



SUBSEA:
Subtropical Underwater
Biogeochemistry &
Subsurface Export Alliance

Fieldwork Summary
2025 – 2026

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SUBSEA Fieldwork Summary (2025–2026)

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Introduction to SUBSEA Fieldwork Activities

Fieldwork is a major component of SUBSEA research. Our empirical observations are based in both the North Pacific Subtropical Gyre (NPSG) and the South Atlantic Subtropical Gyre (SASG). The subtropical ocean gyres are some of Earth's largest biomes and responsible for globally important fluxes of carbon (C). Warming associated expansion of the low nutrient gyres has led biogeochemical models to predict declining primary production across the subtropics; however, time-series observations document multi-decadal scale increases in primary production. In the NPSG, these trends appear mostly linked to increased productivity in the lower photic waters. The NPSG and SASG ecosystems offer novel opportunities for comparative studies that SUBSEA is leveraging to help improve models describing plankton biogeochemistry of the subtropical gyres.

The photic zone of subtropical oceans have historically been characterized as two independent layers: a well-illuminated upper layer with persistently low nutrients sitting above a dim, subsurface layer hosting a deep chlorophyll maximum (DCM), below which nutrient concentrations increase rapidly (Figure 1). While ocean C cycle predictions increasingly rely on satellite observations, only the thin canopy (< 30 m) of the photic zone is visible to ocean color satellites, leaving the remaining ~100 m of the productive photic zone invisible to remote sensing. Foremost among the SUBSEA research questions are: what processes couple biogeochemistry in these two layers and to what extent does organic matter exported from the well-lit upper layer subsidize plankton productivity in the dim, lower portion of the photic zone?

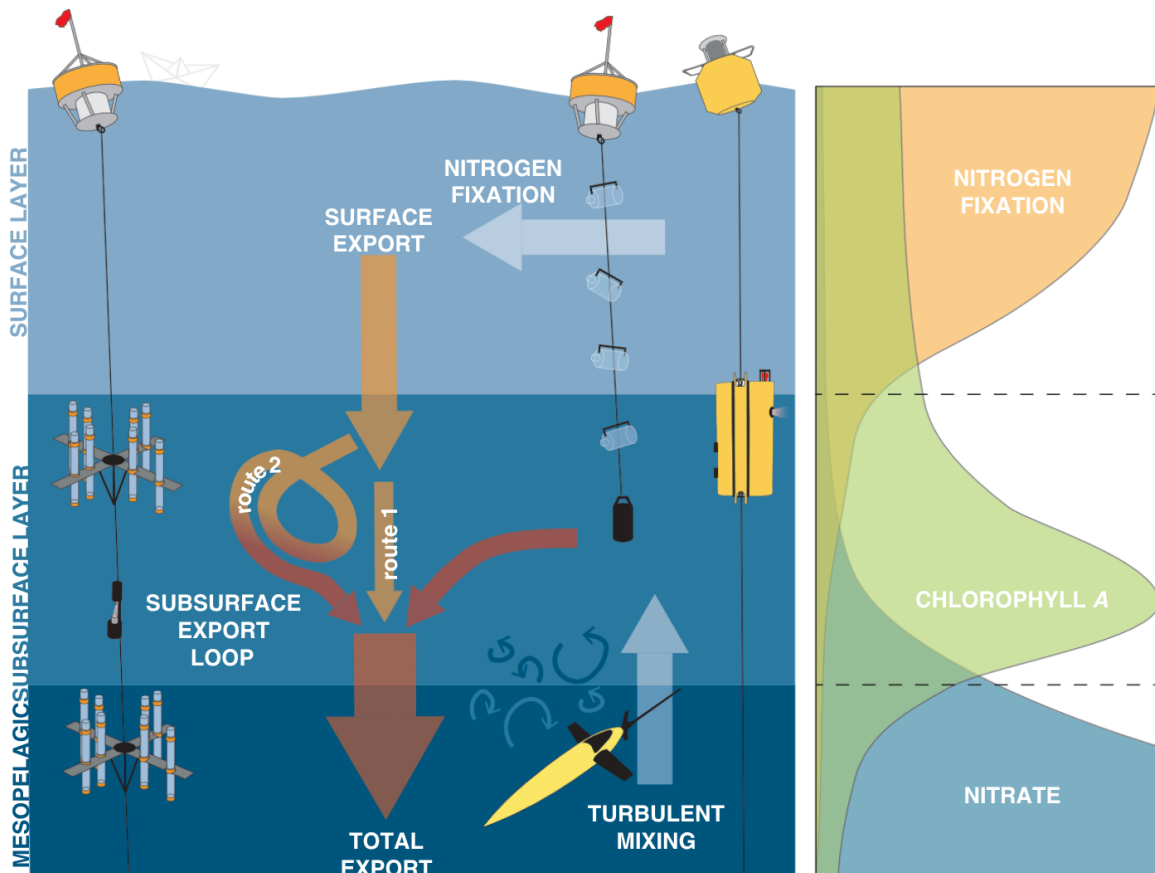


Figure 1: The conceptual two-layer model of the subtropical ocean photic zone. The pathways and magnitudes of N fluxes supporting plankton production and export differ among the two layers, with the well-lit upper photic zone partly supported by N_2 fixation and the lower layer partly supported by turbulent exchange of nitrate from below. SUBSEA has identified two possible routes for material export from the well-lit upper ocean: 1) direct sinking of N_2 fixation supported new production, and 2) N_2 fixation supported new production undergoes remineralization within the photic zone with the resulting recycled nutrients supporting nitrate supported production at depth. SUBSEA is using a suite of technologies and tools to quantify sources of nutrient supply and cycling in the upper ocean. Figure courtesy of Benedetto Barone.

To answer these questions, we have devised a field-based sampling program around identifying processes and quantifying fluxes associated with those processes:

- (1) C, nitrogen (N), phosphorus (P), silicon (Si), and metals export and import fluxes (particulate and dissolved phases) to and from the photic zone,
- (2) Fluxes of nutrients, including metals, within the upper and lower layers of the photic zone, to constrain differential residence times of nutrients in the upper ocean,
- (3) Use of natural abundance and isotope tracer analyses (^{15}N and ^{13}C) of sinking and suspended particles, including compound-specific analyses (e.g., carbohydrates, amino acids), for information on particle transformation processes and to determine rates of N and carbon recycling,
- (4) Gene-based analyses of plankton abundances and distributions for insight into time and space variability in plankton catalyzing key nutrient cycling processes (e.g., N_2 fixation, nitrification).

The resulting suite of field-based measurements is providing an unprecedented view of elemental movement and transformation by biological processes through the upper ocean, and providing novel information on how such processes ultimately determine the magnitude of carbon export into the ocean interior.

In the first two years of the SUBSEA project (2025 & 2026) we successfully completed ten field campaigns (Table 1). Our work in the NPSG has been made possible through close collaboration with the Hawai‘i Ocean Time-series (HOT) program. SUBSEA routinely participates in the near-monthly HOT program cruises to Station ALOHA, providing a time-resolved perspective on coupled interactions between upper and lower photic zone processes. Our on-going sampling on HOT cruises began in February 2025 (HOT 356). Our fieldwork in the SASG was made possible through a dedicated SUBSEA cruise aboard the R/V *Falkor(too)* in February-March 2026. This cruise provided a late summer sampling opportunity across biogeochemical gradients in photic zone phosphate and chlorophyll. Moreover, we were fortunate to sample the decaying stages of a spatially extensive phytoplankton bloom. This cruise provided an opportunity for SUBSEA to test several ideas fundamental to our project, including how variation in phosphate and metal availability influenced N_2 fixation, how variability in the depth of photic zone influenced nutrient recycling, and the time scales over which biomass and fluxes (e.g., production, export, N_2 fixation) are linked within open ocean ecosystems.

Table 1: Summary of SUBSEA fieldwork activities (2025-2026) including HOT and Falkor(too) cruises

	Dates	Departure & Arrival	Vessel	Master of the Vessel	Chief Scientist
HOT 356	February 10 – 14 2025	Honolulu, HI – Honolulu, HI	R/V <i>Sikuliaq</i>	Captain John Hamill	Dan Sadler, University of Hawai‘i at Mānoa
HOT 357	March 10 – 15 2025	Honolulu, HI – Honolulu, HI	R/V <i>Sikuliaq</i>	Captain John Hamill	Dan Sadler, University of Hawai‘i at Mānoa
HOT 358	September 5 – 11 2025	Honolulu, HI – Honolulu, HI	R/V <i>Kilo Moana</i>	Captain Matt Nugent	Fernando Carvalho Pacheco, University of Hawai‘i at Mānoa
HOT 359	October 6 – 10 2025	Honolulu, HI – Honolulu, HI	R/V <i>Kilo Moana</i>	Captain Matt Nugent	Fernando Santiago- Mandujano, University of Hawai‘i at Mānoa
HOT 360	October 22 – 27 2025	Honolulu, HI – Honolulu, HI	R/V <i>Kilo Moana</i>	Captain Matt Nugent	Carolina Funkey, University of Hawai‘i at Mānoa
HOT 361	November 19 – 23 2025	Honolulu, HI – Honolulu, HI	R/V <i>Kilo Moana</i>	Captain James Clifford	Tully Rohrer, University of Hawai‘i at Mānoa
HOT 362	December 19 – 23 2025	Honolulu, HI – Honolulu, HI	R/V <i>Kilo Moana</i>	Captain Carolina Gamarra	Tully Rohrer, University of Hawai‘i at Mānoa
HOT 364	July 25 – 31 2026	Honolulu, HI – Honolulu, HI	R/V <i>Kilo Moana</i>	Captain Carolina Gamarra	Tully Rohrer, University of Hawai‘i at Mānoa
Fkt260303	March 3 – April 6 2026	Rio de Janeiro – Salvador de Bahia, Brazil	R/V <i>Falkor (too)</i>	Captain Peter Reyonolds	Matt Church, University of Montana
HOT 365	August 20 – 25 2026	Honolulu, HI – Honolulu, HI	R/V <i>Kilo Moana</i>	Captain Matt Nugent	Tully Rohrer, University of Hawai‘i at Mānoa

SUBSEA HOT Cruises Cumulative Report

Cruise Summary

SUBSEA field research uses time-resolved ocean observations and comparative process studies to quantify pathways and fluxes associated with the flow of C and nutrients through the upper ocean. We have been fortunate to have strong connections to the Hawai‘i Ocean Time-series (HOT) program, affording opportunities to access time-varying ocean processes in the North Pacific Subtropical Gyre (NPSG). The NPSG is one of Earth’s largest contiguous biomes, and since 1988, the HOT program has provided high quality ocean measurements required for defining variability in ecosystem properties and fluxes in this ecosystem. With nearly four decades of sustained observations at the field site Station ALOHA (22°45'N, 158°W; Figure 2), HOT provides a wealth of contextual measurements and historical data that SUBSEA leverages for our research. In return, SUBSEA has introduced new tools and approaches to Stn ALOHA that collectively are providing novel information on the functioning of the upper ocean ecosystem. Of particular interest to SUBSEA, we have focused our work on discerning coupled interactions between the well-lit but nutrient poor upper photic zone, and the dim but more nutrient enriched lower waters. Hence, SUBSEA’s work aboard the near-monthly HOT cruises is providing new information needed to close nutrient and C budgets and link elemental fluxes to variations in plankton diversity.

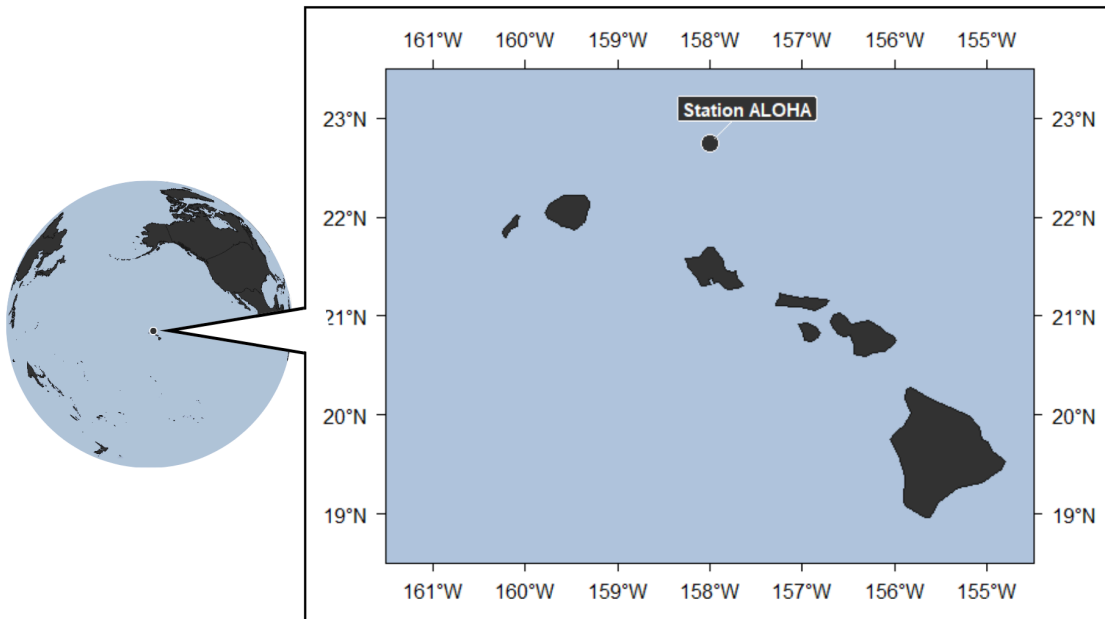


Figure 2: Location of Station ALOHA. Figure courtesy of Katie Coates.

The NPSG fits the classic two layer conceptual model of the gyres, including a low nutrient upper layer sitting atop a light-limited, nutrient-enriched DCM layer. Primary production is tightly coupled to loss processes, making the upper ocean highly retentive with respect to elemental cycling; e.g., <10% of net primary production is exported out of the photic zone. Despite low productivity and export, recurrent summer blooms of N_2 -fixing cyanobacteria are regular features of the NPSG

ecosystem. These blooms have an important influence on upper ocean biogeochemistry and particle export. Most often these blooms involve diatom–diazotroph associations (Figure 3). Export associated with these blooms appears rapid, occurring in transient pulses of particulate material out of the photic zone during a narrow window of time in the late-summer. Isotopic, elemental, imaging, and molecular analyses all indicate this export pulse is likely largely fueled by N supplied from N_2 fixation. In addition to these summer blooms that are largely relegated to the well-lit layer of the photic zone, there are seasonally recurrent blooms observed in the dimly lit lower photic zone waters as well. In particular, the DCM layer undergoes annual spring blooms, driven by increasing solar energy input. These seasonal increases in light availability to the lower photic zone trigger an expansion of plankton biomass associated with chlorophyll maximum layer, consuming nitrate in the lower photic zone in support of new production.

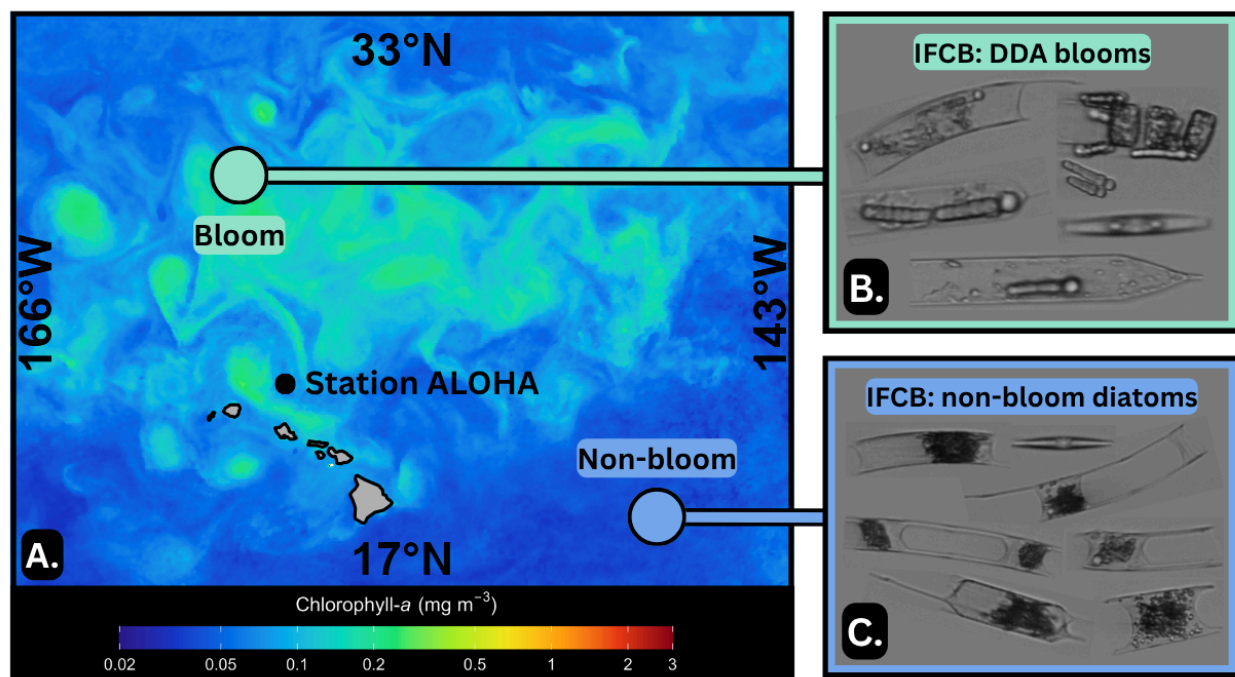


Figure 3. Diatom–diazotroph association (DDA) bloom conditions and associated changes in particle export at Station ALOHA. (A) Near-surface ocean chlorophyll-a concentrations during a DDA bloom sampled during a Hawai‘i Ocean Time-series (HOT) cruise 352 (16–20 August 2024). Station ALOHA is defined as a 6-nautical-mile-radius circle centered at 22°45'N, 158°W. Chlorophyll-a derived from the NOAA CoastWatch Data Portal using the gap-filled DINEOF 2-km daily product averaged over the cruise period. Insets show Imaging FlowCytobot (IFCB) DDA taxa (B), and representative non-bloom diatom assemblages (C) at Station ALOHA during non-bloom conditions. Figure courtesy of Katie Coates.

SUBSEA routinely participates in the near-monthly HOT program cruises to Station ALOHA, providing a time-resolved perspective on coupled interactions between upper and lower photic zone processes. Our on-going sampling on HOT cruises began in February 2025 (HOT 356) and continues to date (as of this writing August 2026, HOT 365).

SUBSEA Measurements & Data Collected at Stn ALOHA

SUBSEA conducts 12 main activities during HOT cruises, including 10 different deployments (Table 2). These activities are as follows:

- (1)** Deployment of a free-drifting SUBSEA sediment trap array (ST). The array includes 12 particle interceptor traps per cross with 5 crosses in total positioned at 75 m, 110 m, 150 m, 225 m, and 300 m. Samples collected include:
 - (a)** Particulate C, N, P, Si, and inorganic C
 - (b)** TDN, TDP, nitrate+nitrite
 - (c)** DNA
 - (d)** Bulk and compound specific C and N isotopes
 - (e)** Trace metals
 - (f)** Particle imaging by In Situ Flow Cytobot (IFCB)
- (2)** Estimation of mixed layer gross O₂ production and respiration based on deployment of dual optode O₂ sensors on SUBSEA ST. Mixed layer O₂ fluxes are estimated based on diel changes in O₂ together with estimates of air-sea gas exchange. Dual optodes are deployed on every SUBSEA ST.
- (3)** Deployment of a 24 hour, free-drifting in-situ array (A), used to quantify:
 - (a)** Upper ocean (0-125 m) rates of N₂ fixation based on ¹⁵N₂ isotope tracer incubations. Volumetric rates of ¹⁵N₂ fixation are measured at 6 discrete depths (5, 25, 45, 75, 100, 125 m) where whole seawater is amended with ¹⁵N₂ tracer and incubated in situ for 24 hrs. Importantly, for SUBSEA sampling, we are collecting particles labeled with ¹⁵N on filters and we are routinely quantifying dissolved N solutes labeled with ¹⁵N.
 - (b)** Primary production via photosynthetic assimilation of ¹³C (24 hrs). A dawn to dusk productivity array using ¹⁴C-bicarbonate assimilation as a metric of primary production is routinely deployed by the HOT program, which targets the same 6 discrete depths (5, 25, 45, 75, 100, 125 m) as the SUBSEA PA. The measurement of ¹³C-bicarbonate assimilation as a metric of primary production during 24-hour incubations allows for the comparison between photoperiod ¹⁴C carbon fixation estimates and the daily ¹³C carbon fixation estimates, which provides a means to estimate nighttime respiration.
 - (c)** Rates of nitrification, including ammonia and nitrite oxidation. ¹⁵N₂ isotopic tracers are measured at 7 discrete depths (25, 45, 75, 100, 125, 150, 200 m).
- (4)** Quantification of vertical gradients in dissolved and particulate nutrient stocks using CTD casts. SUBSEA sampling is largely restricted to the upper 400 m of the water, including highly resolved sampling (~5 m) around the DCM layer. Samples collected include:
 - (a)** Particulate C and N, which are collected from 12 discrete depths, most intensively around the DCM layer, for subsequent analyses of bulk and compound specific stable isotopes (¹³C and ¹⁵N).

- (b) Dissolved nutrients, including total dissolved N and P, ammonium, nitrate, nitrite, silicate and soluble reactive P, which are collected from 24 discrete depths.
 - (c) N and O isotopes ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$), which are subsequently determined from pools of nitrate+nitrite and nitrate-only and N isotopes from total dissolved N. These analyses provide information needed for constraining sources of N supporting biomass in the photic zone and insight into N cycle processes based on isotope fractionation.
 - (d) DNA, collected from 12 discrete depths, used for subsequent analyses of distributions of N_2 fixing microorganisms (based on quantification of *nifH* gene abundances) and ammonia oxidizing archaea (based on quantification of *amoA* gene abundances).
- (5) Deployment of a trace metal clean CTD rosette (TMC) to collect and analyze samples for quantifying concentrations and distributions of dissolved and particulate metals.
 - (6) Deployment of Hyperpro radiometer (HP) to collect vertical profiles of upper ocean bio-optical properties. The instrument is deployed in-situ and measures apparent optical properties for estimation of water leaving radiance, remote sensing reflectance, solar energy flux, and photosynthetically active radiation.
 - (7) Imaging of near-surface ocean plankton using In Situ Flow Cytobot (IFCB) for determination of particle and cell abundances, sizes, and spatial distributions. This instrument is plumbed to the shipboard seawater intake system for continuous measurements and imaging of plankton in the near-surface ocean. In addition, discrete samples, including those subsampled from the SUBSEA sediment trap collections, are analyzed for information on sinking particle morphology.
 - (8) Deployment of a free-drifting Wirewalker profiler (WW) in the upper ocean (0-400 m). The WW is deployed for ~3 days, providing a highly resolved dataset on high frequency temporal variation in ocean physics and biogeochemistry.
 - (9) Deployment of a Seaglider (SG) equipped with a refurbished sensor for quantifying microstructure turbulence. These measurements will be used to estimate turbulent nutrient exchange at the base of the photic zone, which when coupled with high vertical resolution sampling of nutrients can be used to constrain upper ocean N and phosphorus mass balances.
 - (10) Deployment of Vertical Microstructure Profiler (VMP). The microstructure profiler (Rockland VMP-250) was deployed for additional estimates of microstructure turbulence. The instrument was deployed while the vessel was slowly transiting and provided additional estimates of turbulence for closing nutrient budgets.
 - (11) Deployment of high-volume McLane pumps (MP) for collection of size fractionated particulate matter. Dual pump heads are used for collection of organics (Close), and metals and omics (Hawco and Church). Filters for organics include: GF75 (nominal 0.3 μm), GF/C (nominal 1.2 μm), PETE membrane (5 μm), and 53 μm Nitex. Filters for metals and omics are: 0.2 μm , 1.2

μm , 5 μm Supor PES filters, and 53 μm Nitex. Target features include: base of photic zone and trap collection depth (150 m), DCM and trap collection depth (110 m), well-lit photic zone (25 m), upper mesopelagic and trap collection depth (225 m), lower photic zone (135 m), and mid-photoc zone and trap collection depth (75 m).

- (12) Facilitate One-OBVI training (HOT 360). Central to OBVI fieldwork activities in the South Atlantic is the development of a core measurement program, termed One-OBVI. This project is led by Dr. Pierre Marrec at the University of Rhode Island and is designed to provide spatially resolved biogeochemical observations from each of the OBVI cruises. Many of the core One-OBVI measurements and sampling activities rely on protocols developed by the time series programs HOT and BATS. During the initial development of the core measurement program, Dr. Marrec contacted SUBSEA regarding opportunities for training as part of our sampling on HOT cruises. In October 2025, Dr. Marrec joined SUBSEA investigators aboard a HOT cruise, an opportunity that provided training in ocean biogeochemical sampling and afforded an opportunity for sample collection for subsequent comparative analyses.

Table 2: Summary of SUBSEA deployments on HOT cruises. X's denote on which cruise instruments were deployed.

CRUISE #	ST	A	CTD	TMC	HP	IFCB	WW	SG	VMP	MP
HOT 356	X	X	X	X	X	X	X			
HOT 357	X	X	X	X	X	X	X			
HOT 358	X	X	X	X	X	X	X	X	X	
HOT 359		X	X	X	X	X	X			
HOT 360	X	X	X		X	X			X	X
HOT 361		X	X	X	X			X		
HOT 362		X	X		X					
HOT 364	X	X	X		X	X			X	
HOT 365	X	X	X	X				X		X

SUBSEA Participants on HOT cruises

Table 3: List of SUBSEA participants on HOT cruises (2025-2026)

Participant	Position, Affiliation	Role	SUBSEA-HOT cruises
Mattia Da Fieno	Undergraduate Student & Research Assistant, University of Hawai'i	Assist with core SUBSEA high-vertical resolution water column sampling	356, 357, 359, 360, 361, 362, 364, 365
Tully Rohrer	Research Associate, University of Hawai'i	Technical support for Wirewalkers, bio-optical instrumentation, N ₂ fixation measurements	356, 357, 359, 360, 362, 364, 365
Benedetto Barone	SUBSEA Co-PI, University of Hawai'i	Oversee Wirewalker operations, assist with sediment trap processing, and SUBSEA high-vertical resolution water column sampling	356, 357, 358
Nick Hawco	SUBSEA Co-PI, University of Hawai'i	Oversee trace metal sampling, assist with sediment trap processing, and SUBSEA high-vertical resolution water column sampling	356
Hunter Adams	SUBSEA Research Associate, University of Hawai'i	Assist with Wirewalker operations, sediment trap processing, and SUBSEA high-vertical resolution water column sampling	356, 357, 358, 360, 361, 364, 365
Alexis Floback	SUBSEA Post-doc, University of Hawai'i	Oversee trace metal sampling, assist with sediment trap and high vertical resolution sampling	357, 358, 359, 361, 365
Raquel Flynn	SUBSEA Post-doc, University of Cape Town	Oversee analyses specific to assessing rates of N cycling processes (ammonia and nitrite oxidation; ammonia, nitrite and nitrate assimilation, ammonia regeneration)	358

Calvin Swart	SUBSEA graduate student, University of Cape Town	Oversee analyses specific to assessing rates of N cycling processes (ammonia and nitrite oxidation; ammonia, nitrite and nitrate assimilation)	358
Rhea Foreman	SUBSEA Researcher, University of Hawai'i	Sediment trap processing, including an experiment to examine influence of pre-screening particle interceptor traps; assist with core SUBSEA sampling	360, 364
Wing Kwan (Esther) Mak	SUBSEA Post-doc, University of Montana	Conduct N amendment experiment to examine interaction between light and N substrate availability on plankton growth in the DCM layer	357, 360, 365
Hilary Close	SUBSEA Co-PI, University of Miami	Oversee McLane pump deployments and assist with processing high vertical resolution CTD particulates	360, 365
Jason Freisen	SUBSEA Graduate student, University of Miami	Assist with McLane pump deployments and processing high vertical resolution CTD particulates	360
Lauren Manck	SUBSEA Post-doc, University of Montana	Assist with sediment trap and high vertical resolution sampling	357
Jamison Ward	SUBSEA technician, University of Montana	Assist with sediment trap and high vertical resolution sampling	357
Pierre Marrec	OBVI Researcher, University of Rhode Island	Receive training on core HOT program biogeochemical sample collections for One-OBVI sampling efforts.	360

Preliminary Research Highlights

Our work at Stn ALOHA is providing novel perspectives on the functioning of the two layer photic zone habitat. Among our primary objectives is to better constrain the pathways and flows of elements through the upper ocean. Doing so will improve elemental mass balances and assessment of C and nutrient import and export in the open ocean. Toward this objective, our core work on HOT cruises

includes a combination of high quality nutrient determinations, sediment trap export fluxes, Seaglider based microstructure turbulence estimates, and near-monthly quantification of N_2 fixation. Collectively, SUBSEA has assembled arguably the most robust suite of time-resolved measurements needed for establishing upper ocean mass balances, with a particular focus on N, anywhere in the world's oceans. In the highlights below, we describe our work toward quantifying sources and sinks of nutrients to the upper ocean, including via physical and biological processes. Preliminary observations for our work at Stn ALOHA are highlighted below for the following SUBSEA themes: (1) physical & biological controls on biogeochemistry, (2) nutrient distributions & processes, and (3) export.

(1) Physical & Biological Controls on Biogeochemistry:

We have successfully deployed and recovered a free-drifting Wirewalker profiler on multiple HOT program cruises. This wave-powered profiler drifts for multiple days (3-4 days) through the upper 400 m of the water column while the research vessel conducts work at Stn ALOHA. The platform is equipped with sensors to measure dissolved O_2 , fluorescence (chlorophyll and CDOM), transmissometry, temperature, conductivity, backscatter, and irradiance. Below we provide highlights from 3 HOT cruises (356, 357, 358) where SUBSEA deployed the Wirewalker to demonstrate the rich temporal context provided by use of this platform (Figures 4-6). Use of this instrumented platform is providing an unprecedented look at how high frequency variations in habitat structure (e.g., solar energy input, isopycnal heaving) couple or uncouple plankton biology and biogeochemistry in the upper and lower photic of the photic zone. For example, on HOT 356 (February 2025), the surface mixed layer was exceptionally deep (>130 m), penetrating the chlorophyll maximum layer and entraining chlorophyll into the mixed layer (Figure 4). The Wirewalker time series provided an unprecedented view into the time scales over which this mixed layer fluorescence signal persisted. On HOT 357 (March 2025), the Wirewalker time series revealed a mixed layer that was actively shoaling, with increases in mixed layer chlorophyll fluorescence associated with the onset of this stratification (Figure 5). Moreover, the DCM was a broad and intense feature situated between 100 m and 150 m, and it coincided with elevated beam attenuation consistent with increased particle load. On HOT 358 (September 2025), the Wirewalker time series revealed a decoupling in the particle maximum and the chlorophyll maximum, with beam attenuation (a proxy for particle load) elevated in the surface mixed layer, with the top of the chlorophyll maximum sitting nearly 50 m deeper (Figure 6).

