natural setting, a wild-caught microalgal consortium was treated with these agents, resulting in efficient suppression of harmful wild cyanobacterial growth and simultaneous promotion of specific eukaryotic microalgae. Considering the global ubiquity of *P. protegens* family and its potential application as a biopesticide for infectious diseases in agriculture, the identification of similar bacterial groups in this study suggests their promise as safe and globally applicable biological countermeasures for the control of cyanobacterial blooms.

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Survival and chronological changes of blue-green algae in the sediment core from Lake Hachiro, Japan

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Dredging to remove benthic *Microcystis* spp. has been considered as a countermeasure against harmful algal blooms. However, there is a report that *Microcystis* spp. were found to be alive in the bottom sediment after 7 years, and there is concern that these cells may return to the lake water after dredging. Therefore, the objective of this study was to determine vertical changes in sedimentary facies and *Microcystis* spp. viability in sediment cores in Lake Hachiro, Japan. The average weight deposition rate calculated from the slope of the specific activity of ^{210}Pb was 0.26 g・cm^{-2}・year^{-1}, and the deposition age of each layer was estimated together with the results for ^{137}Cs. Lake Hachiro was desalinated in 1961 when the tidal gate was closed to the Japan Sea, which was estimated to be at a depth of 28-30 cm. The depth below 32 cm was estimated to be the pre-declining sediment. Similarity analysis of the bacterial flora showed that they were significantly different at depths greater than 20 cm, consistent with the frequency distribution of brackish and saltwater diatoms such as *Melosira moniliformis*. On the other hand, the recruitment of *Microcystis* spp. was confirmed to 24 cm. The depth of 24 cm was estimated to be 1971, indicating that *Microcystis* spp. have retained survivability for more than 40 years. At depths deeper than 26 cm, *Desulfatiglans* spp. was dominant, suggesting that the long-term survivability of *Microcystis* spp. was predicted to be related to the bacterial flora.

Cyanobacterial HABs

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The occurrence of cyanobacterial toxins in Alberta recreational waters

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The occurrences of cyanobacteria (blue-green algae) blooms and the presence of its toxins (microcystin; MCs) in fresh water has been reported worldwide. Based on public awareness, the Alberta Health and Alberta Health Services have conducted seasonal monitoring of cyanobacteria bloom since 2009. Microcystins (MCs) is one of important indicator because they are highly potent hepatotoxins that diversify in forms and health consequences. As part of the Alberta Cyanobacteria Monitoring Program, a total of 2,626 (91% composite, 8% grab) water samples were collected from recreational beach areas across Alberta in late spring and summer months from 2015 to 2022. All water samples were screened for MCs using the protein phosphatase inhibition assay (PPIA). Liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS) was used to analyze 1,298 water samples for MC congeners quantification. During the monitoring period, the PPIA results showed the average of 3.1% of composite and 28% of grab samples had MC concentrations exceeding 10μg/L (the Canadian Recreational Water Guideline), mostly in northern and central Alberta lakes from July to early September each year. Multiple MC congeners were detected in some water samples. MC-LR was found to be the most dominant and prevalent, followed by MC-LA, and dME-MCLR. Our results showed the geological distribution and the temporal trend of the MCs occurrence in Alberta recreational water during spring and summer months. This monitoring program will assist in the development of policies and procedures for safe use of beaches and better public health management.

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Expanded monitoring of cyanobacterial toxins in various water sources using a targeted LC/MS/MS analysis….More Toxins…More Information!

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Liquid Chromatography/Tandem-Mass Spectrometry (LC/MS/MS) is a powerful tool for the targeted analysis of various analytes in a wide variety of matrices. What is especially attractive about LC/MS/MS is its sensitivity and selectivity. Using a small particle (sub 2um particle size) column and a simple gradient method and that was developed for the original toxins (6 microcystins at the time, anatoxin and cylindrospermopsin) it has been expanded to include a variety of additional microcystins, variants of anatoxin and cylindrospermopsin as well as several dermatoxins, various anabenopeptins, euglenopyhycin, okadaic acid and others. We will show examples of the usefulness of additional toxin screening for specific cases where regular, traditional toxin, analysis may not have detected this. Currently this targeted method screens for over 30 compounds in a single 14-minute run. Results of sample analysis from a wide variety of lakes, rivers and ponds will be discussed using this expanded method with some interesting results found.

Cyanobacterial HABs

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The beta-lactamase activity at the community level confers beta-lactam resistance to bloom-forming *Microcystis aeruginosa* cells

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Many freshwater cyanobacteria including *Microcystis aeruginosa* lack many known antibiotic resistance (AR) genes, but both axenic and xenic *M. aeruginosa* strains exhibited high AR against many antibiotics including colistin, trimethoprim, and kanamycin under our tested concentrations. Interestingly, axenic PCC7806, not xenic NIBR18 and NIBR452 strains, displayed susceptibility to both ampicillin and amoxicillin, indicating that associated bacteria in the phycosphere could confer such AR to xenic strains. Fluorescence and scanning electron microscopic observations revealed their tight association, leading to possible community-level beta-lactamase activity. Combinatory treatment of ampicillin with a beta-lactamase inhibitor, sulbactam, abolished ampicillin resistance in xenic stains. The nitrocefin-based assay confirmed the presence of significant community-level beta-lactamase activity. The qPCR array analysis detected the presence of beta-lactam AR genes belonging to the *bla*OXA-51 or *bla*OXA-23 groups in the bacterial community. Our tested low concentration and high beta-lactamase activity might create a balance between the competitive advantage of dominant species and the opportunities for less competitive species, which resulted in higher bacterial diversity under ampicillin-treated conditions. Non-PCR based metagenome data of xenic NIBR18 culture also revealed the dominance of *blaOXA*-related AR genes followed by the *blaAST* and *blaFAR* genes. Alleviation of ampicillin toxicity could be observed only in axenic PCC7806 cocultured with beta lactamase-possessing other freshwater bacteria. Our study suggested *M. aeruginosa* develops resistance to old-class beta-lactam antibiotics through a form of altruism, where associated bacteria provide protection to axenic *M. aeruginosa* cells.

Cyanobacterial HABs

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Particle-attached and free-living bacterial communities in response to *Microcystis* blooms

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Globally, freshwater ecosystems are at risk, and water quality has declined due to the vast spread of *Microcystis*. Understanding the factors that promote *Microcystis* growth is essential for controlling *Microcystis* blooms. Bacterial lifestyles can be divided into two categories: particle-attached (PA; > 3 μm) and free-living (FL; 0.2 - 3.0 μm). However, nothing is known about how PA and FL bacteria react to *Microcystis* blooms. We studied the stability, assembly process, and co-occurrence patterns of PA and FL bacterial communities at diverse bloom stages using high-throughput sequencing of the 16S rRNA gene. We discovered that PA bacteria differed phylogenetically from their FL counterparts. Bacterial populations were significantly impacted by *Microcystis* blooms. According to the time decay relationship model, *Microcystis* blooms may increase the stability of both PA and FL bacterial communities. The PA and FL bacterial communities showed two different community assembly mechanisms. Homogeneous selection was the main assembly process that affected the PA bacterial community during *Microcystis* blooms, whereas drift accounted for the majority of the turnover of the FL bacterial population. Both the PA and FL bacterial populations could be divided into modules corresponding to various stages of *Microcystis* blooms. Functional analysis suggested that the PA bacterial community responds to *Microcystis* blooms more quickly than the FL bacterial community. Our findings underscore the necessity of investigating bacterial lifestyles in order to understand their roles in managing *Microcystis* blooms.

Cyanobacterial HABs
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Alleviation of H_2O_2 toxicity by extracellular catalases in phycosphere of *Microcystis aeruginosa*

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Unmanagable amounts of environmental H_2O_2 lead to a menacing challenge for catalase-less freshwater bacteria including toxic bloom-forming *Microcystis aeruginosa* to survive particularly under high-light conditions. The highest extracellular catalase activity-possessing *Pseudoduganella aquatica* HC52 was chosen among culturable 36 symbiotic isolates from the phycosphere in freshly collected *M. aeruginosa* cells. A zymogram for catalase activity revealed only one extracellular catalase out of four putative catalase-genes (*katA1*, *katA2*, *katE*, *srpA*) in the newly sequenced genome of *P. aquatica* HC52. Secreted catalase analyzed using a LC-MS/MS was found to be the leaderless KatA1 lacking a typical signal peptide although underlying mechanism for its secretion is unknown. Cell counting and fluorescence-activated cell sorting confirmed HC52 cell viability in our H_2O_2 condition, ruling out cell lysis as the cause of catalase release. The expression of secreted KatA1 protein appeared to be inducible in the presence of H_2O_2, and to a lesser extent, the extracellular outer membrane vesicles (OMV) were also observed to contain secreted KatA1. Transmission electron microscope and nanosight analyses demonstrated the presence of OMV of different sizes near cells, suggesting the potential release of catalase-containing OMV into the surrounding environment. High-light conditions could kill catalase-less axenic *M. aeruginosa* cells. Either *P. aquatica* cells or secreted catalases in the supernatant enabled the growth of *M. aeruginosa* to be sustained. Based on our findings, we propose that the release of extracellular catalase by *P. aquatica* HC52 enhances the tolerance of *M. aeruginosa* to H_2O_2, thereby facilitating the formation of *M. aeruginosa* blooms in high-light conditions.

Cyanobacterial HABs
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Unraveling the bacterial community composition and chemical space during a freshwater cyanobacterial harmful algal bloom using a multi-omics approach

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Cyanobacterial specialized metabolites contain a high degree of chemical diversity due to their non-ribosomal peptide synthetase and polyketide synthetase (NRPS & PKS) biosynthetic systems, and these metabolites serve as an important source in drug discovery. However, microcystins, peptidic cyanobacterial toxins produced during certain cyanobacterial harmful algal blooms (cyanoHABs), pose a threat to human and environmental health. Many other cyanotoxins including anatoxin and cylindrospermopsin threaten aquatic ecosystems due to their ability to bioaccumulate and contaminate clean drinking water. Together this suite of cyanobacteria toxins primarily impact the liver potentially causing hepatotoxicity and non-alcoholic fatty liver disease, but they can also impact vital organs including the kidney and the reproductive system. In this study, we utilized a multi-omics approach: metabolomics and genomics to reveal the bacterial species composition and specialized metabolite content during cyanobacterial blooms over a three-month period in a lake system in Providence, Rhode Island, USA. Extracts of bloom material were dosed against hepatocytes to better understand the toxicological profile and relevant human health impact from a multi-toxin event. This approach – examining community composition and toxin profile - will allow connections to be made between species presence, toxin presence, and cytotoxic bioactivity; ultimately helping with monitoring for contamination of drinking water and identifying any potentially new cyanotoxins.

Cyanobacterial HABs

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Spatiotemporal diversity of bloom-forming cyanobacteria throughout the Kissimmee chain of lakes (Florida, USA)

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Cyanobacteria are important members of the aquatic environment, as they are the base of the food chain, produce oxygen through photosynthesis, and are involved in nutrient cycling. Cyanobacterial community structure can vary spatially and temporally due to several factors, (e.g., temperature, light, nutrients, presence of aquatic vegetation). The aim of this study was to assess cyanobacterial community structure and determine environmental parameters that influence community structure and bloom-forming and/or toxic genera (e.g., *Microcystis*) in the Kissimmee Chain of Lakes, Lake Istokpoga, and northern Lake Okeechobee. Sampling occurred across seven lakes over seven outings. The community was determined using metabarcoding of the 16S rRNA. Limnological parameters (e.g., temperature, pH, and conductivity) were determined *in situ*, while chlorophyll-a was also determined in the lab. The cyanobacterial community was dominated by toxigenic bloom-forming cyanobacteria including *Aphanizomenon*, *Cuspidothrix*, *Dolichospermum*, *Microcystis*, *Raphidiopsis*, and *Sphaerospermopsis*, although many non-blooming forming taxa (e.g., *Cyanobium*) were also present. Cyanobacterial taxonomic richness increased throughout the Kissimmee Chain of Lakes, being the lowest in the northern region and increasing downstream throughout the lakes, being highest in Lake Okeechobee. There were significant differences in the cyanobacterial community structure between many of the lakes. In particular, cyanobacterial communities from Lake Okeechobee were significantly different than those within the larger lakes (i.e., Lake Kissimmee, Lake Tohopekaliga). These data highlight the cyanobacterial diversity within the headwaters of Lake Okeechobee, revealing a great abundance of toxigenic bloom-forming cyanobacteria present.

Cyanobacterial HABs

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Characterization of cyanobacteria and microcystins at recreational activity points in the Billings Reservoir (São Paulo-Brazil)

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Microcystins, the target of this work, are the most commonly found and studied cyanotoxins in the world. The main objective was to identify and quantify cyanobacteria and microcystins present in water samples from the Billings reservoir in places of recreational activities. The Billings reservoir is the largest reservoir in the metropolitan region of São Paulo and where some leisure activities are carried out, such as: fishing, swimming, and spots activities. Water samples were collected at four points in the reservoir: one point in Central Body I and three points in Central Body II, in regions with navigation, fishing, and bathing activities. Water samples were used to determine cyanobacteria composition, microcystin genes present in the cyanobacteria community by PCR, and microcystin quantification by Elisa test. The presence of cyanobacterial taxa was verified at all sampled points, and presence of taxa potentially producing microcystin in some: *Aphanocapsa*, *Dolichospermum*, *Microcystis*, *Planktothrix*, *Synechococcus*, *Synechocystis*, and *Woronichinia*. The mcyB gene was found in all analyzed samples, and the mcyE gene was not detected in just one sample. The presence of microcystins was detected in all analyzed samples and the concentration ranged from 0.22 µg/L to 5.42 µg/L. Although total microcystin concentrations are below the limits indicated by the World Health Organization for recreational activities, the high biovolume of cyanobacteria, including microcystin-producing taxa, may represent a risk to human health in these activities.

Cyanobacterial HABs

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The influence of environmental conditions on *Microcystis* abundance and colony size during blooms across North America quantified using a novel imaging technique

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Microcystis is the most cosmopolitan genus of HAB-forming cyanobacteria and can produce toxins that impact both humans and wildlife. *Microcystis* typically forms colonies that may facilitate bloom formation as colony formation facilitates surface water aggregation and larger colonies may permit more efficient nutrient cycling and resistance from grazing. While changes in temperature, nutrients, grazer pressure, and turbulence have been hypothesized as triggers that influence the size of *Microcystis* colonies, challenges associated with accurate and precise quantification of *Microcystis* colony sizes has limited the understanding of how these conditions truly impact colonies. Here, we describe the development of a novel, automated imaging process that quantifies *Microcystis* colony sizes and biovolume permitting an assessment of how environmental factors alter colony size distribution and biomass of *Microcystis* during HABs. Regarding zooplankton, we exposed *Microcystis* populations from several lakes across North America, including Lake Erie, to two species of *Daphnia*, *D. magna* and *D. pulex*. While *D. magna* and *D. pulex* were effective at grazing smaller sized colonies (~50 -100 µm diameter) of *Microcystis*, larger colonies (>100 µm) experienced little grazing pressure. Given that disaggregation of colonies has been considered as an approach to reducing *Microcystis* bloom impacts and intensity, our findings suggest such approaches may allow indigenous zooplankton communities to better control *Microcystis* blooms. Experiments involving the impact of nutrient availability and temperature on colony size distributions will also be discussed.

Cyanobacterial HABs

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Interspecific relationships between 2-MIB-producing and non-producing cyanobacteria in a brackish lake

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Musty odor outbreaks in water reservoirs and lakes are a major problem worldwide, resulting in economic losses for deodorization of tap water and deterioration of fishery product quality. In many cases, cyanobacteria are the primary producers of musty odor substances, and there is concern that climate change and eutrophication will enhance the growth of cyanobacteria, leading to increased occurrence of 2-Methylisoborneol (2-MIB). However, the concentration of 2-MIB, one of the main causes of musty odor, is not always high during cyanobacteria outbreaks, suggesting that 2-MIB production may be influenced by interspecific relationships among cyanobacteria. In this study, we used molecular ecological techniques to investigate the relationship between 2-MIB-producing and non-producing species in the cyanobacterial community of Lake Ogawara, a brackish lake in Aomori Prefecture, Japan. DNA metabarcoding of the 2-MIB synthase gene and isolation of cyanobacteria suggested that *Pseudanabaena* are the only 2-MIB producers in the lake. DNA metabarcoding analysis of bacterial community composition using 16S rRNA gene showed a positive but weak correlations between the proportions of cyanobacteria and *Pseudanabaena* in the bacterial community and the density of 2-MIB-producing *Pseudanabaena* in the lake water. This result suggests that the dominance of 2-MIB-producing cyanobacterial species is related to interspecific competition within the microbial community from the phylum level to the strain level.

