

Autonomous Tracking and Sampling of the Deep Chlorophyll Maximum Layer in an Open-Ocean Eddy by a Long-Range Autonomous Underwater Vehicle

Yanwu Zhang ¹, Senior Member, IEEE, Brian Kieft, Brett W. Hobson, John P. Ryan, Benedetto Barone, Christina M. Preston, Brent Roman, Ben-Yair Raanan, Roman Marin III, Thomas C. O'Reilly, Carlos A. Rueda, Douglas Pargett, Kevan M. Yamahara, Steve Poulos, Senior Member, IEEE, Anna Romano, Gabe Foreman, Hans Ramm, Samuel T. Wilson, Edward F. DeLong, David M. Karl ², James M. Birch, James G. Bellingham, and Christopher A. Scholin

Abstract—Phytoplankton communities residing in the open ocean, the largest habitat on Earth, play a key role in global primary production. Through their influence on nutrient supply to the euphotic zone, open-ocean eddies impact the magnitude of primary production and its spatial and temporal distributions. It is important to gain a deeper understanding of the microbial ecology of marine ecosystems under the influence of eddy physics with the aid of advanced technologies. In March and April 2018, we deployed autonomous underwater and surface vehicles in a cyclonic eddy in the North Pacific Subtropical Gyre to investigate the variability of the microbial community in the deep chlorophyll maximum (DCM) layer. One long-range autonomous underwater vehicle (LRAUV) carrying a third-generation Environmental Sample Processor (3G-ESP) autonomously tracked and sampled the DCM layer for four days without surfacing. The sampling LRAUV's vertical position in the DCM layer was maintained by locking onto the isotherm corresponding to the chlorophyll peak. The vehicle ran on tight circles while drifting with the eddy current. This mode of operation enabled a quasi-Lagrangian time series focused on sampling the temporal variation of the DCM population. A companion LRAUV surveyed a cylindrical volume around the sampling LRAUV to monitor

spatial and temporal variation in contextual water column properties. The simultaneous sampling and mapping enabled observation of DCM microbial community in its natural frame of reference.

Index Terms—Autonomous underwater vehicle (AUV), eddy, Environmental Sample Processor (ESP), phytoplankton, sampling, tracking.

I. INTRODUCTION

OCEANIC life depends upon photosynthetic production of organic matter by microscopic organisms. Photosynthesis requires light and nutrients, and in the open ocean it is limited by low concentrations of nutrients in shallow water that receives the most sunlight. At the base of the nutrient impoverished surface layer (~100-m depth), nutrient concentrations increase across the strong density gradient of the pycnocline. This creates a vertically limited layer in which photosynthetic microbes can access both nutrients from below and light energy from above. With its locally enhanced concentration of the photosynthetic pigment chlorophyll, this layer is referred to as the deep chlorophyll maximum (DCM) [1], [2]. The DCM is a ubiquitous feature of open-ocean stratified ecosystems. Physical processes that alter the vertical distributions of nutrients and DCM microbes shape the functioning of open-ocean ecosystems and global biogeochemical cycles [3].

Among the physical processes influencing the DCM are eddies, vortical circulations that affect vertical transport. Global analyses of eddies using satellite altimeter data [4], [5] show that eddies in our study region, north of the Hawaiian Islands (see Fig. 1), are responsible for approximately half of the variance in sea level anomaly (SLA). These eddies have a mean radius scale of ~100 km and a mean westward zonal propagation speed of ~5 cm/s. Eddy circulation can be cyclonic or anticyclonic (counterclockwise and clockwise in the northern hemisphere, respectively). In the context of this study, cyclonic eddies are of particular importance because of the consequences of their circulation, including upward transport of nutrients and DCM populations, which enhances both nutrient and light resources for photosynthesis and thus productivity and biomass, and changes in species composition and export of organic matter to the deep sea [6], [7]. Furthermore, the interacting eddy field

Manuscript received December 20, 2018; revised May 16, 2019; accepted May 28, 2019. Date of publication July 22, 2019; date of current version October 13, 2020. This work was supported in part by the National Science Foundation (OCE-0962032 and OCE-1337601), in part by the Gordon and Betty Moore Foundation (Grant #3777 to E. F. DeLong; Grant #3794 to D. M. Karl; Grant #2728 to C. A. Scholin), in part by the David and Lucile Packard Foundation, and in part by the Schmidt Ocean Institute for *R/V Falkor* Cruise FK180310. The work of E. F. DeLong and D. M. Karl was supported in part by the Simons Foundation Grant #329108. (Corresponding author: Yanwu Zhang.)

Associate Editor: R. Bachmayer.

Y. Zhang, B. Kieft, B. W. Hobson, J. P. Ryan, C. M. Preston, B. Roman, B.-Y. Raanan, R. Marin III, T. C. O'Reilly, C. A. Rueda, D. Pargett, K. M. Yamahara, J. M. Birch, and C. A. Scholin are with the Monterey Bay Aquarium Research Institute, Moss Landing, CA 95039 USA (e-mail: yzhang@mbari.org; bkieft@mbari.org; hobson@mbari.org; ryjo@mbari.org; preston@mbari.org; bren@mbari.org; byraanan@mbari.org; maro@mbari.org; oreilly@mbari.org; carueda@mbari.org; pargett@mbari.org; kyamahara@mbari.org; jbirch@mbari.org; scholin@mbari.org).

B. Barone, S. Poulos, A. Romano, G. Foreman, H. Ramm, S. T. Wilson, E. F. DeLong, and D. M. Karl are with the University of Hawaii at Manoa, Honolulu, HI 96822 USA (e-mail: benedetto.barone@gmail.com; poulos@soest.hawaii.edu; ritchiea@hawaii.edu; gabe@hawaii.edu; hramm@hawaii.edu; stwilson@hawaii.edu; edelong@hawaii.edu; dkarl@hawaii.edu).

J. G. Bellingham is with the Woods Hole Oceanographic Institution, Woods Hole, MA 02543 USA (e-mail: jbellingham@whoi.edu).

Digital Object Identifier 10.1109/JOE.2019.2920217

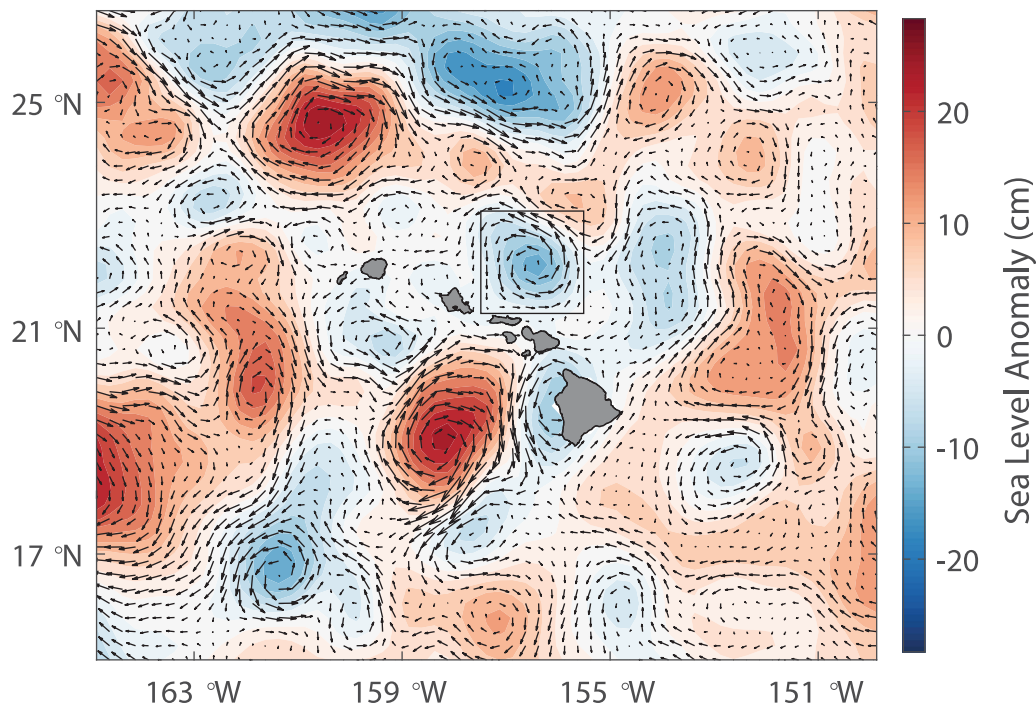


Fig. 1. SLA and geostrophic current velocity around the Hawaiian Islands on March 28, 2018. SLA is negative in cyclonic (counterclockwise) eddies, and positive in anticyclonic (clockwise) eddies. Range of current speeds: 0 ~ 0.72 m/s. On the day represented by this map, a four-day quasi-Lagrangian study was initiated within the cyclonic eddy (marked by the box) located immediately north of the central islands. Data source: Copernicus Marine Environment Monitoring Service (CMEMS).

(see Fig. 1) can create enhanced stirring and vertical circulations within frontal zones, which influence productivity and export of carbon from the surface mixed layer [8].