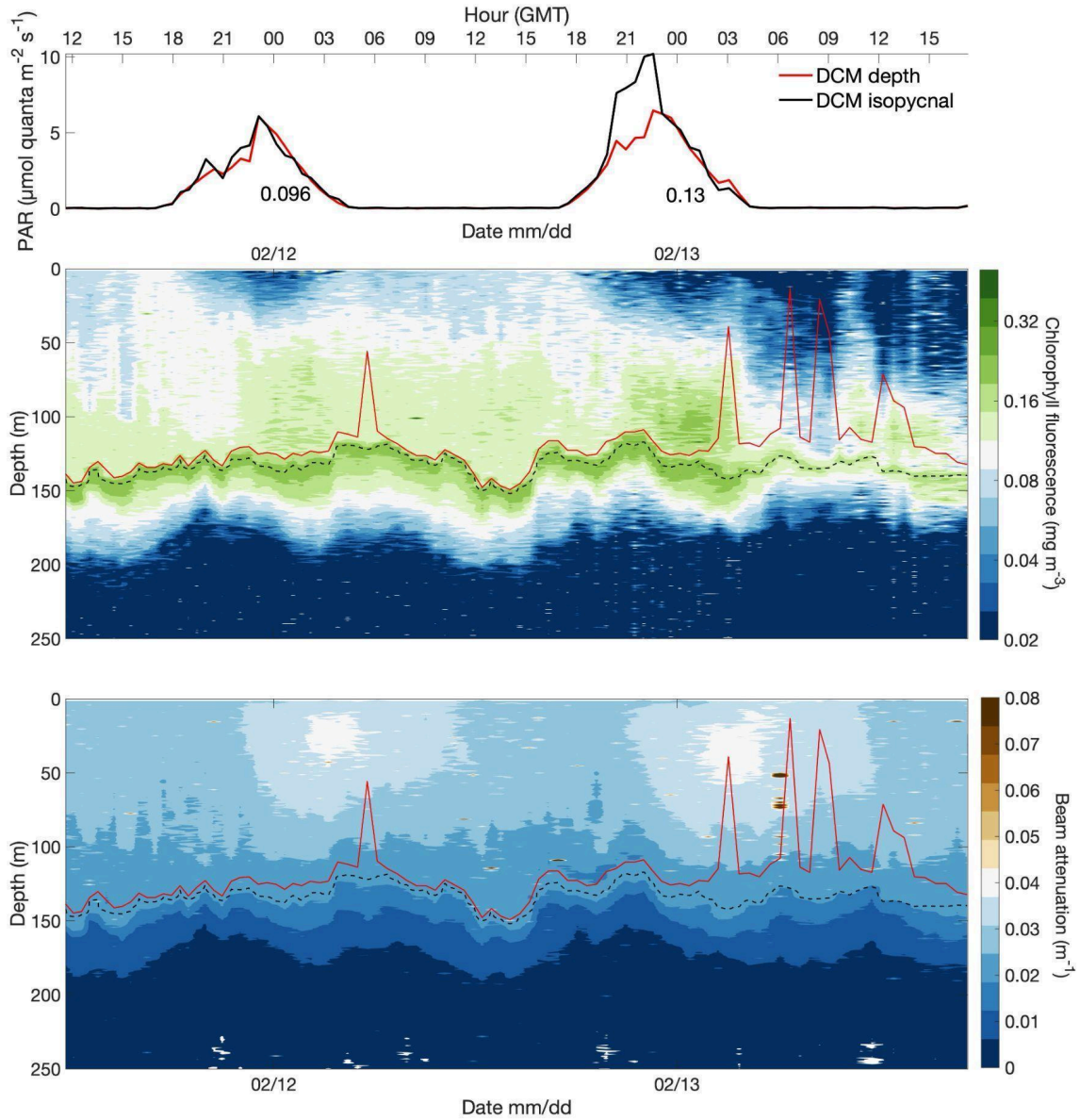


Figure 4. Bio-optical observations measured during HOT 356 (February 2025) by the wave-powered drifting profiler (Wirewalker) at Station ALOHA. This cruise was characterized by unusually deep surface mixing that eroded the deep chlorophyll layer. The DCM had low fluorescence and beam attenuation, and the DCM was isolated to a deep layer with low light intensity. The top panel depicts the time series of photosynthetically available radiation on the median depth of the DCM and on the median isopycnal surface of the DCM. Numbers below the daily peaks indicate the integrated daily irradiance on the DCM isopycnal with units of $\text{mol photons m}^{-2} \text{ d}^{-1}$. The center panel depicts the time series of chlorophyll fluorescence in the upper 250 m of the water column. The bottom panel depicts the time series of beam attenuation in the upper 250 m. The dashed black line and red line in the lower two panels depict the median DCM isopycnal and the base of the surface mixed layer (based on a 0.03 kg m^{-3} offset from the potential density at 10 m depth), respectively. Figure courtesy of Benedetto Barone.

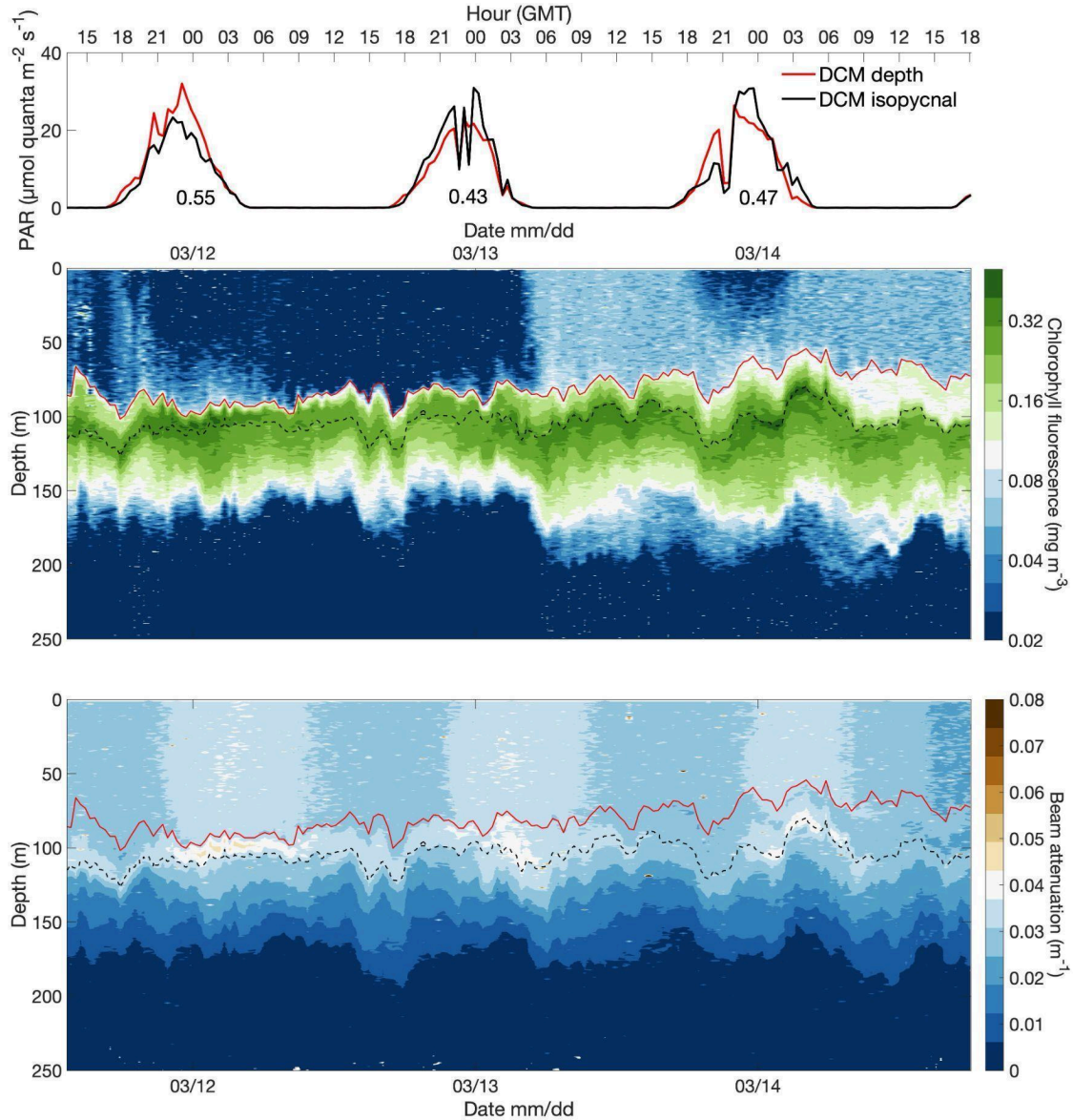


Figure 5. Bio-optical observations measured by a wave-powered drifting profiler (Wirewalker) in March 2025 at Station ALOHA, during cruise HOT-357. Surface mixing extended to approximately 100 m depth at the beginning of the cruise, but the base of the mixed layer became shallower towards the end of the cruise. The DCM was also a maximum in beam attenuation, and it was observed in a layer with relatively elevated light intensity. The top panel depicts the time series of photosynthetically available radiation on the median depth of the DCM and on the median isopycnal surface of the DCM. Numbers below the daily peaks indicate the integrated daily irradiance on the DCM isopycnal with units of $\text{mol photons m}^{-2} \text{d}^{-1}$. The center panel depicts the time series of chlorophyll fluorescence in the upper 250 m of the water column. The bottom panel depicts the time series of beam attenuation in the upper 250 m. The dashed black line and red line in the lower two panels depict the median DCM isopycnal and the base of the surface mixed layer (based on a 0.03 kg m^{-3} offset from the potential density at 10 m depth), respectively. Figure courtesy of Benedetto Barone.

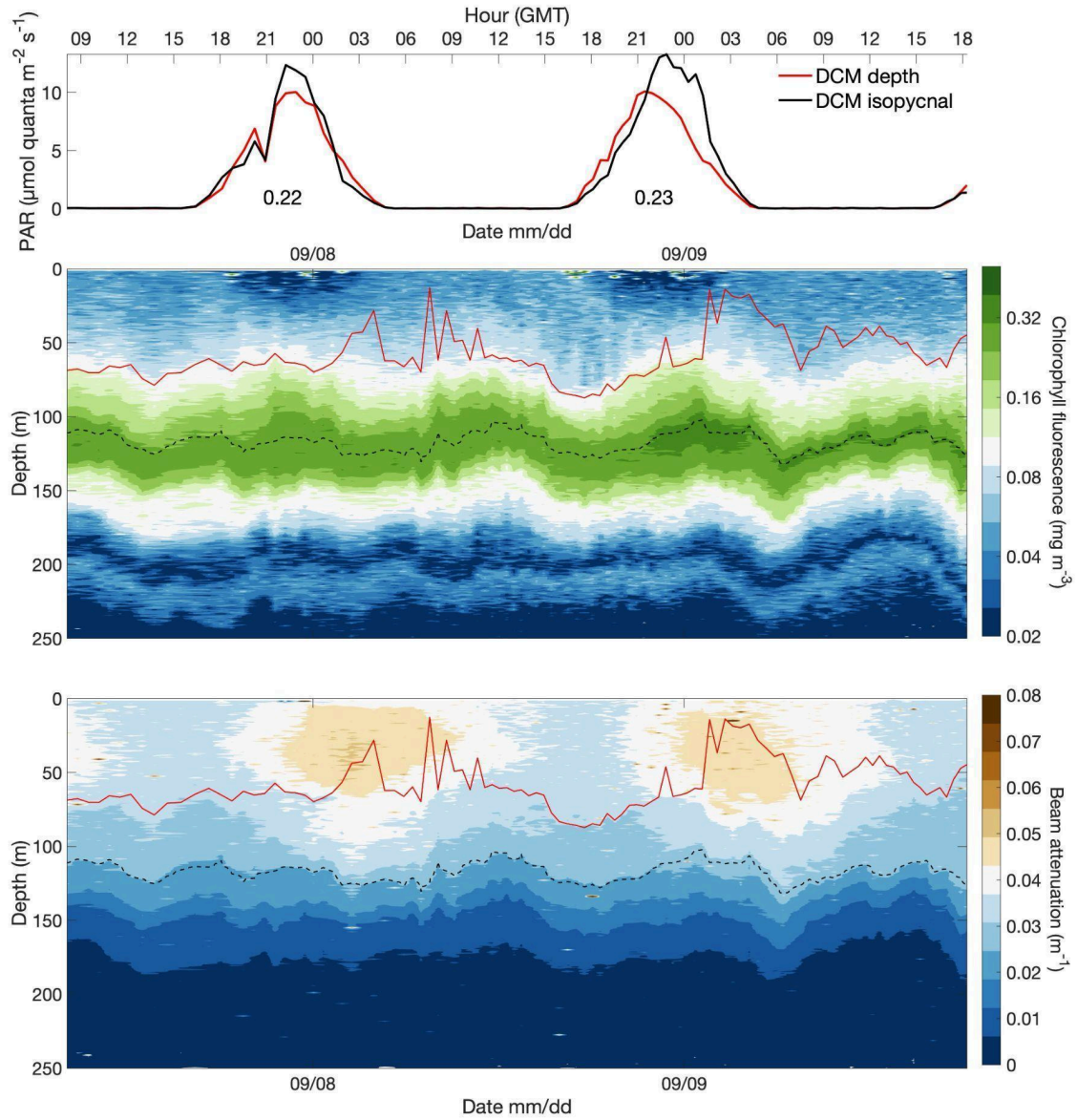


Figure 6. Bio-optical observations measured by a wave-powered drifting profiler (Wirewalker) in September 2025 at Station ALOHA, during cruise HOT-358. The maximum beam attenuation was found in the upper 50 m, vertically decoupled from the DCM. The top panel depicts the time series of photosynthetically available radiation on the median depth of the DCM and on the median isopycnal surface of the DCM. Numbers below the daily peaks indicate the integrated daily irradiance on the DCM isopycnal with units of $\text{mol photons m}^{-2} \text{ d}^{-1}$. The center panel depicts the time series of chlorophyll fluorescence in the upper 250 m of the water column. The bottom panel depicts the time series of beam attenuation in the upper 250 m. The dashed black line and red line in the lower two panels depict the median DCM isopycnal and the base of the surface mixed layer (based on a 0.03 kg m^{-3} offset from the potential density at 10 m depth), respectively. Figure courtesy of Benedetto Barone.

Additional insights into physical-biogeochemical coupling stem from SUBSEA deployment of a Seaglider equipped with sensors to measure temperature, conductivity, pressure, dissolved O_2 , fluorescence, backscatter (470 nm and 700 nm), and of particular relevance to SUBSEA, a sensor for estimating microstructure shear. This latter sensor enables calculation of diapycnal diffusivity, which when coupled with information on nutrient concentration gradients obtained from our high vertical

resolution CTD water sampling (see Nutrient Distributions & Processes below), provide robust assessment of nutrient import and export into and out of the photic zone. Internal tides and near-inertial wind energy input support diapycnal mixing at Stn ALOHA; however, such mixing is generally very weak. Our glider-based determinations of diapycnal diffusivity point to very low turbulent supply of nutrients to the photic zone (Figure 7), suggesting vertical resupply of nitrate and nitrite from interior waters is weak and that nitrate-fueled new production within the DCM could stem from remineralized N within the photic zone waters. Our glider time-series is providing an unprecedented view of spatio-temporal variability in physical-biological coupling in this relatively well-studied ecosystem.

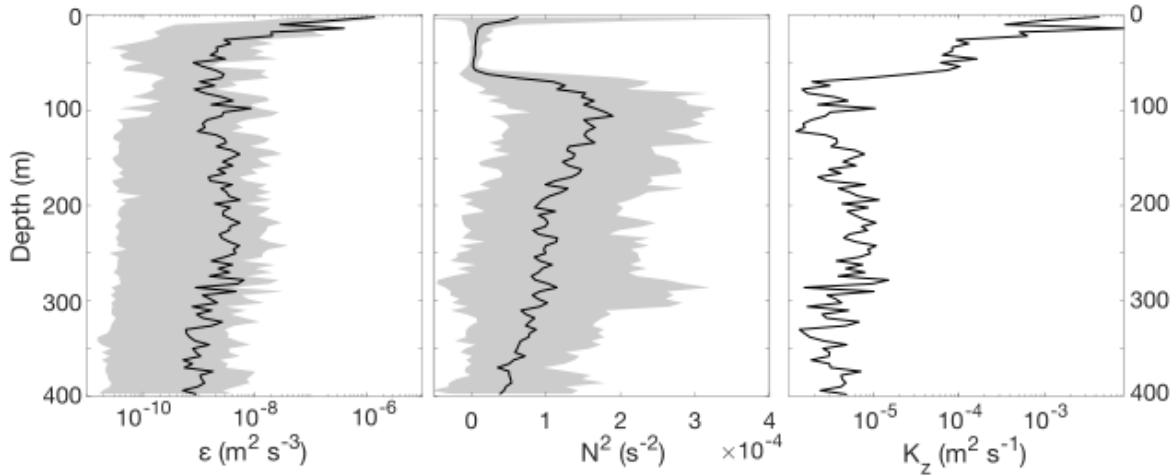


Figure 7: Seaglider based estimates of microstructure shear (panel A), buoyancy frequency (panel B) and diapycnal diffusivity (panel C) at Station ALOHA, HOT 358 (September 2025). Diapycnal diffusivity was calculated from 67 Seaglider vertical profiles. Figure courtesy of Benedetto Barone.

SUBSEA is also leveraging an on-going time series of N₂ fixation rate measurements at Stn ALOHA (Figure 8) to quantify biologically sourced N supply to the upper ocean. We have begun quantifying fluxes of fixed N that are rapidly transferred into the dissolved phase over course of the N₂ fixation incubation. Such transfer could occur via cell excretion of organic N or ammonia, or catalyzed by various food web processes such as grazing. We find that approximately 30% of the N₂ fixed during a 24 hr incubation can be shuttled into the dissolved N pool. Such work continues to emphasize the importance of N₂ fixation as a source of new N to this ecosystem and points to underestimation of N₂ fixation by traditional isotopic tracer methods that consider only particle associated fixation.

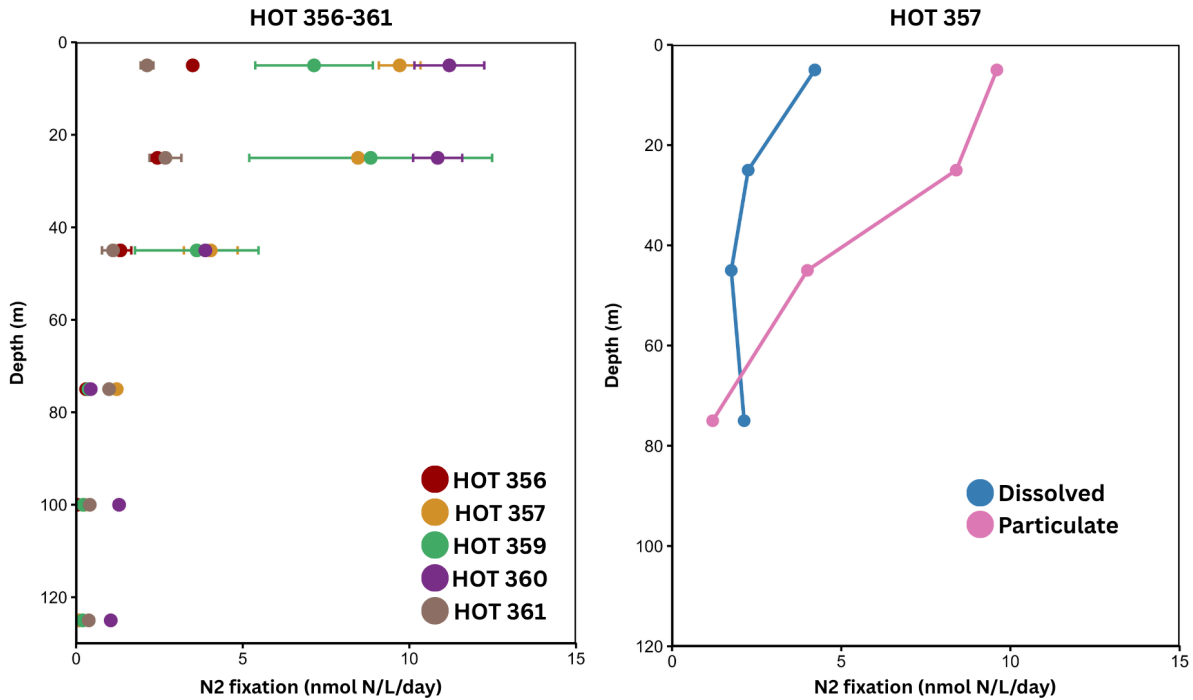


Figure 8: Rates of N_2 fixation (right panel) during 5 HOT cruises where SUBSEA sampled in 2025 and vertical profile of N_2 fixation associated with particulate and dissolved phases. N_2 fixation rate measurements conducted on a free-drifting array with incubations over a 24 hr period. Figure courtesy of Angelique White & Rhea Foreman.

(2) Nutrient Distributions & Processes:

SUBSEA initiated sampling of high vertical resolution CTD cast for subsequent analyses of dissolved and particulate nutrient concentrations through the upper 400 m of the water. Dissolved nutrients sampled at 24 discrete depths, with highly resolved sampling around the DCM layer. Samples collected included total dissolved N and P, ammonium, nitrite, nitrate, silicate, and soluble reactive P. Measurements of ammonium and nitrite concentrations have not been systematically conducted at Stn ALOHA, so these analyses are providing new insights into cycling of these N pools. In addition, the high vertical resolution profiles are allowing robust quantification of vertical gradients in nutrient elements needed for estimating export of organic matter out of the photic zone and import of inorganic nutrients into the photic zone. As expected, in the well-lit portion of the photic zone, dissolved organic N is the dominant pool of N, with inorganic N substrates (nitrate, nitrite, and ammonium) collectively comprising <1% of the total dissolved N pool (Figure 9). With increasing depth, nitrate+nitrite pools increase in concentration, becoming dominant pools of N toward the base of the photic zone. Importantly, nitrite comprised upwards of 80% of the nitrate+nitrite pools at the top of the nutricline (Figure 9), emphasizing the potential importance of this largely overlooked source of N to the lower photic zone.

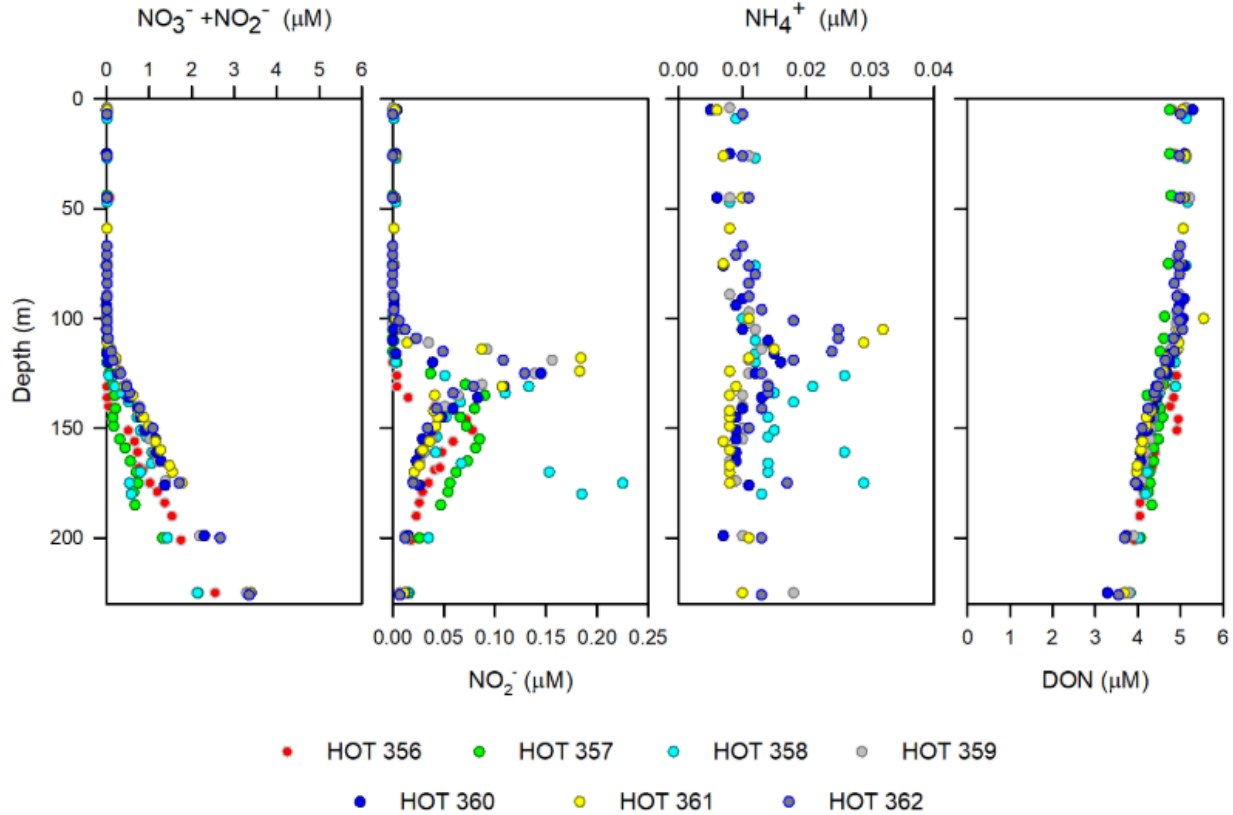


Figure 9: SUBSEA analyses of nitrogenous nutrients at Stn ALOHA. On each HOT cruise, SUBSEA collects high vertical resolution profiles for subsequent determinations of dissolved and particulate nutrient stocks. The resulting profiles are used to calculate vertical gradients in nutrients, which when coupled with estimates of microstructure turbulence (Seaglider and VMP measurements) provide constraint on import and export of nutrients from the photic zone. Figure courtesy of Rhea Foreman.

We are also collecting samples for subsequent analyses of N isotopes ($\delta^{15}\text{N}$) associated with nitrate, nitrite, and total dissolved N. Accompanying the steep concentration gradients in nitrate+nitrite, the $\delta^{15}\text{N}$ signature of both nitrate and total dissolved N pools increases below the photic zone (Figure 10). The total dissolved N $\delta^{15}\text{N}$ in the well-lit upper ocean, where this pool is dominated by dissolved organic N, is similar to the $\delta^{15}\text{N}$ signature of nitrate in the lower photic zone. These findings imply that nitrification depends on an isotopically light source of N, for example from either N_2 fixation and heavily recycled ammonium or organic N.

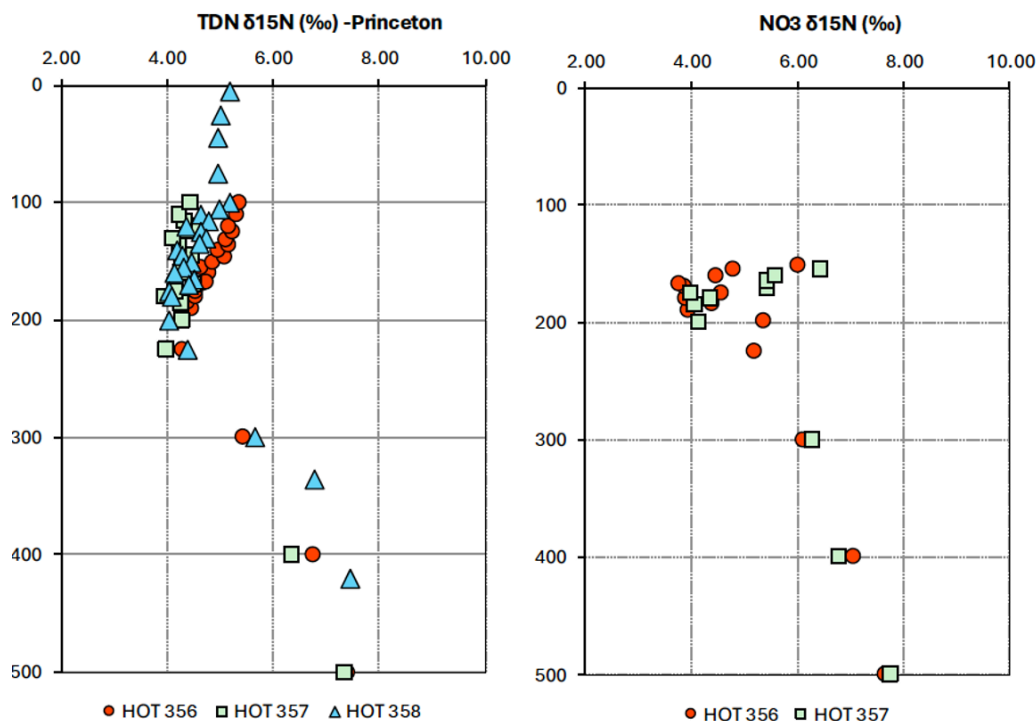


Figure 10: $\delta^{15}\text{N}$ of (a) total dissolved N (TDN) pool and (b) NO_3^- only from samples collected on the high resolution casts from HOT 356 (red circles), HOT 357 (green squares) and HOT 358 (blue triangles). Note that data is not shown for the $\delta^{15}\text{N}$ of NO_3^- for HOT 358. Figure courtesy of Raquel Flynn.