Cyanobacterial HABs

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Cyanobacterial water blooms in Lake Biwa -revisiting 40 years of history

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In 1983, cyanobacterial water bloom first appeared in Lake Biwa, the largest lake in Japan and an ancient lake. For 40 years since then, cyanobacterial water blooms have appeared almost every year in the South Basin of Lake Biwa. The cyanobacteria that first formed water bloom in Lake Biwa were identified as *Anabaena macrospora* var. *robusta* and *Anabaena macrospora* var. *crassa* at that time. At least some of them are now identified as *Dolichospermum hangangense* and *Dolichospermum smithii*. Later, especially in 1985 and 1986, *Dolichospermum* affine became dominant in cyanobacterial blooms. *Microcystis*-dominant water bloom first occurred in Lake Biwa in 1987. The dominant species was identified as *M. aeruginosa* at that time, but it was probably *M. ichthyoblabe* according to the current taxonomic criteria. Cyanobacterial blooms dominated by *Microcystis novacekii* and *Microcystis wesenbergii* have also occurred in the oligotrophic North Basin of Lake Biwa since 1994. *Oscillatoria kawamurae* and *Aphanizomenon flos-aquae* came to bloom when submerged macrophytes flourished in the South Basin since the late 1990s. Since 2012, *Dolichospermum* species have become increasingly dominant again; *D. flos-aquae* often dominated, but *D. affinis* also dominated in some years. *Dolichospermum minisporum* was first found and immediately formed blooms in Lake Biwa in 2020. Cyanobacterial water blooms continue to occur in the South Basin despite declining nutrient levels. One possible mechanism for this is that previously unknown cyanobacteria species emerged from Lake Biwa one after another, and species suited to the environment of the time became dominant.

Cyanobacterial HABs

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Blooms and the diversity of the toxic Baltic cyanobacterium *Nodulatia spumigena* – from the past to the present

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Nodularia spumigena (Nostocales) has been reported in fresh, brackish, and marine waters worldwide. Global warming, high levels of nutrients, and the ability to fix nitrogen allow this species to dominate over other phytoplankton organisms. In consequence, almost every year in the Baltic, *N. spumigena* forms extensive water blooms. These events pose a serious threat due to the ability of the species to synthesise a potent hepatotoxin called nodularin (NOD). However, these cyanobacteria also produce other classes of bioactive nonribosomal peptides (NRPs) such as anabaenopeptins (AP), spumigins (SPU), aeruginosins (AER) and pesudoaeruginosins (NS). In the presented study, the strain-specific peptide profiles of the Baltic *N. spumigena* population were constructed; and the chemodiversity of the species was explored. The peptide profiles served as a tool to uncover the history of *N. spumigena*, but also to analyse the contemporary seasonal variations in the Baltic population. The study with the application of sediment cores allowed us to correlate the massive past bloom events with climate changes. Although we identified several subpopulations of *N. spumigena* in the Baltic, the obtained results indicated that throughout the millennia, but also in the present times the same two chemotypes (A and B) coexist in the Sea. We also concluded that the dominant subpopulation both in the past and present was *Nodularia spumigena* chemotype B as determined in previous studies.

Toxins, Biosynthesis and Detection Methods

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Mechanistic insights into a divergent PLP-dependent arginine cyclase from a toxic cyanobacterium

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Pyridoxal-5′ -phosphate (PLP) is a widely utilized cofactor that aids in enzyme-facilitated reactions, such as transaminations, epimerizations, and Claisen condensations to produce noncanonical amino acids (ncAAs). Recently, our collaborators and us discovered the biosynthetic pathway of the freshwater cyanotoxin, guanitoxin, from the cyanobacterium *Sphaerospermopsis torques-reginae* ITEP-024. This pathway includes the PLP-dependent cyclodehydratase GntC, which stereospecifically converts (*S*)-γ-hydroxy-L-arginine to L-enduracididine (L-end), a cyclic ncAA. Cyanobacterial L-end biosynthesis suggests a divergent mechanism from the previously characterized actinobacterial cyclic arginine biosynthetic strategies, such as those found in the mannopeptimycin, viomycin, and streptolidine biosynthetic pathways. We elucidate the GntC mechanism using *in vitro* enzyme assays involving a labeled isotopic synthetic substrate, and deuterium isotopic incorporation, revealing that deprotonations at the alpha and beta positions of the substrate occur, which allows for hydroxyl group elimination and subsequent guanidine cyclization. We additionally have employed site-directed mutagenesis to identify key residues needed for GntC catalytic activity. This project seeks to better understand biosynthesis in cyanobacteria, in addition to learning how cyanobacterial biosynthesis diverges from actinobacterial biosynthesis.

Toxins, Biosynthesis and Detection Methods

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Development of nontoxic saxitoxin enantiomers as reference materials

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Saxitoxin (STX), a potent neurotoxin, is one of the natural toxins biosynthesized by toxic dinoflagellates and then accumulated in shellfish via food chains. STX and its analogues in shellfish are monitored by local authorities in many countries by the mouse bioassay (MBA) and other chemical methods. STX is registered as a chemical warfare agent in Schedule 1 of the Chemical Weapons Convention (CWC). This regulation makes difficulties to replace the MBA with chemical analyses in some countries including Japan. Here, we aimed to facilitate chemical analyses by preparing calibration standards using nontoxic enantiomeric STX (*ent*-STX) with the same physicochemical properties as natural STX, based on the fact that the enantiomer of decarbamoylsaxitoxin (dcSTX) was nontoxic (Hong and Kishi, 1992). Enantioselectively synthesized *ent*-STXs (*ent*-STX and *ent*-dcSTX) were equivalent to natural STXs in terms of the calibration curves and the MS/MS fragmentations. Furthermore, the toxicities of *ent*-STXs by cell-based assay using Neuro2A cells and whole-cell patch clamp recording showed extremely low affinity to voltage-gated sodium channels on cells (Watanabe *et al*, 2022). In addition, *ent*-STX at a concentration of 100 mouse unit (MU)/ml did not show any symptoms in mice by the MBA. Finally, we tested *ent*-STXs using the immunochromatographic strips to successfully discriminate both toxins, natural STX and *ent*-STX. These results demonstrate that *ent*-STXs are nontoxic and can be safely used as reference materials of STXs in the routine shellfish monitoring programs.

Toxins, Biosynthesis and Detection Methods

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New azaspiracid analogues produced by *Azadinium spinosum* isolated from Japanese coastal waters

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Azaspiracids (AZAs) are one of marine toxins that cause food poisoning with a primary syndrome of diarrhea. They are produced by toxic dinoflagellates belonging to the genera *Amphidoma* and *Azadinium*. *Azadinium spinosum* is known to produce AZA1 and AZA2 as the dominant toxin. For investigation of AZAs produced by *A. spinosum*, which was first isolated from the coastal waters of Japan, the methanolic extract of the culture strain was subjected to LC/MS/MS. In positive ion mode LC/MS/MS, AZAs exhibit characteristic product ions on the cyclic amine side. Selected reaction monitoring analysis and precursor ion scan analysis were performed using product ions derived from the C23-C24 cleavage. Furthermore, AZAs were searched by neutral loss scan analysis of the mass spectra of successive dehydrations characteristic of cyclic polyether compounds. The detected ion peaks were identified using MS/MS spectra and compositional formulas. LC/MS/MS analysis revealed four compounds with the product ions derived from the C9-C10, C19-C20, and C23-C24 cleavages, which are characteristic product ions of AZAs. These AZAs produced product ions at m/z 348 or m/z 362 in the C23-C24 cleavage. Four AZAs were determined to be new analogues because their MS/MS spectra did not correspond to known AZAs. Three AZAs were analogues lacking one methyl group attached to the cyclic amine. This is the first report of *A. spinosum* producing AZAs other than AZA1 or AZA2 as the dominant toxins.

Toxins, Biosynthesis and Detection Methods

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Dual-emission ratiometric fluorescent sensor based molecularly imprinted nanoparticles for visual detection of okadaic acid in seawater and sediment

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A dual-emitting ratiometric fluorescence nanosensor based on novel molecularly imprinted polymers (MIPs), which possesses self-referencing ability by nitrobenzoxadiazole (NBD) as recognition signal and new carbon dots (CDs) as interior label, has been prepared for sensitive and visual detection of okadaic acid (OA) in seawater and sediment samples. The analyte can rapidly enhance the fluorescence intensity of NBD at 549 nm, but will not change the CDs' fluorescence signal at 667 nm. The nanosensor has a linear fluorescence enhancement response in the range of 100~1770 ng L^{-1} with a limit of detection of 25 ng L^{-1}, and excellent selective recognition for OA over its interferences. The ratiometric fluorescence nanosensor has been successfully applied to detect OA in seawater and sediment samples. The results show that the nanosensor has high recoveries at four spiking levels of OA ranged from 90.0 % to 107.0 % with the relative standard deviation less than 4.1 % (n=3). More importantly, a reliable visual sense change with two distinct color tonality from bright pink to yellow green can be easily differentiated by the naked eyes during fluorescence analysis, which demonstrates promising potential for the applications of molecularly imprinted sensors in trace analysis of marine environmental matrix.

Toxins, Biosynthesis and Detection Methods

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Broad screening of food and feed supplements for marine and cyanobacterial toxins

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Products of aquatic origin are broadly and increasingly used as dietary supplements for humans and as feed supplements for pets and cattle. The origin of these supplements is diverse, ranging from phytoplankton to seaweed, mussels, fish and krill. This diversity implies that these supplements could be contaminated with a wide range of phycotoxins, both originating from freshwater and marine systems. The aim of our study was to determine the presence of phycotoxins in food and feed supplements of aquatic origin. To this end, we performed a quick literature survey and found that some combinations of supplements and toxins are more intensively studied than others. Next, we optimized and validated our high resolution mass spectrometry (LC-hrMS) method in order to reliably detect both marine and cyanobacterial toxins in a wide range of supplements. Finally we screened over 80 food and feed supplements by LC-hrMS, using a database containing more than 1200 toxins for data processing. When needed and if possible, the LC-hrMS results were confirmed by tandem mass spectrometry (LC-MS/MS).

Toxins, Biosynthesis and Detection Methods

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Toxin analysis of *Kareniaceae* cultures isolated from the northern coastal waters in Japan

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Unarmored dinoflagellates in the Kareniaceae are known to be harmful to a variety of marine organisms due to their toxicity. In 2021, a red tide dominated by *Karenia selliformis*, along with a few occurrences of *K. mikimotoi*, *K. longicanalis*, and *Takayama* sp., caused the death of several fish and sea urchins along the Pacific coast of Hokkaido, Japan. In our present study, brevetoxins (PbTxs) and cyclic imines (CIs) in kareniacean culture strains collected from the Pacific coast of Hokkaido and Mutsu Bay in northern Japan were analyzed using selected reaction monitoring (SRM) liquid chromatography-quadrupole tandem mass spectrometry (LC/MS/MS). PbTxs and CIs were not detectable in the kareniacean cultures and the seawater samples collected from the causative red tide along the Pacific coast of Hokkaido in 2021. The *K. selliformis* strains isolated from Hokkaido in 2021 were found to be closely related to those isolated from the coast of Kamchatka and Chile based on molecular phylogenetic analysis. Interestingly, intraspecific strains from Kamchatka and Chile exhibited high toxicity to some marine organisms. On the other hand, *K. selliformis* cultured strain MoKr600 from the Mutsu Bay produced gymnodimine (GYM)-A and putative GYM-B/-C of CIs. This strain belonged to the same intraspecific phylogenetic group of *K. selliformis* that produces GYM in New Zealand. Our findings suggest that toxin production and intraspecific phylogeny are closely related in *K. selliformis*.

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Aplysiatoxin derivatives from an Okinawan toxic cyanobacterium *Okeania hirsuta*

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Aplysiatoxin and its derivatives have been isolated from the cyanobacteria *Lyngbya majuscula*, *Schizothrix calcicola*, *Oscillatoria nigro-viridis*, *Trichodesmium erythraeum*, and others. It had been reported that aplysiatoxin and its related compounds were the causative agents of severe skin irritation (Swimmer's itch) by accidental skin contact with these noxious cyanobacteria. Successive food poisoning cases caused by the ingestion of a red alga called *Gracilaria coronopifolia* were reported in Hawaii in 1994. The causative agents of these cases were identified as aplysiatoxin and its related compounds. It was demonstrated that the aplysiatoxins were produced by cyanobacteria growing on the red alga *G. coronopifolia*. These were the first reported cases of aplysiatoxin-related food poisoning. Toxic features of aplysiatoxins were explained by their prominent potency for protein kinase C (PKC) activation. It was revealed that aplysiatoxins bind directly to PKC and activate it. Regulation of PKC activity has been recognized as an important therapeutic strategy for various cancers. In a recent series of studies, it was shown that simplified analogues of aplysiatoxins are potential leads for cancer therapy. Therefore, aplysiatoxins are quite interesting compounds for their unique activities. In July of 2010, an outbreak of *Okeania hirsuta* (formerly we had misidentified as *Moorea producens*) occurred in Kuba Beach, Nakagusuku, Okinawa Prefecture, Japan and the responsible village office made people keep out of the beach to ensure the safety of swimmers. This outbreak of *O. hirsuta* lasted for almost one month. In this work, we used a sample collected at that same site and time. From this sample, 24 aplysiatoxin related compounds have been isolated and structure elucidated.[1-6] Fourteen out of 24 compounds were new aplysiatoxin derivatives. These results indicated this *O. hirsuta* sample was quite a chemically rich species. Furthermore, these new aplysiatoxin derivatives gave us a new insight on aplysiatoxins' biosynthetic pathway.

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Prevalence and distribution of phycotoxins in bivalves and phytoplankton from Beibu Gulf, South China Sea

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Phycotoxins produced by toxigenic algae can accumulate and migrate through food chains, potentially threatening human health and marine ecosystem safety. The toxin profile and spatial-temporal distribution of domoic acid (DA) and 11 lipophilic shellfish toxins (LSTs) were systematically analyzed in the bivalve mollusks from October 2018 to October 2020 and phytoplankton samples in August 2021 in Beibu Gulf, South China Sea. Neurotoxin DA was first detected in the mollusks from the investigative regions with a prevalence of 17.7%, peaking at 401 μg kg^{-1}. Cyclic imines (CIs) including GYM-A (46.6%) and SPX1 (15.8%) predominated the lipophilic phycotoxins in shellfish, peaking at 10.1 μg kg^{-1} and 19.6 μg kg^{-1}, respectively. During the investigative period, relatively higher levels of DA occurred in shellfish from March to August, while slightly higher contents of CIs in mollusks appeared in October and December. In the summer 2021, DA, PTX2, hYTX, and GYM-A were dominant in phytoplankton in the gulf. Occurrence and distribution of toxins in phytoplankton showed that the northern gulf suffered more phycotoxin pollution than the other areas in SCS. RDA analysis between phycotoxins and environmental variables showed that surface seawater salinity was the primary driver of toxin abundance with a highly significant negative correlation ($p<0.01$) in the summer. Meanwhile, a preliminary database of toxic and harmful algal species in Beibu Gulf has been established. This study summarized phycotoxin distribution, toxigenic sources and environmental drivers in specific areas, providing a scientific basis for prediction, early warning and ecological risk management of phycotoxins in Beibu Gulf.

