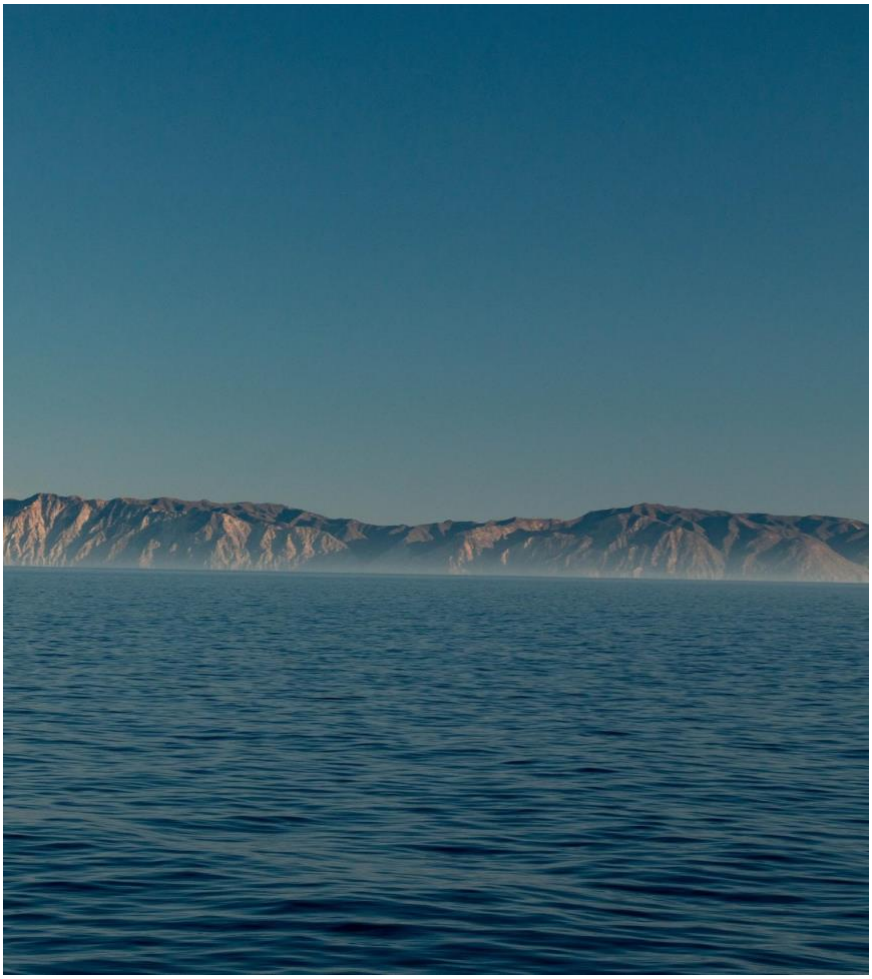
Office of National Marine Sanctuaries
National Oceanic and Atmospheric Administration

NATIONAL MARINE SANCTUARIES CONSERVATION SERIES

2210RL Enhanced CalCOFI



April 2023 | sanctuaries.noaa.gov
National Marine Sanctuaries Conservation Science Series ONMS-23-01

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Suggested citation: White, K., Pound, R., Lampe, R., Hays, A., Schulberg, A., Gold, Z., DeProspero, Delean, B., Freedman, R., & Bowlin, N. (2023). *2210RL Enhanced CalCOFI*. National Marine Sanctuaries Conservation Series ONMS-23-01. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Ocean Service, Office of National Marine Sanctuaries.

Cover photo: Early morning view of the Channel Islands (October 2022). Photo: Zachary Gold/NOAA

About the National Marine Sanctuaries Conservation Series

The Office of National Marine Sanctuaries, part of the National Oceanic and Atmospheric Administration, serves as the trustee for a system of underwater parks encompassing more than 620,000 square miles of ocean and Great Lakes waters. The 15 national marine sanctuaries and two marine national monuments within the National Marine Sanctuary System represent areas of America's ocean and Great Lakes environment that are of special national significance. Within their waters, giant humpback whales breed and calve their young, coral colonies flourish, and shipwrecks tell stories of our nation's maritime history. Habitats include beautiful coral reefs, lush kelp forests, whale migration corridors, spectacular deep-sea canyons, and underwater archaeological sites. These special places also provide homes to thousands of unique or endangered species and are important to America's cultural heritage. Sites range in size from less than one square mile to almost 583,000 square miles. They serve as natural classrooms and cherished recreational spots, and are home to valuable commercial industries.