Studies of how eddies influence open-ocean microbial populations have largely relied on ship-based sampling strategies. While this approach permits synoptic descriptions of eddies and microbial populations, it cannot provide effective sampling of DCM microbial populations in their natural frame of reference, which is moving with ocean currents. Previously developed Lagrangian platforms were used to measure volume transport in the Gulf Stream [9] and Drake Passage [10], track water parcel motion in the convecting layer of the Labrador Sea [11]–[13], and reveal mesoscale dynamics that influence the North Atlantic spring bloom [14]. These passive Lagrangian platforms were not designed to possess mobility for finding an oceanographic feature. There is a growing effort toward enabling AUVs to autonomously detect and track a variety of ocean features, such as the thermocline [15]–[18], internal waves [19], [20], various plumes [21]–[25], intermediate nepheloid layers [26], phytoplankton patches [27], [28], and coastal upwelling fronts [29], [30]. In [31], an AUV demonstrated the ability to perform a Lagrangian-box survey around a drifter. Some AUVs are now equipped with water samplers to take advantage of the vehicle's mobility to collect material while underway [26], [32]–[36].

This study integrates multiple autonomous systems, including surface and underwater vehicles, and a robotic molecular analytical instrument installed in one underwater vehicle, to study DCM microbial ecology in its natural frame of reference on time scales from hours to days, thereby permitting resolution of time-dependent evolution of the microbial population in response to environmental variations. The design of the March–April 2018

SCOPE (Simons Collaboration on Ocean Processes and Ecology) Hawaiian Eddy Experiment is illustrated in Fig. 2, and details are given in Section III.

A drifting second-generation Environmental Sample Processor (2G-ESP, a robotic sample acquisition and analysis system [36], [37]) has been deployed to study microbial ecology off the northern California coast [38] and in the North Pacific Subtropical Gyre [39], [40]. The 2G-ESP was suspended at a fixed depth (23 m) beneath a free-drifting surface float, and took water samples every 2 or 4 h. This drifting ESP was intended for quasi-Lagrangian sampling, but windage from the large surface buoy, and the fixed depth of all samples made it difficult to stay in the areas of greatest biological activity within the water column. In the study presented in this paper, our goal was to accurately follow and observe a plankton community over multiple diel cycles in the DCM layer in a cyclonic eddy. The DCM layer is not only deep, but also undulates in depth due to internal tides and inertial oscillations. Hence, a 2G-ESP suspended at a fixed depth from a surface float cannot accomplish the task. A *Tethys*-class LRAUV equipped with a 3G-ESP and targeted sampling intelligence enables precise and persistent occupancy of the DCM layer.

LRAUV *Aku* containing a 3G-ESP (deployed in the 2018 Hawaiian Eddy Experiment) is shown in Fig. 3. The vehicle is 3.2 m long and 0.3 m in diameter at the midsection. A *Tethys*-class LRAUV can run from 0.5 to 1 m/s using a propeller. Using a primary battery, the vehicle has demonstrated a range of 1800 km (three-week duration) at 1-m/s speed [41]. Long range is realized by minimizing propulsion power consumption through an innovative design of a low-drag body and a high-efficiency propulsion system [42]. In addition, by using a

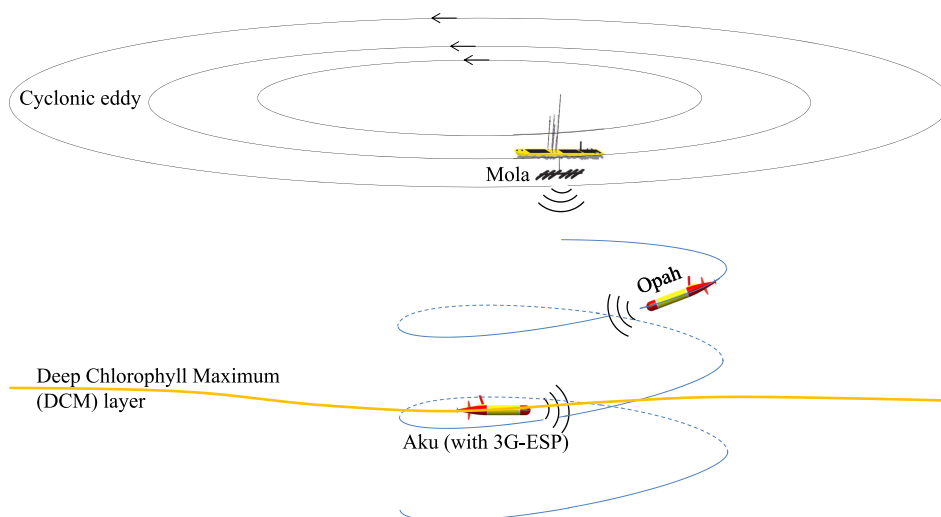


Fig. 2. Illustration of collaborative operation of LRAUVs *Aku*, *Opah*, and Wave Glider *Mola* in the experiment. *Opah* and *Mola* both acoustically tracked *Aku*. *Aku* tracked and sampled the DCM layer (marked by the orange curve). *Opah* spiraled around *Aku* to collect contextual data.

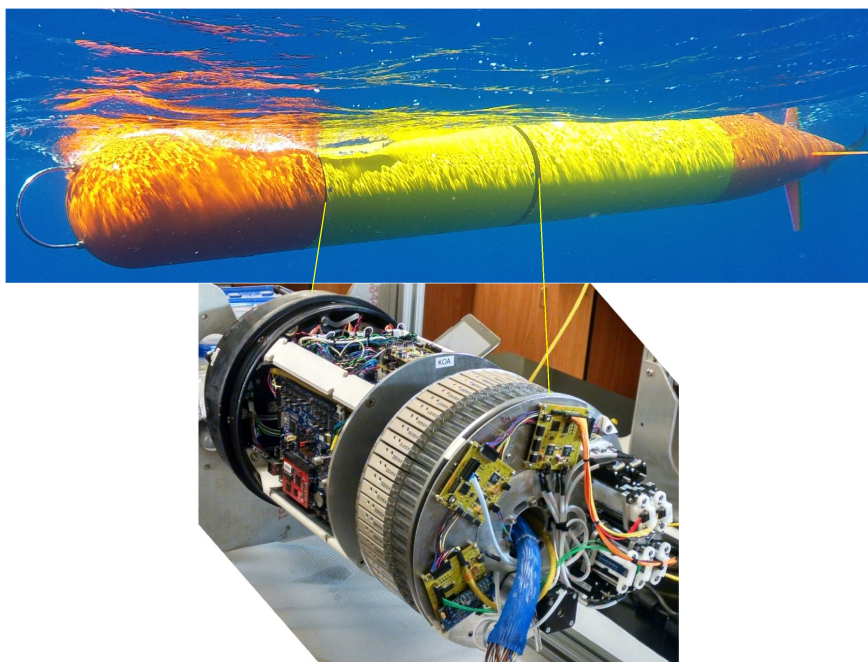


Fig. 3. LRAUV *Aku* deployed in the March–April 2018 Hawaiian Eddy Experiment. The 3G-ESP was installed in the vehicle’s fore-mid section. (The photos were taken by Elisha Wood-Charlson during the experiment.)

buoyancy engine, the vehicle is capable of ballasting to neutral buoyancy and drifting in a lower power mode. The LRAUV thus combines the mobility and speed of propeller-driven vehicles and energy savings of buoyancy-driven vehicles. *Aku*’s science sensors suite (all in the nose section) includes Sea-Bird Scientific (SBE) Glider Payload Conductivity-Temperature-Depth (GPCTD) sensors,¹ a WET Labs BB2FL

fluorescence/backscatter sensor (chlorophyll fluorescence excitation wavelength 470 nm and emission wavelength 695 nm), an Aanderaa 4831F dissolved oxygen sensor, and a LI-COR LI-192SA PAR (photosynthetically active radiation) sensor. The WET Labs fluorescence sensor’s raw count output is converted to chlorophyll concentration using a formula provided by the manufacturer, and the sensor is periodically sent back to the manufacturer for routine calibration. The PAR sensor points to 20° from the vertical (when the vehicle lies horizontal). In this configuration, when the vehicle runs on a yo-yo trajectory of ±20° pitch angles, the PAR sensor will point upward on ascent profiles for accurate light irradiance measurement.

¹The SBE GPCTD sensors are installed on the vehicle’s horizontal center plane and just outside the hull. The temperature measurement range is −5 to +42 °C with a resolution of 0.001 °C. The conductivity measurement range is 0 to 9 S/m, with a resolution of 0.00001 S/m. The depth measurement range is 0 to 350 m, with a resolution of 0.007 m.

The LRAUV software architecture uses state configured layered control [43], which divides the vehicle's operations into a group of behaviors assigned with hierarchical levels of priority. For each AUV mission, the vehicle runs a mission script that invokes appropriate AUV behaviors to achieve a specified goal [41], [44].

The 3G-ESP instrument [35], [36] is installed in the forward pressure housing of the LRAUV. It uses cartridges to collect and process ocean microbial samples. Up to 60 cartridges are installed on a circular wheel, and each cartridge contains the filters and reagents necessary for collecting and processing one sample. The cartridges connect to a central ring of valves that are part of a pumped seawater loop. When the LRAUV mission program triggers a sampling event, the 3G-ESP rotates the motor-driven cartridge wheel to align a designated cartridge with the processing station, where power and actuators can be applied to the cartridge. The pumped seawater loop is flushed clear, and actuators open valves to direct the seawater through the cartridge, concentrating particles and small organisms onto the filters. After a specified volume of water has been filtered, the seawater valves are closed and a valve in the cartridge is moved so the particulate material can be processed with reagents. When processing a cartridge, either a preservative reagent in the cartridge can be added to the sample to preserve the cellular material for later analysis in the laboratory, or the cartridge can prepare the sample for *in situ* detection and quantification of environmental targets. In this study, all particulate samples were preserved onboard for subsequent analyses in a shore side laboratory [45].