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Isolation of GC toxins from dinoflagellate *Gymnodinium catenatum* cultured in the laboratory

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The dinoflagellate *Gymnodinium catenatum* is well-known for its production of PSP toxins. Recently, a new group of PSP toxins, known as hydroxybenzoate toxins (GC1~GC6), was identified in *G. catenatum*. These toxins exhibited relatively higher hydrophobicity compared to the three major PSP groups: carbamoyl, *N*-sulfocarbamoyl, and decarbamoyl toxins, due to the presence of the hydroxybenzoate unit. During the investigation of PSP toxins in Korean waters, the strain of *G. catenatum* isolated from the South Korean sea exhibited a higher concentration of GC toxins compared to the aforementioned three groups. However, the quantitative analysis of GC toxins was hindered by the absence of appropriated standards. Consequently, our primary objective was to isolate GC toxins from cultured *G. catenatum* in order to establish future standards. To achieve this, extensive cultivation of *G. catenatum* cells was conducted in the laboratory, followed by extraction using 0.1 *N* acetic acid and subsequent partitioning into water and methylene chloride. The aqueous layer was then fractioned using a C-18 cartridge, followed by LC-MS analyses. The fractions containing GC toxins were separated using a series of HPLC columns, including HILIC and polar C-18 columns. This poster aims to present the laboratory cultivation, fractionation, and separation procedures employed for isolating GC toxins.

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Seasonal changes in cell density of the genus *Amphidinium* (Dinophyceae) in the Mexican Pacific Transition Zone

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Epibenthic dinoflagellates are fundamental for capturing and transferring energy in different trophic levels of the marine environment. The genus *Amphidinium* is one of the most important groups and contributes significantly to phytobenthos assemblage worldwide. Studying the genus is vital due to its ability to synthesize ichthyotoxins and polyketides that could compromise the stability of the ecosystems they inhabit. In Mexico, recent studies indicated the presence of amphidinols in various *Amphidinium* strains in culture. Therefore, knowing population changes over time is essential. In this study, based on macroalgae samples, we describe the seasonal changes in cell densities of members of the genus *Amphidinium* on the coast of Acapulco, the Mexican Pacific Transition Zone. We found changes in cell density associated with local climate variability, particularly a significant increase during the rainy season (maximum of 5.53×10^3 cell g^{-1}). The average maximum density of *Amphidinium* was related to macroalgae of the genus *Centroceras* (Rhodophyta) in environmentally impacted areas. Salinity and dissolved oxygen were correlated proportionally to the increase in *Amphidinium* density.

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Neuroblastoma (cell-based) cytotoxicity assay: differential protein expression in cells lines based on sensitivity to ouabain and veratridine

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The neuroblastoma cell-based assay (N2a assay) is a sensitive, high-throughput, *in vitro* method for detecting voltage-gated sodium channel (Na_V)-specific toxins (e.g., brevetoxins (PbTx), ciguatoxins (CTX), tetrodotoxins, saxitoxins). The sensitivity and specificity of the assay to compounds activating the Na_V are achieved through the addition of the pharmaceuticals ouabain (O) and veratridine (V). However, these compounds impact the survival of N2a-cells, impacting the utility of the cell based assay. To overcome this drawback, N2a cells were exposed to O and V during growth incubation, and surviving cells exhibiting a lower sensitivity to O and V (OV-LS) were propagated. OV-LS N2a cells were 1.3–2.6-fold more sensitive for detecting Na_V activating marine biotoxins. The underlying difference between the original and the OV-LS cell line was investigated herein using a Western blot technique to determine the protein expression differences between the two cell lines. Cell lines were harvested in exponential growth phase and cell lysis was conducted to extract proteins from the adherent cells. Protein concentrations were measured using a spectrophotometer. Proteins were separated based on molecular weight through gel electrophoresis. Separated bands of proteins were transferred to a membrane. The membrane was incubated with several antibodies specific to the Na_V and ATPase. OV-LS cell lines had lower protein expression for a pan Na channel α subunit and higher protein expression for phosphor-Na K-ATPase $\alpha 1$/beta actin. This study demonstrates that the cell-line protein expression changes after incubation with OV. Based on this study, further improvements to this assay to increase the sensitivity for the detection of marine biotoxins is possible.

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Neurotoxic characterization of an extract obtained from the exudate of *Karenia selliformis* (CREAN_KS02)

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Karenia selliformis is a dinoflagellate globally associated with harmful algal events, and it has caused significant ichthyotoxic events in southern Chile. The toxin profiles of *K. selliformis* can vary depending on various factors, including geographical location, environmental conditions, and genetic diversity within the species. It is primarily associated with the production of neurotoxins known as gymnodimines (GYM). However, the analytical characterization of Chilean strains has not identified any of the expected toxins for this genus such as gymnodimines, brevetoxins, and brevenal. Due to the high mortality rates associated with this species in southern Chile, our study aimed to functionally characterize the neurotoxic metabolites present in the exudate of a culture of the CREAN_KS02 strain, isolated from the Aysén Region (43°S). The toxicity was evaluated on Neuro-2a cells, determining an IC50 of 1.4 μg of exudate extract per mL. Using a Neuro 2a cell-based assay (CBA) to activator toxin detection, a dose-dependent increase in mortality was observed, suggesting the presence of toxins that activate voltage-gated sodium channels. Electrophysiological measurements in cultured mouse hippocampal neurons showed that the extracts altered the function of electrically active cell membranes, exhibiting an effect opposite to that of paralytic toxins such as tetrodotoxin, resulting in a dose-dependent exacerbation of expected activator toxins. Our results suggest the presence of a new neurotoxic compound that is functionally excitotoxic and affects electrically active mammalian cells. Further characterization is necessary due to the potential implications for food safety.

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Nutrient availability may not directly modulate toxin production by *Ostreopsis* cf. *ovata*

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This study explored how N forms and inorganic P, supplied in different concentrations, affected *Ostreopsis* cf. *ovata* toxin content. During the 2018 natural bloom in the NW Mediterranean, the total toxin cell content (up to ca. 57.6 ± 7.0 pg toxin cell^{-1}) varied markedly, with the highest values often coinciding with elevated *O.* cf. *ovata* cell abundance and with low inorganic nutrient concentrations. In culture experiments with a strain isolated from that bloom, phosphate- and nitrate-deficient cells exhibited similar cell toxin variability patterns. In experiments with different nitrogen concentration and forms (nitrate, urea, ammonium, and fertilizer) the highest cellular toxin content were measured in the high-nitrogen availability conditions; among these, urea induced a significantly lower cellular toxin content than the other nutrient sources. Cell toxin content was higher in the stationary than in the exponential phase, under all nutrient conditions tested, i.e., with nutrient sufficient or limited media, high- and low-N concentrations, and independently of the N source. The toxin profile of the field and cultured cells included ovatoxin (OVTX) analogues -a to -g and isobaric PLTX (isoPLTX). OVTX-a and -b were dominant while OVTX-f, -g, and isoPLTX contributed less than 1-2%. Overall, the data suggest that although nutrients determine the intensity of the *O.* cf. *ovata* bloom, the relationship of major nutrient concentrations, sources and stoichiometry with cellular toxin production is not straightforward.

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Isolation, purification, and identification of secondary metabolites of *Coolia malayensis* with cytotoxic activity on human cancer cells

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Marine benthic dinoflagellates have received attention due to the production of bioactive compounds with unique structures and pharmacological potential. The genus *Coolia* comprises eight accepted species but is under constant revision. These benthic dinoflagellates are producers of compounds with potential biotechnological and biomedical applications. In the present study, the anticancer activity of crude extracts from *Coolia malayensis*, isolate CA24 from Veracruz, Mexico, were tested against human carcinoma cells *in vitro*. Cytotoxic activity of methanolic, aqueous, hydroalcoholic, and dichloromethane extracts was tested at a concentration of 100 µg mL^{-1} on different cancer cell lines: PC3 (prostate), HeLa (cervical), MCF7 (breast), and H1299 (lung). Immortalized keratinocytes (HaCat) were utilized as a control group. The hydroalcoholic and dichloromethane extracts showed the highest cytotoxic effect, with >35%, upon all cell lines, except HaCat; the methanolic extract was the least active, with >15% inhibition, and the aqueous extract had no significant cytotoxic effect on any of the evaluated cell lines. A white precipitate from the dichloromethane extract was analyzed with NMR^{1}H and GC-MS. The NMR spectrum showed a lipidic mixture, but the overlapping signals prevented the assignment of each compound. The GC-MS analysis confirmed the presence of fatty acids such as pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl ester (1), hexadecanoic acid methyl ester (2), 6,10,14-trimethylpentadecan-2-one (3), methyl stearate (4), and cholesta-4-6-die-3-ol (5). These results show the cytotoxic activity against cancer cells of these extracts without damaging normal cells, which is conducive to exploring further fractionation and the search for pure compounds with pharmacological potential.

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Branched-chain fatty acids in 7-O-acyl okadaite esters from bivalves

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The okadaites, a group of polyether compounds involved in the human syndrome diarrhetic shellfish poisoning (DSP), include three major parent toxins (okadaic acid and the analogues DTX1 and DTX2) which in bivalves are mostly esterified at C7 with fatty acids (FA). Most of these esters include common saturated and unsaturated FA. Others involve odd FA and multi-branched FA. The major unidentified 7-O-acyl esters with the okadaites in Portuguese bivalves were confirmed here, by high-resolution mass spectrometry (HRMS), to present a side chain with the same exact mass as the OA and DTX2 palmitoyl esters (isopC16:0). A common chain with this exact mass, and abundant in the marine environment, is 4,8,12-trimethyltridecanoic acid (TMTD), an isoprenoid derived from chlorophyll degradation. Although all bivalves assayed contain the isopC16:0 ester, the unknown peaks were more abundant in sand-dwelling bivalves (clams, cockles) than in suspension-feeding bivalves (mussels). In addition to the high abundance of isopC16:0 combined with OA and DTX2 in natural samples, the isopC16:0 ester was also obtained by feeding bivalves with *Prorocentrum lima* containing DTX1. If this metabolization is attributable to the bivalves themselves, to a particular bacterial flora in sand-dwelling bivalves, or both, is a question that remains open.

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Suspect screening of bioaccumulative halogenated natural products in Japanese seabass by GC×GC/HRToFMS

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Several halogenated natural products (HNPs), which have similar chemical structures to those of persistent organic pollutants (POPs), are known to be biosynthesized by marine algal and/or bacterial species living in the coastal environment. These HNPs may possess environmentally persistent and bioaccumulative properties like POPs, but information on the exposure of Japanese marine fish to multiple HNPs are still lacking. The present study screened bioaccumulative HNPs in Japanese seabass (Lateolabrax japonicus) by comprehensive two-dimensional gas chromatography/high-resolution time-of-flight mass spectrometry (GC×GC/HRToFMS). Screening analyses by GC×GC/HRToFMS revealed the accumulation of not only anthropogenic contaminants such as POPs and their structurally related compounds but also HNPs in the muscle tissues of Japanese seabass. Two abundant HNPs, 6-methoxy-2,2′,4,4′-tetrabromodiphenyl ether (6-MeO-BDE-47) and 2,3,3′,4,4′,5,5′-heptachloro-1′-methyl-1,2′-bipyrrole (MBP-Cl_7), were observed on the total current ion chromatograms (TICCs) and identified by comparing with the retention times (${}^{1}t_R$ [min] and ${}^{2}t_R$ [s]) and mass spectrum (m/z) of their reference standards. Considering that Japanese seabass is a carnivorous fish, the species could be chronically exposed to bioaccumulative HNPs via the marine food chains.

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Evaluation of toxicity equivalency and cross-reactivity factors of tetrodotoxin analogues and applicability in the analysis of puffer fish

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Tetrodotoxin (TTX) is one of the most potent marine neurotoxins, responsible for many poisoning accidents and some fatalities. More than 30 TTX analogues have been described, which can contribute, in more or less degree, to the toxicity of samples. Therefore, elucidation of toxicity equivalency factors (TEFs) of TTX analogues is crucial to translate the TTX analogues contents obtained from instrumental analysis techniques, such as liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), into toxicological determinations. Additionally, immunoassays can also be used for the detection of TTXs in samples. However, since they are based on structural determination, elucidation of the cross-reactivity factors (CRFs) is crucial to evaluate their utility to guarantee food safety. Therefore, in this work, several TTX analogues (11-norTTX-6(*S*)-ol, 11-deoxyTTX, 5,11-dideoxyTTX, 6,11-dideoxyTTX and 5,6,11-trideoxyTTX) were obtained and purified from the liver of a toxic pufferfish (*Lagocephalus sceleratus*) from Greece. The TEFs of the TTX analogues were determined with two techniques: a colorimetric cell-based assay (CBA) and an automated patch clamp system, both using Neuro-2a cells. Additionally, the CRFs of the TTX analogues against an anti-TTX monoclonal antibody were evaluated with a competitive magnetic bead-based immunoassay. The application of the TEFs and CRFs to the individual TTX analogues contents in five tissues (gonads, liver, intestinal tract, skin and muscle) of three puffer fish individuals obtained with LC-MS/MS analysis, and comparison of the results with those from the cell-based systems and the immunoassay, respectively, have provided excellent correlations among techniques and contributed to have a wide range of trustworthy analytical tools for TTXs detection.

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Receptor-binding assays for cyclic imine toxins detection on shellfish extracts, an interlaboratory study

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CiTXs are potent antagonists of human nicotinic acetylcholine receptors that are able to cross the intestinal, placental and brain-blood barriers. Pregnant women and patients suffering from neuromuscular junction autoimmune disorders may be particularly vulnerable to these family of neurotoxins. We performed an interlaboratory study to assess the suitability of the microplate-receptor binding assay and the strip test NeuroTorp for the detection of CTXs in shellfish samples. Four laboratories participated in this study: USC, Lugo, Spain, AZTI, Derio, Spain, MicrobiaEnvironment, Argelès-sur-Mer, France and CEA, Saclay, France. ALERTOX-NET partners provided the shellfish samples while CEA provided the detection kits for the study. CiTXs were extracted from shellfish samples and processed for in-vitro and on-site detection. The functional methods we developed can detect all types of CiTXs as a class. The nature of the CiTXs that contaminated the shellfish samples was depicted by UPLC-MS/MS using 8 different CiTX standards. The most frequent contaminants found in this study were 13-desmethyl spirolide-C and pinnatoxin-G. The presence of portimine-A was confirmed in shellfish samples collected in South France. Since cyclic imine toxins are not regulated further monitoring of these emerging toxins is needed to improve evidence gathering of their occurrence in shellfish commercialized for human consumption in Europe.

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A new IOC-UNESCO database on toxins, their chemical and bioactivity characteristics and links to the causative organisms

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Since 2020, a new database has been developed as part of the *Harmful Algal Information System* suite of the IOC UNESCO. The database is supported by a large editorial team, i.e. the Task Team Toxin on Detection, Management and Regulation of the IOC UNESCO's Intergovernmental panel on Harmful Algal Blooms. The database captures toxins known to date produced by microalgae and cyanobacteria relevant to the marine environment. Each toxin is affiliated to a chemically closely related group and the database record contains sections on chemical characteristics, e.g. structures, methods of analysis and regulatory status, producing organisms and potential vector organisms as well as the bioactivity of the toxin. Here we present the 18 toxin groups and some example records.

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Glyco-azaspiracids, novel toxins produced by *Azadinium poporum*

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Classical analytical methods in combination with advances in untargeted metabolomic analyses have dramatically increased our knowledge of the structural diversity of toxins produced by harmful marine microalgae in recent years. However, the enormous genomic complexity of dinoflagellates in addition to chemical analytical blind-spots suggests that the majority of marine toxins remains unidentified. Here, we used a comparative metabolomic analyses to screen extracts from two species of the genus *Azadinium* (*A. spinosum* and *A. poporum*) for azaspiracids (AZAs). AZAs are lipophilic toxins that cause azaspiracid shellfish poisoning (AZP). AZP has been a health hazard in Europe (i.e., *Azadinium* blooms in the North Sea) for many years, but AZAs were also found in coastal waters of the Pacific USA, the Mediterranean, the Atlantic Ocean and Indian Ocean. We found strong differences in the ionization efficiencies and detection thresholds for AZAs when comparing targeted detection on a triple quadrupole (TQMS), a hybrid quadrupole-Orbitrap (HRMS) and a high-resolution time-of-flight (QTOF) platform. Additionally, the QTOF and HRMS both successfully found AZAs through the untargeted data-dependent approach but differed in the assignment of the parental molecules due to differences in in-source fragmentation. The comparative approach led to the discovery of large (>1300 dalton) glyco-AZAs that were found in *A. poporum* (ribotype A2) originally isolated from Denmark and France. The subsequent mass-culturing of *A. poporum* produced sufficient toxin to structurally elucidate the novel AZA. Our approach highlights the advantages of comparative high-resolution analyses of dinoflagellate metabolites for the characterization of toxin chemical diversity.

