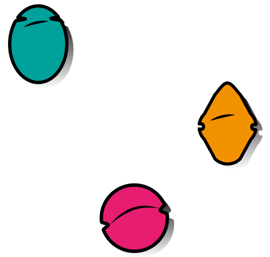

ICHA

19th INTERNATIONAL CONFERENCE ON HARMFUL ALGAE

MEXICO 2021

LA PAZ | baja california sur | october 10-15

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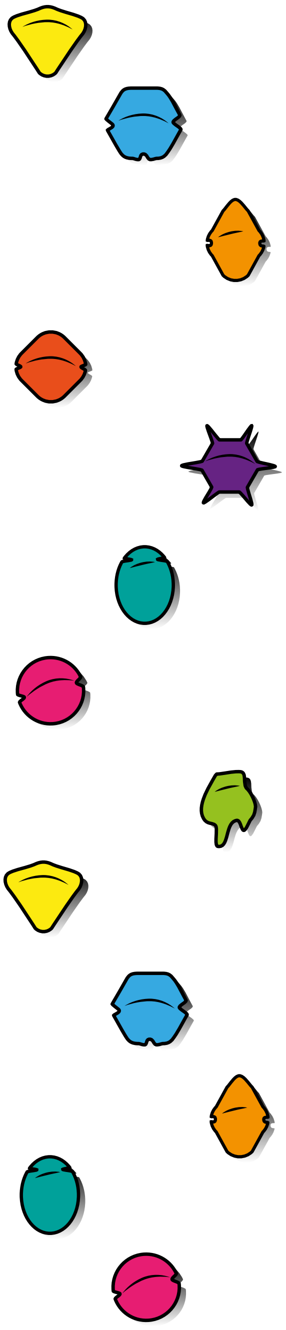
ABSTRACT BOOK



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FIRST VIRTUAL MEETING

INTRODUCTION

Dear colleagues,

We are pleased to present the abstract book of the **19th International Conference on Harmful Algae (ICHA 2021).** This conference had to be postponed for one year due to the global pandemic of coronavirus disease 2019 (COVID-19). Initially, as all the past ICHA conferences, it was planned to be a in-person conference, but due to the traveling restrictions worldwide and the restrictions for hosting scientific conferences in our country we had to change to a virtual format. So, this is our first virtual ISSHA conference held from Mexico. In spite of these complications, we are happy to announce that we received 406 contributions from 47 countries, for which we greatly thank you all. We will have nine plenary talks by world-renowned scientists, 247 oral contributions, 40 speed talks, and 110 posters.

We will also have six special sessions that will be held live and led by well-known leaders covering different aspects of harmful algae from marine and freshwater environments:

- Young Investigator Networking Meeting Session
- HAB Early Warning Systems Session
- Impacts of HABs on Fish Farms: Addressing Industry and Global Insurance Needs
- Control of Cyanobacterial Blooms
- NHABON-NE, a prototype node for a national HAB sensor network in the United States
- New book!! WHO guidance on cyanotoxins

The conference will be held in a virtual platform in three parallel sessions, divided into 49 modules during five days, covering a wide array of topics that are a continuation of previous ICHA conferences with minor changes proposed by the enthusiastic scientific committee members.

This book contains the abstracts of the plenary talks that are presented in the first pages, followed by abstracts that will be presented in the different topics in the following order:

- HABs in changing world
- Freshwater and marine biogeography
- HA biology
- HA ecology
- HA microbiomes
- Cyanobacterial blooms
- Ichthyotoxic HABs
- Ciguatera and benthic HABs
- Algal and cyanobacterial toxins
- Taxonomy and systematics
- Genomics
- Toxicology
- Novel HAB technologies
- HAB prediction
- Surveillance and management
- Wellness of aquatic communities

Within each topic, abstracts have been arranged in oral presentations, speed talks, and posters contributions. An author index is provided at the end of this book.

I want to take this opportunity to thank all the international scientific committees members for helping foresee all the adjustments we had to consider to change from an in-person conference to a virtual one, specially the member of the ISSHA council. I also want to thank each one of you who helped us hold the flame of ISSHA during these difficult and different times until our next conference. These conferences have always been an inspiration for many of us to improve and continue our harmful algae research in our different geographic regions. We hope this new format will allow to transmit the enthusiasm we have always had when we meet with our ISSHA colleagues and share our research.

I want to thank the organizing institutions for support and the facilities they offered to organize this meeting, and the generous sponsors who made this meeting possible and covered the registration fees of 77 participants from 13 countries, many of whom will participate in the Maureen Keller Best Student Award.

And very especially I want to thank Vera Trainer our ISSHA president for her commitment, hard work and her guidance to help us adapt to the virtual world. Thank you Vera on behalf of all of us!

Enjoy the conference!

Christine J. Band Schmidt

Chair of ICHA 2021, La Paz, Mexico.

Photo: Fernando Sánchez Bernal

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TABLE OF CONTENTS

Click on any topic to be directed to the corresponding section

PLENARY TALKS

Forty years living with *Dinophysis*: myths and realities

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Forty years ago, *Dinophysis forti* was identified as the source of diarrhetic shellfish poisoning, *Dinophysis* species were targeted as potential toxins producers worldwide. Further discoveries of their cryptophyte-like pigments (orange autofluorescence), suspicions (confirmed 10 y later) of mixotrophic feeding on ciliate prey, and uncertainties about their life cycles showed this genus to be a fascinating topic for students of dinoflagellate biology, phylogeny and ecology. Within the dinoflagellate order, *Dinophysis* species constitute a unique group, the plastidic specialist non constitutive mixotrophs (pSNCM), in the most recently proposed reclassification of planktonic mixotrophs. To date, only the ciliate *Mesodinium rubrum* fed TPG clade cryptophytes has allowed successful growth of *Dinophysis* in culture, although recent observations suggest there may be alternative trophic pathways. Different species of *Dinophysis* exhibit distinct functional traits and responses to environmental change. Progress in modelling the population dynamics of these highly selective kleptoplastidic mixotrophs is hindered by inappropriate sampling of *Dinophysis* and their potential prey with the same spatio-temporal resolution. Are *Dinophysis* low density slow-growing dinoflagellates with no sexual life, unrelated to water discolorations and never the dominant component of the microplankton community? Are all members of the *D. acuminata* complex the same species? Can we have an early warning of *Dinophysis* bloom development?- Are DSP events increasing in frequency, intensity and geographic distribution? Are they an emerging issue in North America? This talk will summarize key discoveries, recent milestones in the progress of knowledge, and the myths and uncertainties about the biology, population dynamics and toxic events associated with different species of *Dinophysis*.

Ciguatera: Current evidence and management options

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Ciguatera is a foodborne poisoning resulting from the consumption of fish and other marine products naturally contaminated with ciguatoxins (CTXs), neurotoxins produced by benthic dinoflagellates in the genera *Gambierdiscus* and *Fukuyoa*. Historically Ciguatera was limited to tropical areas, especially affecting small islands relying on marine resources as a source of protein and calories. However, with *Gambierdiscus* and *Fukuyoa* expanding to temperate regions, and international fish trade continuously increasing, ciguatera is today, considered a global threat, with cases exceeding that of other HAB poisonings. Food safety measures have been implemented to protect consumers from marine biotoxins and promote fair and sustainable fish trade. As for chemical contaminants, marine biotoxins associated with HABs are generally monitored and controlled, with safety levels established based on risk assessment studies. In addition, this monitoring may be supported by the surveillance of phytoplankton occurrence in harvest areas, and predictive models for early warning systems. Countries affected by ciguatera have adopted measures to control the trade of risky species, as early as the beginning of the twentieth century. These are mostly in the form of bans, solely based on local knowledge of ciguatera prone fishing areas and the fishes most often associated with ciguatera. Since the same measures apply, although decades of scientific research and monitoring data have enriched our knowledge of ciguatera to conceive improved management plan. Scientific knowledge and gaps towards developing and implementing evidence-based management options including CTXs and phytoplankton based monitoring or early warning will be discussed in this presentation.

Disclosure of Interest: None Declared

A hotter, more uncertain future: Can HAB research meet the environmental challenges of our time, and deliver meaningful outcomes?

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Two big environmental challenges of our time are climate change and pollution of marine and freshwaters: both of which impact HAB prediction and management. In order to effectively capture the temporal and spatial scales of these challenges, it is timely to consider strategies for adopting a more complex and definitive suite of experiments, observations, and models. Firstly, better integratiown of laboratory culture and field experiments is needed. For example, a recent analysis of the effect of temperature on cultured cyanoHABs has shown that despite this information being used to inform modelling and prediction, the findings do not link well with global observations of blooms. Overturning paradigms of nutrient utilization by cyanoHABs, to develop new and more meaningful model inputs, is another recent area of study. Meaningful predictions of cyanoHABs have also been hampered by our limited understanding of strain variation and adaptation. More recent studies are shedding light on the highly adaptable nature of cyanoHAB populations as a result of this strain variability. Tackling global environmental challenges also requires whole system and multiple-system studies. Capitalising on global data already available provides one method to determine linkages between global scale processes and cyanoHABs. Improved models and improved linkages between modellers, ecologists and physiologists will improve confidence in predicting impacts of climate change and eutrophication.

Creating large multi-regional community partnerships to monitor Harmful Algal Blooms and shellfish toxins in Alaska

Chris Whitehead

Ocean & Earth Environmental Services, United States.

Native American Tribes and First Nations in the Alaska and British Columbia have a vested interest in protecting traditional natural resources as well as the health of the local community. Regional Tribal networks linked with support from scientist and resource managers have begun detecting harmful algal bloom (HAB) events that pose a human health risk to subsistence and commercial shellfish harvesters. With "eyes on the water" within their communities, Tribes can establish subsistence management plans, and continue the cultural importance of shellfish harvesting. Within each regional network, Tribal partners collects weekly samples at key community harvest sites including phytoplankton identification and quantification, salinity, sea and air temperature, whole water for cellular toxin analysis, and shellfish for biotoxins. The data are uploaded to a shared database and are used by resource managers and subsistence harvesters to make informed decisions on harvest timing and risk. Tribal laboratories within each region provide analytical and technical support to each community within the network. By combining weekly phytoplankton observations and shellfish toxin analysis using approved methods, Tribal organizations are demonstrating **sovereignty through science.**

Socializing HABs knowledge, the Mexican Case

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Communication of risk to stakeholders and decision-makers when there is the probability of the appearance of a harmful algae bloom (HABs) is essential in Early Warning Systems. Also, proper communication and the correct implementation of actions when a HAB occurs is crucial to mitigate the adverse effects of this phenomenon. Knowledge transfer is an important element to prepare society for hazards associated with extreme HABs events. This is especially important considering that HABs have increased in frequency and magnitude in specific locations over the last two decades and have significantly affected regional marine resources. Therefore, socializing HABs knowledge is essential in any HAB national plan for effective management of this phenomenology. National plans to attend HABs must identify trustful sources of information to promote communication, education, and outreach. In this talk, I will present the status of the socialization of HAB knowledge in Mexico as an example of a country with highly diverse coastal environments and diverse and important problems related to HABs of different type: related to toxin-producing species, ichthyotoxic species, and related to degradation of the environment. I will describe recent HABs events and the status of the phycotoxins regulation national program. I will discuss the efforts to address the HAB problems (through a nationwide network and national academic society) in a country with different socioeconomic development in the coastal zone and other ecological and conservation issues.

From genes to ecosystems and back

Uwe John[1,2]

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The genome age has led to major advances in biodiversity research over the last several decades. The progress from classical molecular ecology to differentiation of key species by means of molecular markers and phylogenetic interpretation of their evolutionary relationships has been remarkable. Recent integration of these approaches into environmental genomics makes it possible to study communities and populations in their natural habitat, and thus also to do justice to their complex influencing factors. Metabarcoding and metatranscriptomic approaches are critical to understanding HAB population dynamics. A new promising method metabarcoding PoolSeq will be explained with examples. Transcriptomics and genomics studies have also assisted in metabolic interpretation of the biosynthetic pathways to phycotoxin synthesis. Single-cell genomics applied at the population level allows the exploration of complex intraspecific diversity of species and a mechanistic understanding of the cellular and evolutionary processes of target populations. More specifically, single-cell genomics has the potential to reveal differential trait evolution, including mixotrophy and toxigenicity. These alternative approaches to modern eco-evolutionary genomics are supported with case studies and provide an outlook on HAB research on diversity and biogeography.

Taxonomy and phylogeny of unarmored dinoflagellates in the Kareniaceae found from Asian Pacific

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Marine unarmored dinoflagellates in the Kareniaceae have been reported with harmful red tides responsible for mass mortalities of fish and invertebrates in the Asian Pacific. For unambiguous identification of the harmful kareniacean dinoflagellates, taxonomy and phylogeny of kareniacean dinoflagellates were further investigated using unialgal cultures isolated from Asian Pacific, mainly from Japanese and Philippine coasts. Cell morphologies were observed by LM, SEM and TEM, and pigment compositions were examined by HPLC. Host phylogeny was inferred from LSU rDNA and ITS sequences, and chloroplast phylogeny from psbA sequences. Kareniacean dinoflagellates including five *Karenia* species, seven *Karlodinium* species, six *Takayama* species and other genera (*Asterodinium*, *Brachidinium* and *Gertia*) have so far been found from the Asian Pacific. From this survey, some novel characteristics of this group were revealed. Two kareniaceans not included in previously described genera were also observed, although their cultures were not available. A *Karlodinium* species was described as *K. azanzae*, which showed micropredation on zooplankton. A peridinin-type chloroplast bearing species *Gertia stigmatica*, positioned in kareniaceans with fucoxanthin-type chloroplast derived from the haptophyte, was described. HPLC showed characteristic pigment composition in each clade of *Karenia*, *Karlodinium* and *Takayama*. On the other hand, monophyly of *Karlodinium* was not supported by chloroplast phylogeny inferred from chloroplast-encoded sequences.

Cell-to-cell interactions in the sea

Alexandra Z. Worden

Professor, Ocean Ecosystems Biology Unit, RD3, GEOMAR Helmholtz Center for Ocean Research Kiel and University of Kiel, DE; Senior Scientist and Professor Adjunct Monterey Bay Aquarium Research Institute and University of California Santa Cruz, United States.

We know by now that unicellular eukaryotes, or protists, are incredibly diverse. But what does this diversity mean in terms of metabolisms, ecological roles, and organismal interactions in the sea? These are challenging questions to answer because it is difficult to capture the three dimensional world of a protist at a visualization scale that addresses their dynamics in an appropriate manner. This is an uncomfortable state of knowledge given that the marine biosphere is responsible for half of global primary production – fueling ocean food chains as well as the atmospheric conditions we love so well – and is changing in connection with global climate change. Here we will discuss development of methods and the knowledge they are providing on interactions between both cultured and wild marine protists and other biological entities – including other microbes, like bacteria and archaea, as well as viruses. The discoveries range from new insights into evolutionary biology, ecological roles, and precise currencies of exchange and manipulation that shape the growth and fate of individual protistan species – and ultimately shape how unicellular organisms collectively drive biogeochemical cycling in the ocean.

Chile: Causes, impact and management of a ´hot spot´ for toxic algal blooms

Jorge I. Mardones

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The Chilean coast is a unique and diverse marine ecosystem comprised of the Patagonian fjords and channels in the south, and a region of strong wind-driven coastal upwelling in the north, which fuel extensive primary production. Recent studies and observations have shown that these ecosystems are highly vulnerable to the effects of climatic (i.e., ENOS, SAM) and anthropogenic stressors (i.e., aquaculture), leading to a dramatic increment in the intensity and distribution of harmful algal blooms (HABs) in both northern and southern Chile. For instance, two massive HABs in the Patagonian fjords in 2016 due to severe droughts (also known as "The Godzilla-Red tide events") resulted in the most significant fish farm mortality ever recorded worldwide and a vast socio-environmental impact in the region. Simultaneously, the paralytic shellfish poisoning (PSP) producer *Alexandrium catenella* has been observed migrating equator-ward reaching northern areas and producing extreme PSP events (i.e., a world-record of 143,130 μg STX eq 100 g^{-1} in 2018). These climatic anomalies have recently triggered 'super blooms' of opportunistic new toxic algal genera (i.e., *Karenia*, *Pseudochattonella*, *Heterosigma*), especially affecting important aquaculture areas in southern Chile. Overall, HAB events in Chile have produced, for example, an estimated annual cost in microalgae and toxin monitoring of approximately 7M US$ in 2019 and 93,000 US$ due to hospitalizations in 2018. Further studies need to focus on how projected local-to-global scenarios will modulate scientific capabilities to monitor, model and mitigate future HAB events, and how HABs-environmental uncertainties would affect essential system functions and consequently ecosystems services and wellbeing of coastal communities.

Photo: Tanos Grayeb

HABs IN A CHANGING WORLD

Single-cell microfluidics analyses on revived dinoflagellates reveal common adaptative strategies to decreasing phosphorus concentrations

Raffaele Siano[1], Mathias Girault[2], Claire Labry[1], Cécile Jauzein[1], Thomas Beneyton[2], Lionel Buisson[2], Yolanda Del Amo[3], Jean-Christophe Baret[2]

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Phytoplankton adaptation to environmental changes can be inferred by comparing phenotypes of populations revived from sediments of different ages. Physiological responses to environmental stressor may vary among the cells of a population, therefore to identify population adaptive patterns single-cell phenotypic analyses are needed. Microfluidics may allow phenotypic analyses of a large number of cells, allowing the identification of constant biological traits across cells of a population. To study the adaption of dinoflagellates to the decreasing phosphate availability occurred over the last century in the Bay of Brest (France), we revived toxic (*Alexandrium minutum*) and non-toxic (*Scrippsiella acuminata*) cells from sediments of ages corresponding to a pre-eutrophication period (1940s) and a beginning of a post-eutrophication period (1990s). Alkaline phosphatases are synthesized by dinoflagellates to use organic phosphorus under phosphate-limited conditions. Our aim was to compare the alkaline phosphatase activity (APA) among cells of different ages, using an *ad-hoc* developed microfluidic systems. For both dinoflagellates, single cell APA was relatively comparable among cells of cultures of the same ages, meaning that this trait can be used to compare populations across the time. *S. acuminata* strains had significantly higher APA than *A. minutum* strains. For both species, the APA in the 1990s decade was significantly lower than in the 1940s. Our study suggest that both species have adapted to decreasing concentration of phosphate in the environment, but *A. minutum* would perform better, likely thriving upon internal phosphorus stocks during phosphate-depleted environmental conditions. Beyond the development of microfluidics, this study contributes to explain the recent *A. minutum* ecological success in the studied environment.

Long-term impacts of combined global change drivers on harmful algal blooms in the North Sea

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Harmful algal bloom (HAB) incidences are considered to have increased over the past decades due to human impacts, such as eutrophication, but also due to changes in climate. The North Sea is one of the hotspots for global change, with sea surface temperatures increasing more than the global average over the last 50 years. In addition, major shifts in key nutrients, nitrogen (N) and phosphorus (P), have occurred since the 1980s due to deeutrophication efforts, with stronger decreases in P compared to N. These changes have a strong influence on phytoplankton communities and potential HAB development. To assess the impacts of these changes on HAB species in the North Sea and how different environmental factors influence their abundance, we fitted generalized additive models (GAMs) to empirical observations from 26 sampling points collected over the last ~20 years. We found that temperature, salinity, and P and N, and a spatiotemporal component were all significant predictors for changes in amnesic shellfish poisoning (ASP) toxin-producing diatom, diarrhetic shellfish poisoning (DSP) toxin-producing dinoflagellate, and *Phaeocystis globosa* abundances. Over the years, the magnitude and duration of ASP and DSP abundances increased, with higher densities occurring earlier in the year, especially close to shore. Mean abundances of *P. globosa* stayed the same, with small decreases offshore and increases close to shore. These shifts could be due to changes in temperature and a decline in the abundances of other phytoplankton species. Our results demonstrate how environmental factors may influence HAB development and that HAB occurrences may become more prevalent in the future North Sea.

Plastic-associated harmful phytoplankton assemblages in coastal and off-shore habitats of the Mediterranean Sea

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Plastics (macro, micro and nanoplastics) are durable and persistent pollutants that may alter pelagic and coastal ecosystem functions. Plastics provide a durable substrate that can be colonized by micro- and macro-organisms. They support the growth of potential pathogens and HAB species. Further, plastic debris has been considered to play a key role in the dispersal also of biotoxins. The present study aimed to investigate harmful phytoplankton assemblages that colonized numerous samples of micro -and macro -plastics collected in the Mediterranean Sea by qPCR assay, and to forecast toxin dispersal by quantifying toxin content onto plastic debris. Further, the impact of polystyrene nanoparticles (PS NPs) to the HA diatom *Skeletonema marinoi* was analysed. All plastic samples were positive for the presence of Dinophyceae and Bacillariophyceae communities and some plastic samples were colonized by toxic species of dinoflagellate *Alexandrium pacificum*, *Alexandrium minutum*, *Ostreopsis* cf. *ovata* and diatom *Pseudo-nitzschia* spp. Strains of *A. pacificum* isolated from plastic debris and analysed by LCHRMS resulted to produce PST (paralytic shellfish poisoning). The levels of potential toxins on plastic samples ranged from 101 to 102 ng cm^{-2}. The interactions between HA diatom and nanoparticles demonstrated an increase of both extracellular and intracellular ROS, and significantly reduced colonies length using TEM and SEM techniques. Then, plastics can negatively impact the ecological functioning of oceans. The potential risk of harmful microalgae dispersal associated with plastic pollution was illustrated as well as the potential chemical compounds transfer through the trophic chain with consequent implications for human health and marine ecosystem.

Effects of thirty years of phosphorus reduction and climate change on *Planktothrix rubescens* (Cyanobacteria) in a deep lake south of the Alps (Lake Lugano, CH-IT)

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The transboundary Lake Lugano became severely eutrophic in the mid-20th century, which led to the appearance of blooms of *Planktothrix rubescens*. Thereafter, nutrient and thermal conditions have changed. A restoration programme, started in the ‘80s, reduced phosphorus loads and concentrations, bringing back the lake to mesotrophy, while, climate change has caused a significant rise in surface water temperature (0.6 - 0.9 °C per decade in summer since 1972). Despite the first positive effects of restoration, phytoplankton biomass was not affected since the ‘90s and cyanobacteria remained abundant, especially the filamentous species. Indeed, a wide surface bloom of cyanotoxin-producing *P. rubescens* was observed in the winter of 2019 - 2020, raising concerns about water quality and usage. Because cyanobacteria can be advantaged by warming, the persistent occurrence of *P. rubescens* blooms suggests that any effect of restoration on this species was offset by the rise in temperature, but the environmental drivers of *P. rubescens* abundance in Lake Lugano are poorly understood. In this study, a thirty-year record of biological and physico-chemical data was analyzed to i) quantify the seasonal patterns of variation in *P. rubescens* abundance, and ii) identify the controlling environmental factors. As expected, both nutrients and physical drivers (e.g. temperature and mixing depth) had an important role in controlling the abundance of *P. rubescens*, which supports the assumption that climate change hampered the effects of lake-restoration measures. The results indicate that lake management plans to mitigate ecosystem deterioration and health risks associated with cyanobacteria exposure should account for global warming scenario.

A decadal distribution of *Dinophysis* species and associated shellfish toxins in the Regional Seas of Europe (NE Atlantic, Mediterranean, North and Baltic Seas)

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Several species of *Dinophysis* can naturally generate Harmful Algal blooms (HABs) and are a major concern for public health due to their production of toxins. Knowledge on the chemical contents of these organisms is increasing but still largely understudied due to the genomic complexity of many groups. The CoCliME project, in an attempt to help anticipate the effects of climate change on HABs, reviewed 10 - year data sets from monitoring programs in Sweden, Ireland and France to examine the occurrence of *Dinophysis* species in coastal waters and their toxins in shellfish. *Dinophysis acuminata* was identified as the most distributed species across all study areas, consistent with literature data. *Dinophysis sacculus* was common in the French Mediterranean and Atlantic waters, while *Dinophysis acuta* was frequently observed in Swedish and Irish waters and *Dinophysis norvegica* in Swedish waters. *Dinophysis acuta*, *Dinophysis tripos* and (to a lesser extent) *D. norvegica* were limited by low salinity in the Baltic while *D. acuminata* was found to have the largest salinity range (5 - 35). While some subregions showed increasing or decreasing trends for some species of *Dinophysis*, no overall trend was detected at genus level. Among shellfish species, mussels (*Mytilus edulis* or *Mytilus galloprovincialis*) accumulated significantly (up to 50 - fold) higher levels of okadaic acid than oysters. All 16 bivalve species surveyed were able to metabolize pectenotoxin 2 (PTX2) to its seco acid analog. This confirms that mussels are a relevant indicator species for this toxin group and that shellfish effectively diminish the risk of human exposure to PTXs.

Bloom dynamics of an exceptional red tide of the toxigenic dinoflagellate

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The toxic dinoflagellate *Alexandrium minutum* generally proliferates in semi-enclosed sites such as estuaries, harbours and lagoons, where stratification, restricted circulation and accumulation of resting cysts set suitable conditions for its development. In the Galician Rías (NW Iberian Peninsula), its blooms follow also this pattern. They are recurrent in small, shallow estuarine bays inside the Rías, but rarely detected, and if so in minor amount, out of these areas. However, a massive proliferation of *A. minutum* from June to July 2018 in the Rías Baixas (Vigo and Pontevedra) changed this picture. The bloom initiated in semi-enclosed waters, as previously described for this species, but thereafter spread to the whole embayments where persisted more than one month. It generated a noticeable red tide with disperse patches that became heavily concentrated inside the port of Vigo. During that period shellfish harvesting closures and paralytic shellfish toxins in certain marine invertebrates and fish were reported for the first time in Spain. Meteorological conditions (higher than usual rains/runoff, sustained temperature increment and oscillating wind pattern promoting a series of upwelling-relaxation cycles) fostered optimal circumstances for the outbreak of *A. minutum*: strong vertical stratification and the alternation of retention and dispersion processes. Simulations from a particle tracking model portrayed the observed bloom development phases: onset, transport within the surface layer towards the interior parts of the Ría of Vigo, and dispersion all over the embayment. High concentrations of resting cysts were detected several months after the bloom, which may have favoured flourish of *A. minutum* in the following years, markedly in 2020.

Biogeography and phenology variations of *Ostreopsis* spp. in temperate areas facing ocean warming

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Ocean warming is suggested to be partly responsible for the expansion of *Ostreopsis* spp. in temperate areas. However, the effects of warming waters on species biogeography and phenology still need some evidences. We combined in situ (specific sampling campaign and long-term data series of bloom events) and laboratory approaches to assess and compare the development of *Ostreopsis* species in relation with sea temperature in two European temperature case areas. We analyzed changes in phenology of *Ostreopsis* cf. *ovata* in the NW- Mediterranean Sea, where intense blooms have been observed for the last decades, and the possible expansion of *Ostreopsis* cf. *siamensis* in the Bay of Biscay (NE-Atlantic), in which blooms have been detected so far in the southwestern part of the area, where sea temperatures are the highest. Ocean warming of the Bay of Biscay could potentially promote the extension of the blooming area of *O*. cf. *siamensis* towards higher latitudes, where the species is already present. Sea warming in NW Mediterranean Sea could result in more precocious and late blooms of *O*. cf. *ovata*, with a decrease in intensity in case of excessive sea temperature. In parallel, ecotoxicological experiments carried out at different temperature culture conditions suggest that warming sea temperatures could cause an increase of both *Ostreopsis* species toxicity. This work suggests that the effect of ocean warming on the future development of *Ostreopsis* spp. depend on species and regional areas, therefore risks associated with the development of the genus should be assessed locally.

Planktotrhix rubescens blooms triggered by stratification dynamics and irradiance

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Planktothrix rubescens blooms triggered by stratification dynamics and irradiance *P. rubescens* forms massive metalimnetic blooms, and has become a keystone species in Lake Zurich (Switzerland), a significant drinking water source. Effects of lake warming, i.e. changes in thermal stratification and seasonal deep mixing promoted its increasing importance for the food web since the 1970s. However, since 2009 two massive breakdowns and striking seasonal oscillations of the population were observed. In order to disentangle positive from negative consequences of secular lake warming on cyanobacterial dynamics, we evaluated a high resolution dataset of 320 depth profiles covering a 12-year period. Focus was set on the interplay of deep winter mixing, thermal stratification patterns and light regime as significant environmental drivers for cyanobacterial dynamics. Inter-annual climate variations proofed as decisive in shaping seasonal population oscillations, at times even counteracting long-term effects of secular lake warming. Intensive deep winter mixing and repetitive disruptions of the vernal metalimnion owing to harsh weather conditions each led to massive breakdowns of the populations but pronounced regrowth during thermal stratification. In contrast, years with weak deep mixing due to warming were not sufficient anymore to entrain *P. rubescens* to depths where their gas vesicles would collapse due to hydrostatic pressure. This resulted in high survival of overwintering populations but surprisingly moderate regrowth during stratified seasons. In summary, effects of lake warming were not as unidirectional as previously presumed and predictions of steadily increasing blooms of *P. rubescens* in Lake Zurich could not be confirmed for the last decade.

Evidence for massive and recurrent toxic blooms of *Alexandrium catenella* in the Alaskan Arctic

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The Arctic Ocean is experiencing rapid and dramatic changes in response to climate-driven warming. Many organisms may spread northward as a result of rising temperatures and loss of sea ice, but few present such significant threats to human and ecosystem health as harmful algal bloom (HAB) species. *Alexandrium catenella*, a producer of paralytic shellfish toxins (PSTs), has a long history of causing toxicity in the Gulf of Alaska, yet there is little recognition of this organism as a human health concern north of Bering Strait. Here we describe an exceptionally large *A. catenella* benthic cyst bed and hydrographic conditions across the Chukchi Sea that support germination and development of recurrent, self-initiating, and self-seeding blooms. Two prominent cyst accumulation zones result from deposition promoted by weak circulation. Cyst concentrations are among the highest reported globally for this species and the cyst bed is 6X larger in area than any other recorded. These extraordinary accumulations are attributed to repeated inputs from advected southern blooms and to localized cyst formation and deposition. Over the last two decades, warming has likely increased the magnitude of the germination flux two-fold and advanced the inoculation of the euphotic zone, where conditions are now favorable for bloom development, by 20 days. The region is poised to support recurrent blooms that are unprecedented in scale, increasing exposure and health risks to Alaskan Arctic communities where economies are subsistence based. These observations also highlight how warming can facilitate HAB range expansions into waters where temperatures were formerly too cold.

Environmental effect on the harmful microalgae community assemblages in Johor Strait

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Anthropogenic activities such as the mariculture industries, shipping/cargo port, land reclamation and dredging activities in the Johor strait have led to the water quality deterioration and increase of harmful algal blooms (HABs) events. In this study, the community assemblage of HAB species in the Johor Strait was investigated based on metabarcoding data collected between May 2018 and September 2019, covering 19 stations across the Eastern (EJS) and Western Johor strait (WJS). Subsurface water samples (150 L) were collected monthly from each station, followed by genomic DNA isolation. Amplicon-based sequencing on the 18S ribosomal DNA V9 region was performed using an illumine Miseq platform. Operation taxonomic units (OTUs) were assigned to taxa using the BLASTn against the NCBI database, with an E-value cut-off of 10 - 50 and ≥ 97 % similarity. Environmental physical-chemical parameters were also collected throughout the sampling to study the environmental effects on HAB communities dynamics. This study has revealed 26 HAB taxa, with 12 taxa were new records in the straits. The most common HAB species assemblage, with frequency occurrence > 70 %, consisted of *Heterosigma akashiwo*, *Fibrocapsa japonica*, *Pseudo-nitzschia pungens*, *Dinophysis* spp., *Gymnodinium catenatum*, *Alexandrium leei*, and *Alexandrium tamiyavanichii*. Our result showed that the phytoplankton and HAB species community was rarely exhibited homogenous between the WJS and EJS waters. The HAB assemblages were most likely affected by the environmental variables in the strait. This study provided a comprehensive understanding of the HAB dynamics, and HAB communities shift in the tropical eutrophic coastal ecosystem in Malaysia waters.

Response of dinoflagellate assemblages to climatic factors at the southern end of the California Current

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Quantitative data from two basins in the Pacific margin of Baja California Sur indicate annual to decadal climatic changes. The basins are influenced by the cool northern waters of the California Current and the warm southern waters of the Northern Equatorial Current. The changes are driven by Sea Surface Temperature (SST) and the climatic factors of the El Niño Southern Oscillation (ENSO) and the Pacific Decadal Oscillation (PDO). Dinoflagellate assemblages include heterotrophic (HET) and autotrophic (AUT) taxa concentrations and change synchronously with the climatic variations. Our data indicate changes in dinoflagellate concentrations at a semiannual scale from 1968 to 2009 in San Lazaro Basin (SLB) and biannual records from 1811 to 2009 in Magdalena Basin (MGB). HET taxa dominate the assemblages in both basins, where the most abundant taxon is the heterotrophic *Brigantedinium* sp. Results illustrate mixed oceanographic features and reflect the SST warming, suggesting tropicalization since ~1990. Influence of the California Current is reflected by the temperate to sub-polar autotrophic *Ataxodinium choane*. However, the thermophilic species *Stelladinium stellatum, Dubridinium caperatum, Votadinium calvum*, and *Quinquecuspis concreta* have increased since1996 reflecting the influence of the tropical Northern Equatorial Countercurrent. Ever since 1811, the highest (lowest) concentrations are observed during intervals with positive (negative) SST anomalies, supporting a regional increase in marine productivity during warmer periods. Our data indicate that in the southern part of the California Current, marine productivity will increase following the current global warming. Furthermore, despite the perceived effects of the warming, we did not observe the toxic species which occur commonly in locations to the south.

Are harmful marine microalgal blooms and their societal impacts increasing? A 30 year global data analysis

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Here we mine the global data bases of HAEDAT (Harmful Algae Event Database; 9,503 events) and OBIS (Ocean Biodiversity Information System; 6 million microalgal records) from the period of 1985 to 2018 to test the widely stated contention of increased frequency and distribution of harmful algal blooms (HABs). Total global numbers of HAEDAT events showed a statistically significant increase over time, while the global number of geographic grids with one or more HAB events also increased but this was only weakly statistically significant. Because of large differences in the level of monitoring, HAEDAT trends were examined on a region by-region basis and corrected for sampling effort using OBIS microalgal species records as a proxy. Based on these adjusted data, no evidence was found of a statistically significant, uniform global increase in the number of HAB events over the period considered. When expressed relative to monitoring effort, the number of HAEDAT events increased in five regions, showed no change in two regions, but decreased in two other regions. Within regions, trends of increase or decrease in HAEDAT case numbers varied and represented different HAB types, while other types decreased or stabilised. Our analysis points to intensified monitoring efforts associated with increased aquaculture production, and the regional emergence of new HAB syndromes or impacts, as key drivers of the increasing number of records of HAB events. Broad statements on global HAB trends increasing are not supported by the present meta-analyses, but trends need to be considered regionally and at the species level.

DNA metabarcoding assessment on phytoplankton community and diversity from East Coast of Peninsular Malaysia

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Maricultures industries have expanded rapidly in the countries but underlying risk due to HABs in particular the concern on the safety and security of our seafood supply was not assessed in many of the aquacultures sites. In this study, phytoplankton composition along the east coast of Peninsular Malaysia was analysed using next generation sequencing approach. Subsurface seawater samples were collected at 18 stations during the southwest monsoon in August 2019 using UMT Research Vessel Discovery. Plankton samples were filtered with 16 µm - mesh plankton net and preserved in buffer saline ethanol for metabarcoding analysis, with primer 1391F and EukB targeting SSU rDNA V9 region and Illumina MiSeq platform. Sequences obtained were obtained and annotated using a closed reference database, Protist Ribosomal Reference Database PR2, E-value cutoff 10 - 50, with ≥ 97 % sequence similarity threshold. Diatom was dominated by Chaetocerotales (33.0 %), Coscinodiscales (27.0 %), and Cocconeidales (19.9 %) meanwhile dominant dinoflagellates order was made up of Peridiniales (49.2 %), Gonyaulacales (16.8 %), and Suessiales (11.8 %). Twenty-five harmful dinoflagellates species and six harmful diatom species were confirmed, with ten species represented new records, including paralytic shellfish toxin producer (eight *Alexandrium* species, *Gymnodinium catenatum*), six *Pseudo-nitzschia* species known to cause ASP, (four potentially toxic *Dinophysis* species and three *Prorocentrum*), *Karenia brevis* and *Karenia. selliformis*, *Lingulodinium polyedrum* and *Gonyaulax spinifera*, and ichthyotoxic species *Karlodinium veneficum* and *Margalefidinium polykrikoides*. The HAB dataset obtained in this study will be a useful reference for mariculturist and as the baseline information for long-term changes in the areas.

Difference of the sensibility for anthropogenic chemicals, herbicides and antibiotics, between diatoms and harmful phytoflagellates

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A largest semi-enclosed sea in Japan, the Seto Inland Sea, has been under oligotrophication and experienced decreases of diatom occurrences while the phytoplankton niches are often dominated by harmful phytoflagellates. The oligotrophication and nutrient requirement of the phytoplankton species do not fully explain this phenomenon since the area and the season affected with nutrient-rich riverine water are yet under diatom senescence. Under a hypothesis where anthropogenic chemicals might affect the phytoplankton florae, two diatoms and four harmful phytoflagellates were exposed to herbicides and antibiotics those actually detected in the coastal seawater. The herbicides, diuron and bromacil, indiscriminately deterred growths of all the tested species, and no specific taxon-related susceptibility was found. The antibiotics, clarithromycin and clindamycin, were, however, distinctively toxic to the diatoms. Values giving EC50 of the daily cell division rate in *Skeletonema costatum* and *Chaetoceros lorenzianus* were 22.9 - 72.8 µg L^{-1}, while those of phytoflagellates of *Karenia mikimotoi*, *Prorocentrum shikokuense*, *Chattonella marina* var. *antiqua* and *Heterosigma akashiwo* were above 100 µg L^{-1}. Another antibiotic, azithromycin, were less toxic, and the highest tested concentration (1000 µg L^{-1}) gave a significant growth inhibition (less than 50 % of the daily cell division rate) only in *C. lorenzianus*. These results suggest that diatoms are more susceptible 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.

What factors control *Dinophysis acuta* population dynamics in the Chilean Fjords?

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Dinophysis acuta is considered the main producer of diarrhetic shellfish poisoning (DSP) toxins and pectenotoxins in Southern Chile. Unlike *D. acuminata*, blooms (> 10^3 cells L^{-1}) of *D. acuta* exhibit a short (summer) growth season. Nevertheless, environmental conditions associated with their interannual variability have not been explored. An exceptional bloom (650,000 cells L^{-1}) in 2018 was associated with a favourable combination of meteorological and hydrographic processes of multiple scale. Between 2018 and 2021, mid-summer (February) cruises were carried out every year along a 25 - km transect in Puyuhuapi Fjord -a "hotspot" for *Dinophysis* in Southern Chile- to describe vertical distribution of phytoplankton and fine-resolution measurements to depict water column microstructure. Data from the IFOP monitoring provided logistic support and were used to date the initiation and seasonal growth of *D. acuta.* Despite considerable interannual variability, maximal cell densities (118,000 and 35,800 cells L^{-1}in 2018 and 2019 respectively) were always found off the head of the Magdalena Sound, aggregated in subsurface (6 m) thin layers by the pycnocline. In 2020, cell densities did not exceed 200 cells L^{-1} in the same transect. Macroscale hydroclimatic conditions during 2018 - 2021 agreed with the persistent trend of negative precipitation anomalies and positive values of the Southern Atlantic Mode observed the last four decades. Here we examine differences in meso and microscale factors —density of winter populations, potential ciliate prey, water column structure— in addition to hydrodynamic features of the area. Our objective was to interpret the interannual variability in contrasting years in an attempt to describe the "optimal environmental window" for *D. acuta* blooms in the Chilean fjords.

Elevated pCO_2 and temperature significantly enhance cyanobacterial N_2-fixation in eutrophic freshwater ecosystems

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Harmful cyanobacterial blooms (cHABs) are recurring with increasing intensity due to climate change and eutrophication. While the positive effects of elevated temperature and nitrogen on cyanobacteria are well studied, the effect of elevated pCO_2 in tandem with elevated temperature and nitrogen amendment on cHAB taxa has never been characterized. Understanding how both climate change factors and eutrophication affect cHABs is critical for understanding how they will respond in a changing world, particularly with respect to growth and major ecosystem processes such as dinitrogen (N_2)-fixation. Here, we characterized cyanobacterial growth and N_2 - fixation in response to changes in temperature, pCO_2, and nitrogen enrichment during cHABs within lakes in NY, USA comprised of diazotrophs. Elevated temperature and nitrogen enrichment significantly enhanced cyanobacterial growth either under ambient and/or elevated pCO_2 that reflected cHAB origin, community composition, and season. In addition, cyanobacteria grown under elevated pCO_2 and temperature conditions exhibited significantly higher N_2-fixation rates relative to cyanobacteria grown under ambient pCO_2 and elevated temperature conditions, regardless of nutrient levels. Exogenous nitrogen amendment under elevated temperature and/or pCO_2 also enhanced N_2- fixation relative to that of diazotrophs grown under ambient pCO_2 conditions, strongly indicating that N_2-fixation is sensitive to the carbon-nitrogen balance. For all experiments and factors, rates of N_2-fixation were inversely proportional to cyanobacterial growth rates, suggesting that diazotrophy did not contribute to the intensification of these cHABs. Taken together, these data demonstrate how acidification can regulate cyanobacterial growth and N_2-fixation, and how temperature and nitrogen availability can become important drivers of cHABs under elevated pCO_2.

Intraspecific variation in *Pseudo-nitzschia seriata*: Temperature effecting growth and toxicity

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Phytoplankton make up the basis of the marine food web, and changes in abundance can have pronounced effects on higher trophic levels. Ecosystems worldwide are facing effects due to climate change, and it is therefore important to investigate the implications for phytoplankton. Increase in water temperature is a central effect of global warming, particularly in cold - water areas like the Arctic. When assessing the effect of increased water temperature on phytoplankton, the amount of physiological intraspecific variation may be considered a key factor for the survival of phytoplankton in a changing environment. We addressed two major questions: how will increasing temperatures affect the cold-water diatom *Pseudo-nitzschia seriata*? How is the response modulated by physiological intraspecific variation of the species? The response of *P. seriata* is of particular interest because it is known to produce the neurotoxin domoic acid. We exposed 50 strains of *P. seriata* to three temperatures (2, 6 and 10 °C). Preliminary results show a pronounced variation in growth response to the different temperatures among strains. There is an apparent variation in maximum growth rate (e.g. ranging from 0.22 to 0.99 cell divisions day^{-1} at 6 °C), and also the temperature at which different strains had the highest growth rate and maximum cell density showed considerable variation. For all strains production of domoic acid was measured, however, results of the toxin analyses are still being processed. Additionally, temperature-induced morphology changes will be examined.

Harmful Algal Blooms in a changing environment: *Alexandrium catenella* and Paralytic Shellfish Toxin for the past 50 years in Chile

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The first records of *Alexandrium catenella* and Paralytic Shellfish Toxin (PST) were in the fjords Southern area. Since 1972, an apparent expansion from South to North have occurred (55° to 36°S), but historical and biological data, point out the contrary. Many blooms and PST outbreaks have occurred, causing 35 fatal cases and about three hundred intoxications, but since 2000 no fatal cases have occurred. Some features are: (i) characteristic interannual variability in *A. catenella* density and shellfish toxicity; (ii) the existence of temporal-spatial differences between macro-regions and within them; (iii) a latitudinal increase of the annual highest toxicity records, but since 2015 this tendency has disappeared (e.g. 143,130 µg STX eq 100 g^{-1} in 2018, middle fjords area); (iv) differences in the periods when *A. catenella* density increases and PST outbreaks occur; (v) *A. catenella* may be the numerical phytoplankton assemblages dominant during blooms events (> 300,000 cells L^{-1}), but usually is < 1 % of the total numerical abundance; (vi) the Pacific Ocean cell densities are lower than those from the fjords; (vii) the Summer-Autumn bloom of 2016 in the Northern fjords, that finally went out to the Pacific Ocean; (viii) the bloom of 2018 served to show that in some areas, density increments are explained by a motile stage passive transport due to S or SE winds. Finally, the variabilities of *A. catenella* abundance and PST concentrations in shellfish, considering oceanographical and meteorological data for the last 15 years, suggest that climatic and oceanographic variables may be the main explaining factors of observed patterns.

Paralytic shellfish toxins in Arctic food webs

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Climate change-related ocean warming and reduction in Arctic Sea ice extent, duration and thickness increases the risk of cyst germination and toxic blooms of the dinoflagellate, *Alexandrium catenella*. This algal species produces neurotoxins that impact marine wildlife health and cause the human illness known as Paralytic Shellfish Poisoning (PSP). This study reports Paralytic Shellfish Toxin (PST) concentrations quantified in Arctic food web samples which include phytoplankton, zooplankton, pelagic fish, benthic worms, and benthic clams collected in summer 2019 during anomalously warm ocean conditions. PSTs were detected in all trophic levels with concentrations above seafood safety regulatory limits in clams collected offshore on the continental shelf in the Beaufort, Chukchi, and Bering Seas. These findings raise concerns regarding the potential for increasing PST exposure risks and health impacts to Arctic residents and marine wildlife as ocean warming and sea ice reduction continue.

Combined effects of temperature and light intensity on growth, metabolome and ovatoxin content of one *Ostreopsis* cf. *ovata* strain from the Mediterranean Sea

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The toxic species *Ostreopsis* cf. *ovata* is spreading yearly along the Mediterranean coast and has been related to human illness and unusual mortality of marine organisms. The enhancement of its proliferations in this temperate area has been linked to global changes and its consequences such as the increase of temperature or light intensities. To investigate the effects of these parameters, an experimental design using batch cultures of pre-acclimated cells was implemented. The combined effects of temperature (23, 27 and 30 °C) and light intensity (200, 400 and 600 µmol m^{-2} s^{-1}) on the growth, metabolome and ovatoxin (OVTX) content were explored on one strain of *O.* cf. *ovata* isolated from the French Mediterranean Sea. Growth was significantly affected by both parameters and higher growth rates were measured under 400 and 600 µmol m^{-2} s^{-1} and especially at 27 °C (0.48 d^{-1}). Metabolomic analyses highlighted a clear effect only for temperature that may correspond to two different strategies of acclimation to suboptimal temperatures. Unfortunately, only 8 significant features modified by the temperature and/or light condition were annotated, reflecting the lack of knowledge about the metabolome of such non-model organisms. Concerning OVTX content, temperature showed a significant and negative effect with higher values at 23 °C (29 – 36 pg $cell^{-1}$). In a context of global changes, these results suggest that the increase of temperature might favor the proliferation of less toxic cells. However, in the light of the intraspecific variability of *O.* cf. *ovata*, further studies with will be necessary to test this hypothesis.

Harmful Algal Blooms and Ocean Acidification: Defining a Research Agenda

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Harmful algal blooms (HABs) and ocean acidification (OA) are threats to marine ecosystems and human communities. HABs and OA have common drivers in coastal areas and often co-occur in space and time. Although many studies have provided insight into HABs dynamics, only a few of these have included OA. Similarly, OA studies have examined biogeochemistry and impacts to marine resources and have started to expand into multi-stressor studies, but less is known about the influences of HABs-OA interactions and cascading impacts to coastal ecosystems and economies. The NOAA Ocean Acidification Program and National Centers for Coastal Ocean Science Competitive Research Program held a virtual workshop to identify research needs at the intersection of HABs and OA in the United States. This presentation will focus on the grand challenges, research priorities, and research products highlighted at the workshop. Research areas of high priority include modeling for prediction, attribution, and sensitivity testing; leveraging monitoring assets to measure HABs and OA parameters simultaneously; data management to foster integration; data product development; and enhanced communication and outreach efforts. Importantly, the workshop identified a need for increased collaboration between HABs and OA scientists. Interdisciplinary approaches will be required to disentangle the complexities of HABs-OA interactions and address stakeholder needs.

Understanding the contribution of sediment nutrient fluxes to the proliferation of HABs formed by multiple genera of dinoflagellate

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This project documented the occurrence of multiple HAB events caused by *Alexandrium catenella* and *Dinophysis acuminata* in Northport Harbor, Cold Spring Harbor, and Shinnecock Bay, NY, U.S.A. Sediment fluxes of ammonium were large these systems and sediment fluxes represented 28 %, 48 %, and 50 - 60 % of the total nitrogen entering Northport Harbor, Weesuck Creek (of Shinnecock Bay), and Cold Spring Harbor during bloom periods, demonstrating these were significant and often the dominant source of nitrogen fueling these HABs. To complement field sampling, mesocosm experiments were conducted to assess the ability of sediment fluxes from muds and sands to influence the occurrence of HABs formed by *A. catenella.* Mesocosms with muddy sediments that had high rates of ammonium fluxes resulted in significantly higher levels of *A. catenella* compared to mesocosms with sandy sediments and sandy sediments amended with inorganic nutrients. A laboratory experiment with sediment porewater from muddy sediments demonstrated that nutrients from sediment pore water yielded growth rates of *A. catenella* cultures that were significantly greater growth rates of cultures grown on standard algal media with inorganic nutrients. Collectively, this study demonstrates that sediment nutrient fluxes can promote estuarine HABs, particularly those caused by *A. catenella*.

New records of intensive blooms of *Alexandrium minutum* (Dinophyceae) in the Ionian Sea (Italy)

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In Italy *Alexandrium minutum* is recurrently found at low concentrations in areas monitored for the presence of mussel farms, such as the Western Adriatic and Sardinia coasts; mussels positive to PSP toxins have been occasionally detected, and rarely exceeded the law limit for mussel commercialization. Conversely, the Sicilian coast represents a hot spot of *A. minutum* blooms repeatedly occurring in high density. This work attests the presence of extensive blooms detected for the first time in the Ionian coast of Calabria at Roccella Ionica: the first occurred in 2018, followed by a larger event in 2020 (79 and 261 x 10^6 cells L^{-1}, respectively); blooms occurred in March in the harbour dockyard, an area not monitored for toxic algae presence due to the absence of mussel farms, causing a yellow/brown water discoloration, while no PSP symptoms were reported. In order to understand if these blooms could cause harmful consequences, 5 clones were isolated from the 2018 event for molecular identification at the species level and analysis of the toxin content and profile. All clones were confirmed to belong to the *A. minutum* group and resulted to be PSP toxins producers with four out of five clones displaying high toxin levels. The toxin profile displayed slight differences among clones; however, GTX1, 4 were the prevalent analogs as previously observed in the Mediterranean area. Despite in Italy *A. minutum* blooms represent rare events, coastal areas interested by intensive toxic blooms are increasing especially in the South, posing some concern and evidencing the need of a better monitoring and management activity of sheltered coastal zones.

Effects of the plastic component bisphenol A on growth and toxigenicity of the dinoflagellate *Gymnnodinium catenatum* in laboratory cultures

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Plastic pollution is an increasingly severe problem in coastal and oceanic ecosystems. Plasticizers, catalysts, and additives, e.g., bisphenol A (BPA) widely used in plastic manufacture, and some components pose risks to human health and marine ecosystem functioning. In particular, BPA, a known environmental xenoestrogen capable of disrupting the endocrine systems of marine fauna, can be readily absorbed by diverse organisms, from bacteria and fungi to plants and animals. Ocean primary producers such as phytoplankton could also act as vectors of plastic micro- and/or nano-particles and xenobiotics. The marine dinoflagellate *Gymnodinium catenatum* is a significant global producer of paralytic shellfish toxins (PSTs), i.e., saxitoxin analogs. This dinoflagellate is frequently present on the Pacific coast of Mexico, where cell growth and bloom toxicity might be sensitive to xenobiotics such as BPA. To test this hypothesis, the effect of BPA on the growth and toxigenicity of cultured *G. catenatum* strain GCBAPAZ-8 from Bahía de La Paz, Baja California Sur, Mexico, was assayed at two BPA concentrations (0.6 and 2.5 $\mu g\ L^{-1}$). The growth rate in exponential phase decreased at the higher BPA dosage but final cell yield and toxin composition were apparently unaffected. The most abundant analogs were N-sulfocarbamoyl C1/2 >carbamoyl GTX2/3>decarbamoyl dcGTX2/3. Maximum toxicity (10.5 pg STXeq $cell^{-1}$) occurred after exposure to the higher BPA dosage. This preliminary study highlights the importance of continuing research on the xenobiotic and synergistic effects on growth and toxigenicity of harmful algal bloom species exposed to plastic pollution in coastal waters.

Microcystin-LR occurrence in twenty-four water reservoirs of the Eastern of Cuba

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For its drinking water supply, Cuba depends mainly on water stored in reservoirs. In Cuba, information on the occurrence of cyanobacteria and cyanotoxins is emerging from scientific research, i.e. from initial surveys and ongoing monitoring programs. The goal of this research is to present trophic status of 24 reservoirs of Eastern of Cuba, linking it with cyanobacteria occurrence and cyanotoxin levels. Were detected 38 Cyanobacteria species, being 23 (61 %) potentially toxics. The most frequent species were *Aphanocapsa* sp., *Aphanothece* sp., *Synechocystis aquatilis*, *Merismopedia punctate* and *Microcystis* spp. was the best represented genus with six toxic species. Microcystin-LR were detected in the 38 % of studied reservoirs with values higher than limit established by WHO (1 µg L^{-1}). The main environmental factors which trigger the growth of toxic species of cyanobacteria and cyanotoxins occurrence were water temperature and TN:TP ratio. The levels of microcystin-LR became a risk in most of the reservoirs, with the highest levels in Cautillo, La Yaya, Mícara and Hatillo, representing an imminent risk in terms of safe water supply.

Reddish-brown bloom of the dinoflagellate *Prorocentrum cordatum* in the brackish lake "Lago delle Nazioni" (Italy)

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Lago delle Nazioni is a semi-artificial brackish lake, located along the Western Adriatic coast. During winter 2019 - 2020 an exponential reddish-brown discoloration of the water caused by an intense bloom of the dinoflagellate *Prorocentrum cordatum* (Ostenfeld) was observed with densities up to 4.7×10^7 cells L^{-1}, and amounted to 99 % of the entire phytoplankton community, corresponding to a chloropyll *a* peak of 251 $\mu g\ L^{-1}$. The event occurred with low concentrations of inorganic nitrogen (N) and phosphorous (P) in the water, resulting in an N:P molar ratio far lower than the Redfield one. The lack of dissolved N led to hypothesize that mixotrophy and organic matter inputs could have played an important role in driving the bloom, as the lake is surrounded by agricultural crops. Since recent studies have reported the presence of tetrodotoxins (TTXs) in bivalves linked to *Prorocentrum*, the strain has been isolated and LC-MS analyzed, where no TTXs were detected. *Artemia* sp. assay confirmed the lack of toxic effects. Moreover, our strain was tested in bioassays using different N sources (urea, NO^{3-} and NH^{4+}) to investigate a potential bloom-trigger condition. Although growth rates did not differ among treatments, our results showed a higher N assimilation in the Urea condition (21 pg N $cell^{-1}$) compared to NO^{3-} and NH^{4+} (14 and 16 pg N $cell^{-1}$, respectively). Consequently, the cellular C:N ratio was lower with urea (9:1) than with nitrate (12:1), indicating the efficient utilization of organic N source, which may have contributed to the unusual bloom of this species during the winter season.

Potentially toxic species of the genus *Dinophysis* reported from the Pacific coast of Costa Rica

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The marine phytoplankton of the Caribbean coast of Costa Rica has been little studied. This coast has a different behavior of water masses than the Pacific coast of our country. Three main groups of phytoplankton were observed in this study. The most abundant group was the Bacillariophyceae class and in less abundance the dinoflagellates. Samples for phytoplankton analysis were collected from the coast of Limón, Caribbean coast of Costa Rica. The taxonomic composition and the number of microalgae present in the samples were determined. Seventy species of microalgae were recorded, of which 32 belong to the diatom group, 31 to the dinoflagellates and some species of radiolarians and coccolithophorids. The group of microalgae with the highest volume of organisms were the dinoflagellates and sometimes *Dinophysis caudata* on occasions was a significant increase and was observed in high abundance. The dominance of the genera *Tripos* and *Dinophysis* was observed. The most abundant diatom species were *Bacteriastrum*, *Thalasionema*, Coscinodiscus and *Rhizosolenia*, being *Thalasionema nitzchioides* the most representative. It can be observed that there was a dominance of planktonic dinoflagellates in all collection stations. Comparing the samplings over time, we see that there was a decrease in the number of *Chaetoceros* species with a dominance of the diatoms *Thalassiosionema* and *Cosninodiscus*. Finally, it is important to recognize that productivity is increasing during the rainy season due to the great diversity of species observed during sampling.

Population dynamics of the raphidophycea *Heterosigma akashiwo* in the intensive culture systems of *Argopecten purpuratus,* scallop shell, in Guaynuná Bay

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In the present study, the population dynamics of the raphidophyse *Heterosigma akashiwo* (Y. Hada ex Y. Hara & M. Chihara, 1987), responsible for noxious flowering in Guaynuná Bay (Casma, Ancash, Peru) during January and March of 2018 by *in situ* measurements of temperature and dissolved oxygen. Due to the continuous upwelling processes where nutrient-rich waters are located, the evolution of *H. akashiwo* was monitored in Guaynuná Bay. For the present monitoring, the study area comprised 6 stations: in order to reveal the cellular concentration of the algal bloom at different depths, based on (cells L^{-1}) and its relationship with environmental parameters. The material was collected between January 22 and February 1, 2018, and comes from 6 stations: 01-A-GUA, 01-B-GUA, 01-C-GUA, 01-A-SAL, 01-A- TOR and FERRAN-130. 90 water samples were obtained from all stations at the surface level (0 m), medium (0 - 5 m) and bottom (5 - 10 m), with the help of the diver. Twenty three phytoplankton taxa were identified, belonging to 4 families and 18 genera, with a high abundance of raphidophyceae (75 %) followed by dinoflagellates (15 %) and silicoflagellates (2 %). Population concentrations ranged from 1.3 x 10^6 to 97.6 x 10^6 cells L^{-1} with variations in surface temperature (SST) from 17.7 to 18.3 ^{0}C and dissolved oxygen from 6.01 to 10.54 mL L^{-1}. The raphidoficea *Heterosigma akashiwo* is responsible for the death of fish in different parts of the world and is potentially toxic due to the production of lipophilic toxins and phycotoxins.

New species blooms and emergent toxins in Uruguayan coasts related to climate change

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Climate-driven changes in coastal oceanographic and ecological systems are becoming evident and in some cases exacerbated by localized human activities, impacting on the distribution of marine populations and triggering changes in circulation and nutrients availability and hence the occurrence of Harmful Algal Blooms. There is no doubt that the problems caused by HABs are growing worldwide. For this reason, Uruguay integrates the network REMARCO in the study of HABs as one of the main environmental stressors studied by the Project OIEA RLA 7025. HABs are expected to increase as sea surface temperature rise. Warming expands the seasonal bloom opportunity and the range of distribution of species. Climate-driven change in species distributions is one of the main concerns in addition to the new toxins that could arise. Using long-term data we found in the last decade blooms of two dinoflagellates toxic species that have not been in bloom before. One of them Protoceratium reticulatum is a Yessotoxin producer, and the other, Dinophysis tripos is a Pectenotoxin producer. The last decade warming was noticeable on our coast and since changes in species composition were found. In spring 2017 a huge P. reticulatum bloom was found concomitant with lipophilic toxin. D. tripos had shown higher abundance since 2016 but until the moment we didn't found toxicity associated. In this study, a high temporal resolution monitoring on the oceanic coastal area of Uruguay will be used to understand how the emergence of harmful blooms and new toxins may by promoted by changes in sea surface temperature.

Photo: Tanos Grayeb

FRESHWATER AND MARINE HAB BIOGEOGRAPHY

Ancient DNA and microfossils reveal dynamics of three harmful dinoflagellate species off Eastern Tasmania, Australia, over the last 9,000 years

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Harmful algal blooms (HABs) have significantly impacted the seafood industry along the Tasmanian east coast over the past three decades. To investigate the long-term history of regional HABs, a combination of palynological and sedimentary ancient DNA (sedaDNA, including the use of hybridization capture) analyses was applied to marine sediment cores from inshore (up to 145 years old) and offshore (~ 9,000 years) sites at Maria Island, southeast Tasmania. Analyses focused on the paralytic shellfish toxin (PST) producing dinoflagellates *Alexandrium catenella* and *Gymnodinium catenatum*, and the red-tide dinoflagellate *Noctiluca scintillans*. Both palynology and sedaDNA revealed the presence of *A. catenella* throughout the cores, with a bloom-phase of *Alexandrium* ~ 15 years ago (inshore), indicating the recent stimulation of a cryptic endemic population. *G. catenatum* cysts were found spanning the last 30 years, supporting previous evidence of a 1970s introduction via shipping ballast water, and sedaDNA revealed the presence of a *G. catenatum*-like species inshore and offshore, however, unambiguous species identification was hindered by limited reference sequence coverage of *Gymnodinium*. sedaDNA detected *N. scintillans* inshore from ~ 30 years ago to present, and offshore in recent sediments, matching first observations of this species in Tasmanian coastal waters in 1994 accredited to climate-driven range expansion. This study provides new insights into the long-term distribution and abundance of three HAB species off Tasmania, with further research being required to identify the role of previously unnoticed species and the environmental factors driving bloom phases through time.

Cyanobacterial akinete distribution, viability, and toxin records in sediment archives from the Northern Baltic Sea

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Cyanobacteria of the order Nostocales, including Baltic Sea bloom-forming taxa *Nodularia spumigena*, *Aphanizomenon flosaquae*, and *Dolichospermum* spp., produce resting stages, known as akinetes, under unfavorable conditions. These akinetes can persist in the sediment and germinate if favorable conditions return, simultaneously representing past blooms and possibly contributing to future bloom formation. The present study characterized akinete survival, germination, and potential toxin production in brackish water sediment archives from the Gulf of Finland to understand recent bloom expansion, akinete persistence, and cyanobacteria life cycles in the northern Baltic Sea. Results showed that akinetes can persist in and germinate from northern Baltic Sea sediment up to > 40 and > 200 years old, at coastal and open-sea locations respectively. Akinete abundance and viability decreased with age and depth of vertical sediment layers. The detection of potential hepatotoxin production from akinetes and revived cultures was minimal and restricted to the coastal sediment core. Phylogenetic analysis of culturable cyanobacteria from the coastal sediment core indicated that the majority of strains likely belonged to the benthic genus Anabaena. Potentially planktonic species of *Dolichospermum* could only be revived from the near-surface layers of the sediment. Results of germination experiments supported the notion that, in comparison with *Nodularia* and *Aphanizomenon* spp. akinetes, *Anabaena*/*Dolichospermum* spp. akinetes play a more significant role in their life cycle and bloom initiation strategies. Further research is recommended to accurately quantify akinetes and create a higher rate of toxin gene detection from brackish water sediment samples in order to further describe species-specific benthic archives of cyanobacteria.

Dynamics of historical dinoflagellate communities from regions experiencing novel blooms of *Alexandrium pacificum*

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Toxic *Alexandrium* blooms are frequent in the North Island of New Zealand, and in recent years have resulted in cases of paralytic shellfish poisoning after the consumption of contaminated shellfish. *Alexandrium pacificum* was unknown from the coastal waters of the South Island until 2011, when a bloom developed in Queen Charlotte Sound accompanied by widespread contamination of shellfish. This species has subsequently bloomed annually and has recently been detected in nearby Pelorus Sound, an extremely important aquaculture region. In 2018, 250 mussel farms were closed for harvesting in the biggest shut down seen by industry for 25 years. Previous sediment cores from Queen Charlotte Sound identified historical *A. pacificum* cysts, corresponding with an introduction during mid - 1970s. It was hypothesized that unusual environmental conditions enabled the formation and spread of an intense bloom. In this study, we used a combination of molecular and palynological analyses applied to sediments cores from Pelorus Sound to investigate historical communities of harmful dinoflagellates and associated environmental conditions. Dinoflagellate, bacterial and eukaryotic communities all showed similar patterns with discrete shifts in structure over time. Sequence reads identified as Dinophyceae dominated most samples down the cores. Molecular techniques (metabarcoding and droplet digital PCR) verified the presence and high abundance of *A. pacificum* in the last few decades. Other toxic dinoflagellates were detected including *Dinophysis*, *Karenia*, and *Prorocentrum*, all common bloom forming species in Pelorus Sound. Paleo-environmental conditions and associated community structure will help to identify the factors driving this increase in *Alexandrium* blooms, with the aim to predict plankton community responses under different future climate scenarios.

Environmental factors promoting the recurrent *Alexandrium minutum* blooms in a semi-enclosed tropical lagoon of Malaysia

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The paralytic shellfish producing species *Alexandrium minutum* was first reported to occur in Geting Lagoon, north-eastern Peninsular Malaysia in 2001 when an unprecedented paralytic shellfish poisoning (PSP) event was reported from the area. From then on there was no record of PSP reported, until 2015 an outbreak recurred for several months. This study was a continued monitoring effort to investigate the occurrence of *A. minutum* in the lagoon. Field sampling was conducted from January 2018 to October 2020 to examine the triggering factors influencing the phytoplankton dynamics and the recurrence of *A. minutum* bloom. In August 2020 bloom of *A. minutum* recurred and last for at least three months. Here, we reported the weekly *A. minutum* abundances in relation to the physical chemical parameters collected. The results showed that the inner-most part of the lagoon demonstrated a significant bloom density, reaching the density up to 1.5×10^7 cell L^{-1}, coincided with high phosphate level of > 4.15 µM. Seawater temperature was recorded in the range of 30.2 – 30.5 °C, salinity ranged from 10.4 –11.8, and dissolved oxygen levels ranged 5.68 – 8.00 mg L^{-1}. Notably, an intense fluctuation of water parameters was recorded during the early stage of bloom termination. The results of this study suggested that nutrient enrichment, particularly phosphate, plays an important role in the recurrent bloom of *A. minutum* in the lagoon.

Effects of recurrent Harmful Algae Blooms in the Northern Gulf of California, Mexico

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A Harmful Algal Bloom (HABs) is an accumulation in the algae population that causes a negative impact. Paralytic Shellfish Toxins (PSTs) are a group of neurotoxins that accumulate in shellfish and planktivorous fish. Consuming contaminated shellfish results in Paralytic Shellfish Poisoning (PSP) and can cause death from cardiorespiratory arrest. The fishery of the geoduck clam *Panopea globosa* is one of the most important economic activities in the Northern Gulf of California (NGC), Mexico. In 2010, the health authority COFEPRIS began monitoring PSTs in geoduck extracted from the NGC and implemented a sanitary ban due to the detection of toxins. In January 2015, an intense and extensive HAB of the PSTs producer *Gymnodinium catenatum* was detected near San Felipe Baja, California. This HABs was associated with large bird and marine mammal die-offs, the accumulation of PSTs in *P. globosa*, in concentrations up to 16,000 µg STXeq kg^{-1}, and the intoxication of at least five people after consuming contaminated wild bivalves. Since then, HABs of this dinoflagellate have occurred in January every year in the same region. The recent increase in the number and intensity of the HABs in the NGC is causing non-quantified impacts on the wildlife, coastal economic activities, and public health and is making it necessary to understand factors that can be developing the events. The present work describes the HABs that occurred in the NGC in recent years (2015-2021) and their effects on wildlife, public health, and shellfish fisheries in the area.

Distribution, abundance and toxin variability of azaspiracid producing Amphidomataceae in North Atlantic and North Sea waters

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Azaspiracids (AZA) are a group of lipophilic toxins, which are produced by a few species of the marine nanoplanktonic Amphidomataceae. In 2018, a survey was performed to strengthen knowledge on distribution and abundance of AZA-producing species and their respective toxins around Ireland and in the North Sea. *Azadinium poporum* was rarely detected and very low in abundance. *Azadinium spinosum* was present all-around Ireland with a peak density of 8.3 x 10^4 cells L^{-1} and AZA abundances up to 1,274 pg L^{-1}. *Amphidoma languida* was also widely present and appeared in remarkably high abundance (1.2 x 10^5 cells L^{-1}; AZA: 618 pg L^{-1}) in the central North Sea. Moreover, 82 new strains of *Az. spinosum* and *Am. languida* were established. All ten new *Am. languida* strains conformed in morphology and toxin profile (AZA-38/-39) with previous records from the area. Five strains assigned to *Az.* cf. *spinosum* conformed in morphology with *Az. spinosum*, but differed significantly in sequence data and lacked any AZA. For *Az. spinosum*, strains of two distinct ribotypes, which consistently differed in their toxin profile were present. Moreover, within ribotype A there was strain-specificity in presence/absence of AZA-2/-33. Toxin profiles of all strains were consistent and stable over time (up to 18 month). Total AZA cell quotas were highly variable both among and within strains, with quotas ranging from 0.1 – 63 fg AZA $cell^{-1}$. Overall, the data provide a more differentiated perspective of distribution, abundance and diversity of AZA production in the area of the world ocean that is most affected by AZA.

Global biogeography of harmful algal bloom events

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Harmful algal blooms (HABs) are natural occurrences that may negatively impact aquatic ecosystems and human health. Depending on the species, many toxins can be produced, potentially causing different illnesses, i.e., Paralytic Shellfish Poisoning (PSP), Amnesic Shellfish Poisoning (ASP), Diarrhetic Shellfish Poisoning (DSP), Neurotoxic Shellfish Poisoning (NSP), Cyanotoxin effects (CTX) or Ciguatera Fish Poisoning (CFP). These toxic events can cause morbidity and mortality in marine wildlife via direct bioaccumulation of toxins in filter-feeding organisms as well as through consumption of contaminated prey by higher trophic level organisms. Trophic transfer of toxins during HABs also presents risks to human and overall ecosystem health. Additionally, HABs events are responsible for shellfish harvesting closures, leading to major economic and sociological impacts. In the present study, we aim to identify the hotspots of the different HABs groups worldwide and discuss patterns of bloom frequency and duration. To this end, data were collected from the Harmful Algae Event Database (HAEDAT), compiled, filtered, and standardized for the period between 1978 and 2018. This database compiles the data fed through monitoring programs worldwide by ICES and PICES member states. European waters appear to constitute the major hotspot regarding most toxins analyzed. Analysis of the total number of events reported revealed that most events reported PSP occurrences, followed by DSP, with NSP occurrences being the least abundant. The results of the present work contribute to a better understanding of the dynamics, geographical distribution, and temporal patterns of HABs events globally over the last four decades.

Diversity of HAB species in Samborombón Bay (Río de la Plata estuarine front, Buenos Aires, Argentina): first records and morphological description of ichthyotoxic species

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Samborombón Bay is located on the south of the Río de la Plata estuarine front (35°27’ S – 56°4´ W to 36°22’ S – 56°3´ W), where the waters of the river mix with the South Atlantic Ocean. This creates particular ecological conditions that allow it to support valuable artisanal and coastal fisheries, and function as a nursery ground in the case of the whitemouth croaker (*Micropogonias furnieri*), a highly economic important species for Argentina and Uruguay. Phytoplankton from this area, particularly harmful algae, have been scarcely studied. In this work a preliminary list of potentially harmful species is presented. Surface samples were collected once per month, from December to March, during two summer seasons (2020 and 2021), in four stations along the bay. In addition, at each site values of temperature, salinity, and other environmental variables were taken. Sample analyses were performed with an inverted microscope. Cultures were obtained from non-fixed samples for molecular and toxinological purposes in the future. Potentially harmful species belonging to the genera *Pseudo-nitzschia*, *Akashiwo*, *Gymnodinium*, *Dinophysis* and *Prorocentrum* were found in 46 % of the samples from three of the studied stations. Moreover, fish-killing algae such as *Fibrocapsa japonica*, *Heterosigma akashiwo*, and *Chattonella marina* were registered and morphologically described. The relationship of the occurrence of these genera with environmental variables was explored. Although the abundance of HABs species has been relatively low, they are potentially bloom-forming due to the characteristics of the estuary. The present study includes the first records of ichthyotoxic species for this area.

Does *Chrysophaeum taylorii* Lewis & Bryan represent a threat for coastal ecosystems of Eastern Mediterranean?

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The mucilage phenomenon has been highlighted in recent years by the occurrence of increasingly severe planktonic or benthic algal blooms in the world seas. The mass development of marine mucilaginous aggregates in the coastal ecosystems has a great ecological impact due to significant role in the benthic and pelagic food web and negative effect on marine ecosystem. *Chrysophaeum taylorii* is a marine chrysophytes belonging to Sarcinochrysidales. This species is known as one of the mucilage producer species in the benthic areas. The distribution of this species was given from tropical regions of the Atlantic and Pacific Oceans. So far benthic mucilage caused by Sarcinochrysidales (Chrysophyceae) was observed several times in the warm temperate region of the western Mediterranean, but it has never been observed more east than the Tyrrenian Sea. In this study, *C. taylorii* is reported for the first time in the Eastern Mediterranean Sea. This new record contributes to the algal flora in Turkish seas in terms of species richness, and also supplies new information about spreading areas of *C. taylorii*, defined as "a nuisance algae" due to its mucilaginous colonies in the benthic marine ecosystems, in the Mediterranean Sea. Monitoring studies are of great importance in terms of determination of the change in the Mediterranean and revealed the potential causes and consequences of these changes. If the occurrence of benthic mucilage is continues and more widely spreads around the coastal areas of the Mediterranean Sea in future, benthic ecosystem may be damaged and serious environmental and economic losses may occur.

Biogeography and seasonal distribution of harmful algae in the Baltic Sea and the Kattegat-Skagerrak investigated using metabarcoding and microscopy

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Harmful algae affect e.g., the aquaculture industry and tourism along the coasts of Sweden. Metabarcoding revealed previously unknown diversity and distribution of harmful algae. In a project aimed at adding metabarcoding methodology to the Swedish National Marine Monitoring Program phytoplankton samples for analyses of phyto and microzooplankton using metabarcoding and microscopy (Utermöhl method) were collected during a year. Sampling was carried out approximately once a month at ~ 15 locations covering a salinity gradient of 3 to 30. Illumina was used for 16S and 18S metabarcoding. Longer sequences were analyzed in a subset of samples using Pac-Bio. Phycotoxin producing dinoflagellates from the genera *Alexandrium*, *Azadinium*, *Dinophysis*, *Gonyaulax*, *Lingulodinium* and *Protoceratium*, were observed. Representatives of the diatom genus *Pseudo-nitzschia*, producer of domoic acid, were also observed. Toxin producing cyanobacteria include *Nodularia spumigena*. Fish killing genera include *Karenia*, *Karlodinium*, *Pseudochattonella*, *Heterosigma*, *Chrysochromulina* and *Prymnesium*. The geographical and seasonal distribution of the HAB taxa will be presented at the genus, species or at the Amplicon Sequence Variant (ASV) level together with a discussion on the advantages and disadvantages of the methods used.

Presence of dissolved domoic acid in the Bay of Todos Santos, Baja California, Mexico

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Domoic acid (DA) is the potent neurotoxin responsible of the amnesic shellfish poisoning (ASP) in humans and has been associated with marine mammals and seabirds mortalities. Particulate DA (pDA), the toxin content in the phytoplankton fraction, is regularly monitored to assess the presence of this toxin in the environment. However, DA can as a dissolved fraction (dDA). The permanence, transformation, and degradation of dDA in the environment have not been studied. Therefore, we evaluated the temporal presence of dDA, its relationship with pDA and species responsible for the accumulation of these toxin fractions in Todos Santos Bay, Baja California from July 2017 to February 2020. We detected two clear conditions. When *Pseudo-nitzchia* cf. *australis* abundance was low or this species was no detected, maximum dDA concentrations were up to 650 ng L^{-1} and maximum pDA concentration was 50 ng L^{-1}. The second, and most important condition was when *P*. cf. *australis* abundance was high. During blooms of this species as in May 2018, maximum concentration of pDA was 54,450 ng L^{-1} and 10,790 ng L^{-1} for dDA. This was the most intense bloom registered in the region. The highest pDA concentration was 17 times higher than that documented when low abundances of *P*. cf. *australis* were present. The present work documents the importance of dDA in the environment. This fraction was detected in 54.7 % of the analyzed samples and represented 70 to 100 % of the total DA. This suggests that dDA is ubiquitous and persistent in the marine environment, probably related to a low degradation rate.