We previously designed and field tested an algorithm for an LRAUV to autonomously detect and track the depth of the chlorophyll peak, and sample at that depth [46]. However, the chlorophyll peak's depth varies over time because of the phytoplankton's vertical migration and internal waves [47], which was also seen in our experiment [46].

Based on the underlying physics of the chlorophyll maximum layer in an eddy, we developed a new method for an LRAUV to accurately track and sample the DCM layer. In the 2018 Hawaiian Eddy Experiment, a 3G-ESP LRAUV *Aku* ran the algorithm to track the DCM layer in a cyclonic eddy for four days and acquired 38 ESP samples. The algorithm is presented in Section II. The experiment is described in Section III. We conclude and outline future work in Section IV.

II. AUTONOMOUS DETECTION, TRACKING, AND SAMPLING OF THE DCM LAYER

A. Design Principle

Horizontal and temporal variations of the DCM layer depth tend to follow those of an isopycnal layer [47], [48]. When density variation is dominated by temperature variation, an isopycnal can be effectively tracked by tracking an isotherm. Hence, we developed an algorithm to enable an LRAUV to autonomously track and sample the DCM layer by locking onto the isotherm corresponding to the chlorophyll peak. The algorithm comprises the following key components.

B. Lowpass Filtering of Chlorophyll Measurement

To remove spurious peaks due to sensor noise, the raw chlorophyll measurement is lowpass filtered by a moving-average window of duration τ_{LP} . Given the chlorophyll sensor's sampling interval τ_{s_chl} , the length of the lowpass filter window is $L = \lceil \tau_{LP}/\tau_{s_chl} \rceil + 1$ samples, where $\lceil \cdot \rceil$ rounds up to the nearest integer. The real-time lowpass filtering of chlorophyll runs as follows:

$$\text{Chl}_{LP}(l) = \frac{1}{L} \sum_{i=0}^{L-1} \text{Chl}(l-i) \quad (1)$$

where l is the current sample index, $\text{Chl}(l)$ is the raw chlorophyll measurement, and $\text{Chl}_{LP}(l)$ is the lowpass filtered signal.

The raw temperature measurement is lowpass filtered by the same moving-average window of duration τ_{LP} . Given the temperature sensor's sampling interval τ_{s_temp} , the length of the lowpass filter window is $M = \lceil \tau_{LP}/\tau_{s_temp} \rceil + 1$ samples. The real-time lowpass filtering of temperature runs as follows:

$$T_{LP}(m) = \frac{1}{M} \sum_{i=0}^{M-1} T(m-i) \quad (2)$$

where m is the current sample index, $T(m)$ is the raw temperature measurement, and $T_{LP}(m)$ is the lowpass filtered signal.

The raw depth measurement is also lowpass filtered by the same moving-average window of duration τ_{LP} . Given the depth sensor's sampling interval τ_{s_depth} , the length of the lowpass filter window is $N = \lceil \tau_{LP}/\tau_{s_depth} \rceil + 1$ samples. The real-time lowpass filtering of depth runs as follows:

$$z_{LP}(n) = \frac{1}{N} \sum_{i=0}^{N-1} z(n-i) \quad (3)$$

where n is the current sample index, $z(n)$ is the raw depth measurement, and $z_{LP}(n)$ is the lowpass filtered signal.

Note that the lowpass filter introduces a delay of $\tau_{LP}/2$ in Chl_{LP} , T_{LP} , and z_{LP} . Compared with chlorophyll, the temperature and depth measurements are much less noisy. Despite their lower noise levels, we apply the same lowpass filter to temperature and depth as to chlorophyll to synchronize T_{LP} and z_{LP} with Chl_{LP} , as will be elaborated in Section III-B.

C. Autonomous Detection of the DCM Layer

The AUV performs the following steps to autonomously find the peak chlorophyll layer and the corresponding isotherm, and then stay on that isotherm. These steps are illustrated in Fig. 4, labeled with the corresponding step number.

- 1) The AUV descends from the surface to $Dep_{DeepBound}$ (a deep bound that is sufficiently deeper than the anticipated DCM layer depth). On the descent, the AUV seeks Chl_{LP_max} (the peak of the Chl_{LP} signal) and the corresponding temperature $T_{LP_ChlPeak}$. Because Chl_{LP} and T_{LP} carry the same delay of $\tau_{LP}/2$ (due to the same lowpass filter), $T_{LP_ChlPeak}$ truly marks the temperature of the chlorophyll peak, as will be seen in Fig. 7 in Section III-B.

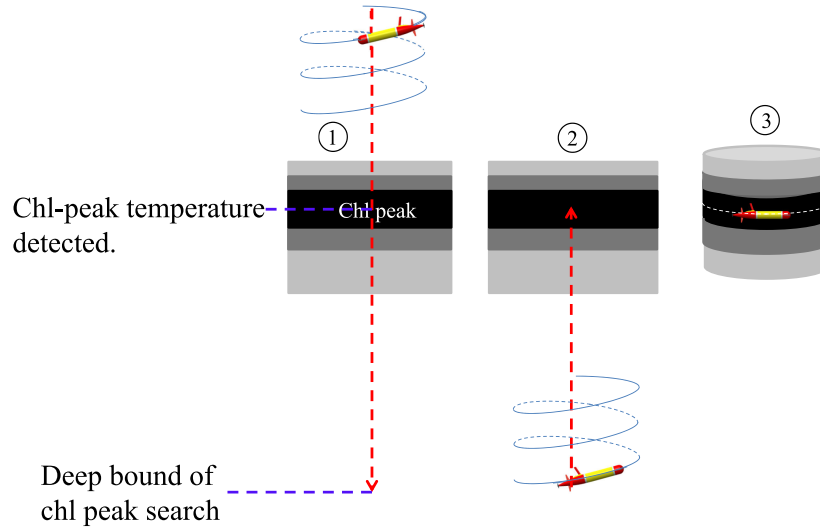


Fig. 4. Illustration of the algorithm for autonomous detection of the DCM temperature and tracking that isotherm. The gray scale level represents the chlorophyll signal level. The darkest layer represents the DCM.

The vehicle can descend in spiral mode (propeller turned ON with a nonzero rudder angle) or drift mode (propeller turned OFF; adjusting buoyancy).

- 2) When reaching depth $Dep_{DeepBound}$, the vehicle turns to an ascent (in spiral mode or drift mode). To confirm the turn from descent to ascent, the AUV checks the following two conditions [18]: first, the depth has decreased four times in a row. Second, the depth has decreased from the maximum depth by more than 1 m. Once the turn is confirmed, the vehicle reports the peak signal value Chl_{LP_max} of the entire descent leg and the corresponding temperature $T_{LP_ChlPeak}$.
- 3) On the ascent, when the AUV reaches temperature $T_{LP_ChlPeak}$, it stops ascending and follows the targeted water mass by temperature. The isotherm tracking algorithm is given in Section II-D.

D. Isotherm Tracking Algorithm

We previously designed an AUV autonomous isotherm tracking algorithm [49]. In an initial vertical search, the vehicle records the depth corresponding to the target temperature T_{target} and holds that depth. During depth holding, if the measured temperature $T_{measured}$ goes beyond a tolerance range (e.g., $T_{target} \pm 0.2$ °C), the vehicle ascends or descends to reacquire the target temperature. In each reacquisition maneuver, a lock-out time (several minutes) is allowed for any depth overshoot to damp down. In an experiment in Monterey Bay in June 2015, an LRAUV ran the algorithm to track a targeted temperature for 13 h. The standard deviation of temperature was 0.11 °C; 95% of the temperature points fell within $T_{target} \pm 0.25$ °C. In this method, the AUV holds depth until the temperature error is larger than the tolerance range. This introduces a latency in responding to temperature discrepancy.

Therefore, we improved the approach so that the temperature error is continuously fed back to the controller for achieving a

more responsive and accurate isotherm tracking, as illustrated in Fig. 5. In each control cycle of duration Δt , a projected temperature T_{proj} is calculated based on the discrepancy between the target temperature T_{target} and the measured temperature $T_{measured}$, as well as the rate of temperature change on the vehicle's vertical maneuver \dot{T} . The difference between T_{proj} and $T_{measured}$ produces a depth adjustment z_{adj} , which is subtracted from the measured depth $z_{measured}$ to give the commanded depth $z_{commanded}$. The AUV maneuvers (by adjusting attitude when in flight mode) to attain $z_{commanded}$.

III. EXPERIMENT

A. Experimental Design

During March and April 2018, two LRAUVs along with one Liquid Robotics Wave Glider were deployed to the north of Hawaiian Islands to investigate the diel variability of the microbial community in the DCM layer residing in a cyclonic eddy [50], as shown in Fig. 2. LRAUV *Aku* carried a 3G-ESP. *Aku* ran the presented algorithm to autonomously find and track the DCM layer, and trigger 3G-ESP water sampling. During *Aku*'s submerged tracking, Wave Glider *Mola* acoustically tracked it to provide safety assurance and the functionality of terminating *Aku*'s mission. LRAUV *Opah* also acoustically tracked *Aku* and spiraled around it to measure the contextual water properties.