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Development of a cyanobacterial matrix certified reference material for multiple classes of cyanotoxins

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Some cyanobacterial dietary supplements are prepared using biomass harvested from lakes and ponds where multiple species co-occur. This can result in the contamination of products with cyanotoxins that present a risk for the consumer. Reference materials are therefore required to assist in the development, validation and quality control of analytical methods used to assess raw material and for product testing in both research and regulatory environments. Here, we report the development and characterization of a cyanobacterial matrix certified reference material (CRM) for multiple classes of cyanotoxins. The CRM was prepared by blending biomass from a variety of toxic cyanobacterial strains with a typically-consumed non-toxic strain (*Aphanizomenon* sp.). The freeze-dried, homogenized material was aliquoted into over 2000 identical units and characterized for toxin concentration, homogeneity and stability using multi-class extraction and LC-MS methods. A suite of class-specific methods was optimized and used to assign certified values to toxins for which CRM calibration solutions are available, using a combination of isotope dilution, standard addition and external standard calibration. These include five microcystins (MCs), eight saxitoxins (STXs), nodularin-R, anatoxin-a and cylindrospermopsin, with preliminary concentrations ranging from 1 to 35 µg/g dry weight. Finally, non-target analysis high-resolution mass spectrometry methods will be used to characterize the rich profile of over 50 non-certified cyanotoxins and other cyanobacterial secondary metabolites present. The CRM will be valuable for use in quality control of dietary supplement testing methods, but can also play a role in a broad range of cyanotoxin measurement applications.

Toxins, Biosynthesis and Detection Methods

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Differential and co-expression analysis of toxic and non-toxic strains of the marine dinoflagellate *Alexandrium minutum*

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Saxitoxin, a neurotoxic alkaloid produced by cyanobacteria and dinoflagellates, poses potential risks to public health and local economies. However, the molecular basis of saxitoxin production in dinoflagellates remains poorly understood. Here, saxitoxin production in *Alexandrium minutum* was characterized by comparing toxic and non-toxic strains at the genetic and systemic levels. A total of 52 publicly available RNA-seq samples originally designed for a genetic linkage study were used to investigate transcriptome-level differences associated with saxitoxin production. Differential expression analysis showed upregulation of several transcripts in toxic relative to non-toxic strains. Notably, this included three transcripts homologous to *sxtA1*, *sxtS*, and *sxtI* – genes known to be directly involved in the saxitoxin biosynthesis pathway in *C. raciborskii*. Additionally, several transcripts homologous to ion channels were also found to be upregulated in toxic strains. Network analysis showed two (ME52 and ME66) and three modules (ME189, ME133, and ME146) that were positively and negatively correlated with the toxicity phenotype, respectively. Analysis of the hub transcripts in the positively correlated modules showed terms related to ion homeostasis, cellular reproduction and development. For negatively correlated modules, hub transcripts were associated with cell cycle, cell response to different stressors, cell-cell signaling and interaction. Overall, this study provides new insights on the possible underlying molecular mechanisms of saxitoxin production in dinoflagellates.

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Variation in the growth and toxin production of *Gymnodinium catenatum* under different laboratory conditions

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The chain-forming dinoflagellate *Gymnodinium catenatum* is the only known gymnodinioid dinoflagellate that produces paralytic shellfish toxins (PST). Dense blooms caused by the dinoflagellate have been frequently reported in coastal waters of Fujian, China since 2017. While there is still limited understanding of the major physiological characteristics of *G. catenatum* isolated from Fujian coastal waters, the growth and toxin production of the *G. catenatum* strain were examined in batch cultures with different levels of irradiance, temperature, salinity, nitrate, and phosphate conditions. The results indicated that the highest maximum cell density of the strain was achieved at 70 μmol m^{-2} s^{-1}, with the highest growth rate at 120 μmol m^{-2} s^{-1}. The strain grew well within the temperature range of 15 - 30 °C, with maximum growth rate and cell density achieved at 20 °C. The dinoflagellate also showed higher tolerance to salinity variation (20 - 40), with the highest growth rate at salinity 25. Meanwhile, *G. catenatum* showed higher demand for nitrogen and phosphorus as indicated by its higher half-saturation constant. A decrease in nitrate and phosphate greatly inhibited the growth of *G. catenatum*. The toxin profile of the *G. catenatum* strain was conservative and dominated mainly by the N-sulfcarbamoyl C-toxins (>95 %), indicating its hypotoxicity. The cellular toxicity increased with the algal growth, with the highest cellular toxicity observed at the stationary growth phase. The cellular toxicity of *G. catenatum* also responded to environmental variations including lower temperature (15 °C), lower salinity (20), nitrate-repletion, and phosphate-depletion conditions which enhanced the cellular toxicity, while irradiance exerted non-significant influence. The present study depicted the physiological characteristics of the particular *G. catenatum* strain and provided valuable insight on the ecophysiology of *G. catenatum* in natural coastal waters.

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Metabolomic and transcriptomic characterization of the ability to produce PST in *Alexandrium minutum* using recombinant progeny

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Paralytic Shellfish Toxins (PST) are produced by certain species of cyanobacteria and dinoflagellates. Part of the PST biosynthetic pathway has been elucidated in cyanobacteria, and the implication of certain *sxt* genes has been confirmed by experimental studies. Many studies have identified potential homologs of *sxt* genes in dinoflagellate transcriptomes, linking their expression or gene copy number to the toxic phenotype. Other studies have shown that certain cyanobacterial biosynthetic intermediates are present in toxic strains of *Alexandrium*. However, there are no studies showing the link between *sxt* genes and metabolites involved in PST synthesis in *A. minutum*. We used a family of *A. minutum* strains, resulting from a recombinant cross between two toxic x non-toxic parent strains to identify genes and metabolites produced during toxin synthesis. By conjointly analysing metabolomic and transcriptomic datasets, we confirmed that the expression of the *sxtI*, *sxtG* and long-form *sxtA* genes correlated with the toxic phenotype in *A. minutum*, as did a domain analogous to *sxtS*, and other unidentified genes. In addition, we detected a number of metabolites correlated with the toxic phenotype. Among them, a metabolite with a mass-to-charge ratio m/z 236.1139 (-MS), which could not be identified, was strongly correlated to this phenotype. Finally, Int-C', a synthesis intermediate involved in PST synthesis in *A. tamarense* and toxic cyanobacteria, could also be involved in toxin production in *A. minutum*. Our results are consistent with parts of the biosynthetic pathway already identified in dinoflagellates and cyanobacteria, and we present new candidates, potentially specific to *A. minutum*.

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Effects of lipophilic phycotoxin okadaic acid on the early development and transcriptional expression of marine medaka *Oryzias melastigma*

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The lipophilic okadaic acid (OA)-group toxins produced by some species of *Dinophysis* spp. and *Prorocentrum* spp. marine dinoflagellates have been frequently and widely detected in natural seawater environments. Effects of dissolved OA on the embryonic development and 1-month old larvae of marine medaka (*Oryzias melastigma*) were explored and discussed in this study. Significantly increased mortality and decreased hatching rates occurred for the medaka embryos exposed to OA at 1.0 μg/mL. Diverse malformations were also observed in the embryos exposed to OA and the heart rates significantly increased at 11 d post fertilization. The ROS and CAT enzyme activity were significantly increased in 1-month old larvae, and AChE activity was significantly increased with a dose-dependent pattern. Differentially expressed genes (DEGs) were enriched in 11 KEGG pathways with Q value < 0.05 in 1-month old medaka larvae exposed to OA at 0.38 μg/mL (1/10 LC_{50}) for 96 h, which were mainly related to cell division and proliferation, and nervous system. Most of DEGs involved in DNA replication, cell cycle, nucleotide excision repair, oocyte meiosis, and mismatch repair pathways were significantly up-regulated, while most of DEGs involved in synaptic vesicle cycle, glutamatergic synapse, and long-term potentiation pathways were markedly down-regulated. This transcriptome analysis demonstrated that a risk of cancer developing was possibly caused by OA due to DNA damage in marine medaka larvae. In addition, the neurotoxicity of OA was also testified for marine fish, which potentially cause major depressive disorder (MDD) via the up-regulated expression of *NOS1* gene.

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Crassostrea virginica early life stage survival and toxin accumulation after exposure to different *Dinophysis* species

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The impact of lipophilic phycotoxins on the viability of oyster larvae is under investigation due to the need for sustainable aquaculture in a changing climate. This study aimed to explore the effects of different toxigenic *Dinophysis* species on the survival and toxin accumulation in 7-day-old oyster larvae. Four different bioassays were conducted using live *Dinophysis* cells, cell lysate, dialysis separation, and purified toxins to represent realistic toxin concentrations in both hatcheries and natural environments. After 48 hours, the mortality rate of the larvae and toxin levels in the larvae and surrounding environment were assessed. The highest mortality rates were observed in bioassays with live *D. caudata* (28%) and *D. fortii* (25%) cells, which also produced the highest concentrations of hydroxyPTX2 and PTX2, respectively. In contrast, bioassays using lysate and dialysis methods did not cause significant mortality compared to control treatments. *D. acuminata*, which produced lower amounts of PTX2, *D. ovum*, which only produced okadaic acid (OA), and exposure to purified OA had no discernible impact on larval survival. However, exposure to pure PTX2 significantly reduced larval viability, albeit to a lesser extent than exposure to live *D. caudata* and *D. fortii* cells. These findings suggest that the increased mortality rates in oyster larvae can be attributed to direct contact with live *Dinophysis* cells that produced higher concentrations of PTXs or other active compounds. Therefore, the presence of live *D. fortii* and *D. caudata* cells in the oyster larvae's diet can severely affect their survival, leading to significant economic and environmental repercussions.

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Portimine from *Vulcanodinium rugosum* induces severe pyroptosis in primary skin cells through ribotoxic stress response (RSR)

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Vulcanodinium rugosum is a marine dinoflagellate known for producing cyclic imine toxins: pinnatoxins (PnTXs) and portimines. Recently, blooms of the dinoflagellate have been reported worldwide, associated with cases of acute dermatitis in bathers and fishermen. Due to its cytotoxicity in various cell lines, portimine A has been strongly suspected of being a causative agent. This study aims to characterize the activity of dinoflagellate extracts and purified toxins on human skin cells. Immortalized human keratinocytes (HaCaT) were used to assess cytotoxicity, while A549-ASC-GFP reporter cells were employed to evaluate the inflammatory response through the activation of the NLRP1 inflammasome. While significant cytotoxicity and NLRP1 activation were obtained for extracts and portimine A, no such effects were observed for PnTXs. Using different inhibitors of MAP kinase-driven phosphorylation (PLX4720, SB203580, bortezomib, and selonsertib), the action of portimine A on NLRP1 was then investigated. Portimine A triggered a ribotoxic stress response, leading to the activation of the stress kinases Zakalpha and, subsequently, P38 and JNK. This kinase cascade ultimately results in the activation of the NLRP1 inflammasome, leading to pyroptotic cell death and severe inflammation, with the release of IL-1β/IL-18 cytokines (confirmed by ELISA on primary keratinocyte supernatants). Additionally, portimine A was tested on a collection of knockout yeasts, and the 60S ribosomal export protein NMD3 was identified as a potential target of the toxin. This study provides a comprehensive understanding of the mechanism of action of portimine A, i.e. inflammation through ribotoxicity, clarifying its likely role in the reported cases of *Vulcanodinium*-related dermatitis.

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Toxicity and depuration of Okadaic acid in mice exposed by voluntary feeding

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Diarrhetic Shellfish Toxins' (DSTs) worldwide distribution comprises a constant threat to consumers health as well as negatively impact the economy of seafood-farming areas. Okadaic acid (OA) group of toxins comprise a recurrent cause for harvesting banning in some European coasts due to the frequency of harmful algal blooms. The legal limit is of 160 μg OA equivalents/kg shellfish meat, meaning seafood products with toxin concentrations under that cut-off, may reach the consumer. Diarrhetic Shellfish Poisoning symptoms can develop as early as 30 min after shellfish contaminated with DSTs, lasting for 2-3 days. Data of toxin distribution in the body and clearance of these toxins remain limited. Therefore, this work aims to study in mice the pharmacokinetic of a single dose of OA for 5 days by using Certified Reference Material. In order to minimize mice stress as well as keep a resemblance with human intoxication, toxin administration was by voluntary feeding. To do so, animals were habituated to the food for a week to ensure full dose ingestion. These preliminary results showed time dependent decrease in toxin concentration and clinical signs comprising mainly diarrhea. No deaths or relevant changes in body weights or necropsy findings were observed. This information is of interest to determine the risks of low-level exposure scenarios.

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14 day-Neurotoxicity evaluation of Brevetoxin 3

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Brevetoxin 3 (PbTx3) is the analog mostly found in shellfish meat, which can lead to Neurotoxic Shellfish Poisoning. Monitoring programs' development and/or changes in microalgae physiology/distribution dynamics, detection of emerging toxins like PbTxs in non-previously detected areas have risen concerns regarding lack of legislation and consumers health. Brevetoxins act on voltage-gated sodium channels site 5 facilitating the firing and resulting in neurological symptomatology. The severity of Neurotoxic Shellfish Poisoning can vary from paresthesia, vertigo, muscle weakness and ataxia to seizures and coma in most severe cases. Still no deaths have been reported in humans. Our aim was to evaluate the neurotoxicity induced by PbTx3 in mice following the Test n° 424 guideline from the Organization for Economic Co-operation and Development (OECD). After a single administration of the toxin, we have evaluated symptomatology, and performed functional tests the day prior administration, at 7 and 14 days after treatment. As a result, we observed that the highest tested doses developed neuromuscular symptoms shortly after, whereas they were not observed at 24 h. No differences between males and females regarding functional tests or symptoms were detected. Hence, ataxia and muscle weakness should be considered as reversible adverse effects in humans intoxicated by ingestion of seafood with PbTxs, suggesting an acute poisoning with neurological symptoms and fully recovery rather than long lasting signs, though further research to ensure this premise should be carried out.

Toxicology

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Toxicological investigations of emerging cyanotoxins and cyanopeptides on fish cell lines

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With a high variability of taxa and bioactive secondary metabolite contents, cyanobacteria become a widespread problem that tends to worsen over time. *Microcystis* is one of the most commonly occurring genus of cyanobacteria in freshwaters worldwide. While there are numerous ecotoxicological studies on its cyanotoxin toxicity, especially microcystins, the effects of other secondary metabolites such as cyanopeptides are still understudied. Moreover, the hepatotoxicity induced by microcystins has been firmly established, but their recently highlighted neurotoxicity and immunotoxicity still need to be characterized. This study aims to evaluate the effects of various cyanopeptides (microginins, aeruginosins, or microcyclamides) on a freshwater model and to compare their levels of toxicity with the well-characterized microcystin-LR. Common carp brain (CCB) and leukocyte (CLC) cell lines were exposed for 24 and 48h to bloom-cultured extracts of *Microcystis* producing either microcystins or aeruginosins with few microginins or microcyclamides. The analysis of cell mortality was performed by flow cytometry. Exposures to purified microcystin-LR and *Microcystis* biomass extracts producing only microcystins revealed cytotoxic effects on CCB with EC50 values estimated at 148.05 mg.L^{-1} and 782.32 mg DW.L^{-1}, respectively. Exposure to a biomass extract (660 mg DW.L^{-1}) of the *Microcystis* strain producing mostly aeruginosins and few microginins showed higher toxicity in CCB than exposure to an equivalent biomass extract (700 mg DW.L^{-1}) of the strain producing only microcystins (eq 10 mg MC.L^{-1}). Our results indicate the toxicity of some emerging *Microcystis* secondary metabolites, that need to be further assessed.