Because of considerable differences in settings, resources, and threats, each national marine sanctuary has a tailored management plan. Conservation, education, research, monitoring, and enforcement programs vary accordingly. The integration of these programs is fundamental to marine protected area management. The National Marine Sanctuaries Conservation Series reflects and supports this integration by providing a forum for publication and discussion of the complex issues currently facing the National Marine Sanctuary System. Topics of published reports vary substantially and may include descriptions of educational programs, discussions on resource management issues, and results of scientific or historical research and monitoring projects. The series facilitates integration of natural sciences, socioeconomic and social sciences, education, and policy development to accomplish the diverse needs of NOAA's resource protection mandate. All publications are available on the Office of National Marine Sanctuaries website.

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Report Availability

Electronic copies of this report may be downloaded from the Office of National Marine Sanctuaries website.

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Abstract

This report summarizes the objectives, methods, and operations from the October 2022 enhanced CalCOFI research cruise, 2210RL Enhanced CalCOFI. California Cooperative Oceanic Fisheries Investigations (CalCOFI) is a multi-agency partnership that studies California's marine environment and includes the National Oceanic and Atmospheric Administration's National Marine Fisheries Service, Scripps Institution of Oceanography, and the California Department of Fish and Wildlife. The research cruise collected data and samples from 16 locations in the California Current between San Francisco and San Diego, including locations within Channel Islands National Marine Sanctuary, Monterey Bay National Marine Sanctuary, the proposed Chumash Heritage National Marine Sanctuary, and the Morro Bay Wind Energy Area. Physical data and samples of ichthyoplankton, water chemistry, and eDNA (using five different protocols to compare methodologies) were collected. Additionally, seabirds and marine mammals were observed while transiting between locations. This expanded sampling effort will help with the designation of the proposed Chumash Heritage National Marine Sanctuary and track potential impacts from offshore wind energy development.

Key Words

CalCOFI, marine environment monitoring, environmental DNA, plankton sampling, water sampling, marine mammals, seabirds, proposed Chumash Heritage National Marine Sanctuary

Chapter 1:
Introduction, Objectives, and Operating Area

Introduction

California Cooperative Oceanic Fisheries Investigations (CalCOFI) has a long history of collecting contemporaneous biological, physical, and chemical time series data, making it an invaluable asset for resource management and ecosystem research. Recent marine zoning management efforts for protected areas and proposed wind energy areas have increased the need for the types of data collected by CalCOFI at different spatial scales, and for new technologies to effectively monitor marine ecosystems. In an attempt to meet the resource management needs of state and federal governments, National Oceanic and Atmospheric Administration (NOAA) and Scripps Institution of Oceanography (SIO) researchers expanded the CalCOFI mission to 16 locations identified as important by federal agencies. During this enhanced CalCOFI cruise, "2210RL Enhanced CalCOFI," physical data, ichthyoplankton, and water chemistry samples were collected at each location. Environmental DNA (eDNA) samples were also collected using five different protocols to compare methodologies. Additionally, seabirds and marine mammals were observed while transiting between desired locations. This expanded sampling will support the designation of the proposed Chumash Heritage National Marine Sanctuary (CHNMS) and track potential impacts from offshore wind energy development. Mission goals included:

- Resurveying historic CalCOFI stations to understand how climate shifts have modified oceanographic conditions, altered habitat use by protected resources, and impacted fisheries;
- Strategically adding new sample stations to enhance characterizations of national marine sanctuaries and provide data in critical habitats on a spatial scale relevant to sanctuary management;
- Resurveying and increasing the frequency of sampling at key locations to characterize and monitor areas nominated to be a new national marine sanctuary on the Central Coast; and
- Supporting wind energy implementation by testing new technologies for vessel-based data collection in areas designated for green offshore energy development in preparation for assessing the environmental impacts of oceanic wind farms.

