Southern California Bight
2023 Regional Marine Monitoring Program
(Bight '23)

Ocean Acidification Workplan

Prepared by:
Bight '23 Water Quality, Ocean Acidification, and Hypoxia Committee

Prepared for:
Commission of Southern California Coastal Water Research Project
3535 Harbor Blvd, Suite 110
Costa Mesa, CA 92626

June 2024

CONTENTS

Table of Figures

Table of Tables

I. INTRODUCTION

The Southern California Bight 2023 Regional Marine Monitoring Program (Bight '23) Program is organized into five technical components: 1) Ocean Acidification and Hypoxia (OAH); 2) Sediment Quality (formerly Contaminant Impact Assessment/Coastal Ecology); 3) Microbiology; 4) Bioaccumulation, 5) Submerged Aquatic Vegetation, 6) Estuaries Bioassessment; and 5) Trash. This workplan provides a summary of the program design for OAH; separate workplans are available for the other elements of Bight '23 here.

In the introduction, we describe the environmental setting, the history of coastal water quality monitoring through the Bight program, and the impetus for the proposed Bight '23 OAH study, detailed in this workplan.

History of the Bight Regional Monitoring Program and Offshore Water Quality Study Component

The Southern California Bight (Bight; Figure I-1) is an open embayment along the coast between Point Conception and Cape Colnett (south of Ensenada), Baja California. Complex bathymetry and currents have resulted in a diversity of habitats and marine organisms, including more than 500 species of fish and several thousand species of invertebrates. The Bight is a major migration route for marine bird and mammal populations and is ranked among the most diverse ecosystems in north temperate waters. In addition to its ecological value, the coastal zone of the Bight is a substantial economic resource. The Los Angeles/Long Beach Harbor complex is the largest commercial port in the United States, while San Diego Harbor is home to one of the largest U.S. Naval facilities in the country. In addition to being the home to more than 20 million people (NRC 1990), Southern California receives more than 100 million visitors to its beaches and coastal areas annually.

Climate change and local factors are greatly influencing the coastal water quality of the Bight. The conversion of open land into impervious surfaces has caused extensive alterations of coastal streams and rivers (NRC 1990). This "hardening of the coast" changes both the timing and rate of runoff releases to coastal waters and can affect water quality through addition of sediment, toxic chemicals, pathogens, and nutrients. Besides input of urban runoff via storm drains and channelized rivers and streams, numerous municipal wastewater treatment facilities, power-generating stations, industrial treatment facilities, and oil platforms discharge to the Bight via ocean outfalls. Superimposed on these stressors, global climate change is causing ocean warming and deoxygenation of surface waters (hypoxia; H), and the absorption of rising atmospheric CO_2 is causing declining pH (ocean acidification; OA). These combined stressors could exceed the tolerances of Bight organisms, causing ecosystem disruption and declines in

populations of recreational and commercial fish and shellfish, with important links to coastal economies.

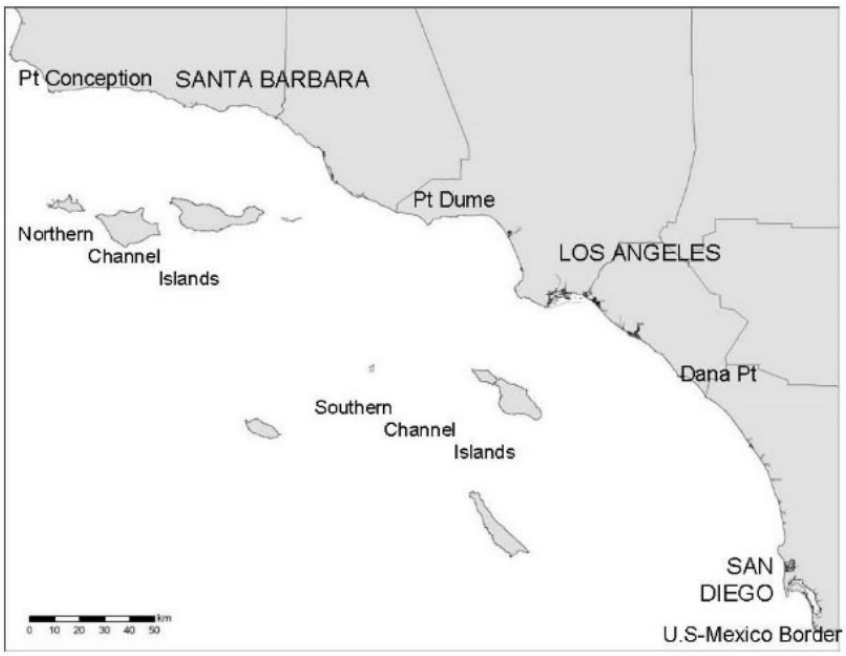


Figure I-1. Map of the Southern California Bight.

History of Bight Regional Water Quality Monitoring

To understand the cumulative impacts of global and local water quality impacts on the Bight, a cooperative, multi-agency regional monitoring program has been established that looks at the health of the Bight ecosystem as a whole. Prior to the inception of the Bight Regional Monitoring Program, coastal monitoring was conducted primarily around individual discharges related to National Pollutant Discharge Elimination System (NPDES) permits and was intended to assess compliance of waste discharge with state and federal regulations, which set water quality standards for effluent and receiving waters. While these monitoring programs are providing important information to evaluate impacts near individual discharges, they do not provide regional context to assess the cumulative impacts of contaminant inputs and to evaluate relative risk among different types of stressors needed by managers. The Bight Program was designed to fill this need. Other benefits derived from the Bight Program included the development of new technical tools and increased standardization and comparability in field and laboratory methods that could only be developed with regional data sets and participation by multiple organizations.

The Bight Regional Monitoring Program is organized into technical components, each focusing on research with clear management implications. All Bight programs to date have contained a component related to offshore water quality. This component of the Bight Regional

Monitoring Program focuses on assessing condition of the water column in the near coastal ocean and exploring the influence of climate change and land-based discharges on coastal water quality. Bight '23 is a collaboration among many organizations with different perspectives and interests, including a combination of regulators and dischargers, ensures that an appropriate set of regional-scale questions will be addressed by the study.

To date, there have been six previous regional monitoring surveys focused on offshore water quality. Early surveys conducted in 1994, 1998 and 2003 focused on topics from regional dissolved oxygen assessments, mass loading and impacts of stormwater and dry weather discharges of rivers on ambient oxygen, bacteria, toxicity, and remotely sensed and *in situ* chlorophyll-a, while latter studies have focused more specifically on chemical and biological outcomes from eutrophication and climate change. The Southern California Bight 2008 Regional Monitoring Program (Bight '08) Offshore Water Quality Study provided evidence that on small scales relevant to the development of algal blooms, anthropogenic nitrogen loads were equivalent to upwelled nitrogen loads in the heavily urbanized regions of the Bight (Howard et al. 2014). The Bight '13 Offshore Water Quality study was the first study to investigate the status of ocean acidification in the Bight and collect data that could support the investigation of local and global drivers. The Bight '18 Offshore Water Quality study focused on OA. It expanded on the 2013 study to incorporate biological impact metrics into overall assessment. This program successfully implemented collection and analysis of pteropod and larval crab shell dissolution as a biological metric of OA impacts. This survey was also done cooperatively with the NOAA West Coast Ocean Acidification (WCOA) Cruise in Summer 2021, which allowed for a super-regional assessment of shell condition. Preliminary data shows that the Bight generally had less moderate to severe dissolution compared to the rest of the U.S. West Coast. Pteropods were sampleable more consistently than crabs, leading to their emergence as the primary biological indicator of choice for regional monitoring. Crab larvae can be retained as a secondary indicator, if preferred.

Context for Studies of Ocean Acidification and Hypoxia in the Bight

The Bight is situated on the southern portion of the California Current System, one of the world's four large eastern boundary upwelling systems (Chavez and Messie 2009). Such systems are most at risk to global changes in OA and hypoxia, wherein changes in ocean chemistry related to elevated atmospheric CO_2 have led to increases in oceanic dissolved CO_2 concentration, as well as concomitant decreases in pH and aragonite saturation state (Ω_{arag}) (Orr et al. 2005, Zeebe 2012). These changes have been documented both offshore and in the nearshore environments of the Bight (Feely et al. 2008, 2012, 2016; McLaughlin et al. 2018), and are strongly associated with coastal upwelling, which transports subsurface waters with high levels of carbon dioxide (CO_2) and low pH to nearshore surface waters (Fassbender et al. 2011, Harris et al. 2013). Ship-based surveys of the U.S. West Coast routinely encounter hypoxia (Chan et al. 2008, McClatchie et al. 2010) and OA hot spots, where large regions of the

continental shelf are undersaturated with respect to aragonite (Ω_{arag} <1) in shallow nearshore waters (Feely et al. 2008, 2016).

The West Coast is already vulnerable to OAH due to seasonal upwelling which draws waters that are naturally low in O_2, pH, and carbonate saturation states (Ω) onto the shelf and into the photic zone. These deep-water masses are subject to large-scale climatic changes occurring globally, related to uptake of atmospheric CO_2, as well as natural climatic variations of the Pacific Basin. Upwelled waters are also nutrient-rich and can support high levels of biological productivity in the Bight. These natural nutrient fluxes are modulated in nearshore waters by a variety of terrestrial sources of nutrients, dominated by wastewater treatment discharges (Sutula et al. 2021, Figure I-3). Because upwelled water is already low in pH and O_2, the West Coast is particularly sensitive to additional perturbations in these parameters. Nutrient additions can indirectly affect the O_2 and CO_2 content of seawater via primary production and respiration. Nutrients from land-based inputs can stimulate phytoplankton blooms which assimilate dissolved CO_2 during photosynthesis, reducing acidity and elevating O_2 (Kessouri et al. 2021a, 2021b, 2024). When this algal organic material is respired, oxygen is consumed and CO_2 released (thereby lowering O_2 and increasing acidity). Because primary production occurs during daylight hours, shallow coastal ecosystems can experience large diel swings in O_2, pCO_2, pH, and variability which may exceed the physiological tolerances of sensitive species. Offshore, where primary production is limited to the euphotic zone (where light penetration is sufficient to support photosynthesis), surface blooms may result in acidification and hypoxia at depth when organic matter sinks and is respired below the euphotic zone, thus compressing habitat for pelagic species (Frieder et al. in review).

In McLaughlin et al. (2018), the ambient monitoring results of the Bight '13 study found that a substantial portion of Southern California continental shelf waters exhibit water column aragonite saturation states (a key measure of acidified conditions) that fall within a range critical for biological organisms (Fabry et al. 2008, Hofmann et al. 2010, Barton et al. 2015, Bednaršek et al. 2017). For three quarters of the year, more than 80% of the upper water column (depths less than 100 m, within the diel vertical migration for pteropods) have waters with Ω_{arag} that could result in pteropod shell dissolution (McLaughlin et al. 2018). Furthermore, waters below critical thresholds were observed in surface waters which may indicate that pelagic calcifiers (pteropods) and intertidal shellfish populations in Southern California may be adversely affected by acidification conditions along the coast.

The Bight '18 Program established a model monitoring program for biological impacts assessment in the Bight and provided preliminary estimates of the biological effects of these low saturation states, showing that mild dissolution was pervasive throughout the Bight and a small percentage of individual pteropods had moderate to severe dissolution. Carbonate state thresholds of adverse effects have been published for pteropods, decapods and echinoderms, based on expert consensus (Bednarsek et al. 2019, 2020, 2021). The Oregon Department of Environmental Quality has incorporated a combination of aragonite saturation state and shell dissolution into their impaired waters assessments (ODEQ 2024; https://www.oregon.gov/deq/wq/Documents/ir24OAHfs.pdf), and California intends to update their integrated assessment methodologies to incorporate the chemical and biological effects of OA. However, these approaches are based on single stressors and there are no standardized protocols to incorporate multiple stressors (declining dissolved oxygen, rising temperatures, etc.) into a comprehensive assessment of the biological impacts of OAH. Assessment endpoints provide a framework for consistent interpretation of chemical monitoring data. Continued regional assessment of biological impacts and adoption of broad indicators to evaluate multiple stressors on important Bight indicator taxa, coupled with a strong chemical monitoring program, is needed to inform an interpretive framework for monitoring data and is the focus of the Bight '23 OAH element.

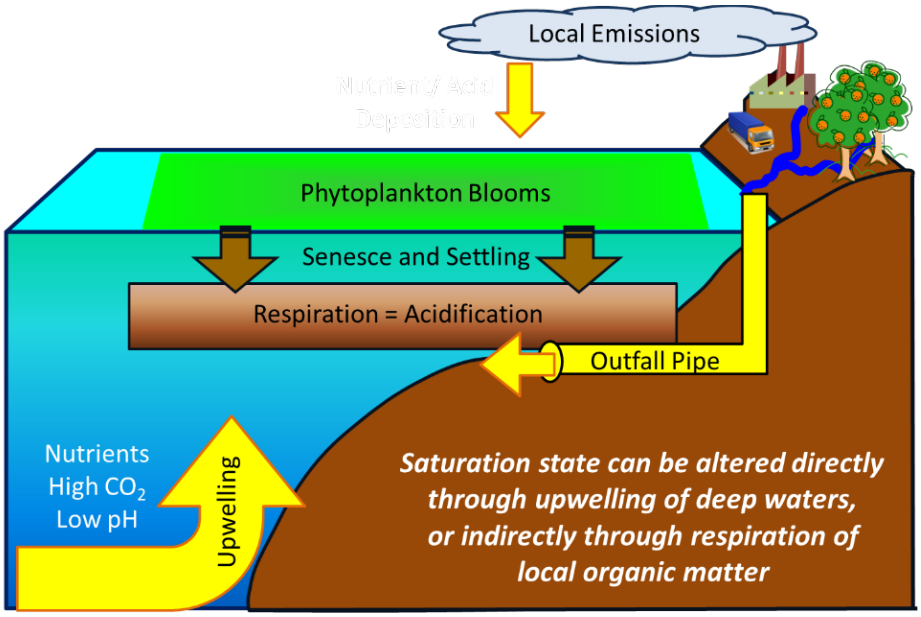


Figure I-2. Conceptual model of impacts of nutrient inputs on coastal ocean acidification.

II. 2023 Program Study Design

The Bight '23 OAH study builds upon previous successes of Bight '13 and '18 studies to assess the status of OA chemical and related biological effects. OAH chemistry, OA-specific impacts on shell dissolution in calcifying zooplankton, and environmental DNA (eDNA) will be assessed on a quarterly (chemistry, eDNA) to biannual basis (shell dissolution) over a two-year period, starting in Fall 2024. In addition, the study will incorporate a larger-scale collaboration with the NOAA 2025 West Coast Ocean Acidification survey, which will help continue the super-regional comparison of Bight condition compared to the rest of the U.S. West Coast.

Table II-1. Participants in the Bight '23 Regional Marine Monitoring Program, Ocean Acidification and Hypoxia component

Aquatic Bioassay and Consulting Laboratories (ABC Labs)
California Cooperative Oceanic Fisheries Investigations (CalCOFI)
Chevron USA Products Company
City of Los Angeles Environmental Monitoring Division (CLAEMD)
City of Oxnard
City of San Diego (CSD)
Los Angeles Regional Water Board
Los Angeles County Sanitation Districts (LACSD)
National Oceanic and Atmospheric Administration (NOAA), Pacific Marine Environmental Laboratory (PMEL)
National Oceanic and Atmospheric Administration (NOAA), Southwest Fisheries Science Center
Ocean Protection Council (OPC)
Orange County Sanitation District (OC San)
San Diego Regional Water Board
Santa Ana Regional Water Board
Southern California Coastal Ocean Observing System (SCCOOS)
State Water Board

A. Study Objectives

The overall goal of the Bight '23 OAH Study is to determine the extent and magnitude of chemical and biological impacts related to carbonate chemistry in the Bight. There are three principal questions for this component:

1. What is the extent and magnitude of low pH, low aragonite saturation state (Ω_{Ar}), and low oxygen in Bight shelf waters (chemical indicators of OAH)?

2. What is the extent and magnitude of biological impacts related to carbonate chemistry stress on Bight species in the pelagic environment (biological indicators of OA)?
3. What is the diversity of fish and zooplankton in the Bight and (over the long term) how are these biological communities responding to OAH and warming in the Bight (eDNA indicators of OAH)?

The first question will be addressed by a chemical monitoring program for assessing status and trends in carbonate chemistry and oxygen in the Bight. The second will be addressed by a biological monitoring program, with indicators and endpoints reflecting carbonate chemistry exposure. The third will be addressed by characterizing biological communities from eDNA samples that are compared to ship-based measurements of OAH and warming conditions.

