Southern California Bight
2018 Regional Marine Monitoring Program
(Bight '18)

Ocean Acidification Workplan

Prepared by:
Bight'18 Ocean Acidification Committee

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

May 23, 2019

CONTENTS

I. INTRODUCTION

A. Setting

The Southern California Bight (SCB; Figure I-1) is an open embayment in 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 SCB 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 SCB 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 US Naval facilities in the country. In addition to being the home to more than 20 million people, (NRC 1990), Southern California receives over 100 million visitors to its beaches and coastal areas annually. The combination of resident and transient populations has resulted in highly developed urban environment that has greatly altered the natural landscape. The conversion of open land into impervious surfaces has included dredging and filling over 75% of bays and estuaries (Horn and Allen 1985) and extensive alterations of coastal streams and rivers (Brownlie and Taylor 1981, 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 SCB.

At the same time, the SCB 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 most at risk to global changes in ocean acidification (OA), 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 the depth of 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 SCB (Feely et al., 2008, 2012, 2016; McLaughlin et al. 2017), and are strongly associated with coastal upwelling, which transports subsurface waters with high levels of carbon dioxide (CO_2) and low pH to surface waters nearshore (Fassbender et al. 2011, Harris et al. 2013). Ship-based surveys of the west coast routinely encounter OA hot spots, where large regions of the continental shelf are undersaturated with respect to aragonite ($\Omega_{arag} <1$) in shallow nearshore waters (Feely et al. 2008, 2016) (Figure I-2).

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

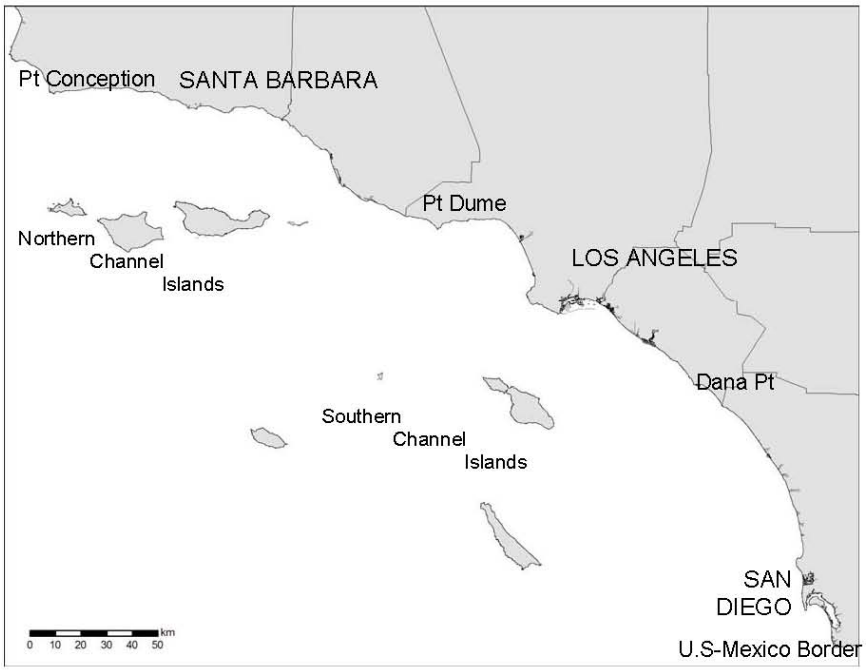
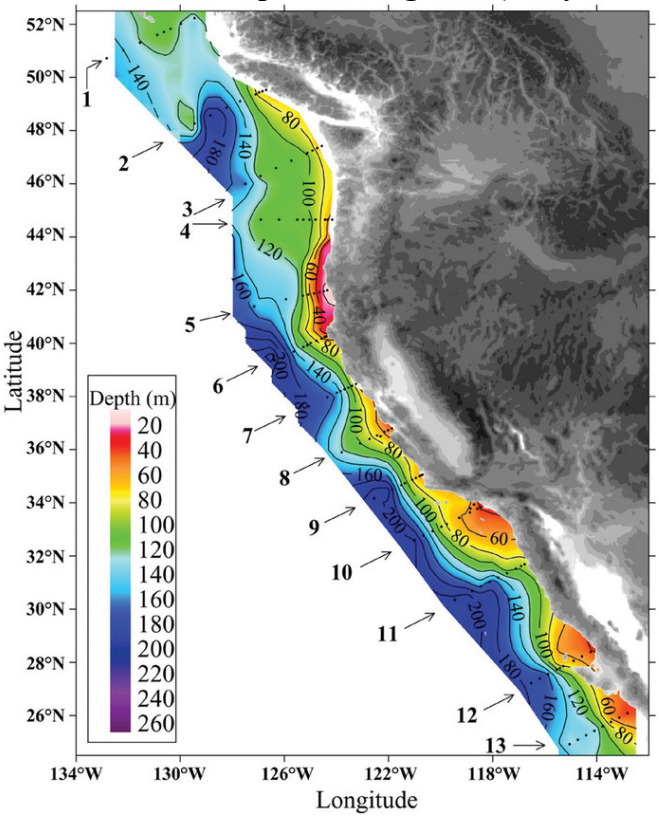


Figure I-2. Map of the U.S. West Coast showing the depth where waters are under-saturated with respect to aragonite (Feely et al. 2008)



B. History of Bight Regional Water Quality Monitoring

To understand the cumulative impacts of anthropogenic discharges to the SCB, a cooperative, multi-agency regional monitoring program has been established that looks at the health of the SCB 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 the 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 the regionally-based information 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.

To date, there have been five previous regional monitoring efforts addressing environmental concerns at larger spatial scales in the SCB. 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 both direct and indirect impacts of wastewater plumes on coastal water quality. This program builds on the existing collaborations between the large discharging agencies to bring additional partners and expand the variety of parameters measured and questions addressed.

The first Offshore Water Quality Assessment was associated with the 1994 Southern California Bight Pilot Project (SCBPP), which included 12 agencies that sampled over 260 sites along the continental shelf between Point Conception and the United States/Mexico border. Findings showed natural latitudinal differences (e.g., colder water in the northern strata) and that over 99% of the coastal waters met California Ocean Plan objectives for dissolved oxygen and light transmittance.

In 1998, 64 agencies undertook the Southern California Bight 1998 Regional Monitoring Program (Bight'98) and sampled sites between Point Conception and Punta Banda, Mexico that included new habitats such as ports, bays, and marinas. The Bight'98 water quality surveys looked at both dry and wet weather water quality and the relative inputs of offshore ocean outfalls versus urban stormwater runoff at over 500 stations.

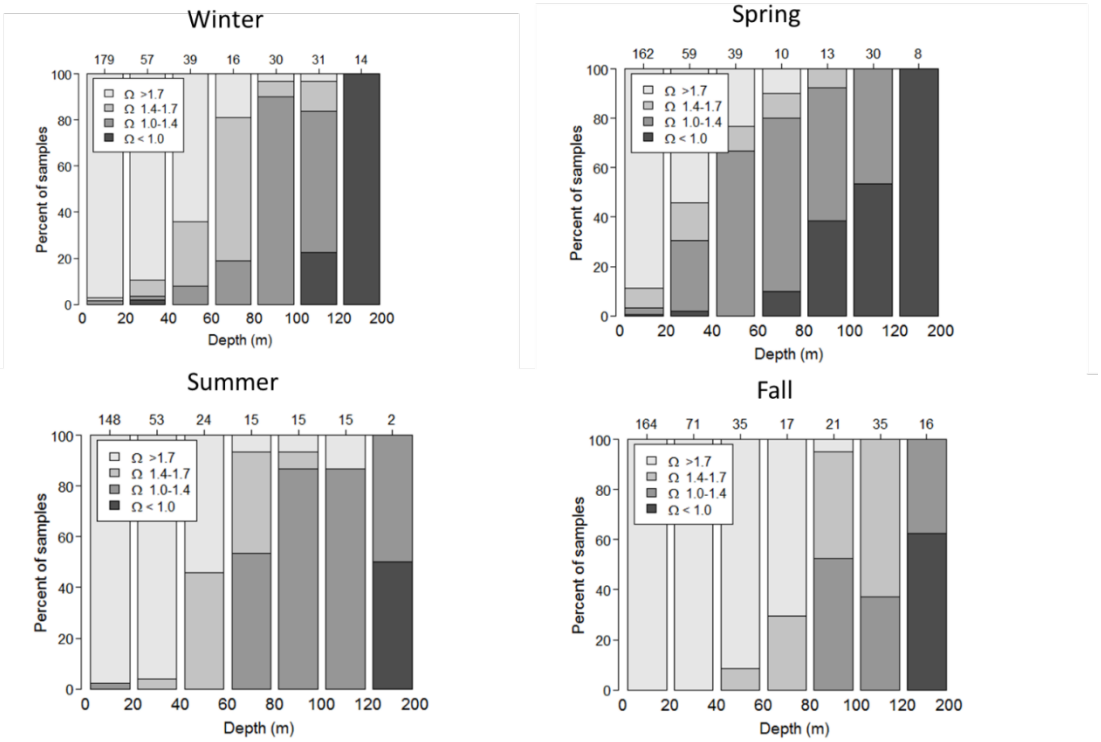
The Southern California Bight 2003 Regional Monitoring Program (Bight'03), was comprised of 65 agencies that sampled between Point Conception and the United States/Mexico border. To better characterize stormwater flows, the Bight'03 water quality survey sampled four major SCB river systems at nearly 200 stations. Sampling occurred over multiple days (3-5) after a rainfall event and collected discrete samples for bacteria, toxicity, chlorophyll, and phytoplankton both at the source and within the stormwater plumes with the goal of correlating

these measures with standard satellite imagery (e.g., ocean color). While the offshore turbidity plumes observed by satellites were found to be extensive in time and space, the measured water quality impact (e.g., toxicity and indicator bacteria exceeding recreational standards) was typically <10% of this area and declined rapidly within 1-3 days following the rainfall event.

The Southern California Bight 2008 Regional Monitoring Program (Bight '08) was comprised of 65 agencies sampling the same geographic area as in 2003. 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 SCB (Howard et al. 2012). The discharged effluent of publicly owned treatment works (POTWs) was the main anthropogenic constituent that comprised the anthropogenic nitrogen loads, whereas riverine runoff and atmospheric deposition were determined to be 1-3 orders of magnitude smaller (Howard et al. 2012). Additionally, the results indicated that the extent of surface algal blooms has increased over the last decade, with chronic blooms documented in areas of the SCB co-located with major inputs of anthropogenic nutrients, as well as longer residence times of coastal waters. The Bight '08 study also provided new insights into algal bloom development in that upwelling was documented to transport a subsurface algal bloom closer to shore and into surface waters, resulting in bloom intensification.

The Southern California Bight 2013 Regional Monitoring Program (Bight '13) was comprised of 34 organizations, sampling the same regions as the previous two programs, with the inclusion of some new habitats. The water quality component of this program was further divided into three research areas: an assessment of acidified waters in the SCB, an assessment of spatial and temporal patterns in subsurface chlorophyll a, and direct measurements of key rates and processes related to nutrient and carbon cycling (process studies). This program 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. For three quarters of the year, greater 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 (Figure I-3, McLaughlin et al. 2017). Furthermore, waters below critical thresholds were observed in surface waters which may indicate that pelagic calcifers (pteropods) and intertidal shellfish populations in Southern California may be adversely affected by acidification conditions along the coast. The study also found that global forcings had a significant impact on chlorophyll a in the SCB as well as on the key rates and processes, but that local impacts may also play a role at smaller scales in the nearshore environments.

Figure I-3. Seasonal variability documented in the Bight '13 Program. Percent of samples within Ω_{arag} threshold categories for each depth bin for the continental shelf. Depths are on the bottom axis. Number of samples in each depth bin is on the top axis.



C. Relationship Between Water Quality and Ocean Acidification in the SCB

The West Coast is vulnerable to OA due to seasonal upwelling which draws water masses that are naturally low in dissolved oxygen (DO), 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 adsorption 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 SCB. These natural nutrient fluxes are modulated in nearshore waters by a variety of anthropogenic and terrestrial sources, dominated by wastewater treatment discharges (Figure I-4).

