

AUVs for Ecological Studies Of Marine Plankton Communities

Intelligent Algorithms on Dorado and Tethys AUVs Enable Precise Water Sampling for Plankton Research

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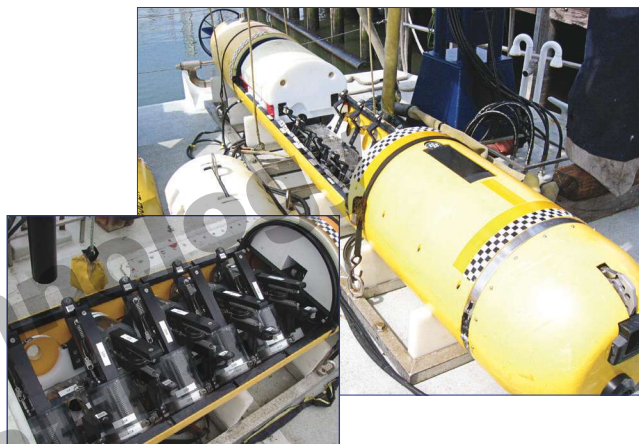
Marine plankton are central to processes sustaining life on Earth. For example, as primary producers, they regulate atmospheric gases, and phyto- and zooplankton, which occupy the lowest trophic levels, fuel marine food webs, supporting larger organisms, including economically important species for fisheries around the world.

Coastal plankton communities exhibit widespread spatial and temporal variation in abundance, diversity and behavior. These variations are often in response to similarly variable shifts in local environmental processes, both physical (e.g., wind-driven circulation and mixing) and chemical (e.g., nutrient influxes from upwelling, riverine or other terrestrial outflow sources).

Traditional methods, like tow-netting, are invaluable for documenting overall plankton community diversity and continue to provide comprehensive data sets. These efforts, however, have also revealed that advancing plankton ecology studies requires precise methods to sample spatially and temporally variable plankton communities.

High-precision sampling methods have made it possible to identify invertebrate larvae transported in sediment plumes, phytoplankton concentrated into thin layers and zooplankton collected against steep density gradients generated by upwelling fronts. These accomplishments address the paramount ecological issues of population connectivity and effective planning of marine protected areas, harmful algal bloom development and monitoring, and upwelling mediated production and aggregation of zooplankton, including local invertebrate larval retention and supply.

Because a single method of observation cannot comprehensively describe the complex and dynamic interplay between coastal oceanic processes and biological outcomes, multiscale, multidisciplinary experiments are necessary. Such experiments simultaneously deploy multiple ocean-



(Right) The Monterey Bay Aquarium Research Institute's AUV Dorado, with Gulper midsection cover removed.

(Left) The Gulper water sampling mechanisms.

observing assets, including moorings, ships, drifters, gliders, AUVs, autonomous in-situ water sample analyzers and remote sensing instrumentation. The resulting data sets enable insights into complex ecological relationships.

Dorado AUV

The Monterey Bay Aquarium Research Institute's (MBARI) Dorado AUV combines synoptic environmental data acquisition, high-intake-rate water sampling and adaptive decision-making software to collect plankton at fine spatial and temporal scales. The Dorado is outfitted with a suite of sensors measuring temperature, salinity, nitrate, oxygen, chlorophyll fluorescence, optical backscattering, bioluminescence and particle-size distribution, as well as 10 Gulper water samplers. The sensors' measurements reveal a synoptic view of water-column properties along the vehicle's sawtooth-shaped flight path and are precisely associated with each of the 10 water samples collected during an AUV mission.

When triggered to fire by the AUV's decision-making software, each spring-loaded sampler collects 1.8 liters of seawater in less than two seconds through ports in the hull of the vehicle and stores these samples for analysis upon return to shore. The rapid sample intake is designed to break through the boundary layer formed by passage of the ve-

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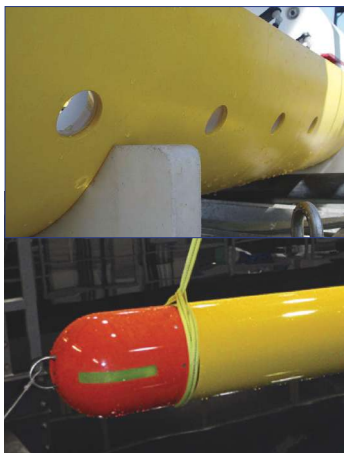
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(Top) Water sampling ports in the Dorado AUV's hull.