In addition to sampling for inorganic and organic dissolved nutrients, we also collected particulate carbon and N samples from the high vertical resolution CTD profiles. Particulates are collected from 12 discrete depths, most intensively around the DCM layer, for subsequent analyses of bulk stable isotopes (^{13}C and ^{15}N). Particulate organic C concentrations demonstrate depth and time variability, with peak concentrations at the chlorophyll maximum in March, a pattern consistent with the previously described spring bloom in the lower photic zone. There are also strong shifts in the bulk ^{13}C signature of suspended particles, with particles enriched in ^{13}C in the surface ocean and depleted in the chlorophyll maximum (Figure 11). This pattern has previously been observed in other subtropical gyres and likely reflects a combination of light limited photosynthetic production and the vertical shift in the photosynthetic community from smaller cyanobacteria in the well-lit waters to somewhat larger eukaryotes in the lower photic zone.

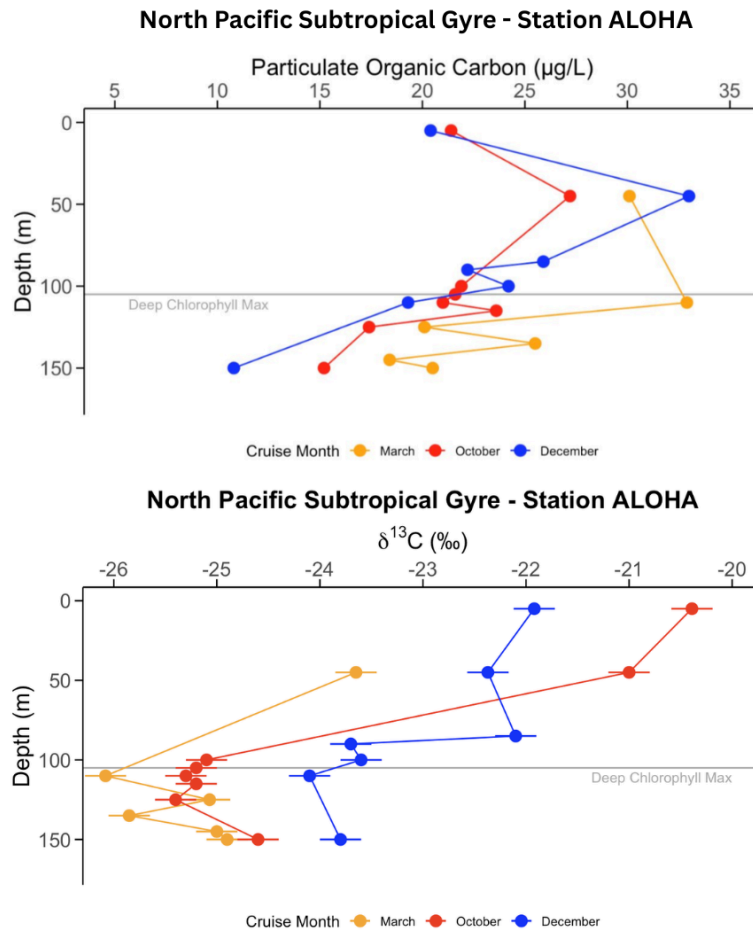


Figure 11. Seasonal comparison of particulate organic C and bulk $\delta^{13}\text{C}$ -POC depth profiles at Stn ALOHA. Figure courtesy of Close Lab.

(3) Export:

SUBSEA fabricated a sediment trap array that is used for HOT cruises and our work in the South Atlantic. The array was designed to enable estimation of export fluxes at 5 discrete depths, using VERTEX-style particle interceptor traps, with collection depths located above, within, and below the chlorophyll maximum layer (75, 110, 150, 225, 300 m). The resulting depth-resolved flux estimates are providing new information on the magnitude and shape of the C, N, P, Si, and metal flux attenuation within, and below, the photic zone. Unexpectedly, our emerging time series from Stn ALOHA is revealing elevated C and N fluxes near the base of the well-lit upper ocean, where fluxes appear upwards of 2-fold greater than fluxes at the base of the photic zone (Figure 12). The apparent vertical attenuation in flux within the photic zone challenges mass balances of key elements; in particular, such results suggest that either remineralized byproducts (or recycled production) associated with this flux attenuation are retained within the upper ocean over time scales shorter than our monthly sampling resolution, or that advective processes spatially decouple the two layers of the photic zone.

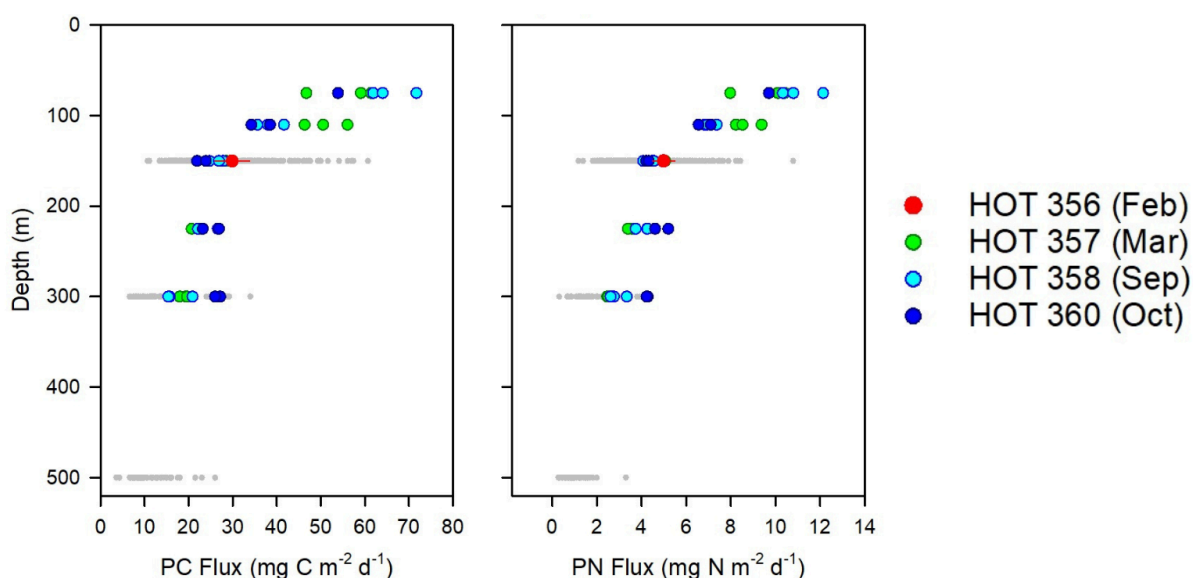


Figure 12: Vertically resolved particulate C and N fluxes on three HOT cruises where SUBSEA deployed particle interceptor traps. The resulting flux attenuation is comparable to historical HOT program flux estimates at 150 m, 300 m, and 500 m (grey symbols). SUBSEA's sampling reveals elevated fluxes in the photic zone which challenges elemental mass balances for this ecosystem. Figure courtesy of Matthew Church.

In addition to our work quantifying photic zone particle export and flux attenuation, we have also examined the contribution of N and P in the dissolved phase of the sediment trap solutions, presumably due in part to particle solubilization on entering the sediment traps (Figure 13). Surprisingly, we observe a large fraction of the N and P within sediment traps gets partitioned into the solute phase. In the case of N, inclusion of this dissolved N increases the implied export by up to 60%, implying a substantial underestimate of export when considering only the particulate phases of elements.

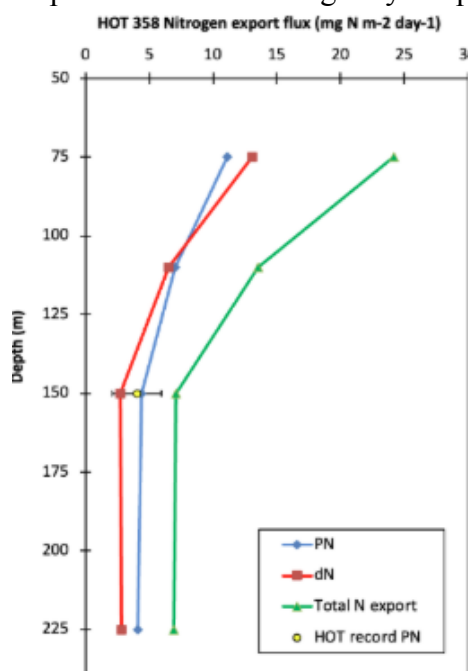


Figure 13: Export of N during HOT 358 that accounts for particulate N (PN), as well as the dissolved N (dN) found in trap brines. Only PN is typically quantified, which results in an underestimation of total export by a factor of ~2. Our preliminary estimates suggest this deficiency in reported export values is also true for C and possibly even more severe for P. The averaged HOT data for PN export at 150 m ($\pm 1\sigma$) is shown for reference. Figure courtesy of Rhea Foreman.

SUBSEA routinely collects vertically resolved profiles to quantify particulate and dissolved trace metal concentrations, together with analyses of trace metal fluxes from sediment traps. This work suggests the main source of Fe to the NPSG stems from dust deposition, of which only a small proportion is labile (bioavailable), while most remains in the refractory phase (Figure 14, a). Below the DCM, the proportion of sinking particles containing labile Fe increases, likely reflecting particle disaggregation or inputs of new sinking particles from the DCM, in contrast to the export of macronutrients (Figure 14, b). In the well-lit portion of the photic zone (0-75 m), Fe appears to cycle over longer time scales (~4 months) compared to the lower photic zone and mesopelagic waters (~2-3 months). Intriguingly, similar calculations for N imply much shorter upper ocean residence times (1-2 weeks), suggesting differential retention of elements in the photic zone, presumably reflecting in part differences in biological demand for nutrients.

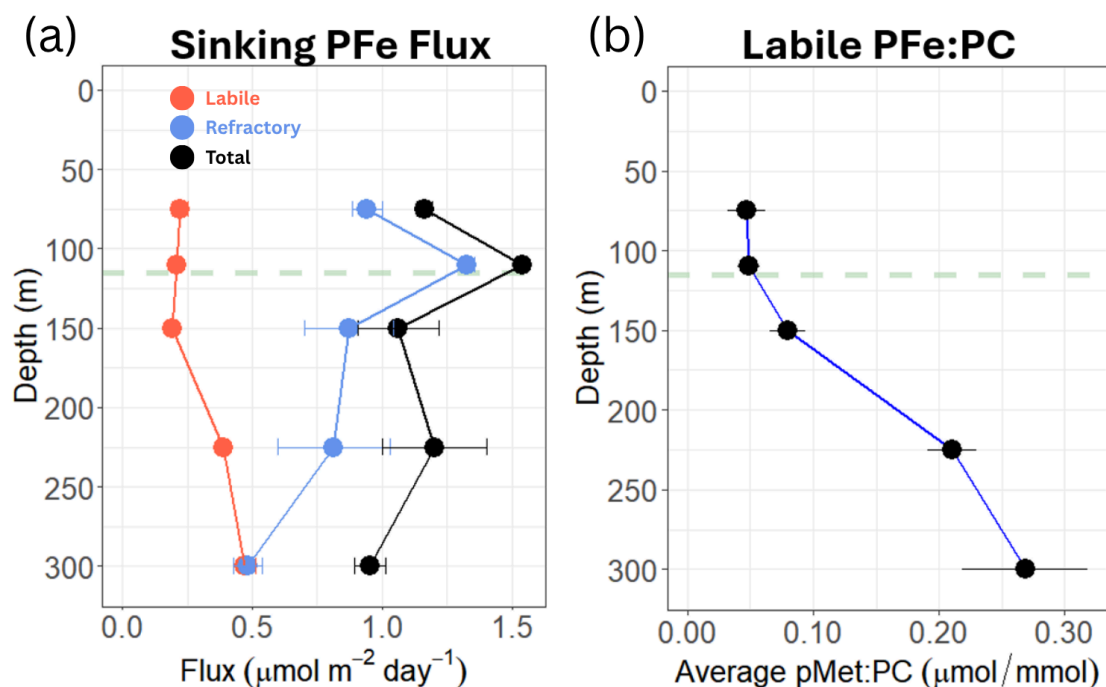


Figure 14. Sediment trap total, refractory, and labile Fe fluxes (a) and C-normalized labile particulate Fe in sinking particles (b). The green dashed line reflects the depth of the chlorophyll maximum layer. Figure courtesy of Alexis Floback.

Cruise Summary

The objectives of SUBSEA are designed to resolve both the sources of material supporting subsurface productivity and the processes controlling organic matter cycling and export. The first SUBSEA expedition (FKt260303) aboard Schmidt Ocean Institute's R/V *Falkor(too)* provided the project's first intensive investigation of these processes in the subtropical South Atlantic. The expedition was conducted from March 3 to April 6, 2026, sailing from Rio de Janeiro to Salvador da Bahia, Brazil (Figure 15). It was the first of two planned SUBSEA expeditions to the region and was designed to characterize the movement and transformation of C and nutrients across the photic zone and into the upper mesopelagic ocean. Particular emphasis was placed on identifying the sources and transformations of N that support plankton production, quantifying the magnitude and composition of particle export, and determining how much exported material is regenerated as it moves through the water column.

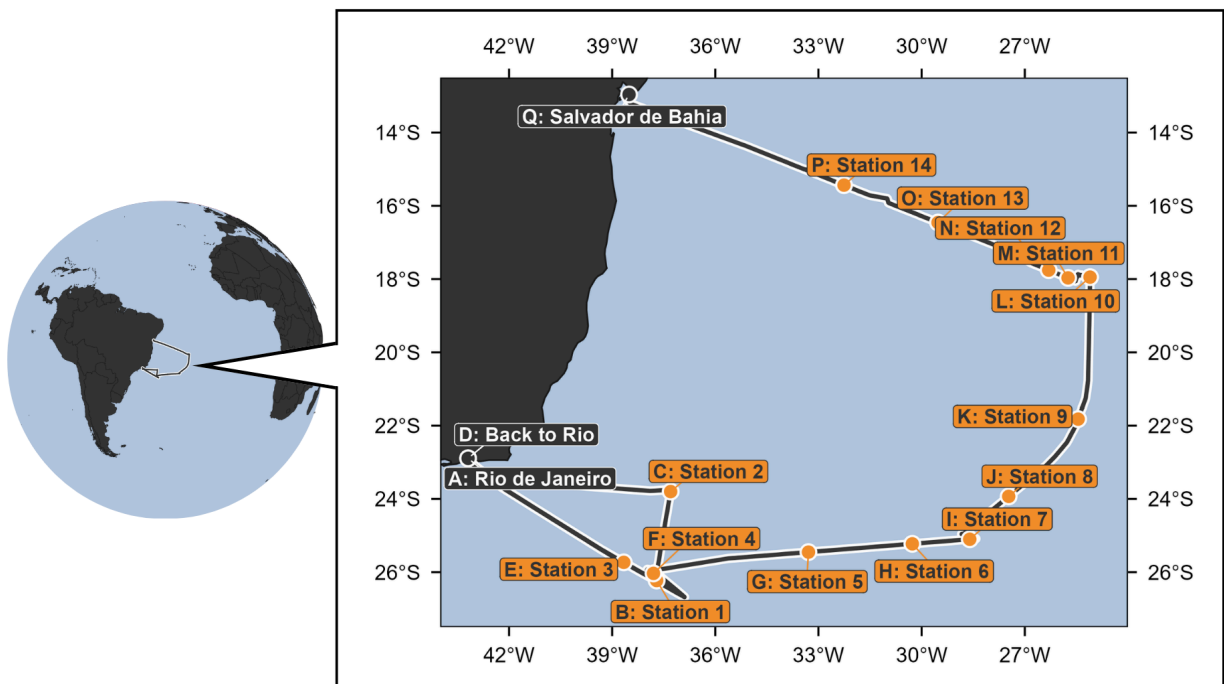


Figure 15: Cruise track of Fkt260303, with sampling stations highlighted in orange, and port locations highlighted in grey.
Figure courtesy of Katie Coates.

To address these questions, SUBSEA combined measurements of nutrient and trace-element inventories, plankton productivity, particle composition, N_2 fixation, nitrate supply, N transformations, and particle and dissolved solute export with observations from autonomous and shipboard platforms. For example, N assimilation, mineralization, and nitrification were measured and used to examine the recycling of N within the subsurface photic zone, while measurements of N_2 fixation and diapycnal nitrate flux were quantified as sources of new N. Sinking and suspended particles were collected, counted, imaged, and chemically characterized across multiple depths to determine their abundance,

size, composition, origin, and degradation state. Shipboard experiments further examined how changes in light and nutrient availability regulate surface and subsurface plankton metabolism and quantified nutrient regeneration, bacterial growth, and respiration associated with sinking material. Together, these complementary observations were designed to constrain the pathways linking nutrient supply, biological production, particle transformation, and export through the subtropical water column.

The cruise combined four longer process stations with eight shorter survey stations selected to resolve regional gradients in surface PO_4^{3-} and chlorophyll, while a quasi-Lagrangian sampling strategy at the longer stations allowed temporal assessment of changes in upper-ocean biogeochemistry and particle flux to be examined while following a water mass. Sampling and observations incorporated the ship's CTD, underway systems, and sensors, together with SUBSEA sediment traps, in situ pumps, in situ arrays, SVP drifters, Wirewalker profilers, day/night net tows, bioptic sensors, a Seaglider, an in-situ flow cytobot, and a membrane inlet mass spectrometer.

Cruise Background

The FKt260303 sampling strategy was designed to resolve biogeochemical variability across multiple spatial and temporal scales. Cruise-track selection was informed by satellite altimetry, historical nutrient observations, and satellite ocean-color products. Sea-surface height anomaly (SSHA) was evaluated prior to and during the expedition to minimize sampling within strong mesoscale eddies and preferentially target conditions representative of the broader gyre. Historical altimetry indicated very low variability in SSHA within the targeted region, and SSHA remained comparatively uniform along the cruise track during the expedition.

Within this physical context, station locations were selected to span gradients in surface-ocean PO_4^{3-} and chlorophyll, two properties expected to influence productivity and the recycling potential of particles. Surface PO_4^{3-} was a particularly important consideration in cruise design because P availability can regulate N_2 fixation and therefore the supply of newly fixed N to the upper photic zone. The expedition targeted low- PO_4^{3-} waters to examine nutrient cycling and productivity under conditions where P availability may constrain N_2 fixation, while also sampling contrasting PO_4^{3-} regimes across the gyre. Historical surface PO_4^{3-} measurements collected between 1972 and 2021 (Global Ocean Data Analysis Project and World Ocean Atlas) were used to identify potential sampling regions. However, these observations were spatially sparse, and a 1° climatology constructed from the historical measurements indicated substantial spatial heterogeneity in surface PO_4^{3-} (Figure 16). The limited spatial coverage and apparent variability of the historical dataset reinforced the value of obtaining new, spatially resolved observations during FKt260303 and informed the placement of longer stations across regions expected to differ in surface PO_4^{3-} concentrations, including Stn 4 in a region of historically relatively low PO_4^{3-} and Stn 10 in a region of historically relatively high PO_4^{3-} .

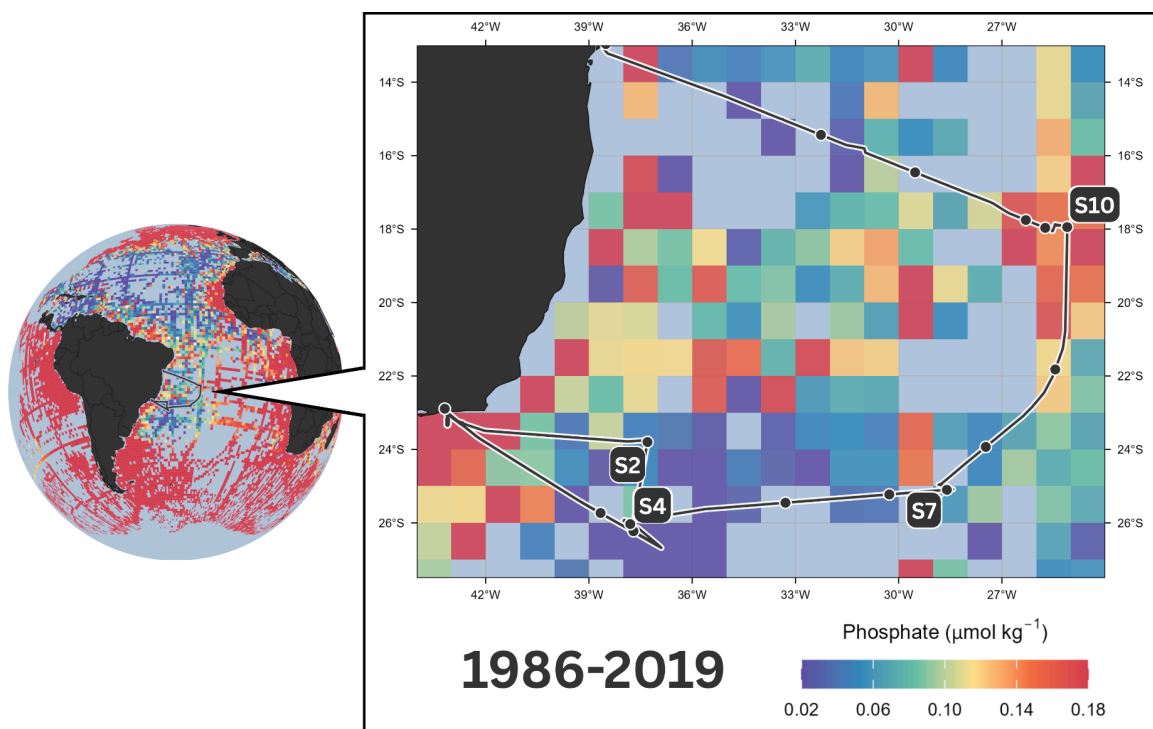


Figure 16: Historical surface phosphate (PO_4^{3-}) concentrations and the FKt260303 cruise track in the South Atlantic Subtropical Gyre. Surface PO_4^{3-} climatology represents the statistical mean of historical measurements within $1^\circ \times 1^\circ$ grid cells. The FKt260303 cruise track is overlaid, with longer-duration stations (Stns 2, 4, 7, and 10) labeled to illustrate their placement across regions with contrasting historical surface PO_4^{3-} concentrations. Data source: World Ocean Atlas 2023, NOAA National Centers for Environmental Information (NCEI). Figure courtesy of Katie Coates.

Near-real-time PO_4^{3-} measurements collected during the expedition were used to evaluate whether the targeted gradient had been captured and to inform subsequent cruise-track decisions. Preliminary measurements indicate substantial spatial variability in surface PO_4^{3-} across the study region (see Preliminary Research Highlights, page 40). Although the complete suite of underway and depth-resolved nutrient measurements remains under analysis, these initial observations indicate that the cruise successfully sampled across a pronounced gradient in surface P availability and provide new spatial resolution in a region where historical nutrient observations are limited.

Satellite chlorophyll observations provided a second criterion for cruise-track selection. Chlorophyll concentration influences light attenuation and therefore the vertical extent of the photic zone: low-chlorophyll waters permit deeper light penetration, increasing the vertical distance through which sinking particles may be remineralized and recycled before leaving the photic zone. The original cruise plan therefore preferentially targeted persistently low-chlorophyll waters characteristic of the subtropical gyre. Satellite observations monitored during the expedition, however, revealed a region of elevated chlorophyll that provided an opportunity to sample a contrasting biological regime (Figure 17, panel a). This feature was first identified in March, and was first sampled during the initial western portion of the expedition (Stn 2). We subsequently sampled the feature farther east later in the cruise (Stn 7), providing observations across different portions of the feature. This sampling at two stations also provided observations across different apparent stages of the feature, as by the time the eastern portion of the feature was sampled, the feature was in an apparent stage of decay (Figure 17, panel b).

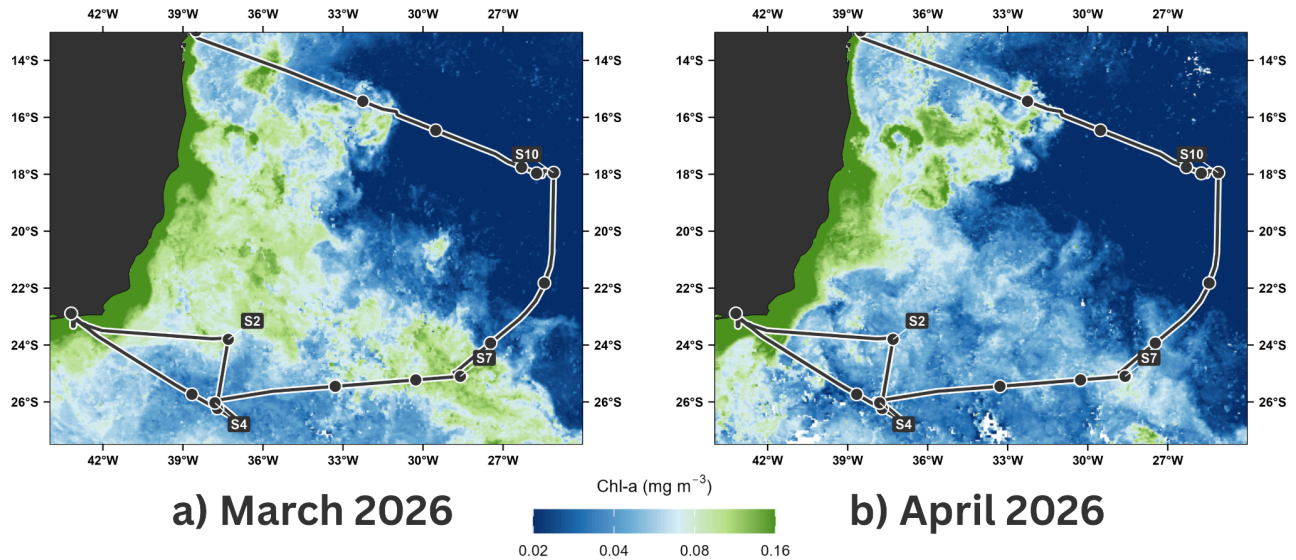


Figure 17: Satellite chlorophyll-*a* observations illustrate the contrasting biological regimes sampled during FKt260303. (a) Mean chlorophyll-*a* across Aqua MODIS 8-day composites from 3–31 March 2026, showing the elevated-chlorophyll feature first sampled during the western portion of the expedition at Stn 2. (b) Aqua MODIS 8-day chlorophyll-*a* composite for 1–8 April 2026, showing the feature later in the expedition near Stn 7, when it appeared to be in a stage of decay. Satellite periods were restricted to the FKt260303 expedition (3 March–6 April 2026); the 1–8 April composite was included because it overlaps the final six days of the expedition. The FKt260303 cruise track is overlaid on both panels. Data source: Aqua MODIS chlorophyll-*a*, 8-day composites, NOAA CoastWatch ERDDAP. Figure courtesy of Katie Coates.

Retrospective satellite observations indicate that this elevated-chlorophyll feature developed several months prior to FKt260303 and that similar features have recurred within the region in previous years (Figure 18). Its persistence is notable within the otherwise oligotrophic South Atlantic Subtropical Gyre and raises questions regarding the nutrient sources and physical processes capable of sustaining elevated phytoplankton biomass over extended periods. Potential mechanisms include enhanced N_2 fixation, spatial variability in Fe availability, and interactions with regional bathymetry; however, the processes responsible for the development and persistence of these features remain unresolved. Sampling across both the elevated-chlorophyll feature and surrounding low-chlorophyll gyre waters provided an opportunity to evaluate how contrasting ecosystem states influence photic-zone structure, nutrient cycling, and particle export.

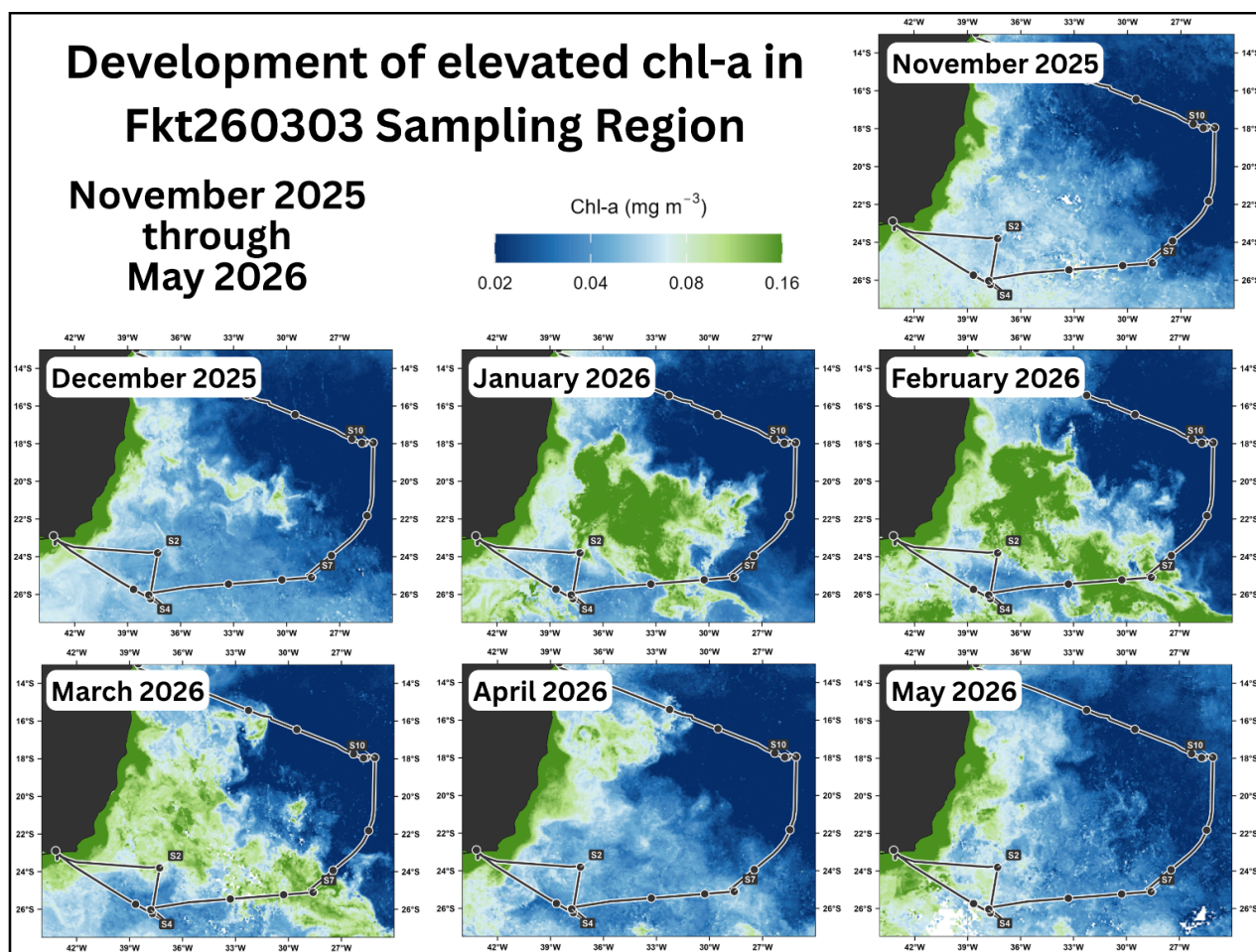


Figure 18: Seasonal evolution of satellite chlorophyll-*a* in the South Atlantic Subtropical Gyre before, during, and after FKt260303. Panels show mean chlorophyll-*a* concentrations derived from Aqua MODIS 8-day composites for November 2025, December 2025, January 2026, February 2026, March 2026, April 2026, and May 2026. The retrospective observations indicate that the elevated-chlorophyll feature sampled during FKt260303 developed several months prior to the expedition and persisted within the otherwise low-chlorophyll waters of the subtropical gyre. The FKt260303 cruise track and station locations are overlaid on each panel for spatial reference, with Stns 2, 4, 7, and 10 labeled. Data source: Aqua MODIS chlorophyll-*a*, 8-day composites, NOAA CoastWatch ERDDAP. Figure courtesy of Katie Coates.