Toxicology

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Apoptosis and oxidative stress of mouse breast carcinoma 4T1 and human intestinal epithelial Caco-2 cell lines caused by gymnodimine-A

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Gymnodimine-A (GYM-A) is an emerging phycotoxin produced by dinoflagellates that has been frequently reported in shellfish. This toxin class is known as “fast-acting toxin” due to causing rapid death on mice injected intraperitoneally. However, it was unexplored about its cytotoxicity. In this study, four different cell lines were employed in the viability assessment of GYM-A-treated cells by MTT assay. Results showed that GYM-A was cytotoxic in concentration dependent manner to each cell type tested, with mean IC_{50} values ranging between 1.39-2.79 μmol L^{-1}. Generally, the normal cell lines (3T3 and L929 cells) were more sensitive to GYM-A than tumor cells (Caco-2 cells), but breast cancer 4T1 cells were most sensitive to GYM-A. Results of Annexin V-FITC/PI staining suggested that the loss of cell viability was attributed to the apoptosis in 4T1 and Caco-2 cells. Furthermore, the collapse of mitochondrial membrane potential and caspases activation were observed in GYM-A-treated cells, further indicating that apoptosis was involved in the cytotoxicity of GYM-A. Reactive oxygen species (ROS) and lipid peroxides (LPO) levels were markedly increased in 4T1 and Caco-2 cells exposed to GYM-A of 2 μmol L^{-1}. Additionally, unusual ultrastructure impairment on mitochondria and mitophagosomes were found in GYM-A-treated cells. These results suggested that an ROS-mediated mitochondrial pathway in apoptosis and mitophagy was implicated in the cytotoxic effects induced by GYM-A. This is the first report to explore the cytotoxic mechanisms of GYM-A through apoptosis and oxidative stress, and it will provide theoretical foundations for the potential therapeutic applications of GYM-A.

Toxicology

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Effects of pharmaceutical antibiotic macrolide Clarithromycin on cosmopolitan benthic dinoflagellate *Amphidinium carterae* (Genotype 2)

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Amphidinium carterae (Genotype 2) is a cosmopolitan benthic dinoflagellate that can be found on various substrates in the marine environment. *A. carterae* is toxic to some marine organisms and it is also the producer of bioactive compounds like Amphidinolides, a compound of macrolide and Amphidinols. They have properties like antitumor, antibacterial, antifungal, and anti-diabetes, making them potentially important to the pharmaceutical industry. Clarithromycin is one of the widely used pharmaceutical macrolide antibiotics. High demand for this antibiotic and the waste causing accumulation of Clarithromycin in the marine environment. Clarithromycin was found to be harmful to microalgae. There is a lack of knowledge regarding the effects of Clarithromycin on benthic dinoflagellate *A. carterae* especially their survival and their production of bioactive compound productions including algal toxins. This study aims to investigate the effects of *A. carterae* exposing CLM at various environmental concentrations over a long-term exposure with 3 generations. This is the first report on the effect of Clarithromycin on benthic dinoflagellate *A. carterae* isolated in Hong Kong waters. *A. carterae* strain named SKLMP_Ve021 (Genotype 2), was exposed to six concentrations of CLM ranging from 0.01 to 1000 ng/L, and the control groups (one with 0.05% acetone as the solvent control and the other one without addition of Clarithromycin and acetone) for three generations. Each generation was collected at its exponential phase. Endpoints of morphological and physiological changes, analysis of antioxidant responses and other enzyme activities that affect the algal survival, and the changes in CLM concentrations in algal culture medium, as well as intracellular and extracellular portions of *A. carterae* were examined. Production of bioactive compounds including biotoxins produced by algal cells after exposures will be determined by UPLC-Q-TOF-MS/MS. Algal cells exposed to the two highest concentrations of Clarithromycin, 100 and 1000 ng/L, survived for only two generations, with only 5-8 % (n=4) cell density of the control group without any addition of Clarithromycin and solvent. Decreased maximum photosynthetic efficiency and some variation of enzyme activities were observed as the exposure concentration increased. More results regarding Clarithromycin concentration in the intracellular, extracellular, and cultured medium as well we the production of bioactive compounds and biotoxins will be obtained soon. This study provides a better understanding of the effects of Clarithromycin on cosmopolitan benthic dinoflagellate *A. carterae*, highlighting the threats of emerging pharmaceutical contaminants to benthic microalgae.

Toxicology
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Long-term study of domoic acid in the population of warty venus from a semi-enclosed bay in the Middle Adriatic Sea

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Domoic acid (DA) is the amino acid that causes Amnesic Shellfish Poisoning (ASP). Concentrations of domoic acid that cause poisoning in humans, manifested by gastrointestinal and neurological symptoms, are above the MDK (20 mg DA eq./kg). Domoic acid, like other marine toxins that accumulate in shellfish, is thermostable and cannot be removed during preparation. Therefore, continuous monitoring of domoic acid in shellfish is very important. The research station for domoic acid in natural populations of the warty venus, *Venus verrucose* (Linnaeus, 1758) is located in the semi-enclosed Kaštela Bay in the middle of the Adriatic Sea. Kaštela Bay is an area of relatively high primary production. Anthropogenic and natural influences contribute to this characteristic. The bivalve warty venus lives throughout the Adriatic Sea, mainly on sandy and muddy bottoms, feeding on plankton and usually living buried in the sand. The content of domoic acid in the soft tissue of the warty venus was determined by liquid chromatography with mass spectrometry (LC-MS /MS), Agilent Technologies Triple Quad. The analyzed warty venus samples were collected once a week for 5.5 years (January 2018 - June 2023). The percentage of samples in which the concentration of domoic acid is below the detection limit is 94.6%, and the range of measured domoic acid in 5.4% of the samples is 0.16 - 13.30 mg DA eq./kg. Domoic acid was determined in samples collected during the winter months of 2018, 2019, and 2023.

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Change in the profile of paralytic shellfish toxins (PSTs) in zooplankton during toxic dinoflagellate *Alexandrium* emergence during spring season

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Investigated Paralytic shellfish toxin (PST) intoxication in the zooplankton near Jinhae Bay following the emergence of the toxic dinoflagellate *Alexandrium* in the spring. The suspended matter of *Alexandrium* contained PST toxin that showed an increasing trend before decreasing with rising water temperatures. Zooplankton samples collected in January initially contained low levels of PST toxin, which gradually increased reaching a peak in March. The correlation between the toxin content of zooplankton and *Alexandrium* cell density in suspension ($r=0.70$; $p<0.05$; $n=35$) and that correlation between the toxin content of zooplankton and *Alexandrium* toxin content ($r=0.75$; $p<0.05$; $n= 32$), indicated that PST toxification in zooplankton was caused by *Alexandrium* in seawater. The predominant toxins in the suspended matter and zooplankton were N-sulfocarbamoyl group C1+2 and carbamate group GTX1+4, and the trace component was GTX2+3. However, GTX1+4 detected in zooplankton accounted for 70% of the total toxin compositions, which was higher than that in suspended matter. This suggests that toxic dinoflagellates that containing PST toxins can be transformed into strong toxins in zooplankton and can spread to higher trophic levels. Comparing this result with the National Institute of Fisheries Science report. PST was detected in bivalves about a week after its detection in zooplankton. Therefore, this study provides basic data for predicting the occurrence of PST.

Toxicology

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Different effects on growth of *Scenedesmus quadricauda*, *Scenedesmus dimorphus*, and *Scenedesmus obliquus* under microplastics stress

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Microplastics are a concerning type of emerging pollutant in various environmental compartments and pose a significant threat to the aquatic ecosystem. Microalgae, which are among the most abundant microorganisms in aquatic environments, are particularly vulnerable to pollutants and can serve as an early warning signal of ecosystem impacts. Changes in microalgae can have far-reaching effects such as reducing oxygen production and food supply for higher trophic levels, leading to imbalances in the ecosystem. While several studies have investigated the effects of microplastics on microalgae, the toxicity and mechanisms of microplastics on microalgae, especially non-model species, remain poorly understood. In this study, *Scenedesmus quadricauda*, *Scenedesmus dimorphus*, and *Scenedesmus obliquus*, microalgal isolates from mangrove in Shenzhen, China, were exposed to microplastics with three different sizes (0.08, 1, and 10 μm) for 14 days to monitor their responses. The results showed that exposure to microplastics had different effects on the growth of the three species. *S. quadricauda* showed growth enhancement due to the stimulation of chlorophyll a content that facilitates cell growth. Oxidative stress-induced lipid peroxidation caused growth inhibition in *S. dimorphus*, and *S. obliquus* showed no effect on growth, probably due to a higher yield of extracellular polymeric substances which protect the cell from the microplastic's physical damage. Different responses of the three microalgae species under the same stress exerted by microplastics highlight the potential of ecological imbalances caused by microplastic contamination. Further studies are needed to fully understand the risk of microplastics to not only microalgae but also the ecosystem.

Surveillance and Management

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Annual changes in the occurrence of vegetative cells and abundance of cysts of *Alexandrium catenella* and *A. pacificum* in Osaka Bay, Japan

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The toxic dinoflagellate *Alexandrium catenella* (Group I) has proliferated in Osaka Bay during the spring season. In order to assess the growth potential of the toxic plankton in Osaka Bay, the annual fluctuations in the appearance of *A. catenella* and *A. pacificum* vegetative cells and *A. catenella*/*A. pacificum* cyst (*Alexandrium* cyst) abundance were monitored from 1994 to 2022, 2007 to 2022, respectively. In addition, species composition of the cysts was investigated using quantitative PCR from 2013 to 2015. The annual maximum cell density (AMD) of *A. catenella* has been increasing to the level of 10^4 cells mL^{-1} since 1990s. In contrast, *A. pacificum* occasionally proliferated, but the cell density remained at a level of 10^2 cells mL^{-1}. *Alexandrium* cysts were confirmed at high density (up to 10^3-10^4 cysts g^{-1}). In May, there was a significant positive correlation between the cyst abundance and AMD of *A. catenella* in the same year. But, no correlation was identified between cyst abundance in October and AMD of *A. catenella* in the following year. Quantitative PCR results showed that *A. catenella* cysts accounted for more than 90%. Thus, it was inferred that *Alexandrium* cysts present in Osaka Bay are almost *A. catenella*, with a stock of $>10^3$ cysts g^{-1} at any time. In conclusion, considering the high cyst abundance, the growth potential of the toxic plankton is possibly high. But the scale of the outbreak in following year is assumed to be more dependent on the growth environment rather than the abundance of the cysts.

Surveillance and Management

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Biotoxins profile in mussels of the Northern-Central Adriatic Sea in the last decade (2012-2022): major contamination events and correlation with toxic phytoplankton

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The Northern-Central Adriatic Sea is one of the most eutrophic areas of the Mediterranean Sea, and represents an important aquaculture area, accounting for about 90% of the Italian shellfish production. Since the 1970s, HAB species have frequently occurred in this area, posing a serious health risk to consumers. Therefore, according to European legislation, seawater and shellfish in production and relaying sites are regularly monitored. This study presents an analysis of over 2000 samples collected from 2012 to 2022 as part of the official monitoring in 10 production areas along the Emilia-Romagna Region coast. During these 10 years, no PSP toxins were above the regulatory limit in mussels, despite the presence of *Alexandrium* spp. in seawater. Instead, several toxic events related to lipophilic toxins occurred, with a clear correspondence between the main phytoplanktonic species involved in the production of each type of toxin and toxin presence in mussels. Yessotoxins, which represented the main toxins detected in the preceding decade, were found in mussels mainly during winter periods from 2012 to 2015 and reached levels well above the regulatory limit, despite its increase in 2013 (from 1 to 3.75 mg/kg p.e.). A different toxin profile related with the producing species *Gonyaulax spinifera*, *Protoceratium reticulatum*, and *Lingulodinium polyedra* has been observed. Diarrhoetic toxins levels increased in mussels together with the increase in seawater of several *Dinophysis* spp. cells, for which seasonal trends were observed. Multiple toxic events attributable to these toxins have been identified, each resulting in the temporary closure of production areas.

Surveillance and Management

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CYANOTECH: A sustainable and innovative management system for toxic cyanobacteria blooming of surface waters with combined energy production, sustainable agriculture, and food safety

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Blooming of toxic cyanobacteria in surface waters has become more persistent and prevalent globally. Besides their aesthetic and environmental effects, a number of economic sectors including tourism, fishery, food, health, and water industries, experience annual loses in the range of millions USD. With so many diverse sectors of modern living being affected by the same problem, it is crucial to develop and apply innovative and sustainable management systems for toxic cyanobacteria that can be easily adapted into current infrastructures. CYanoTech is a two-year project that proposes a novel, sustainable, and innovative management system for mitigating the effects of toxic cyanobacteria blooming in surface waters while combining energy production and promoting sustainable agriculture, and food safety. The CYanoTech system comprises of removal of the excess aquatic biomass (cyanobacteria cells and algae) from water with a low energy non-mechanical separation technology, the treatment of the aquatic biomass for the production of energy and marketable products (fertilizers), and the application of treated and untreated surface water in hydroponic cultures that produces safe for consumptions crops (cyanotoxins-free crops). The energy produced will compensate the total energy needs of the applied treatment processes, further reducing the system's overall carbon footprint, making it self-sustainable. Life-Cycle-Analysis will be used to prove the system's sustainability and market accessibility.

Surveillance and Management

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Integrated system for the surveillance and management of harmful algal blooms in coastal areas of Cienfuegos province, Cuba

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Harmful algal blooms (HABs) can negatively affect the biological diversity of marine ecosystems, human health, economy, and tourism. In Cienfuegos Bay, located in south-central Cienfuegos province, Cuba, different bloom events have occurred, with negative consequences for the health and economy of the locality. Several national and regional projects from the International Atomic Energy Agency have built capacities in Cuba to establish early warning systems for assessing the occurrence of HABs. Our objective was to design and implement a monitoring and management system in Cienfuegos Bay to have well-defined intersectoral action mechanisms during HAB events based on the principles of the Integrated Coastal Zone Management. During 2021, several workshops were conducted focusing on the system design, using Health and Environmental specialists' criteria for managing these events in the territory to guarantee the coordinated participation of these two sectors. The integrated system was divided into five stages: Surveillance, Communication, Activation, Management, and Impact Assessment. The implementation of this system contributes to mitigating the negative impacts of HABs in different development sectors in the Cienfuegos province and may serve as an experience applicable to other regions of Cuba and the Americas.

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Pseudo-nitzschia blooms associated with rapid onset of amnesic shellfish toxin contamination in mussels from Scottish coastal waters

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Pseudo-nitzschia is present in the waters around the coast of Scotland throughout the year and despite the frequent occurrence of blooms (defined as exceeding 50,000 cells/L), shellfish contamination with domoic acid is relatively rare. Although it is not possible to accurately identify *Pseudo-nitzschia* to species level using light microscopy, cells may be split into size categories, with those of valve width <3 μm assigned to the *P. delicatissima* group, and those >3 μm to the *P. seriata* group. In 2022, the spring blooms were mostly composed of *P. delicatissima* group cells, with little or no toxicity detected in shellfish. However, in some regions, blooms detected in mid-summer (June and July) were mostly composed of *P. seriata* group cells and resulted in the rapid onset of amnesic shellfish toxin (AST) contamination in mussels at four harvesting sites. A maximum AST concentration of 95 mg/kg was recorded in mussels from Braewick Voe (Shetland Islands) in July. These *Pseudo-nitzschia* blooms were short-lived in duration and toxin levels declined to below the regulatory limit one week later. Subsequent analysis using scanning electron microscopy revealed *P. australis* to be the likely causative organism. Autumn blooms containing a mix of *Pseudo-nitzschia* species, including *P. fraudulenta*, occurred at some sites in the Shetland Islands in September, but were not associated with any toxicity. A regulatory monitoring programme is in place to protect public health and while there have been no reported incidences of human AST poisoning in the UK, the potential for such an event does exist.