(Bottom) The long-range AUV Tethys.

vehicle through water and to overcome avoidance behavior exhibited by some target organisms, such as copepods, which feed on phytoplankton.

Feature Detection and Sampling

Originally, the Dorado was tasked with collecting water samples at pre-programmed geographic locations, or waypoints. While this method was useful for interpreting relationships between water sample biology and associated environmental conditions, sampling was essentially random with regard to environmental conditions immediately surrounding the AUV.

Comparative analysis of the collected biological and associated environmental data enabled the definition of requirements for subsequent software development, resulting in algorithms that allow the vehicle to interpret environmental data independently in real time and use that information to make decisions about where and when to collect water samples. In a similar fashion, the AUV is also now capable of identifying features of biological interest, and traversing and sampling them with precision.

Intermediate Nepheloid Layers

Intermediate nepheloid layers (INLs) are episodic sediment transport events mediated by bottom boundary layer dynamics. They are thought to play a role in benthic invertebrate larval transport. Multiple algorithms have been successfully developed and applied to identify and sample INLs with the Dorado.

Information from the AUV's sensor suite is used to differentiate INL signatures from other signals present in the surrounding water column in order to sample them selectively. For example, aggregations of phytoplankton produce

particle backscatter similar to INLs but also return high values for chlorophyll fluorescence. Conversely, sediment-derived backscatter from INLs coincides with low chlorophyll signals. Molecular analysis of INL water samples from missions conducted in January

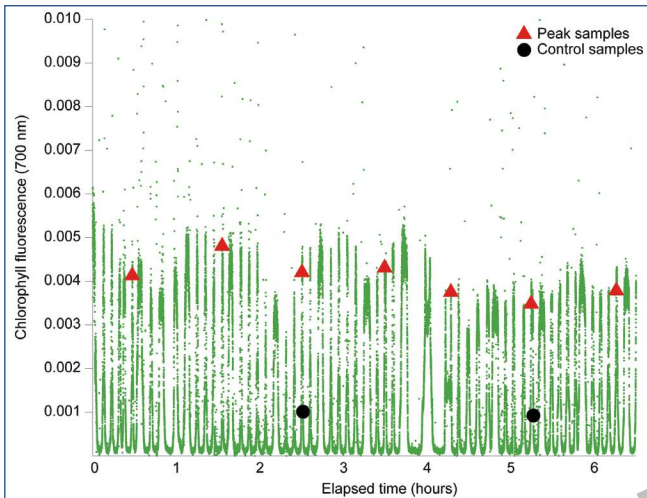
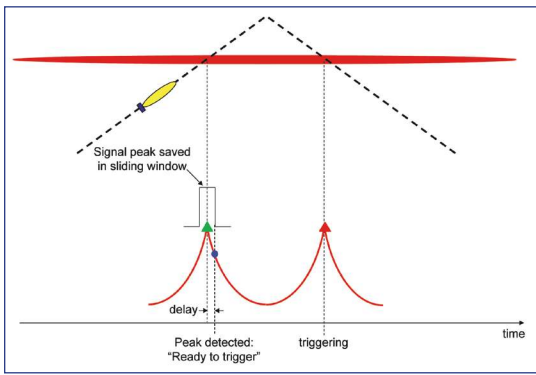
and November 2008 in Monterey Bay, California, demonstrated the presence of invertebrate larvae (i.e., polychaete worms, barnacles and mussels) in these features. Such ecological data can help inform studies of population connectivity, relevant to planning and managing marine protected areas.

Thin Phytoplankton Layers

In addition to forming large-scale blooms in response to processes such as wind-forced upwelling of nutrients, phytoplankton can aggregate into layers ranging in thickness from fractions of a meter to several meters. Precisely sampling these layers, which are detectable by high-chlorophyll signal, is now possible with the Dorado, thanks to the development of an algorithm that can adaptively identify and capture chlorophyll peaks.