Alexandrium minutum and *Gymnodinium catenatum* bloom dynamic in the Galician Rías

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Alexandrium minutum and *Gymnodinium catenatum* are associated with paralyzing toxins in Galician Rias (NW Spain). This upwelling area supports the mussel culture on suspended ropes (270,000 t year^{-1}). Both autotrophic dinoflagellates produce cysts and have developed toxic episodes and red tides in the Rías due to slow growth in situ from the internal part to the outside in the axis that the river forms with the external platform. In addition, *G. catenatum* appears suddenly, due to advection associated with downwelling. Intensive monitoring has not detected the coexistence of these dinoflagellates in the Rías. The voracious *Noctiluca scintillans* can control flowering in both. The small tecate *A. minutum*, especially develops in localized points of the Ría de Ares and the estuary of Baiona. It is very common for it to appear after upwelling and accumulating in the front that forms between the fresh water flowing out of the rivers and the salty water of the estuary. It then forms blooms with the strong pycnoclines, both due to salinity and temperature, as happened in 2018 when it produced an unusual red tide in Vigo, disappearing due to subsidence. The nude *G. catenatum*, a maker of large chains, has developed episodes that have started in the internal part of the Ría de Pontevedra, with export to the external part but the most common is that, in certain years with very warm autumns, *G. catenatum* suddenly penetrates the estuaries from south to north with the downwelling water from the surface and sinks into the estuaries, thus producing the accumulation of toxins also in infaunal mollusks.

Distribution and blooms of *Akashiwo sanguinea* in a tropical estuary: The role of bottom-up dynamics during wet and dry periods

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The incidence and frequency of harmful algal blooms have increased worldwide over the past few decades. These events may pose serious threats to ecosystems through changing trophic structures and some species can even cause human health concerns due to toxicity. The mixotrophic dinoflagellate *Akashiwo sanguinea* is a non-toxic species, but its blooms can still be harmful. This paper discusses whether and how ecological stoichiometric fluctuations (N, P, SiO_2 and their ratios) and environmental descriptors influence the abundance (in biovolume) and spatial dynamics of *A. sanguinea* during two wet and dry periods in a tropical estuarine system. Cluster ordination of log-transformed biovolume formed three groups based on ecological criteria. The variables were then also clustered and constrained to the biovolume discontinuities as factors. The highest biovolume accumulation was during the dry season of 2013, followed by both wet seasons. The main factors associated with distributional patterns throughout the sites were a combination of specific environmental conditions regarding mainly salinity, temperature and nutrient availability.

Updates on HABs and their consequences in FANSA region

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In FANSA region, HABs are very frequent and diverse in terms of causative species, distribution, and impacts. Several marine and continental toxic or noxious species have been reported over the entire region, including Atlantic (Argentina, Uruguay and Brazil) and Pacific coasts (Chile, Peru and Ecuador), and inland water bodies. In the last years, successive episodes of bivalve contamination with lipophilic toxins from *Dinophysis* spp. have occurred in Argentina, Uruguay, Peru and southern Brazil, as well as massive fish kills by *Pseudochattonella* and *Karenia*, and *Alexandrium* blooms in southern Peru and southern Chile. Recently, April 2021, in this same place there was a mortality of salmon in cultures due to *Heterosigma* sp. and two species of *Leptocylindrus*. The region has also been subjected to mass mortality of invertebrates and whale calves, associated to the presence of yessotoxins in northern Chile and of PSTs and domoic acid in Argentina, respectively. Detection of PSP in Peru, YTX and PTX in Argentina, and the high diversity of toxigenic benthic dinoflagellates in Northeast Brazil are also of special concern. Finally, cyanobacteria and cyanotoxins constitute a major issue in the region. In all FANSA countries, cyanotoxins have been reported in drinking water sources, coastal recreational waters and lakes, where microcystins, followed by cylindrospermopsins and saxitoxins are the most frequent toxins detected. In the last years, massive *Microcystis* and *Dolichospermum* blooms have affected freshwater resources and coastal waters in eastern Argentina, Uruguay and Peru, and blooms of *Trichodesmium* have been recurrently observed in oceanic waters near the Brazilian continental slope.

Seasonal succession of phytoplankton community in the tropical semi-enclosed Johor Strait of Malaysia

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The occurrence of harmful algal bloom in aquaculture areas has posed serious threats not only to seafood safety but also to the economy of the affected countries. Therefore, understanding the occurrence and distribution of harmful phytoplankton in the area is crucial in developing a comprehensive monitoring plan to minimize economical losses. A field study was carried out between 2017 and 2018 to investigate the pattern of seasonal succession in the phytoplankton community its relationship with environmental factors along the tropical semi-enclosed Johor Strait. The in situ physical (temperature, salinity, pH and dissolved oxygen), macronutrient (nitrate, ammonia, nitrite, silicate, phosphate), chlorophyll *a* were determined. Bloom-forming diatoms included *Chaetoceros*, *Coscinodiscus*, *Eucampia*, *Guirnadia*, *Navicula*, *Pseudo-nitzschia*, *Rhizosolenia*, *Skeletonema* and *Thalassiosira* were preponderant and dominated alternately or co-exist together during bloom events. Meanwhile, dinoflagellate *B. quinquecornis* and *Scrippsiella* were the dominant dinoflagellates, causing two blooms during inter-monsoon in 2018. This finding showed that nitrate and silicate was the trigger factor for the bloom-forming of diatoms, and phosphate was important for dinoflagellates succession during the time. The presence of shellfish toxins producing dinoflagellates (*Alexandrium*, *Prorocentrum*, *Dinophysis*) and diatom (*Pseudo-nitzschia*); and fish-killing species (*Karlodinium*, *Karenia*, *Chattonella*) were encountered in the strait especially in the aquaculture area. This study also served as a follow-up study to monitor harmful species in the strait following the incidents of massive fish kills in 2014 - 2015 and this information will contribute better insight into the spatial-temporal pattern of harmful algal blooms in Johor Strait water.

Dinoflagellate cyst assemblages from the South central coast of Chile (~36°- 43° S)

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The study of dinoflagellate cyst assemblages along the open coast to the Pacific Ocean have been scarce in comparison to the cyst study at the fjord system (~41°–55°S). Here, in order to determine the diversity, abundance and distribution of cysts in the South central coast of Chile (~36° – 43° S), 63 sampling stations were established at 2, 5 and 10 nm from the coastline, which were sampled twice using a HAPs corer or a Van Veen drag between August 2020 and January 2021. Surface sediment samples were placed in black bags and stored at 4°C, until processing. Samples were processed as described by Matsuoka and Fukuyo (2000). Full and empty cysts were counted and identified using biological or paleontological nomenclature for their designation. A total of 62 taxa of dinoflagellate cysts representing 19 genera, 46 species and 11 unidentified form were identified. The toxin-producing species *Alexandrium catenella*, *Alexandrium ostenfeldii* and *Protoceratium reticulatum* were identified. *Alexandrium catenella* cysts were recorded from 39.2° S to the South for the first time along the Chilean coast. Usually, the highest cyst abundances were recorded in stations at 10 nm from the coast, and the lowest abundances closer to the coast. Cyst assemblages were more diverse and abundant in the active upwelling system off Concepción (35° – 37°S), recording in this area the presence of *A. ostenfeldii* cysts. All assemblages are characterized by the dominance of heterotrophic species, being the genus *Protoperidinium* the most diverse of the study area with 16 species.

Early detection of HABs species in the Tumaco Bay

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The economy in the municipality of Tumaco, Nariño-Colombia is characterized by agriculture, logging, tourism, fishing, extraction of crustaceans and mollusks considered hydrobiological resources that constitute the major source of economic and food income, depending directly or indirectly up to 30 % of the population. These activities may be affected by interannual changes in the ocean-atmospheric dynamics of the Colombian Pacific basin, the transfer of microorganisms by ballast water and allochthonous deposits from rivers. These factors favor Harmful Algal Blooms (HABs) that affect water quality. The objective of the research is to identify the HAB-forming species, as well as the richness, distribution and abundance found in Tumaco Bay. Preliminary results indicate that 45 FANs species have been identified, 17 harmful and 28 toxin-producing, among them, the most abundant species, the diatom of the genus *Pseudo-nitzschia* (Domoic acid) and the dinoflagellates *Prorocentrum micans* (Toxin not known), *Dinophysis caudata* (Dinophysitoxins), *Alexandrium complex* (Saxitoxins), *Gymnodinium catenatum* (Saxitoxins), *Akashiwo sanguinea* (Surfactants), *Phalacroma rapa* (Okadaic acid), *Margalefidinium* (Ichthyotoxins) and *Karenia* (Ichthyotoxins). The latter are new records for the bay of Tumaco. The information gathered in this research is intended to be the basic input for prevention, regulation and prediction of future HABs events in Tumaco Inlet.

Photo: Tanos Grayeb

HA BIOLOGY

Cyst dormancy cycling controls *Alexandrium catenella* bloom termination in the Nauset Marsh estuary

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Dinoflagellate cysts can alternate between states of dormancy and quiescence many times in their lifetime, controlling when they can germinate to initiate and renew blooms. In the PSP species *Alexandrium catenella*, cycles have a period of about one year, a rhythmicity that is driven by two opposing temperature relationships. The first is chilling-mediated passage through dormancy and the second is warming-mediated passage through quiescence. The two relationships are well described by chilling units and growing degree days, both simple heuristics commonly used in horticulture. Critical parameters of the chilling and growing degree day relationships were estimated by fitting experimental data from Gulf of Maine cysts to develop a simple model of dormancy cycling and explore the effects of warming on *A. catenella* bloom phenology. Increases in mean temperature and temperature seasonality drive changes in the timing, synchronicity, and intensity of germling fluxes from cyst beds. Comparison of outputs to bloom phenology in the Nauset Marsh (Cape Cod, MA, U.S.A.) reveals how dormancy induction prevents revival of blooms after sharp encystment-driven declines. In the absence of dormancy induction, blooms quickly recover, taking advantage of physicochemical conditions that remain favorable for continued vegetative cell division and bloom development. The result mirrors recent measurements of high cellular N and P quotas through the course of encystment events. In Nauset, sexual induction of vegetative cells is the immediate driver of most bloom declines but blooms quickly re-develop so long as cyst beds remain quiescent and able to produce new vegetative cells.

Degree day-based cyst germination rates and *in situ* germling production of *Alexandrium catenella*

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Blooms of many dinoflagellates, including several harmful algal bloom species, are seeded and revived through germination of benthic resting cysts. Temperature is a key determinant of the germination rate, and temperature–rate relationships are therefore fundamental to understanding cyst bed persistence and germling cell production. Rate differences within and between populations also underlie cysts' ability to adapt to environmental change. This study conducted sediment slurry germination experiments with *Alexandrium catenella* cysts at different temperatures and developed an approach using degree-days (DDs; a metric which uses temperature and time) to measure and compare rates. As temperature increased, the median days until germination decreased hyperbolically. The median DDs until germination and the variance (slower and faster cysts) were consistent across all temperatures, demonstrating this metric's efficacy at capturing the diversity of a population's response. Cysts from the Nauset Marsh estuary (Cape Cod, MA) germinated faster than cysts from the Gulf of Maine, a difference that advances germling production in Nauset by more than a week, all else being equal. Derived germination rates were also used to explore data from Plankton Emergence Trap (PET) deployments in Nauset. Observations from PETs and cyst bed samples indicate that a miniscule fraction of the total cyst bed germinates to inoculate the annual Nauset bloom. Comparison between observed and modeled germling production suggests that as temperatures warm seasonally, *in situ* germination is increasingly suppressed, presumably by hypoxia. This research provides a framework for comparing germination by different populations and for investigating relationships between climate and germling production.

Physical factors and production of saxitoxin analogues in *Gymnodinium catenatum* and *Alexandrium pacificum* cultures

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In vitro production of the common saxitoxin analogues C1+2 and B1 in the dinoflagellates *Gymnodinium catenatum* and *Alexandrium pacificum* was related with several natural physical parameters, such as temperature and oscillations in geomagnetic activity or solar activity, such as the X-ray output derived from the 11-year sunspot cycle. The disulfated C1+2 toxin increased significantly with temperature and X-ray levels in *G. catenatum*. In *A. pacificum*, C1+2 presented only a low increase with temperature and decreased significantly with X-ray levels. Toxin relation with geomagnetic activity was not clearly observed in *G. catenatum*, while in *A. pacificum* a J-shaped hormetic dose response was observed centered around the 7-20 nT interval. Increasing oxidative stress also increased toxin levels, either triggered by an external extremely weak low-frequency magnetic field or hydrogen peroxide. Ultraviolet light increased toxin content only in the highest doses assayed. The cellular concentrations of the disulfated C1+2 over B1 presented average ratios of *circa* 9-10 for both species. Toxin increase after exposures conducing to increased oxidative stress was mostly attributed to C1+2, while B1 was reduced in some cases, in particular in *A. pacificum*. The relevance of sulfation observed in both microalgae is in accordance with the general dominance of sulfated analogues in toxin producing marine dinoflagellates and can be related to oxidative stress. If saxitoxin analogues can play a multifunctional role, remains elusive so far.

Physiological, genetic and toxicological variabilities in *Alexandrium catenella* strains from southern Chile (41°– 55°S)

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Ten strains of *Alexandrium catenella* isolated from the Chilean fjords (41° - 55 °S) were cultivated at a standard temperature (15 °C), salinity (34) and luminic intensity (60 µmol m^{-2} s^{-1}) to assess physiological, genetic and toxicological responses. The growth rates were higher at the northern area of the fjords (Los Lagos region) (0.54 div d^{-1}) and lower at the southernmost area of the fjords system (Magellan region) (0.22 div d^{-1}). DNA extraction was performed amplifying the no coding (ITS1 + 5.8S rDNA + ITS2) and encoding (SSU) regions of nuclear ribosomal DNA, which showed an intraregional genetic diversity among strains. These strains belong to Clade I of the phylogenetic tree of the *A. tamarense* complex. The toxin profiles were characterized mainly by carbamates (GTX1-4, GTX2-3, GTX5) and sulfocarbamoil (C1 y C2). Since STX and neoSTX were not detected in most strains, the related genes to paralytic toxins synthesis (sxt4) were assessed to test if are transcriptionally active in the studied strains.

Potentially toxic microalgae and their toxins in the Gulf of Naples (Mediterranean Sea)

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The high diversity of potentially toxic microalgae, their ecological characteristics and toxin content variability require that a site-based approach be taken to assess their possible impacts and health risks. Here we present an account of the diversity of toxin-producing microalgae in the Gulf of Naples (Campania region), a densely populated area in the Mediterranean Sea, along with information on their toxins. The study was based on phytoplankton samples collected at the LTER-MC site over the last 35 years, and on 61 microalgal strains analyzed through HPLC-time-of-flight mass spectrometry (HPLC-TOF) and mouse bioassay. In addition, liposoluble toxins were analyzed in 14 net samples collected in spring and summer 2017-2019. The list of potentially toxic species of the Gulf of Naples consists of 54 species. Among these, toxin production was confirmed from culture material of 9 species belonging to the genera *Alexandrium*, *Azadinium, Prorocentrum*, *Protoceratium* and *Pseudo-nitzschia*. In natural phytoplankton samples, the most frequent toxins were domoic acid, okadaic acid and yessotoxin, most likely produced by *Pseudo-nitzschia* spp., *Dinophysis sacculus* and *Protoceratium reticulatum*, respectively, which were identified in light microscopy in the same samples. In the benthos, ovatoxins produced by *Ostreopsis* cf. *ovata* were frequently detected in cultivated strains and invertebrates. These results confirm that the Gulf of Naples, as other Mediterranean areas, despite few reports of cases of impact, harbors a great diversity of toxic species and toxins, which should be taken into account in view of future expansion of aquaculture and tourism activities.

The effects of the light intensity on the growth of *Amphidinium* sp. (Dinophyceae)

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The genus *Amphidinium* is notable for producing amphidinols, which promote potent anticancer, antifungal, and hemolytic activities, and are potential sources of new drugs. Also, produce other metabolites with pharmaceutical interest like fatty acids and carotenoids. Light affects microalgae growth, which provides the energy to support their metabolism; high photosynthetic efficiency is indispensable in large cultivation systems; an increase in illumination stimulates growth; however, if present in excess, has an inhibitory effect causing a reduction in duplication rate. This study evaluated the influence of irradiance on *Amphidinium* sp. on growth, proximal composition, pigment content, and photosynthesis in batch cultures. Five irradiances were used: 50, 100, 150, 200, and 250 µmol photon $m^{-2}s^{-1}$. *Amphidinium* has the lowest growth rate and photosynthesis efficiency in the lowest irradiance, increasing irradiance the growth rates and photosynthesis efficiency increase. However, the highest organic dry weight and lipid content per cell was in the lowest irradiance. Chlorophyll *a* and *c* decrease as the irradiance increases, but carotenoids increase when the irradiance increases. *Amphidinium* is known to grow well at low irradiances in cultures; however, in this study, we describe that it can grow well in higher light intensities having high biomass and high growth rates. Light is a limiting factor, wherein irradiances below 100 µmol photon m^{-2} s^{-1} cause *Amphidinium* to have low growth.

Interaction between the green macroalga *Ulva prolifera* and three microalgae and effects of decaying *Ulva prolifera* on the photosynthesis and antioxidant system of microalgae

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Green tides formed by *Ulva prolifera* have occurred in the Yellow Sea of China every spring since 2007. Millions of tons of green algae accumulated and decomposed at the late stage of green tides, significantly affecting the growth of other species of phytoplankton. Interaction between *U. prolifera* and three microalgal species (*Skeletonema costatum*, *Prorocentrum donghaiense*, and *Nitzschia clusters*) under different irradiance was studied. Combined with the field investigation of irradiance at various depths, the depth ranges at which *U. prolifera* and the three microalgal species can coexist were evaluated. The effects of *U. prolifera* at different decaying stages on the growth of two diatom species, *Skeletonema costatum* and *Chaetoceros curvisetus*, were also investigated. *Ulva* filtrates significantly inhibited the cell densities of *S. costatum* in all decay stages, allelopathy played a dominant role in the growth. The results indicated that *U. prolifera* is better adapted to a wide range of light intensity than the microalgae. Growth of the microalgae in co-culture was significantly inferior to that in pure culture, and ecophysiological differences were found between these three species. The effects of different *U. prolifera* biomass on microalgae in different decomposition stages were different, which provided a basis for the potential ecological consequences caused by green tides.

Cryopreservation of three morphologically different marine dinoflagellates: *Vulcanodinium rugosum, Alexandrium pacificum* and *Durusdinium trenchii*

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Dinoflagellates are ecologically diverse eukaryotic microorganisms that are important components of marine ecosystems. Some species produce toxins and form harmful algal blooms that can be detrimental to humans, and marine organisms. Two well described toxic dinoflagellates are *Vulcanodinium rugosum*, which can produce pinnatoxin E, F and G, and *Alexandrium pacificum*, which produces saxitoxins. *Durusdinium trenchii* belongs to the family Symbiodiniaceae, which do not produce toxins, but form crucial endosymbiotic associations with invertebrates, such as corals. Strains of these dinoflagellates are maintained in culture collections worldwide. This is labor intensive, and there is an ongoing risk of contamination or loss of culture. Cryopreserving will help to maintain long-term viability, molecular integrity and reduce costs. In this study, we assessed 12 cryoprotectants agents (CPAs) at different concentrations, and a combination of dimethyl sulfoxide (DMSO) and non-penetrating CPAs to determine which had the least impact on cell health. We then tested plunge-in freezing and controlled-rate freezer methods during cryopreservation. The best CPAs tested were DMSO, glycerol and propylene glycol all at 15 % final concentration. Despite optimizing the CPAs and exploring a range of cryopreservation protocols we were unable to successfully cryopreserve *V. rugosum* and *A. pacificum*. *Durusdinium trenchii* was successfully preserved using 15 % DMSO with an optimized controlled-rate freezer protocol. To our knowledge this is the first time a strain from this species has been cryopreserved. Future work aims to explore encapsulation vitrification and encapsulation-dehydration to attempt to cryopreserve *V. rugosum* and *A. pacificum*.

Strategies for *in vitro Microcystis* bloom propagation and community analysis

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The success and pervasiveness of the *Microcystis* genus stems from a meroplanktonic life cycle that features dense pelagic blooms in the summer and the benthic sedimentation of vegetative cells in the winter. Annual accretion of hibernating *Microcystis* cells in the sediment generates a genetically diverse endogenous seeding source for future bloom formation events where environmental parameters drive ecotype selection. Phenotypic plasticity and colonial disaggregation under culturing conditions have previously prevented *in-vitro* modelling of the *Microcystis* life cycle. This study presents a novel culturing method for the accurate modelling of toxigenic *Microcystis* bloom formation. *In-vitro* propagation models were established using sediment and filtered site water supplemented with BG11 media collected from an Australian lagoon with documented biannual *Microcystis* blooms. Toxigenic capacity of the *in-vitro* blooms was confirmed using PCR of mycE. Serial observations of cell density, morphology and colony structure made using fluorescent and scanning electron microscopy demonstrate that propagation models using site water enriched with 50 % BG11 had the highest growth rate and the highest number of cells per colony. Metagenomic sequencing of the 16S rRNA V13 hypervariable regions determined the relative abundance of cyanobacterial species in the in-vitro blooms as 43 - 56 %, comparable to the species dominance during naturally occurring blooms. This is the first reported incidence of successful *Microcystis* bloom initiation under laboratory conditions and will enable for the study of genetic mechanisms of propagation and bloom termination.

The combination of oligotrophication and strong light leads to fading diatoms and blooms of *Chattonella marina* var. *antiqua*

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The Seto-Inland Sea, the largest semi-enclosed sea in Japan, has been experiencing oligotrophication. This oligotrophication has caused a decrease in diatoms and drastic changes in species composition, while there has been constant suffering from harmful red tides, e.g., *Chattonella* species, mostly in the summer season. Continuous culture experiments for two diatoms, *Skeletonema costatum* and *Chaetoceros lorenzianus*, with a combination of PAM fluorometry revealed that under nutrient depletion, stagnation of photochemical quenching coefficient (qP) and an increase in photoprotective function (NPQ) were observed, especially under phosphorus depletion. However, most notably in *S. costatum*, NPQ was not sufficiently induced under nitrogen depletion, indicating that the species could experience photoinhibition. Similar but fewer reactions were observed in *C. lorenzianus*, suggesting differences in resistance to nutrient depletion and susceptibility to excess light. On the other hand, the Raphidophyta *C. marina* var. *antiqua* showed a higher qP and resulting higher electron transportation rates in PSII even under severe phosphorus depletion, and these rates were also achieved by minimal NPQ induction enabling the acceptance of more light. The recent trends in the Seto-Inland Sea, where not only oligotrophication but also stronger light in the summer season due to increases in global solar radiation and transparency occur, could produce unfavorable conditions more for *S. costatum* than for *C. lorenzianus*. Conversely, *C. marina* could adapt to nutrient depletion and use strong light efficiently. This assumption might partly explain the recent decreasing trends in *Skeletonema*, which has been replaced by *Chaetoceros*, and increasing occurrences of *Chattonella* red tides.

Tolerance to temperature and salinity stress of three *Dinophysis* species in culture

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Blooms of mixotrophic dinoflagellate species of *Dinophysis*, in particular *D. acuminata*, produce okadaic acid related toxins and pectenotoxins and are the main agents of shellfish harvesting bans in North Eastern Atlantic waters. In the Galician Rías, a system subject to large variations in temperature, salinity and water column structure due to freshwater inputs and the upwelling regime, *D. acuminata*, *D. acuta* and *D. caudata* exhibit markedly distinct spatio-temporal patterns. It is supposed that combinations of hydrodynamics and biological traits control these patterns. Monospecific cultures of the three species were exposed to combinations of temperature (T) and salinity (S) following a factorial design to identify and quantify the direct physiological effects (growth and toxins). Response surface methodology (RSM) showed that optimal conditions for growth at exponential and final phases were 19 - 16.7 ºC, S > 37 for *D. acuminata*; 19 º- 20.5 ºC, S > 37 for *D. caudata*, and 17.9 - 16.8 ºC, S 29.3 28.7 for *D. acuta*. In contrast, *D. acuminata* and *D. acuta* died at salinities of 5 - 9.7 whereas *D. caudata*, could only tolerate salinity values > 21. A fixed temperature (12.5 ºC) and two salinities (21, 35.5) reflecting two contrasting conditions found in the Galician Rías under oceanic or estuarine influence were selected to study toxin production in *D. acuminata* and *D. acuta*. After 10 days of incubation, intracellular okadaic acid levels were similar in both conditions. In conclusion, the three studied species tolerated sudden changes in temperature within the range found in the Galician Rías, but salinity clearly indicated that, unlike other estuarine dinoflagellates common in the area (e.g *Alexandrium minutum*), these species are acclimated to neritic conditions.

The influence of Dissolved Organic Matter on growth rate of the Cyanobacteria (*Chrysosporum ovalisporum*)

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Cyanobacterial blooms can have negative impacts on aquatic ecosystems by altering their trophic structure and functionality. Monitoring of physical chemical parameters is essential to understanding possible triggers which correlate with algal blooms. One important parameter which is often overlooked is dissolved organic matter (DOM). DOM plays a major role in abiotic and biotic processes within aquatic ecosystems. Studies comparing the effect of different DOM at varying concentrations on the growth and toxin production of cyanobacteria and possible role in the development of harmful algal blooms are currently lacking. This study aims to determine how different concentrations and types of DOM affect the growth rate of *Chrysosporum ovalisporum*. Fourteen days bioassays comparing growth rate of *C. ovalisporum* in presence of four natural DOMs collected from Australian water bodies at 3 realistic environmental concentrations (2, 10 and 20 mg L^{-1}) and control (without DOM: synthetic water) were conducted. The four DOMs differed in key characteristics as such as source (allochthonous and/or autochthonous), aromaticity, abundance of key components such as proteins, humic and fulvic acids. DOM type and concentration were shown to influence growth rate of *C. ovalisporum*. Significantly higher growth rates were recorded in presence of one DOM, then the other treatments. This suggests that some kinds of DOM are capable of stimulating growth of the harmful alga *C. ovalisporum* and may play a role in bloom formation.

Growth curves and identification of dinoflagellate strains of the genus *Prorocentrum* (Dinophyceae) from Bahía de La Paz, B.C.S., Mexico

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In Bahía de La Paz, the presence of harmful species of the genus *Prorocentrum* have been registered, nevertheless few studies have focused on their ecophysiology and identification. The objective of this work was to identify at species level four strains of *Prorocentrum* and cultivate them in two culture media. This study will allow us to improve the monitoring of HAB to decide on which species focus attention. Morphological and molecular identification was carried out with light microscopy, scanning electron microscopy, and also by using partial sequences of the 28S region of rDNA LSU. Growth curves were followed in f/2 and modified GSe media with vermicomposting extract, maintaining a temperature of 24 ± 1 °C, salinity of 34, light intensity of 120 - 150 µmol photon m^{-2} sec^{-1} and a photoperiod of 12:12 h light:dark. Strains were identified as *P. rhathymum* / *P. mexicanum*, *P. koreanum* and *P. cassubicum*. The maximum biomass for *P. rhathymum* / *P. mexicanum* was 52,177 ± 1,415 cells mL^{-1}, for *P. rhathymum* / *P. mexicanum* 52,830 ± 4,592 cells $mL^{-1}$and for *P. koreanum* 101, 974 ± 9,138 cells mL^{-1}in GSe medium; with exponential growth rates of 0.599 ± 0.001 div day^{-1}, 0.360 ± 0.003 div day^{-1}, and 0.714 ± 0.005 div day^{-1}, respectively. For *P. cassubicum* the highest maximum biomass (74,312 ± 2,008 cells mL^{-1}) and growth rate (0.464 ± 0.006 div day^{-1}) were observed in f/2 medium. Toxin profile analyzes are required to complement the characterization of these species. The correct taxonomic identification of *Prorocentrum* species is essential information for harmful algae monitoring programs.

Life cycle study of the toxic dinoflagellate *Alexandrium monilatum*

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The toxic dinoflagellate *Alexandrium monilatum* (Howell) Balech (= *Gonyaulax monilata* Howell) has a recent history in lower Chesapeake Bay of forming large late summer blooms almost annually since 2007. The complete life cycle and physicochemical parameters controlling transitions between life phases of this species have yet to be described. Using a FlowCam and light microscopy, morphologically different life stages were visually documented. Both asexual and sexual cell forms were observed from *A. monilatum* bloom samples. During asexual reproduction, *A. monilatum* vegetative cells divide by binary fission and form long chains up to 60+ cells in length. Long chains were the dominant form observed in bloom samples. In sexual reproduction, smaller, lighter-colored cells (gametes) pair up and fuse, forming a larger, darker-colored planozygote. The planozygote eventually becomes stationary, the flagella and theca are shed, and a hypnozygote (cyst) is formed. Sexual reproductive phases were observed in bloom samples approaching termination. Results from preliminary experiments suggest that the transition to sexual stages may be induced by a reduction in nutrients and furthermore, cysts mature more quickly and have a higher germination success rate in higher incubation temperatures (25° C) compared to lower temperatures (15 °C, 20° C).

Taxonomy, autecology and toxicology of *Alexandrium affine* isolated from Isla San José, Gulf of California

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The morphology, molecular phylogeny, growth kinetics and toxicology of an *Alexandrium* strain from Isla San José, southwest Gulf of California were characterized. Strain identification was studied based on Kofoidian tabulation and LSU sequencing. Static cultures in f/2 + Se medium were prepared by fivefold, and incubated for 28 days in a temperature gradient (21, 23, 25, 27 and 29 °C). Individual aliquots were collected every third day for cell counting, saxitoxin and nutrient analyses. Phylogenetic analyses of *Alexandrium* (D1/D2 region, LSU rDNA), showed that strain from Isla San José (AAJQ-1) clusters with *Alexandrium affine* sequences from other parts of the world. Instantaneous growth rate was related to temperature in all treatments. The highest cell density (6,724 cells mL^{-1}) and maximum instant rate (0.22 day^{-1}) occurred at 27 °C during the exponential phase. Temperature range for optimal growth was 25 - 27 °C. Cell density showed negative correlation with nutrients at temperatures ≥ 23°C (nitrate), and 27 °C (orthophosphate). In this study *A. affine*, commonly considered as nontoxic species, showed a low saxitoxin production. The strain AAJQ-1 shows an affinity to tropical waters, its growth was influenced by temperature and was based on available nitrate and orthophosphate. Toxins production by this strain was not related to the temperature gradient, however, was favored by nitrates at temperatures above 23 °C, while with orthophosphates at 27 °C. Finally, the results suggest that nitrite at high temperature favors saxitoxin production, which has not been sufficiently documented.

Effects of phosphorus limitation and nitrogen enrichment in the dinoflagellate *Alexandrium tamiyavanichii* Balech (1994) in growth kinetics, toxin production, and nutrients dynamic

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Alexandrium tamiyavanichii is a toxic dinoflagellate that produces paralyzing shellfish poisoning (PSP) toxins. A strain of *A. tamiyavanichii* isolated from La Paz Bay, Mexico, was used to evaluate phosphorus limitation (PL) and nitrogen enrichment (NE) in the growth kinetics and PSP toxin production. The growth conditions were 24°C ± 0.3°C, 100 µmol of photons m^{-2} s^{-1} and 12:12 h light-dark cycles for 33 days, in GSe culture medium. Both PL and NE culture conditions of *A. tamiyavanichii*, yield a 3.4 and 2.3 times respectively cell density as compared to the standard control culture conditions. Also, the stationary phase was absent in all treatments. PSP toxin's highest concentration was found in control culture (309 fmol $cell^{-1}$) on day 21. Furthermore, saxitoxin (STX) was the major analogue found in all culture conditions (> 40 % mol). This work describes for the first time the toxin composition of *A. tamiyavanichii* strain isolated from Mexican coasts. It is concluded that PL and NE treatments promoted the population growth but did not affect PSP toxins production.

Effects of temperature and phosphate limitation on the growth of three species of the genus *Alexandrium* (Dinophyceae) from Mexican Pacific Ocean

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Under specific environmental conditions, some species of the genus *Alexandrium* may form Harmful Algal Blooms. To determine the effect of phosphorus concentration in the culture medium (f/2 + Se and f/2 + SeWPA: without phosphate addition), and temperature (15, 20, and 25 °C) on the growth of *Alexandrium* sp. (ALEXMZN) from Cuastecomate, Jalisco; *A. affine* (AAMV - 1) from Mazatlán, Sinaloa; and *A. margalefii* (AMCQ-1) from Concepcion, Baja California Sur, two simultaneous experiments were performed. The maximum cell abundance in f/2 + Se medium was 32,783 ± 106, 13,043 ± 309, and 8,005 ± 244 cell mL^{-1}, for *Alexandrium* sp., *A. affine* and *A. margalefii,* at 25, 20 and 20 °C, respectively; moreover, the maximum growth rates were 0.77, 0.73 and 0.49 day^{-1}, in the same species and temperature order. The f/2 + Se medium stimulated the growth of the evaluated species from 2 to 6 times more than the f/2 + SeWPA medium; further, the maximum cell abundance was related to the decrease in phosphorus and nitrogen concentration in the stationary phase. The highest growth of the evaluated species corresponded to the temperature of their place of isolation, *Alexandrium* sp. showed affinity to warm waters, unlike to *A. affine* and *A. margalefii* whose origin is the subtropical zone. Unexpectedly, the species of tropical origin *Alexandrium* sp. in f/2 + SeWPA medium grew better at 15 °C and was more affected at 25 °C than the other species. These differential responses bolster the need to perform more autecological studies to *Alexandrium* species.

Temperature rules growth but salinity rules toxicity in an *Alexandrium minutum* culture from an estuarine area in NW Spain

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The dinoflagellate *Alexandrium minutum* is responsible of paralytic shellfish poisoning (PSP) episodes in Western Europe, commonly in areas directly affected by significant freshwater inputs, as the Ría de Vigo (NW Spain), a coastal embayment with estuarine influence characterized by seasonal upwelling. Because pronounced environmental short-term changes characterized these ecosystems, tolerance windows for *A. minutum* against temperature (T) and salinity (S) stress were studied using a factorial approach. For this purpose, a gradient matrix with five temperatures (12.5, 14.4, 19, 23.6, and 25 ºC) and five salinities (5, 9.7, 21, 32.3 and 37) were assayed on a clonal *A. minutum* strain. As a result, the optimal values of temperature for growth were determined in the range of 19.0 - 20.6 ºC, while salinity tolerance was wide and of limited influence for growth. The impact of these environmental conditions in toxin production (HPLC analyses) and cell biovolume (Imaging Flow Cytometry analyses), was also checked on selected conditions according with the previously determined highest and lowest growth points (temperatures 15 ºC and 25 ºC, and salinities 10 and 30). Clonal toxin profile was characterized by pre-dominantly production of GTX4, followed by GTX3 and smaller amounts of GTX1 and GTX3, but toxin production was focused only on GTX4 as another gonyautoxins could not always be quantified. The results showed an increasing content of GTX4 per cell when decreasing salinity and no significant response to temperature. Biovolume remained stable throughout temperature and salinity conditions. These results demonstrate that *A. minutum* is an estuarine specie able to support sudden changes in salinity, an adaptive advantage to outcompete other species.

Characterization of *Alexandrium tamarense* strains from the same blooming population in a Mediterranean lagoon (Sa Praia Lagoon, Sardinia, NW Mediterranean)

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In this study, several *Alexandrium tamarense* (Lebour) Balech emend. John strains were morphologically, molecularly and toxicologically characterized. The variability of some morpho-functional traits was also investigated. Six *A. tamarense* strains (C3, D3, D5 - D8) were established from the same bloom at Sa Praia Lagoon (Sardinia, NW Mediterranean Sea) in February 2020. The morphology of strains was defined and the sequences of the 28S region were obtained. All strains were also tested for the presence of the RNA regions (stxA1 and sxtA4) noted as involved in the PSTs production. In addition, three (C3, D6, D7) of the six strains were tested for Paralytic Shellfish Toxin production. The growth rates were determined for each strain and cell size and volume were measured at the start and at the end of the growth experiment. *Alexandrium tamarense* strains from Sa Praia Lagoon showed the same morphology and all cells showed a ventral pore. The 28S sequences for the six strains were identical. Two strains showed the presence of the sxA1 and sxA4 regions (C3 and D3), two showed only one of the two (D6 and D7) and two lacked them (D5 and D8). The toxins analyses were negative for the three analyzed strains. Growth rates, cell size and volume were significantly different among some of the strains. The obtained results suggest a certain intraspecific variability for some of the analyzed traits, as already observed for other species of the genus.

Growth of *Coolia malayensis* (Dinophyceae) with extracts of the macroalga *Dictyota dichotoma* (Phaeophyceae)

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Epibenthic dinoflagellates can form algal blooms that can impact the ecosystem, economy and human health. *Coolia* represents a genus of epibenthic dinoflagellates of particular interest due to the ecological associations it has with other toxigenic dinoflagellates and its toxin production (e.g., cooliatoxin). Information on the diversity of *Coolia* in Mexican waters is scarce. Two isolates of *Coolia* (CMBAPAZ-1 and S/I 72) from Bahía de La Paz, southern Gulf of California, were cultured, and the effects of macroalgal extracts (*Dictyota dichotoma*) on their growth were determined. Morphological traits and phylogenetic analyses of the 28S rDNA, allowed to identify both strains as *Coolia malayensis*. The strains were cultured in GSe medium with and without macroalgal extracts: (1) GSe, (2) GSe with a crude extract of *D. dichotoma*, (3) GSe with a carbohydrate fraction of *D. dichotoma* and (4) GSe with a non-precipitable fraction of *D. dichotoma*. There were no significant differences ($p < 0.05$) in the cell densities of both strains in GSe medium (1) compared with the other treatments. The growth rate and maximum cell density were reported in strain S/I 72 (0.139 ± 0.003 div day^{-1} and 43,428 ± 4,560 cells mL^{-1}, respectively) in GSe medium with the addition of the non-precipitable fraction of *D. dichotoma* (4). Our results confirm that the extract and fractions of the macroalga did not favour the growth of either strains of *C. malayensis*. However, the possibility that this macroalga may provide nutrients for the growth of *C. malayensis* is not excluded.

First detection of the toxic dinoflagellates *Prorocentrum rhathymum* and *Prorocentrum* cf *consutum* in Rapa Nui (Easter Island)

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Some benthic species of *Prorocentrum* have been shown to produce okadaic acid (OA), dinophysistoxin-1 (DTX-1), dinophysistoxin-2 (DTX2) and water - soluble OA esters, which cause diarrheic shellfish poisoning (DSP). During a survey of a mesophotic coral reef in Rapa Nui, some species of this genus have been detected in mat samples obtained at 80 m depth. Clonal cultures of two species were established, and their morphology and toxicity were studied. Examination by SEM of the strain D008 - 10 showed oval cells of 28 - 31 mm in length which were in very good overall agreement with descriptions of *P. rhathymum*. The valvar surface was smooth, except for two sets of pores, one in posterior radial rows and another constituted of large pores located in a depression. The periflagellar area was a triangle constituted by seven or eight plates of different size and an apical wing-shaped spine ended in two tips. Strain D008 - 10 fitted well with the descriptions of *P. consutum*, with ovoid cells of 40 - 46 mm in length. The valvar surface was smooth with scattered small pores and marginal row of depressions. The periflagellar area was a deep apical excavation formed by nine plates. In both strains, toxin analysis by LC- HR/MS revealed a toxic profile dominated by OA followed by DTX2. This is the first detection of toxic dinoflagellates in this remote island and suggests a potential risk for human health.

Bioactive compounds from marine phytoplankton (Project BLUEBIOLAB)

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The objectives of the BLUEBIOLAB (Interreg-POCTEP; Spain-Portugal) are the creation of a transboundary laboratory of scientific excellence in marine biotechnology, to reinforce and internationalize the R & D capacities in the territory. For this purpose, several subprojects were launched for the period 2020 - 2022, related with marine biotechnology, aquaculture and biodiversity. The Oceanographic Center of Vigo (IEO, CSIC) participates in one of these initiatives, entitled "Bioactive compounds from marine photosynthetic organisms and biomedical potential", and led by UMINHO (Braga, Portugal) in consortium with CIIMAR, UdV and IIM-CSIC. Among the objectives of this initiative is the obtaining of microalgae cell extracts to search for bioactive compounds by means of biological activity assays. At present, cultures of 35 species of microalgae belonging to 7 classes (from the CCVIEO culture collection at IEO) have been performed: *Dinophyceae* class (22 species), *Bacillariophyceae* (4), *Cryptophyceae* (3), *Prasinophyceae* (1), *Raphidophyceae* (1), *Euglenophyceae* (1), *Prymnesiophyceae* (1), *Dictyochophyceae* (1), and *Chlorophyceae* (1). The cultivation parameters were selected to optimize the growth of each species. Culture biomass harvested for each strain was extracted following a protocol based on H_2O - MeOH and CH_2Cl_2-MeOH. Final fractions obtained were solubilized in DMSO and kept at -80 ºC until carrying out the corresponding bioassays. In this regard, the antiviral activity is now being evaluated against spring viraemia of carp virus (SVCV), and antibacterial and anti-obesity activities will be subsequently evaluated. The bioassays will be performed using two approaches: *in vitro* cell cultures and *in vivo* assays using zebrafish as animal model. Those extracts showing biological activity will be analyzed by liquid chromatography coupled to mass spectrometry for compound identification.

Photo: Tanos Grayeb

HA ECOLOGY

Alexandrium minutum use chemical weapons against the parasite *Amoebophrya* sp. (Syndiniales)

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Parasites of the genus *Amoebophrya* sp. are important contributors to marine ecosystems and can be determining factors in the demise of blooms of Dinophyceae, including toxic red tides. Yet they rarely lead to the total collapse of Dinophyceae blooms. The addition of resistant Dinophyceae (*Alexandrium minutum*) or its exudate into a well-established host-parasite culture (*Scrippsiella acuminate*-*Amoebophrya* sp.) mitigated the success of the parasite and increased the survival of the sensitive host. Effect were mediated via water-borne molecules without the need of a physical contact. Severity of the anti-parasitic defenses fluctuated between *A. minutum* strains and was enhanced with increasing resistant host cell concentrations, but never totally prevented the parasite transmission. The survival time of *Amoebophrya* sp. free-living stages (dinospores) decreased in presence of *A. minutum* and the number of progeny drastically decreased. Integrity of the membrane of dinospores was altered by *A. minutum* which provided a first indication on the mode of action of these anti-parasitic molecules. These results demonstrate that extracellular defenses are an effective strategy against parasites that does not only protect the resistant cells but also have the potential to affect the whole surrounding community.

Diversity of parasitic chytrids associated with dinoflagellate blooms on the Catalan coast

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To understand bloom dynamics, biological mechanisms play a key role in phytoplankton behavior. Factors such as parasitism and saprophytism can impair bloom development and enhance the decline of natural Harmful Algae Blooms (HAB) populations, as well as allow the transfer of organic matter to other trophic levels. An example of this dynamics is the parasitic phytoplankton-fungi relationship. However, it is mostly unknown in the marine environment. For this reason, here, we evaluated the diversity of Chytridiomycota during blooms of 6 different dinoflagellate species occurring in several coastal locations in the NW Mediterranean Sea. For this purpose, we combined metabarcoding, microscopy observations and live cultivation of chytrids from seawater and sediment samples. Our results showed that chytrids represented an overall abundance of 1.4 % in the eukaryotic community with a total of 77 amplicon sequence variants (ASVs). Furthermore, the chytrid community varied significantly among sites, mainly depending on the blooming host. The highest diversity and abundance of chytrids were found in *Ostreopsis* and *Alexandrium* blooms as well as having a higher diversity and abundance mainly in sediment compared to seawater. ASVs corresponding to the chytrid species *Dinomyces arenysensis* co-occurred with the dinoflagellate *Ostreopsis* sp. in all locations where present. Such relationship was also confirmed by lab experiments. Therefore, our results suggest a diverse and wide geographic distribution of chytrid communities during dinoflagellate coastal bloom, in some cases, specific distribution of chytrids species is strongly dependent on its host distribution.

Changes in distributions of phytoplankton functional groups distribution, pigment composition and the realized niche of *Dinophysis acuminata* at the onset of an upwelling event

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Dinophysis acuminata is the major cause of lengthy harvesting bans in shellfish growing areas in European Atlantic waters. In North Western Iberian shelf waters, the growing season of *D. acuminata* lasts throughout the upwelling season (ca. from March to September). Once environmental conditions trigger initial population growth, abundance fluctuations are coupled to the event-scale dynamics of coastal upwelling-relaxation/downwelling cycles. The "ASIMUTH-Rias" cruise (17 – 21 June 2013) in the Galician Rias (Pontevedra and Vigo) and adjacent shelf, during a DSP outbreak, aimed to explore small-scale physical processes associated with late spring blooms (> 10^3 cells L^{-1}) of *D. acuminata*. The cruise coincided with the initiation of an upwelling pulse following relaxation and deepening of a previously formed thin layer of centric colony-forming diatoms. In this work, a niche (*sensu* Hutchinson) approach based on an Outlying Mean Index (OMI) analysis was used to describe the realized niche of *D. acuminata* during transient conditions. The OMI analysis was applied to fine-scale measurements of physical properties, HPLC-derived pigment composition, and phytoplankton functional groups in cross-shelf transects and in a 36 h study at a fixed station. Although having a wide niche breath, the realized niche of *D. acuminata* (cell maximum 5×10^3 cells L^{-1} between 3 and 5 m) was characterized by outflowing warmer (15 - 16 °C) waters with high turbulence ($\varepsilon < 10^{-6}$ m^2 s^{-3}), high light intensity, and low nitrate concentrations. These results show that during a transient upwelling-initiation scenario, mid-shelf waters provided a more suitable habitat for *D. acuminata* and the accompanying dinoflagellate populations than waters inside the rías.

First culture of *Dinophysis acuminata* from southern Chile: ecophysiology, toxin production and phylogeny

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In Chile *Dinophysis acuminata* is a conspicuous mixotrophic dinoflagellate inhabit on Ocean Pacific Coast (OPC) and Patagonian fjords (PF). In this study, for the first time, we evaluated the effects of light and temperature on the growth and the toxin productivity in the culture strains of *D. acuminata* isolated from southern Chile. The phylogenetic analysis was also carried out using the COI marker. Strains were isolated in 2019 - 2020 from the OPC and PF and were maintained in culture by feeding *M. rubrum* as prey. High growth rate and cell abundance were observed at 45 than at 90 and 125 mmol m^{-2} s^{-1}. However, the growth was not affected by the temperature modification (12 °C and 15 °C). Toxins screening revealed the presence of pectenotoxins 2 (PTX2) in six strains isolated from OPC and PF, but okadaic acid was not detected. PTX2 concentration was not affected by temperature and light, showing values ranged from 3.920 to 14.077 pg $cell^{-1}$. The Chilean *D. acuminata* sequences were clustered in the "*Dinophysis acuminata* complex" clade showing among sequences genetic distances ranged from 0.000 and 0.001. These results may indicate that the tolerance and plasticity to environmental driving can explain the species persistence on the Pacific Ocean and Patagonian fjords.

Use of the imaging flowcytobot to assess differential grazing by zooplankton during *Dinophysis acuminata* blooms on Long Island, New York

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Blooms of *Dinophysis acuminata* are annual events in estuaries of Long Island, NY, co-occurring with other plankton species. The complexity of planktonic interactions during blooms requires a robust assessment of plankton community composition and dynamics, a need fulfilled by the Imaging FlowCytobot (IFCB) that records live images of plankton > 10 µm. The IFCB was used to track plankton dynamics during blooms in 2019 and 2020 while concurrently assessing grazing by copepod (*Acartia tonsa*) adults and nauplii. Algal bloom intensity and communities differed between years with the 2020 bloom being less dense than the 2019 bloom. The addition of *A. tonsa* had significant effects at the onset of blooms, but showed reduced effect as the bloom progressed. Nauplii were shown to reduce growth of *Mesodinium* in 2019 and increase growth of *Dinophysis* in both years suggesting that they may influence bloom development. The addition of adult copepods yielded an increase in *Dinophysis* during peak bloom in 2019 and no significant effects on *Dinophysis* growth throughout 2020, suggesting that *A. tonsa* adults are not primary grazers of *Dinophysis*. Adults, however, caused a significant decrease in *Mesodinium* growth at the onset of the bloom in 2020 which could reduce *Dinophysis* bloom intensity. In this study, the use of imaging cytometry to assess differential grazing shows grazing by *A. tonsa* does not directly contribute to bloom decline, but may indirectly modulate bloom intensity.

Toxin production in mutualistic dinoflagellates: potential insights for coral-algal symbiosis

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Dinoflagellate toxin production varies according to growth phase, nutrient availability, temperature, and light; parameters that also influence maintenance of functioning animal-algal symbioses. For coral-algal mutualisms, host-symbiont signaling via exchange of secondary metabolites such as toxins may be an important component in dysbiosis. Toxins have been isolated from several species in the family Symbiodiniaceae, but how toxin production in this family correlates to phylogeny, ecology (mutualistic, opportunistic, or exclusively free-living lifestyle) or abiotic factors has not been thoroughly examined. We measured the photophysiology and hemolytic capabilities of seven symbiotic and three non-symbiotic species of cultured Symbiodiniaceae under nutrient limitation and thermal stress. Toxicity was not detected in three symbiotic species, while the other four species demonstrated toxicity changes that varied with treatment. These findings showcase the diversity in response to stress within symbiodiniaceans and make the case for future study in the function of these toxic compounds as they pertain to cell survival and proliferation outside of, or within, a host. These toxicity results combined with the vast genetic resources that exist for this group make Symbiodiniaceae a strong candidate system for studying the genetic basis of dinoflagellate toxins.

Resolving the physiological dynamics and drivers of brown tides (*Aureococcus anophagefferens*) using metatranscriptomics

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Harmful brown tide blooms attributed to pelagophyte, *Aureococcus anophagefferens*, occur for extended periods in estuaries in the United States, China, and South Africa. This study tracked brown tide bloom and identify drivers and biochemical pathways important for supporting the bloom by generating metatranscriptomes from *in situ* samples. Transcriptomes were also generated from field experiments by amending natural seawater from the bloom (beginning, during peak and decline) with nutrients. During the initiation of the brown tide, *Aureococcus* transitioned from 5 % to > 85 % of eukaryotic phytoplankton in two weeks and displayed a transcriptional profile consistent with this rapid growth with an overrepresentation of transcripts associated with DNA polymerase, pyrimidine metabolism, and repair systems, as well as the transport of lipids, metals, and vitamin B12. Multiple lines of evidence (transcriptional, stoichiometric, orthogonal, empirical) indicated the supply of phosphorus (P) limited the growth of *Aureococcus* at this time. Upon achieving a high biomass (10^6 cells mL^{-1}), the bloom population transitioned to a maintenance stage that persisted for nearly a month during which the transcription of genes associated with carbon fixation, the ribosome, central carbohydrate metabolism, fatty acid biosynthesis, and amino acid metabolism were overrepresented. Concurrently, this stage of the bloom was more nitrogen- (N) than P-limited, with both macronutrients eliciting significant transcriptional changes in the alga. As such, this study demonstrates how both N and P can modulate brown tide dynamics during the progression of a bloom.

DMSP release during dinoflagellate blooms in the northern Gulf of Mexico: Implications of a chemo-attractant

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With the increasing likelihood of harmful algal blooms occurring in nearshore communities, there is a growing need to understand the chemical dynamics of these bloom communities and their physiological and behavioral effects on the surrounding food web. In 2015, a *Karenia brevis* bloom advected to the northern Gulf of Mexico coast, impacting fisheries and local economies. Water samples were collected from six fixed sites and from a multitude of opportunistic sites around Mobile Bay and Mississippi Sound over the course of two months. The dinoflagellate community was enumerated via microscopy and whole water samples were analyzed for total dimethylsulfoniopropionate (DMSPt), a known fish attractant. We found significant positive correlations between dinoflagellate abundance and DMSPt, and specifically between DMSPt and abundance of those dinoflagellate genera/species which are known to be toxic. Given our current understanding of the attractiveness of DMSPt to a variety of organisms, the high levels of DMSP (peak 580 nM, mean 63 nM) associated with these communities highlight the need to understand how the olfactory landscape produced during these transient blooms impact fish behavior and might exacerbate harmful effects on these nearshore marine communities. Through this spatio-temporal study of a harmful dinoflagellate/algal bloom in the northern Gulf of Mexico, we explore the linkages between the changing bloom community and chemical attractant (DMSPt) which they released.

Dynamic photo-physiological responses of dinoflagellate *Karenia* to short-term changes in temperature and nitrogen substrates

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On the West Florida Shelf, annual blooms of neurotoxin producing harmful dinoflagellate, *Karenia brevis* (and other *Karenia* species) cause negative effects on the environment, economy, and human health of Florida. Blooms primarily occur in the fall months and may overwinter into winter and spring, but they rarely persist throughout the hot summer months (> 30 °C). During January 2021 when *K. brevis* occurred in low-moderate concentrations in Southwest Florida, surface sampling from 3 stations within the bloom was conducted. Here, using these natural samples, the photosynthesis-irradiance responses were examined using Phyto-PAM fluorometry. Treatments included controls and exposures to different temperatures (15, 20, 25, 30 °C) with and without 10 µM of different nitrogen (N) forms (nitrate, ammonium, urea). Although there were variations between stations, most samples showed lower values of fluorescence parameters after one hour exposure to 30 °C compared to those at 15, 20, 25 °C, especially when samples were also enriched with nitrate, suggesting that 30 °C might be physiologically stressful for *Karenia*. Rapid light-response curves were variable depending on N forms, but the extent of variability with temperature was always lowest with urea, implying dynamic response of photosynthesis to changes in N forms. These results also suggest that dependence on N form may change with temperature, and availability of chemically-reduced forms of N may be favored at the highest temperatures that *Karenia* may experience.

Photosynthesis of *Karenia brevis* -and that of its competitors- in a nearshore bloom

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Blooms of *Karenia brevis* frequently occur on the West Florida Shelf, generally originating offshore following accumulations of the nitrogen-fixing cyanobacterium *Trichodesmium*. In early 2021, an unusual bloom developed, seemingly initiated nearer shore, thus more dependent on land-based nutrient sources than those derived from *Trichodesmium* for its early development. During the bloom, *Karenia* co-occurred with green algae, but as the bloom progressed and moved northward, the main co-occurring taxa were picocyanobacteria. Using pulse-amplitude-modulated (PAM) fluorometry, the photosynthesis of *Karenia* and its competitors was measured in natural samples, and in samples manipulated with nutrients and with temperature. Photosynthesis-irradiance curves for *Karenia* consistently showed higher ETRmax and less photoinhibition than those of the green algae, and a greater response to N enrichment. As the bloom progressed, the ETRmax rates of *Karenia* declined, consistent with the response previously shown in the winter temperature manipulations, and the green algal signal was replaced with that of picocyanobacteria. Photosynthesis relationships of the PE-cyanobacteria were highly variable due to low concentrations, but generally showed higher photoinhibition than *Karenia*. As of the time of this submission, the *Karenia* bloom is beginning to intercept nutrients from a very large fertilizer spill in Tampa Bay. These results suggest that *Karenia* may be well positioned to be the better competitor in these nutrient enriched conditions, and these results suggest that the bloom of *Karenia* may persist until the recently discharged nutrients become dispersed and diluted.

Competitive interactions as a mechanism for chemical diversity maintenance in *Nodularia spumigena*

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Nodularia spumigena is a bloom-forming diazotrophic cyanobacterium inhabiting brackish waters worldwide. This species produces non-ribosomal peptides (NRPs), including the hepatotoxin nodularin, often referred to as cyanotoxin. Several known classes of NRPs have various biological activities, although their modes of action are poorly understood. In the Baltic *N. spumigena*, there is a high NRPs chemodiversity among strains, allowing their grouping in specific chemotypes and subgroups. Therefore, it is relevant to ask whether the NRPs production is affected by intraspecific interactions between the co-existing strains. Using a novel approach that combines culture technique and liquid chromatography-tandem mass spectrometry for the NRP analysis, we examined *N. spumigena* strains under mono- and co-culture conditions. The test strains were selected to represent *N. spumigena* belonging to the same or different chemotype subgroups. In this setup, we observed physiological and metabolic responses in the test strains grown without cell contact. The changes in NRPs levels to co-culture conditions were conserved within a chemotype subgroup but different between the subgroups. Our results suggest that intraspecific interactions may promote a chemical diversity in *N. spumigena* population, with higher NRPs production compared to a single-strain population. Studying allelochemical signalling in this cyanobacterium is crucial for understanding toxicity mechanisms and plankton community interactions in the Baltic Sea and other aquatic systems experiencing regular blooms.

Cell death and metabolic stress in *Gymnodinium catenatum* induced by allelopathy of *Chattonella marina* var. *marina, Gymnodinium impudicum* and *Margalefidinium polykrikoides*

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Allelopathy between phytoplankton species can induce cell stress and when the caspase-type protease enzymes are activated, they can promote apoptosis or induce programmed cell death (PCD) signals in the cells. *Chattonella marina* var. *marina*, *Margalefidinium polykrikoides* and *Gymnodinium impudicum* have an allelopathic effect on *Gymnodinium catenatum*, the physiological responses of these interactions are unknown. We evaluated whether the allelopathic effect promotes cellular stress and activates PCD in *G. catenatum*. Cultures of *G. catenatum* with a cell density of 500 cells mL^{-1} were exposed to *C. marina* var. *marina*, M. *polykrikoides* and *G. impudicum* cell-free media, during 24, 48 and 72 h. The mortality, superoxide radical $O_2^{\bullet-}$ production, thiobarbituric acid reactive substances (TBARS) levels, superoxide dismutase (SOD) activity, protein content, caspase-3 activity, and preliminary identification of metabolic changes by nuclear magnetic resonance (NMR), were evaluated in *G. catenatum*. The cell-free media from three species caused a mortality from 59 to 69 % in *G. catenatum* at 72 h. The maximum production of ($O_2^{\bullet-}$) occurred with the cell-free medium of *C. marina* var. *marina*, while the highest TBARS production, and the highest SOD activity were induced by the culture medium of *G. impudicum*. The cell-free media of *M. polykrikoides* caused an increase in the total protein content. All treatments increased caspase-3 activity; in addition, changes were observed in the metabolism of *G. catenatum*. We conclude that the allelopathic effect on *G. catenatum* is related to cellular stress and the activity of caspase-3 as a possible executor of PCD.

Allelopathic effects of *Margalefidinium polykrikoides* on *Pyrodinium bahamense* under different nutrients conditions

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Co-occurring of *Margalefidinium polykrikoides*, a fish killer and toxic *Pyrodinum bahamense* have been commonly observed in the coastal waters of Sabah, Malaysia. During most of the events, *M. polykrikoides* will dominate the bloom and suppress *P. bahamense*. The reason for this condition is unknown. Therefore, this study explores the interaction of these species and try to understand under which nutrient conditions *M. polykrikoides* will become dominant over *P. bahamense*. To achieve this, the allelopathic abilities of *M. polykrikoides* on *P. bahamense* were investigated in different ratio of Nitrogen (N) and Phosphorus (P) concentrations in bi-algal and filtrate conditions. The bioassays were tested via bi-algal experiments at three ratios: 1:1 (500 cells mL^{-1} of each species, *M. polykrikoides* and *P. bahamense*), 1: 5 (100 cells mL^{-1} of *M. polykrikoides* and 500 cells mL^{-1} *P. bahamense*) and 5:1 (500 cells mL^{-1} *M. polykrikoides* and 100 cells mL^{-1} *P. bahamense*). For culture filtrates condition, three different volumes of *M. polykrikoides* filtrates (10, 20 and 50 mL) harvested at exponential phase were combined with 150 mL *P. bahamense*. Result indicates *M. polykrikoides* inhibit the growth of *P. bahamense* and caused morphological changes of *P. bahamense* in bi-algal culture. The best inhibition of *P. bahamense* by *M. polykrikoides* was observed at nitrate concentration at 30 µM and phosphorus concentration at 5 µM. This information will increase our understanding in bloom mechanism of these two important harmful algal blooms (HABs) species.

A look at two studies that investigate how allelopathy, mixotrophy, and altered nutrient regimes may influence HAB formation

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Here we present work from two recently conducted studies, one experimental and the other theoretical, that involve aspects of allelopathy, mixotrophy, and altered nutrient regimes which have implications for HABs. Using laboratory experiments that involved plankton bioassays and time series interaction experiments it was demonstrated that the ecological role of harmful chemicals produced by *Prymnesium parvum* (strain ZZ181) may be altered by the N:P ratio the culture is exposed to. When grown in media where N:P was balanced at the Redfield ratio, mortality of the phytoplankton species *Rhodomonas salina* was low and related to direct cell-to-cell contact and phagotrophic feeding, suggesting the chemicals may play a role in micropredation. Differently, when grown under imbalanced N:P (reduced nitrate) conditions chemicals produced by the culture induced widespread *R. salina* mortality that was not associated with cell-to-cell contact, suggesting an ecological role resembling broadcast allelopathy. Using a dynamic numerical plankton model we investigated the effects of phytoplankton nutrient acquisition strategy (mixotrophy vs. autotrophy) and system enrichment on seasonal plankton dynamics. Simulation results demonstrated that with enrichment the yearly maximum population density of a theoretical mixotroph was less predictable and high density populations could be achieved. Though allelopathy was not included in the model, we suggest that deleterious chemicals produced by the mixotroph population would have reinforced its bloom trajectory once cell density surpassed some threshold allowing the chemicals to reach an effective concentration in the environment. These studies demonstrate how altered nutrient regimes in aquatic systems may contribute to the growing HAB problem.

Trait-based approaches to understand impacts of multiple global change factors on harmful algae

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Global environmental change involves shifts in a wide range of environmental factors including elevated CO_2 levels, warming and changes in nutrient availabilities. This interplay of environmental stressors complicates our understanding on the impacts of global change for harmful algal blooms. Many cellular processes rely on the availabilities of nutrients, while temperature is a major driver for metabolic rates, and CO_2 for photosynthesis and carbon acquisition. Together, these environmental factors determine a wide range of cellular traits. Inspired by trait-based approaches and ecological stoichiometry, this talk will explore the combined effects of CO_2, temperature, and nutrient availabilities on various traits, notably growth, nutrient acquisition, and toxin synthesis in harmful algae. Results demonstrate that both CO_2 and temperature may enhance nutrient acquisition in freshwater cyanobacteria and marine dinoflagellates, and thereby enhance their competitive ability for nutrients. Moreover, CO_2 and temperature driven changes in carbon and nutrient acquisition affected cellular elemental ratios, with largely predictable patterns in cellular toxin quota and composition. Together, these findings contribute to a mechanistic understanding on the impacts of combined global change stressors on harmful algal physiology and toxicity.

Conditions promoting *Pseudo-nitzschia* spp. blooms in the eastern English Channel and southern North Sea: lessons from the realized ecological niches

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Pseudo-nitzschia spp. are cosmopolitan marine diatoms. *Pseudo-nitzschia* spp. blooms occasionally represent a threat to human health and economic activities since some of them produce domoic acid, a neurotoxin responsible for amnesic shellfish poisoning. Understand the causes of these blooms would help to avoid the negative impacts on seafood consumers and would prevent the economic losses due to the harvesting and sale restrictions. However, since our understanding of factors controlling *Pseudo-nitzschia* spp. blooms is still limited, this remains a challenging task. Analyzing available long-term monitoring data could be one of the keys to this direction. In this study, we investigated the conditions promoting the blooms of *Pseudo-nitzschia seriata* and *Pseudo-nitzschia delicatissima* complexes by analyzing data series acquired from 1992 to 2020 in the framework of French coastal monitoring programs. Since blooms may be the result of complex interplays between *Pseudo-nitzschia* species, environmental conditions and the other species composing the phytoplankton community, a multivariate approach based on the Hutchinson's ecological realized niche concept has been used. This approach not only revealed the role of environmental conditions but also that of biotic interactions. *P. seriata* and *P. delicatissima* showed different seasonal dynamics and environmental requirements. *Pseudo-nitzschia delicatissima* occupied a niche favored by spring conditions while *P. seriata* had affinity for spring-summer conditions. *Pseudo-nitzschia seriata* occupied a niche more marginal than *P. delicatissima* and was more tolerant. In the particular case of the eastern English Channel and southern North Sea, *P. seriata* and *P. delicatissima* interacted differently with *Phaeocystis globosa* blooms and were restricted by different biotic interactions with Diatoms.

Copepods and *Pseudo-nitzschia,* the mystery of domoic acid unraveled

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The past decades of research on the production of domoic acid (DA) by the diatom genus *Pseudo-nitzschia*, have left one major question: Why is DA produced? - or in other words, what evolutionary benefit does DA provide the diatoms. The metabolic pathway of DA has been unraveled, and we know that DA content varies tremendously with abiotic and biotic factors, like grazers, nutrients, and strains. We expect that DA is an expensive secondary metabolite. The lipid chemical cues copepodamides, revealing the presence of grazers, induce or increase production of DA. Still, we have not understood the evolutionary benefit of DA. We have addressed this black hole by utilizing the DA-induction-traits of copepodamides, to induce *Pseudo-nitzschia seriata* strains to a hypothetical maximum DA production. Afterward, we have been feeding the toxic *Pseudo-nitzschia seriata* cells to copepods fixated in an aquarium in the focus of a high-speed camera connected to a computer. The results have turned out quite interesting. Firstly, the induction was successful. The non-induced *Pseudo-nitzschia* cultures had no or very low cellular DA content, while the induced cultures had DA levels around two pg. DA cell^{-1}. Secondly, the major results imply that DA provides efficient protection against grazing by copepods, as the majority of DA-containing cells were rejected after brief handling by the copepods. These results provide a key contribution to understanding *Pseudo-nitzschia* and copepod interactions and an explanation of the evolutionary benefit of DA production.

Effects of nitrate and ammonium on uptake and assimilation of nitric oxide by *Heterosigma akashiwo*

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The globally distributed harmful algal species, *Heterosigma akashiwo*, forms blooms annually in Delaware's inland bays, U.S.A., where it often outcompetes other algal species. Laboratory culture experiments examining the growth and physiological response of *Heterosigma* to nitrogen sources have focused on its ability to utilize nitrate, ammonium and urea. Other nitrogen sources, such as nitric oxide (NO) may be an important but overlooked source of nitrogen to *Heterosigma* productivity in the natural environment. Previous research shows that *Heterosigma* is able to use NO as a nitrogen source, due to a novel hybrid nitrate reductase enzyme, NR2-2/2HbN (NR2), which couples nitric oxide dioxygenase activity with nitrate reductase activity. Assimilation of NO via NR2 enzyme activity, however, may be constrained by the presence of ammonium. Here, we investigated the capacity of *Heterosigma* to utilize NO as a source of nitrogen in the presence of other nitrogen sources. *Heterosigma* was grown in varying ratios of nitrate: ammonium, and spiked with 15N-labeled NO. Samples were collected to evaluate the uptake rate and assimilation of 15N into biomass, expression of genes involved in nitrogen assimilation, and NR activity. Results of this investigation revealed significant differences in the expression of key genes as well as 15N assimilation into biomass for cultures spiked with 15N-NO, supporting the hypothesis that NO may be a source of N for this species even in the presence of other N sources. Results will contribute to our understanding of factors that fuel blooms of *H. akashiwo* in coastal regions.

Microbial community metabolic balance during a harmful algal bloom of *Lingulodinium polyedra* and *Noctiluca scintillans* bloom, implications on the mixotrophic state of the southern coast of California

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During May of 2020 a dense algal bloom of *Lingulodinium polyedra* was present all along California and Baja California coast followed by an intense bloom of *Noctiluca scintillans*. Bloom initiates during May and stays until July 2020. To address the question of how microbial community metabolic balance (O_2 consumption and production) changes during the bloom of two mixotrophic species at Todos Santos Bay, Baja California, we performed an incubation experiment with three different water samples from three sites: I) *L. polyedra* dominated, II) two dinoflagellates dominated and iii) *N. scintillans* dominated. During temperature regulated conditions (18 ^{o}C), microbial community (mixotrophic community and prokaryotic) respiration rates and primary production (13C) were estimated using 1L polycarbonate, and BOD bottles, with sensitive patches SP-PSt3-NAU-YOP (PreSens, Regensburg, Germany) during a time series of 48 h. Samples for chlorophyll *a* and total organic carbon (TOC) were taken. Our results showed significant differences ($p < 0.05$) between sites, oxygen consumption rate varied from 82.40 to 378.40 µM O_2 L^{-1} day^{-1}, prokaryotic community respiration changed from 18 to 78 µM O_2 L^{-1} day^{-1}, and net primary production average was 76 µM O_2 L^{-1} day^{-1}, additional data about adding VitB12 and nutrients (NO_3, PO_4, trace metals) our results suggest important responses in terms of metabolism activity and reflects the relevance of mixotrophic HAB species present in the coast of Baja California.

Examination of the Factors driving *Karenia brevis* bloom expansion and termination: The ECOHAB: Life and Death of *K. brevis* Blooms Program

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The factors governing the initiation and development of *Karenia brevis* blooms in the eastern Gulf of Mexico, with the exception of certain outlier years, are fairly well understood, whereas the physical, chemical and biological factors underlying bloom termination are not. The NOAA funded ECOHAB: Life and Death of *K. brevis* Blooms program is addressing two critical aspects of *K. brevis* bloom ecology: the role of extreme events in magnifying directly or indirectly the intensity and duration (i.e. expansion) of blooms, and the factors that ultimately lead to bloom decline. Initial historical bloom analysis has identified several distinct reoccurring patterns in bloom expansion and termination and is examining the role of both local physical drivers and regional influences in driving these patterns. Combined with results from a monthly shelf sampling program, data will be used to develop and populate new statistical and mechanistic models of *K. brevis*, the latter to be developed within an open source physical and biogeochemical framework, and incorporate mixotrophy and bacterial and viral dynamics. Such knowledge is absolutely critical for effective bloom management and prediction, minimization of bloom-related economic damage to marine industries and tourism, and the development of targeted mitigation efforts.

Allelopathic inhibitory effect of the macroalga *Pyropia haitanensis* (Rhodophyta) on harmful bloom-forming *Pseudo-nitzschia* species

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The blooms of harmful microalgae represent a prominent threat to fisheries, public health, and economies throughout the world. Several approaches to control the microalgae blooms has been described, but they seem to have some negative effect on environment and the present organisms. Therefore, it is necessary to develop environmentally friendly techniques to control microalgal blooms. Recent studies have shown that certain macroalgae release allelochemicals that can inhibit the growth of bloom-forming microalgae. In present study, we found that macroalga *Pyropia haitanensis* significantly inhibited the growth of harmful bloom-forming microalgae *Pseudo-nitzschia pungens* and *Pseudo-nitzschia multiseries*. Allelopathic potency of *P. haitanensis* has not been reported previously by other researchers. The inhibitory-effect of the live thali of *P. haitanensis* was highest, followed by that of dry powder, water-soluble extract, and culture medium filtrate. The *Pseudo-nitzschia* species died 96 h after exposure to 5–10 g fresh-weight L^{-1} of *P. haitanensis* live thalli. Furthermore, a water-soluble extract of *P. haitanensis* significantly suppressed the growth of *P. pungens* and *P. multiseries*, thereby indicating that *P. haitanensis* contains stable allelopathic substances that cause the observed inhibitory-effects. Our Gas Chromatography-Mass Spectroscopy (GC-MS) based analysis with methanol extract of *P. haitanensis* reveals the presence of certain allelopathic metabolites. On the basis of these findings, we conclude that the macroalga *P. haitanensis* would have potential utility in controlling the blooms of *Pseudo-nitzschia* species.

What have we learned about the *Peridinium quadridentatum* blooms in Veracruz, Mexico?

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There have been 19 years since the first *Peridinium quadridentatum* bloom, a non-toxic dinoflagellate, was recorded in Veracruz, Mexico. Since thence, recurrent blooms have occurred in Veracruz coast, specifically in Veracruz Port and Boca del Rio, the most important cities that constitute an extended urban coastal zone. Before the first bloom of this dinoflagellate, the toxic *Karenia brevis* was the microalgae causing recurrent blooms in this area. What we have learned about the blooms of *P. quadridentatum* after 13 years of observations and different planned studies about its ecology is the following: blooms occur mainly from July to September (during the rainy season); blooms occur in patches in different sites along coastal urban zone; they occur during the most exposition of sun light time: 12:00 h to 18:00 h and vanishes; they occur for few days in different time intervals. Although the coastal area is characterized by high nutrients concentrations, is limited by phosphorous, so special conditions are necessary for the blooming: diminution of salinity, warm temperature of water, high incidence of sunlight, and a balance between N-P concentrations no limited by N. We believe that the benthic-planktonic habit of this dinoflagellate is favorable for prevailing in coastal zone, and it is advantageous over other microalgae during the rainy period.

Protoceratium reticulatum bloom in NW Iberia mid-shelf waters

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The yessotoxin (YTX)-producing dinoflagellate *Protoceratium reticulatum* is a cosmopolitan species occasionally observed in Portuguese coastal waters. In September 2019, and for the first time, a bloom was detected during a cruise carried out offshore F. Foz, Portugal (latitude 40º 13´ N). The study sampled a cross shelf section 3 times in one week, and revealed the bloom was already present at mid-shelf in stratified warm waters and was separated from the coast by coastal upwelling waters. SST and chlorophyll *a* satellite images indicated that two days prior to the cruise there was a short but strong upwelling event with a large and strong patch of chlorophyll a identified in the leeward side of the upwelling plume rooted at cape Mondego. The *P. reticulatum* bloom coincided with the northern side of this patch. It was distributed above the pycnocline, in waters with temperatures from 14 º to 17 ºC, reaching maxima of 2,250 cells L^{-1} at the surface (17 ºC). With the observed upwelling relaxation conditions until the end of the cruise, the bloom approached the coast being observed in low numbers at the most coastal station. Pairs of fusing cells and cysts of *P. reticulatum* were observed in the water column, mainly at the end of the cruise. *Protoceratium reticulatum* co-occurred within a dense mixture of diatoms and dinoflagellates, particularly other HAB species such as a dense bloom of *Dinophysis acuta* (168 x 10^3 cells L^{-1}), *Pseudo-nitzschia seriata* group (740 x 10^3 cells L^{-1}) and *Pseudo-nitzschia delicatissima* group (1,360 x 10^3 cells L^{-1}).

Harmful phytoplankton species in coastal and deep waters around Cozumel, Mexican Caribbean

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The distribution and density of harmful algal blooms (HABs) species are related to environmental factors. We studied the relationship between bloom-forming phytoplankton species and physicochemical and hydrological factors in the Mexican Caribbean. Samplings were performed in oceanic (0 – 200 m depth; daytime) and neritic (0 – 20 m depth; circadian cycles) epipelagic waters to the east and west of Cozumel Island. In the oceanic region, the highest densities of the HABs species *Alexandrium* spp. (2,111 cells L^{-1}), *Phalacroma rotundata* (56 cells L^{-1}), *Karenia* sp. (333 cells L^{-1}), *Prorocentrum lima* (56 cells L^{-1}), and *Prorocentrum rhatymum* (56 cells L^{-1}) were recorded on the surface, and at the depth of maximum fluorescence, associated with high temperature, lower salinity, and low nutrient concentrations. The circadian cycles in the coastal zone, showed the highest densities of the HABs species *Gambierdiscus* sp. (40 cells L^{-1}) and *Phalacroma rotundata* (40 cells L^{-1}) during both day and night, *Karenia* sp. (60 cells L^{-1}) and *P. lima* (60 cells L^{-1}) only during the night, and *P. rhatymum* only during the day. The occurrence of the harmful dinoflagellates, *Prorocentrum hoffmanianum* and *Pyrodinium bahamense*, was also recorded. The phytoplankton community was dominated by the nitrogen-fixing cyanobacteria *Trichodesmium*. The oceanic eastern side of the island had a clear surface mixed layer, but on the western side the mixed layer was less defined. The vertical structure of temperature and salinity showed a dynamic uplift in the Cozumel Channel (western side) with effects on phytoplankton density. Environmental variables, especially stratification, seem to control the distribution of HABs species.

Ability of Asian clams (*Corbicula fluminea*) to feed on and differentiate between toxic bloom-forming cyanobacteria and other algae

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Cyanobacterial harmful algae blooms (cHABs) are increasingly disrupting freshwater ecosystems and introducing toxins into aquatic food webs. The invasive and edible Asian clam *Corbicula fluminea* can be found in waterways afflicted with cHABs across the United States. This study focused on how quickly *C. fluminea* could become a possible vector of cHAB toxins by investigating feeding on cHAB species. We conducted laboratory experiments to quantify *C. fluminea* clearance rates and particle selection using algal cultures (microcystin-producing *Microcystis aeruginosa*, saxitoxin-producing *Dolichospermum circinale*, and using the non-toxic green microalga *Raphidocelis subcapitata* as a control). The first aim was to investigate clearance rates of each algae species at three concentrations mimicking typical dense bloom conditions and smaller blooms: 1 x 10^6 cells mL^{-1}, 5 x 10^5 cells mL^{-1}, and 2.5 x 10^5 cells mL^{-1}. The second aim was to explore particle selectivity with five ratios of *Microcystis* or *Dolichospermum* and *Raphidocelis* in the above cell concentrations/biovolumes. Clearance rates were statistically higher ($p < 0.05$) on algal type in both the *Microcystis* and *Dolichospermum* monoculture experiments compared to the control algae. Clams did not produce pseudofeces in the mixed *Microcystis* experiments, indicating ingestion of cells, but did produce pseudofeces in the *Dolichospermum* experiments, indicating possible cell rejection. Understanding how rapidly *C. fluminea* consumes cyanobacterial cells and toxins has implications for human health and could be used for management decisions on harvesting this species.