I. Chemical Monitoring Program. The chemical monitoring program will be accomplished by capturing the data from quarterly NPDES regulatory monitoring, in order to answer the main monitoring question:

1. What is the extent, magnitude and duration of Ωarag at biologically important OA thresholds on the SCB shelf?

That ship-based monitoring consists of, among other measures, vertical profiling of conductivity, temperature, and depth (CTD) casts, alongside sensors that measure O_2, pH, chlorophyll-a (chl-a) and other parameters. Because the CTD potentiometric pH is prone to error, discrete samples for pH and alkalinity will be measured to improve the pH calibration and to translate pH to biologically relevant measures of dissolved inorganic carbon and aragonite saturation state.

II. Biological Monitoring Program. The biological monitoring program has two components. The first focuses on sensitive calcifying taxa and has two main monitoring questions:

1. What percent of Bight pelagic waters (upper 200 m) show evidence of calcifying taxa exposure to OA?
2. What is the correlation between observed biological impacts and carbonate chemistry?

The first question will be addressed through ship-based pelagic sampling of calcifying zooplankton species using net tows, followed by laboratory analysis of shell dissolution, an OA specific measure of effect. The second question will use the metrics from the first question to see how well these endpoints correlate with carbonate chemistry.

III. eDNA Monitoring Program. The second biological monitoring component is focused on biological community composition and has one near-term and one long-term monitoring question:

1. Near-term (Bight 2023 study): What is the biological diversity of fish in whole water samples and zooplankton in bongo tow samples?
2. Long-term: How does eDNA-based data provide insights into biological community response to OAH et al. environmental conditions?

eDNA samples will be taken from both water samples and bongo net tows to characterize biological communities, prioritizing fish populations in the water samples and zooplankton communities in the net tow samples. The ability to detected biological community response to environmental gradients in OAH will be limited by sample size in the short term (this Bight study), but the study provides an opportunity to establish a long-term baseline to better investigate this question. Resources are not available to comprehensively sequence all eDNA samples, so samples will be archived at SCCWRP and a priority subset analyzed based on gradients in OAH and warming.

B. Sampling Design

The sampling design for Bight '23 OAH study will be divided into three main components: 1) Chemical monitoring for extent and magnitude of threshold carbonate chemistry and oxygen conditions, 2) Biological monitoring for extent and magnitude of impacts on sensitive taxa in the Bight, and 3) eDNA analysis of water samples and bongo tow net samples for biological community assemblages and OA-specific biological effects, respectively. Table II-2 shows the seasonal timing and II-3 summarizes the targeted number of samples by sample type for the Bight '23 OAH survey.

Table II-2. Timing of Bight '23 OAH sampling effort by season and indicator type. The coordination with the NOAA cruise occurs August 2025.

Season	Timing of Quarterly Chemistry and eDNA Samples			Timing of Biannual Chemistry-Biological Samples			
	CTD	pH & TA Bottle	Water eDNA	CTD	Zooplankton eDNA	Zooplankton SEM	pH & TA Bottle
November 2024	X	X	X	--	--	--	--
February 2025	X	X	X	X	X	X	X
May 2025	X	X	X	--	--	--	--
August 2025	X	X	X	X	X	X	X
November 2025	X	X	X	--	--	--	--
February 2026	X	X	X	X	X	X	X

May 2026	X	X	X	--	--	--	--
August 2026	X	X	X	X	X	X	X

Table II-3. Summary of sampling effort across agencies and indicator types. Sampling effort represents the minimum commitment, with the exception of City of San Diego.

Season	No. of Quarterly Samples			No. Biannual Chemistry-Biological Samples				
	CTD Casts	pH & TA Bottle[1]	Water eDNA[2]	CTD	Water eDNA[3]	Zooplankton eDNA	Zooplankton SEM	pH & TA Bottle[4]
CSD	71	23	6	3	18	3	3	6
OC San	78	7	6	3	18	3	3	6
LACSD	48	7	6	3	18	3	3	6
CLA	55	7	6	3	18	3	3	6
City of Oxnard	25	4	3	3	18	3	3	6
Total Across Agencies/Quarter	277	48	27	15	90	15	15	30
Total Across Agencies/2 years	2216	384	216	60	360	60	60	120

[1]Includes, at minimum 3 regular (surface, chlorophyll-a max, and bottom) on first and last days of quarterly sampling, for a total of 6 samples, plus one field duplicate per quarter. The exceptions are: 1) City of San Diego, who have pledged a more rigorous pH and TA bottle collection and 2) The City of Oxnard, who conducts all their sampling on one field day, rather than two.

[2] Includes a single eDNA sample conducted at surface, chlorophyll-a max, and bottom at two sites per quarter

[3] Includes triplicate eDNA samples conducted at surface and bottom of the bongo trawl at three sites per quarter (2 quarters per year).

[4] Includes 2 regular (surface, bottom of bongo trawl) at three sites. All quality assurance replicates are conducted during the routine quarterly compliance monitoring at occurs at the same time as biological sampling, so none are included during the biological sampling).

1. Chemical Monitoring

Magnitude and Extent. The magnitude and extent of low O_2, pH and low Ω_{arag} in the upper 200 m of the Bight shelf waters will be assessed quarterly concomitant with routine NPDES monitoring conducted by the Publicly Owned Treatment Works (POTW) agencies along established continental shelf grids (Appendix A). Water column profiles for temperature, salinity, chlorophyll-a, O_2 and pH have been regularly monitored by the POTWs for decades. CTD potentiometric pH profiles have accuracy issues, so potentiometric pH sensor measurements will be recalibrated with discrete bottle samples in which pH is measured spectrophotometrically in the laboratory. Total alkalinity in seawater can be estimated either by an algorithm (e.g., Alin et al. 2012) or in discrete water samples. The combination of pH and total alkalinity (TA) in the discrete sampling allows for calculation of Ω_{arag} for all water-column profiles collected during POTW quarterly monitoring. This adoption of improved carbonate chemistry monitoring provides not only a comprehensive understanding of acidification along the Southern California shelf, but also allows for comparison with other U.S. West Coast

datasets that use the same methods (i.e., Bednarsek et al. 2014). Procedures for collection of pH and TA bottle samples is specified in Appendix B. Data quality targets are specified in Appendix C.

A total of 8 quarters will be sampled, starting in November 2024 (Table II-2). A map of the monitoring grids is provided in Figure II-1 (Appendix A). Each agency has established in-house CD calibration procedures. The CTD calibration protocols for each agency can be accessed from the CTD Users Group webpage: https://www.sccwrp.org/ctd/.

Putting the Bight into a West Coastwide Context. In August 2025, NOAA Pacific Environmental Laboratory (PMEL) will be repeating their survey of the U.S. West Coast. The PMEL cruise stations do not extend onto the Bight shelf, an area well covered by POTW agencies. PMEL transect lines will help place the carbonate and oxygen chemistry of the Bight shelf into a larger, regional context. Exact locations will be determined and approved by the committee before sampling commences.

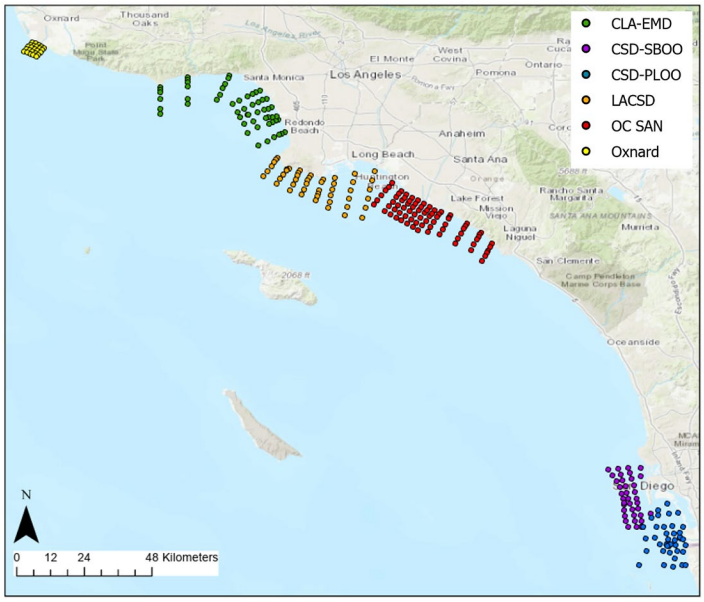


Figure II-1. Map of the POTW CTD stations.

Detailed Description of *In situ* Calibration of pH Sensors. The glass-electrode, potentiometric pH sensors used by the POTWs were found to have large uncertainties associated with the calibration in NBS buffers and difficulties with their pressure compensation (McLaughlin et al. 2017). These problems caused a depth dependent bias in the pH data measured by the sensor. This study also found that a significant improvement in data quality could be achieved using an *in-situ* calibration of the sensor with bottle measurements, improving uncertainty from ± 0.5 pH units to ± 0.1 pH units (McLaughlin et al. 2017). Consequently, the

POTW agencies have agreed to continue implementing the *in-situ* calibration methods as part of the Bight '23 program. The corresponding pH measured by the glass electrode sensor at the depth where bottle samples are collected for pH will be recorded, then the CTD profiles will be recalibrated.

A draft web application was developed to correct for bias in the potentiometric pH profile by generating the depth dependent regression between spectrophotometric pH from the bottle samples and the glass-electrode pH. Regressions were generated for the first and final days and the linear difference in the calibration equation between the two days will be used to account for any instrument drift between days. The *in-situ* calibration provides pH within ± 0.1 pH units at 95% confidence. SCCWRP will update and finalize the functionality of the pH tool for use in the Bight OAH Information Management Portal (Section III) and regulatory compliance applications according to internal guidelines for quality control, review and documentation of SCCWRP data tools and products.

Starting in the November 2024, during each quarterly monitoring cruise, a minimum of 3 water samples will be collected on the first and last day of sampling: 1) at the surface, 2) at the chlorophyll-a maximum or mid-depth if there is no chlorophyll-a maximum, and 3) at the deepest sampling possible depth for that station, hereafter called "bottom". Stations where pH and TA bottles are selected by the agencies, balancing the criteria that include: 1) deepest stations and 2) logistics of sampling. Two pH/TA duplicate samples are required for QA/QC during each sample quarter. The QA/QC should be taken as a replicate of one of the samples collected for the calibration of the pH sensor

Additional samples are required if the sampling is split over multiple weeks to appropriately characterize any potential sensor drift. If sampling is required in a second week, pH bottle samples should be collected at the surface and at the bottom for the first and last sampling days respectively to capture sensor drift with time and pressure.

If a glass electrode needs to be replaced or recalibrated with NBS standards during the quarterly sampling event, the *in-situ* calibration will need to be calculated for each new/recalibrated electrode independently. In this case, a minimum of 3 new calibration samples should be collected on the first day the electrode is utilized (surface, chlorophyll-a max and bottom). In the event of a probe failure, the *in-situ* calibration on the failed probe should be done with just the three samples from day 1.

Water samples for pH and total alkalinity (TA) should be collected according to Dickson et al. (2003), using a seawater sampling bottle on either a rosette or on a line with a messenger. Seawater from the sample bottle should be collected into glass bottles (not plastic) using an appropriately sized drawing tube (Tygon or similar) placed into the bottom of the bottle. Pyrex

bottles are recommended, particularly if samples will not be analyzed within 1 week of collection. Bottles should be overfilled at least 50% and care should be taken when filling the bottle and slowly removing the drawing tube to eliminate any bubbles in the bottle. A 1% headspace should be created before the bottle is sealed. Samples should be treated with mercuric chloride as a preservative. Bottle caps or stoppers should be sealed with either grease, electrical tape, parafilm or similar, to minimize gas exchange. Bottle samples should be stored at or below room temperature (but not frozen) until analysis. TA and pH can be measured from a single 500 mL bottle or may be analyzed on separate 250 mL bottles (filled from the same Niskin). Each agency should check with their analytical laboratory for their preference on how water samples should be collected and preserved in the field. An intercalibration will be held prior to sample initiation to determine comparability of pH and TA measurements amongst the laboratories participating in the sample analysis. Details of the intercalibration are provided in Appendix C and a recommended procedure for water sample collection in Appendix B.

Derivation of Carbonate System Parameters. The carbonate system parameter thought to be most closely tied to biological impacts on shelled organisms is Ω_{arag}. Two carbonate parameters are needed to calculate Ω_{arag}, so one additional measure is required beyond pH. TA was selected because it can be estimated by temperature and salinity, both of which are measured by the POTW CTDs and neither of which experience pressure-dependent variability. Alin et al. (2012) developed an algorithm to derive TA from temperature and salinity data; however, these relationships are known to be region-specific and can change over time. Therefore, these algorithms were customized for the Bight using the Bight '13 acidification dataset and will be routinely evaluated against bottle measurements of TA at the Bight '18 data analysis (underway) and at the end of the Bight '23 study (measurements conducted on the same bottle samples as those collected for the pH calibration, Table II-3).

These algorithms are expected to provide TA within ± 80 µmol/kg at the 95% confidence interval. Interestingly, because TA within the Bight is relatively consistent (the Bight has no major freshwater sources of alkalinity), the average TA collected during the Bight '13 study appears to be as robust a measurement of TA as that generated by the Alin et al. (2012) algorithm. The algorithm-estimated TA and the average TA can be compared to bottle measurements of TA collected from bottle samples during each survey and the closest match can be applied to the dataset to estimate TA for all CTD observations collected during the survey, allowing for calculation of $\Omega_{arag.}$ The remaining parameters of the carbonate system, the partial pressure of CO_2 (pCO_2), dissolved inorganic carbon (DIC), and Ω_{arag} can be computed using the same tool and the program CO2calc.

As with pH, bottle samples for TA will be analyzed either by the agency or an outside contract laboratory. All participating laboratories participate in chemistry intercalibration before sampling starts to ensure interlaboratory comparability (Appendix D). As noted in the previous

section, the same 500 mL sample can be analyzed from both pH and TA or two separate bottles can be collected for each analyte. Each agency should check with its analytical lab on the preference for how samples should be collected.

Products: Short-term products from this element include cumulative distribution functions of aragonite saturation state, pH, and dissolved oxygen in the Bight and stacked bar graphs of the distribution of aragonite saturation states by depth bins with data separated seasonally and annually. Longer-term products are to develop time-series of the spatial distribution of carbonate chemical parameters and oxygen in the Bight.

2. OA-specific Biological Effects Assessment

Magnitude and Extent of Impacts. The magnitude and extent of biological impacts on the upper 200 m of the water column in the Bight will be assessed at 15 sites biannually for two years via ship surveys along 5 transects, starting in **February 2025** and ending **August 2026** (Tables II-2 and II-3). Biological effects surveys (2 ship days per agency) should occur within a month of the quarterly surveys conducted for the carbonate chemistry status and trends element. Biannual surveys were selected because seasonal upwelling is known to affect the aragonite saturation state of Bight waters and therefore the biological effects may be expected to have a seasonal component. A two-year program was chosen for characterization of interannual variability in the assessment.

Site Selection. A map of the sampling sites is provided in Figure II-2. For Bight '18, sites were selected using a systematic approach in coastal waters approximately 5-10 nautical miles offshore and in water depths greater than 250 m. This habitat was selected because pteropods are known to vertically migrate throughout the water column to depths up to 200 meters. Site transects will be oriented approximately perpendicular to the coast.

Sites were selected using a systematic approach with stations at an increasing distance from shore, while maximizing each agency's ability to sample the sites within 2 sampling days. This approach allows for complete coverage of the nearshore Bight, while still being tractable for the participating agencies. A list of sample sites and agencies responsible for sampling these sites is provided in Appendix A.