Prior to the experiment, the University of Hawaii scientists studied satellite SLA maps to identify eddies and plan ship tracks to transect the targeted eddy. A cyclonic eddy to the northeast of Molokai was selected for study, as shown in Fig. 6. The sea surface sloped downward toward the eddy center (hence the most negative SLA at the center) to balance the Coriolis force exerted on the eddy current by the Earth's rotation. While *R/V Falkor* transected through the eddy, the onboard scientists identified the eddy center by observing in real time the

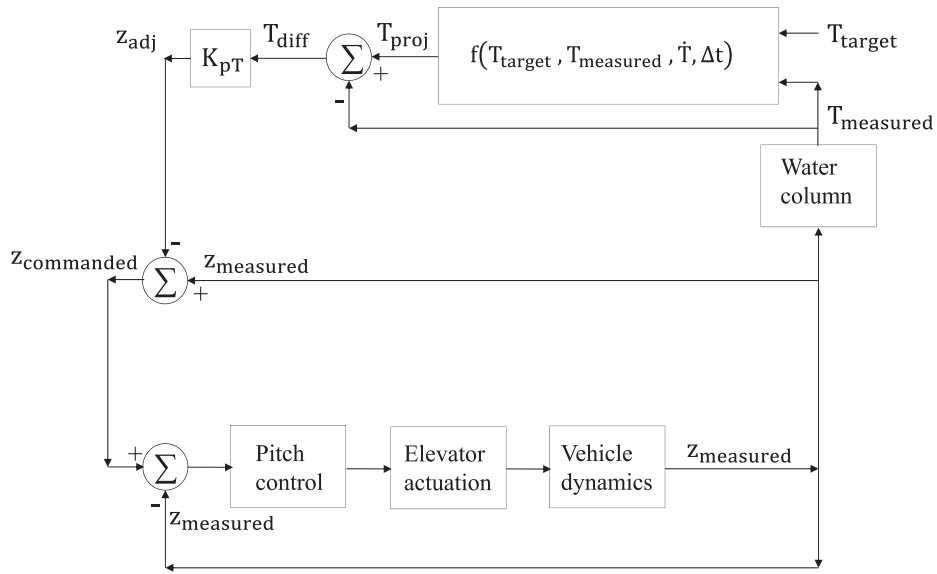


Fig. 5. Diagram of LRAUV’s control mechanism for isotherm tracking.

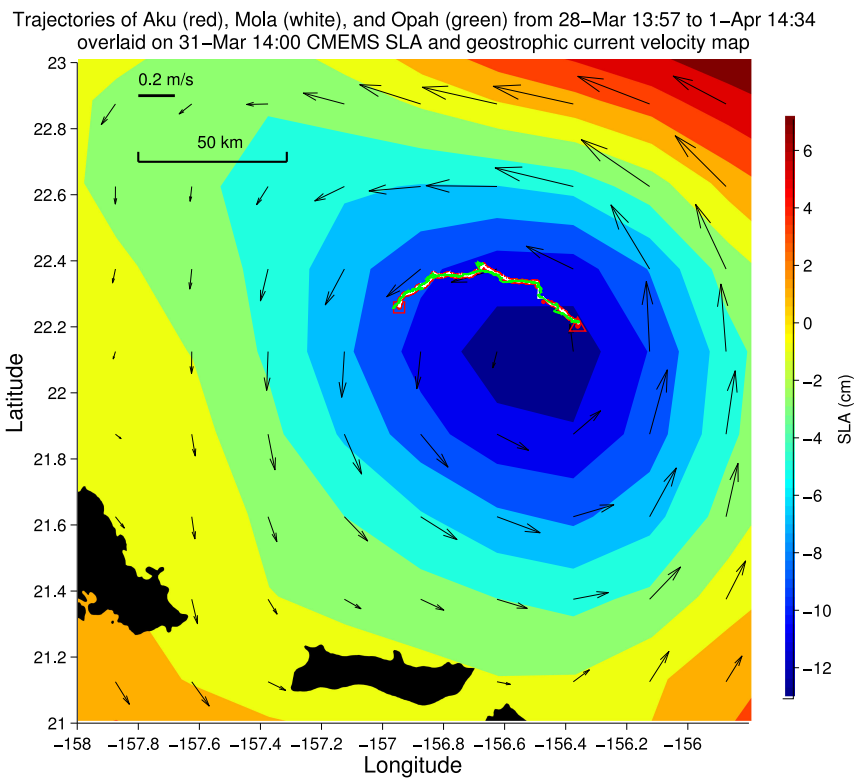


Fig. 6. Trajectories of *Aku*, *Opah*, and *Mola* during the four-day mission, overlaid on the CMEMS SLA and geostrophic current velocity map. The triangle and the square mark the start and the end of the mission, respectively. Time is in Hawaii Standard Time (HST). HST = Coordinated Universal Time (UTC) – 10:00.

diminishing current velocity measured by the shipboard Acoustic Doppler Current Profiler (ADCP) and by tracking the depth of isopycnal surfaces measured with the underway Conductivity-Temperature-Depth (CTD) sensors deployed from the ship’s stern. At the eddy center, we deployed *Aku*, *Opah*, and *Mola* to kick off the experiment which comprised two legs. In leg 1 from March 17 to 21, *Aku* took 36 ESP samples from the DCM

layer in three segments (the vehicle surfaced between segments). At the end of this leg, we recovered the vehicles, and retrieved the ESP samples. On March 28, we redeployed the vehicles at the eddy center for leg 2 through April 2, in which *Aku* took 46 ESP samples from the DCM layer in three segments. In the longest segment from March 28 13:57 to April 1 14:34 (all times in HST), *Aku* tracked the DCM layer to the north of the eddy

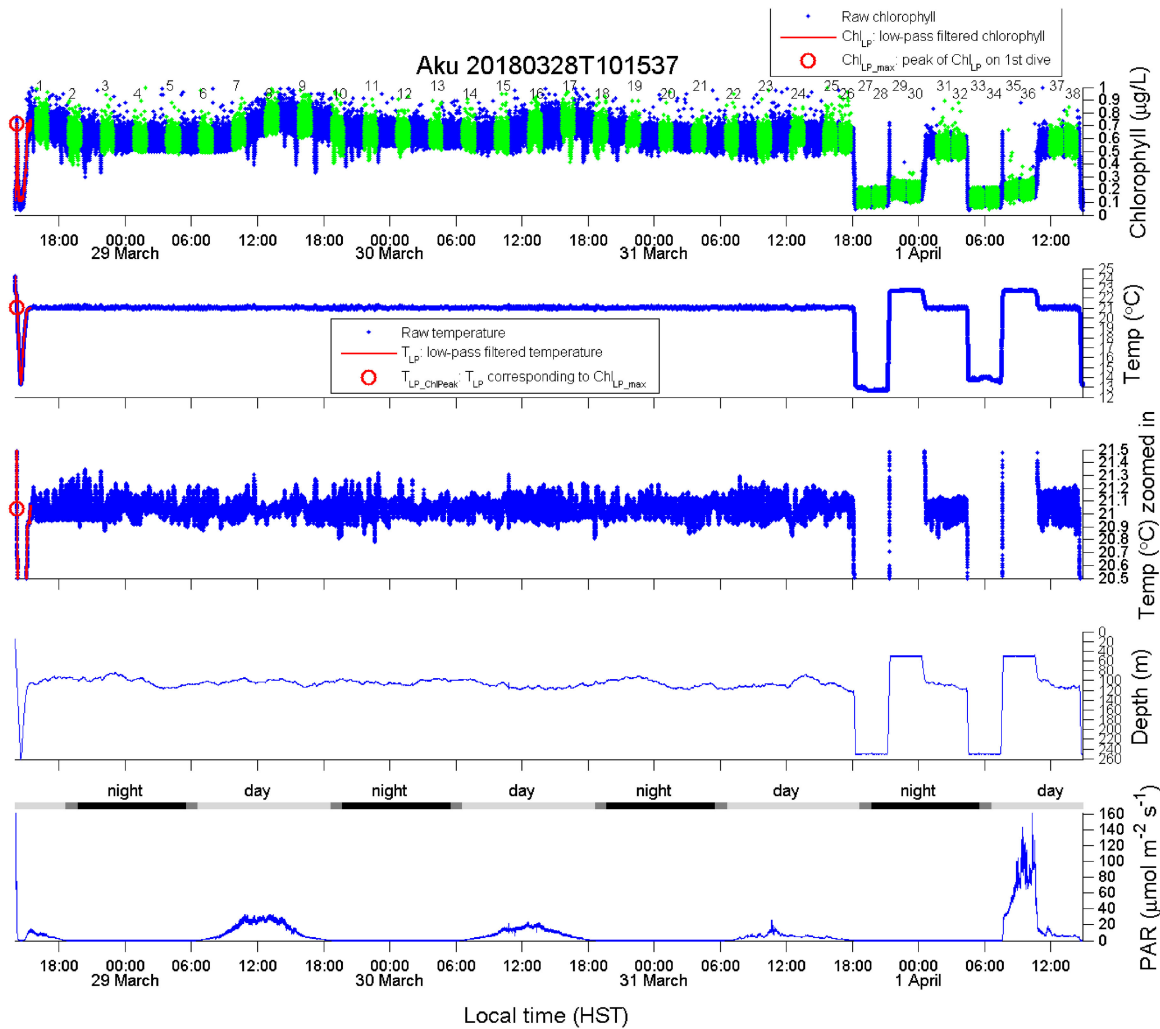


Fig. 7. *Aku*-measured chlorophyll (first panel), temperature (second and third panels), depth (fourth panel), and PAR (fifth panel) during the four-day mission. In the first three panels, the lowpass filtered signal is plotted only on the initial dive and the succeeding ascent.

center for four days without surfacing, and took 38 ESP samples. This longest nonstop segment is reported below.