The cruise design therefore integrated intensive, quasi-Lagrangian process studies with higher-resolution spatial surveys across gradients in P availability and chlorophyll. Together with frequent sampling of the shipboard underway seawater system and underway CTD observations, this approach provided spatial context for the process-station measurements while resolving variability not captured by station-based sampling alone. These complementary observations were designed to constrain elemental budgets within the upper ocean, quantify N inputs through N₂ fixation and diapycnal nutrient supply, measure losses through sinking and dissolved organic matter export, and determine the origins and transformations of material exported from the photic zone. Targeted process experiments further examined mechanisms regulating nutrient assimilation and regeneration, N₂ fixation, plankton productivity, and particle remineralization.

The cruise combined four approximately 96-hour process stations with eight shorter survey stations distributed across the SASG. Extended occupations at the process stations provided sufficient

time to conduct rate measurements and incubation experiments and to deploy and track free-drifting observational and experimental platforms. The intervening survey stations increased spatial resolution across the cruise track. One-OBVI Core Measurements were conducted at both process and survey stations, providing a standardized suite of observations with which to place the intensive process studies in a broader regional context.

Sampling during the process stations followed a quasi-Lagrangian framework designed to distinguish temporal changes in biogeochemistry from spatial variability associated with movement through the surrounding water masses. At each process station, a suite of drifting assets, including sediment traps, a productivity array, a Wirewalker profiler, and a Surface Velocity Program (SVP) drifter, were deployed and tracked for the duration of the occupation, while a Seaglider provided complementary autonomous observations. Ship operations were centered on following the drifting sediment traps, with five sediment-trap deployments completed over the course of the expedition (Figure 19). This approach allowed repeated measurements to be made within approximately the same drifting water mass while resolving changes in particle flux, productivity, water-column structure, and biogeochemistry over several days.

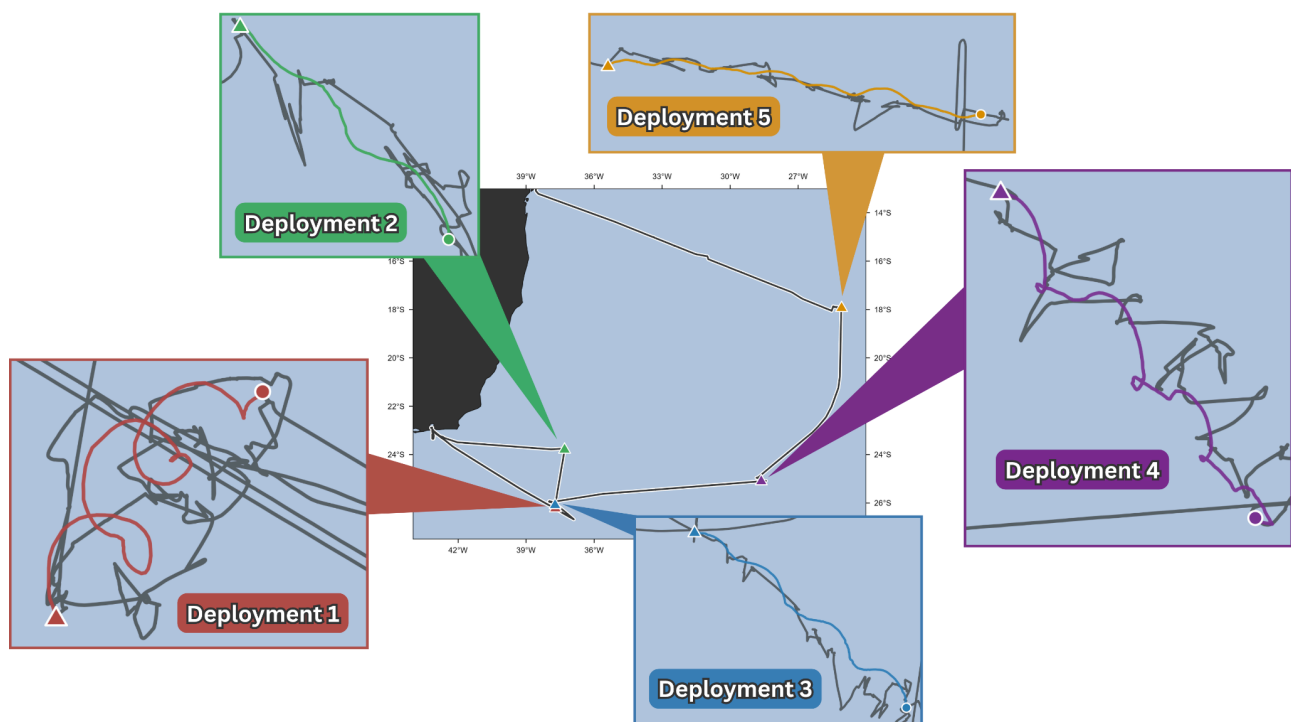


Figure 19: Track of the five sediment-trap deployments conducted during FKt260303 (in colors green, orange, purple, blue, & red), shown along the expedition cruise track (in grey). Deployment locations represent the positions of the drifting sediment-trap arrays as recorded by their satellite beacons. Sediment traps were deployed during longer-station occupations (Stn 1, 2, 4, 7, & 10) and tracked as they drifted with the surrounding water mass. Data source: Preliminary sediment-trap beacon position data collected during FKt260303. Figure courtesy of Katie Coates.

This expedition also establishes the observational foundation for comparisons with other subtropical systems and for the second planned SUBSEA expedition in the South Atlantic. Ultimately, integration of these measurements with autonomous observations and ecosystem process models will

allow SUBSEA to better resolve the mechanisms governing C and N fluxes through the photic zone and assess how differences in nutrient availability among subtropical gyres influence productivity, recycling, and export.

SUBSEA Measurements & Data Collected

The resulting cruise design combined longer-duration process stations with shorter survey stations distributed across the gradients in chlorophyll and PO_4^{3-} targeted during FKt260303 (Figure 20). Process stations were occupied for extended periods to support intensive, process-oriented sampling and experiments designed to identify mechanisms regulating nutrient cycling and productivity. These longer occupations enabled deployment of longer-duration observational and experimental assets, including sediment traps, Seagliders, and McLane in situ pumps, as well as repeated sampling and targeted incubation experiments. In contrast, survey stations were occupied for shorter periods and prioritized collection of standardized OBVI Core biogeochemical measurements and trace-metal-clean seawater for dissolved metal measurements, providing greater spatial resolution of biogeochemical conditions along the cruise transect. Between stations, frequent underway CTD deployments further resolved spatial variability in upper-ocean physical and biogeochemical properties. Station locations, classifications, occupation dates and durations, and coordinates are summarized in Table 4. Together, the process stations, survey stations, and underway observations provided complementary temporal and spatial perspectives, placing intensive process-level measurements within the broader biogeochemical gradients sampled across the cruise track.

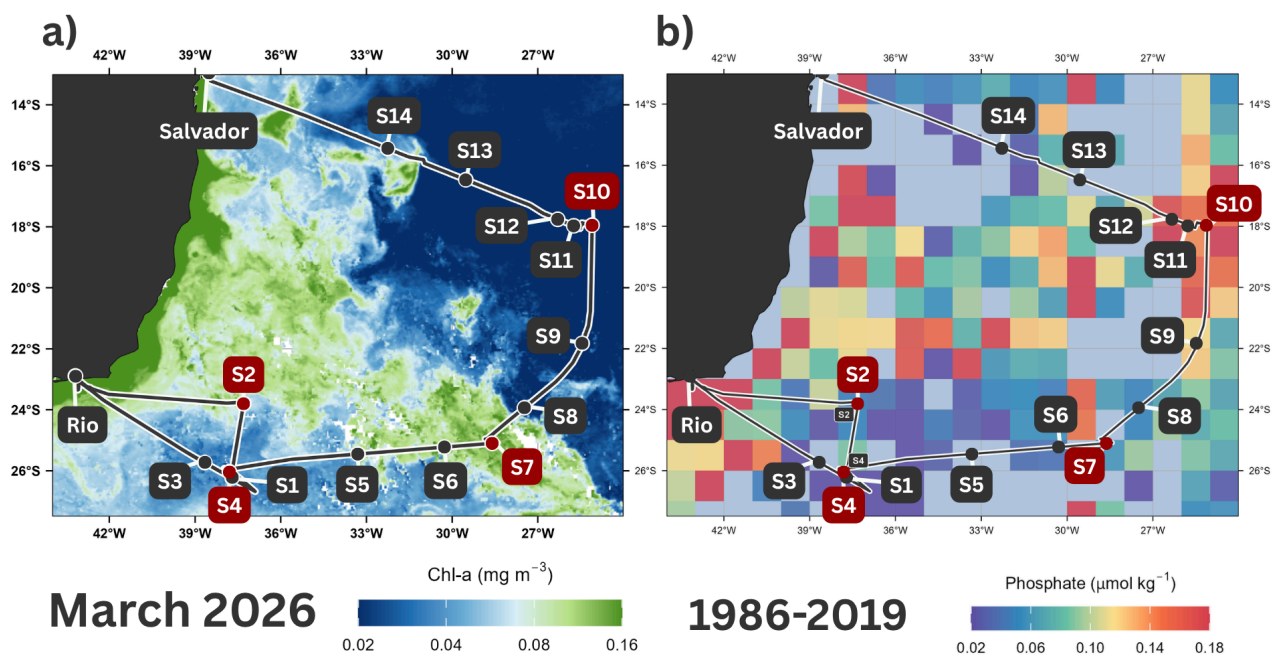


Figure 20. Final FKt260303 station locations in relation to the chlorophyll-*a* and historical surface phosphate (PO_4^{3-}) gradients used to inform cruise design in the South Atlantic Subtropical Gyre. Longer-duration process stations (Stns 2, 4, 7, and 10) are shown in red, while shorter survey stations are shown in grey. (a) FKt260303 cruise track and station locations

overlaid on mean chlorophyll-*a* concentrations across Aqua MODIS 8-day composites from 3–31 March 2026, illustrating the elevated-chlorophyll feature sampled at Stns 2 and 7 and surrounding lower-chlorophyll gyre waters. Data source: Aqua MODIS chlorophyll-*a*, 8-day composites, NOAA CoastWatch ERDDAP. (b) Cruise track and station locations overlaid on historical surface PO₄³⁻ concentrations. Surface PO₄³⁻ climatology represents the statistical mean of historical measurements within 1° × 1° grid cells and illustrates the placement of stations across contrasting historical PO₄³⁻ regimes. Data source: World Ocean Atlas 2023, NOAA National Centers for Environmental Information (NCEI). Figure courtesy of Katie Coates.

Table 4: Summary of stations occupied during FKt260303.

Station	Station Type	Feature	Hours Occupied	Dates Occupied	Coordinates
1	Long: Process	Low Phosphate	69	3/6 – 3/8	-26.227144, -37.706217
2	Long: Process	Western Bloom	40	3/9 – 3/10	-23.799483, -37.299183
3	Short: Survey		5	3/14	-25.731641, -38.662314
4	Long: Process	Low Phosphate	89	3/15 – 3/18	-26.031828, -37.798844
5	Short: Survey		4.5	3/20	-25.541122, -33.283535
6	Short: Survey		2.5	3/21	-25.225968, -30.270903
7	Long: Process	Eastern Bloom	96	3/21 – 3/25	-25.100000, -28.600000
8	Short: Survey		2.5	3/26	-23.930618, -27.467479
9	Short: Survey		6.5	3/27	-21.963639, -25.095520
10	Long: Process	Low Chlorophyll	102	3/28 – 4/1	-17.945767, -25.102083
11	Short: Survey		6	4/1 – 4/2	-17.967100, -25.740900
12	Short: Survey		27	4/2	-17.754700, -26.304500
13	Short: Survey		3	4/3	-16.456300, -29.522400
14	Short: Survey		5.5	4/4	-15.435200, -32.257900

Science operations were conducted continuously throughout the 35-day expedition. Across the expedition, the team completed 10 types of deployments (Appendix Table 1), collected 11,082 samples (Appendix Table 2), including 80 CTD-rosette casts (Appendix Table 3), 55 underway CTD deployments, 22 trace-metal-clean rosette casts, 24 vertical microstructure profiles, 16 bongo-net tows (day and night), 14 HyperPro hyperspectral light profiles, and 8 McLane in situ pump deployments. Autonomous and free-drifting operations included 5 sediment-trap array deployments, 4 Seaglider deployments, 3 free-drifting productivity array deployments, 3 Wirewalker deployments, and 3 Surface Velocity Program (SVP) drifter deployments. Together, these operations provided repeated depth-resolved measurements at process and survey stations, quasi-Lagrangian observations during extended station occupations, and higher-resolution spatial coverage along the cruise track.

SUBSEA conducted 17 major activities during FKt260303. These activities are as follows:

- (1)** Deployment of a free-drifting SUBSEA sediment trap array (ST). The array includes 12 particle interceptor traps per cross with 5 crosses in total positioned at 75 m, 110 m, 150 m, 225 m, and 300 m. Samples collected include:
 - (a)** Particulate C, N, P, Si, and inorganic C
 - (b)** TDN, TDP, nitrate+nitrite
 - (c)** DNA
 - (d)** Bulk and compound specific C and N isotopes
 - (e)** Trace metals
 - (f)** Particle imaging by In Situ Flow Cytobot (IFCB)
 - (g)** Optical sediment trap (OST) at 110 m and 150 m

- (2)** Estimation of mixed layer gross O₂ production and respiration based on deployment of dual optode O₂ sensors on SUBSEA ST. Mixed layer O₂ fluxes are estimated based on diel changes in O₂ together with estimates of air-sea gas exchange. Dual optodes are deployed on every SUBSEA ST.

- (3)** High frequency deployment of an underway CTD (uCTD) profiler (deployed every 1-4 hrs while the vessel was transiting between sampling stations) to capture biogeochemical and physical gradients through the upper 250 m. The profiler included sensors for temperature, conductivity, dissolved O₂, turbidity, and fluorescence.

- (4)** Deployment of a 24 hour, free-drifting in-situ array (A), used to quantify:
 - (a)** Upper ocean (0-125 m) rates of N₂ fixation based on ¹⁵N₂ isotope tracer incubations. Volumetric rates of ¹⁵N₂ fixation are measured at 6 discrete depths (5, 25, 45, 75, 100, 125 m) where whole seawater is amended with ¹⁵N₂ tracer and incubated in situ for 24 hrs. Importantly, for SUBSEA sampling, we are collecting particles labeled with ¹⁵N on filters and we are routinely quantifying dissolved N solutes labeled with ¹⁵N.
 - (b)** Primary production via photosynthetic assimilation of ¹³C (24 hrs), targeting the same 6 discrete depths (5, 25, 45, 75, 100, 125, 150 m).
 - (c)** Gross primary production (GPP) via ¹⁸O-H₂O isotope tracer incubations and net community production (NCP) based on O₂/Ar (15, 25, 45, 75, 100, 125 m).
 - (d)** Rates of nitrification, including ammonia and nitrite oxidation. ¹⁵N₂ isotopic tracers are measured at 7 discrete depths (25, 45, 75, 100, 125, 150, 200 m).

- (5)** Quantification of vertical gradients in dissolved and particulate nutrient stocks using CTD casts. SUBSEA sampling is largely restricted to the upper 400 m of the water, including highly resolved sampling (~5 m) around the DCM layer. Samples collected include:
 - (a)** Particulate C and N, which are collected from 12 discrete depths, most intensively around the DCM layer, for subsequent analyses of bulk stable isotopes (¹³C and ¹⁵N).
 - (b)** Dissolved nutrients, including total dissolved N and P, ammonium, nitrate, nitrite, silicate and soluble reactive P, which are collected from 24 discrete depths.

- (c) N and O isotopes ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$), which are subsequently determined from pools of nitrate+nitrite and N isotopes from total dissolved N. These analyses provide information needed for constraining sources of N supporting biomass in the photic zone and insight into N cycle processes based on isotope fractionation.
 - (d) Photosynthetic and photoprotective pigments collected from 12 discrete depths in the upper 200 m for subsequent high performance liquid chromatography analyses.
 - (e) Collection of samples for subsequent analyses of particle light absorption using filter pad absorption technique. Samples collected from 12 discrete depths in the upper 200 m.
 - (f) DNA, collected from 12 discrete depths, used for subsequent analyses of distributions of N_2 fixing microorganisms (based on quantification of *nifH* gene abundances) and ammonia oxidizing archaea (based on quantification of *amoA* gene abundances).
- (6) Deployment of a trace metal clean CTD rosette (TMC) to collect and analyze samples for quantifying concentrations and distributions of dissolved and particulate metals.
 - (7) Hyperpro radiometer (HP) for vertical profiles of upper ocean bio-optical properties. The instrument is deployed in-situ and measures apparent optical properties for estimation of water leaving radiance, remote sensing reflectance, solar energy flux, and photosynthetically active radiation.
 - (8) Daytime and nighttime vertically integrated (0-150 m) zooplankton net tows. Tows were size fractionated (5 size classes) for subsequent determination of dry mass for information on migratory biomass. Additional net tows targeting the DCM for collection of foraminifera for Sigman lab (Princeton).
 - (9) Imaging of near-surface ocean plankton using In Situ Flow Cytobot (IFCB) for determination of particle and cell abundances, sizes, and spatial distributions. This instrument is plumbed to the shipboard seawater intake system for continuous measurements and imaging of plankton in the near-surface ocean. In addition, discrete samples, including those subsampled from the SUBSEA sediment trap collections, are analyzed for information on sinking particle morphology.
 - (10) Deployment of a free-drifting Wirewalker profiler (WW) in the upper ocean (0-400 m). The WW is deployed for ~3 days, providing a highly resolved dataset on high frequency temporal variation in ocean physics and biogeochemistry.
 - (11) Hyperspectral backscatter, AC-S, LISST, fluorescence, and dissolved O_2 from shipboard underway seawater system for information on particle absorption, number, size, and optical characteristics and dissolved O_2 .
 - (12) Deployment of a Seaglider (SG) equipped with a refurbished sensor for quantifying microstructure turbulence. These measurements will be used to estimate turbulent nutrient

exchange at the base of the photic zone, which when coupled with high vertical resolution sampling of nutrients can be used to constrain upper ocean N and phosphorus mass balances.

- (13) Deployment of Vertical Microstructure Profiler (VMP). The microstructure profiler (Rockland VMP-250) was deployed for additional estimates of microstructure turbulence. The instrument was deployed while the vessel was slowly transiting and provided additional estimates of turbulence for closing nutrient budgets.
- (14) Deployment of high-volume McLane pumps (MP) for collection of size fractionated particulate matter. Dual pump heads are used for collection of organics (Close), and metals and omics (Hawco and Church). Filters for organics include: GF75 (nominal 0.3 μm), GF/C (nominal 1.2 μm), PETE membrane (5 μm), and 53 μm Nitex. Filters for metals and omics are: 0.2 μm , 1.2 μm , 5 μm Supor PES filters, and 53 μm Nitex. Target features include: base of photic zone and trap collection depth (150 m), DCM and trap collection depth (110 m), well-lit photic zone (25 m), upper mesopelagic and trap collection depth (225 m), lower photic zone (135 m), and mid-photoc zone and trap collection depth (75 m).
- (15) SUBSEA accommodated the One-OBVI core measurement team (Dr. Pierre Marrec and Mattia Da Fieno) as part of the collective OBVI effort to improve spatially resolved biogeochemical observations in the SASG.
- (16) Vertical profiles for subsequent analyses of dissolved and particulate metabolomics collected from CTD casts by members of the Ingalls lab.
- (17) A suite of in situ and shipboard experiments were conducted (see SUBSEA Experiments below) to evaluate plankton responses to light, nutrients (including metals), fluxes of C and N by different processes, and stoichiometry of organic matter decomposition.

SUBSEA Experiments

We also have a heavy focus on process level understanding. Many of the SUBSEA research teams conducted targeted experiments (Appendix Table 4) designed to identify the mechanisms driving nutrient cycling and productivity. This included using isotopes as tracers of N cycling and primary production. For example, a series of experiments were conducted using shipboard incubators and free-drifting arrays to assess rates of various N assimilatory (e.g., nitrate and ammonium uptake, N_2 fixation) and dissimilatory (e.g., ammonia and nitrite oxidation, ammonium regeneration) processes (Fawcett). Several of these experiments examined rates of assimilation of nitrate, nitrite, and urea by different size classes of plankton and for subsequent flow cytometric sorting of ^{15}N labeled cells. Other N centric experiments sought to elucidate fates of different nitrogenous substrates using metabolomic approaches to trace assimilation into particulate and dissolved organic matter pools (Ingalls). Other experiments, using $^{18}\text{H}_2\text{O}$, examined photosynthetic oxygen production as a function of light in both the surface and subsurface layers (del Valle). Phytoplankton Fe uptake was quantified in a series of

experiments using seawater collected from both the surface ocean and DCM (Hawco). Other collaborative experiments, aimed to map functionally important organisms onto biogeochemical processes; for example, experiments were conducted to examine potential competitive interactions for nitrogenous substrates between phytoplankton (e.g., *Prochlorococcus*) and ammonia and nitrite oxidizers (Church). In addition, collaborative experiments were conducted to examine potential nutrient limitation of primary production, phytoplankton biomass accumulation, and N_2 fixers (Hawco, Hall, Church). From some of these nutrient amendment experiments, samples were collected to examine possible changes in single cell stoichiometry (Hall).

Recognizing the potential importance of zooplankton and vertical migration as controls on C and nutrient export and remineralization, a series of experiments were conducted to improve understanding of decomposition pathways and fluxes associated with fecal pellet decomposition. Katie Coates (Church lab) led the design and execution of these experiments. Zooplankton were collected from 0-150 m integrated nighttime net tows and the animals were size fractionated into 4 different size classes. Size fractions were then incubated in filtered seawater, using meshes to exclude animals from their fecal pellets (Figure 21). The pellets were collected, split, and splits were added to filtered and whole seawater collected from 340 m and 265 m (the approximate position of the deep scattering layer and 75 m above the scattering layer). Samples were collected from the beginning of each incubation for initial determination of nutrients, dissolved and particulate organic matter concentrations, and bacterial abundances and diversity. After 96 hr of incubation, incubations were subsampled for this same suite of properties to examine how pellet decomposition influences the elemental transformations and the partitioning of nutrient elements into particulate and dissolved phases.

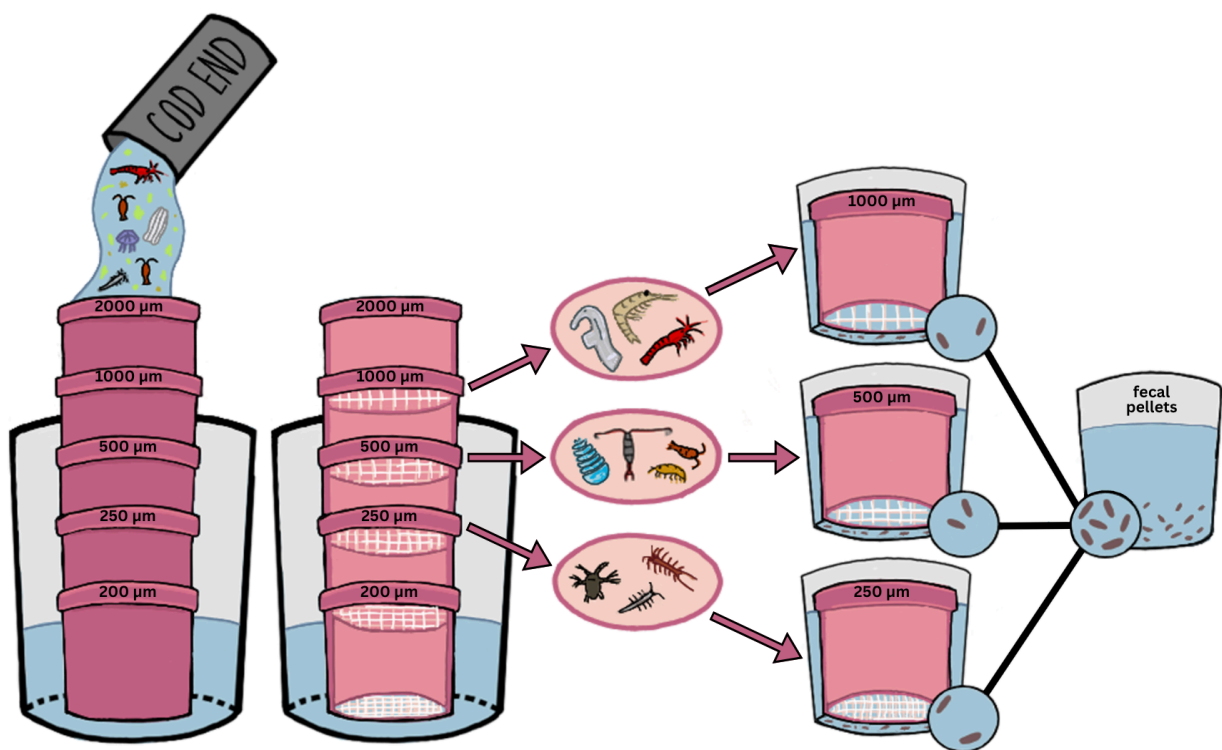


Figure 21: Methodology for fecal pellet decomposition experiments. Figure displays collecting zooplankton and setting up incubations to exclude animals from their fecal pellets. Figure courtesy of Katie Coates.

FKt260303 SUBSEA Participants

Table 5: SUBSEA participants on FKt260303.

Lab Group	Science Group	Team Member	Position, Affiliation
White Lab Group	Physical & biological controls on biogeochemistry, Biomass & plankton diversity, Export	Team Lead: Dr. Angelicque White	SUBSEA Co-PI, University of Hawai‘i at Mānoa
		Tully Rohrer	Technician, University of Hawai‘i at Mānoa
		Mattia Da Fieno	Technician, University of Hawai‘i at Mānoa
Church Lab Group	Nutrient distributions & processes, Biomass & plankton diversity, Export	Team Lead: Dr. Matthew Church	SUBSEA Co-PI, Chief Scientist, University of Montana
		Dr. Esther Mak	Postdoc, University of Montana
		Jared McGourty	Graduate Student, University of Montana
		Katie Coates	SUBSEA Project Manager, University of Montana
Del Valle Lab Group	Productivity	Team Lead: Dr. Daniela del Valle	SUBSEA Co-PI, Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)
		Dr. María Luz Torres Alberto	Postdoc, INIDEP
		Micaela Gonella	Graduate Student, SOI-POGO Fellow, CONICET
Close Lab Group	Nutrient distributions & processes, Export	Team Lead: Dr. Hilary Close	SUBSEA Co-PI, University of Miami
		Jason Freisen	Graduate Student, University of Miami

		Dailen Jeng	Graduate Student, University of Miami
Fawcett Lab Group	Nutrient distributions & processes, Biomass & plankton diversity	Dr. Raquel Flynn	Research Scientist, University of Cape Town, South Africa
		Calvin Swart	Graduate Student, University of Cape Town, South Africa
Barone Lab Group	Physical & biological controls on biogeochemistry, Export	Team Lead: Dr. Benedetto Barone	SUBSEA Co-PI, University of Hawai‘i at Mānoa
		Hunter Adams	Technician, University of Hawai‘i at Mānoa
Hawco Lab Group	Physical & biological controls on biogeochemistry, Nutrient distributions & processes, Export	Team Lead: Dr. Nicholas Hawco	SUBSEA Co-PI, University of Hawai‘i at Mānoa
		Dr. Alexis Floback	Postdoc, University of Hawai‘i at Mānoa
Letelier Lab Group	Productivity, Physical & biological controls on biogeochemistry	Team Lead: Dr. Ricardo Letelier	SUBSEA Co-PI, Oregon State University

		Kelly George	Technician, Oregon State University
Hall Lab Group	Productivity	Team Lead: Dr. Robert Hall	SUBSEA Co-PI, University of Montana
		Dr. Deepika Sahoo	Postdoc, University of Montana
Ingalls Lab Group	Nutrient distributions & processes, Plankton diversity & biomass	Team Lead: Dr. Frank Xavier Ferrer Gonzalez	Postdoc, University of Washington
		Susan Garcia	Graduate Student, University of Washington
Core One-OBVI Group	Nutrient distributions & processes	Team Lead: Dr. Pierre Marrec	Field Coordinator, University of Rhode Island
		Mattia Da Fieno	Technician, University of Hawai'i at Mānoa

Preliminary Research Highlights

Collectively our observations from the FKt260303 expedition provide an integrated view of nutrient supply, biological production, particle transformation, and export within the SASG and establish a foundation for comparison with observations from the North Pacific and the second SUBSEA South Atlantic expedition. Although it has only been 4 months (at the time of this writing) since we disembarked from the vessel, we have made great progress analyzing samples and beginning to synthesize our observations. In this section, we present preliminary observations to (1) characterize variability in the near-surface ocean, demonstrating that we were successful in sampling across biogeochemically distinct regions of the SASG. After, we highlight contrasting (2) variability in the vertical structure of biogeochemistry across the transect, with a focus on the longer process stations. Notably, we were able to sample across spatially distinct regions, including a region where the mixed layer was shallow and PO_4^{3-} was low (Stn 4), a decaying phytoplankton bloom where the chlorophyll maximum shoaled upwards and the photic zone was relatively shallow (Stn 7), and a site of exceptionally low chlorophyll and a very deep photic zone (Stn 10). The dynamic range of biogeochemistry sampled during the FKt260303 cruise captures nearly the full biogeochemical range of conditions typically observed at Stn ALOHA (e.g., surface ocean PO_4^{3-} across the spatial transect in the SASG varied from $<0.02 \mu\text{M}$ to $>0.1 \mu\text{M}$, while at Stn ALOHA phosphate varies from $<0.02 \mu\text{M}$ to $0.18 \mu\text{M}$), potentially facilitating space-for-time comparative analyses of these two ecosystems (see Lessons Learned from NPSG & SASG Comparisons). The design and successful execution of our cruise plan is enabling us to test how variation in upper ocean biogeochemistry influences nutrient recycling, productivity, and export in and from the subsurface photic zone.