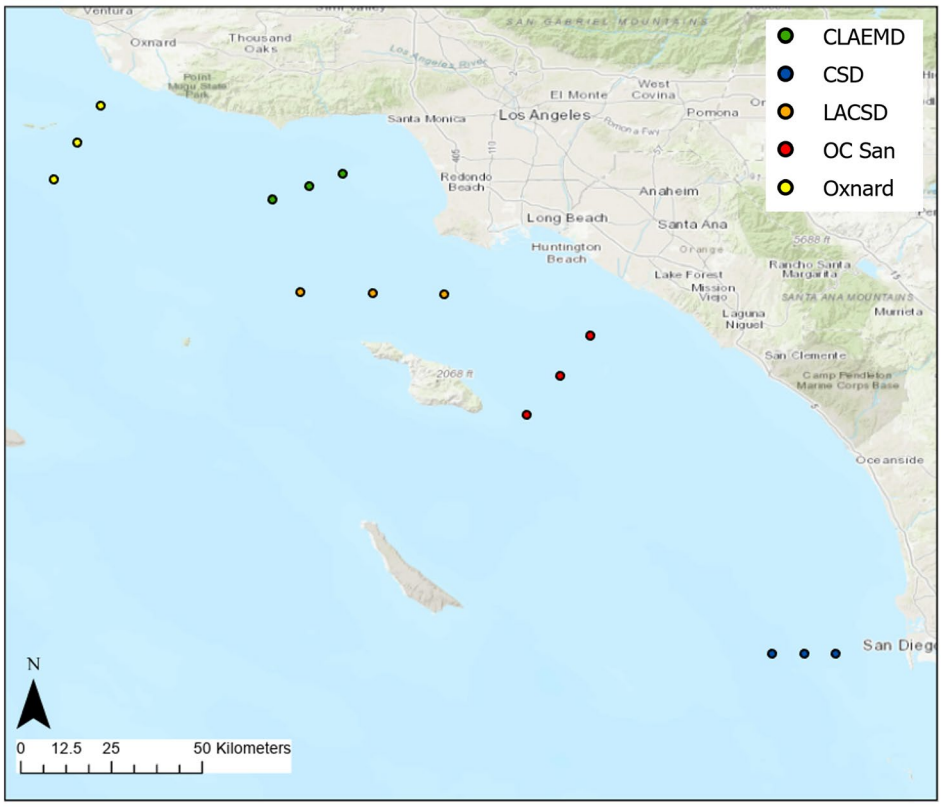


Figure II-2. Map of sampling stations for OA pelagic water biological effects

Shell Dissolution Indicators, Metrics, Sample Collection and Analysis. The Bight '18 study identified pteropods (provisionally, *Limacina spp.*) and crab larvae (Pacific Sand Crab, *Emerita analoga*) sampling in a 200 µm net tow to be in sufficient abundance at all stations and all quarters to be reliable indicator taxa for OA assessment. Because crab larvae were not reliably captured, the Bight '23 program will continue to focus pteropods. Biological samples will be archived in order to preserve the opportunity for analysis of crab larvae carapace dissolution, if the opportunity emerges.

Shell dissolution is a key OA metric because environmental carbonate chemistry is thought to be the only stressor responsible for the observed response. Thus, this measurement combined with the pteropod abundance data will form the cornerstone of the OA specific biological impact assessment. Following the original Bight '18 design, the design of the biological effects assessment is to co-locate biological indicators with measurements of ocean chemistry, allowing us to relate biological response to chemical exposure. To link the biological sampling with a chemical sampling, a CTD cast will be conducted once on station, before the tow, to collect a profile of the water column structure for physical and chemical parameters. Bottle samples for pH and total alkalinity will be collected from the surface and at the targeted bottom of the tow depth (up to 200 m) with a Niskin bottle (or similar) deployed with the CTD sensor package (Table II-3). These bottle samples will be used to calibrate the CTD pH sensor

(as described in the previous section) and provide direct chemical measurements that can be correlated with observed impacts to the biological indicators.

Pteropods are sampled as "zooplankton." Zooplankton samples will be collected using a double ringed bongo-net tow that is pulled obliquely through the water column from a depth of 200 m (see Appendix E). This design will enable us to capture the target organisms residing at a variety of depths depending on their vertical migration habits. The contents of both cod ends will be preserved in separate sampling jars (one for OA biological analysis and one for eDNA) within 8 hours of sampling for assessment of shell condition in 90% ethanol buffered to pH 8.2 with ammonium hydroxide. The initial ethanol should be decanted and replaced with buffered ethanol after 24 hours of sampling.

From each sample, the pteropod will be sorted and their abundance counted as described in Appendix F, using a Folsom splitter when necessary. Gastropods (including pteropods) settle to the bottom and will be decanted for abundance and scanning electron micrograph (SEM) sampling preservation. A voucher specimen will be collected from each agency/quarter to verify genomic taxonomy.

A targeted number of 15 pteropod per sample will be chosen for shell dissolution by SEM, using the methods in Appendix H. Scoring and reporting will follow expert recommended expert best practices per April 2024 workshop recommendations (Frieder et al. in prep).

Products: The short-term key graphic from this element is to compare the percent of individuals (pteropods) with moderate and severe dissolution relative to the Ω_{arag} in the paired water sample. Comparisons of findings will be made with Bight '18 and OPC supported monitoring by season within the Bight and supra regional comparisons with Central Coast (ACCESS) and WCOA (west coast wide). Longer-term products will contribute to a time-series of the spatial distribution of the biological effects of OA in the Bight and how that varies with oceanographic conditions captured.

3. eDNA-based Monitoring

This study component will analyze water and net tow eDNA samples to characterize biological communities. Analysis of water from Niskin bottles will focus on fish (Appendix G), while analysis of bongo net tow samples will focus on zooplankton community diversity and relative abundance(Appendix F). The water eDNA sampling protocol will follow the Smith-Root eDNA sampling protocol found in Appendix G. Zooplankton eDNA sampling follows the protocol of Appendix E and F.

Quarterly Monitoring Stations**:** Collection and filtration of 1-2 liters of water for eDNA samples from CTD niskin bottles will be paired with collection of ~500 ml water for pH and TA samples. Sampling will consist of 6 samples per quarter (2 stations x 3 depths; surface, chlorophyll-a max, and bottom) for CSD, LACSD, OC San and CLA, while Oxnard samples all CTD stations in one day therefore will only sample one station (3 samples) for pH/TA/eDNA. This translates a total of 27 samples per quarterly event across all agencies, for a total of 216 samples over the two-year period (Table II-3).

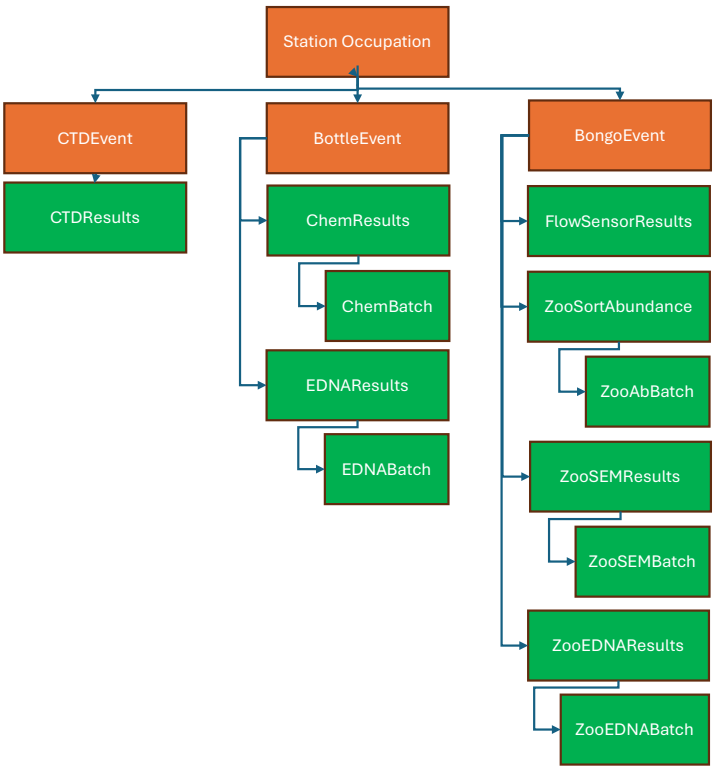
Biological Monitoring Stations. At biological monitoring stations, triplicate water samples are collected for eDNA filtration at the same depths as for pH and TA (surface and bottom). This triplicate sampling represents the quality assurance required to characterize variability in community characterization. This translates to 18 eDNA samples (3 stations *2 depths * triplicate samples) for each of five agencies, for a total of 90 per quarter, for four quarters (360 samples).

Zooplankton samples are collected from bongo tows, then split to provide samples for 1) eDNA of zooplankton whole community relative abundance and 2) zooplankton wet biovolume, pteropod abundance and shell dissolution. This translates to 1 sample per station and 3 stations per agency, across 5 agencies, for a total of 15 stations per biannual sampling event (Table II-2). This translates to 60 eDNA samples across the 2 years of sampling.

Products: The short-term key graphic from this element is to compare the eDNA fingerprint of fish (water samples) and bongo tows (zooplankton) with OAH chemistry. Findings will be compared with MBON, CalCOFI, and WCOA (west coast-wide surveys). Longer-term products will contribute to a time-series of the spatial distribution of the biological diversity and relationship to effects of OAH and warming in the Bight.

III. Bight '23 OAH Information Management

The Bight '23 IM strategy is to maintain a high level of data quality assurance and quality control from field collection through laboratory analysis to data submission and subsequent data analysis. The Bight '23 Data Portal organizes the collection, analysis, and retrieval of Bight '23 data. In the project's first phase, collection, this portal links to the workgroups associated with Bight '23. The Bight '23 IM Plan focuses on Sediment Quality, but provides an overview of the approach that each committee will take to ensure the final dataset is complete and of the highest quality. In the sediment quality portal, data checkers include logic checks, completeness checks, range checks, syntax checks, duplicate checks, qualifier checks, checks for calculated parameters, and QA/QC checks.



The Bight '23 cycle represents the first time that Bight participants will utilize a portal for submitting their data. The portal landing page exists and can be accessed through this link: Bight 2023 OA | SCCWRP Data Portal. The portal is being built in conjunction with a subcommittee to make it more comprehensive, according to the vision in Figure III-1. The Event (in Orange) and CTD and Chemistry results (first two columns, green boxes) are prioritized first, and the Bongo results for each of the component are the second priority, with a rough schedule for completion in Table II-4

Figure III-1. Vision for Bight '23 OAH Portal Architecture.

Table II-4 Proposed Schedule for Completion of Data Submission and IM Portal Functionality and Schedule for Data Uploads to the Portal.

Data Type	Data SubType	Deadline for IM Portal Functionality	Schedule for Data Uploads to Portal
Field	Station Occupation CTD Event Bottle Event Bongo Event	October 15, 2024	3 months from sampling date
CTD	CTD Results	October 15, 2024	3 months from sampling date
Bottle chemistry	Chemistry Results and Chemistry Batch	October 15, 2024	6 months from sampling date
Biological	Zooplankton Abundance/Biovolume Results and Batch	January 1, 2025	6 months from sampling date
	Gastropod SEM Results and Batch	January 1, 2025	6 months from sampling date
	e-DNA /Results and Batch	January 1, 2025	To be determined by need for archive versus sequencing

POTW agencies will input their data into the Excel templates and submit them through the Bight OAH data portal to include all station occupation and event information, field sampling data (e.g., CTD casts), OA bottle chemistry data. SCCWRP will be submitting zooplankton sorting, scanning electron micrograph, and e-DNA data. CTD pH potentiometric data is not accurate alone, so a pH tool to calibrate the potentiometric data from the Niskin bottle data has been developed. When pH bottle data are included in the template of data uploaded, the pH tool automatically recalibrates the CTD potentiometric pH data. The pH tool R code can be found on the SCCWRP GitHub: https://github.com/SCCWRP/phcorrection. The IM committee has agreed to guide an update of the pH so that it has improved functionality for use by both individual agencies to submit their data for compliance monitoring and for application to Bight '23 submitted data.

All files will be run through the appropriate data checkers. If a file fails one of the data checks, the error will be highlighted on the Excel form with a note on how to correct the error, and it will be recorded on the webpage. Once all errors have been corrected, the file can be re-uploaded through the checkers (which should now be satisfied) and into the database. This process should expedite the data submittal process, allowing for more rapid access to the final dataset. SCCWRP can provide a training session on how to use the Excel templates and the data portal. This session will demonstrate the data submission process and allow users direct experience in using the data portal before submitting their data.

IV. References

Barton, A., G. Waldbusser, R. Feely, S.B. Weisberg, J. Newton, B. Hales, S. Cudd, B. Eudeline, C. Langdon, I. Jefferds, T. King, A. Suhrbier, and K. McLaughlin. 2015. Impacts of coastal acidification on the Pacific Northwest shellfish industry and adaptation strategies implemented in response. Oceanography. 28:146–159. dx.doi.org/10.5670/oceanog.2015.38.

Bednaršek, N.A, T. Klinger, C.J. Harvey, S. Weisberg, R.M. McCabe, R.A. Feely, J. Newton, and N. Tolimieri. 2017. New ocean, new needs: Application of pteropod shell dissolution as a biological indicator for marine resource management. Ecological Indicators 76:240–244

Chan, F., J.A. Barth, J. Lubchenco, A. Kirincich, H. Weeks, W.T. Peterson, and B.A. Menge. 2008. Emergence of anoxia in the California Current large marine ecosystem. Science 319(5865):920, https://doi.org/10.1126/science.1149016.

Chavez, F.P. and Messié, M., 2009. A comparison of eastern boundary upwelling ecosystems. *Progress in Oceanography*, *83*(1-4), pp.80-96.

Dickson, A.G., J.D. Afghan, G.C. Anderson. 2003. Reference materials for oceanic CO2 analysis: a method for the certification of total alkalinity. Marine Chemistry, 80: 185-197.

Fabry, V.J., B.A. Seibel, R.A. Feely, and J.C. Orr. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. ICES Journal of Marine Science 65: 414–432.

Fassbender, A.J., C.L. Sabine, R.A. Feely, C. Langdon, and C.W. Mordy. 2011. Inorganic carbon dynamics during northern California coastal upwelling, Continental Shelf Research. 31:1180–1192, doi:10.1016/j.csr.2011.04.006.

Feely, R.A., S.R. Alin, B. Carter, N. Bednaršek, B. Hales, F. Chan, T.M. Hill, B. Gaylord, E. Sanford, R.H. Byrne, C.L. Sabine, D. Greeley, and L. Juranek. 2016. Chemical and biological impacts of ocean acidification along the west coast of North America. Estuarine, Coastal and Shelf Science 183:260-270

Feely, R.A., C.L. Sabine, R.H. Byrne, F.J. Millero, A.G. Dickson, R. Wanninkhof, A. Murata, L.A. Miller, and D. Greeley. 2012. Decadal changes in the aragonite and calcite saturation state of the Pacific Ocean Global Biogeochemical Cycles. 26: 1–5

Feely, R.A., C.L. Sabine, J.M. Hernandez-Ayon, D. Ianson, and B. Hales. 2008. Evidence for upwelling of corrosive acidified water onto the continental shelf. Science. 320:1490–2

Frieder, C., Kessouri, F., Ho, M., Sutula, M., Bianchi, D., McWilliams, J. C., et al. (2023). Effects of urban eutrophication on pelagic habitat capacity in the Southern California Bight (preprint). Preprints. https://doi.org/10.22541/essoar.167870388.82984110/v1

Harris, K. E., M. D. DeGrandpre, and B. Hales. 2013. Aragonite saturation state dynamics in a coastal upwelling zone, Geophysical Research Letters, 40, 2720–2725. doi:10.1002/grl.50460.

Hofmann, G.E., J.P. Barry, P.J. Edmunds, R.D. Gates, D.A. Hutchins, T. Klinger, and M.A. Sewell. 2010. The effect of ocean acidification on calcifying organisms in marine ecosystems: An organism-to-ecosystem perspective. Annual Review of Ecology, Evolution, and Systematics 41:127–147, http://dx.doi.org/10.1146/annurev.ecolsys.110308.120227.

Howard, M.D.A., M. Sutula, D. Caron, Y. Chao, J.D. Farrara, H. Frenzel, B. Jones, G. Robertson, K. McLaughlin, A. Sengupta. (2014) Anthropogenic Nutrient Sources Rival Natural Sources on Small Scales in the Coastal Waters of the Southern California Bight. Limnology and Oceanography. 59: 285-297

Kessouri, F., Sutula, M., C., Bianchi, McWilliams, J. D., Ho, M., , et al. (2024). Cross-shore transport and eddies promote large scale response to urban eutrophication. Nature Science Advances Mar 27;14(1):7240. doi: 10.1038/s41598-024-57626-6.

Kessouri, F., McLaughlin, K., Sutula, M. A., Bianchi, D., , McWilliams, J. C., et al. (2021a). Configuration and validation of an oceanic physical and biogeochemical model to investigate coastal eutrophication: case study in the Southern California Bight. . Journal of Advances in Modeling Earth Systems, 13(12), p.e2020MS002296.