B. LRAUV *Aku*'s Detection, Tracking, and Sampling of the DCM Layer

From March 28 13:57 to April 1 14:34, *Aku* autonomously found the DCM and continuously tracked the DCM layer without surfacing, as shown in Fig. 7. During the four-day tracking, *Aku* took 38 ESP samples: 24 samples in the layer at 3-h intervals, followed by 14 samples in, below, and above the layer for comparison. In the first panel, the raw chlorophyll is shown by the blue dots, and the lowpass filtered chlorophyll (on the initial dive and the succeeding ascent) is shown by the red line. The red circle marks the peak of the lowpass filtered chlorophyll found on the initial dive. The green dots mark the ESP sampling duration of each sample. In the second panel, the raw temperature is shown by the blue dots, and the lowpass filtered temperature is shown by the red line. The red circle marks the lowpass filtered temperature corresponding to the chlorophyll peak. In the third panel, temperature is zoomed in for examining *Aku*'s isotherm

tracking accuracy. The fourth panel shows *Aku*'s depth trajectory. We see that the tracked isotherm undulated in depth. In the fifth panel, *Aku*'s PAR measurement clearly shows four daily cycles. Note that the very high PAR peak on 1 April was due to *Aku* ascending to a much shallower depth (50 m) in daylight. Details of DCM detection, tracking, and sampling are given below.

1) *Autonomous Detection of the DCM Layer*: A close-up view of the initial dive and the succeeding ascent is shown in Fig. 8. *Aku* spiraled from the surface down to $Dep_{DeepBound} = 260$ m to seek the DCM layer. The vehicle's rudder angle was set to 13° and the vehicle speed was 1 m/s (with the vertical component of 0.14 m/s). At 102.36-m depth, the vehicle found the peak chlorophyll $Chl_{LP_max} = 0.72 \mu\text{g/L}$ and the corresponding temperature $T_{LP_ChlPeak} = 21.04^\circ\text{C}$. All values were lowpass filtered output from an 8-s moving-average window.

The WET Labs BB2FL fluorescence/backscatter sensor's sampling frequency was 2 Hz. Hence, the 8-s sliding window averaged 16 chlorophyll measurements to produce the lowpass filtered output at each time instant. This was sufficient to smooth out the noise, as shown in the upper left panel of Fig. 8. At the

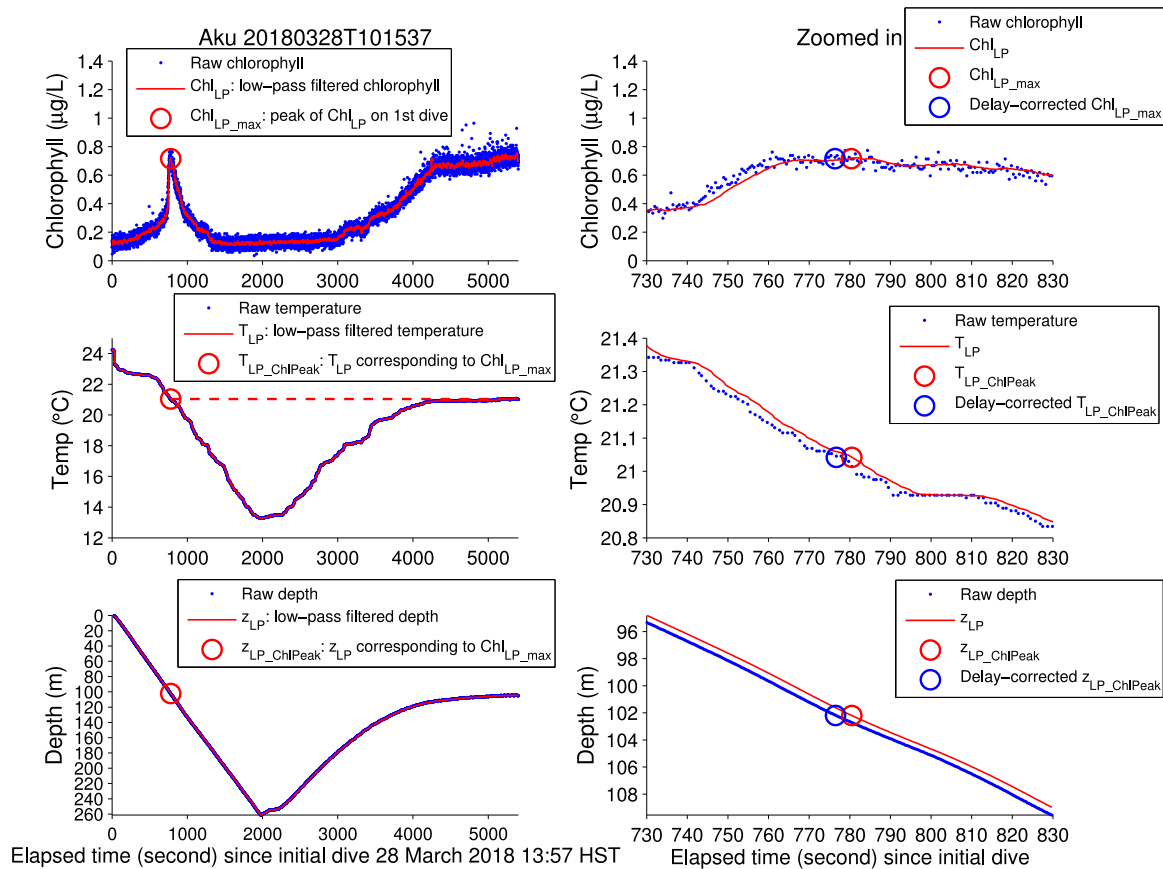


Fig. 8. *Aku*-measured chlorophyll (upper), temperature (middle), and depth (lower) on the initial dive and the succeeding ascent.

vehicle's 0.14 m/s vertical speed, the 8-s time window was equivalent to a 1.1 m depth window. The DCM thickness on *Aku*'s initial dive was 8 m (when chlorophyll dropped to 90% of the peak level). The 8-s lowpass sliding window's thickness was small compared with the DCM layer thickness, thus well preserving the chlorophyll signal. The SBE GPCTD temperature sensor and Keller depth sensor's sampling frequencies were 1 and 2.5 Hz, respectively. Hence, the 8-s sliding window averaged 8 temperature measurements or 20 depth measurements.

The purpose of applying the same lowpass filter to temperature and depth as to chlorophyll was to synchronize T_{LP} and z_{LP} with Ch_{LP} . Because they carried the same delay of $8\text{ s}/2 = 4\text{ s}$, $T_{LP_ChIPeak}$ truly marked the temperature of the chlorophyll peak, as shown in the right panels of Fig. 8. In the upper right panel, Ch_{LP} (red line) has a 4-s delay relative to the raw chlorophyll (blue dots). The red circle marks Ch_{LP_max} . With a 4-s displacement (to correct the delay), the red circle falls back onto the raw chlorophyll and is recolored blue. In the middle right panel, T_{LP} (red line) has a 4-s delay relative to the raw temperature (blue dots). The red circle marks $T_{LP_ChIPeak}$ that corresponds to Ch_{LP_max} . The 4-s delay-corrected $T_{LP_ChIPeak}$ (blue circle) falls back on the raw temperature. Delay-corrected Ch_{LP_max} and $T_{LP_ChIPeak}$ are vertically aligned in the two panels, both lying on the raw chlorophyll's peak. This verifies that $T_{LP_ChIPeak}$ corresponded to the chlorophyll peak.

2) *Autonomous Tracking and Sampling of the DCM Layer*: *Aku* locked onto the 21.04 °C peak-chlorophyll isotherm for three days to track and sample the DCM layer, as shown in the second and third panels of Fig. 7. The standard deviation of temperature was 0.06 °C; 98% of the temperature points fell within 21.04 °C \pm 0.15 °C. The isotherm tracking accuracy improved by a factor of two over the previous algorithm (in Section II-D). The depth of the peak-chlorophyll isotherm undulated between 83 and 124 m depths. The large depth undulation of the DCM layer manifests the importance of enabling the LRAUV to track the peak-chlorophyll isotherm rather than a certain depth.

Aku triggered the 3G-ESP sampling every 3 h. For each sample, filtration took 65 min and processing took 12 min. A 100-min wait was inserted before the next sampling to make the inter-sample spacing 3 h. Thus, 24 samples were acquired inside the DCM layer in three days. On the fourth day, *Aku* switched to a different sampling sequence to acquire 14 ESP samples from inside, below and above the DCM layer for comparison: 2 in DCM; 2 at 250-m depth; 2 at 50-m depth; 2 in DCM; 2 at 250-m depth; 2 at 50-m depth; 2 in DCM. In total, 38 samples were acquired in four days. All particulate samples were preserved to support transcriptomic analysis after vehicle recovery.