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HABreports & My-HABs online early warning of HAB events for the aquaculture industry in Scotland and Malaysia

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Since 2014 SAMS has operated an online risk assessment and early warning system for harmful algal blooms in Scottish waters. This is composed of weekly HAB risk assessments in the form of bulletins for the Shetland Shellfish industry and an open-access web portal www.HABreports.org that provides a site-specific HAB/biotoxin risk index and mathematical model-based forecasts of future HAB events. We are now in the process of developing this system to provide model-based alerts for the Scottish salmon industry using cell counts undertaken daily at fish farm sites to initiate the models. This has involved cross industry collaboration to produce an accepted list of target taxa. We are now integrating into HABreports real-time data from two Imaging Flow Cytobots deployed in the Shetland Islands. A parallel project (My-HABs) has allowed the development of a HAB alert system for the Malaysian state of Sabah. The My-HABs system also includes a mobile phone-based alert for registered users, downloadable from App Stores. This system we are now seeking to implement in Scottish waters.

Surveillance and Management

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Joint FAO-IOC-IAEA technical guidance for the implementation of early warning systems for harmful algal blooms

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Harmful algal blooms (HABs) have significant impacts on food safety and security through contamination or mass mortalities of aquatic organisms. Indeed, if not properly controlled, aquatic products contaminated with HAB biotoxins are responsible for potentially deadly foodborne diseases and when rapidly growing, HAB consequences include reduced dissolved oxygen in the ocean, dead zones, and mass mortalities of aquatic organisms. Improving HAB forecasting is an opportunity to develop early warning systems for HAB events such as food contamination, mass mortalities, or foodborne diseases. Surveillance systems have been developed to monitor HABs in many countries; however, the lead-time or the type of data (i.e. identification at the Species-level, determination of toxicity) may not be sufficient to take effective action for food safety management measures or other reasons, such as transfer of aquaculture products to other areas. Having early warning systems could help mitigate the impact of HABs and reduce the occurrence of HAB events. In this regard, FAO took the lead in the development of a Joint FAO-IOC-IAEA Technical Guidance for the Implementation of Early Warning Systems for HABs. The document will guide competent authorities and relevant institutions involved in consumer protection or environmental monitoring to implement early warning systems for HABs present in their areas (marine and brackish waters), specifically for those affecting food safety or food security (benthic HABs, fish-killing HABs, pelagic toxic HABs, and cyanobacteria HABs).

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Citizen science participation increases the coverage and speediness in harmful microalgae blooms detection

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Monitoring HABs requires a well established connection between scientists and environmental, alimentary and even health authorities. It is a well-structured coalition whose central limitations are the lack of coverage of certain areas mainly due to budget-driven sampling constraints and, secondly, the slow response time due to the reaction time between the problem is reported, the samplers arrive at the site, and the samples reaches the laboratory. Some of these constraints could be overwhelmed by the participation from the general public acquiring and uploading in situ or ex situ (saving the position and date of each sample and uploading the observations in delayed mode) microphotographs of microalgae. The harmful benthic dinoflagellate *Ostreopsis* proliferates each summer in Mediterranean beaches covering the marine bottom (macroalgae, phanerogams, pebbles and sand) with a self-produced brown sticky mucilage. This mucus could be easily recognized by the naked eye when people is trained to recognize it. Our approach in the framework of the project "WHO'S THERE?" took advantage of the high number of people visiting the Mediterranean beaches to become our eyes there. A pilot trial has been developed to train volunteers to recognize the mucilage containing microalgae and providing them by different cheap portable microscopes. Citizen observations as microphotographs are uploaded in an online app and validated by scientists. Citizen observations increase the speediness in HAB detection and become a useful and complementary tool to established monitoring programmes. Once the sampling protocol has been adapted, it is also suitable for monitoring phytoplankton blooms.

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Citizen science of red tide monitoring using smartphone

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Red tide, as one of the environmental problems of coastal waters, is still important to be solved. For this, many citizens should learn the real state of red tide and discuss measures against it. In the present study, we develop a smartphone-based red tide monitoring program for citizen science. In the program, a user (citizen) takes a picture of red tide. We develop a machine learning application to estimate the chlorophyll a concentration from the hue value of the picture. Chlorophyll a concentration from the picture with time and location information via GPS have been accumulated on the internet server for further AI analysis. These accumulated data will also be made available to the public. Encouraging the participation of various stakeholders, including citizens, in reforming science is essential to achieving the "14 Life below water" of the SDGs. We hope that our program will be considered as one of the casual tool to participate in marine science. We welcome you to join our monitoring program. Please visit our website for more information (https://sites.google.com/view/wanted-red/).

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Occurrence and risk assessment of saxitoxin group toxins in South Korean seafood

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Saxitoxin (STX) is paralytic shellfish toxins produced by dinoflagellates, and co-contaminated with various analogues. In Korea, there is an increasing importance in safety management due to the continuous occurrence of seafood toxin exceeding the regulatory limit. Therefore, this study characterized the occurrence of saxitoxin (STX)-group toxins in aquatic products distributed throughout Korea and then performed a risk assessment for aquatic product consumption. Total 235 Korean seafood samples from three sea areas (West, and South) were analyzed by hydrophilic interaction chromatography (HILIC)-tandem mass spectrometry to determine STX-group toxins. The STX-group toxins were detected in 55 samples, but no sample exceeded the regulatory limit. Contamination levels were high in bivalves such as Japanese geoduck 92.3-513.9 μg/kg) and Mytilus galloprovincialis 1.7-228.0 μg/kg). The result of evaluating exposure and risk level of STX group with deterministic approach, the acute dietary exposure values of the consumer group were 0.2490-0.2501 μg STX eq/kg bw/day and 0.0133-0.0318 μg STX eq/kg bw/day, respectively. They were lower than the ARfD of 0.70 μg STX eq/kg bw. There were limitations in terms of the sample size for monitoring and the number of subjects surveyed for food consumption in this study. Therefore, if the risk assessment is conducted based on the data that increase the number of monitoring samples and extend the subject of food consumption survey in the future, it will be possible to estimate the risk of STX-group toxins more accurately.

Surveillance and Management

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Occurrence and risk assessment of okadaic acid, dynophysistoxin-1, dynophysistoxin-2, and dynophysistoxin-3 in South Korean seafood

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The okadaic acid (OA)-group toxins, including OA, dynophysistoxin-1 (DTX1), dynophysistoxin-2 (DTX2), and dynophysistoxin-3 (DTX3), cause diarrheic shellfish poisoning in humans. To manage OA-group toxins more strictly, the subject of regulation in Korea was recently revised from only the sum of OA and DTX1 to the sum of OA, DTX1, DTX2, and DTX3. Therefore, our study characterized the occurrence of OA, DTX1, DTX2, and DTX3 in seafood distributed across South Korea, after which a risk assessment of seafood consumption was conducted. To examine the occurrence of OA-group toxins in South Korean seafood, a total of 217 samples consisting of 16 bivalve and 7 non-bivalve species collected from three representative coastal areas in 2021 were analyzed via liquid chromatography-tandem mass spectrometry. OA, DTX1, and DTX3 were detected in 2.3%, 4.1%, and 9.2% of the examined seafood samples and exhibited a positive mean concentration of 11.3, 16.4, and 40.9 μg/kg, respectively. However, DTX-2 was not detected in any of the seafood samples. Based on the occurrence data, the estimated acute exposure to OA-group toxins through the dietary intake of seafood in the Korean population and consumer groups was 28.3%–37.9% lower than the acute reference dose (ARfD) of 0.33 μg OA equivalents/kg bw. However, for the scallop consumers aged 7–12 years, acute exposure to OA-group toxins exceeded the ARfD, indicating a possible health risk. These results suggest that the inclusion of DTX3 in the new regulatory limits is appropriate to protect Korean seafood consumers from exposure to OA-group toxins.

Surveillance and Management

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Seasonal dynamics of *Alexandrium pseudogonyaulax* in Oslofjorden, Norway estimated by qPCR and light microscopy cell counts

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The mixotrophic dinoflagellate *Alexandrium pseudogonyaulax* (Biecheler) Horiguchi ex K.Yuki & Y. Fukuyo 1992, was first recorded in the monitoring programs of Oslofjorden and Skagerrak in 2009. Since then, it has been recorded regularly in June, July, and August and in 2023 it appeared already at the end of May. *A. pseudogonyaulax* produces the toxin goniodomin A (GDA) that is known to be cytotoxic and is associated with fish mortalities. Fish kills due to *A. pseudogonyaulax* has so far not been observed in Norway, but swimmers in Oslofjorden have reported skin discomfort when cell densities have been high. We have developed a qPCR assay that targets *A. pseudogonyaulax* and it is tested on samples collected in Oslofjorden and compared with light microscopy cell counts. Furthermore, we explore the spatiotemporal occurrence of *A. pseudogonyaulax* and its correlation to environmental factors such as nutrients, temperature, and salinity. Our study contributes to the understanding of the ecological dynamics of *A. pseudogonyaulax* and potential implications for the Oslofjorden and Skagerrak ecosystems.

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Toxic *Dinophysis* species in aquaculture areas of North Western Adriatic Sea: dynamic and a molecular qPCR approach

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Dinophysis spp. is a cosmopolitan genus found mostly related to water column stratification. It can cause DSP (Diarrhetic Shellfish Poisoning) by producing toxins that can accumulate in mussel tissues. In Italy, when accumulation exceeds the regulatory limit (160 μg OA eq./kg of tissue eq.), the production is suspended until toxin values return to harmless concentrations, causing significant economic losses to the aquaculture sector. This study aims to analyse data from North Adriatic Sea monitoring activity. *Dinophysis* spp. abundances and okadaic acid events from 1999 to 2022 were analysed to look for inter-annual trends and seasonality. It also applies a molecular qPCR assay for quantification of *Dinophysis* spp. in aquaculture sites. The preliminary data analysed trend in both *Dinophysis* spp. abundance and toxic events. Moreover, the investigation conducted on *Dinophysis* spp. seasonal occurrence showed a clear abundance seasonality that don't correspond to relative toxicity determined in farmed mussels. The qPCR assay was applied to phytoplankton field samples using genus-specific primer pair targeting the 5.8S-ITS rDNA and a newly generated standard curve. Mean rDNA copy number per cell was obtained by single-cell analysis performed on different site-specific individuals of *Dinophysis*. The cell numbers obtained by qPCR were compared to the traditional method of microscopy count. The results showed that the qPCR assay is a more rapid and sensitive method to quantify the target genus than traditional light microscopy determination. Implemented approaches for monitoring of HAB species is fundamental to understand both ecological dynamic and mitigation action in target areas of mussel farms.

HABs in a Changing World

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Adding a paleo-perspective to harmful algal blooms (HABs) along the West Florida Coast: Assessing the influence of anthropogenic factors

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Whether harmful algal bloom (HAB) occurrences have increased globally due to climate change and anthropogenic stressors is still debated. Monitoring data, although crucial for understanding HAB trends, often cover limited timeframes. To unravel patterns and assess the influence of human factors, an investigation of HAB-inducing species over longer timescales is essential. Paleoecological research offers means to achieve this objective by examining historical records. Our project aims at providing a paleo-perspective to HABs on the western coast of Florida, a recognized hotspot for HABs of the dinoflagellate species *Pyrodinium bahamense* and *Karenia brevis*. Through a multiproxy approach, involving palynology (dinoflagellate cysts), lipid biomarkers (i.e., sterols), and sedimentary DNA (sedaDNA) analysis, we investigate surface sediments and sediment cores from Tampa Bay and Charlotte Harbor estuaries. Preliminary palynological findings of surface sediments in Tampa Bay reveal a widespread distribution of *P. zoharyi* cysts, with certain areas exhibiting dominance. Additionally, examination of a sediment core from Middle Tampa Bay indicates an increasing abundance of *P. zoharyi* cysts over the past ca. 50 years. Ongoing lipid biomarker and sedaDNA analysis are aiming to also detect the presence of *K. brevis* (preservable cyst yet to be discovered), as well as other phytoplankton taxa. A better understanding of historical events will improve short- and long-term prediction of future *P. bahamense* and *K. brevis* blooms, aiding in the development of effective management strategies for minimizing HABs and their impacts. Furthermore, it sheds light on complementary methods for tracking HABs over time, when palynology may have limitations.

HABs in a Changing World

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Vertical distribution of HAB cysts in the sediment of Uranouchi Inlet using metabarcoding

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Uranouchi Inlet, situated on the Pacific coast of southwestern Japan, is a highly enclosed inlet known for yellowtail farming since the 1960s. Later, from the 1970s, harmful algal blooms (HABs) have frequently occurred in the inlet, leading to substantial fish and shellfish mortality rates. It is well documented that many HAB species form resting cysts that accumulate in the sediment, potentially preserving the history of HAB events. However, the vertical distribution of HAB species cysts in the sediment of Uranouchi Inlet remains poorly understood. To address this gap, core sediment samples were collected from the inlet on August 22, 2016, using a 57-cm core sampler, resulting in 19 samples at 3 cm intervals. Metabarcoding analysis was performed on each sample using Illumina MiSeq, and HAB species sequences were identified via BLAST searches with reference to the IOC-UNESCO Taxonomic Reference List of Harmful Microalgae. The dating of each sample was carried out using Pb-210 and Cs-137 radiometric methods (Takahashi et al., 2021). The findings revealed the presence of eight HAB species spanning from the oldest samples (1920s) to the present, with notable shifts in species distribution occurring around the 1977–1988 period. These changes in HAB species composition corresponded to the development of aquaculture and the resulting eutrophication. The vertical metabarcoding analysis of sediment core samples collected from an aquaculture farm provides insights into how environmental changes in the Anthropocene era may have influenced the community composition of HAB species.

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Plastics as a new vector for marine toxins

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Plastic debris is a considerable part of marine litter posing risks to the world's oceans health. Since lipophilic toxins have low polarity and tend to remain in seawater after toxic algae blooms, their interaction with plastics has been recently studied. The present study tested the relevance of those interactions by investigating the adsorption isotherms of okadaic acid (OA) onto expanded (EPS) and non-expanded polystyrene (PS) particles. Circular 6 mm fragments of EPS and PS (at a dose of 4 g L^{-1}) were exposed for 48 hours to synthetic seawater spiked with 5, 10, 15, 20, 30, and 40 ng mL^{-1} of OA. A solid phase extraction procedure was used to determine dissolved OA concentration in spiked seawater before LC-MS/MS quantification. Six adsorption isotherm models were adjusted to the experimental data to examine possible sorption mechanisms. The most appropriate isotherms were found to be Sips and Krishnamurti models for both EPS and PS ($R^2 \geq 0.947$). Comparing these two models by Akaike's Information Criterion shows that Krishnamurti is the most robust model for the adsorption of OA by PS with a probability of 99.61 % (Evidence Ratio 256.4), while EPS has a 79.37 % of chance (Evidence Ratio 3.847). These models point to an adsorption process governed by electrostatic interactions showing that EPS and PS can sorb dissolved OA at concentrations as higher as 40 ng mL^{-1}. These findings point toward the role of plastics as a vector for toxins in the marine environment.

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Interaction between toxic *Alexandrium pacificum* exposure and increased water temperature in *Perna canaliculus* spat

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Alexandrium spp. is a globally distributed harmful algal bloom (HAB)-forming genus of dinoflagellate which can produce a range of biotoxins including paralytic shellfish toxins (PSTs) and other unelucidated bioactive extracellular compounds (BECs). Since 2011, blooms of *Alexandrium pacificum* have been occurring annually in the Marlborough Sounds of New Zealand, a significant farming location for the key aquaculture species, *Perna canaliculus* (= Green-lipped mussels, GLM). In the years since blooms of *Alexandrium* spp. began in the Marlborough Sounds, there has also been a major reduction in wild spat fall of GLM (juvenile mussels) in this area. GLM are not only exposed to annual blooms of *A. pacificum,* but also to increasing ocean temperatures and marine heatwaves, further adding to a complex environment for GLM populations. In this work, GLM spat were exposed to either typical (17°C) or warmer temperatures (22°C) and fed a lower or higher ration of paralytic shellfish toxin-producing *A. pacificum* cells. GLM spat exposed to a combination of high HAB exposure and warm temperatures had the highest mortality rate, with 39% mortality compared to 0-15% in other treatments. Exposure to HABs at 17°C had a more negative effect on growth than when combined with high temperatures. Byssal plaque production was significantly reduced when HAB exposure and warm temperatures were combined, indicating a potential impact on the ability of GLM spat to anchor themselves to a substrate. These results, combined with histological and transcriptomic analyses, will further enhance our knowledge of how multiple stressors could impact GLM spat populations.