Kessouri, F., McWilliams, J. C., Bianchi, D., Sutula, M., Renault, L., Deutsch, C., et al. (2021b). Coastal eutrophication drives acidification, oxygen loss, and ecosystem change in a major oceanic upwelling system. Proceedings of the National Academy of Sciences, 118(21), e2018856118. https://doi.org/10.1073/pnas.2018856118

Liu, X., M.C. Patsavas, R.H. Byrne. 2011. Purification and Characterization of meta-Cresol Purple for Spectrophotometric Seawater pH Measurements. Environmental Science & Technology, 45: 4862-4868.

McClatchie, S., R. Goericke, R. Cosgrove, G. Auad, and R. Vetter (2010), Oxygen in the Southern California Bight: Multidecadal trends and implications for demersal fisheries, Geophys. Res. Lett., 37, L19602, doi:10.1029/2010GL044497.

McLaughlin, K., Nezlin, N.P., Weisberg, S.B., Dickson, A.G., Booth, J.A., Cash, C.L., Feit, A., Gully, J.R., Johnson, S., Latker, A., Mengel, M.J., Robertson, G.L., Steele, A. and Terriquez, L. (2017), An evaluation of potentiometric pH sensors in coastal monitoring applications. Limnol. Oceanogr. Methods, 15: 679-689. https://doi.org/10.1002/lom3.10191

McLaughlin, K., N.P. Nezlin, S.B. Weisberg, A. Dickson, J.A.T. Booth, C.L. Cash, A. Feit, J.R. Gully, M.D. Howard, S. Johnson, A. Latker, M.J. Mengel, G.L. Robertson, A. Steele, L. Terriquez. 2018. Seasonal patterns in aragonite saturation state on the southern California continental shelf. Continental Shelf Research 167:77-86

NRC. 1990. Managing troubled waters. National Research Council. National Academies Press. Washington, DC. 125 pp.

Orr, C.J., Fabry, V.J., Aumont, O., Bopp, L., Doney, S.C., Feely, R.A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R.M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R.G., Plattner, G.-K., Rodgers, K.B., Sabine, C.L., Sarmiento, J.L., Schlitzer, R., Slater, R.D., Totterdell, I.J., Weirig, M.-F., Yamanaka, Y., Yool, A., 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature. 437: 681–686, doi:10.1038/nature04095.

Sutula, M., Ho, M., Sengupta, A., Kessouri, F., McLaughlin, K., McCune, K., & Bianchi, D. (2021). A baseline of terrestrial freshwater and nitrogen fluxes to the Southern California Bight, USA. Marine Pollution Bulletin, 170, 112669. https://doi.org/10.1016/j.marpolbul.2021.112669

Zeebe, R.E. 2012. History of seawater carbonate chemistry, atmospheric, and ocean acidification Annual Reviews of Earth Planetary Sciences. 40:141–65.

APPENDIX A – List of Sampling Stations

Table A1. Quarterly routine compliance monitoring stations. Data from stations specified with a frequency less then quarterly will be utilized if pH and TA bottles samples are taken within the same week of sampling.

Agency	AgencyStationID	Latitude	Longitude	AgencyFrequency
CLAEMD	3201	33.85417	-118.40612	Quarterly
CLAEMD	3202	33.84862	-118.41778	Quarterly
CLAEMD	3203	33.84528	-118.42638	Quarterly
CLAEMD	3204	33.83695	-118.44055	Quarterly
CLAEMD	3205	33.82388	-118.46362	Quarterly
CLAEMD	3206	33.81110	-118.49278	Quarterly
CLAEMD	3301	33.89305	-118.42722	Quarterly
CLAEMD	3302	33.88917	-118.43638	Quarterly
CLAEMD	3303	33.88555	-118.44667	Quarterly
CLAEMD	3304	33.87945	-118.45695	Quarterly
CLAEMD	3305	33.86833	-118.49333	Quarterly
CLAEMD	3306	33.85112	-118.52722	Quarterly
CLAEMD	3401	33.90250	-118.43250	Quarterly
CLAEMD	3402	33.90000	-118.44722	Quarterly
CLAEMD	3403	33.90110	-118.46000	Quarterly
CLAEMD	3404	33.89693	-118.46860	Quarterly
CLAEMD	3405	33.88722	-118.50638	Quarterly
CLAEMD	3406	33.87917	-118.53555	Quarterly
CLAEMD	3501	33.93138	-118.44805	Quarterly
CLAEMD	3502	33.92777	-118.46027	Quarterly
CLAEMD	3503	33.92388	-118.47250	Quarterly
CLAEMD	3504	33.91667	-118.49417	Quarterly
CLAEMD	3505	33.90917	-118.52527	Quarterly
CLAEMD	3505B	33.90880	-118.52320	Quarterly
CLAEMD	3506	33.90000	-118.54972	Quarterly
CLAEMD	3601	33.95973	-118.46625	Quarterly
CLAEMD	3602	33.95555	-118.47777	Quarterly
CLAEMD	3603	33.94943	-118.49027	Quarterly
CLAEMD	3604	33.94027	-118.50977	Quarterly
CLAEMD	3605	33.92777	-118.53555	Quarterly
CLAEMD	3606	33.91667	-118.55833	Quarterly
CLAEMD	3701	33.98610	-118.48610	Quarterly
CLAEMD	3702	33.98000	-118.50000	Quarterly
CLAEMD	3703	33.97417	-118.51000	Quarterly
CLAEMD	3704	33.96667	-118.52555	Quarterly

Agency	AgencyStationID	Latitude	Longitude	AgencyFrequency
CLAEMD	3705	33.95360	-118.55360	Quarterly
CLAEMD	3706	33.94250	-118.57500	Quarterly
CLAEMD	3801	34.03333	-118.58333	Quarterly
CLAEMD	3802	34.02583	-118.58750	Quarterly
CLAEMD	3803	34.00583	-118.59722	Quarterly
CLAEMD	3804	33.99333	-118.60417	Quarterly
CLAEMD	3805	33.97222	-118.61417	Quarterly
CLAEMD	3806	33.95610	-118.62360	Quarterly
CLAEMD	3901	34.02750	-118.71667	Quarterly
CLAEMD	3902	34.01943	-118.71667	Quarterly
CLAEMD	3903	34.01110	-118.71667	Quarterly
CLAEMD	3904	33.99750	-118.71667	Quarterly
CLAEMD	3905	33.96027	-118.71667	Quarterly
CLAEMD	3906	33.94277	-118.71667	Quarterly
CLAEMD	4001	33.99527	-118.80527	Quarterly
CLAEMD	4002	33.98833	-118.80527	Quarterly
CLAEMD	4003	33.98055	-118.80527	Quarterly
CLAEMD	4004	33.95833	-118.80527	Quarterly
CLAEMD	4005	33.92805	-118.80527	Quarterly
CLAEMD	4006	33.91250	-118.80527	Quarterly
CSD-PLOO	F26	32.59377	-117.31220	Quarterly
CSD-PLOO	F27	32.61178	-117.32138	Quarterly
CSD-PLOO	F28	32.62929	-117.32372	Quarterly
CSD-PLOO	F29	32.64782	-117.32493	Quarterly
CSD-PLOO	F30	32.66567	-117.32483	Quarterly
CSD-PLOO	F31	32.68467	-117.32835	Quarterly
CSD-PLOO	F32	32.70142	-117.33417	Quarterly
CSD-PLOO	F33	32.72047	-117.33992	Quarterly
CSD-PLOO	F34	32.73890	-117.34937	Quarterly
CSD-PLOO	F35	32.75770	-117.36338	Quarterly
CSD-PLOO	F36	32.77678	-117.37457	Quarterly
CSD-PLOO	F01	32.63768	-117.24032	Quarterly
CSD-PLOO	F02	32.75697	-117.27273	Quarterly
CSD-PLOO	F03	32.78183	-117.27242	Quarterly
CSD-PLOO	F04	32.59453	-117.26875	Quarterly
CSD-PLOO	F05	32.61168	-117.26965	Quarterly
CSD-PLOO	F06	32.63083	-117.27360	Quarterly
CSD-PLOO	F07	32.65113	-117.27999	Quarterly
CSD-PLOO	F08	32.67215	-117.28298	Quarterly
CSD-PLOO	F09	32.68555	-117.28632	Quarterly
CSD-PLOO	F10	32.70542	-117.29066	Quarterly

Agency	AgencyStationID	Latitude	Longitude	AgencyFrequency
CSD-PLOO	F11	32.72554	-117.29463	Quarterly
CSD-PLOO	F12	32.74658	-117.30207	Quarterly
CSD-PLOO	F13	32.76538	-117.30720	Quarterly
CSD-PLOO	F14	32.78156	-117.31142	Quarterly
CSD-PLOO	F15	32.59410	-117.28645	Quarterly
CSD-PLOO	F16	32.61183	-117.29007	Quarterly
CSD-PLOO	F17	32.63002	-117.29417	Quarterly
CSD-PLOO	F18	32.64977	-117.29833	Quarterly
CSD-PLOO	F19	32.66785	-117.30683	Quarterly
CSD-PLOO	F20	32.68542	-117.31097	Quarterly
CSD-PLOO	F21	32.70380	-117.31869	Quarterly
CSD-PLOO	F22	32.72273	-117.32090	Quarterly
CSD-PLOO	F23	32.74188	-117.33042	Quarterly
CSD-PLOO	F24	32.76122	-117.33645	Quarterly
CSD-PLOO	F25	32.77895	-117.34358	Quarterly
CSD-SBOO	I28	32.59383	-117.26400	Quarterly
CSD-SBOO	I29	32.59450	-117.22300	Quarterly
CSD-SBOO	I30	32.59533	-117.19700	Quarterly
CSD-SBOO	I31	32.59550	-117.17200	Quarterly
CSD-SBOO	I33	32.62383	-117.23700	Quarterly
CSD-SBOO	I34	32.63000	-117.21600	Quarterly
CSD-SBOO	I35	32.63667	-117.18200	Quarterly
CSD-SBOO	I36	32.63917	-117.15400	Quarterly
CSD-SBOO	I37	32.64800	-117.21633	Quarterly
CSD-SBOO	I38	32.66883	-117.18667	Quarterly
CSD-SBOO	I12	32.53283	-117.18300	Quarterly
CSD-SBOO	I13	32.53750	-117.21200	Quarterly
CSD-SBOO	I14	32.54300	-117.18400	Quarterly
CSD-SBOO	I15	32.53783	-117.18900	Quarterly
CSD-SBOO	I16	32.53783	-117.18300	Quarterly
CSD-SBOO	I17	32.53783	-117.17800	Quarterly
CSD-SBOO	I18	32.53617	-117.16100	Quarterly
CSD-SBOO	I20	32.55700	-117.25700	Quarterly
CSD-SBOO	I21	32.56067	-117.22700	Quarterly
CSD-SBOO	I22	32.55333	-117.18500	Quarterly
CSD-SBOO	I23	32.55083	-117.16500	Quarterly
CSD-SBOO	I27	32.57417	-117.19100	Quarterly
CSD-SBOO	I1	32.47333	-117.27700	Quarterly
CSD-SBOO	I10	32.51667	-117.15600	Quarterly
CSD-SBOO	I11	32.51333	-117.13700	Quarterly
CSD-SBOO	I2	32.47333	-117.19900	Quarterly

Agency	AgencyStationID	Latitude	Longitude	AgencyFrequency
CSD-SBOO	I3	32.46700	-117.16800	Quarterly
CSD-SBOO	I4	32.47167	-117.14000	Quarterly
CSD-SBOO	I5	32.47167	-117.13000	Quarterly
CSD-SBOO	I6	32.49350	-117.16300	Quarterly
CSD-SBOO	I7	32.51667	-117.25300	Quarterly
CSD-SBOO	I8	32.51667	-117.20200	Quarterly
CSD-SBOO	I9	32.51167	-117.17900	Quarterly
CSD-SBOO	SBOO-RTM	32.53187	-117.1863	Quarterly
CSD-PLOO	PLOO-RTM	32.67012	-117.32463	Quarterly
LACSD	RW-OS-2501	33.72783	-118.12017	Quarterly
LACSD	RW-OS-2502	33.69900	-118.12783	Quarterly
LACSD	RW-OS-2503	33.67017	-118.13533	Quarterly
LACSD	RW-OS-2504	33.64133	-118.14283	Quarterly
LACSD	RW-OS-2505	33.61250	-118.15033	Quarterly
LACSD	RW-OS-2506	33.58100	-118.15900	Quarterly
LACSD	RW-OS-2601	33.72050	-118.18433	Quarterly
LACSD	RW-OS-2602	33.69400	-118.19050	Quarterly
LACSD	RW-OS-2603	33.66750	-118.19667	Quarterly
LACSD	RW-OS-2604	33.64100	-118.20300	Quarterly
LACSD	RW-OS-2605	33.61467	-118.20917	Quarterly
LACSD	RW-OS-2606	33.58817	-118.21550	Quarterly
LACSD	RW-OS-2701	33.70767	-118.24667	Quarterly
LACSD	RW-OS-2702	33.68867	-118.25117	Quarterly
LACSD	RW-OS-2703	33.66950	-118.25567	Quarterly
LACSD	RW-OS-2704	33.65050	-118.26000	Quarterly
LACSD	RW-OS-2705	33.63133	-118.26450	Quarterly
LACSD	RW-OS-2706	33.61217	-118.26900	Quarterly
LACSD	RW-OS-2801	33.70283	-118.28433	Quarterly
LACSD	RW-OS-2802	33.69333	-118.28900	Quarterly
LACSD	RW-OS-2803	33.66850	-118.29683	Quarterly
LACSD	RW-OS-2804	33.65767	-118.30133	Quarterly
LACSD	RW-OS-2805	33.64850	-118.30400	Quarterly
LACSD	RW-OS-2806	33.63700	-118.30917	Quarterly
LACSD	RW-OS-2901	33.71433	-118.32350	Quarterly
LACSD	RW-OS-2902	33.70700	-118.32983	Quarterly
LACSD	RW-OS-2903	33.69850	-118.33567	Quarterly
LACSD	RW-OS-2904	33.68783	-118.33900	Quarterly
LACSD	RW-OS-2905	33.67100	-118.34617	Quarterly
LACSD	RW-OS-2906	33.65417	-118.35433	Quarterly
LACSD	RW-OS-3001	33.73217	-118.36033	Quarterly
LACSD	RW-OS-3002	33.72233	-118.36317	Quarterly

Agency	AgencyStationID	Latitude	Longitude	AgencyFrequency
LACSD	RW-OS-3003	33.71467	-118.36600	Quarterly
LACSD	RW-OS-3004	33.70100	-118.37133	Quarterly
LACSD	RW-OS-3005	33.68500	-118.38100	Quarterly
LACSD	RW-OS-3006	33.66683	-118.39067	Quarterly
LACSD	RW-OS-3051	33.73633	-118.39433	Quarterly
LACSD	RW-OS-3052	33.73317	-118.40050	Quarterly
LACSD	RW-OS-3053	33.73000	-118.40250	Quarterly
LACSD	RW-OS-3054	33.71900	-118.41100	Quarterly
LACSD	RW-OS-3055	33.70500	-118.42200	Quarterly
LACSD	RW-OS-3056	33.68967	-118.43317	Quarterly
LACSD	RW-OS-3101	33.77100	-118.43017	Quarterly
LACSD	RW-OS-3102	33.76500	-118.43533	Quarterly
LACSD	RW-OS-3103	33.75733	-118.44100	Quarterly
LACSD	RW-OS-3104	33.74533	-118.44983	Quarterly
LACSD	RW-OS-3105	33.72883	-118.46117	Quarterly
LACSD	RW-OS-3106	33.71250	-118.47550	Quarterly
OCSAN	2103	33.58482	-117.94463	Monthly
OCSAN	2104	33.56998	-117.95690	Monthly
OCSAN	2105	33.55515	-117.96917	Monthly
OCSAN	2106	33.54033	-117.98142	Monthly
OCSAN	2183	33.59502	-117.96240	Monthly
OCSAN	2184	33.58018	-117.97467	Monthly
OCSAN	2185	33.56537	-117.98692	Monthly
OCSAN	2186	33.55053	-117.99918	Monthly
OCSAN	2203	33.60522	-117.98017	Monthly
OCSAN	2204	33.59038	-117.99243	Monthly
OCSAN	2205	33.57557	-118.00470	Monthly
OCSAN	2206	33.56073	-118.01697	Monthly
OCSAN	2223	33.61540	-117.99785	Monthly
OCSAN	2224	33.60058	-118.01013	Monthly
OCSAN	2225	33.58577	-118.02243	Monthly
OCSAN	2226	33.57095	-118.03472	Monthly
OCSAN	2303	33.62562	-118.01560	Monthly
OCSAN	2304	33.61082	-118.02790	Monthly
OCSAN	2305	33.59600	-118.04020	Monthly
OCSAN	2306	33.58118	-118.05248	Monthly
OCSAN	2351	33.63585	-118.03335	Monthly
OCSAN	2352	33.62103	-118.04565	Monthly
OCSAN	2353	33.60622	-118.05795	Monthly
OCSAN	2354	33.59140	-118.07023	Monthly
OCSAN	2403	33.64608	-118.05120	Monthly