The chlorophyll fluorescence and PAR levels in the DCM layer measured by *Aku* are shown in the first and fifth panels of Fig. 7, respectively. The DCM chlorophyll fluorescence level

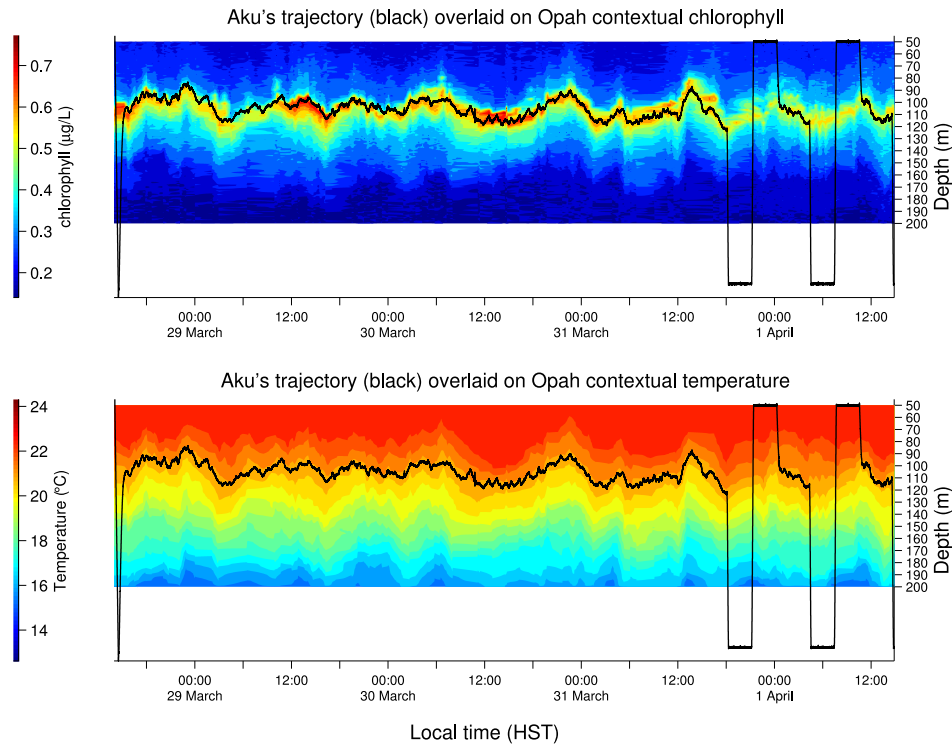


Fig. 9. *Aku*'s depth trajectory overlaid on *Opah*-measured contextual chlorophyll (upper) and temperature (lower), respectively.

exhibited diel variation, and the daily highest level was reached around 15:00 (local time), following the daily peak in PAR. The daily chlorophyll fluorescence peak level also showed day-to-day variation co-varying with the PAR level. These variations in chlorophyll fluorescence may have been associated with population growth, variation of cell pigmentation levels [48], and nonphotochemical quenching, which can occur at low PAR levels [51]. The diel pattern of the DCM chlorophyll level is similar to that observed in a separate experiment in the North Pacific Ocean (27.7° N, 139.5° W) measured by an autonomous profiler [52].

On the 21.04° C isotherm of the DCM, *Aku* ran on tight circles (circle radius ~ 10 m) at 13° rudder angle and 1 m/s speed while drifting with the eddy current. The trajectories of *Aku*, *Opah*, and *Mola* during *Aku*'s four-day mission are shown in Fig. 6. *Mola*'s trajectory is given by its continuous GPS tracking on the sea surface. *Opah*'s trajectory is estimated by underwater dead-reckoned navigation that is corrected by periodic GPS fixes on the surface. *Aku*'s trajectory is estimated by combining *Mola*'s own location and *Aku*'s acoustic range and bearing from *Mola* (with a horizontal positioning error of about 50 m).

In 74-h continuous tracking of the DCM layer, *Aku* drifted 72 km in the eddy current at an average drift speed of 0.27 m/s. Concurrently, a GPS-tracked drifter comprising a surface float and a drogue at 120-m depth was deployed near *Aku*. In the same 74-h duration, the drifter drifted 71 km at an average drift speed of 0.27 m/s. Another reference was *Falkor*'s shipboard ADCP measurement near *Aku*'s route. The ADCP-measured Earth-referenced current velocity at the 103-m depth bin (nearest DCM's mean depth of 105 m) in this duration was 0.25 m/s. The

closeness between *Aku*'s drift speed and that of the drifter as well as the ship ADCP-measured eddy current velocity shows that *Aku* largely followed the DCM water mass in a quasi-Lagrangian mode.

C. LRAUV *Opah*'s Contextual Mapping Around *Aku*

Mola, *Opah*, and *Aku* were each equipped with a Teledyne Benthos directional acoustic transponder that integrates an acoustic modem and an ultra-short baseline acoustic positioning system. *Opah* acoustically tracked *Aku*, while spiraling up and down between 50 and 200 m depths around *Aku* to measure the contextual water properties. During the four-day mission, the distance between the two vehicles varied from 30 m to 3 km, averaging 840 m. It is useful to know how representative the water column structure mapped by *Opah* was in relation to *Aku*, considering the distance between them and the DCM structure. This requires synoptic mapping data of the eddy, as acquired by *Opah* and *Aku* on two 100-km cross-eddy yo-yo transects (north-south and east-west, respectively) prior to *Aku* sampling mission. The average DCM thickness (when chlorophyll dropped to 90% of the peak level) was 13 m. At 3-km distance, the average difference of DCM depths was 7 m, smaller than the DCM thickness. This indicates that *Opah* water column data accurately represented the vertical structure around *Aku* during the sampling mission.

In the upper panel of Fig. 9, *Aku*'s depth trajectory is overlaid on *Opah*-measured contextual chlorophyll. The overlap of *Aku*'s depth and *Opah*-measured chlorophyll-maximum depth confirms that *Aku* precisely tracked the DCM layer. In the lower

panel, *Aku*'s depth trajectory is overlaid on *Opah*-measured contextual temperature, which shows that *Aku* stayed on the targeted isotherm corresponding to the DCM.

IV. CONCLUSION AND FUTURE WORK

In the 2018 SCOPE Hawaiian Eddy Experiment, a 3G-ESP LRAUV ran our targeted sampling algorithm to autonomously detect, track, and sample the DCM layer in a cyclonic eddy for four days and acquired 38 water samples from inside, below, and above the DCM layer. Molecular analysis of the samples is underway, aimed at understanding the function, activity, and environmental sensitivities of microbial populations over four consecutive diel cycles. The result is expected to shed light on how eddy physics affects biological processes and ocean productivity over time.

In the Hawaiian Eddy Experiment, all cartridges were of "archival" type, i.e., the samples were preserved for lab analysis after the LRAUV was recovered. We are currently working on another type of cartridge that will allow on-board processing of filtered material, creating a homogenate for downstream *in situ* analysis [36]. To perform the *in situ* analysis, reactive reagents are added to the filtered material, which is then heated to release the genetic material and proteins. Additional reagents are added to the sample, and the mixture is pushed from the cartridge to a detection instrument embedded within the 3G-ESP. The detection instruments (under development) will target environmental toxins or nucleic acids. Real-time molecular detection and reporting opens exciting possibilities for 3G-ESP LRAUVs to react to genomic findings and accordingly modify missions to maximize scientific gains.

Multivehicle collaboration allowed continuous sampling and contextual mapping in a moving eddy field, enabling quasi-Lagrangian observation of DCM microbial ecology [53]. The Wave Glider and the contextual-mapping LRAUV acoustically tracked the sampling LRAUV, but there was no data exchange between them. We are in the process of testing intervehicle acoustic messaging. Exchange of key information (e.g., chlorophyll level and ESP status) will greatly improve efficiency, flexibility, and persistence of autonomous targeted sampling missions.

ACKNOWLEDGMENT

The authors would like to thank the *R/V Falkor* crew for the field support. The SLA and geostrophic current velocity data came from the CMEMS (<http://marine.copernicus.eu/>). M. Godin, R. McEwen, and M. J. Stanway contributed to the LRAUV isotherm tracking and depth control code. The LRAUV's buoyancy engine was designed by J. Erickson. On board *R/V Falkor*, A. White and F. Henderikx helped with using the real-time shipboard ADCP data to locate the eddy center. R. Tabata, T. Burrell, E. Shimabukuro, and T. Clemente deployed the underway CTD during the initial eddy survey. P. Den Uyl assisted with 3G-ESP sample processing. The authors would also like to thank E. Firing and J. Hummon for processing the shipboard ADCP data, and L. Fujieki for providing access to the processed ADCP data. The authors thank M. Chaffey,

E. Mellinger, D. Klimov, and T. Hoover for contributions to the LRAUV development, W. Ussler and S. Jensen for contributions to the 3G-ESP development, and K. Gomes for managing the MBARI Oceanographic Decision Support System. B. Watkins, E. Wood-Charlson, and T. Hoffman provided operational and logistical support during the experiment. M. Salisbury helped with refining Fig. 1, and K. Lance drew the Wave Glider symbol used in Fig. 2. The authors would also like to thank D. Au for his support and advice. The authors appreciate the very helpful comments from the three anonymous reviewers for improving the paper. This work is a contribution of the SCOPE and the Center for Microbial Oceanography: Research and Education.

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Yanwu Zhang (S'95–M'00–SM'05) was born in 1969 in Shaanxi Province, China. He received the B.S. degree in electrical engineering and the M.S. degree in underwater acoustics engineering from Northwestern Polytechnic University, Xi'an, China, in 1989 and 1991, respectively, the M.S. degree in electrical engineering and computer science from the Massachusetts Institute of Technology (MIT), Cambridge, MA, USA, in 1998, and the Ph.D. degree in oceanographic engineering from the MIT/Woods Hole Oceanographic Institution Joint

Program, Woods Hole, MA, USA, in June 2000.