Objectives

Overall cruise objectives were to survey biotic and abiotic environments and pelagic fish stocks in the California Current between San Francisco and San Diego. Specific objectives included:

1) Estimating the distribution and abundance of coastal pelagic fishes and krill via multi-frequency acoustic backscatter sampling using the Simrad EK80 and Simrad ME70 echo sounders
2) Estimating the physical oceanographic habitats for target species by sampling sea-surface temperature, salinity, and chlorophyll *a* using a thermo-salinometer and fluorometer

3) Sampling profiles of seawater for nutrients, chlorophyll *a*, temperature, salinity, eDNA, and RNA using a conductivity, temperature, and depth (CTD) sensor with a water sampling rosette and other instruments
    - Sampling current profiles using a Teledyne Marine acoustic doppler current profiler
    - Testing current and potential future ocean eDNA protocols, including: **1)** the current CalCOFI protocol (the NOAA-CalCOFI Ocean Genomics Project [NCOG; DNA and RNA], **2)** the rockfish cruise protocol (Rockfish Recruitment and Ecosystem Assessment Survey [RREAS-eDNA]), **3)** Global eDNA Marine Collection and Analysis Program (GEMCAP), **4)** passive filtering, and **5)** aerosol filtering
4) Estimating the distributions and abundances of phytoplankton, zooplankton, and ichthyoplankton species; continuously examining eukaryotic phytoplankton with an Imaging FlowCytobot (IFCB); and sampling zooplankton and ichthyoplankton using two methods: CalCOFI Bongo Oblique (CalBOBL) and Manta (neuston) net tows
5) Continually collecting the following information from the study area: air temperature; barometric pressure; wind speed and direction using an integrated weather station; surface water temperature and salinity; seabird observations during daylight hours

Personnel

Table 1. 2210RL Enhanced CalCOFI cruise personnel information.

Name	Title	Affiliation
Freedman, Ryan	Chief Scientist	Office of National Marine Sanctuaries
Hays, Amy	Fisheries Scientist	Southwest Fisheries Science Center (SWFSC)
Concha-Saiz, Nicolas	Fisheries Scientist	SWFSC
Pound, Rachel	Biological Oceanographer	SIO
Walsh, Kamran	Student	SIO
Russell, Tammy	Student	SIO
Gold, Zack	eDNA Specialist	University of Washington/SIO/Pacific Marine Environmental Laboratory
Inskeep, Dylan	Fisheries Scientist	California Department of Fish and Wildlife
Huang, Harrison	Fisheries Scientist	California Department of Fish and Wildlife
Lampe, Robert	Student	SIO/J. Craig Venter Institute
Schulberg, Anne	Student	SIO/ J. Craig Venter Institute

Cruise Statistics

Table 2. General sampling and observation statistics.

Category	Statistics
Cruise dates	October 10–October 20, 2022
Weather delays	0
CTD deployments (water sampling)	16 (one per station)
Maximum CTD depth	515 m
NCOG (DNA + RNA) eDNA samples	99
Passive filter eDNA samples	165
GEMCAP eDNA samples	80
RREAS-eDNA samples	131
Aerosol eDNA samples	16
Total eDNA samples	491
IFCB plankton samples (underway)	671
IFCB plankton samples (discrete)	28
CalBOBL (total deployments)	16 (one per station)
CalBOBL survey time (total)	~300 mins
CalBOBL survey time (average per deployment)	~21 mins
Manta net (total deployments)	16 (one per station)
Manta net survey time (total)	~245 mins
Manta net survey time (average per deployment)	~15 mins
Marine mammal and seabird observation hours	51 hrs 48 mins
Number of marine mammal species observed	15
Number of seabird species observed	54

Operating Area

The CalCOFI survey covered an area from San Diego to Monterey extending approximately 150 miles offshore. Figure 1 shows the planned locations of all 18 sample stations: five within Channel Islands National Marine Sanctuary (CINMS; stations 1–5), five within the proposed CHNMS (stations 6–9, 13), four within Monterey Bay National Marine Sanctuary (MBNMS; stations 10–11, 14–15), and one within the Bureau of Ocean Energy Management's Morro Bay

Wind Energy Area (station 12). A total of 16 stations were sampled. Stations 17 and 18 were not sampled.

