Southern California Bight
2013 Regional Marine Monitoring Survey
(Bight'13)

Nutrients Field Operations Manual

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Prepared for:
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I. Introduction

A. Background and Study Objectives

The overall goal of the Bight'13 Nutrients Study is to determine the extent to which anthropogenic nutrients are altering ecological conditions in the SCB. There are three main areas of interest that will be the focus of the Bight '13 Nutrient study, (1) historical analysis of algal blooms, (2) acidification and low pH (aragonite saturation state), and (3) ecological processes (biological productivity, concentrations of dissolved oxygen and nutrient cycling). Previous studies have documented some effects to primary production however, the specific role of anthropogenic or local nutrients in the decline in dissolved oxygen concentrations and low pH have not been examined in the SCB. Primary productivity and nutrient cycling can have direct and indirect effects on dissolved oxygen and pH measurements at local scales and it is unclear the extent to which these processes are influenced by anthropogenic nutrients. The Bight '13 Nutrients Program is designed to focus on these aspects.

There are three specific objectives of the program:

1. Determine the frequency, spatial extent and seasonality of algal blooms (high chlorophyll features), in the SCB.
2. Develop or refine existing algorithms to estimate total alkalinity and aragonite saturation; determine the spatial patterns and seasonality of pH and aragonite saturation state in the SCB.
3. Determine how anthropogenic nutrient inputs affect ecological processes and rates that drive biological productivity, concentrations of dissolved oxygen, and aragonite saturation state.

Objective 1 will be accomplished by analyzing historical chlorophyll data collected as part of routine monitoring by the POTWs, CalCOFI and SPOTs programs. Additionally, when available, discrete chlorophyll *a* samples collected during previous programs will be used to create a calibration coefficient that will be used to correct the chlorophyll fluorescence datasets. This analysis will focus on the trends of algal blooms with respect to the (1) frequency, (2) spatial extent and (3) seasonality.

Objective 2 purpose is to improve the quality of pH data collected during POTW ocean monitoring programs. The collection of discrete samples of pH and alkalinity at a subset of stations during the POTW NPDES monitoring programs will be used to calibrate *in situ* pH CTD measurements and refine an existing or develop a new empirical model to estimate total alkalinity and aragonite saturation and to determine the 2-year spatial and seasonal patterns in the SCB.

Objective 3 will be accomplished by conducting biogeochemical field experiments to determine key rates of primary production and respiration processes and nitrogen and carbon cycling. The use of stable isotopes to trace nitrogen sources in dissolved seawater as well as in algal and bacterial biomass will also be utilized. These chemical

and biological rates will be used to populate, refine and validate models used by University of California, Los Angeles collaborators. These models will be used to determine if anthropogenic nutrients are having significant effects on primary production, hypoxia and acidification.

II. Conceptual Overview of Field Survey

The sampling design for Bight'13 Nutrients field program will focus on objectives 2 & 3 (Objective 1 is an analysis of existing historical data and therefore is not part of the field survey). The two main field study components are 1) spatial patterns of pH and aragonite saturation, and 2) biogeochemical process studies and nutrient source tracking.

A. Spatial patterns of pH and aragonite saturation state

The spatial patterns of pH, total alkalinity and the calculated parameter, aragonite saturation state, will be determined quarterly from new data collected over 2 years. CTD pH, temperature, salinity, and dissolved oxygen will be combined with discrete samples of pH and total alkalinity to generate a Bight-wide synoptic evaluation of pH and aragonite saturation state, each quarter for two years. Discrete pH samples will be used as *in situ* calibration points to correct CTD glass electrode pH measurements. Discrete total alkalinity samples will be used to ground-truth (and adjust as necessary) a published algorithm for estimation of *in situ* total alkalinity from CTD salinity and temperature records. The end result will be calibrated profiles of pH and total alkalinity collected synoptically throughout the SCB. Aragonite saturation state will be calculated from pH and total alkalinity data using a freely available software package (CO2Calc).

Discrete samples will be collected at ~25% of stations and at 2 to 3 depths per station for alkalinity and pH, coincident with pH, salinity, temperature, and dissolved oxygen profiles collected using a water column profiling package.

The goal of this study component is two-fold:

1) To generate synoptic maps each quarter of pH and aragonite saturation state to assess spatial variability and seasonal trends in acidification in the SCB.
2) To pilot methods to improve quality of pH data, as well as evaluate the potential of collecting data on aragonite saturation state (a master variable to understand impacts of acidification) in POTW ocean monitoring programs. The collection of discrete samples of pH and total alkalinity at a subset of stations during the POTW NPDES monitoring programs, and the results of the *in situ* calibration exercise, will be used to develop and evaluate these protocols.

B. Biogeochemical Process Studies and Nutrient Source Tracking

The combination of field observations and experimental studies will be used to characterize the sources and fates of nitrogen in an effluent impacted area, the San Pedro Area (LACSD and OCSD monitoring locations) as well as a minimally-impacted area (Camp Pendleton) and an offshore site. Figure 1 shows a map of the sites to be sampled for both biogeochemical experiments and rates and nutrient source tracking and Table 3 lists the measurements that will be collected. Collectively, these datasets will be used to elucidate the sources and fates of nutrients at times when effluent discharge is anticipated to constitute minor (spring upwelling period) or major (late summer/fall) components of the total nutrient budget. The use of stable nitrogen isotopes offer a direct means of source identification because different sources of nitrogen (*e.g.* effluent, atmospheric nitrogen, chemical fertilizers etc.) often have distinct isotopic compositions or signatures. Thus utilizing the isotopic composition of nitrate and ammonia in the SCB can be used to identify point and non-point sources of nitrogen to the bight and/or the biological transformation of nitrate.

The goal of this study component to understand the influence of effluent nitrogen on coastal waters and how the nature of this nitrogen affects the response of the biological community. The study objectives are:

1. Identify the relative contribution of different nitrogen sources that are being utilized by phytoplankton and bacteria in effluent impacted area (Los Angeles and Orange Counties), and contrast those results with minimally-impacted regions, both along the coastline (Camp Pendleton) and offshore (i.e. mostly natural nutrient sources; anthropogenic inputs minor).
2. Conduct process studies to determine key rates of primary production, respiration, nitrogen uptake and nitrification in effluent impacted (Los Angeles and Orange Counties) and minimally-impacted regions (Camp Pendleton and offshore).

Data generated from these study components will be used in a future study to calibrate oceanographic models of SCB ecological response from natural and anthropogenic nutrient inputs. These models will be used to quantify the extent to which anthropogenic nutrients are affecting primary production, acidification and hypoxia, as well as which regions are most at risk. They will also be used to analyze management scenarios to understand the effects of anthropogenic nutrient load reductions relative to climate change scenarios.

III. Program Organization

A. Program Chain of Command

Table 1 List of personnel and contact information

Name	Organization	Contact Information	Area
George Robertson	Orange County Sanitation District	(714) 593-7468 grobertson@ocsd.com	Co-Chair, Nutrients Committee CTD Field Surveys
Meredith Howard	SCCWRP	(714)-755-3263 mhoward@sccwrp.org	Co-Chair, Nutrients Committee, Program Coordination Historical algal bloom analysis subcommittee lead, Biogeochemical process study and source tracking
Karen McLaughlin	SCCWRP	(714)-755-3242 karenm@sccwrp.org	Acidification subcommittee lead, biogeochemical process study and source tracking
Mike Mengel	Orange County Sanitation District	(714) 593-7465 mjmengel@ocsd.com	CTD Field Surveys
Ashley Booth	City of Los Angeles	(310) 648-5317 ashley.booth@lacity.org	Analyst for historical algal bloom analysis

B. Permits

There are no additional permits needed for this field study.

IV. Quality Assurance/Quality Control Procedures

The QA/QC procedures are described in detail in each section.

V. Spatial patterns of pH and aragonite saturation state

A. Study Design

The aragonite saturation state of the SCB (and thus acidification status) will be calculated from measurements of pH and total alkalinity collected on POTW monitoring events. Measurements will be collected synoptically each quarter for two years. Discrete measurements of pH and total alkalinity at a subset of locations (~25% of monitoring sites) and a subset of depths (2-3 at each substation) will be used to calibrate *in situ* measurements collected from the ships CTD package. Sub-stations and depths were selected to be representative of the region, focusing specifically on generating several transects from the nearshore to offshore in each region that were spatially distributed around each outfall.

Discrete pH measurements made in the laboratory will be used to calibrate glass electrode CTD data, thereby improving its accuracy for to develop spatial maps of pH in the SCB. Discrete total alkalinity and salinity measurements made in the laboratory will be used to calibrate an

empirical model which estimates total alkalinity from *in situ* temperature and salinity. This empirical model will be applied to CTD salinity and temperature data to generate detailed maps of total alkalinity in the SCB. The corrected pH and estimated total alkalinity data will be used to develop comprehensive maps of aragonite saturation state (a calculated parameter) for the SCB.

B. Description of Field Teams and Activities

i. Personnel and chain of command

Field sampling will be conducted by each agency, after training by SCCWRP personnel.

ii. Communications and important contact information

All questions regarding the acidification subcomponent field sampling and data analysis should be directed to Karen McLaughlin at SCCWRP. Contact information for McLaughlin is as follows: email: karenm@sccwrp.org; Tel: (714) 755-3242; Cell: (949) 786-7047.

Table 2 Agency Lead contact information for B'13 Nutrient Surveys

Name	Agency Representing	Contact Information	Area
Ashley Booth	City of Los Angeles, Environmental Monitoring Division	(310) 648-5317 Ashley.booth@lacity.org	CTD Field Surveys
Scott Johnson	City of Oxnard/ Aquatic Bioassay Laboratories, Inc.	(805) 643-5621 scott@aquabio.org	CTD Field Surveys Contract Ship – North (Santa Barbara)
Mike Kelly	City of San Diego Metropolitan Wastewater Department, Environmental Monitoring and Technical Services Division	(619) 758-2342 mkelly@sandiego.gov	CTD Field Surveys
George Robertson	Orange County Sanitation District	(714) 593-7468 grobertson@ocsd.com	CTD Field Surveys
Alt. Mike Mengel Vessel: Nerissa		(714) 593-7465 mjmengel@ocsd.com (714) 307-9146	
Alex Steel	Los Angeles County Sanitation Districts Ocean Monitoring & Research Group	(562) 699-7411 x2812asteele@lacsd.org	CTD Field Surveys

C. Field Data and Discrete Sample Collection

i. Station locations and sample schedule

CTD casts will be collected at every station in each agencies' regular monitoring grid. Discrete samples will be collected at a subset of these stations (~25%) and at a subset of depths at each station (2-3). All field sampling will take place between May 2014 and April 2016. Sampling will occur quarterly for 2 years. Agencies may elect to collect additional, event based samples during wet weather at major river mouths as resources allow.

The ship-based indicators to be sampled are listed in Table 3. Discrete samples will be analyzed by an outside contract laboratory (Andrew Dickson's Laboratory at Scripps Institution of Oceanography).

Table 4 summarizes the number of stations for sample collection by agency and the total number of discrete samples that will be collected. Appendix 1 lists all of the NPDES stations for each agency and indicates the stations and depths where pH and alkalinity samples will be collected.

Table 3 List of ship-based indicators to be collected during the regular NPDES permit monitoring surveys.

Component	**Indicator/Analyte**
CTD profile	Temperature Salinity Dissolved oxygen Turbidity Fluorescence (for chlorophyll *a* and colored dissolved organic matter, CDOM) pH
Discrete water samples	Total Alkalinity pH

Table 4. The number of stations at which discrete samples will be collected for the spatial patterns of pH and aragonite saturation component of the study during the NPDES permit monitoring programs.

Responsible Agency	Number of CTD Stations for each survey	Number of stations for discrete sample collection	Number of discrete samples per survey	QA/QC (5%)	Total number of samples to be collected (Quarterly for 2 years)
City of Oxnard/ ABC Labs	45	9	24	2	208
City of Los Angeles	54	12	28	2	240
LACSD	48	11	24	2	208
OCSD	66	15	39	2	328
City of San Diego	76	17	38	2	320

ii. Equipment

For *in situ* watercolumn physio-chemical profiles, a standard CTD package (SeaBird or equivalent) is required with probes for measurement of depth, temperature, salinity, dissolved oxygen, pH, and chlorophyll fluorescence. Quality Assurance and Quality control procedures for CTD casts are described in Section VII.