Agency	AgencyStationID	Latitude	Longitude	AgencyFrequency
OCSAN	2404	33.63125	-118.06347	Monthly
OCSAN	2405	33.61643	-118.07573	Monthly
OCSAN	2406	33.60160	-118.08800	Monthly
OCSAN	1701	33.49697	-117.74590	Quarterly
OCSAN	1702	33.48634	-117.75200	Quarterly
OCSAN	1703	33.47454	-117.75874	Quarterly
OCSAN	1704	33.46785	-117.76254	Quarterly
OCSAN	1705	33.45723	-117.76859	Quarterly
OCSAN	1706	33.44091	-117.77799	Quarterly
OCSAN	1801	33.53235	-117.78242	Quarterly
OCSAN	1802	33.52651	-117.78596	Quarterly
OCSAN	1803	33.51924	-117.79010	Quarterly
OCSAN	1804	33.51020	-117.79516	Quarterly
OCSAN	1805	33.49637	-117.80461	Quarterly
OCSAN	1806	33.47696	-117.81412	Quarterly
OCSAN	1901	33.56137	-117.82757	Quarterly
OCSAN	1902	33.55275	-117.83240	Quarterly
OCSAN	1903	33.54603	-117.83637	Quarterly
OCSAN	1904	33.52978	-117.84557	Quarterly
OCSAN	1905	33.51350	-117.85475	Quarterly
OCSAN	1906	33.49715	-117.86403	Quarterly
OCSAN	2001	33.58892	-117.87820	Quarterly
OCSAN	2002	33.57925	-117.88380	Quarterly
OCSAN	2003	33.57608	-117.88573	Quarterly
OCSAN	2004	33.55982	-117.89513	Quarterly
OCSAN	2005	33.54355	-117.90438	Quarterly
OCSAN	2006	33.52745	-117.91373	Quarterly
OCSAN	2041	33.59949	-117.90946	Quarterly
OCSAN	2042	33.59022	-117.91550	Quarterly
OCSAN	2043	33.58181	-117.92109	Quarterly
OCSAN	2044	33.56585	-117.93145	Quarterly
OCSAN	2045	33.55021	-117.94167	Quarterly
OCSAN	2046	33.53466	-117.95184	Quarterly
OCSAN	2101	33.60305	-117.92915	Quarterly
OCSAN	2102	33.59385	-117.93677	Quarterly
OCSAN	2181	33.61462	-117.94587	Quarterly
OCSAN	2182	33.60453	-117.95440	Quarterly
OCSAN	2201	33.62488	-117.96385	Quarterly
OCSAN	2202	33.61502	-117.97190	Quarterly
OCSAN	2221	33.63498	-117.98180	Quarterly
OCSAN	2222	33.62537	-117.98957	Quarterly

Agency	AgencyStationID	Latitude	Longitude	AgencyFrequency
OCSAN	2301	33.64287	-118.00107	Quarterly
OCSAN	2302	33.63422	-118.00825	Quarterly
OCSAN	2349	33.65317	-118.01892	Quarterly
OCSAN	2350	33.64445	-118.02610	Quarterly
OCSAN	2401	33.66533	-118.03505	Quarterly
OCSAN	2402	33.65570	-118.04322	Quarterly
OCSAN	2451	33.69125	-118.06573	Quarterly
OCSAN	2452	33.67898	-118.07640	Quarterly
OCSAN	2453	33.66645	-118.08673	Quarterly
OCSAN	2454	33.65163	-118.09910	Quarterly
OCSAN	2455	33.63683	-118.11125	Quarterly
OCSAN	2456	33.62197	-118.12352	Quarterly
Oxnard	A001	34.138081	-119.212132	Quarterly
Oxnard	A002	34.132012	-119.218126	Quarterly
Oxnard	A003	34.125161	-119.224458	Quarterly
Oxnard	A004	34.117883	-119.231060	Quarterly
Oxnard	A005	34.110413	-119.237707	Quarterly
Oxnard	B001	34.136325	-119.201523	Quarterly
Oxnard	B002	34.129138	-119.208035	Quarterly
Oxnard	B003	34.122454	-119.214308	Quarterly
Oxnard	B004	34.115150	-119.220927	Quarterly
Oxnard	B005	34.107873	-119.227089	Quarterly
Oxnard	C001	34.132179	-119.192064	Quarterly
Oxnard	C002	34.124922°	-119.198634	Quarterly
Oxnard	C003	34.117716	-119.205188	Quarterly
Oxnard	C004	34.110515	-119.211638	Quarterly
Oxnard	C005	34.103261	-119.218082	Quarterly
Oxnard	D001	34.126674	-119.183273	Quarterly
Oxnard	D002	34.119997	-119.189477	Quarterly
Oxnard	D003	34.113029	-119.195943	Quarterly
Oxnard	D004	34.105505	-119.202504	Quarterly
Oxnard	D005	34.098363	-119.208956	Quarterly
Oxnard	E001	34.120332	-119.175354	Quarterly
Oxnard	E002	34.114238	-119.181209	Quarterly
Oxnard	E003	34.107623	-119.186815	Quarterly
Oxnard	E004	34.100469	-119.193520	Quarterly
Oxnard	E005	34.093656	-119.199672	Quarterly

Table A2. Sample Locations for Paired OA Chemistry and Biological Assessment

Agency	AgencyStationID	B23-OA-StationID	Longitude	Latitude
Oxnard	4-3-1	B23-OA-OXN-B1	-119.248304	34.060626
Oxnard	4-3-2	B23-OA-OXN-B2	-119.306661	33.969193
Oxnard	4-3-3	B23-OA-OXN-B3	-119.365343	33.879037
CSD	B23-OA-4-19	B23-OA-CSD-B1	-117.4293800	32.7037200
CSD	B23-OA-CSD-16nm	B23-OA-CSD-B2	-117.5083700	32.7036900
CSD	B23-OA-CSD-20nm	B23-OA-CSD-B3	-117.5873800	32.7036200
OC San	14	B23-OA-OCS-B1	-118.0377880	33.4902729
OC San	24	B23-OA-OCS-B2	-118.1123483	33.3910656
OC San	25	B23-OA-OCS-B3	-118.1934580	33.2951129
LACSD	B23OA-4-11	B23-OA-LCS-B1	-118.3983210	33.5940890
LACSD	LACSD OAH mid	B23-OA-LCS-B2	-118.5761600	33.5964800
LACSD	LACSD OAH far	B23-OA-LCS-B3	-118.7544000	33.5999700
CLAEMD	B23OA-4-7	B23-OA-CLA-B1	-118.6510062	33.8912931
CLAEMD	B23OA-B-4	B23-OA-CLA-B2	-118.7325833	33.8620333
CLAEMD	B23OA-B-5	B23-OA-CLA-B3	-118.8245000	33.8281667

Sample Locations for PMEL Inshore Extension (Paired OA Chemistry and Biological Assessment)

[To Be Determined by Spring 2025]

APPENDIX B − Procedure for Collecting Carbonate Chemistry Water Samples

- **Conduct a CTD Cast.** A CTD cast should be done once on station. The CTD should record water column profiles of temperature, salinity, dissolved oxygen, chlorophyll fluorescence, CDOM, and pH to maximum station depth possible.

- **Collect Water Samples for pH and TA.** A bottle sample for pH and total alkalinity (TA) should be collected from the surface, chlorophyll-a maximum, and deepest possible, not to exceed 200 m. Bottles should be triggered on the upcast, rather than downcast. The temperature, depth, pressure and salinity at that point should be recorded in the field data sheet. Record which Niskin bottle that each sample came from, including whether replicates are from the same Niskin or a different one.

  Bottle samples should be collected into 250- or 500-mL glass bottles (not plastic). Pyrex bottles are recommended (Fisher Scientific 02-940D), particularly if the time until sample processing will be greater than 1 week. One 500 mL bottle can be used to analyze both pH and TA or alternatively two 250 mL bottles can be collected (1 for pH and 1 for TA). Bottles should be overfilled at least 50%, making sure no air bubbles are trapped in the bottle. Once the bottle is filled to the brim, create a ~1% space (based on the total volume of the container) and preserve the sample with mercuric chloride (0.02% of the sample volume, or 120 microliters for 500 mL). Apply a thin strip of Apiezon L grease around the bottom of each bottle's stopper (see picture at right for proper greasing method).

  After sampling, insert a stopper into the neck of the sample bottle and twist to spread the grease evenly and form a good seal. Seal the glass stopper with grease, place a collar and rubber band to secure the bottle stopper, and store bottle samples at room temperature (not frozen) until analysis. The Dickson Lab protocol is provided below.

Dickson Protocol

Samples are to be collected in Pyrex reagent bottles and sealed using a greased glass stopper secured with a rubber band and clip. Samples are to be poisoned with a small volume of saturated mercuric chloride (HgCl2) solution. Remember that time is of the essence when sampling for DIC. Please be sure to carefully read these instructions BEFORE you start sampling and be sure to move quickly between the steps described herein.

Before drawing the first sample, the following items should be removed from the box of equipment and supplies and prepared for use:

1) The polyethafoam block, which has holes for holding a single bottle and stopper.

2) The 20 mL syringe and its ~2-inch Tygon tube. This will be used to withdraw enough water from the sample to create a ~1% headspace. *There is a short piece of tubing on the tip of the syringe. Push the 2-inch tube into this piece of tubing.*

3) The grease dispensing "gun" and 30 mL syringe of grease and delivery tip. Install the 30 mL syringe of grease onto the dispensing "gun". Remove the orange cap from the end of the syringe and replace it with the green delivery tip. Note: If no gun present, delivery from the Apiezon grease tube directly or from a 20 ml syringe is acceptable.

4) The kimwipe-wrapped sticks to wipe water from the neck of the bottles.

5) The Eppendorf pipette and a delivery tip. Install the tip onto the pipette. The Eppendorf has been set to a volume of 120 micro liters (about 0.02% of the sample volume).

6) Saturated mercuric chloride ($HgCl_2$) solution.

7) The plastic bottle containing the Tygon drawing tubes soaking in seawater. There are three sizes of tubing. Determine which size will be needed to draw samples from the Niskin bottles.

8) A data sheet.

9) Open the blue plastic box and remove the first two layers of polyethafoam. These must be returned to the box after all samples have been drawn.

10) The box of kimwipes.

Sample Drawing

1) Remove the first bottle from the blue box. The box has a tag in the corner from which the first bottle should be removed. You will notice the bottle has been marked to show an ~1% airspace (important).

2) Remove the greased stopper from the bottle and with a kimwipe, remove as much grease as possible. Using the grease gun, apply four thin beads of grease to the entire length of the frosted portion of the stopper.

3) Put the re-greased stopper into the polyethafoam hold. The greased portion of the stopper should be up.

4) Using a regular kimwipe, wipe the grease off the neck of the bottle.

5) Using the appropriately sized drawing tube, draw the first sample. Since the bottles have been cleaned and dried, there is no need to rinse the bottle before filling. Run water out the drawing tube, pinching the tube to eliminate any air bubbles that may adhere to the sides of the tubing. With the tubing pinched between your fingers, insert the end of the tube to the bottom of the bottle. Start the flow slowly until the bottom of the tube is covered with water; then, increase the flow until the bottle is filled as fast as the water comes out. Overflow the bottle at least 50%. It is easy to do this by counting the seconds it takes to fill the bottle, then restarting the count until the bottle has overflowed the appropriate volume. Pinch off the tubing so that no water comes out of the Niskin. Slowly lower the bottle to remove the drawing tube, leaving the bottle full to the brim. The displacement of the removed tube will leave adequate headspace in the bottle (~ 1% air space).

Poisoning the sample (addition of Mercuric chloride, $HgCl_2$)

1) Depress the top of the pipette to the first stop position. Put the tip of the pipette into the small glass vial of saturated mercuric chloride solution. Let out the top slowly to fill the tip. Look at the tip to be sure the tip is filled with solutions. If not, eject the solution back into the vial and try again.

** Do NOT put the tip into the sample

2) With the tip of the pipette as close as possible to the surface of the water in the sample bottle, push down the top to the first stop, then depress further to the second stop to "blow out" the remaining solution in the tip. Set the pipette to one side.

[**Special note**: If the tip does not fill correctly, replace the tip with a spare and proceed with the poisoning Once the stopper has been replaced and secured, the clogged tip should be discarded.]

3) Using one of the kimwipe-wrapped sticks, wipe any droplets of liquid that have adhered to the greased neck of the bottle. **This is extremely important.** If all the water is not removed, the subsequent seal will not be satisfactory.

Replace and secure the stopper

1) Remove the stopper from the polyethafoam block and put it into the bottle. Push the stopper straight down and watch as the grease oozes to the sides. Allow the grease to spread until there is no air space between the strips, then twist the stopper to complete the seal.

2) Stretch the band over the top of the stopper. Secure the band in place using one of the white clips.

3) Mix the sample by inversion at least five times.

4) Put the sample into the sample box for storage at room temperature, in the dark.

5) Dispose of any waste (kimwipes, pipette tips) into a toxic waste container labeled for $HgCl_2$.

Recording data on the data sheet

The columns on the data sheet should be completed with the information available. Be sure to record the number on the bottle label in the appropriate column. Please note that additional labeling of the bottle in not necessary as the number on the bottle label serves to distinguish the samples from one another.

Note that the laboratory will need salinity data to appropriately process the samples, so make sure to fill out the COC with appropriate information.

Some additional notes

The beads of Apiezon-L grease can be applied to the stopper using a 20 mL syringe, without needle. You can use a green tip on this syringe or not.

Should the Eppendorf pipettor fail, the mercuric chloride can be added to the sample bottle using one of the 4.5 mL plastic disposable pipettes, which have little tygon tubing caps. Remove the cap from the pipette and put the end into the bottle of mercuric chloride and fill it about half full. Add three drops of mercuric chloride to the sample bottle. If you have to use this plastic pipette, be sure to make a note of this on the data sheet. Before you actually use it to add drops to a sample, you should practice dispensing drops back into the mercuric chloride bottle. You will find that with just a little practice, adding the mercuric chloride a drop at a time is relatively easy.

A box of small kimwipes has been sent to be used for general wiping. For example, with use, the piece of tygon tubing used to collect the sample from the Niskin will accumulate some grease

from the neck of the greased bottles. As needed, use these wipes to remove the grease. Note: it is much easier to wipe the grease off the tube when the grease is warm rather than cold.

You can also use a wipe to remove any residual liquid from the tube on the end of the syringe that is used to remove enough water from a sample bottle to create an airspace. You do not want to transfer any liquid or salt from one sample to the next by failing to wipe this clean after each use.

If you spill some of the mercuric chloride, first, put on a pair of rubber gloves. Blot up the spill with 1 or 2 of the kimwipes. Put the kimwipe(s) into the gallon bag labeled "mercuric chloride clean-up wipes". This bag will be removed from the ship at the end of the cruise. Then, use a sponge to wipe down the area where the mercuric chloride spilled. Rinse the sponge thoroughly with tap water. As diluted, this very low concentration of mercuric chloride can be discharged over the side of the ship. When finished with this clean-up, be sure to wash your hands.

APPENDIX C − Procedures for the Laboratory Analysis of Alkalinity and pH in Discrete Water Samples

Overview of Procedures

Total alkalinity (TA) is determined by potentiometric titration (Dickson et al. 2003) using the Dickson titrator setup. pH is determined by spectrophotometric measurements using meta-cresol purple as an indicator (Liu et al. 2011). Following analysis, bottles should be cleaned for re-use by rinsing with DI water and combusting at 450°C for four hours in a muffle furnace.

The rationale for the pH and TA accuracy and precision quality assurance targets is given in Appendix D.