Spatio-temporal variability of harmful potential diatoms and its relationship with distribution of vitamin B12 (particulate and dissolved) in the northwest Mexican Pacific coast

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To associate the relationship between the presence of vitamin B12 (VitB12) with the spatio-temporal variability of potentially harmful diatoms present in Todos Santos Bay, Baja California, monthly samplings were carried out (June-November 2020 and February-March 2021), surface (2 m) and thermocline depths (20 m) samples were taken for taxonomic identification, chlorophyll *a* and dissolved and particulate VitB12 determination. To describe the spatial-temporal variability of hydrographic variables we analyzed CTD RBR profiles (temperature, oxygen, fluorescence and PAR sensors). Two genera of diatoms were considered, *Pseudo-nitzschia*, potentially producing domoic acid (DA) and *Chaetoceros*, potentially harmful. *Pseudo-nitzschia* genus was representative in July and November 2020 with abundances of 48 x 10^3 cells L^{-1} and 26 x 10^3 cells L^{-1} respectively with abundances of 41 x 10^3 cells L^{-1} and 16 x 10^3 cells L^{-1} in stations located at the entrance of the Bay where it's influenced by colder waters. Both genera were more abundant at 20 m, the average chlorophyll concentration was 13.19 µg L^{-1} at 15 m in July and 5.5 µg L^{-1} at 20 m in November. The concentration of VitB12 dissolved and particulate VitB12 at 20 m was 2.4 pM, 2.8 pM respectively, which coincides with the presence of *Pseudo-nitzschia* in two stations. Our results suggest that the distribution of particulate VitB12 is related to the presence of species of diatoms with harmful potential in the study area, July data was obtained after the presence of a dense *Lingulodinium polyedra* bloom which may influence de dynamics of particulate and dissolve VitB12 and its availability during phytoplankton succession.

Allelopathic inhibition of toxic *Pseudo-nitzschia* spp. by the kelp, *Saccharina latissima,* and other seaweeds

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Anthropogenic forcing is increasing the range, frequency, and intensity of harmful algal blooms (HABs) around the world. *Pseudo-nitzschia* is a globally distributed genus of diatoms known for production of domoic acid (DA), which causes amnesiac shellfish poisoning (ASP). *Pseudo-nitzschia* HABs overlap temporally with the growing season for *Saccharina latissima*, the most cultivated seaweed in North America, which has previously shown algicidal activity against the dinoflagellate HAB, *Alexandrium catenella*. In this study, we investigate the effects of *Saccharina latissima* and other seaweeds against toxic *Pseudo-nitzschia* spp. First, we performed 3-7 day co-culture assays of toxic *Pseudo-nitzschia* strains isolated from the Pacific Northwest and the Gulf of Maine with and without live thalli of *S. latissima* and other seaweeds. We found that *S. latissima* significantly reduced *Pseudo-nitzschia* cell density by 72 % in 3 days and 90 % in five days. Second, we performed bottle incubations of *Pseudo-nitzschia* bloom water and found that *S. latissima* significantly reduced *Pseudo-nitzschia* cell density by 69-75 % in four days. Data on the effects of *S. latissima* on *Pseudo-nitzschia* bloom toxicity will be presented, both in terms of cellular DA content when co-cultured with *S. latissima,* and in shellfish DA content when co-cultured with *S. latissima* on a *Pseudo-nitzschia* diet. Finally, to validate the hypothesis that the algicidal effects of *S. latissima* on *Pseudo-nitzschia* are allelopathic, LC-MS will be conducted to quantify allelopathic compounds, such as phlorotannins and other polyphenols, in the filtered culture media of *Pseudo-nitzschia*/seaweed co-culture assays.

The harmful effect of the green dinoflagellate *Lepidodinium chlorophorum*: from water discolorations to impact on cultivated bivalves

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The marine green dinoflagellate *Lepidodinium chlorophorum* cause summertime seawater discolorations along Southern Brittany (NE Atlantic, France). Freshwater inputs, neap tidal cycle and sea-surface temperature warming can promote this species blooms. Inorganic nutrients provided by bacterial remineralisation might contribute to sustain the bloom development for a month or more. Long-lasting blooms have effects on tourism and the recreational use of the coastal area, but also on local economy since high biomasses of the species have sometimes been associated with mass mortalities of cultivated oysters. Anoxia following these events would likely be associated with these mortalities, although other high-biomass phytoplankton blooms occurring in the area are without any specific effect on oysters. The specific harmful effect of *L. chlorophorum* might originate from high secretion of Extracellular Polymeric Substances (EPS), a biological trait particularly developed by this dinoflagellate. We demonstrated that *L. chlorophorum* produced a large amount of carbon-rich Transparent Exo-polymer Particles (TEP) and that the soluble fraction of EPS produced by this species was mainly composed of sulphated galactan both in culture and in the field. The EPS production could confer an ecological advantage to *L. chlorophorum* by enhancing bacteria remineralisation and sustaining the bloom, but it might have physiological negative consequences on bivalves. Eco-physiological experiments on the interaction between *L. chlorophorum* and oysters have been carried out to verify whether EPS production might alter the palatability of oysters to this dinoflagellate. Our studies might show how a cellular physiological feature can have adverse effects on marine coastal resources and local economy.

Growth interaction between epibenthic dinoflagellates *Amphidinium thermaeum* and *Coolia malayensis* under culture conditions

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Benthic ecosystems are regulated by secondary metabolites, which in turn are regulated by space and substrate type. In this work, growth interactions of the epibenthic dinoflagellates *Amphidinium* and *Coolia* were evaluated. Isolates from Bahía de La Paz, Gulf of California were cultured in GSe medium at 24 ± 1 °C, at a salinity of 34, under a 12:12 h L:D cycle at 150 µmol photons m^{-2} s^{-1}. Growth curves were performed in 300 mL flasks, 1000 and 240 µL plates. Growth interaction cultures were followed in 200 µL with the following cell biovolume proportions: 1:1, 1:2, and 2:1 for 12 days. Molecular identification was performed by amplifying the D1-D3 region of the LSU-rDNA, and identified as *A. thermaeum* and *C. malayensis*. The highest biomass of both species was observed in 1000 µL in comparison to 240 µL and 300 mL ($5.58 \times 10^4 \pm 1.18 \times 10^4$ and $1.77 \times 10^4 \pm 1.34 \times 10^4$ cells mL^{-1}; $9.72 \times 10^3 \pm 8.57 \times 10^2$ and 133 ± 72 cells mL^{-1}; $1.7 \times 10^4 \pm 2.01 \times 10^3$ and $9.78 \times 10^3 \pm 2.52 \times 10^3$ cells mL^{-1}, respectively). None of the species were morphologically affected by the interaction; growth rates were similar to the controls (0.4 - 0.5 div day^{-1}). *A. thermaeum* predominated in all proportions. Cell densities decreased compared to the controls in both species, which was more evident in the 1:1 proportion ($2.59 \times 10^4 \pm 1.06 \times 10^4$ and 163 ± 131 cells mL^{-1}, respectively). These species do not seem to be involved in allelopathic mechanisms; however, they compete for space resulting in a decrease in cell density. Estimating the growth and effects of epibenthic dinoflagellates is challenging due to their adhesion capacity, swimming speed, mucilage production, and life cycle complexity.

The Ability of Native and Invasive Rhodophytes (*Gracilaria tikvahiae* and *Dasysiphona japonica*) to Inhibit Harmful Algal Blooms Caused by *Aureococcus anophagefferens* (Pelagophyceae)

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Intense harmful algal blooms (HABs) caused by *Aureococcus anophagefferens* have spread globally and are a threat to coastal ecosystems and shellfish. Although some HAB mitigation techniques such as algal biocides can be effective, many such as approaches can have potentially long-term negative consequences on the environment. Therefore, it is important to investigate alternative HAB mitigation techniques that are ecologically safe and/or commercially viable. Here, we report on experiments using naturally occurring densities of the macroalgae, *Gracilaria tikvahiae*, *Dasysiphonia japonica*, and *Ulva lactuca* designed to assess effects on the brown tide-forming pelagophyte, *A. anophagefferens*. During these experiments, varying densities of these macroalgal species were co-cultured with varying strains and cell densities of *A. anophagefferens*. The co-effects of nutrients, bacteria, and pH were also assessed and *A. anophagefferens* cell concentrations and photosynthetic efficiency were quantified. In nearly all experiments, *G. tikvahiae* and *D. japonica* significantly reduced *A. anophagefferens* cell densities in a dose-dependent manner, with the effectiveness of each macroalgal species varying depending on *A. anophagefferens* cell densities, nutrient levels, and macroalgal concentrations. The algacidal effects of D. *japonica* and *U. lactuca* were significantly stronger than *G. tikvahiae*. Our findings, in conjunction with the well-known nutrient removal capacity of macroalgae, suggest that the use of the rhodophytes and chlorophyte seaweeds may be a promising mitigation strategy for HABs in Long Island's coastal ecosystems.

Photo: Tanos Grayeb

HA MICROBIOMES

History of a couple: *Alexandrium minutum* and its associated bacterium in both natural and cultivated populations

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Dinoflagellate-bacteria interactions consist in species-specific relationships that vary at population and biogeographical scales. These local interactions can be favourable or detrimental to the dinoflagellate blooms and need to be characterized by both *in situ* observations and experimental work. *Alexandrium minutum* blooms are seasonally observed in the Bay of Brest (NE-Atlantic France) and the influence of bacteria on local populations phenology have never been studied. Size-fractioned metabarcoding analyses of environmental DNA have been performed during three years of *A. minutum* blooming seasons to identify the regularity of the co-occurrence of specific bacteria with the dinoflagellate across the blooming time. In parallel, metabarcoding analyses of bacterial communities were carried out on cultivated *A. minutum* strains. Plankton and bacteria diversity analyses was assessed by Amplicon Sequence Variant (ASV) both *in situ* and in culture and recurrent relationships between *A. minutum* and bacteria were inferred by co-occurrence network analyses. An ASV provisionally affiliated to the bacterial genus *Fluviicola* (Flavobacteriales order) was regularly co-occurrent with *A. minutum* in the > 20 µm fraction. The same ASV was found in high abundance in cultures, corroborating the hypothesis of the existence of a regular and intimate biological relationship between both microorganisms. The positive or negative nature of this interaction for the dinoflagellate bloom development has been assessed during a one-month culture experiment. Our data sustain the hypothesis of a natural association between the toxic dinoflagellate and bacterium within phycosphere and question about the chemical and evolutionary explanations allowing its stability in time.

Long-term composition of 16S-based bacterial communities associated with algal bloom events in northern Chile

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Algal blooms are a natural phenomenon that commonly occurs in marine ecosystems affecting the economy and public health worldwide. On the coasts of Atacama Desert, northern Chile, seawater desalination plants have been installed to supply drinking water for human consumption. Currently, there is an increasing interest in the occurrence of marine algal blooms because can strongly affect the operations of desalination plants. In this context, studies on the marine algal blooms and their microbial interactions are relevant to understand their composition and dynamic as well as to design strategies to mitigate their impact on desalination plants. In this work, we used 16S-based high-throughput DNA sequence analyses (Illumina) to study the total bacterial community associated with algal bloom events of both *Prorocentrum micans* and *Ceratium* sp. occurred in January 2019 and *Akashiwo sanguinea* occurred in December 2019 on the coast of northern Chile, where desalination plants are located. We considered samples weekly taken from December 2018 to January 2020 in two locations. Libraries of 16S rRNA genes were built, sequenced, and used to identify the composition of bacterial communities associated with alga, including free-living and algal-attached bacterial cells. Algal-attached cells were those collected and filtered with 1.0 um pore-size membranes, and free-living were those collected by filtering with 0.2 µm pore-size membranes. Differences between (1) bloom events and no bloom events, and (2) free-living cells and attached cells were evidenced. We found that attached Cyanobacteria-relative abundance changed during the bloom events and Epsilonproteobacteria increased its relative abundance. We also observed a lesser bacterial diversity in samples collected during algal bloom events.

Comparison of bacterial communities, core microbiota and metabolic potential among geographically distinct populations of the benthic dinoflagellate *Prorocentrum lima*

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Marine benthic species of the dinoflagellate genus *Prorocentrum* produce a diversity of polyketide-derived phycotoxins, primarily associated with diarrhetic shellfish poisoning (DSP). The toxin function is unknown but may involve interactions with microeukaryotes and bacteria within the phycosphere and upon epibenthic substrates. There is increasing evidence of bacteria affecting *in situ* growth rates of benthic dinoflagellates and perhaps in determining cell toxin production via chemical communication. In this study, we compared the bacterial communities associated with *Prorocentrum lima* populations from two geographically distant sites on Mexican coasts to identify core members. The metabolic potential in this bacterial subset was then investigated to decode the main interactions between epibenthic *Prorocentrum* and microbiota. Natural *P. lima* isolates were obtained from Baja California Sur, northeast Pacific Ocean, and Veracruz on the Gulf of Mexico. Monoclonal *Prorocentrum* cultures were established focusing on conditions for optimal growth and DSP toxin production determined by liquid chromatography coupled to tandem mass spectrometry. Profiles of bacterial communities associated with dinoflagellate clones were obtained by high-throughput sequencing of 16S V3 - V4 amplicons. Alpha - (α) and gamma - (γ) proteobacteria phyla dominated the community, wherein more than thirty bacterial orders were identified. Core microbiota of *P. lima* cultures included members of Caulobacterales, Rhodobacterales, and Rhizobiales. Key metabolic pathways involving nutrient production and metabolite exchange were identified. Our results show a complex bacterial community and suggest potential allelochemical interaction with core microbiota. We discuss the role and value of such metabolic capabilities in the function, fitness, and toxigenicity of *P. lima*. Moreover, this study provides a template for further elucidation of ecological interactions in natural populations.

Microbial population dynamics and diversity during a toxic *Karenia* bloom

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Harmful algal blooms (HABs) have important implications for the health and functioning of aquatic ecosystems. Recent findings suggest that interactions between microorganisms and blooming algae during bloom events significantly impact their physiology, alters ambient chemistry, and shapes ecosystem diversity. Consequently, investigating the microbial community structure and succession at specific bloom stages and shedding light on interactions among taxa is important. Here, we employ DNA metabarcoding to identify patterns in a harmful dinoflagellate *Karenia brevis* bloom and associated ecological communities in the Gulf of Mexico. Five barcodes were used in this study that target bacteria, archaea and microeukaryotes. For the latter, four different barcodes were selected to provide a comprehensive view of eukaryotic phytoplankton, other protists and fungi. The abundance and diversity of these microbial groups were examined in relation to various environmental factors (e.g., salinity, temperature, inorganic nitrogen, and inorganic phosphate). Our results demonstrate the importance of multi-marker DNA metabarcoding for biomonitoring the community succession in algal bloom trajectories and reveal the dynamics of the interaction between microorganisms and environmental factors during HABs.

Harmful algae are fished by virus with baits?

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HaV is an only member of double-stranded DNA virus (Raphidovirus), which infects a noxious bloom-forming raphidophyte *Heterosigma akashiwo*. Previously, we collected a number of *H. akashiwo* clones and its dsDNA virus (HaV) clones, and conducted cross-reactivity tests between them. As a result, their host-virus relationship was shown to be highly specific at the clonal level. HaV01 and HaV53 were originally isolated by using *H. akashiwo* strain H93616 as host, but they showed a different host range to each other. HaV53 was shown to have a giant protein (492 kDa in molecular weight) which has a parallel β-helix structure on the N-terminal side found in the pectate lyase superfamily, and a highly repetitive region composed of 74-amino-acid repeat sequence (74aa) on the C-terminal side. Later, the 74aa was also detected in HaV01. It is notable that each 74aa slightly differed not only in amino acid sequence within each clone, and the compositional diversity significantly differed between HaV01 and HaV53. Here, a fascinating hypothesis is that the 74aa has an affinity to polysaccharides on the host cell surface, and the pectate lyase domain works as a kind of "can opener" to help the initial infection step of HaV to *H. akashiwo*; i.e., an HaV clone may fish its suitable host cell by using a variety of "baits (74aa)". If this is correct, it might be possible to explain the "gradiently-different host range" of HaV clones. In this paper, we predicted partial conformation of the giant protein *in silico*.

The potential of cyanophages to modulate photosynthesis in bloom-forming cyanobacteria

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Cyanophages kill up to 20 % of the world's cyanobacterial population every day, but little is known about the physiology of infection. Our goal is to comprehensively investigate the cyanophage infection process. Metabolism modification of the freshwater cyanobacterium *Aphanizomenon flos-aquae* infected with the vB-AphaS-CL131 cyanophage suggests that the virus maintains the host's vital processes and adapts them to its needs. *In vivo* chlorophyll fluorescence measurements demonstrated a partial reduction of the photosystem II efficiency and a subsequent decrease of electron transport around PS II due to infection. The expression of selected host genes involved in photosynthetic electron transport and those involved in carbon metabolism (Calvin cycle and pentose phosphate pathway) was affected. Such rearrangements resulted in increased ATP and NADPH concentrations which suggests their reduced consumption by the host or/and redirection of these products to virus progeny synthesis. The performed experiments provide an insight into the mechanisms of cyanophage infection and research is being continued to describe the overall model of freshwater cyanobacterial phage infection.

Micro-scale genomic diversity of the bloom-forming cyanobacterium *Aphanizomenon gracile* in a drop of water

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Aphanizomenon gracile is a species of harmful cyanobacteria blooming worldwide with severe consequences for fresh and brackish water ecosystems. Although abiotic factors sustaining bloom events are well known; the biotic interactions involved in the population regulation remain unclear. Here, comparative omics of four monoclonal strains of *A. gracile* isolated from a single drop of water reveals extensive heterogeneity of chemotypes and gene contents, despite constrained genome size and high similarity indices. These variations are remarkably associated with horizontal gene transfers (HGT) of biosynthetic gene clusters (BCG), and a novel siphophage infecting *A. gracile* appears to participate to this genotypic diversification. In spite of high variability in heterotrophic taxa relative abundances, *A. gracile* cyanospheres displayed an apparent functional redundancy implying biosynthesis of public goods. Altogether, these results suggest that a bloom would constitute a hot-spot for *A. gracile* genotype diversification driven by cyanophages, where losses and gains of BCGs compels cyanobacteria individuals to cooperate together and with heterotrophic bacteria in a black queen hypothesis compatible way.

Parasites impacting community composition in diatom spring blooms

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Recurring phytoplankton blooms can promote the development of an active parasite community, which infects the dominant species for reproduction and might contribute to the decline of the bloom. An infection strategy characterized by low host specificity and wide host ranges could be common, as it enables the parasites to opportunistically infect any emerging high-biomass phytoplankton species. We tested this hypothesizes by monitoring the diatom spring bloom in a temperate lake using amplicon sequencing and performed infection experiments with different eukaryotic, unicellular parasites and several cultures of different diatom species isolated from the bloom. Chytridiomycota displayed the highest relative abundance of all parasitic lineages, particularly in large size fractions (> 10 µm) suggesting their association to larger cells. Chytridiomycota reached their highest relative abundance short after the peak in diatom reads. In contrast, Perkinsida were the dominant parasites in small size fractions (< 10 µm) of some samples, challenging the identification of associations with specific micro-phytoplankton taxa. The infection experiments revealed significant differences in host specificity and range among different Chytridiomycota species, illustrating successful infection of many diatom species by certain parasite taxa and high specialization by other chytrid species. This study highlights complex host-parasite interactions in recurring freshwater diatom blooms.

Biology of four Perkinsozoan parasitoids infecting marine dinoflagellates: an indispensable prerequisite for use as a biological control agent

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Perkinsozoan parasitic protists of dinoflagellates (i.e., Parviluciferaceae members) have long received attention as a potential biological control agent of harmful dinoflagellates. However, little is known about the biological characters of Parviluciferaceae parasitoids. Here, we examined the parasitoid generation time, prevalence, host range, and zoospore size, swimming speed, infectivity, survival, and the number of zoospore of four parasitoids (*Tuberlatum coatsi*, *Dinovorax pyriformis*, *Parvilucifera infectans*, and *Parvilucifera multicavata*) under the same dinoflagellate host (*Alexandrium pacificum*) and growth conditions. While *T. coatsi* produced the smallest and the most abundant numbers of zoospores, *D. pyriformis* produced big but the fewest number of zoospores. Survival and infectivity of zoospores from each parasitoid decreased over time and zoospores of *T. coatsi* had a relatively longer survival and infectivity than those of other parasitoids. Parasitoid generation times also differed among Parviluciferaceae parasitoids, with the total generation time of *D. pyriformis* the shortest. All parasitoids had broad host range in host dinoflagellates and had different host range with variable host susceptibility. The biological data obtained from this study would be an indispensable prerequisite when applied to control the harmful algal blooms in the field.

A multi-omic approach for a better understanding of the interactions between *Alexandrium minutum* and its microbiome

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Phytoplankton-bacteria interactions occur in the microenvironment surrounding algal cells known as the phycosphere. The transformation of algal-derived organic matter, the exchange of metabolites and infochemicals govern phytoplankton-bacteria relationships, which span from mutualism to competition. With the emergence of high throughput molecular tools, changes of bacterial communities' composition as a function of the phytoplankton species/strains presence or the different phases of the development of a bloom have been highlighted, suggesting that the physiology and the metabolism of phytoplankton cells would have an influence on the microbiome's diversity and structure. With the aim to test this hypothesis, here the bacterial community composition (comparing free-living and phytoplankton-attached fractions), physiology and metabolome were investigated for *Alexandrium minutum* and a self-selected natural microbiome over 48 days of co-cultivation. *Flavobacteriacea*, *Rhodobacteracea*, *Marinobacteracea*, *Alteromonadacea* and *Methylophagaceae* were the most abundant families associated with this *A. minutum* strain. The fraction and growth phase were identified as structuring factors of the bacterial community. *Flavobacteriaceae* tended to be attached to the *Alexandrium* cells reaching up to 80 % of this sub-population at the end of the exponential growth phase, whereas *Marinobacteracea* and members of Alpha-proteobacteria (*Rhodobacteracea* and *Hyphomonodacea)* enriched the free-living fraction, respectively during the exponential growth phase and the stationary phase. Interestingly, the factor time induced an evolution of the metabolome of both fractions. Altogether, dynamic of the microbiome associated with *A. minutum* strain depended on the ability of its members to use different metabolites excreted by *Alexandrium* during its growth and to interactions among the heterotrophic bacteria.

Fate and interaction between *Microcystis* and its microbiome in estuary

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Due to climate changes and eutrophication, blooms of predominantly toxic freshwater cyanobacteria are intensifying and are likely to colonize estuaries, thus impacting benthic organisms and shellfish farming representing a major ecological, health and economic risk. However, little is known about the fate of natural colonies of *Microcystis* by salinity increase. In natural environment, *Microcystis* form large mucilaginous colonies with numerous heterotrophic bacteria embedded in the mucilage. This micro-environment, known as phycosphere, is the place of numerous biotic interactions that influence the development of both cyanobacterial and bacterial communities, as well as the production and fate of toxins. The aim of this PhD thesis is to study the impact of a salinity increase on the cyanobacterial genotypic composition, the physicochemical characteristics of the mucilage and the production of specific metabolites (toxins and compatible solutes) along a French freshwater-marine gradient at different phases of a bloom. We will also determine how these changes influence the structure and the composition of the bacterial community associated with *Microcystis* and their function in toxins degradation. The project will combine *in situ* monitoring and *in vitro* experiments at the community (batch experiment) and single-colony levels (microfluidic experiment). We will present preliminary results of the first field season.

The ecological network and it´s research progress in algal-bacterial interactions

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The relationship between algae and bacteria play critical roles in marine ecological processes, including symbiotic relationship, matter metabolism, element cycle and coevolution. Previous studies have summarized a variety of performance among the algal-bacterial interaction, including mutual benefit, symbiosis, antagonism and mutual harm. However, due to the complexity and diversity between this relationship, traditional methods cannot fully exploit the hidden information behind algal-bacterial symbiont. With the development of Omics technology and the influence of global change on marine ecosystem, the research perspective of algal-bacterial relationship has risen to systemic ecology level gradually. The rapid-blooming of high-throughput sequencing technology and the establishment of big data analysis ability bring us new opportunities to study the algal-bacterial interactions deeply. Among these technical methods, ecological-network is the key member, which affects the establishment of symbiotic relationship, the occurrence of interactive behavior and the proceeding trend of ecological events. Here, in this paper, we reviewed the research advances of algal - bacterial ecological network based on big data. At first, we introduced the ecological network' formation conditions, network properties and construction methods. Secondly, network' function, co-occurrence mode of algae bacteria relationship (molecular network and metabolic network), as well as the ecological significance were performed. Finally, the idea of establishing the knowledge network database of algal-bacterial interactions and the graphic database of photosphere biological profiles are proposed. The aim of this paper is to excavate the holistic information behind algal-bacterial relationship, and provide new cues for further revealing the molecular mechanisms of algal-bacterial interaction and its multi-dimensional roles in phycosphere niche.

Photo: Tanos Grayeb

CYANOBACTERIAL BLOOMS

Nitrogen and *Microcystis* blooms: considering the importance of exogenous vs endogenous supplies

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During the past two decades there has been accumulating evidence demonstrating that the growth and toxicity of harmful algal blooms formed by *Microcystis* can be controlled by nitrogen (N). Cellular toxin quotas of *Microcystis* have been found to depend upon exogenous N supplies and observations from Lake Erie demonstrate that microcystin concentrations peak in parallel with inorganic N and are significantly lower during years of reduced inorganic nitrogen loading and concentrations. Experiments in multiple lakes across North America have demonstrated that N loading can promote cyanobacterial biomass and concentrations of microcystin. Still, during bloom events, *Microcystis* forms large colonies known to harbor diverse heterotrophic bacterial assemblages and there has been growing interest in understanding how the microbes within this phycosphere may influence blooms. Here we present data demonstrating that enrichment of these colonies with N can significantly alter the bacterial community composition. Moreover, bacterial communities associated with *Microcystis* colonies were found to be enriched in pathways involved in N cycling, with taxa potentially capable of N_2 - fixation being four-fold more abundant within *Microcystis* colonies relative to the pelagic bacterial community. In addition, the relative abundance of these specific taxa within colonies was inversely proportional to concentrations of inorganic nitrogen. Nitrogen assimilation experiments performed with bloom water from North American lakes and *Microcystis* colonies demonstrated that the N uptake rates and preferences of *Microcystis* colonies differed from whole plankton communities. The relative importance of differing N sources and microbes in supplying N to *Microcystis* colonies will be discussed.

Cyanotoxins in pelagic and benthic zones of lakes in the Italian perialpine region

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Cyanobacteria constitute a health hazard in freshwater environments due to the ability to produce toxic metabolites. Animals and humans can be harmed upon exposure to water containing high levels of cyanotoxins that can occur during intense blooms. *Planktothrix rubescens* and *Tychonema bourrellyi* are the main responsible of toxins production (microcystins and anatoxins, respectively) in pelagic environments of the Italian subalpine lakes. In particular, in Lake Garda, the largest Italian water basin, *T. bourrellyi* has become year after year dominant over the other toxigenic cyanobacteria, leading to pelagic concentrations of anatoxin-a higher than microcystins'. This shift has important implications for the management of the lake, considering that chemical, physical and toxicological characteristics of anatoxin-a and microcystins are very different. Besides the risks connected to the presence of toxins in the pelagic zone, a new issue has emerged from recent investigations conducted in the frame of the Eco-AlpsWater project (financed by the EU-Interreg Alpine Space programme): remarkable quantities of homoanatoxin-a have been found in biofilms collected from rocks in different sites of Lake Garda shores. Further studies are on the way to identify the producing organism(s). This finding is the first report of cyanotoxins in benthic mats in the area, highlighting the necessity of more deep studies on the occurrence of toxic benthic cyanobacteria in lacustrine (and riverine) environments in the Italian perialpine region.

Interspecific protection against oxidative stress: how the Black Queen interferes with hydrogen peroxide treatments of cyanobacterial blooms

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Oceanographic studies have shown that heterotrophic bacteria can protect the marine cyanobacterium *Prochlorococcus* against oxidative stress caused by hydrogen peroxide (H_2O_2), through an eco-evolutionary interaction that has become known as the 'Black Queen Hypothesis'. Could a similar interspecific protection play a role in toxic cyanobacterial blooms of freshwater ecosystems? In a series of laboratory experiments and lake studies, we demonstrate that freshwater cyanobacteria are sensitive to H_2O_2 but can be protected by less sensitive species such as green algae. Our laboratory experiments show that green algae degrade H_2O_2 much faster than cyanobacteria. Consequently, the cyanobacterium *Microcystis* was able to survive at higher H_2O_2 concentrations in mixtures with the green alga *Chlorella* than in monoculture. The level of interspecific protection provided to *Microcystis* depended on the density of *Chlorella*. These lab results were confirmed by H_2O_2 treatments of two lakes with different densities of green algae. Our findings have implications for the mitigation of toxic cyanobacterial blooms, which threaten the water quality of many eutrophic lakes and reservoirs worldwide. In several lakes, H_2O_2 treatments have been successfully applied to suppress cyanobacterial blooms. Our results demonstrate that high densities of green algae can interfere with these lake treatments, as they may rapidly degrade the added H_2O_2 and thereby protect the bloom-forming cyanobacteria. Hydrogen peroxide treatments of cyanobacterial blooms are thus recommended only if the densities of green algae are low.

Cyanobacterial blooms interannual variations in a Mexican tropical crater lake

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Santa María del Oro crater lake has become one of the most visited sites in Nayarit, Mexico, and it is used for tourism, agriculture, livestock and water supply. In 2015, a Cyanobacterial Harmful Algal Bloom (CyanoHABs) was detected, for which monitoring was started, carrying out horizontal trawls and bottle samples. For species identification, we used a phase-contrast microscope and 16S rRNA gene sequencing, while quantification was performed a Sedgwick Rafter chamber. The CyanoHAB in the lake become recurrent and allowed us to follow changes in species composition and density between 2015 to 2021. In 2015 and 2016, the dominant species was *Limnoraphis robusta*, which formed "scums" with a pale green coloration. Its maximum densities was in February with 70 thousand cells mL^{-1} followed by *Microcystis aeruginosa* with 710 colonies mL^{-1}. While from 2017 to 2018, the dominant species was *M. aeruginosa* with uniform distribution and lemon green colour. Its maximum densities were in March 2018 with 802 colonies mL^{-1}, while *L. robusta* considerably reduced its population. The blooms decreased notably its intensities from 2019 to 2020. It was until 2021 that two previously unknown species for the lake bloomed. In February, we detected an *Auxenochlorella protothecoides* bloom with a density of 10 thousand cells mL^{-1}, while in April, the dominant species was *Synechococcus* sp. Changes in the dominant species observed in the 2018 - 2019 bloom represented a health risk for lake users, mainly due to microcystin production. Also, lake tourism suffers negative impacts due to visually awful scum and odors.

Accumulation of cyanobacterial toxins in shellfish and irrigated crops

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Ingestion of contaminated food, such as aquaculture products, algal supplements and irrigated crops, might lead to human exposure to cyanobacterial toxins. In the Netherlands, crops cannot be irrigated with water in which the cyanobacterial toxin concentration exceeds 1 µg L^{-1}. However, more data are needed to underpin this guideline. We tested whether lettuce, beetroot and strawberries accumulate cyanobacterial toxins (microcystins, cylindrospermopsins and homoanatoxin-a) when irrigated with water containing cyanobacteria. Although the crops were exposed to toxin concentrations up to 170 µg L^{-1}, no toxins were detected in the edible parts of beetroot and strawberries. Only lettuce which was irrigated on the plant contained detectable amounts of microcystins. The fate of the toxins (adsorption to the soil, accumulation in inedible parts, biotransformation) is still under investigation. Cyanobacterial toxins can also accumulate in shellfish. In the Netherlands, some of the official production areas are located in a delta which receives an influx of freshwater. We performed a survey on the presence of cyanobacterial toxins in shellfish from these areas. Microcystins and anatoxins were only detected in a few samples, but cylindrospermopsins were detected in 60 of the 165 samples. The highest detected cylindrospermopsin concentration (sum cylindrospermopsin and 7-deoxy-cylindrospermopsin) was 64 µg kg^{-1}. Phytoplankton samples were taken at the same locations and times as the shellfish, but cylindrospermopsins were only detected in one of these samples. Also during routine phytoplankton monitoring, no high abundances of cyanobacteria were observed. The source of cylindrospermopsins in Dutch shellfish areas is therefore still unknown.

Cyanobacterial harmful algal bloom dynamics in the northern Gulf of Mexico: Implications for coastal risk management

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During 2019, the Bonnet Carré Spillway was opened to protect New Orleans from Mississippi River flooding and remained open for a record 118+ days. Throughout this period, low salinities persisted across coastal Mississippi for several months and resulted in blooms of *Microcystis* spp. and other potential HAB species along the Mississippi coastline. While these genera have been identified locally in freshwater, this event was the first time that cyanobacterial HABs and their associated toxins have been reported in the northern Gulf of Mexico. The objective of this work was to examine how environmental conditions such as water temperature, salinity, dissolved oxygen, total suspended solids, and nutrients, affected in-situ toxin production through time. A west – east salinity gradient persisted throughout the study as well as many zones of hypoxia that extended from the surface through the water column. Dense blooms of cyanobacteria were not observed during sampling and presence of *Microcystis* could not be confirmed via 16S rRNA across the area. However, microcystins were detected by ELISA and LC/MS, and persisted at concentrations > 1 ppb total microcystin even in the absence of visible colonies or mcyB/E. Water movement in these coastal waters was influenced by meteorological conditions and west winds persisted throughout the summer of 2019, likely promoting spatiotemporal movement of toxin. With climate disturbances predicted to increase over the next several decades, prolonged openings of spillways may also increase in frequency. These data have allowed a deeper understanding of cyanobacterial bloom dynamics in freshwater dominated coastal systems and will directly inform on response efforts for future events.

Tropical cyanobacterial diversity along a trophic gradient in the New River, Belize

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The New River is situated in northern Belize and has a trophic gradient due to several points of anthropogenic influence including agricultural, urban, and industrial run-off. In the fall of 2019 this system experienced a severe drought, leading to fish kills and reports of harmful algal blooms (HABs) along much of the river. A study was conducted to determine the trophic state, cyanobacterial community structure, and cyanotoxin concentrations along the river from the non-impacted lagoon downstream through impacted areas and into Corozal Bay. The trophic state varied spatially along the river, starting from oligotrophic in the lagoon and increasing in trophic state near impacted areas, with *in situ* chlorophyll *a* concentrations typically exceeding 20 µg L^{-1} in these areas. Cyanobacterial community structure, and cyanotoxin concentrations varied spatially along the river and cyanobacterial HABs were seen in various locations along the river as both planktonic blooms and dislodged benthic mats. 16S metagenomic analysis detected that community structure varied along the trophic gradient. *Synechococcus* dominated throughout much of the river, though toxigenic species, such as *Microcystis aeruginosa* were also found along much of the river along with microcystins detected in several locations.

Phytoremediation of ANTX-a, CYN and MC-LR by the *Lemna trisulca* macrophyte

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Using plants to improve water quality and restrict or remove cyanobacterial blooms is not as fast as chemical or physical processes, but it is more environmentally friendly and can be used without applying advanced technology. In this study, *Lemna trisulca* was tested as a phytoremediation agent for three common cyanotoxins; anatoxin-a (ANTX-a), cylindrospermopsin (CYN), and microcystin LR (MC-LR). The initial amount of cyanobacteria used in experiments was close to the highest values confirmed in the natural environment (chlorophyll *a* equivalents-minimum 0.2 mg L^{-1}). Co-cultivation of plant with *Dolichospermum flos-aquae* caused a release of the intracellular pool of ANTX-a into the medium and the adsorption of 92 % of the toxin by the plant (after 14 days, the total amount of toxin decreased 3.17 times). Common cultivation with *Raphidopsis raciborskii* caused a 2.77-time reduction in the total CYN concentration (62 % of the total pool of CYN was associated with the plant). After two weeks of co-cultivation of plant and *Microcystis aeruginosa*, the MC-LR concentration decreased more than 310 times. The attachment of cyanobacteria cells or MC-LR adsorption to the macrophyte has not been demonstrated. The macrophyte also influenced the growth and development of cyanobacteria. Overall, two weeks of co-cultivation reduced the biomass of *D. flos-aquae*, *M. aeruginosa*, and *R. raciborskii* by 8, 12, and 3 times, and chlorophyll *a* concentration in comparison to the control decreased by 17.5, 4.3, and 32.6 times, respectively. The obtained results indicate the potential of *L. trisulca* for limiting the development of harmful cyanobacterial blooms and their toxicity.

Occurrence of a single-species cyanobacterial bloom in a lake in Cyprus: monitoring and treatment with hydrogen peroxide releasing granules

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Excess loads of nutrients (from urban and industrial waste and agricultural activities) that find their way into surface waters can cause rapid and excessive growth of phytoplankton species, leading to the formation of cyanobacterial harmful algal blooms. The presence of toxic metabolites (cyanotoxins) produced from cyanobacteria drastically decreases water quality and negatively affects aquatic ecosystem, human and animal health. Hence, their presence must be closely monitored and effectively mitigated. The present study focused on monitoring St. George Lake, located in the Athalassa National Forest Park of Cyprus, for its water quality characteristics and its trophic condition. Moreover, a new methodology to combat cyanobacterial blooming was applied, by using novel metallic peroxide granules as source of hydrogen peroxide instead of liquid hydrogen peroxide. During the recorded blooming period, the nutrient load of the lake was much higher than the remaining monitoring period which inferred long term contamination and the lake was characterized as eutrophic. Microscopic analysis indicated a single species cyanobacterial bloom of *Merismopedia* sp., comprising 99 % of the phytoplankton bio-volume. The presence of microcystin synthase encoding gene was documented, however microcystins were not detected by LC/MS/MS. Treatment with liquid hydrogen peroxide in concentrations 1 to 5 mg L^{-1} had no effect on the phycocyanin fluorescence (Ft) and quantum yield of PSII (Fv/Fm) indicating an ineffective treatment for the dense *Merismopedia* bloom (10^6 cells mL^{-1} ± 20 %). Metallic peroxide granules tested for their H_2O_2 releasing capacity, showing that CaO_2 released higher H_2O_2 concentration and therefore have better mitigation efficiency than MgO_2 granules.

Exploring the origin of tetrodotoxin (TTX) in New Zealand bivalves: could cyanobacteria be responsible?

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Tetrodotoxin (TTX) is a potent neurotoxin that has been responsible for many human intoxications and deaths globally. The distribution of TTX and its analogues is remarkably diverse, and the toxin has been detected in organisms from marine, freshwater and terrestrial environments. Increasing detections of TTX in aquaculture species not typically associated with TTX, such as edible bivalves, has drawn considerable attention to the toxin. This has reinvigorated scientific interest regarding its source in the marine environment. My PhD research study focused on exploring the source of TTX in *Paphies australis*, an endemic clam of New Zealand found to accumulate high concentrations of TTX. *Paphies australis* are a popular food source, are largely sessile and commonly found in subtidal habitats, making them a highly amendable organism to investigate the source of TTX. A multiple line of evidence approach was used to investigate potential TTX producers, including histological and analytical techniques to explore the micro-distribution of TTX within the organs of *P. australis*, aquaria studies to investigate the depuration and uptake of TTX, and field studies to explore the variation in TTX concentrations and bacterial communities from different *P. australis* populations. The key results will be presented, in particular the metabarcoding analyses demonstrating that marine picocyanobacterial (e.g., *Cyanobium*, *Synechococcus*, *Pleurocapsa*, and *Prochlorococcus*), were found in all samples collected from sites containing the highest amount of TTX and were present in the core microbiome of TTX-bearing clams. These results indicate that marine cyanobacteria should be further investigated as potential TTX producers.

Macronutrient and B vitamin dynamics of Chowan River (North Carolina, U.S.A.) CyanoHABs

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Cyanobacterial harmful algal bloom (CyanoHAB) proliferation impacts ecosystem and human health on a global scale. The Chowan River-Albemarle Sound Estuary, NC has been plagued by a resurgence of toxigenic CyanoHABs since 2015 after prolific CyanoHABs in the 1970s and 1980s. Non-N_2-fixing and N_2-fixing cyanobacteria compose Chowan River (CR) blooms, but in summer 2020 N_2-fixing *Dolichospermum* spp. were dominant. To understand the nutritional drivers of these CyanoHABs, we investigated N_2-fixation rates using acetylene reduction assays in addition to nitrogen (N), phosphorus (P), and B vitamin limitation using F/200 vitamin addition bioassays in the summer of 2020. Cyanobacteria are generally thought to not require external B vitamins, though the opportunistic use of external vitamins may increase metabolism, or, alternatively, increased supply of B vitamins may stimulate the cyanobacterial 'microbiome' and in-turn stimulate cyanobacterial metabolism. The CR CyanoHABs exhibited N-limitation with a secondary vitamin limitation in NH_4 addition treatments. The secondary vitamin limitation is likely due to B vitamin additions boosting nondiazotrophic populations by increasing chlorophyte relative abundance and reducing cyanobacterial relative abundance. Furthermore, given the high N_2-fixation rates in the CR, further research is required to 1) understand if N reduction thresholds will be effective in reducing the severity of the blooms and 2) evaluate the budgetary importance of N_2-fixation relative to external N inputs and N regeneration in the CR. The results demonstrate the importance of N in supporting CR CyanoHABs and the need to better understand multiple nutritional drivers of the recurring blooms.

Assessing the role of nutrient loading in promoting harmful cyanobacteria blooms in New York City´s central and prospect park lakes

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In the last decade, cyanobacterial harmful algae blooms (cHABs) have occurred in the Lake in Central Park in Manhattan, NY, USA, and Prospect Park Lake in Brooklyn, NY, U.S.A., during the summer months. Combined, these parks receive more than 50 million visitors annually, a number exceeding any U.S. National Park by more than three-fold. This study investigated the seasonal dynamics of the phytoplankton community assemblages, the types and concentrations of cyanotoxins produced, and the interaction between both community assemblage and toxin production with nutrient dynamics in the Lake in Central Park and Prospect Park. We also conducted nutrient enrichment and nutrient dilution experiments to identify how nutrients altered phytoplankton community composition including microcystin- and saxitoxin-producing cHABs. The majority of experiments (15 of the 20) demonstrated that nitrogen enrichment yielded a significant increase in blue-green algae compared the unamended control and treatments. In both lakes cyanobacteria quickly dominated over green algae, remaining well above bloom levels throughout October. Levels of the microcystin-producing genes, mcyE/ndaF were consistently elevated throughout the summer months ($> 5 \times 10^4$ c mL^{-1}) in both lakes, and saxitoxin-producing genes, sxtA unexpectedly had multiple spikes ($> 3 \times 10^4$ c mL^{-1}) as well. Understanding the species composition and toxicity of the blooms in relation to the nutrient dynamic of these systems will aid in the development of local government's management strategies to limit the public health risk associated with cHABs in the Lake in Central Park and Prospect Park Lake.

Toxigenic cyanobacterial bloom in Pastoria Lagoon, Oaxaca, Mexico

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The risk to human and ecosystem health due to the increasing occurrence of cyanobacterial blooms is of great concern due to the ability of many cyanobacteria to produce toxins. The Chacahua-Pastoria Lagoon system (Oaxaca, Mexico), used for fisheries and as recreational purposes, have been affected by a persistent cyanobacterial bloom (38.4 - 1,660 x 10^3 cells L^{-1}) between September 2017 and September 2018, high nutrient loads (TN:P ratio 73), dissolved oxygen was severely depleted (< 1 mg L^{-1}) and a massive death of ichthyofauna (> 8 ton) was recorded. Different group of blue green algae were registered in the period (individual cells, filaments, clumps), predominating in September 2017 at a salinity of 15 *Cyanobacterium aponinum*, in February at a salinity of 13 *Anabaenopsis elenkinii*, in march at a salinity of 25 *Roseofilum reptotaenium*, and *Limnothrix* sp. in May at a salinity of 19. Phytoplankton densities were correlated to environmental parameters (Ratio TN:P, total N and temperature). *Microcystin* toxigenicity was evaluated in cyanobacterial cultures by the microcystin synthase gene, the mcyG gene, was amplified in three of the strains except *Limnothrix* sp., and the mcyE gene was detected in *C. aponinum* and *Limnothrix* sp. The neurotoxin β-N-methylamino-L-alanine (β-MAA) was detected in environmental samples (41 µg b-MAA g^{-1}) and cyanobacterial cultures (216 to 2,260 µg b-MAA g^{-1}). The nutrient concentrations suggest that agricultural practices will need to be designed in this region, to reduce the cyanobacterial dominance. The presence of cyanotoxins in the Pastoria Lagoon, highlight the need to further analyses.

Tracking a novel cyanobacterium bloom in the Indian River Lagoon, Florida, U.S.A., during the Summer and Fall of 2020

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A bloom of an unidentified nano-sized cyanobacterium (~3 - 4 µm x 5 µm) was observed in the northern Indian River Lagoon, Florida, U.S.A. from August through December 2020, causing greenish water discoloration. Microscopic enumeration revealed concentrations of > 10^6 cells mL^{-1} in the northern and central Indian River, Banana River, and Mosquito Lagoon basins (maximum of 3 x 10^6 cells mL^{-1}). Bloom concentrations (2 x 10^5 cells mL^{-1}) were first observed in the northern Indian River and Banana River where it persisted longer than in other basins, but cell abundance rapidly declined throughout the system as water temperatures decreased in December. Inspection of archived samples suggested this taxon had been in the system since at least mid-June. Bloom development and decline was readily observed by satellite imagery (Sentinel-2, Landsat-8). Cells were round to oblong, often with two or more cells aligned in a chain. Live cells had an elongated aerotope that became inconspicuous upon preservation. Flow cytometry indicated that this cyanobacterium had low chlorophyll *a* and high phycocyanin-like fluorescence. Toxin analysis at five time points between 9/24 and 12/03 returned non-detected concentrations of anatoxin-a and cylindrospermopsin and trace levels of total microcystin-LR in four samples and saxitoxin in three samples, likely unrelated to the bloom. Direct sequencing and PacBio 16S amplicon sequencing revealed a novel cyanobacterial ribotype in bloom samples (i.e., > 75 % of 16S amplicons). A complete metagenome assembled genome was retrieved, and phylogenetic analysis placed this organism adjacent to *Prochlorothrix* spp. Ongoing phylogenomic and functional annotation will complement other identification via cell ultrastructure and pigment analyses.

Two Decades of Cyanobacteria Bloom Assessment and Change Detection in Lakes of The Contiguous United States using Satellite Observations

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Continuous monitoring and assessment of Cyanobacterial Harmful Algal Blooms (CyanoHABs) is a critical need for timely management of water quality and policymaking. This study assessed CyanoHAB magnitude in freshwater lakes across the Contiguous United States (CONUS) using satellite observations. CyanoHAB magnitude was estimated as the temporal mean of satellite-derived areal CyanoHAB biomass from the MERIS and OLCI instruments on Envisat and Sentinel-3 spacecrafts. CyanoHAB magnitudes in all CONUS lakes resolvable at 300 x 300 m resolution were calculated for MERIS (2008-2011) and OLCI (2016 - 2020) time series. In total, 2,357 lakes were evaluated, with most of these lakes in Minnesota, Maine, Michigan, Texas, Florida, and Wisconsin. About 16 % of all the lakes had CyanoHAB magnitude of potentially high risk (based on World Health Organization guidelines). These CONUS lakes were further ranked based on median bloom magnitude over the years. Ranking of lakes across the country provides actionable insight, which can be helpful to prioritize lake management strategies.

Freshwater cyanotoxins: a threat to marine food security

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Freshwater cyanotoxins (FHABs) are impacting the coastal ocean and are an emerging threat to the marine food web. These cyanotoxins can concentrate in the tissues of filter-feeding shellfish. The Salish Sea, Washington, U.S.A. and British Columbia, CAN is home to numerous shellfish species which are harvested for commercial, recreational, subsistence, and cultural uses. The Coast Salish Peoples and Lummi Nation have been shellfish harvesting for millennia, and access to safe, sustainable shellfish is a food security priority for their communities. Bellingham and Lummi Bay, WA, are often plagued by shellfishery closures related to both water quality and presence of marine toxins. Both bays have freshwater inputs from local rivers, exposing shellfish beds to potential freshwater toxins. We present a multiyear dataset (2018–2021) with more than 100 instances of cyanotoxins (microcystins, anatoxin-a, and cylindrospermopsin) in marine waters. Periodic testing of marine shellfish harvested in both bays have also indicated low, but quantifiable toxins in the marine food web. As global temperatures rise and blooms of toxin-producing cyanobacteria are predicted to increase, these data are being used to identify underlying environmental drivers. We have preliminarily used a mixed effects model to investigate if there are statistically significant predictors that are influencing freshwater toxin production in northwest Washington. We aim to produce data which can be used by our sovereign tribal partners to guide policy for marine species that are exposed to freshwater biotoxins.

Seasonal variation of *Microcystis aeruginosa* and factors related to blooms in a deep warm monomictic lake in Mexico

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The occurrence of cyanobacterial blooms has increased globally over the last decades, with the combined effect of climate change and eutrophication as its main drivers. The seasonal dynamic of cyanobacterial blooms is a well-known phenomenon in lakes and reservoirs in temperate zones. Nevertheless, in the tropics, most studies have been performed in shallow and artificial lakes; therefore, the dynamics of cyanobacterial blooms in deep and eutrophic tropical lakes is still under research. We studied the seasonal variation of the phytoplankton community and the factors associated with *Microcystis aeruginosa* blooms along the water column of Lake Alberca de Tacámbaro, a warm monomictic crater lake located in Mexico, during 2018 and 2019. According to previous studies performed in 2006 and 2009 - 2010, this lake was mesotrophic-eutrophic, with Chlorophyta and Bacillariophyta as the dominant groups of the phytoplankton community. During 2018 and 2019, the lake was eutrophic and occasionally, hypertrophic. The dominant species was *M. aeruginosa*, forming blooms at different depths, and mostly, during autumn and winter months. These findings suggest that *M. aeruginosa* in Lake Alberca de Tacámbaro displays seasonal and spatial population dynamics. Total phosphorus, dissolved inorganic nitrogen, water temperature and photosynthetically active radiation were the environmental factors related to *M. aeruginosa* blooms. Our results suggest that the changes in the structure of the phytoplankton community through time, and *M. aeruginosa* blooms in Lake Alberca de Tacámbaro, are mainly related to changes in land use from forest to farmland in areas adjacent to the lake, which promoted its eutrophication in the last years through runoffs.

Microcystins temporal variations in a tropical crater lake

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The Santa María del Oro crater lake is one of the major water resources and tourist icons in the state of Nayarit, Mexico. Since 2015 the lake has suffered from seasonal blooms of *Limnoraphis robusta* and *Microcystis aeruginosa*. The last specie is known to produce microcystins, which are hepatotoxins associated with mortality in animals and humans, and classified as probable carcinogen. Therefore, the aim of this work was to monitor dissolved microcystins in Santa María del Oro crater lake. We collected surface water from the lake between 2015 and 2020. Microcystins detection and quantification were performed to filtered water with ELISA tests (MCs equivalents, QuantiPlate™ Kit for Microcystins, EnviroLogix™). Our findings indicate the seasonal cyanobacteria spring blooms. The distribution of MCs was heterogenous. The highest MCs concentrations were observed in March 2017 and 208 at the east (2.84 µg L^{-1}), north (2.59 µg L^{-1}), and center of the lake (2.42 µg L^{-1}) associated with blooms of *M. aeruginosa*. In 2018 we detected an *M. aeruginosa* intense bloom (average 802 col mL^{-1}) that results in an average MCs concentration of 2.60 µg L^{-1}. MCs high concentration and spatial distribution are associated with *M. aeruginosa* blooms and represent a potential health risk.

Potentially toxic planktic cyanobacteria from the Abreus reservoir, central-southern Cuba

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Toxic cyanobacteria represent a risk for drink water quality from reservoirs due to their potential ability to produce toxins and the associated threats for human health they may pose. Taxonomic composition and abundance of cyanobacteria and related physicochemical parameters were surveyed during 2018 in Abreus Reservoir, central-southern Cuba. Cyanobacterial flora included 34 species grouped in seven families and fourteen genera; eleven species were new records for this reservoir. *Microcystis* and *Raphidiopsis* were the most frequent genera during all studied period. Cyanobacterial blooms were detected in September, where *Microcystis* sp. and *Raphidiopsis* sp. were the dominant species, with densities of 2.9×10^7 cells L^{-1} and 2.2×10^7 cells L^{-1}, respectively. There was also high abundance of other cyanobacteria such as *Raphidiopsis gangetica* (1.4×10^7 cells L^{-1}), *Aphanocapsa* sp. (7.9×10^6 cells L^{-1}) and *Pseudanabaena* sp. (7.2×10^6 cells L^{-1}). The abundance of most important genera, including *Microcystis*, *Aphanocapsa*, *Raphidiopsis* and *Dolichospermum*, was positively correlated with the water temperature and transparency. A preliminary study on cyanotoxins in the reservoir showed the presence of microcystin for the first time in the plankton of Abreus reservoir, with concentrations between 0.018 $\mu g\ L^{-1}$ and 0.031 $\mu g\ L^{-1}$. So, these values reported in the Abreus reservoir are low, but it is a warning sign due to the occurrence of harmful cyanobacterial blooms in the reservoir.

Blooms of the cyanobacteria *Limnoraphis* cf. *birgei* in a volcanic lake of El Salvador, Central America

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In March 2018, the proliferation of a filamentous cyanobacteria was detected for the first time in Lake Coatepeque, a deep, oligotrophic, volcanic lake in western El Salvador, Central America. Since that year, five events of proliferation of this cyanobacteria have been recorded, which had an impact on the inhabitants' activities that depend on the lake's water. Sampling campaigns were conducted both during bloom and non-bloom conditions between in May 2018 and March 2021 in Lake Coatepeque. In these campaigns, surface samples were collected using a sampling bottle at five sites in the lake and physico-chemical data was also registered. Material was characterized by optical microscopy; cell abundance was quantified using a Sedgwick-Rafter camera on an inverted microscope and with the aid of an ocular reticle. In the five blooms events, the causative organism was observed forming uniseriate filaments of 21 - 24 µm and trichomes 18 - 22 µm wide, with aerotopes irregularly distributed along the trichome and with a firm yellowish hyaline sheath. According to the morphological characteristics, the species corresponds to the description of *Limnoraphis birgei*, however, molecular and biochemical data is needed to confirm the species identity. Proliferations of *Limnoraphis* cf. *birgei* generated brown surface agglomerations, which in some cases reached cell concentrations exceeding 900,000 cells mL^{-1}. No human or animal intoxications were reported. This is the first report of occurrence and proliferation of *Limnoraphis* in El Salvador and one of the few cases reported worldwide.

Diversity of 2-MIB-producing *Pseudanabaena* in a brackish lake

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2-Methylisoborneol (2-MIB) is the substance responsible for the musty odor of lakes and dams. It is produced primarily by cyanobacteria and causes significant economic losses worldwide. Therefore, it is important to better understand the ecology of the producers to develop measures to prevent the outbreaks. *Pseudanabaena* is known as one of the major cyanobacterial 2-MIB producers. However, not all *Pseudanabaena* produce 2-MIB. Productivity can vary not only at the species level but also at the strain level, and hence it is difficult to quantify 2-MIB-producing *Pseudanabaena* in environmental samples by optical microscopy. Molecular biology techniques enable specific quantification of cyanobacteria based on target genotype. Using these techniques, the diversity of 2-MIB-producing *Pseudanabaena* was investigated for the first time in a cool-temperate brackish lake, namely Lake Ogawara. 2-MIB-producing *Pseudanabaena* quantified by qPCR accounted only 8 % of total *Pseudanabaena* quantified by optical microscopy. Isolation of *Pseudanabaena* strains and amplicon sequencing of 2-MIB synthase gene from Lake Ogawara revealed the presence of at least 3 strains (3 OTUs) of 2-MIB-producing *Pseudanabaena*. The OTU composition changed interannually: In 2017, *Pseudanabaena* cinerea (OTU-1) accounted for > 98 % of the total reads of amplicon sequencing. In 2020 when 2-MIB occurred, OTU-1 accounted for < 11 %, and *Pseudanabaena* sp. (OTU-2) was dominant with > 62 %, and the OTU composition changed over time. This study is the first to show temporal changes in the density of *Pseudanabaena* capable of producing 2-MIB at the strain level, which has important implications for 2-MIB outbreak control.

Adaptation of the photosynthetic apparatus in *Raphidiopsis raciborskii* strains of various origin and toxicity

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Raphidiopsis raciborskii has attracted attention as it spreads in freshwater estuaries of varied temperature and light conditions in diverse regions worldwide. While proliferation of species to new habitats is connected with the phenotypic plasticity of an organism (acclimation) and/or ability of ecotype selection (adaptation), the factors that determine the omnipresence of *R. raciborskii* remain largely unknown. Therefore, environmental adaptation of the *R. raciborskii* photosynthetic apparatus was examined. To minimize phenotypic variability, five cyanobacterial species (three non-toxic Polish strains and two toxic Australian strains) were grown under standardized photon flux densities and the same temperature (40 µmol m^{-2} s^{-1} at 20 °C). Concentration of photosynthetic pigments (chlorophyll *a*, carotenoids, phycocyanin, allophycocyanin) was determined a qualitative and quantitative analysis of carotenoid composition was conducted and photosynthetic performance (chlorophyll fluorescence *in vivo*) was analysed. The photosystem II quantum efficiency (Fv/Fm ratio), the photochemical (qP) and non-photochemical quenching (NPQ) parameters and dark and light levels of plastoquinone reduction did not differ significantly among the investigated strains. However, considerable variations in pigment accumulation were observed. The Australian CS-505 strain expressed substantially higher concentration of chlorophyll *a* and total carotenoids. The ratio of photoprotective carotenoids (myxoxanthophyll) to chlorophyll *a* was higher in CS-505 and a Polish strain from BiskupiÅskie Lake. These strains have also shown the highest phycocyanin/allophycocyanin and phycobilisome/photosystem I ratios. The results indicate that the ability to accumulate specific pigments involved both in light harvesting (phycocyanins) and in photoprotection (myxoxanthophyll) may play an important role in the adaptation of the photosynthetic apparatus in *R. raciborskii* to new environmental conditions.

ICHTHYOTOXIC HABs

Insights into the toxicity of prymnesins, toxic metabolites of the microalgae *Prymnesium parvum*

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The microalga *Prymnesium parvum* is capable of forming blooms that lead to devastating fish kills worldwide. The suspected causative agents are large ladder frame polyethers called prymnesins. Currently, they are divided into three groups based on the number of carbon-atoms in the aglycon backbone (A-, B- and C-types). Since little is known about the molecular mechanisms of these compounds, the toxic potential of the three prymnesin-types was explored in *in-vitro* studies. For the toxicological evaluations, two adherent primary cell lines were selected: the fish gill cell line RTgill-W1 and the human colon epithelial cell line HCEC-1CT. Extracts of different *P. parvum* isolates were investigated for their ability to affect cell viability and membrane integrity. Furthermore, the prymnesin profile was determined by liquid chromatography coupled to high resolution mass spectrometry. As shown in previous studies, each *P. parvum* isolate was only capable of producing one prymnesin type, but several different congeners, resulting in complex mixtures. These varied in the degree of chlorination, saturation and the number of attached sugar moieties. Both the fish gill and the human intestinal cell lines were affected by *P. parvum* extracts with slightly higher sensitivity of the RTgill-W1. The mixture of A-type prymnesins proved to be the most potent ones with half maximal effective concentrations (EC_{50} values) in the low nM range, closely followed by C-type toxins while B-type toxins were the least toxic ones. Furthermore, we could show that the chloride channels of the cell membrane play an important role in the toxicity of prymnesins.

Strain-specific effects of the toxic dinoflagellate *Alexandrium minutum* on Pacific oyster at various life stages: implication of bioactive extracellular compounds

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The dinoflagellate genus *Alexandrium* comprises species that produce potent neurotoxins known as paralytic shellfish toxins (PST), and bioactive extracellular compounds (BEC) of unknown structure and ecological significance. The toxic bloom-forming species, *Alexandrium minutum*, is distributed worldwide and adversely affects many bivalves including the commercially and ecologically important Pacific oyster, *Crassostrea gigas*. In France, recurrent *A. minutum* blooms can co-occur during *C. gigas* spawning and larval development, and could endanger recruitment. The effects of *A. minutum* on *C. gigas* have often been attributed to its production of PST without testing separately the effects of BEC, which can also be produced by this microalga. We investigated the sensitivity of the different oyster life stages to *A. minutum* by experimentally exposing gametes, embryos, larvae and adult oysters to *A. minutum* strains at environmentally realistic concentrations. Three strains of *A. minutum* producing either only PST, only BEC, or both PST and BEC were tested. All oyster life stages tested were affected by *A. minutum*. The toxic dinoflagellate inhibited fertilization and embryogenesis, and strongly decreased larval growth and settlement. Adult oysters exposed to *A. minutum* produced larvae of smaller size which showed higher mortalities during settlement. Both PST and BEC produced by *A. minutum* are likely implicated in the deleterious effects observed. The BEC-producing strain appeared particularly toxic for gametes and oyster external tissues, implying that non-PST-producing strains could also alter oyster reproduction. This study provides a better understanding of the toxicity of *A. minutum* on oyster and highlights the significant role of BEC in this toxicity calling for further chemical characterization of these substances.

A study of the fish-killing *Chrysochromulina leadbeateri* bloom in northern Norway, May-June 2019

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The bloom of *Chrysochromulina leadbeateri* (Haptophyta) in Northern Norway in May-June 2019 was the most toxic and extensive bloom of this species ever recorded, killing over 13,000 tonns of farmed salmon. The bloom was studied as part of a research cruise (R/V Heincke, HE533) with the aim to investigate the diversity, distribution and functional ecology of protists along the northern Norwegian coast from Lofoten to Tanafjorden. Biological and physico-chemical samples and measurements were collected allowing analyses of the blooming organism, its distribution and ecology. Cultures of *C. leadbeateri* were isolated from the bloom and used to determine the morphology, taxonomic identity, and phylogeny of the responsible organism, and were compared with a *C. leadbeateri* strain isolated from an earlier bloom in 1991. Metabarcoding (SSU rRNA gene), microscopy cell counts and flow cytometry were used to determine the abundance of the species along the coast and in five fjord systems, and correlate its distribution with biotic and abiotic factors. The new strains of *C. leadbeateri* were genetically identical in rRNA genes to the strain from the 1991-bloom. However, metabarcoding revealed significant intraspecific genetic variability of *C. leadbeateri* in field material. Highest abundance of *C. leadbeateri* was found in Balsfjorden, with up to 27 million cells L^{-1}. The abundance *C. leadbeateri* showed positive correlations with phosphate and nitrate concentrations, negative correlation with salinity and a slightly positive correlation with temperature. Ongoing work is focusing on physiological and genetic profiling of the isolated *C. leadbeateri* strains with the aim to improve our understanding of factors promoting toxicity and bloom formation.

Hemolytic Activity in relation to the Photosynthetic System in *Chattonella marina*

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The harmful raphidophycean, *Chattonella marina*, is known to have hemolytic effects on many marine organisms and resulting in massive ecological damage worldwide. Previous studies confirmed that the hemolytic activity (HA) of *C. marina* was initiated by light and highly related to the chlorophyll *c2* biosynthesis pathway. Therefore, to better understand the toxigenic mechanism of this flagellate, current study investigated the response of HA and H_2O_2 production of *C. marina* in relation to the photosynthetic system in molecular level by altering the light-harvesting antenna pigments under light of blue (463 nm), green (520 nm) and red (633 nm). Response of *C. marina* growth showed no difference, however, significantly higher Fv/Fm, Y (II) and ETR were investigated under red and blue, co-related to the high HA. Quantitative PCR confirmed the result of upregulated psbA, psbC, and antioxidant enzyme 2-cysteine peroxiredoxin (2-Cys Prx) in *C. marina*. In contrast, significant low Fv/Fm, Y (II) and ETR in related to low production of HA, as well as gene of psbA and psbC, and enzyme 2-Cys Prx were found in green-light-treated *C. marina* cells. These results suggest that the photosynthesis, especially the photoreaction stage, plays an important role in the ichthyotoxic of *C. marina*. Further investigation will be conducted on quantification of pigments and antioxidative system of *C. marina*.

Phylogeny, lipid composition, pigment signature, ichthyotoxicity and growth of the fishkiller *Heterosigma akashiwo* from Chilean Patagonia

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Heterosigma akashiwo is widely distributed in coastal ecosystems where it has caused massive events of fish mortality and shellfish damage. In April 2021, high cell abundances of *H. akashiwo* (> 70,000 cells mL^{-1}) killed > 6,000 tons of salmon in the Chilean fjords. This study investigated the molecular phylogeny, pigment composition, fatty acid profile, cell growth and ichthyotoxicity of *H. akashiwo* from Chilean waters. A phylogenetic reconstruction based on the large sub-unit ribosomal nucleotide showed that the Chilean strain belongs to the *H. akashiwo* clade. The genetic distance was 0.00 related to strains from Japan, Australia, U.S.A., China and New Zealand. The pigment signature showed that the CREAN_HA03 strain is mainly dominated by fucoxanthin (48.4 %), chlorophyll *c2* (18.3 %), chlorophyll *a* (14.7 %) and violaxanthin (9.8 %). A factorial T-S growth experiment showed a µmax of 0.35 d^{-1} at 17 °C and 35 in salinity and reached a maximum cell abundance of ~50,000 cells mL^{-1} at 12 °C and a salinity of 25. The fatty acid profile exhibited a high abundance of polyunsaturated fatty acids; 20.94 % palmitic acid (16:0), 13.04 % EPA (20:5ω3), and 10.44 % stearidonic acid (18:4ω3), which have all been described as highly cytotoxic against fish gill tissue. In cell-based bioassays, Chilean *H. akashiwo* was only toxic to the RTgill-W1 cell line following exposures to high cell densities (> 40,000 cells mL^{-1}). The present study pioneers research on the fish-killer *H. akashiwo* from Chilean waters. Further characterization of more Chilean *H. akashiwo* isolates is needed to understand the precise ichthyotoxic mechanisms and environmental drivers that trigger massive bloom events in Chilean Patagonia.

Ichthyotoxicity and underlying mechanisms of French *Karlodinium veneficum* strains using *in vitro* fish gill cell-based bioassays

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The dinoflagellate *Karlodinium veneficum* has previously been associated with massive fish kills, causing economic losses to the global fish farming industry. In France, *K. veneficum* is regularly detected, but its toxicity remain poorly characterised. Therefore, this study aimed to screen the ichthyotoxic potency of selected French *K. veneficum* strains against the rainbow trout fish gill cell line (RTgill-W1) and delineate the underlying mechanisms. The *in vitro* cytotoxic effect of the algal intracellular (lysed cells) and extracellular contents (supernatant) extracted in their culture media and methanol was assessed. Among the cultured strains investigated at the stationary phase, a French Mediterranean and a French Atlantic *K. veneficum* strain induced over 50 % loss of cell viability. A high level of intra-specific variability of ichthyotoxic activity was observed among the French strains, and cytotoxicity of the intracellular and extracellular fractions were strain dependent. In general, the methanolic extracts were found to be more cytotoxic than their corresponding extracts in the culture media. The potential involvement of oxidative stress that may account for gill cell damage, superoxide production by these microalgae and their ability to induce apoptosis/necrosis is being examined using High Content Analysis. Moreover, the cytotoxicity of each strains at different growth phases will also be evaluated. Overall, results from this work contribute towards a better understanding of the potential ichthyotoxic risk posed by *K. veneficum* in France, and have important implications for the mitigation and management of algal bloom impacts by the aquaculture industry.

In vitro evaluation of allelochemical compounds produced by two bloom-forming ichthyotoxic microalgae

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Ichthyotoxic algal blooms cause millionaire losses to aquaculture and wild fisheries worldwide, therefore they are known as Fish Killing Algae (FKA). Despite efforts to control, monitor, and mitigate these events, they still cannot be predicted, nor avoided. However, the potentially responsible ichthyotoxic-related species and molecules identified so far fail to explain the massiveness of these events. In this study, the effect of exudated allelopathic compounds during the co-culture of the raphidophyte *Heterosigma akashiwo* and the dinoflagellate *Alexandrium catenella* were evaluated. Besides, their ichthyotoxicity were assessed using a cell bioassay with fish cells (CHSE214). The allelochemicals effect was greater for co-cultures (direct contact) between *A. catenella* and *H. akashiwo* compared to exposure of exudates from monocultures. The maximum growth inhibition was observed at 16 h of direct interaction with no survival of *Heterosigma*. *Alexandrium catenella* was able to generate allelochemical effect both in *H. akashiwo* and in the non-toxic microalga *Rodhomona salina* (enhanced by direct contact). On the other hand, *H. akashiwo* did not affect *A. catenella* nor *R. salina*. The *in vitro* model for ichthyotoxicity evaluation coincidentally showed a greater toxic effect during the 24 h exposure of the exudates from the co-cultures which generated 100 % decrease in cell viability of the fish cell cultures (24 h exposure), ruling out a toxic effect associated with saxitoxin (saxitoxin was used as control treatment). These preliminary results reinforce the hypothesis that competition between toxic strains increases the allelopathic effect and could be the indirect responsible of their ichthyotoxicity.

Phylogenetic characterization and inter-strain variability in toxicity of *Heterocapsa* spp. (Peridiniales, Dinophyceae) from temperate south coast of Australia

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Harmful algal bloom (HAB) forming species and populations exhibit substantial intraspecific functional trait variation. This results from large scale adaptive strategies that are an outcome of numerous individual cells/clones. Phenotypic variability amongst populations can buffer immediate detrimental effects of environmental fluctuations, with more diverse populations expected to survive changing conditions more efficiently than their uniform counterparts. In temperate waters of South Australia (~34 °S), a *Karenia mikimotoi* Miyake & Kominami *ex* Oda bloom occurred, causing high mortality of benthic marine organisms, in which cryptic species of *Heterocapsa* Stein were present. *Heterocapsa* species have been associated with large-scale mortalities of bivalves and have known to cause considerable damage to aquaculture industries, but have rarely been reported from Australian waters. In this study, twenty one monoclonal *Heterocapsa* isolates were established from the bloom sample which were subsequently identified using microscopy and ribosomal markers (SSU, ITS/5.8S and LSU D1-D3 rDNA regions) as *Heterocapsa ovata* Iwataki & Fukuyo. The isolates were then tested for toxicity on the cell line RTgill-W1 initiated from the gill fragments of rainbow trout (*Oncorhynchus mykiss*). Various culture fractions (whole cells, supernatant and lysed cells) were used in the bioassay yielding high inter-strain variability. Results from this study expand on our understanding of adaptation and clonal trait variability in toxic algal species which enables us to predict the dynamics of future phytoplankton populations under changing climatic regimes.

Insights into the toxic potential of the Chilean strains fish-killing microalgae *Karenia selliformis* and *Heterosigma akashiwo*

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Fish-killing algae (FKA) species generate considerable economic losses to the aquaculture industry due to massive fish mortalities. Several mechanisms of damage to fish have been proposed so far, one of them is the production of phycotoxins with fish-killing potential. In Chile and at a global level, an evident increase in FKA blooms has been observed, frequently associated with the dinoflagellate *Karenia selliformis* and the raphydophyte *Heterosigma akashiwo*. Both species have been reported as neurotoxin-producers, which may explain fish mortality. In this study, the cytotoxic and neurotoxic potential of two Chilean strains of *H. akashiwo* (CREAN_HA03) and *K. selliformis* (CREAN_KS02) at the stationary growth phase was evaluated. Cytotoxicity (MTT test) was assessed on biomass and exudates, and the neurotoxic potential was measured using the cell-based Neuro2a assay, in order to search for blocking (TTX-like) or activating (brevetoxin-like) voltage-gated sodium channels (VGSC) toxins. Neither cytotoxic nor neurotoxic effects were observed in *H. akashiwo* extracts, which contrasts with previous results from our laboratory using a different strain from New Zealand (CCMP302). On the contrary, biomass and exudates extracts from *K. selliformis* showed cytotoxicity with an IC50 of 28 µg mL^{-1} and 0.83 µg mL^{-1}, respectively. Exudate extracts using the Diaion HP20 resin did not show any effect on VGSC, which suggests that neurotoxic mechanisms are not involved in the ichthyotoxicity of *K. selliformis* (at evaluated conditions). These results have important implications for the understanding of FKA toxic mechanisms.

Morphology and molecular characterization of *Karlodinium australe* in Southern part of Malaysia water

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Blooms of *Karlodinium* have been reported to cause the mass fish mortality in Johor strait, Malaysia, with serious economic impacts to the aquaculture industries. Recurrence of blooms of this species highlighted the needs to investigate further on the distribution of this species in Malaysian waters. In this study, we investigated the distribution of the *K. australe* in Malaysian water based on the morphological and molecular methods, and studied the ichthyotoxicity of strains of *K. australe* species. A total of 15 clonal cultures were established from Johor Strait, Malaysia and Telaga Air, Sarawak. These clonal cultures were analysed morphologically and confirmed all the cultures are *K. australe*. The identification of these strains was further supported by the molecular phylogeny of internal transcribed space (ITS) and large-subunit (LSU) ribosomal DNA. In fish bioassay experiment, fish mortality was observed in the different types of treated *K. australe* cells (filtered, unfiltered, lysed and filter lysed). Our result showed the presence of water-soluble ichthyotoxin in the cultures of *K. australe*. The detailed histological examination of fish gill showed different degree of the damage due to the toxin. Culture of *K. australe* from the two locations also showed different degree of toxicity. This is the first report of the *K. australe* in Sarawak and its ichthytoxicity was confirmed for the first-time.

Ichthyotoxic microalgae from the French coasts: investigating links between biological toxicity, chemodiversity and genetic diversity within the genus *Karlodinium*

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Ichthyotoxic microalgal blooms have occasionally affected the French coasts these last decades, causing fauna mortalities. A previous study in France highlighted the high diversity of Kareniaceae, a family of dinoflagellates that includes several ichthyotoxic species. Particularly, the species *Karlodinium veneficum*, *K. armiger* and *K. gentienii* are regularly detected. Blooms of the two first species were reported to affect aquaculture industry through fish and shellfish kills in other countries, but the ichthyotoxic risk associated with French strains remains unknown and needs to be clarified. To achieve such aim, this study compared ichthyotoxic activity, chemodiversity and genetic diversity of cultured French strains with those of foreign strains with recognized toxicity. Genetic diversity was studied by sequencing the whole ribosomal operon region (rDNA), using combined next generation sequencing techniques (Illumina and Oxford Nanopore Technology), which ameliorated inter- and intra-specific discrimination compared to usual, shorter, ITSor LSU markers. Among 18 strains, *K. veneficum* could be separated into three clades, mostly associated with their geographical origin. Bioassays targeting fish and shellfish cells were used to estimate ichthyotoxicity of *Karlodinium* strains. Results indicate that French strains can be as cytotoxic as foreign strains known to be ichthyotoxic, with a high intraspecific variability. First results of LC - MS/MS analysis of toxins in *K. armiger* and *K. veneficum* strains identified karmitoxin, characterized karlotoxins analogues, and potential new analogues which structures must still be elucidated. These analyses will allow determining the relationship between toxin profile, ichthyotoxic activity and genetic diversity, and will participate in better assessing inter- and intraspecific variability of ichthyotoxicity within the *Karlodinium* genus.