Discrete sample collection of pH and total alkalinity will require a Niskin water sampler (or similar) deployed on either a depth calibrated line or on a rosette paired with the CTD package. A sample collection kit will be provided by the Dickson Laboratory for collection of water samples from the Niskin bottles.

iii. Sample preparation/collection protocols

Standard Operating Procedures for collection of discrete samples, including pre-sampling preparation, all field collection procedures, and post collection processing to store acidification samples for laboratory analysis are detailed in Appendix 2. All samples will be treated with mercuric chloride for preservation. A sample collection training will be held at SCCWRP prior to the first sampling period in order to ensure proper collection of all samples.

iv. Field data sheets

Field data collection consists of recording the sampling date, station ID, and depths of discrete sample collection, general observations (e.g. contamination in the samples), and sample ID number. The original field data sheets will be maintained at SCCWRP.

v. Sample delivery and holding times

Samples preserved in mercuric chloride will be stored in provided storage containers and stored at room temperature until they can be transported to SCCWRP or directly to the Dickson Laboratory at Scripps. Karen McLaughlin will be responsible for coordinating subsequent delivery of all samples to the Dickson Laboratory as necessary, accompanied by a chain of custody form. Because the samples are preserved, there is no prescribed holding time. However, samples should be received by the Dickson Lab within two weeks of sample collection to insure the data is reported in a timely fashion.

Mercuric Chloride is Hazardous Material, all participants should be aware of the risks in handling this substance (see MSDS in Appendix 3). Extreme caution should be exercised when handling the stock solution of saturated mercuric chloride. The volume of saturated solution has been limited to qualify for the hazardous materials small quantity exception (solutions less than 30mL); however to comply with safety standards, all personnel transporting the substance must be aware of the fact that they are transporting a hazardous material and carry a copy of the MSDS. Saturated solutions must be transported in secondary containment (provided). Seawater samples preserved with mercuric chloride also meet the small quantity exception for highway transport (mercuric chloride in water solutions at concentrations of 0.004% by weight or less or less). Boxes containing the saturated mercuric chloride solution as well as boxes of preserved seawater samples should be labeled (to identify compliance with U.S. Department of Transportation regulations):
"This package conforms to 49 CFR 173.4 for domestic highway or rail transport only."

vi. Laboratory Analysis

Laboratory analysis of pH and total alkalinity will be done at Andrew Dickson's Laboratory at Scripps Institution of Oceanography, using established analytical methods. The Dickson lab shall analyze each sample for its pH, measured at 25 °C using a spectrophotometric pH method together with the indicator dye: m-cresol purple (Carter et al., Limnology and Oceanography Methods, Vol. 11, pp. 16-27, 2013). They shall also determine the total alkalinity of the sample using a previously published open-cell titration method (Dickson et al., Marine Chemistry, Vol. 80, pp. 185–197, 2003), and shall determine the salinity of the sample using a standard comparison of its conductance ratio to that of standard seawater.

vii. Submission of laboratory analytical results and field data

The Dickson Laboratory will submit an electronic copy of the analytical results to SCCWRP within 3 months of sample acquisition. Data format will be pre-determined Bight '13 data formats.

D. Quality Assurance/Quality Control Procedures

Standard quality assurance/ quality control procedures apply for the collection, analysis and data management of acidification samples. QAQC procedures are detailed below for each applicable component of data collection and management.

i. Field Data Collection

Duplicate samples will be collected for a minimum of 5% of total samples to estimate the precision of the measurement equipment.

ii. Sample Processing and Laboratory Analysis

Samples arriving at SCCWRP for transport to Scripps will be transferred through chain of custody forms. All samples will bear identification labels that match information entered onto field data sheets by field crews. Labeling information for all samples will include the following:

- Project name (Bight ’13 Nutrients)
- Date
- Time
- Sampling Site and Depth
- Collector’s initials
- Sample ID number
- Parameters to be analyzed
- Preservation

Water samples will be accompanied by hard copy of field data sheets that identify the appropriate site information. Hard copies of the field data sheets will be maintained in a project notebook by the sub-component coordinator (McLaughlin). Field data sheets and chain of custody forms (CoCs) will be filled out by field teams. CoCs will accompany the samples delivered to the laboratories.

Water samples will be preserved on ship immediately after collection. Once sample containers are filled, they will be preserved by addition of mercuric chloride, capped, placed in the shipping containers provided and stored at room temperature and out of direct sunlight. Samples should be transported to SCCWRP or directly to the Dickson lab within 2 weeks of collection. For each set of samples that arrive from an agency, a set of field duplicate will be prepared for each analyte. Additional QA samples will be requested of the laboratory, including laboratory duplicates and equipment blanks.

Unless otherwise stated, containers for total alkalinity and pH discrete samples will be Pyrex 500 mL glass bottles with matching coarse glass frits that must be sealed with apezion grease and secured with a rubber band fastener. Table 5 gives the volumes, containers and holding times associated with the acidification parameters.

Table 5. Sample Handling and Custody

Parameter	Matrix	Container	Volume	Initial Preservation	Holding Time
pH and total alkalinity	Whole Water	Pyrex Glass Bottle	500 mL (~1% headspac e)	Preserve with 120 uLof mercuric chloride, store at room temperature	6 months at room temperatur e

Transport of the samples to the analytical laboratory will be coordinated by Karen McLaughlin to ensure that all samples are handled properly and delivered to the Dickson lab in a timely manner. CoCs will be reviewed by personnel at the receiving laboratories to ensure that no samples have been lost in transport. All sample material will be properly and safely disposed of by the analytical laboratory once analyses are completed and all analytical quality assurance/quality control procedures have been reviewed and accepted.

Any failures (e.g., instrument failures) that occur during data collection and laboratory analyses will be the responsibility of the field crew or laboratory conducting the work, respectively. Laboratories will be responsible for conducting analyses, or implementing appropriate preservation measures, within holding times as arranged ahead of time by the Project Manager. Laboratory turnaround times will be sufficiently timely to allow for QA checks of the data, entry into the database, analysis, and reporting by the project team in order to meet project planning and deliverable deadlines.

The Dickson lab shall analyze each sample for its pH, measured at 25 °C using a spectrophotometric pH method together with the indicator dye: m-cresol purple (Carter et al., Limnology and Oceanography Methods, Vol. 11, pp. 16-27, 2013). They shall also determine the total alkalinity of the sample using a previously published open-cell titration method (Dickson et al., Marine Chemistry, Vol. 80, pp. 185–197, 2003), and shall determine the salinity of the sample using a standard comparison of its conductance ratio to that of standard seawater. Once all these analyses have been completed, they will send a report detailing the results, and indicating their likely uncertainty (based on their laboratory quality control procedures). Laboratory analysis data quality objectives for precision, accuracy, and percent recovery have been defined in the Table 14. These quality assurance objectives will be used as comparison criteria during data quality review by SCCWRP to determine if the minimum requirements have been met and the data may be used as intended.

Table 6. Data quality objectives for discrete samples

Constituent	Method	Units	Precision / RPD	Accuracy (value/%)	Recovery[1] (%)	Complete-ness (%)
pH	Carter et al. 2013	pH units	25	80-120%	80-120%	90
Total Alkalinity	Dickson et al. 2003	□mol/kg	25	80-120%	80-120%	90
Salinity	Dickson et al. 2003	ppt	25	80-120%	80-120%	90

[1]RPD Relative Percent Difference

iii. Data management

Quality assurance procedures for data management are specified in SCCWRP's Bight Information Management Plan. These procedures will be followed to ensure that data collected under the study are of highest quality possible.

VI. Biogeochemical Process Rates and Nutrient Source Tracking

A. Study Design

The combination of field observations and experimental studies will be used to characterize the sources and fates of nitrogen in an effluent impacted area, the San Pedro Area (LACSD and OCSD monitoring locations) as well as a minimally-impacted area (Camp Pendleton) and an offshore site.

B. Description of Field Teams and Activities

i. Personnel and chain of command

Field sampling will be conducted by SCCWRP personnel under the direction of Meredith Howard and Karen McLaughlin. Meredith and Karen will coordinate and supervise all field sampling operations, develop field sampling protocols, provide training, as necessary, to additional SCCWRP personnel, and act as the primary contact persons for all biogeochemical process rates and nutrient source tracking field sampling activities.

ii. Communications and important contact information

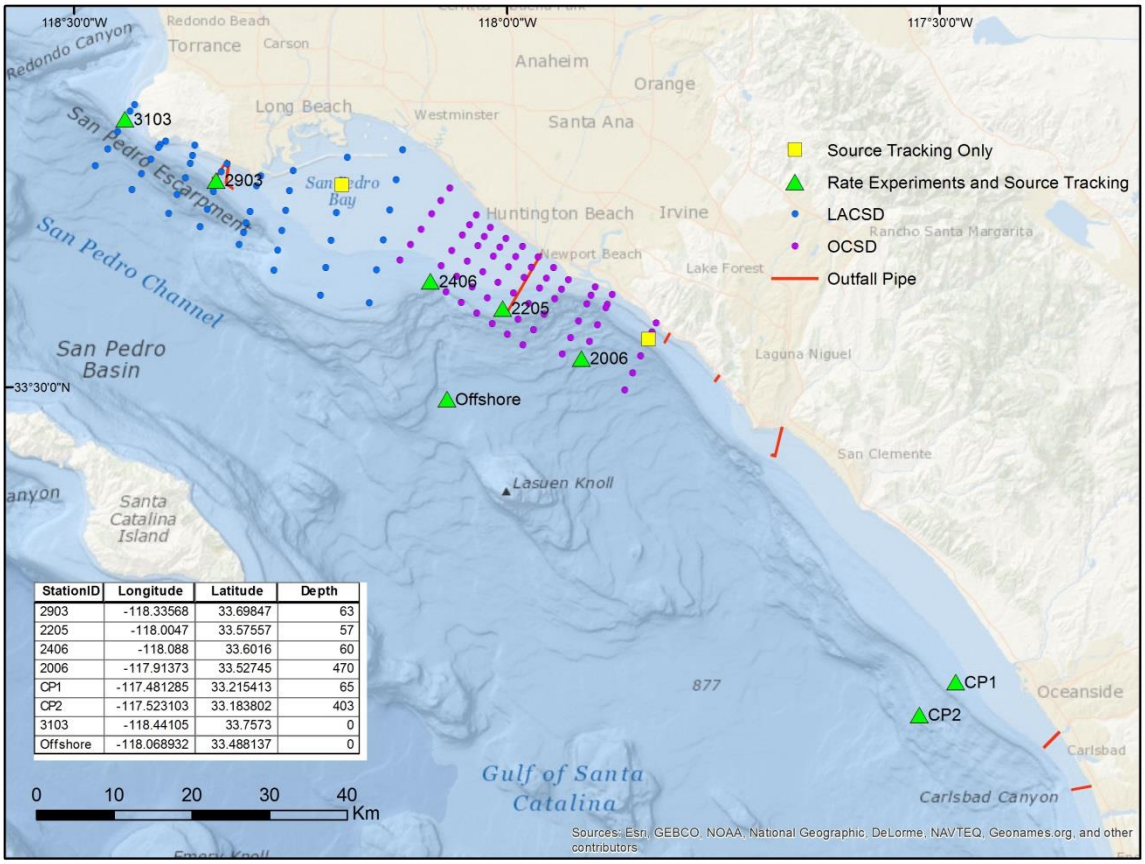
All questions regarding field sampling and data analysis should be directed to Meredith Howard at SCCWRP. Contact information for Meredith is listed in Table 1.

C. Field and Discrete Data Collection

i. Station locations and sample schedule

Survey cruises will be conducted twice a year during the spring and the fall period beginning in the Fall 2014 and ending in the Fall 2016. Cruises will be conducted aboard the POTW vessels if logistically possible (City of San Diego will provide shiptime to sample the Camp Pendleton area). SCCWRP personnel will collect all samples for this study component. The 3 areas will be sampled in 3 different weeks in order to allow for cleaning and maintenance of experiment equipment. These cruises will not be part of the regular NPDES permit survey cruises.
Figure 1 is a map of the tentative station locations for the study. The OCSD grid shows all of the potential stations, however, rate experiments will only be conducted at 2 stations within the OCSD grid, mainly 2205 and either 2406 or 2006, to be determined on the day of sampling depending on the direction of the currents. Table 7 summarizes the number of stations and depths in each area for each study component and Table 8 summarizes the indicators to be collected.