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Nutrient recycling through microalgae cultivation in food waste treatment

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Hong Kong produces about 3,600 tons of food waste every day, about one-third of which comes from industry and commerce. The waste has a high content of organic matter and water. Besides, leachate will be generated during collection, transportation and landfill operations, which poses a serious burden on the environment. However, food waste leachate is rich in nutrients such as nitrogen and phosphate, which are essential for the growth of microorganisms such as microalgae. Therefore, this study aimed to assess the feasibility of using microalgal cultures to treat food waste leachate. Eight potential microalgal species were investigated. Monoclonal cultures of these species were grown with different concentrations of food waste leachate (0%, 10%, 30%, 50%, 80%, and 100%), while microalgae grown in L1 medium served as a control. Experiments were performed under a 12:12 h light-dark cycle with a light intensity of 6,000 lux and a constant temperature of 24±1°C. Comparing the results at day 6 and day 0, the cell densities of WWS (an unknown microalgal species), *Cyanobacterium aponinum* and *Dunaliella tertiolecta* increased by approximately 800%, 383% and 300%, respectively. The three microalgal species were found to be tolerant of up to 10% food waste leachate. Of the eight species screened, *C. aponinum* had the highest growth rate (0.314 cells d^{-1}) when cultured in 10% food waste filtrate, a 20% increase compared to the second-highest species, WWS. These results demonstrate integrating microalgal culture into waste treatment for nutrient recovery offers a potential avenue for energy recovery from food waste.

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Prolonged periods of nutrient limitation as a driving factor for *Pseudo-nitzschia* blooms

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As climate change is predicted to increase water stratification, phytoplankton are likely to face prolonged periods of nutrient scarcity. To better comprehend the dynamics of the HABs in the future climate, this study evaluates the response of two widespread diatom species, pennate toxigenic diatom *Pseudo-nitzschia pungens* and non-toxic centric diatom *Skeletonema costatum*, to nutrient re-infusion after short (~2 weeks) and prolonged (~4 weeks) periods of nutrient limitation. Growth responses, in terms of lag times and growth rates, were measured at different temperatures between 9°C to 25°C. Differences in responses were expected because unfavorable conditions are known to trigger the resting stage in *S. costatum*, but not in *P. pungens*. Both species initially exhibited optimal growth at similar rates, with negligible lag times, except at 9°C. However, following a short nutrient limitation period, both diatoms exhibited 2-4 days of lag times at growth rates that matched (*P. pungens*) or fell below (*S. costatum*) initial optimal conditions. Further, after a 4-week prolonged nutrient limitation, *P. pungens*' lag time decreased to about 1-2 days, but growth was roughly halved compared to initial optimal rates, whereas *S. costatum* did not grow within 10 days. The lag times after nutrient replenishment decreased with increasing temperature for *P. pungens* for both short and prolonged nutrient depletion, but not for *S. costatum*. The findings suggest that *P. pungens* is more adaptable to long-term nutrient depletion conditions than *S. costatum*, though the specific mechanisms remain to be understood.

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Biological features of *Phaeocystis globosa* in Beibu Gulf and implications on the bloom dynamics

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Haptophyte *Phaeocystis globosa* is a common causative species of harmful algal blooms in the South China Sea (SCS). It has a polymorphic life cycle alternating between solitary cells and gelatinous colonies. *Phaeocystis* blooms are usually dominated by the colonial morphotype with a diameter of hundreds of microns to several millimeters, while the diameter of *P. globosa* colonies in the SCS can reach 3 cm. Large-scale *P. globosa* blooms featured by “giant colony” have erupted in the Beibu Gulf of the SCS since 2011, posing serious threats to local nuclear power facilities and the marine environment. Based on the studies of *P. globosa* isolates from the Beibu Gulf, we found that the giant-colony-forming *P. globosa* possess unique characteristic pigment 19'-butanoyloxyfucoxanthin (But-fuco) and genetic information, which significantly differ from the bloom-forming *P. globosa* in the North Sea of Europe. The distribution and dynamics of *P. globosa* blooms in the Beibu Gulf were investigated by pigment analysis. We found the blooms of *P. globosa* mainly distributed in the coastal waters of Guangxi and a region west to Leizhou Peninsula, which might develop from different origins. Lower temperature (<20°C) during wintertime is a critical factor to trigger the formation of *P. globosa* blooms. During the blooms, diatoms had positive correlation with *P. globosa* colony abundance, while cyanobacteria had negative correlation. The diagnostic pigments for colony-forming *P. globosa*, diatoms and cyanobacteria, as well as temperature, could serve as potential early-warning indicators for the formation of *P. globosa* blooms in the Beibu Gulf.

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Investigation and database of Harmful algae and algal toxins in coastal China: HAATC Project

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The “Harmful Algae and Algal Toxins in Coastal Waters of China: Investigation and Database” project (HAATC) intends to determine the overall distribution of marine harmful microalgae and macroalgae, and marine algal toxins (in phytoplankton and shellfish) through a large-scale investigation in the coastal waters of China. Continuous investigation have been carried out more than three years in seven key sea areas, which are HAB&farming zones from south to north coast. Biotic and abiotic processes were observed during harmful red tides and green tides events. It also aims to establish an open database and query platform of harmful algal blooms in China for understanding the long-term evolution of HAB occurrence under the influence of intensified human activity and global change. It is funded (US$2.3 million) by the Ministry of Science and Technology of China for 5 years starting on the 1st Jan 2019. This HAATC research consortium, led by chief scientist Prof. Tian YAN, includes approximately 100 scientists and students. More than 4 years of work have shown the increased distribution of HAB species (including cysts) and algal toxins in coastal waters of China, the increased occurrence of macro-algae blooms (mainly by Ulva prolifera and Sargassum horneri) in the Southern Yellow Sea, increased amount of causative species being identified, and new records of algal toxins such as azaspiracid, cycloimines and neurotoxins β-N-methylamino-l-alanine have been detected. Eventually, the project will provide systematic basic data and techniques for the rapid and accurate identification of harmful algae species to support the research, monitoring, and control of harmful algal blooms, as well as seafood safety management in China.

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Anthropogenic chemicals may promote a shift from diatoms to harmful phytoflagellates

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Phytoplankton composition has drastically changed in the Seto Inland Sea of Japan over last several decades, which could be primarily controlled by recent climate change and/or decreased nutrient level of the sea. Additionally, increased human-related anthropogenic substances may influence such changes, since significant levels of antibiotics and herbicides have been detected in the seawater, and some of them are revealed to be toxic to various phytoplankton. In this study, two diatoms (*Skeletonema costatum* and *Chaetoceros lorenzianus*) and two phytoflagellates (*Prorocentrum shikokuense* and *Heterosigma akashiwo*) were exposed to herbicides and antibiotics those actually detected in the coastal seawater, to know differences of susceptibility for the anthropogenic chemicals in those species. The herbicides, diuron and bromacil, indiscriminately impeded growths of all the tested species, and no specific susceptibility was found. The antibiotics, clarithromycin and clindamycin, were, however, distinctively toxic to the diatoms. Values giving EC_{50} based on growth rate in diatoms *S. costatum* and *C. lorenzianus* were 17.1-105.4 μg L^{-1}, however, the values in dinoflagellate *P. shikokuense* and phytoflagellate *H. akashiwo* tended to be higher than those of diatoms. Another antibiotic, azithromycin, was less toxic, giving higher EC_{50} values (611-828 μg L^{-1}) in the diatoms, but no growth inhibition was observed in *P. shikokuense* and *H. akashiwo* even at the highest concentration tested (1000 μg L^{-1}). These results indicate that diatoms are more sensitive to anthropogenic chemicals, especially to antibiotics, than phytoflagellates, and this fact may partly explain the phenomenon in which harmful phytoflagellates have overtaken diatoms in the Seto Inland Sea.

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Researches on the opportunistic red tide ciliate, *Mesodinium rubrum,* in Korea - Unique klepto-organelle chains and sustainable communities -

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Mesodinium rubrum is an obligate mixotroph requiring TPG clade plastid for photosynthesis, sustainable growth, and even cosmopolitan blooming. *M. rubrum* red tide in Korea was first reported from Nakdong River Estuary in 1982. Thereafter, occurrence and species succession (1988), successful establishment of first temperate strains of *M. rubrum* (MR-MAL01, 2004) as well as first *Dinophysis acuminata* strain (DA-MAL01, 2006), and biological and ecophysiological characteristics of the Korean strains has been serially reported. Several novel marine natural products including PTX-2, a lipophilic shellfish toxin and a potential anticancer agent, were isolated from mass-cultured *Dinophysis acuminata* strains. The origin of the klepto-organelle chains (Cryptomonad-*Mesodinium*-*Dinophysis*) for the related researches in Korea was a strain of TPG-clade cryptomonad, *Teleaulax amphioxeia* (CR-MAL01). The CR-MAL01 strain is quite unique in terms of trophic mode (photosynthesis and bacterivory on eubacteria including *Synechococcus*), cell organelles (plastid and nucleus) functioning inside *Mesodinium* (MR-MAL01) when sequestered and even when secondarily sequestered by *Dinophysis* (DA-MAL01), quality prey organism for larvae aquaculture, and ‘common’ natural prey for many mixo- and heterotrophic protists in marine ecosystem. Populations of the three protistan components along the klepto-organelle chains might possibly be sustainably kept owing to the ecologically unique flexibility of TPG cryptomonads. Over the last 10 years, *Mesodinium rubrum* became listed as the fourth frequent HAB species in Korean seas, which necessitates further long-term community monitoring, biotechnological and ecophysiological exploration, and ecosystem health consequences tracking for the three opportunistic component groups along the klepto-organelle chains.

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Effects of predicted increasing temperatures and decreasing salinities on *Nodularia spumigena*

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In the Baltic Sea, summer blooms are regularly dominated by diazotrophic filamentous cyanobacteria. In the southern basin, this includes nodularin producing *Nodularia*, having ecological consequences for food web efficiency and an economic impact on the region. Predicted higher temperatures could benefit *Nodularia* and even facilitate a northward migration, whereas decreased salinity might hamper growth. While most studies only assess short term acclimation, we investigated the adaptation of two Baltic *Nodularia spumigena* strains (KAC11, KAC66) to increasing temperatures (16, 18 and 20°C) and decreased salinities (7, 5, 3psu) from ambient bloom initiation conditions (16 °C, 7psu) for more than eight years in culture. The experimental cultures were kept in nitrogen replete media, and already after three years the two strains displayed different adaptation strategies. KAC11 showed increased growth rates and toxicity but reduced filament length, while KAC66 showed no effect. In nature, its diazotrophic capability gives *N. spumigena* the advantage over co-occurring phytoplankton in nitrogen deplete summer waters. Therefore, the current experiment assesses growth rates and toxin production in nitrogen deplete conditions. During blooms, *N. spumigena* forms surface scum due to interlocking long filaments filled with gas vacuoles. Thus, filament length and the presence of gas vacuoles was determined microscopically, since changes in these characteristics may affect the development of harmful blooms independent of individual growth rates. When fully analysed, this study will provide unique insight form a long-term adaptation setup, knowledge that can be used for prediction/modelling distribution and growth season of future *Nodularia* blooms in the Baltic Sea.

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Intense blooms of *Phaeocystis globosa* in the coastal waters of China are caused by a unique ecotype

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Haptophyte *Phaeocystis globosa* often causes intense harmful algal blooms (HABs) in the coastal waters worldwide. The blooms in the coastal waters of China are featured by centimeter-sized giant colonies, the unique diagnostic pigment, etc. Previous studies showed that *P. globosa* has complex genetic diversity and differentiated biological traits. In this study, a high-resolution molecular marker *rbcS-rpl*27 was developed based on the chloroplast intergenic spacer of *P. globosa*. Based on the sequences of this marker, 19 geographical strains were divided into four genotypes associating with colony size and the composition of diagnostic pigment. Type I, Type Ⅱ and Type Ⅲ were featured by small colonies (less than 3 mm) and 19’-hexanoyloxyfucoxanthin (Hex-fuco) and 19’-butanoyloxyfucoxanthin (But-fuco). Several strains from the South China Sea (SCS) belonged to Type Ⅳ featured by giant colonies (more than 1 cm) and But-fuco. It was suggested that these SCS strains should be a unique “giant-colony” ecotype of *P. globosa*. More than 1,000 sequences of *rbcS-rpl*27 obtained from *P. globosa* colonies and phytoplankton in the SCS during the period 2016-2021. Phylogenetic analysis indicated that >95% of the sequences from *P. globosa* colonies belonged to Type Ⅳ and possessing But-fuco as their diagnostic pigment, implying these colonies were comprised of the “giant-colony” ecotype. Whereas, the genetic diversity of solitary cells was much higher. The colonies of *P. globosa* that caused blooms in the Southern Yellow Sea in winter 2021 also belonged to Type Ⅳ and characterized by But-fuco as the diagnostic pigment. Therefore, the intense blooms of *P. globosa* in the coastal waters of China were mainly caused by this “giant-colony” ecotype. These results will help to understand the intraspecific diversity of *P. globosa* and the formation mechanism of bloom.

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Phytoplankton composition and monitoring of HABs in the marine waters of Cancabato Bay in Leyte, Philippines

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Cancabato Bay is a source of food and livelihood of people living in its coasts. The Bureau of Fisheries and Aquatic Resources (BFAR) have been conducting monthly monitoring of the bay due to its numerous harmful algal bloom (HAB) occurrences that cause Paralytic Shellfish Poisoning (PSP) posing a threat to humans. Shellfish bans are imposed when the regulatory limit of 60 μg/STX equivalent/100g shellfish meat is exceeded. This paper aimed to evaluate the presence of *Pyrodinium bahamense* with the water quality assessments of the bay using both phytoplankton and physicochemical parameters as indicators, from October 2022 to January 2023. Physicochemical parameters and water samples were collected in three sampling stations within Cancabato Bay. Physicochemical parameters such as pH, salinity and dissolved oxygen were within normal range. However, salinity, TSS, phosphates, nitrates, and temperature fluctuated in other months and were not within environmental standards due to external factors such as rains, wind, and sediment runoffs. A total of 56 genera of phytoplankton were recorded during the whole sampling period. Diatoms dominated the phytoplankton composition, composed of *Chaetoceros*, *Skeletonema* and *Leptocylindrus* species. Presence of toxic producing phytoplankton such as *Pyrodinium bahamense* were prevalent in all sampling periods. Moreover, the study observed a *Skeletonema* bloom that reached 2.03 x 10^6 cells/L during November 2022. Based on the Integrated Phytoplankton Index (IPI), the values for water quality in Cancabato Bay were interpreted as "low". The phytoplankton composition in the bay could be significant in understanding its relationship with the occurrence of *Pyrodinium bahamense*.

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Harmful algal blooms variations in response to annual and inter-annual environment changes in the eutrophic Haizhou Bay, Yellow Sea, China

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In recent years, serious eutrophication has intensified in Haizhou Bay, Yellow Sea, China, resulting in frequent occurrence of diverse harmful algal blooms (HAB). From 2020 to 2022, HABs has a tendency to expand throughout a whole year and many newly recorded dinoflagellate blooms were found. Different blooms could occurred simultaneously, such as *Takayama acrotrocha* and *Gymnodinium impudicum* respectively caused blooms in high dissolved organic nitrogen (DON) and dissolved inorganic nitrogen (DIN) sea area in September 2020. HABs in Haizhou Bay in 2020 were dominated by dinoflagellate, mainly *Karlodinium veneficum* in May, *T. acrotrocha* and *G. impudicum* in September, and *Heterocapsa globosa* in November, while diatoms took advantage in 2021, correspondingly *Skeletonema* sp. and *K. veneficum* in May, and *Pseudo-nitzschia delicatissima* in June, and *Skeletonema* sp. in August. The results of DON in 2020 (38.87±12.81 μmol/L) was about three times higher than that of 2021 (11.43±4.54 μmol/L), indicating that DON may be the key nutrient triggering dinoflagellate blooms. HABs are closely related to terrestrial nitrogen and phosphorus input and intensive mariculture activities.