Mortality and histopathology in sheepshead minnow (*Cyprinodon variegatus*) larvae exposed to *Dinophysis acuminata* and pectenotoxin 2 during a 96 h bioassay

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Marine coastal and estuarine ecosystems frequently experience harmful algal blooms of toxic phytoplankton which co-occur with reproduction and spawning periods of mollusks and finfish. Toxic species of the genus *Dinophysis* synthesize lipophilic diarrheic toxins, including okadaic acid and dinophysistoxins. These toxins can accumulate in filter-feeding bivalves and cause diarrheic shellfish poisoning in their human consumers. Additionally, some *Dinophysis* species also produce macrocyclic polyethers, i.e. pectenotoxins (PTXs). The effects of *Dinophysis* and its toxins on marine animals are still poorly studied. We exposed sheepshead minnow larvae (*Cyprinodon variegatus*) to PTX2 or either *D. acuminata* culture, culture filtrate, cell lysate or cells resuspended in clean media, producing primarily PTX2 using a 96 h toxicity bioassay. No mortality was observed when larvae were exposed to *D. acuminata* (from 5 to 5,500 cells mL^{-1}), whereas exposure to PTX2 (from 250 to 4,000 nM) induced 8 to 100 % mortality by 96 h. However, in both experiments, histopathology and transmission electron microscopy revealed gill damage, including intercellular edema, necrosis and sloughing of the gill respiratory epithelia, and damage to the osmoregulatory epithelium, including hypertrophy, proliferation, redistribution and necrosis of chloride cells (= ionocytes). Tissue damage in gills is hypothesized to be caused by the interaction of PTXs2 with the actin cytoskeleton of the gill epithelia. We suggested that death is due to loss of respiratory and osmoregulatory functions in the fish larvae. The minor pathology observed in challenge of larvae to *Dinophysis* culture and fractions thereof, may be attributable to cell-to-cell contact between *D. acuminata* and fish gills.

Hemolytic activity in relation to the Calvin cycle in *Phaeocystis globosa*

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Phaeocystis globosa blooms were reported frequently in China Coast recent years, causing massive fish mortalities and economic loss. The major ichthyotoxic effects may drive by reactive oxygen species (ROS) or hemolytic toxins, either separately or synergistically. Previous study indicated that the hemolytic compounds of *P. globosa* were initiated by light and would potential be hemolytic glycolipid. Therefore, the physiological processes of *P. globosa*, involving photosynthetic process, i.e. light - induced photosynthetic system, Calvin cycle, sphingolipid metabolism, O-mannan biosynthesis, together with hydrogen H_2O_2 production and hemolytic activity (HA) were investigated under variable environmental conditions. Results showed that the HA was generally produced with the cell division. The heathier *P. globosa*, higher Fv/Fm, Y (II) and rETR, the higher production of HA. Stress would not be the driver of HA of *P. globosa*. Hemolytic compounds would not involve in the PSII electron transfer chain, xanthophyll cycle and process of the light-harvesting antenna pigments, however, highly related to the three stages of Calvin cycle by the evidence of up-regulated gene expression in RBCL, PGK, TK and PRK ($p < 0.05$). Together the result, the hemolytic compounds in *P. globosa* would most be possibly related to the Calvin cycle in the dark reaction of photosynthesis, which ultimately leading to changes in its carbohydrates metabolism.

CIGUATERA AND BENTHIC HABs

Early Warning Systems for *Gambierdiscus*, a Benthic HAB

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Ciguatera poisoning (CP) is a long-neglected foodborne disease affecting tropical regions of the Pacific and Indian Oceans and the Caribbean Sea. It was raised at the 32nd Session of the Codex Committee on Fisheries and Fishery Products (FAO) in 2016. In 2017, CP was an agenda item at the 11th Session of the Codex Committee on Contaminants in Food. The committee requested scientific advice, so late 2018 a group of experts met to advise FAO-WHO on the development of risk management options for CP. In parallel to this an interagency global ciguatera strategy was developed among FAO, IOC and IAEA. In this framework, an expert team is developing guidance for the implementation of CP Early Warning Systems (EWSs). The EWS approach includes monitoring *Gambierdiscus* species (benthic dinoflagellates) that produce the toxins responsible for CP. Advances in *Gambierdiscus* taxonomy, better understanding of their global distribution and toxicity and species specific molecular identification and enumeration methods make this possible. Key to standardizing cell based EWS for *Gambierdiscus* are how, when and where to sample. Unlike planktonic species, no standardized sampling method for benthic species exists. Traditionally, macrophytes are collected and cells abundances normalized to grams wet weight of macrophyte. There are myriad arguments against this collection technique, including ignoring the use of more accurate, surface-area normalized cell densities. Artificial substrates, rather than macrophytes, are proposed so cell abundances can be normalized to surface areas. We hope to open a dialog among interested colleagues and invite them to develop proof of concept projects comparing traditional macrophyte sampling methods with artificial substrates.

Molecular-based assays, strip tests and biosensors for the detection of *Gambierdiscus* and *Fukuyoa* genera and of the ciguatoxin-producing species *G. australes* and *G. excentricus*

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Dinoflagellates of the genera *Gambierdiscus* and *Fukuyoa* are known to produce several bioactive compounds, including the potent neurotoxic ciguatoxins (CTXs), which are able to accumulate in fish and through the food web. If fish contaminated with CTXs is ingested by humans, it can result in an intoxication named ciguatera. Within the two genera of dinoflagellates, only some species are able to produce toxins, and *G. australes* and *G. excentricus* have been highlighted to be the most abundant and toxic. Although the genera *Gambierdiscus* and *Fukuyoa* are endemic to tropical areas, their presence in subtropical and temperate regions has been recently recorded. In this work, three primer sets modified with oligonucleotide tails have been designed within the D8D10 and D1-D3 regions of the 28 S LSU ribosomal DNA. Then, they have been successfully exploited for the detection of the *Gambierdiscus* and *Fukuyoa* genera and for *G. australes* and *G. excentricus* species, through the combination of the recombinase polymerase amplification technique and a colorimetric sandwich hybridization assay. Furthermore, a remarkable limit of detection of a single cell was achieved. Additionally, the species-specific primers sets were used for 1) the development of a strip test based on PCR and lateral-flow technique and 2) the development of the first PCR based biosensor which allowed the simultaneous detection of *G. australes* and *G. excentricus* species in field samples. These developments provide rapid and cost-effective strategies for detection of both genera and two toxic species, which will contribute to ciguatera risk assessment, guaranteeing seafood safety.

Chemodiversity and chemotaxonomy, the use of metabolomics to explore *Gambierdiscus* genus in the Atlantic Ocean

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The genus *Gambierdiscus* was identified as producing the toxins responsible for causing ciguatera food poisoning over 40 years ago. Historically, the presence of *Gambierdiscus* was endemic in the tropical area and the Caribbean Sea but nowadays its presence has also been reported in Mediterranean Sea, Madeira and the Canary Islands, possibly as a result of climate change and other environmental changes. Adapted monitoring tools and prevention of public health risks and economic impacts rely on knowledge on toxicity and metabolites produced by these microalgae. This study describes the use of targeted and untargeted analyses using mass spectrometry to explore the chemodiversity of the genus *Gambierdiscus*. Lyophilized cell pellets (n = 4-5, 0.2-3.1 x 10^6 cells) of 13 different strains of *Gambierdiscus* species predominantly found in the Atlantic Ocean (i.e. *G. belizeanus*, *G. caribaeus*, *G. carolinianus*, G. excentricus and *G. silvae*) were obtained from four laboratories (U.S.A., Brazil, Spain and France). Samples were extracted (methanol 90 %) and analysed using both low-resolution mass spectrometry (targeting ciguatoxins, maitotoxins and related polyethers) and high-resolution mass spectrometry (untargeted analysis). Data were processed using MzMine2, Workflow4Metabolomics and MetaboAnalyst for peak picking, data treatment and statistics, respectively. The presence of more than 14 strain dependant biomarkers (including maitotoxin (6-45 pg eq cell^{-1}) and gambierones (0.1-34 pg eq cell^{-1}) was evaluated for their use as complementary tools for taxonomy. Chemodiversity was also explored using molecular networks to gain new insights into the knowledge of secondary metabolites produced by *Gambierdiscus* species and the presence of gambierones-, maitotoxin- and ciguatoxin-like compounds in the 13 strains of *Gambierdiscus*.

Phylogeny-related toxin profiles in *Ostreopsis* cf. *ovata* strains from different geographic locations

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The benthic marine dinoflagellate *Ostreopsis* cf. *ovata* produces isobaric palytoxin (PLTX) and their analogues – ovatoxins (OvTX) – affecting marine fauna and human health. However, toxin profile and concentration vary enormously among *O.* cf. *ovata* strains, with intracellular toxin contents ranging from undetectable to hundreds of pg cell^{-1}. Here we examined previously reported (in GenBank) and new genetic sequences (ITS region, D1-D3 and D8-D10 domains of LSU rDNA) from several *O.* cf. *ovata* strains, comparing the toxin profiles among each clade and subclade identified by phylogeny. Six different subclades of *O.* cf. *ovata* were assigned: Subclades A and B, with sequences from Atlantic, Mediterranean and West Pacific strains; Subclades C and D, with sequences from Indian and Pacific strains; Subclade E, with sequences from Indian, Pacific, West Atlantic and Caribbean strains; and Subclade F, with sequences from West Atlantic and Caribbean strains. Low ($\leq$ 1.8 pg PLTX eq cell^{-1}) intracellular toxin concentrations were reported for strains belonging to Subclades C, D and F, and undetectable levels (< 0.01 pg PLTX eq cell^{-1}) for Subclade E. There is no current information on subclade B toxin content. Noteworthy, all highly toxic (6.1 – 450 pg PLTX-eq. cell^{-1}), new and previously sequenced *O.* cf. *ovata* strains are associated with the genetic Subclade A. Strains within this subclade exhibited two markedly distinct toxin profiles: the most frequent one dominated by OvTX-a (56 – 62 %) and OvTX-b (29 – 31 %), with minor proportions of OvTX-c to -f; and another lacking OvTX-b and -c, with greater proportions (80 – 86 %) of OvTX-a and lower amounts of OvTX-f and/or -e.

Environmental, human health and socioeconomic impacts of *Ostreopsis* spp. blooms in the NW Mediterranean

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This presentation summarizes the research conducted by the CoCliME project to ascertain the impacts of *Ostreopsis* (mainly *O.* cf. *ovata*) blooms in the NW Mediterranean beached and to design strategies to prevent, mitigate and, if necessary, adapt to the impacts of these events in the future. Noxious effects of *Ostreopsis* proliferations in the Mediterranean include massive mortalities of benthic organisms. Still, the involved deleterious mechanisms are poorly understood, requiring standardized ecotoxicology tests. Also, the chemical nature of the harmful compounds synthesized by *Ostreopsis* is unknown. There is a need to understand the toxin transfer through the marine food webs, and to evaluate the real risk of seafood poisonings in the area. The main effects on human health are, by now, associated to direct exposure to seawater with high *Ostreopsis* cell concentrations and to inhalation of aerosols containing unknown irritative chemicals produced under certain circumstances during the blooms. A six - year epidemiologic study confirmed the mild acute symptoms, affecting mainly the ophthalmologic, digestive, respiratory and dermatologic systems but the risk of chronic exposure as well. Beach monitoring and surveillance systems in summer (such as the surveillance Network on the French Mediterranean coast since 2006) constitutes an effective strategy to prevent *Ostreopsis* impacts on human health. Nowadays, the general public has a poor knowledge about the occurrence of *Ostreopsis* blooms. However, considering hypothetical future scenarios of increased *Ostreopsis* blooms, a large part of coastal recreational and tourist activities could be negatively impacted. Tourist and residents might continue to go to the beaches during *Ostreopsis* proliferations, and this be exposed to associated health risks.

Gambierdiscus species community structure and spatiotemporal dynamics in St. Thomas (USVI) and the Florida Keys (U.S.A.)

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Of the human poisoning syndromes associated with harmful algal blooms (HABs), ciguatera poisoning (CP) has the most significant human health and economic impacts globally, and is often an ongoing and chronic problem in endemic regions. CP differs from other HABs in that poisoning events are not associated with large-scale planktonic blooms of a single species, but are thought to be linked to one or more highly toxic species or strains present in a cryptic assemblage. The recent development of species-specific fluorescent in situ hybridization (FISH) probes provides a powerful new tool for investigating *Gambierdiscus* community structure, and for monitoring the abundances of the most toxic – and thus the most important – taxa. Here we present findings from field studies carried out in St. Thomas, USVI and the Florida Keys, U.S.A., which used FISH probes to characterize *Gambierdiscus* species abundance and community dynamics at each location, and over multiple years of sampling. Within each location samples were analyzed from multiple sites comprising varying depths, habitats, and algal substrates to investigate community structure over small spatial scales as well as linkages with environmental factors. This approach enabled the quantitative determination of community over spatiotemporal gradients, as well as the selective enumeration of species known to exhibit high toxicity. The investigation revealed community structuring between St. Thomas and Florida sites that was largely driven by *Gambierdiscus silvae* and *G. carolinianus*, as well as temporal variation in species composition. Results of these field studies will be presented and discussed within the context of regional patterns of toxicity and ecological drivers.

First insights on species diversity, toxicity and toxin profiles of dinoflagellates genera *Gambierdiscus* and *Fukuyoa* in the French West Indies (Caribbean Sea)

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Historically, the incidence rate of ciguatera poisoning (CP) in the French West Indies is known to be higher in islands north of Martinique than in the more southern islands. To better understand the link between the incidence rate and the distribution of benthic dinoflagellates associated with ciguatoxin production (*Gambierdiscus* and *Fukuyoa*) in this area, a survey was carried out in 2020. Epiphytic cells of *Gambierdiscus*/*Fukuyoa* were collected along a north-south gradient from Saint Barthelemy and Saint Martin, over Guadeloupe to Martinique. Isolates from these field samples were used to establish monospecific cultures of *Gambierdiscus* and *Fukuyoa* using F/10K medium in order i) to identify the different species using a morpho-molecular approach, ii) to assess their toxicity by neuroblastoma (neuro-2a) cell based assay and iii) to characterize their toxin profiles using liquid chromatography coupled to tandem mass spectrometry (LC - MS/MS). During this study, the species *G. belizeanus*, *G. caribaeus*, *G. carpenteri*, *G. ribotype* 2 and *G. silvae* have been identified using qPCR assays and scanning electron microscopy (SEM). The identity of *F. yasumotoi* was confirmed by sequencing of the LSU rDNA D1–D3 region. Preliminary results confirmed a high biodiversity of the genera *Gambierdiscus* and *Fukuyoa* in the French West Indies. Neuro2a cell-based assays revealed mostly non-specific toxicity to CTXs and LC - MS/MS analyses allowed for the comparison of toxin profiles of species collected at different localities. Further investigations are necessary in the Caribbean area to identify the *Gambierdiscus*/*Fukuyoa* species producing CTX algal precursors of CTX1 responsible for CP as reported in the Pacific Ocean.

Gambierdiscus occurrence and distribution in Madeira and Selvagens Islands (NE Atlantic, Madeira, Portugal)

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Ciguatera poisoning is one of the most common types of food poisoning associated with the consumption of fish. Despite being frequent in tropical and subtropical areas, is considered an emerging threat to seafood safety in Europe. Some ciguatoxins (CTX) are produced by epiphytic benthic dinoflagellates *Gambierdiscus* and *Fukuyoa* and others are products of fish metabolism resulting from biotransformation of precursor compounds produced by these dinoflagellates. These dinoflagellates were detected relatively recently in the southern waters of Europe, such as Canary Islands-Spain and Madeira-Portugal, and in temperate regions of the Mediterranean Sea. In Canary Islands several species were reported, like *Gambierdiscus australes*, *G. caribaeus*, *G. carolonianus*, *G. excentricus* and *G. silvae*. Since the presence of *Gambierdiscus* species in Madeira and Selvagens have been poorly investigated and ciguatoxins have been detected in several fish species in subsequent years, the aim of this work was to investigate the *Gambierdiscus* distribution and species composition these Islands. In Selvagens, *Gambierdiscus* was widely spread in both Selvagem Grande and Selvagem Pequena, and identified as *Gambierdiscus australes*. On the other hand, in Madeira, *Gambierdiscus* was only observed in the north coast of the island with morphological characters pointing to *G. excentricus*, which has to be confirmed with molecular analysis. Additionally, several potentially toxic benthic/epiphytic dinoflagellates were observed co-existing with *Gambierdiscus* belonging to the genera *Ostreopsis*, *Prorocentrum*, *Amphidinium*, and *Coolia*. This work highlights the potential of these Islands as incubation and proliferation sites of ciguatera-causing dinoflagellates and other toxic benthic microalgae.

Ciguatera poisoning in Germany: Outbreak trace-back and ciguatoxin-like compounds in Red Snapper (*Lutjanus bohar*)

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A globalized seafood trade can carry the potential for risks that, historically, were geographically localized, such as ciguatera seafood-poisoning (CP). To protect consumers, human health food and safety authorities determined guidelines and requested methods for detecting marine biotoxins in seafood implicated in CP outbreak cases. Following a CP outbreak in Germany, a product trace-back investigation was conducted. Fifty two pieces of fish (9.3 kg total), verified as *Lutjanus bohar* by DNA barcoding, were recovered; representing 0.85 % of the total amount distributed throughout Germany and 0.34 % distributed in the European Union. Muscle-tissue extracts (semi-purified) were analyzed for marine biotoxins in the class Ciguatoxin (CTX). All extracts were deemed positive for 'CTX-like toxicity', having a composite concentration of 0.22 - 10.54 ppb CTX3C equivalents (Eq.), as determined by the mouse neuroblastoma cytotoxicity assay. Extracts were then analyzed by LC-MS/MS, and chromatograms revealed the (potential) presence of several marine biotoxins of the class of CTX in 90 % of the current samples. CP outbreaks have been reported after exposure to ~1 ng CTX1B kg^{-1} human body weight. Determined from the average CTX concentration, the average portion (179 ± 37 g) contained 454 ± 385 ng CTX3C eq. (maximum level detected was 2148 ng CTX3C eq.). Given the findings of this investigation, it is unclear whether the toxin concentrations (exceeding the guidance level) determined here were extraordinarily anomalous compared to the larger lot, the other fish were below a level capable of causing an acute CP intoxication, or whether more cases went unreported.

Sulphated cyclic polyethers produced by toxic *Gambierdiscus* species

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The genus *Gambierdiscus* has been demonstrated to produce a complex array of bioactive, ladder-shaped polyether secondary metabolites, which have varying levels of toxicity. These include ciguatoxins (CTXs), maitotoxins, gambieric acids, gambierol, gambieroxide and gambierones. While CTXs are thought to be the causative agents of ciguatera poisoning (CP), the contribution of other secondary metabolites produced by *Gambierdiscus* is not yet understood. Two recently described species from Rarotonga in the Cook Islands, *G. cheloniae* CAWD232 and *G. honu* CAWD242, displayed high acute toxicity to mice yet did not produce any known CP toxins. A representative isolate from each species was bulk cultured in order to purify and characterize several bioactive compounds using toxicity-guided fractionation, liquid chromatography-tandem mass spectrometry and nuclear magnetic resonance spectroscopy. The toxicity of these novel and known secondary metabolites was evaluated to better understand how these *Gambierdiscus* species might contribute to CP. In addition, isolates of fourteen microalgal species from three genera of benthic dinoflagellates commonly found in assemblages on tropical reef systems; *Gambierdiscus* (10), *Coolia* (2) and *Fukuyoa* (2), were quantitatively analysed for gambierone and 44methylgambierone production (expressed as cell quotas), and qualitatively analysed for additional bioactive secondary metabolites. This presentation will describe the isolation of sulphated ladder-shaped polyether secondary metabolites from *G. cheloniae* CAWD232 and *G. honu* CAWD242. New toxicity information on these, and other known compounds, allows an assessment of how different secondary metabolites produced by *Gambierdiscus* might contribute to CP. Production levels of gambierones by co-habitating benthic dinoflagellates will also be discussed.

Variations in toxin content and metabolomic profile of two genetically similar populations of *Gambierdiscus excentricus* from Brazil

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Benthic dinoflagellates were sampled from seaweeds and artificial substrates along the eastern coast of Brazil. *Gambierdiscus* spp. were present in 80 % (12/15) and 20 % (1/5) of the samples from NE and SE regions (tropical), respectively, while absent in the South (subtropical). Four monoclonal cultures were established from NE and two from SE. All strains were found to belong to *Gambierdiscus excentricus*, exhibiting similar cell size and morphology, as well as identical genetic sequences in the ITS region, and D1-D3/D8-D10 domains of LSU rDNA. Even though, as measured by LC-MS/MS and confirmed in high-resolution mass spectra (HRMS), NE strains (n = 1-3 replicates each) contained significantly higher intracellular MTX4 levels than those of their SE (n = 1-5) counterparts (63.1 ± 14.1 vs. 36.1 ± 13.1 pg cell^{-1}). Conversely, a potential analog of gambierone (same –ESI HRMS accurate mass; different retention time), was more frequent and slightly more abundant in SE strains. Ciguatoxins, MTX1, MTX2 and MTX3 (44 - methyl gambierone) were not detectable in any strain. Non-targeted metabolomics revealed the occurrence of 493 (1 %) markedly distinct (> 10-fold change) compounds between the two regional groups of strains at $p < 0.05$, and 169 (0.35 %) at $p < 0.001$ significance level. Some compounds were undetectable in one group of strains while highly abundant in another, and could be used as indicators of either SE (e.g. mass: 4958.3003 and 935.4601 in –ESI; 1071.6866 and 794.6051 in +ESI) or NE strains (mass: 2217.1750 and 696.4251 in –ESI). To judge from analogs detected, toxicity risks associated with *G. excentricus* appear higher in NE Brazil, with warmer, clearer and less turbulent coastal waters.

Cell immunolocalization of ciguatoxins in the benthic dinoflagellate *Gambierdiscus australes*

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Gambierdiscus species have been recognized as the producers of ciguatoxins (CTXs) which, after biotransformation in fish, are responsible of ciguatera fish poisoning (CFP).Cells from a culture of *Gambierdiscus australes* (clone IRTA-SMM-16-286) were fixed in paraformaldehyde and sectioned in a cryostat. Whole cells and sections were TritonX-100 permeabilized, BSA-blocked and incubated with 8H4 anti -CTX antibody, which binds to the right wing of CTX1B, CTX3C, 54-deoxyCTX1B and 51-hydroxyCTX3C, and then with a secondary Alexa Fluor 488-conjugated anti-mouse IgG. Cells and sections were mounted on slides and observed with a confocal microscope. Whole cells showed a strong positive reaction at cell surface, in correspondence with thecal pores (or poroids), while no reaction was detected inside the cells, as the antibody did not seem to be able to pass through the amphiesma. The observation of sections confirmed the positive reaction at cell surface and revealed positive reactions inside the cytoplasm. Small fluorescent dots grouped together in a reticulate pattern were visible between chloroplasts. Single small dots were observed also on nuclear surface. However, the strongest reaction was observed in two or three larger cytoplasmic globular structures (8 - 10 µm wide), which appeared to be formed by tiny filaments. The results confirm the reactivity of 8H4 anti - CTX antibody with *G. australes* toxins and suggest an involvement of cell endomembrane system in toxin production. Further research will be necessary to verify this hypothesis and to understand if the positive reaction observed at cell surface represents an effective release of toxins outside the cell.

Matrix effect evaluation on the detection of CTXs by Neuro-2a assay in shark samples and two other fish species from La Reunión

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Ciguatoxins (CTXs) are toxins produced by dinoflagellates of the genera *Gambierdiscus* and *Fukuyoa*, which are epiphytic microalgae. CTXs are transferred throughout the food webs and over 400 species of fish have been reported to be involved in Ciguatera Fish Poisoning (CFP), the most common cause of food poisoning of marine origin which is produced by these CTXs. The carnivorous fish at the top of the food webs (shark, barracuda, among others) have been shown to have the highest concentration of CTXs. Unfortunately, there is no standardization of methodologies for the detection of CTXs due to the different CTX structures, the varying methods of diagnosis along with the limited availability and high cost of reference material. The complexity of the matrix is also a critical point that limits the standardization of methods for CTX evaluation. In this work, we present an optimization of the methodology of the extraction methods implemented for the detection of CTXs. The reduction of matrix effects by clean-up protocols results on the reduction of the limit of detection of the Neuro-2a cell-based assay (Neuro-2a assay) when implemented in flesh and liver samples of shark and two other fish species from La Reunión. The proposed optimization of the extraction, clean-up and Neuro-2a assay protocol allowed to detect 0.01 µg kg^{-1} of CTX-1B in shark including *G. cuvier* and *C. leucas*, *L. bohar* and *V. louti*, avoiding matrix effects. Finally, an inter-comparative exercise between two laboratories including six flesh samples and six liver samples from shark was performed and an excellent correlation was obtained.

Distribution of Caribbean ciguatoxins in pinfish (*Lagodon rhomboides*) during uptake and depuration: implications for trophic transfer

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Ciguatoxins (CTXs) produced by marine benthic dinoflagellates bioaccumulate in fish and cause ciguatera poisoning (CP) in humans. CTXs are considered persistent contaminants, but evidence has been primarily from CTX measurements in wild-caught fish from CP-endemic regions without information on exposure conditions, including time since the last CTX meal. Without understanding the kinetics of CTX in fish, modeling its distribution in food webs remains a major gap in managing CP. In this study, we analyzed the retention of CTX in major tissue compartments of a marine omnivorous fish, *Lagodon rhomboides*. Fish were exposed for 20 days to Caribbean CTX-1 by diet followed by depuration periods up to 99 days. Sampling of whole muscle, liver, and other pooled viscera at various intervals for toxin analyses by in vitro mouse neuroblastoma assay revealed differing kinetic patterns between tissues. Most notably was a decrease in viscera CTX concentration with depuration time that was mirrored by increasing muscle CTX concentration. These trends suggest a compartmental redistribution of accumulated CTXs during depuration may occur. Estimated cumulative CTX burdens at the end of the uptake phase inferred that accumulated CTX was not eliminated from fish during the depuration period (99 days). These results reveal that most accumulated CTX can be retained at least 3.3 months after fish are removed from a CTX source and support prior studies on retention in wild fish. This work should be considered when developing models of CTX cascades between dinoflagellates and resident high site fidelity fish and has implications for analyzing fish that emigrate away from CTX sources for long periods.

Gambierdiscus species and ciguatoxins in eastern Australia in an era of ocean climate change

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Ciguatera Poisoning (CP) is common in Australia and the Pacific region. In Australia, it is estimated that there is a reporting rate of ~10, indicating ~300 cases occur annually. Prior to 2014, fish caught south Queensland (29 °S) accounted for only one CP event. Since then, fish caught in southerly regions (to 32°S) now account for 31 cases of CP (from seven events). Increasing cases may be associated with the climate change-related southward intrusion of the East Australian Current. *Gambierdiscus* spp. were first cultured from Australia in 1991, but knowledge of genetic diversity and CTX production of *Gambierdiscus* has expanded since. Few *Gambierdiscus* species produce CTX-4A, 4B, the analogs that bioconvert to CTX-1B in the presence of fish enzymes. In Pacific countries neighbouring Australia, *G. polynesiensis* produces high levels of CTX-4A, 4B, and its abundance is significantly correlated with CP in reef fish, but it has yet to be detected from Australian waters. Four new *Gambierdiscus* species were found from Australia (*G. lapillus*, *G. honu*, *G. holmesi*, *G. lewisii*) as well as *G. carpenteri* and *Fukuyoa* spp., but none produce known CTXs. We are investigating *Gambierdiscus* species in eastern Australia and their toxin production, in combination with fish toxicity. A diversity of *Gambierdiscus* species have been detected and cultured, suggesting that north-eastern Australia is particularly species rich for the genus. This work builds on our knowledge of CTX causative and vector species to provide the information needed to manage increasing risks of CP in Australia.

The benthic dinoflagellate genus *Prorocentrum* in Aotearoa/New Zealand coastal waters: genetic diversity, distribution, and diarrhetic shellfish toxin production

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In Aotearoa/New Zealand coastal waters, the species composition, distribution, and toxicity of benthic *Prorocentrum* species have not been well characterized. In this study macroalgal samples were collected around New Zealand mainly between 2018 and 2019. More than 80 clonal isolates were established and molecular phylogenetic analysis of the large-subunit ribosomal DNA D1–D2 region revealed that the isolates belonged to four species (*P*. aff. *foraminosum*, *P*. *lima* complex, *P*. *malayense*, and *P*. *tsawwassenense*). The strains of *P*. aff. *foraminosum* and *P. lima* complex were divided into two clades (1 and 2) and three subclades (1e, 4a, and 4b) respectively. *Prorocentrum lima* complex subclade 4a was widespread ranging from subtropical to temperate zones. In contrast, *P. lima* complex subclade 1e and *P. malayense* were restricted to subtropical zone, while the other species/clades/subclade were restricted to temperate zone. The production of diarrhetic shellfish toxins [okadaic acid (OA) and dinophysistoxins (DTXs)] by the strains was assessed using liquid chromatographytandem mass spectrometry. The results revealed that all strains of *P*. aff. *foraminosum* clade 2, *P. lima* complex subclades 1e, 4a, and 4b produced OA, while a strain of *P*. aff. *foraminosum* clade 1 did not produce OA. Strains of *P*. aff. *foraminosum* clades 1 and 2, *P. lima* complex subclades 1e and 4b produced DTX-1, while those of *P. lima* complex subclade 4a produced either no or low quantities of DTX-1. Strains of *P*. *malayense* and *P*. *tsawwassenense* did not produce detectable concentrations of either OA or DTX-1.

Using 18S rDNA metabarcoding to determine the vertical and horizontal distribution and diversity of *Gambierdiscus* spp. (Gonyaulacales) in Japan

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In the subtropical and temperate regions of Japan, four species/phylotypes of *Gambierdiscus* have been reported from shallow depths (< 3 m). The occurrence of *Gambierdiscus* from deep waters has not yet been investigated. Here, we describe the vertical as well as the horizontal distribution of *Gambierdiscus* in Japanese coastal waters by 18S rDNA metabarcoding with a newly developed primer set, which was specific to Alveolata supergroup. We extracted environmental DNA from the adherent fractions of 89 macroalgal samples that were collected from two water depths (3 and 30 m) in Kochi Prefecture (abbreviated KON) as a temperate site and Okinawa Prefecture (OUN) as a subtropical site from January 2015 to February 2018. MiSeq sequencing was carried out and the obtained data were analyzed using Mothur and BLAST search. A maximum likelihood molecular phylogenetic tree was constructed using the obtained sequences and those of reported *Gambierdiscus* spp. As a result, six known species/phylotypes of the genus *Gambierdiscus* and four 'new phylotypes' which has not been reported in the world were found. All the new phylotypes were distributed in subtropical region. Among them, one phylotype was found specifically in deep waters. The results of canonical correspondence analysis (CCA) showed that *G. scabrosus* had a positive correlation with high seawater temperature while one new phylotype was positive with low temperature. However, *G. silvae* had no correlation with any environmental factors. This study clarified the inclusive diversity and distribution, especially vertical distribution, of *Gambierdiscus* in Japan.

Overview of the Australian ciguatera poisoning research strategy and status update on implementation

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Ciguatera poisoning (CP) accounts for the majority of seafood safety related outbreaks in Australia, with more than 1,650 cases reported since 1965. The Australian seafood safety & market access program, SafeFish, therefore facilitated and coordinated a multidisciplinary working group to develop a national ciguatera research strategy for Australia. No routine monitoring of ciguatoxins currently occurs in Australia due to a lack of validated analytical methods and ciguatoxin standards, as well as problematic migratory fish species that may acquire toxins from distant locations. Previously confined to the tropical waters of Australia, human poisoning events are now increasingly being reported in temperate New South Wales. It is not yet clear whether this represents a range extension of the causative *Gambierdiscus* and/or ciguatoxic Spanish mackerel. Less than 20 % of ciguatera cases are thought to currently be reported in Australia and awareness among recreational fishers and health care workers, alongside consistent national reporting was identified as a priority in the field of epidemiology. A recent survey of recreational fishers has revealed that although most individuals in ciguatera endemic areas are aware of the risk, key information on the fish species at risk of carrying ciguatoxins and ciguatera symptoms is lacking. 12 % of respondents (n = 236) identified as having suffered from ciguatera before, with 40 % not having been diagnosed by a health care worker. Australian efforts to implement the research strategy and raising awareness are continuing, with the Australian notifiable disease network, OzFoodNet, preparing nationally consistent ciguatera case questionnaires to link toxicological and epidemiological parameters.

Global occurrences and trends of Ciguatera Poisoning (CP)

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A review of Ciguatera Poisoning global occurrences and trends was conducted in the framework of the Global HAB Status Report Initiative launched in June 2021. This talk summarizes the main findings, while highlighting some major issues that currently hamper global efforts to capture the true burden of ciguatera. First, analysis of CP records available from HAEDAT and HABMAP-OBIS databases clearly reflects a significant weakness in data entry of CP events and *Gambierdiscus*/*Fukuyoa* spp. occurrence records, respectively. Hence this review was based mainly on data collated from scientific and grey literature. Second, the true incidence of CP remains difficult to ascertain globally due to the failure to recognize CP symptoms, a nearly universal under-reporting of the illness, and gaps and weaknesses in collection of CP epidemiological data. Despite these short comes, regional specificities were apparent with regards to outbreak occurring preferentially as large disease clusters vs. isolated cases, causative fish families, as well as reports of singular types of CP implicating sharks or marine invertebrates. Too, time-series data available for a limited number of CP endemic countries/regions revealed none of these areas showed identical trends (e.g. stable IR in the Caribbean vs. geographical expansion in Macaronesia and East/Southeast Asia), suggesting CP occurrences in a given region likely depend on data collection/reporting capabilities and a complex combination of trend drivers specific to each country, including socio-cultural determinants, tourism and fish trade activities, and climate change. Hopefully, the regional and international initiatives that have emerged recently will foster the implementation of a proactive surveillance of ciguatera at a global level.

Determination of optimal culture conditions for toxin production by a *Prorocentrum lima* complex strain with a high yield of diarrhetic shellfish toxins

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Diarrhetic shellfish poisoning is caused by the consumption of shellfish contaminated with diarrhetic shellfish toxins (DSTs) such as okadaic acid (OA) and dinophysistoxins (DTXs). To prevent the occurrence of human intoxication, the detection of DSTs in shellfish is critical. An instrumental method using liquid chromatography with tandem mass spectrometry has been recently employed for the monitoring of OA and DTX levels in shellfish. For such analysis, reference materials (RMs) of OA and DTXs are essential. The demand for RMs, especially for DTX1, has recently increased. Production of the RMs has been performed by mass-cultivation of a *Prorocentrum lima* strain producing DTXs and OA, indicating that the efficiency of production depends on the toxin production capacity of the strain used. Recently, we reported that approximately 200 clonal strains of *P. lima* complex were established from the Japanese coastal waters and separated phylogenetically into several clades/subclades. In this study, *P. lima* complex subclade 1e strain MIO12P demonstrated the highest DTX1 yield (1.27 µg mL^{-1}) in the world at a small-scale (test-tube) culture level. The culture medium suitable for toxin production by the strain MIO12P was metals mix SWII medium, and the optimal temperature and salinity for toxin production were 25 °C and 30, respectively. The DTX1 yield of this strain cultured at the mass-scale culture level (100 L) was almost identical to that at the small-scale culture level. Thus, strain MIO12P has the potential to be used for the sustainable production of DTX1 as an RM source for application in chemical and biochemical analyses.

Morphology, growth, toxin production, and toxicity of cultured marine benthic dinoflagellates from Brazil and Cuba

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The present study investigated selected benthic dinoflagellates isolated from different regions from the Western Atlantic, with respect to their morphology, growth, toxicity, and toxin production in culture. A total of nine strains of benthic dinoflagellates belonging to three genera were cultivated: *Amphidinium massartii*, *Amphidinium operculatum* (2 strains), *Coolia malayensis* (2strains), *Prorocentrum hoffmannianum* (2 strains), and *Prorocentrum mexicanum*, whose morphological and genetic characterizations matched previous descriptions, and *Prorocentrum* sp., morphologically related to *P.* cf. *norrissianum*. The two strains of *C. malayensis* from Brazil attained the highest growth rates (0.42 - 0.47 day^{-1}), but the lowest cell densities (2.2 - 2.9 × 10^4 cells mL^{-1}) in culture. The highest cell densities were recorded for *A. massartii* from Cuba (3.8 × 10^5 cells mL^{-1}). All species/strains investigated exhibited moderate toxicity to larvae of the brine shrimp *Artemia salina*; *A. massartii* being the most toxic species and *Prorocentrum* sp. the least one. Additionally, extracts of *Prorocentrum* species (*P. hoffmannianum* and *Prorocentrum* sp.) tested positive in mouse bioassays following intraperitoneal injection. Moderate to high concentrations of okadaic acid (OA), but no dinophysistoxins (DTXs), were found in both *P. hoffmannianum* strains from Cuba; but no diarrheic toxins were detected in either *P. mexicanum* from Cuba or *Prorocentrum* sp. from southern Brazil. Finally, five novel amphidinols were detected in cultures of both *A. massartii* (Cuba) and *A. operculatum* (Brazil) by LC-MS/MS, with molecular weights of 1440.8 (two isomers), 1360.8, 1287.7, and 984.6. These findings clearly indicate the need to include benthic species among the harmful microalgae surveyed in regional monitoring programs of phytoplankton.

Perception of risk of ciguatera among coastal communities, naval military and biomedical personnel of Colima (Mexican Tropical Pacific)

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Sporadic cases of ciguatera fish poisoning (CFP) have been described in the Mexican Tropical Pacific, but no investigation has been carried out on its origin and impact. In this study, we conducted an epidemiological survey (N = 282) to assess the perception of risk of CFP among coastal communities (n = 153), naval military personnel (n = 94) and biomedical (n = 35) of Colima. Surveys were conducted in people 18 - 80 years of age, 72 % men and 28 % women. 91 % don´t know what ciguatera is, only 9% mentioned knowing what ciguatera is and describe its presence in the spring-summer months. The fish involved in the problem between 2008 and 2019 were "barracuda" (*Sphyraena* spp) 29 %; "puffer fish" (Tetraodontidae) 29 %; "guajo" (*Acanthocybium solandri*) 17%; "catfish" (Ariidae) 8 % and other unidentified fish 12.5 %. The appearance of signs and symptoms in people occurred between 2 and 6 h after the consumption of fish, lasting from two to seven days and consisted of gastroenteric, neurological and cardiovascular problems. 93 % went to medical attention, however, no specific palliative treatment was performed (antidiarrheal, antibiotics, serum, antihistamines and analgesics), and a minority used traditional medicine such as infusions, ointments and essences of medicinal plants. The groups with the best knowledge of what ciguatera is where students, sailors/fishermen and merchants. The lack of knowledge of this syndrome among biomedical personnel (n = 35) in the area stands out, only a doctor and a nurse have information about this intoxication. Therefore, research and scientific divulgation of this issue is necessary for the protection of public health and its socio-economic impacts.

Influence of resources and regulators on potentially toxic benthic dinoflagellates abundance: evidence from different coastal systems of the Colombian Caribbean

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Taking into account that resources such as inorganic nutrients and regulators such as salinity and temperature have been identified as modulators of dinoflagellate populations, our objective was to understand the influence of these environmental factors on the abundance of potentially toxic epiphytic dinoflagellates that grow in association with the seagrass *Thalassia testudinum*. We evaluate the composition and abundance of dinoflagellates from coastal systems with different environmental characteristics of the Colombian Caribbean. Leaves of *T. testudinum* were collected to obtain the dinoflagellates. Salinity, temperature, dissolved oxygen, pH, nutrients and total suspended solids (TSS) were measured, and precipitation data and the Oceanic Niño Index (ONI) were obtained. Dinoflagellates were detached from the leaves, morphologically identified by analyzing their thecal plates arrangements, and quantified using a Sedgewick-Rafter chamber. The information was analyzed using standard statistics and regression models. Fourteen species of potentially toxic epiphytic dinoflagellate belonging to four genera were recorded, being *Prorocentrum* the most representative in number of species. With the statistical relationships between the most abundant species and the main environmental variables, fundamental niche models were proposed in which cells could proliferate. The temperature hypothesis gains strength as one of the main modulators of dinoflagellate abundance observed in the Caribbean, especially regarding *Prorocentrum* species. This assumption is supported by the high positive correlation between El Niño Southern Oscillation and sea surface temperature in the Caribbean during 2015. The degree of risk to human health due to the presence of these potentially toxic epiphytic dinoflagellates will not be resolved until their toxicity discarded.

Biodiversity, Biogeography and Chemical Ecology of Toxigenic Benthic Marine Dinoflagellates from Mexican Coastal Waters

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Benthic dinoflagellates are essential functional components of benthic food webs and are implicated in species interactions, especially with microeukaryotes and bacteria on epibenthic substrates. Dense cell aggregations can cause harmful events, particularly in tropical and subtropical coastal zones; these events are then referred to as “benthic harmful algal blooms” (bHABs). Many benthic dinoflagellates produce polyketide-derived polyether toxins, responsible for diverse seafood poisoning syndromes, such as diarrhetic shellfish poisoning and ciguatera fish poisoning. In Mexico, bHAB events have received recent public health and scientific attention, but many species lack molecular identification, and phylogeographic and toxigenic information remains limited. Our multidisciplinary project on bHAB taxa includes morphological and molecular characterization, phylogeography, and investigation of allelopathic and pharmacological bioactivity, supplemented with microbiome analysis. Thus far, we have completed the first phylogenetic study of *Amphidinium* and registered two species (*A. theodorei* and *A. massartii*) for the first time from Mexico. Preliminary studies on the in vitro cytotoxic properties of *A. operculatum* and *Coolia malayensis* are also underway. We characterized the phylogeography and diversity of the genus *Prorocentrum* from Mexican coasts and investigated the potential allelochemical effects of associated bacteria on the growth and toxigenicity of members of the *Prorocentrum lima* species complex. The focus on functional diversity and species interactions yields valuable insights in evaluating toxin risk in the design and implementation of monitoring systems for bHABs.

Effect of adding macroalgal extracts on the growth of *Gambierdiscus scabrosus* and *G. silvae* isolated from Japan

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Gambierdiscus scabrosus and *Gambierdiscus silvae*, which mouse bioassay has shown to be highly toxic, are thought to be the causative agents of ciguatera fish poisoning in Japan. However, ciguatoxin was not detected in the two species using LC - MS/MS analysis, which suggests that these two species produce unknown toxins. To purify and to identify the toxins, mass-cultured cells are needed, but to do this, determining the conditions under which *G. scabrosus* and *G. silvae* grow well is important. Recently, the medium, temperature, and salinity conditions suitable for their growth were clarified. In addition to the physico - chemical conditions, Bomber et al. (1989) reported that adding the lipid fraction extracted from macroalga promoted the growth of *Gambierdiscus toxicus*. However, the effect of macroalgal extracts on the growth of the Japanese species had not been examined. In this study, we investigated the effects of adding lipophilic fractions extracted from three species of macroalgae on the growth of *G. scabrosus* and *G. silvae*. The results, showed that adding the lipophilic extract from *Tricleocarpa cylindrica* (Rhodophyta) with 6.2 eq. algae fw mg mL^{-1} and the extract from *Delisea japonica* (Rhodophyta) with 1.6 eq algae fw mg mL^{-1} significantly enhanced the growth of *G. scabrosus* and *G. silvae* and that of *G. silvae*, respectively ($p < 0.05$). The results suggest that adding the macroalgal extracts to the medium may be useful for mass-culturing of the two Japanese *Gambierdiscus* species.

Genetic markers responsible for ciguatoxin production in *Gambierdiscus polynesiensis* (Dinophyceae)

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Dinoflagellates play significant ecological roles in aquatic ecosystems, however marine dinoflagellates can produce toxins that impact fisheries and aquaculture industries worldwide. *Gambierdiscus* produce the polyether ladder compounds ciguatoxins (CTX) and maitotoxins (MTX) which can cause ciguatera fish poisoning (CFP) associated with human illness. As yet, we have limited information regarding the genetic regulation of harmful algal toxin production, especially concerning species that produce CTX such as *G. polynesiensis*. In this project, the expression of genetic markers associated with CTX production by *G. polynesiensis* under different environmental and growth conditions will be determined. These results will help in identifying regulatory factors for toxin biosynthesis in *G. polynesiensis*. The study will allow for an increased understanding of the causes of toxin production and the potential for the development of novel CFP monitoring tools in the future.

Morphology, distribution and genetic diversity of the benthic dinoflagellate genus *Ostreopsis* in Korea

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Species belonging to the toxic dinoflagellate genus *Ostreopsis* are widespread, occurring from tropical to temperate waters. As mainly benthic/epiphytic species, they would be expected to show distinct geographical patterns. In this study, ribosomal DNA (rDNA) sequences from partial nuclear LSU D8-D10, 5.8S, and ITS regions were determined for 169 isolates of *Ostreopsis* species collected from three coastal sites (i.e., Jeju Island, Chuja Island, and Pohang) within Korea. The phylogenetic tree inferred from the LSU rDNA D8-D10 sequences showed that Korean *Ostreopsis* species corresponded to either *Ostreopsis* sp. 1 or sp. 6, with *Ostreopsis* sp. 1 being relatively predominant regarding their distribution. While *Ostreopsis* sp.1 occurred throughout all the three sampling sites within Korea, *Ostreopsis* sp. 6 was confined to the northern part of Jeju Island. When further investigated, the genetic diversity of *Ostreopsis* sp. 1 in Korea based on ITS sequences showed a total of 21 haplotypes. The presumed ancestral haplotype H3, was also present in the Japanese and Russian populations. Although the overall demographic history of all the Korean populations of *Ostreopsis* sp. 1 could not be clearly identified, probably owing to a mixture of different regional demographic patterns within Korea, each *Ostreopsis* population showed a characteristic demographic pattern at a regional scale. While the Jeju Island population showed a signal in agreement with population equilibrium, the Chuja Island and Pohang populations showed distribution patterns that are expected in a sudden population expansion model. The results from this study provide a basis for a better understanding of the distribution and genetic structure of the Asian *Ostreopsis* populations.

Gambierdiscus species from Brazil: abundance, morphology and molecular phylogeny

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Ciguatera is a worldwide illness caused by the consumption of fish and shellfish that have accumulated ciguatoxins produced by certain species of *Gambierdiscus*. There are no official records of the disease in Brazil and *Gambierdiscus* (*G. excentricus*) has been registered in the country only recently, in 2015, at Rio de Janeiro state. The identity, distribution, and abundance of *Gambierdiscus* species is largely unknown along the Brazilian coast, which has been mostly uninvestigated. The aim of this study was to investigate *Gambierdiscus* species occurrence and abundance in Brazil, as well as to describe the morphology and molecular phylogeny of isolated strains. Macroalgae samples were collected as substrates for *Gambierdiscus* from eighteen locations along the tropical and subtropical Brazilian coast and from three oceanic islands in the South Atlantic Ocean. Over 600 samples were analyzed. Strains of *Gambierdiscus silvae* and *Gambierdiscus belizeanus* were isolated from the Trindade and Fernando de Noronha islands, respectively. *Gambierdiscus* abundance varied between zero and 1.2 x 10^4 cells g FW^{-1} macroalgae. In general, *Gambierdiscus* was a minor component of the benthic dinoflagellates assemblage and abundances were low, but slightly higher at the oceanic islands relative to the coast. Ciguatera is an expanding public health threat and therefore there is a need to improve our knowledge on its causative organism in Brazil. This study contributes with new information on this important dinoflagellate genus in the country.

Evaluating Fiji´s CFP Incidence and Factors Relevant to its Occurrence

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Ciguatera Fish Poisoning (CFP) is a threat to livelihoods, trade and human health. However, Fijians continue to catch, sell and consume ciguatoxic fish as is evident from the increasing number of CFP incidence recorded by the Ministry of Health (MoH). For ten years from 2008-2017, recorded incidence of CFP increased from 854 to 1,775 cases. However, MoH records are limited to the cases that visited Health Centres and Hospitals. But not all affected people visit a Health Centre leading to under-reporting, particularly in rural and isolated communities. Such gaps in data triggered the development of a three-prong project: (i) evaluating the incidence of CFP in communities, (ii) determining the identification and distribution of ciguatera causative organisms and other Harmful Alga Blooms (HABs), and (iii) developing a framework for a CFP predictive model. To evaluate the issue of under-reporting, a CFP notification booklet has been developed and is being piloted with Community Health Workers that live in rural communities to record the incidence of CFP as it occurs. The identification and distribution of ciguatera causative organisms and other HABs involves sampling microalgae for microscopy and assessment of the environmental conditions in which they thrive. The CFP modelling effort involves using available environmental data and CFP incidence from sites around Fiji to look for ecologically relevant correlations that could be developed into an early warning systems for CFP. Collectively these projects will improve our understanding regarding the occurrence of CFP, the environmental conditions that are associated with CFP occurrence, as well as provide key information to inform the relative stakeholders and authorities.

Ciguatoxin analysis towards risk assessment of ciguatera in Cuba: achievements to date and current challenges

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Ciguatera is the most significant of all non-bacterial seafood poisoning problems in Cuba. It poses a risk mainly for subsistence-based fisheries, which represents a primary source of local income and food supply. Several IAEA technical cooperation projects and research initiatives have allowed gaining knowledge and capacities for sampling, morphological identification, and culturing of *Gambierdiscus* species, and for ciguatoxin analysis in cells and seafood using the radioligand Receptor Binding Assay (r-RBA). This work reports progress and challenges on the implementation of r-RBA in Cuba, from the collection of toxic fishes in known hot spots to the conduction of internal validation studies. The performance of the assay was characterized using the Plate CHAMELEON V counter (Hidex, Finland), not previously used to analyze algal toxins. Optimization of counting measurements (i.e. backgroung variability, counting time and selection of the scintillant cocktail) as well as adapting the assay protocol to this new technology were required. The feasibility of in situ preparation of brain homogenates was discussed as a way to overcome the difficulties of local importation processes. Matrix effect was assessed using locally caught freshwater fish species, not expected to be contaminated with ciguatoxins. Preliminary results revealed the need to optimize the extraction protocol for high content fat fish and hence avoid false-positive samples.

Experimental evidence of ciguatoxin accumulation and depuration in carnivorous lionfish

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Ciguatera poisoning is globally the most common non-bacterial food intoxication associated with fish consumption. This foodborne illness finds its origin in potent neurotoxins, the ciguatoxins (CTXs), which are produced by dinoflagellates of the genera *Gambierdiscus* and *Fukuyoa*, and can be transferred to marine organisms through the food chain. In this study, we developed an experimental model to assess the trophic transfer of CTXs from herbivorous parrotfish, *Chlorurus microrhinos* to carnivorous lionfish, *Pterois volitans*. Juvenile lionfish fed with CTX naturally-contaminated parrotfish fillets received during six weeks 0.11 ng CTX3C eq g^{-1} d^{-1} or 0.035 ng CTX3C eq g^{-1} d^{-1} as assessed by the radioactive receptor binding assay (r-RBA) or neuroblastoma cell based assay (CBA-N2a), respectively. Control fish received a non-ciguatoxic fish diet throughout the experiment. CTX-exposed fish exhibited no behavioral signs of intoxication during the experiment. No CTX was detected in muscular tissues using r-RBA. In the liver of some lionfish, 9.43 and 9.77 ng CTX3C eq g^{-1} were estimated at the end of the exposure and depuration periods, respectively. When pooled liver extracts of lionfish were analyzed by the CBA-N2a, 0.89 and 0.37 ng CTX3C eq g^{-1} were found during the same period, at last days of exposure and depuration stages, respectively, suggesting slow elimination of CTXs. Unfortunately, no CTX-related compound could be detected by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) due to limited sample size and low sensitivity. These preliminary results obtained from juvenile lionfish should be pursued in adults to strengthen knowledge on CTX transfer and kinetics along the food web.

Preliminary results on the characterization of marine benthic dinoflagellates (*Gambierdiscus* spp., *Coolia* spp., *Ostreopsis* spp., *Prorocentrum* sp, *Amphidinium* sp.) from New Caledonia (South West Pacific)

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Gambierdiscus and *Fukuyoa* are producers of neurotoxins associated with ciguatera poisoning (CP). However, other toxic epiphytic dinoflagellates are often found in the same habitat, such as the genus *Ostreopsis*, which produces palytoxin analogues (neurotoxin), or the genus *Prorocentrum*, which produces diarrhetic toxins (okadaic acid and derivatives). These toxins could contribute to the toxic cocktail associated with ciguatera and explain the variability of observed symptoms. In New Caledonia, there is currently a lack of knowledge about ciguatera poisoning and about the microalgae associated to this foodborne illness. In this study, live samples were collected from seaweed and sand on the west coast of New Caledonia. Several strains were isolated and cultured using F/10K, L1 or L1/10 medium enriched with soil extract when necessary. Among 16 isolates, a morpho-molecular approach allowed for identification of two *Gambierdiscus* spp., four *Coolia* spp., two *Ostreopsis* spp., an *Amphidinium* sp. and a *Prorocentrum* sp. Pulse amplitude modulated fluorometry was also used to assess and differentiate their photo-physiological capacity. As most of these organisms are known to produce several groups of bioactive compounds, toxicity has been screened using the neuroblastoma cell-based assay (CBA-N2A). Toxin profiles of microalgae extracts were characterized by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Preliminary results of this study provide an overview of marine benthic dinoflagellate species occurring in New Caledonia.

Lipophilic toxins analyses by liquid chromatography-high resolution mass spectrometry reveal the okadaic acid production in a new *Prorocentrum* species of the *P.* cf. *lima* complex

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The genus *Prorocentrum* includes, up to date, 79 predominantly marine species with planktonic and/or epibenthic members that inhabit inert and/or living substrates. Some *Prorocentrum* species are responsible of harmful algal blooms and around a dozen, generally epibenthic, have been shown to produce biotoxins such as okadaic acid (OA), dinophysistoxins (DTXs), borbotoxins, fast-acting and hemolytic toxins. In this study a new marine benthic *Prorocentrum* species from the "*P.* cf. *lima* complex" isolated from the Parque Nacional Natural Tayrona (tropical region; Colombian Caribbean Sea), was tested for toxins production. This study describes the analyses of okadaic acid (OA) DTX1, DTX2 and pectenotoxin 2 (PTX2) in cultures of strain INVMYZ0002. At the early exponential phase aliquots were taken for toxins analysis and cell counts. Cellular toxins were recovered with a double 100 % MEOH extraction protocol and subsequently purified by solid phase extraction (SPE). The extracts were also hydrolyzed in order to determine Total OA Group levels. Extracellular toxins from culture supernatants were concentrated and purified by SPE. Intracellular and extracellular extracts were analyzed by liquid chromatography-high resolution mass spectrometry. OA was quantified in both intracellular and extracellular extracts. DTX1, DTX2 and PTX2 were not detected in those extracts. Analysis of cellular hydrolyzed extracts revealed the presence of OA esters, but DTX1 and DTX2 were not detected. Our findings highlight the importance of investigating Diarrhetic Shellfish Toxins and their related harmful algae species in Parque Nacional Natural Tayrona in order to prevent adverse health effects.

Epibenthic dinoflagellates of the southern Gulf of California: species composition and abundance (2015-2019)

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With the aim of studying the epibenthic dinoflagellate taxocoenosis, 153 quantitative samples were collected from different substrates (mainly attached green, brown and red macroalgae, also sediments, mangrove roots and floating macroalgae) at 10 sites along the western coast of the southern Gulf of California (eight sites in Bahía de La Paz from Playa El Califín (PC) to Playa El Tecolote (PT) and two sites beyond the bay) in May 2015, June 2016 and 2018, and December 2019. In total, about 23 dinoflagellate species from the genera *Prorocentrum* (8), *Ostreopsis* (3), *Sinophysis* (3), *Amphidinium*, *Blixaea*, *Bysmatrum*, *Cabra*, *Coolia*, *Durinskia*, *Gambierdiscus*, *Fukuyoa* and *Plagiodinium* (one species each) were found. The maximum cell abundance was observed at PC in June 2016: 14,440 cells g^{-1} of macroalgae wet weight (*Padina* with cyanobacteria clumps). Usually, *Prorocentrum lima*, *P. rhathymum* and *P. sculptile* were dominant (up to 76 %). *Gambierdiscus* was dominant only once (64 %; PT, December 2019). Occasionally, *Ostreopsis* dominated (up to 94 %; PT, December 2019). In all 13 macroalgal samples taken at PT in 2019, *Gambierdiscus* and *Ostreopsis* occurred; abundance reached 5,697 cells g^{-1}. The same level of cell abundances in May-June and December can be explained by similar seasonal characteristics (warm and dry), particularly the moderate water temperature (21.5 - 25.0 °C) and low precipitation. Two mangrove root samples contained rare cells of *P. lima*, *P. rhathymum* and *Coolia* (Playa El Tesoro I). Among all sampling sites, PT can be considered a locality of maximum risk for human health, probably due to the richest subaquatic vegetation as a habitat.

Distribution of potentially toxic epibenthic dinoflagellates in Venezuela

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Epibenthic dinoflagellates have been identified as part of the community in recent reports about harmful algal blooms in Venezuela. This can be considered a potential risk and is of concern due to the apparent geographic expansion of these organisms and the frequency with which these phenomena are occurring in the Caribbean. The coastal marine zone in Venezuela has a great diversity of habitats and substrates that seem to favor the presence of these microalgae, which are potential producers of biotoxins and represent a health risk for shellfish consumers who live near coastlines. To evaluate the distribution of epibenthic dinoflagellates associated with various substrates in Venezuela, we used historical data from 1994 to 2020. *Prorocentrum lima* was the genus with the highest number of taxa and the most frequent potentially toxic species was *Prorocentrum lima*. We observed that variations in the abundance of dinoflagellates are possibly dependent on environmental conditions and the geographic location of the sampled areas. For example, the coastal marine zone in eastern Venezuela is favored by seasonal coastal upwelling. Also, there are interactions in the coastal zone with coastal lagoons, such as La Restinga lagoon in Margarita Island. These conditions contribute to the development of potentially toxic species and some substrates (macroalgae and seagrasses), which remain lush throughout the year and proved to be preferred by epibenthic dinoflagellates for their colonization.

Ostreopsis cf. ovata *blooms* at Rio de Janeiro state, Brazil in summer 2020

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Blooms of *Ostreopsis* cf. *ovata* have been commonly reported at Armação dos Búzios (AB) and Arraial do Cabo (AC) in Rio de Janeiro state, Brazil, since 1998 and were associated to massive deaths of the sea urchin *Echinometra lucunter*. This study aimed to follow epibenthic dinoflagellates abundances associated to macroalgae during summer 2020 at AB and AC to gain insight on factors that act as triggers of *O*. cf. *ovata* blooms in the region. Macroalgae (*Sargassum* sp. and red calcareous *Jania* sp. and *Amphiroa* sp., n = 4) were collected from 1m depth at 4 stations (Anjos-AC, Forno-AC, Forno-AB, Tartaruga-AB) between December 2019 and February 2020, once a month. Seawater temperature was continuously monitored at a nearby station at AC. Cell abundances were determined using Sedgewick-Rafter and sedimentation chambers and observation in an inverted microscope. *Ostreopsis* cf. *ovata* abundances were high throughout summer 2020 and varied between 5,086 cells g^{-1} FW *Sargassum* at Forno-AC in December and 37,184 cells g^{-1} FW calcareous macroalgae at Forno-AB in February. A biofilm of O. cf. ovata was seen covering calcareous macroalgae at Anjos-AC in January, when abundances were 8,815 cells g^{-1} FW calcareous macroalgae. No major coastal upwelling event that frequently enriches shallow waters in the region, occurred during the sampling period, but sewage spill and decomposing seagrasses may have contributed as nutrients sources to support these blooms. This is a touristic destination that can receive 600,000 people only in a Brazilian Carnival holyday and high *O*. cf. *ovata* abundances are of concern in relation to human health.

Occurrence of potentially toxic benthic dinoflagellates in two coastal areas of Central Cuba

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Benthic dinoflagellates of the genera *Gambierdiscus*, *Prorocentrum* and *Ostreopsis* were sampled in March and October 2019 at two coastal areas (Rancho Luna and Cayo Santa María) located in the south and north region of central Cuba respectively). Different host species were collected in order to evaluate possible preferential associations between macrophyte species and benthic dinoflagellates. Abundance of the three genera was higher in March at both sites and, in general, Rancho Luna presented greater abundance compared to Cayo Santa María. The most abundant genus in Rancho Luna was *Ostreopsis*, while *Prorocentrum* showed the highest abundance in Cayo Santa María. In both sites, the genus with the lowest abundance was *Gambierdiscus*. Phaeophyceae hosted the highest total abundance of benthic dinoflagellates at both sites, while the lowest total abundance was observed on Ulvophyceae. Host preferences was observed among the different macrophytes collected. *Ostreopsis* was most abundant on the seagrass *Thalassia testudinum* and on *Dictyota* spp. while the highest abundance of *Prorocentrum* was found on *Liagora* spp. The genus *Gambierdiscus* showed the highest abundance on *Dictyota* spp. and *Lobophora* spp. and was least abundant on *Rhipocephalus* spp. and *Chondria* spp., however it was present on 10 of the 12 genera of macrophytes studied. These results increase the knowledge about the ciguatoxin-source dinoflagellates that are present in Cuba and contribute with the monitoring efforts in potentially ciguatera risk areas.

BHAB occurrence in the south coast of Madeira Island, Portugal: from 2018 to 2020

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Ciguatera fish poisoning episodes have been recently reported in the Madeira Archipelago, requiring actions to protect both public health and economic sectors such as tourism and fisheries. Surveys on the occurrence of BHAB species were performed during 3 consecutive years (2018 - 2020), at Cais do Carvão Bay, Funchal, Madeira. Samples collected with artificial substrates were analyzed for the presence of BHAB species, the seasonal succession of the BHAB community and the effects of abiotic factors on BHAB dynamics. The BHAB community was characterized by the presence of five genera: *Gambierdiscus*, *Ostreopsis*, *Coolia*, *Prorocentrum*, and *Amphidinium*. The abundance of the different genera showed marked differences. *Ostreopsis* displayed cell abundances several orders of magnitude higher than the other genera and was recorded at all sampling dates. The second most abundant genus was *Coolia*, followed by *Prorocentrum* and *Amphidinium*. *Gambierdiscus* showed always the lowest cell densities. During the study period, while *Gambierdiscus* reached maximum abundances of 0.43 x 10^3 cells 100 cm^{-2} (September 2018), *Ostreopsis* cell densities were considerably higher, reaching a maximum of 841.05 x 10^3 cells 100 cm^{-2} (September 2018). The occurrence of higher *Ostreopsis* and *Gambierdiscus* cell densities may be linked to an increase in water temperature during summer. In all summer months *Ostreopsis* abundances exceeded, in several sampling dates, the limits of level of concern for respiratory syndrome outbreaks (20 x 10^3 cells 100 cm^{-2}) as referred in the literature. These results confirm Cais do Carvão Bay, as a potential high-risk area for BHAB development, especially concerning the genus *Ostreopsis*.

Benthic dinoflagellates assemblage and anthropic stressors within the Fernando de Noronha Archipelago, Brazil

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The Fernando de Noronha archipelago (03º 54 'S, 32º 25' W) is located 545 km away from the Brazilian coast. It is a top touristic destination and receives around 83,020 tourists per year and has about 3,100 inhabitants. The abundance of benthic dinoflagellates associated to macroalgae was determined at four stations, two of them (1, 2) located close to an urbanized area and one to a sewage discharge (station 1), while stations 3 and 4 were in more pristine zones that can be reached only by boat. Benthic dinoflagellates were identified using light and scanning electron microscopy. The genera *Prorocentrum*, *Ostreopsis*, *Gambierdiscus*, *Coolia*, *Amphidinium*, *Sinophysis* and *Cabra* were found. At stations 1 and 2, abundances were 22,449 and 55,968 cells g^{-1} FW macroalgae (mainly *Ostreopsis lenticularis*), considerably higher than at stations 3 and 4 (mean 3,144 cells g^{-1} FW macroalgae). While at stations 1 and 2 *Ostreopsis* represented more than 97 % of total dinoflagellate abundance, at station 3 *Prorocentrum* corresponded to 78 % of total abundance. At station 4, relative abundances were: *Prorocentrum* (31 %), *Ostreopsis* (40 %) and *Gambierdiscus* (21 %). An *Ostreopsis* bloom was found at stations subjected to more intense anthropic impacts while higher species richness and lower abundances were registered at the more preserved sites. Although this is a limited set of data, the benthic dinoflagellates assemblage well reflected the pattern of biodiversity loss at human impacted sites relative to near pristine areas within this remote located archipelago at the South Atlantic Ocean.

Comparing the distribution and abundance of benthic dinoflagellates in the Florida Keys: 1985 versus 2021

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Ciguatera Poisoning (CP) is a foodborne illness associated with seafood consumption from tropical areas. Tens of thousands of people are afflicted with CP each year and the distribution of this illness is increasing worldwide. CP is caused by ciguatoxin, the precursors of which are produced by some members of the benthic dinoflagellate genus, *Gambierdiscus*, and possibly other compounds produced by other co-occurring dinoflagellates which inhabit dead coral surfaces and benthic macroalgae. The toxins accumulate in the tissues of fish that eat the macroalgae and are transferred up the food chain. As climate change increases sea surface temperatures (SST) and impacts coral reefs, the habitat of toxic dinoflagellates (macroalgae) could potentially expand, increasing cases of CP worldwide. As a follow up to the pioneering work of Bomber (1985), a study was designed to assess the abundance of *Gambierdiscus* and co-occurring benthic dinoflagellates in the Florida Keys, to determine if they have increased over the past four decades. The dinoflagellates and associated environmental parameters are being monitored over a 14-month period using four of the same locations and time points utilized by Bomber (1985). It is predicted that dinoflagellate densities will have increased, due to environmental changes over the past 40+ years. The current status of the project will be presented herein.

ALGAL AND CYANOBACTERIAL TOXINS

Paralytic Shellfish Toxins: a complex group in constant (bio) transformation

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Paralytic shellfish toxins (PSTs), saxitoxin and its analogues or simply saxitoxins (STXs) are a large and diverse group of marine biotoxins responsible by paralytic shellfish poisoning (PSP). All members of the group share a common core structure, but the combination of different functional groups (R1 – R4) defines the subgroup to which each analogue belongs (e.g. carbamate, N-sulfacarbamoyl, decarbamoyl, benzoate), influences its toxicological action and determines the greater or lesser toxicity of each member. STXs are prone to biotransformations within living organisms, affording analogues with higher or lesser toxicity. Such biotransformations may be mediated by different agents (e.g. enzymes) in different living organisms, undergoing chemical processes that could lead to diverse outcomes. With this presentation we propose to highlight the most recent discoveries related to STXs biotransformations in different living organisms (dinoflagellates, bivalves and humans), focusing on M-toxins, and relate the (bio) transformations to toxicity.

The occurrence of emerging marine toxins in shellfish from The Netherlands

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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. In the last decade the European Food Safety Authority (EFSA) prepared a number of opinions on unregulated toxins such as tetrodotoxins (TTX), cyclic imines (CIs), brevetoxins (PbTXs) and palytoxins (PlTXs). The most frequent mentioned conclusion in these EFSA opinions was the need for methods and lack of occurrence data. Therefore we developed various analytical methodologies and performed a number of surveys on samples from shellfish production areas in the Netherlands. After positive findings in a survey of TTX it became clear that these more tropical related toxins also might occur in temperate regions. Currently, for TTX a liquid chromatography tandem mass spectrometric (LC-MS/MS) method, a monitoring program and national legislation (44 µg TTX kg^{-1}) are established. For CIs and PbTXs LC-MS/MS methods have been developed and one year surveys were organized. The CIs are a class of “toxins” consisting out of spirolides, gymnodimines and pinnatoxins, which are known to occur also in more temperate areas. CIs were found in multiple production areas at relative low concentrations. For PbTXs no detectable concentrations were observed. For PlTX, large molecule with a mw of 2680 Da, a sensitive LC-MS/MS method based on lithium cationization has been developed. For PlTX still a survey should be organized. During the presentation both the methodologies as well as the (seasonal) occurrence will be discussed.

Novel benthic vectors of paralytic shellfish toxins

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Paralytic Shellfish Toxins (PSTs) are a regulated group of potent neurotoxins, which are extremely toxic to mammalian nervous systems. Accumulation of PSTs in bivalve molluscs is a well explored risk to human health. In January 2018, a large easterly storm hit the East coast of the U.K., which resulted in mass strandings of benthic organisms. Dogs subsequently consumed washed up organisms, leading to illness and, in some cases, fatalities. The benthic organisms responsible were tested for the presence of marine toxins which contained high concentrations of PSTs (> 10,000 µg STX eq kg^{-1}). Subsequent work conducted in the North Sea highlighted widespread PSTs presence in a large range of taxa across a wide geographical fetch. Novel vectors of PSTs were identified, with the common sunstar (*C. papposus*) and sea chervil (*A. diaphanum*) highlighted as key species (concentrations > 1,000 µg STX eq kg^{-1}). Two toxin profiles were identified that were geographically driven. Another investigation assessed the PSTs presence in the common sunstar from multiple locations around the U.K. Results confirmed the ubiquitous presence of PSTs in this species with total toxicities reaching 11,500 µg STX eq kg^{-1}. Inter-organ analysis of sunstars showed highest concentrations in female gonads with toxicities exceeding 40,000 µg STX eq kg^{-1}. In conjunction, the depuration of PSTs in sunstars was analysed via a depuration experiment. Results highlighted no significant depuration of PSTs over 14 days, hinting at either active storage or internal/symbiotic production. To conclude, the UK coast hosts multiple different benthic organisms that are capable of accumulating PSTs, sometimes to dangerous levels and that sunstars are especially prone to containing PSTs.

Bioaccumulation and mode of action of 28-O-palmitoyl ester of pinnatoxin-G and portimine-A on nicotinic acetylcholine receptors

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Phycotoxin bioaccumulation implies that shellfish are tolerant or develop resistance mechanisms against such harmful compounds. A well-illustrated example is the molecular mechanism behind saxitoxin resistance in natural *Mya arenaria* populations. In contrast, the bioaccumulation of cyclic imine toxins seems not to be deleterious for shellfish under natural and *in vitro* conditions. Further, they are able to metabolize them generating a series of acyl derivative compounds. Cyclic imine toxins are potent antagonists of muscle and neuronal nicotinic acetylcholine receptors which are vectored to humans by shellfish that bioaccumulates them. Our aim was to monitor the presence of 28-O-palmitoyl ester of pinnatoxin-G and portimine-A in mussels collected in the Mediterranean Lagoon of Ingril (France), and to determine their mode of action against nicotinic acetylcholine receptors. Ingril is known for its recurrent blooms dominated by *Vulcanodinium rugosum*, a dinoflagellate that produces pinnatoxins and portimines. Both, the acylated derivative of pinnatoxin-G and portimine-A, were seen for the first time to be bioaccumulated by native mussels according to microplate-receptor binding assay and UPLC-MS/MS. In addition, electrophysiological recordings showed that the acylation of pinnatoxin-G at the secondary OH at C-28, near to the spirocyclic group, reduced by ~300 times the affinity of 28-O-palmitoyl ester of pinnatoxin-G for muscle-type nicotinic acetylcholine receptors when compared to its precursor toxin. Portimine-A, whose potent cytotoxic behavior was tested against four different human cell lines, is also a relatively weak antagonist of neuronal α7 nicotinic acetylcholine receptor. Portimine-A bioaccumulation may represent a different toxicological risk than pinnatoxins, spirolides or gymnodimines, given its potent apoptotic and cytotoxic properties.

Guidance value for brevetoxins in French shellfish

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Brevetoxins (BTXs) are responsible for neurotoxic shellfish poisoning (NSP) after ingestion of contaminated shellfish, characterized by neurological, gastrointestinal and/or cardiovascular symptoms. BTXs are cyclic polyether toxins classified into groups A and B, based on their backbone structure. More than 70 analogs/metabolites have been reported, most of them issued from shellfish metabolism. The chemical structure is only available for about 30 analogs. The main producer of BTXs is the dinoflagellate *Karenia brevis*. In addition, *K. papilionacea* has been shown to produce BTXs under laboratory conditions. BTX-like compounds are also produced by *K. mikimotoi* and *K. bicuneiformis*, and by some raphidophytes, i.e. *Chattonella marina*, *C. antiqua*, *Heterosigma akashiwo* and *Fibrocapsa japonica*. BTXs are currently not regulated in France or in Europe. In 2018, they have been included in the French network for monitoring the emergence of marine biotoxins in shellfish (EMERGTOX) and in November 2018 were detected for the first time in France in mussels from a lagoon in the Corsica Island (Mediterranean Sea). Consequently, Anses (the French Agency for Food, Environmental and Occupational Health & Safety) was mandated to provide a guidance value for BTXs in French shellfish. Anses set up a working group to update the state of knowledge related to BTXs. Toxicological data were too limited to derive an oral acute reference dose (ARfD). However, based on human case reports, two lowest-observed-adverse-effect levels (LOAELs) were identified. A guidance value of 180 µg eq BTX-3 kg^{-1} shellfish meat was set, considering a protective default portion size of 400 g.

Chasing a moving target: The intriguing diversity of goniodomins

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Currently four *Alexandrium* species are known to produce goniodomins (GD): *A. hiranoi*, *A. monilatum*, *A.* pseudogonyaulax and *A. taylorii*. Whereas *A. hiranoi* seems to be constrained to the Northwestern Pacific and *A. monilatum* to the Americas, records of *A. pseudogonyaulax* and *A. taylorii* suggest a more global distribution. To date only goniodomins A (GDA) and B (GDB) haven been described. Analysis of GD profiles of the four species revealed that GDA was the most abundant variant in cell extracts of most strains, but several other GD variants were present in all strains. However, one *A. taylorii* strain originally isolated from Japanese coastal waters produced the putative 34-desmethyl-GDA as the primary compound in the GD group. The observation that extracellular GD profiles differed completely from those obtained by cell extraction prompted stability testing of GDA, which showed rapid conversion of GDA into different variants. Monitoring data and field data of surveys performed in 2016 and 2020 on the Eastern North Sea, Danish Limfjord and the Western Baltic Sea indicate an expansion of *A. pseudogonyaulax* in Northern European waters. This is of particular concern as *A. pseudogonyaulax* and the other three species are suspected to be ichthyotoxic. This is in agreement with the fact that extracellular compounds of all four species cause cell lysis of co-occurring protistan species. However, extracellular GD did not significantly contribute to protistan cell lysis and thus other bioactive extracellular compounds (BEC) must be involved in this effect.

Naturally released metabolites of *Microcystis aeruginosa* impact on *Daphnia magna*

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In some urgent scenarios, cyanobacterial blooms are chemically treated, for example by application of H_2O_2. Following an H_2O_2 treatment in a lake, a negative impact on zooplankton was observed, the populations, however, recovered. Cyanobacteria themselves release harmful compounds and to disentangle impact of those compounds from impact of feeding on cyanobacteria, we focused on the environmental realistic scenario of the naturally released mixture without crushing cyanobacterial cells or their uptake as food. *Daphnia magna* were exposed to two concentrations of cell free cyanobacterial spent medium from *Microcystis aeruginosa* (higher and lower ecologically-relevant concentrations of cyanobacterial metabolites). Microcystin-LR and 11 metabolites have been detected, of which five were quantified. Survival, gene expression marking digestion and metabolism, oxidative stress response, cell cycle and molting and activities of detoxification and antioxidant enzymes were followed for seven days. *Daphnia magna* suffered from oxidative stress as gene-expressions of stress markers and both catalase and glutathione S-transferase enzyme activities significantly decreased, suggesting enzyme exhaustibility after three and seven days. Expression of energy allocation marker was increased after three days but decreased after seven days exposure. Cell cycle was impacted time-dependently but differently by the two concentrations, along with an increasing downregulation of myosin heavy chain responsible for cell arrangement and muscular movements. Deregulation of nuclear hormone receptor genes indicate that *D. magna* hormonal steering including molting seemed impaired despite no detection of microviridin J. *D. magna* survival was impaired over time, in a concentration dependent manner. Our results confirm that besides microcystin-LR, other secondary metabolites contribute to negative impact on *D. magna* survival and stress response.

A new un-targeted data dependent acquisition-based approach for the identification of unknown fast-acting toxins and their metabolites in Mediterranean shellfish

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Cyclic imines (CI), also known as fast-acting toxins, are listed among the emerging toxins of European concern as they cause marked neurotoxic effects in mice by intraperitoneal injection. Their detection in raw and processed shellfish within the European borders has increased significantly in recent years, to the point of raising even more worries for consumer safety. However, no regulation exists for this family of toxins due to the lack of robust toxicological data, and consequently the European Food Safety Authority has required more scientific efforts before establishing a maximum permitted level in seafood. In this study, a new data dependent liquid chromatography – high resolution mass spectrometry (LC-HRMS) method has been conceived and successfully applied in combination with targeted studies for an in - depth investigation of the metabolic profile of shellfish. This approach allowed to discover a number of novel gymnodimine fatty acid ester metabolites, as well as to implement a new LC-HRMS-based strategy, named "backward analysis", for discovery and identification of new analogues. The implemented analytical workflow has widened the structural diversity of gymnodimine family through the inclusion of five new congeners, gymnodimine -F, to -J for which a tentative structural characterization was carried out. In parallel, the study of toxin accumulation in Mediterranean and Galician mollusks revealed the presence of gymnodimine A in Tunisian shellfish, pinnatoxin G in mussels from the Atlantic coast of Spain (Rías Baixas) and, for the first time, in mussels from Sardinia (Tyrrhenian Sea, Italy); in the former 13 desmethyl spirolde C co-occurred with pinnatoxin G.