Longer (96 hr), quasi-Lagrangian stations were used to examine stocks and fluxes associated with key biogeochemical properties and processes. For the purposes of this report, we focus most of discussion of findings from 3 process stations: Station 4: selected as a low phosphate, shallow mixed layer site; Station 7: selected based on satellite ocean color as a “bloom” station; and Station 10: selected in part based on historical satellite ocean color climatology as representative of a site with exceptionally low chlorophyll concentrations.

(1) Variability in the Near-Surface Ocean

Across the sampling transect we observed striking spatial variability in near-surface ocean PO_4^{3-} concentrations, with phosphate concentrations varying from below the limits of detection ($\sim 0.02 \mu\text{M}$) to nearly $0.2 \mu\text{M}$ over the span of only a few hundred kilometers. This was particularly notable in the southwestern portion of the cruise transect (e.g., Stn 3 and 4) where within the span of single day (e.g., March 14, 2026) of the vessel transiting, phosphate concentrations spanned the full dynamic range observed throughout the expedition (Figure 22).

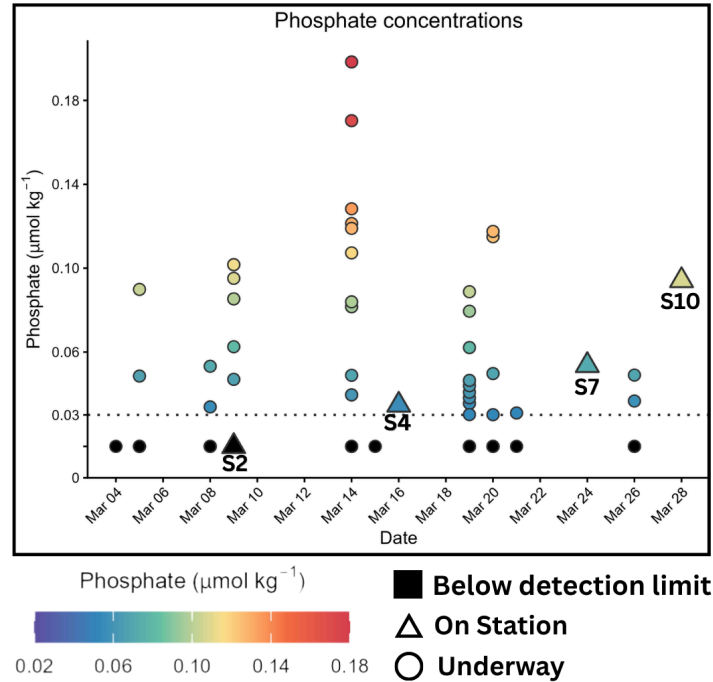


Figure 22: Shipboard determinations of near-surface ocean phosphate concentrations during the FKt260303 expedition demonstrating relatively high spatial variation in phosphate concentrations, in some cases changing from below detection to nearly $0.2 \mu\text{M}$ over a few hundred kilometers. Samples were collected from the shipboard seawater intake while the vessel was transiting (underway) and while occupying major stations (on station). Concentrations color coded to highlight variability in phosphate. Note that high variability within a day reflects spatial variation in phosphate while the vessel was transiting. The dashed line depicts minimum limit of detection ($0.03 \mu\text{mol kg}^{-1}$); symbols below limit of detection depict samples measured but phosphate too low to quantify. Figure courtesy of Katie Coates.

Using a shipboard $p\text{CO}_2$ equilibrator system whose installation was facilitated via a collaboration between SUBSEA investigator Dr. Daniela del Valle and Denis Pierrot (NOAA) and Carla Berghoff (INIDEP), we assessed spatial variability in $p\text{CO}_2$ and these measurements were used to calculate $\Delta f\text{CO}_2$ in the near surface waters along the entire cruise track (Figure X). Regions where $\Delta f\text{CO}_2$ is higher act as stronger net source of CO_2 to the atmosphere. $\Delta f\text{CO}_2$ was lowest in the southern portion of the transect coincident with a decaying phytoplankton bloom, with $\Delta f\text{CO}_2$ increasing into the low chlorophyll central gyre (Figure 23).

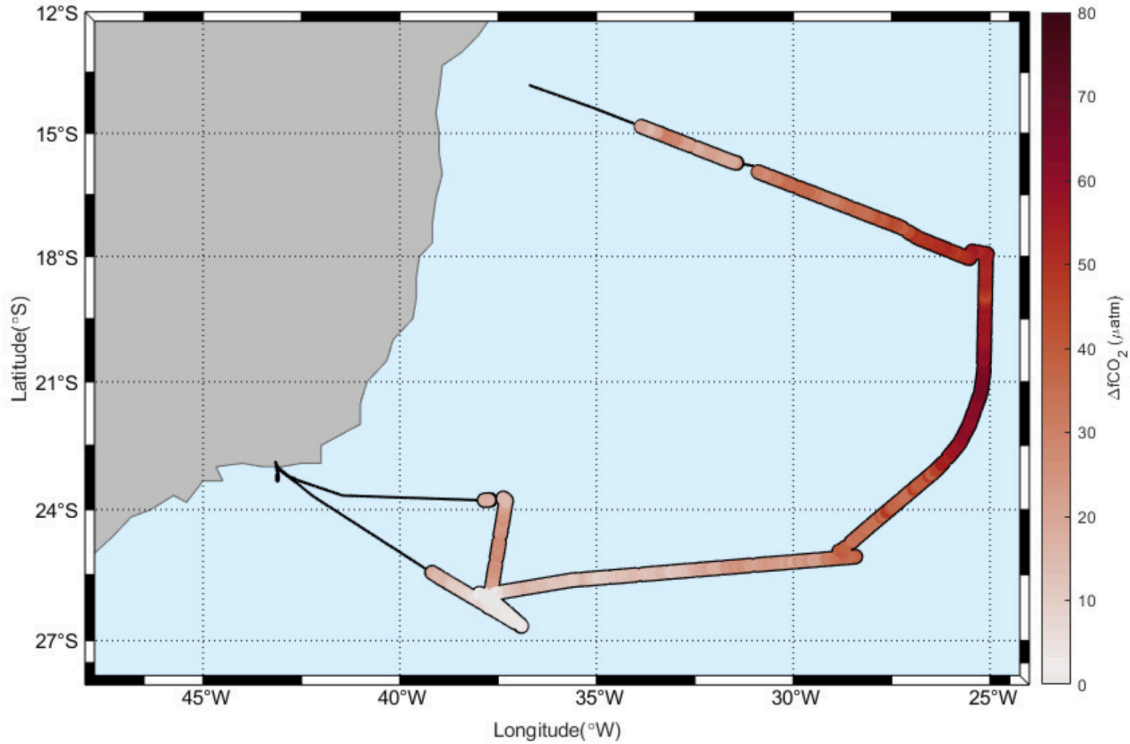


Figure 23: Underway CO₂ fugacity ($\Delta f\text{CO}_2 = f\text{CO}_{2,\text{sw}} - f\text{CO}_{2,\text{atm}}$; μatm) along the SUBSEA cruise track. Positive values (red) indicate CO₂ outgassing from the ocean to the atmosphere. Surface $f\text{CO}_2$ was continuously measured underway and interpolated against atmospheric $f\text{CO}_2$ derived from the General Oceanic underway $p\text{CO}_2$ system. In collaboration with Denis Pierrot and Carla Berghoff. Figure courtesy of María Luz Torres Alberto.

We also used spatial differences in the ratio of $\Delta\text{O}_2:\text{Ar}$, measured at sea, as a metric to estimate net community production across the sampling region. Regions where $\Delta\text{O}_2:\text{Ar}$ were positive reflect net photosynthetic production of O₂. Sampling from the shipboard underway seawater intake system provided a near-continuous determination of $\Delta\text{O}_2:\text{Ar}$ (Figure 24).

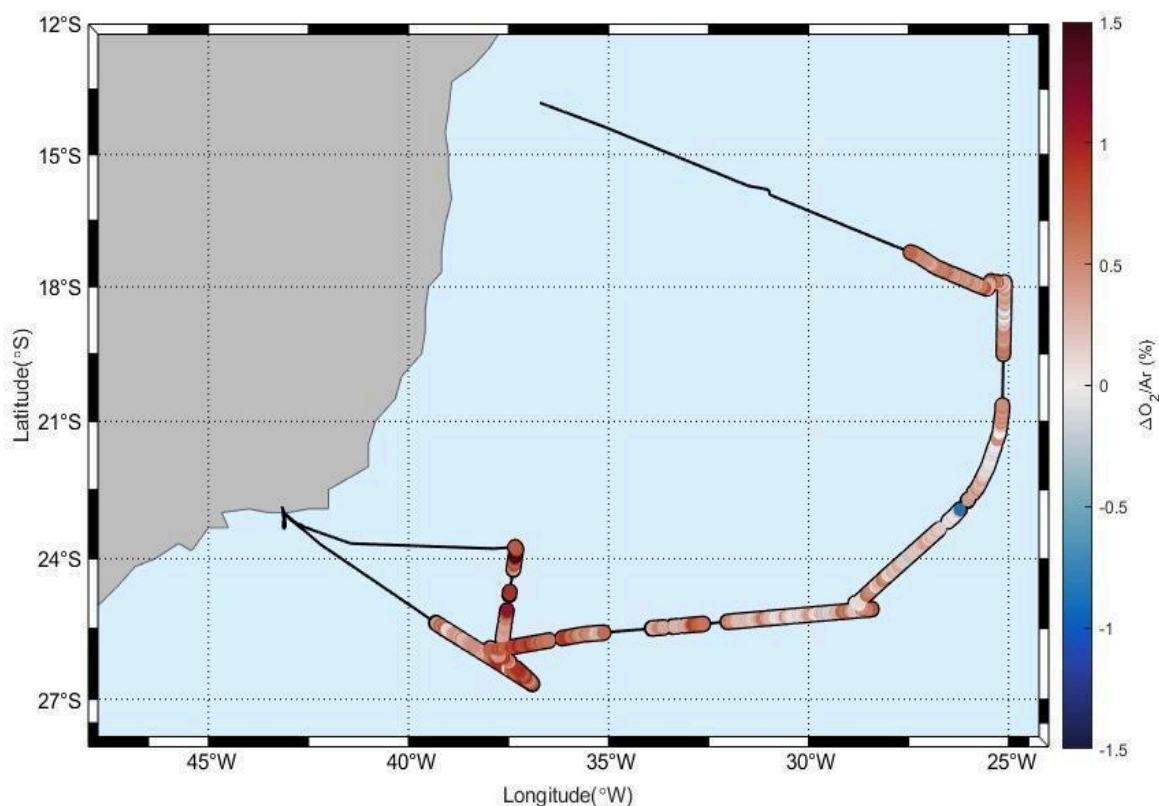


Figure 24. Underway dissolved O_2 -to-argon ratios ($\Delta(O_2/Ar)$; preliminary results) along the SUBSEA cruise track. $\Delta(O_2/Ar)$ represents the biologically driven O_2 supersaturation or undersaturation relative to an inert-gas reference (Ar), allowing biological changes in O_2 to be distinguished from physical variability associated with gas solubility and air–sea equilibration. Positive values indicate net autotrophy (net O_2 production), whereas values near zero or negative indicate net O_2 consumption or a minimal biological signal. $\Delta(O_2/Ar)$ was measured semi-continuously using membrane inlet mass spectrometry (MIMS) coupled to the underway seawater system. Figure courtesy of María Luz Torres Alberto.

In general, $\Delta O_2:Ar$ varied from neutral (near zero) to weakly positive, consistent with tightly coupled photosynthetic production and respiration across most of the sampling transect. $\Delta O_2:Ar$ was greatest at those stations where phytoplankton biomass was elevated, consistent with net photosynthetic production of organic matter within the mixed layer. We also measured $\Delta O_2:Ar$ from seawater collected within the mixed layer from CTD rosette cast profiles at discrete sampling stations. From these measurements, net biological fluxes of O_2 (i.e., community production) were calculated (Figure 25). Discrete estimates of mixed layer net community production varied from $<1 \text{ mmol } O_2 \text{ m}^{-2} \text{ d}^{-1}$ to upwards of $5 \text{ mmol } O_2 \text{ m}^{-2} \text{ d}^{-1}$, with the mean mixed layer fluxes varying approximately two-fold across the sampling transect.

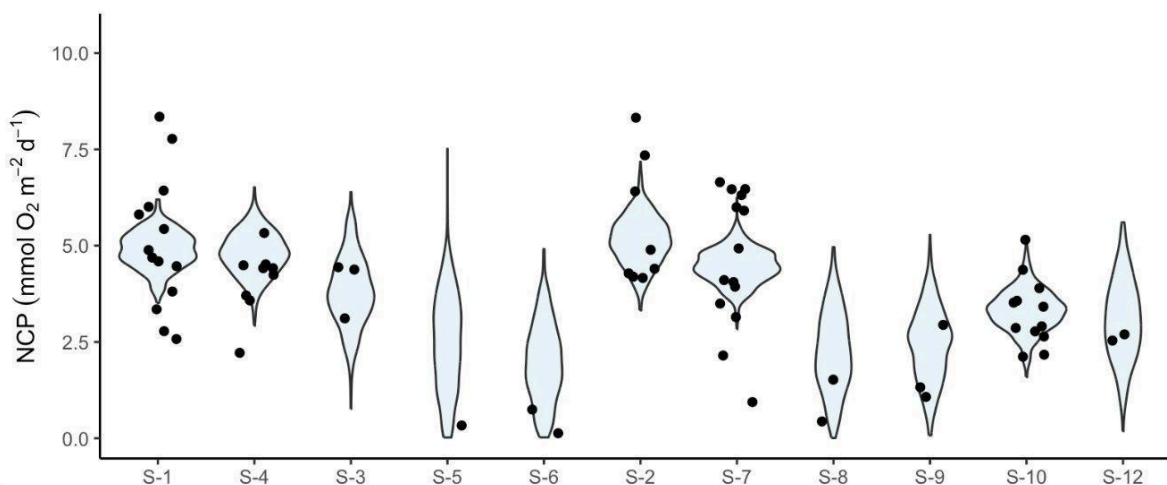


Figure 25. Net community production (NCP) in the mixed layer varied spatially across SUBSEA cruise. Points are samples from CTD casts (5-29 m) and measured by the O₂:Ar method. Blue violins are posterior probabilities from a multilevel Bayesian model and represent the uncertainty interval around the mean NCP at any one site. Sites with 2-3 points have more uncertainty than the intensively sampled core sites. Figure courtesy of Bob Hall.

To examine the extent to which nutrients (including Fe) limited primary production, we conducted a series of nutrient amendment experiments at each main process station using seawater collected from both the near-surface ocean and the DCM. These factorial experiments tested whether additions of N, P, or iron (or combinations of these elements) resulted in changes to chlorophyll concentrations, gross O₂ production (determined based on ¹⁸O₂), cell abundances, and plankton diversity (based on 16S and 18S rRNA gene analyses). We find spatial differences in limiting nutrients, with PO₄³⁻ limiting gross O₂ production in waters where near-surface ocean phosphate concentrations were low (i.e., Stn 1; Figure 26).

$$\log(GOP) = \beta_0 + \beta_N N + \beta_P P + \beta_{NP} NP + \varepsilon$$

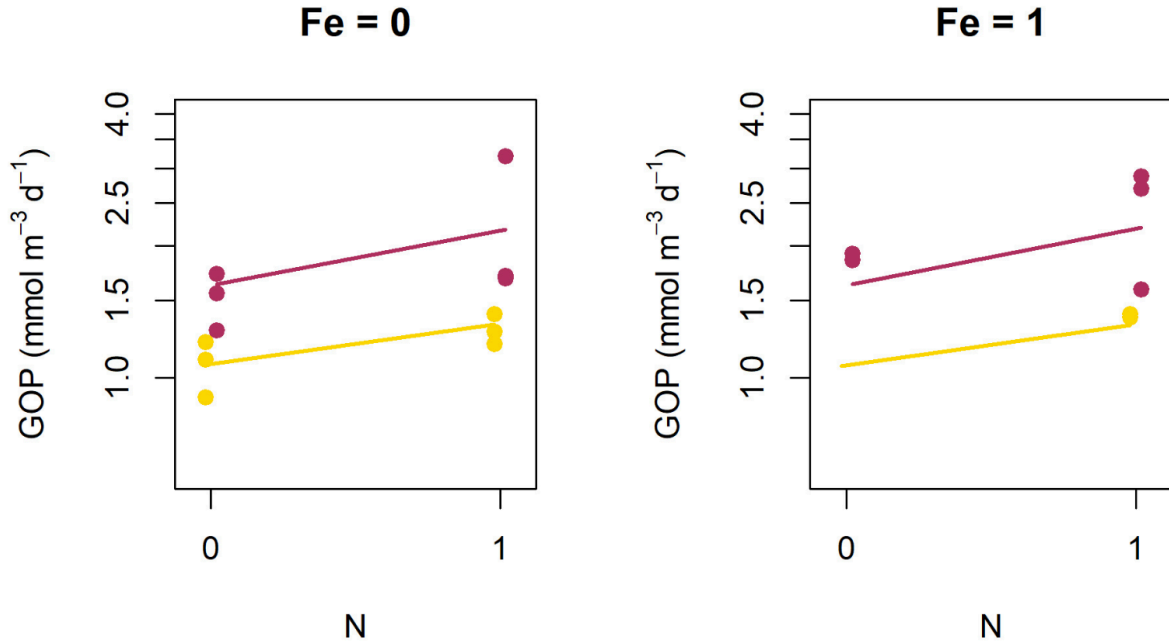


Figure 26. Factorial model demonstrating positive P effect on GOP at the surface waters (5 m) at station 1 in the South Atlantic Ocean. Yellow represents $P = 0$ and maroon represents $P = 1$. Figure courtesy of Deepika Sahoo.

We also examined high frequency spatial variation in particle and plankton distributions in the near-surface ocean using an IFCB that was plumbed to the shipboard seawater intake system. The resulting observations provide rich biological context for evaluating the types of organisms underlying spatial patterns in particle distributions. For example, based on IFCB derived cell abundances, the decaying phytoplankton bloom sampled at Stn 7 appeared at least in part attributable to increased abundances of diatoms, in particular centric diatoms (Figure 27). Notably, coinciding with increased centric diatoms, smaller pennate diatoms became less prevalent in the decaying bloom waters.

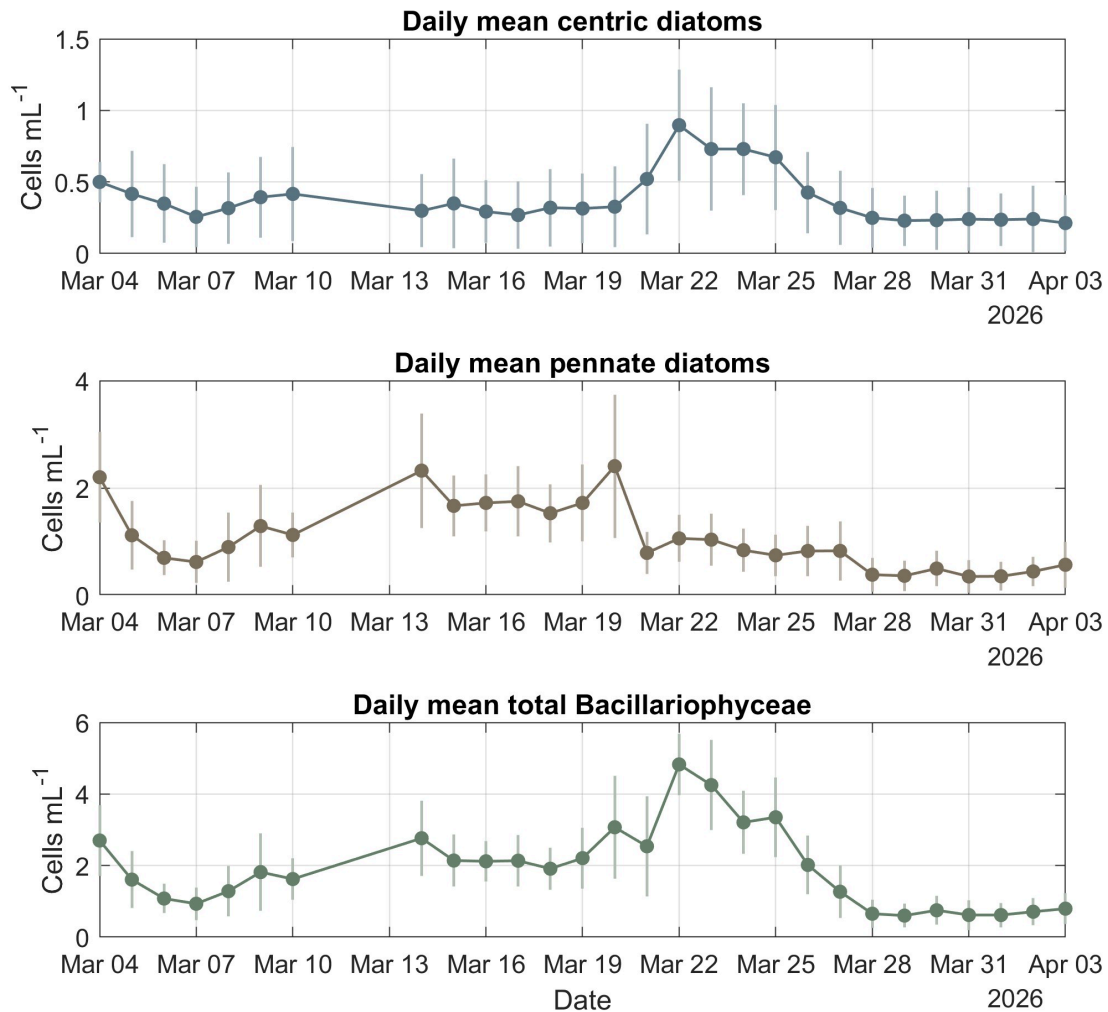


Figure 27. Diatom abundances varied spatially in the near-surface waters along the cruise transect. Use of the IFCB plumbed to the shipboard seawater intake system provided information on particle and phytoplankton abundances and sizes. Elevated total diatom abundances (bottom panel), driven in part by increases in centric diatoms (top panel), were observed in the phytoplankton bloom that was sampled along the southeastern portion of the transect (e.g., Stn 7). Figure courtesy of Angelique White.

(2) Depth-Resolved Variability in Physical Habitat

Whenever the vessel was transiting, we regularly profiled an underway CTD (uCTD) system, providing highly resolved vertical structure in upper ocean physical and biogeochemistry. The uCTD included temperature, conductivity, pressure, chlorophyll fluorescence, and dissolved O_2 sensors. The resulting profiles demonstrated several key features of sampling region (Figure 28): (1) Across much of the southern portion of the transect (i.e., Stn 1-8), the depth of the chlorophyll fluorescence maximum shoaled and in some cases chlorophyll fluorescence was elevated in the near-surface ocean (e.g., Stn 4 and 7). (2) The depth of the fluorescence maximum deepened to ~150 m in waters where near-surface phosphate was relatively high but chlorophyll was low (i.e., Stn 10). (3) The surface mixed varied from <25 m to ~50 m, with the mixed layer shoaling in the southern sites and deepening to the northwest. (4) There was persistent subsurface O_2 maximum throughout the sampling transect, and the vertical

position of this feature deepened coincident with observed spatial variations in mixing and light. Apparent oxygen utilization (AOU), a derived proxy for respiratory O_2 consumption, increased in the interior waters of the northern portion of the transect. These findings are consistent with older waters with longer ventilation time scales.

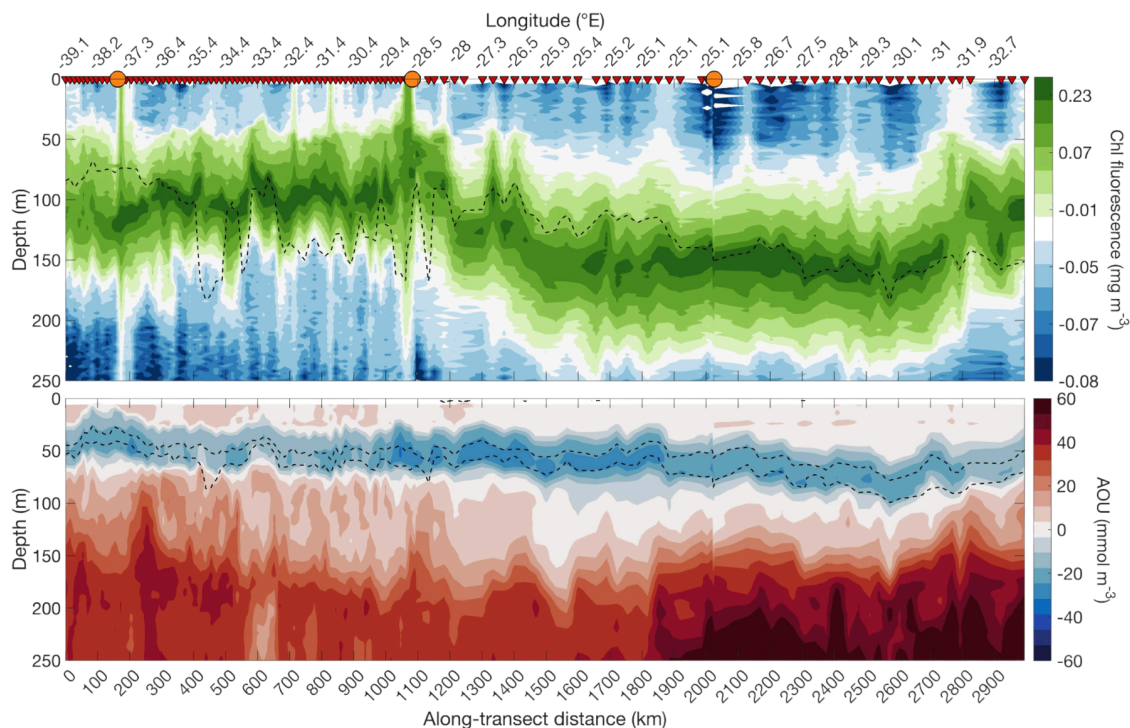


Figure 28. Spatial distribution of chlorophyll fluorescence and AOU along the FKt260303 cruise transect from underway CTD (uCTD) profiles. Orange symbols at top of panel represent positions of process stations 4, 7, and 10, respectively (left to right). Red triangles depict locations of discrete uCTD profiles. Dashed black line in the top panel depicts depth of the 25.5 kg/m^3 isopycnal surface. Dashed black lines in the bottom panel depict 24.5 and 25 kg/m^3 isopycnal surfaces. Figure courtesy of Benedetto Barone.

At the start of each long station, we deployed a series of assets, including the Wirewalker, which free-drifted during the duration of the station occupation. The resulting profiles provided rich time-resolved context for physical and biogeochemical variability at each station. At Stn 4, the vertical position of the chlorophyll maximum was relatively invariant, remaining around 100 m over the duration of time on station (Figure 29). The vertical position of the chlorophyll maximum closely followed the modest vertical oscillations in isopycnals over the time series. Beam attenuation, a metric used to track particle concentration, was elevated immediately below the mixed layer, but above the DCM layer.