From 2000 to 2004, he was a Systems Engineer working on medical image processing with the General Electric Company Research and Development Center, Niskayuna, NY, USA, and then a Senior Digital Signal Processing Engineer working on digital communications with Aware, Inc., Bedford, MA, USA. Since December 2004, he has been with the Monterey Bay Aquarium Research Institute, Moss Landing, CA, USA, first as a Senior Research Specialist and then as a Senior Research Engineer. He leads the project of targeted sampling by autonomous vehicles, designs adaptive sampling algorithms for marine ecosystem studies, and participated in the development of the Tethys-class long range AUVs. Since 1996, he has participated in more than a dozen field experiments running the *Odyssey IIB*, *Dorado*, and *Tethys* AUVs. He was a finalist of the MIT Technology Review Magazine's 100 young innovators (TR100) in 1999 when he was a Ph.D. student.

Dr. Zhang is a member of Sigma Xi. In 2018, he was the recipient of the Visiting Fellowship of Antarctic Gateway Partnership from University of Tasmania, Australia.

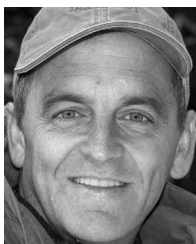


Brian Kieft received the B.S. degree in computer science from Hope College, Holland, MI, USA, in 2001.

He worked with the avionics industry, developing and testing subsystems for military aircraft from 2001 to 2006. In 2006, he joined the Monterey Bay Aquarium Research Institute, Moss Landing, CA, USA, as a Software Engineer. He has worked on various platforms, including mooring controllers, benthic instruments, wavegliders, and several AUVs and their associated payloads. He currently works on development of the Tethys class AUV a long-range, upper-water-

column AUV designed primarily for biological sensing. Apart from development, he also takes part in mission planning and payload integration for ongoing collaborative field programs and engineering tests.

Mr. Kieft has been actively involved in updating and teaching the IEEE tutorial *AUV Technology and Application Basics* since 2011. He also Co-Chairs the wave glider users group.



Brett W. Hobson received the B.S. degree in mechanical engineering from San Francisco State University, San Francisco, CA, USA, in 1989.

He began his ocean engineering career with Deep Ocean Engineering in San Leandro, San Jose, CA, USA, developing remotely operated vehicles. In 1992, he helped start and run Deep Sea Discoveries where he helped develop and operate deep towed sonar and camera systems offshore the United States, Venezuela, Spain, and Philippines. In 1998, he joined Nekton Research, Durham, NC, USA, to design and

deploy bioinspired underwater vehicles for Navy applications. After the sale of Nekton Research to iRobot in 2005, he joined the Monterey Bay Aquarium Research Institute (MBARI), Moss Landing, CA, USA, where he leads the Long Range Autonomous Underwater Vehicle (AUV) program overseeing the development and science operations of six mega-meter range AUVs. His team has also developed MBARIs long-endurance seafloor crawling Benthic Rover. He teaches AUV tutorials for IEEE/MTS OCEANS Conferences each year, holds one patent, and is currently Co-PI on NASA and NSF projects aimed at developing novel underwater vehicles for ocean science.



John P. Ryan was born in Lafayette, IN, USA, in 1965. He received the B.S. degree in biology from the University of Massachusetts, Boston, MA, USA, in 1988, and the M.S. and Ph.D. degrees in biological oceanography from The University of Rhode Island, Narragansett, RI, USA, in 1993 and 1998, respectively.

He began a Postdoctoral Research Position with the Monterey Bay Aquarium Research Institute (MBARI), Moss Landing, CA, USA, in fall 1998, transitioned to MBARI Scientist in 2001, and is currently Senior Research Specialist. His research explores relationships between marine life, ranging from microscopic algae to whales, and their environment.

Dr. Ryan was the recipient of a fellowship by the Office of Naval Research in support of his M.S., and a NASA New Investigator Research grant in support of his Postdoctoral Research.



Benedetto Barone received the M.S. degree in environmental sciences from the Parthenope University of Naples, Naples, Italy, in 2005, and the Ph.D. degree in marine sciences and engineering from the Federico II University and the Stazione Zoologica Anton Dohrn of Naples, Naples, Italy, in 2010.

He is currently the Balzan Research Fellow with the Center for Microbial Oceanography: Research and Education with the University of Hawaii, Honolulu, HI, USA. He is focused on understanding the ecological and biogeochemical dynamics of the open

ocean including phytoplankton photosynthesis and particle export. He is particularly interested in assessing the importance of physical biological dynamics associated with turbulent mixing and mesoscale eddies. He uses autonomous vehicles to collect bio-optical observations that serve as proxies for the ecological characteristics of marine environments.



Christina M. Preston received the B.S. degree in biology from James Madison University, Harrisonburg, VA, USA, in 1992, and the Ph.D. degree in ecology, evolution, and marine biology, from the University of California, Santa Barbara, CA, USA, in 1998.

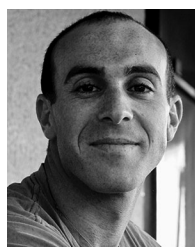
She was a Postdoctoral Scholar with Hopkins Marine Station, Stanford University, Pacific Grove, CA, USA. She is currently a Research Specialist with the Monterey Bay Aquarium Research Institute, Moss Landing, CA, USA. Her current research activities involve developing molecular methods to identify and quantify plankton *in situ* using underwater robots.



Brent Roman was born in Canton, IL, USA, in 1963. He received the B.S. degree in computer and information sciences from the University of California at Santa Cruz, Santa Cruz, CA, USA, in 1985.

His career as a Software Engineer, began as a teenager working on automated concrete ready-mix batching systems coded in 8085 assembly language. He paid for university by coding video tape editor controllers in Z-80 assembly. After the B.S. degree, he did a six-year stint marketing CAE software. In 1990, he returned to product development, working

on a small team creating a prototyping tool to aid in the design of automotive and aerospace digital servo control systems. In 1997, he joined the Santa Cruz Operation where he specialized in use of Unix and Linux on small PCs and embedded systems. He joined the Monterey Bay Aquarium Research Institute, Moss Landing, CA, USA, in 2000, where he has focused on distributed control systems, communications, and software development for the Environmental Sample Processor.



Ben-Yair Raanan received the B.S. degree in marine science from the Ruppin Academic Center, Israel, in 2011, and the M.S. degree in marine science (Physical Oceanography Laboratory) from Moss Landing Marine Laboratories, San Jose State University, San Jose, CA, USA, in 2014.

He joined the Monterey Bay Aquarium Research Institute, Moss Landing, CA, USA, as an Associate Engineer, in 2014. He currently focused on developing and testing new capabilities for the *Tethys*-class long-range AUVs. His research interests include soft-

ware design for autonomous systems, hydrodynamic modeling, and machine learning, and artificial intelligence.



Roman Marin III received the B.S. degree in marine biology and the M.S. degree in marine sciences from the University of California at Santa Cruz, Santa Cruz, CA, USA, in 1993 and 1998, respectively.

He participated in the development of the first, second, and third generations of the Environmental Sample Processor (ESP) at the Monterey Bay Aquarium Research Institute, Moss Landing, CA, USA. He currently works on autonomous biomolecular analyzers, and supports ESP operations by collaborating institutions in the United States and overseas.



Thomas C. O'Reilly received the B.A. degree in earth science from the University of Chicago, Chicago, IL, USA, in 1977, and the M.A. degree in geochemistry from Washington University, St. Louis, MO, USA, in 1983.

He is currently a Software Engineer with Monterey Bay Aquarium Research Institute (MBARI), Moss Landing, CA, USA. He develops applications for autonomous underwater, surface, and aerial vehicles including acoustic target geolocation and tracking, aerial surveys of the sea surface, communications

and coordination between robotic platforms, and improved methods for sensor payload integration. He also develops desktop and mobile tools for viewing and processing bathymetric datasets as well as "crowd-sourced" marine biology applications.



Carlos A. Rueda was born and raised in Colombia. He received the B.S. degree in system engineering from the Universidad Autnoma de Manizales, Manizales, Colombia, in 1994, and the Ph.D. degree in computer science from the University of California (UC), Davis, CA, USA, in 2007.

While at UC Davis, he developed data processing and numerical analysis algorithms for a variety of applications ranging from the spatial interpolation of meteorological parameters over extended geographical areas, to the efficient inversion of models for estimation of vegetation properties from remotely sensed data. After holding a Postdoctoral Position with UC Davis Computer Science Department, he joined the Monterey Bay Aquarium Research Institute, Moss Landing, CA, USA, as a Software Engineer in 2008. His areas of expertise and interest include scientific data management, visualization, data integration and interoperability, programming languages, and semantic web.

After holding a Postdoctoral Position with UC Davis Computer Science Department, he joined the Monterey Bay Aquarium Research Institute, Moss Landing, CA, USA, as a Software Engineer in 2008. His areas of expertise and interest include scientific data management, visualization, data integration and interoperability, programming languages, and semantic web.



Douglas Pargett received the B.S. degree in mechanical engineering and the M.S. degree in engineering management from Santa Clara University, Santa Clara, CA, USA, in 1993 and 2005, respectively.

He is currently a Mechanical Engineer. He develops ocean research instruments and platforms for surface to deep ocean operation. He has more than 20 years of experience developing novel equipment and systems, but also likes to utilize the newest technologies such as additive manufacturing so that MBARI can truly push the envelope of ocean science and technology. He has a Professional Engineering license in California.

He has a Professional Engineering license in California.



Kevan M. Yamahara received the B.S. degree in civil engineering from California State Polytechnic University, Pomona, CA, USA, in 2006, and the M.S. and Ph.D. degrees in environmental engineering from Stanford University, in 2008 and 2011, respectively.