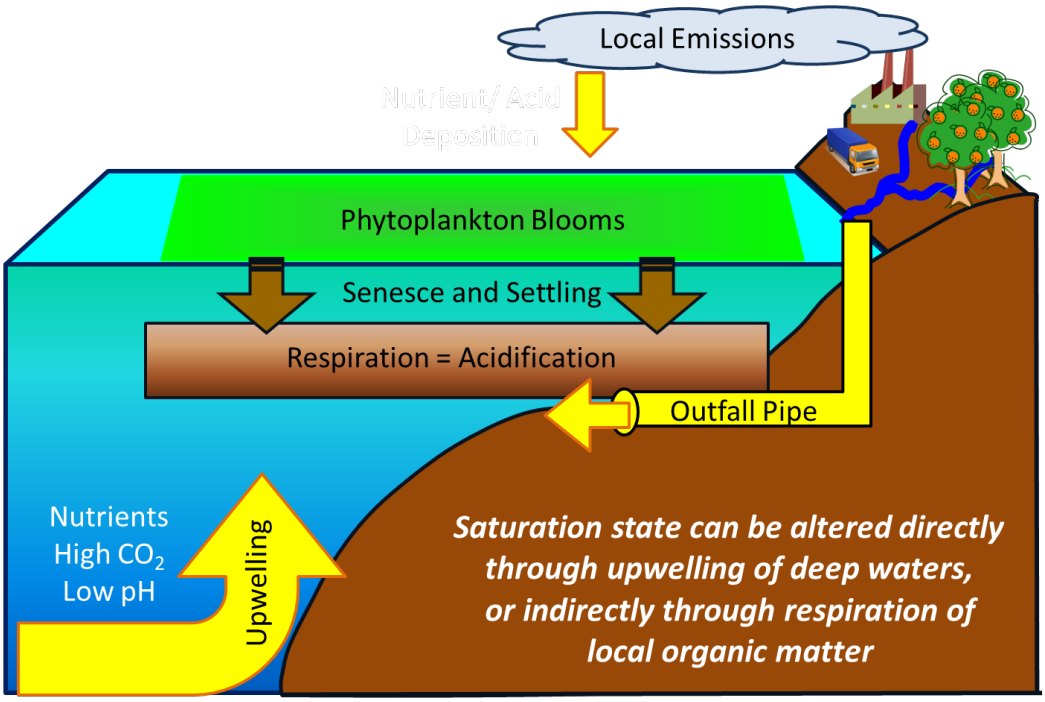
Because upwelled water is already low in pH and DO, the West Coast is particularly sensitive to additional perturbations in these parameters. Nutrient additions can indirectly affect the DO and CO_2 content of seawater via primary production and respiration. Nutrients from wastewater inputs may stimulate phytoplankton blooms which assimilate dissolved CO_2 during photosynthesis, reducing acidity and elevating dissolved oxygen. When this algal organic material is respired, oxygen is consumed and CO_2 released (thereby lowering DO and increasing acidity). Because primary production occurs during daylight hours, shallow coastal ecosystems can experience large diel swings in DO, pCO_2 and pH; 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 at below the euphotic zone, potentially pushing these deeper water past ecological tipping points for pelagic species.

Primary productivity and nutrient cycling (including oxygen demanding processes like nitrification) can have direct and indirect effects on ecological condition of coastal waters. The California Ocean Plan establishes criteria for the amount of influence anthropogenic wastewater dischargers are permitted to have on coastal water ecosystems. These include criteria for nutrients ("shall not cause objectionable growth or degrade indigenous biota"), dissolved oxygen ("shall not be depressed by more than 10% of that which would occur naturally"), and pH ("shall not be changed more than 0.2 pH units"). However, how anthropogenic nutrients influences each of these is not well understood.

The Bight '13 Program provided the first comprehensive study of carbonate chemistry on the SCB continental shelf. This study found levels of carbonate chemistry thought to present an impairment to the health of many marine calcifers (Fabry et al. 2008, Hofmann et al. 2010, Barton et al. 2015, Bednaršek et al. 2017). Indeed, there is a growing body of evidence that OA may affect species distributions and the health of nearshore marine ecosystems in the SCB (Kroeker et al. 2013, Sato et al. 2017). However scientific consensus is lacking on the thresholds of OA parameters that can result in the decline of marine organisms (assessment endpoints). Assessment endpoints provide a framework for consistent interpretation of chemical monitoring data. Initial biological endpoints have recently been proposed, however their relevancy for SCB waters is unknown. A regional assessment of biological impacts on important SCB 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 '18 Ocean Acidification element.

Figure I-4. Conceptual model of impacts of nutrient inputs on coastal ocean acidification.



D. 2018 Program

The proposed Southern California Bight 2018 Regional Marine Monitoring Program (Bight '18) is a continuation of the successful cooperative regional-scale monitoring program. Bight '18 builds upon previous successes and expands on the 2013 program by including new participants and answering questions on biological impacts. Twenty-four organizations have agreed to participate (Table I-1). Cooperative interactions 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.

The Bight '18 Program is organized into five technical components: 1) Ocean Acidification; 2) Sediment Quality (formerly Contaminant Impact Assessment/ Coastal Ecology); 3) Microbiology; 4) Harmful Algal Blooms; 5) Trash. The Water Quality group chose to divide into Ocean Acidification and Harmful Algal Blooms, because the study design and approaches were sufficiently different to warrant separation. The OA element will focus on enhancements to chemical monitoring to improve data quality and develop the first SCB assessment of biological impacts of OA. This work plan provides a summary of the program design for this component. Appendices detail intercalibration requirements for chemical measurements and the sampling SOP for biological impact assessment. Separate work plans are available for the other elements of Bight '18.

TABLE I-2. Participants in the Bight '18 Regional Marine Monitoring Program, Ocean Acidification component.

Aquatic Bioassay and Consulting Laboratories (ABCL)
Chevron USA Products Company
City of Los Angeles Environmental Monitoring Division (CLAEMD)
City of Oxnard
City of San Diego
Los Angeles Regional Water Quality Control Board
Los Angeles County Sanitation Districts (LACSD)
Los Angeles Waterkeeper
MBC Applied Environmental Sciences
National Oceanic and Atmospheric Administration (NOAA), Pacific Marine Environmental Laboratory (PMEL)
National Oceanic and Atmospheric Administration (NOAA), Southwest Fisheries Science Center
Ocean Protection Council
Orange County Coastkeeper
Orange County Sanitation District (OCSD)
Reef Check
San Diego Regional Water Quality Control Board (SDRWQCB)
Santa Ana Regional Water Quality Control Board
Santa Barbara Channel Keeper
Santa Monica Bay Restoration Commission
Southern California Coastal Ocean Observing System (SCCOOS)
State Water Resources Control Board
University of California, Irvine
University of California, Santa Barbara

II. Study Design

A. Study Objectives

The overall goal of the Bight'18 Ocean Acidification Study is to determine the extent and magnitude of chemical and biological impacts related to carbonate chemistry exposure in the SCB. There are two principal questions for the Ocean Acidification component:

1. What is the extent, magnitude, and duration of low pH and low Ω in SCB shelf waters (chemical indicators of OA)?
2. What is the extent and magnitude of biological impacts related to carbonate chemistry stress on SCB species in the pelagic environment (biological indicators of OA)?

The first question will be addressed by a chemical monitoring program for assessing status and trends in carbonate chemistry in the SCB. The second will be addressed by a biological impact's assessment, with indicators and endpoints that link directly back to carbonate chemistry.

The chemical monitoring program has two main monitoring questions:

1. What percent of SCB continental shelf waters in the upper 100 m fall within specific thresholds of OA (based on Ω_{arag}) and how does this vary by season and year?
2. What is the duration of low Ω_{arag} events on the SCB continental shelf and how does this vary by depth?

The first question will be addressed by enhancing protocols for NPDES regulatory monitoring of carbonate system parameters, specifically using bottle measurements for an in situ calibration for pH and applying algorithms to profiling data for the estimation of total alkalinity, dissolved inorganic carbon, and aragonite saturation state. The second question will be answered through a compilation and statistical analysis of available SCB mooring data.

The biological impacts assessment has two main monitoring questions:

1. What percent of the SCB pelagic waters (upper 200 m) show evidence of exposure or sub-lethal impacts of 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 separation of selected indicator taxa and laboratory analysis of biological metrics for acidification. The second question will use the physiological and biochemical metrics analysis from the first question to see how well these endpoints correlate with carbonate chemistry.

B. Sampling Design

The sampling design for Bight '18 Ocean Acidification Program will be divided into two main components: 1) Chemical monitoring for extent, magnitude and duration of carbonate chemistry, and 2) Biological monitoring for extent and magnitude of impacts on sensitive taxa in the SCB. Biological monitoring should occur as soon as is feasible to the chemical monitoring so that the biological monitoring results from the along shore transect can be used to interpret the chemical exposure as measured in the coastal grids.

1. Chemical Monitoring

Magnitude and Extent.

The magnitude and extent of low pH, low Ω_{arag} in the upper 100 m of the SCB 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. Water column profiles for temperature, salinity, dissolved oxygen and pH have been regularly monitored by the POTWs for decades. The Bight '18 study will implement recommendations from the Bight '13 acidification study to improve carbonate chemistry measurements using existing technology at 370 sites in the POTW sampling grids. A map of the monitoring grids is provided in Figure II-1.

The goal of this element is to field-test improvements to pH calibration methods to evaluate whether they can be adopted for routine monitoring so that long-term histories of spatial and seasonal changes in Ω_{arag} can be generated. The first recommendation from the Bight '13 program was to implement a regular, in situ calibration of the potentiometric pH sensor with bottle samples measured spectrophotometrically. The second recommendation was to estimate total alkalinity in seawater using either an algorithm for assessment of total alkalinity in seawater (e.g., Alin et al., 2012) or alternative estimate (e.g., average historical alkalinity). The combination of pH and total alkalinity allows for calculation of Ω_{arag} for all water column profiles collected during POTW quarterly monitoring. Adoption of improved carbonate chemistry monitoring would not only provide a comprehensive understanding of acidification of the Southern California shelf, but also allows for comparison with West Coast datasets.

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 (Figure II-2); improving uncertainty from ± 0.5 pH units to ± 0.1 pH units (McLaughlin et al. 2017). Consequently, the POTW agencies agreed to field-test the recommendation for an in situ calibration of glass electrode pH sensors during the Bight '18 program to assess feasibility of incorporation into routine monitoring.

Starting in the Spring of 2019, during each quarterly monitoring cruise, a minimum of 3 water samples will be collected on the first day of sampling (at the surface, thermocline or mid-depth if there is no thermocline, and at the deepest sampling depth for that cruise, here after called "bottom") and two (surface and bottom) on the final day. Calibration samples should be

collected at the first station sampled with a depth greater than the deepest water sample collected during monitoring (generally 100 m or 200 m depth depending on the agency) on day one and the last station with a depth greater than the deepest sampling depth on the last day of sampling; this will allow for the most time to pass between calibration samples to verify the drift in sensors while also maximizing the depth differences for the pressure correction. 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, the sampling for the first week should be conducted as stated above then for the second week two bottle samples on day 1 and a two on the final day (surface and bottom) should be analyzed. One sample is required for QA/QC from each sample quarter. The QA/QC should be taken as a duplicate of one of the samples collected for the calibration; although which station receives the QA sample should be randomly assigned by each agency.

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 2 new calibration samples should be collected on the first day the electrode is utilized (surface and bottom), though 3 is preferred if resources allow. 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. The in situ calibration on the replacement/recalibrated probe should be done with the 2 samples from day 1 and 2 samples from the last day of sampling.

Water samples for pH and total alkalinity (TA) should be collected 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. There will be an intercalibration before sampling starts to determine comparability of pH and TA measurements amongst the laboratories participating in the sample analysis. Details of the intercalibration are provided in Appendix A and a recommended procedure for water sample collection in Appendix E.

The corresponding pH measured by the glass electrode sensor at the depth where bottle samples are collected for pH will be recorded. A web application has been developed to generate the depth dependent regression (between spectrophotometric pH from the bottle samples and the glass-electrode pH) to correct for bias in the sensor measurement. Regressions will be 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, at 95% confidence, pH within ± 0.1 pH units. A protocol for using the application is provided in Appendix C. The number of bottle samples is indicated in Table II-1.

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 on 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 SCB using the Bight '13 acidification dataset and will be routinely evaluated against bottle measurements of TA (measurements conducted on the same bottle samples as those collected for the pH calibration, Table II-1). These algorithms are expected to provide TA within ± 80 umol/kg at the 95% confidence interval. Interestingly, because TA within the SCB is relatively consistent (SCB 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 the algorithms. SCCWRP has developed an online tool which provides both an estimated TA and the average TA, these two values 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 analysis collected during the survey, allowing for calculation of $\Omega_{arag.}$

As with pH, bottle samples for TA will be analyzed either by the agency or an outside contract laboratory and there will be an intercalibration before sampling starts to determine interlaboratory comparability (Appendix A). 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. The online tool will estimate the TA from the CTD temperature and salinity data as well as the average total alkalinity; users can select the method that most closely matches the TA data collected for the calibration. The tool will then generate water column profiles of TA from the POTW monitoring datasets. 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.

Putting the SCB into Context. During the summer of 2020, the 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 SCB shelf, therefore, POTW agencies will extend the lines onto the SCB shelf into their regulatory monitoring grids. These lines will be a subset of their existing grid lines and bottle samples will be collected every 5 – 10 m depth and analyzed by PMEL for DIC and TA, from which Ω_{arag} will be computed. These transect lines will help place the carbonate chemistry of the SCB shelf into a larger, regional context. Exact locations will be determined and approved by the committee before sampling commences.