TA Measurements: General quality assurance procedures for each batch (day) of measurement

1. Start the batch with three measurements of a 'junk' seawater sample at the start of the day to check for precision. The standard deviation of the three measurements should be < 8 µmol/kg. Obvious outlier measurements can be thrown out and additional measurements can be made to reach precision target.
2. Once precision target is reached, run a certified reference material (CRM) sample (bottle opened < 4 days). The resulting measurement should have an absolute difference of <20 µmol/kg to the reported TA of the CRM.
3. Once accuracy target is reached. Proceed to sample measurements. Sample should be measured within 4 days of opening the bottle for pH measurement, i.e., pH measurement should be done prior to TA measurement. Ideally, the TA measurement should be made < 24 hours of the bottle being opened for pH measurement. Sample salinity can be obtained from CTD measurement.
4. Rerun sample if the measurements look questionable (i.e., < 2,100 µmol/kg or >2,300 µmol/kg). Proceed to next sample if the second measurement gives consistent results with the first measurement (i.e., < 20 µmol/kg difference between the two measurements)
5. Run another CRM measurement at the end of the batch to check for possible drift in accuracy. At least two CRM measurements should be made per batch (day).

pH Measurement: General procedures for each batch (day) of measurement

1. Start the batch with three measurements of a freshly opened CRM (opened < 1 hour) at the start of the day. The standard deviation of the three measurements should be < 0.008 and the average of the three measurements should be < ± 0.02 difference than the expected pH for the CRM. Obvious outlier measurements can be thrown out and additional measurements can be made to reach precision and accuracy target. The

expected pH of the CRM can be calculated through the CO2Sys excel calculator posted on James Orr's GitHUB page (https://github.com/jamesorr/CO2SYS-MATLAB).

2. Proceed to sample measurements when both the accuracy and precision target of the CRM measurement has been reached.
3. Measure temperature of sample (report to the 10th degree). Salinity of sample can be obtained from the CTD salinity measurement when samples were collected.
4. Sample pH is calculated through the equation (equation 18 in Liu et al 2011)

$$pH_T = a + b/T + c^* \ln T - d^*T + \log((R - e_1)/(1 - R^* e_3/e_2))$$

where

$a = -246.64209 + 0.315971^*S + 2.8855 \times 10^{-4}{}^*S^2$

$b = 7229.23864 - 7.09137^*S - 0.057034^* S^2$

$c = 44.493382 - 0.052711^*S$

$d = 0.0781344$

$e_1 = -0.007762 + 4.5174 \times 10^{-5}{}^*T$

$e3/e2 = -0.020813 + 2.60262 * 10^{-4}{}^*T + 1.0436 * 10^{-4}{}^*(S - 35)$

$R = (\text{Abs}(578) - \text{Abs}(730))/(\text{Abs}(434) - \text{Abs}(730))$

S = salinity in ppt

T = temperature in K

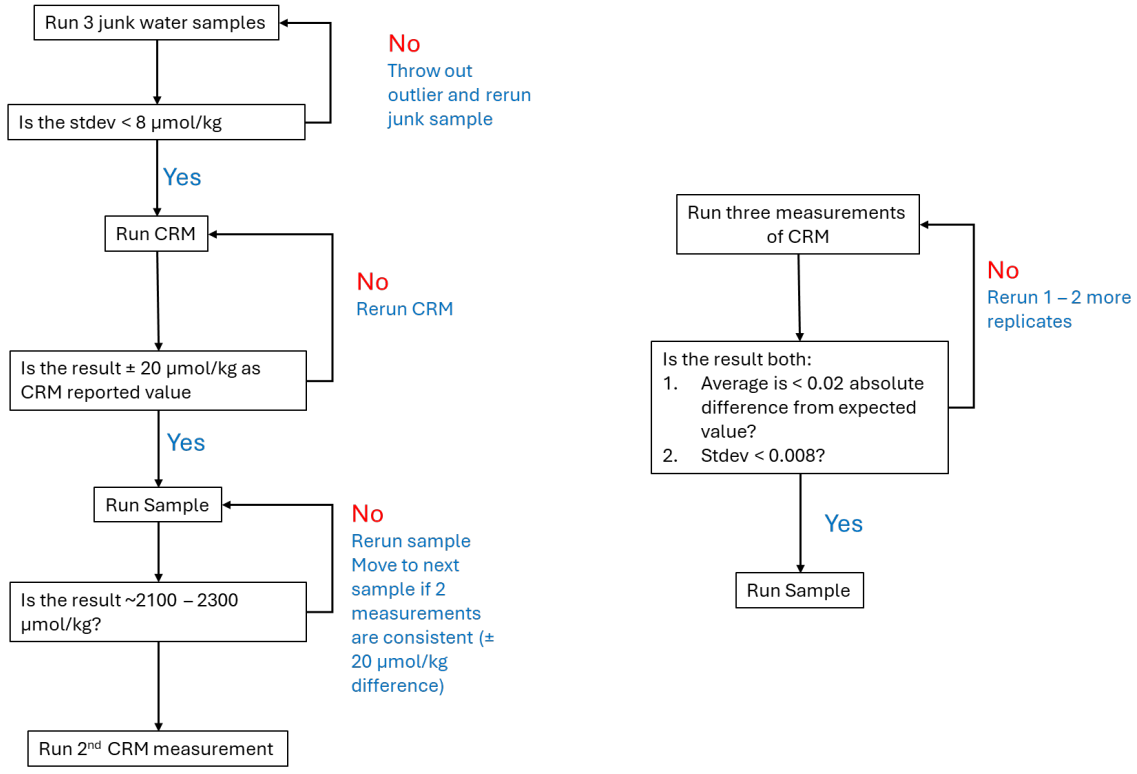


Figure D1. Workflow of total alkalinity measurement (left) and pH measurement (right).

APPENDIX D − OA Chemistry Laboratory Intercalibration Exercise

Purpose: The purpose of the interlaboratory calibration exercise is to establish that pH and total alkalinity samples analyzed by different laboratories are directly comparable and that uncertainties in the measurements are within a range that allows for interpretation of biological data (e.g., uncertainty in the measurement is not so large as to render the data useless for interpreting biological impacts).

Intercalibration Study Design:

Standard: A standard reference material will be provided to laboratories participating in the intercalibration for pH and total alkalinity. This reference material consists of natural seawater sterilized by a combination of filtration, ultra-violet radiation, and addition of mercuric chloride. The characteristics of this seawater (salinity, pH, pCO2, total alkalinity) are similar to those expected during the study. The reference material is supplied by Dr. Andrew Dickson's Marine Physical Laboratory at the Scripps Institution of Oceanography. The batch is #212.

Procedure: Participating laboratories will be required to analyze the seawater standard in triplicate for pH and total alkalinity. Three values will be averaged. Obvious outliers (due to instrument error or sample contamination) may be eliminated and reanalyzed.

Accuracy Target: Absolute Allowable Uncertainty
C-CAN recommends uncertainty for aragonite saturation state to be ± 0.2 (McLaughlin et al. 2015). This corresponds to a relative uncertainty of ~ 10%. However, half of this uncertainty, ~ 5% or 0.1 units, is attributable to uncertainty associated with constants. To attain an Overall Uncertainty of 0.2, then the remaining allowable uncertainty for combined measurement uncertainty is 0.1.

Therefore, our accuracy target for pH and TA is chosen so that the combined relative uncertainty for saturation state does not exceed 5% (Table 1). **The paired uncertainties in bold are the recommended pairing for measurement accuracy relative to a controlled reference material (CRM).** It is worth noting that a relative uncertainty in pH contributes much greater to uncertainty in saturation state than that of alkalinity.

Above calculations for a seawater sample at 20 °C, pH = 7.8973 and Ω_{Arag} = 1.9831

Table B1. Relative (Absolute) Uncertainty for measured parameters pH and TA and the combined relative (absolute) uncertainty in calculated saturation state.

Pairing	pH	TA	Ω_{Arag}
pH + TA	0.3% (0.02)	0.5% (10)	4.5% (0.09)
pH + TA	**0.3% (0.02)**	**1% (20)**	**5% (0.1)**
pH + TA	0.4% (0.03)	0.5% (10)	7% (0.13)
pH + TA	0.5% (0.04)	0.5% (10)	9% (0.17)

Precision Target

We chose a precision target expressed as the standard deviation of three measurements (sample or CRM) that would give us 95% confidence that the mean is within the allowable uncertainty for pH and TA (Table 1). We assume the confidence interval would be calculated with a student's T distribution (appropriate for small sample size). This yields a std. deviation of 0.008 for pH and 8 for TA.

Parameter	**pH**	**TA**
Standard deviation	0.008	8
Confidence Interval (n=3, alpha=0.05)	0.02	20

Evaluation: The average pH of the three CRM measurements must have a standard deviation within ± 0.008 pH units and be within ± 0.02 pH units of the expected value. The expected value can be calculated through the CO2Sys excel calculator on James Orr's Github (https://github.com/jamesorr/CO2SYS-MATLAB). The average total alkalinity must have a standard deviation within ± 8 µmol/kg and be within ± 20 µmol/kg of the reported value.

APPENDIX E − Standard Operating Procedure for Bongo Net Sampling

A. Pre-Tow Water Column Sampling

Before the net tow is conducted, the water column chemical sampling should be conducted. Water column chemistry will be used to interpret biological data. Chemical sampling includes CTD casts for water column profiles, discrete water bottle samples for pH and TA for high quality carbonate chemistry data.

- **Conduct a CTD Cast.** A CTD cast should be done once on station. The CTD should record water column profiles of temperature, salinity, dissolved oxygen, chlorophyll fluorescence, CDOM, and pH to 150 m depth (100 m if line is an issue).

- **Collect Water Samples for pH and TA (consistent with Appendix B).** A bottle sample for pH and total alkalinity (TA) should be collected from the surface and from 150 m (or as deep as you can go with your line). Bottle samples should be collected into 250- or 500-mL glass bottles (not plastic). Pyrex bottles are recommended (Fisher Scientific 02-940D), particularly if the time until sample processing will be greater than 1 week. One 500 mL bottle can be used to analyze both pH and TA or alternatively two 250 mL bottles can be collected (1 for pH and 1 for TA). Bottles should be overfilled at least 50%, making sure no air bubbles are trapped in the bottle. Once the bottle is filled to the brim, create a ~1% space (based on the total volume of the container) and preserve the sample with mercuric chloride (0.02% of the sample volume, or 120 microliters for 500 mL). Seal the glass stopper with grease, place a collar and rubber band to secure the bottle stopper, and store bottle samples at room temperature (not frozen) until analysis. The Dickson Lab protocol is provided in Appendix B as an example.

Net Preparation

The Bongo net can be deployed off the stern of the boat using an A-frame, as pictured below aboard the *M/V Nerissa*, or the net can be deployed off one side of the boat using a crane. A line with weight (~100 lb) should be securely attached to the lower end of the tow yoke on the Bongo net, and the tow line from the boat should be securely attached to the upper end of the tow yoke. As shown in the picture below, the line with the weight should trail behind the frame; the tow line from the boat should be in front of the frame. Cod ends should be checked to ensure they are properly secured to the net. Put a pressure sensor on the yoke to record depths achieved. A flow meter should also be mounted at the mouth of one of the nets to estimate the volume of water filtered by the net (Figure

below, right panel). The flow meter should be mounted in a flexible position so that it can orient itself according to the flow.

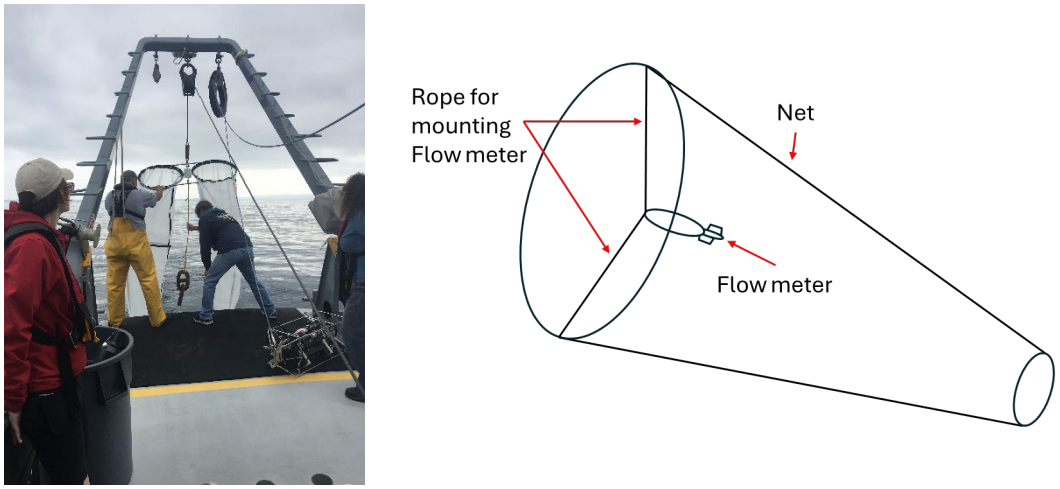


Figure E1. Photo and schematic showing the deployment of the bongo net, with the rope for mounting the flow meter. The 200-micron net dimensions are 60 cm X 200 cm (conical or cylindrical) x 11 cm diameter cod end aperture. The cod end assembly consists of 11 cm diameter w/ detachable lower section with lateral apertures and lined with 200-micron mesh, threaded coupler with band clamp zinc ballast weight.

Towing

The 200-micron net will be towed at 4 depths for approximately 45 minutes total: (1) ~150 m for ~15 minutes, (2) ~100 m for ~10 minutes, (3) ~50 m for ~10 minutes, (4) ~25 m for 10 minutes, depth permitting. During the tow, the boat should be going slowly, 1.5 to 2 knots is recommended. Adjust amount of line let out to accommodate for line angle to achieve target depth (see Wire Angle table below). In test runs on the *M/V Nerissa*, wire angle was ~60° and a 2:1 scope was required with 106 lb weight to achieve depth.

For any particular boat, net, and current conditions, the goal is to adjust the total weight of the net (using added weights) and keep wire angle between 45° and 60° at 1.5-2 kts ship speed—too little drag or too much weight on the net will cause the net to sample too deep; too much drag or too little weight will keep the net too shallow. This is something you may need to play with first to optimize. Try not to decrease boat speed to <1.5 kts or strongly swimming organisms will be under sampled.

Record the time, latitude, longitude at the location for net-overboard and net-onboard. Record the counter position (e.g., 6-digit number, [XXXXXX])of the flow meter for net-overboard and net-onboard.

1. Net Overboard:
    a) Begin to let out the tow wire while two people help guide the net into the water. On the *M/V Nerissa*, extra attention was needed to ensure that the net was not pulled under the boat or into the propeller. To prevent the net from getting under the boat:
        i. Have two people hold the cod ends until first the weights and then the frame of the net are lowered into the water.
        ii. Next, toss the cod ends as far out as possible, away from the propeller.
        iii. During this time, the boat should be slowly moving forward to decrease the likelihood the net will get pulled under the boat.
    b) Once the net is in the water and straightened out (not tangled), let out the wire to the desired length.
    c) Record time, latitude, longitude, and bottom depth of net overboard, and flow counter start time.

2. Begin the towing at each targeted depth

Record tow start time, depth, wire angle and length of wire out, and boat speed, and tow end time and depth

Tow at 150 m:
Estimate the wire angle and use the table to determine how much wire length is needed to reach 150 m while towing at 1.5 – 2 knots. Wire should be released at 30m/min until the target depth is reached. Tow for ~15 minutes.

Tow at 100 m
After 15 minutes of towing at 150 m, bring the net up to 100 m at 30 m/min using the wire angle and the table below. Tow for ~10 minutes.

Tow at 50 m
After 10 minutes of towing at 100 m, bring the net up to 50 m at 30 m/min using the wire angle and table below. Tow for ~10 minutes.

Tow at 25 m
After 10 minutes of towing at 50 m, bring the net up to 25 m at 30 m/min using the wire angle and table below. Tow for ~10 minutes.

Tow End

After 10 minutes of towing at 25 m bring the net up to the surface at 30 m/min and prepare to bring the net onboard. The net should be removed from the water quickly once it reaches the surface.

3. Net onboard:
   a) While the net is hanging from A-frame or crane, secure the weight and spray down the net with a filtered seawater hose, washing down all material stuck in the mesh into the cod ends.
   b) To recover the net, similar to deployment, two people guide the weight and the net onto the deck.
   c) Record time, latitude, longitude, and bottom depth of net onboard. Record the counter position of the flow meter.