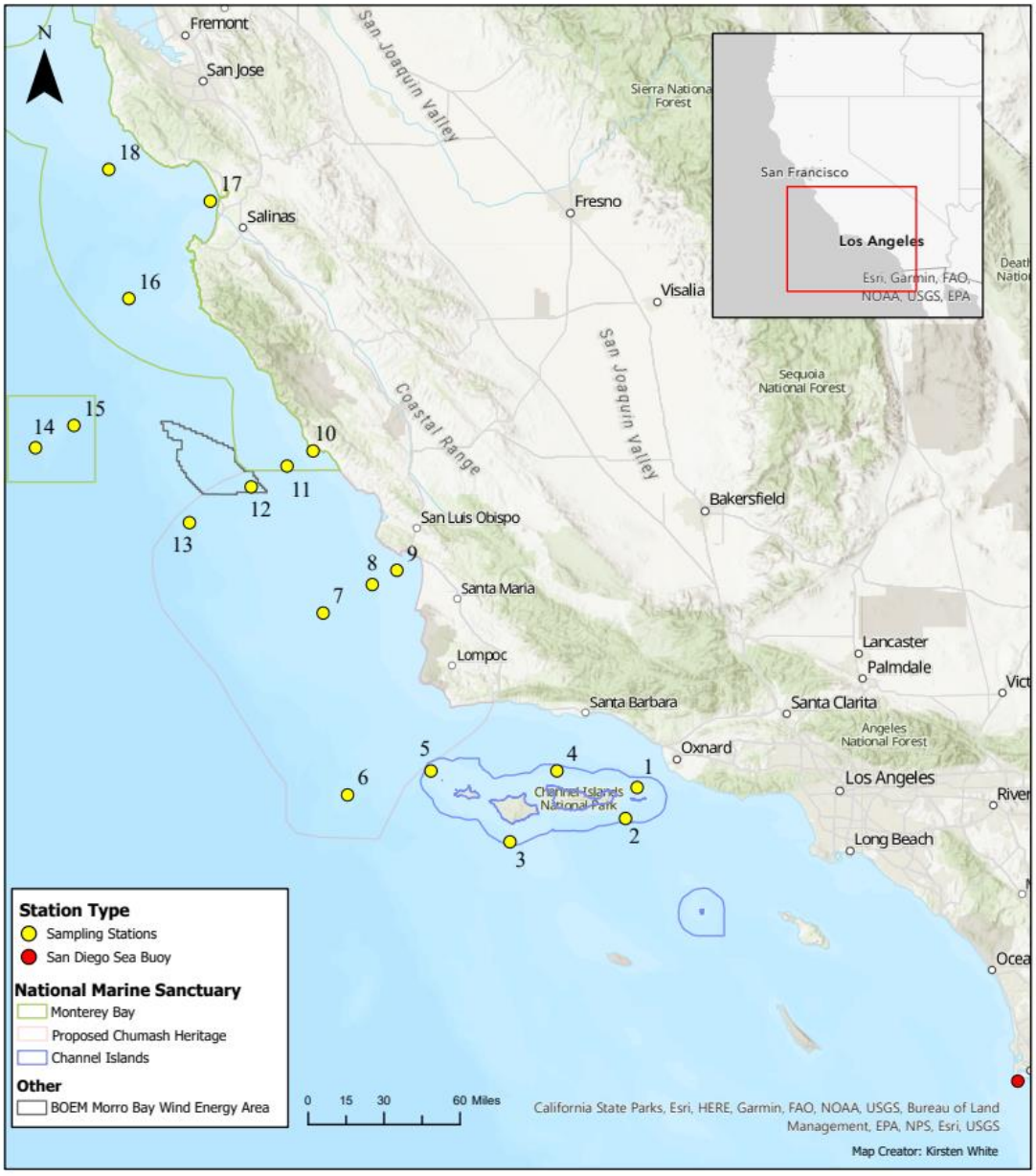

Figure 1. Proposed project map. NOAA Ship *Reuben Lasker* set sail from San Diego (near the SD Sea Buoy station) and navigated north. The map depicts the location of all original 18 proposed sampling locations throughout CINMS, proposed CINMS, MBNMS, and Morro Bay Wind Energy Area.