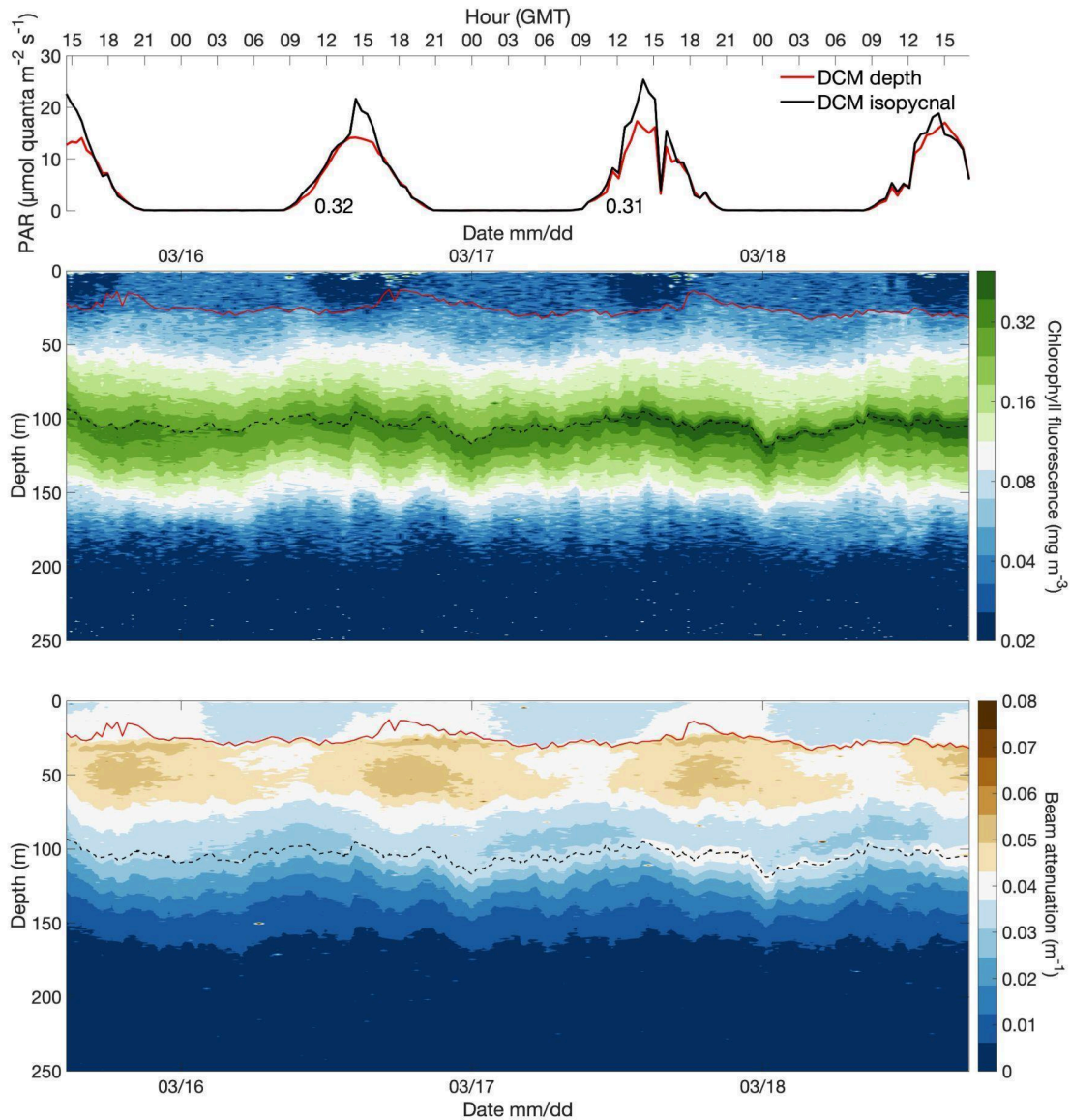


Figure 29. Bio-optical observations measured by a wave-powered drifting profiler (Wirewalker) in March 2026 at Station 4, onboard R/V Falkor(too) in the South Atlantic Subtropical Gyre. This station was characterized by a shallow mixed layer. Beam attenuation had a peak between the mixed layer and the DCM. Top panel depicts the time series of photosynthetically available radiation on the median depth of the DCM and on the median isopycnal surface of the DCM. Numbers below the daily peaks indicate the integrated daily irradiance on the DCM isopycnal with units of $\text{mol photons m}^{-2} \text{ d}^{-1}$. Center panel depicts the time series of chlorophyll fluorescence in the upper 250 m of the water column. Bottom panel depicts the time series of beam attenuation in the upper 250 m. The dashed black line and red line in the lower two panels depict the median DCM isopycnal and the base of the surface mixed layer (based on a 0.03 kg/m^3 offset from the potential density at 10 m depth), respectively. Figure courtesy of Benedetto Barone.

Deployment of the Wirewalker at Stn 7 provided insight into conditions associated with a decaying phytoplankton bloom (Figure 30). The mixed layer remained relatively shallow ($\sim 25 \text{ m}$), consistent with conditions observed at Stn 4. Although the fluorometer malfunctioned in the early period of observations, the initial observations confirmed upward shoaling of the fluorescence maximum. Moreover, the beam attenuation time series confirmed a pronounced particle maximum within the mixed

layer and extending into waters just above the chlorophyll maximum. Photon flux to the chlorophyll maximum was greater at Stn 7 than at Stn 4, consistent with upward shoaling of the fluorescence maximum. Moreover, time-variability in light flux to the chlorophyll maximum closely followed variations in light at the mean isopycnal layer hosting the chlorophyll maximum.

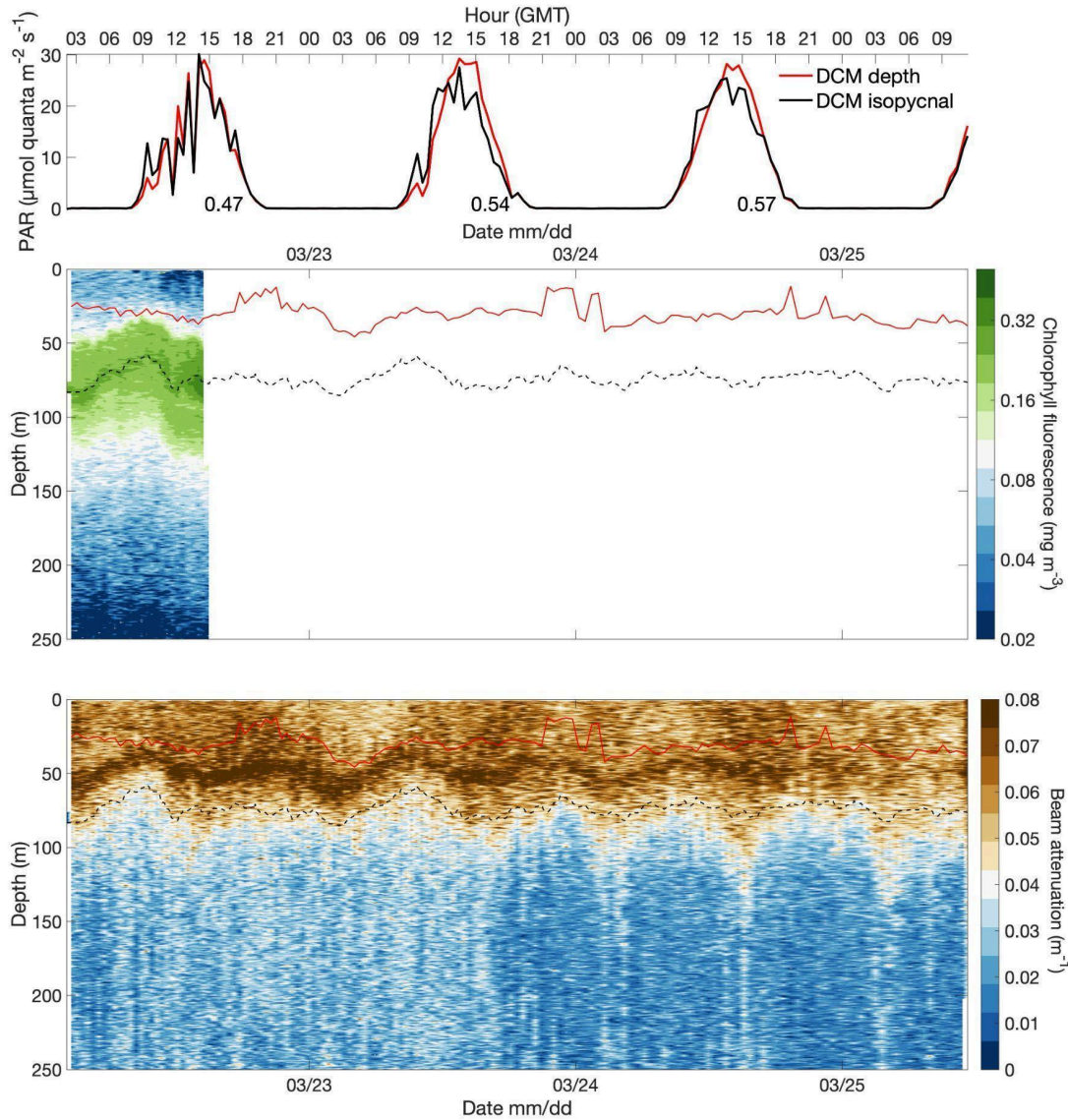


Figure 30. Bio-optical observations measured by a wave-powered drifting profiler (Wirewalker) in March 2026 at Station 7, onboard R/V Falkor (too) in the South Atlantic Subtropical Gyre. This station was coincident with a phytoplankton bloom observed from satellite. Unfortunately, the fluorometer stopped recording only several hours into the deployment. Beam attenuation had a peak slightly shallower than the DCM, which became less intense during the deployment. Top panel depicts the time series of photosynthetically available radiation on the median depth of the DCM and on the median isopycnal surface of the DCM. Numbers below the daily peaks indicate the integrated daily irradiance on the DCM isopycnal with units of $\text{mol photons m}^{-2} \text{ d}^{-1}$. Center panel depicts the time series of chlorophyll fluorescence in the upper 250 m of the water column. Bottom panel depicts the time series of beam attenuation in the upper 250 m. The dashed black line and red line in the lower two panels depict the median DCM isopycnal and the base of the surface mixed layer (based on a 0.03 kg m^{-3} offset from the potential density at 10 m depth), respectively. Figure courtesy of Benedetto Barone.

The time series from the Wirewalker deployment at Stn 10 confirmed the exceptional optical clarity of the waters in the northern portion of the sampling transect. The chlorophyll maximum at this site was very deep (150 m) and particle load (as reflected based on beam attenuation) was very low (Figure 31). As a result of the very deep nature of the feature, light flux to the DCM layer was the lowest of any of the sampled stations, and variability in light reaching this feature closely tracked the relatively modest vertical oscillations in isopycnals.

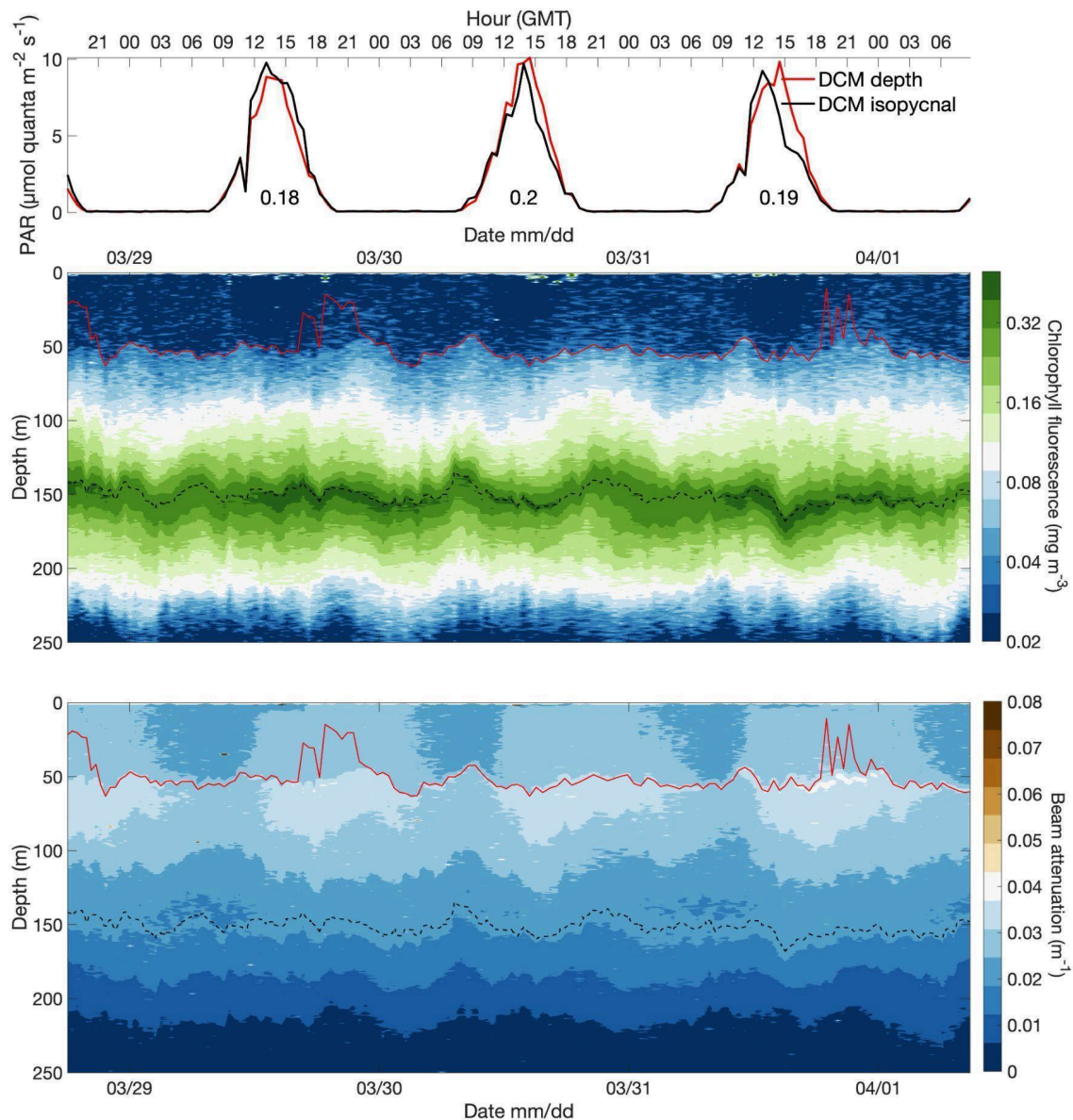


Figure 31. Bio-optical observations measured by a wave-powered drifting profiler (Wirewalker) in March 2026 at Station 10, onboard R/V Falkor (too) in the South Atlantic Subtropical Gyre. This station was characterized by low surface fluorescence and a deep DCM (~150m). Beam attenuation was low and peaked just below the base of the mixed layer (~50 m). Top panel depicts the time series of photosynthetically available radiation on the median depth of the DCM and on the median isopycnal surface of the DCM. Numbers below the daily peaks indicate the integrated daily irradiance on the DCM isopycnal with units of $\text{mol photons m}^{-2} \text{ d}^{-1}$. Center panel depicts the time series of chlorophyll fluorescence in the upper 250 m of the water column. Bottom panel depicts the time series of beam attenuation in the upper 250 m. The dashed black line and red

line in the lower two panels depict the median DCM isopycnal and the base of the surface mixed layer (based on a 0.03 kg m^{-3} offset from the potential density at 10 m depth), respectively. Figure courtesy of Benedetto Barone.

Spatial variability in solar energy input to the upper ocean was examined at each of the process stations using a hyperspectral radiometer (Hyperpro). Daily integrated irradiance reaching the base of the mixed layer was greatest within the high PO_4^{3-} low chlorophyll waters (Station 10; Figure 32), consistent with low particle load. In regions where phytoplankton biomass was elevated (i.e., Stn 7), light flux within the mixed layer was reduced and slightly shifted to longer wavelengths, consistent with light attenuation due to absorption and scattering by particles. In contrast, at the depth of the chlorophyll maximum, daily integrated light was greatest at Stn 7, where phytoplankton biomass was elevated, but the chlorophyll maximum layer had shoaled upward, with light flux to the DCM lowest at Stn 10 where the depth of this feature extended to 150 m.

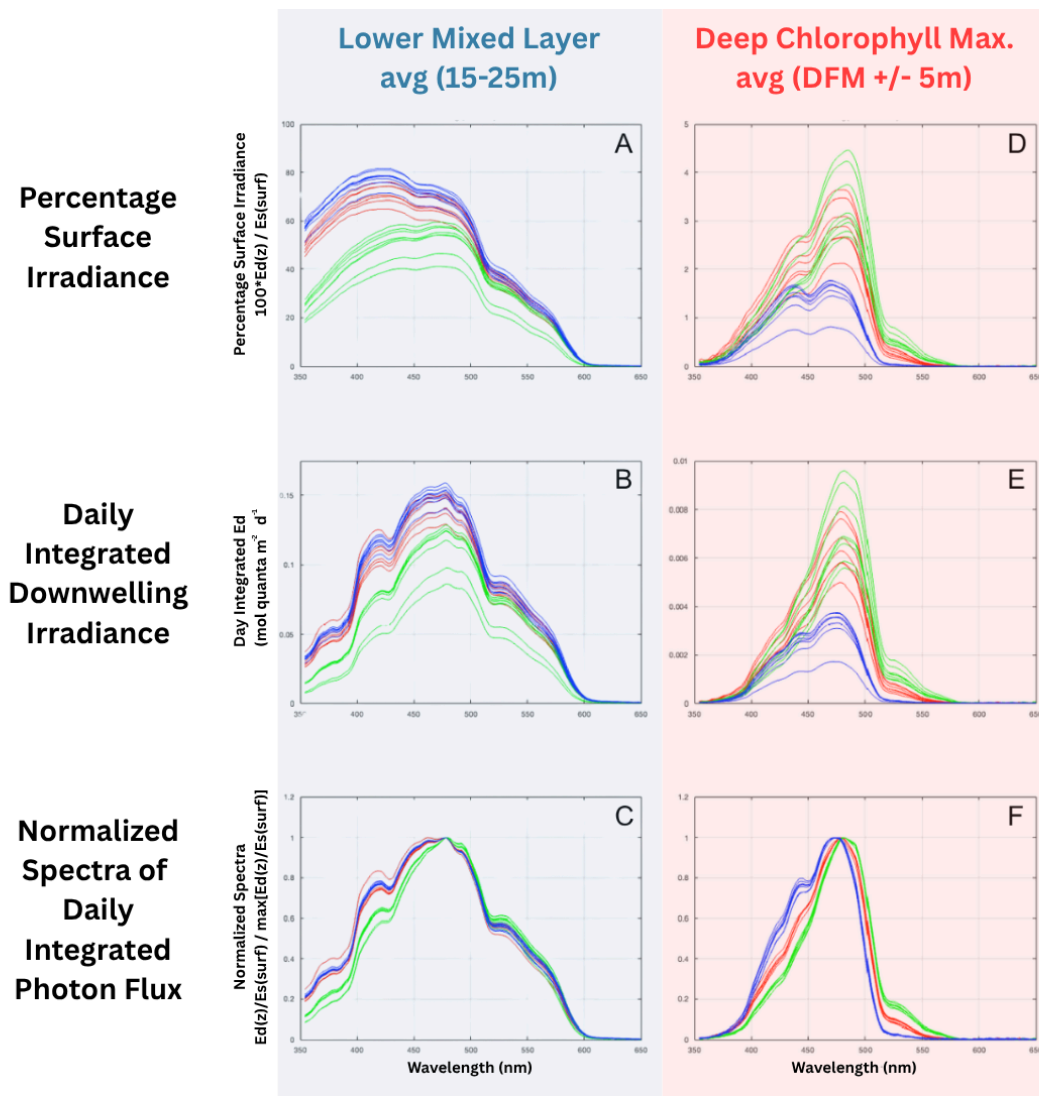


Figure 32: Spectral distribution of photosynthetically available radiation in the lower mixed layer (A, B, and C); and at the depth of the Deep Fluorescence Maximum (D,E,F) at Station 4 (red), Station 7 (green), and Station 10 (blue). A and D = Daily integrated spectral photon flux at depth; B and E = Spectral distribution of the percentage surface irradiance reaching

the lower mixed layer and Deep Fluorescence Maximum, respectively; *C* and *F* = Normalized spectra of the daily integrated photon flux at depth. Figure courtesy of Ricardo Letelier.

(3) Depth-Resolved Variability in Biogeochemistry

Some of the clearest examples highlighting differences in the vertical structure of biogeochemistry can be observed from comparisons of CTD-derived fluorescence, dissolved O₂, and nutrients at each of the longer process stations. The cruise targeted two different process stations (Stn 2 and 7) which were in regions that satellite ocean color observations indicated were actively blooming. Stn 2 was located along the western edge of the bloom where chlorophyll concentrations remained elevated (Figure 33). In contrast, Stn 7 was located along the eastern flank of the bloom region and at the time of our occupation. Figure 33 depicts satellite derived chlorophyll concentrations during the week we occupied each station (panel b) alongside the monthly (March 2026) composite chlorophyll product (panel a) which was used to select station locations.

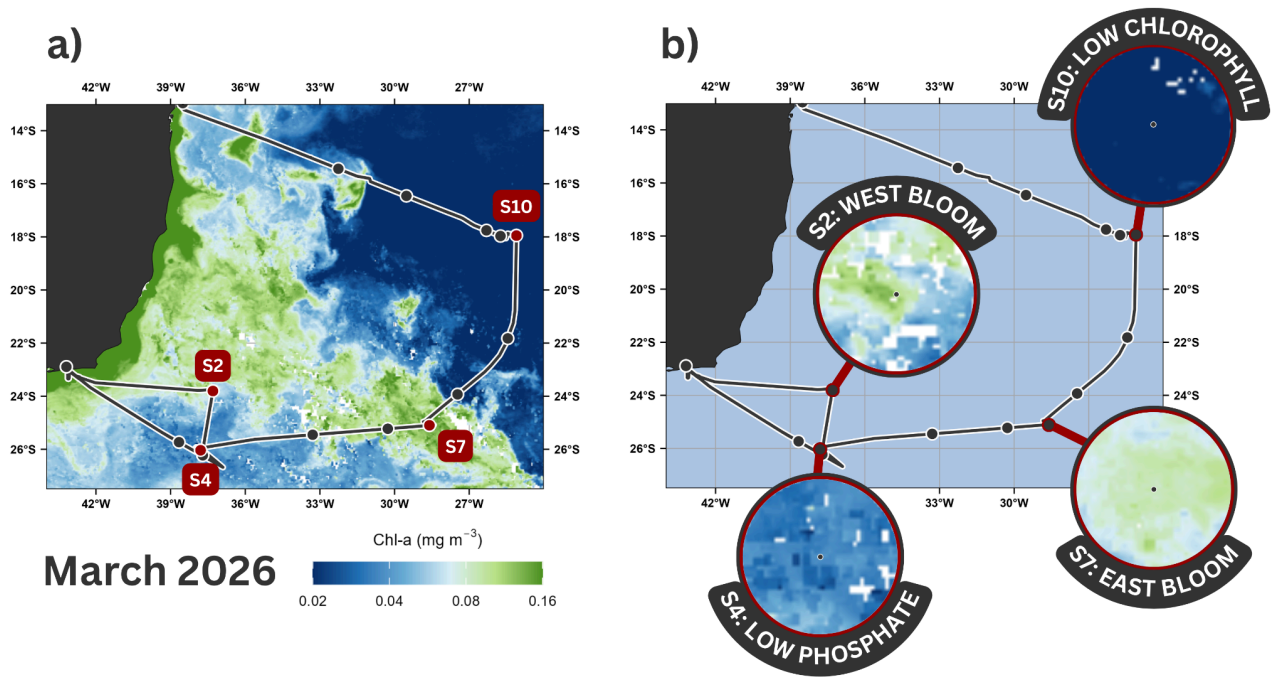


Figure 33. Satellite chlorophyll-a conditions across the FKt260303 cruise track and during occupation of the four longer stations. (a) March 2026 chlorophyll-a conditions along the cruise track, derived from Aqua MODIS 8-day composite observations, with Stns 2, 4, 7, and 10 highlighted. (b) Local chlorophyll-a conditions within a 50-nautical-mile radius of each process station. Circular insets show the mean of available daily Aqua MODIS chlorophyll-a observations over a 7-day window encompassing each station occupation: 7–13 March for Stn 2, 13–19 March for Stn 4, 20–26 March for Stn 7, and 27 March–2 April for Stn 10. Station locations are indicated by the central grey points. Together, the panels illustrate the contrasting chlorophyll regimes sampled across the expedition, including elevated chlorophyll at Stns 2 and 7 and lower chlorophyll conditions at Stns 4 and 10. Data source: Aqua MODIS chlorophyll-a, NOAA CoastWatch ERDDAP; 8-day composite product `erdMH1chla8day_R2022NRT` for panel (a) and daily product `erdMH1chla1day_R2022NRT` for panel (b). Figure courtesy of Katie Coates.

Notably, at both of these stations, CTD-based vertical profiles of fluorescence indicated relatively shallow chlorophyll maximum layers (mean depth of the DCM was 62 m at Stn 2 and 82 m at Stn 7; Figure 34, panel a). Moreover, when examined as a function of density, the chlorophyll maximum at Stn 2 resided on a much shallower isopycnal than other stations. In addition, both Stn 2 and 7 demonstrated considerably more variability in fluorescence throughout the upper photic zone layer than other sampled sites, a finding we attribute to submesoscale patchiness in the vertical structure of the bloom. In comparison, both Stn 4 (low PO_4^{3-} waters) and Stn 10 (high PO_4^{3-} but optically clear waters) demonstrated deeper chlorophyll maximum layers (113 m and 153 m, respectively) more typical of the subtropical ocean gyres. At these stations, cast to cast variability in fluorescence at the chlorophyll maximum layer appeared closely linked to isopycnal heaving (Figure 34, panel b).

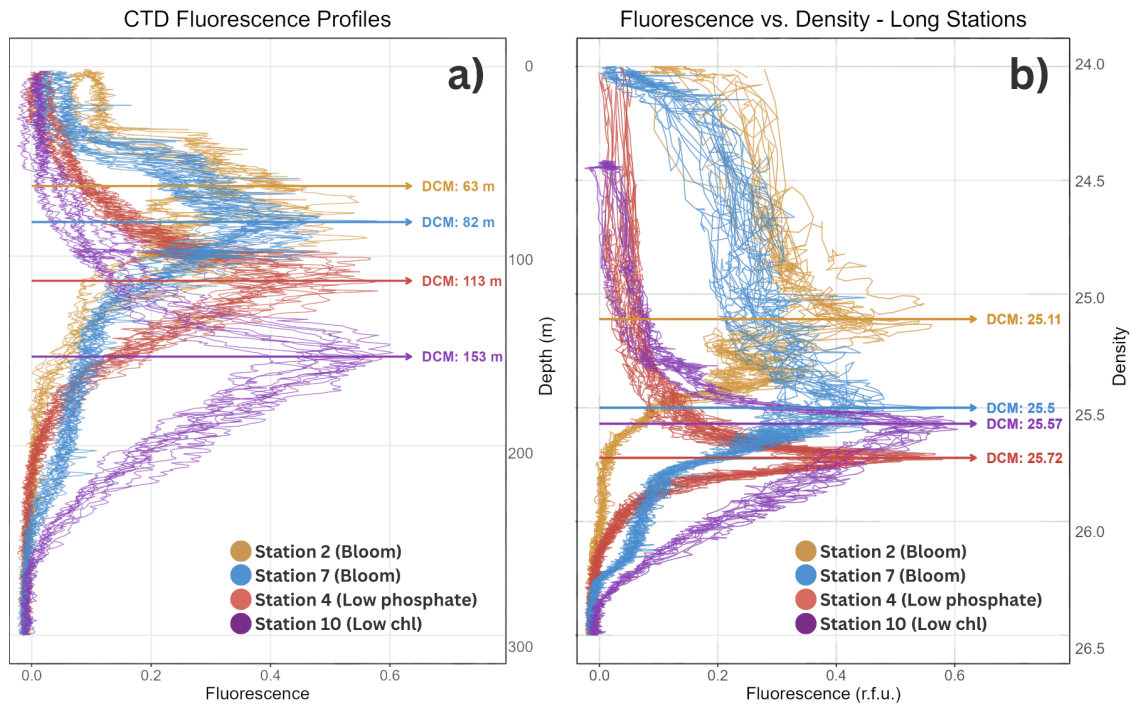


Figure 34. Vertical structure of fluorescence at the four longer stations (2, 4, 7, & 10) during FKt260303. CTD-derived fluorescence profiles illustrate contrasting chlorophyll maximum depths and vertical structure. a) fluorescence plotted as a function of depth from all CTD casts on station. b) fluorescence plotted relative to seawater density from all CTD casts on station, illustrating differences in the density surfaces associated with the chlorophyll maximum among stations. Data are preliminary and fluorescence measurements are uncalibrated. Figure courtesy of Katie Coates.

Another interesting feature of the two bloom stations (Stn 2 and 7) is revealed by examining vertical distributions of dissolved O_2 relative and fluorescence. At both bloom sites, the subsurface maximum in dissolved O_2 coincided with the vertical position of peak fluorescence, suggesting the fluorescence maximum reflected the distributions of phytoplankton biomass (Figure 35). In contrast, at the non-bloom stations (e.g., Stn 4 and 10), the subsurface O_2 maximum was vertically decoupled and shallower than the fluorescence maximum. These findings point to the fluorescence maximum as likely indicative of a photoadaptive response by phytoplankton growing at low light fluxes, manifest through

increases in chlorophyll per cell. The shallower dissolved O₂ maximum is consistent with net photosynthetic production of O₂ occurring below the mixed layer but still above the compensation depth.

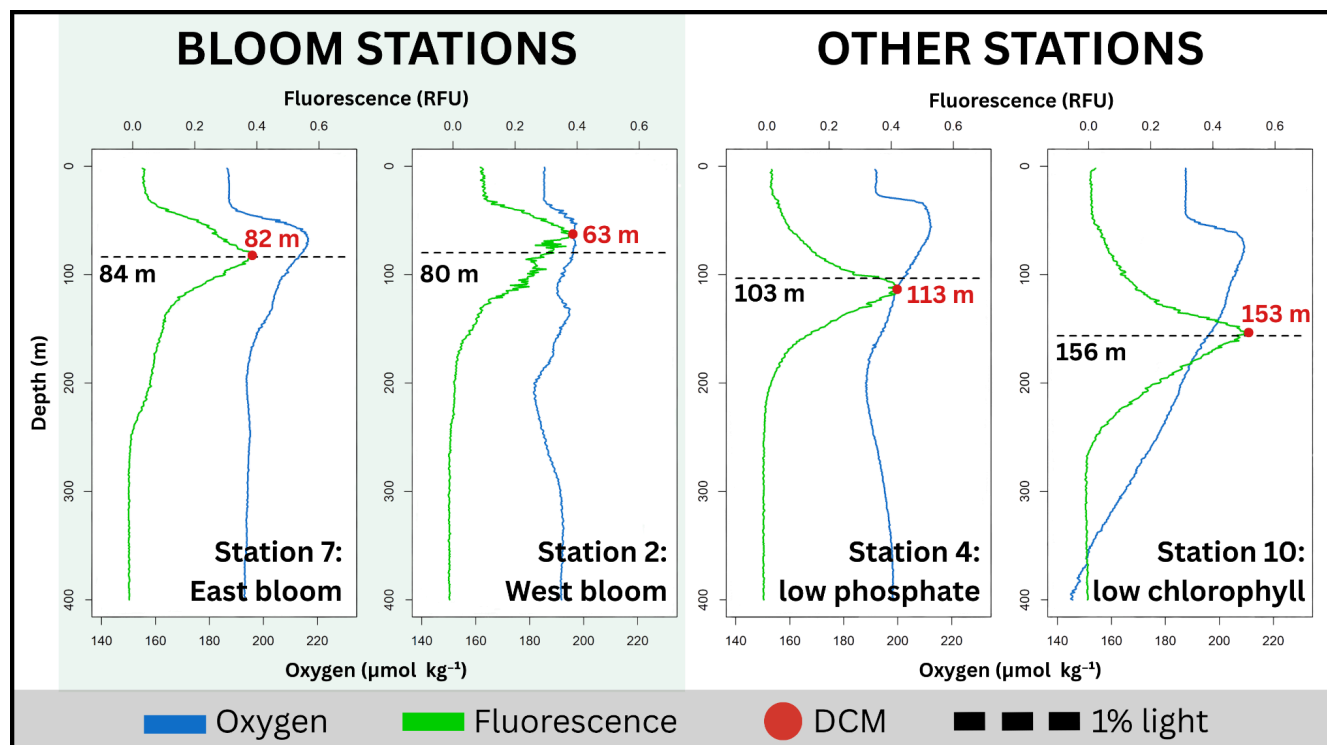


Figure 35. Vertical distributions of CTD-derived fluorescence and dissolved O₂ at the four longer stations during FKt260303. At the “bloom stations” a high-chlorophyll feature was observed (Stns 2 and 7), compared to the “other stations” (Stns 4 and 10) where such a feature was not observed. Oxygen profiles are shown in blue, fluorescence profiles are shown in green, the DCM is displayed as a red dot and its depth is labelled in red, and the depth of 1% light is plotted as a dotted black line as well as labelled. Data are preliminary and fluorescence and dissolved O₂ measurements are uncalibrated. Figure courtesy of Katie Coates.