Keep in mind that the taxa we are targeting migrate throughout the upper 100 to 200 meters water depth so tow depths and length of time are approximate and are designed to maximize chances of capturing targeted taxa. The goal of the tow is to be within ± 30 m of 150 m target depth for the 15-minute-deep tow and all subsequent depths are evenly distributed throughout the water column. The idea is to capture a general idea of which taxa are present and migrating throughout the water column, not to sample specific depths. The only time a net tow would constitute a failure and should be repeated would be if the net hits the bottom. In this case, rinse the net out well and redeploy.

Sample Processing

Once the net is onboard, remove the cod ends. The cod ends thread onto the plastic secured by the hose clamp (no screwdriver required). Each cod end will ultimately be collected into a 1 gallon wide-mouth HDPE plastic sample jar (supplied by SCCWRP). Both sides will be preserved with Ethanol (supplied by the agency) and buffered with provided ammonium hydroxide solution. Samples are collected by decanting cod ends carefully into coolers. This step is important because it minimizes both physical (keeps shells from breaking) and physiological stress (minimizes adding sampling stress on measured physiological stress related to acidification, hypoxia, or temperature) on the organisms.

To collect the cod end in 1 gallon sample jars:

1. Prepare two coolers by filling them with ~6" of seawater (ideally filtered, but surface water from the station is sufficient). Smaller ~28-quart coolers are easier to manage.
2. Take one cod end and while it is still attached to the net, hold it over the cooler and unscrew, gently allowing the contents of the cod end and any extra material in this end

portion of the net to be collected in the seawater within the cooler. Perform an initial rinse of the cod end by dipping it in seawater in the cooler.

3. Using a pitcher filled with seawater, rinse out the cod end three times (fill with ~200 mL of filtered seawater, gently swirl, pour in cooler). Use a squirt bottle filled with filtered seawater to rinse the last remaining sample out of both the end of the net and the cod end.
    a. Note: Pteropods can look like tiny grains of sand stuck to the PVC/mesh, if visible at all.
4. Repeat steps 2-3 with the other cooler and cod end.
5. Allow each cooler to settle. The time it will take a sample to settle varies depending on how much material is in the sample but is generally in the order of about 1 minute, not to exceed 5 minutes. Not all material may settle, and you should begin processing even if there is some floating material. Coolers may be gently swirled to force heavier material to the bottom.
6. Decant off the top of the water, minimizing disturbance to the settled material (the pteropods will have settled into the bottom of the cooler). This can be accomplished using a 2L pitcher (provided) to remove the water.
7. Once the cooler has been decanted to the lowest amount possible, pour the remaining sample from the cooler into the 2L pitcher. Allow the sample to settle. Pour off the top potion of water from the pitcher, being careful not to disturb the plankton sample.
8. When enough of the sample has been decanted, pour it into the 1-gallon bottle. Rinse out the pitcher using the squirt bottle of filtered seawater, paying particular attention to any "specks" remaining on the plastic. Let the bottle rest until the material settles. Continue to decant until the sample is down to ~500 mL (1/5 of the sample jar). This is because buffered ethanol must be added in a 1:4 ratio of sample to ethanol.
9. Steps 5-8 should be repeated for both coolers/samples, resulting in (at least) two separate samples, each in their own sample jar.
10. Fill the remaining sample volume with preservation solution, 190 proof ethanol in a 1-part sample, 4-parts ethanol ratio
11. Ensure each sample jar is labeled, and any previously written label was not been removed by ethanol.
12. Samples can be stored at room temperature, not in direct sunlight. A cooler with a few ice packs is recommended.
13. Rinse out the beaker and cod ends with filtered seawater hose to prepare for the next tow.

Sample Storage

Samples should be buffered with ammonium hydroxide solution to raise the pH above 8.5 as soon as possible following collection (not to exceed 24 hours) to prevent shell dissolution from the preservation solutions. Use disposable 1 ml pipette tips to dispense a few drops of ammonium hydroxide, then use the pH test strips to test. If you exceed the pH of 9, then lower

with HCl solution. Buffering can occur in the laboratory after samples are returned. Once buffered, samples can be maintained in the refrigerator until analysis.

Table E1. Wire Angle Table. Match up wire angle with target depth to find out how many meters of line to put out.

Wire angle →	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
Target depth (m) ↓																
5	5	5	5	5	6	6	6	7	7	8	9	10	12	15	19	29
10	10	10	10	11	11	12	12	13	14	16	17	20	24	29	39	58
12	12	12	12	13	13	14	15	16	17	19	21	24	28	35	46	69
15	15	15	16	16	17	17	18	20	21	23	26	30	35	44	58	86
17	17	17	18	18	19	20	21	22	24	26	30	34	40	50	66	98
20	20	20	21	21	22	23	24	26	28	31	35	40	47	58	77	115
25	25	25	26	27	28	29	31	33	35	39	44	50	59	73	97	144
30	30	30	31	32	33	35	37	39	42	47	52	60	71	88	116	173
35	35	36	36	37	39	40	43	46	49	54	61	70	83	102	135	202
40	40	41	41	43	44	46	49	52	57	62	70	80	95	117	155	230
45	45	46	47	48	50	52	55	59	64	70	78	90	106	132	174	259
50	50	51	52	53	55	58	61	65	71	78	87	100	118	146	193	288
55	55	56	57	59	61	64	67	72	78	86	96	110	130	161	213	317
60	60	61	62	64	66	69	73	78	85	93	105	120	142	175	232	346
65	65	66	67	69	72	75	79	85	92	101	113	130	154	190	251	374
70	70	71	72	74	77	81	85	91	99	109	122	140	166	205	270	403
75	75	76	78	80	83	87	92	98	106	117	131	150	177	219	290	432
80	80	81	83	85	88	92	98	104	113	124	139	160	189	234	309	461
85	85	86	88	90	94	98	104	111	120	132	148	170	201	249	328	489
90	90	91	93	96	99	104	110	117	127	140	157	180	213	263	348	518
95	95	96	98	101	105	110	116	124	134	148	166	190	225	278	367	547
100	100	102	104	106	110	115	122	131	141	156	174	200	237	292	386	576
105	105	107	109	112	116	121	128	137	148	163	183	210	248	307	406	605
110	110	112	114	117	121	127	134	144	156	171	192	220	260	322	425	633
115	115	117	119	122	127	133	140	150	163	179	200	230	272	336	444	662
120	120	122	124	128	132	139	146	157	170	187	209	240	284	351	464	691
125	125	127	129	133	138	144	153	163	177	194	218	250	296	365	483	720
130	130	132	135	138	143	150	159	170	184	202	227	260	308	380	502	749
135	136	137	140	144	149	156	165	176	191	210	235	270	319	395	522	777
140	141	142	145	149	154	162	171	183	198	218	244	280	331	409	541	806
145	146	147	150	154	160	167	177	189	205	226	253	290	343	424	560	835
150	151	152	155	160	166	173	183	196	212	233	262	300	355	439	580	864
155	156	157	160	165	171	179	189	202	219	241	270	310	367	453	599	893
160	161	162	166	170	177	185	195	209	226	249	279	320	379	468	618	921

APPENDIX F − Standardized Operating Procedure for Laboratory Processing of Zooplankton Bongo Tow Samples

June 2024 Version

Introduction and Purpose

The Southern California Bight Regional Monitoring Program has been conducting monitoring to assess the biological effects of ocean acidification and hypoxia since 2018. This standardized operating procedure (SOP) establishes the methodology by which zooplankton bongo tow samples are processed to prepare them for e-DNA analysis and assessment of dissolution in the shells of calcifying zooplankton. Measurement of zooplankton biovolume and confirmation of gastropod taxonomy with genomic vouchers will be instituted in Bight '23.

The samples consist of paired bongo tows, which will produce two samples per station (Cod end #1 and #2). The SOP consists of two major components which processes these two samples: #1) e-DNA sample processing and zooplankton wet biomass analysis, #2) gastropod abundance and picking for scanning electron microscopy samples (Figure 1), with an archive of the sample preserved for assessment of crab larvae carapace dissolution if resources and collaborator are identified.

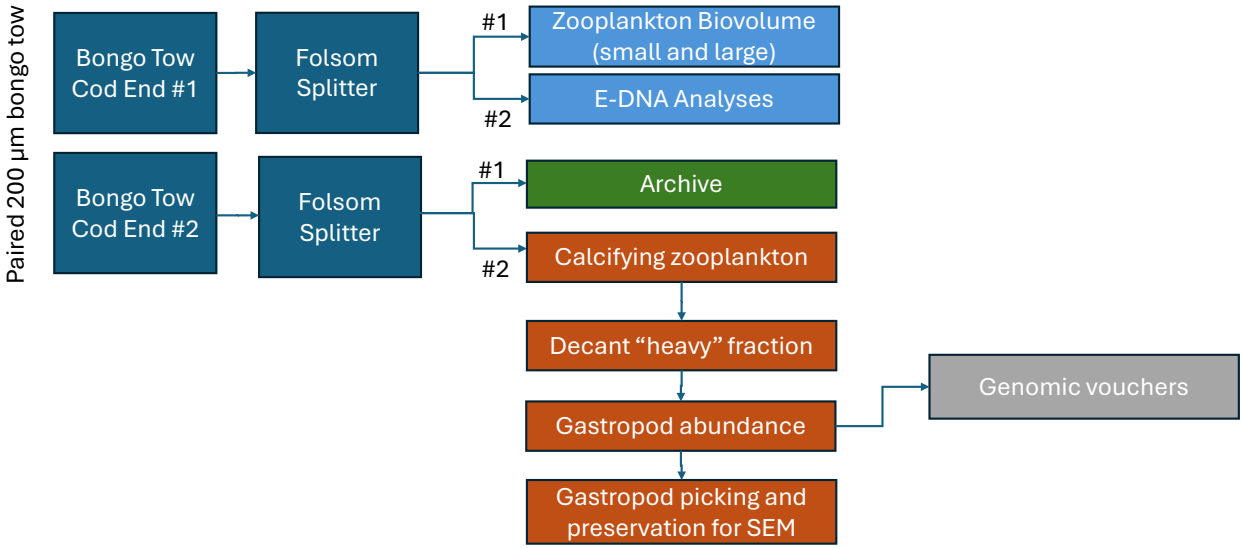


Figure F1. Schematic of sample processing of zooplankton samples

Part I: Sample Splitting and e-DNA and Zooplankton Biovolume Processing

A. <u>Sample processing list</u>

- Data sheet
- Folsom splitter

- Two graduated cylinders
- Nested 200 and 500-micron drainage socks
- 90% ethanol, buffered to > 8.4

B. Sample preparation and splitting

Decant the contents of Bongo Tow bottle #1 into the Folsom Splitter. Agitate the sample, then apportion into the chambers. Chamber 1 should be decanted for wet biomass estimation, described below. Chamber 2 should be decanted to a bottle preserved for e-DNA analyses. E-DNA samples are topped off with 90% ethanol, buffered to > 8.4, then stored in archive for further sample processing. Sample processing consists of homogenizing the sample with a blender, then DNA extraction and sequencing.

Decant the contents of Bongo Tow bottle #2 into the Folsom Splitter, IF splitting is required. Agitate the sample, then apportion into the chambers. One of the two Chambers should be decanted for gastropod abundance and SEM analysis, as described in Part II below.

C. Zooplankton wet biomass

Zooplankton wet biovolume can be determined by ash-free dry mass or wet biovolume. We chose to utilize wet biovolume to be consistent with CalCOFI. Ohman and Wilkinson (1989) establish the relationship for zooplankton wet biovolume to AFDM for fractions > 200 to < 505, and > 505 microns in the southern California Current System.

Zooplankton wet biovolume is determined using the method of Ahlstrom and Thrailkill (1963). The split sample is placed into a graduated cylinder and the volume noted. The sample is stirred, then decanted into a mesh "sock" constructed of a 200-micron sleeves. The filtrate volume is captured in a separate graduated cylinder and volumed noted.

Part II: Sample Processing for Pteropod Abundance and Shell Dissolution

A. Equipment List

- medium plastic petri dishes
- 5 small plastic petri dishes
- 2-L plastic separatory funnel with large bore (>10 mm) stopcock, e.g. this one
- 10 ml serological pipette (new for each station)
- Folsom splitter
- Data sheet
- Training and reference materials for gastropod identification
- P200 pipette tips and pipette
- Shorty glass vials for SEM sample preservation
- Training resources for gastropod identification

Note: You should be able to work through these steps using the buffered ethanol already present in the sample. This ethanol has been raised to a pH above 8.5 to prevent any potential shell dissolution from occurring. If you do end up having a reason that requires the use of pure 190 proof ethanol from the squirt bottle, and you add more than about 10 ml, check the pH of the solution using the pH test strips to ensure the solution maintains a pH above 8.5. If it is lower than this, ammonium hydroxide will need to be added.

B. Gastropod Abundance and SEM Sample Preparation

1. Review training resources to identify the five targeted groups of gastropods: 1) *Limacina helicina*, 2) other *Limacina spp.*, 3) *Heliconoides spp*., and 4) other whorled shelled gastropods.
    a. Quick reference guide in the front of the Bight binder
    b. The PowerPoint of gastropod picking groups in the Bight drive
    c. Bound copy of the Northeastern pacific gastropod ID guide.
2. Select the sample that you will be picking and document appropriate information in the data sheet.
3. Settle the sample. The goal is to have the shelled gastropods (pteropods and other gastropods with whorled shelled) settle to bottom of separatory funnel to decrease picking time. Agitate the sample, then pour into a large bore separatory funnel. Set a timer for 2 mins and allow the sample to settle for that time. Decant the settled layer into a 200 ml graduated centrifuge tube and estimate and record the total sample volume.
4. Pour off the buffered ethanol from the sample into a beaker – this will be our working stock of buffered ethanol with pH > 8.5. Leave about 2 cm of buffered ethanol above content in sample bottle. Use a dropper to transfer some buffered ethanol into the small petri dish, cover, and set aside.
5. Take the centrifuge of shelled zooplankton, gently swirl each petri dish, then decant about 30 mls at a time into medium size petri dishes until all the material is decanted. Allow time for the sample to settle in the petri dish, then **pour off the top of the material back into the centrifuge tube**. This is meant to target the gastropods – they will stick to the bottom of the dish, so this step serves to eliminate some of the copepods and other animals that are not of interest. You will need to have a good amount of ethanol in the petri dish to truly decant the sample. If there are a significant number of pteropods and other small, shelled organisms in the sample, you should be able to see a "line" of tiny beige dots at the back of the waterline when you are pouring. This is a good sign that pteropods have been targeted properly. Resuspend the remaining material in the excess buffered ethanol that was set aside. Pour buffered ethanol from beaker into each petri dish to resuspend material to make it easier to examine under the light microscope.
6. From each petri dish with sample, under the microscope, pick the gastropods in three "rounds," as detailed below.
7. First round: **Swirl petri dish to collect all shelled-organisms into the center of the petri dish**, then using the grid on the petri dish, zig zag through the swirled area of the

petri dish to identify any gastropods, covering the entire grid. Use pick to push all "junk" away from the center to expose shells. Start at one side of the grid and work your way through, covering the entire dish.

8. When you see a gastropod, use the dissecting needle to push any copepods or detritus away from the shell, then, use **a pipette or small fine paint brush to remove it**. Place it in the designated smaller petri dishes filled with buffered ethanol, corresponding to its taxonomic grouping.
    a. Cut the tip off the P200 to collect gastropods. Make sure the pipette volume is set to something the pipette tip can still handle so that you DO NOT flood the pipette (set to 50). The gastropods are tiny and easy to lose in pipettes.
    b. We are going to use these gastropods to look at the shell dissolution on the SEM. It is important to touch them as little as possible (while still giving a confident ID), and to be gentle with them.
    c. When transferring the gastropods to the small petri dish and later to the vials, it is best to submerge the tip of the pipette in liquid so the pteropods are gently placed in the ethanol (aka try to avoid them falling into the ethanol).
9. Second round: "Zig-zag" through petri dish to look for gastropods missed in initial pick. The gridded petri dishes make it easier to keep your place. Place the organisms that you find in the same small petri dish.
10. Third round: Repeat the second round, making sure to follow the grid lines. If ≤3 gastropods are found on the third round, stop picking. If >3 gastropods are found on your third round, do a fourth round, etc.
11. **When all medium petri dishes have been picked, transfer each of the contents of the small petri dishes to a small glass sample vial, labeled with sample number and taxonomic grouping, preserved with buffered ethanol. Record the number found while picking by taxonomic grouping** on the data sheet. Collect microscope photos of each representative taxonomic grouping and a voucher vial for genomic verification.
12. Rinse contents from three petri dishes into new waste material beaker, parafilm, and set aside.
13. **Quality assurance.** A senior staff member in charge of quality assurance will then review each of the vials of picked organisms, confirm the identification, or reallocate organisms to the appropriate vials if needed, then confirm the abundance of the targeted taxonomic groups. Once all stations are completed, a genomic voucher sample will be collected for *Limacina helacina*, *Limacina spp., and Heliconoides spp.* from each agency/quarter for a check on the accuracy of identification by microscopy.