Figure 1. Map of the station locations for the biogeochemical rate experiments and the nutrient source tracking samples for Los Angeles County Sanitation Districts (LACSD), Orange County Sanitation District (OCSD), and Camp Pendelton (CP).



StationID	Longitude	Latitude	Depth
2903	-118.33568	33.69847	63
2205	-118.0047	33.57557	57
2406	-118.088	33.6016	60
2006	-117.91373	33.52745	470
CP1	-117.481285	33.215413	65
CP2	-117.523103	33.183802	403
3103	-118.44105	33.7573	0
Offshore	-118.068932	33.488137	0

Table 7. List of number of stations in each location for the process studies and source tracking components.

Category	Number of Stations (number of depths)		
	Source Tracking	Process Study Rates (Nitrification, production, respiration)	Nitrogen uptake Rates
Effluent Impacted			
LACSD Grid	3 (4)	2 (4)	1 (1)
OCSD Grid	3 (4)	2 (4)	1 (1)
Minimally Impacted			
Camp Pendleton	2 (4)	2 (4)	1 (1)
Offshore	1 (4)	1 (4)	1 (1)
Total	9 (16)	7 (16)	4 (4)

ii. Discrete Samples

Source Tracking Analysis: Discrete samples for nitrogen concentrations in different pools (dissolved inorganic, dissolved organic, and particulate), and for stable isotopic composition of the dissolved and particulate fractions will be collected on the ship surveys in order to determine the nutrient sources present in coastal waters as well as which sources are being utilized by phytoplankton and bacteria. These samples will be collected at 9 stations, 6 in the effluent impacted area (3 in each of the LACSD and OCSD grids), 2 in the minimally-impacted area, and 1 from an offshore location. Discrete samples will be collected at 4 depths at each station (where possible). The isotopic source tracking samples will be used with CTD colored dissolved organic matter (CDOM) data in order to determine where the effluent plume was located during the ship surveys. The relative contribution of nitrogen from effluent and upwelling in the SCB will be assessed by comparing the natural abundance stable isotopic composition of *in situ* waters (i.e. seawater samples) with those of the distinct nutrient sources. POTW effluent will be collected on the day of the cruises and the upwelling source sample will be collected at the offshore station. Atmospheric deposition samples were collected as part of a different study. Figure 1 shows the locations of the sampling sites, Table 8 summarizes the indicators to be collected and Table 7 summarizes the number of stations and depths in each area for each study component.

Samples to be analyzed for the isotopic composition of nitrate samples will be filtered through a 0.45 µm filter and the filtrate stored frozen until analysis. The preparation and isotope analysis ($\delta^{15}N$, $\delta^{18}O$) of dissolved nitrate in water will be

assessed by using a bacterial denitrification assay (Casciotti et al. 2002). Isotope ratios of $^{15}N/^{14}N$ and $^{18}O/^{16}O$ will measured using a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer at the Stable Isotope Facility at the University of California, Davis.

Samples to be analyzed for the isotopic composition of ammonium will be filtered through a 0.45 µm filter and acidified with ammonium-free sulfuric acid to a pH of 2 and frozen until extraction. Ammonium will be extracted onto glass fiber filter "traps" (Holmes et al. 1998, Hannon and Böhlke 2008) and the isotope ratios of $^{15}N/^{14}N$ will be measured using a coupled Costech Elemental Analyzer with a Finnigan Delta Plus Advantage in Continuous Flow Mode at the Marine Science Institute at the University of California, Santa Barbara.

Particulate organic matter collected from unfractionated seawater will be filtered onto pre-combusted glass fiber filters. The isotope ratios of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ will be measured using an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility at the University of California Davis. Samples are combusted at 1000°C in a reactor packed with copper oxide and lead chromate. Following combustion, oxides are removed in a reduction reactor (reduced copper at 650°C). The helium carrier then flows through a water trap (magnesium perchlorate). N2 and CO2 are separated using a molecular sieve adsorption trap before entering the IRMS.

Sample collection protocols are described in Appendix 4.

Table 8. List of indicators to be collected for the nutrient source tracking

Component	Indicator/Analyte
CTD profile	Temperature Salinity Dissolved oxygen Turbidity Fluorescence (for chlorophyll *a* and CDOM) pH
Discrete Samples	Dissolved inorganic nutrients (NO_3, NH_4, PO_4) Particulate nitrogen and carbon Total dissolved nitrogen and phosphorus $\delta^{13}C$ and $\delta^{15}N$ of particulate matter ($P^{15}N$) $\delta^{18}O$ and $\delta^{15}N$ of dissolved nitrate (Dissolved $^{15}NO_3$ abundance) $\delta^{15}N$ of ammonia (Dissolved $^{15}NH_3$ abundance)
Nutrient Source Samples	Effluent (NO_3, NH_4, $^{15}NO_3$, $^{15}NH_3$) Atmospheric samples ($^{15}NO_3$, $^{15}NH_3$) Deep offshore water samples (representative of upwelled water) ($^{15}NO_3$, $^{15}NH_3$)

iii. Rate Experiments

Process Studies: Three main types of bottle incubation experiments will be conducted with seawater collected during each survey cruise, designed to identify the fate of nitrogen in the San Pedro shelf ecosystem.

Nitrification Rates

Nitrification rates will be determined by measuring the accumulation of ^{15}N in the dissolved nitrate pool following addition of isotopically-enriched ammonium to bottle incubations (Santoro et al. 2010). Water samples will be collected into 500 mL acid-washed polycarbonate bottles which have been wrapped in black tape. An enriched (99%) tracer of $^{15}NH_4Cl$ will be added to a final concentration of 100 nM to two bottles and a third bottle without the tracer acts as a control. Bottles will be incubated in the dark to minimize nitrogen uptake by phytoplankton and as close to *in situ* temperature conditions as possible. Subsamples of 50 mL each will be collected at four time points (0, 12, 24, and 36 hours), filtered through 0.45 µm filters and frozen until analysis for dissolved nitrate, nitrite, ammonium and the isotopic composition of nitrate ($\delta^{15}N$, $\delta^{18}O$)

as described above. Nitrification rates will be determined by modeling the ^{15}N and ^{14}N contents of the combined nitrate and nitrite pool with inputs from the labeled ammonium pool and outputs through nitrate and nitrite uptake as described in Santoro et al. (2010). Data fitting will be done using non-linear least squares regression using the ^{15}N and ^{14}N values measured at each time point in Matlab (Mathworks).

Primary Production and Respiration Rates

Short-term incubations of natural plankton communities will be conducted to determine rates of primary production and respiration using radioactive ^{14}C labeled compounds, following the same protocols listed on the CalCOFI website (http://data.calcofi.org/). Seawater for experiments will be collected at 7 stations total (the same as the nitrification rate experiments) at 2 depths. These experiments will provide rates of primary production and rates of respiration and will be used in conjunction with the nitrogen source tracking results to determine if differences in rates exist between effluent impacted and non-impacted regions.

Triplicate samples (two light and one dark control) were drawn from each productivity sample depth into 250 ml polycarbonate incubation bottles. At the end of the incubation, the samples will be filtered onto Millipore HA filters and placed in scintillation vials and radioactivity will be determined using a scintillation counter in the SCCWRP isotope laboratory.

Nitrogen Uptake Rates

Short-term incubations of natural plankton communities will be conducted to determine the uptake rates of ^{15}N-labeled nitrogenous compounds (ammonium, nitrate) to characterize the nitrogenous physiology of the biological community as a whole and determine specific rates of uptake of the common nitrogen forms present (nitrate, ammonia). Experiments will be conducted within 24 hours of collection at *in situ* temperatures as described in Kudela and Cochlan, 2000. One station and one depth at the deep chlorophyll maximum will be selected in each region (LACSD grid, OCSD grid, Camp Pendleton area and offshore) in each season for this experiment. These experiments will be conducted at a site where nitrification, primary production and respiration are also performed.

iv. Field data sheets and sample labels

Field data collection consists of recording the station ID, depths, and sample volumes collected. The original field data sheets will be maintained at SCCWRP. The field data sheet is included in Appendix 6.

D. Sample Processing, Laboratory Analysis and Quality Assurance

Laboratory analysis of atmospheric samples will be done at one of the contract laboratories utilized by Bight 13 participating agencies, using established analytical methods as described previously.

Water samples will be accompanied by hard copy of field data sheets that identify the appropriate site information. Hard copies of the field and laboratory data sheets will be maintained in a project notebook by the field coordinator (Lisa), and by laboratory personnel, respectively. Field data sheets and chain of custody forms (CoCs) will be filled out by field teams. CoCs will accompany the samples delivered to the laboratories. Samples will be stored frozen at SCCWRP and will be shipped to the contract laboratories within 1 month of completion of the field work in that season (i.e. once all areas have been sampled in a season). Additional QA samples will be requested of the laboratory, including matrix spikes, laboratory duplicates, and equipment blanks.

E. Data management

Quality assurance procedures for data management are specified in SCCWRP's Bight Information Management Plan. These procedures will be followed to ensure that data collected under the study are of highest quality possible.

VII. CTD Data Collection and Vertical Profiles

i. Safety

Collection of samples in field surveys is inherently hazardous and this danger is compounded in bad weather. The safety of the crews and equipment is of paramount importance and each person working on board a vessel should take responsibility for his or her own safety as well as for those around them. Safety awareness by the captain and crew is the greatest single factor that will reduce accidents at sea. Each survey crew should follow established rules and provisions within their respective agency's safety program. Field personnel should be aware of Safety Data Sheets (SDS) for any hazardous materials that they are likely to encounter.

Sampling should be canceled or postponed during hazardous weather conditions. The final decision is made by the vessel captain, who is responsible for the safety of everyone on board. As with any field program, the first priority is the safety of the people on board, followed by the safety of the equipment, and the recovery of the data.

ii. Important Contact Information

Contact information for each agency involved in the offshore water quality surveys are listed in Table 2.

iii. Navigation

Accurate location of sampling sites is important to the success of all monitoring surveys. For standard operations, differential global positioning system (DGPS) is required; standard GPS is acceptable as a back-up in the event that the DGPS is down.

iv. Site Acceptability Criteria

The location of each station will be designated in advance as a set of coordinates (latitude and longitude). Most of these sites are either visited routinely by the various agencies are have been part of previous regional projects (e.g., Bight'98). In the advent a station has not been sampled previously, than upon arrival at the site, the depth at the station depth will be determined by fathometer. This will be regarded as the nominal station depth for all subsequent sampling at the station during the Bight'13 survey and will be used for calculating station acceptability if the station must be moved.

v. Cruise and Site Data

A specific set of cruise and site data should be recorded for every CTD survey. The following data should be recorded for each cruise: (1) date; (2) vessel; and (3) vessel crew, cruise leader and scientific party.

Each agency is responsible for maintaining a station log during each cruise. Coordinates of each station must be based on North American Datum 1983 (NAD 83) and should be expressed in degrees, minutes, and thousandths of a minute. Each vessel must also have a fathometer. Depth should be recorded for each station in meters and included with the navigation data. All samples must be collected within 100 m radius of the nominal station co-ordinates; a 50 m radius is the optimal distance. Coordinates, distance from nominal, and fathometer readings should be recorded electronically, if possible.

The following data shall be recorded at each sampling site:

1) Date;
2) Coordinates (latitude and longitude in degrees, minutes, and thousandths of a minute) of sampling site;
3) Time;
4) Depth (in meters);
5) Weather observations (sky, wind speed, and wind direction should be measured on an 8 point compass in degrees from magnetic north);
6) Sea conditions (swell height and direction, period).

Additional specific data to be collected are discussed under the Water Column Profiling section below.

A. Field Database Management

A field computer system has been developed for the Bight‘13 that includes the forms with all of the fields for all of the field data sheets. This system employs tablets, however, the use of the field computer system is optional during the Bight‘13 survey.

Data can either be entered into the computer while at sea, or it can be taken from the data forms at a later time. Although hard copies of all field data sheets are mandatory, these can either be hand-written or hard copy printouts from the computer.

The data entered into each field of the electronic forms is checked automatically by the software and it provides a warning when the data do not fall within an expected range. After entering the data into the field computer system, it will be printed out to hard copy and checked by the cruise leader against the original handwritten data sheets. Once the data have been checked, corrected (if necessary), and accepted by the cruise leader/agency lead (see Table 2), no further access to the data will be granted.