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Morphological plasticity of *Alexandrium minutum* investigated using Imaging Flow Cytobot

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Alexandrium minutum is a toxic dinoflagellate frequently reported to form blooms in different areas globally across seasons, emphasizing their adaptability and resilience to changing conditions. This is evident in their capacity to successfully colonize diverse habitats and persist over extended periods of time. Morphological plasticity has been suggested as one of the survival strategies exhibited by dinoflagellates but conventional microscopy alone limits real-time observation of the physical changes in individual cells in both culture and field setting. Here, Imaging Flow CytoBot (IFCB) was combined with light microscopy and SEM to determine variations in cell morphology (size and shape) in response to varying physico-chemical conditions. We hypothesized that the N:P ratio would be positively correlated with cell morphology since xxx. However, no significant differences were observed in morphology between those cells grown in ambient and elevated temperature and salinity conditions. Corresponding changes in toxicity was also investigated via Enzyme-linked immunosorbent assay for saxitoxin to understand potential effect of cell size. Notably, cell size was observed to be directly positively correlated with toxicity, indicating that toxin storage might be a function of the volume of the cell. Our method suggests that the IFCB can be utilized not only for monitoring the presence of HABs species but also for detecting the morphological plasticity of Alexandrium induced by its response to varying nutrient conditions.

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The global success of toxigenic *Pseudo-nitzschia* species; what does it mean for future ocean ecosystems?

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Species of the toxigenic genus *Pseudo-nitzschia* are found in nearshore coastal and coastal upwelling waters but also comprise a significant presence in oceanic iron-limited subarctic and tropical regions. Although they represent only ~4% of pennate diatoms, *Pseudo-nitzschia* is one of the most cosmopolitan genera of the world's oceans, are among the most diverse, and have a relatively even distribution across all ocean provinces. The factors contributing to the broad success of *Pseudo-nitzschia* spp. include a matrix of life cycle, physiological and morphological attributes that poise them to better take advantage of the transition to favourable growth conditions when they arise. Although we still are early in the progression of changing ocean conditions, there are emerging trends to suggest that the frequency of toxigenic blooms of *Pseudo-nitzschia* species are likely to increase in coastal and offshore waters under future ocean conditions. The underlying mechanistic reasons for this increased alarm will be presented as a framework of cellular/environmental interactions that can help to evaluate the potential impacts of climate change on toxic blooms of *Pseudo-nitzschia*.

Socio-economic Impacts

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Socio-environmental impacts of harmful algal blooms in Mexico

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The impact of Harmful Algal Blooms (HABs) in Mexico from 2003 to 2022, there is 321 events registered of HABs, which caused a total of 11,886 days of closures due to ASP, DSP, NSP and PSP toxins or by non-toxic algal blooms that could affect the human health. In this same period, 54 events of marine fauna mortality such as fish, octopus, marine mammals and birds have been registered, also seizure and destruction of marine products (bivalve mollusks and fishes) were recorded. In the Mexican coasts, 17 HAB events of ASP with an impact on marine mammals and birds were registered. Moreover, in the Gulf of Mexico, *Karenia brevis* have caused 20 HAB events related to NSP toxins leading to at least 9 cases of human intoxication due to respiratory irritation. For the Mexican Pacific Ocean (MPO), there are 38 HAB events of DSP formed by *Dinophysis* spp. that caused precautionary or sanitary closure. Besides for the MPO, there are 174 HAB events causing PSP and formed mainly by *Pyrodinium bahamense* and *Gymnodinium catenatum* representing the greatest health risk in our country because of 27 cases of human poisoning and two children decease in Chiapas in 2022. Although, there are no records of Ciguatera (CFP) in the official Mexican Health System, there are 160 cases of CFP caused by *Gambierdiscus* spp. and *Fukuyoa* spp. in Baja California Sur, Yucatan and Quintana Roo leading to 27 foreigners in Mexico poisoned and two adult deceases in 2020 (provided by Erick Núñez Vázquez).

Socio-economic Impacts

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Perceptions and preparedness of local coastal communities towards harmful algal blooms in Chilean Patagonia: A survey-based study

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Chilean Patagonia is significantly affected by harmful algae blooms (HABs), yet there is limited research on how local coastal communities perceive these events. This study presents a survey conducted in three HAB affected Provinces in southern Chile, using AI techniques for sampling and online platforms for data collection. The survey had a large sample size (n=1718). Results show that 79% of respondents have experienced at least one HABs episode in their lifetime, with health and economic dimensions being the most affected. The overall level of preparedness for future episodes is generally low, with participants feeling more prepared for economic risks than for health risks. Some 66% of respondents believe that is likely or very likely that a HABs episode will occur in their area within the next five years; 56% express a high level of concern about such an event. Respondents were also divided into two groups based on the variability of their responses. The first group (37%) with participants from the northern Patagonia region (Chiloe Island and Llanquihue Province), whose income is heavily dependent on marine resources, showed greater knowledge of HABs symptoms and associated risks. In contrast, the second group (63%) included people from the three provinces with lower economic dependence on the sea and varying levels of knowledge about HABs. These finding highlight the importance of understanding local perceptions of HABs for policy-making and interdisciplinary research. Such knowledge can contribute to the development of more informed responses and interventions to mitigate the impacts of future HABs events.

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Enhancement of lipid production by microalgal isolates from mangrove through environmental stresses

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Microalgae has sparked great interest in its rich lipid contents, resulting in tremendous potential as the principal material for producing renewable energy and other commercial products. However, the lipid productivity of microalgae is not always sufficient to sustain the economic viability of microalgal biomass facilities. Therefore, previous studies have explored the enhancement of lipid production by exposing microalgae to environmental stresses. The present study aims to investigate the effects of different environmental stresses, such as high salinity, nitrogen deficiency and phosphate deficiency, on the lipid production of four microalgae, including *Scenedesmus quadricauda*, *Scenedesmus obtusus*, *Scenedesmus dimorphus* and *Characiochloris* sp., isolated from a mangrove in Shenzhen, China. In the present study, four microalgal species were exposed to different concentrations of salinity (0, 10, 20, and 30 ppt), nitrate, and phosphate (100, 75, 50, and 25% of original) for 14 days. Cell density was monitored while lipid content and biomass were measured on day 14. Results showed nitrogen and phosphate deficiency only lead little effect on lipid production while lipid production significantly enhanced under high salinity in all microalgal species, compared to the control. Although substantial inhibition of cell growth was observed under salinity stress, the highest biomass productivity with 39.6 $mgL^{-1}day^{-1}$ was observed from *S. quadricauda* under 30 ppt salinity level. This optimal condition showed 1.66 times biomass productivity and 11 times fluorescence intensity of lipid content compared to the control. These findings suggest that high salinity stress is a promising tool for enhancing lipid production in certain microalgal species, with *S. quadricauda* showing particularly promising results.

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Exploring the human dimensions of harmful algal blooms through a well-being framework to advance ecosystem assessment and management in a changing world

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There has been considerable investment of effort to identify, characterize, track, and predict the direction and magnitude of harmful algal bloom (HAB) response to climate change. In comparison, far less effort has been devoted to understanding how human communities respond to HABs in a changing world. HABs alter social-ecological interactions and can have negative consequences for human well-being. This is especially true for fishing communities because their resource-based economies operate at the interface of the natural environment and society. Identifying the components of human well-being that are most affected by HABs can inform choices about climate-ready management strategies in and across complex systems. This study uses a framework for considering human well-being in management contexts to explore the effects of HABs of *Pseudo-nitzschia* spp. on US West Coast fishing communities. We find that HABs, and the management strategies to address them, affect almost every domain of human well-being; however, less than half of these effects can be considered by federal disaster response and recovery policies that provide relief to impacted communities. Moreover, much of the data used to measure the effects of HABs that are eligible for consideration by these policies are not consistently collected which could lead to inequitable access to disaster relief. Our analysis may provide a starting point for communities to develop a suite of high-quality indicators of human well-being to aid in decision support, assessing the effectiveness of management actions and the equity of management outcomes, and tracking adaptation to system dynamics and external pressures.

Emerging Issues
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Impacts of agricultural microplastics on the growth of marine phytoplankton in Monterey Bay, California (USA)

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Microplastics (MPs) are ubiquitous in various marine ecosystems, spanning from coastal waters to deep depths, and have been documented to harm organisms that reside in these habitats. The increase in MPs can be attributed to inadequate disposal and insufficient recycling practices for plastic material. The Monterey Bay (California, USA) is surrounded by coastal agricultural fields where plastic materials are used to prevent soil erosion, maintain temperatures and humidity, and reduce weeds. During the removal of macroplastics from the fields mechanical degradation can occur, producing MPs that can persist in the soils and make their way into water bodies. Limited research has been undertaken to investigate agricultural MPs in the marine environment, particularly their impact on marine phytoplankton, a key component of marine ecosystems. The objective of this study is to evaluate the impacts of agricultural MPs on the growth of two HAB (harmful algal bloom) and two non-HAB species of marine phytoplankton within controlled experimental settings. Agricultural plastic samples were collected from a farm located in the surrounding watershed. The impacts of these agricultural plastic samples will be tested using controlled mesocosms with known inputs of phytoplankton cells and MP concentrations. HAB species response (growth and toxin production) will be analyzed through cell counts, pulse amplitude modulated (PAM) fluorometry, chlorophyll, nutrient concentrations, and toxin assays. The results of this study will contribute to a better understanding of the response of marine phytoplankton (including HAB species) to the presence of agricultural MPs in Monterey Bay.

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Harmful algae in the waters of four mangrove ecosystems of Eastern Visayas, Philippines

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The regulating services of mangrove ecosystems include absorption of nutrients that would prevent occurrence of harmful algal blooms (HABs) in the marine environment. We assess the extent of this regulating service in the mangrove ecosystems in the islands of Samar and Leyte, two large islands that comprise the Eastern Visayas political region. Waters near and inside mangrove ecosystems, three in Leyte, and one in Samar islands were collected and assessed for phytoplankton, focusing on harmful algae. A 20 μm, 30m diameter, 1m plankton net. and a 2.2-L Van Dorn sampler were used to collect the samples. Physicochemical data were also recorded. The following potential marine HAB causing genera were found in samples from concentrated 1-L samples in all four sites; *Chaetoceros*, *Dinophysis*, *Prorocentrum*, *Pseudo-nitzschia*, and *Skeletonema*. The dinoflagellate, *Pyrodinium bahamense* was found in three out of four sites using this method. It was not found in the 1-L samples from Carigara, Leyte. But these were found in samples from the plankton net, in the same site, in a "brackish water station" at a salinity of 15, which is still within the tolerable range for this dinoflagellate. The freshwater HAB causing cyanobacteria found were *Leptolyngbya* sp. and *Oscillatoria* sp. The efficiency of nutrient and HAB regulating services, as well as their mechanisms is an avenue to be explored in these times of mangrove destruction and increasing HAB occurrences.

Emerging Issues
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Nanoparticle-assisted photosynthesis stimulation in dinoflagellates

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Dinoflagellates are key ecosystem components and extraordinary secondary metabolite producers. Recent studies have focused on evaluating their cytotoxic, antiproliferative, antibiotic, and antifungal effects as potential sources of drugs. Research on dinoflagellate-produced molecules for drug discovery requires large biomass availability, but dinoflagellates grow slowly, and it is difficult to achieve high cell densities in culture. In addition, active metabolites are often produced in very low concentrations. To overcome these limitations, we are exploring what we have named nanoparticle-assisted photosynthesis stimulation to enhance the photosynthetic dinoflagellates' processes through light-harvesting nanoparticles (NPs). The use of low-cost materials whose NPs show useful absorption/luminescence properties, such as silicon (Si), rare elements, molybdenum iodine, or even plasmonic excitation, may enhance pigment and other compound production. In a first essay, silicon nanoparticles (SiNPs) were synthesized by laser ablation of solids in liquids (LASL), followed by laser fragmentation down to a < 10 nm nanoparticle size. These SiNPs were added to dinoflagellate *Levanderina fissa* cultures. Our preliminary results showed a significant increase in the average growth rate, from 0.10 to 0.14 cell divisions day^{-1}, a reduced generation time from 7 to 5 days, and an increase in total cell biomass, from 43 to 52x10^{3} cell mL^{-1}. Furthermore, we observed a beneficial effect on chlorophyll concentration since it increased significantly from 0.25 mg m^{-3} to 0.78 mg m^{-3} when incorporating NPs into the culture system. In conclusion, NPs can positively impact biomass production for research and, eventually, commercial purposes.

Emerging Issues
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Detection and quantification of invasive *Alexandrium pseudogonyaulax* in the Baltic Sea using ddPCR

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Supported by increasing Sea Surface Temperature, warm water adapted phytoplankton species are currently expanding in the Baltic Sea. Among them goniodomin producing and potentially fish-killing *Alexandrium pseudogonyaulax* increasingly occurs at high concentrations in coastal brackish waters of the Southern Baltic, founding permanent populations here and representing a new threat to co-occurring biota and ecosystem services. To rapidly detect and quantify the species for risk assessment and monitoring of bloom development a quantitative molecular assay was developed based on digital droplet PCR (ddPCR) using previously published 28S rDNA primers. The assay was successfully tested on *A. pseudogonyaulax* cultures revealing a sensitive detection potential of less than 1 cell/L, which was confirmed on field samples from a coastal monitoring site. Besides mirroring microscopically determined bloom dynamics measured at Heiligendamm monitoring station, the ddPCR assay was able to detect *A. pseudogonyaulax* far below the microscopic detection limit. It thus represents a sensitive method for early warning that provides precise quantification without standard, and enables high throughput of samples due to short processing times. The assay was used to analyze samples from several cruises performed in successive years, mapping the distribution of *A. pseudogonyaulax* in the Southern Baltic Sea. We present the results of the assay in the context of environmental conditions, measured toxicities and presence of seed banks, and assess the progression of expansion during the past years.

Emerging Issues

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Exploring the bioremediation potential by microalgae

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Microalgal biomass is believed to be the sustainable substitute of fossil fuel as it can be derived into various kinds of materials with economic value such as feedstock for biofuel, food, nutraceuticals, aquaculture feed and bioplastics. While studying these microalgae technology is popular, bioremediation by microalgae, utilizing microalgae to treat of wastewater and pollutants, draws relatively less attention although bioremediation has high potential to be a source of low-cost microalgal biomass. The bioremediation potential of microalgae on special wastewater is discussed in the present study. In an experiment, anaerobic digestate, a type of heavy nutrient load wastewater generated in food waste treatment, was examined as an alternative source growth medium for cultivating *Chlorella* sp., a local isolate. The biomass productivity of the microalgae cultivated in diluted anaerobic digestate (10% V/V) was comparable to that cultivated in BG11 medium while the microalgae could survive in anaerobic digestate up to 75% (V/V). In another study, *Scenedesmus dimorphus*, an isolate from a local mangrove, showed good bioremediation performance on chlorpyrifos, a common pesticide leading to pollution. The microalgae could deal with a high concentration of 10 mg/kg chlorpyrifos, 10 times of that of the level in common contaminated water. In a period of 7 days, 96.0% removal and 86.5% degradation of chlorpyrifos were achieved. These results reflected that microalgae have high potential to be applied in bioremediation of different wastewaters.

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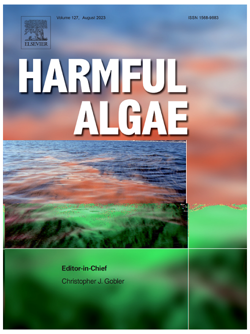

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