Timeline

Upon arrival at each sample station, the order of sampling operations was: 1) CTD deployment, 2) Manta net tow, and 3) CalBOBL (Bongo net tow). After removal of the Bongo net, the cruise departed for the next station. Table 3 outlines general spatial and timeline information for the sampling stations.

Altogether, execution at each station was smooth, controlled and efficient. Distances between stations usually ensured a >1 hr transit to work through science objectives, but sometimes the 12 hour limitation made it difficult to complete work near the end of operation hours. Eventually as staff became comfortable with operations, the pace increased and staff were able to complete stations more efficiently.

Table 3. Data on sample station date, location, and time. Station numbers align with those indicated in Figure 1. Location indicates the general area in relation to the greater regions of interest (CINMS, proposed CHNMS, MBNMS, and the Morro Bay Wind Energy Area). Time frame indicates time of arrival and departure for each station on a 24-hour clock in Pacific Standard Time. Total time indicates the duration spent sampling from arrival time to departure time. Note that station 6 was skipped on October 15 and completed instead on October 19.

Date	Station	Location	Time Frame	Total (hrs)
10/13/22	1	Rodriguez Seamount, CINMS	12:13–14:25	2:12
10/14/22	2	North of San Miguel Island, CINMS	17:27–19:10	1:43
10/14/22	3	Northeast of Santa Cruz Island, CINMS	11:20–13:04	1:43
10/14/22	4 (initiated)	East of Anacapa Island, CINMS	15:25–15:45	0:20
10/15/22	4 (completed)	East of Anacapa Island, CINMS	16:35–17:15	0:40
10/15/22	5	Western edge of CINMS	18:28–20:14	1:46
10/15/22	7	Santa Lucia Bank, proposed CHNMS	11:24–13:12	1:48
10/15/22	8 (initiated)	Santa Lucia Bank, proposed CHNMS	15:04–15:58	0:54
10/16/22	8 (completed)	Santa Lucia Bank, proposed CHNMS	16:03–16:36	0:33
10/16/22	9	Santa Lucia Bank, proposed CHNMS	17:40–19:03	1:22
10/16/22	10	Southern MBNMS	11:11–12:54	1:43
10/16/22	11 (initiated)	Southern MBNMS	14:48–15:53	1:05
10/17/22	11 (completed)	Southern MBNMS	16:14–16:22	0:08

Date	Station	Location	Time Frame	Total (hrs)
10/17/22	12	BOEM's Morro Bay Wind Energy Area	17:40–19:13	1:33
10/17/22	13	Western proposed CHNMS	20:15–21:22	1:06
10/17/22	14	Davidson Seamount, MBNMS	11:14–12:53	1:39
10/17/22	15	Davidson Seamount, MBNMS	14:33–15:54	1:21
10/18/22	16	MBNMS	20:10–21:53	1:43
10/19/22	6	Southern proposed CHNMS	19:57–21:35	1:37

Operations

Planning, Preparation, and Staffing

Project planning was successful, despite limitations on time for planning prior to sailing and working hours due to OMAO crew limitations. Additionally, a number of planning issues endangered the mission's success, including: vessel staffing, COVID exposure, and logistical issues with medical clearance process. The mission was at risk until the day of departure, which exacerbated planning difficulties. Due to staffing limitations, CINMS brought their NOAA Corps Officer to ensure operations could proceed with the required number of crew per OMAO's guidance. However even with this augmentation, OMAO staffing constraints restricted science operations to a 12-hour cycle (1200–2400). This approach ensured that biological sampling was not biased to day or night samples. Various members of the deck and survey departments volunteered to fill unfamiliar roles, helping to ensure the project's success. The wardroom and scientific party did an excellent job adjusting to staffing/working hour constraints to maximize project efficiency and station completion percentage. Overall communication was outstanding.

Underway

Certain information was collected continuously throughout the research cruise. The NOAA Ship *Reuben Lasker* provided: a thermosalinometer calibrated to continuously measure surface water temperature and salinity; an acoustic doppler current profiler to sample current profiles in five-minute averages; and an IFCB connected to the ship's underway seawater supply to image the eukaryotic phytoplankton community approximately every 25 minutes. Lastly, during daylight hours, a seabird observer was positioned at the flying bridge to identify and count seabirds during project transects.