B. Water Column Profiling

i. Purpose

Water-column profiles are collected to characterize depth-related gradients in temperature, salinity, hydrogen ion content (pH), percent light transmittance, dissolved oxygen (DO), and fluorescence (chlorophyll *a* and CDOM). For instance, high chlorophyll fluorescence values may indicate the presence of phytoplankton. Water-column profiles can describe whether stratification (layering) is present and, if so, the depth of the thermocline or pycnocline. Variation in these parameters at the same depth among stations may indicate anthropogenic or natural perturbations of the environment.

ii. Equipment

A conductivity-temperature-depth profiler (CTD) with additional sensors will be used to provide a continuous (8-24 scans/second) water-column profile of temperature, salinity, dissolved oxygen, pH, percent light transmittance, irradiance, and fluorescence (for Chlorophyll *a* & CDOM) with depth. This instrument must meet the program performance specifications for temperature, salinity, DO, pH, transmissivity, and pressure (Table 9).

Table 9 The program performance specifications for temperature, salinity, DO, pH, transmissivity, and pressure

Parameter	Initial Accuracy/Sensitivity	Resolution
Conductivity	0.0003 S/m	0.00004 at 24 Hz
Temperature	±0.001 °C	0.0003 °C at 24 samples/sec
Pressure	0.01%	0.0001%
Dissolved Oxygen	2% of saturation	Not available
pH	0.1 pH	Not available
Light Transmittance	1.25 mV	Not available
Irradiance	$1x10^{17}$quanta/(cm^2·sec)	Not available
Chlorophyll *a* fluorescence	≥0.03 µg/L	Varies per sensor
CDOM fluorescence	0.100 ppb QSD	Varies per sensor
*Values obtained from manufacturer's specifications (SeaBird Electronics, WETLabs, and Biospherical Instruments.		

iii. Training

Any individual who will be maintaining, calibrating, or operating the CTD should be trained in each of these operations. Prior to performing these operations unsupervised, an individual should demonstrate proficiency in that operation to a senior, experienced agency staff member. Proficiency should be evaluated based on successfully completing the operation following written procedures and demonstrating an understanding of the equipment. Additionally, the individual should be evaluated on his/her ability to troubleshoot common problems. All training and demonstration of proficiency should be documented. An agency using and deploying a CTD should be an active participant in the Southern California CTD Users Group.

C. CTD Pre-Cruise Checkout and Calibration

i. Pre-cruise Equipment Checkout

A pre-cruise equipment checkout and calibration will be conducted prior to starting the cruise. This inspection should include the following:

1) A visual inspection of the CTD for any obvious defects;
2) A check of all metal components for corrosion, cleaning or replacing as necessary;
3) An inspection and cleaning of all connections with contact cleaner, as necessary;
4) Verification that the plugs are secure and waterproof and lubricated with silicone;
5) An inspection of all cables for nicks, cuts, abrasions, or other signs of physical damage;
6) A test of the CTD to see if connections and software work properly;

7) Cleansing and/or replacement of all accessory tubing as necessary; and
8) Checking battery status for all units using RAM data storage.

ii. Pre-cruise Calibrations

A pre-cruise calibration will be conducted less than or equal to 72 hours prior to starting the cruise for pH, percent light transmittance, and pressure. There is no required lab calibration for DO, conductivity, temperature, irradiance, and fluorescence (chlorophyll-*a* and CDOM). Verification that the proper sensor coefficients are in the configuration file should be made before proceeding. A CTD calibration data sheet will be prepared at that time, with all required information entered on that sheet.

Hydrogen Ion Content (pH):
The pH sensor may be calibrated by using commercially available buffer solutions. When sampling in the ocean it is best to use three buffers of pH 7, 8, and 9. The manufacturer's specifications should always be followed during calibration of the probe. For example, when calibrating the sensor, it may be necessary to make an electrical connection between the body of the pH sensor module and the buffer solution. This connection may be made using any convenient piece of wire. One end of the wire is attached to one of the screws attaching the zinc anode. The other end of the wire is immersed in the solution. It is important that the buffer is thermally equilibrated with the water bath; this is best accomplished by keeping the CTD in the water bath and using a holding bracket for the cup of buffer. The water temperature, pH, and voltage output for each of the three buffers is then recorded. These values are entered into the manufacturer's software and checked against three buffers, recording the pH values.

Transmissometer:
This calibration is performed in air. The transmissometer should be calibrated according to manufacturer procedures. The CTD software should be modified to reflect any changes that are made during the calibrations

Pressure Offset:
This determination should also be performed in air. The pressure sensor is checked before use, recording air sea-level values. The pressure reading in air at sea level should be a negative number between 0.00 and -0.60 db. If out of this range, adjustments should be made **to the offset value** and the manufacturer software should be rerun to achieve a value between 0.00 and -0.60 db. If this does not correct the displayed pressure value, the unit should be serviced. The pressure output and any changes made are recorded on the calibration sheet.

Following calibration, the sensors and equipment should be disturbed as little as possible.

iii. Factory Calibration and Maintenance

Maintenance and calibration of the CTD and/or specific sensors should be documented, including dates of most recent servicing. All sensors shall be calibrated by the manufacturer annually. Preventative maintenance should be conducted on the CTD unit periodically. Upon return from the factory, enter any new factory calibration coefficients and input where appropriate.

The temperature and conductivity sensor calibration should be conducted by the National Oceanic and Atmospheric Administration/National Regional Calibration Center (NOAA/NRCC) lab and certified and inspected by the manufacturer. Certification should be provided when the sensor is returned.

iv. Post-cruise calibration

Each agency lead is responsible for deciding whether post-cruise calibrations are within acceptable limits. The time between last cast and the completion of the postcalibration should be not more than 72 hr.

Hydrogen Ion Content (pH):
Agreement between sensor output and known values should be within 0.1 pH unit. The only similarity between the post-cruise and pre-cruise calibration of the pH sensor is that the sensor is checked against the three buffers with no adjustments being made. The water temperature should be recorded, as well as the pH and voltage output for each buffer. Agreement should occur between each sensor value and the known buffer value, and should be within 0.15 pH units. If agreement is out of this range, the unit should be recalibrated and the new calibration coefficients used for processing the data.

*Transmissivity and Pressure***:**
Post-calibration of the transmissometer and pressure sensors are the same as those performed in the pre-calibration. If the pressure reading in air at sea level is not a negative number between 0 and -0.60 db, record the pressure output and any changes on the calibration sheet.

v. CTD Deployment

The CTD should be deployed with a means of data storage, such as a deck or RAM (random access memory -- an internal recording instrument) unit. The instrument should be set for a scan rate of no fewer than eight scans/sec. If there is a risk of obtaining less than three scans/m, the deployment descent rate should be decreased.

The recommended optimum speed is 0.75-1 m/sec. The CTD descent rate should not be less than 0.25 meter/second or greater than 1.5 m/sec. If deploying real-time, some manufacturer software allows this rate to be monitored by displaying and viewing the lowering rate variable. If RAM is used during deployment, the rate should be monitored with a meter wheel and timer. Descent rates should always be slower than 1.5 m/sec to minimize spiking of sensor output.

The objective of water-column profiling is to collect water-column data for every meter. Therefore, to avoid omissions in data from a given meter of depth, it is recommended that the scan rate should not be less than 8 scans/sec and a descent rate less than 1 m/sec is to be used. Optimal scan value and descent rate are dependent upon sea surface conditions during deployment and should be evaluated and adjusted accordingly.

Before beginning a cast, the CTD sensors are brought to thermal equilibration with the ambient seawater. If applicable, the pump should be activated and bubbles should be purged from any tubing. This is best accomplished by lowering the CTD a few meters and (if capable) monitoring salinity and DO values to ensure their stabilization. In either case, a 3-min equilibration upon initial power-up at the first station and 90 sec at each station thereafter is the minimum soak time for thermal equilibration and sensor stabilization. After sensor stabilization and at least the 3 min or 90 sec, the CTD is raised so the top of the unit is at the water surface and profiling is begun.

Downcast data will be used for data processing; however, data should be logged throughout the entire cast to allow for recovering missed or poor quality cast data (e.g., 1 m surface values). The CTD should be deployed to within 2 meters of the bottom or to 75 meters if the station is deeper than this depth.

iv. CTD Cast Acceptability

The goal of monitoring surveys is to collect water-column profiles at all stations. During field sampling, cast acceptability should be determined immediately following the first cast of the day (it is recommended that this be done following each cast for real time data) in one of two ways:

1) All parameters can be displayed graphically to determine if any grossly anomalous readings occurred. Graphs can be scaled to illustrate obviously anomalous values that lie outside the control limit range for each parameter (10); or

2) A range-checking computer program can be used to evaluate the presence of anomalous values based on predetermined criteria (i.e. range acceptability checks).

Casts should also be evaluated by comparison of values obtained at previous or nearby stations.

Table 10 CTD parameter ranges. Source: 1998-2008 Central Bight Water Quality combined Winter and Spring surveys.

Parameter	Range	Mean
Conductivity (S/m)	1.1 – 4.5	3.9
Temperature (°C)	6.2 – 19.3	12.3
Dissolved Oxygen (mg/L)	0.5 – 15.2	6.4
Salinity (psu)	18.1 – 37.3	33.6
Transmissivity (%)	0.1 – 99.5	83.2
Density (σ-theta)	13.8 – 32.2	25.4
pH	6.5 – 8.6	8.0
CDOM	0.0 – 60.9	2.0
Chlorophyll-a	0.0 – 122.0	3.8

If anomalous values are present, the cause should be investigated and remedied before proceeding. If damage to the CTD (due to striking the bottom or some other event) is suspected, review that cast as described above to ensure acceptability. Further review of the subsequent cast in a like manner will ensure that all sensors are functioning properly. If a sensor is replaced during the day, a replicate cast should be made with the new sensor, at the last station at which the malfunctioning sensor was known to have been working properly. If a sensor is replaced, all coefficients for that sensor should be entered and saved in the configuration file. All activities relating to the occurrence of these types of events (e.g., repeated casts, damaged equipment and remedies, replaced sensors, etc.) should be noted in the field logbook. If feasible, a station should be resurveyed when an unacceptable profile is obtained.

v. CTD Quality Assurance/Quality Control

No field quality control (QC) of any of the parameters is required beyond the cast acceptability check described above or the range checks. Dissolved oxygen, pH, pressure offset, and transmissivity performance are carefully monitored and calibrated prior to and immediately following a survey. This evaluation is deemed sufficient to assure the quality of the performance. Conductivity and temperature are evaluated and calibrated on a strict factory maintenance schedule and traceable to NOAA/NRCC standards. Their performance and integrity from calibration to calibration are reliable to such a level that field QC is deemed unnecessary. The typical ranges are guidelines only and any value outside of them should be evaluated relative to the entire cast and the entire day's survey; legitimate values may exist outside of these ranges but the vast majority of values will fall within these ranges.

All data will be checked to be certain all data and configuration files are present and properly named. All data files should contain proper and complete header information.

This check should be verified and documented by field personnel. All data will be reviewed graphically and statistically for single point outliers (spikes) as well as trends.

vi. CTD Data

If CTD data are to be submitted to another agency, data will be output using a mutually agreed upon format. The format will be defined in the Bight'13 Information Management Plan. The three primary data types will be cast event data, bin-averaged cast data, and discrete sample depth data.

The following header information, parameters, units, and format is an example of the cast event data output:

1) Station ID
2) Latitude
3) Longitude
4) Sampling organization
5) Sample date
6) Cast time
7) Station depth
8) CTD method
9) Equipment
10) Cast number

The following header information, parameters, units, and format is an example of the water quality depth samples data output:

1) Station ID
2) Sample date
3) Sample number
4) Sample depth
5) Sampling organization
6) Comments

The following header information, parameters, units, and format is an example of the cast event data output:

1) Station ID
2) Sample Date
3) Start Of Cast Time
4) Temperature, ITS 90, °C
5) Conductivity, S/m
6) pH. Standard pH units
7) Beam Transmission, %
8) Beam Attenuation, 1/m
9) Elapsed Time, seconds
10) Chlorophyll, ug/L

11) Chlorophyll Voltage, volts
12) CDOM, ug/L
13) CDOM Voltage, volts
14) Depth, meters
15) Dissolved Oxygen, mg/L
16) Oxygen Saturation, mg/L
17) Percent Oxygen Saturation, %
18) Salinity, practical salinity units (PSU)
19) Density, sigma-theta, kg/m^3
20) Cast Portion, E (equilibration) ,D (downcast) , U (up-cast)
21) Comments

Hard copies of all sensor and equipment factory maintenance, pre- and postcruise calibration sheets, and CTD field data sheets should be maintained and made available upon request. Additionally, raw CTD files should be archived. These should include all data files, configuration files, header files, and any mark files created.