Products: Short-term products from this element include cumulative distribution functions of aragonite saturation state, pH, and dissolved oxygen in the SCB 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 in the SCB.

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

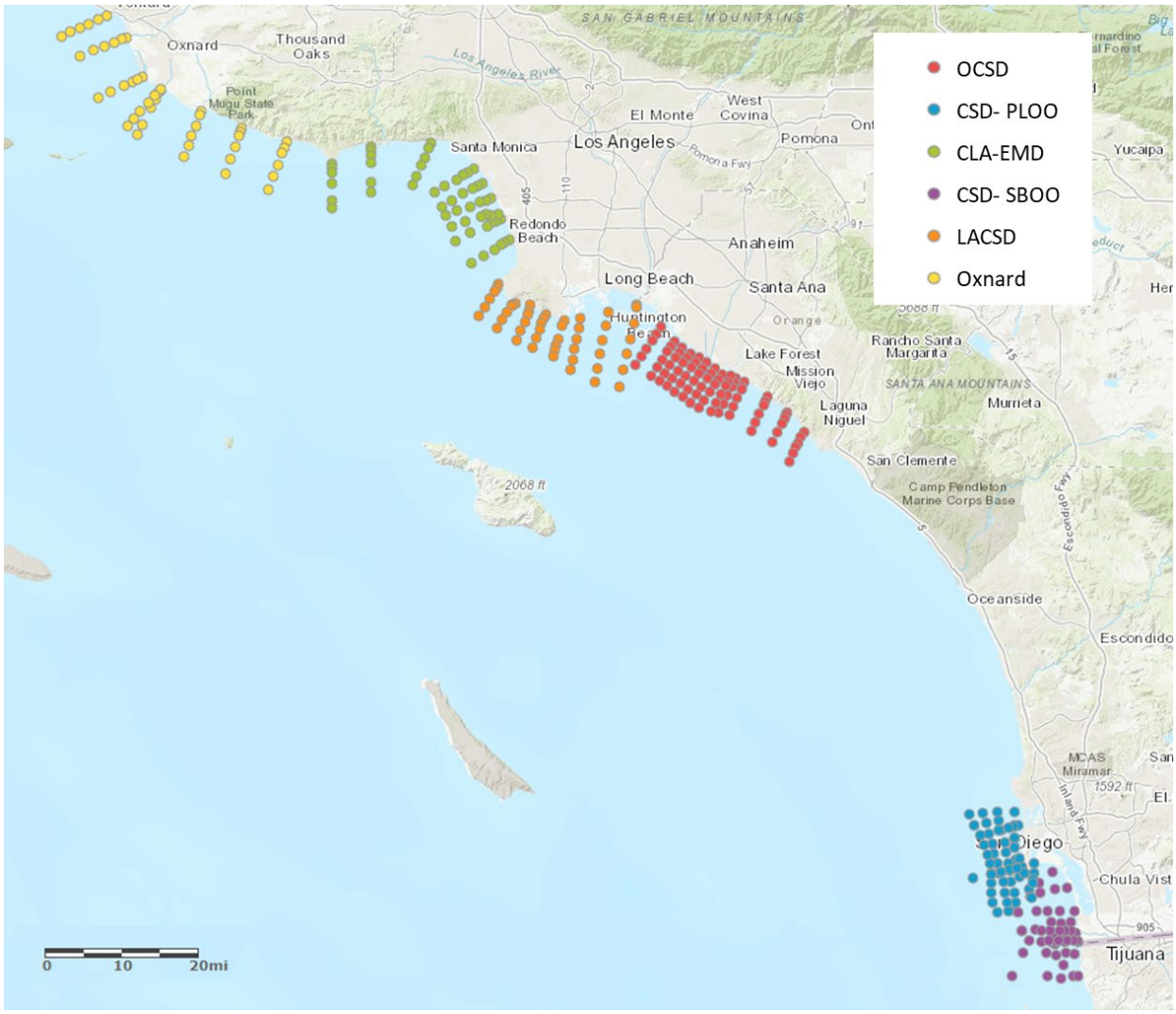


Table II-1. Number of discrete samples that will be collected for calibration of the pH sensors by agency between Spring 2019 and Winter 2021.

Agency	# Bottle Samples Per Sample Event	# Events	Total # Bottle Samples
Oxnard	6	8	48
CLAEMD	6	8	48
LACSD	6	8	48
OCSD	6	8	48
City of San Diego	14	8	112
Total			304

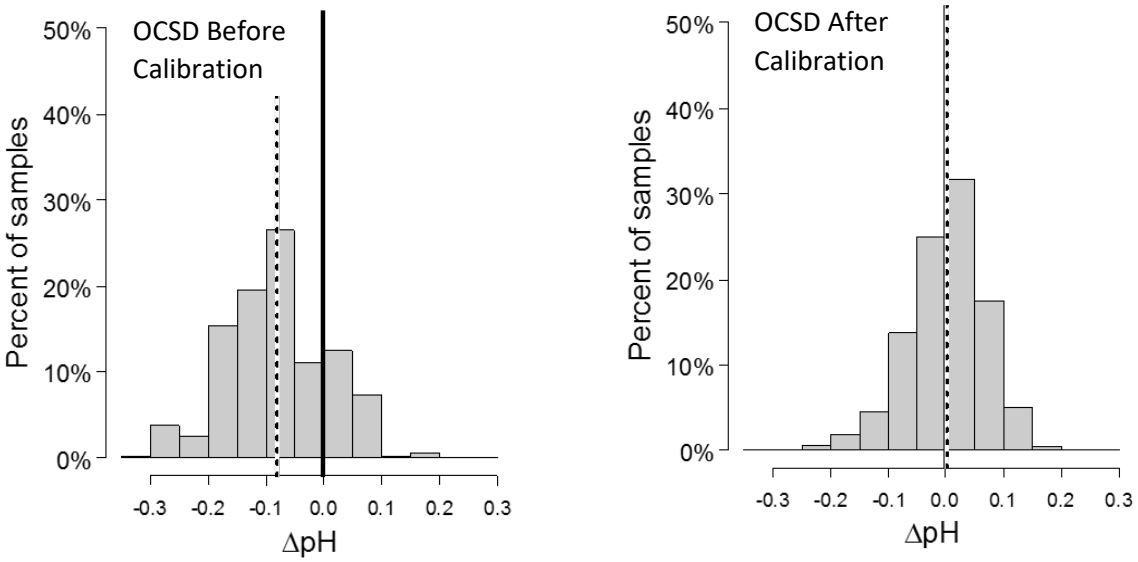


Figure II-2. Distribution of differences between bottle pH and electrode pH for paired samples collected during the Bight '13 survey. The difference between bottle samples and electrode pH was significantly reduced when an in situ calibration was applied to the CTD pH dataset.

Duration of Exposure.

Analysis of moored sensor data. The duration of low pH, low DO, and low Ω_{arag} in the SCB will be determined through analysis of mooring data. Available mooring datasets from throughout the SCB will be collated and analyzed for magnitude and duration of exposure. Table II-2 provides a list of available moorings for the SCB.

Products: Products from this component include frequency maps of continuous duration of exposure across all moorings in the SCB, as well as means and standard deviations of exposure by time or site/strata annually/seasonally as box and whiskers plots.

Table II-2. Existing mooring data in the SCB.

Mooring location	**Owner/Operator**
San Pedro Shelf	SMBRC/LACSD
OCSD outfall	OCSD
Del Mar	Scripps
South Bay ocean outfall	City of San Diego/ Scripps
Point Loma ocean outfall	City of San Diego/ Scripps
Santa Barbara LTER	UCSB
California Current Ecosystem, Point Conception	NOAA/Scripps
Rocky Reefs throughout the SCB	Reef Check

Sterns Wharf	SCCOOS
Santa Monica Pier	SCCOOS
Newport pier	SCCOOS
Scripps pier	SCCOOS

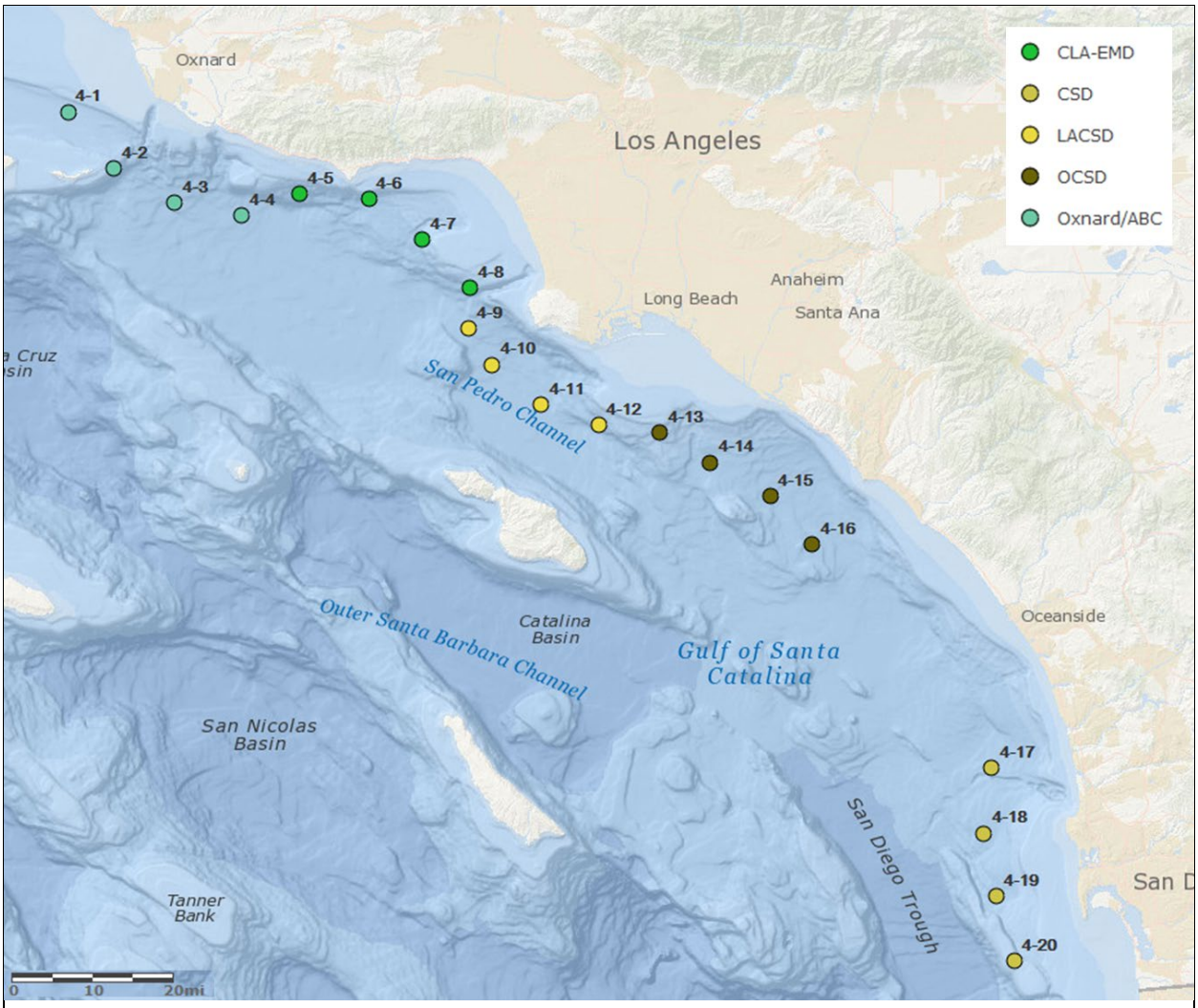
2. Biological Impacts Assessment

Magnitude and Extent of Impacts

The magnitude and extent of biological impacts on the upper 150 m of the water column in the SCB will be assessed at 20 to 30 sites quarterly for two years via ship surveys, starting in spring 2019 and ending in winter 2020. Biological impact surveys (2 ship days per agency) should occur within a month of the quarterly surveys conducted for the carbonate chemistry status and trends element. Quarterly surveys were selected because seasonal upwelling is known to affect the aragonite saturation state of SCB waters and therefore the biological impacts may be expected to have a seasonal component. A two-year program was chosen for two reasons: 1) indicator taxa and endpoints for the SCB are not established so a two-year program allows for 1 year of piloting taxa and metrics and a second year of refinement (see workflow below) and, 2) a two-year study allows for some characterization of interannual variability in the assessment.