The Scientific Computing System is the ship's onboard data management system and served as the main data collection system which recorded timestamps of all relevant data collection efforts and physical water parameters (e.g. temperature, salinity, chlorophyll content) in the flow through systems. Upon completion of the project, all data will be provided to SWFSC.

Chapter 2:
Water Sampling

CTD/Rosette Setup and Operations

NOAA Ship *Reuben Lasker* provided a CTD/rosette package equipped with 24 10-liter hydrographic bottles. At each station, the CTD was lowered to approximately 515 meters, depth permitting, to measure physical parameters and collect water samples. Samples were collected at discrete depths for analyses of chlorophyll *a*, salinity, nutrients, and eDNA and RNA. Prior to each CTD cast, passive filters were attached to the rosette to collect an eDNA profile.

Sample bottles were collected following the standard CalCOFI protocol. At each station, one bottle was used to collect a sample at depth (515 meters or at least 10 meters above the sea floor in shallower locations) and two bottles were used to collect samples 10 meters below the surface. Depth permitting, additional samples were collected at: the chlorophyll *a* maximum (two bottles), 100 m (two bottles), and 170 m (one bottle). Three to five samples were typically collected at each station, with 1–2 sample bottles used at each depth.

Data Processing and Analysis

Water samples were collected from Niskin bottles that were attached to the CTD rosette. Salinity was sampled from each Niskin bottle. Chlorophyll was sampled from Niskin bottles collected at a depth of 200 meters or shallower. Nutrients samples were collected at each depth.

Salinity

A Guildline Instruments Portasal™ Salinometer (8410A) was used to make precise conductivity comparisons between the water samples and a reference water standard. From these comparisons, salinities were calculated and logged using PC-based software that averages data to meet replicate criteria. Concurrent with the water sampling, a Sea-Bird Electronics CTD profiled in situ data. Data processing software was used to compare sampled salinity to *in situ* CTD measurements.

Chlorophyll

Seawater samples of a known volume were filtered (<10 psi) onto GF/F filters. The filters were then placed into 10-ml screw-top culture tubes containing 8.0 ml of 90% acetone. After 24–48 hours, the fluorescence of the samples was read on a fluorometer. Following that, samples were acidified, allowing the chlorophyll phaeopigments (i.e., phaeophytin) to degrade, and a second reading was taken. The readings prior to and after acidification were then used to calculate concentrations of both chlorophyll *a* and phaeopigments. The method used was based on those developed by Yentsch and Menzel (1963), Holm-Hansen et al. (1965), and Lorenzen (1967). Note that concentrations of phaeopigments are not a good measure of chlorophyll *a* degradation products present in the sample because chlorophyll *b* present in the sample will also be measured as phaeopigments.

Nutrients

Water samples were collected at sea, then immediately frozen to be processed for nutrients in the lab at a future date. Nutrient analysis will be performed on a QuAAtro continuous segmented flow autoanalyzer (SEAL Analytical). In this analysis, a sample of seawater enters a reagent stream within a manifold on the analyzer, where it undergoes a series of reactions that ultimately produce a colored compound. These compounds absorb light at a specific wavelength. A monochromatic beam of light is passed through the sample and the absorbance is measured. The machine is calibrated with a series of known standards and a standard curve is produced. The intensity of the color produced by the unknown sample is proportional to the concentration of the analyte present. The product of the ammonia method is a fluorescent species; however, the same basic principle applies, and the intensity of fluorescence is directly related to concentration. The methods for silicate and total oxidized nitrogen are modified versions of those described by Armstrong et al. (1967) and Gordon et al. (1992). The phosphate determination employs a modification of the method described by Murphy and Riley (1962), and ammonia is analyzed based on the Kerouel and Aminot (1997) fluorometric method.

eDNA Passive Filters

Upon recovery of the CTD rosette at each station, eDNA passive filters were immediately removed, stored in a Ziploc® bag, and frozen to be analyzed at a future date.