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IX. Appendices

Appendix 1
Station locations of NPDES Permit Stations and Aragonite Saturation Discrete Sample Collection

Appendix 1
Table 1-1 Station locations of NPDES Permit Stations and Aragonite Saturation Discrete Sample Collection

Bight'13 POTW/NPDES Sampling Station List.							
Area	**Station Name**	**Latitude**	**Longitude**	**Depth**	**Acidification Samples ***	**Location**	**Responsible Agency**
Central	4701	34.27123	-119.31041	10		Ventura River	City of Oxnard (ABC Labs)
Central	4702	34.26350	-119.32909	20		Ventura River	City of Oxnard (ABC Labs)
Central	4703	34.25557	-119.35091	20		Ventura River	City of Oxnard (ABC Labs)
Central	4704	34.24853	-119.37058	20		Ventura River	City of Oxnard (ABC Labs)
Central	4705	34.24054	-119.39239	30		Ventura River	City of Oxnard (ABC Labs)
Central	4706	34.23303	-119.41258	30		Ventura River	City of Oxnard (ABC Labs)
Central	4601	34.23065	-119.26730	10		Santa Clara River	City of Oxnard (ABC Labs)
Central	4602	34.22732	-119.27850	20		Santa Clara River	City of Oxnard (ABC Labs)
Central	4603	34.22166	-119.29413	30		Santa Clara River	City of Oxnard (ABC Labs)
Central	4604	34.21452	-119.31484	30		Santa Clara River	City of Oxnard (ABC Labs)
Central	4605	34.20637	-119.33997	30		Santa Clara River	City of Oxnard (ABC Labs)
Central	4606	34.19531	-119.37207	35		Santa Clara River	City of Oxnard (ABC Labs)
Central	4501	34.15659	-119.22992	10			City of Oxnard (ABC Labs)
Central	4502	34.15167	-119.24178	20	*		City of Oxnard (ABC Labs)
Central	4503	34.14807	-119.25161	20			City of Oxnard (ABC Labs)
Central	4504	34.13992	-119.27199	20	*		City of Oxnard (ABC Labs)
Central	4505	34.12876	-119.30299	30			City of Oxnard (ABC Labs)
Central	4506	34.11839	-119.32968	60	*		City of Oxnard (ABC Labs)
Central	4401	34.13350	-119.19300	15		Port Hueneme/Oxnard Outfall	City of Oxnard (ABC Labs)
Central	4402	34.12550	-119.19900	20	*	Port Hueneme/Oxnard Outfall	City of Oxnard (ABC Labs)
Central	4403	34.11770	-119.20500	25		Port Hueneme/Oxnard	City of Oxnard (ABC Labs)

Bight'13 POTW/NPDES Sampling Station List.							
Area	**Station Name**	**Latitude**	**Longitude**	**Depth**	**Acidification Samples ***	**Location**	**Responsible Agency**
						Outfall	
Central	4404	34.10180	-119.21600	40	*	Port Hueneme/Oxnard Outfall	City of Oxnard (ABC Labs)
Central	4405	34.07941	-119.25044	100		Port Hueneme/Oxnard Outfall	City of Oxnard (ABC Labs)
Central	4406	34.06687	-119.26411	100	*	Port Hueneme/Oxnard Outfall	City of Oxnard (ABC Labs)
Central	4391	34.13118	-119.18943	10			City of Oxnard (ABC Labs)
Central	4392	34.12417	-119.19680	15			City of Oxnard (ABC Labs)
Central	4393	34.11637	-119.20182	25			City of Oxnard (ABC Labs)
Central	4394	34.10033	-119.21225	35			City of Oxnard (ABC Labs)
Central	4395	34.06795	-119.23137	100			City of Oxnard (ABC Labs)
Central	4396	34.05077	-119.24043	100			City of Oxnard (ABC Labs)
Central	4301	34.09760	-119.10000	15		Mugu Lagoon/Calleguas Creek	City of Oxnard (ABC Labs)
Central	4302	34.08870	-119.10200	20	*	Mugu Lagoon/Calleguas Creek	City of Oxnard (ABC Labs)
Central	4303	34.07960	-119.10300	65		Mugu Lagoon/Calleguas Creek	City of Oxnard (ABC Labs)
Central	4304	34.06160	-119.10700	100	*	Mugu Lagoon/Calleguas Creek	City of Oxnard (ABC Labs)
Central	4305	34.03021	-119.12659	100		Mugu Lagoon/Calleguas Creek	City of Oxnard (ABC Labs)
Central	4306	34.00905	-119.13779	100	*	Mugu Lagoon/Calleguas Creek	City of Oxnard (ABC Labs)
Central	4201	34.06532	-119.01072	10			City of Oxnard (ABC Labs)
Central	4202	34.05740	-119.00988	30			City of Oxnard (ABC Labs)
Central	4203	34.04640	-119.01457	60			City of Oxnard (ABC Labs)
Central	4204	34.03222	-119.01830	100			City of Oxnard (ABC Labs)

Bight'13 POTW/NPDES Sampling Station List.							
Area	**Station Name**	**Latitude**	**Longitude**	**Depth**	**Acidification Samples ***	**Location**	**Responsible Agency**
Central	4205	34.00423	-119.03314	100			City of Oxnard (ABC Labs)
Central	4206	33.97667	-119.04532	100			City of Oxnard (ABC Labs)
Central	4101	34.04100	-118.92400	30			City of Oxnard (ABC Labs)
Central	4102	34.03200	-118.92600	60			City of Oxnard (ABC Labs)
Central	4103	34.02280	-118.92700	100			City of Oxnard (ABC Labs)
Central	4001	33.99528	-118.80526	20			City of Los Angeles (Hyperion)
Central	4002	33.98833	-118.80526	100			City of Los Angeles (Hyperion)
Central	4003	33.98055	-118.80526	100			City of Los Angeles (Hyperion)
Central	4004	33.95833	-118.80526	100			City of Los Angeles (Hyperion)
Central	4005	33.92805	-118.80526	100			City of Los Angeles (Hyperion)
Central	4006	33.91250	-118.80526	100			City of Los Angeles (Hyperion)
Central	3901	34.02750	-118.71667	10		Malibu Creek	City of Los Angeles (Hyperion)
Central	3902	34.01943	-118.71667	20		Malibu Creek	City of Los Angeles (Hyperion)
Central	3903	34.01110	-118.71667	30		Malibu Creek	City of Los Angeles (Hyperion)
Central	3904	33.99750	-118.71667	60		Malibu Creek	City of Los Angeles (Hyperion)
Central	3905	33.96027	-118.71667	100		Malibu Creek	City of Los Angeles (Hyperion)
Central	3906	33.94277	-118.71667	100		Malibu Creek	City of Los Angeles (Hyperion)
Central	3801	34.03333	-118.58333	10			City of Los Angeles (Hyperion)
Central	3802	34.02583	-118.58750	20			City of Los Angeles (Hyperion)
Central	3803	34.00583	-118.59722	45			City of Los Angeles (Hyperion)
Central	3804	33.99333	-118.60417	60			City of Los Angeles (Hyperion)
Central	3805	33.97222	-118.61417	100			City of Los Angeles (Hyperion)
Central	3806	33.95610	-118.62360	100			City of Los Angeles (Hyperion)
Central	3701	33.98610	-118.48610	10			City of Los Angeles (Hyperion)

Bight'13 POTW/NPDES Sampling Station List.							
Area	**Station Name**	**Latitude**	**Longitude**	**Depth**	**Acidification Samples ***	**Location**	**Responsible Agency**
Central	3702	33.98000	-118.50000	20			City of Los Angeles (Hyperion)
Central	3703	33.97417	-118.51000	30			City of Los Angeles (Hyperion)
Central	3704	33.96667	-118.52555	45			City of Los Angeles (Hyperion)
Central	3705	33.95360	-118.55360	60			City of Los Angeles (Hyperion)
Central	3706	33.94250	-118.57500	100			City of Los Angeles (Hyperion)
Central	3601	33.95973	-118.46625	10		Ballona Creek	City of Los Angeles (Hyperion)
Central	3602	33.95555	-118.47777	20		Ballona Creek	City of Los Angeles (Hyperion)
Central	3603	33.94943	-118.49027	30		Ballona Creek	City of Los Angeles (Hyperion)
Central	3604	33.94027	-118.50977	45		Ballona Creek	City of Los Angeles (Hyperion)
Central	3605	33.92777	-118.53555	60		Ballona Creek	City of Los Angeles (Hyperion)
Central	3606	33.91667	-118.55833	100		Ballona Creek	City of Los Angeles (Hyperion)
Central	3501	33.93138	-118.44805	10		Hyperion Outfall	City of Los Angeles (Hyperion)
Central	3502	33.92777	-118.46027	20		Hyperion Outfall	City of Los Angeles (Hyperion)
Central	3503	33.92388	-118.47250	30		Hyperion Outfall	City of Los Angeles (Hyperion)
Central	3504	33.91667	-118.49417	45		Hyperion Outfall	City of Los Angeles (Hyperion)
Central	3505	33.90917	-118.52527	60		Hyperion Outfall	City of Los Angeles (Hyperion)
Central	3506	33.90000	-118.54972	80		Hyperion Outfall	City of Los Angeles (Hyperion)
Central	3401	33.90250	-118.43250	10			City of Los Angeles (Hyperion)
Central	3402	33.90000	-118.44722	20			City of Los Angeles (Hyperion)
Central	3403	33.90110	-118.46000	30			City of Los Angeles (Hyperion)
Central	3404	33.89693	-118.46860	45			City of Los Angeles (Hyperion)
Central	3405	33.88722	-118.50638	55			City of Los Angeles (Hyperion)
Central	3406	33.87917	-118.53555	60			City of Los Angeles (Hyperion)
Central	3301	33.89305	-118.42722	10			City of Los Angeles (Hyperion)

Bight'13 POTW/NPDES Sampling Station List.							
Area	**Station Name**	**Latitude**	**Longitude**	**Depth**	**Acidification Samples ***	**Location**	**Responsible Agency**
Central	3302	33.88917	-118.43638	20			City of Los Angeles (Hyperion)
Central	3303	33.88555	-118.44666	30			City of Los Angeles (Hyperion)
Central	3304	33.87945	-118.45695	45			City of Los Angeles (Hyperion)
Central	3305	33.86833	-118.49333	60			City of Los Angeles (Hyperion)
Central	3306	33.85112	-118.52722	80			City of Los Angeles (Hyperion)
Central	3201	33.85416	-118.40612	10			City of Los Angeles (Hyperion)
Central	3202	33.84861	-118.41778	30			City of Los Angeles (Hyperion)
Central	3203	33.84528	-118.42638	100			City of Los Angeles (Hyperion)
Central	3204	33.83695	-118.44055	100			City of Los Angeles (Hyperion)
Central	3205	33.82388	-118.46362	100			City of Los Angeles (Hyperion)
Central	3206	33.81110	-118.49278	100			City of Los Angeles (Hyperion)
Central	3101	33.77100	-118.43017	10			LACSD
Central	3102	33.76498	-118.43538	30	*		LACSD
Central	3103	33.75730	-118.44105	60			LACSD
Central	3104	33.74527	-118.44977	300	*		LACSD
Central	3105	33.72877	-118.46123	770			LACSD
Central	3106	33.71254	-118.47551	790	*		LACSD
Central	3051	33.73632	-118.39430	13			LACSD
Central	3052	33.73315	-118.40043	30			LACSD
Central	3053	33.72995	-118.40247	60			LACSD
Central	3054	33.71893	-118.41098	300			LACSD
Central	3055	33.70497	-118.42198	800			LACSD
Central	3056	33.68966	-118.43313	830			LACSD
Central	3001	33.73217	-118.36030	10			LACSD
Central	3002	33.72238	-118.36315	30			LACSD
Central	3003	33.71462	-118.36592	60			LACSD
Central	3004	33.70098	-118.37132	300			LACSD
Central	3005	33.68492	-118.38107	590			LACSD
Central	3006	33.66679	-118.39075	800			LACSD
Central	2901	33.71430	-118.32345	10		LACSD Outfall	LACSD
Central	2902	33.70693	-118.32983	30		LACSD Outfall	LACSD
Central	2903	33.69847	-118.33568	60	*	LACSD Outfall	LACSD