Site Selection. A map of the sampling sites is provided in Figure II-3. Sites were selected using a systematic approach in coastal waters approximately 5 - 10 nautical miles off shore and in water depths greater than 250 m. This habitat was selected because many of the sensitive taxa to be investigated are known to vertically migrate throughout the water column to depths up to 200 meters. A total twenty sites yields a 90 % CI of about ± 15% (assuming a binomial probability distribution and p= 0.2). This level of desired precision is acceptable because differences in response of less than 10-15% among subpopulations are unlikely to result in modifications to the pilot monitoring program for OA and would likely not impact management decisions regarding OA for SCB pelagic waters.

Sites were selected using a systematic approach with regularly spaced stations along a specified distance from shore (5-10 miles), while maximizing each agencies ability to sample the sites within 2 sampling days. This approach allows for complete coverage of the SCB, while still being tractable for the participating agencies. Ecological condition between sites will be estimated by kriging. A list of sites and the agencies responsible for sampling these sites is presented in Figure II-3; a list of sample sites is provided in Appendix B.



4 sampling sites per agency. 20 sites total

Figure II-3. Map of the pelagic water biological impacts of OA assessment sites (orange: sites sampled by City of Oxnard, red: sites sampled by CLA-EMD, green: sites sampled by LACSD, purple: sites sampled by OCSD, blue: sites sampled by CSD).

Sample Collection. Zooplankton samples will be collected using a double ringed bongo-net tow that is pulled obliquely through the water column from a depth of 150 m (see Appendix D). This design will enable us to capture a several sensitive zooplankton taxa residing at a variety of depths depending on their vertical migration habits. Because this is the first-time biological monitoring for OA is being conducted in the SCB, several candidate taxanomic groups have been selected as potential indicators. Candidate taxa include: pteropods, crustacean larvae (crabs, lobsters), echinoderm larvae (urchins). Indicator taxa will be selected from the candidates based on regional representation. To evaluate biological impacts on the candidate taxa, we will apply a clear metric of OA impact (shell dissolution as apparent under scanning electron microscopy) and we will investigate the use of sub-lethal metrics of physiological stress (as an early warning marker). The contents of one of the two net cod ends will be preserved in ethanol for assessment of shell condition, while the contents of the second cod end will be preserved with a solution for molecular metrics of physiological stress (Zymo DNA/RNA Shield).

Coccolithophores will also be collected at each of the biological monitoring stations as a part of a special study lead by UCLA (Ina Benner and Robert Eagle) to determine the feasibility of using them as a proxy for pH changes. At each station, 4 liters of water will be collected from the surface and preserved with buffered formaldehyde (2%). Bottles with the preservative will be provided. Samples should be stored on ice in the dark until they can be retrieved by the UCLA team.

One of the design attributes of Bight '18 biological impacts 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 100 m with a Van Dorn Bottle (or similar) deployed with the CTD sensor package. 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.

Study Phasing. This study will have three phases: 1) a local pilot phase, to develop field and laboratory methods, 2) a year 1 regional assessment pilot, to characterize regional taxa/species diversity and refine metrics, and 3) a year 2 assessment, during which interannual variability in impacts is assessed and the SCB is placed into larger context. The year 2 assessment will coincide with the NOAA PMEL 2020 summer U.S West Coast and SCB indicators and metrics will be incorporated into this larger study based on the year 1 results.

Phase 1. In Fall 2018/Winter 2019, a pilot project and training will be conducted by SCCWRP in collaboration with OCSD to develop the field methods for the pelagic sampling. This pilot will be conducted offshore of OCSD's regular sampling grid in the central region of the SCB. Samples collected during this effort will be evaluated for taxa/species present to provide an initial estimate of organisms we are likely to collect during the regional assessment. These samples will also serve as the sample set to begin development of the laboratory methods for analysis of candidate metrics (Table II-3). Field training for all agencies expecting to participate in the regional assessment will be conducted in the winter of 2019.

Phase 2. During year 1 of the regional assessment, Spring 2019 through Winter 2020, samples will be collected from the 20-30 sites selected for the regional assessment and preserved for analysis of biological impacts. Subsamples from each site will be used to evaluate the ranges of candidate taxa/species. Subsamples will be selected based two criteria: 1) they should represent the largest range of chemical exposures to low pH waters for that survey period, and 2) should represent the spatial range of the study area. Presence/absence of taxa in those subsamples will establish which taxa are widely distributed throughout the SCB and the exposure range will determine how sensitive those taxa are to exposure to low pH waters. Those calcifying taxa/species found to be widely distributed throughout the SCB will be prioritized for analysis of candidate metrics. Candidate metrics of physiological stress will be compared to both water chemistry (exposure) as well as shell dissolution measured with SEM.

Phase 3. During year 2 of the regional assessment, Spring 2020 through Winter 2021, samples will be collected from the all sites for the regional assessment and preserved for analysis of biological impacts based on distributions and sensitivities determined in phase 1. Species distributions will also be evaluated; however, indicator/ metric pairings from year 1 will be prioritized over new indicators/metrics. Sampling will be coordinated with the NOAA-PMEL summer 2020 cruise so that sensitive indicators and metrics are comparable between the two programs.

A conceptual diagram of the workflow is shown in Figure II-4.

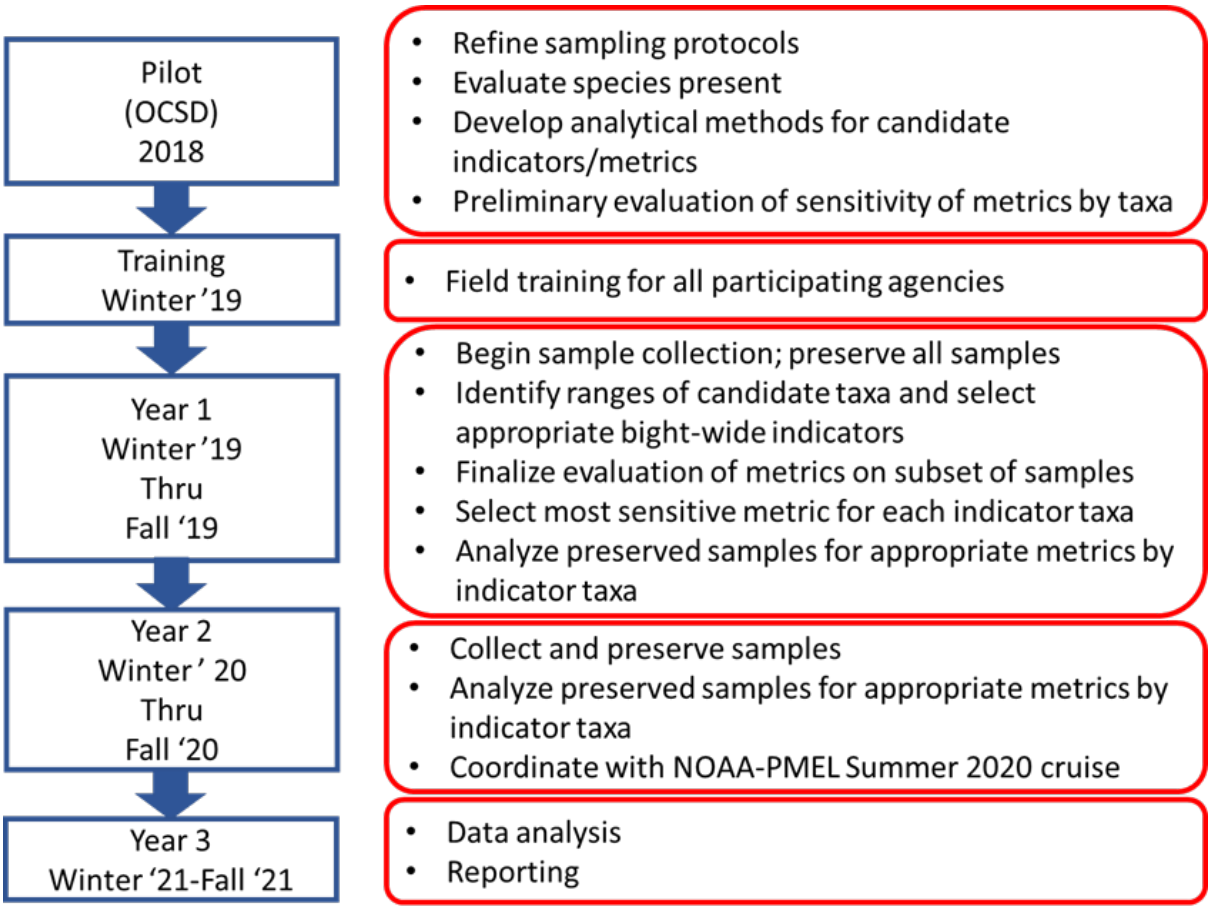


Figure II-4. Conceptual diagram of workflow for the pelagic water biological impacts of OA assessment

Indicators and Metrics.

Indicators. The Bight '18 study will be the first time a comprehensive assessment of biological effects due to acidification has been conducted in the SCB. Consequently, the distribution of sensitive pelagic taxa in SCB waters, and the physiological and biochemical metrics that best characterize biological impacts on these taxa, are poorly understood. Therefore,

the pelagic biological impact assessment has a phased approach to characterize species distributions of sensitive taxa and relevant metrics for assessing biological impacts of OA.

To better focus the pilot and year 1 sampling, 3 candidate taxonomic groups have been selected based on previous studies along the U.S. West Coast and other regions: pteropods, crustacean larvae, echinoderm larvae. Specific species for each taxonomic group that will be used during the biological impacts assessment will be selected based on two criteria: 1) they are widespread throughout the SCB and 2) they are sensitive to impacts of acidification. For purposes of pre-screening, sensitivity is largely determined based on whether the taxa precipitate calcium carbonate structures and have been shown in the literature to have exhibited biological impacts from OA stress.

Metrics. For ocean acidification, dissolution is a key metric because environmental carbonate chemistry is thought to be the only stressor responsible for the observed response. Thus, this measurement combined with the presence/absence data will form the cornerstone of the biological impact assessment. However, such measurements are laborious. Furthermore, the gold standard method to assess dissolution, Scanning Electron Microscopy (SEM), requires a specialized microscope that is not directly accessible to most Bight Regional Monitoring Program participants. Thus, there was broad interest in developing additional methods to assess biological impacts of OA using readily available laboratory methods. New metrics for acidification will be evaluated as a special study component to the survey by comparing replicate samples measured using the new protocols to the same samples assessed for dissolution using SEM to determine efficacy. Candidate metrics are indicated in Table II-3.

Table II-3. Candidate metrics for acidification biological impacts assessment, the stressor each metric assesses, the measurement method, and the availability of this method for use in the assessment.

Metric	Stressor	Method	Method Availability
Presence/Absence of taxonomic groups*	Measure species distributions relative to exposure	Light microscopy	SCCWRP, POTW labs
Shell Dissolution (% individuals displaying mild and severe dissolution; % individuals displaying re-calcification)	Measure extent to which an organism's shell has dissolved and/or re-calcified due to acidification	Scanning Electron Microscopy (SEM)*	SCCWRP
		Light/ fluorescence microscopy	SCCWRP, POTW labs
Targeted Gene Expression (% of indicator taxa expressing targeted genes)	Measure expression of 3-4 targeted genes related to OA stress; Indicator of organismal stress	PCR/ digital PCR	SCCWRP, POTW labs
Oxidative Stress at Cellular Level	Measure enzymatic activity involved in	Biochemical assay –	SCCWRP, POTW labs

(% of indicator taxa demonstrating stress)	stress response related to OA	spectrophotometric measurement	
Stable $\delta^{13}C$ and $\delta^{18}O$ ratios in calcium carbonate structures (cumulative distribution function of isotope ratios)	Fractionation of carbon in carbonate is a proxy for dissolution Heavier isotopes are favored during precipitation (shells are heavier than DIC) Lighter isotopes are favored during dissolution, i.e., dissolved shells are heavier than undissolved	Isotope Ratio Mass Spectrometry (IR-MS)	Outsourced to academic labs

*Shell dissolution as measured by SEM and presence/absence of taxonomic groups will serve as the cornerstone metrics for the biological impact assessment. Other candidate metrics will be compared to these two because they have clear relationships with OA exposure, existing protocols for the candidate taxa, and proposed endpoints.

III. 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

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

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

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.

Horn, M.H. and Allen, L.G., 1985. Fish community ecology in Southern California bays and estuaries. Fish community ecology in estuaries and coastal lagoons: towards an ecosystem integration, pp.169-190.

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

Kroeker, K. J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.S., Duarte, C.M., Gattuso, J.-P. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Global change biology 19(6): 1884-1896.

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.

Sato, K.N., L.A. Levin, K.C. Schiff. 2016. Habitat compression and expansion of sea urchins in response to changing climate conditions on the California continental shelf and slope (1994-2013). Deep-Sea Research II 137:377-389.

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

APPENDIX A - **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 #177.

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.