The vertical distributions of nutrients also varied among the process stations. We were fortunate to have an optical nitrate sensor (SUNA) mounted to the CTD rosette, providing time-resolved, repeated vertical profiles of nitrate whole on station. Although the lower detection limit for this instrument prevented it from acquiring meaningful data in the upper layer of the photic zone, the instrument provided insight into variability in the depth of nitracline, and enabled assessment of coupling between distributions of nitrate, light, fluorescence, and dissolved O₂. For example, in comparing the bloom stations (Stn 2 and 7), both the chlorophyll and O₂ maxima occurred at depths shallower than the nitracline (Figure 36). In contrast, at the non-bloom stations (Stn 4 and 10), the nitracline depth vertically coincided with the chlorophyll maxima.

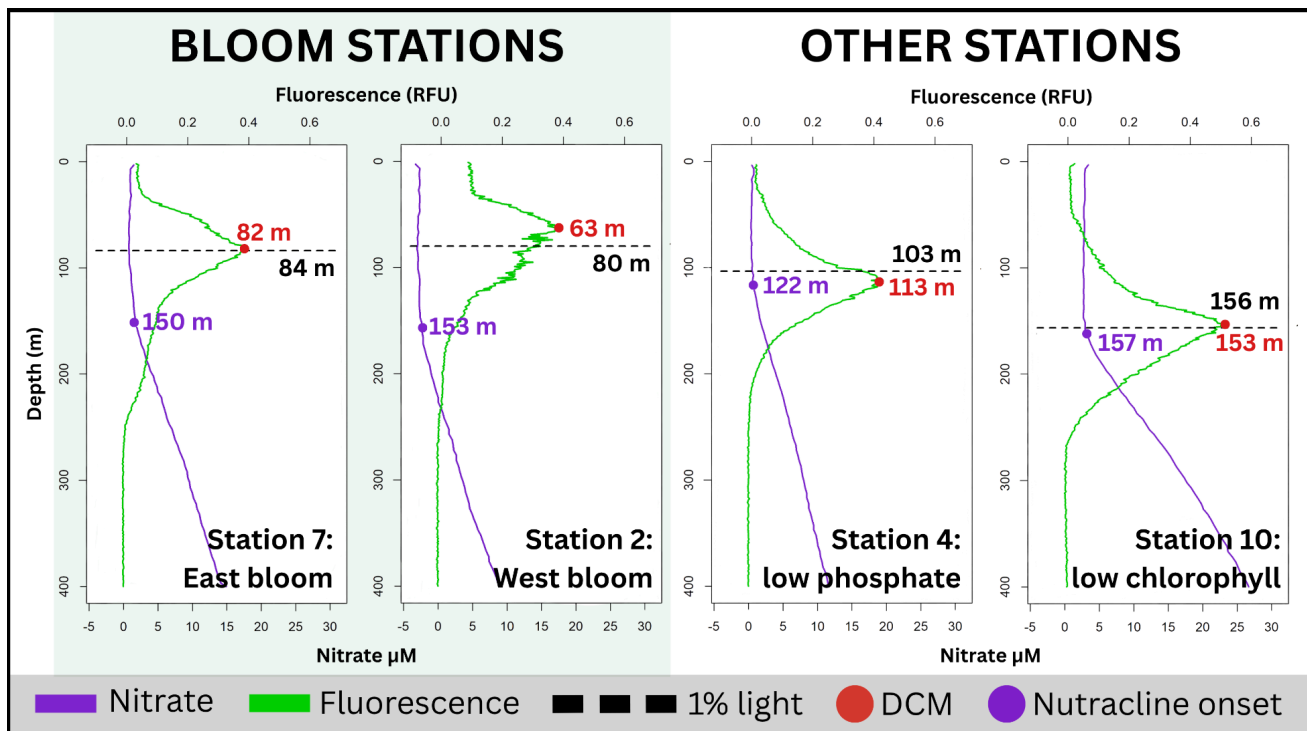


Figure 36. Vertical distributions of CTD-derived fluorescence and nitrate measured by the rosette-mounted SUNA optical nitrate sensor at the four longer stations during FKt260303 (Stns 2, 4, 7, & 10). At the bloom stations (Stns 2 and 7), the fluorescence and dissolved O_2 maxima occurred above the nitracline. In contrast, at the non-bloom stations (Stns 4 and 10), the fluorescence maximum occurred at approximately the same depth as the nitracline. Nitrate profiles are shown in purple, fluorescence profiles are shown in green, the depth in meters of the 1% light level is marked by a black dotted line as well as labelled, the DCM is labelled in red, and the nutracline onset is labelled in purple. SUNA measurements in the upper photic zone were limited by the instrument's lower detection limit. Data shown are preliminary and uncalibrated. Figure courtesy of Katie Coates.

Concentrations of dissolved and particulate trace elements also varied along the cruise transect. Interestingly, particulate P concentrations were elevated in the southern portion of the cruise transect (e.g., Stn 4) where surface ocean PO_4^{3-} was low, suggesting active biological consumption may have reduced phosphate concentrations in the near-surface ocean in these waters prior to our arrival. Overall, particle-bound metals (e.g., cobalt, cadmium, nickel) tended to be elevated in the photic zone and decreased with depth; however, particulate manganese demonstrated an opposite pattern, with concentrations greatest in the dim mesopelagic waters, likely reflecting sunlight catalyzed reduction of manganese oxides back to the dissolved Mn^{2+} phase in the surface ocean (Figure 37).

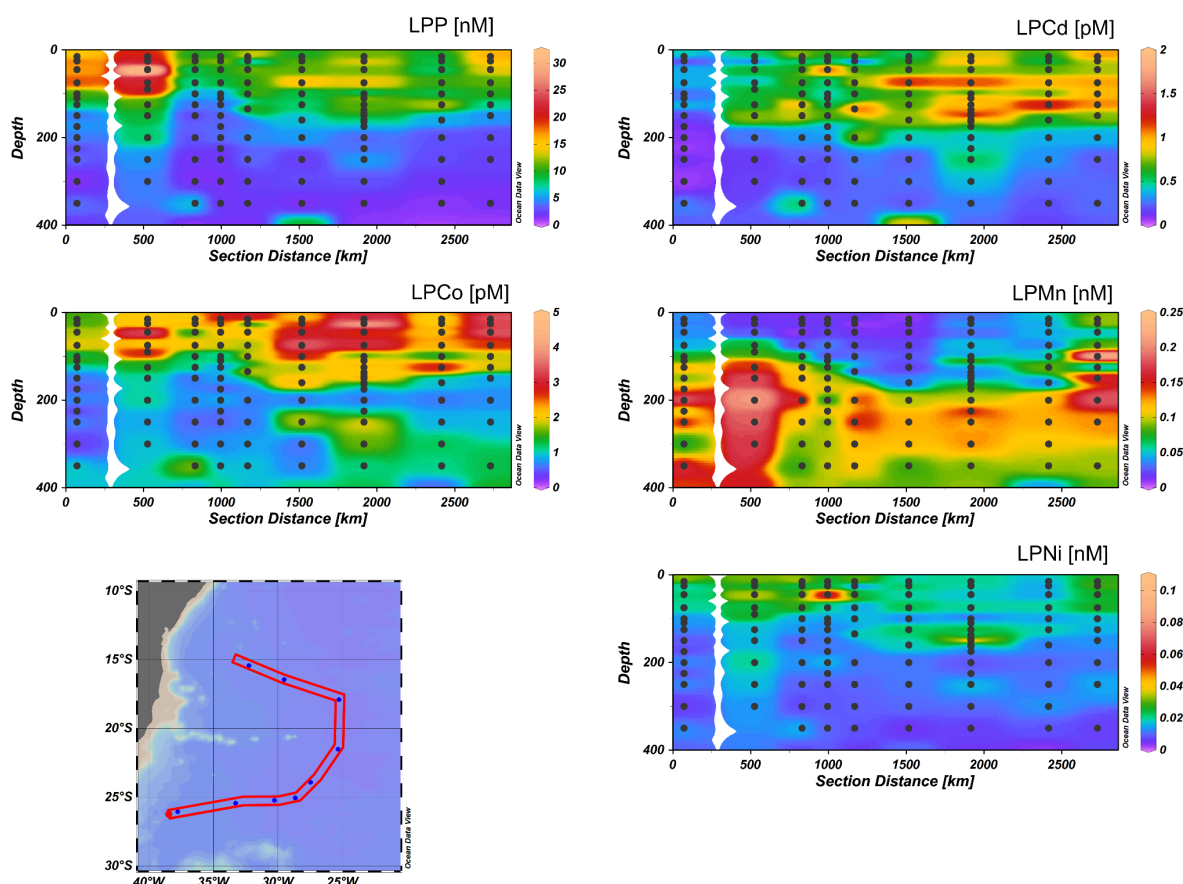


Figure 37. Sections of the distribution labile particulate P and biologically-associated trace metals cadmium, cobalt, manganese, and nickel. Color is concentration and x axis is the section distance following the map from station 4 to 14. Most elements show highest concentrations in the upper 100 m where there is highest biomass, except for particulate Mn, which experiences depletion within the euphotic zone due to photo-dissolution of Mn oxides. Figure courtesy of Alexis Floback.

Vertical distributions of particulate C and N were examined based on analyses of discrete seawater samples collected from the high vertical resolution CTD casts focused around the DCM layer. These samples are also being used for analyses of bulk and compound specific stable isotopes (^{13}C and ^{15}N). Particulate organic C concentrations were nearly twice as high in the upper photic zone in the decaying phytoplankton bloom (Stn 7) compared to the high phosphate site (Stn 10). At both Stn 7 and 10, particulate organic C concentrations decreased below the chlorophyll maximum (Figure 38), and as observed at Stn ALOHA, despite vertical differences in the position of the chlorophyll maximum layer and in daily integrated light flux to these features, the bulk ^{13}C of suspended particles consistently decreased within this layer (Figure 38). Such observations suggest this shift in isotopic signatures likely reflects vertical differences in the photosynthetic community rather than reflecting a physiological response to light limitation.

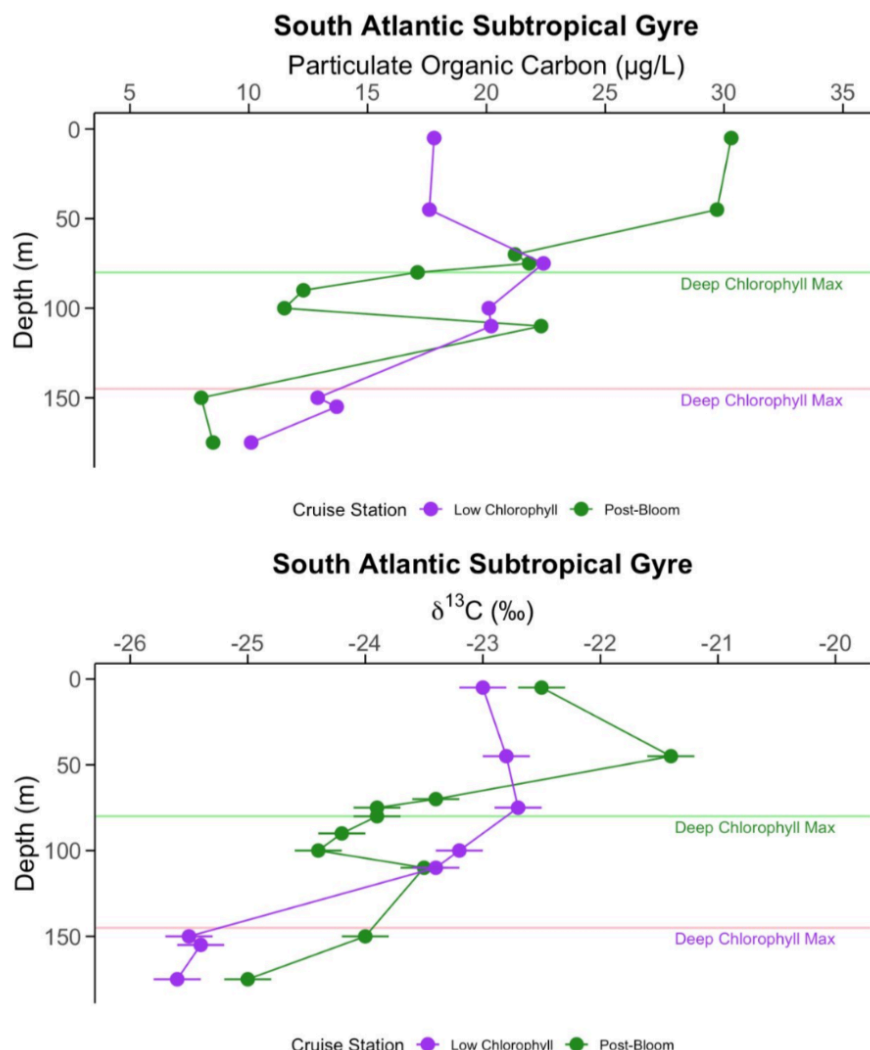


Figure 38: Bulk particulate organic C (POC) concentrations (top) and bulk $\delta^{13}\text{C}$ depth profiles of particulate organic C at contrasting stations in the South Atlantic (bottom). Low chlorophyll, Station 10; Decaying bloom, Station 7. Figure courtesy of Close Lab.

At three of the main process stations we quantified rates of N_2 fixation and C fixation using ^{15}N and ^{13}C -bicarbonate as tracers, respectively. These measurements were conducted from triplicate seawater samples collected in the pre-dawn hours at 6 discrete depths (5, 25, 45, 75, 100, 125 m). Seawater was subsampled from the CTD rosette, inoculated with the stable isotope tracers, and incubations were conducted over 24 hrs on a free-drifting array. We found very low rates of N_2 fixation, with rates mostly below the limits of detection at all sites aside for Stn 7 (the decaying bloom site; Figure 39); however even at Stn 7 N_2 fixation rates were considerably lower than typically observed at Stn ALOHA (e.g., mixed layer rates were $<2 \text{ nmol N L}^{-1} \text{ d}^{-1}$ in the SASG compared to $3\text{--}4 \text{ nmol N L}^{-1} \text{ d}^{-1}$ at ALOHA). Similarly, rates of C fixation were also low at all stations. Depth-integrated (0–125 m) C fixation varied between $202 \text{ mg C m}^{-2} \text{ d}^{-1}$ and $294 \text{ mg C m}^{-2} \text{ d}^{-1}$; in comparison, the mean rate of primary production at Stn ALOHA is $527 \text{ mg C m}^{-2} \text{ d}^{-1}$.

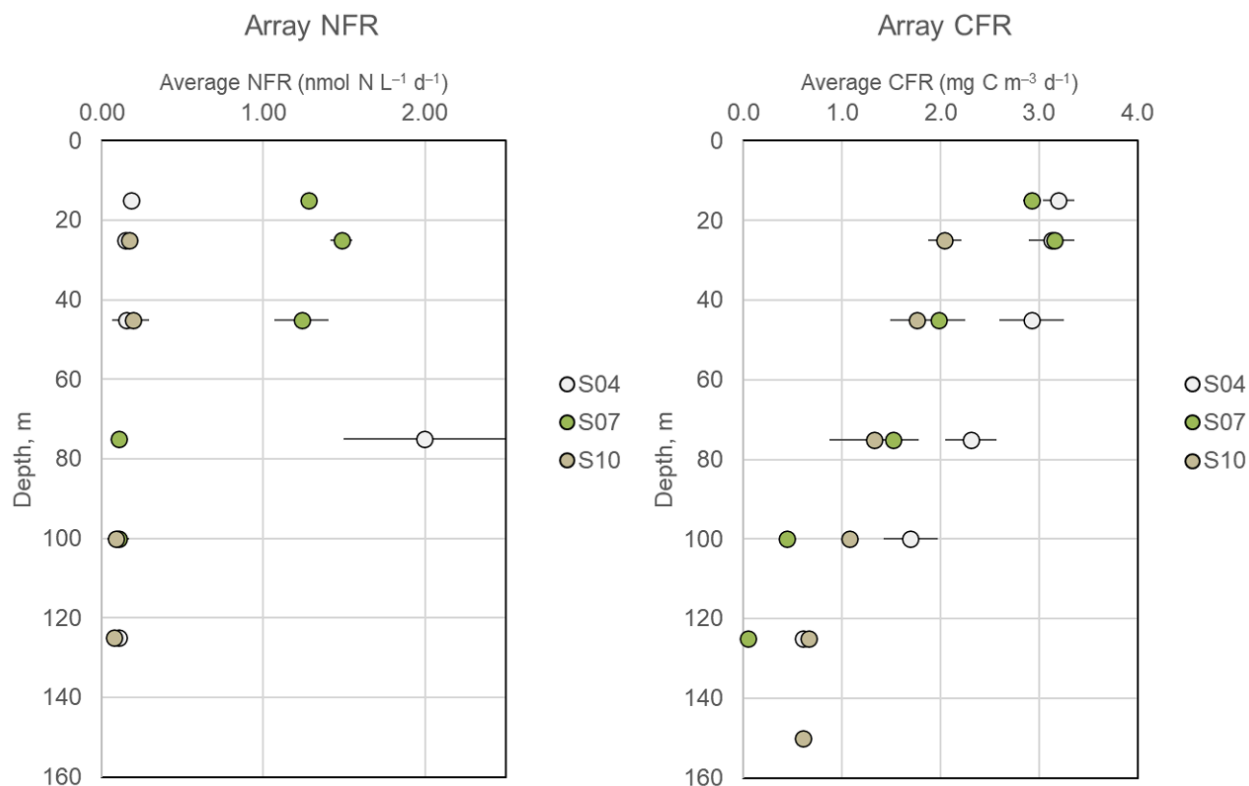


Figure 39. Vertical structure in rates of N_2 and C fixation at three SUBSEA process stations in the SASG. Rates of both N_2 and C fixation were low at all sites. Figure courtesy of Angelicque White.

Despite very low rates of both N_2 and C fixation, droplet digital polymerase chain reaction quantification of N_2 fixing cyanobacteria revealed relatively high abundances of several diazotrophs along the southwestern portion of the cruise transect, with particularly high abundances of the diatom endosymbiont *Richelia spp.* within the decaying bloom (Stn 7; Figure 40).

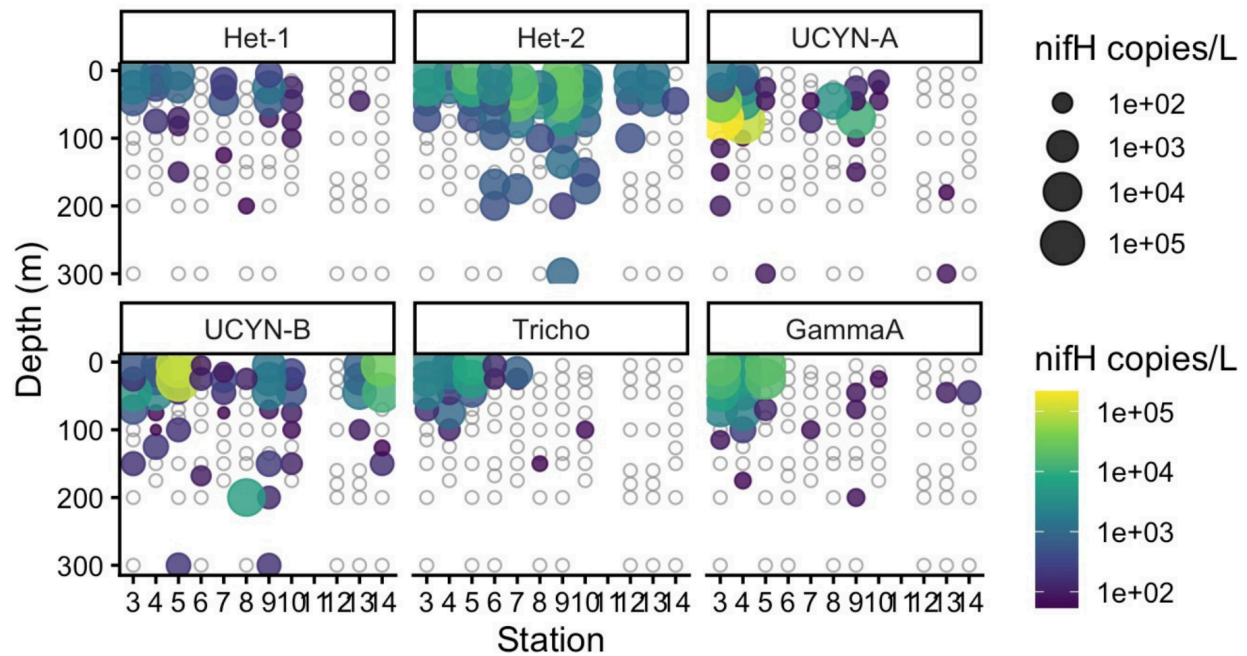


Figure 40. Droplet digital PCR analyses of *nifH* gene abundances arranged by station and depth. Positive detections are log-scaled; open grey circles are samples below the limits of detection. Het-1 and Het-2 reflect abundances of *Richelia* associated with different diatoms (*Rhizosolenia* and *Hemiaulus*, respectively); UCYN-A reflects gene abundances of the nitroplast of *Atelocyanobacterium thalassa*; UCYN-B reflects *Crococosphera*; Tricho reflects *Trichodesmium* gene abundances; GammaA reflects gene abundances of a diatom symbiont belonging to the order Rhizobiales. Figure courtesy of Esther Mak.

We also examined spatial variation in plankton metabolism (gross O_2 production and respiration) at each of the process stations. Gross O_2 production was quantified using $^{18}H_2O$ labeled water, measuring the $^{18}O_2$ byproduct of photosynthesis. Respiration was estimated based on changes in $O_2:Ar$ in dark bottle incubations. All incubations were hung from a free-drifting array and incubated at the discrete depths of sample collections over 24 hrs. Despite relatively large differences in particle concentrations (e.g., see Wirewalker beam attenuation), rates of gross O_2 production and respiration were relatively invariant across sampling sites (Figure 41). Rates of gross O_2 production and respiration were lowest at Stn 10; however, at all stations, these two processes appeared closely coupled, resulting in only small differences in net community production (Figure 41). Both gross O_2 production and respiration were generally both elevated in the well-lit portion of the photic zone, with rates of both decreasing into the subsurface layer. However, at all depths production and respiration were closely balanced (i.e., net community production = 0).

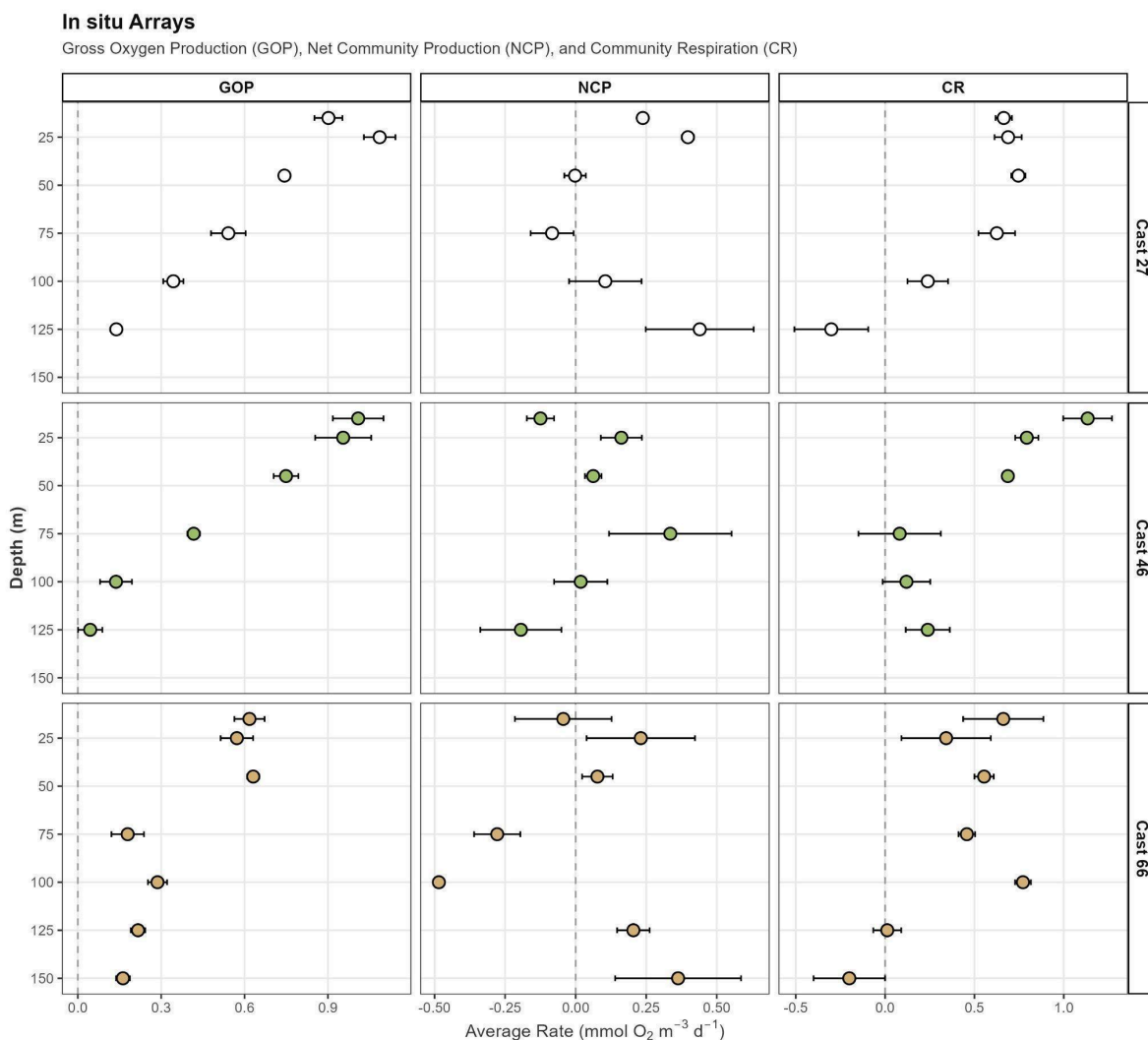


Figure 41. Depth profiles of gross O_2 production (GOP), net community production (NCP), and community respiration (CR) measured using in situ incubation arrays during Casts 27 (st 4), 46 (st 7), and 66 (st 10). Points represent average rates at each incubation depth, with error bars indicating variability among replicate measurements. The dashed vertical line indicates zero net O_2 production. Positive NCP values indicate net autotrophy, whereas negative values indicate net heterotrophy. Rates are expressed as $\text{mmol O}_2 \text{ m}^{-3} \text{ d}^{-1}$. Figure courtesy of María Luz Torres Alberto.

We deployed the SUBSEA sediment trap array at 5 different stations (Stn 1, 2, 4, 7, 10) during the FKt260303 cruise. At 3 of these stations (Stn 4, 7, 10) this included particle interceptor traps at 5 discrete depths (75, 100, 150, 225, 300 m), while at Stn 1 and 2 traps were deployed at 2 discrete depths (75 and 150 m). Samples were collected for analyses of particulate organic and inorganic C, particulate N, particulate P, particulate Si, stable isotopes of C and N (^{13}C and ^{15}N) for bulk and compound-specific analyses, subsamples for analyses of total N and P in trap solutions, particle imaging by IFCB, and DNA. At the time of this writing, these samples are actively being processed in respective SUBSEA laboratories. When available, these data will be invaluable for helping examine spatial variability in material export and variations in flux attenuation.

Reflections on SUBSEA Fieldwork in the NPSG & SASG 2025–2026

By nearly any measure, SUBSEA has had a productive set of field campaigns over the past 1.5 yrs. We successfully completed 11 cruises, including a logistically complex 35 day cruise in the SASG and integration of SUBSEA research into 10 HOT program cruises in the NPSG. The emergent datasets are providing a wealth of information needed to address our primary research questions: 1) How are biogeochemical processes coupled between the two photic zone layers found in the subtropical gyre ecosystems and what processes (biological and physical) strengthen or weaken this coupling? And 2) To what extent does particle remineralization fuel primary production and export from the subsurface photic zone layer? Our fieldwork is addressing these questions through a combination of time and space resolved sampling.

For our work in the NPSG, we rely on near-monthly HOT program cruises, providing seasonally resolved observations on the functioning of the photic zone habitat. For the SASG, our fieldwork is restricted to the austral summer, when the SASG is stratified and most nutrient depleted. In the SASG, we sampled across spatially resolved biogeochemical gradients to assess the functioning of the two photic zone layers. Together, we are hopeful our observations will lend themselves to comparative analyses of these gyres. Our work has already provided new insights into the functioning of these ecosystems. For example, we find a substantial fraction of N fixed by diazotrophs moves through dissolved N pools on short time scales (<24 hrs). In addition, our analyses of dissolved N and P in sediment traps suggests particle solubilization can lead to substantial underestimation of export based solely on particle fluxes.