Bight'13 POTW/NPDES Sampling Station List.							
Area	**Station Name**	**Latitude**	**Longitude**	**Depth**	**Acidification Samples ***	**Location**	**Responsible Agency**
Central	2904	33.68783	-118.33900	300		LACSD Outfall	LACSD
Central	2905	33.67094	-118.34618	555		LACSD Outfall	LACSD
Central	2906	33.65409	-118.35430	775		LACSD Outfall	LACSD
Central	2801	33.70288	-118.28438	10			LACSD
Central	2802	33.69327	-118.28908	30			LACSD
Central	2803	33.66845	-118.29680	60			LACSD
Central	2804	33.65767	-118.30128	300			LACSD
Central	2805	33.64854	-118.30404	540			LACSD
Central	2806	33.63708	-118.30919	630			LACSD
Central	2701	33.70775	-118.24666	26		Los Angeles Harbor/Dominguez Channel	LACSD
Central	2702	33.68864	-118.25112	26		Los Angeles Harbor/Dominguez Channel	LACSD
Central	2703	33.66953	-118.25559	28		Los Angeles Harbor/Dominguez Channel	LACSD
Central	2704	33.65042	-118.26005	50		Los Angeles Harbor/Dominguez Channel	LACSD
Central	2705	33.63131	-118.26452	220		Los Angeles Harbor/Dominguez Channel	LACSD
Central	2706	33.61220	-118.26898	80		Los Angeles Harbor/Dominguez Channel	LACSD
Central	2601	33.72043	-118.18426	19		Long Beach Harbor/Los Angeles River	LACSD
Central	2602	33.69398	-118.19050	23		Long Beach Harbor/Los Angeles River	LACSD
Central	2603	33.66752	-118.19674	23		Long Beach Harbor/Los Angeles River	LACSD
Central	2604	33.64107	-118.20299	32		Long Beach Harbor/Los Angeles River	LACSD
Central	2605	33.61461	-118.20923	47		Long Beach Harbor/Los Angeles River	LACSD
Central	2606	33.58816	-118.21547	62		Long Beach Harbor/Los Angeles River	LACSD
Central	2501	33.72787	-118.12025	10	*	San Gabriel	LACSD

Bight'13 POTW/NPDES Sampling Station List.							
Area	**Station Name**	**Latitude**	**Longitude**	**Depth**	**Acidification Samples ***	**Location**	**Responsible Agency**
						River/Alamitos Bay	
Central	2502	33.69904	-118.12779	20		San Gabriel River/Alamitos Bay	LACSD
Central	2503	33.67021	-118.13533	26	*	San Gabriel River/Alamitos Bay	LACSD
Central	2504	33.64139	-118.14287	33		San Gabriel River/Alamitos Bay	LACSD
Central	2505	33.61256	-118.15041	44		San Gabriel River/Alamitos Bay	LACSD
Central	2506	33.58102	-118.15908	60	*	San Gabriel River/Alamitos Bay	LACSD
Central	2451	33.69125	-118.06573	10			OCSD
Central	2452	33.67898	-118.07640	17			OCSD
Central	2453	33.66645	-118.08673	22			OCSD
Central	2454	33.65163	-118.09910	30	*		OCSD
Central	2455	33.63683	-118.11125	36			OCSD
Central	2456	33.62197	-118.12352	42	*		OCSD
Central	2401	33.66533	-118.03505	10		Bolsa Chica Inlet	OCSD
Central	2402	33.65570	-118.04322	16		Bolsa Chica Inlet	OCSD
Central	2403	33.64608	-118.05120	21		Bolsa Chica Inlet	OCSD
Central	2404	33.63125	-118.06347	29		Bolsa Chica Inlet	OCSD
Central	2405	33.61643	-118.07573	37		Bolsa Chica Inlet	OCSD
Central	2406	33.60160	-118.08800	60		Bolsa Chica Inlet	OCSD
Central	2349	33.65317	-118.01892	10			OCSD
Central	2350	33.64445	-118.02610	14			OCSD
Central	2351	33.63585	-118.03335	21			OCSD
Central	2352	33.62103	-118.04565	29			OCSD
Central	2353	33.60622	-118.05795	37			OCSD
Central	2354	33.5914	-118.07023	123			OCSD
Central	2301	33.64287	-118.00107	10			OCSD
Central	2302	33.63422	-118.00825	15	*		OCSD
Central	2303	33.62562	-118.01560	21			OCSD
Central	2304	33.61082	-118.02790	29	*		OCSD
Central	2305	33.59600	-118.04020	38			OCSD
Central	2306	33.58118	-118.05248	114	*		OCSD
Central	2221	33.63498	-117.98180	10			OCSD

Bight'13 POTW/NPDES Sampling Station List.							
Area	**Station Name**	**Latitude**	**Longitude**	**Depth**	**Acidification Samples ***	**Location**	**Responsible Agency**
Central	2222	33.62537	-117.98957	15			OCSD
Central	2223	33.61557	-117.99785	22			OCSD
Central	2224	33.60058	-118.01013	31			OCSD
Central	2225	33.58577	-118.02243	47			OCSD
Central	2226	33.57095	-118.03472	135			OCSD
Central	2201	33.62488	-117.96385	10		Santa Ana River	OCSD
Central	2202	33.61502	-117.97190	16	*	Santa Ana River	OCSD
Central	2203	33.60522	-117.98017	25		Santa Ana River	OCSD
Central	2204	33.59038	-117.99243	39	*	Santa Ana River	OCSD
Central	2205	33.57557	-118.00470	57		Santa Ana River	OCSD
Central	2206	33.56073	-118.01697	185	*	Santa Ana River	OCSD
Central	2181	33.61462	-117.94587	10			OCSD
Central	2182	33.60453	-117.95440	15			OCSD
Central	2183	33.59502	-117.96240	36			OCSD
Central	2184	33.58018	-117.97467	51			OCSD
Central	2185	33.56537	-117.98692	114			OCSD
Central	2186	33.55053	-117.99918	247			OCSD
Central	2101	33.60305	-117.92915	10			OCSD
Central	2102	33.59385	-117.93677	26	*		OCSD
Central	2103	33.58482	-117.94463	110			OCSD
Central	2104	33.56998	-117.95690	143	*		OCSD
Central	2105	33.55515	-117.96917	280			OCSD
Central	2106	33.54033	-117.98142	309	*		OCSD
Central	2021	33.59618	-117.89803	10			OCSD
Central	2022	33.58805	-117.90270	53			OCSD
Central	2023	33.57993	-117.90737	165			OCSD
Central	2024	33.56352	-117.91718	300			OCSD
Central	2025	33.54752	-117.92655	390			OCSD
Central	2026	33.53167	-117.93582	432			OCSD
Central	2001	33.58892	-117.87820	10	*	Newport Harbor/San Diego Creek	OCSD
Central	2002	33.57925	-117.88380	60		Newport Harbor/San Diego Creek	OCSD
Central	2003	33.57608	-117.88573	100		Newport Harbor/San Diego Creek	OCSD
Central	2004	33.55982	-117.89513	345		Newport Harbor/San Diego Creek	OCSD

Bight'13 POTW/NPDES Sampling Station List.							
Area	**Station Name**	**Latitude**	**Longitude**	**Depth**	**Acidification Samples ***	**Location**	**Responsible Agency**
Central	2005	33.54355	-117.90438	410		Newport Harbor/San Diego Creek	OCSD
Central	2006	33.52745	-117.91373	470		Newport Harbor/San Diego Creek	OCSD
Central	1901	33.56137	-117.82757	10		Crystal Cove State Beach	OCSD
Central	1902	33.55275	-117.83240	60	*	Crystal Cove State Beach	OCSD
Central	1903	33.54603	-117.83637	100		Crystal Cove State Beach	OCSD
Central	1904	33.52978	-117.84557	405	*	Crystal Cove State Beach	OCSD
Central	1905	33.51350	-117.85475	510		Crystal Cove State Beach	OCSD
Central	1906	33.49715	-117.86403	550	*	Crystal Cove State Beach	OCSD
South	F01	32.63768	-117.24032	19			City of San Diego
South	F02	32.75697	-117.27273	19		Mission Bay	City of San Diego
South	F03	32.78183	-117.27242	18			City of San Diego
South	F04	32.59453	-117.26875	60			City of San Diego
South	F05	32.61168	-117.26965	60			City of San Diego
South	F06	32.63083	-117.27360	60			City of San Diego
South	F07	32.65113	-117.27999	62			City of San Diego
South	F08	32.67133	-117.28515	63	*	Point Loma Outfall	City of San Diego
South	F09	32.68555	-117.28632	60			City of San Diego
South	F10	32.70542	-117.29066	60			City of San Diego
South	F11	32.72554	-117.29463	60	*		City of San Diego
South	F12	32.74658	-117.30207	61		Mission Bay	City of San Diego
South	F13	32.76538	-117.30720	60			City of San Diego
South	F14	32.78156	-117.31142	59	*		City of San Diego
South	F15	32.59410	-117.28645	80			City of San Diego
South	F16	32.61183	-117.29007	80	*		City of San Diego
South	F17	32.63002	-117.29417	80			City of San Diego
South	F18	32.64977	-117.29833	80			City of San Diego
South	F19	32.66785	-117.30683	81		Point Loma Outfall	City of San Diego
South	F20	32.68542	-117.31097	80			City of San Diego
South	F21	32.70380	-117.31869	81			City of San Diego
South	F22	32.72273	-117.32090	80			City of San Diego
South	F23	32.74188	-117.33042	81			City of San Diego
South	F24	32.76122	-117.33645	81			City of San Diego

Bight'13 POTW/NPDES Sampling Station List.							
Area	**Station Name**	**Latitude**	**Longitude**	**Depth**	**Acidification Samples ***	**Location**	**Responsible Agency**
South	F25	32.77895	-117.34358	79	*		City of San Diego
South	F26	32.59377	-117.31220	98			City of San Diego
South	F27	32.61178	-117.32138	98	*		City of San Diego
South	F28	32.62929	-117.32372	99			City of San Diego
South	F29	32.64782	-117.32493	98			City of San Diego
South	F30	32.66567	-117.32483	96	*	Point Loma Outfall	City of San Diego
South	F31	32.68467	-117.32835	97			City of San Diego
South	F32	32.70142	-117.33417	99			City of San Diego
South	F33	32.72047	-117.33992	99	*		City of San Diego
South	F34	32.73890	-117.34937	99		Mission Bay	City of San Diego
South	F35	32.75770	-117.36338	99			City of San Diego
South	F36	32.77678	-117.37457	98	*		City of San Diego
South	I1	32.47333	-117.27700	60	*		City of San Diego
South	I2	32.47333	-117.19900	32	*		City of San Diego
South	I3	32.46700	-117.16800	27	*		City of San Diego
South	I4	32.47167	-117.14000	18			City of San Diego
South	I5	32.47167	-117.13000	14			City of San Diego
South	I6	32.49350	-117.16300	26			City of San Diego
South	I7	32.51667	-117.25300	52			City of San Diego
South	I8	32.51667	-117.20200	36			City of San Diego
South	I9	32.51167	-117.17900	29			City of San Diego
South	I10	32.51667	-117.15600	19			City of San Diego
South	I11	32.51333	-117.13700	13			City of San Diego
South	I12	32.53283	-117.18300	28	*		City of San Diego
South	I13	32.53750	-117.21200	38		Tijuana Outfall	City of San Diego
South	I14	32.54300	-117.18400	28			City of San Diego
South	I15	32.53783	-117.18900	31			City of San Diego
South	I16	32.53783	-117.18300	28		Tijuana Outfall	City of San Diego
South	I17	32.53783	-117.17800	25			City of San Diego
South	I18	32.53617	-117.16100	19			City of San Diego
South	I19	32.53633	-117.12900	10		Tijuana Outfall	City of San Diego
South	I20	32.55700	-117.25700	55	*	Tijuana River	City of San Diego
South	I21	32.56067	-117.22700	41	*		City of San Diego
South	I22	32.55333	-117.18500	28		Tijuana River	City of San Diego
South	I23	32.55083	-117.16500	21			City of San Diego
South	I24	32.55667	-117.14500	11		Tijuana River	City of San Diego
South	I25	32.56117	-117.14800	9			City of San Diego
South	I26	32.57450	-117.14700	9			City of San Diego