***Evaluation*:** The average pH of the measurements must have a standard deviation within ± 0.03 pH units and be within ± 0.05 pH units of the reported value. The average total alkalinity must have a standard deviation within ± 30 µmol/kg and be within ± 50 µmol/kg of the reported value. Some level of uncertainty is expected in the measurements. Results from the Bight '13 study evaluating pH and total alkalinity on duplicates collected in the field had absolute differences in pH lower than 0.05 and total alkalinity of lower than 50 µmol/kg (Figure A1), so it should be easily achievable on laboratory duplicates. Uncertainties in pH and total alkalinity translate into uncertainty in aragonite saturation state. Uncertainty within 0.05 pH units and 50 µmol/kg in total alkalinity equates to an uncertainty of about 0.3 in aragonite saturation state.

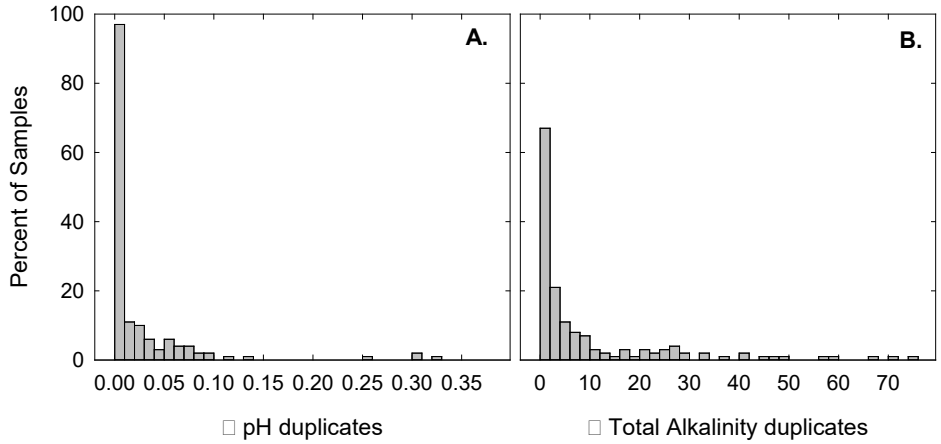


Figure A1. Absolute difference in field duplicates for pH and total alkalinity for all duplicates collected during the Bight '13 Acidification program.

APPENDIX B - Sample Locations

20 Site Sampling Scheme- 4 Sites Per Agency

Agency	Station	ID	Latitude	Longitude
Oxnard/ABC	B18OA-4-1	4-1	34.11990	-119.40494
Oxnard/ABC	B18OA-4-2	4-2	34.01945	-119.30837
Oxnard/ABC	B18OA-4-3	4-3	33.95766	-119.17819
Oxnard/ABC	B18OA-4-4	4-4	33.93523	-119.03607
CLA-EMD	B18OA-4-5	4-5	33.97368541	-118.9126162
CLA-EMD	B18OA-4-6	4-6	33.9643418	-118.7653046
CLA-EMD	B18OA-4-7	4-7	33.89129311	-118.6510062
CLA-EMD	B18OA-4-8	4-8	33.80393012	-118.5490934
LACSD	B18OA-4-9	4-9	33.731735	-118.552292
LACSD	B18OA-4-10	4-10	33.666505	-118.502832
LACSD	B18OA-4-11	4-11	33.594089	-118.398321
LACSD	B18OA-4-12	4-12	33.556814	-118.275623
OCSD	B18OA-4-13	4-13	33.544089	-118.145192
OCSD	B18OA-4-14	4-14	33.49027292	-118.0377884
OCSD	B18OA-4-15	4-15	33.42849801	-117.9106432
OCSD	B18OA-4-16	4-16	33.3421953	-117.8207463
CSD	B18OA-4-17	4-17	32.936947	-117.439508
CSD	B18OA-4-18	4-18	32.818817	-117.456712
CSD	B18OA-4-19	4-19	32.703722	-117.429378
CSD	B18OA-4-20	4-20	32.58679	-117.3915026

APPENDIX C – **Web Application for In Situ Calibration of CTD pH Data**

Correction of pH measurements collected by CTD profilers:
Interactive web application *pH_correction_v201*

Introduction

Water quality monitoring in the ocean often includes data collected by conductivity-temperature-depth (CTD) profilers. These instruments are equipped with potentiometric glass electrodes measuring seawater acidity (pH). The accuracy and stability of these measurements is poor (McLaughlin et al., 2017) but may be corrected using a limited number of discrete bottle water samples collected in parallel with CTD casts and analyzed in laboratories using spectrophotometric method. This approach has been automated in an interactive web application. The program is written using R "shiny" technology (Chang et al., 2017) and the package of Seawater Carbonate Chemistry calculations "seacarb" (Gattuso et al., 2018).

Data organization

CTD data

CTD data collected by the agencies monitoring water quality around four major Publicly Owned Treatment Works (POTW) outfalls in southern California are stored in the format called "Central Bight Master Database". The monitoring program includes quarterly surveys of CTD casts at fixed stations. The collected CTD data are stored in Excel files, typically one year of observations in one file. Monitoring surveys are associated with seasons (Winter, Spring, etc.). For the analysis described in this document, Excel data must be exported to CSV (comma separated) format, because R-based software does not always accurately read MS Excel files. We recommend using one CSV file for each survey to avoid large files, because the 'Shiny' file system used in this application has a default limit of 5Mb in the size of files uploaded to the server. Each CTD file must contain the columns indicated in Table 1:

Table C.1. CTD parameters used for pH correction. "Parameter" is the variable name in the R program code.

Parameter	ParamNameStarts	Format	Comment
Season	Season	character	Identifies the survey
Agency	Agency	character	Identifies the location
Date	Date	%m/%d/%Y	Day
Time	Time	%H:%M	Local time
StnID	Station	character	Standard station
FieldRep	Field	numeric	Replicated profile at the same station
Z	Depth	numeric	Depth (m)
TdegC	Temperature	numeric	Temperature (deg. C)

Spsu	Salinity	numeric	Salinity (psu)
Sigma	Density	numeric	Specific density (kg m^{-3})
pH.nbs	pH	numeric	pH measured by CTD (NBS scale)

The column names must start from the string ParamNameStarts (case sensitive). For example, the Z (depth) parameter may be "Depth", "Depth (m)", etc., but not "depth" or "Z(m)". If the CTD file contains several columns that fit the same ParamNameStarts condition, the first column is selected.

Casts are identified by the Station identification code (StnID) and field replica (FieldRep). Station IDs must correspond to the Station IDs in the Central Bight Database (the table of station coordinates is stored in the file "Stn_coords.csv"). For example, the 2nd cast at station 2505 should be coded as StnID = 2505 and FieldRep = 2. Do not use station IDs that are not available from the "Stn_coords.csv" table, i.e., in this example do not use the station ID = 25052.

Columns FieldRep and Time may be absent. If FieldRep is not found (it happens when all only one profile was collected at each station), it is automatically set to 1; missing Time is automatically set to 12:00 (noon).

If the column names or the formats of Date and Time in your CTD file are different from the default values (Table 1) but you prefer not to change them, you can use this file after editing the CTD column names and formats directly in the web application (see **Editing Settings**).

The "character" and "numeric" formats are recognized automatically, only the Date and Time formats are used for reading the CTD data file. If the format of your data is different from the format in the Settings tab (e.g., "%H%M%S" instead of "%H:%M"), edit it before reading the data file, otherwise you will receive an error message.

Bottle (BB) data

Bottle (BB) data file includes the results of chemical measurements of seawater collected in discrete bottle samples in parallel to CTD casts. Each BB file must contain the columns in Table 2. The rules of organization of BB data file are similar to CTD file.

Table 2. Parameters measured in bottle samples used for pH correction. "Parameter" is the variable name in the R program code.

Parameter	ParamNameStarts	Format	Comment
Agency	Agency	character	Identifies the location
Date	Date	%m/%d/%Y	Day
Time	Time	%H:%M	Local time
StnID	Station	character	Standard station
FieldRep	Field	numeric	Replicated profile at the same station
Z	Depth	numeric	Depth (m)
TdegC	Temperature	numeric	Temperature in laboratory

Spsu	Salinity	numeric	Salinity measured in laboratory in bottle sample
pH	pH	numeric	pH measured in laboratory in bottle sample (total scale)
TA	TA	numeric	Total Alkalinity measured in laboratory in bottle sample (µmol/kg)

The columns FieldRep, Time and TdegC may be absent. When missing, FieldRep is automatically set to 1, Time is set to 12:00 (noon) and TdegC (the temperature in laboratory) is set to 25.

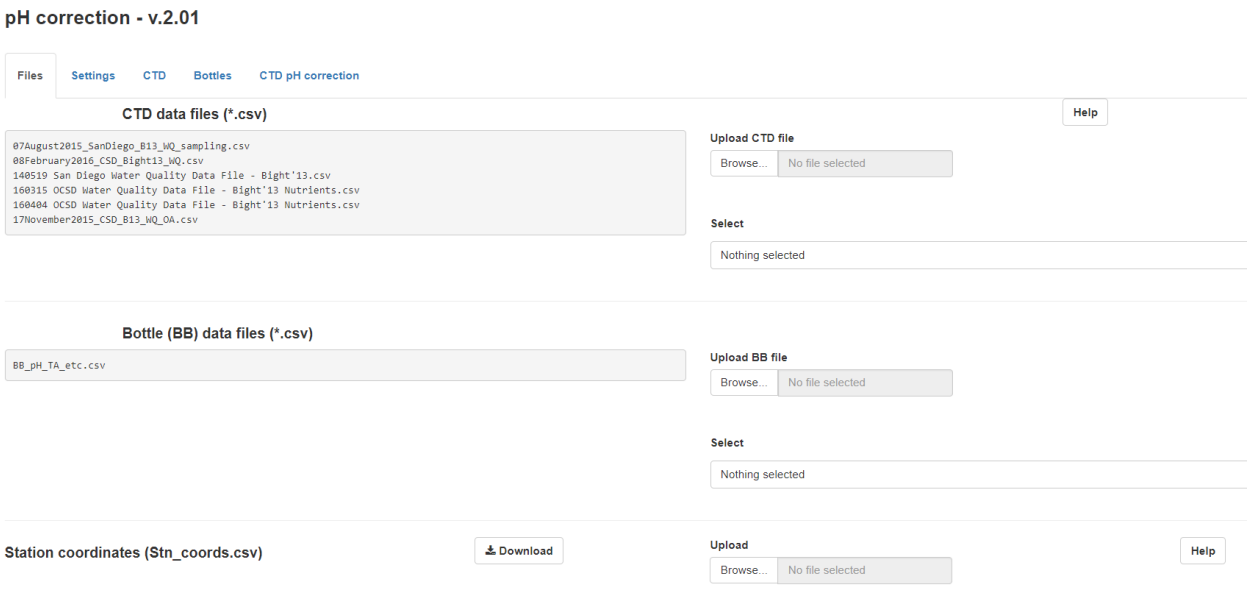
The information from CTD and bottle samples is stored in different files because this data is often obtained by different researchers and organized differently. For example, in Bight'13 program (used in this manual as an example) all laboratory pH measurements obtained during several seasons were stored in one data file.

Starting interactive web application

In your web browser, go to the web address: https://sccwrp.shinyapps.io/ph_correction_v201/

Uploading data to the server

You will start in the "Files" tab.