Biochemical performance of cockles, mussels and razor shells contaminated by paralytic shellfish toxins

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In general, paralytic shellfish toxins (PSTs) accumulated in bivalves in values above the limit imposed by regulatory authorities occur during short periods, but in some cases, it may be extended from weeks to months. The present study examines whether cockles (*Cerastoderme edule*), mussels (*Mytilus galloprovincialis*) and razor shells (*Solen marginatus*) naturally exposed to a bloom of *Gymnodinium catenatum* activated or suppressed biochemical responses as result of the presence of PSTs in their soft tissues. Toxins (dcSTX, dcGTX2 + 3, dcNEO, C1 + 2, C3 + 4, GTX5 and GTX6) and a set of biomarkers (GLY, glycogen; SOD, superoxide dismutase; PROT, protein; CAT, catalase; GPx, glutathione peroxidase; GST, glutathione S - transferases; LPO, lipid peroxidation; reduced (GSH) and oxidized (GSSG) glutathione contents; AChE, acetylcholinesterase activity and ETS, electron transport system activity) were determined in specimens harvested weekly in Aveiro lagoon, Portugal, along thirteen weeks. This period included three weeks in which bivalve toxicity exceeded largely the regulatory limit and the subsequence recovery period of ten weeks. Biochemical performance of the surveyed species clearly indicated that PSTs induce oxidative stress and neurotoxicity, with higher impact on mussels and razor shells than in cockles. The antioxidant enzymes CAT and GPx seemed to be the biomarkers better associated with toxin effects.

Biodiversity, toxicity and biotechnological potential of cyanobacteria from Cabo Verde islands

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Cyanobacteria are a diverse group of gram-negative photosynthetic prokaryotes with a great ecological importance. They colonize a wide heterogenous range of ecosystems and are a major component of the phytoplankton. Their ability to produce toxins as secondary metabolites may lead to the development of harmful blooms causing huge economic losses in the fishing and aquaculture industries and poisoning incidents to humans due to their accumulation in the food-chain. However, cyanobacteria have also attracted an increasing interest owing to the production of a large number of natural bioactive compounds (e.g. alkaloids, non-ribosomal peptides, polyketides) with promising biotechnological and biomedical applications. Cabo Verde is a group of volcanic islands located in the Tropical Eastern Atlantic, in the Sahel region of Africa. The environmental characteristics of this archipelago provide niche conditions for a wide variety of cyanobacteria but scarce information exists at the date. Here, a metagenomic approach was selected in order to analyze the environmental samples collected at different locations in São Vicente and Santo Antão islands in the framework of the ENMERTOX project. This culture-independent methodology will constitute an accurate representation of the natural diverse community, and open access to untapped genetic resources for the screening and identification of genes encoding new bioactive compounds. Applying this state-of-the-art sequencing technology and analytical tools, we determined the taxonomic biodiversity in our samples, the presence of genes of interest and explore the production of toxins and secondary metabolites with biotechnological potential. Risk assessment will be done taken into account the food chain implications of these cyanobacteria.

High-Throughput Analysis of Anatoxins in Benthic Cyanobacterial Mat Field Samples by Direct Analysis in Real Time-High Resolution Tandem Mass Spectrometry (DART-HRMS/MS)

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Improved analytical methods are required to enable large-scale field studies of the occurrence of anatoxins (ATXs) in benthic cyanobacterial mats that are increasingly linked to animal fatalities worldwide. A rapid method for analysis of anatoxin-a, homoanatoxin-a and dihydroanatoxin-a by direct analysis in real time-high resolution tandem mass spectrometry (DART-HRMS/MS) with a total run time of under 2 min per triplicate analysis is presented. Sample preparation was simplified to only require cell lysis, homogenization and centrifugation. Isotope dilution calibration with 13C4-anatoxin-a corrected for the significant and variable matrix effects and showed excellent quantitative agreement with a LC-HRMS reference method. Estimated LODs were approximately 5 µg L^{-1} and are considered acceptable in the context of new WHO guidelines for short-term exposure to anatoxin-a in drinking (30 µg L^{-1}) and recreational (60 µg L^{-1}) water. The developed method was applied to over 150 field samples collected in 2019 from the Wolastoq (Saint John River) in New Brunswick, Canada, along with end-point PCR for the presence of anaC, a critical gene for ATX biosynthesis. ATXs were detected in 88 % of samples with total ATX concentrations as high as 1,600 mg kg^{-1}. The anaC gene was detected in 90 % of ATX positive and 30 % of ATX negative samples, highlighting differences in the information provided by the two techniques. Important spatial and temporal trends in ATX levels are revealed that will inform management and study of this highly impacted river in the future. The developed methodology is broadly applicable to other quantitative or screening applications where high-throughput analysis of ATXs is required.

UPLC/MS/MS Detection for the Analysis of Cyanobacterial Toxins in Recreational Waters… Expanded Toxins

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Liquid Chromatography/Tandem-Mass Spectrometry (LC/MS/MS) is a powerful tool for the analysis of various analytes in a wide variety of matrices. What is especially attractive about LC/MS/MS is its sensitivity and selectivity. Microcystins, in particular, represent an emerging class of algal toxins of concern to the drinking water industry. Other potential toxins such as anabaenopeptins, have also been discovered in bloom samples. In this paper we investigate the use of smaller column packing (sub 2 µm particles) to both improve the selectivity, speed, sensitivity and resolution to screen for many of these toxins using a generic gradient method. Specifically microcystins, anatoxin-a, cylindrospermopsin as well as newer toxins, such as anabaenopeptins, deoxy cylindrospermopsin and micropeptin using Ultra-Performance Liquid Chromatography (UPLC®) combined with tandem mass spectrometry. We investigated the analysis of a wide variety of freshwater samples from throughout the United States. Examples showing additional toxins detected in freshwater samples will be presented.

Hemiaminal type saxitoxin analogues found in the scallop, *Patinopecten yessoensis*

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Two hemiaminal type saxitoxin analogues, M5-hemiaminal (HA) and M6-HA, were newly found in the scallop, *Patinopecten yessoensis*, by MS-based screening of new paralytic shellfish toxins (PSTs). M5-HA was isolated from the hepatopancreas of *P. yessoensis*, and the structure was determined based on NMR spectroscopic data, as well as comparison of spectral data with that of FD-STX, which is the previously reported synthetic STX analogue with similar hemiaminal structure. Another new analogue, M6-HA, was also identified in the *P. yessoensis* by using LCMSMS. M6-HA was suggested to be the carbamoyl N-sulfate analogue of M5-HA by the result that M5-HA was produced from M6-HA by acid hydrolysis. Furthermore, PSTs in the hepatopancreas of *P. yessoensis*, cultured in a bay located in North-eastern Japan, were chronologically analyzed in 2018. The highest concentrations of M1 (11-hydroxy gonyautoxin-5), M3 (11, 11-dihydroxy gonyautoxin-5), and M5-HA were observed two weeks after C-toxins reached their highest concentrations. This result suggested that M1, M3, and M5-HA are metabolites of C-toxins, and the metabolic pathways from C2 to M1, M3, and M5-HA are proposed.

Oxidative stress in marine annelids (*Laeonereis acuta*) induced by short-term exposure to *Prorocentrum* cf. *lima*

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We evaluated the accumulation of okadaic acid (OA), and it's potentially effects on *Laeonereis acuta* exposed to the OA - producing benthic dinoflagellate *Prorocentrum* cf. *lima*. Nontoxic microalgae, *Tetraselmis* sp., were used as a control diet. Algal cells were supplied to animals kept in an agar medium. To assess the significance of the observed effects, our experimental design treated the algal species, algal cell densities and exposure time. Toxin accumulation was measured by LC-MS/MS and responses of the organisms were assessed through oxidative stress biomarkers (glutathione-S-transferase-GST, catalase-CAT, reduced glutathione-GSH, Glutamate cysteine ligase-GCL and lipid peroxidation-LPO) in homogenates after 12, 24 and 72 h. Over the experiment, worms exposed to the toxic dinoflagellate gradually accumulated OA in their tissues (up to 83.79 ± 35.90 ng g^{-1}), with toxin levels directly related to *P*. cf. *lima* cell density. The most significant responses in animals fed with *P*. cf. *lima* were activation of CAT (affected by time × species of algae), increased LPO levels (affected by algae species × time × dose) and decreased GSH and GCL levels (affected by algae species and by time × dose × algae species). Higher LPO levels at 12 and 24 h of exposure, together with the activation of CAT and decreased GSH and GCL levels, clearly indicated oxidative stress in worms exposed to *P.* cf. *lima*. In addition, the increase in LPO levels, together with the activation of GST in 72 h for both high concentrations of *Tetraselmis* sp. and *P*. cf. *lima*, indicated that high cell density is a significant stress factor over time.

Improved extraction of multiclass cyanotoxins from soil and sensitive quantification with on-line purification liquid chromatography tandem mass spectrometry

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Cyanotoxins are associated with harmful cyanobacterial blooms, but also exist in biological soil crusts and soils irrigated with cyanobacteria-contaminated water. To achieve an accurate analysis of cyanotoxins in soil, effective extraction, purification and determination methods are imperative. The most challenging aspect is extracting cyanotoxins from soil, due to their tendency to bind strongly to the soil matrix. We used a methanolammonium acetate solution to efficiently extract 17 cyanotoxins (microcystins, cylindrospermopsin, anatoxins, anabaenopeptins and cyanopeptolin) from soil. The extract was purified by on - line solid - phase extraction coupled with ultra-high-performance liquid chromatography tandem mass spectrometry. The optimized procedure involved two ultrasonication cycles of 15 min with four mL of methanol and 200 mM ammonium acetate, which recovered 60 % to > 90 % of the added cyanotoxins from five soils with diverse organic matter, pH and texture. The method improved extraction by up to 10 times compared to a methanol/water solution. Linearity, accuracy and precision were validated on matrix-mixed soil with surrogate microcystin and cylindrospermopsin internal standards. Limits of detection were 0.001 - 0.3 ng g^{-1}, depending on the cyanotoxins. The method was used to analyze cyanotoxins in 25 field - collected soils from Quebec, Canada. Out of the 25 soil samples, 11 soils had at least one cyanotoxin, and up to eight different cyanotoxins were detected in one soil. The sum of all microcystins congeners was from 0.02 to 31 ng microcystins g^{-1} soil. We also detected anabaenopeptin, the first reported occurrence of this cyanotoxin in soil.

Spatial distribution and tracing source of phycotoxins in the South China Sea, China

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Marine phycotoxins severely threaten ecosystem health and mariculture. This study investigates the spatial distribution and source of diverse phycotoxins in the South China Sea (SCS), during four cruises between 2019 and 2020. Pectention-2 (PTX2) occurred with highest (93.5 %) detection rate (DR) during all cruises, especially in the Pearl River Estuary (PRE) in June 2019, peaking at 1,342 pg L^{-1}. Although gymodimine-A was ubiquitous, the highest DR and concentration were 26.7 % and 43 pg L^{-1}, respectively. Homo-yessotoxin (hYTX) and domoic acid (DA) were found during three cruises in August 2020, and high DR of hYTX (67.7 %, 29.3 %) and DA (29.0 %, 29.3 %) in the PRE and Guangdong coast, respectively, in June 2019 and 2020, peaking at concentrations of 777 pg hYTX L^{-1} and 38514 pg DA L^{-1}. Trace amounts of STX-group toxins including gonyautoxins-1-4 were frequently detected in the PRE and Guangdong/Hainan coasts, especially the former in June and October 2019 with DRs of 58.1 % and 46.7 %, respectively. The phycotoxin distribution demonstrated that DA-producing microalgae were concentrated close to the PRE and Guangdong coast, while hYTX producing microalgae occurred relatively far offshore. Microalgae producing PTX2- and STX-group toxins were more widely distributed in the SCS. High-throughput sequencing suggested that *Alexandrium pacificum* and *Gonyaulax spinifera* were the source of STX-group toxins and hYTX, respectively, while *Pseudo-nitzschia cuspidata* was the main source of DA. Widely distributed PTX2, hYTX, and DA were reported for the first time in the SCS. Associated ecosystems and human health risks need to be considered.

UPLC-MS/MS-based metabolomics and molecular networking reveal the chemical arsenal of three *Gambierdiscus* strains from the Philippines

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Gambierdiscus spp. are known producers of toxic compounds implicated in ciguatera fish poisoning (CFP), one of the most common foodborne diseases associated with seafood consumption in the world. Here, we present a comparative metabolomics analysis of three *Gambierdiscus* strains isolated from the Philippines, *G. carpenteri* Gam1BOL080513, *G. balechii* GtoxSAM092414, and *G. balechii* Gtox112513. Extracts of these strains were analyzed using ultrahigh pressure liquid chromatography-mass spectrometry (UPLC-MS) and tandem MS (MS/MS) analysis. The metabolomics profiling showed that all three *Gambierdiscus* strains produced maitotoxin-3 as the major toxin component. Global metabolite profiling through the GNPS molecular networking platform allowed us to identify the major compound classes and obtain insights into the chemodiversity of secondary metabolites from *Gambierdiscus* spp. A total of 45 clusters were identified, with terpene glycosides, carbohydrate derivatives, polyketides and macrolides being the most represented.

In vitro glucuronidation of Caribbean ciguatoxins (C-CTX-1/-2) in reef fish: First identification of conjugated CTX metabolites by UPHLC-HRMS (/MS)

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Ciguatoxins (CTXs) are potent neurotoxins associated with ciguatera poisoning, a leading cause of seafood-borne illness throughout the world. CTX precursors are produced by *Gambierdiscus* spp. and subsequently transferred through marine food webs, undergoing biotransformations that yield several related derivatives. Identifying CTX biotransformation pathways is a key step towards predicting the ecotoxicological fate of CTXs in marine ecosystems. Most evidence of CTX biotransformation comes from studies on the Pacific suite of CTXs, while pathways for Caribbean CTX (C-CTX) biotransformation remain unknown. To fill this knowledge gap, we investigated the *in vitro* biotransformation of C-CTX-1/-2 across several fish and mammalian species. Metabolism assays focused on Phase I (CYP) functionalization and Phase II (UGT) conjugation reactions, and UHPLC-HRMS (/MS) was used to aid in the identification of potential metabolites. While no Phase I products were observed, we identified two glucuronide product peaks forming in the MS-spectra of incubations with fish, but not mammalian, microsomes. Chemical reduction experiments confirmed that these metabolite peaks were comprised of four distinct glucuronide products, and excluded the C56 hydroxyl group as the conjugation site. Glucuronidation of CTXs has not been described before and this work provides the first evidence of C-CTX biotransformation thus far. Yet, the detection of glucuronidation products across all fish species tested indicates that this could be a prevalent detoxification mechanism in marine fish, with differing capacities potentially contributing to variable fish toxicities. The absence of glucuronidation observed here in both rat and human microsomes suggests that alternate biotransformation pathways could dominate in mammals, and may partly underlie human sensitivity to C-CTX poisoning.

The Regulation and Expression of Cylindrospermopsin by Cyanobacteria *Raphidiopsis raciborskii* AWT205

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Cyanobacterial harmful algal blooms (HABs) producing the hepatotoxin cylindrospermopsin (CYN) are the second most frequently reported HABs globally (second to microcystin). While the genetics and biosynthesis of CYN production have been well characterized in the cyanobacteria *Raphidiopsis raciborskii* and *Chrysosporum ovalisporum*, many aspects of the regulation of CYN production are yet to be resolved. This study investigates the regulation of CYN production in the Australian strain *R. raciborskii* AWT205 through *in silico*, transcriptional, and protein binding analysis. *In silico* analysis of the cylindrospermopsin biosynthetic cluster (cyr) reveals several putative promoters and transcription factor binding sites, in particular preceding cyrA, cyrC, cyrD and between cyrK and cyrH. Biotinylated probes of these promoter regions were then used to capture DNA-binding proteins through DNA-affinity Capture Assays (DACA). These assays showed the binding of global regulatory protein AbrB to the cyr cluster, supporting reports of the environmental regulation of CYN production through nitrogen availability. However, transcriptional studies observed a lack of correlation between cyr gene expression and the production of CYN analogues. The results of this study have contributed to clarifying the mechanisms by which the cyr cluster may be regulated at a transcriptional level. Nonetheless, the study demonstrated that the control of CYN production cannot be solely attributed to transcriptional regulation and alluded to additional mechanisms of regulation.

Localization of the saxitoxin biosynthetic enzyme, SxtA, in the *Alexandrium* chloroplasts, and its mutations in mRNA 3’UTR might lead toxicity reduction in a non-toxic mutant

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SxtA is the enzyme that catalyses the first step of saxitoxin biosynthesis. An immunofluorescent detection by a confocal laser scanning microscopy (CLSM) using an anti-SxtA peptide antibody revealed that compartmentalization of the immune-reactive protein in the cells of STX-producing dinoflagellates, *Alexandrium catenella* (Group I), *A. pacificum* (Group IV) and *Gymnodinium catenatum*, while no signal was detected in non-toxic species of *A. insuetum* and *Prorocentrum triestinium*. Co-localization of SxtA1 and SxtA4 with RuBisCO II in the CLSM observation and an ultrastructural confirmation of the antibody binding site by an immunoelectron microscopy strongly suggest association of SxtA with the chloroplasts. We also performed comparative analysis of toxic and non-toxic sub-clones of *A. catenella* (Group I) to elucidate possible mutation responsible for toxicity loss in the non-toxic sub-clone. Although copy numbers of the sxtA4 gene were indistinguishable in the toxic and the non-toxic sub-clones, mRNA and protein expression were significantly reduced in the non-toxic sub-clone, and sequence variation at the 3’ untranslated region (3’UTR) of the sxtA4 mRNA was found. Based on these findings, we propose that mutation in the sxtA4 mRNA 3’UTR leads to down-regulation of STX biosynthesis post-transcriptionally, thereby explaining the significant decrease in toxicity of the non-toxic *A. catenella* (Group I) sub-clone.

Contrasting high and low toxin strains of the Florida red tide dinoflagellate *Karenia brevis* by redox proteomics reveals differences in protein expression and global disparities in cysteine oxidation

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The Florida red tide organism, *Karenia brevis*, produces a suite of toxins known as the brevetoxins. These toxins are responsible for massive fish kills, marine wildlife poisonings, respiratory irritation among beachgoers and a syndrome called neurotoxic shellfish poisoning that results from the consumption of contaminated shellfish. The endogenous function of the brevetoxins is uncertain and has been the subject of considerable research and speculation. We have recently reported that two of the brevetoxins (PbTx-2 and -3) are inhibitors of the mammalian selenoprotein, thioredoxin reductase (TrxR). We further posit that brevetoxins are regulators of *K. brevis* TrxR. We have cloned and expressed TrxR from *K. brevis* and have determined that PbTx-2 inhibits the reduction of KbTrxR substrates with an IC50 in the micromolar range. We have further identified sites of TrxR adduction in both rat and K. brevis TrxR. Interestingly, PbTx-2 adducts different residues in these orthologs. TrxR regulates cellular redox homeostasis through its principal substrate thioredoxin (Trx). If PbTx-2 regulates TrxR activity, it follows that strains with differences in PbTx-2 cellular content should have observable differences in cellular redox status. Redox proteomics using iodo-Tandem Mass Tags can not only be used to identify differences in expression, but also differences in the redox status of the proteome. Comparative redox proteomics of two strains of *K. brevis* that differ in toxin content by ten-fold show large and significant differences in their proteomes with the proteome of the low toxin *K. brevis* more highly reduced.

Molecular networking as a novel approach to investigate the toxin diversity of harmful microalgal species of the genera *Dinophysis* and *Azadinium*

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Several species of dinoflagellates can naturally form Harmful Algal blooms (HABs) and are a major concern for public health due to their production of toxins. Knowledge on the chemical contents of these organisms is increasing but still largely understudied due to the genomic complexity of many groups. Classical analytical methods based on liquid chromatography coupled to mass spectrometry (LC-MS/MS) are routinely used to target known toxins in samples of interest. However, such targeted approaches can only detect previously known analogs and thus are not useful for the detection of novel compounds. In parallel, High Resolution Mass Spectrometry (HRMS) has emerged aiming at untargeted detection of analytes. The introduction of Molecular Networking based on HRMS allows to compare large scale data-dependent MS/MS spectra to highlight spectral similarities related to compound structural similarities. After a thorough optimization of acquisition parameters, this approach was applied on four French strains of *Dinophysis* corresponding to three major *Dinophysis* species (*D. acuta*, *D. caudata* and the "*D. acuminata* complex" species *D. acuminata* and *D. sacculus*) and led to the discovery of five potential new metabolites chemically related to PTX. This dereplication approach appeared as a powerful tool to identify unknown compounds based on MS fragmentation information and was further extended to *Azadinium* as a key species and model organism in an attempt to detect metabolites chemically related to known azaspiracids.

Response of fatty acids and lipid metabolism enzymes during accumulation, depuration and esterification of diarrhetic shellfish toxins in mussels (*Mytilus galloprovincialis*)

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In bivalves, free forms of diarrhetic shellfish toxins (DSTs) can be esterified with fatty acids to form acyl esters (DTX3). However, the effects of esterification of DSTs on fatty acid metabolism in mollusks remain poorly understood. In this study, mussels (*Mytilus galloprovincialis*) were fed *Prorocentrum lima* for ten days followed by an additional ten days depuration to track the variation in quantity and composition of DST acyl esters and fatty acids, and in the activities of lipid metabolism enzymes. A variety of esters of Okadaic Acid (OA) and DTX1 were mainly formed in the digestive gland (DG), although trace amounts of esters also appeared in muscle tissue. A large relative amount of OA (60 % to 84 %) and DTX1 (80 % to 92 %) was esterified to DTX3 in the DG. The DG and muscle tissues showed pronounced differences in fatty acid content and composition during both feeding and depuration periods. In the DG, fatty acid content gradually decreased in parallel with increasing accumulation and esterification of DSTs. The decline in fatty acids was accelerated during depuration without food. This reduction in the content of important polyunsaturated fatty acids, especially docosahexaenoic acid and eicosapentaenoic acid, would lead to a reduction in the nutritional value of mussels. Enzymes involved in lipid metabolism were actively involved in the metabolism of fatty acids in the DG, whereas their activities were weak in muscle tissue during the feeding period. This study will lay a foundation for the study of the relationship between toxin metabolism and lipid metabolism in shellfish.

Accumulation of cyanobacterial toxins in shellfish and irrigated crops

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Ingestion of contaminated food, such as aquaculture products, algal supplements and irrigated crops, might lead to human exposure to cyanobacterial toxins. In the Netherlands, crops cannot be irrigated with water in which the cyanobacterial toxin concentration exceeds 1 µg L^{-1}. However, more data are needed to underpin this guideline. We tested whether lettuce, beetroot and strawberries accumulate cyanobacterial toxins (microcystins, cylindrospermopsins and homoanatoxin-a) when irrigated with water containing cyanobacteria. Although the crops were exposed to toxin concentrations up to 170 µg L^{-1}, no toxins were detected in the edible parts of beetroot and strawberries. Only lettuce which was irrigated on the plant contained detectable amounts of microcystins. The fate of the toxins (adsorption to the soil, accumulation in inedible parts, biotransformation) is still under investigation. Cyanobacterial toxins can also accumulate in shellfish. In the Netherlands, some of the official production areas are located in a delta which receives an influx of freshwater. We performed a survey on the presence of cyanobacterial toxins in shellfish from these areas. Microcystins and anatoxins were only detected in a few samples, but cylindrospermopsins were detected in 60 of the 165 samples. The highest detected cylindrospermopsin concentration (sum cylindrospermopsin and 7-deoxy-cylindrospermopsin) was 64 µg kg^{-1}. Phytoplankton samples were taken at the same locations and times as the shellfish, but cylindrospermopsins were only detected in one of these samples. Also during routine phytoplankton monitoring, no high abundances of cyanobacteria were observed. The source of cylindrospermopsins in Dutch shellfish areas is therefore still unknown.

Seasonal variability of lipophilic toxin events at a "hotspot" in the Chilean fjords

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Blooms of toxin-producing microalgae are recurrent in the Chilean Fjords. Diarrheic shellfish poisoning (DSP) toxins (OA and congeners) and pectenotoxins produced by *Dinophysis* species, mainly *D. acuta* and *D. acuminata*, are accumulated by filter-feeders affecting public health and shellfish exports. The seasonality and the environmental conditions associated with these events, and the species-specific toxin profiles and contribution to shellfish toxicity are poorly known in the southernmost Chilean fjords. From January 2018 to July 2019, monthly surveys were carried out at a fixed sampling station in Puyuhuapi Fjord -a "hotspot" area for *Dinophysis* in Southern Chile- to describe water column structure, analyze phytoplankton communities and toxin profile and contents in seawater. Maximal concentration of DSP toxins (1,052 ng mL^{-1}) and PTXs (540 ng mL^{-1}) were associated with bloom development of *D. acuta* -cell maxima of 452,000 and 35,800 cells L^{-1} in February (austral mid-summer) 2018 and 2019, respectively. These maxima formed subsurface (6 - 8 m) thin layers by the pycnocline. *Dinophysis acuminata* was present in lower densities, with cell maxima of 6,600 and 4,400 cells L^{-1}. This species seemed to have a much lower contribution to shellfish toxicity and related only to PTXs. Concentrations of its putative prey, *Mesodinium rubrum* did not exceed 400 cells L^{-1}. Peaks of yesso toxins (YTX 1,400 ng mL^{-1}) were associated with the co-occurrence of *Protoceratium reticulatum* in February 2019. Winter cells density was very scarce, but toxins (mainly OA) were present throughout the year. These preliminary results show that maximal risk of DSP contamination in the southernmost Chilean fjords is linked to *D. acuta* blooms during maximal thermal stratification in summer.

Degradation and detoxification of cylindrospermopsin: 3D HepG2 cells as perspective model for hepatotoxicity evaluation

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Cyanobacteria are bloom-forming phototrophic prokaryotes that produce various toxic compounds, i.e. cyanotoxins. In recent years, due to increased eutrophication, massive harmful cyanobacterial blooms and global toxicity outbreaks occurred more frequently. This consequently increased the risk for cyanotoxincontaminated drinking water, which includes the potent hepatotoxin cylindrospermopsin (CYN). Here we investigated mechanisms of degradation of CYN by advanced oxidation processes (AOPs), namely Fenton and Fenton-like oxidation (i.e. Fe^{2+}/H_2O_2, Co^{2+}/peroxymonosulfate (PMS), and Ag+/ peroxydisulfate. We complemented the instrumental analyses with the assessment of the residual toxicity of CYN degradation products using an advanced 3-dimensional spheroid model of the human hepatic cell line HepG2. The spheroid cultures were adapted for the 96-well microplates and the multiparametric evaluation of spheroid size, growth and viability. The most effective CYN removal was achieved by Fe^{2+}/H_2O_2, driven by •OH radicals, and $Co^{2+/}$PMS, primarily driven by $SO^{4-•}$ radicals. However, underlying degradation mechanisms were similar for •OH and $SO^{4-•}$ and involved the same reaction processes. *In vitro* toxicity was substantially reduced during both of these AOP treatments. Although the results indicated that some of the formed degradation products may still have exhibited toxicity, the overall reduction of the toxicity together with the proposed degradation pathways showed that degradation of CYN significantly reduced its hepatotoxicity. Thus, the present study demonstrates that hepatospheroids are a cost-effective, and perspective model for the assessment of potential hepatotoxicity of CYN or other cyanotoxins, and their eventual degradation (by-) products.

Marine toxic species from the S and SE Brazil: field studies of phytoplankton composition and phycotoxins

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On the tropical (< 23 °S) and sub-tropical (> 23 °S) regions of Brazil, temperature, salinity, and mixture layer depth (MLD) have shown to provide conditions to potentially toxic taxa of dinoflagellates, diatoms, and cyanobacteria to thrive. This study aims to discuss the occurrence of toxin-producing species across the continental shelf and slope of S and SE zones of Brazil, highlighting new information. Independent datasets from research cruises performed on the shelf break (2012) and over the continental shelf (2018 - 2019) were used. Basically, species composition was derived from net and/or bottle samples and analyzed with an inverted microscope, while pigment and phycotoxins were obtained through High Performance Liquid Cromatography. Saxitoxins (STX)-producing dinoflagellates like *Gymnodinium* sp. and *Alexandrium* spp. occurred both in tropical and sub-tropical shelf waters and were linked to higher concentrations (3.59 - 4.29 μg STX L^{-1}) whereas on the shelf slope, lower concentrations were related only to *Trichodesmium* spp. (2.6 μg STX L^{-1}). Toxin concentrations varied and analogues content differed markedly between zones. For instance, gonyautoxins-2, -3, and -4 and neo-STX were found only in SE zone. Contrastingly, low amounts of domoic acid (DA) were detected across S and SE zones in 2018 - 2019 (0.12 - 0.30 μg AD L^{-1}). DA was related to *Pseudo-nitzschia pungens*, at least on the SE, while < 23 °S either *P. seriata* complex or *P. delicatissima* complex could be producing DA within subtropical waters. Despite environmental influence, potentially toxic species can have a pivotal role on phytoplankton community composition and toxin/analogue temporal differentiation and spatial distribution.

First evidence of biotransformation of AZA-38 and -39 in mussels (*Mytilus edulis*) fed with *Amphidoma languida* (Amphidomataceae)

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Azaspiracids are produced by several species of dinoflagellates from the family Amphidomataceae. Within the genus *Amphidoma*, *Am. languida* is the only species known so far to produce azaspiracids, all the other toxin producers within the family are belonging to the genus *Azadinium*. Strains from the NE Atlantic have been found to produce AZA-38 and -39. Recent biotoxin data from the Irish monitoring program suggests that several shellfish species could ingest and accumulate AZA-38 and -39. Successful large scale-culturing of *Am. languida* now for the first time allowed a 3-day feeding experiment using mussels (*Mytilus edulis*), which showed that azaspiracids from *Am. languida* accumulate in shellfish tissue. Mussels were fed with an algal suspension of *Am. languida* strain from the North Sea at cell concentrations of 30,000 cells mL^{-1} and mussels were harvested after 24 h periods. Cell counts of the algal suspension were performed at multiple time points within the first 6 h to assess rate of feeding. Unreported mussel metabolites of AZA-38 and -39 were identified that are formed by similar metabolic pathways, which undergo AZA-1 and -2 in blue mussels. Using UHPLC-HRMS levels of AZA-38, -39 and analogues of both were quantified at each time point. AZA-38 and -39 levels increased daily and combined, reached 89.4 $\mu g\ kg^{-1}$ of tissue on the third day. Although toxin concentrations in the tissue never reached regulatory limits, the study demonstrates that toxins from *Am. languida* can readily accumulate and transform in shellfish.

Impact of Zooplankton on Anatoxin-a Production by the Freshwater Cyanobacteria, *Dolichospermum*

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Blooms of the filamentous freshwater cyanobacteria *Dolichospermum* can produce potent neurotoxins such as anatoxin-a and saxitoxin that can cause death in both humans and wildlife. Zooplankton grazing has been identified as an important toxin production trigger for various harmful algal bloom species. Exposure to zooplankton such as *Daphnia* has been shown to cause up to a five-fold increase in intracellular toxin production in freshwater cyanobacteria such as *Microcystis*. Despite the potency of grazer-induced toxin production, no known study to date has investigated how grazer exposure impacts *Dolichospermum* toxin production. Here, we assessed whether exposure to *Daphnia* altered intracellular anatoxin-a concentrations in *Dolichospermum*. We directly exposed two different species of *Daphnia*, *D. magna* and *D. pulex* to an anatoxin-a producing *Dolichospermum* over several days. Results indicated that exposure to both *D. magna* and *D. pulex* significantly increased the intracellular anatoxin-a content of *Dolichospermum*. Results regarding the dependence of grazer controlled increases in anatoxin-a concentrations on biotic and abiotic factors will also be discussed. Accounting for grazer impact on toxin production will provide valuable information on how zooplankton communities may impact algal toxin concentrations in natural ecosystems and can provide insight regarding the ecological cause of toxin production in HABs.

Interactions of *Noctiluca scintillans* with the toxic dinoflagellates *Dinophysis acuta* and *Alexandrium minutum*: toxins and allelopathic effects

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The heterotrophic dinoflagellate *Noctiluca scintillans*, a common red tide producer in the Galician Rías (NW Iberian Peninsula), was isolated from a summer bloom in Vigo. Individual cells were collected and kept in culture to examine the interactions with toxic dinoflagellates responsible of DSP and PSP events in the area (*Dinophysis acuta* and *Alexandrium minutum*). Experimental design included separate incubations of *N. scintillans* (1 cell mL^{-1}) with two levels of *D. acuta* (11 and 32 cells mL^{-1}) and *A. minutum* (958 and 2,873 cells mL^{-1}), for 3 days at 19 °C respectively. Control cultures of the three species, with the same cell densities, were incubated under equal conditions. Daily cell counts were performed in triplicate samples for each treatment and control cultures. Triplicate samples for Paralytic and Diarrhetic Shellfish toxins were taken at day 3. Contrasting results were found for each toxic species. *Dinophysis acuta* cell numbers were lower in the cultures exposed to *N. scintillans* than in the corresponding controls, suggesting ingestion of *D. acuta* by *N. scintillans*. Regarding the *A. minutum* experiments, deleterious effects (death of *N. scintillans* cells) were observed in both levels assayed. Methanolic extracts of cellular DSTs were analyzed by LC-HRMS. Toxin profile of *D. acuta* was characterized by the dominance of PTX2. Low OA levels were detected but not quantified due to the presence of interferences, therefore only PTX2 results were evaluated. An increase of PTX2 levels in *D. acuta* cultures exposed to *N. scintillans* was observed compared to blank cultures, suggesting a potential relationship with defense mechanisms against grazing.

Biomarkers of brevetoxin exposure in lymphoblast cells

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Exposure to the Florida red tide dinoflagellate *Karenia brevis*, has been linked to oxidative stress in marine life. *Karenia brevis* produces a suite of neurotoxins collectively known as the brevetoxins which are responsible for the poisoning of marine animals and humans. Our studies with the most abundant of the brevetoxins, PbTx-2 have established that PbTx-2 increases the expression of the inflammatory cytokine IL-8, and the reduced GSH content in lymphoblast cells. Both are associated with oxidative stress. Redox proteomics and metabolomics were carried out on lymphoblast cells treated with PbTx-2 and compared to control cells. Differences in metabolites, gene expression and cellular redox status were observed. We shall further examine the ability of antioxidants to alleviate the brevetoxin induced oxidative stress. These findings may be used to develop a biomarker profile for brevetoxin exposure in marine animals and could lead to a mechanism-based treatment for exposure to brevetoxins.

A fast and high sensitivity liquid chromatography mass spectrometry method coupled with a pre-concentration online system for the analysis of lipophilic toxins directly in phytoplankton cultures or se

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Prevalence and incidence of the marine toxins (PSP, ASP, and lipophilic toxins) including the so-called emerging toxins (these are, gymnodimines, pinnatoxins, or spirolides among others) have increased in recent years all over the world. Climate change, which is affecting the distribution of their producing phytoplankton species, is probably one of the main causes. Early detection of the toxins present in a particular area and linking the toxins to their causative phytoplankton species are key tools to minimize the risk they pose for human consumers. The development of both types of studies requires fast and high sensitivity analytical methods. In the present work, we have been developed a high sensitivity LC-MS/MS methodology, using a column with fused core particles technology, coupled with a pre-concentration online system, for the determination of toxins directly in phytoplankton cultures or seawater samples. With the method developed fourteen lipophilic toxins can be concentrated more than 25x and quantified in 3.6 min. This analysis method will be useful in the detection of trace levels of toxins and searching and isolating the potential phytoplankton producers of the type of toxicities detected in the challenge samples.

New insights on bioavailability and toxicity of microcystins produced by cyanobacteria using *in silico* ADMET methodology

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In silico methodologies have been used in the discovery of new drugs to evaluate their safety as well as in predicting potentially toxic effects not yet analyzed by *in vivo* methodologies. The software ADMET Predictor™ was used in this work to predict toxic effects of microcystins variants MC-LR, MC-YR, MC-RR, and MC-HarHar. Most of the models in this tool were assessed in rodents and humans. Noteworthy results in rodents include that all analyzed variants were carcinogenic; the respiratory sensitivity model differentiated microcystins into two categories (sensitizing [MC-LR and -YR] and non-sensitizing [MC-HarHar and -RR]); median lethal dose (LD_{50}) by oral ingestion indicated that MC-HarHar was the most toxic variant and MC- LR was the less toxic one. Main results in humans showed that variants MC-LR and MC-RR were considered to induce phospholipidosis and MC-LR has the highest probability of crossing lipoprotein barriers (MC-LR> YR> -HarHar> -RR). The permeability of microcystins in rabbit cornea allows inferring that the same event could happen in humans. Considering the bioavailable models under evaluation, microcystins lead to more biological effects in rats than humans, indicating significant differences between the experimental models. This result pointed out that extrapolating rodent data to humans ought to be conducted with caution. Our results indicate that ADMET in silico simulations proved to be a useful tool in adding new information regarding toxicity and bioavailability of microcystin variants as well as in contributing to the reduction of experiments using animals.

Multi-class Cyanobacterial Toxin Screening using Liquid Chromatography-High Resolution Mass Spectrometry

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Cyanobacteria can produce diverse classes of toxins including microcystins (MCs), anatoxins (ATXs), cylindrospermopsins (CYNs), and saxitoxins (STXs). These hepatotoxins and neurotoxins cover a range of polarities and molecular weights (160-1400 Da), generally requiring multiple preparation and chromatographic techniques for evaluation by mass spectrometry. We present the development of a single untargeted liquid chromatography - high resolution mass spectrometry screening method, encompassing all of the aforementioned cyanobacterial toxin classes. High resolution MS/MS settings were established on a Q Exactive mass spectrometer for untargeted analysis using data-dependent MS/MS acquisition with positive/negative polarity switching. Hydrophilic interaction liquid chromatography with gradient elution gave good retention of relatively non-polar microcystins (MC-LA = 5 min), while polar saxitoxins eluted before 25 min (30 min total run time). Identification was achieved using retention time matching and spectral comparison with reference materials. Combined toxin extraction methods were investigated using an in-house blend of toxic cyanobacteria. Linearity, precision, and detection limits were evaluated using calibration curves prepared in cyanobacterial extracts. Limits of detection range from 7 ng mL^{-1} ([Dha7] MC-LR and ATX) to 20 ng mL^{-1} (dcSTX). Recoveries were evaluated using spiked algal extracts and in-house reference materials. The method was applied for screening cultures of toxic and non-toxic cyanobacteria, as well as bloom and benthic algal samples collected in Canada. Conditions were also found to be suitable for detection of other classes of polar marine algal toxins including domoic acid and tetrodotoxin, offering potential utility in estuarine environments impacted by both marine and freshwater phycotoxins.

The global distribution of microcystins and their detection methods

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Freshwater cyanobacterial blooms are a global problem. The most common bloom taxon, *Microcystis*, occurs on every continent and produces a complex array of microcystins, with at least 279 known congeners. These toxins cause mortality and morbidity in humans, livestock, and other animals, so knowledge of their presence is important. However, the variety of congeners can make microcystins difficult to detect. Although there are several detection methods in use, no single method yet developed can detect all known congeners from a single sample. This in-depth meta-analysis examined reports of microcystins from environmental samples across the globe, recording their location, timing, methods used to detect them, cyanobacterial genera present, and whether any target organisms were found with the toxin in their tissues. We also examined the global the distribution of microcystin reports compared with the human population distribution. The results demonstrated that field sampling of microcystins has been concentrated in a few regions, while large areas of high human population density have few or no studies examining the presence of microcystins. The results also support the efficacy of using methods that detect the ADDA moiety, as ADDA was present in > 99 % of the reported microcystins. Our analysis revealed a need for more research on the occurrence of congeners other than microcystin-LR, the most commonly monitored congener, a need for more studies on certain cyanobacterial genera, and more field sampling of plant and animal tissue for the presence of microcystins.

Development of a dietary supplement reference material for multiple classes of cyanobacterial toxins

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Some algal dietary supplements are prepared using cyanobacterial biomass obtained from open air lakes, where multiple algal species can co-occur. Studies have shown that this can result in source materials and final products containing cyanobacterial toxins that present a risk for human health. Reference materials are therefore required to assist in the development, validation and quality control of analytical methods used for raw material and product testing for both research and regulatory purposes. Following a successful feasibility study, a project has been initiated to prepare a certified reference material (CRM) by blending biomass of a variety of toxic, cultured cyanobacterial species with a typically-consumed non-toxic species (*Aphanizomenon* sp.). The toxin profile of this material will include microcystins (MCs), anatoxins (ATXs), cylindrospermopsins (CYNs) and saxitoxins (STXs). The freeze-dried, homogenized matrix will be aliquoted into units representative in composition of an *Aphanizomenon* dietary supplement. This presentation summarizes current project progress including strain selection, bulk culturing and processing, toxin profiling and CRM design. The strategy for final material characterization will be outlined with consideration for exhaustive toxin extraction, analysis by liquid chromatography-mass spectrometry and approaches for matrix effect compensation including isotope dilution and standard addition. Certified values will be assigned where equivalent calibration CRMs are available and information values will be provided for additional cyanotoxins and secondary metabolites of interest.

Comparison of four Microcystin kits from Beacon Analytical System Inc.

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Cyanobacterial harmful algal blooms (CyanoHABs) occur frequently throughout the world. *Microcystis aeruginosa* is one of the algae responsible for producing CyanoHABs toxins. Current test kits reported their results as MC-LR equivalents based on the ELISA protocol. It is important to note that quantification is not consistent among eighty Microcystin variants. The Beacon Microcystin plate kits are competitive enzyme-labeled immunoassay with the limit of detection 0.1 ppb. Beacon Microcystin LR plate kit Cat. # 20-0068 was quantify MC-LR and thus was most sensitive to this variant with some cross-reactivities (CR) with other MC congeners (RR, YR, LA, LY, LF, LW and Nodularin). Beacon Microcystin BX plate kit Cat. # 20-0300 have higher CR than Cat. # 20-0068. Recently we developed two more microcystin plate kits with MMPB and ADDA related haptens. We tested the CR of these four kits with calibrators from ENZO Bioscience (LR, RR, YR, LA, LY, LF, LW and Nodularin). The results show: MC-LR as 100 %, CR with RR/NOD/LW/LY/LA/YR/LF, for # 20-0068: 78 %, 127 %, 88 %, 72 %, 77 %, 105 %, and 76 %; microcystin-BX: 85 %, 72 %, 74 %, 63 %, 49 %, 89 %, and 63 %; MMPB format: 102 %, 156 %, 272 %, 272 %, 142 %, 93 %, and 315 %; ADDA related format: 58 %, 98 %, 210 %, 117 %, 237 %, 107 %, and 124 %. We tested eight of recreational water samples for Microcystins, the recoveries are 63 % to 99%, 65% to 125%, 78 % to 118 %, 94 % to 250 %.

Diatom cells and ocean waters ontain the building blocks for Domoic Acid Toxin

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Pseudo-nitzschia is a diatom genus in coastal oceans worldwide. Some *Pseudo-nitzschia* species biosynthesize domoic acid (DA), which accumulates in shellfish and is neurotoxic to mammals. DA biosynthesis was recently decoded, establishing the dab cluster of transcriptionally co-regulated genes (dabA, dabB, dabC, dabD) that encode enzymes that perform all but one step of the biosynthesis. By synthesizing stable isotope di-labeled intermediates of each proposed biosynthetic intermediate and feeding them to a live culture of *Pseudo-nitzschia*, we established which intermediates are on-pathway to making DA by screening for production of di-labeled DA. We have probed for these steps in both the toxic *Pseudo-nitzschia australis* and the non-toxic *Pseudo-nitzschia delicatissima* to understand species-specific differences in metabolic capability. Confirmation that each of these biochemically established steps are functional *in vivo* allows for future probing for the final hypothetical biosynthetic gene which encodes an isomerase enzyme to produce the hallmark toxin, DA itself. Understanding the metabolic capability of a non-toxic *Pseudo-nitzschia* also allows insight into the lack of universality of the dab gene cluster.

New methods for the characterization of cyanotoxins in lakes: identification of new congeners and abundance of anabaenopeptins

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The growing number of cyanobacteria proliferation episodes is a sign of the increasing eutrophication of water bodies. These harmful cyanobacteria produce cyanotoxins where the toxicity is historically associated with microcystins. However, other bioactive cyanopeptides are equally abundant, and over 500 cyanopeptides have thus been structurally identified, including over 279 microcystins. There is therefore a lack of knowledge about their production and their ecotoxicological impact. An analytical method based on high-resolution mass spectrometry has been developed to target twelve microcystins and two anabaenopeptins in lake water samples during cyanobacterial bloom episodes. Then, a non-targeted analytical method was used and the data obtained were compared to an in-house database including a theoretical list of microcystins and anabaenopeptins. Up to eleven microcystin congeners were detected at concentrations varying from ng/L to ug/L, and samples with high microcystins concentrations systematically contained anabaenopeptins. Three microcystins and six anabenopeptins were identified from the database, including four new congeners. In parallel, a commercial kit for the analysis of total anabaenopeptins, based on an enzyme-linked immunosorbent assay (ELISA), was utilized on samples from cyanotoxins-contaminated lakes with. The presence of false positives was observed in the ELISA-results when compared to mass spectrometry due to the presence of other cyanopeptides, but 73 % of the contaminated samples still contained anabaenopeptins. Considering these results, many microcystin congeners can go under the radar if they are not appropriately monitored. Anabaenopeptins can be abundant in toxic cyanobacteria blooms, indicating that their presence could affect the toxicological interpretation of harmful cyanobacterial blooms if not followed.

TAXONOMY AND SYSTEMATICS

Toxic diatoms in Antarctic waters? – diversity and toxicity

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The Southern Ocean (the ocean encircling Antarctica) is one of the most productive marine ecosystems, covering about 10 % of the global ocean surface, and serving as feeding ground for sea birds and mammals. Diatoms make up a crucial part of the basis of the food web, and hence composition and toxicity of diatoms are important. The diatom *Pseudo-nitzschia* plays a prominent role in the Antarctic marine ecosystem, and on top of that, some of the species have the potential to produce the neurotoxin domoic acid. In spite of this, *Pseudo-nitzschia* diversity and toxicity have scarcely been explored in Antarctic waters - toxic strains have not previously been recorded from Antarctic waters, and morphological, molecular and toxinological characterizations of Antarctic species are surprisingly limited. On two separate cruises to the Southern Ocean, monoclonal strains of *Pseudo-nitzschia* were established. Morphological and molecular analyses identified two *Pseudo-nitzschia* species new to Science, in addition to several previously reported species. For the first time, domoic acid was confirmed present in *Pseudo-nitzschia* strains from Antarctic waters. The results underline the need for further studies of the Antarctic toxic diatom flora and related toxins, especially in the light of the ongoing global climate changes.

Taxonomic investigation of *Pseudo-nitzschia pungens* varieties and proposal of a new species

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Pseudo-nitzschia pungens is a cosmopolitan pennate diatom that has been given much attention in domoic acid studies. It comprised three taxonomic varieties: *P. pungens* var. *pungens*, *P. pungens* var. *cingulata*, and *P. pungens* var. *aveirensis*. The varieties are differentiated based on the variation in the cingular band structure and the phylogenetic positions. In this study, 15 isolates of *Pseudo-nitzschia* that resembled *P. pungens* were established from the western coast of Sarawak, Malaysia Borneo. The frustule morphology was observed under transmission electron microscopy. Our results revealed two morphotypes. A morphotype is morphologically similar to *P. pungens* var. *pungens* and another morphotype exhibits a unique valve shape, which inflate in the middle and gradually tapering to both apices. Also, it composed mostly round oval poroids in the valvocopula. For this, the latter merits a separate taxonomic classification at species level. The species delineation was supported by the phylogenetic inferences of the large subunit and internal transcribed spacer (ITS) of ribosomal RNA gene. The inferences revealed a distinct lineage that form a sister clade with *P. pungens* varieties. ITS2 transcript comparisons of the new species revealed up to seven hemi-compensatory base changes when compared to *P. pungens* varieties. Our results of mating experiments showed sexual incompatibility between the new species and other *P. pungens* strains, further supported the new species identity.

Bloom of *Pseudo-nitzschia pungens* with the possible description of a new variety associated with a massive mortality event of fish and invertebrates

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The occidental coast of Baja California is an important zone for species with high value fisheries such as lobsters and shrimps. In June - July 2019 a massive oyster mortality was reported in a small fishery village Las Barrancas and was associated to a phytoplankton bloom. Besides oyster mortality, lobster and abalone were found stranded in the beach. The bloom specie was *Pseudo-nitzschia* sp. in an approximate extension of 10 km along the coast. Mortality could be associated with a drop in oxygen in the area and not with the production of domoic acid. Cells were isolated for SEM and phylogenetic analysis. DNA, extracted from cultures grown exponentially phase. The analyses were performed only in ITS2 of the ITS region and the secondary structure of ITS2 was predicted. Cell morphology agree with those of *P. pungens* var *cingulata* with slight variation. The phylogenetic trees based on ITS2 revealed the same clades of *Pseudo-nitzschia* species and cluster with *P. pungens* and *P. multiseries* as sister clade with values over 94 % of similarity for the analyzed ML, MP and MB. The secondary structure of ITS2, a four-helix structure (I–IV) with an additional helix, IIa, was identified, 13 single nucleotide polymorphisms (SNPs) and 1 CBC (Compensatory Base Change) in the helix IV. It is possible that it is a new variety of *P. pungens*.

Phenological segregation suggests speciation by time in the cryptic marine diatom *Pseudo-nitzschia allochrona* sp. nov.

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The mechanisms underlying the emergence of new marine microbial species are poorly understood in the plankton realm, where the absence of physical barriers and relatively homogeneous environmental conditions constitute a hindrance to allopatric and ecological speciation. The cryptic diatom *Pseudo-nitzschia allochrona*, discovered in the Gulf of Naples (GoN), is proposed here as a possible case of speciation by temporal segregation. This new species of the *P. delicatissima* - complex distinctly differs from other species based on four phylogenetic markers (18S rDNA, LSU rDNA, ITS, and rbcL), while the ITS2 secondary structure and mating experiments support its reproductive isolation from its closest relative, *P. arenysensis*. Domoic acid was not detected in two strains of the species. Numerous isolates (> 250 over > 10 ys) and metabarcoding data from the area show that *P. allochrona* invariably occurs from early summer through autumn, while the other *P. delicatissima*-like species (*P. delicatissima*, *P. dolorosa* and *P. arenysensis*) are only recorded in winter - spring. Data from a multi-decadal time-series indicate temperature and nutrient levels as the main factors differentiating the ecological niches of *P. allochrona* from that of the sister species of the GoN. Based on its peculiar and recurrent seasonality, we propose that *P. allochrona* may have evolved in sympatry by occupying a phenological niche different from its closest relatives, whereby the temporal dimension would offer good chances for isolation in a spatially homogeneous environment. This case of possible speciation by time shows the relevance of combining ecological time series with molecular information to shed light on the eco-evolutionary dynamics of marine microorganisms.

Using Molecular Criteria When Describing Dinoflagellate Species

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Background and Methods Dinoflagellates are best known for species which form harmful algal blooms (HABs). Adverse ecological, economic and health impacts of these HABs, along with evidence for morphologically similar species having significantly different toxicities, have prompted numerous taxonomic investigations regarding these species. Research has shown morphological characters alone are sometimes insufficient to delineate species. This has led to increased reliance on molecular characters - primarily ribosomal (rDNA) gene sequences – when defining dinoflagellate species. Despite this increasing reliance on genetic data, no codified rules exist for evaluating when and how rDNA gene phylogenies should be used in describing dinoflagellate species or whether multigene gene phylogenies would prove more reliable. To begin addressing the rDNA versus multigene phylogeny issue, we obtained transcriptomes for seven *Gambierdiscus* species and constructed phylogenies for these and closely related species using rDNA sequences as well as two independent multigene phylogenies. We also reviewed rDNA phylogenies from > 120 different genera (> 450 publications, > 500 species) to document how successfully various rDNA regions delineated described species. Conclusions The transcriptomic results show the D1-D3 rDNA region phylogenies reliably identifies *Gambierdiscus* and *Alexandrium* species as well as multigene phylogenies. The literature review further demonstrates D1 - D3 rDNA phylogenies, when corroborated by a corresponding ITS / 5.8S phylogeny, successfully delineated > 95 % of the reviewed species. Based on these findings, we propose a systematic approach for using morphology, in combination with D1-D3 / ITS rDNA phylogenies, to define species and to recognize and address cases (mainly recently diverged species) when rDNA sequences fail to discriminate species.

Diversity and production of diarrhetic shellfish toxins by benthic *Prorocentrum* species from Brazil

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Benthic *Prorocentrum* species are commonly associated with the production of various marine toxins. As many taxonomical problems have been reported within this genus, comprehensive field surveys in coastal areas with high species diversity, like the Brazilian coastline, can help solving some of these issues. Here we investigated the morpho-molecular taxonomy phylogeny (using ITS region and D1-D3 of LSU rDNA), and diarrhetic toxin production of 23 strains of *Prorocentrum* spp. isolated from Northeast and Southeast Brazil. Samples were collected from variable sites at different depths (down to 40 m), searching for the most contrasting morphotypes during monoclonal culture establishment. As a result, *P. leve* and *P. panamense* (one strain each) were reported for the first time in Brazil. We also evaluated the phylogeny and morphology of *P. borbonicum* (n = 2 strains), *P. caipirignum* (n = 4), *P. hoffmannianum* (n =2), *P. mexicanum* (n = 3), two clades of *P. lima* complex (n = 8) and the unidentified species *Prorocentrum* sp. type 2 (n = 2). Okadaic acid and/or DTX-1 were detected in all evaluated strains and species, except in *P. borbonicum* (no toxin assessment in *P. leve*). Toxin profile varied among species, with *P. caipirignum*, *P. hoffmannianum*, *P. mexicanum* and *Prorocentrum* sp. type 2 producing only okadaic acid. This latter species was morphologically distinct from other similar species belonging to the *P. lima* complex, which was also supported by genetic divergence. A major effort at global scale must be taken to solve the taxonomical problems within *P. lima*, *P. caipirignum*, *P. hoffmannianum* and similar species; obtaining strains from poorly explored locations should help in this task.

Diversity and distribution of non-toxigenic species of *Azadinium* (Dinophyceae) in Irish waters, North East Atlantic, including the characterization of two new species

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The diversity and distribution of *Azadinium* species in Irish waters is poorly understood. Here we present a morphological and molecular characterization of two new non-toxigenic *Azadinium* species described from the North East Atlantic from multiple new strains isolated during the 2018 Heincke 516 survey: *Azadinium galwayense* sp. nov. and *Azadinium perfusorium* sp. nov. *Azadinium galwayense* differed from other *Azadinium* by a characteristic combination regarding the presence and location of the ventral pore (vp; on the right side of the pore plate), of a pyrenoid (located in the episome), and by a pentagonal shape of the median anterior intercalary plate 2a, and lack of contact between plates 1'' and 1a. *Azadinium perfusorium* shared the same vp position as *Az. galwayense* and differed by a characteristic combination of a pyrenoid located in the hyposome, a tetragonal shape of plate 2a, and a relatively large size of the two lateral anterior intercalary plates. Molecular phylogeny confirmed the distinctiveness of these two new species and their placement in *Azadinium*. Also, one strain of *Azadinium caudatum* var. *margalefii* (first strain for the area) confirmed non toxigenicity of Atlantic populations of this species. Another strain was designated as *Azadinium* cf. *zhuanum* with similarities to *Az. zhuanum* described from China but differed from the type strain in nucleus position, by the dominant number of apical plates, and by significant differences in rRNA gene sequences. The present findings significantly increased knowledge on the diversity of *Azadinium* species in the North East Atlantic.

Characterization of toxin-producing strains of the *Dinophysis acuminata* - complex, *D. acuta*, *D. caudata* and *D. tripos* isolated from French coastal waters

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Among dinoflagellates, several species of the genus *Dinophysis* produce potent lipophilic toxins and have been implicated as the causative organism of Diarrhetic Shellfish Poisoning (DSP) in French coastal waters since 1983. A set of 30 monoclonal cultures including strains of the *D. acuminata*-complex, *D. acuta*, *D. caudata* and *D. tripos* have been isolated from French coasts including the English Channel, the Atlantic Ocean and the Mediterranean Sea between 2015 and 2019. Molecular identification based on the sequences of the mitochondrial gene cox1 revealed two groups of strains differing by only one substitution in the D. acuminate complex, but SEM analysis of various strains showed a large range of morphological variations. Based on geographical origin and morphology, strains of the subclade A were ascribed to '*D. acuminata*' while those of the subclade B were ascribed to '*D. sacculus*'. Nevertheless, the distinction into two separate species remains questionable and was not supported by our genetic data. The LC-MS/MS analysis showed species-conserved toxin profiles for *D. acuta* (DTX2, OA, PTX2), *D. caudata* (PTX2) and *D. tripos* (PTX2), irrespective of geographical origin. While two different toxin profiles were observed within the *D. acuminata*-complex: the strains from the English Channel and the Atlantic Ocean putatively identified as '*D. acuminata*' (subclade A) produced only OA while strains from Mediterranean Sea/Atlantic Ocean putatively identified as *D. sacculus* (subclade B) contained PTX2 as the dominant toxin, with OA and C9-esters also being present. This difference in toxin profiles has consequences in terms of public health, and on their respective environmental impact.

New potentially toxic epiphytic dinoflagellates from the Great Barrier Reef, Australia

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The benthic and epiphytic dinoflagellate flora of Australian temperate and tropical waters has been historically under-sampled. With a few notable exceptions, most studies target specific species/groups, leaving most epiphytic genera undescribed and uncultured. During field collections targeting CTX-producing species of *Gambierdiscus*, an opportunity arose to culture and examine other epiphytic dinoflagellates. Here we describe the morphology and phylogeny of 32 cultures isolated from reef epiphyte communities ranging from 18.4 to 18.7 °S in latitude, bordering Orpheus, Pelorus and Curacoa Islands, and Bramble Reef on Australia's Great Barrier Reef (Queensland). Our collection included six genera and eleven species including *Coolia tropicalis*, *C. areolata*, *Ceratocorys malayensis*, *Prorocentrum lima* and *P. sculptile*, and five new or currently undescribed species of *Amphidinium* (1), *Gymnodinium* (1), *Ostreopsis* (2), and *Prorocentrum* (1). Phylogenetic analyses of LSU - rRNA demonstrated that *C. areolata* sequences cluster close to but distinct from *C. canariensis*. While morphologically similar to *Amphidinium operculatum*, *Amphidinium* OIRS46 was highly divergent from other *Amphidinium* (max. 82 % identity), branching near. The base of trees positioned between *A. operculatum* and *A. herdmanii*. Cultures of *Gymnodinium* (OIRS8, OIRS39) were photosynthetic highly dorso-ventrally flattened, and composed primarily of two-cell chains. Phylogenetic analyses clustered this species among other *Gymnodinium* (*sensu stricto*), allied with *G. dorsalisulcum*. All *Ostreopsis* strains clustered into one of three distinct rRNA genotypes; two *Ostreopsis ovata*-related genotypes or a sub-cluster of *Ostreopsis siamensis*. The high proportion of new records and undescribed species from a small opportunistic collection confirm the considerable undocumented species diversity of the benthic and epiphytic dinoflagellate flora in Australian tropical waters.

A Rapid fluorescence approach on differentiation of typical dinoflagellate of East China Sea

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Attempts to classify phytoplankton populations on the base of absorption or fluorescence spectra were successfully deployed in the past few years, however, the results are in a rough discrimination of big classes of phytoplankton. To better character each genu of dinoflagellates, especially the typical dinoflagellate at East China Sea, *Prorocentrum donghaiense*, *Amphidinium carterae*, *Alexandrium tamarense*, *Scrippsiella trochoidea*, *Karenia mikimotoi* as well as the diatom *Skeletonema costatum* were selected for developing a rapid fluorescence method on differentiation of these algal populations in the lab. Initial fluorescence excitation spectra were measured (under variable environmental conditions and different growth stages) under 3Dfluorometer. Then fingerprints of these algae were characterized by seven-point excitation spectrum with the excitation wavelengths of 405, 435, 470, 490, 535, 555 and 590 nm and slope values that were chosen according to the hierarchical clustering method. The correct discrimination ratios (CDRs) of each single sample can exceed 95 % after corrected by Chemtax (a program based on least square regression and steepest descent algorithm). As for the mixed two species samples, the CDRs could reach over 90.0 % for the dominant species when the concentration as low as 500 cells/ml. Finally, non-negative Least Square method were used to quantify the concentration of each species. The relative error of concentration of single algae was 8.7 % - 17.6 % and two mixed samples of 9.6 % - 30.7 %. The overall concentration detection error was no more than one order of magnitude, indicating that this method could provide an important technical support for monitoring these algal blooms. Further in situ progress will be conducted.

Harmful algal bloom of the dinoflagellate *Blixaea quinquecornis* (Abé) Gottschling in bays of North - Central Peru

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The first record of a harmful algal bloom caused by the dinoflagellate *Blixaea quinquecornis* (Abé) Gottschling, 2017, in coastal waters of the Peruvian sea, is presented. This species has been traditionally considered to be distributed in tropical, marine, and estuarine areas. The positive species identification was done by using Light (LM), Scanning Electron Microscopy (SEM), and Differential Interference Contrast (DIC) microscopy. The thecal formula is: pp, 3', 2a, 7'', 5c, 4s, 5''' and 2''''. *Blixaea quinquecornis* was found in Sechura, Miraflores and Paracas bays during the 2014-2016 period, mainly with densities of 3.2×10^5 cells L^{-1} (Miraflores), 2.11×10^5 cells L^{-1} (Paracas), and 4.11×10^4 cells L^{-1} (Paracas) in autumn of 2016. *B. quinquecornis* was found alternating with an algal bloom produced by the atecate dinoflagellate *Akashiwo sanguinea*. The physicochemical variables associated with the bloom of *B. quinquecornis* were SST (18.5 to 26.6 °C), salinities (34.7 to 35.325), pH (7.32 to 12.5), and dissolved oxygen (3.8 to 12.5 mL L^{-1}). The bloom of *B. quinquecornis* in Miraflores Bay was positively associated with the increase in SST, salinity and negatively with the concentration of nitrates, while the algal blooms of *A. sanguinea* were positively correlated with the concentration of phosphates and oxygen. The record of the *B. quinquecornis* blooms in the 3 bays coincided with unusual warming events in the sea associated with the El Niño phenomenon 2015 - 2016.

Molecular identification of *Alexandrium pseudogonyaulax* from Bahía de La Paz, Mexico

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The Gulf of California is recognized as an area with a high primary productivity. Dinoflagellate species of the genus *Alexandrium* such as *A. catenella* and *A. ostenfeldii*, that produce hydrophilic and lipophilic toxins inhabit in this region. In April 2019, from a water sample collected in southern Bahía de La Paz individual cells were identified morphologically as *Alexandrium pseudogonyaulax*. To corroborate the identification, monoclonal cell cultures were grown with GSe medium, at 24 ± 1°C at a salinity of 34, under a 12:12 h L:D cycle at 150 µmol photons $m^{-2}s^{-1}$. Molecular identification was performed amplifying rDNA regions: 28S, 18S and 5.8S, with rDNA universal primers. The length of the sequences was 682 bp, 1657 bp, and 493 bp, respectively. The algorithms Maxima Parsimonia Maximum Likelihood and Bayesian Inference were used to perform phylogenetic analyzes. The best substitution model selected for 28S and 5.8S was TrN + G, and GTR + G for 18S sequences. The strain from Bahía de La Paz was included within the *A. pseudogonyaulax* clade, with all the molecular markers. *Alexandrium pseudogonyaulax* produces Goniodomin A, a lipophilic toxin with hemolytic activity, this species is recognized as ichthyotoxic. In Mexico *A. pseudogonyaulax* has been reported in the Pacific coast and the Gulf of California, although there is no other molecular data or toxinology studies in this species. This strain will be used to determine its toxin profile, toxicity in animal and cellular models, as well as for allelopathic interactions with other microorganisms.

Morphology, pigment composition and phylogeny of an unarmored dinoflagellate *Gertia* sp., a peridinin-containing species in the Kareniaceae

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The unarmored dinoflagellate family Kareniaceae possesses the haptophyte-type chloroplast, instead of the peridinin-type chloroplast common in dinoflagellates. Recently, we reported a new genus and new species *Gertia stigmatica*, a peridinin-containing species in the Kareniaceae. This contradictory combination of host and chloroplast has made the origin of two chloroplast - types in the family uncertain. Here, we observed the second existence to be described as a new species in *Gertia*. The isolate collected from a Japanese coast was observed under LM and EM. Its photosynthetic pigment was analyzed by HPLC, and its phylogeny was inferred from nucleus-rDNA and chloroplast-encoded psbC gene. This species differs from *G. stigmatica* in a larger cell size (14 – 21 µm vs 6 – 10 µm) and the absence of stalked pyrenoids and eyespot. A straight furrow of the apical structure complex, characteristic in the Kareniaceae, justified the placement of the species in this family. Comprehensive LM and TEM identification of cell components did not obviously support a previous maintenance of the haptophyte - type chloroplast in this species, although an organelle accumulating polyphosphate novel in dinoflagellates was identified at the dorsal side of the cell. The chloroplast was very similar to that of peridinin - type seen in other dinoflagellates; brown color, reticulate shape, peripheral position of the cell, composition of three-grouped thylakoids, variation and relative amount of photosynthetic pigment, and molecular evolutionary rate of chloroplast-encoded psbC. These results support that *Gertia* inherited the peridinin-type chloroplast from a common ancestor of the Kareniaceae, while other kareniaceans of at least two lineages replaced the chloroplast with that of haptophytes independently.

Morphology and molecular characterization of *Heterocapsa* (Dinophyceae) from Malaysian water, with the description of three novel species

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A total of eighty-six strains of *Heterocapsa* were established from Malacca strait and South China Sea, Malaysia. Based on the combined morphological and molecular characteristics, two species, *H. bohaiensis* and *H. rotundata*, and three new species (*H. borneoensis* nom. nud., *H. iwatakii* nom. nud., and *H. pseudopygmaea* nom. nud.) were discovered. *Heterocapsa borneoensis* is morphologically closely related to *H. bohaiensis*, but differs in the cell shape, nucleus shape and body scale. *Heterocapsa iwatakii* differs from other species in the cell size, cell shape, presence of the red body in its cytoplasm, and the size of body scale. *Heterocapsa pseudopygmaea* shares identical cell morphology with *H. pygmaea*. The species delineation was further supported by the phylogenetic inferences of the large subunit and small subunit ribosomal rDNA, and the internal transcribed spacer (ITS) region. The results revealed that *H. borneoensis* as a sister species to *H. bohaiensis* while *H. iwatakii* are closer to *H. horiguchii* and *H. ovata*. Pairwise comparison of the ITS2 transcripts revealed the presence of hemiâ and compensatory base changes. *Heterocapsa borneoensis* showed only one Hemi-CBC when compared to *H. bohaiensis*, with no CBC. *Heterocapsa iwatakii* was supported with 2-3 CBCs and 4-10 Hemi-CBCs when compared to its closely related species. The presence of one CBCs and five Hemi-CBCs between *H. pseudopygmaea* and *H. pygmaea* supported the cryptic diversity of *H. pygmaea*. This study documented, for the first time, the occurrence and diversity of *Heterocapsa* species in Malaysian water.

Morphology and phylogeny of an undescribed suessiacean dinoflagellate from a tidal pool in Japan

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Marine dinoflagellates in the Suessiaceae have been found in the Asian Pacific, and also from specimens of harmful algal blooms that had caused massive fish mortalities. In this study, morphology and phylogeny of an undescribed suessiacean dinoflagellate were examined using unialgal cultures established from a tidal pool in Jogashima Island, Misaki, Japan. Cell morphology was examined by light microscopy, SEM and TEM, and phylogenetic position was inferred from LSU rDNA and ITS region. Immotile form, spherical to ovoid in shape and often covered by a thick wall, was dominant and forming aggregates attached to the culture tube. SEM showed scarce tube-like processes with terminal hairs ornamented on the immotile cell wall. Motile cells were ellipsoidal with blunt apical and roundish antapical ends, and cingulum was located at the middle of the cell. Mean cell sizes were 16.3 µm long and 11.9 µm wide. Motile cells had a nucleus in the epicone, peripherally distributed chloroplasts, usually one pyrenoid in the hypocone, and an eyespot in the sulcal region. The apical structure complex was straight and composed of an elongated knob vesicle, typical in the Suessiaceae. Phylogenetic trees confirmed the placement of this species in the Suessiaceae, but not in any established genera. The dominant immotile form, as observed in our species, has not been reported in most species in the Suessiaceae.

Genetic characterization of *Prorocentrum lima* species complex strains from coastal and oceanic areas in Brazil

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The dinoflagellate *Prorocentrum lima* is a cosmopolitan species complex, that synthesizes okadaic acid (OA) and dinophysistoxins (DTXs), responsible for diarrhetic shellfish poisoning. The aim of this study was to genetically characterize seven strains of *P. lima* isolated from the Brazilian coast and oceanic islands, from six locations between latitudes 3°50′25″S and 20°31´29´´S. The ITS (Internal Transcribed Space) and LSU (Large SubUnit) rDNA loci were sequenced, and phylogenetic reconstructions were performed using Maximum Likelihood (ML) and Bayesian Inference (BI). Considering the ITS and LSU loci, four clades of P. lima were found, in agreement with previous studies. Phylogenetic analyses showed that the Brazilian sequences clustered in three clades (1, 2 and 3) within the *P. lima* species complex. Five strains grouped within clade 1, along with strains from Japan and a large group of sequences acquired from several regions in the Atlantic, Pacific and Indian Oceans. One strain clustered in clade 2, with strains from Brazil, Bermuda (U.K.), the Caribbean Sea and the Gulf of Mexico. Another strain grouped within clade 3, that has been considered to be *Prorocentrum arenarium*, before being proposed as a synonym of *P. lima*. No strain grouped in clade 4, that includes strains isolated from the North Atlantic Ocean and the Mediterranean Sea. Phylogenetic analyzes based on ITS and LSU revealed high genetic diversity in *P. lima* strains isolated from Brazil, with representatives from three major clades of this species complex.

Morphological and molecular diversity of athecate dinoflagellates from Bahía Todos Santos, Baja California, Mexico

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The athecate dinoflagellates are mainly characterized by having an amphysm with fine vesicles. They are a diverse and abundant group in temperate and mainly tropical seas around the world, where the Gymnodiniales order represents the most diverse and best studied group, with approximately 638 taxonomically valid species, distributed in 11 families and 71 genera. The objective of this study was to contribute to the knowledge of athecate group in Todos Santos Bay, Baja California, Mexico, through an updated list of athecate dinoflagellates, including the photographic record, morphological description of each species and identification through the use of two molecular markers (18s and 28s). Twelve monthly samplings were carried out, between the period from 2019 to 2020, 150 samples were obtained and observed fresh under light microscopy. Through morphological identification, a total of 49 morpho-species of athecates were obtained, of which 42 represent new records for the area and 21 new records for Mexico. Molecular identification of 34 partial sequences of rDNA of athecate dinoflagellates were obtained, 17 sequences correspond to marker 18s and 17 sequences to marker 28s, 27 sequences correspond to the order Gymnodiniales, four to the order Syndiniales, two to the order Noctilucales and one to the order Suessiales. Photographic record of 11 species is presented. This work suggest that there is an important diversity of these organisms in the bay, so it is necessary to continue working on morphological data, molecular characters and scanning electronic microscopy that allow us to confirm the presence and its prevalence of potential harmful dinoflagellates in the site of study.

Morphology and molecular characterization of *Alexandrium pseudogonyaulax* from Malaysian Borneo, with descriptions of two new species

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Alexandrium pseudogonyaulax and its closely related species possess common thecal characteristics like indirect connection of first apical (1') plate and the apical pore complex (APC) and oblique posterior sulcal plate (s.p.). The species identification is very much relied on the minute differences in the shape of the 1' and 6" plates. Seven strains of *A. pseudogonyaulax* were established from Malaysia Borneo and underwent microscopic observation and molecular characterization. *A. pseudogonyaulax* was identified and two new morphotypes, herein designated as *Alexandrium ogatae* nom. nud. and *A. limii* nom. nud. *Alexandrium ogatae* differs from *A. foedum* by the shape of plate 1', 6'', 2'''', and s.p. *Alexandrium limii* differs from A. taylorii in 6'', s. a., and position of ventral pore. The species delineations were further supported by the phylogenetic inferences using the large subunit rDNA and internal transcribed spacer (ITS), including the ITS2 secondary structure. The results showed that *A. ogatae* and *A. limii* were clustered together *A. pseudogonyaulax*, *A. taylorii*, and *A. hiranoi*. The species lineages were also supported by high genetic divergences. The pairwise comparisons of the ITS2 transcripts revealed the presence of hemi and compensatory base changes with the closely related species.

CICCM: new entries and new descriptions of historical cultures

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The Cawthron Institute Culture Collection of Micro-algae (CICCM) includes more than 600 unique micro-algae and cyanobacteria isolates from tropical, temperate, and Polar Regions and from both marine and freshwater environments. Half the collection is cryopreserved, and the other half is regularly sub-cultured. Live cultures, DNA extracts and chemical compounds may be purchased for research purposes and all have a full description and toxin profile accompanying them (see www.cultures.cawthron on-line). To date, isolates have enabled the description of new species, the preparation of biotoxin standards and the carrying out of toxicity studies amongst other research endeavours. For example, extracts of mass cultures of *Alexandrium pacificum* have been supplied to pharmaceutical companies for the preparation of paralytic shellfish toxin standards. Some of the additions to the CICCM since 2019 will be discussed. These include 84 dinoflagellate isolates (17 genera, 44 species), 32 diatoms (3 genera, 16 species), 10 cyanobacteria (5 genera, 9 species), and a chlorophyte. New descriptions of older cultures in the collection will also be highlighted, for example the rappemonad, *Pavlomulina ranunculiformis*.

Morphological, molecular and toxinological analyses of strains of *Alexandrium catenella, Protoceratium reticulatum* (Dinophyceae) and *Pseudo-nitzschia multiseries* (Bacillariophyceae) isolated from Buenos Aires

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In the framework of an ongoing monitoring program of harmful microalgae from the marine coastal waters of Buenos Aires Province, series of cultures have been isolated and different potentially toxigenic species characterized by morphological and molecular analysis based on LSU rDNA sequence data. Established strains were *Alexandrium catenella* (LPCc001 LP21/07/2015; LPCc002 BSB4/08/2016; LPCc004 LP27/09/2016; LPCc008 RJ21/07/2015), *Protoceratium reticulatum* (LPCc021 VG17/10/2017) and *Pseudo-nitzschia multiseries* (LPCc036 MAZ29/01/2019; LPCc037 MAZ29/01/2019). The four Argentinean strains of *A. catenella* group in the ribotype I of *A. tamarense/catenella* clade, *Protoceratium reticulatum* and *Pseudo-nitzschia multiseries* coincide in the clades of each species with strains from other areas. The toxinological profile of the target strains were studied using LC - MS/MS for lipophilic (LSTs) and amnesic toxins (ASTs) and UHPLC-FLD equipment for paralytic toxins (PSTs). Strains of *A. catenella* are characterized by the dominance of gonyautoxins (GTX1, 4, 2, 3) and N-sulfocarbamoil toxins (C1, 2), minor proportion of decarbamoilgonyautoxins (dcGTX2, 3) and traces or the absence of saxitoxin (STX), decarbamoil-saxitoxin (dcSTX) and neo-saxitoxin (Neo). In the case of *P. reticulatum*, yessotoxin (YTX) and traces of 45-hydroxy yessotoxin (45OH YTX) were detected and in *P. multiseries*, domoic acid (DA) and epi-domoic acid (epi-DA) were identified. Several differences about the morphological details and the toxinological profiles of the strains of *A. catenella*, *P. reticulatum* and *P. multiseries* analyzed in this study and others previously described from Argentinean waters were discussed. Differences in morphological details and toxinological profiles have been observed between the strains characterized in this study and others previously published from the Argentine Sea.