Thin phytoplankton-rich layers are difficult to sample with a moving AUV because a delay in chlorophyll peak detection (unavoidable by any real-time peak detection algorithm) of even a few seconds will result in water-sample collection occurring past the physical chlorophyll peak target. To solve this problem, an AUV peak-capture algorithm learns from environmental data in real time. Within each vertical profile, the vehicle registers the maximum chlorophyll signal on its first pass through a thin layer. On its second pass, the AUV triggers a Gulper as soon as the measured chlorophyll reaches the chlorophyll peak signal recorded on the first pass, thus accurately acquiring a peak-chlorophyll water sample without delay.

This approach has enabled high spatial- and temporal-resolution studies of phytoplankton bloom dynamics and ecology through the consistent sampling of chlorophyll peak maxima.



(Top) A graphical depiction of the AUV Dorado chlorophyll peak capture algorithm.

(Bottom) The AUV Dorado ran the chlorophyll peak capture algorithm in a thin-phytoplankton-layer survey in October 2010. Seven Gulpers were triggered at chlorophyll fluorescence peaks (i.e., red triangles), and two Gulpers were triggered at lower-than-average fluorescence (i.e., black circles).

Upwelling Fronts

Wind-driven Ekman transport of coastal surface layers results in upwelling, bringing colder, nutrient-rich, vertically

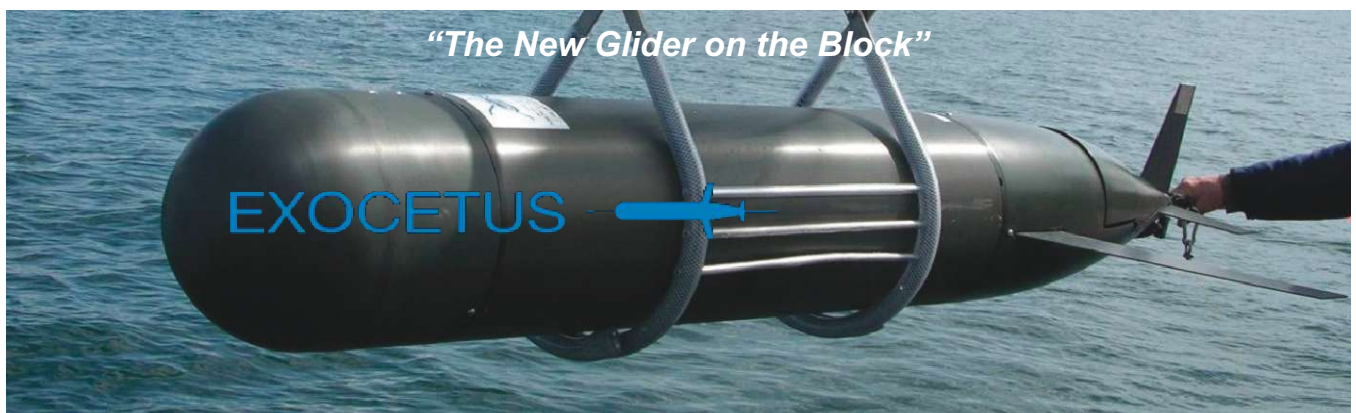
homogenized waters into contact with warmer, less saline, stratified waters. The steep density gradients that commonly form at the interface of upwelled and stratified waters, called the upwelling front, are thought to concentrate and transport a variety of plankton. Upwelling fronts are postulated to play a role in local retention of larvae from broadcast spawning organisms and have been correlated with recruitment periods for ecologically and economically important fish and invertebrate species.

From March to June 2009, the Dorado collected water samples from upwelling-stratified water interfaces while sampling at preprogrammed waypoints in Monterey Bay. Biological results from these upwelling front samples included elevated molecular signals for calanoid copepods and provided impetus for the development of upwelling front detection and sampling software for the AUV. As a result, the Dorado can now autonomously identify and sample three water types within upwelling environments: newly upwelled water in the core of an upwelling filament, the upwelling front and the stratified water beyond the upwelling front. Precise sampling of the anatomy of upwelling frontal environments is enabling examination of the complex relationships between environmental processes and plankton diversity and abundance.