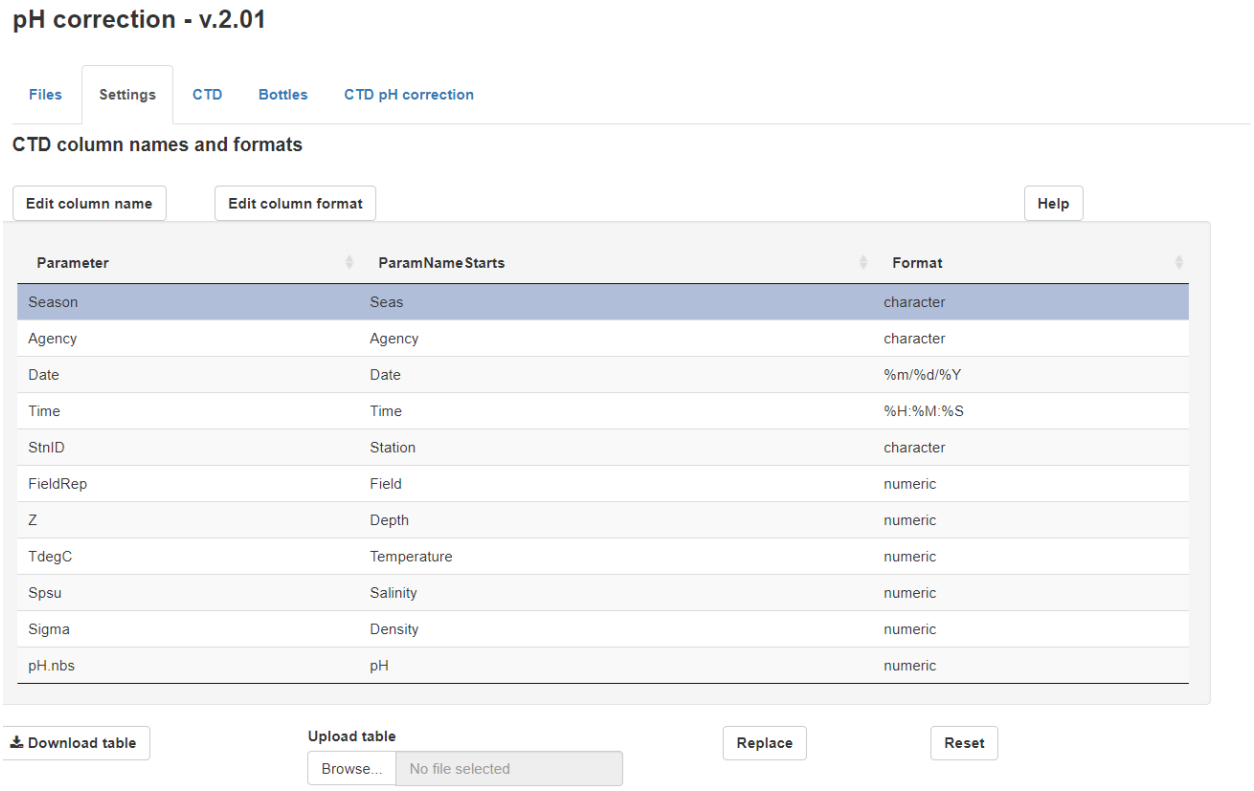


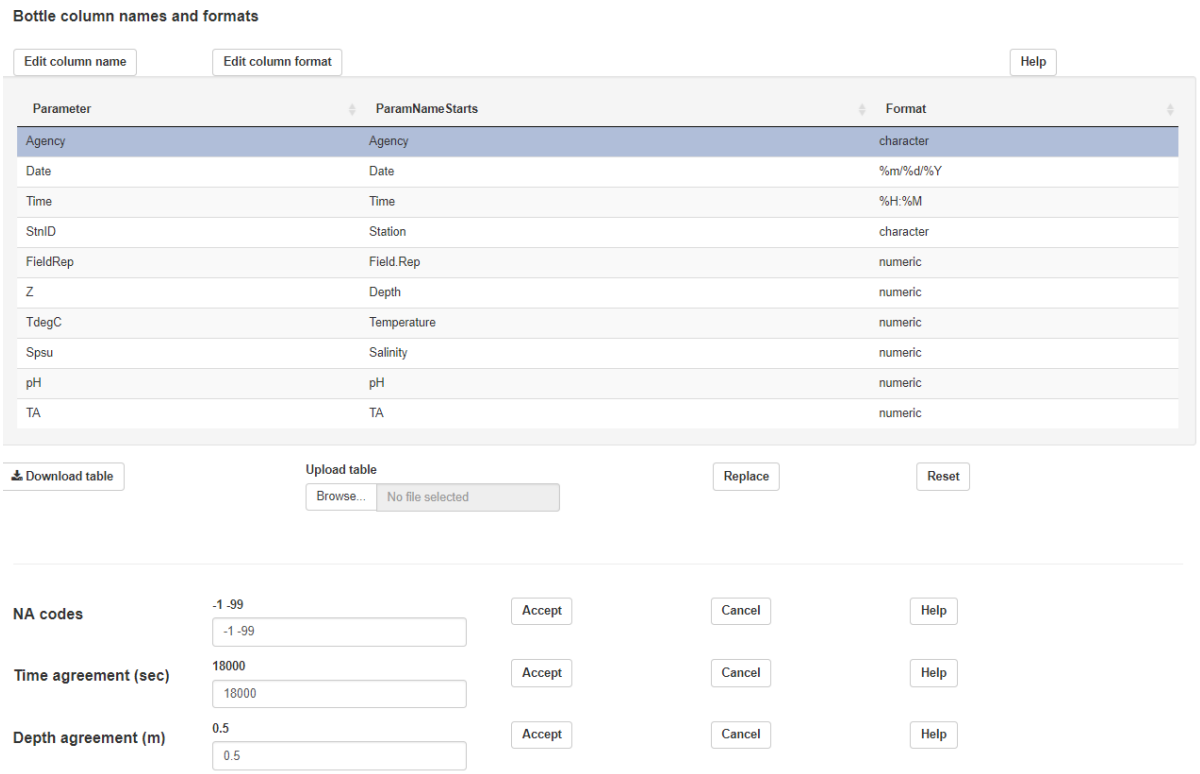
When the application is opened, the active tab is Files. You can upload CTD and BB (bottle) data files by clicking 'Upload CTD file'. Click 'Select' to select one file: you can either download it or delete it from the server.

The file with station coordinates (Stn_coords.csv) must include five columns: Station, Agency, Latitude, Longitude and Depth. You can upload/download this file. When you need to add new stations to the list, it makes sense to download the file Stn_coords.csv on your computer, edit it (add stations) and upload it.

Editing settings

Click Settings tab.





If the column names in the CTD or BB files you are going to analyze are different from default, you can edit them from here.

- Select the row in the table
- Click Edit column name or Edit column format
- Edit the element of the table
- Click Accept or Cancel

All formats may be edited, but it makes sense only for Date and Time. The formats of other parameters are recognized automatically.

Another option to change the column names is to upload the entire table (CTD, BB or both).

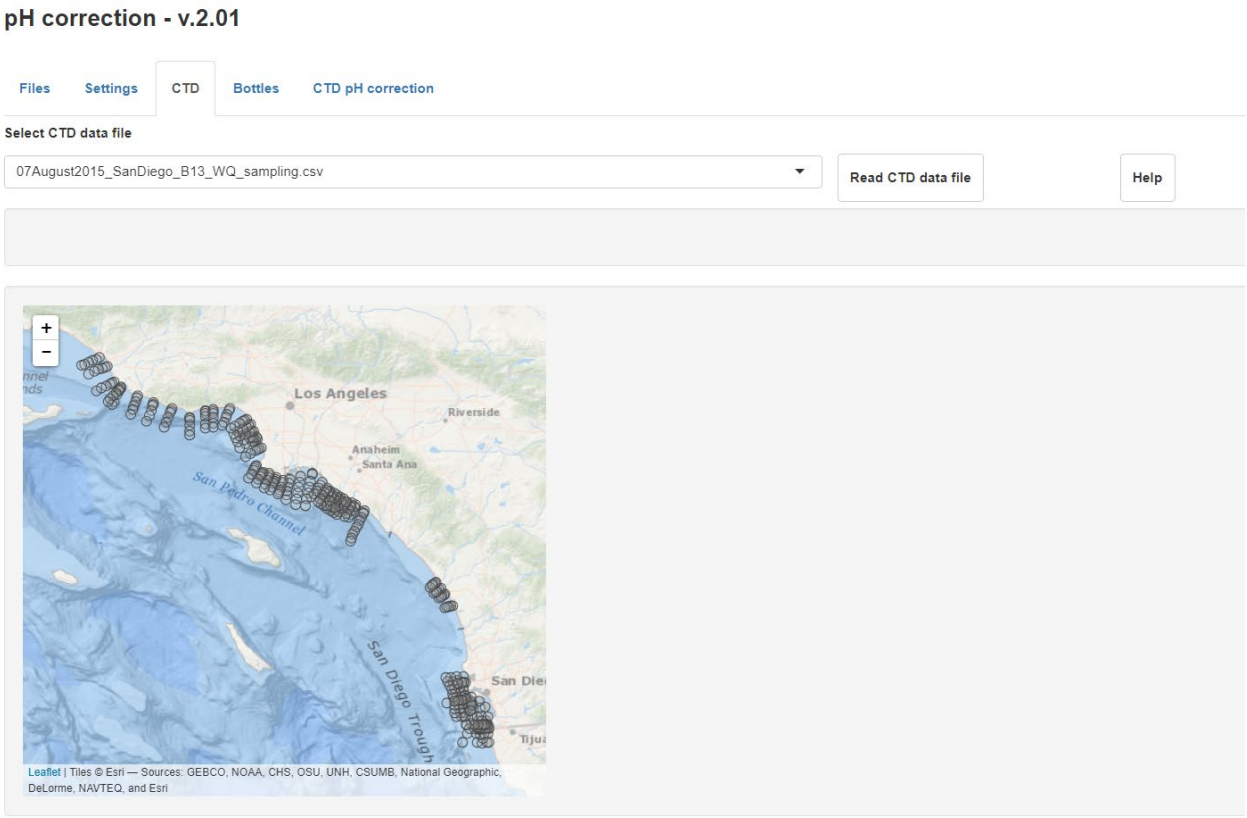
You can click Download table, use this table as a template to enter the column names in your file, then click Upload table and Replace. To return to default settings, click Reset.
At the bottom of the page you see three additional parameters which you can edit. NA_codes (default -1 and -99) are digital codes indicating missing data. The program replaces them with NA (no data) code. Edit these values if you use other "missing data" codes (e.g., -999, 9999, etc.).

Time agreement (sec) is the allowable difference in the sampling time between CTD casts and bottle (BB) samples. The goal of using this parameter is to avoid disagreement between the Time values stored in CTD and BB data files. For example, in BB table the Time column may be

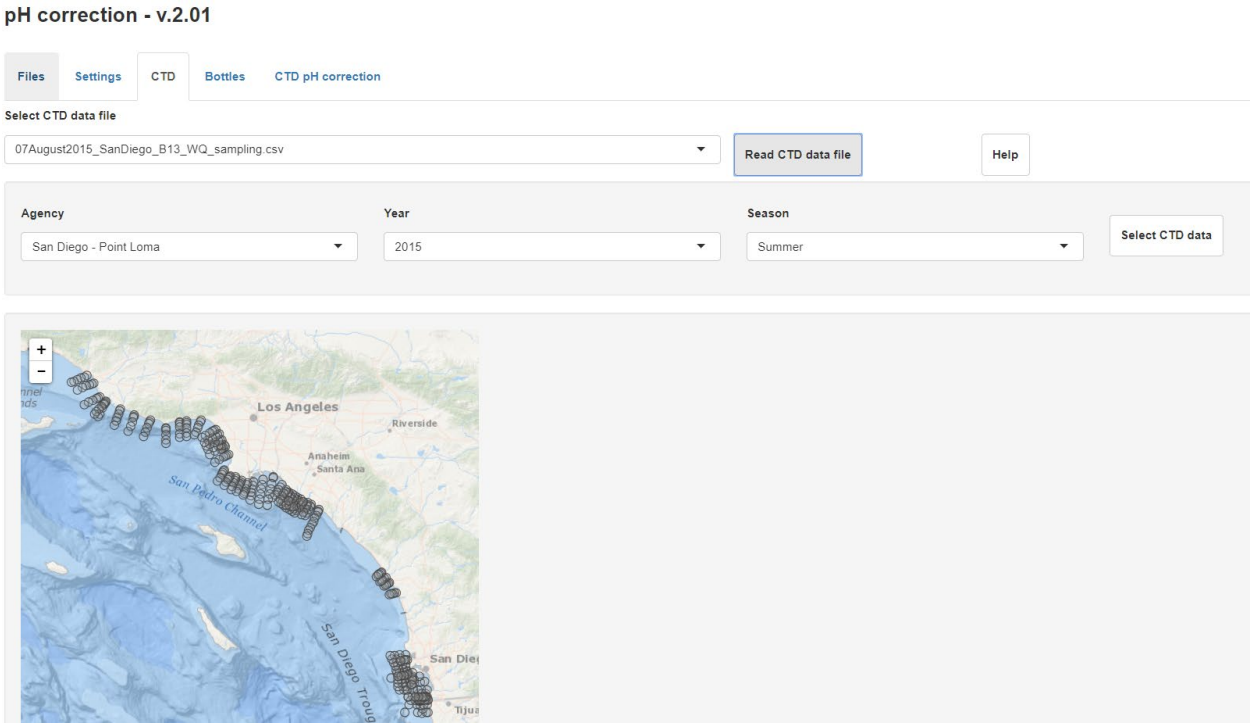
absent (Day, StnID and Z completely identify each bottle sample). If the Time column is not found during reading BB file, it is set to 12:00 (noon). To connect BB samples to CTD casts (which Date and Time columns contain real sampling time), make Time agreement large enough (e.g., 18000 seconds = 5 hours), and all CTD samples collected between 7:00 A.M. and 5:00 P.M. will be found and associated with BB samples. Depth agreement (m) is the allowable difference between the sampling depths stored in the CTD and bottle (BB) datasets. Typically, CTD casts are interpolated to profiles of 1m vertical resolution. The default value of Depth agreement is 0.5 m, that means that for each BB sample only one (the nearest) CTD sample will be found and used for analysis. If you increase the Depth agreement value, several CTD samples will be averaged and used for comparison with BB samples.
If the values in the Tables 1 and 2 fit the settings, the CTD and BB data files would be successfully read and analyzed. If not, the program will report and error.