The culture collection of cyanobacteria and microalgae at the French museum of Natural history

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Created in the 1930s, the collection of cyanobacteria and microalgae cultures of the "Muséum national d'histoire naturelle" (MNHN) includes now more than 1800 live non-axenic strains isolated mainly from freshwater ecosystems in France. Since its origin, the collection has been dedicated to research and contributes to biodiversity, taxonomy, genomics, and research of bioactive compounds. Enrichment of the collection is most often associated with specific research projects. In particular, the collection contains many strains of various genera of cyanobacteria that form blooms of ecological concern, some of which produce cyanotoxins. Through research, expertise in ecotoxicology, and partnerships with industry, the MNHN collection is both a repository of biodiversity and a resource for the life sciences. The maintenance of the collection requires a significant labour force and a reflection on the modalities of strains conservation. The maintenance of living cells inevitably leads to the genetic drift of organisms, all the more important if it is about micro-organisms whose generation time is short. Alternatives, or complementary approaches such as cryopreservation or conservation in a protective atmosphere can constitute progress. However, the experience in the collection shows that it is often necessary to that it is often necessary to adapt the protocols to the different taxa, strains or cell lines. Rich of its past, the recent evolution of this collection allows it to respond to the current challenges of fundamental and applied research and conservation of biological resources.

Morphological and molecular characterization of the HAB - producing dinoflagellate *Lingulodinium polyedra* in Todos Santos Bay, Baja California

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Harmful Algal Blooms (HABs) are frequent in coastal ecosystems, where they often cause fish mortality and widespread damage to ecosystem function, as well as risk to human consumers of seafood contaminated by their biotoxins. *Lingulodinium polyedra* is a thecate bioluminescent dinoflagellate distributed widely from subtropical to arctic areas, with blooms often associated with coastal upwelling zones. This species forms high magnitude blooms ("red tides") from southern California in the U.S. to Baja California in Mexico. Several dinoflagellate species, including *L. polyedra*, cause recurrent HABs in Todos Santos Bay, Baja California, and this species is recognized as harmful because its dense blooms can cause deleterious effects on pelagic food webs. Many *L. polyedra* strains can produce yessotoxins (YTXs) and perhaps uncharacterized ichthyotoxins, but there is insufficient knowledge in Mexico on *L. polyedra* HAB etiology, just as on their molecular and toxinological features. This study aims to determine the molecular identity and global phylogenetic relationships with *L. polyedra* clonal cultures from the May 2020 event in Todos Santos Bay. Morphological analysis by light and scanning electron microscopy will be referenced by DNA bar-coding of the LSU rDNA gene. Toxigenicity of the strains, specifically for YTXs, will be confirmed by LC-MS/MS and compared with allelopathic activity against other ecologically relevant dinoflagellate species in mixed culture experiments.

Species of the planktonic dinoflagellate genus *Alexandrium* (Dinophyta) from the tropical and subtropical Mexican Pacific Ocean

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All species of the dinoflagellate genus *Alexandrium* Halim (Dinophyta) are marine and truly planktonic forms, many species are widely distributed and some are recognized to form “red tides” and Harmful Algal Blooms (HAB), and to produce saxitoxins causing Paralytic Shellfish Poisoning (PSP) in humans. The purpose of this work was to recognize the species of *Alexandrium* from coasts of the tropical and subtropical Mexican Pacific Ocean. Based on samples obtained following several methods and from various localities of the tropical and subtropical Mexican Pacific, from coasts of Baja California Sur (Gulf of California) to Chiapas (Gulf of Tehuantepec), we have found and identified nine species (morphospecies) of *Alexandrium*: *Alexandrium affine*, *A. concavum*, *A. globosum*, *A. margalefii*, *A. minutum*, *A. monilatum*, *A. pseudogonyaulax*, *A. tamarense* and *A. tamiyavanichii*. These species are described and illustrated by light microscopy, after thecal dissociation, and epifluorescence microscopy, using Calcofluor. Additional observations of certain species were made by Scanning Electron Microscopy (SEM). The species *Alexandrium affine*, *A. minutum*, *A. monilatum*, *A. pseudogonyaulax*, *A. tamarense* and *A. tamiyavanichii* have been flagged as potentially toxic. This is the first record of *Alexandrium* globosum, and the first morphological documentation of *A. pseudogonyaulax* from the study area (Mexican Pacific Ocean). Currently, there are 16 species of *Alexandrium* listed for the Mexican Pacific, but we believe that this number will increase when more studies dedicated to the genus can be undertaken, including cultures and molecular biology.

GENOMICS

Inferring phytoplankton community composition during *Pseudo-nitzschia* blooms using a metatranscriptomic approach

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Pseudo-nitzschia blooms and the resulting toxicities are a global problem. *Pseudo-nitzschia* species are difficult to distinguish morphologically and extremely high taxonomic skills are required to identify them by light microscopy. This is particularly critical because although many species produce domoic acid, major toxicity events are mainly due to a few species often co-occurring with less problematic species. The understanding of toxicity events can therefore only be achieved by analyzing the community composition at the species level. Several molecular techniques may be used to infer community composition, either focusing on very specific taxonomic groups (for instance using qPCR), or on entire communities but with limited taxonomic resolution (for instance metabarcoding). These methods often require specific molecular development and/or targeted sequencing approaches. Here, we use non-targeted community wide RNA sequencing (metatranscriptomic) to identify the composition of the active community. For this purpose, we aligned natural metatranscriptomic samples on two databases: PR2 (18S) enabling an exhaustive characterization of the community, but at low taxonomic resolution for diatoms, and diat_barcode (rbcL) to obtain a better taxonomic resolution for diatoms and especially *Pseudo-nitzschia* species. With the multiplication of non-targeted sequencing projects and the improvement of reference databases, such an approach offers the promise to characterize with a single dataset the microbial community composition and functions during harmful algal blooms without the need of specific protocol development or targeted sequencing.

Interrogating molecular differences between two *Alexandrium* species through quantitative proteomics

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The genus *Alexandrium* is one of the primary causes of HAB occurrence worldwide. *Alexandrium minutum* and *Alexandrium tamutum* are two closely-related species in terms of morphology. These two species used to be both taxonomically classified as *A. minutum*. With recent advances in molecular taxonomy, the two species were formally re-classified as distinct species. However, limited studies on the molecular biology and biochemistry of the two species have been reported. In our study, we report comparative analyses of the two species based on its proteome using isobaric tag for relative and absolute quantitation (iTRAQ) mass spectrometry, toxin analysis and immunostaining. A total of 1,436 proteins were identified using iTRAQ-mass spectrometry. Of these proteins 88% were both expressed by both species. Annotations of commonly expressed proteins are involved in various biochemical pathways such as amino acid biosynthesis, purine metabolism, carbohydrate degradation, carbohydrate metabolism, to include others. Differentially regulated proteins such as upregulation of S-adenosylhomocysteine, a negative feedback inhibitor of S-adenosylmethionine, was observed. It is hypothesized that this contributes to lower toxicity of one species. Toxin analysis showed that *A. minutum* produced saxitoxin analogues such as gonyautoxins 1, 4 and 2, 3. On the other hand, no detectable toxins were observed for *A. tamutum*. Quantitative immunostaining using primary antibody against *A. minutum*, with mouse secondary antibody conjugated with FITC revealed statistically significant difference (alpha = 0.05) between the two species. This study provides knowledge on the molecular underpinnings of *Alexandrium* species and provides contemporary techniques to differentiate these two HAB-forming species.

The seasonal transcriptomic profile of the cockle *Ceratodesma edule* exposed to diarrhetic shellfish toxins

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Bivalves constitute an important source of protein for human consumption, but some accumulated biotoxins such as Diarrhetic Shellfish Toxins (DSTs) constitute a risk to human health. The cockle *Cerastoderma edule* is one of the most affected species in Portugal, mainly in the summer. However, the molecular process underlying the accumulation and depuration phases of DSTs in this species is still unclear. Herein, we performed a transcriptomic analysis of two tissues from wild cockles sampled in Ria de Aveiro, Portugal, in two different seasons (without DTSs and exposed). The total RNA of two tissues: gills and digestive glands, were conducted for paired-end RNA sequencing using an Illumina NovaSeq 6000 platform. The *de novo* assembly performed with Trinity v2.10.0 returned 684,723 contigs, N50 of 1049, and 98.53 % completeness. Altogether, 1,098 Differentially Expressed Genes (DEGs) were identified ($p < 0.01$), of which 209 DEGs were common in both tissues, 353 DEGs exclusive for the digestive gland and 536 unique for the gills. Some biomarkers previously reported among known stress and detoxing-DSTs related-genes were identified within DEGs. Among them we identified Glutathione peroxidase, glutathione S-transferase Mu 3, superoxide dismutase and cytochrome P450 2U1-like (involved in antioxidant system), ABC transporter family members (Metabolic Detoxification/Xenobiotic Transmembrane Transporter Activity), apoptosis regulator protein 1 and CREB-binding protein (transcription regulators), actin 1 isoform X2, actin beta/gamma 1 and Tubulin alpha chain (cytoskeleton, actin related proteins), Hsp90 protein (Heat shock protein HSP 90 (HSP90), and complement C1Q-like proteins (immune responses). This study provides the first transcriptomic profile of *C. edule*, giving new insights about its molecular responses facing seasonally DSTs-Blooms exposures.

Spatial distribution of harmful species along Chilean coast using metabarcoding

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Along the coast of Chile, harmful algal blooms (HABs) have increased in frequency and intensity during the last 50 years, the most studied correspond to events caused by the dinoflagellate *Alexandrium catenella* which has apparently expanded northward its distribution since its first detection in the Magallanes region 53 °S (1972), however, in recent years, blooms of emerging species such as *K. selliformis*, *P. verruculosa* and *H. akashiwo* have been pointed out as responsible of marine fauna mortalities with important economic loses to aquaculture. Despite this, the molecular diversity of phytoplankton communities along the Chilean coast has not been fully described, although massive sequencing technologies have been proposed as one of the main sources of innovation in the detection and quantification of microalgae due to the high-resolution level of organisms at low concentrations, cryptic complexes or smaller species. In this work we describe the molecular diversity in phytoplankton assemblages along the southern coast of Chile between 36 °S to 53 °S using metabarcoding as the identification tool. Spatial patterns of diversity were identified in contrasting areas (exposed Pacific Ocean and fjords) where the main harmful genera were identified (*Alexandrium*, *Dinophysis*, *Protoceratium*, *Karenia*, *Karlodinium*, *Pseudochattonella*, *Pseudo-nitzschia*, *Chaetoceros* among others). The implementation of metabarcoding as a complementary tool to the ongoing monitoring programs of harmful microalgae is discussed.

Diversity and seasonal dynamics of the planktonic microalgae community and occurrence of harmful species in a mariculture farm in Arraial do Cabo / RJ, Brazil

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The mariculture farm in Arraial do Cabo / RJ, Brazil (22 ° 57 ‘’ S, 42 ° 00 ‘’ W) is an important cultivation area for local extractive farmers. Still, it lacks studies on phytoplankton’s distribution and seasonal dynamics, including species causing harmful blooms. There is only one study on potentially harmful taxa carried out in the years 2013/2014 and the occurrence of DSP toxins in mussel meat, recorded in January, March, and May / 2014. Here we present the results of the seasonal dynamics of planktonic microalgae, applying traditional and environmental DNA - metabarcoding technique (V - 9 region), focusing on potentially harmful species in the area for twelve months (Jun / 2018 to May / 19) associated with some environmental data. It is the first study evaluating HABs-related species identification using a combination of morphological and molecular approaches for this area. Based on the analysis of a total of 24 samples distributed at two collection points, 66 (microscopy) and 154 (metabarcoding) phytoplankton taxa were identified, with Bacillariophyta with the highest contribution of taxa, using both techniques. Among the potentially harmful species (microscopy and metabarcoding) were registered: *Akashiwo sanguinea, Asterionellopsis glacialis, Chaetoceros affinis, Cjaetoceros cf. lorenzianus, Chaetoceros decipiens, Cerataulina pelagica, Cylindrotheca closterium, Guinardia delicatula, Guinardia flaccida, Ginardia striata, Leptocylindrus danicus, Leptocylindrus minimus, Noctiluca scintillans, Scrippsiella trocoidea* e *Skeletonema costatum;* and among the toxin-producing taxa: *Alexandrium cf. tamarensis, Alexandrium cohorticula, Alexandrium ostenfeldii, Amphidoma languida, Amphora coffeiformis, Dinophysis cf. acuminata, Fibrocapsa japonica, Gambierdiscus cf. yasumotoi, Gonyaulax spinifera, Ostreopsis cf. ovata, Pfiesteria-like dinoflagellate, Pseudo-nitzschia delicatissima, P seudo-nitzschia pseudodelicatissima* and *Pseudo-nitzschia seriata.*

Assessment of harmful microalgal assemblage in the Semerak Lagoon, Kelantan, Malaysia by 18S rDNA metabarcoding

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Harmful algal bloom (HAB) events have become recurring nuisance in the coastal waters with tremendous economic impact to mariculture activities and public health. In this study, 18S rDNA metabarcoding was adopted in the assessment of HAB species from December 2018 to November 2019 in the Semerak Lagoon, an important mariculture site in the state of Kelantan, Malaysia. A total of 107 water samples at 1 m depth were collected from nine sampling stations, followed by DNA extraction and sequencing of the V9 hypervariable region of 18S ribosomal RNA gene using Illumina MiSeq platform. Operational taxonomic units (OTUs) were assigned to taxa by BLASTn search against National Center for Biotechnology Information (NCBI) database with cut off at > 97 % for similarity. Nineteen HAB taxa were detected from 177 OTUs, with nine new records in this area including *Gonyaulax spinifera*, *Gymnodinium catenatum*, *Margalefidinium polykrikoides*, *Polykrikos hartmanii*, *Chattonella* spp., *Fibrocapsa japonica*, *Heterosigma akashiwo*, *Phaeocystis* spp., and *Prymnesium parvum*/*calathiferum*. HAB taxa with most abundance was represented by bloom forming *Akashiwo sanguinea* and icthyotoxic *H. akashiwo* in September and November 2019, respectively. Therefore, metabarcoding is a powerful tool in the discovery of novel HAB species and evaluating minor changes in phytoplankton composition in the coastal water.

Coexistence of *Microcoleus* sp. genotypes with and without anatoxin-production potential in benthic mats from the Wolastoq (Saint John River, Canada)

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In recent years, the presence of toxigenic benthic cyanobacteria in riverine ecosystems has become an increasing concern around the world. In 2018, the death of three dogs in the Fredericton area (New Brunswick, Canada) was attributed to anatoxins (a potent class of neurotoxins), after they ingested benthic microbial mats found along the shore of the Wolastoq (Saint John River). Samples of the mats ingested by the dogs and from the vicinity were collected and analyzed by LC-MS, PCR screening for anatoxin biosynthesis genes, and 16S rRNA phylogeny. Here, we present the metagenomic analysis of four of the mat samples and of 15 non-axenic cyanobacterial isolates obtained from the same material. Total DNA from the 19 samples was sequenced individually using Illumina technology (150 bp paired-end reads). From one of the mat samples and 12 of the isolates, we recovered 13 highly similar (likely members of the same species) cyanobacterial genomes that belong to genus *Microcoleus*, whereas we were unable to recover complete genomes from the remaining samples. With our comparative analyses we identified the genetic repertoire to produce anatoxin-a and dihydroanatoxin-a in nine of them, allowing us to classify these genomes into those with toxin potential and those without. Further analysis revealed the coexistence of the two strains with similar relative abundance in a mat identified by the dog owners as having been ingested by the dogs, whereas other mats collected in the vicinity only contained strain lacking the toxin cluster.

Molecular mechanisms underlying the diversity of PST production in *Alexandrium minutum*: A genetic approach

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Dinoflagellates of the genus *Alexandrium* produce Paralytic Shellfish Toxins (PSTs). These PSTs are also produced by cyanobacteria, notably in *Cylindrospermopsis raciborskii* for whom the PSTs synthesis, modification and transport have been shown to rely on a cluster of 26 genes (Kellmann et al., 2008). Amongst the genus *Alexandrium*, 1/3 of the species are toxic. The toxicity profile of each strain seems to be fixed, and Sako et al. (1992) showed that it appears to be inherited in a Mendelian manner in the offspring produced by sexual reproduction between two strains of *A. catenella*. Regarding the genes encoding PSTs, Hackett et al. (2013) were able to find only 13 homologs of cyanobacterial sxt genes in *A. tamarense*, suggesting a biosynthetic pathway at least partially different from that of cyanobacteria. The genes and biosynthetic pathway of PSTs in dinoflagellates, as well as the modalities of transmission of this phenotype are therefore not yet fully elucidated. We propose to investigate the molecular bases of PST production using a family of strains initiated from four *A. minutum* parental strains. They display a genetic, morphological and toxicity profile (two toxic, two non-toxic) variability. These parental strains have been crossed in order to generate a recombinant offspring from sexual reproduction. The parents and their descendants were sequenced and genotyped using several 100 000s SNPs and a genetic linkage map was generated. We quantified the toxicity profiles of the offspring strains and explore the molecular mechanisms (gene expression and genetic differences) that may underly the observed diversity.

Analysis of *mcy* genes in a *Microcystis* population reveals a systematic pattern of gene loss in non microcystin-producing strains

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Fresh-water cyanobacteria of the genus *Microcystis* are common producers of the hepatotoxin microcystin. However, the diversity in microcystin production is notable: not all strains produce microcystins. Microcystin biosynthesis are coded for by a gene cluster consisting of ten genes (*mcy*A-J). The inability of strains to produce microcystins are due to lack of the whole gene complex. However, studies has shown that the mcy gene complex can be partially retained. Here, we investigated the link between microcystin phenotypes and the corresponding genotypes in *Microcystis botrys*, one of the most common *Microcystis* species in Southern Sweden. We hypothesized that microcystin-producing and non-producing strains separate into distinct groups, based on the presence/absence of the mcy gene complex. *M. botrys* colonies were isolated during a bloom. Established cultures were sequenced by Illumina, and microcystin phenotypes analyzed by LC-MS/MS. Reads were assembled and annotated, and the resulting amino acid sequences used for mcy gene identification, multiple sequence alignment, and subsequent tree-building. We examined whether the inability of strains to produce microcystins is due to absence of the mcy gene complex, either partially, or complete. Preliminary results show that none of the strains in our dataset lack the whole mcy gene complex. Moreover, based on trees built on individual mcy genes, microcystin-producing and non-producing strains do not clearly separate into distinct groups. Instead, we observed a pattern in which most non-producing strains lacked *mcy*F and *mcy*J. However, some inconsistencies were found, that will be presented. Furthermore, our results indicate that genes commonly used in qPCR-protocols (e.g. *mcy*B) are in fact present also in many non-producing strains.

Functional and phylogenetic comparisons of the chloroplast transcriptomes in *Karenia* and *Takayama* from the Gulf of Mexico

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The Kareniaceae are believed to be a monophyletic class of dinoflagellates that have acquired a haptophyte derived chloroplast. As a taxonomic group, they are renowned for harmful algal blooms and production of unique toxins (e.g., brevetoxins, karlotoxins, gymnocin-B). Links between their unique chloroplast and toxin production is of growing interest. Across the group, their ecological success, is likely variably related to their tertiary plastid. For example, ranging from a strong reliance on predation (e.g. *Karlodinium*), through to predominantly photoautotrophic (e.g. *Karenia brevis*). Here we sequenced (Illumina) in triplicate, the polyuridylated transcriptomes, of five species of *Karenia* (*K. brevis, K. mikimotoi*, *K. papilionaceae*, *K. umbella*, *K. asterichroma*), and two species of *Takayama* (*T. tasmanica* and *T. pulchellum*). Using this new data, we phylogenetically assessed the Kareniaceae via nuclear rRNA and chloroplast genes. Via nuclear 28S - rRNA, we observe that *K. brevis* and *K. mikimotoi* are most closely related, despite their morphological differences, and *K. umbella* is the most basal *Karenia* species. Plastid-wide comparisons revealed which genes are most divergent across the group, and how their relative expression varies across taxa - relating to their function. Assemblies yielded a median of ~2,600 unique transcripts per sample. Translating these into protein and searching (BLASTP) against a haptophyte chloroplast gene database at expect values of e-80, we retrieved 41 chloroplast genes, of which 14 were found in all seven species. Ultimately linking these genetic patterns to life history strategies across the class could reveal how different species are selectively evolving their chloroplast genomes to optimally suit their ecological niches and reveal potential relationships to toxin production.

Metagenomic comparison of *Planktothrix agardhii* isolates from a Lake Erie embayment

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Planktothrix agardhii is a filamentous cyanobacteria species that dominates harmful algal blooms in Sandusky Bay, Lake Erie and other freshwater basins across the world. *Planktothrix agardhii* isolates were obtained from early (June) blooms via single filament isolation; Eight have been characterized from 2016 and 12 additional isolates have been characterized from 2018 for a total of 20 new cultures. These novel isolates were processed for metagenomic sequencing, where sequencing reads were used to generate scaffolds and contigs which were annotated with DIAMOND BLAST hit, Pfam, and GO. Analyses include whole genome alignment to generate phylogenetic trees and comparison of genetic rearrangements between isolates. Nutrient acquisition and metabolism (i.e. nitrogen, phosphorus, carbon) was compared across isolates. Secondary metabolite production was genetically explored including microcystins, two types of aeruginosin clusters, anabaenopeptins, cyanopeptolins, microviridins, and prenylagaramides. Two common and four unique CRISPR-cas islands were analyzed for similar sequences across all isolates and against known *Planktothrix*-specific cyanophage, PaV-LD. Overall, the uniqueness of each sequence despite the same sampling location in approximately the same time of the harmful algae boom lifecycle belies the unexplored diversity of cyanobacteria.

Is sxtA4 gene discriminative for toxic *Alexandrium* species? What can we learn from its variability in copy number, expression and transcript isoform associated with toxin content?

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While sxtA4 gene seemed to be the most reliable gene for discriminating PSP producing dinoflagellates, we investigated whether sxtA4 copy numbers was related to the toxin content in 20 European strains of *Alexandrium minutum* and *Alexandrium pacificum*. Further, we analyzed the expression rate and transcript isoforms among 11 *A. minutum* strains to better connect the molecular processes toward toxin production. First, the toxin content varied between strains with distinguished toxin profiles between *A. minutum* and *A. pacificum*. In addition, variability in the number of copies of sxtA4 genes was observed between species and also diverse within species (ie between strains), for *A. minutum* and *A. pacificum*. Then, we observed significant correlation between sxtA4 copy number and the toxin content in *Alexandrium* species along with a moderate correlation between copy number and the genome size. Moreover, the sxtA4 copy number was significantly correlated with the expression in *A. minutum*, as expected for constitutive gene expression. Besides, within the strain panel, two strains were identified as non-toxic and evidenced as harboring a unique sxtA4 copy. Their sxtA4 expression rate was lower and the transcript isoform was different than in toxic species. On the contrary, toxic species presented variable RNA sequences between strains and between gene copies within strains. Briefly, the presence of sxtA4 gene may occur in some non-toxic *Alexandrium* species, however with a different form, and involving constitutive gene expression moderated by regulation mechanisms.

Life cycle controls in the planktonic diatom *Pseudo-nitzschia multistriata*

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Over the past years, many studies have been conducted to explore the life cycle of the diatom *Pseudo-nitzschia multistriata*, a moderately toxic species observed occasionally at several sites in world oceans. Building on a large body of information, our current, multidisciplinary project is addressing questions related to the regulation of life cycle transitions, to genome variation among different strains, and to the impact of the life cycle on population dynamics. Genome sequencing and previous transcriptomics analyses of different stages of the life cycle led to the identification of key genes required for sexual reproduction, including mating type related genes and the mating type determining gene. We are now also investigating whether epigenetic mechanisms are at play in the control of the mating type determination locus and in specific phases of the life cycle. New transcriptomic analyses are revealing that the general cell growth arrest occurring during mating is associated to downregulation of genes related to major metabolic functions, from the early phases of mating. Single cell photophysiology also pinpoints an inhibition of photosynthesis in the arrested population, which is exacerbated in gametes/zygotes. Numerical simulations revealed that growth arrest affects the balance between parental cells and their siblings, possibly favoring the new generation. Finally, the opportunity to resequence the genome of *P. multistriata* strains collected over different years and at different geographical locations is allowing to quantify the extent of genome variation in natural populations, to define constraints acting on the *P. multistriata* genome and to integrate, expand and enrich an ongoing multiyear population genetics study.

Long-term stability of genome structure of *Dolichospermum* spp. in Lake Stechlin, Germany

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The cyanobacterium *Dolichospermum circinale* has been a long-term resident of Lake Stechlin, Germany. However, in recent decades, shifts in the phosphorus loading and phytoplankton population have increased biomass of *Dolichospermum* during summer blooms. To investigate the historic change in genomic features of *Dolichospermum* spp. in Lake Stechlin, akinetes were isolated from a sediment core representing the period of 1980 – 2017 and sequenced via single-cell amplified genomes. Ten akinetes from each of ten 1 cm sediments were isolated and sequenced, representing five-year average populations. Comparison of these genomes and genomes from extant isolates revealed a single ADA genotype with remarkable genome stability, without gene gain or loss events in response to recent environmental changes, however SNP analysis suggests shifts in strain dominance within the population. The genome's core characteristics imply a lifestyle adapted for the deep chlorophyll maxima: additional light-harvesting and P scavenging genes. Overall, the results show that there is little change within a population after the initial local adaptation despite the remarkable diversity between extant populations from different geographic locations and the dramatic in-lake changes in P concentrations.

Genetic analysis revealed large genetic breaks among Pacific Rim populations in *Alexandrium catenella*

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The geographic range of shellfish poisoning attributable to *Alexandrium catenella* appears to be increasing on both regional and global scales. The molecular ecological study of genetic relationships using genome-wide single nucleotide polymorphisms (SNPs) may reveal the dispersal mechanism of this species. In this study, MIG ISSR (multiplexed Genotyping inter simple sequence repeats) - seq was employed to reveal the genetic relatedness among Pacific Rim populations. Using loci which were detected in > 70 % individuals and isolates with < 50 % missing loci, we obtained 349 SNPs in 165 samples isolated from Atsukeshi (eastern Hokkaido), Sendai, Osaka, Hiroshima Bays (Japan), Jinhae Bay (Korea), Bering Sea, Chukchi Sea, Puget Sound (USA) and Chile. Pairwise FST values showed significant differences among most of the pairwise samples except between Osaka & Jinhae Bay and Osaka & Hiroshima Bay. In the principal coordination analysis, samples from Japan and Korea, the Bering Sea, and the Chukchi Sea (seven samples) were positioned closely, but Puget Sound and Chilean samples were distant from the Japan and Alaska samples. Also, results of the bar plot by STRUCTURE analysis suggested that when K = 3, each cluster was grouped as 1) Japan and Korean except for Akkeshi Bay, 2) Akkeshi Bay and the Bering/Chukchi Sea, 3) Puget Sound and Chilean samples. These data suggested the presence of large genetic breaks between the Bering Sea and Puget Sound populations and genetic connectivity between Akkeshi Bay and the Bering/ Chukchi Sea populations.

TOXICOLOGY

Exploring the possibility of the steatogenic potential of cyanotoxins using 3D *in vitro* liver model

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The present study focusses on addressing the issue of assessing hazardous effects of hepatotoxic cyanobacterial toxins (cyanotoxins), chiefly cylindrospermopsin (CYN) and microcystin-LR (MC-LR), where advanced 3D culturing techniques show a promising approach to study the underlying mechanisms linked to the development of metabolic disorders induced by these cyanotoxins. While organic anion transporting polypeptides (OATPs) are crucial for MC-LR toxicity, cytochrome P450s (CYP450s) has been implicated in CYN toxicity. We used a 3D scaffold-free spheroid culture system of human hepatocellular carcinoma cell line, HepG2, to study the alterations in the key cellular and molecular events linked to the development of liver steatosis after CYN and MC-LR exposures. We exposed 2-weeks old HepG2 spheroids with improved hepatocyte characteristics and functions (albumin secretion, OATPs and CYP450s expression) to low, non-cytotoxic concentrations of CYN (≤ 1 µM) and MC-LR (≤ 10 µM) for 48 h. While CYN (1 µM) decreased expression of genes involved in fatty acid synthesis (ACLY, ACCA, FASN, SCD1), fatty acid oxidation (CPT1A) and uptake (APOB) and increased expression of genes involved in triglyceride synthesis (DGAT1/2) and lipogenesis (SREBP1c), MC-LR (≤ 10 µM) did not alter the expression of these genes. On the contrary, MC-LR reduced expression of OATP1B1 / 1B3, connexin 32 (Cx32) and increased expression of biliary duct/ liver progenitor cell marker, Connexin 43 (Cx43). It suggests possible selective elimination of OATP- and Cx32-expressing hepatocytes, causing activation of cytoprotective mechanisms and a major shift in the cell population towards less differentiated and/or non-parenchymal cells. Overall, 3D HepG2 serves as a suitable model for the evaluation of the steatogenic potential of cyanotoxins.

Characterization of Microcystin-Induced Toxicity on Primary Human Hepatocytes

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The amino acid composition of microcystins (MCs) can determine the structural, physical, and chemical characteristics including hydrophilicity and is thought to influence the toxicokinetic and toxicodynamic profiles of each congener. In this study, the effects of the more hydrophobic (LA, LF, LW, LY) and the more hydrophilic (LR, RR, WR, YR) congeners on cell viability, reactive oxygen species (ROS) production, glutathione (GSH) depletion, and protein phosphatase 2A (PP2A) inhibition were determined using primary human hepatocytes (HH). Twenty four hours after plating, HH were exposed to LR, LW, LA, LF, LY, WR, YR, or RR (0 to 20 µM) for up to 24 h. EC50s from cell viability studies were used to determine the cytotoxic potency of each MC congener: the rank order for cytotoxic potency was LY > LW > LA > WR > LF > LR > YR > RR. HH were assayed for ROS, GSH or PP2A activity after exposure to each congener at their respective EC50 (≤ 0.2 µM) concentrations, as determined in the viability studies. ROS and GSH were unchanged; however, PP2A activity was inhibited by every congener. The decreased PP2A activity correlated with decreased cell viability ($r^2 = 0.83$; $p = 0.002$). This study shows that the more hydrophobic congeners caused a greater decrease in cell viability, compared to the more hydrophilic congeners. Correlations in cell viability and PP2A activity inhibition suggest that PP2A inhibition is an important marker of cytotoxicity in HH exposed to low concentrations of MCs. Coupled with kinetic data on MC uptake and distribution, our results provide an enhanced understanding of the potential toxic effects produced by these congeners *in vivo*. [This abstract does not represent EPA policy.]

Pinnatoxins, an emergent class of marine toxins interacting with nAChRs. Pharmacological characterization, biodistribution and musculo-skeletal effect of these neurotoxic agents

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Cyclic imines, produced by various species of marine dinoflagellates, constitute a widely distributed group of phycotoxins. The warming of ocean temperatures and the increases in nutrients has driven an intensification of harmful algal bloom events. During these active dinoflagellate proliferations, phycotoxins may accumulate in shellfish tissues and can be transferred into fish, marine mammals and ultimately to humans. Pinnatoxin-G (PnTx-G) is an emerging marine toxin belonging to the cyclic imines family, and is considered as a fast-acting toxin in the mouse bioassay. PnTx-G is produced by the cosmopolitan dinoflagellate *Vulcanodinium rugosum* and is regularly detected in European coastal environments and in contaminated shellfish samples, and may represent a human health risk. In this work, the ability of tritiated PnTx-G to interact with neuronal- and muscletypes of nicotinic acetylcholine receptors was first evaluated. The biodistribution of [3H]-PnTx-G, using highresolution digital radio-imaging after oral or intravenous administration to rats, revealed the presence of the radiolabeled toxin in various peripheral organs, as well as in the central nervous system, highlighting its property to cross both the intestinal and the blood-brain barrier. The ability of [3H]-PnTx-G to cross the materno-fetal barrier was demonstrated by detecting the toxin in rat embryos, after injection of the radiolabeled toxin to pregnant rats. The use of mass spectrometry and a human perfused ex vivo cotyledon model disclosed the PnTx-G cross over the human materno-fetal barrier. In conclusion, this work shows that PnTx-G crosses very efficiently the intestinal, blood-brain and placental barriers. Further work is necessary to determine if the crossing is receptor-mediated.

Cytotoxicity of dinoflagellates from the Mexican Pacific Ocean: Inhibition of hNav1.7 by Saxitoxin, a therapeutically relevant sodium ion channel

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The hNav1.7 is a voltage-gated sodium channel associated with human neuropathic pain and the search for natural compounds with inhibitory properties of hNav1.7 might lead to chemical agents with therapeutic potential. In this work the action of the Saxitoxin (STX), a potent neurotoxin, on the functional activity of hNav1.7 was characterized. STX was obtained from marine dinoflagellates strains collected from the Mexican Pacific Ocean. Each culture was subjected to: freeze-thaw to induce cell lysis, cleaning with a solid phase extraction cartridge, evaporation for concentration, and chromatographic analysis. Inward currents were recorded by patch-clamp in whole-cell configuration from a cell line heterologously expressing hNav1.7. The results showed that, under a controlled voltage, STX at 1 micromolar blocked in a completely reversible manner, 15 % of the amplitude of the sodium macroscopic current without affecting its kinetics. In addition of its voltage dependence and its rapid kinetics, the unambiguous identification of active hNav1.7 channels was confirmed by its 100 % blockade at 100 nM Tetrodotoxin (TTX). Utilizing two independent methods of noise analysis (Variance Analysis and Spectral Density Distribution), the biophysical features of the single channel activity were determined. The TTX decreased the open probability and the effective number of functional channels, leaving the single channel current and conductance unaffected. Natural toxic metabolites such as STX and TTX, target sodium ion channels in cell membranes, affecting marine biota and humans through the consumption of contaminated shellfish, but they also represent an opportunity to apply them therapeutically.

Gene expression of the Na+/K+-ATPase ß2 subunit isoform correlates with cells sensitivity to palytoxin: an *in vitro* toxicogenetic study

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Palytoxins (PLTXs) are highly toxic compounds identified in marine *Palythoa* zoanthids, *Ostreopsis* dinoflagellates and *Thricodesmium cyanobacteria*. Human poisonings ascribed to PLTXs are associated with oral, inhalation, skin and/or ocular exposures to these toxins. Epidemiological and molecular evidences suggest a variable inter-individual sensitivity to PLTXs, possibly related to genetic-dependent differences in the expression of the subunits' isoforms of Na+/K+-ATPase, the molecular target for these toxins. To identify the Na+/ K+-ATPase subunits isoforms related with variable cells sensitivity to PLTX as well as the genetic markers associated with an increased risk of toxic effects in humans, purified peripheral blood monocytes from 60 healthy donors were used. *In vitro* cells sensitivity to PLTX (MTT test) was expressed as toxin concentration reducing cells viability by 50 % (EC50) and percentage of cell viability reduction (Emax) induced by the highest toxin concentration (10^{-7} M). Sensitivity parameters demonstrated a high inter-individual variability (median EC50 = 2.7×10^{-10} M; interquartile range: 0.4 - 13.2×10^{-10} M - median Emax = 92.0 %; interquartile range: 87.594.4 %). Spearman's analysis showed significant positive correlations between the β2-encoding ATP1B2 gene expression and Emax values (rho = 0.30; p = 0.025) as well as between Emax and the ATP1B2/ATP1B3 expression ratio (rho = 0.38; p = 0.004). A significant negative correlation between Emax and the ATP1B1/ ATP1B2 expression ratio (rho = -0.30; p = 0.026) was also recorded. In conclusion, the results of this toxicogenetic study suggest inter-individual differences in sensitivity to PLTX: individuals with high β2 isoform gene expression levels (alone or with respect to the β1 and/or β3 isoforms) could be highly sensitive to the toxic effects of PLTX.

Environmental samples of LPS isolated from fresh water HABs as well as LPS from axenic cyanobacterial cultures activate proinflammatory effects in keratinocytes and immune cells

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Cyanobacterial water blooms (cyanoHABs) are a worldwide problem nowadays. They produce not only various cyanotoxins but also lipopolysaccharides (cyanoLPSs). Last years, evidences of pro-inflammatory effects of cyanoLPSs have appeared. Therefore, we decided to study effects of cyanoLPSs isolated from environmental samples of cyanoHABs as well as cyanoLPSs isolated from axenic cultures of selected cyanobacterial species commonly found in fresh water cyanoHABs. One of the most probable exposures is via skin during recreational activities. Therefore, human keratinocytes HaCaT were used. After 24 h of exposure, two from four environmental samples had no effect on cytokine production but the other two induced production of interleukin (IL) 6, IL-8, CCL-2 and -20 similarly or even more than *Escherichia coli* LPS used as a positive control. Moreover, cyanoLPS isolated from axenic *Aphanizomenon flos-aquae* was also active. Surprisingly, all studied cyanoLPSs accelerated wound closure in the scratch assay except of cyanoLPS isolated from axenic *Microcystis aeruginosa*. Furthermore, three environmental samples and axenic samples from *A. flos-aquae*, *Planktothrix aghardii*, and *Dolichospermum curvum* induced production of TNFα by mice macrophages. Interestingly this effect was not affected by inhibition of Toll-like receptor 4 (TLR4) which is known to be the main receptor for *E. coli* LPS action. Taken together, environmental mixtures of LPS produced by cyanoHABs can have similar proinflammatory effects *in vitro* as *E. coli* LPS. Moreover, also pure cyanoLPSs can be active in the means of proinflammatory effects. Surprisingly, TLR4 probably is not the main receptor for these LPSs.

Toxicity of palytoxin, ovatoxin-a, ovatoxin-d and extracts of *Ostreopsis* cf. *ovata* on a panel of cell cultures

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Blooms of the dinoflagellate *Ostreopsis* cf. *ovata* regularly occur along the Mediterranean coastline where they have been involved in human intoxications through inhalation and contact. Although the ovatoxins (OVTX), analogs of the palytoxin (PlTX), are suspected to be responsible for the symptoms, little information on the OVTX toxicity is available due to the difficulty to have significant yields out of purification. In this study, the toxicity of (i) *Ostreopsis* cf. *ovata* crude extracts, (ii) fractions without OVTXs, (iii) purified OVTX a and d, and (iv) commercial PlTX was assessed on a panel of cell types from intestine (Caco2), lung (A549), liver (HepaRG) and nervous system (Neuro2A and EGC= enteric glial cells). Viability (MTT assay) and inflammatory response (IL8 release) were monitored. Besides, intestinal barrier integrity (TEER and LY crossing) was measured also on differentiated Caco2 cells mimicking the intestinal barrier. Except on A549 lung cells, the crude extracts from the two *O*. cf. *ovata* strains showed a similar response on the different cell types. No difference of toxicity between OVTX-a and –d was observed on the different cell types and it appeared equivalent to PlTX. Overall, the non-differentiated Caco2 cells were always less sensitive. On differentiated Caco2 cells, in contrast to crude extracts, no toxicity was observed with OVTX-a and -d up to 20 ng mL^{-1}. Barrier integrity was also affected by PlTX and OVTX-a but no effect was observed with OVTX-d up to 5 ng mL^{-1}. In contrast, an inflammatory response was induced both with crude extracts and with OVTX-a and -d.

Molecular interactions of yessotoxin in the voltage-gated sodium channel Nav1.5: an *in silico* biomedical approach

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Yessotoxin (YTX) and its analogs are polyketide-derived polycyclic toxins produced by at least three species (*Protoceratium reticulatum*, *Lingulodinium polyedra*, and *Gonyaulax spinifera*) of marine dinoflagellates. Toxicological studies indicate low or negligible oral toxicity, but moderate effects in mammalian test subjects by intraperitoneal administration of YTX in high dosages. The mode of action is not fully defined although modifications in secondary messengers in signaling pathways, induction of apoptosis, immunoresponse effects, and cardiotoxicity are reported consequences of high-level YTX exposure. Preliminary clinical trials have shown promise in pharmacological and toxicological applications including as potential anti-inflammatory, antiallergic, anti-viral, and anticancer therapeutants. Over-expression or aberrant function of ion channels (channelopathy) is related to several human illness syndromes, e.g., cell metastasis in breast and prostate cancer. The diverse action mechanisms of YTXs are capable of modifying intracellular signaling pathways, including such voltage-gated sodium channels, specifically NaV1.5. In the search for innovative therapeutics, we evaluated the affinity of YTX for the NaV1.5 channel with *in silico* modeling tools. This approach allowed us to identify these interactions and determine their respective free-binding energies. Our results showed significant interactions and low binding free energies (ΔG), between -6.79 and -10.32 Kcal mol^{-1} for YTX in the NaV1.5 protein model. Certain amino acid residues in Domains I and II were reached, indicating that this toxin is a potential NaV 1.5 modulator. This study constitutes the first reported *in silico* approach for exploration of polyketide-derived dinoflagellate toxins in pursuit of evaluating their therapeutic potential.

Investigating human and environmental risk of *Ostreopsis ovata* and *Prorocentrum lima* by an integrated *in vitro* approach

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Dinoflagellate blooms have become a serious issue in marine systems worldwide. These events can be harmful for the environment and human health due to the production of some of the mightiest toxins in marine environment. *Ostreopsis ovata* and *Prorocentrum lima* are toxic species common in the Mediterranean Sea. The former, known producer of palytoxin-like compounds, is responsible for serious health problems on people exposed to dense blooms. The latter, often associated to *O. ovata* blooms, is able to produce, among other toxins, okadaic acid linked to shellfish poisoning. This study applies a comprehensive *in vitro* toxicological approach to investigate the effect of toxins produced by these two species, evaluating the risks for environment and humans. To preserve all the toxins, experimental treatments were prepared by means of cell lysis. Ecotoxicological effect was evaluated by measuring the mortality rate of *Artemia franciscana* nauplii exposed to treatments with different concentrations. The human risk was evaluated by *in-vitro* skin and eye irritational tests and multielectrode array (MEA)-based neuronal assay. Results show a high mortality rate in *A. franciscana* produced by both species (LC50 = 104.5 cells mL^{-1} for *O. ovata* and LC50 = 2,356.3 cells mL^{-1} for *P. lima*), validating the treatments preparation protocol. Surprisingly, skin and eye irritation tests did not show any significant effect, suggesting that other cellular pathways may be involved. On the other hand, MEAs-based analysis showed a significant inhibition of spontaneous electrical activity at low concentrations after exposure to *O. ovata* (IC50 MFR = 4.5 cells mL^{-1}) and *P. lima* (IC50 MFR = 30 cells mL^{-1}), not attributable to neuronal death.

Sensitization of receptors and ion channels, mediating pain and pruritus in sensory neurons, by the Pacific-ciguatoxin-2 and the Brevetoxin-1

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Ciguatera fish poisoning (CFP) and neurotoxic shellfish poisoning (NSP) syndromes are induced by consumption of seafood contaminated by ciguatoxins and brevetoxins, respectively. The common disabling and persistent sensory symptoms of the two syndromes include paraesthesia, cold dysesthesia, painful disorders (and pruritus in CFP). No curative treatment is available and the pathophysiology is not fully elucidated. Previous work in the laboratory showed that P-CTX-2 induces neuropeptide release from a co-culture of sensory neurons and keratinocytes participating to neurogenic inflammation. In a mouse model, CTX-induced cold allodynia is mediated by voltage-gated sodium channels (Nav)-dependent TRPA1 sensitization. Here, we performed single cell calcium video imaging in sensory neurons from newborn rats to study in vitro the ability of Pacificciguatoxin-2 (P-CTX-2) and brevetoxin-1 (PbTx-1) to sensitize sensory receptors and ion channels. In addition, we studied the neurotrophin release in sensory neurons co-cultured with keratinocytes after exposure of P-CTX2. Our results show that P-CTX-2 and PbTx-1 induced the sensitization of transient receptor potential A1, transient receptor potential V4, protease-activated receptor 2, tetrodotoxin resistant (TTX-r) Nav channels, masrelated G protein-coupled receptor C and A3 in rat sensory neurons. P-CTX-2 increased the release of nerve growth factor and brain-derived neurotrophic factor in the co-culture supernatant, suggesting that those neurotrophins may contribute to the sensitization of the aforementioned receptors and channels. Our results suggest the potential role of sensory receptors and ion channels sensitization in the induction or maintenance of sensory symptoms in CFP and NSP.

Negative impacts of lipophilic toxins on zebrafish development, immune system and tissue regeneration

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The increase in distribution and frequency of Harmful Algal Blooms (HABs) worldwide are cause of social and environmental concern. In southern Chile, HABs episodes have caused countless losses due to high fish mortalities in salmonid farms, affecting local economies and threatening food safety. The most damaging episodes of HABs in the region are associated to paralytic shellfish poison (PSP), but increasingly, episodes caused by species producing Diarrheic Shellfish Poison (DSP) have been detected. The sub-lethal toxic effects on mammals and the mechanisms of action described to date for DSP are diverse although their impact on fish have been neglected. Using the small freshwater teleost zebrafish (*Danio rerio*) as a model, we tested the effects of four lipophilic biotoxins: Okadaic acid, Dinophysis toxin-1, Dinophysis toxin-2, and Yessotoxin-1. We assessed the effects of sub-lethal concentrations on swim bladder inflation, tactile response and locomotor activity on larvae, and the results suggest teratogenic impacts and/or neurotoxicity. Also, using fish expressing fluorescent proteins in specific cell types and whole-mount histology, we detected changes in the distribution pattern of neutrophils and the amount of Goblet cells, suggesting an activation of the immune response. Finally, taking advantage of the zebrafish regenerative capacity, we used a tail-fin amputation protocol and detected a negative impact on tissue regeneration. These effects all together can have complex implications for environmental safety, biodiversity and the salmon industry, since small amounts of toxins can probably increase susceptibility to other threats for fish, such as environmental pathogens.

Temporal and spatial exposure of fish from Scottish coastal waters to domoic acid and paralytic shellfish toxins

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Biotoxin exposure from harmful algal blooms (HABs) has resulted in widespread morbidity and mortality in marine life, including top marine predators such as sea birds, cetaceans and seals. There is therefore a need for an improved understanding of the trophic transfer, and persistence of toxins in marine food webs. For the first time, the concentrations of two toxin groups of commercial and environmental importance, domoic acid (DA) and saxitoxin (including Paralytic Shellfish Toxin (PST) analogues), were measured in the viscera of 40 different fish species caught in Scotland between February and November, 2012 to 2019. Overall, fish had higher concentrations of DA compared to PSTs, with a peak in the summer/autumn. Generalized linear modelling revealed that whole fish concentrations were highest in pelagic species including Atlantic mackerel and herring, key forage fish for many marine predators. The highest DA concentrations were measured along the east coast of Scotland and in Orkney. PSTs showed highest concentrations in early summer, consistent with phytoplankton bloom timings. The detection of multiple toxins in such a range of demersal, pelagic and benthic fish prey species suggests that both the fish, and by extension, piscivorous marine predators, experience multiple routes of toxin exposure. Risk assessment models to understand the impacts of exposure to HABs toxins on marine predators therefore need to consider how chronic, low-dose exposure as well as acute exposure during a bloom could lead to mortalities. The potential synergistic, neurotoxic and physiological effects of multiple toxins require investigation in order to appropriately assess the risks of HABs toxin exposure on marine predators.

Sub-chronic feeding study of saxitoxin using mice

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Regulatory limits for shellfish toxins are often set using the results of acute toxicity testing (one-off dose) whereas in reality, people who eat shellfish often do so regularly. It is therefore important to determine the effect of shellfish toxins over an extended time period. We investigated the effect of saxitoxin dihydrochloride (STX.2HCl) to mice in a 21-day feeding trial. To mimic human feeding behaviour mice were fed saxitoxin-laced food at twice daily 'meal times'. Four treatment groups were used (five female and five male per group) including a control group and three groups fed STX.2HCl at doses of 250, 490 and 715 µg kg^{-1} day^{-1}. Mice were individually caged with food consumption and bodyweight measured daily. In addition, motor control, blood pressure/heart rate and grip strength were measured weekly. Following the 21-day feeding period a blood sample was taken from each mouse for haematology/blood chemistry and the major organs were weighed. Histology sections of tissues were examined by a specialist veterinary pathologist. No adverse effects were observed. Using the current EU regulatory limit of 800 µg STX.2HCl kg^{-1} shellfish flesh, the EFSA high portion size of 400 g and an adult bodyweight of 70 kg, the amount of saxitoxin that can be safely ingested is 4.6 µg kg^{-1} bodyweight. Taking into account the 100-fold safety factor used to extrapolate from animal data to human, mice in this experiment were fed up to 1.5 times this dietary intake for 21 consecutive days without adverse effects. The current saxitoxin regulatory limit therefore seems adequate.

Effect of dissolved metabolites of *Gymnodinium catenatum* (Graham, 1943) on the shrimp *Litopenaeus vannamei* (Boone, 1931): A histological study

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The direct ingestion of toxic species or organisms contaminated with phycotoxins are common forms of poisoning. A second mechanism of poisoning is by the contact or absorption of dissolved toxins through membranes and gills, which allow their uptake through the circulatory system reaching tissues and organs. Of these mechanisms, the less studied one is the effect of dissolved toxins which may be linked to unexplained mortalities of marine organisms. In this work, shrimp juveniles (2.1 g wet weight) were exposed to extracts obtained from 10^4, 10^5 and 10^6 cells L^{-1} of the paralytic shellfish toxin (PST) producer *Gymnodinium catenatum*. Bioassays lasted 10 h, time when mortality initiated. Shrimp exposed to extracts presented erratic movements, swam towards the water surface, and remained close to air diffusers. The main tissue damages were: 1) severe atrophy in gills, 2) nervous system edema grade 1 in 10^4 cells L^{-1} treatment and grade 2 in 10^5 and 10^6 cells L^{-1} treatments, and 3) muscle atrophy. Damages are more evident when shrimps are exposed to more concentrated extracts. Tissue damages, although similar to those recorded when shrimps are exposed to *G. catenatum* cells, are less severe with the exception of the atrophy in gills. Results suggest that dissolved metabolites, including PST, may cause tissue damages and that they may be a potential factor involved in the shrimp mortality in culture ponds and larvae rearing laboratories. Paralytic shellfish toxins may cause these damages, however other dissolved metabolites produced by *G. catenatum* cannot be disregarded for the observed behavioral and tissue damages.

Ichthyotoxicity of mixed blooms of *Dinophysis, Alexandrium* and *Cochlodinium*

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Harmful algal blooms (HAB) cause several environmental as well as economic impacts on coastal ecosystems and aquaculture. In spite of being studied for decades, the effects of HAB on aquatic organisms are not fully elucidated. The majority of studies on HAB have considered the effects of their monoblooms on aquatic organisms. Mixed blooms, in spite of their frequent occurrence (unpublished data), have not received appropriate attention. Among the most widespread species associated with hardship to the aquaculture industries species of the genera *Alexandrium*, *Dinophysis* and *Cochlodinium* do form mixed blooms in several regions of the globe. Since 2014, we have been evaluating the effects of ichthyotoxic HAB on the full life cycle of valuable aquaculture species of shellfish in simulated monoblooms, and since 2017, in mixed blooms. In previous research presented at ICHA2014 and 2016, we reported part of the results of our research on simulated monoblooms of *Dinophysis caudata*, *Alexandrium* spp., *Heterocapsa*, *Chattonella* and *Heterosigma*. In ICHA2018, we presented our preliminary data on mixed blooms of several ichthyotoxic dinoflagellates and raphidophytes on larvae of the Japanese pearl oyster. At the upcoming ICHA2021, we will be presenting the recent results of our studies on the effects of mixed blooms of *Dinophysis* spp., *Alexandrium catenella* and *Cochlodinium polykrikoides* on gametes, fertilization, embryogenesis, and several larval stages of the Japanese pearl oyster. The synergy and antagonism in mixed blooms of ichthyotoxic HAB will be further discussed in light of our previous and recent results, and based on extensive analyses of the global occurrence of mixed blooms of ichthyotoxic HAB.

Response of the metabolome of *Dreissena polymorpha* exposed to four strains of the cyanobacteria *Microcystis aeruginosa* producing microcystins together or not emerging cyanopeptides

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Cyanotoxins produced by freshwater cyanobacteria constitute a source of growing concern worldwide because of their impact on animal and human health. Besides the cyanotoxins, cyanobacteria can produce around 600 oligopeptides grouped into different classes (e.g. anabaenopeptins, aeruginosins, microginins, cyanopeptolins, microcyclamides), whose ecological relevance and potential impairs for biota have been poorly investigated. The freshwater bivalve *Dreissena polymorpha* was fed with four strains of the cyanobacteria *Microcystis aeruginosa* producing different contents of secondary metabolites during ten days, followed by six days of depuration. The four cyanobacterial metabolite profiles contained microcystins, with or not cyanopeptolins, or aeruginosins with microginins, or microcyclamides with anabaenopeptides. We followed the kinetics of cyanobacterial metabolites in the digestive gland of *D. polymorpha*, and evaluated the molecular effects on the mussel endometabolome via an untargeted metabolomic approach by chromatography coupled to high-resolution mass spectrometry. Results showed that aeruginosins and anabaenopeptins were present in the bivalve after two days of exposures but were probably further metabolised as their concentrations significantly decreased at day ten of exposure. The metabolic profile of *D. polymorpha* exposed to cyanobacteria producing aeruginosins was significantly modified after two days of exposure but differences were less marked after ten days. Mussels exposed to cyanobacteria producing anabaenopeptides with high amounts of microcyclamides contained microcyclamides in their tissues and exhibited a specific endometabolome signature at both days two and ten of exposure. The signature of exposure to cyanobacteria producing microcystins was only visible at ten days of exposure but without microcystins in tissues. These results suggest different dynamics of metabolisation and of physiological effects induced by various cyanopeptides in *D. polymorpha*.

Effects of the marine toxin palytoxin on the model organism *Artemia franciscana*

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Palytoxin and its analogous are non-protein marine toxins with a worldwide distribution. They have been found in several marine organisms including soft corals, dinoflagellates and cyanobacteria. Besides strong evidences demonstrating its toxic potential in humans, literature data suggest that palytoxin can have a negative impact also at the environmental level, including marine organisms. Considering the potential ecotoxicological impact of palytoxin, this study was aimed to investigate its effects on *Artemia franciscana*, a micro-crustacean of the Artemiidae family frequently used as model organism in (eco) toxicological studies. Exposure of *Artemia* (two developmental stages) to environmental-relevant concentrations of palytoxin (0.1 - 10 nM) showed that the adults were more sensitive than the early stage (nauplii stage I), in term of mortality. Hence, *Artemia* adults were chosen to investigate the possible toxic mechanism after 12 h exposure to the toxin: palytoxin exposure induced a slight, but significant, increase of reactive oxygen species with an altered activity of gluthatione S-transferase, an enzyme correlated with the detoxification capability. Moreover, palytoxin did not significantly alter protein content, and, even if an altered locomotion was observed, no change in cholinesterase activity was detected. In conclusions, these results demonstrate that environmental-relevant concentrations of palytoxin could have a negative impact on *A. franciscana* population, suggesting a potential ecotoxicological impact of the toxin.

Experimental studies on paralytic shellfish toxins in Southern Rock Lobster

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Paralytic shellfish toxin (PST) risks in the Tasmanian and New Zealand Southern Rock Lobster (SRL) industries (collectively valued at AUD 365M) are actively managed. Experimental studies were conducted to inform public health risk, supply chain susceptibility and understanding of physiological impacts to SRL. Adult male lobsters fed highly toxic mussels rapidly accumulated PST in the hepatopancreas, reaching a maximum of 9.0 mg STX.2HCl eq kg^{-1}, then depurated at a rate of 7 % per day. PST were not detected at significant levels in the haemolymph but were found in antennal glands and gills in measurable quantities. This first report of PST in lobster antennal glands and gills represents possible excretion routes for PST. Apparent feed intake increased with PST concentration, indicating SRL will continue to feed during bloom periods. Exposure to PST did not result in SRL mortality nor significant changes in a comprehensive range of behavioural, health, haemolymph biochemical parameters or nutritional measures. Lobsters showed a hyperglycaemic response to PST exposure indicative of stress, but the lack of other impacts suggest limited gross impact on SRL performance. In a separate controlled experiment, SRL were exposed to highly toxic cultures of *Alexandrium catenella* at field relevant concentrations (2×10^5 cells L^{-1}) over a period of 21 days. No PST toxin accumulated and no negative impact on lobster health was observed. We conclude that there is no risk of PST accumulation, nor risk to survival or quality at the point of consumption through exposure to toxic algal cells.

Gambierol blocks a K+ current component affecting action potential duration in fetal adrenomedullary chromaffin cells. Is this action sufficient to modulate catecholamine release?

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The genera *Gambierdiscus* and *Fukuyoa* produce numerous ladder polycyclic ether compounds including gambierol, characterized by a transfused octacyclic polyether core, and the identified ciguatoxins, responsible for ciguatera poisoning. Gambierol inhibits voltage-gated K+ (Kv) channels in various cells, and motor nerve terminals and enhances acetylcholine release. The purpose of this work was to study the effects of gambierol on cultured rat fetal adrenomedullary chromaffin cells. These excitable cells have different types of Kv channels and release catecholamines. Perforated patch-clamp current-recordings revealed that gambierol (100 nM) only blocked a small component of the total outward K+ current, and did not affect calciumâactivated K+ channels (KCa) and ATP-sensitive K+ (KATP) channels. When KATP and KCa channel activation were blocked, the gambierol concentration inhibiting 50 % of the K+ current-component (IC50) was 7.6 ± 1.10 nM (n = 9). Gambierol also slowed the kinetics of K+ current activation. The recording of all-or-none action potentials in chromaffin cells disclosed that gambierol (50 nM) prolonged by 33 % their repolarizing phase. Gambierol did not modify the membrane potential following 15-second depolarizing current-steps, and did not increase the number of exocytotic catecholamine release events, as determined by simultaneous patch-clamp and single-cell amperometry recordings. Under, the same conditions gambierol enhanced catecholamine secretion provided KCa and KATP channels were blocked. In conclusion, the specific inhibition of Kv channels by gambierol is not enough to modulate catecholamine-secretion, emphasizing the key role played by KCa and KATP channels.

Characterization of mechanism domoic acid uptake of the mussel (*Mytilus galloprovincialis*) digestive gland

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The raft cultures of the mussel *Mytilus galloprovincialis* are frequently affected by the accumulation of the amnesic shellfish poisoning toxin domoic acid (DA). This species is characterized by a fast uptake and release of this toxin. In this work the main characteristics of its uptake mechanism have been studied by incubation of digestive gland thin slices in media with different composition and DA concentration. DA uptake seems to follow a Michaelis - Menten kinetics with very high estimated KM (1,722 µg DA mL^{-1}) and a Vmax of 71.9 µg DA g^{-1} h^{-1} which is similar to those found for other amino acids in invertebrates. Replacement of NaCl from the incubation media by Cl-choline (Na+-free medium) did not significantly reduce the uptake, but replacement by sorbitol (Na+-free and Cl--depleted medium) did. A new experiment replacing all chlorides by their equivalent gluconates (Na+- and Cl-- free medium) show and important reduction in the uptake that should be attributed to the absence of chloride, pointing to a Na+-independent, Cl--dependent transporter. In media with Na+ and Cl-, neither decreasing the pH nor adding cyanide (a metabolic inhibitor) had significant effect on DA uptake, suggesting that the transport mechanism is not H+- or ATP-dependent. In a chloride depleted medium, notwithstanding, lowering pH or adding cyanide increased the uptake, suggesting that other anions could, at least partially, substitute chloride.

Immune response of *Crassostrea corteziensis* (Bivalvia: Ostreidae) exposed to *Prorocentrum koreanum* (Dinophyceae: Prorocentrales) from Gulf of California

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The native oyster *Crassostrea corteziensis* is reared in NW Mexico and is naturally exposed to potentially toxic phytoplankton, such as *Prorocentrum koreanum*, a planktonic species which toxicity is globally unknown. To evaluate the immune response, juvenile oysters were exposed to three treatments: 1,000, 2,000 and 3,000 cells mL^{-1} of a *P. koreanum* strain (PKPV-1), three individuals were collected at 1, 3 and 24 h; 3, 5 and 7 days. The control group and the remaining individuals of the treatments were fed with *Chaetoceros muelleri* for two more weeks for their detoxification. The total hemocytes count (THC), its viability, and their phagocytic activity were assessed according to cell density and time of exposure. The THC decreased with increasing cell density, and it was reduced by tenfold from hour one to day seven of exposure to dinoflagellate. Hemocyte viability decreased at seven days of exposure. Phagocytosis was higher in the treatments with respect to the control, increased with time of exposure and decreased after the detoxification period. *Crassostrea corteziensis* resisted these effects the first three days. Lipophilic toxins were not detected by UPLC–MS/MS in this *P. koreanum* strain neither in exposed oysters. The results suggest that the observed effects of *P. koreanum* on the immune response of *C. corteziensis* were due to 1) the production of mucus by the dinoflagellate; 2) differences on the abundance, ingestion, and digestion of cells between treatments; and 3) feeding behavior controlled by the mechanism of closing-opening of valves in the mollusk.

PSP concentration in the mollusk *Concholepas concholepas* (Loco) and detoxification measures

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Harmful algal blooms of toxin-producing microalgae are recurrent in Southern Chile. Paralytic Shellfish Poisoning (PSP) outbreaks pose the main threat to public health and the fishing industry in the Patagonian fjords. The aims of this study is to know the individual and spatial variability of the PSP concentration in *Concholepas concholepas* from two geographic areas of the Aysén Region —Ovalada and Concoto Islands— to determine the effect of pigment removal and freezing process in the detoxification. 150 specimens (≥ 10 cm length) were collected in each geographic area. The live specimens were transferred to the processing plant, where they were measured, gutted and the foot was divided into two equal parts, the pigment was manually removed in one of them. The PSP concentration of each individual was determined by mouse bioassay and High Performance Liquid Chromatography. The toxin concentration was variable in the two geographic areas, 48 and 146 µg STX eq 100 g^{-1} (CV = 43.8 %) in Ovalada and <30 µg STX eq 100 g^{-1} and 153 µg STX eq 100 g^{-1} (CV = 52.5 %) in Concoto. The toxicological profile showed a dominance of Saxitoxin (> 95 %), neoSTX and GTX2 in some individuals. The removal of pigment produced up to 90 % of reduction in PSP concentration and could be used as a good detoxification tool. The freezing process in the muscle with pigment did not show a clear pattern. There is a significant reduction ($p < 0.05$) of PSP concentration from Ovalada, but not in individuals from Concoto. This difference could be related to the smaller size of the individuals collected from Ovalada.

The potential impact of water blooms and associated bacteria on human health concerning their composition

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Lipopolysaccharides (LPS) of natural cyanobacterial water blooms (CWB-LPS) are a mixture of both cyanobacteria (CyanoLPS) and heterotrophic Gram-negative (G-) bacteria. While G- bacterial LPS are potent inflammatory agents (endotoxins) causing serious human health effects through activation of Toll-like receptor 4 (TLR4), the biological activity of CyanoLPS or CWB-LPS has been understudied. We investigated how are the proinflammatory effects of CWB-LPS affected by a cyanobacterial bloom composition, namely, by the presence of specific cyanobacterial and associated bacterial species. Microscopy, flow cytometry, 16S rRNA gene amplicon sequence analysis, TaqMan-Probe qPCR, chlorophyll and phycocyanin contents and Pyrogene rFC assay (endotoxin activity) have been used to describe > 15 microbial communities (cyanobacteria, bloom-associated heterotrophic and free-living heterotrophic bacteria) of studied reservoirs in the Czech Republic. Complex CWB-LPS were isolated from cyanobacterial bloom samples, their proinflammatory *in vitro* effects have been evaluated using Pyrogen assay and by the production of proinflammatory cytokines in human cells. Results showed that proinflammatory effects of CWB-LPS are likely caused by both LPS from bloomassociated G- heterotrophic bacteria and CyanoLPS and their pyrogenic activity depended on present cyanobacterial species. The pyrogenic activity of CyanoLPS, unlike G- bacterial LPS (*E. coli*), did not predict their potential to activate immune cells. Moreover, another mechanism than the TLR4 - signalling pathway is likely involved in the proinflammatory activity of CyanoLPS.

NOVEL HAB TECHNOLOGIES

Co-deployed, advanced sensor technologies yield unprecedented insight into *Pseudo-nitzschia* toxicity and growth dynamics in Gulf of Maine, U.S.A.

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The NHABON - NE (National Harmful Algal Bloom Observing Network – New England) comprises a fleet of advanced phytoplankton sensors for harmful algal bloom surveillance in New England, U.S.A., providing unprecedented situational awareness and decision support for resource managers. An Imaging FlowCytobot (IFCB) and Environmental Sample Processor (ESP) were co-deployed shoreside and configured to measure particulate domoic acid (pDA) when *Pseudo-nitzschia* spp. (PN) were present, as a means of addressing the increasing regional threat of Amnesic Shellfish Poisoning. A high-resolution time-course of PN toxicity and growth dynamics over a fall 2020 HAB event in Harpswell Sound, ME, captured an entire bloom cycle and revealed changes in toxicity of the PN population that may influence how quickly and to what extent shellfish become contaminated with DA. Maxima for pDA and PN of 921 ng L^{-1} and 118,352 cells L^{-1} (mostly *P. pungens*/*P. seriata* morphotypes), respectively, were recorded. Throughout early bloom phases, increasing pDA tracked closely with PN cell concentration; however, during the termination phase these variables became uncoupled, resulting in elevated cellular toxin quotas (maximum 44 pg DA $cell^{-1}$), although pDA associated with detritus potentially contributed. Data from co - deployed mussel bags (analyses underway) will show how shellfish toxicity varied with PN toxicity and cell concentration, and help to determine whether many low toxicity cells or few high toxicity cells are more effective in contaminating shellfish. These findings and their potential management implications are being communicated to state shellfish managers and the aquaculture industry as the initial phase of a dialog aimed at maximizing the value of IFCB - ESP data as a decision support tool.

Advances in the detection of toxic algae using electrochemical biosensors

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Harmful algal blooms (HABs) are becoming more frequent as climate changes, with tropical toxic species moving northward, e.g. up the Iberian Peninsula. Monitoring programs, detecting the presence of toxic algae before they bloom, are of paramount importance to protect aquatic ecosystems, aquaculture, human health and local economies. Rapid and reliable species identification methods using molecular barcodes coupled to biosensor detection tools have received increasing attention as an alternative to the legally required but impractical standard microscopic counting-based techniques. Our electrochemical detection system for the determination of these toxic algae has been improved moving from the conventional sandwich hybridization protocols using different redox mediators and signaling probes modified with different labels to a novel strategy involving the recognition of heteroduplexes by selective commercial antibodies further labeled with bacterial antibody binding proteins conjugated with multiple enzyme molecules. Although each development has increased sensitivity, the most significant (a 100-fold increase in signal) has been produced with this latest strategy. Our newest results involve the use of magnetic microbeads (MBs) and amperometric detection at screen-printed carbon electrodes (SPCEs) to detect the RNA of our target toxic species. With these improvements, our current system is able to detect as low as 5 cells per liter for some species, by using a fast, simple and cheap methodology that can be integrated in easy-to-use portable systems. Our long-term goal is to apply this optimized protocol in a laboratory on a chip (LOC) with up to 200 electrodes for the simultaneous detection of all toxic species.

Near real-time surveillance of Harmful Algae and their toxins in dynamic coastal shelf environments using the environmental sample processor

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Efforts to identify *in situ* the mechanisms underpinning the response of harmful algae to climate change demand frequent observations in dynamic and often difficult to access environments. Increasingly, resource managers and researchers are looking to fill this data gap using unmanned systems. To this end, the Environmental Sample Processor (ESP) was integrated into an autonomous platform to provide near real-time surveillance of harmful algae and the toxin, domoic acid, on the Washington State, USA continental shelf over a three-year period (2016 - 2018). The ESP mooring design accommodated the necessary subsystems to sustain ESP operations, supporting deployment durations of up to 7.5 weeks. The combination of ESP observations and a suite of contextual measurements from the ESP mooring and a nearby surface buoy permitted an investigation into toxic *Pseudo-nitzschia* spp. bloom dynamics. Preliminary findings suggest a connection between bloom formation and nutrient availability that is modulated by wind-forced coastal-trapped waves. In addition, high concentrations of *Pseudo-nitzschia* spp. and elevated levels of domoic acid observed at the ESP mooring location were not necessarily associated with the advection of water from known bloom initiation sites. Work in progress on this project will involve modifications to the ESP to improve communications as well as increase sample capacity and deployment duration by 50 %. These improvements will enable the ESP to sample and provide data to resource managers for continuous periods of up to six months.

Smart biosensors for the detection of ciguatoxins

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Ciguatoxins (CTXs) are lipophilic marine toxins produced by microalgae of the genera *Gambierdiscus* and *Fukuyoa*. Ciguatoxins accumulate into fish and through the food webs, and are responsible for ciguatera fish poisoning (CFP) when reaching human consumers. In this work, two biosensors for the detection of CTXs were developed, both based on the use of three monoclonal antibodies, two capture (3G8, 10C9) and one detector (8H4), in a sandwich configuration. In the first immunosensor, the capture antibodies were conjugated to magnetic beads and measurements were taken with a conventional potentiostat. In the second one, the capture antibodies were immobilised on multi-walled carbon nanotube-modified electrodes and a portable potentiostat connected to a smartphone was used for electrochemical signal recording. Immunosensors attained limits of detection at the pg mL^{-1} level and, when applied to the analysis of spiked fish samples, they were able to detect 0.01 µg kg^{-1} of CTX1B, the guidance level proposed by the United States Food and Drug Administration (US FDA). They were also applied to the analysis of naturally contaminated fish from la Réunion (France) and Japan, providing results that correlate with mouse bioassay (MBA), cell-based assay (CBA) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The approach was also applied to the analysis of *Gambierdiscus* and *Fukuyoa* extracts, providing useful information about their toxin profile. The developed biosensors are highly sensitive, rapid and easy-to- use bioanalytical tools. They can contribute to guarantee fish safety and consumer health protection, and to understand the ecology and impact of ciguatera.

The application of Nanopore sequencing to the study of phytoplankton. All the thrills with smaller bills

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The mass sequencing of environmental DNA (Metabarcoding) offers great opportunity for the study of phytoplankton but has, historically come at significant financial cost, both in terms of the hardware and the consumables required. The availability of third generation/long read sequencing from Oxford Nanopore Technologies (ONT) has the potential to remove these financial barriers. With platforms starting at ~$ 1,000 that can generate long sequence data in real time and being portable, the application of this technology has many exciting opportunities to the study of harmful algae. There have, however, been significant hurdles to overcome, notably read accuracy is relatively low in comparison to other technologies (~85 - 95 %) and handling the large volumes of data can be challenging. This presentation introduces nanopore sequencing products and how bioinformatic pipelines were used to convert low accuracy raw sequences into polished consensus sequences (> 99 %) from both reference cultures and complex environmental samples. Other challenges associated with the assay are also discussed as well as a strategy to provide ongoing improvements in the future.

Using qPCR and high-resolution sensor data to model a multi-species *Pseudo-nitzschia* (Bacillariophyceae) bloom in southeastern Australia

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Harmful algal blooms, including those caused by the toxic diatom *Pseudo-nitzschia*, can have significant impacts on human health, ecosystem functioning and ultimately food security. In this study, we characterized a bloom of *Pseudo-nitzschia* that occurred in a south-eastern Australian oyster-growing estuary in 2019. Using light microscopy, molecular (ITS/5.8S and LSU D1-D3 rDNA regions) and toxicological evidence, we observed a multispecies bloom consisting of *Pseudo-nitzschia* cf. *cuspidata*, *P. hasleana*, *P. fraudulenta* and *P. multiseries*, with *P.* cf. *cuspidata* being the only species that produced domoic acid (3.1 pg DA per cell). We developed a rapid, sensitive and efficient quantitative real-time polymerase chain reaction (qPCR) assay to detect species belonging to the *P. pseudodelicatissima* complex Clade I, to which *P.* cf. *cuspidata* belongs. This assay indicated that *P.* cf. *cuspidata* or closely related strains, may have dominated the *Pseudo-nitzschia* community during the bloom. Using high-resolution water temperature and salinity sensor data, we then modelled the relationship between light microscopy determined abundance of *P. delicatissima* group and environmental variables (temperature, salinity, rainfall) within the estuary. General Linear Models (GLMs), explaining between 9 and 54 % of the deviance, suggested that water temperature (increasing) and/or salinity (decreasing) data were generally more predictive of high cell concentrations than rainfall data, previously used for management purposes. We conclude that the combination of rapid molecular methods such as qPCR and real-time sensor data modelling can provide a more rapid and effective early warning of harmful algal blooms of species of *Pseudo-nitzschia*, resulting in more beneficial regulatory and management outcomes.

First Imaging FlowCytoBot observations of the phytoplankton community in Scotland coastal waters

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Underpinning marine food webs and supporting the carbon cycling, phytoplankton are important for all coastal ecosystems. Nevertheless, depending on environmental factors, Harmful Algal Blooms (HABs) can develop. Aquaculture is important for food supply, and particularly in rural areas, for income and local economy. This leads to the need to understand HABs to best manage coastal resources. The Shetland Islands are an important hub for Scottish aquaculture and hence need protection from HABs. Current microscope based HAB monitoring methods are time consuming and labour demanding, limited to weekly monitoring and slowing the early warning processes. Moreover, changing climate may increase the difficulty in forecasting HAB trends in the future. To capture and understand these rapid changes, higher resolution time series are needed. This lack of large dataset production can be overcome with new emerging technologies, such as the Imaging FlowCytoBot (IFCB), developed by WHOI and marketed by McLane labs, which can provide hourly monitoring of the phytoplankton community. Here we present the first observations of the phytoplankton community in Shetland coastal waters using an IFCB. The automated classification software, focused on currently monitored phytoplankton species, enables fast enumeration and identification of the cells present. This first high-resolution dataset will be compared to the data produced by the current weekly monitoring programme. The potential for the IFCB data to feed forecast and predictive models coupled with environmental variables, using approaches such as Random Forest or Long Short-Term Memory, will be evaluated.

Rapid and sensitive detection of *Karenia mikimotoi* by loop-mediated isothermal amplification combined with a lateral flow dipstick

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Harmful algal blooms occur frequently in various seas across the world, causing huge economic losses in aquaculture, serious damage to marine ecology, and threats to human safety. Because of these risks, a rapid detection method for key toxic and harmful red tide algal species is needed, to allow for red tide early warnings as well as daily monitoring. In this study, loop-mediated isothermal amplification (LAMP) was combined with a lateral flow dipstick (LFD) to establish a rapid biological detection method for a key red tide algae *Karenia mikimotoi*. In this method, the internal transcribed spacer (ITS) sequence was used as the template, and the corresponding specific primers were designed using the online software PrimerExplorer V5. Biotin was labeled on the 5 'end of the inner primer FIP, and the LAMP reaction was carried out under determined optimal conditions. The lowest concentration of *K. mikimotoi* DNA tested using LAMP was 3.3 × 10^{-1} pg μL^{-1}. A 6-FAM labeled probe was also designed, and the results were displayed on the LFD after hybridization of the amplified product with the probe. The results demonstrated that this new method has good specificity, high sensitivity, is simple in operation, and produces results fast, indicating that it has promising prospects for use in detecting and monitoring red tide.

Development and comparison of IFCB classifiers in two different environments in coastal California

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The Imaging FlowCytobot (IFCB) is emerging as a valuable new tool in phytoplankton ecology and Harmful Algae Bloms (HAB) monitoring. After development and testing of a classifier for image identification, an IFCB provides round-the clock, real-time observation of the phytoplankton community in its deployment location. Development of the classifier is time-consuming and labor intensive, and requires trained taxonomists with knowledge of the local phytoplankton. In this study, IFCBs were deployed at Pier 17 in the San Francisco Bay, and the Santa Cruz Wharf in Monterey Bay. We investigate how the performance of the classifiers developed in these specific locations compare to a combined, "regional" classifier for identifying common California HAB-forming taxa, including *Pseudo-nitzschia* spp., *Alexandrium* spp., and *Dinophysis* spp. Additionally, data collected from side by-side IFCB deployments at the Santa Cruz Wharf were used to compare the performance of the instruments to one another. This allowed us to determine how well images from two different instruments represent the phytoplankton community at a single site, and evaluate whether or not a classifier is inherently tied to the instrument it was built on. This work will provide valuable insight into the development of the HAB-monitoring network of IFCBs along the California coast.