Robotic Cooperation

Using multiple intelligent AUVs can enhance the precision of observational targeting. Designed for long-endurance missions, the Tethys AUV has led cooperative multi-AUV operations that include the Dorado. The Tethys' persistence and algorithms permit localization and tracking of targeted phenomena within a complex and rapidly changing environment.

Following the Tethys' lead, the Dorado can direct more extensive sensing and sampling capabilities to the localized target. This robotic teamwork has been applied to track biological patches containing toxic phytoplankton populations, as well physical and biological processes in fronts, e.g., phytoplankton bloom tracking and sampling conducted in October 2010, and upwelling front tracking and sampling in June 2011, both conducted in Monterey Bay.



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Water-Sample Processing

In addition to direct enumeration and morphological examination of organisms in Gulper water samples with light microscopy, a variety of molecular methods are used to identify the taxonomy and relative abundance of phytoplankton, zooplankton and bacterioplankton. AUV samples are also commonly analyzed for nutrients (e.g., phosphate, silicate, nitrate), chlorophyll or other chemistries in shore-based laboratories for comparison with data collected by AUV and additional data.

Organisms from multiple trophic levels present in individual water samples can be size fractionated and collected by serial filtration with decreasing porosity. Filters are then frozen or chemically preserved for subsequent molecular analysis. For example, comparatively large zooplankton (larger than 100 micrometers) can be separated from toxin-producing algal-bloom forming species of phytoplankton (0.65 to 100 micrometers) or marine bacteria (0.22 to 5 micrometers) present in a single sample.

Subsequent molecular analysis of filter-collected organisms involves chemical lysis, followed by various methods at the researcher's discretion. Molecular probe-based techniques, such as the sandwich hybridization assay, assess the presence and relative abundance of target RNA from organisms of interest, while reverse transcriptase amplification of RNA transcripts, followed by quantitative polymerase chain reaction of bacterial functional genes, provides information regarding the regulation of ocean biochemical cycling.

Several methods are frequently used together. A sandwich hybridization assay can be used to assess harmful algal bloom species abundance and diversity, while replicate filters and liquid filtrate can quantify harmful algal bloom toxicity by high-performance liquid chromatography analysis of toxin concentrations. Zooplankton collected in these samples can also be analyzed to quantify the transfer of toxin to the next higher trophic level, which has detrimental consequences for humans and larger marine life.

Conclusions

Among myriad technologies available in the rapidly growing field of ocean-observing networks, water sampling AUVs equipped with adaptive decision-making software based on

intelligent processing of environmental signals offer novel opportunities to investigate plankton ecology. Recent signal-processing and software-engineering developments for AUVs have taken these already versatile platforms to a new level of autonomous, adaptive sampling of marine ecological niches, such as thin phytoplankton layers and upwelling fronts. These phenomena are often highly spatially and temporally variable, precluding precise sampling by traditional means and driving requirements for present and future engineering efforts. Thanks to AUV innovations, monumental advances in the study of plankton ecology are underway.

Acknowledgments

The David and Lucile Packard Foundation and MBARI provided funding and facilities for this research. The authors extend sincere thanks to their collaborators at MBARI and elsewhere.

References

For a list of references, contact Julio Harvey at jharvey@mbari.org. ■

After receiving his Ph.D. at the University of California, Santa Cruz, in 2004, Dr. Julio Harvey worked with the University of Washington to develop molecular methods to detect marine invasive species. Since 2008, his work at the Monterey Bay Aquarium Research Institute has integrated molecular genetic detection with robot-mediated adaptive sampling.

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Dr. John Ryan received a Ph.D. in biological oceanography from the University of Rhode Island in 1998. He is a senior research specialist at the Monterey Bay Aquarium Research Institute, focusing on studies of coastal ocean processes using observational and modeling approaches.

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