1. Reading and visualizing CTD data

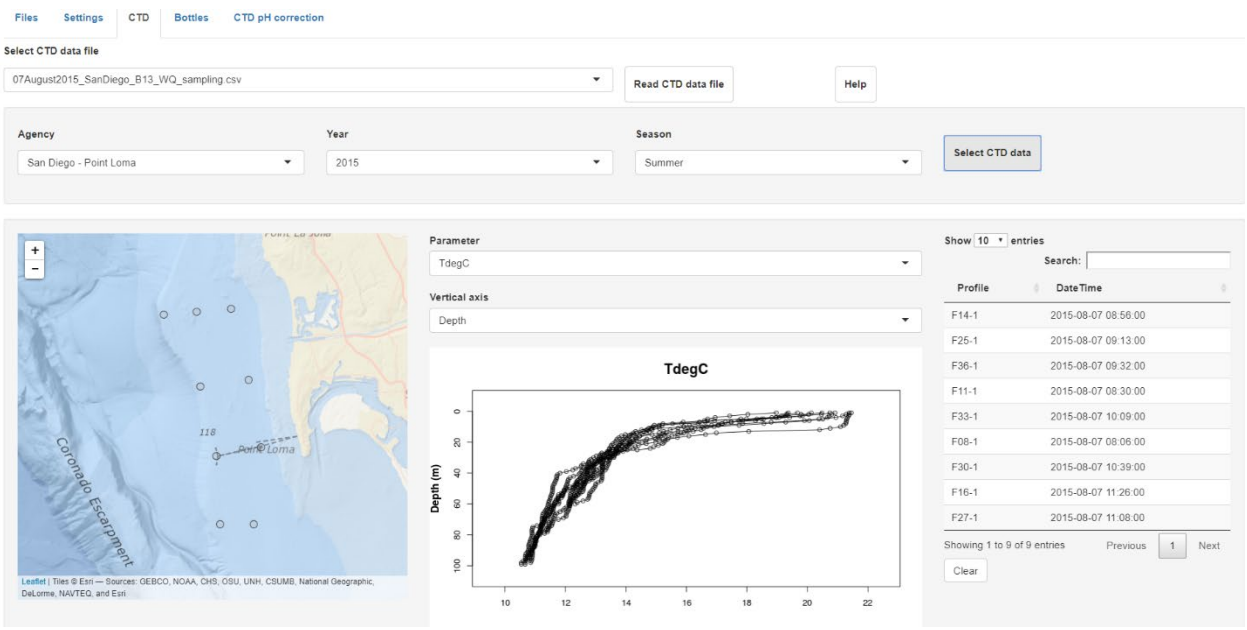
Go to CTD tab.



Select one CTD data file and click the Read CTD data file button. After reading the dataset, three drop-down menus appear. Select Agency, Year and Season you wish to analyze. Click Select CTD data.



Keep in mind that if the CTD file contains stations not found in the station list, these data will not be included into the selected dataset. Warning message will be shown.

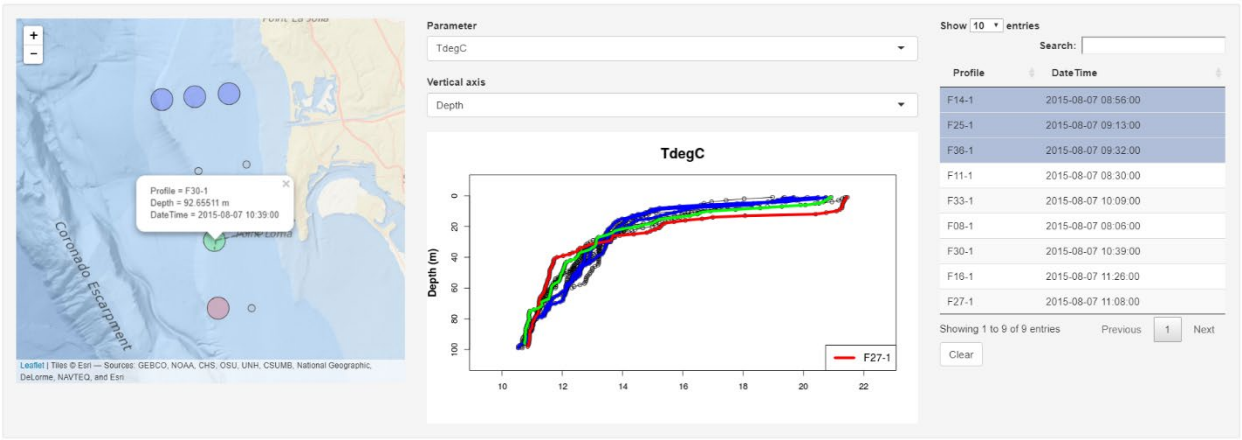


You see three panels: the map of stations (left), the plot with vertical profiles (center) and the table with the list of profiles (including profile IDs and Date-Time).

Below these panels you see the table with the list of CTD parameters which will be used for analysis and some statistics (the total numbers of samples, stations, profiles, and the range of variations of each parameter).

Parameter	Samples	Stations	Profiles	Min	Max
Profile	732	9	9	F08-1	F36-1
DateTime	732	9	9	2015-08-07 08:06:00	2015-08-07 11:26:00
Z	732	9	9	1	99
TdegC	732	9	9	10.54	21.45
Spsu	732	9	9	33.194	33.727
Sigma	732	9	9	23.116	25.865
pH.nbs	732	9	9	7.81	8.24

Data visualization



Clicking one profile at the map, you

- see the information about the station in the pop-up window,
- select the station (green circle)
- see the profile in the plot (green line)

Clicking one profile at the plot, you

- select the profile (red)
- see its location at the map (red circle)

Clicking multiple profiles in the table, you

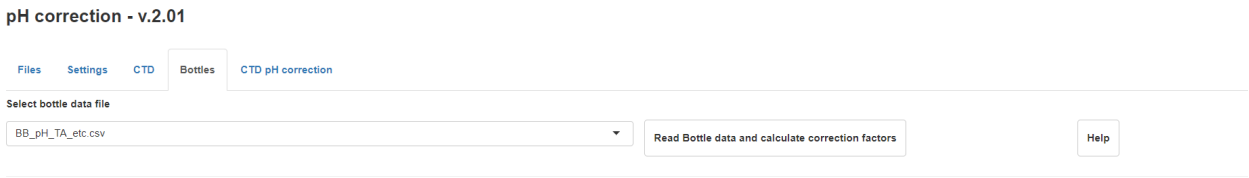
- select them in the table
- see the profiles in the plot (blue lines)
- see their locations at the map (blue circles)

You can analyze different parameters. Select them in the drop-down menu 'Parameter'. When you just read the CTD file and did not calculate new parameters, you can select one of the four parameters: TdegC, Spsu, Sigma and pH.nbs). Vertical axis may be Depth (m) or Specific Density (kg m^{-3}).

When the number of profiles is large, use Search box to quickly access the profiles you need. For example, if you wish to see the profile over the PLWTP outfall (station F30), enter "F30" in the "Search" box and click the profile "F30-1" in the remaining table. Also, you can sort profiles by the station IDs (in alphabetic order) and by Date-Time. It may be useful to sort the table in the order the profiles were collected and clicking them one by one see the route of the vessel and how vertical profiles changed with time.

pH data correction

After CTD data are ready for analysis, you can read the bottle (BB) dataset. Click Bottles tab.



Select the BB data file and click the button Read Bottle data and calculate correction factors.

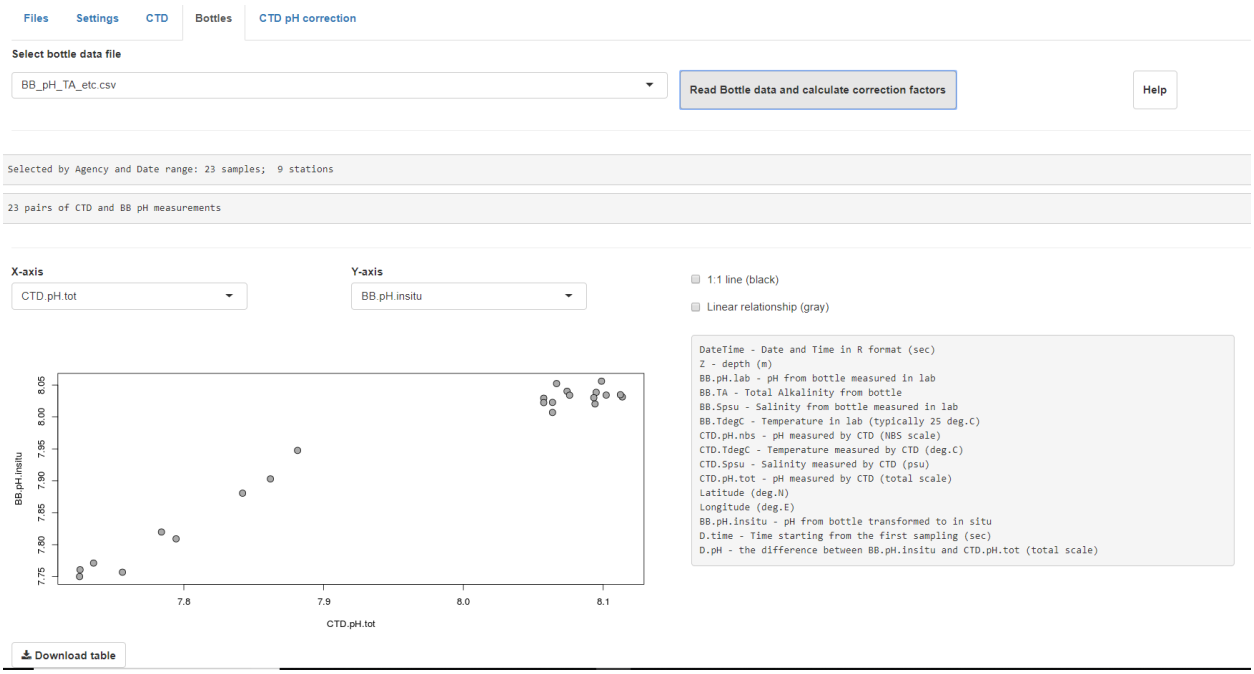
After BB data are read, the program constructs a table with the values which would be used to calculate pH correction factors. The table contains the measurements from BB file (Z, BB.pH.lab, BB.TA.lab, BB.Spsu.lab, BB.TdegC.lab) selected using the same Agency you selected for CTD data and the day/time within the period when CTD data were collected. The data which contain NA (not available) code in any field of BB file are excluded from the table (warning message is generated).

The values found in the CTD dataset (based on the same date-time, profile and depth) and included in the table are: CTD.pH.nbc, CTD.TdegC and CTD.Spsu. The samples for which CTD data are not found are excluded and a warning message is generated demonstrating the list of stations where no CTD data are found. Typically, it results from inconsistencies in time and depth in the BB table. Check these data and re-upload the data file.

Latitude and Longitude for each measurement are taken from the default station list (file Stn_coords.csv). If the station is not found in the list, it is excluded from the analysis (warning message is generated).

The following parameters are calculated:

- BB.pH.insitu - pH measured in the bottle samples transformed to in situ temperature and pressure.
- CTD.pH.tot – CTD.pH.nbs measured in "NBS" scale is transformed to "total" scale.
- D.pH - the difference between BB.pH.insitu and CTD.pH.tot
- D.time - Time starting from the first sampling (sec). We need it for assessment of sensor drift.

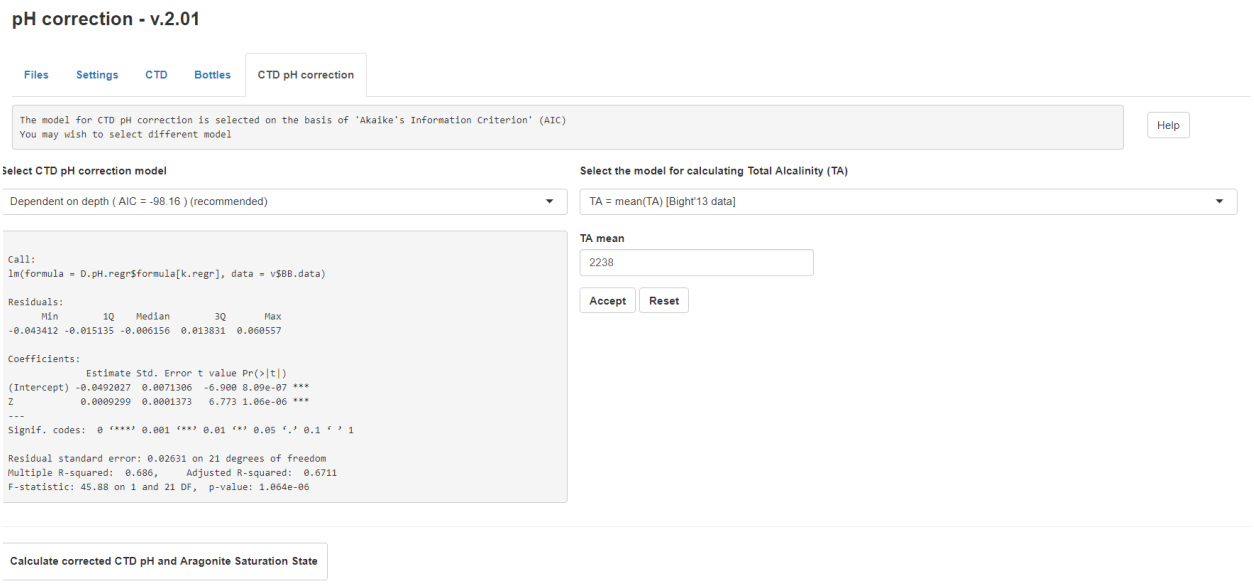


After BB data are read and the pH correction factors are calculated, you can visually analyze the relationship between each pair of parameters.