OA-producing dinoflagellate *Dinophysis acuminate* induces behavioral responses in the mussel *Mytilus galloprovincialis*

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The okadaic acid (OA)-producing dinoflagellate *Dinophysis acuminate* is a well-known microalga that causes diarrhetic shellfish poisoning (DSP) in many coastal regions of the world. In this laboratory study, we applied high-frequency valvometry to follow the valve movements of mussels *Mytilus galloprovincialis* feeding on toxic *D. acuminate* (n = 34 mussels, shell length = 60.1 ± 2.0 mm, xÌ ± SD) or a morphologically similar, but toxin-free dinoflagellate, *Prorocentrum micans* (n = 31 mussels, shell length = 60.0 ± 1.6 mm). Dinoflagellate inoculations were conducted in four sequential "pulses" intended to increase cell concentrations in a stepwise manner up to ~14,000-20,000 cells L^{-1} over a 6 h period. Rising concentrations of the toxic *D. acuminate* induced no immediate response from the mussels, whereas *P. micans* prompted an increase in shell opening over a short period of time (~ 9 h). More striking behavioral features emerged ~12-24 h following *D. acuminate* exposure, and these features persisted over a 3-day period when mussels had accumulated 43-64 µg OA equivalent per kg of meat (EU regulatory limit is 160 µg kg^{-1} shellfish meat). It was found that OA - contaminated mussels were more frequently and widely opened than control mussels, suggesting that OA altered the contraction of the adductor muscle. These observations are consistent with growing evidence that bivalves are sensitive to low concentrations of harmful microalgae. Deploying in situ valvometry sensors in recurrent HAB areas constitutes a promising tool to use bivalves as early warning sentinels.

High resolution melting: An innovative approach for molecular characterization of the diatom *Pseudo-nitzschia* spp.

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The diatom *Pseudo-nitzschia* species are common components of marine phytoplankton. The *Pseudo-nitzschia* spp. are extensively studied with regard to the ecological, morphological and genetic aspects, and amnesic shellfish poisoning (ASP) toxin content. Currently, differentiation of species requires time-consuming optical and electron microscopy analyses to distinguish taxonomic features that allow species identification, while cryptic species can still remain to be misidentified. Molecular approaches can play a key role in solving taxonomic problems, as well as geographical distribution, and level of genetic population differentiation. Further, the correct identification of *Pseudo-nitzschia* species has relevant implications for monitoring and management uses considering that toxigenic species may be morphologically similar or identical to non-toxigenic ones. The high resolution melting (HRM) PCR analysis is a powerful technique able to accurately genotype individuals from monoclonal cultures. The HRM analysis is faster, and less expensive than alternative approaches, such as microarray or microsatellites. In this study, strains of the various *Pseudo-nitzschia* species were PCR amplified for subsequently HRM analysis for species-specific discrimination. The distinct melting temperature profiles obtained by this analysis were associated to different species. The HRM assay has been applied for assigning the species of unknown strains (n = 31) of *Pseudo-nitzschia* spp.: 12 strains were identified as *P. calliantha*, 11 strains were identified as *P. delicatissima* / *P*. cf. *arenysensis*; seven strains were identified as *P. pungens* and one strain as *P. multistriata*. The Tm values among species were significantly different by Kruskal-Wallis test (Hc = 70.96, $p < 0.001$). This innovative molecular HRM-curve analysis approach can be used for genotyping and/or species identification of *Pseudo-nitzschia* species.

Quantification of multiple harmful algal bloom species from Long Island Sound using molecular, imaging, and microscopy: 2020-2021

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Long Island Sound (LIS) receives substantial inputs of nitrogen (N) from the nearby New York City (NYC) metropolitan area mainly due to runoff and combined sewer overflow systems. Despite known linkages between N and harmful algal bloom (HAB) development, the extent to which LIS phytoplankton assemblages vary across spatial (area, depth) and temporal (weeks, months) scales remain less characterized. This study leverages long-term, year-round LIS water quality and hypoxia monitoring conducted by the Connecticut Department of Energy and Environmental Protection (CTDEEP). Samples were acquired from five Western LIS stations, in addition to several Central and Eastern LIS stations at two depths (surface and sub-chlorophyll maximum). Here we describe evaluations of physical water quality (salinity, turbidity, temperature, oxygen), chlorophyll *a* and nutrients (N, P, Si) from these surveys. Phytoplankton community composition was analyzed using microscopy and molecular (sandwich hybridization assay, SHA) approaches. Microphytoplankton cell biovolume was also measured to assess the relative biomass contributions of different genera. SHA was used to quantify *Alexandrium catenella*, and results were compared to microscopy counts in both field and laboratory settings. Preliminary observations of summer 2021 phytoplankton assemblages using a FlowCam 8100 obtained courtesy of a student instrument award (May - Aug 2021), highlighting any detections of key HAB species known to bloom in the region (such as *Scrippsiella* spp., *A. catenella*, *Prorocentrum* spp., and / or *Dinophysis acuminata*) will be provided. Results will refine our understanding of the spatiotemporal transitions in relevant environmental metrics, phytoplankton assemblages, can inform biogeochemical and HAB prediction models, and interpret remote sensing data.

A decade of domoic acid in Monterey Bay - SPATT observations give new insight on toxin variability

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The risk of amnesic shellfish poisoning from annual *Pseudo-nitzschia* sp. blooms and subsequent domoic acid events has persisted in Monterey Bay on the central coast of California for nearly three decades. Over this time, harmful algae bloom monitoring has experienced rapid advancement in methods and technologies designed to predict toxic blooms and mitigate losses. One such innovation, solid phase adsorption toxin tracking (SPATT), is now widely applied to measure *in situ* dissolved toxins, often complementing toxin quantification from shellfish. Here we show ten years of domoic acid measured from SPATT in Monterey Bay and investigate trends in relation to key ecological and physical factors. Of the three SPATT resins deployed (HP20, SP207, and SP700), results indicate subtle differences that may affect how well domoic acid from SPATT models extractions from shellfish. In recent years, SPATT samples contained domoic acid when levels in mussels were below the limit of detection, and all three resins showed increasing toxin concentration over time. Monitoring data from the Santa Cruz Wharf repository revealed positive correlations between SPATT, *Pseudo-nitzschia* sp. cell counts, and domoic acid grab samples and negative relationships between domoic acid and nutrient concentrations, especially phosphate. To our knowledge, this is the longest dataset for detecting domoic acid by SPATT. Continued analysis may give insight to present and future toxin trends as well as greater understanding of the ecological role of domoic acid in Monterey Bay.

Toxic microalgae detection by near infrared spectroscopy and hyperspectral imaging

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Most of coastal countries are affected by out-of-control proliferation of microalgae - harmful algal blooms (HABs), which represent a public health concern and lead to economic losses. Surveillance programs rely on optical microscopy for identification and quantification of toxic algae, which is time consuming and requires specialized and highly qualified operators. Furthermore, species with a similar appearance can only be distinguished unequivocally through observation under electron microscopy or by molecular techniques. The purpose of this work is development of a rapid and cost-effective approach to detection and identification of toxic microalgae based on NIR and FT-MIR spectroscopy and NIR hyperspectral imaging. These methodologies were applied to the identification and quantification of toxic microalgae species in water samples in the presence of a mixed phytoplankton assemblage. The toxic species *Gymnodinium catenatum* and the nontoxic species *G. nolleri*, with morphological affinities, were cultivated in the laboratory. Mixtures of the two species and natural seawater collected at the Aveiro lagoon were prepared to obtain different cell concentrations corresponding to the warning and closure thresholds established in EU regulation. After filtration of samples, phytoplankton was measured using NIR and FT-MIR spectroscopy and NIR imaging. Spectroscopy and imaging data were processed using multivariate and multi-way methods, respectively. IR spectroscopy and especially NIR imaging were found to be capable of identifying and quantifying toxic microalgae in the presence of other non-toxic species.

Harmful Algal Bloom detection using ocean colour satellites in coastal waters of the English Channel.

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The frequency and magnitude of Harmful Algal Blooms (HABs) is increasing globally, and therefore poses high risk to human health and shellfish industries. HABs often start in one area and evolve to become problematic in another. Each HABs species has a preferred set of environmental conditions for growth and by understanding these conditions locally, better prediction of the timing and location of HABs may be possible. HABs can be detected using ocean colour satellite data. As part of the project S-3 EUROHAB we have developed a web alert system for the detection of local HABs events in coastal and shelf areas of the French-English Channel. The system provides near real time risk indicator maps of three HABs species (*Karenia mikimotoi*, *Phaeocystis globosa*, *Pseudo-nitzschia* spp.) and a suite of other environmental parameters (including chlorophyll *a*, particulate inorganic carbon and turbidity, rainfall, wind speed and direction, sea water temperature, salinity and ocean mixed layer thickness) using data from the Copernicus European satellites. In this talk we assess the extent of HABs risk events using satellite data over the past decade. In relation to this, we also assess changes in phytoplankton biomass in English Channel coastal waters over the past 20 years, and the reasons for this. We also demonstrate the use of satellite data to track the development, magnitude and spread of HABs and assess the environmental drivers that cause them. Multiple satellite data are combined to develop proxies for HAB species that occur at very low cell density.

Photo: Fernando Sánchez Bernal

HAB PREDICTION

Predicting *Pseudo-nitzschia* Harmful Algal Blooms along the California Coast in a Changing World

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Pseudo-nitzschia species, cosmopolitan diatom, produce domoic acid (DA), a neurotoxin which has been shown to negatively impact wildlife, fisheries, and put human life at risk through amnesic shellfish poisoning. Environmental stressors are hypothesized to stimulate DA production and accumulation. Silicate limitation, in diatoms, results in decreasing primary metabolism, i.e., DNA synthesis and gene expression, alleviating energy for DA production. Changes in light levels have also been shown to adjust biosynthetic properties in *Pseudo-nitzschia*. To quantify and estimate the feedbacks between DA production and environmental conditions, we designed a simple mechanistic model, which is validated against batch and chemostat experiments. Similar to observations, our model shows that as nutrients (i.e., silicon, iron, and phosphorus) become limiting DA production increases. Under Si limitation, we found an approximate doubling in DA production compared with N limitation. Additionally, DA production was found to have a positive relationship with light intensity based on adjustments in primary metabolism and photosynthesis. These results support the idea that the relationship with nutrient limitation and light is based on direct impacts on biosynthesis and biomass accumulation in *Pseudo-nitzschia*. The model is deigned to be easily embedded in three-dimensional ocean ecosystem models. We show preliminary results from numerical simulations with a regional physical biogeochemical model of the California Current, and compare results to a large compilation of DA observations along the U.S. West Coast.

‘MyRedTides’ a fast and easy web application for sharing Harmful Algal Bloom information in Sabah coastal waters, Malaysia

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Harmful algal bloom (HAB) was first reported in Sabah coastal waters in 1976. The first event was caused by *Pyrodinium bahamense* and later in 2005, *Margalefidinium polykrikoides* started to occur. Since then, these two species have occurred alternately in the risk areas mainly in the West Coast of Sabah and caused huge impact to fisheries industry as well as human health problems. To manage this problem, monthly monitoring has been conducted by the Department of Fisheries Sabah at the affected areas which include 13 districts i.e. Kota Kinabalu, Tuaran, Kota Belud, Sipitang, Tawau, Papar, Kota Marudu, Kudat, Kuala Penyu, Beaufort, Sandakan, Lahad Datu and Semporna. In every district, water samples and shellfish are collected and sent to Likas Fisheries Complex for cell density and toxicity analyses. Data collected from each district are combined to prepare a full report and disseminate to all Fisheries district officers for further action. The whole process of decimating the whole result, took about three days. In order to organize and updating data analysis and also to shorten and fast disseminating of HAB data, a Web Apps developed by ArcGIS is being used to develop an application known as MyRedTides for the Department of Fisheries Sabah. Through this application, HAB data can be shared via online public information system and will be used to map and updating HAB information as well as to be employed as early alerting system.

Improving Forecasts for *Karenia brevis* in the Gulf of Mexico

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Brevetoxins produced by *Karenia brevis* can be aerosolized and lead to severe respiratory irritation, with documented health risks for people with asthma. Economic impacts also occur as even healthy people avoid beach businesses when a “red tide” is reported to be in a region. However, the distribution of brevetoxin aerosol impact varies greatly, depending on patchiness of blooms and wind speed and direction. Locating and forecasting the blooms and respiratory risk are then necessary to reduce the health and economic impacts. Previously, manual and qualitative evaluation determined the location and respiratory risk of these blooms at coarse resolutions (daily and county scale). We have substantially improved forecast and nowcast timeliness with an automated capability that combines new resources and models. These include a chlorophyll fluorescence algorithm (red - band difference) applied to Sentinel - 3 satellite products, the HABscope (a microscope deployed by volunteers with digital video analysis), existing water sampling networks, respiratory irritation risk models, and a bloom intensification forecast.

Investigating environmental proxies to predict *Dinophysis* spp. blooms along the coasts of the English-French Channel

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In a context where Harmful Algal Blooms represent an important issue for shellfish production around the globe, the ongoing Interreg funded S3 - EuroHAB project aims at creating a web alert system to monitor, through satellite remote sensing data, the presence and dispersion of selected harmful species (*Pseudo-nitzschia* spp., *Karenia* spp., *Phaeocystis* spp.) in the English-French Channel. From this project, a specific collaboration study around *Dinophysis* occurrence and DSP toxic episodes has been undertaken using temporal datasets from both side of the Channel. *Dinophysis* cells can contaminate shellfish at low levels of cell abundance making its forecast from satellite difficult. Therefore, our objective is to find an environmental proxy for *Dinophysis* occurrence by investigating the conditions that influence its appearance. The case study areas cover coastal sites within St Austell Bay and Lantivet Bay on the English side of the Channel plus an offshore site south of Plymouth and coastal sites within the Bay of Seine in France. These sites were chosen for comparison as they represent areas heavily affected by *Dinophysis* blooms or areas without any bloom occurrence. On both sides of the Channel, the toxic episodes caused by *Dinophysis* are becoming more frequent, causing regular shellfish farm closures. From an analysis of seasonal affinities, results indicate bloom occurrences specific to summer and early autumn but rare during the rest of the year. At coastal monitoring stations, common correlations indicates a link between *Dinophysis* blooms and wind direction, water temperature, daylight and water stratification on both sides of the Channel.

The temporal dynamics of *Dinophysis* blooms is linked to that of the providers of its plastids

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The dinoflagellate *Dinophysis* spp. is responsible for causing diarrheic shellfish poisoning (DSP) and consequently severe economic damage to shellfish aquaculture around the world. Most *Dinophysis* are mixoplankton. *Dinophysis* acquires phototrophy through a food chain linkage between a cryptophyte (often the constitutive mixoplanktonic *Teleaulax*), the mixoplanktonic ciliate *Mesodinium*, and *Dinophysis* itself. Thus, *Mesodinium* relies exclusively on *Teleaulax* as a source of its chloroplasts and *Dinophysis*, in turn, receives its chloroplasts exclusively from *Mesodinium*. Despite the importance of this chain of events for *Dinophysis* blooms, the temporal dynamics of the blooms of *Teleaulax* and *Mesodinium* as precursors for *Dinophysis* blooms have not been explored in simulation models explicitly describing the exchange of plastids and the mixoplanktonic activity of the three organisms. Using a nitrogen-based *Teleaulax*-*Mesodinium*-*Dinophysis*model we explored these dynamics operating under different ecological settings. The model included *Synechococcus* as prey for *Teleaulax* and another autotrophic alga as an additional competitor and prey species. Nutrient load, mixed layer depth and irradiance all greatly influenced the species succession, biomass of each species and the potential for *Dinophysis* to form blooms. Varying ecological conditions affected the potential for *Dinophysis* blooms up to three months later. The model provides a test bed for hypotheses on the bloom formation of *Dinophysis*, showing the complexity of its relationship with its environment, and aiding monitoring and forecasting of *Dinophysis* HAB events. Future monitoring of *Dinophysis* would likely be enhanced by monitoring also for its precursor prey species, *Teleaulax* and *Mesodinium*, which are rarely accorded the same monitoring effort.

Synoptic-to-intraseasonal modulation of phytoplankton abundance in the Inner Sea of Chiloé, northwestern Patagonia (42.5° - 43.5°S)

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The Inner Sea of Chiloé (ISC), in northwestern Patagonia, has experienced large HABs in the past decade, impacting human health and affecting the large aquaculture industry of the region. Using a novel method to analyze the synoptic-to-intraseasonal variability, we explore changes in phytoplankton abundance and their concurrent meteorological and oceanographic conditions. We used high resolution satellite normalized fluorescence line height (nFLH) from the MODIS-Aqua sensor as a proxy for phytoplankton abundance, besides atmospheric and oceanographic variables from the ERA5 and GLORYS reanalysis. Specifically, we focus on events of high phytoplankton abundance (EHPA), which are defined as those cases when nFLH exceeds the 95th percentile threshold; each event was characterized by its first date of occurrence (called day 0). We detected 16 EHPA between 2003 and 2019 in the southern ISC. EHPA occurred under the influence of a migratory anticyclone that induced persistent cloudless conditions preceding day 0, leading in turn to enhanced photosynthetically active radiation (PAR) starting around day -8, and positive sea surface temperature (SST) anomalies between days -4 and 4. We hypothesize that EHPA are mainly modulated by i) mixing and advection that could contribute to a greater availability of nutrients on the sea surface prior and during the anticyclonic anomalies, respectively; and ii) increased thermal stratification related to positive PAR and SST anomalies that would promote phytoplankton growth. Furthermore, we show that the Madden-Julian Oscillation modulates the conditions associated with EHPA, a fact that indicates an enhanced predictability of these cases.

A mesoscale temporal assessment of a *Microcystis* sp. bloom using unmanned aircraft system (UAS) hyperspectral imagery and satellite-derived algorithms

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Remote sensing technologies are known to provide advantages for comprehensive, spatiotemporal coverage to assess water quality, including estimating cyanobacterial biomass. Emerging technologies like hyperspectral sensors and Unmanned Aircraft Systems (UAS) offer the potential to monitor cyanobacteria blooms at higher spectral, spatial, and temporal resolutions. Therefore, we used a hyperspectral sensor (Headwall Photonics Nano-Hyperspec) onboard a UAS (Multirotor G4 SkyCrane) to monitor a developing *Microcystis* sp. bloom in a controlled mesocosm study with coincident ground sampling. This approach allowed for the simultaneous evaluation of 45 spectrally-derived water quality algorithms across three water quality indicators (chlorophyll *a*, phycocyanin, and turbidity) and two cyanobacterial life stages. Of the algorithms evaluated, 22 exhibited relatively strong correlations (R2 > 0.7) for at least one water quality indicator in at least one growth stage. For all water quality indicators, 14 (turbidity) to 15 (chlorophyll *a* and phycocyanin) algorithms had < 15 % difference between growth stages. The greatest variability between performance metrics by growth stage was observed in algorithms having blue or violet wavebands. Of those 22 algorithms with strong correlations, 6 algorithms were portable across water quality indicators (< 15 % difference between growth stages). Collectively, this indicates that not only can cyanobacterial growth stage have an impact on overall algorithm performance, but also that some algorithms could be more effective generalizable predictors of cyanobacterial biomass. This research highlights the importance of understanding how sensors/imagers and spectrally-derived algorithms are impacted by water quality indicators and cyanobacterial growth stages.

Pathways of harmful algae towards the Scottish Shetland Islands – An unexpected journey?

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A particle tracking model is presented and used to explore advection of harmful algal blooms to the West coast of the Shetland Islands at the very North of Scotland. The movement of particles was modelled using surface velocities taken from a configuration run of Atlantic Margin Model, 1.5 km resolution (AMM15). Results of the model are compared to results of *Dinophysis* spp. cell counts measured during the routine coastal monitoring of potentially harmful algal species around Shetland. Particle tracking model results appear to recreate previously hypothesised westward advection of harmful algal cells during 2006, 2013, and 2018, when exceptional *Dinophysis* spp. abundances were measured from the Shetland coast. This provides evidence for the previously posed hypothesis that exceptional abundances of *Dinophysis* spp. are due to cells being advected to the Shetland coast from the West, rather than local growth. Estimates of the water structure modelled by AMM15 are compared to water structure data measured from CTD transects completed during 2017 and 2018. With drifter tracks these comparisons are used to show that although the model does not perform perfectly close to shore, major structures and currents are recreated relatively accurately.

PST events and density of *Alexandrium catenella* at the Beagle Channel (55° S), a singular ecosystem

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The Beagle Channel is an ecosystem located in the southern most area of Chile, characterized by a strong longitudinal gradient, with influence of the Pacific Ocean by the West and the Atlantic Ocean by the East, and at least three large semi-basins that receive freshwater and sediment discharges from glaciar (West side) and river systems (East side). Although it is a remote territory in the South-America, it has a political-strategic and scientific importance. From the point of view of harmful algal blooms, this channel has been included in monitoring activities within the Magellan region since 1997, and then along all the fjords system since 2006. First, the channel was monitored through seven sampling stations, and since 2011 through 10 sampling sites. Part of the obtained results are presented. In each sampling station, qualitative and quantitative phytoplankton samples were collected. Shellfish samples by scuba diving are collected and the PST toxicity was estimated by mouse bioassay. During each cruise, the oceanographic and meteorological conditions, in each sampling sited, has been also recorded. Since 2006, six important PST events have occurred during Spring-Summer, but two occurred during autumn. At a macroscale, these events matched with an increase of *Alexandrium catenella* density, and with microscale lags depending on the sampling station. Maximum toxicity occurred at the middle area of the channel with 9,000 µg of STX eq 100 g^{-1} and 11,000 cells L^{-1} on water column. The results are contrasted and discussed with those obtained for the 1997-2005 period, considering the channel physical characteristics and the oceanographic and meteorological conditions.

HABreports.org: Operational Early warning system (EWS) for harmful algal blooms in Scotland

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Here we present details of an operational early warning system for harmful algal blooms. Toxin producing phytoplankton pose a serious and ongoing obstacle to the growth of the shellfish aquaculture industry. Since 2014 Scientists at the Scottish Association for Marine Science (SAMS), have been producing weekly Harmful Algal Bloom (HAB), risk assessments in the form of bulletins for the Shetland Shellfish industry. These have been enhanced by the addition of an open access web portal. The system consists of a combination of official control monitoring phytoplankton and biotoxin results, satellite derived chlorophyll concentrations and sea surface temperatures, forecasts of surface sea currents, and wind direction. The site also provides Scottish shellfish farmers with an assessment of harvesting risk in the form of a traffic light index. As part of the Interreg Atlantic Area funded project PRIMROSE high resolution particle tracking models have been added providing early warning of HABs that may be advected to coastal sites from offshore waters. We are currently in the process of developing an operational, model based system for fish farming.

Ireland's Harmful algal bloom bulletin – short term predictive forecasting of HAB events and development of an Early Warning System

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Closures of shellfish aquaculture production areas due to the occurrence of toxigenic Harmful Algal Bloom events in coastal waters has been a long established problem for shellfish producers. Therefore, there is often a demand from regulatory authorities and the shellfish industry for the implementation of Early Warning Systems (EWS) to provide forecasting information on the probabilistic likelihood of the occurrence of HAB events. These EWSs are becoming an essential tool in providing warnings to the shellfish industry to mitigate and/or reduce socio-economic impacts. From 2013, the Marine Institute, Ireland, has generated and published a weekly HAB bulletin to the Irish aquaculture industry and relevant stakeholders which is publicly available to download. This bulletin provides a short-term (3-5 days) predictive forecast on the likelihood of the onset of or duration of a HAB event. This prediction is primarily based on the in situ monitoring of shellfish and water samples from the national monitoring programmes, which reviews the current and historical data, incorporating observed trends and patterns of known toxigenic HAB species and biotoxin concentrations, with additional inputs from satellite data (chlorophyll *a* and sea surface temperature) and predictive 3 day forecast hydrodynamic models. The data, tables and summary graphics compiled for the bulletin are generated from automated scripts, which are then reviewed and interpreted by an expert evaluator who provides expert opinion and summary text describing the national forecast for the current production week. This presentation provides in depth details on the generation of the Irish EWS HAB bulletin, and how it is used by shellfish producers, regulatory authorities and other stakeholders.

Predicting *Dinophysis* Blooms in the Gulf of Mexico

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Increasingly common blooms of toxic *Dinophysis* species represent a threat to human health and shellfish fisheries in the United States. The dependence of mixotrophic kleptoplastidic *Dinophysis* species on specific prey, the marine ciliate *Mesodinium rubrum*, further complicates predicting and mitigating the impacts of *Dinophysis* blooms. A decade of high temporal resolution monitoring of the phytoplankton community along the Texas, United States coast with Imaging FlowCytobots (IFCB) has revealed seasonal *Dinophysis ovum* blooms of varying intensity that occur between January and April in the Gulf of Mexico. Previous analysis of IFCB field data revealed a weak time-lagged relationship between *D. ovum* and *Mesodinium* abundance. Recent laboratory culturing experiments have indicated temperature and prey biovolume are important factors influencing *Dinophysis* biomass and growth rates. Utilizing data from laboratory culturing experiments and the Texas Observatory for Algal Succession Timeseries, we developed a model of *Dinophysis* and prey population dynamics in the Gulf of Mexico. The model results indicated prey biomass, rather than abundance alone, was a more reliable predictor of *D. ovum* bloom onset and intensity. We also found temperature played an important role in the onset of *D. ovum* blooms, specifically overlap of *D. ovum* and its prey. The influence of future warming on *D. ovum* bloom dynamics in the Gulf of Mexico will determine whether increases in *D. ovum* bloom frequency or intensity are likely to occur.

Predicting Risk and Impact of Harmful Algal Events on the Aquaculture Sector

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Harmful Algal Blooms (HABs) are natural phenomena produced when toxin-producing microscopic algae grow excessively and form bloom populations. Only a few species of algae can produce natural biotoxins, when conditions in natural water bodies are favourable, i.e. abundance of nutrients, sea surface temperature increase or sea currents change, these organisms can increase their abundance generating a HAB event. Accumulation of toxins produced during HABs can concentrate in the flesh of shellfish and finfish presenting a concern for the health of organisms themselves and human consumers. The Atlantic coast of Europe is often affected by such blooms causing economic losses to aquaculture industries due to closures and death of organisms. While we cannot prevent these blooms, industry, scientists and government agencies can be better prepared, by having the ability to forecast when HAB events might happen which is a very valuable tool. The main objective of PRIMROSE is the development of a trans-national alert across Europe's Atlantic Arc combining data acquired from different partners to develop a HAB/microbial contamination risk forecast. The Interreg funded PRIMROSE Project builds on existing monitoring programmes carried out in the partner regions to estimate early warnings on harmful blooms, shellfish toxins and microbial contamination to comply with EU regulations and guarantee seafood safety and ecosystem sustainability. We are designing an improved predictive model joining historical data sets, data from biomonitoring programs, satellite sensed data, ocean circulation patterns and mathematical models. The new systems will provide an easily understandable forecast, which will be standardized and partly automated, to have accurate information on HABs and associated climate impacts.

Sensitivity of a satellite algorithm for harmful algal bloom discrimination to the use of laboratory bio-optical data for training

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Early detection of dense harmful algal blooms (HABs) is possible using ocean colour remote sensing. Some algorithms require a training dataset, usually constructed from satellite images with a priori knowledge of the existence of the bloom. This approach can be limited if there is a lack of in situ observations, coincident with satellite images. A laboratory experiment collected biological and bio-optical data from a culture of *Karenia mikimotoi*, a harmful phytoplankton dinoflagellate. These data showed characteristic signals in chlorophyll specific absorption and backscattering coefficients. The bio-optical data from the culture and a bio-optical model were used to construct a training dataset for an existing statistical classifier. MERIS imagery over the European continental shelf were processed with the classifier using different training datasets. The differences in positive rates of detection of *K. mikimotoi* between using an algorithm trained with purely manually selected areas on satellite images and using laboratory data as training was overall less than 1 %. The difference was higher, less than 15 %, when using modelled optical data rather than laboratory data, with potential for improvement if local average chlorophyll concentrations are used. Using a laboratory-derived training dataset improved the ability of the algorithm to distinguish high turbidity from high chlorophyll concentrations. However, additional in situ observations of non-harmful high chlorophyll blooms in the area would improve testing of the ability to distinguish harmful from non-harmful high chlorophyll blooms.

Optical properties and satellite detection of *Pseudo-nitzschia* spp. and *Alexandrium minutum*

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The detection of phytoplankton blooms from satellite data can really provide timely information on emerging risks but the development of species or taxa-specific algorithms from satellite data is still challenged by a number of factors. In this study, we present a set of new algorithms for the detection and monitoring of *Pseudo-nitzschia* spp. and *Alexandrium minutum* blooms from satellite data over the Galician coast (NW Iberian Peninsula). Sentinel-3 OLCI and Sentinel-2 MSI images were used for the development of the algorithms. Our algorithm for the detection of *Pseudo-nitzschia* spp. is based on support vector machines (SVM) and was trained and tested using a match-up library of 260 OLCI images and 4,607 *Pseudo-nitzschia* spp. records. The new *Pseudo-nitzschia* spp. algorithm shows high overall accuracy and specificity. The *Pseudo-nitzschia* spp. detection model outperforms linear models and shows a very weak correlation with chlorophyll-*a*. We showcase the application of our model and Sentinel-3 images for the monitoring of *Pseudo-nitzschia* spp. in periods when the in-situ monitoring was not possible due to COVID-19 restrictions. This study also suggests new methods for the detection of *A. minutum* from Sentinel-2 MSI data. *In-situ* data of inherent optical properties (such as phytoplankton absorption, non-algal particle absorption particle backscattering) and hyperspectral remote sensing reflectance were collected during the blooms of *A. minutum* in the ria de Vigo and were used for the development and parameterization of the models.

Remote sensing of ciliate cell number in red tides as pre-alert indicator of *Dinophysis* harmful algal blooms

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Mesodinium rubrum is a globally-distributed photosynthetic marine ciliate known to form spectacular burgundy red tides in estuaries, fjords, and upwelling zones. Though *M. rubrum* does not produce toxins, its blooms are generally classified as harmful due to their impact on water quality (i.e., oxygen depletion, modification of foodweb dynamics). *M. rubrum* has been identified as a prey for *Dinophysis* spp., a dinoflagellate responsible for the diarrheic shellfish poisoning (DSP) toxin. Detection, sampling, and quantification of *M. rubrum* red tides is however notoriously challenging due to the delicateness of the cells, to the speed at which this ciliate can swim, aggregate, disaggregate, and/or be consumed, and to the bloom patchiness. Here, we present a novel detection and quantification method based on high resolution satellite remote sensing. Using laboratory measurements of *M. rubrum*'s inherent optical properties (absorption, scattering and backscattering coefficients), a radiative transfer model was adapted to simulate *M. rubrum* remote sensing reflectance (Rrs). A specific inversion algorithm was then developed to estimate *M. rubrum* biomass in terms of either chlorophyll *a* concentration or cells number. The algorithm was applied to several red tide case study using Sentinel-2 and Sentinel-3 images, and validated against in situ measurements. As *Dinophysis* is a *M. rubrum* obligate feeder, these results open new perspectives for the early warning of DSP events.

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

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Forecast tools are being developed to mitigate human health risks and negative economic effects of shellfish closures due to seasonal blooms of *Alexandrium catenella*, the toxic dinoflagellate that causes paralytic shellfish poisoning along the Pacific and Atlantic coastlines of the U.S. and Canada. *A. catenella* overwinters as a benthic resting cyst in the sediment and germinates into the water column in the spring and summer, making cells available for filter feeding by shellfish. Previous studies have mapped the winter distribution of *A. catenella* cysts in Gulf of Maine and Puget Sound surface sediments as a way of providing shellfish growers with an early warning system of potential hotspots for blooms of this harmful alga (HAB). However, the current protocol for cyst enumeration by fluorescent microscopy is time consuming and requires highly specific training. This MERHAB project, funded by NOAA's National Centers for Coastal Ocean Science (NCCOS), is developing new quantitative polymerase chain reaction (qPCR) and fluorescent in situ hybridization (FISH) assays for *A. catenella* cysts that will be evaluated against the standard microscopy protocol with the goal of producing more rapid and accurate cyst abundance data. Surface sediment samples from the Gulf of Maine, Puget Sound and Alaska were collected in winter of 2020 for cyst mapping, interlaboratory microscopy technique comparison, and molecular method development, calibration, and testing. Cyst distribution maps from all three regions will be presented, along with an interlaboratory comparison of cyst concentrations using the standard microscopy method. Preliminary results from molecular method development will also be presented.

Forecasting biotoxin contamination in mussels via artificial neural network modelling

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Harmful algal blooms are among the most severe ecological marine problems worldwide. Under favourable climate and oceanographic conditions, toxic microalgae species may proliferate and produce biotoxins that accumulate in shellfish, threatening the health of seafood consumers. In Portugal, in order to minimize the risk of poisoning by contaminated shellfish consumption, the Portuguese Institute of Sea and Atmosphere routinely monitors this product, prohibiting the harvest and commercialization of shellfish whenever the concentration of biotoxins exceeds the safety limits. Since this prohibition frequently leads to significant negative economic impacts, it becomes necessary to develop strategies that predict shellfish contamination. In this study, we used historical data of *in situ* measurements of biotoxin concentration in mussels (*Mytilus galloprovincialis*) and cell counts of toxic phytoplankton species in the seawater from shellfish production areas of the Portuguese coast, together with oceanographic and meteorological time series, to forecast the biotoxin shellfish contamination at least one week in advance. We developed forecasting methods based on artificial neural network models to achieve this goal, including feed-forward, convolutional, and long short-term memory (LSTM) neural networks. The developed models allowed to achieve promising forecasting results, with the best accuracies being obtained using LSTM networks. These results might be regarded as the first pivotal steps towards developing a model based forecasting tool, which will allow the production sector to anticipate the harvesting prohibition, enabling the development of strategies to mitigate the economic losses inherent to this situation.

Time series analysis of the *Karenia brevis* blooms in western Florida and its correlated parameters

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Blooms of the toxigenic dinoflagellate *Karenia brevis* occur regularly in the Gulf of Mexico, especially in western Florida, USA. Here, time series data from 1998 to 2020 were investigated in terms of relationships between *K. brevis* abundance and El Niño – Southern Oscillation (ENSO) and its rate of change, as well as temperature, precipitation, river flow, and salinity. This dataset gives us a broad view of the factors correlated with higher abundances of this species since this period includes times of substantial blooms (~ 1.4×10^6 cells L^{-1}) and also times characterized by background cell concentrations (~1.0×10^3 cells L^{-1}). El Niño brings wet and cool weather to South Florida, including greater frequency of storms, while La Niña brings dry and warm weather. The greatest *K. brevis* abundances observed in the study were between the years 2004 – 2005 and 2017 – 2018, which occurred when hurricane events followed drought periods. High correspondences were observed between cell counts and river discharge, indicating that oscillations in river flow can play a significant role in determining the amount of continental water–and its associated nutrients– that reaches the marine ecosystem that ultimately, sustains coastal *K. brevis* blooms. These time series analyses will help to inform new models of bloom formation and termination in western Florida waters.

Intelligent ASVs to explore water bodies and support HABs detection, prediction and early warning

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Harmful Algae Blooms (HABs) are dynamic biological processes that occur inside many water bodies and become visible as they expand into the water surface. They should be anticipated/detected as soon as possible, to warn the authorities about dangerous situations. Early warning systems in use today, are not enough to capture HAB temporal-space evolution, because their probes, placed at fixed locations, cannot provide the quantity and distributed data required to understand what is happening. Autonomous Surface Vehicles (ASVs, a kind of robotized boats) can be used instead as mobile sensor platforms to take measurements of the variables of interest at different locations and depths of the water body. Our research goes one step beyond and aims to develop an Artificial Intelligent aLERT (AILERT) system based on intelligent ASVs capable of deciding where and how to take measurements for building models that predict HABs evolution and alert the authorities about them. Our ASVs are equipped with on-board computers and software designed for mixed-initiative work, which lets human operators intervene when desired, while ASVs usually function autonomously as a fleet, performing coordinated exploration tasks supported by different principles. For instance, these tasks can be achieved from a systematic water covering/monitoring perspective, by pre-planning ASV and probe trajectories using the information provided by the simulations and/or the probability distribution of the HABs, or with data-driven controllers that adapt ASVs displacements to the information extracted in real-time from the measurements taken by on-board probes. During our presentation, we will provide an overall view of our system, with details of the already-developed elements.

Recent advances in the long-term satellite monitoring of the cyanobacteria blooms in the Baltic Sea

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In search of the optimal method of remote sensing of the cyanobacteria blooms in the Baltic Sea, a performance of CI index has been evaluated and the method was applied to investigate almost twenty years of variability of the summer toxic blooms in the Baltic Sea, formed by three main taxa: *Nodularia* sp., *Aphanizomenon flos aquae* and *Dolichospermum* sp. Spectral shape analyses adopted to derive CI index gives it an advantage over common methods which are based on the chlorophyll a absorption or on the chlorophyll a fluorescence characteristic that require full atmospheric correction. Confirmed here relation to the in situ measured phycocyanin concentration proves that the index efficiently identifies cyanobacteria-dominated blooms in the Baltic Sea and comparison with the SST maps proved that it detects near-surface filamentous cyanobacteria aggregates that modify various surface water properties. The index has been applied in the processing of the MODIS AQUA satellite data acquired in years 2002 – 2018. The time series analyses revealed no long-term trends of the blooms intensity or the number of days with the presence of surface aggregates. However, a negative correlation between the date of the last notification of a bloom event in the season and the maximum area of the bloom was spotted, likely due to phosphorus limitation. In turn, relation between the number of days with surface aggregates and daily PAR dose suggests profound impact of meteorological conditions, and demonstrates importance of further HABs monitoring in the age of global warming.

Habitat suitability model for *Alexandrium catenella* in the Gulf of Alaska

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The dinoflagellate *Alexandrium catenella* produces a suite of neurotoxins (saxitoxins) which can accumulate in shellfish and cause paralytic shellfish poisoning. *Alexandrium catenella* blooms occur seasonally in the coastal waters of the Gulf of Alaska, Bering Sea, and Chukchi Sea where it has shown to be present in a wide range of temperatures (7° - 15 °C) and salinities (4 - 30), however it has an optimal growth rates with temperatures of 10° - 13 °C and salinities of 18 - 23. Here we will discuss a habitat suitability model for *A. catenella* using satellite derived sea surface temperature (SST) as the initial environmental input. Other environmental parameters will be added to the model to better mirror the regional distribution of *Alexandrium* cells and shellfish toxicity. NASA and NOAA CoastWatch will provide data on potential factors of interest, such as salinity, precipitation, runoff, air temperature, wind stress and direction, water absorption and clarity, cloud cover, irradiance, and heat flux. Available field data including toxin levels and cell abundances will be used to evaluate the resultant model. As the regional climate is projected to continue warming, and more unusual events like marine heatwaves become more common characteristic features of the North Pacific, the model will be useful to predict the potential increase in habitat area for toxic *A. catenella* blooms in Alaska.

Temporal and spatial variability of HABs affecting Scottish shellfish aquaculture in Scotland

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Shellfish aquaculture has become a crucial commercial business in rural areas in Scotland. The presence, or increased abundance, of certain species of phytoplankton can negatively impact through the closure of farms to safeguard food consumption. The occurrence, intensity and distribution of Harmful Algal Blooms (HABs) is perceived as an increasing problem worldwide. Such increases are not evident in all studies, suggesting that local or regional variability is likely important in modulating any overall trend. This study summarises the Scotland wide temporal and spatial patterns of a 15-year time series of the incidence of harmful phytoplankton and shellfish biotoxins collected as part of the Food Standards Scotland regulatory monitoring programme. The study found a high temporal variability with no increasing trend of HABs of main concern or toxic events incidence. Seasonality was identified as an important factor regulating HABs incidence, but this varied according to the genus and biotoxin. Principal component analysis was able to discriminate locations that experienced blooms at different times of the year. This analysis also demonstrated blooms of *Dinophysis* and *Alexandrium* and their toxins typically occurred at a regional scale, with other genera being more likely to be regulated by local conditions. Spatial clusters and risk areas were identified by the use of k-means. The study also confirms the complex interaction between phytoplankton and synthesised biotoxins. These temporal and spatial patterns provide baseline information about the current and recent historical situation of HABs in Scotland, and have the potential to be used by shellfish industry for management and prevention of economic losses and food poisoning events.

The Black Tide (Kuroshio) Potentially Induces *Karenia mikimotoi* Red Tides

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The red tide of dinoflagellate *Karenia mikimotoi* can result in multi-million-dollar loss in mariculture. The Seto Inland Sea, located in western Japan, was once described as "Dying Sea" due to its serious pollution and nearly recorded 300 red tide events at its peak. Since the late 1970s, the annual number of red tide events has declined in this sea, but that of *K. mikimotoi* red tide persists even in recent years. A great number of *K. mikimotoi* red tide events in the eastern Seto Inland Sea occurred between 1995 and 1997 (10 – 17 events per year while the average was approximately three). Similar to the Gulf Stream in the Atlantic, a western boundary current, named the Kuroshio (meaning "black tide" in Japanese), flows south of this area. We statistically modeled the annual number of *K. mikimotoi* red tide events in the eastern Seto Inland Sea, using the environmental data for 29 years (1991 – 2019). A sea level difference between two coastal stations was used as an indicator of the Kuroshio path type (KPI: Kuroshio Path Index). The KPI in winter and the number of events in the previous year were both significant predictors of the number of *K. mikimotoi* red tide events ($p < 0.05$). The model with both predictors showed significant improvement over the model with only the latter ($p < 0.05$). Although the representation by the number of red tide events should be carefully considered, our finding indicates that the nearshore path of the Kuroshio in winter induces the large number of *K. mikimotoi* red tides.

Influence of environmental factors on the abundance of toxic dinoflagellates (*Alexandrium* and *Dinophysis*) in French Mediterranean Lagoons: a result of ten years monitoring network

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French Mediterranean lagoons are frequently subject to shellfishes' contaminations by Diarrheic Shellfish Toxins (DST) and Paralytic Shellfish Toxins (PST). To highlight the influence of the main environmental factors (temperature, salinity and turbidity) on the abundance of the major producers of these toxins, *Dinophysis* spp. and *Alexandrium* spp., and the link with shellfish contamination, we analyzed a 10 years set of data collected from 2010 to 2019 in two shellfish farming lagoons, Thau and Leucate. Decisional trees analysis revealed that the highest risks of *Dinophysis* spp. and *Alexandrium* spp. bloom events occurred at temperature < 16.3 °C for a salinity < 27.8, and at temperature from 8, 6 °C to 16.1 °C for a salinity > 39.2, respectively. The highest risks of shellfish's contaminations by DST and PST occurred during the set of conditions promoting bloom events. The predictions were confirmed by Zero Inflated Negative Binomial (ZINB) models which allowed us to understand whether environmental factors impact *Alexandrium* species presence and/or their abundance. We provided tools which could help sanitary management of shellfish farming areas.

Modelling of diarrheic shellfish poisoning in *Mytilus edulis* harvest in Bantry Bay, Ireland

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Diarrhetic Shellfish Poisoning (DSP) results from toxins produced by microalgae dinoflagellates. The occurrence of DSP toxins is mainly related to *Dinophysis* and *Prorocentrum* species. Shellfish contamination with marine biotoxins not only pose a threat to human health, but also lead to financial loss to aquaculture industries from the temporary closure of production areas. In this study, we developed a Bayesian Network (BN) model for forecasting the short-term variations of DSP toxins in mussels from Bantry Bay, Southwest Ireland. Regular phytoplankton concentration in seawater and DSP toxin concentration in mussels collected from ten production sites in Bantry Bay as well as sea surface temperature were used as inputs to a BN model. The model was trained with data from 2014 to 2018 and validated with data from 2019. Validation consisted of predicting the DSP toxin concentration at one production site using the model parameters from the other locations as input values. Model validation showed that the prediction accuracy was higher than 86 %. Sensitivity analysis indicated that in general, DSP toxin concentration was more relevant than phytoplankton concentrations. This initial work has demonstrated the utility of the BN approach. Further work is ongoing to use the model for scenario testing and to increase the number of environmental parameters used as inputs to the model.

Monitoring harmful algal blooms in Spain and Chile with the Sentinel-2 and Sentinel-3 satellites

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The objective of this study is to evaluate the potential of the Ocean and Land Color Instrument (OLCI) and the Multispectral Instrument (MSI) on-board the Sentinel-3A/B and Sentinel-2A/B satellites, respectively, to monitor HABs in coastal environments. The satellite imagery has been used to map the spread of a bloom of the dinoflagellate *Lingulodinium polyedra* in Spain (2019) and large blooms of the dinoflagellate species *Cochlodinium* sp. in Chile (2020). Current methods of tracking HABs by the Regional Agencies consist of traditional ship-based approaches and determination of phytoplankton species and associated toxins in water, fish and shellfish. However, these approaches are labour intensive and costly and do not provide synoptic views of the bloom condition and evolution. A well-validated algorithm, the normalized difference chlorophyll index, available for operational use on coastal waters, has been applied using satellite products. Results indicate that both missions are a powerful tool providing valuable spatiotemporal information of HABs; whereas Sentinel-3 at 300 m allows daily operational monitoring, Sentinel-2 at 10 m allows enhanced mapping of small and heterogeneous blooms. This study shows the benefits of the optical satellites from the European Copernicus programme in terms of their spectral, spatial and temporal capabilities for enhanced algal bloom detection. The value added by the available products in terms of frequency and synoptic observations is of paramount importance for ecological and management purposes at regional and national scales, which can potentially foster optimal decision-making and save economic resources.

Photo: Fernando Sánchez Bernal

SURVEILLANCE AND MANAGEMENT

History of Harmful Algal Blooms (HABs) in Indonesia: Factors involved in outbreaks in Lampung Bay

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In Indonesia, the world's largest island country, HABs have been observed since the 1970s. The first major outbreak (in Lewotobi, November 1983) caused four deaths and 191 hospitalizations after people consumed toxic fish. Since then, HABs have become more frequent and more widespread in Indonesia, consistent with global trends. Based on documented events, there are three main species that impact fisheries, the economy and public health: *Noctiluca scintillans*, *Pyrodinium bahamense* var. *compressum* and *Margalefidinium polykrikoides*. Blooms of these species occur in waters surrounding large population centers that have experienced eutrophication (e.g., Ambon Bay, Jakarta Bay, and Lampung Bay). Lampung Bay, in particular, has undergone extensive development and is a major economic hub, hosting a large shipping harbor and a significant aquaculture industry. *M. polykrikoides* blooms have been recorded in the inner-middle harbor as well as Legundi Island. *M. polykrikoides* was recently observed in high abundance within marine plastic debris (up to 6,843 cells L^{-1}) in Legundi Island. Here, we analyse time-series observations of phytoplankton in Lampung Bay to assess factors involved in *M. polykrikoides* bloom formation. To determine why blooms have recently appeared at Legundi Island, we also systematically investigate the role of marine plastic debris in dispersing HAB vegetative cells and temporary resting cysts in Lampung Bay, undertaking a survey of different debris types and determining whether cells disassociate from debris to inoculate the water column.

Harmful algal blooms along central Guatemala´s Pacific coast

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Along the Pacific Guatemalan coast, sporadic studies date back to 1987 when 193 human intoxications (including 22 deaths) caused by the dinoflagellate *Pyrodinium bahamense* were reported. In recent years, harmful algal blooms along central Guatemala's Pacific coast have been caused by *Margalefidinium polykrikoides* and *P. bahamense*; however, the reports do not mention accompanying species. To fill this gap, in 2019 sampling of the phytoplankton community and studies of spatial-temporal changes in physical-chemical variables (temperature, salinity, pH, dissolved oxygen, ammonium, phosphates, sulphates, total dissolved solids and chlorophyll *a*) were initiated. In November 2019, *P. compressum* reached an abundance of 10,078 cells L^{-1}. In September 2020, *M. polykrikoides* vegetative cells reached 1,242,000 cells L^{-1} and its cysts 1,530,000 cells L^{-1}. In November 2020, *Noctiluca scintillans* bloomed (up to 1,000,000 cells L^{-1}). Since January 2021, monthly monitoring was launched at three sampling stations with site depths from 9 to 40 m at 3-5 km from the coastline. In March 2021, a *M. polykrikoides* bloom was detected (1,200,000 cells L^{-1}). In January-March 2021, at a water temperature of 28.4 °C, an average of 46 diatom (mainly *Skeletonema*, *Chaetoceros* and *Rhizosolenia*) and dinoflagellate species (mostly *Tripos*, *Protoperidinium* and *Prorocentrum*) was observed in the Mesoamerican subduction zone and San José Underwater Canyon in front of the Port of Quetzal. During this period, the diatom contribution to the community diminished from 68 % to 42 %. The sampling area is impacted by the port and the Marialinda and Achiguate River discharges. Monitoring is underway in collaboration with the Port of Quetzal authorities and the Guatemalan Pacific Navy Command.

Mitigation of harmful cyanobacterial with metallic peroxide granules: pH effect on hydrogen peroxide release kinetics and toxicity study on invertebrates

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For the mitigation of blue-green algae several chemical treatments have been applied over the years including harmful pesticides and algaecides. With their usage been prohibited in many U.S. states and the European Union, alternative treatments that are more environmentally friendly needed to be apply. Hydrogen peroxide (HP) is currently being applied in contaminated with cyanobacteria sites, since studies have shown that cyanobacteria are more sensitive to HP treatment than green algae. However, dense blooms require high doses of HP that distress the remaining ecosystem (including zooplankton and phytoplankton). To address this problem, slow releasing HP metallic granules were used in this study as an alternative approach to liquid solution HP application. Specifically, calcium peroxide (CaO_2) and magnesium peroxide (MgO_2) granules were applied in surface water matrix from the Kouris Reservoir in Cyprus) to examine: (a) their HP releasing properties with varying pH values, (b) their mitigation efficiency on *Microcystis* sp. bloom in comparison with HP liquid application and (c) their toxicity on *Gammarus* sp. in a range of concentrations. Results showed that in acidified environments granules have higher HP releasing capacity. Moreover, treatment of *Microcystis* sp. with CaO_2 outperformed MgO_2 and it can be safely applied in concentrations < 3 g L^{-1} granules as an alternative method to direct HP application.

Developing an *Ostreopsis* Early Warning System: the joint engagement of environmental agencies, beach users and scientists

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In the last twenty years, blooms of the harmful benthic dinoflagellate *Ostreopsis*, affecting human and environmental health, have become a common seasonal phenomenon in many temperate areas. Therefore, implementing an Early Warning System (EWS) of benthic harmful algal blooms (BHABs) has been needed. In the Mediterranean, where routine monitoring programmes were mainly focused on phytoplankton, several administrations have been conducting BHABs monitoring using scientific approaches in coordination with research centres. The key to an effective EWS is samples need to be collected and processed soon and the results should be quickly communicated to the users. In the frame of the CoCliME project, an easy and reliable protocol was co-designed and implemented by scientists and stakeholders. Scientists elaborated and distributed a benthic sampling kit and provided simple training sessions on sampling. The need of benthic samples, implying the collection of *Ostreopsis* substrate (generally macroalgae), is important in order to evaluate the stock of toxic microalgae. Results (*Ostreopsis* abundance) were shared on coordinated online platforms, between scientists and public agencies. Microscopy cell counts were conducted at the research centres, but can also be done by trained personnel and associations (e.g. Surfrider Foundation) elsewhere. This protocol has been successfully tested in Catalonia and France during the summers 2019 and 2020. It was proven to be an effective EWS for authorities to quickly raise recommendations on the health risks associated to *Ostreopsis* blooms in Mediterranean beaches. The protocol also contributes to elaborating time series for elucidation of climate change effects on *Ostreopsis* blooms.

Payment for Ecosystem Services–an efficient approach to reduce eutrophication?

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Excess nutrient loads not only impair aquatic ecosystems by decreasing their biodiversity, but also cause direct and indirect economic losses. The project CPES (Channel Payments for Ecosystem Services) develops PES schemes remunerating the farmer for activities improving water quality by reducing emissions of nutrients (both, nitrogen and phosphorus) or silt from agricultural activities. Catchment wide approaches are tested in six case studies at both sides of the channel. One of the six case studies concerns Lac au Duc, suffering from recurrent cyanobacterial blooms. Long term data series were evaluated following nutrients and climate conditions, connecting them to the observed cyanobacterial dynamics, as their inter-annual variance seems to increase. The main sources of excess phosphorus entering the lake were analysed in the lake's catchment to be merely of farming origin but vary according to hydrogeological characteristics and agricultural practices between the sub-catchments. While reduction of phosphorus is the most efficient measure, this requires time, therefore the possibility to apply curative actions was assessed and hydrogen peroxide selected to be tested–with limited success. Long term prevention possibilities to ameliorate agricultural practices include e.g. permanent cover or anti-erosive hedges. We are now in the process to merge the monetary needs of the farmers to ameliorate their land management to the motivation of potential buyers to construct PES contracts, which may follow other interest such as carbon footprint. This project requires thus a multi-facetted approach combining expertise of ecology, hydrogeology, agro-economy, moreover the experiences of intermediate and farmers' associations, and non-the-least, the agricultural skills of the farmers!

Mitigation of harmful algal blooms and their toxins

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Every year the UK shellfish industry suffers economic losses (£25-30 k per week) as a consequence of harvesting closures following high toxin levels accumulating in shellfish tissues due to filter-feeding of some harmful algal bloom (HAB) species. Rigorous regulatory monitoring programs are in place to protect public health but there is a clear need for mitigation strategies in the field to support shellfish growers. For the first time advanced oxidation by photocatalysis has been used to demonstrate the potential for toxin and cell destruction at lab scale and will underpin development of a scalable curtain to protect shellfish production sites by limiting the HAB cell numbers. A TiO_2/UV system completely destroyed okadaic acid at a concentration of 10 mg mL^{-1} in sea water in 30 minutes, with no remaining toxicity when samples were assessed for protein phosphatase inhibition. First efforts to destroy cells of *Alexandrium catenella* with a range of treatments (TiO_2/UV, H_2O_2, H_2O_2/ UV, ZnO/UV) resulted in 35 % and 50 % destruction of cells following treatment for 3 h with ZnO/UV and TiO_2/UV respectively. Intra and extracellular paralytic shellfish toxins (PSTs) were analysed by LC-MS/MS to confirm reduction in PSTs. TiO_2/UV was immobilised and evaluated in tank trials where mussels were challenged with *A. catenella* to demonstrate the application of a photocatalytic curtain to reduce accumulation of toxins.

Optimization of alginate-based carrier matrices for algicide delivery

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Blooms of *Karenia brevis* are common in the Gulf of Mexico, and are referred to as "Florida Red Tide", and heavily impacts the West Coast of Florida, U.S.A. These toxic blooms of *K. brevis* present major human and ecosystem health concerns that threatens the livelihood of local economies and coastal communities. Therefore, it is essential to develop environmentally sustainable and species-specific control strategies to manage red tide events in this region with minimal impacts to non-target organisms. Previous research characterized the algicidal activity of a novel bacterial-mediated algicidal exudate, produced by *Shewanella* sp. IRI-160, with demonstrated activity against *K. brevis*. The next phase of this research is to optimize the algicide delivery for use in coastal systems in Florida. The hypothesized approach is to embed either live bacteria or algicide into a porous polymer matrix to allow for sustained release of the product over time. Alginate is an excellent candidate for this application since it is: (1) a natural polymer derived from brown seaweeds and bacteria; (2) relatively low-cost; (3) reported to be non-toxic; and (4) highly biodegradable. In this investigation, we fabricated an array of alginate beads using various physical and chemical conditions (alginate molecular weight and concentration, calcium concentration, drop height, diameter, etc.) to optimize algicide delivery and ensure a consistent rate for future field applications. The various matrices were prepared with Coomassie Blue (a surrogate for future algicide/bacteria experiments) and subjected to diffusion studies (static and dynamic) in distilled water as well as seawater. Moreover, the addition of a chitosan-based outer layer was explored to increase alginate bead stability.

Evaluation of the effectiveness and feasibility of different oxidants for the inactivation of harmful phytoplankton and associated bacteria

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Harmful Algal Blooms in coastal areas can cause significant impacts on ecosystem and human health. Together with these blooms, *Vibrio* spp. have been also reported, suggesting that HABs may enhance the bacterial growth of these pathogenic species, increasing health risk and eco-hazards. Accordingly, efficient solutions that can mitigate both harmful phytoplankton and associated bacteria are encouraged. This study assessed the biocide efficacy of two different oxidants divided by their action mode: i) H_2O_2 and ii) persulfate salts (in form of $S_2O_8^{2-}$ or HSO_5^{-}). These oxidants are also widely applied in the so-called Advanced Oxidation Processes, where strong radicals may be generated and can accelerate the inactivation practices. Their use has been shown promising results in freshwaters, although are less studied in seawater. As target microorganisms, *Anabaena* sp. and *Prymnesium parvum*, together with *Vibrio alginolyticus* were selected. In a series of laboratory experiments, we tested the efficacy of these oxidants (0-3 mM) to inactivate these three different species.

Effect of hydrogen peroxide on natural phytoplankton and bacterioplankton in a drinking water reservoir: Mesocosm-scale study

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CyanoHABs are increasingly reported worldwide, presenting a challenge to water treatment and concerning risks to human health and aquatic ecosystems. Oxidative processes comprise efficient and safe methods for water treatment. Hydrogen peroxide (H_2O_2) has been proposed as a sustainable solution to mitigate cyanobacteria since this group presents a higher sensitivity compared to other phytoplankton, with no major risks to the environment at low concentrations. Here, we evaluated the effects of a single H_2O_2 addition (10 mg L^{-1}) over 120 h in mesocosms introduced in a reservoir located in Brazilian semi-arid region. We followed changes in physical and chemical parameters and in the bacterioplankton composition. H_2O_2 efficiently suppressed cyanobacteria, green-algae, and diatoms over 72 h, leading to an increase in transparency and dissolved organic carbon, and a decrease in dissolved oxygen and pH, while nutrient concentrations were not affected. After 120 h, cyanobacterial abundance remained low and green-algae became dominant. 16SrRNA sequencing revealed that the original cyanobacterial bloom was composed by *Planktothrix*, *Cyanobium* and *Microcystis*. Only *Cyanobium* increased in relative abundance at 120 h, suggesting regrowth. A prominent change in the composition of heterotrophic bacteria was observed with *Exiguobacterium*, *Paracoccus* and *Deinococcus* becoming the most abundant genera after H_2O_2. Our results indicate that this approach is efficient in suppressing cyanobacteria, improving water quality in tropical environments. Monitoring changes in abiotic parameters and the relative abundance of specific bacterial taxa could be used to anticipate the regrowth of cyanobacteria after H_2O_2 degradation and to indicate where in the reservoir H_2O_2 should be applied so the effects are still felt in the water treatment plant intake.

The economic impact of harmful algal blooms on the productivity of Scottish shellfish farms

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Shellfish production is an important activity for the economy of many countries. The health implications of human consumption of shellfish contaminated by HAB generated biotoxins are well known and typically managed by regulatory shellfishery closures. Such management action has an economic consequence for the aquaculture industry, that is recognized, but poorly quantified. Our research therefore evaluated HAB generated economic impacts on Scottish shellfish aquaculture using a Cobb-Douglas production function approach. Using publicly available data we evaluated how variation in production was related to factors including labour, capital, climate variables and the concentration of HABs and biotoxins. Results demonstrated that diarrhetic shellfish toxins generated by the genera *Dinophysis* had a significant economic impact. A 1 % change in the production of these biotoxins reduces shellfish production by 0.66 %, with an average yearly negative variation in production of 15 % (1,080 ton) and an economic loss (turnover) of GBP 1.37 m per year (in 2015 currency) over a turnover of GBP 12 m. Coupling the production function approach to a multivariate time series model capturing the statistical relationship between algal concentration and climatic variables can then help forecast the impact of harmful algae on shellfish production. This provides producers and the regulators with the economic information to plan mitigating measures to limit damages to production by comparing the costs of these measures with the benefits of reducing the loss of production as calculated by our approach.

High resolution Sentinel-2 satellite imagery to support management agencies in monitoring algal blooms during COVID

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Toxic algal blooms in both fresh and saltwater systems are a global problem, often affecting small lakes and coastal regions. While satellite detection has been successful for species that dominate the phytoplankton community and have a large chlorophyll or fluorescence signature, they often occur in small embayments and lakes where spatial resolution of the imagery becomes a problem. During the past year of COVID, field sampling for blooms was often impossible, making satellite detection of algal blooms critical for early warning of toxic events. As a result, 20 m imagery obtained from the Copernicus Sentinel-2 was processed and delivered by the National Oceanic and Atmospheric Administration for the state of Virginia, U.S.A. and smaller reaches of the Chesapeake Bay tributaries. The products provided information to guide sampling efforts and alert the state of the occurrence of possible HABs. A strategy has been developed for further refinement of the algorithm for bloom detection in order to better support state environmental and public health managers, aquaculture operators, and those responsible for safe drinking water. Sentinel-2 data sets are provided by the Copernicus Program and provisionally distributed through NOAA's CoastWatch program. Products will be validated and applied to expanded coverage as resources permit.

Monitoring harmful algal bloom species and their appearance in the Tokyo Bay, Japan, based on metabarcoding and high-throughput sequencing

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During the recent decade, metabarcoding and high-throughput sequencing (HTS) has facilitated increased detection of biodiversity, including harmful algal bloom (HAB) species. In this study, the presence of HAB species and their appearance patterns were investigated by employing molecular and light microscopy-based monitoring in Tokyo Bay, Japan. The potential co-appearance patterns between the HAB species as well as with other eukaryotes and prokaryotes were investigated using correlation and association rule-based time-series analysis. In total, 40 unique HAB species were detected, including 12 toxin-producing HAB species previously not reported from the area. More than half of the HAB species were present from summer to autumn, and no structuring or succession patterns associated with the environmental conditions could be detected. Statistically significant ($p < 0.05$) associations were found among the HAB species and other eukaryotic and prokaryotic species, including genera containing growth-limiting bacteria. However, significant correlations between species differed among the years, indicating that variability in environmental conditions between the years may have a stronger influence on the phytoplankton community structure and interspecies interactions, than the variability during the sampling season. The association rule-based time-series analysis allowed the detection of a previously reported negative relationship between *Synechococcus* sp. and *Skeletonema* sp. Overall, the results support the usefulness of metabarcoding and HTS-based phytoplankton monitoring, as it facilitates more precise species identification compared to light microscopy as well as provides input for investigating potential interactions among different species/groups through simultaneous detection of multiple species/genera.

Shellfish poisoning associated with neurological disorders: cases registered by the French Poison Control Centers from January 2012 to December 2019

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In June 2019, a paralytic shellfish poisoning (PSP) case related to the consumption of mussels contaminated by saxitoxins below the regulatory threshold drew the attention of the National Agency for Food, Environmental and Occupational Health Safety (Anses) of probable unnoticed human poisonings by neurotoxic phycotoxins. Therefore, a retrospective study of cases intoxicated by bivalve shellfish (oysters, mussels, scallops) recorded by the French Poison Control Centres (PCCs) from 2012 to 2019 reported 619 cases of food poisoning, among which 22 % (n = 134) had at least a neurological symptom (headache, dizziness, paresthesia). The review of the 134 medical files led to the suspicion of 14 cases of PSP and one case of amnesic shellfish poisoning. The diagnosis was based on the observation of the patient symptoms and the contamination data of the shellfish production areas (collected by the French Research Institute for Exploitation of the Sea, IFREMER) or the notifications of the European Rapid Alert System for Food and Feed. However, no research of marine neurotoxins was performed in the blood or urine of these 15 patients. PCCs, ANSES and IFREMER developed a specific guide to collect information when a person reports neurological signs after shellfish consumption (symptoms, quantity consumed, production area), and advise actions to take for further investigations (keep leftovers of the meal and go to the emergency room for biological sampling). A daily monitoring of shellfish poisoning cases registered in the PCCs database was also set up in order to rapidly detect any suspicious case, alert the competent authorities and warn the population.

ScillyHAB: A multi-disciplinary survey of Harmful Marine Phytoplankton and Shellfish Toxins in the Isles of Scilly, utilizing Citizen Science in a remote offshore U.K. territory during the COVID-19 pandemia

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The Isles of Scilly (IOS) is an archipelago of 145 islets, 45 km southwest of England. Whilst no commercial shellfish fisheries exist, bivalves are consumed recreationally. Shellfisheries in SW England suffer periodic HABs, resulting in closures of harvesting areas due to PSP and DSP toxins above permitted limits. With IOS never monitored for HABs and/or shellfish toxins, there was a need to assess the presence of such hazards for risk assessment, early warning of developing blooms or potential presence of emerging HAB species. A monitoring program started March 2020, however, one week later the U.K. entered lockdown due to the COVID pandemic. Consequently, a team of local volunteers was recruited to carry out water sampling and shellfish collection and supplied with a portable inverted light microscope. Fixed water samples and shellfish were analysed at CEFAS. Microscopy confirmed the presence of HAB genera well known in mainland waters: *Pseudo-nitzschia*, *Karenia*, *Dinophysis acuminata* and *D. acuta*, *Alexandrium*, *Procentrum cordatum* and *P. lima*. Nanopore sequencing (MinION) was used to generate consensus sequences confirming the presence of *Azadinium*, *Dinophysis*, *Pseudo-nitzschia* and *Alexandrium* species. Shellfish analysis confirmed domoic acid, saxitoxins, dinophysis toxins, azaspiracids and tetrodotoxin, albeit at concentrations well below regulatory/guidance limits and those determined in shellfish harvested in SW England. Overall, this work demonstrated the presence of both low concentrations of regulated and non-regulated marine toxins and HAB genera/species in an island region of the U.K. previously unassessed for these hazards, using a variety of monitoring tools and citizen-input during a highly challenging time for field-based scientific investigation in remote regions.

Pseudo-nitzschia blooms associated with amnesic shellfish toxins in Scottish coastal waters: a case study from the Shetland Islands

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The potentially harmful genus *Pseudo-nitzschia* is frequently detected in shellfish harvesting areas around the Scottish coast, with dense blooms observed in most years. In Scotland, the regulatory monitoring programme uses a *Pseudo-nitzschia* spp. trigger level of 50,000 cells L^{-1} to schedule additional testing of shellfish for the presence of amnesic shellfish toxins (ASTs). However, testing for ASTs does not occur when harvesting sites are closed due to the presence of other toxin groups. The prevalence of ASTs in Scottish shellfish, and hence the associated risk, may therefore be underestimated. *Pseudo-nitzschia* blooms were widespread around the Shetland Islands from July to September in 2020, although limited official control AST testing occurred because temporary harvesting bans were in place, due to the presence of diarrhetic shellfish toxins. In this study, additional AST testing was undertaken on common mussels collected from these sites during the *Pseudo-nitzschia* bloom period. The results show that amnesic shellfish toxins were present and, in one case, exceeded the maximum permitted level. Using light microscopy, it was possible to split the *Pseudo-nitzschia* into size categories, with cells of valve width < 3 μm assigned to the *P. delicatissima* group, and those > 3 μm to the *P. seriata* group. A higher toxin content per cell has been associated with the *P. seriata* group and the discrimination of *Pseudo-nitzschia* into these categories may be useful, both as an early warning for harvesters, and to focus regulatory testing on sites at potentially greater risk.

Scallop contamination by domoic acid: is surface water monitoring for *Pseudo-nitzschia* sufficient?

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Shellfish farming is highly dependent on the quality of the marine environment. Over the last decades, in France, this industry was subjected to an increase of harmful algal blooms of *Pseudo-nitzschia* and its toxin domoic acid (DA). Among the highly affected bivalves is the king scallop (*Pecten maximus*), which unlike most bivalves, takes very long time to depurate DA, sometimes up to years. *Pseudo-nitzschia* monitoring in the surface water and *P. maximus* contamination does not correlate. Shellfish can be contaminated when *Pseudo-nitzschia* is not detected in surface water. Conversely, *Pseudo-nitzschia* sampling does not always cause scallop contamination. The aim of this study is therefore to evaluate if the contamination of scallops can be partly explained by the presence, at the bottom of the water column of *Pseudo-nitzschia* cells, or dissolved DA. Since 2011, bi-monthly survey has been deployed in the Bay of Brest (France) to monitor *Pseudo-nitzschia* cells and DA in surface and bottom waters, as well as DA in king scallops, thus allowing to compare their contamination with the presence of DA in the surface and bottom water as well as with the presence or not of *Pseudo-nitzschia*. The first results demonstrate that DA can be detected in the bottom water and also in shellfish even though there is no sign of *Pseudo-nitzschia* at the surface. Furthermore, DA in scallops seems also correlated to the toxin in the bottom water. These results highlight the need to extent monitoring to the detection of *Pseudo-nitzschia* and domoic acid in bottom water.

Microplate genoassay for the detection of *Pseudo-nitzschia* in environmental samples

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Molecular detection systems for several toxic algae species have been extensively developed in research but are rarely a part of routine monitoring. Moreover, the current detection systems are in no case predictive, although there is a need for more effective systems to cope with the worldwide increase of harmful algal bloom (HAB) events and the expansion of coastal activities and aquaculture. Here we present a 28S RNA-based immunoassay in a microplate format that reliably semi-quantifies the diatom genus *Pseudo-nitzschia* in a variety of matrices. We have tested the assay on several *Pseudo-nitzschia* strains isolated from the Northern Adriatic Sea, to generate calibration curves and establish sensitivity standards for four different molecular probes. Then, the assay was applied to environmental time-series and the response compared to microscopic cell abundance counts. The assay signal correlates well with cell abundance, while providing an additional layer of data since RNA concentration is a proxy for cell activity. By comparing sequential signals, especially in a high-frequency sampling strategy, predictive trends can be discerned. Our tool thus offers an assessment of the activity and state of a given *Pseudo-nitzschia* population, including species-specific setups. Given the microplate format, the assay is able to process 84 samples in a matter of hours. Our assay thus offers a molecular system, that can be easily integrated into a *Pseudo-nitzschia* monitoring strategy for either aquaculture, research, or water monitoring and was already implemented in several local aquaculture facilities with a positive response from the industry.

An outbreak of paralytic shellfish poisoning (PSP) in Southern Oman linked to a bloom of *Gymnodinium catenatum*

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At least 18 patients had to receive medical treatment at the hospital in Mirbat (Dhofar Governorate, Oman) in October 2020 after eating oysters (*Saccostrea cuccullata*) and mussels (*Perna perna*) that were harvested along the coast. The symptoms, such as paraesthesia in the lips, tongue and extremities, mild cramps, dizziness, were indicative of paralytic shellfish poisoning (PSP). Seawater and oyster samples were collected the day after, at the same locations along the coast of Mirbat where people harvested the shellfish and later became intoxicated. Seawater samples were preserved in Lugol's and analysed by inverted microscopy. DNA was extracted from one of the Lugol's preserved samples that appeared to be mono-specific, for sequencing. Oyster samples were analysed for lipophilic marine biotoxins by liquid chromatography with mass spectrometry detector, domoic acid and epidomoic acid by liquid chromatography with UV-VIS spectrophotometry detector (LC-UV-VIS), and paralytic shellfish poisoning toxins (PSP) by HPLC-FLD with pre-column oxidation. All oyster samples collected contained PSP toxins in the range 9,312 - 36,718 µg STX 2-HCI equivalents/Kg. Neither lipophilic toxins, nor domoic acid were detected. The abundance of *Gymnodinium catenatum* in the water samples was in the range 2 x 10^5 - 2 x 10^6 cells L^{-1}, which is largely over the warning level used in different countries. Initial analysis of the DNA sample obtained provided evidence of the identification of *G. catenatum* as well as the presence of a novel species of *Parvilucifera*, closely related to *P. multicavata*. In spite of the fact that there were no fatalities, this event highlights the need for the implementation of a HABs monitoring program along the coast of Oman.

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Paralytic Shellfish Toxins in Fish and Invertebrates of Southcentral and Southwest Alaska

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In Alaska, paralytic shellfish poisoning (PSP) is caused by ingestion of seafood products containing saxitoxins, potent neurotoxins produced by the dinoflagellate *Alexandrium catenella*. PSP is usually caused by consumption of toxin-containing bivalves (mussels, clams, etc.), but there is growing evidence that toxins can also be transferred to other biota during *Alexandrium* blooms, including species that do not feed directly on shellfish. Here, we report preliminary results from two concurrent projects examining the potential occurrence of PSP toxins in marine fish and invertebrates across southcentral and southwest Alaska. Samples were collected during 2014 - 2020 at sites in Lower Cook Inlet, Prince William Sound, the Kodiak Islands, the Alaska Peninsula, the Aleutians and the Pribilof Islands, and were analyzed for PSP toxin concentrations by ELISA and HPLC. Resulting data indicate toxin concentrations in forage fishes reached the highest levels in Dolly Varden (Salvelinas), Pacific Herring (Clupea) and Pacific Sand Lance (Ammodytes), with concentrations several times the U.S. regulatory limit of 80 µg STX eq 100 g^{-1} in some specimens. Toxins were also present in tissues of five salmon species and other predatory fishes, with maximum concentrations in organs (kidney, liver and digestive tract) and much lower toxin levels in muscle tissue and roe. Among invertebrates, the highest toxin concentrations were recorded in bivalve consumers (crabs, sea stars, predatory snails), but with appreciable levels in amphipods, urchins, tunicates and other species. Regional differences in toxin levels among biota were examined, with implications for high level marine predators, human health and the Alaskan seafood industry.

Mapping the development of *Dinophysis* spp. HABs using a novel molecular qPCR assay

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Diarrhetic shellfish toxins, produced by some species of *Dinophysis*, can accumulate in shellfish at high levels, representing a food safety issue for consumers. This risk is managed by routine monitoring programs in shellfish producing areas. We examined the dynamics of a *Dinophysis* bloom from the Manning River estuary (New South Wales, Australia (Latitude: -31.8667, Longitude: 152.6333), which occurred in February 2019 (max abundance 5,300 cells L^{-1}, *D. acuminata*). Using light microscopy, cells were identified to the closest possible taxon to determine the abundance of individual *Dinophysis* species. We then designed specific primers for a quantitative real-time polymerase chain reaction (qPCR) assay based on the ITS1/5.8S/ITS2 ribosomal region and evaluated its specificity, efficiency, and sensitivity. The assay amplified three *Dinophysis* species with efficiencies of 92.4 % for *D. caudata*, 91.3 % for *D fortii*, and 91.5 % for *D. acuminata*, and no cross-reactivity with other closely related species. Finally, the qPCR results from environmental samples were then compared with microscopy-based results from the same area across the same time period. The use of novel molecular tools, in combination with microscopy, has the potential to aid in rapid and comprehensive understanding of HAB development, and act as an early warning monitoring system for food safety.

Control of six different *Microcystis aeruginosa* strains and their toxic metabolites by phycocyanin-enhanced UV-A irradiation

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Climate change and anthropogenic pollution give rise to more frequent and severe cyanobacterial blooms. Toxic cyanobacteria challenge potable water treatment worldwide. In many cases, retrofitting water treatment plants is impractical, thus, alternative mitigation strategies such as in-reservoir treatments need to me investigated. Traditionally, UV-C (250 nm) is considered germicidal, however, this photolytic treatment cannot be applied *in situ* because it is non-selective and would harm other biota. UV-A (365 nm) can be an option for targeted cyanobacterial and toxin removal due to the presence of intracellular phycocyanin, a pigment specific to cyanobacteria, that can enhance the photolytic effects of UV-A irradiation. Six *Microcystis aeruginosa* strains (SCIENTO, NIES 1099, B2666, PCC 7820, 7813 and 7806) were exposed to UV-A irradiation for seven days. Cell number, intra- and extracellular microcystin concentration and photosynthetic activity were monitored. UV-A treatment significantly affected (up to 90 % removal) those *Microcystis aeruginosa* strains containing the most phycocyanin. A direct correlation between phycocyanin concentration per cell and removal efficiency was observed. Photosynthetic activity analysis showed significantly decrease activity after 24 h of irradiation with samples showing no photosynthetic activity by the end of the experiment ($F_V/F_M' = 0$). Intra- and extracellular microcystin concentrations were markedly decreased in UV-A treated samples with a combined microcystin removal of 86 %. UV-A irradiation was demonstrated to be an effective tool in the mitigation of *Microcystis aeruginosa* and associated toxic metabolites.