Our field-based observations provide a robust framework for characterizing the surface and subsurface photic layers in the subtropical gyres. We find that the subsurface layer is characterized by increased stratification and very low diapycnal diffusivity, suggesting vertical input of nutrients to the base of the photic zone is low. Moreover, the subsurface hosts a persistent DCM, although the intensity and vertical positioning of this feature varies on a variety of time scales from diurnal to seasonal. Notably, the DCM often appears vertically decoupled from the more particle-enriched waters below the mixed layer. Despite expectations, particle flux is lower in the subsurface layer than the overlying waters. Residence times of Fe are shorter in the subsurface layer than in the overlying waters, implying more rapid export of Fe from the deeper photic zone waters. Plankton community structure shifts coincide with the vertical position of the DCM, a transition that imprints unique geochemical signatures on organic matter in the subsurface layer. Finally, the subsurface habitat has persistent but variable concentrations of remineralized nutrients (including NH_4^+ , NO_2^- , and NO_3^-) and these dim waters sustain elevated rates of key N remineralization pathways (including ammonia and nitrite oxidation).

In comparison, we find the well-lit photic zone layer retains several defining characteristics that include elevated rates of primary production, respiration, and N_2 fixation. The surface layer also often hosts elevated particle concentrations, often observed at the base of the mixed layer but above the DCM. Export fluxes out of the well-lit layer exceed those observed at the base of the photic zone, suggesting active remineralization, solubilization, or disaggregation and slowing of particle sinking during transit through the DCM layer. However, all of these mechanisms of particle degradation would require storage of nutrients imported via sinking particles from the overlying waters. To date, there is little evidence

suggesting long-term accumulation of such nutrient pools in the gyres. Hence, SUBSEA's fieldwork has provided new information necessary for characterizing the photic zone habitat - clearly our next steps are to use this information to improve understanding of time and space coupling between these layers.

Our work has yielded a few findings that we find interesting and demanding of our continued attention. Perhaps most fundamentally, our sediment trap measurements indicate C and N flux out of the well-lit layer is greater than that exported from the base of the photic zone. Such observations challenge assumptions of steady state and complicate mass balances for the upper ocean. Our observations imply that either remineralized byproducts or organic matter (or both) are stored on time scales currently longer than our observational window, or that there is some other process, perhaps physical advection, removing material from the lower layer. Hence, even at monthly scales of sampling, we still do not appear to be adequately resolving time-coupling between two layers. This is further complicated by differential residence times of elements in the photic zone; for example, while N appears to turnover relatively quickly (1-2 weeks), Fe cycles for intermediate time scales (2-3 months), while other nutrients (e.g. phosphate) have effective residence times of ca. 1 year.

Another related finding that demands additional attention centers on improving understanding of time scales coupling plankton biomass to biogeochemical fluxes. Our work in the SASG, in particular our sampling of a decaying phytoplankton bloom, revealed that despite elevated abundances of phytoplankton, including diatom-diazotroph assemblages, we observed low rates of both C- and N₂ fixation. These results suggest that biomass accrual can be decoupled from ecosystem fluxes. This relates to the issue of residence times previously highlighted and begs a number of questions such as over what time scales will biomass persist in the upper ocean after plankton are no longer metabolically active and contributing to ecosystem processes? And how long does organic matter produced from N supplied via N₂ fixation cycle through the upper ocean before it is exported? One thing we will be discussing for future cruise planning is whether we should occupy our sampling stations (using quasi-Lagrangian approaches) for at least the time scale of elemental turnover times.

We are also considering whether our sediment trap findings might reflect methodological biases. Use of sediment traps to estimate photic zone particle fluxes has been previously challenged by issues related to zooplankton swimmers (e.g., cryptic swimmers and gelatinous zooplankton) which can result in overestimation of particle flux. In addition, particle interceptor traps like the ones SUBSEA uses have been shown to both under- and over-collect particles due to hydrodynamic shear in the upper ocean. The relatively strong upper ocean currents have been shown to horizontally advect particles faster than downward sinking speeds, a problem exacerbated by attaching traps to a fixed, slow moving array. Finally, upper ocean currents could introduce advective flows that effectively decouple export from the upper layer to lower layer. As a result, knowledge of vertical variations in the trap collection area complicates a 1-D assessment of particle fluxes. In an effort to address possible methodological issues with sediment trapping in the upper ocean, on two recent HOT cruises (HOT 364 and 365) we tested use of Marine Snowcatchers as tools to estimate fluxes from the upper and lower layers, respectively, independent of the use of sediment traps. Results from use of these tools are pending.

One area of SUBSEA research that we have initiated that we are excited to continue to explore focuses on particle degradation and the stoichiometry of organic matter remineralization. Our efforts on this topic began with a series of experiments on the SASG cruise, designed to identify the mechanisms underlying degradation of zooplankton wastes, specifically fecal pellets. Experiments were designed to

examine the stoichiometry of fecal pellet decomposition. Recognizing that the stoichiometry of production and remineralization control carbon export potential, future SUBSEA efforts should be directed towards examining potential stoichiometric differences in organic matter production and remineralization within the upper and lower layers. If C fixed per unit nutrient assimilated is identical in both layers, there should be no net C removal associated with subsurface recycling of surface-derived organic matter. However, if the stoichiometry of subsurface layer production or remineralization differs from the surface, recycling of nutrients from the upper layer could support additional new production in the lower layer.

Our sampling in two subtropical gyre ecosystems offers new opportunities for comparative synthesis. We have already begun thinking about various comparative analyses that link our time-resolved work in the NPSG to the spatially-focused sampling within the SASG. Our work in both of these ecosystems captured a relatively large dynamic range in upper ocean biogeochemistry, including spanning times and places where P concentrations, phytoplankton biomass, and Fe supply were both low and high. These conditions offer some intriguing commonalities which we hope to use to facilitate cross-system comparisons on the functioning of these gyres.

Appendix

Appendix Table 1: Summary of SUBSEA instruments used on Fkt260303. X's denote whether the instrument was deployed itself or utilized an underway system. For sensors that were deployed on another instrument, the instrument it was deployed on is listed. If an instrument was deployed, the number of deployments is listed in the “#” column.

INSTRUMENT	PARAMETER	DEPLOYMENT	#	UNDERWAY SYSTEM	LAB GROUP
Conductivity, Temperature, & Depth (CTD) rosette	Temperature, Salinity, Pressure, O ₂ , Fluorescence, Beam transmissometry, Photosynthetically Active Radiation (PAR)	X	80		Schmidt Ocean Institute
Trace Metal CTD (TMC) rosette	Pressure	X	22		Hawco
24-hour Free-drifting In-situ Array (A)	¹³ C-primary production, ¹⁵ N ₂ fixation, rates of N cycling processes	X	3		White, Fawcett
Sediment Trap Array (ST)	Particulate C, N, P, Si, PIC, ¹⁵ N-PN, ¹³ C-POC, IFCB, DNA, TDN, TDP, and trace elements	X	5		Hawco, Close, Church, Barone, White
McLane In-situ Pump (MP)	Particulate C, N, P, bulk and compound specific ¹⁵ N-PN, ¹³ C-POC, and trace elements	X	8		Close, Hawco, Church
Sea Bird Scientific Optical Dissolved Oxygen Sensor (SBE63)	Dissolved O ₂			X	Core OBVI
SUNA Optical Nitrate Sensor (depth rated 500 m)	Nitrate	Deployed on CTD			
SUNA Optical Nitrate Sensor (depth rated 1000 m)	Nitrate	Deployed on CTD			
Hyperpro II Spectral Radiometer (HP)	Surface and downwelling UV/Visible irradiance & upwelling radiance spectra	X	14		Letelier
Membrane Inlet Mass Spectrometer (MIMS)	O ₂ /Ar			X	del Valle
Seaglider (SG)	Temperature, Salinity, Pressure,	X	4		Barone

	O ₂ , chlorophyll, particle backscattering, μ -structure				
Wirewalker (WW)	Temperature, Salinity, Pressure, O ₂ , chlorophyll, particle backscattering, beam-c, light	X	3		
Surface Velocity Program (SVP) Drifter	Surface currents	X	3		
Vertical Microstructure Profiler VMP-250 (VMP)	Temperature, Salinity, Pressure, μ -structure	X	24		
Rinko Underway CTD Profiler (uCTD)	Temperature, Salinity, Pressure, O ₂ , chlorophyll, turbidity	X	55		
Oxygen Optodes	O ₂ , Temperature	Deployed on Sediment Trap			
Spectral Absorption and Attenuation Sensor (AC-S)	Attenuation & absorption			X	White
ECO Optical Sensor	Fluorometer chlorophyll			X	
Hyper-Spectral Backscatter Instrument (Hyper BB)	Hyperspectral backscatter			X	
Submersible Particle Size Analyzer (LISST-200X)	Particle number & size			X	
Optical Sediment Trap (OST)	Attenuation	Deployed on Sediment Trap			
Deep Submersible Particle Size Analyzer (LISST-DEEP)	Particle number & size	Deployed on CTD			
In-situ Flow Cytobot (IFCB)	Plankton imaging & counts			X	
LI-COR Spectrometer (LI-180)	Photosynthetically Active Radiation (PAR)	Mounted on AUV van			

ECHO microscope	Plankton imagery				
HOBO Temperature Logger	Temperature	Used in incubators			
1 m diameter Bongo Net, 202 µm mesh	0-150 m integrated tows for size-fractionated zooplankton dry mass determination, Foraminifera collections from the DCM	X	16		Close, Fawcett
1 m diameter, 53 µm mesh	Surface tow for size-fractionated zooplankton collections for incubations	X			White, Church
Multibeam (EM124, EM712, EM2040)	Seafloor mapping	Hull Mounted			Schmidt Ocean Institute
38 kHz & 300 kHz Acoustic Doppler Current Profiler (ADCP)	Vertically-resolved currents	Hull Mounted			Schmidt Ocean Institute
Sinrad Echosounder (EK80)	Acoustic backscatter	Hull Mounted			Schmidt Ocean Institute

Appendix Table 2: Summary of SUBSEA samples collected on Fkt260303.

PARAMETER	FROM	# SAMPLES COLLECTED	ANALYSIS LOCATION (TYPE)	LAB GROUP
pH	CTD	171	Onboard (spectrophotometric)	Core OBVI
	Underway	91	Onboard	
Dissolved O₂	Underway	90	Onboard	Core OBVI
	CTD	178	Onboard (Winkler titrations)	
Dissolved Inorganic Carbon & Total Alkalinity	CTD	163	Southern Ocean Carbon-Climate Observatory - South Africa	Core OBVI
DIC d13C	CTD	60	University of Miami - Swart Lab	Close

Dissolved Organic Carbon	CTD	244	High temperature combustion; DOM Analytical Lab Services - University of California, Santa Barbara - Prof. Craig Carlson	Core OBVI
POC/PN isotopes	CTD	60	University of Miami (Close Lab, acidified, d13C), and University of Cape Town (Fawcett Lab, d15N)	Close, Fawcett
N isotope	CTD	587	University of Cape Town	Fawcett
Fluorescence-activated cell sorting (FACS) + N isotope analysis	CTD	80	University of Cape Town	Fawcett
Particulate Organic Carbon & Nitrogen (including 13C and 15N isotopes)	CTD	206	Sigman Research Laboratory - Princeton University - Prof. Daniel Sigman	Core OBVI
Size -fractionated particulate N	CTD	120	University of Cape Town	UCT
PC/PN	CTD	44	Flathead Lake Bio Station - University of Montana - Prof. Matt Church	Church
	ST (PITs)	61	SOEST Isotope Lab - University of Hawai'i at Mānoa	
Size fractionated PC/PN	Underway	45	University of Hawai'i at Mānoa - Prof. Benedetto Barone	Barone
PC/PN, PIC, PPO4	ST (from PITs)	100	University of Hawai'i at Mānoa - Prof. Benedetto Barone	Barone
Particulate Phosphorus	CTD	254	Flathead Lake Bio Station - University of Montana - Prof. Matt Church	Core OBVI, Church
	ST (PITs)	61		Church
Soluble Reactive Phosphorus concentrations	Underway	102	University of Hawai'i at Mānoa - Rhea Foreman	Church
Particulate Si	CTD	40	Flathead Lake Bio Station - University of Montana - Prof. Matt Church	Church
	ST (PITs)	56		
Total Dissolved Nitrogen & Phosphorous	CTD	267	Sigman Research Laboratory - Princeton University - Prof. Daniel Sigman	Core OBVI
	CTD (colorimetric)	267	Fawcett Lab - University of Cape Town - Prof. Sarah Fawcett	

Inorganic nutrients and total N/P	CTD	240	University of Hawai'i at Mānoa - Prof. Benedetto Barone	Barone
Inorganic Nutrients ($\text{NO}_3^- + \text{NO}_2^-$, NH_4^+ , TDN, SRP, TDP, Si)	CTD	82	University of Capetown	Fawcett
Ammonium concentration	CTD	538	University of Cape Town	Fawcett
Ammonium regeneration rates (PON)	Nutrient amendment incubation experiment	289	University of Cape Town	Fawcett
Ammonium regeneration rates (isotope)	Nutrient amendment incubation experiment	289	University of Cape Town	Fawcett
Kinetic nitrogen uptake rates (nitrate & ammonium)	Nutrient amendment incubation experiment	672	University of Cape Town	Fawcett
Size-fractionated nitrogen uptake rates (ammonium, nitrate, nitrite and urea)	Nutrient amendment incubation experiment	360	University of Cape Town	Fawcett
Urea concentration	CTD	10	University of Cape Town	Fawcett
FACS N uptake rates (nitrate & ammonium)	Nutrient amendment incubation experiment	60	University of Cape Town	Fawcett
Nitrogen uptake rates - primary productivity array (A)	Nutrient amendment incubation experiment	60	University of Cape Town	Fawcett
Filtrate from N₂-fixation incubation	Incubation bottles	60	University of Hawai'i at Mānoa - Prof. Benedetto Barone	Barone
N₂ Fixation samples	CTD - Productivity Array (A) Incubations	72	White Lab - University of Hawai'i at Mānoa - Prof. Angelique White	White
Nitrification rates - Productivity	Nutrient amendment incubation	168	University of Cape Town	Fawcett

Array (A)	experiment			
Biomarkers, bulk & compound-specific isotopes, size-fractionated POM	CTD	12	University of Miami - Close Lab - Prof. Close	Close
	MP	184	University of Miami - Close Lab - Prof. Close (bulk POC, PC, PN, bulk d13C, d15N, compound-specific biomarker & isotopes), Flathead Lake Bio Station - University of Montana - Prof. Matt Church (P)	Close, Church
DOM	CTD	18	University of Miami - Close Lab - Prof. Close	Close
Quantitative zooplankton biomass, C & N	Bongo Net	53	University of Miami - Close Lab - Prof. Close	Close
Chl-a	Underway	138	Onboard	Core OBVI
	CTD	137	Onboard (fluorometric; 1 replicate), University of Rhode Island Graduate School of Oceanography (2 replicates)	
	Nutrient amendment incubation experiment	201	Onboard	Hawco
		230	Flathead Lake Bio Station - University of Montana - Prof. Matt Church	Church
Chl-a (>5 µm)	CTD	125	Onboard ((fluorometric; 1 replicate), University of Rhode Island Graduate School of Oceanography (1 replicate)	Core OBVI
Flow Cytometry	CTD	282	White Lab - University of Hawai'i at Mānoa - Prof. Angelique White	Core OBVI
		58	University of Cape Town	Fawcett
	CTD (Hi-res casts)	192	White Lab - University of Hawai'i at Mānoa - Prof. Angelique White	White
	Nutrient amendment incubation experiment	230	Flathead Lake Bio Station - University of Montana - Prof. Matt Church	Church
Pigments - HPLC	CTD	44	HPLC Facility - Oregon State University - Prof. Ricardo Letelier	Core OBVI
Phytoplankton pigments	CTD	113	Oregon State University - Prof. Ricardo Letelier	Letelier
Phytoplankton absorption spectra	CTD	84	Oregon State University - Prof. Ricardo Letelier	Letelier

IFCB	CTD (Hi-res casts)	8	Onboard	White
O2/Ar	Underway	422,400 discrete data	Onboard	del Valle
	CTD	173		
13C productivity samples	CTD & A	18	UC Davis	White
Productivity response of microbes to nutrient amendments	Nutrient amendment incubation experiment	201	Flathead Lake Bio Station - University of Montana - Deepika Sahoo	Hall
C:N:P stoichiometry	Nutrient amendment incubation experiment	36	Colorado State University	Hall
Dissolved trace metal samples	TMC	144	University of Hawai'i at Mānoa - Hawco Lab	Hawco
Particulate suspended trace metal samples	TMC	144	University of Hawai'i at Mānoa - Hawco Lab	Hawco
Total Dissolvable trace metals	TMC	144	University of Hawai'i at Mānoa - Hawco Lab	Hawco
Size-fractionated particulate metals	MP	184	University of Hawai'i at Mānoa - Hawco Lab	Hawco
Size-fractionated archive	MP	184	Stored at University of Hawai'i at Mānoa	Hawco
Sinking particulate trace metals	ST	72	Stored at University of Hawai'i at Mānoa (Meshed & Open PITS)	Hawco
Particulate and Dissolved Metabolites	Underway	18	University of Washington	Ingalls
	CTD	288		
Metabolites	ST	5	University of Washington	Ingalls
Total hydrolysable amino acids and nucleobases (THAA/THNB)	CTD	96	University of Washington	Ingalls

Vitamins (Particulate & Dissolved)	CTD	144	University of Washington	Ingalls
Proteins	CTD	9	University of Washington	Ingalls
DNA	CTD	500	Flathead Lake Bio Station - University of Montana - Prof. Matt Church	Church
		62	University of Cape Town	Fawcett
	Underway	100	Flathead Lake Bio Station - University of Montana - Prof. Matt Church	Church
	Nutrient amendment incubation experiment	400		
Interfaces and filtrate from PIT	ST (PITs)	75	University of Hawai'i - Prof. Benedetto Barone	Barone
15N MIMS initial mixing samples	CTD - Productivity Array (A) Incubations	18	White Lab - University of Hawai'i at Mānoa - Prof. Angelique White	White

Appendix Table 3: Summary of CTD casts performed during Fkt260303.

Cast	Station	Date Time UTC	Latitude (°N)	Longitude (°E)	Max Depth (m)	SUNA 500	SUNA 1000
1	1	3/5/26 22:08	-26.2220	-37.6118	401	X	X
2	1	3/6/26 0:16	-26.1577	-37.7067	401	X	X
3	1	3/6/26 5:14	-26.2227	-37.5737	401	X	X
4	1	3/6/26 7:56	-26.1954	-37.7312	401	X	X
5	1	3/6/26 9:29	-26.2063	-37.6375	1002		X
6	1	3/7/26 0:02	-26.1681	-37.6261	402	X	X
7	1	3/7/26 8:01	-26.0642	-37.5560	400	X	X
8	1	3/7/26 9:33	-26.1595	-37.6567	1001		X
9	1	3/8/26 0:01	-26.1986	-37.7340	400	X	X
10	1	3/8/26 7:01	-26.1462	-37.4043	400	X	X

11	1	3/8/26 8:57	-26.2119	-37.6500	1001		X
12	2	3/9/26 7:33	-23.7701	-37.1601	401	X	X
13	2	3/9/26 15:32	-23.7803	-37.2615	400	X	X
14	2	3/9/26 18:00	-23.7476	-37.2214	401	X	X
15	2	3/9/26 21:32	-23.6289	-37.0821	401	X	X
16	2	3/10/26 0:02	-23.7752	-37.1262	401	X	X
18	2	3/10/26 6:15	-23.6933	-37.1552	1004		X
19	2	3/10/26 9:55	-23.7507	-37.1549	402	X	X
20	2	3/10/26 21:34	-23.7088	-37.2331	407	X	X
21	3	3/14/26 7:42	-25.6995	-38.6141	401	X	X
22	3	3/14/26 10:53	-25.6552	-38.5662	1003		X
23	3	3/15/26 9:21	-26.0671	-37.7012	400	X	X
24	4	3/15/26 19:03	-25.9985	-37.6262	402	X	X
25	4	3/15/26 23:59	-26.0114	-37.5784	401	X	X
26	4	3/16/26 3:07	-26.0465	-37.6387	452	X	X
27	4	3/16/26 4:57	-26.0071	-37.5437	401	X	X
28	4	3/16/26 9:01	-26.0617	-37.7035	1001		X
29	4	3/16/26 16:38	-26.0795	-37.6104	400	X	X
30	4	3/17/26 0:07	-26.0730	-37.7068	400	X	X
31	4	3/17/26 8:58	-25.9678	-37.7494	402	X	X
32	4	3/17/26 22:00	-26.0168	-37.7270	451	X	X
33	4	3/17/26 23:49	-26.0162	-37.7646	402	X	X
34	4	3/18/26 7:07	-25.9839	-37.8117	401	X	X
35	4	3/18/26 9:11	-25.9756	-37.7535	1002		X
36	4	3/18/26 13:00	-25.9572	-37.7601	401	X	X
37	5	3/20/26 6:57	-25.4197	-33.2422	400	X	X

38	5	3/20/26 9:55	-25.4261	-33.2339	1001		X
39	6	3/21/26 6:56	-25.1884	-30.2260	1001		X
40	6	3/22/26 0:22	-25.0720	-28.6158	401	X	X
41	7	3/22/26 9:00	-25.0709	-28.5741	1001		X
42	7	3/22/26 13:59	-25.0861	-28.6171	401	X	X
43	7	3/22/26 19:10	-25.0750	-28.6211	1000		
44	7	3/22/26 22:00	-25.0526	-28.5352	401	X	X
45	7	3/23/26 1:59	-25.0501	-28.6436	401	X	X
46	7	3/23/26 3:57	-25.0178	-28.6481	401	X	X
47	7	3/23/26 8:59	-25.0264	-28.5825	400	X	X
48	7	3/23/26 19:06	-25.0475	-28.6127	400	X	X
49	7	3/23/26 21:58	-25.0225	-28.6138	601		X
50	7	3/24/26 0:05	-25.0322	-28.6627	402	X	X
51	7	3/24/26 8:58	-25.0408	-28.6368	401	X	X
52	7	3/24/26 19:04	-25.0030	-28.6323	400	X	X
53	7	3/24/26 23:59	-24.9955	-28.6658	401	X	X
54	7	3/25/26 6:55	-24.9865	-28.6995	401	X	X
55	7	3/25/26 9:01	-24.9346	-28.5601	1002		X
56	8	3/26/26 6:52	-23.8829	-27.4268	1001		X
57	9	3/27/26 6:58	-21.4896	-25.2689	401	X	X
58	9	3/27/26 9:47	-21.5178	-25.3324	41	X	
59	9	3/27/26 11:26	-21.4107	-25.2066	1001		X
60	10	3/28/26 21:57	-17.9459	-25.0712	401	X	X
61	10	3/28/26 23:56	-17.9120	-25.1036	501		X
62	10	3/29/26 8:57	-17.9353	-25.1084	401	X	X
63	10	3/29/26 13:56	-17.9300	-25.1572	401	X	X

64	10	3/29/26 22:01	-17.9064	-25.0977	401	X	X
65	10	3/30/26 2:00	-17.9061	-25.1606	400	X	X
66	10	3/30/26 4:29	-17.9044	-25.1043	401	X	X
67	10	3/30/26 8:49	-17.9013	-25.2164	1008		X
68	10	3/30/26 21:59	-17.8259	-25.2161	429	X	X
69	10	3/31/26 0:04	-17.9104	-25.2751	401	X	X
70	10	3/31/26 8:58	-17.8587	-25.3139	399	X	X
71	10	3/31/26 10:55	-17.9047	-25.3166	401	X	X
72	10	4/1/26 0:03	-17.8951	-25.2132	401	X	X
73	10	4/1/26 5:46	-17.8767	-25.3566	413	X	X
74	10	4/1/26 7:21	-17.8916	-25.3855	403	X	X
76	11	4/1/26 21:36	-17.9360	-25.6731	5003		
77	12	4/2/26 6:53	-17.7195	-26.2916	1002		X
78	13	4/3/26 6:31	-16.4430	-29.3536	1116		X
79	14	4/4/26 6:24	-15.4276	-32.1936	1002		X
80	14	4/4/26 11:04	-15.3644	-32.4569	401		X

Appendix Table 4: Summary of SUBSEA experiments performed on Fkt260303.

EXPERIMENT TYPE	PARAMETER / PROCESS MEASURED	#	SHORT DESCRIPTION	LAB GROUP
Deckboard Incubation	Gross Oxygen Production	16	Samples of 5m and/or DCM were spiked with $18\text{O-H}_2\text{O}$ and incubated in the corresponding deckboard incubator.	del Valle
	Rates of ammonium regeneration	5	Collection of seawater from the CTD at 25 m and the DCM; added 1 At% 15N -ammonium and incubated. Subsamples for ammonium isotope and PON analysis were collected at 3, 6, 12, 24, 48, 72, 96, 120 and 144 hours.	Fawcett
	Rates of size-fractionated nitrogen uptake	5	Collection of seawater from the CTD at 25 m and the DCM; added 99 At% 15N -ammonium, nitrate, nitrite and urea at 100 μM to yield $[\text{N}]$ of 20 nM. Incubated for 4-6 hrs and terminated via filtration	Fawcett

			onto 0.3, 1.2 and 5 um GF/F filters.	
	Rates of FACS nitrogen-uptake	5	Collection of seawater from the CTD at 25 m and the DCM; added 99 At% 15N-ammonium and nitrate at 100 uM to yield [N] of 20 nM. Incubated for 4-6 hrs and terminated via filtration onto 0.45 um PES filters.	Fawcett
	Kinetic nitrogen uptake rates of nitrate and ammonium	4	Collection of seawater from the CTD at 25 m and the DCM; added 99 At% 15N-nitrate and ammonium at 100 uM, and 30 At% 15N-nitrate and ammonium at 1 mM to yield [N] of 10, 20, 50, 100, 250, 500 and 1000 nM. Incubated for 4-6 hrs and terminated via filtration onto either 0.3 um or 5 um GF/F filter to determine size-fractionated estimates of Vmax and Ks for phytoplankton.	Fawcett
Nutrient Amendment (Deckboard Incubation)	Chlorophyll	4	Experiments led by Alexis Floback and Nick Hawco. Church lab collected DNA samples from time zero and time final on-deck incubations. Sampling coordinated with other groups (Hawco/Floback, Hall/Sahoo, etc.)	Hawco
	Microbial DNA (for 16S rRNA gene amplicons)	4	Esther Mak led experiments. Collection of water from the DCM using TMC; added NH4 and incubated for 96 hrs at 1% and 3% surface light.	Church
Fe Uptake (Deckboard incubation)	Fe uptake	4	Fe uptake experiments incubated for 24 hours for surface and DCM water collected from TMC for each main station, with an additional depth (50-75m below DCM) at stations 4 and 7. Station 1 included a surface incubation from water from the crane+carboy.	Hawco
NH4 additions at the DCM	Microbial DNA and RNA, picoplankton abundances	4	Esther Mak led experiments. Collection of water from the DCM using TMC; added NH4 and incubated for 96 hrs at 1% and 3% surface light.	Church
Nitrogen addition experiment at 5 m and the DCM	Chlorophyll, picoplankton abundances	5	Esther Mak conducted experiments involving water collection at 5 m depth and from the DCM; ammonium, nitrite, and nitrate were added, and samples were incubated for 48 hours to assess community response.	Church
Fe addition to high SRP water	Microbial DNA and RNA	1	Experiment led by Jared McGourty. Surface seawater was collected from the shipboard underway system at a site where SRP concentrations approached 100 nM, and Chl-A concentrations were increasing. A second site was sampled where SRP was high, but the Chl-A concentration was greatly diminished; Fe was added (2 nM final concentration), and bottles were incubated for 14 days in a surface seawater incubator.	Church
P vs E incubation	Gross Oxygen Production	11	Samples of a given depth were spiked with 18O-H2O and incubated across a gradient of	del Valle

			irradiances.	
GOP of nutrient amendment incubation	Gross Oxygen Production	4	Samples from nutrient amendment incubation experiments were spiked with $^{18}\text{O}_2$ labelled water and incubated for 7-8 hr on deck incubator.	Hall
C:N:P stoichiometry of individual microbes	C:N:P stoichiometry	36	Samples from nutrient amendment incubation experiments were collected on 3 mm Ni grids to analyze the microbial C:N:P stoichiometry.	Hall
Nitrogen Fate Experiment	Metabolomics	1	Seawater incubations amended with ^{15}N -labeled substrates (NO_3^- , NH_4^+ , and urea) and sampled over time (T0, T6, T12, T24) to track nitrogen incorporation into particulate and dissolved metabolite pools.	Ingalls
Methylated Experiment	Metabolomics (GBT + B12)	2		Ingalls
	Metabolomics (DMSP + B12)	1		
Zooplankton decomposition	Microbial DNA, RNA, TOC, NH_4 , TN, TP, FCM, PC/PN	1	Katie Coates led experiments. Collection of zooplankton from 0-150 m integrated nighttime net tows; size fractionate zooplankton, add 10 animals per experimental bottle, incubate experimental bottle with controls for 96 hours with whole seawater from 340 m (the approximate position of the deep scattering layer), 265 m (75 m above), and 415 m (75 m below).	Church
Zooplankton fecal pellet decomposition	Microbial DNA, RNA, TOC, NH_4 , TN, TP, SRP, FCM, PC/PN, PPO_4	3	Katie Coates led experiments. Collection of zooplankton from 0-150 m integrated nighttime net tows; size fractionate zooplankton, exclude animals from fecal pellets. Collect fecal pellets, incubate for 96 hrs with filtered SW and whole seawater from 340 m and 265 m (the approximate position of the deep scattering layer and 75 m above the scattering layer).	Church
Primary productivity array (A)	Rates of bulk ammonium and nitrate uptake	3	Collection of seawater from the CTD at 25, 45, 75, 100 and 125 m; added 99 At% ^{15}N -ammonium and nitrate at 100 μM to yield $[\text{N}]$ of 20 nM. Incubated for 24 hrs and terminated via filtration onto 0.3 μm GF/F filters.	Fawcett
	Rates of ammonium and nitrite oxidation	3	Collection of seawater from the CTD at 25, 45, 75, 100, 125, 150 and 200 m; added 99 At% ^{15}N -ammonium and nitrite at 100 μM to yield $[\text{N}]$ of 20 nM, and ^{14}N -nitrite and nitrate at 100 μM to yield $[\text{N}]$ of 1 μM . Incubated for 24 hrs and terminated via filtration onto 0.3 μm GF/F filters.	