You can select the parameters for X and Y axes and add to the scatterplot two lines: 1:1 and linear regression (checkboxes).

If you wish to look at this data in more detail, click the Download table button to download it on your computer.

Click CTD pH correction tab.



The program suggests four models to calculate pH correction factors:

1) Independent of depth and time
2) Dependent on depth
3) Dependent on time (sensor drift)
4) Dependent on depth and time

The coefficients for each model are calculated from BB data (D.pH as a function of Z and D.time) and the models are compared using the Akaike's Information Criterion (AIC), which is used as an estimator of the relative quality of statistical models for a given set of data (Akaike, 1973). You can compare the models by selecting them from the drop-down menu and see their statistics (the output of the R-function summary.lm) in the panel to the right. The model with minimum AIC is selected as recommended for pH correction.

To calculate the Aragonite Saturation State, we need pH and one more parameter of the seawater carbonate system. The second parameter we have chosen is Total Alkalinity (TA), which can be estimated using four options:

1) Mean TA calculated from Bight'13 data (2238)
2) Mean TA calculated from the analyzed dataset
3) TA calculated from Temperature and Salinity using the Simone Alin's approach (Alin et al., 2012) from Bight'13 data
4) TA calculated using the same equation and Temperature and Salinity from the analyzed dataset.

Make a selection using the drop-down window to the right. It you prefer to use different coefficients, correct them and click 'Accept'. Click "Reset' to go back to calculated coefficients. In practice, the resulting Aragonite Saturation State (Omega) depends mostly on pH and only slightly on TA.

Click Calculate corrected CTD pH and Aragonite Saturation State button. Corrected pH and Aragonite Saturation State (Omega) are calculated for all selected CTD measurements.

Corrected pH is calculated as: CTD.pH.corr = CTD.pH.tot + CTD.D.pH, where CTD.D.pH is predicted from the model selected in the left drop-down menu. If you selected the model which AIC is not minimal, the program will warn you before calculating.

The Aragonite Saturaton State (Omega) is calculated from CTD.pH.corr and TA.calc using the R package 'seacarb' (Gattuso et al., 2018). TA.calc is calculated using the equation and coefficients from the right panel.

After CTD.pH.corr and Omega are calculated, the button Download results appears at the bottom of the screen. Click it to download the results to your computer. The table includes the following CTD data:

Agency, Season, Profile, StnID, DateTime, Z, TdegC, Spsu, Sigma, pH.nbs, pH.tot, pH.corr, Omega.

The difference between pH, pH.tot, pH.corr is that pH was measured by CTD sensor in NBS scale, pH.tot is pH transformed to "total" scale, and pH.corr is pH.tot plus the correction factor. The file is stored in ".csv" format. The file name starts from "CTD_" and includes the Agency, Year and Season.

After pH.corr and Omega are calculated, you can see their vertical profiles at the plot (CTD tab). You also can compare BB.TA (measured in bottle samples) with BB.TA.calc (calculated using the selected equation and coefficients) at the XY plot (Bottle tab).

References

Akaike, H. (1973). Information theory and an extension of the maximum likelihood principle. In B. N. Petrov & F. Csáki (Eds.), *2nd International Symposium on Information Theory, Tsahkadsor, Armenia, USSR, September 2-8, 1971* (pp. 267–281). Budapest: Akadémiai Kiadó.

Alin, S. R., Feely, R. A., Dickson, A. G., Hernandez-Ayon, J. M., Juranek, L. W., Ohman, M. D., & Goericke, R. (2012). Robust empirical relationships for estimating the carbonate system in the southern California Current System and application to CalCOFI hydrographic cruise data (2005-2011). *Journal of Geophysical Research-Oceans, 117*(C05033). doi:Doi 10.1029/2011jc007511

Chang, W., Cheng, J., Allaire, J. J., Xie, Y., & McPherson, J. (2017). shiny: Web Application Framework for R. R package version 1.0.3. Retrieved from https://CRAN.R-project.org/package=shiny

Gattuso, J.-P., Epitalon, J.-M., Lavigne, H., & Orr, J. (2018). seacarb: Seawater Carbonate Chemistry. R package version 3.2.6. Retrieved from https://CRAN.R-project.org/package=seacarb

McLaughlin, K., Nezlin, N. P., Weisberg, S. B., Dickson, A. G., Booth, J. A. T., Cash, C. L., . . . Terriquez, L. (2017). An evaluation of potentiometric pH sensors in coastal monitoring. *Limnology and Oceanography: Methods, 15*(8), 679–689. doi:10.1002/lom3.10191

APPENDIX D- 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, and samples for the calcifying phytoplankton coccolithophores.

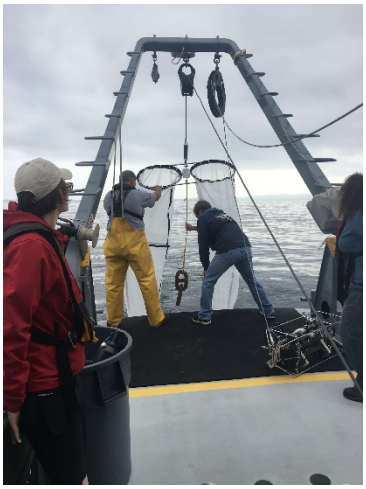
1. **Conduct a CTD Cast.** A CTD cast should be done once on station. The CTD should record water column profiles of temperature, salinity, dissolved oxygen, chlorophyll fluorescence, CDOM, and pH to 150 m depth (100 m if line is an issue).

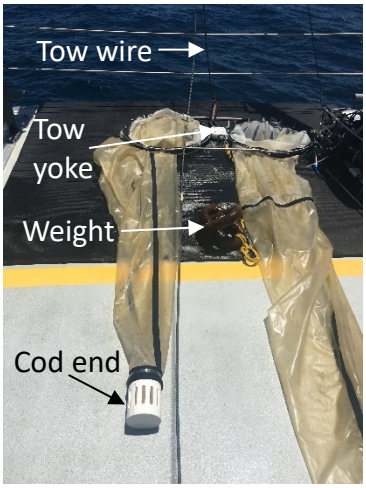
2. **Collect Water Samples for pH and TA.** A bottle sample for pH and total alkalinity (TA) should be collected from the surface and from 150 m (or as deep as you can go with your line). Bottle samples should be collected into 250 or 500 mL glass bottles (not plastic). Pyrex bottles are recommended (Fisher Scientific 02-940D), particularly if 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 or seal the lid with either electrical tape or parafilm to minimize gas exchange and store bottle samples at room temperature (not frozen) until analysis. The Dickson Lab protocol is provided in Appendix E as an example.

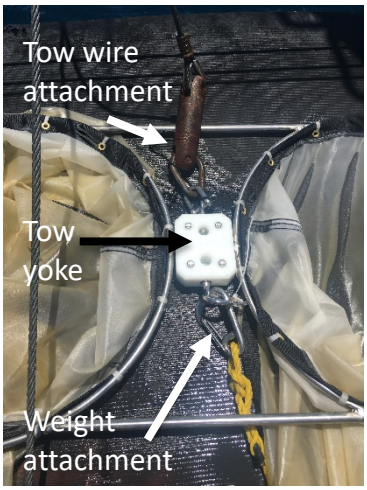
3. **Collect Water for Coccolithophore Study.** Four liters of water should be collected at the surface for the Coccolithophore special study. If a rosette with a live CTD wire is available, please also collect and preserve 4 liters of water at the deep chlorophyll maximum. Coccolithophore samples should be collected in bottles with buffered formaldehyde (2%), provided UCLA, and stored on ice until they can be retrieved by the UCLA team.

B. 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. SCCWRP can provide nets and weights. 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.







C. Towing

The 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 at 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.

1. **<u>Net Overboard:</u>**
   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 and 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.

2. **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.

3. **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.

4. **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.

5. **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.

6. **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.

7. **Net onboard:**
   a) While the net 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.

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. Tows do not need to be repeated if the maximum depth achieved is 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. Most likely the only time a net tow would need to be repeated would be if the net hits the bottom. In this case, rinse the net out well and redeploy.

D. 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 250 mL wide-mouth HDPE plastic sample jar (supplied by SCCWRP). One will be preserved with Ethanol (supplied by the agency) and the other with Zymo DNA/RNA Shield (supplied by SCCWRP). 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. The objective is not to collect a quantitative sampling off all organisms present in the net, but to focus collection on the shelled organisms in the sample.

To collect the cod end in 250 mL 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 (e.g., https://www.coleman.com/hard-thermoelectric-coolers/28-quart-cooler/Cooler28Q.html.) 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 the 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 the both the end of the net and the cod end.
    a. Note: pteropods and crab larvae 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 on 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 250 mL 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 material settles. Continue to decant until the sample is down to ~50 mL (1/5 of the sample jar) for ethanol or 100 mL

for Zymo fluid. If there are more organisms than will fit in these volumes, multiple jars will need to be used. This is because ethanol must be added in a 1:4 ratio of sample to ethanol and Zymo in a 1:1 ratio.

9. Steps 5-8 should be repeated for both coolers/samples, resulting in (at least) two separate samples, each in their own 250 mL sample jar.
10. Fill the remaining sample volume with preservation solution
    a. One cod end is preserved in 190 proof ethanol in a part sample, 4 parts ethanol ratio
    b. The other cod end is preserved in RNA preservation solution (Zymo DNA/RNA shield) in a 1 part sample to 1 part Zymo ratio.
11. Ensure each sample jar is labeled, and any previously written label has 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 beaker and cod ends with filtered seawater hose to prepare for the next tow.

E. 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 1 day) to prevent shell dissolution from the preservation solutions. Buffering can occur in the laboratory after samples are returned. Once buffered, samples can be maintained in the refrigerator until analysis.

Wire Angle Table: Match up wire angle with target depth to find 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

Bight '18 Ocean Acidification Biological Impacts Assessment
Field Data Sheet

Station Occupation:

Date	
Agency	
Site ID	
Latitude	
Longitude	
Depth (m)	
Arrival Time	

Weather (check one)				**Sea State**	
Clear		Rain		Calm	
Overcast		T-storm		Choppy	
Partly Cloudy		Fog		Rough	
Drizzle		Fog+Drizzle		Confused	
Hazy		Smoky			

Water Column Chemistry

CTD Cast

Max Depth (m)	
Time Complete	

Water Samples (check if collected)

Sample	**pH**	**TA**	**Coccoliths**
Surface			
100 m			

Bongo Net Tow Location

Net Position	**Time (hh:mm)**	**Latitude**	**Longitude**	**Depth**
Net Overboard				
Net Onboard				

Net Configuration

Approximate Depth	**150 m**	**100 m**	**50 m**	**25 m**
Estimated Net Angle				
Wire Out				
Time Towed				
Boat Speed				

Tow Failure (Y/N)?	
Re-tow (Y/N)?	

Comments:_____

_____

_____

APPENDIX E- Dickson Laboratory Procedure for Collecting Water Samples for Carbonate Chemistry

Overview of procedures

Samples are to be collected in Pyrex reagent bottles and are sealed using a greased glass stopper secured with a rubber band and clip. Samples are to be poisoned with a small volume of a 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 two 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.*

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 from 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 being 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. Once again, pinch off the tubing so that water only slowly comes out of the Niskin. Slowly lower the bottle to remove the drawing tube, leaving the bottle full to the brim.

Creating an ~1% air space

Place the bottle in the polyethafoam holder and using the 20 mL syringe, draw out one full syringe volume. by slowly pulling the piston up to slightly above the 20 mL line (where it will stop). Set the syringe aside and proceed quickly to the next step.

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 has filled with solution. 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 adheared 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.

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.

Some additional notes

Should the dispensing grease gun fail, the beads of grease can be applied to the stopper using the 20 mL syringe, which has also been filled with the Apiezon-L grease. 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 don't 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.