APPENDIX G – eDNA Water Sampling Protocol

eDNA Sampling Protocol: Smith-Root citizen scientist sampler

Demonstration Video: https://youtu.be/BupozR1fnlg
Smith-Root citizen scientist manual: https://www.smith-root.com/support/downloads/edna-citizen-scientist-sampler-manual

Supplies:

- Smith-Root citizen scientist sampler*
- Smith-Root 0.45 um self-preserving filters
- Nitrile gloves
- MilliQ water for filter blank (~100 ml)

If not sampling directly from a stream or other water source:

- Sterilized 1 L bottle or large Whirl-Pak bags for water sample collection**
- DNAway or 10% bleach for cleaning bottles

* Before heading to the field, ensure the sampler is fully charged. There is a charging cord included for the lead acid battery.

** Bottles should be sterilized before use and between sampling sites to prevent cross-contamination. To sterilize, soak in acid wash (1% solution of hydrochloric or nitric acid), rinse three times in DI H2O, and autoclave. Alternatively, soak in bleach solution (final concentration 1-5%) and rinse three times with DI H2O.

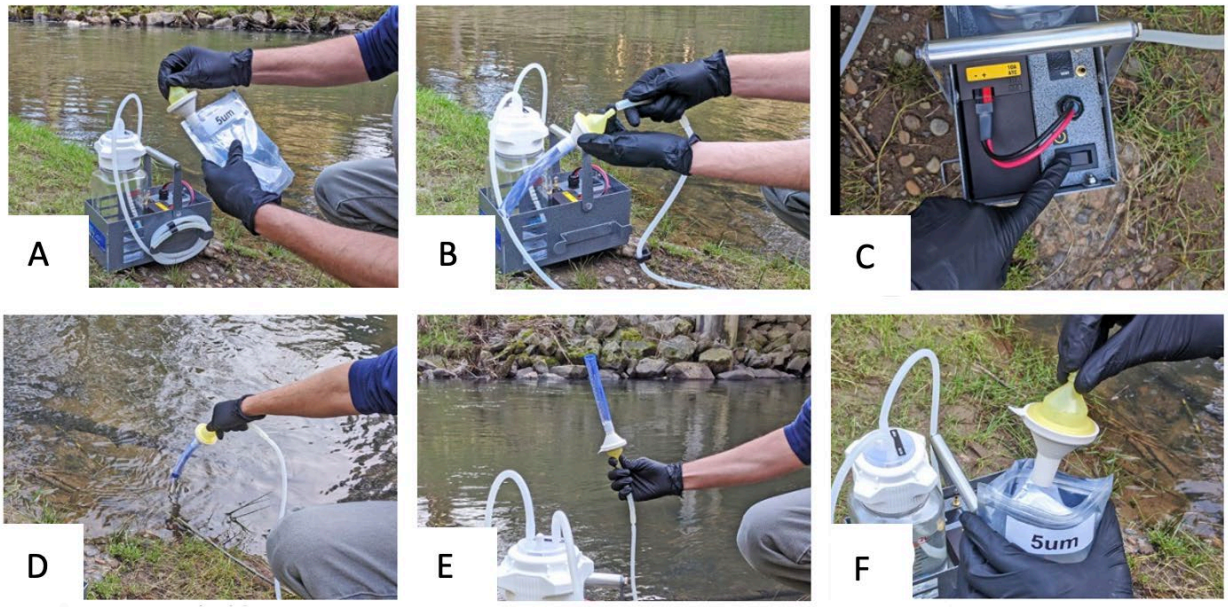


Figure G1. Overview of Smith-Root sampler filtration steps. Images from Smith-Root.

Water collection:

1. Skip this step if sampling directly from a stream or other water source (as in Figure 1D). Wearing gloves, rinse sample bottle three times with ~10 ml of sample water; discard rinse water offshore or away from sampling site. Fill bottle with 1 L of sample water. If bottles are limited, large Whirl-Pak bags can be used instead.

Filtering:

2. Wearing gloves, open an individually wrapped Smith-Root filter pouch.
3. Without touching the snorkel, attach it to the smooth end of the filter housing (**Figure 1A**).
4. Attach the sampler hose to the ridged end of the filter (**Figure 1B**).
5. Turn on the vacuum pump (**Figure 1C**).
6. Insert the snorkel into the sample water (**Figure 1D**).
7. When 2 L has been filtered (or when filter clogs), invert the filter housing so the snorkel is facing the sky. Continue to run the vacuum pump for 20 seconds to air dry the filter (**Figure 1E**). Record final volume in field data sheet.

After filtering:

8. Remove the snorkel from the filter housing and discard the snorkel.
9. Place the filter housing back in the pouch (**Figure 1F**).
10. Label pouch with Station, Date, Depth, Replicate, and Volume filtered.
11. Discard the filtrate in the collection vessel.
12. Repeat all steps for as many replicates as requested (default = 3 replicates).

Blanks:

13. Filter ~100 ml of MilliQ water through a clean filter as above. Process one blank for each sampling day.

Filter storage:

14. Smith-Root self-preserving filters are stable at room temperature for six months.

Cleaning the sampler:

15. Thoroughly wipe down the Smith-Root sampler after each field day with a wet paper towel. Note that seawater can corrode the metal parts of the sampler.

APPENDIX H − Standardized Operating Procedures for Scanning Electron Micrograph of Gastropod

The procedure for SEM of gastropod shells consists of three basic steps: 1) removal of the organic periostracum, 2) sample preparation through sputter gold coating, and 3) SEM imaging.

Removal of Organic "Periostracum"

The periostracum of gastropods is essentially composed of fibrous proteins, carbohydrates and lipids, plus a biofilm that varies with the ambient environment. This organic coating must be removed in order to clearly image the dissolution of the shell. Samples are placed in 2.5 % bleach, diluted with milliQ water, buffered to > 8.2 for 40 to 60 minutes. Samples are placed in a desiccant chamber located inside the chemical hood to thoroughly dry before proceeding to the next step.

Mounting and Sputter Coating

Follow the instructions on the wall about dehydrating/gold coating, air blowing, and turning upside down, then, lowering the sample.

II. **Mounting the gastropods on the SEM stub**

1. Prepare the stub by adding the double-sided tape to a stub and putting it in an empty stub box, making sure to label the bottom of the stub with the station ID, quarter, and two duration/net size (only if relevant).
    a. Always keep an empty stub box in the Bio lab for prepping.
    b. The stub is small to write on so just try your best. This should be a last resort for identification, as the stub box itself will be labeled in the end
2. **Using a damp paintbrush, pick up the pteropods and place on the stub.** Pteropods must be placed whorl side up.
    a. You will need to use the external fiber optic lights to place the pteropods on the stub.
    b. This process is hard and takes a lot of practice, especially if the pteropods are very small. Start off with samples with lots of extra pteropods to practice plating.
    c. Be careful when the pteropods become drier after time, as when dry out they tend to "bounce" out of the watch glass and become impossible to find and recover. It can be helpful to occasionally add a drop of water to keep the pteropods you are not currently working with damp. When you are ready to work with a pteropod, the trick is to have it dry enough that you can dip the paintbrush in water, wipe it, and have the pteropod then stick. However, if it is too dried out already, it may fly away.
    d. Try your best to place pteropods in straight lines, so they are easier to find under the SEM.
    e. Use the Bio lab prep log document to record a "stub map." Essentially, label a box with the stub's information and draw a circle with the general locations of the pteropods numbered on it.

3. **Gold coat** using the sputter coater in the Bio lab. Follow the following Ted Pella protocol.
4. Check that the sample has been mounted on a stub suitable for use on the sample stage tables. Any solvent-based adhesive should have been allowed to dry out thoroughly. The sample should be a suitable shape to allow conducting path to form during coating
5. Check that the valve on the argon gas supply is open and that the regulator gauge is reading about 0.3bar (see 4.2)
    a. *NOTE: if the unit is used frequently using the same conditions, steps 6, 7, and 8 can be omitted.*
6. Check the timer value on the digital display. If necessary, press PAUSE/TEST and reset the process time.
7. Check the preset sputter current value by pressing *SET mA* and adjust if necessary. For initial tests 40mA can be used.
8. Select MANUAL. Press FLUSH, wait for 3-5 seconds and press LEAK. Wait for the pressure to stabilize. Check the pressure and adjust if necessary. For initial tests 0.08mb can be used.
9. Select AUTO and press CYCLE.
10. When the timer resets the coating is complete. Switch off the unit. Lift the top-plate to remove the sample
11. If the coating thickness is unsuitable, change the process time for future samples. In the first instance do not adjust power or gas pressure.
    a. If the stubs visually appear more green/gray than gold and/or are not imaging well (known as "charging"), it is likely it might be time to replace the gold target.
    b. Mark where the pteropods are and an orientation mark, along the station ID on the top of the stub.
12. **Ready to image!** Place the finished stub in the next slot in the most recent stub box. Label the top of the box using a mini-Sharpie with the Sample ID, date, and initials of the preparer.

SEM Imaging of Gastropods

1. Ensure the sample has been gold coated. Blow a little compressed air on the sample to remove dust. Turn the sample upside down to ensure no organisms fall off. Finally, twist the sample holder up so it is slightly taller than the sample level, then load the sample**.**
2. Take an overview photo of each shell or carapace, around 600-900x. When taking each overview picture, ensure where the whorl-line starts at the center of the pteropod is clear and in focus. Make sure to change the label before each photo of a new gastropod.
    a. Add a whorl line to the photo (using the measuring tool), making sure to draw and label this whorl line on the worksheet for each pteropod (picture 1)
    b. If you need help finding the gastropod on the stub, use the "stub map" from the bio lab log to help find the gastropod.
3. Take the thumb-drive (with the overview photos) and add them to the blank worksheet. Print out this worksheet with all overview photos and whorl lines and go back to the SEM.

4. Before scoring dissolution on the gastropod, using the whorl-count line method, denote how many whorls are on the pteropod, marking this down on the worksheet (picture 2).
5. Starting at the protoconch for the first whorl (center of the pteropod), take **at least** 2 photos per whorl, denoting what type of dissolution (Type >1, Type 1, Type 2, Type 3) is present on the **entire** whorl.
    a. **Note: Aim to take most photos at magnification of 6,000 and 12,000x. This will allow easier comparison of photos between gastropods and between sites.**
    b. If a whorl has many kinds of dissolution, do not be hesitant to take 3-4 pictures. If another sheet is needed to write down the picture numbers and dissolution, please mark that on the worksheet
6. Once the whorl has been fully assessed and the pictures effectively represent the entire whorl, write down total dissolution and dissolution type present on that whorl (out of 100%)
7. Repeat steps 5 – 7 until total number of whorls has been covered
8. Take a last picture of the growing edge, noting if it is intact or not, and if spikes are present (if this edge has dissolution, it counts as part of the last whorl).
9. Take a photo of the entire shell and using the SEM measuring tool, record the length of the entire shell, from the growing edge to the other side (picture 1).

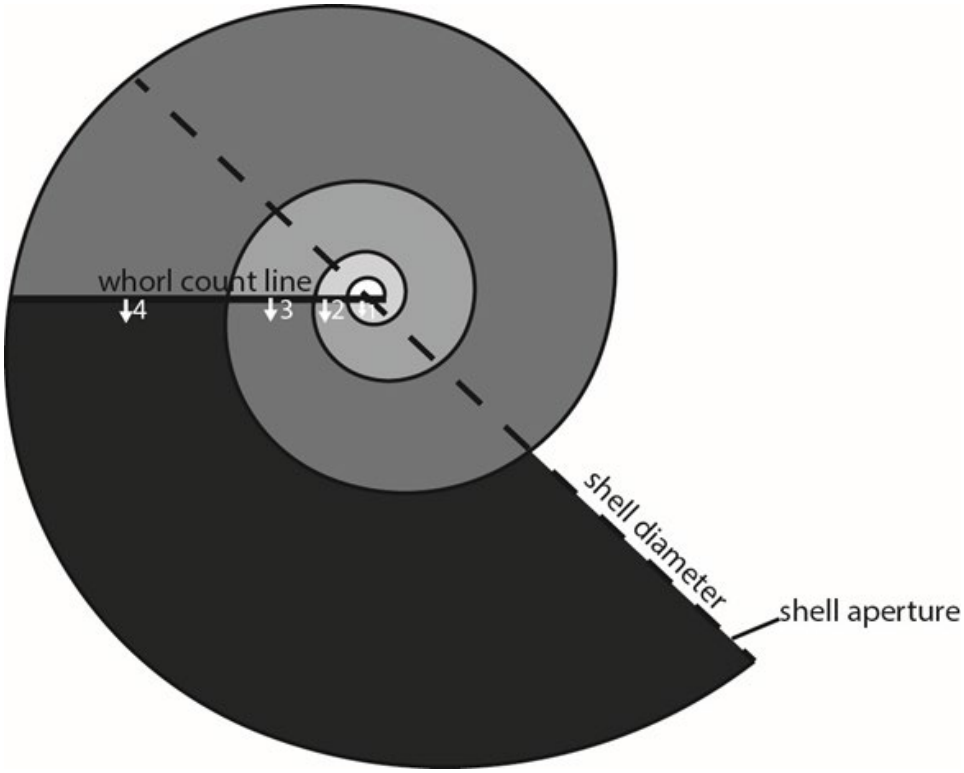


Figure H1. The whorl count line can be recreated using the SEM measuring tool

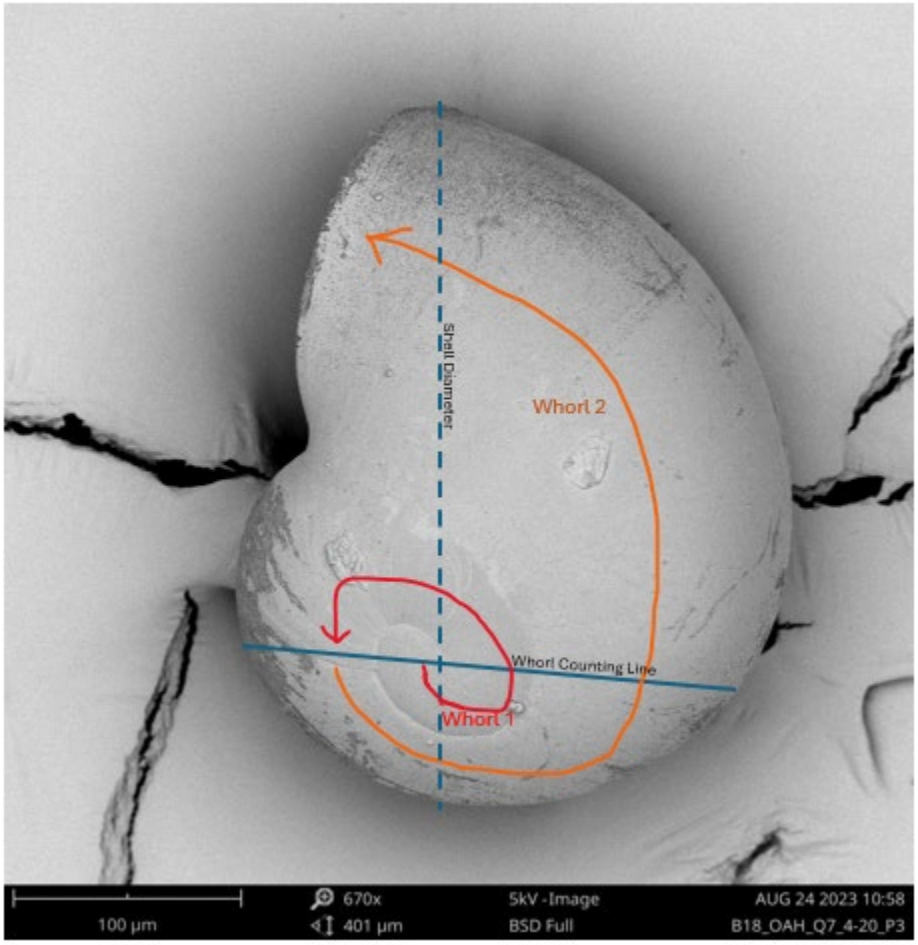


Figure H2. Many pteropods are imaged at an angle, please account for this when counting whorls