Chapter 3:
Plankton Sampling

Setup and Operations

Imaging FlowCytobot

The IFCB—a continuous and automated imaging flow cytometer—has capabilities to visualize the eukaryotic phytoplankton community (8 - 150 µm). The IFCB sampled 5 mL directly from the ship's uncontaminated seawater supply in the laboratory approximately every 25 minutes. Particles with chlorophyll fluorescence within each sample were imaged automatically. Discrete samples were also collected from the CTD-rosette at 10 m and the chlorophyll maxima when different than 10 m. Integrating the data to be used as part of the larger California Imaging FlowCytobot Network– that includes now regular CalCOFI IFCB deployments– is underway. The data will be publicly available online on the network's dashboard (Woods Hole Oceanographic Institution, 2019).

CalCOFI Bongo Oblique

The CalBOBL frame included two 71-cm ringed openings with a 505-micron mesh net attached. The net was connected to a wire with a 75-lb weight affixed to the underside of the frame. Upon deployment, the net was dropped to a terminal depth of 212 meters below the surface (or 30 meters above the sea floor, depending on the local maximum depth). A buffer of 30 meters was provided to avoid contact with the seafloor. The net was towed at a speed of 1.5 knots to ensure an oblique wire angle of 45 degrees throughout the tow. A General Oceanics flowmeter was mounted on the starboard side net to calculate the volume of water strained throughout the tow. The flowmeter was an essential component in quantifying the density of eggs and larvae in the sampling area. The nets collected samples of ichthyoplankton. Additionally, passive filters were attached to the nets to collect eDNA samples.

Manta Net

The Manta net frame also had a 505-micron mesh net attached and was connected to a wire supported by twin floats. Upon deployment, the Manta net was towed along the surface (neuston layer) at a speed of 1–1.5 knots for 15 minutes. A General Oceanics flowmeter was also mounted in the opening to calculate the volume of water strained throughout the tow. The Manta nets collected ichthyoplankton samples within the neuston layer. Passive filters were also attached to the Manta net for eDNA sample collection.

Sample Processing

CalBOBL samples were collected from the starboard and port sides. When each CalBOBL and Manta tow was completed, the frames were brought back to the surface and each net was rinsed with seawater to consolidate the ichthyoplankton sample into the detachable codend. Following that, the CalBOBL codends from the starboard and port, plus codends from the Manta tow were removed and taken into the preservation area in the on-board fish lab. The starboard side CalBOBL samples were preserved in a quart-sized jar and affixed with a 5% buffered

formalin/sodium borate/seawater mixture. The port side CalBOBL samples were preserved in a pint-sized jar and affixed with a 100% buffered ethanol solution. After 24 hours, the samples were drained and replenished with a new, fresh mixture of buffered ethanol. The Manta net samples were preserved in a pint-sized jar and affixed with a 5% buffered formalin/sodium borate/seawater mixture.

Upon conclusion of the cruise, samples were processed and sorted for fish eggs and larvae at the SWFSC Larval Fish Lab. Eggs and larvae were identified to the lowest possible taxa. The passive filters outfitted on the Bongo and Manta nets were removed and placed in a plastic Ziploc® bag for processing once the cruise was completed.

Metadata from the CalBOBL and Manta collections were processed and entered into the SWFSC CalCOFI database. In the future, these data will be used by the stock assessment team to help manage coastal pelagic species, such as sardine and anchovy. These data are made public through the ERDDAP CoastWatch West Coast server.

Chapter 4:
Environmental DNA

Introduction

Five different eDNA sample methods were used on the research mission, including: 1) NCOG, 2) RREAS-eDNA, 3) GEMCAP, 4) passive filtering, and 5) aerosol filtering. This mission aimed to carry out an intensive intercalibration of these different eDNA protocols. The goal of the intercalibration effort was to identify best practices for NOAA ocean eDNA protocols and harmonize datasets so that water samples collected as part of one ocean observing program can be integrated into analyses of other programs. An additional goal was to demonstrate the value of novel eDNA sampling methods.

Dr. Andrew Allen at Scripps Institution of Oceanography and J. Craig Venter Institute leads the NCOG project. Dr. Allen has collected and analyzed eDNA samples from CalCOFI since 2014. The samples collected during this cruise using NCOG methodology will be integrated into the existing and ongoing CalCOFI genomics time-series data set. Additionally, all eDNA sample processing using the other protocols will be conducted in Dr. Andrew Allen's laboratory at the J. Craig Venter Institute. Data requests should be submitted to Dr. Allen and Dr. Zachary Gold, Director of Genomics at NOAA's Pacific Marine Environment Laboratory.