Bight'13 POTW/NPDES Sampling Station List.							
Area	**Station Name**	**Latitude**	**Longitude**	**Depth**	**Acidification Samples ***	**Location**	**Responsible Agency**
South	I27	32.57417	-117.19100	28	*		City of San Diego
South	I28	32.59383	-117.26400	55			City of San Diego
South	I29	32.59450	-117.22300	38		San Diego Bay	City of San Diego
South	I30	32.59533	-117.19700	28			City of San Diego
South	I31	32.59550	-117.17200	19			City of San Diego
South	I32	32.59467	-117.13800	10			City of San Diego
South	I33	32.62383	-117.23700	30	*		City of San Diego
South	I34	32.63000	-117.21600	19		San Diego Bay	City of San Diego
South	I35	32.63667	-117.18200	19			City of San Diego
South	I36	32.63917	-117.15400	11			City of San Diego
South	I37	32.64800	-117.21633	12		San Diego Bay	City of San Diego
South	I38	32.66883	-117.18667	11			City of San Diego
South	I39	32.57233	-117.16750	18			City of San Diego
South	I40	32.55383	-117.13617	10			City of San Diego

Appendix 2
Sample Collection Protocols for Aragonite Saturation and pH Component

Appendix 2 Sample Collection Protocols for Aragonite Saturation and pH Component

Instructions for DIC/ALK Sampling Program(s)
Dissolved Inorganic Carbon (DIC) or Alkalinity (ALK)

Packing list for the sample collection:
Instructions
1 pipetter
A centrifuge tube full of tips (some of you may have a baggie of extra tips as well, you may reuse tips)
A centrifuge tube for used tips
Tygon tubing for the Niskins (you may use your own tubing)
A foam sampling platform
Kimwipes on sticks
A box of kimwipes
A grease gun with grease
A 25 mL syringe and tubing for pulling the headspace
Bags of black rubber bands and white locking strips
30 mL bottle of saturated mercuric chloride solution (double bagged)
A small baggie for cleaning up mercuric chloride spills
Gloves

Overview of procedures

As a gas is to be analyzed, samples for DIC/ALK should be among the first collected.

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 ($HgCl_2$) solution.

If at any time during the cruise, you run into a problem and/or want to discuss aspects of the sampling procedure, please call Karen McLaughlin (714-755-3242).

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.

Thank you very much for assisting with this sample collection.

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 appropriate 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.

Appendix 3
SDS Mercuric Chloride

Material Safety Data Sheet
Mercuric chloride MSDS

Section 1: Chemical Product and Company Identification

Product Name: Mercuric chloride

Catalog Codes: SLM3604

CAS#: 7487-94-7

RTECS: OV9100000

TSCA: TSCA 8(b) inventory: Mercuric chloride

CI#: Not applicable.

Synonym: Calochlor; Mercury (II) Chloride; Bichloride of Mercury

Chemical Name: Mercuric chloride

Chemical Formula: HgCl2

Contact Information:

Sciencelab.com, Inc.
14025 Smith Rd.
Houston, Texas 77396

US Sales: **1-800-901-7247**
International Sales: **1-281-441-4400**

Order Online: ScienceLab.com

CHEMTREC (24HR Emergency Telephone), call: 1-800-424-9300

International CHEMTREC, call: 1-703-527-3887

For non-emergency assistance, call: 1-281-441-4400

Section 2: Composition and Information on Ingredients

Composition:

Name	CAS #	% by Weight
Mercuric chloride	7487-94-7	100

Toxicological Data on Ingredients: Mercuric chloride: ORAL (LD50): Acute: 1 mg/kg [Rat.]. 6 mg/kg [Mouse]. DERMAL (LD50): Acute: 41 mg/kg [Rat].

Section 3: Hazards Identification

Potential Acute Health Effects:
Extremely hazardous in case of ingestion, of inhalation. Very hazardous in case of skin contact (irritant, permeator), of eye contact (irritant). Corrosive to eyes and skin. The amount of tissue damage depends on length of contact. Eye contact can result in corneal damage or blindness. Skin contact can produce inflammation and blistering. Inhalation of dust will produce irritation to gastro-intestinal or respiratory tract, characterized by burning, sneezing and coughing. Severe over-exposure can produce lung damage, choking, unconsciousness or death. Inflammation of the eye is characterized by redness, watering, and itching. Skin inflammation is characterized by itching, scaling, reddening, or, occasionally, blistering.

Potential Chronic Health Effects:
Very hazardous in case of skin contact (irritant), of eye contact (irritant), of inhalation. CARCINOGENIC EFFECTS: Classified POSSIBLE by IRIS, 3 (Equivocal evidence.) by NTP. A4 (Not classifiable for human or animal.) by ACGIH. MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells. Mutagenic for bacteria and/or yeast. TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Classified Reproductive system/toxin/female, Reproductive system/toxin/male

[POSSIBLE]. The substance is toxic to brain, peripheral nervous system, skin, central nervous system (CNS), eye, lens or cornea. The substance may be toxic to the reproductive system. Repeated or prolonged exposure to the substance can produce target organs damage. Repeated exposure of the eyes to a low level of dust can produce eye irritation. Repeated skin exposure can produce local skin destruction, or dermatitis. Repeated inhalation of dust can produce varying degree of respiratory irritation or lung damage. Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs. Repeated or prolonged inhalation of dust may lead to chronic respiratory irritation.

Section 4: First Aid Measures

Eye Contact:
Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Cold water may be used. Get medical attention immediately.

Skin Contact:
In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Cover the irritated skin with an emollient. Cold water may be used.Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention immediately.

Serious Skin Contact:
Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.

Inhalation:
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.

Serious Inhalation:
Evacuate the victim to a safe area as soon as possible. Loosen tight clothing such as a collar, tie, belt or waistband. If breathing is difficult, administer oxygen. If the victim is not breathing, perform mouth-to-mouth resuscitation. WARNING: It may be hazardous to the person providing aid to give mouth-to-mouth resuscitation when the inhaled material is toxic, infectious or corrosive. Seek immediate medical attention.

Ingestion:
If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.

Serious Ingestion: Not available.

Section 5: Fire and Explosion Data

Flammability of the Product: Non-flammable.

Auto-Ignition Temperature: Not applicable.

Flash Points: Not applicable.

Flammable Limits: Not applicable.

Products of Combustion: Toxic fumes of mercury, chloride fumes.

Fire Hazards in Presence of Various Substances: Not applicable.

Explosion Hazards in Presence of Various Substances:
Risks of explosion of the product in presence of mechanical impact: Not available. Risks of explosion of the product in presence of static discharge: Not available.

Fire Fighting Media and Instructions: Not applicable.

Special Remarks on Fire Hazards: When heated to decomposition it emits toxic fumes.

Special Remarks on Explosion Hazards:

Mixture of Mercuric Chloride with Potassium and metalic halides produces strong explosion on impact. Mixture of Mercuric Chloride with sodium and halide compouns produces a strong explosion in impact.

Section 6: Accidental Release Measures

Small Spill: Use appropriate tools to put the spilled solid in a convenient waste disposal container.

Large Spill:
Corrosive solid. Poisonous solid. Stop leak if without risk. Do not get water inside container. Do not touch spilled material. Use water spray to reduce vapors. Prevent entry into sewers, basements or confined areas; dike if needed. Call for assistance on disposal. Be careful that the product is not present at a concentration level above TLV. Check TLV on the MSDS and with local authorities.

Section 7: Handling and Storage

Precautions:
Keep locked up.. Keep container dry. Do not ingest. Do not breathe dust. Never add water to this product. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, metals, acids, alkalis.

Storage: Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above 23°C (73.4°F).

Section 8: Exposure Controls/Personal Protection

Engineering Controls:
Use process enclosures, local exhaust ventilation, or other engineering controls to keep airborne levels below recommended exposure limits. If user operations generate dust, fume or mist, use ventilation to keep exposure to airborne contaminants below the exposure limit.

Personal Protection:
Splash goggles. Synthetic apron. Vapor and dust respirator. Be sure to use an approved/certified respirator or equivalent. Gloves.

Personal Protection in Case of a Large Spill:
Splash goggles. Full suit. Vapor and dust respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

Exposure Limits:
TWA: 0.025 (mg (Hg)/m) from ACGIH (TLV) [United States] SKIN TWA: 0.1 (mg/m3) from ACGIH (TLV) [United States] TWA: 0.05 (mg/m3) from OSHA (PEL) [United States] Inhalation TWA: 0.01 (mg/m3) from Ocupational Reproductive Guidelines - Screening Method, Jankovic and Drake Inhalation TWA: 0.1 (mg/m3) from OSHA (PEL) [United States] Inhalation3 Consult local authorities for acceptable exposure limits.

Section 9: Physical and Chemical Properties

Physical state and appearance: Solid. (Crystals solid.)

Odor: Odorless.

Taste: Not available.

Molecular Weight: 271.5 g/mole

Color: White.

pH (1% soln/water): Not available.

Boiling Point: 302°C (575.6°F)

Melting Point: 276°C (528.8°F)

Critical Temperature: Not available.

Specific Gravity: 5.44 (Water = 1)

Vapor Pressure: Not applicable.

Vapor Density: Not available.

Volatility: Not available.

Odor Threshold: Not available.

Water/Oil Dist. Coeff.: Not available.

Ionicity (in Water): Not available.

Dispersion Properties: See solubility in water, methanol, diethyl ether.

Solubility:
Easily soluble in cold water, hot water. Soluble in methanol, diethyl ether.

Section 10: Stability and Reactivity Data

Stability: The product is stable.

Instability Temperature: Not available.

Conditions of Instability: Incompatible materials, light, excess heat, organic matter.

Incompatibility with various substances: Reactive with oxidizing agents, metals, acids, alkalis.

Corrosivity: Non-corrosive in presence of glass.

Special Remarks on Reactivity:
May decompose on exposure to light. Reacts with sodium, potassium and their alloys. Incompatible with Acids, Albumin, Alkalis, alkaloid salts, ammonia, antimony, arsenic, borax, bromides, carbonates, copper, formates, gelatin, hypophosphites, iron, lead, lime water, metals, phosphates, postassium, reduced iron, sodium, sulfates, sulfides, tannic acid, and vegetable astringents.

Special Remarks on Corrosivity: Not available.

Polymerization: Will not occur.

Section 11: Toxicological Information

Routes of Entry: Inhalation. Ingestion.

Toxicity to Animals:
Acute oral toxicity (LD50): 1 mg/kg [Rat.]. Acute dermal toxicity (LD50): 41 mg/kg [Rat].

Chronic Effects on Humans:
CARCINOGENIC EFFECTS: Classified POSSIBLE by IRIS, 3 (Equivocal evidence.) by NTP. A4 (Not classifiable for human or animal.) by ACGIH. MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells. Mutagenic for bacteria and/or yeast. DEVELOPMENTAL TOXICITY: Classified Reproductive system/toxin/female, Reproductive system/toxin/male [POSSIBLE]. Causes damage to the following organs: brain, peripheral nervous system, skin, central nervous system (CNS), eye, lens or cornea. May cause damage to the following organs: the reproductive system.

Other Toxic Effects on Humans:
Extremely hazardous in case of ingestion, . Very hazardous in case of skin contact (irritant, permeator). Hazardous in case of skin contact (corrosive), of eye contact (corrosive), of inhalation (lung corrosive).

Special Remarks on Toxicity to Animals: Not available.

Special Remarks on Chronic Effects on Humans:
May affect genetic material and cause adverse reproductive effects (fetotoxicity, developmental abnormalties, fertility). Found in human breast milk.