He is currently a Research Specialist with the Monterey Bay Aquarium Research Institute (MBARI), Moss Landing, CA, USA. He began working with MBARI in 2012 as an Early Career Fellow with the Center for Ocean Solutions to develop rapid methods for microbial water quality monitoring. He joined MBARI in 2015 to continue his work with the Environmental Sample Processor group. At MBARI, he focuses on development and application of new technologies for biological monitoring of the marine environment. He is currently working on a team developing autonomous, *in situ* instrumentation for water quality (fecal indicators and source-tracking methods) and advancing environmental DNA monitoring in aquatic systems. He also works closely with the Monterey Bay Aquarium to conduct exploratory studies using MBARI's technology for conservation and management applications.

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Steve Poulos (SM'07) received the B.S. degree in electrical engineering from San Diego State University, San Diego, CA, USA, in 1977.

He was with the Aerospace industry working on Boeing's Air Launched cruise missile project. He came to the University of Hawaii, Honolulu, HI, USA, in 1984 and for more than 30 years he has been involved in the design and development of data acquisition systems, for shipboard, geophysical, and oceanographic research. He is currently a Special Projects Engineer with the Department of Oceanography.



Anna Romano received the B.S. degree in marine biology from Hawaii Pacific University, Honolulu, HI, USA, in 2005, and the M.S. degree in biological oceanography from the University of Hawaii, Mānoa, HI, USA, in 2008.

Afterward, she worked for the private sector contributing to algae biofuel research for Cellana and Synthetic Genomics. In 2014, she was a Research Associate with Dr. Edward F. DeLong's Laboratory, University of Hawaii. She joined the team to develop high throughput workflows using robotics to prepare marine microbial samples for genetic sequencing. In addition to her laboratory role, she became a part of the University of Hawaii and Monterey Bay Aquarium Research Institute LRAUV collaboration in 2015. During this time, she trained with Dr. Christina Preston to learn third-generation Environmental Sampler Processor operations and contribute to validation experiments of the new technology.

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Gabe Foreman received the B.S. degree in physics from the University of California, San Diego, CA, USA, in 1997.

He worked at quantum magnetics in research and development of explosive detection until 2002. From 2002 to 2007, he worked with the University of Hawaii as a Marine Research Systems Engineer aboard the University's research vessels, *R/V Ka'imikai O Kanaloa* and *R/V Kilo Moana*, logging more than 750 days at sea. From 2007 to 2015, he worked on a wide variety of projects ranging from

the design and construction of multiple algae-to-biofuel facilities, alternative energy and energy storage, to instrumented buoy design. He is currently working as a Marine Research Engineer for the Simons Collaboration on Ocean Processes and Ecology with the University of Hawaii, Manoa, HI, USA. His interests include autonomous and unmanned vehicles, underwater robotics, and sensor development.



Hans Ramm received the B.A. degree in physics from Whitman College, Walla Walla, WA, USA, in 1972, the B.S. degree in electrical engineering and computer science from the University of Colorado, Boulder, CO, USA, in 1986, and the M.S. degree in oceanography from the University of California, San Diego, CA, USA, in 1995.

He is currently a Marine Research Engineer with the Oceanography Department, the University of Hawaii, Manoa, HI, USA. His primary responsibility is the maintenance and operation of the group's autonomous ocean vehicles, including Seagliders, long-range AUVs, and a Wave Glider. Previously, he has developed atmospheric and oceanographic research instrumentation and supported research and development efforts while working at NOAA, University Corporation for Atmospheric Research, Scripps Institution of Oceanography, Lockheed Martin and Science Applications International Corporation. Most of those efforts were focussed on applying acoustic techniques to study fluid flow and characterize underwater acoustic signals.

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Samuel T. Wilson was born in Suva, Fiji, and grew up in England. He received the B.Sc. Honors degree in marine biology from the University of Wales, Swansea, U.K., in 1999, and the Ph.D. degree in marine biogeochemistry from the Scots Association for Marine Science, Oban, U.K., in 2007.

He began a Postdoctoral Research Position with the University of Hawaii, Honolulu, HI, USA, in summer 2007 and in 2012 transitioned to a researcher position. His research focuses on trace gases in the marine environment from the perspective of biogeochemical cycles, microbial metabolism, and air–sea exchange.



Edward F. DeLong was born and raised in Northern California. He received the B.S. degree in bacteriology from the University of California at Davis, Davis, CA, USA, in 1982, and the Ph.D. degree in marine biology from Scripps Institute of Oceanography, University of California at San Diego, San Diego, CA, USA, in 1986.

He is currently a Microbial Oceanographer focusing on central questions in marine microbial genomics, biogeochemistry, ecology, and evolution. A large part of his efforts have been devoted to the study of microbes and microbial processes in the ocean, combining laboratory, and field-based approaches. Development and application of genomic, biochemical, and metabolic approaches to study and explore microbial communities and processes is primary area of interest. He is also interested coupling the use of autonomous robotic sensors and samplers with genomic technologies, to derive highly resolved spatial and temporal maps of microbial community gene distributions and gene expression datasets in four dimensions in the oceans water column.

Dr. DeLong is a Fellow of the American Academy of Microbiology, the American Academy of Arts and Science, the U. S. National Academy of Science, the European Molecular Biology Association, and the American Association for the Advancement of Science.



David M. Karl received the B.A. degree in biology from the State University College at Buffalo, NY, USA, in 1971, the M.S. degree in oceanography from Florida State University in Tallahassee, FL, USA, in 1974, and the Ph.D. degree in oceanography from Scripps Institution of Oceanography, La Jolla, CA, USA, in 1978.

He is currently the Victor and Peggy Brandstrom Pavel Professor of Oceanography and Director of the Daniel K. Inouye Center for Microbial Oceanography: Research and Education (C-MORE) with the University of Hawaii, Honolulu, HI, USA. He has made significant contributions in environmental microbiology and helped to establish the discipline of microbial oceanography. He has logged more than 1000 days conducting research at sea including 23 expeditions to Antarctica. In 1988, he cofounded the Hawaii Ocean Time-series program that has conducted sustained physical, biogeochemical, and microbial measurements and experiments at Station ALOHA on approximately monthly intervals for the past 30 years. In 2006, he led a team of Scientists with the establishment of a new NSF-supported Science and Technology Center, the University of Hawaii. C-MORE conducts collaborative research on marine microorganisms from genomes to biomes, and has a vital training mission to help prepare the next generation of microbial oceanographers. In 2014, he and the University of Hawaii colleague Edward F. DeLong established the Simons Collaboration on Ocean Processes and Ecology to enhance our understanding of pathways and controls on microbially mediated matter and energy flow in the open sea.



James M. Birch received the B.S. degree in zoology from the University of North Carolina, Chapel Hill NC, USA, in 1983, and the M.S./Ph.D. degrees in biology from the University of Michigan, Ann Arbor, MI, USA, in 1995.

He is currently the Director of the SURF Center with the Monterey Bay Aquarium Research Institute, Moss Landing, CA, USA. The SURF Center (Sensors; Underwater Research of the Future) is a program that shepherds the development, deployment, and advancement of the Environmental Sample Processor (ESP), a robotic, autonomous microbiology laboratory that automates sample acquisition, processing, and analysis. Versions of the ESP have been deployed around the world's oceans, and recently have been used as a sampling instrument in our nations freshwater streams collecting data on invasive species and helping with fish stock assessments. His research interests include technology development for the ocean sciences with crossover capabilities to freshwater/terrestrial realms.



James G. Bellingham received the B.S./M.S. degrees in physics and the Ph.D. degree in physics from the Massachusetts Institute of Technology, Cambridge, MA, USA, in 1984 and in 1988, respectively.

He is currently the founding Director of the Center for Marine Robotics, Woods Hole Oceanographic Institution (WHOI), Woods Hole, MA, USA. He was the Chief Technologist with the Monterey Bay Aquarium Research Institute (MBARI), Moss Landing, CA, USA, before moving to WHOI. He is a leader in the field of marine autonomous robotics, having created a large number of robotic systems and pioneered their use at sea. He started the MIT Autonomous Underwater Vehicle (AUV) Laboratory at Sea Grant in 1988, led the multi-institutional Autonomous Ocean Sampling Network program through the mid and late 1990s, cofounded Bluefin Robotics Corporation in 1997, and led MBARI's Engineering Department in the 2000s. He has spent considerable time at sea, leading more than 20 AUV expeditions in environments ranging from the waters off Antarctica to the central Arctic. Although his concepts of small, high-performance vehicles, and distributed operations were controversial in the 1990s, many of the component technologies and approaches he championed are widely embraced today.

Dr. Bellingham is a recipient of many awards, including the Lockheed Martin Award for Ocean Science and Engineering, the 14th Robert Bruce Wallace Lecture at MIT, and the Antarctica Service Medal. He served on a number of advisory boards and councils, including the Naval Research Advisory Committee and the Strategic Advisory Group for Battelle's National Security Division.



Christopher A. Scholin received the bachelor's degree in biology from the University of California, Santa Barbara, CA, USA, in 1984, the master's degree in molecular biology and immunology from Duke University, Durham, NA, USA, in 1986, and the Ph.D. degree in biological oceanography from the Massachusetts Institute of Technology/Woods Hole Oceanographic Institution Joint Program, Woods Hole, MA, USA, in 1992.

He is currently the President/CEO of the Monterey Bay Aquarium Research Institute, Moss Landing, CA, USA. His research at MBARI focuses on development of ecogenomic sensors for detecting water-borne microorganisms and environmental DNA using molecular probe technology.

Dr. Scholin serves on the Board of Trustees of the Monterey Bay Aquarium Foundation and the Consortium for Ocean Leadership.