Potentially toxic phytoplankton in coastal bays along Peruvian coast

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In the present work, we evaluated the occurrence of potentially toxic microalgae and their relationship with environmental variables (sea surface temperatures, wind velocity, rainfall, river flow, and the Peruvian Coastal Thermal Index as an indicator of El Niño Southern Oscillation) in three coastal bays (Sechura, Guaynuná, and Paracas) from the Peruvian coast. Phytoplankton data were obtained from the Programa de Control de Moluscos Bivalvos developed by the Organismo Nacional de Sanidad Pesquera (2011 – 2019). Seawater was collected using a divisible hose sampler. The species analyzed are recognized as toxigenic in the IOC's list of harmful microalgae species. The species count was carried out according to the Utermöhl method. The most abundant species were *P. delicatissima* and *seriata* complexes in Sechura, *H. akashiwo* and *Prorocentrum cordatum* in Guaynuná, *P. cordatum* and *P. delicatissima* complex in Paracas. The most frequent species in all sites were *P. delicatissima* and *seriata* complexes, *P. cordatum*, and *Dinophysis acuminata*. Differences were observed in the relationships between the species and the environmental variables. In general, the presence of diatoms was favored by high wind velocities. The presence of dinoflagellates, mainly *A. ostenfeldii*, was associated with warm temperatures, greater rainfall, or river flows, and a decrease in wind velocity (Paracas). Potentially toxic species can be found year-round, although they occur more frequently with the highest abundances during the summer in Sechura and Guaynuná, and in autumn in Paracas. Species that represent a potential risk due to their high frequencies and abundances were mainly *Pseudo-nitzschia* species and *P. cordatum.*

A multi-year study on the detection and distribution of domoic acid contamination of shellfish in production areas from Los Lagos Region, Chile (2000-2021)

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Domoic acid, a marine neurotoxin causative of Amnesic Shellfish Poisoning (ASP) was first detected in Chilean shellfish in January 1997. The presence of domoic acid (DA) in shellfish extracts was determined by reverse-phase HPLC-UV. DA concentrations exceeded in some instances the maximum permitted level (MPL) of 20 mg Kg^{-1} of tissue. Highest levels were found in mussels (*Mytilus chilensis*). DA presence was associated with blooms of diatoms of the *Pseudo-nitzschia* genus. Since the diatoms *P. australis* and *P. pseudodelicatissima* are frequent phytoplankton components with a wide distribution in Chilean coastal waters, a permanent ASP monitoring program was implemented by the National Fisheries Service (Sernapesca) in 2000. Analyses of live shellfish samples collected from production areas from Los Lagos Region (41°30' to 42°30' S) are reported from January 2000 to May 2021. A total of 94 from 57,882 samples (0.16 %) were found to contain DA above 20 mg Kg^{-1}. Another 1360 samples (2.35 %) contained DA below MPL (0.01 to < 20 mg Kg^{-1}). Diatom blooms and domoic acid were detected every year. However, toxicity levels above MPL were observed only in 9 of 22 years of continuous monitoring. Regular increases in toxicity were observed mainly during the transition from spring to summer (October to March) with peaks during December-January. High detoxification rates of DA from mussels (T1/2 = 4 ± 1 days) allowed the development of contingency plans during the blooms that minimized harvest closures. Despite its recurrent nature, the risk observed to date has been low, with no reported cases of poisoning in humans, marine mammals, or birds.

Vertical distributions of *Ostreopsis* cf. *ovata* in NW Mediterranean Sea: impact on monitoring strategy

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Ostreopsis cf. *ovata* is a benthic dinoflagellate it has also already been described as free in the water column or aggregated at the sea water surface. However, the mechanisms of its resuspension have been poorly documented. To study this resuspension, a high frequency temporal monitoring was conducted in the Villefranche bay (France) to determine the abundance of (1) epibenthic cells attached to macroalgae, (2) planktonic cells in the water column and (3) cells aggregated at the sea water surface. This monitoring was realized over three consecutive years (2018, 2019 and 2020) and at different phase of the bloom (exponential – 2020, peak – 2019 and decline phase – 2018). Strong variations in *O*. cf. *ovata* abundances was observed over a 24 h cycle with a peak of abundances during the day and a strong decrease at night for all the three years monitored except in 2020 when the benthic abundances did not differ significantly between night and day. Moreover, the peak of abundance was first reached for epibenthic cells and later for planktonic and sea - surface cells. Monitoring of *O*. cf. *ovata* is currently based on a single sampling per day without precise indications of time of sampling and shows great variability in *O*. cf. *ovata* abundance. Our observations clearly indicate that time and place of sampling constitute a great source of variability in the determination of *O*. cf. *ovata* abundance and have to be considered when designing new monitoring strategies.

An extraordinary 2021 *Heterosigma akashiwo* (Raphidophyte) bloom in Chile: large-scale farmed salmon mortality associated to unusual environmental conditions

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During early Austral Autumn, Comau fjord (42 ºS) experienced an extraordinary HAB event that led to losses of more than 6,000 ton of farmed salmon during an extremely low rain – high solar radiation period. Field sampling and monitoring activities were carried out at the bloom site in the vicinity of farming cages close to the Loncochalgua river mouth (Los Lagos region). CTD data from the fjord transect, where some of the highest mortalities were reported, showed a pronounced stratification with high chlorophyll, low salinity, high temperature and high dissolved oxygen values at the surface (~ 70 µg L^{-1}; salinity ~ 24; 17 °C; > 10 mg L^{-1}), with a shallow pycnocline (mostly in the upper 10 m of the water column). At surface, cell abundances of *H. akashiwo* reached up to ~70,000 cells mL^{-1} and significantly decreasing abundances along with increasing depth. Hydrodynamic modeled particulate dispersion (Party - MOSA) showed high retention times in the Comau fjord, suggesting that this physical driver might have an important role in enhancing cell aggregation processes (patchiness) at the very surface layer of the water column. Finally, the end of the bloom was associated with cloudy - rainy weather. These important observations highlight the need of having differentiated strategies to tackle these more and more frequent fast - developing HAB events in Chilean Patagonia.

Harmful Algae Monitoring on San Jorge Bay in Antofagasta, Chile

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San Jorge Bay is located on the coast of Antofagasta, adjacent to the Atacama Desert in northern Chile. The area is experiencing an increasing number of Harmful Algae Blooms (HABs), and phytoplankton-derived toxins contaminate the local bivalves, leading to economic damages to the country. However, the information on HABs in this area is currently limited. The most recent HAB in San Jorge Bay occurred in November 2018 with multi-dominant phytoplankton species and was one of the largest HABs in Chilean history, resulting in massive jellyfish and squid mortalities. We started monitoring the San Jorge Bay during this HAB occurrence and continued it for 16 months, collecting information on abiotic and biotic parameters at six sampling stations. Through three cases of HABs that occurred during the study period, we evidenced that water temperature was the most influential driving factor for the three HABs, and 18â was the optimum temperature for the local phytoplankton growth. We also confirmed that San Jorge Bay is nitrogen-limited. The phytoplankton composition varied between sampling stations and sampling dates, suggesting that each geography has unique microbiological characteristics. Additionally, we used 18S rRNA metabarcoding analysis to confirm in San Jorge Bay the presence of several prominent toxin-producing phytoplankton, such as *Dinophysis acuminata*, *Alexandrium ostenfeldii*, *Pseudochattonella farcimen*, *Pseudochattonella verruculosa*, and *Pseudo-nitzschia australis*. These results contain valuable information for characterizing the local HABs and for strategizing the mitigation of ecological and economic damages caused by local HABs.

Monitoring of toxic dinoflagellate *Pyrodinium bahamense* and shellfish toxicity in Sabah

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Blooms of *Pyrodinium bahamense* have been reported almost annually in Sabah, with more than 200 reported paralytic shellfish poisoning (PSP) cases with > 20 deaths to date. The scale of blooms varied yearly and affected different districts of Sabah. High incidence of *P. bahamense* blooms was recorded onset and during the dry seasons (January to April), which were often observed after periods of heavy precipitation. The highest incidence of *P. bahamense* blooms in 2004-2005, coincided with the El Niño episode. However, no trend of increase in PSP was documented over the past three decades. The most recent PSP outbreak occurred in 2013, with more than 60 victims and four casualties. This unprecedented incident was believed due to ignorance of the shellfish ban. While the existing monitoring program and high public awareness have resulted in a lower number of PSP incidents, there is room for improvement in the current plankton and shellfish monitoring program. It is suggested that sampling frequency during the months of higher *P. bahamense* occurrences should be increased to weekly. A rapid test kit should be used to replace the existing mouse bioassay, and shellfish toxicity screening facilities are to decentralize, with more laboratories covering areas far away from the center. Analytic methods should be adopted for routine toxin testing. Other known shellfish toxins (DSP, NSP, ASP, etc.) should also be monitored. Malaysian HAB reports, a project funded by UK BBSRC & SAMS to develop an online portal, will be beneficial to facilitate early warning and to disseminate information to the public.

Evaluation of a virus-based control method reducing the damages by the harmful dinoflagellate *Heterocapsa circularisquama*

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In Japan, bivalves aquaculture species such as cultured oysters Crassostrea gigas and pearl oysters Pinctada fucata are severely threatened by blooms of the harmful dinoflagellate, *Heterocapsa circularisquama*. HcRNAV is a single- stranded RNA virus that specifically infects *H. circularisquama*. Given that high concentrations of HcRNAV remain in bottom sediment following a *H. circularisquama* bloom, dispersal of sediments containing HcRNAV was expected to provide a promising tool to mitigate blooms of this dinoflagellate. A laboratory experiment demonstrated the effectiveness of a) HcRNAV alone, and b) sediments with HcRNAV, in reducing *H. circularisquama* densities. For the first time, a field test was implemented in an active oyster farm on Sado Island, Japan in which sediment containing HcRNAV collected from nearby waters was dispersed into 15,000 L floating mesocosms during a *H. circularisquama* bloom. In the treatment spraying with sediment containing HcRNAV, the density of H. circularisquama decreased significantly until it reached only ~1 % of the initial after five days, while that of HcRNAV increased significantly by Day 2 (one-way ANOVA, n = 3, $P < 0.01$). These results indicate that the virus + sediment treatment was very effective in mitigating the *H. circularisquama* bloom under field conditions. Only a very low sediment concentration achieved these results, thereby reducing potential ecological impacts. Given the high diversity and mutation potential of HcRNAV, and the variable host response, it is recommended that field application of this mitigation strategy use indigenous sediment. This study provides the first field test of viral control of HAB worldwide.

Comparative accumulation and elimination of diarrhetic shellfish toxins by commercial bivalve species from Brazil

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Uptake and detoxification of diarrhetic shellfish toxins (DSTs) were compared in clams (*Anomalocardia flexuosa*), oysters (*Crassostrea gigas*, *Crassostrea gasar*), mussels (*Perna perna*) and scallops (*Nodipecten nodosus*). Bivalves were exposed in the laboratory to DST - containing *Prorocentrum lima* or *Dinophysis acuminata* complex cells. After 24 h of exposure, *C. gasar*, *A. flexuosa*, *C. gigas*, *P. perna* and *N. nodosus* attained average whole - body DST levels equivalent to 7.5, 8.5, 23.4, 51.1 and 660 ng g^{-1}, respectively. When normalized by the amount of DST available in suspension, toxin uptake by scallops was 7, 13 - 17 and 860 times greater than that of mussels, oysters and clams, respectively. However, in scallops, toxins were largely restricted to the digestive glands (DGs), which accumulated ~300 - fold higher DST concentrations relative to non-digestive tissues; DGs of mussels, clams and oysters contained 10 - 30 - fold higher DST levels than other tissues. After 168 h of depuration on similar non-toxic diets, only 2 % of the maximum toxin concentrations were detected in clams and mussels and 4.5 % in scallops, whereas oysters still retained 16 % (*C. gigas*) to 35 % (*C. gasar*). Clams eliminated DSTs at faster exponential rates (0.029 h^{-1}) than mussels (0.023 h^{-1}), scallops (0.014 h^{-1}) and oysters [*C. gigas* (0.010 h^{-1}); *C. gasar* (0.004 h^{-1})]. In general, *N. nodosus* scallops and *P. perna* mussels exhibited greater DST uptake capacity than oysters and clams, with the latter eliminating toxins at faster rates, resulting in lower toxin loads. These findings are relevant for the management of DST - contaminated bivalves in Brazil, where DSP episodes and shellfish harvesting closures are frequent.

Toxic marine microalgae and associated phycotoxins in shellfish- 14 years of data from the Italian coasts

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Along the Italian coasts, the presence of toxins of algal origin in wild and cultivated shellfish has been reported since the 1970s. In this study, we use data gathered by the Veterinary Public Health Institutes and the Italian Environmental Health Protection Agencies from 2006 to 2019 to investigate toxicity events and relate them to the distribution of toxic species. Among detected toxins (OA and analogues, YTXs, PTXs, STXs, DAs, AZAs), OA and YTX have been those most frequently reported in cases of seafood contamination. Levels of those toxins exceeding regulatory limits were associated with high abundances of *Dinophysis* spp. in case of OA and of *Gonyaulax spinifera*, *Lingulodinium polyedra* and *Protoceratium reticulatum* in case of YTX. Seasonal blooms of *Pseudo-nitzschia* spp. have occurred all along Italian coast, but DA has only occasionally been found in shellfish, with values always below the regulatory

limit. *Alexandrium* have been recorded in many more areas than those where STXs have been recorded, only rarely exceeding regulatory limit. *Azadinium* have been sporadically recorded and AZAs have been sometimes detected but always in low amounts. Among the emerging toxins, PLTX-like toxins have often accumulated in wild mussels and sea-urchins from rocky shores due to the occurrences of *Ostreopsis* cf. *ovata*. Overall, Italian coastal waters harbor a high number of potentially toxic species but rare cases of intoxications have occurred so far, with a few HAB hotspots mainly related to DSP toxins, reflecting the conditions of the whole Mediterranean Sea.

Rapid, portable, multiplexed detection of harmful algal toxins in freshwater

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Harmful algal blooms are a significant threat to fresh waters necessitating routine testing to protect humans from exposure to contaminated drinking and recreational waters and for forecasting and modeling. Since toxin profiles change spatially and temporally there is significant need for rapid tests that can provide real-time, local answers. Currently, the four classes of toxins that are typically monitored in freshwater are microcystins (MC), cylindrospermopsins (CYN), saxitoxins (STX) and anatoxin- a (ATX-a). There is currently no method of measuring STX in freshwaters in the field and only a semi-quantitative test strip for ATX-a. A rapid, portable multiplexed test would reduce the time and cost associated with collecting critical data while improving human safety by ensuring that all four toxin classes are always monitored. The LightDeck technology enables portable, multiplexed detection of toxins and has been demonstrated in a duplex MC/CYN panel and is being expanded to include STX and ATX toxin classes.

Dark post-treatment after UV irradiation as a method for increasing the efficacy of cyanobacteria inactivation

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Synechococcus and *Anabaena* are two genera of cyanobacteria with a worldwide distribution and some species associated with blooms and biotoxin production. The International Maritime Organization (IMO) approve the Ballast Water Management Convention (BWMC) in 2004. The D-2 standards of the BWMC limit the maximum concentration of viable organisms in the discharged ballast water. For 2024, all the ships should have a Ballast Water Management System (BWMS) to reach the D-2 standards. The most common approved BWMSs are UV based. In our experiment *Synechococcus* sp. and *Anabaena* sp. were subjected to UV irradiation (a collimate beam reactor with a UV-C mercury lamp) and after that, half of the samples were immediately exposed to light and the other half were kept in dark for five days to see if the photoreactivation was avoided and the efficacy of the treatment was increased. The results showed that *Synechococcus* sp. has a high resistance to UV irradiation, notably higher than *Anabaena* sp. On the other hand, in both cyanobacteria, the inactivation rate was higher in the samples subjected to a dark post-treatment, although the statistical analysis indicated that these differences were not significant. These results indicate that these cyanobacteria do not undergo photoreactivation, therefore storage in the ballast tanks after the UV irradiation, hardly increases the inactivation efficacy. These findings have importance in ballast water management and the avoiding of spreading harmful organisms like some species of cyanobacteria.

ZEBRAMARTOX: Zebrafish-based toxilogical tool for the screening of marine toxins for assessing seafood safety

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Global climate change favors increasing frequency, intensity, and geographical coverage of known harmful algal blooms (HAB), thus the risk of food contamination with toxins must be rigorously controlled. In Chile, the National Integrated Red Tide Program has prevented poisoning events by a timely detection of potential toxic outbreaks. The official methods for detection of paralytic shellfish toxin (PST) includes analytical techniques that require equipment and specialists not available in every laboratory, and the mouse bioassay. At large-scale HAB events, the demand for MBA experimental animals increases, which is opposed to the reduction of animal experimentation for ethical reasons. To face this challenge, we used zebrafish as a biological model of paralysis and Saxitoxin analytical standard for developing a complementary toxicological tool that will allow the initial screen of shellfish samples suspected to contain PST before applying the official methods. Using both a simple touch response and a semi-automated assay, we can evaluate the locomotor response of zebrafish larvae and detect diluted PST at concentrations around regulatory limit (80 µg STX 100 g^{-1}). In order to standardize this bioassay for detection of PST directly on shellfish extracts and lighten the burden on official laboratories, we have evaluated the effect of pH of extracts, matrix interference and different solvents on zebrafish larvae locomotor activity and touch response. This bioassay could strengthen control systems by using a combined approach: a functional assay, that provides information about sample toxicity quickly and cheaply, and a confirmatory method, which identifies the profile of toxins in the sample.

Humorous content effectiveness in marine biotoxins' risk communication through social media

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Background: Social media are largely integrated into citizens' daily routines and constitute powerful communication tools, often exploited by regulatory authorities as information distribution channels for risk communication purposes. Food safety issues frequently raise public concern, requiring management through trustworthy information provision, ideally disseminated by digital means. Risk communication strategies entailing use of humor in social media are increasingly becoming popular, aiming to raise public awareness and induce risk perception. Objective: Assessment of the effectiveness of humor-mediated risk communication strategies regarding the food safety hazard of marine biotoxins in a multinational audience. Methods: A multinational survey targeting adult English-speaking social media users was conducted, also involving participants with food safety and marine biotoxin expertise, by means of an online questionnaire including: (a) demographical data, educational/professional background, food safety-related attitude and social media usage; and (b) respondents' perception on various effectiveness parameters, examining three groups of materials relevant to marine biotoxins (conventional, humorous and combination), as risk communication scenarios. Statistical analysis of data examined relationships among variables through chi-square and Spearman rank correlation tests. Results: Majority of the 218 participants indicated humorous materials as of lower effectiveness than conventional ones; however, the former were scored as more "Likeable" or sharable, especially by those more familiar with food safety and/or marine biotoxins. Interestingly, participants perceived materials combining both humorous and conventional elements as comparably effective to those purely conventional. Conclusions: Materials combining both informative and humorous elements may constitute potentially valuable tools in food safety/marine biotoxins' risk communication through social media, by further content optimization.

Spatiotemporal distribution of paralytic shellfish poisoning (PSP) toxins in shellfish from southern South America (Beagle Channel) during 2005-2017

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The Beagle Channel is a sub-Antarctic semi-estuarine environment at the southern limit of South America, where harmful algal blooms of *Alexandrium catenella* associated with paralytic shellfish poisoning (PSP), have been reported since 1886. PSP outbreaks in this area affect public health, marine wildlife, and ecosystem services, particularly fisheries management and mussel aquaculture activities. Within the framework of the Shellfish Toxicity Monitoring Program carried out in Beagle Channel eastern coastal waters, we analysed the spatiotemporal patterns of PSP toxins in the Magellan mussel *Aulacomya ater* and the blue mussel *Mytilus edulis*, measured by the mouse bioassay, for 12 years (2005 – 2017). PSP outbreaks occurred mostly during summer and their duration was significantly longer than those detected during spring and winter. A marked interannual variability in their magnitude was also found. Most intense outbreaks were detected during 2009 - 2013, reaching up to 5600 µg STX eq 100 g^{-1} tissue, determining closure of shellfish fisheries of up to 200 days. Moreover, the highest toxicity values were observed in the mussel culture areas, and the lowest ones in natural mussels' beds. PSP toxicities for the Magellan mussel were significantly higher than for the blue mussel. PSP dynamic models suggest that mussels from the Beagle Channel have lower detoxification rates than those from elsewhere in the world. The present study helps to understand the dynamic of PSP outbreaks in this little-known area, aiding to prevent economic losses, and contributing to protect human health and marine ecosystems.

Toxigenic microalgae and co-ocurrence of toxins in Patagonian gulfs of Argentina

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In coastal waters of Chubut Province, Argentina several species of toxigenic microalgae have been recorded. Among those, *Alexandrium catenella* has caused poisonings and human deaths since 1980 resulting in closures of mollusk fisheries, due to paralytic shellfish toxins (PST). Dinoflagellates of the genera *Dinophysis* and *Prorocentrum*, have been reported as producers of lipophilic toxins (okadaic acid (OA) and pectenotoxins (PTX)), whereas diatoms of the genus *Pseudo-nitzschia* are known to produce domoic acid (DA). In recent years, in addition to PST outbreaks, fishery closures have also been imposed due to lipophilic toxins above regulatory limits in shellfish. Phytoplankton analyses were performed during 2018 at six sites located in the North Patagonian gulfs (San Matías, San José and Nuevo). Between February and June, the presence of toxins and toxic species was assessed. DA was detected in 47 % of the samples, while PTX2 and/or PTX-2sa were detected in 79 % of the samples. The co-occurrence of both toxins was observed in all months with the exception of March. *Pseudo-nitzschia pungens*, *P. fraudulenta* and *P. calliantha* as well as *D. tripos* and *D. acuminata* were identified as potential producers of these toxins. This study expands the knowledge about the simultaneous presence of multiple phycotoxins in the North Patagonian gulfs, as well as raising questions about the possible impacts of such occurrences for public health. This study was carried out in the frame of the Provincial Plan for Prevention and Control of Red Tides.

Monitoring cyanobacterial blooms using NDCI index derived from satellite images in the Patos Lagoon, Brazil

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Cyanobacteria are photosynthetic, diverse and cosmopolitan microorganisms. Some cyanobacteria are highly toxic to animals. The rapid growth of the cyanobacterium *Microcystis aeruginosa* can produce visible blooms which are recurrent in the Lagoon and its estuary every summer. Certain climate features can intensify these events during the hottest period of the year, causing impacts on recreation, fishing, tourism, water sports, leisure and water supply in the lagoon cities. The general objective of this work is to use satellite images as a tool to monitor cyanobacterial blooms, especially *Microcystis* in the lagoon. Based on these images, we intend to estimate pigment values and colonies abundance to support the safety of mentioned activities in the lagoon. Samples were taken on "São Lourenço do Sul" and "Tapes" beaches with 5 L bottles. Measurements of localization (eTrexH Garmin), water temperature (Hg thermometer) and salinity (Kasvi refractometer) were taken *in situ*. Samples obtained were quantified in colonies and cells with Utermöhl sedimentation chamber by invert microscopy ZEISS AXIO VERT. Chlorophyll *a* analyses were made at the laboratory with a visible spectrophotometer (CARY50-VARIAN). These data were associated with satellite images obtained MSI/Sentinel2 (open-data satellite). The results obtained from these correlations, using linear regressions, are positive but with low R values due to a small number of samples (n = 14). The next steps include the improvement of these estimations to use in future applications for public and environmental health, environmental management, applied toxicology, remote sensing and, marine and estuarine microbiology.

Belgian cyanobacterial blooms during the summer of 2019

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The B-blooms 2 project showed the incidence of (toxic) cyanobacterial blooms in Belgium more than a decade ago. However, this was the last major monitoring project since then. In response, Flemish and Walloon regional governments started monitoring programs for toxic cyanobacteria blooms in recreational lakes and ponds used for water contact activities (excluding fishing). The current information about bloom occurrence is fragmented, and monitoring practices differ between the regional authorities. Authorities in Brussels did not initiate monitoring program was. Ten years later, blooms are becoming more common in the temporal regions of Europe, and current monitoring programs might not be sufficient to ensure public health safety. Therefore, Sciensano collaborated with the regional authorities reexamine bloom samples from the summer of 2019 based on toxin content and species present in the bloom. In total, we analyzed 78 samples from 23 different ponds and lakes and other surface waters. We used LC-MS/MS to detect and quantify eight microcystin congeners and nodularin and used PCR and sanger sequencing for species determination. In addition, we examined the presence of the *mcyE*-gene using PCR. After analysis, we found MCs at concentrations between 0.45 µg L^{-1} to 683 µg L^{-1} and detected *mcyE* in 42 samples. MC-RR, MC -LR and MC-LY were most commonly detected. *Microcystis* was the abundant species, although others were also detected.

Trends in cyanobacteria harmful algal blooms in New York State, U.S.A. (2012-2018): frequency, intensity, duration, and the limitations of monitoring data

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The primary objective of this study was to evaluate trends in cyanoHABs occurrence in New York monitoring data from 2012 to 2018. We addressed trends in frequency, intensity, and duration. The statewide dataset included information from several formal monitoring efforts, beach closure reports, as well as public reporting of cyanoHABs in locations statewide. The dataset spanned reporting and monitoring from 846 distinct waterbodies and included at least one cyanoHAB report from 381 waterbodies. Nearly half of those waterbodies (49.6 %) had only one year in which a cyanoHAB was documented. Less than 3 % (11 lakes) had records of cyanoHABs every year of the study period. We noted a significant increase in the number of cyanoHABs reports each year ($P < 0.001$), but not an overall increase in intensity, based on evaluation of chlorophyll levels in mid-lake samples collected on a routine basis. An analysis of duration trends was limited because of the reporting program structures, however, findings indicated that HABs season duration did not lengthen over the study period. This study addressed the limitations of monitoring data for trend analysis based on the structure of the programs and provided retrospective feedback on aspects of monitoring program design that would have improved our ability to detect trends, as well as changes that New York State agencies have made to monitoring programs since 2018 to better detect true occurrence of cyanoHABs.

Historical Blooms of *Pseudo-nitzschia* and outbreaks of domoic acid in the inner sea of Chiloé island, Southern Chile, 2020- 2021

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The Inner Sea of Chiloé (ISC), located in the northern area of fjords in Chile, is one of the most important mussel-growing areas in the world. A prolonged toxic bloom of *Pseudo-nitzschia* occurred between December 2020 and March 2021, leading to the accumulation of domoic acid (DA) in shellfish for more than two months and constituting the longest toxic event ever recorded in this area. The first DA closure of shellfish harvesting areas occurred in Reloncaví sound in late December 2020, owing to a 58 $\mu g\ g^{-1}$ of DA concentration measured in mussels. Subsequent sampling in the area, revealed blooms of *Pseudo-nitzschia* cf. *australis* (0.9×10^6 cells L^{-1}) and *P.* cf. *pseudodelicatissima* (1.1×10^6 cells L^{-1}) and particulate DA up to 10×10^3 ng L^{-1}. Later, DA results obtained during January and February 2021 showed increased accumulation in shellfish at different sites along the eastern coast of ISC, causing precautionary closures with maximum values (140 $\mu g\ g^{-1}$) and with *P.* cf. *pseudodelicatissima* concentrations of 1.5×10^6 cells L^{-1}. Despite the high DA concentrations, no effects on human seafood consumers or wildlife were recorded. We hypothesize that anomalous spatial and temporal extension of *Pseudo-nitzschia* blooms and AD production, could be related to (1) advective processes from the North of ISC following the main circulation patterns, and (2) favorable climate conditions associated to the positive phase of Southern Annular Mode (SAM), that promotes positive temperature and photosynthetic active radiation anomalies, and lower precipitation during the summer of 2021 in the studied area.

Domoic acid and *Pseudo-nitzschia* spp. blooms within a biodiversity hotspot in coastal Mozambique

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Coastal biodiversity hotspots are driven by a delicate balance between nutrients and primary producers. Small changes in the nutrient balance can alter food web structure and trophic energy transfer, allow harmful algal species to establish, and affect the composition, diversity, and biomass of organisms at all trophic levels. This is well-established along coastal ecosystems within developed countries; however, globally, little is known in coastal communities where human populations are rapidly expanding, resources are limited, and the coastal biodiversity is high. Inhambane Province, located in southern Mozambique at the intersection of the Mozambique and Agulhas Currents, represents a unique coastal upwelling system. Coastal upwelling processes provide the ecosystem with balanced nutrient concentrations which sustain coral habitats and a plankton community suitable to support larger marine fauna, notably *Rhincodon typus* (whale shark) and *Mobula alfredi* (reef manta ray), which show high site fidelity and actively feed year-round. Mozambicans rely on the health of this coastal system as a key food source and for economic development through fisheries and tourism. An initial monitoring program was established in partnership with All Out Africa and several U.S. Universities, from 2017 – 2018. During this period, we identified the presence of *Pseudo-nitzschia* spp. and domoic acid in the area. Through an intensive sampling period in the winter of 2018, data suggest blooms and toxins were associated with a coastal upwelling event within the region. Domoic acid concentrations were highest in areas frequented by actively feeding planktivores. These finding suggest further monitoring is needed to ensure human and wildlife health during blooms.

Screening the plankton assemblage in York River using the Imaging FlowCytobot

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Harmful algal blooms (HABs), defined as a rapid increase in algal population, have adverse impacts on the environment. The consequences of HABs vary greatly by algal species, ranging from a reduction in food quality to as severe as animal (or human) mortality. Traditionally, the identification of bloom species has relied on microscopy; however, microscopic identification requires an extensive amount of time and labor. A combination of the Imaging FlowCytobot (IFCB) and the computational artificial neural network (ANN) model has been applied to achieve real-time plankton identification in the York River, Chesapeake Bay, VA. The IFCB combines flow cytometry and photography to capture high-resolution images of suspended particles. These images are manually sorted into algal groups to build a reliable ANN classifier for real-time identification. Continuous physical parameters (e.g., water temperature and turbidity) collected by Virginia Estuarine and Coastal Observing System will be correlated with the algal community structure to determine how these parameters affected the succession of algal communities. Overall, the dominant algal groups had a seasonal variation due to water temperature, salinity, and turbidity differences. Dinoflagellates and diatoms (e.g., *Dinophysis* and *Pseudo-nitzschia*) dominated the winter plankton community, while zooplankton (e.g., *Mesodinium*) and cyanobacteria are the most abundant groups in summer. In spring, the community was composed of the most diverse groups of plankton. The utilization of an IFCB in algal monitoring efforts, reduced the amount of labor required in microscopic identification, making real-time monitoring available to resource managers.

Alexandrium in the Swan Canning Estuary (Western Australia): a new blooming problem for recreational and commercial fishers

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Paralytic Shellfish Toxin (PST)-producing *Alexandrium* has been a significant management issue for fisheries along the eastern coast of Australia since the early 1990's, however Western Australia has not suffered the same impact. *Alexandrium minutum* was first detected in the Swan Canning Estuary in 1983 (Hosja & Deeley 1994) but densities here and around the state have generally remained low. *Alexandrium pacificum* was identified in local coastal waters in 2011 and *Alexandrium* spp. began being detected in other regional monitoring programs from 2014. Between 1994 and 2018, *Alexandrium* was detected 31 times in the Swan Canning Estuary with median and maximum densities of 3.0×10^3 and 4.0×10^4 cells L^{-1}, respectively. In the Austral summer/autumn periods of 2019 and 2020, *Alexandrium* bloomed at unprecedented densities within the Swan Canning Estuary. The initial *A. minutum* bloom impacted over 30 km of the Swan Canning Estuary, lasted 105 days with median and maximum densities of 1.89×10^5 and 14.9×10^6 cells L^{-1}, respectively. The following summer, *A. pacificum* co-occurred with the *A. minutum* bloom, lasting 112 days with median and maximum densities of 48×10^3 and 15.5×10^6 cells L^{-1}. PST levels as high as 8.8 mg STX eq kg^{-1} were measured in mussels (*Xenostrobus securis* and *Mytilus galloprovincialis*) and 1.2 mg STX eq kg^{-1} in the viscera of the blue swimmer crab (*Portunus armatus*); the first known instances of PST consumption guideline (0.8 mg STX eq kg^{-1}) being exceeded in these species in Western Australia. Here we describe the blooms, the response of authorities to the PSP risk and implications for industry and HAB management in Western Australia.

Current status of the study of harmful algal blooms along the coast of Campeche, SE Gulf of Mexico

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The records of harmful algal blooms from 2005 to 2019 in the coastal waters of the state of Campeche, SE Gulf of Mexico, are described. Sampling areas correspond to recreational beaches, areas with anthropogenic activities and oyster banks. Forty - five bloom events were reported, with a minimum of 6.0×10^3 and a maximum of 10×10^6 cells L^{-1} and an average of 8.0×10^5 cells L^{-1}. *Pseudo-nitzschia* spp. were the most frequent in 2005, 2008, 2011, 2017, 2018 and 2019 during the rainy season (June-September) and the beginning of the windy season (October - January), with abundances of 6.0×10^3 to 6.4×10^5 cells L^{-1}, and were widely distributed along the Campeche coast. *Pyrodinium bahamense* also showed a wide distribution in the dry and rainy seasons (2005, 2008, 2010 and 2019), with abundances ranging from 3×10^3 to 3.3×10^5 cells L^{-1}, and its toxicity was detected. Anabaena spp. occurred from Campeche Bay to the oyster banks in Términos Lagoon, with abundances of 3.5×10^4 to 6.6×10^5 cells L^{-1}. The abundance of potentially harmful species showed the highest values (10^6 cells L^{-1}) from 2011 to 2013 during the rainy seasons. However, no trend was apparent during the years monitored. *Karenia brevis* (2011), *Prorocentrum mexicanum* (2010-2012), *P. minimum* (2012 - 2013) and *Cylindrospermopsis cuspis* (2013) caused blooms that resulted in significant economic losses due to the closures by the health authorities, directly affecting the welfare of coastal fishermen and the associated economic structure. Continued monitoring of fishery and aquaculture products is important for ensuring food safety and protecting public health.

Shewanella sp. IRI-160 algicidal activity on *Karenia* spp.: A closer look at the impact of ammonium on efficacy

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Blooms of *Karenia* spp. are a common occurrence in the Gulf of Mexico and present a significant human health threat as they produce potent neurotoxins. These red tides also have adverse effects on the coastal economies and can span hundreds of square miles. Previous research investigated the bacterium, *Shewanella* sp. IRI-160, which was shown to exhibit algicidal activity against *K. brevis*. This bacterium secretes algicidal compounds, "IRI-160AA" that contains high concentrations of ammonium (NH_4^+), which may play a role in its demonstrated algicidal activity. In this study, we investigated the role of NH_4^+ in the efficacy of IRI - 160AA on *K. brevis* and *K. mikimotoi* in laboratory batch cultures. For NH_4^+ conditioning studies, *Karenia* cultures were grown with L1 amended culture medium having 25 to 200 µM of NH_4^+. Specific growth rates were computed using linear regression analysis of the natural log of dilution corrected cell densities (counts or *in vivo* fluorescence). The optimal NH_4^+ concentration for algicide screening assays was determined as the highest NH_4^+ concentration that resulted in a < 10 % deviation from cultures grown under standard conditions. The algicide was screened at 10 % final concentration (v/v) on each of the *Karenia* spp. for 72 h alongside controls that received a matching concentration of ammonium. Algal growth was monitored via cell counts and *in vivo* chlorophyll *a* fluorescence. Additionally, cellular effects of the algicide were confirmed via microscopy and HABscope videography. The results of this work will be used to further elucidate the mode-of-action and active compound(s) of this algicide against *Karenia* spp.

Effects of immobilized algicidal bacterium *Shewanella* sp. IRI-160 and its cell-free filtrate on harmful dinoflagellate *Karenia brevis*

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The toxic dinoflagellate *Karenia brevis* causes persistent blooms in the Gulf of Mexico, U.S.A. Biological controls such as algicidal bacteria may be effective and sustainable approaches for HAB prevention and mitigation. Previous research reported the cell-free filtrate (IRI-160AA) of algicidal bacterium *Shewanella* sp. IRI-160 was effective at controlling the growth of dinoflagellates, including *K. brevis*, while having no negative impacts on other algal species, or on vertebrate and invertebrate marine species when applied at concentrations needed to inhibit the growth of dinoflagellates. Prior research showed that immobilization of *Shewanella* sp. IRI-160 in a porous matrix such as alginate hydrogel may be a viable approach to safely disperse the bacterial algicide for effective HAB control. Preliminary results also indicated alginate beads prepared with the cell-free filtrate, IRI-160AA, were effective in controlling the growth of harmful dinoflagellate *Karlodinium veneficum*. No research has been conducted to investigate the effects or to optimize the algicidal efficacy of immobilized *Shewanella* sp. IRI-160 or the cell-free filtrate, IRI-160AA, on *K. brevis*. Here, the efficacy of alginate beads prepared with either immobilized *Shewanella* sp. IRI-160 or IRI-160AA was evaluated to control the growth of laboratory cultures of *K. brevis*. Results will be presented showing the optimization of immobilized *Shewanella* sp. IRI-160 or IRI-160AA in alginate hydrogel beads for reducing *K. brevis* biomass to 50 %. Recovery of *K. brevis* after treatment will also be examined. Results of this research will provide support for the application of immobilized *Shewanella* sp. IRI-160 and/or its algicide to control *K. brevis* blooms.

News media coverage and public reactions to a *Lingulodinium polyedra* red tide at the Algarve coast (southern Portugal)

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In June 2019, a red tide caused by the dinoflagellate *Lingulodinium polyedra*, whose toxins do not cause harm in humans, developed in the Algarve coast (southern Portugal). The occurrence of algal toxins and consequent interdiction of bivalve harvesting is common in this region, but harmful algal blooms that lead to water discolouration are rare. This study analyses the scientific accuracy of the information communicated by news media, and consequent public reactions, by analysing news pieces shared on Facebook by regional and national news media outlets, and comments to the news posted by individual users. Overall, news pieces had a sensationalized, negative tone, and many lacked scientific accuracy. For instance, some news referred that ingestion of contaminated water, fish, and shellfish leads to gastrointestinal problems, and the toxic substances that the algae produce may contaminate the air and cause respiratory difficulties – which is false in the case of a *L. polyedra* bloom. Many commenters showed an adverse reaction to the event, most likely influenced by the negative portrayal of the red tide by news media. Other Facebook users were quite knowledgeable about the red tide, stemming from their previous experience with these events. Individuals seemed to be aware of the lack of cooperation between authorities and scientists and expressed their mistrust in these stakeholders. As red tides may become a common feature in the Algarve coast, journalists, scientists, and authorities should strive to offer accurate and responsible information to the public.

Development of a red tide communications plan for Florida

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Harmful algal blooms (HABs) of the brevetoxin - producing dinoflagellate *Karenia brevis* occur throughout the Gulf of Mexico and impact Florida's Gulf coast near annually. Despite the frequency of these events and the amount of publicly available information, there persists a gap in knowledge about the organism itself, management actions, and the real and perceived health risks to Florida's residents and tourists. Communication and outreach are essential components of any HAB risk mitigation program. How and what information is disseminated determines how individuals make decisions, ultimately influencing human health, economic, social, and cultural impacts. However, too often, education campaigns are created from the perspective of the practitioner and fail to account for the needs, values, and perspectives of the customer, leading to ineffective messaging and amplification of risk. This project advanced red tide communications by aligning practitioner (i.e. agency) needs with end-user (i.e. resident and visitor) wants regarding preferred red tide information, formats, and delivery modes. This presentation will discuss a multi-method research study that used literature reviews, focus groups, and surveys to evaluate and improve the suite of red tide communication products and delivery modes in Florida. Data was collected from residents, natural resource managers, public health, media, small business, and tourism professionals. This presentation will discuss components of the communication strategy including areas for improvement, and research-guided recommendations for communication dissemination and messaging.

Quality of life in a coastal community exposed to harmful algal blooms (Luanda Bay, Angola)

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Luanda Bay, an important ecosystem located on the coast of Angola, is highly affected by anthropogenic activities that cause several environmental problems, such as eutrophication, harmful algal blooms, and water pollution. Environmental problems can severely affect the quality of life of coastal populations. In this work, we aim to evaluate quality of life and environmental risk perception in this vulnerable coastal ecosystem, using a comprehensive questionnaire applied to residents and other ecosystem users. Respondents were mainly between 25 to 45 years old, and for the most part, either lived and/or worked in Luanda Bay. Results indicated that most respondents considered that they possess a high knowledge about climate change, water pollution (including by plastics), and ingestion of contaminated seafood. However, regarding eutrophication and harmful algal blooms, most participants reported a low/moderate knowledge. Life experience was reported as the most relevant source of information regarding environmental issues, but news media also played a relevant role. Respondents indicated a moderate risk perception towards environmental risks. Residents' perceived quality of life was moderate/good in terms of physical and psychological health, and social relationships, but the environmental component was perceived as weak. Results suggest that improvements in the natural environment are needed to increase the quality of life in this ecosystem. Lack of knowledge regarding harmful algal blooms is concerning and, as bivalve harvesting in Luanda Bay for local consumption is a major economic activity, efforts should be made to better communicate and disseminate information on HABs to this population.

Impact of Anthropogenic Activities on Microbial Eukaryotic Algal Community Structures in the New River, Belize

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Anthropogenic discharge of nutrients and pollutants through time has resulted in deteriorating water quality and hypoxic conditions in New River, Belize. The increase in urban and agricultural areas has also exacerbated the pollution discharge where the conditions alter the aquatic eukaryotic community structure. The New River ranges from oligotrophic to hypereutrophic due to adjacent land usage and near the mouth of the river varies in salinity due to proximity and water exchange with the ocean. Assessing eukaryotic community structure in aquatic reservoirs is important because it can aid in assessing the overall health of an aquatic ecosystem. Evaluating interactions and correlations between environmental data and metagenomic data (18S rRNA) can provide a more in depth look at species dynamics. In this study, we investigated the environmental conditions and eukaryotic algal community structure in the largest river system in Belize and found significant differences along spatial gradients. We observed differences in abundance of photosynthetic eukaryotes along the river system with chlamydomonads, cryptomonads, diatoms and chrysophytes abundant throughout, while prasinophytes, dictyochophytes, porphyridiales, and sphaeroplealeans were also present, but in lower abundance. Heterotrophic cryptomonads were especially dominant in sites with high conductivity, but also appeared in high abundances in the New River lagoon. Chlamydomonads were present in high abundances in the lagoon compared to areas around the mouth of the river and in Corozal Bay. The high abundance of photosynthetic eukaryotic algae demonstrates a highly productive system along the New River, Belize.

The Philippine Early Warning System for Harmful Algal Blooms

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The Philippines has adapted strategies such as quantification of Paralytic Shellfish Poison (PSP) toxin in shellfish meat supplemented by toxic algae population counts in water samples to provide Early Warning System (EWS) for national Harmful Algal Blooms (HABs). Biotoxin quantification is determined through the Receptor Binding Assay (RBA). It is the standard approach used for regulatory closures of shellfish harvest areas and/or coastal waters while HAB microscopic identification and counts provide the information to forecast future HAB. The EWS has been prompted by the country's archipelagic nature and a HABs history, specifically PSP, that span nearly four (4) decades causing significant impacts on public health and the fisheries industry. As an archipelagic State, 62 % of the total number of municipalities are located along the coast where 60 % of the country's population reside. Regular issuance of shellfish bulletins identifying HAB occurrences across the country allow the national, regional and the local government officials, shellfish growers and industries, and the general public to take appropriate actions to minimize HAB impacts. Substantial decrease in PSP cases and fatalities over time was noted during the implementation of the early warning system. The national HAB monitoring and management program is characterized by a management authority led by the national fisheries agency and its regional field units in collaboration with the coastal local government units, an established temporal and spatial monitoring scheme, the use of internationally accepted biotoxin confirmatory tests, an established national PSP regulatory limit, and procedures anchored on national legislation and appropriated budget. All these contribute to the sustainable implementation of the EWS.

Twenty five years of domoic acid monitoring in Galicia (NW Spain): spatial, temporal and interspecific variations

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Prevalence, impact on shellfish resources and interspecific, spatial and temporal variabilities of domoic acid (DA) in bivalves from Galicia (NW Spain) have been studied based on more than 25 years of monitoring results. The maximum prevalence (100 %) and incidence (97.4%) were recorded in *Pecten maximus*, and the minimum ones in *Mytilus galloprovincialis* (12.6 and 1.1 %, respectively). The maximum DA concentrations were 663.9 mg kg^{-1} in *P. maximus* and 316 mg kg^{-1} in *Venerupis corrugata*. After excluding data of the scallop *P. maximus*, DA was found (prevalence) in 13.3 % of the samples of bivalves, and its concentration was over the regulatory limit (incidence) in 1.3 % of them. Most species have DA average concentrations higher than mussels. In general, the prevalence decreased towards the North, but not the magnitude of the episodes. In raft-cultured mussels, the lowest DA values were recorded in the Ría de Arousa. The seasonal distribution was characterized by two maxima, in spring and autumn, with the second one decreasing in importance towards the North. DA levels decreased slightly along the period studied, although this decreasing trend was not linear. A cyclic pattern was observed in the interannual variability, with cycles of 4 and 11 years. Intoxication and detoxification rates were slower than those expected from laboratory experiments, suggesting that the supply of DA during these phases plays an important role.

Photo: Fernando Sánchez Bernal

WELLNESS OF AQUATIC COMMUNITIES

Inclusive partnerships for forecasting and managing HAB risk in coastal communities

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The most proactive approach to resolving current health and climate crises will require a long view, focused on establishing and fostering partnerships to identify and eliminate root causes of the disconnect between humans and nature. We describe the lessons learned through a unique scientific partnership that addresses a specific crisis, harmful algal blooms (HABs), along the northeast Pacific Ocean coast, that blends current-day technology with observational knowledge of Indigenous communities. This integrative scientific strategy resulted in creative solutions for forecasting and managing HAB risk in the Pacific Northwest as a part of the US Ocean and Human Health (OHH) program. Specific OHH projects focused on: (1) understanding genetic responses of tribal members to toxins in the marine environment, (2) knowledge sharing by elders during youth camps; (3) establishing an early warning program to alert resource managers of HABs are explicit examples of proactive strategies used to address environmental problems. The research and monitoring projects with tribal communities taught the collaborating non-Indigenous scientists the value of reciprocity, highlighting both the benefits from and protection of oceans that promote our well-being. Effective global oceans and human health initiatives require a collective action that gives equal respect to all voices to promote forward thinking solutions for ocean health.

Towards a participatory Science: Collaborative risk assessment for people-centered HABs Early Warning System in the Philippines

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Despite the dire effects of Harmful Algal Blooms (HABs) on several coastal communities in the Philippines, such as food insecurity and livelihood displacement, the affected localities are dominantly seen as passive receivers of HAB knowledge and accompanying top-down actions. We present the insights gleaned from initiating a process of participatory science in the context of a people-centered early warning system for HABs. The paper highlights the participatory HAB risk assessment with three Philippine coastal communities differentially affected by HABs in Jiabong, and Sapian in the Visayas, and Bolinao in northern Luzon. This assessment process was aimed to contribute to broader goals of 1) upholding local people's central role in understanding HAB risks, vulnerabilities, and capacities, 2) creating spaces for people's participation in monitoring and information generation, 3) encouraging fishers to help in educating fellow fishers, and 4) enhancing local people's as well as different agencies'/organizations' capacities to respond to HABs. Through participatory tools and iterative community education process, local people examined the HAB hazard and the progressing vulnerabilities that make algal bloom impacts catastrophic. Local participants also integrated and analyzed these conditions to understand broader HAB risks and outline possible community-led actions. The participatory risk assessment process became an integral part of the emerging people-centered HAB early warning system because of its three-pronged function: as participatory research, as community capacity-building, and as a foundation for community planning. We contend that participatory science in collaboration with the affected communities is imperative in ensuring empowering HAB management responses, and is critical for effective and sustainable monitoring, management and mitigation of HABs.

From Bloom to Bust: The footprint of Economic and Social Turbulences due to Florida Red Tide (*Karenia brevis*)

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The true costs of harmful algae blooms (HABs) and the mechanics that determine their socio-economic impacts are for the most part unknown. HABs have become a widespread environmental, economic, and public health problem along numerous waterfront communities in Florida that are now having to live with massive fish kills and respiratory irritation caused by toxins released by the harmful algae. Paradoxically, as public awareness of ongoing HAB events has increased—notably resulting in a beneficial push for intervention to prevent, mitigate, or eliminate bloom impacts—ultimately, this awareness may also induce changes in consumer behavior (e.g., tourists' choice of destination) that risk the socio-economic wellbeing of the region. Thus, communities must find a balance to raise awareness, preparedness, and response to hazards in a way that benefits their economies. Thus, the purpose of this study is to identify direct, indirect, and induced socio-economic impacts caused by HABs, specifically Florida's Red Tide. Concretely, the study focuses on the 2017 - 2019 Red Tide occurrence, for which a state of emergency was declared in several Florida's counties. A comprehensive research framework was employed with triangulation of quantitative and qualitative data sources. Results reveal two types of congruent impacts: a "material" impact and a "socially constructed" impact. This research provides specific insights on the overall economic and social costs of the red tide blooms and hence the potential benefits of reducing the incidence and severity of these blooms.

SHIOHIGARI and Paralytic Shellfish Toxin: Backgrounds and realities of mitigation measures taken at recreational clam picking parks in Osaka prefecture

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Recreational clam picking called SHIOHIGARI has been enjoyed by citizens over a century in Japan. It is a traditional as well as recreational springtime event. Despite the cultural importance of SHIOHIGARI, it is at risk due to the occurrence of toxic algal blooms. Paralytic shellfish toxin (PST) in bivalves exceeding regulatory limits have been detected from shellfishes taken along Osaka prefecture almost every year since 2002. At clamming parks in Osaka, however, they keep opening parks by purchasing non-toxic clams and exchanging the non-toxic ones from those collected by clammers. This paper: i) investigated backgrounds of development of the exchange system and operational procedures at the clamming parks by examining statistics of commercial clam catch over a century and by conducting semi-structured interviews to major stakeholders, and ii) examined citizens' acceptance of mitigation measures taken at the parks under PST detection by conducting a web questionnaire to 440 residents in Osaka prefecture. Key findings are: i) rapid decrease of the clam catch in Osaka prefecture in late 1950's is one of the backgrounds of the operators purchasing clams from outside to address needs of visitors for recreational clamming by the 1960's, ii) the environment of the parks forces the operators to artificially place clams in the parks every day, and iii) two thirds of the respondents are supportive to the mitigation measures taken at the clamming parks. Recovery of number of visitors to the parks has proved the mitigation measures of PST to be effective.

A ten-year experience co-creating sustainable development in the fishing community "El Manglito" in La Paz, B.C.S., Mexico

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A systems approach framework was used to understand the sociocultural complexity of the community and identify the high leverage intervention actions to move the systems towards sustainability. Social methodologies including creation of shared vision, collective reflection and generative dialogue, social capital creation and collective leadership, were applied. The process has gone throughout different stages: 1) Enabling conditions for the ecological restoration, rebuilding fisheries, community building and sustainable economic growth 2) Change in community behavior towards local resource management and sustainable practices; 3) Construction of legal and administrative structures towards economic sustainability, productive diversification and profitability. Different challenges have been faced in the environmental, social and economic dimensions. The creation of a share vision and the use of system approach untapped the energy and aspirations of the community and created needed understanding to led to important outcomes on how to manage open access resources. New internal and external challenges and obstacles have emerged and need to be addressed in order to create a sustainable and resilient sociocultural fishing community. Finally, the creation of scientific knowledge to support management and cultural transformation towards sustainability is also discussed.

Photo: Fernando Sánchez Bernal

EMERGING ISSUES

Harmful or beneficial algae? How organic matter secreted by plankton and neuston algae, including that in the surface microlayer and in sea foam, may be participating in climate regulation

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Plankton and neuston algae produce organic matter (OM), which tends to accumulate in the sea surface microlayer (SML). Fluxes of heat and momentum exchange across the sea-air interface, as do fluxes of matter, including greenhouse gases, aerosols, algae, bacteria *sensu lato*, viruses and other substances. At least at calm to moderate windspeeds, this microbial OM (MOM) in the SML reduces these fluxes. From the standpoint of climate regulation, however, a bigger influence may be exerted by another MOM fraction, the part in ocean foam. Foam covers from < 1 % to > 6 % of different parts of the ocean as a function of foam coverage. Since foam reflects about half of solar radiation incident upon it, it cools the ocean and the Earth. The rheological properties of MOM and its reduction of sea-air exchanges depend on taxonomic composition, abundance and physiological state of the algae, as do the formation and decay rates of the foam. Genomic and ecophysiological control of MOM secretion possibly can regulate air-sea fluxes, the formation and stability of foam, and climate. Changes in abundance and taxonomic composition of these algae-bacteria-virus microbial communities may be making global climate and more local weather more difficult to predict and regulate. By reducing sea-air exchanges and increasing foam coverage, how much does MOM produced in algal blooms contribute to climate regulation?

Potentially toxic plastic particles: Microplastics as a vector of microcystins

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Water quality is of continuing global environmental concern. A bloom of cyanobacteria is an indicator of poor water quality. These hepatotoxic group of cyanotoxins, called microcystins, is the most common cyanotoxin reported in freshwater bodies. There are over 200 microcystin variants, often detected as a mixture in the environment. Other pollutants, such as microplastics, are also commonly detected in freshwater. There is an emerging concern that microplastics can act as a vector for micropollutants when they co-exist in the same environment. This study evaluated a mixture of eight microcystin (MC) variants (initial concentration 5 $\mu g\ mL^{-1}$ each) mixed with two sizes (average particle sizes 5 - 45 µm and 90 - 140 µm) of polypropylene (PP) and polyethylene terephthalate (PET). Additionally, MC-LR and -LF were also placed individually in contact with the smaller particles of PP or PET to evaluate possible competition between MC variants for the adsorption sites on microplastic particles. The smaller size of PP showed the greatest adsorption potential, adsorbing from 83 % (MC-RR) to 100 % (MC-LW, -LF) of the MC variants after 48 h contact. The larger size of PP and both sizes of PET only showed adsorption with the two most hydrophobic variants, MC-LW and MC-LF. In a mixture, lower amounts of either MC-LR or -LF adsorbed on small PET particles compared to when they were individually placed in the solution. MC-LF and -LW appeared to be absorbed preferentially when competing for binding places on microplastics. The conclusion is that microplastics can act as a vector for microcystins in the aquatic environment, potentially entering the food chain.

First evidence for the implication of palytoxin-like compounds in a Haff disease outbreak

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Haff disease, a rare human intoxication syndrome associated with recent ingestion of fish, is characterized by a life-threatening rhabdomyolysis condition causing severe muscle pain, weakness, and usually dark, tea-colored urine. Although the heat-stable toxin responsible for the intoxication is still unknown, palytoxin (PLTX), which may provoke rhabdomyolysis, appear to be a reasonable candidate. In 2020 and 2021, an outbreak of Haff disease hit the northeastern coast of Brazil, leading at least 50 people to hospitalization and one to death after the consumption of marine fish, mostly *Seriola* sp. Using an opportunistic approach, 18 selected samples of fish and meal remains associated to some of these cases were either extracted in aqueous methanol and screened for the presence of PLTX and its analogs – the ovatoxins (OvTX) – by (+MRM) LC-MS/MS and high-resolution mass spectrometry (HRMS), and/or in acetone for mouse bioassays (MBA) following the protocol for DSP toxins. One sample of cooked fish tested positive in MBA, with the death of three out of four challenged Swiss mice within 12 - 24 h following intraperitoneal injection. MRM transition ions indicative of PLTX, OvTX-a, OvTX-b and OvTX-d were found in seven crude extract samples of cooked fish and one of raw fish. HRMS data showed evidence for the presence of PLTX in selected fish samples, with accurate mass error ranging from 42 to 76 ppm (compared to 29 - 61 ppm in *Ostreopsis* cf. *ovata* extract). Altogether, these findings give support to the hypothesis that palytoxin-like compounds are the most likely toxic agents linked to the incidence of Haff disease in Northeast Brazil.

In situ detection of the phycotoxin domoic acid in bivalve tissues: deciphering the subcellular mechanisms involved in its retention in the king scallop *Pecten maximus*

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Domoic acid (DA), the phycotoxin responsible for amnesic shellfish poisoning (ASP), is an extremely dangerous excitatory amino acid naturally produced by at least twenty-six species of the bloom-forming marine diatoms *Pseudo-nitzschia* spp. Filter feeders, such as bivalve mollusks, can accumulate and lengthy retain high amounts of DA in their tissues, threatening human health and leading to extensive-prolonged fishery and aquaculture closures, with severe economic losses. This is particularly problematic for the king scallop *Pecten maximus*, which is the third most important fishery species in France (75 million euros in 2016). This species retains high burdens of DA from months to years compared to other fast-depurator bivalves. The cellular processes at the origin of this retention are still unknown. Recently, we successfully developed an immunohistochemical technique based on the use of an anti-DA antibody for the in situ specific DA-detection in bivalve tissues at a subcellular level. Our results show that in *P. maximus* contaminated (200 – 800 mg DA kg^{-1} of DG; HPLC-UV) during *Pseudo-nitzschia* spp. outbreaks, DA is visualized in the mucus of gills and digestive tract, in mucus-producing cells as well as and in small inclusion bodies (~1 µm) in the digestive gland. The intensity of the staining appeared directly correlated to the toxin load measured by HPLC. Moreover, we aim to analyze the evolution of DA distribution in scallop tissues during contamination and decontamination event. These results are essential to provide a better understanding of the fate of DA in *P. maximus* and the mechanisms involved in the high accumulation and long retention of this toxin.

Anoxic water masses in small ports and inner bays are the possible incubator of harmful algal blooms

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Stratification in inner bays and small ports often generate anoxic water masses in bottom layer in summer, and nutrients (N and P) are eluted from bottom sediments and accumulated in bottom layer. Phytoflagellates performing diurnal vertical migration conduct photosynthesis at surface during daytime and absorb rich nutrients at bottom layer at night. From this viewpoint, we confirmed a *Chattonella* red tide (max 830 cells mL^{-1} on July 31) at Tajiri Port in Seto Inland Sea by the monitoring from June 22 to July 31, 2017. Dissolved oxygen (DO) was less than 1 mg L^{-1} on July 12 and 31 and benthic diatoms completely disappeared. When benthic diatoms are killed by anoxia and nutrients are accumulated in bottom layer, those waters become an incubator of the harmful algal blooms (HABs) such as *Chattonella*. A 3 m - depth mesocosm with sediment placed at the bottom containing diatom resting stage cells (1.5 x 10^5 g^{-1} wet mud) was installed using the port water of early stage of *Chattonella* red tide at the pier during the period of July 24 to 31, 2017. In the mesocosm, diatoms became dominant (> 2,000 cells mL^{-1}), and *C. antiqua* remained low density (< 23 cells mL^{-1}). DO values at the bottom of mesocosm (minimum 2.0 mg L^{-1}) were higher than that of the external bottom water (minimum 0.6 mg L^{-1}). The ports and inner bay areas with oxygen depletion are considered to easily become an incubator of harmful flagellates leading to HABs. Hence more attentions are needed for water way in small ports as a source of anoxic water inducing occurrences of HABs.

Bioactive compounds from marine phytoplankton (Project BLUEBIOLAB)

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The objectives of the BLUEBIOLAB (Interreg-POCTEP; Spain-Portugal) are the creation of a transboundary laboratory of scientific excellence in marine biotechnology, to reinforce and internationalize the R&D capacities in the territory. For this purpose, several subprojects were launched for the period 2020 - 2022, related with marine biotechnology, aquaculture and biodiversity. The Oceanographic Center of Vigo (IEO, CSIC) participates in one of these initiatives, entitled “Bioactive compounds from marine photosynthetic organisms and biomedical potential”, and led by UMINHO (Braga, Portugal) in consortium with CIIMAR, UdV and IIM - CSIC. Among the objectives of this initiative is the obtaining of microalgae cell extracts to search for bioactive compounds by means of biological activity assays. At present, cultures of 35 species of microalgae belonging to seven classes (from the CCVIEO culture collection at IEO) have been performed: Dinophyceae class (22 species), Bacillariophyceae (4), Cryptophyceae (3), Prasinophyceae (1), Raphidophyceae (1), Euglenophyceae (1), Prymnesiophyceae (1), Dictyochophyceae (1), and Chlorophyceae (1). The cultivation parameters were selected to optimize the growth of each species. Culture biomass harvested for each strain was extracted following a protocol based on H_2O-MeOH and CH_2Cl_2 - MeOH. Final fractions obtained were solubilized in DMSO and kept at -80 ºC until carrying out the corresponding bioassays. In this regard, the antiviral activity is now being evaluated against spring viraemia of carp virus (SVCV), and antibacterial and anti-obesity activities will be subsequently evaluated. The bioassays will be performed using two approaches: *in vitro* cell cultures and *in vivo* assays using zebrafish as animal model. Those extracts showing biological activity will be analyzed by liquid chromatography coupled to mass spectrometry for compound identification.

Photo: Fernando Sánchez Bernal

AUTHORS INDEX BY ABSTRACT

Click on any name in the index to be directed to the corresponding abstract

Adam Antosiak[1], Przemysław Malec[2], Sigitas Šulčius[3], Nada Tokodi[4], Dariusz Dziga[1]

Afiqah Hamilton Hanifah[1], Sing Tung Teng[1], Nursyahida Abdullah[1], Law Ing Kuo[2], Po Teen Lim[2], Chui Pin Leaw[2]

Ahlam S. Al Kharusi[1], Asila K. Al Mawali[1], Yahya Al Waihibi[1], Said Al Shigibi[1], Ahmed Al Alawi[1], Karl B. Andree[1], Jorge Diogène[2], María Rambla[2], Margarita Fernández-Tejedor[2]

Ahn Sophia (So Hyun)[1], Patricia M. Glibert[1] and Cynthia Heil[2]

Aifeng Li[1], Chao Liu[1], Ying Ji[1], Lei Zhang[1], Jiangbing Qiu[1], Zhaohui Wang[2], Lei Liu[2], Yunyun Zhuang[3], Tianying Chen[1], Yang Li[4], Biaobiao Niu[4]

Aimee Valle Pombrol[1], Augusto Comas-González[1], David Castro-Rodríguez[1], Alejandro García-Moya[1], Alan E. Wilson[2], Ángel R. Moreira-González[1]

Aini Hannani Naqiah Abdul Manaff, Kieng Soon Hii, Monaliza Mohd Din, Chui Pin Leaw, Po Teen Lim

Alejandra Llanos-Rivera[1], Javiera De la Paz[1], Nicolás Zambrano[2], Francisco P. Chávez[3], Miguel L. Allende[4], Patricio Yañez[2], Allisson Astuya-Villalón[1], Fernando Cruzat[5], Lorena Delgado[6]

Alejandra Rivera[1], Ambbar Aballay[2], Sandra Jara[2], Juan José Gallardo[3], Jorge Mardones[4], Viviana Ulloa[2], Allison Astuya[2]

Alescia Cullen, Nathan Smith, Sarah Ongley, Brett Neilan

Alexandra Z. Worden

Alexis Escarcega[1], Mary Carmen Ruiz de la Torre[1] and María Luisa Núñez Reséndiz[1]

Alexis Fischer[1], Donald Anderson[2], David Kulis[2], Michael Brosnahan[2]

Alisa Rudnitskaya[1], Catarina Moreirinha[1], Maria João Botelho[2], Marta Fraga[3], Ana Amorim[4]

Alison Turnbull[1], Andreas Seger[1], Navreet Malhi[2], Tim Harwood[3], Graeme Knowles[4], Quinn Fitzgibbon[1], Gustaaf Hallegraeff[1]

Allan Santos[1], Dayvson Guedes[2], Mário Barros[3], Samylla Oliveira[2], Ana Beatriz Pacheco[1], Valéria Magalhães[1], Sandra Azevedo[1], Carlos Pestana[4], Christine Edwards[4], Linda Lawton[4], José Capelo[2]

Allison Moreno[1], Clarissa Anderson[2], Raphael Kudela[3], Faycal Kessouri[4], Martha Sutula[4], Jayme Smith[4], Daniele Bianchi[1]

Allisson Astuya Villalón[1], Bárbara López[1], Verónica Avello[1], Juan José Gallardo-Rodriguez[2], Alejandra Aguilera-Belmonte[3]

Amanda Goulart, Magalhães, Vinícius, Nascimento, Silvia, Salgueiro, Fabiano

Amandine Caruana[1], Solène Geffroy[1], Marc-Marie Lechat[1], Mickaël Le Gac[1], Georges-Augustin Rovillon[1], Dominique Marie[2], Estelle Bigeard[2], Florent Malo[1], Zouher Amzil[1], Laure Guillou[2]

Ana Emilia Ramos Santiago[1], Christine J. Band-Schmidt[2], Ignacio Leyva-Valencia[3], Ivonne Santiago-Morales[1], Mauricio Muñoz-Ochoa[2], Yuri B. Okolodkov[4]

Ana Flores-Leñero[1], Javier Paredes-Mella[1], Valentina Vargas[1], Maximiliano Vergara[1], Luis Norambuena[1], Gonzalo Fuenzalida[1], Kim Lee Chang[2], Jorge I. Mardones[1,3]

Ana Martínez, Leonardo Ortega and Silvia Méndez

Andreana Cadaillon[1], Irene R. Schloss[2], Gastón O. Almandoz[3], Marcelo Hernando[4], Leonardo Saravia[5]

Andreas Seger[1], Shauna Murray[2], Hazel Farrell[3], Russell Stafford[4], Richard Lewis[5], Gustaaf Hallegraeff [1], Natalie Dowsett[6], Alison Turnbull[1]

Andrés Ávila[1], Mariela González-Flores[1], Milko Jorquera[1], Joaquín Rilling[1], Carlos Riquelme[2], Henry Cameron[2], Leonardo Guzmán[3], Gonzalo Gajardo[4], Fumito Maruyama[5], Satoshi Nagai[6], Shoko Ueki[7]

Andrew Turner[1], Karl Dean[1], Adam Lewis[1], Zoe Jenkins[2], Beth Bear[1], Frederico Batista[1], David Ryder[1], Ben Maskrey[1], Amy Later[1], Cathy House[1], Nevena Almeida[1], Rob van Ree[1], Cheryl Crisp[1], Kerra Langley[2], Loveday Lewin[2], Robert Hatfield[1]

Ángeles Moroño[1], Juan Blanco[2], Fabiola Arévalo[1], Jorge Correa[1], Covadonga Salgado[1], Araceli E. Rossignoli[2].

Angéline Lefran[1], Anouska Panton[2], Duncan Purdie[2], Tania Hernández Fariñas[1]

Angel Ramón Moreira González[1], Luciano F. Fernandes[2], Hajime Uchida[3], Aya Uesugi[3], Toshiyuki Suzuki[3], Nicolas Chomérat[4], Gwenaël Bilien[4], Thiago Alves Pereira[5], Luiz L. Mafra Jr.[6]

Anna Anschütz[1] and Kevin John Flynn[2]

Anna Junker Olesen[1], Fredrik Ryderheim[2], Bernd Krock[3], Thomas Kiørboe[2], Nina Lundholm[1]

Anna McGaraghan, Kendra Negrey and Raphael Kudela

Anne-Sophie Pavaux, David Velasquez, Kevin Drouet, Anais Lebrun, Alan Hiroux, Sophie Marro, Rodolphe Lemée, Elisabeth Christians, Stefania Castagnetti

Ann Marie Famularo, Jennifer Jankowiak and Christopher Gobler*

Antonella Penna, Antonella Penna, Laura Pugliese, Nadia Marinchel, Francesca Andreoni, Silvia Casabianca

Anusuya Willis[1], Michele Burford[2], Aaron Jex[3], Brett Neilan[4], K. Sivonen[5], D. Fewer[5], H-P Grossart[6], S. Tichkule[3], Jason Woodhouse[6]

Araceli E. Rossignoli and Juan Blanco Pérez

Aramis Olivos-Ortiz[1], María Fernanda Cobarrubias-Chávez[1] and Erick Julián Núñez-Vázquez[2]

Araoz Romulo[1], Nathalie Hue[2], Philipp Hess [3], Denis Servent[4]

Ariel Kaminski, Kucała Małgorzata and Saładyga Michał

Arjun Verma[1], Mona Hoppenrath[2], Kirsty Smith[3], Juan José Aranda Dorantes[4], Clinton Wilkinson[5], Gustaaf Hallegraeff[4], Shauna Murray[1]

Armando Mendoza, Clara Elizabeth Galindo-Sánchez and Sánchez-Saavedra, M. del Pilar

Arturo Picones[1], Rosalba Alonso-Rodríguez[2], Lourdes Morquecho[3], Mauricio Matus-Núñez[1], Oliver C. Lara-Figueroa[1]

Asilah Al Has[1], N. Mohammad-Noor[1], Sitti Raehanah M. Shaleh[2], Shuhadah Mustapha[3], Deny Susanti Darnis[1], Mohd Nor Azman Ayub[4], Aimimuliani Adam[1], Ghaffur Rahim Mustakim[2]

Aubrey Trapp, Kendra Negrey and Raphael Kudela

Audrey Roy Lachepelle Morgan Solliec[1], Christian Gagnon[2] and Sebastien Sauve[3]

Aurelie Boisnoir[1], Vandersea Mark[2], Sibat Manoëlla[1], Lhaute Korian[1], Bilien Gwenael[1], Allenou Jean-Pierre[1], Philipp Hess[1], Chomérat Nicolas[1]

Aurélien Bouquet[1], Mohamed Laabir[2], Nicolas Chomérat[1], Jean Luc Rolland[1], Christelle Reynes[2], Robert Sabatier[2], Tom Berteau[1], Claude Chiantella[1], Eric Abadie[1]

Begoña Ben-Gigirey[1], Edgar Arteaga-Sogamoso[2], José Ernesto Mancera-Pineda[3], Francisco Rodríguez[1]

Bengt Karlson[1], Agneta Andersson[2], Anders Andersson[3], Sonia Brugel[2], Meike Latz[3], Markus Lindh[4], Jenny Lycken[5]

Benitt Colin, Christopher Gobler and Craig Young

Benjamín A. Suárez-Isla, Fernanda Barrera, Juliana Calbuyahue, Daniel Carrasco, Américo E. López-Rivera, Ignacio Rubilar, Carolina Tamayo.

Benjamin Kramer and Christopher J. Gobler

Bente Edvardsen[1], Luka Šupraha[1], Sandra Gran-Stadniczenko[1], Carina Bunse[2], Wenche Eikrem[3], Jan Janouskovec[1], Kerstin Klemm[4], Lars-Johan Naustvoll[5], Helene Reitzel[1], Daniela Voss[6], Sylke Wohlrab[4], Uwe John[4]

Bernd Krock[1], Urban Tillmann[1], Luisa Hintze[1], Kristof Möller[1], Haifeng Gu[2], Satoshi Nagai[3], Kimberly Reece[4], Aaron J. C. Andersen[5], Thomas M. Harris[6]

Boo Seong Jeon and Park Myung Gil

Bora Lee and Park Myung Gil

Bruna Sobrinho[1], Patricia M. Glibert[1], Cynthia A. Heil[2], Ming Li[1]

Bryan John Subong[1], Takeaki Ozawa[1], Rhodora Azanza[2], Arturo Lluisma[2], Lilibeth Salvador-Reyes[2]

Cagle Sierra and Daniel Roelke

Caitlin Romanis, Huiming Zhang, Daniel Nebauer, Brett Neilan.

Callum Whyte, Paola Acre, Dimitry Aleynik, Keith Davidson, Steve Gontarek

Camilla Capelli and Lepori Fabio

Carlos Antonio Poot Delgado[1], Yuri B. Okolodkov[2], Erick J. Núñez-Vázquez[3], Fausto Tafoya del Angel[4], Jaime Rendón-von Osten[5]

Carlos Eduardo J.A. Tibiriçá[1], Damien Réveillon[2], Luiz L. Mafra Jr.[1], Gwenael Bilien[3], Véronique Séchet[2], António Pinto-Almeida[4], A.R. Moreira-González[5], Philipp Hess[2], Nicolas Chomérat[3]

Carlos Rodríguez[1], Papiol Vanesa[1], Mariño-Tapia Ismael[1], Vázquez Gabriela[2], Enríquez-Ortiz Cecilia[1]

Carmen Osorio[1], Allan D. Cembella[2], José Correa-Basurto[3], Lorena María Durán-Riveroll[4]

Caroline Weber[1], Anna Karoline Junker Olesen[2], Bernd Krock[3], Nina Lundholm[2]

Cecilia Teodora Satta[1], Antonella Lugliè[1], Mario Padedda Bachisio[1], Silvia Pulina[1], Monica Cangini[2], Sonia Dall'Ara[2], Stefania Milandri[2]

Cecilio Valadez-Cano[1], Adrian Reyes-Prieto[1], Cheryl Rafuse[2], Daniel Beach[2], Pearse McCarron[2], Janice Lawrence[1]

Cesiah Rebeca Quintanilla García, Oscar Amaya-Monterrosa and Jeniffer Guerra

Chariane Camila Werlang, Márcio Silva de Souza, Luiza Dy Fonseca Costa, João Sarkis Yunes

Charles Tilney and Katherine Hubbard

Cheryl Greengrove[1], Julie Masura[1], Steve Kibler[2], Julie Matweyou[3], Courtney Hart[3]

Christine Edwards[1], Turner, Andrew, D.[2], Lawton, Linda, A.[1], Pestana, Carlos, J.[1], Lewis, Adam[2]

Christopher Bolch[1], Shikder Saiful Islam[1,2], Shanaia Munidasa[1], Gustaaf Hallegraeff[3], Tim Harwood[4], Sam Murray[4], Arjun Verma[5], Shauna A. Murray[5]

Christopher Loeffler, A. Spielmeyer, D. Bodi, K. Kapp, V. Blaschke, O. Kappenstein

Chris Whitehead

Claudia Wiegand[1], Alexandrine Pannard[1], Morgane Le Moal[1], Gorenka Bojadzija-Savic[1], Gerard Gruau[2], Remi Dupas[3], Philippe Legoffe[4], Patrick Latouche[5]

Clay Bennett and Alison Robertson

Cong Fei and Shady A. Amin

Cristina Nordi[1], Cristiane Silva[1], Marcelo Duque[1], Cristina Niero[2]

Cynthia Heil[1], Shady Amin[2], Patricia Glibert[3], Katherine Hubbard[4], Ming Li[3], Joaquin Martínez

Martínez[5], Robert Weisberg[6]

Dania Casanova[1], Lorena Durán[2], Mary Carmen Ruiz Mary[3], Allan Cembella[4]

Daniela Souza, Tatiane Benevides, Lidiane Almeida, Davi Barreto, Mariângela Menezes

Daniel Beach[1], Janice Lawrence[2], Meghann Bruce[3], Jacob Stillwell[2], Cheryl Rafuse[4], Pearse McCarron[4]

Dani Glidewell and K. David Hambright

Dany Domínguez-Pérez[1], José Carlos Martins[1], Daniela Almeida[1,2], Pedro Reis Costa[3], Vitor Vasconcelos [1,4], Alexandre Campos[1]

Dave Clarke, Tara Chamberlain, and Patricia Neira

David U. Hernández-Becerril[1], Maciel-Baltazar, E.[2], Barón-Campis, S. A.[3], Esqueda-Lara, K.[4], Alonso-Rodríguez, R.[1], Morquecho-Escamilla, L.[5], Pichardo-Velarde, J. G.[6]

Deborah Knapp[1], Bieito Fernàndez-Castro[2], Daniel Marty[1], Eugen Loher[1], Oliver Köster[3], Alfred Wüest[4], Thomas Posch[1]

Dedmer van de Waal

Deepak Nanjappa[1], Sonya Dyhrman[2], Matthew Harke[2], Christopher Gobler[1]

Delfina Aguiar Juárez[1], Ricardo I. Silva[2], Eugenia A. Sar[3], Inés Sunesen[3]

Denis Servent[1], Carole Malgorn[1], Mylène Bernes[1], Sophie Gil[2], Christelle Simasotchi[2], Anne-Sophie Hérard[3], Thierry Delzescaux[3], Robert Thai[1], Peggy Barbe[1], Mathilde Keck[1], Fabrice Beau[1], Armen Zakarian[4], Vincent Dive[1], Jordi Molgó[1]

Diana S. Moura[1], Carlos J. Pestana[1], Colin Moffat[1], Jianing Hui[2], John T. S. Irvine[2], Christine Edwards[1], Linda A. Lawton[1]

Diner Bernal[1,2*], Iván Pérez-Santos[1,3,4], Gonzalo Álvarez[5,6], Beatriz Reguera[7], Loreto Lopez[8], Alondra Sandoval[1], Camila Schweter[1], Claudia Aracena[9], Manuel Díaz[10], Michael Araya[6], Francisco Álvarez[5], Patricio A. Díaz[1,11]

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Eleni Keliri[1], Paraskeva Christia[1], Angelos Sofokleous[1], Assaf Sukenik[2], Dariuzs Dziga[3], Ekaterina Chernova[4], Luc Brient[5], Maria G. Antoniou[1]

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