Special Remarks on other Toxic Effects on Humans:
Acute Potential Health Effects: Skin: Causes severe skin irritation. May be fatal if absorbed through skin Eyes: Causes severe eye irritation. It can also be corrosive to eyes. Inhalation: May be fatal if inhaled. Causes respiratory tract irritation and can be corrosive to the throat and mucous membranes. May cause coughing, shortness of breath. May also affect behavior and brain (central and peripherial nervous systems) with vertigo, anxiety, depression, muscle incoordination, and emotioal instability. Ingestion: May be fatal if swallowed. Causes gastrointestinal tract irritation with nausea, vomiting, bloody diarrhea, foul taste, corrosive ulceration, and loosened teeth. May also affect the liver, blood, and urinary system (kidneys, ureter, bladder). Affects the brain, behavior (the central and peripheral nervous systems). Chronic Potential Health Effects: Skin: Repeated skin exposure may cause allergic contact dermatitis. Eyes: May cause Mercurialentis (brown mercury deposits in the lens of the eye), with visual defects. Ingestion: Excessive salivation, muscle weakness, Mercurial Erethism (short term memory loss, personality changes). May cause effects of those of acute ingestion. Inhalation: May cause effects similar to acute inhalation.

Section 12: Ecological Information

Ecotoxicity:
Ecotoxicity in water (LC50): 0.9 ppm 24 hours [Rainbow Trout (juvenille)]. 0.1 ppm 48 hours [Fathead Minnow]. 0.2 ppm 96 hours [Bluegill Sunfish].

BOD5 and COD: Not available.

Products of Biodegradation:
Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

Toxicity of the Products of Biodegradation: The products of degradation are less toxic than the product itself.

Special Remarks on the Products of Biodegradation: Not available.

Section 13: Disposal Considerations

Waste Disposal:
Waste must be disposed of in accordance with federal, state and local environmental control regulations.

Section 14: Transport Information

DOT Classification: CLASS 6.1: Poisonous material.

Identification: : Mercuric chloride UNNA: 1624 PG: II

Special Provisions for Transport: Marine Pollutant

Section 15: Other Regulatory Information

Federal and State Regulations:
California prop. 65: This product contains the following ingredients for which the State of California has found to cause cancer, birth defects or other reproductive harm, which would require a warning under the statute: Mercuric chloride California prop. 65: This product contains the following ingredients for which the State of California has found to cause birth defects which would require a warning under the statute: Mercuric chloride Connecticut hazardous material survey.: Mercuric chloride Illinois chemical safety act: Mercuric chloride New York release reporting list: Mercuric chloride Pennsylvania RTK: Mercuric chloride Florida: Mercuric chloride Michigan critical material: Mercuric chloride Massachusetts RTK: Mercuric chloride Massachusetts spill list: Mercuric chloride New Jersey: Mercuric chloride New Jersey spill list: Mercuric chloride Louisiana RTK reporting list: Mercuric chloride TSCA 8(b) inventory: Mercuric chloride SARA 302/304/311/312 extremely hazardous substances: Mercuric

chloride SARA 313 toxic chemical notification and release reporting: Mercuric chloride; Notification de minims is 1% CERCLA: Hazardous substances.: Mercuric chloride

Other Regulations:
OSHA: Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200). EINECS: This product is on the European Inventory of Existing Commercial Chemical Substances.

Other Classifications:

WHMIS (Canada):
CLASS D-1A: Material causing immediate and serious toxic effects (VERY TOXIC). CLASS D-2B: Material causing other toxic effects (TOXIC).

DSCL (EEC):
R24- Toxic in contact with skin. R28- Very toxic if swallowed. R34- Causes burns. R50/53- Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment. S36/37/39- Wear suitable protective clothing, gloves and eye/face protection. S45- In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). S60- This material and its container must be disposed of as hazardous waste. S61- Avoid release to the environment. Refer to special instructions/Safety data sheets.

HMIS (U.S.A.):

Health Hazard: 4

Fire Hazard: 0

Reactivity: 0

Personal Protection: j

National Fire Protection Association (U.S.A.):

Health: 4

Flammability: 0

Reactivity: 0

Specific hazard:

Protective Equipment:
Gloves. Synthetic apron. Vapor and dust respirator. Be sure to use an approved/certified respirator or equivalent. Wear appropriate respirator when ventilation is inadequate. Splash goggles.

Section 16: Other Information

References:
-Hawley, G.G.. The Condensed Chemical Dictionary, 11e ed., New York N.Y., Van Nostrand Reinold, 1987. -Material safety data sheet emitted by: la Commission de la Santé et de la Sécurité du Travail du Québec. -SAX, N.I. Dangerous Properties of Indutrial Materials. Toronto, Van Nostrand Reinold, 6e ed. 1984. -The Sigma-Aldrich Library of Chemical Safety Data, Edition II. -Guide de la loi et du règlement sur le transport des marchandises dangeureuses au canada. Centre de conformité internatinal Ltée. 1986.

Other Special Considerations: Not available.

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Appendix 4
Nutrient Collection Protocols for Nutrient Source Tracking

Appendix 4 Nutrient Collection Protocols for Nutrient Source Tracking

Table 4-1

Sample	Filter and water needed	Volume required	StorageType	Storage
FILTERS				
CHN	Combusted 25mm GF/F	200mls (color on filter)	Petri dish	Freeze immediately
$P^{15}N$	Combusted 25mm GF/F	200mls (color on filter)	Petri dish	Freeze immediately
FILTRATE (DISSOLVED FRACTION ONLY)				
DIN	Filter SW through a 0.45 MCE filter attached to syringe	Fill bottle 2/3 full (not less than 20mls)	30 mls clear bottles	Freeze immediately
TDN/TDP	Filter SW through a 0.45 MCE filter attached to syringe	Fill bottle 2/3 full (not less than 20mls)	30 mls clear bottles	Freeze immediately
$^{15}NO_3$ Abundance	Use filter tower and 47mm 0.45 um filter	Collect 40 mls of filtrate	60 mls amber bottles	Freeze immediately
$^{15}NH_4$ Abundance	Use filter tower and 47mm 0.45 uM filter	Collect 1.5L of filtrate	2 L bottles	Add H2SO4 to pH of 2 (use pH paper) Freeze immediately

<u>Filter Samples:</u> Particulate organic matter (15N analysis) and particulate nitrogen and carbon

If the volumes filtered need to be adjusted because of the large amount of biomass in the water, the actual filtered volumes need to be recorded on the sample label with sharpie AND on the field cruise sheet.

<u>Particulate sample collection (2 samples per station depth):</u>

- Using clean filter forceps, center a pre-weighted **COMBUSTED** glass-fiber filter (Whatman GF/F) onto the mesh platform of a clean filtering tower apparatus. Avoid touching the filters with hands or anything other than clean forceps. Make sure to keep track of the petri dishes as the filter will need to be placed back into the exact same petri dish for storage and analysis. Two separate filter samples will be collected.
- Mix sample gently but thoroughly to ensure the sample is fully mixed (to get any particulates off the bottom of the bottle). Do not vigorously shake the bottle (that will break cells apart).
- Rinse the graduated cylinder with a small amount of sample water (enough to cover the surface of the cylinder). Then discard the rinse water.
- Carefully measure 200 mLs of the sample into the graduated cylinder.
- Pour the measured sample into the filter reservoir.
- Collect 2 particulate filter samples (each with 200 mLs filtered).
- Gently vacuum filter the sample.

- Remove vacuum as soon as water has passed through filter to avoid damaging or breaking up the cells and particulate material.
- Fold filters onto themselves using forceps and place each filter back into *the same* labeled petri dish it came out of. Label with the station number, date, depth and PN.
- Record all information on field data sheets
- Place samples in the cooler with lots of ice. Samples should be stored frozen (-20^{o}C)
- If it is not possible to filter all of the 200 mLs of the sample, remove the water, discard the filter and try again with a smaller volume (150 mLs).

Particulate Nitrogen Field Blanks

Collect 1 field blanks PER CRUISE. Follow the same procedures as above but Instead of sample water, use DI or MQ water and filter.

Filtering Samples for Nutrient Analysis:

Start by collecting field blanks (1 **per cruise** at any station)

- Wear gloves
- Open Fisher Brand MCE 0.45 µm filter
- Pull plunger out of a clean 60 mL syringe
- Affix MCE filter on the end of the syringe
- Fill the syringe with a few milliliters of distilled deionized water (DDI) and rinse the syringe and the rubber stopper of the plunger. Rinse a total of three times.
- Fill the syringe with DDI water
- Put the plunger back in the syringe, take off the filter to push out the air trapped in the syringe.
- Push ~10 mL through the filter and discard the rinse water
- Open the Dissolved Inorganic Nutrients (DIN) field blank bottle
- Rinse three times with blank water, discarding rinse water each time
- Fill dissolved nutrients FB bottle no more than 2/3 full with blank water.
- Refill the syringe with DDI water as necessary
- Open the TDN/TDP (Total Dissolved Nitrogen/Total Dissolved Phosphorus) sample bottle
- Rinse the TDN/TDP bottle 3 times with **unfiltered** DDI blank water, discarding the rinse each time
- Fill TN/TP bottle no more than 2/3 full with blank **unfiltered** water.

Collect Nutrient Samples (DIN, TDN/TDP) (1 sample per station depth):

- Wear gloves
- Open Fisher Brand MCE 0.45 µm filter
- Pull plunger out of a clean 60 mL syringe
- Affix MCE filter on the end of the syringe

- Fill the syringe with a few milliliters sample water and rinse the syringe and the rubber stopper of the plunger. Rinse a total of three times.
- Fill the syringe with sample water
- Put the plunger back in the syringe, take off the filter to push out the air trapped in the syringe.
- Push ~10 mL through the filter and discard the rinse water
- Open the Dissolved Inorganic Nutrients (DIN) bottle
- Rinse three times with sample water, discarding rinse water each time
- Fill dissolved nutrients FB bottle no more than 2/3 full with filtered sample water.
- Open the TN/TP (Total Nitrogen/Total Phosphorus) sample bottle
- Rinse the TN/TP bottle 3 times with **unfiltered whole** sample water, discarding the rinse each time
- Fill TN/TP bottle no more than 2/3 full with blank **unfiltered whole** sample water.
- Store samples on ice.

Collect Natural Abundance Isotope Samples ($15NO_3$ and $15NH_4$) (1 sample per station depth):

- Wear gloves
- Filter 1.6 Liters through a 0.45 micron 47mm filter membrane using a 47mm field filter tower
- Collect 45-50 mls in 60mls bottle of filtrate for NO_3 isotopes
- Collect 1.5L in 2 L bottle of filtrate for NH_4 isotopes; acidify to pH 2 (check with pH strip) with 5-8 drops of concentrated sulfuric acid
- Store both samples on ice in cooler in dark
-

Appendix 5
Collection procedures for Biogeochemical Process Rate Experiments

Appendix 5 Collection procedures for Biogeochemical Process Rate Experiments

Table 5-1

WHOLE WATER			
Nitrification Incubations	Pour whole water into two triple rinsed 2 Liter bottle	Fill bottle to shoulder	Put in Cooler, keep dark
R/P Incubations	Rinse 9L carboy 3X with SW	Fill 9L Carboy	Put in Cooler, out of sun
Nitrogen uptake incubations	Rinse 2 20L carboy 3X with SW	Fill 20L Cubitainer	Put in cooler, out of sun

- **Nitrification Rates - Incubations**
  - At each depth, collect 3 L of water
  - Triple rinse collection container with seawater, then fill from appropriate depth
  - Store in dark cooler in ice slurry (enough ice to keep at in situ temperature)
- **Respiration and Production Incubations**
  - At each depth, collect 9 L of water
  - Triple rinse collection container with seawater, then fill from appropriate depth
  - Store in dark cooler in ice slurry (enough ice to keep at in situ temperature)
- **15N uptake Kinetics experiments - Incubations**
  - Collect 40 L of water
  - Triple rinse collection container with seawater, then fill from appropriate depth
  - Store in dark cooler in ice slurry (enough ice to keep at in situ temperature)

Appendix 6
Sample Field data sheets for biogeochemical discrete samples.

Appendix 6 Sample Field data sheets for biogeochemical discrete samples.

Site Information			
StationID			
Team Members			
Latitude		Longitude	
Date (mm/dd/yy)			
Time (PST)			
Cast #			

Depth				
CHN				
$P^{15}N$				
DIN				
$^{15}NO_3$Abundance				
$^{15}NH_4$Abundance				
N15 Incubation Water				
R/P Incubation Water				
Nitrogen uptake incubation water				

Niskin #	Depth Collected
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	