Methods

NOAA-CalCOFI Ocean Genomics Project

NCOG eDNA and RNA samples were collected via CTD/rosette casts at each sample station following the methods used on CalCOFI surveys since 2014. Multiple samples were collected at various depths:

- 2 liters were collected for DNA at 10 meters below the surface and at chlorophyll max
- 4–6 liters were collected for DNA or RNA at 10 meters, chlorophyll max, 100 meters, 170 meters, and 515 meters (or 10 meters above the sea floor, depth permitting)

After deployment, the collected seawater samples were filtered onto 0.2-micron sterivex filters for a maximum of 30 minutes. All water was purged from the sterivex with a syringe, wrapped in aluminum foil, and flash-frozen in liquid nitrogen. The volume was recorded based on the volume remaining. Upon completion, lines and bottles were sterilized with Milli-Q water. DNA and RNA will be processed following Allen Lab protocols (Rabines et al., 2020a, 2020b, 2020c, 2020d, 2022). Bioinfomormatic analysis of the DNA will be done with QIIME2 following Allen Lab protocols (Lampe, 2021a, 2021b, 2021c). These data will be integrated into the existing CalCOFI genomics time series (2014–present) led by the Allen Lab at the J. Craig Venter Institute & Scripps Institution of Oceanography.

Rockfish Recruitment and Ecosystem Assessment Survey eDNA

At each station, 3-liter carboy water samples were collected from the CTD/rosette at the depths specified above for NCOG. Equipment required for sample collection included: carboys, peristaltic pumps, swinnex filter holders, tubing, bleach, and Milli-Q. For each station,

preparation for water samples, sea water collection, and water filtration was completed. Preparation included: thorough and meticulous sterilization of the carboys and tubing using bleach and Milli-Q; workstation sterilization; sample data sheet prep; and labeling of cryovial tubes. Details of the preparation process are outlined below. Sea water samples were collected using the carboys (rinsed with sample water) at three depths: 10 m, 100 m, and at the chlorophyll max depth. Afterward, samples were run through tubing and swinnex filters, rolled into a labeled cryovial, transferred to the -80 °C freezer for storage, and recorded on the data sheet. This filtration process also followed careful and specific sterilization guidelines, detailed below.

Water Sample Preparation

Sterilize Carboys and Tubing

- Add one liter of 10% bleach to the carboy; seal and shake.
- Run 250 ml of 10% bleach through tubing and swinnex filter. Hold in a collection bottle.
- Place collected bleach in the next carboy. Repeat for each. Save 10% bleach for three days.
- Rinse carboy with 750 mL of Milli-Q; add another set of 750 mL Milli-Q and run through tubing and swinnex filter into the sink.
- Prepare three swinnex filters for each depth. Pour 1 liter of 10% bleach into the beaker, place three swinnex filters (halved/opened), and rinse sparingly with 50 mL Milli-Q.

Filter Water Samples

- Remove residual bleach or Milli-Q by running one liter of sample water through tubing and swinnex filters.
- Using sterile forceps, disconnect swinnex filters and connect a 0.2-µm PVDF filter.
- Run two liters of sample water through the swinnex filter (collect the sea water to verify the volume). Continually add water until the sample is thoroughly filtered.
- Afterward, gently roll the filter into a labeled cryovial using two sterile forceps. Record the volume of filtered water prior to dumping the waste.
- Transfer cryovials to the -80 °C freezer, recording on the datasheet.
- Note: run a blank once per day.

Global eDNA Marine Collection and Analysis Program

At each station, one-liter samples were collected from the CTD/rosette at the depths specified above for NCOG. The GEMCAP filtering method used water vacuum filtration units. One-liter samples were used per filter for each target parameter. Samples were processed following the steps below:

- Process in numerical order. Quickly rinse filter funnel units with ethanol, followed by either Milli-Q or deionized water.
- Invert funnels, remove filter, and place on the base with the grid side facing up.
- Place the waste line in a graduated pitcher so the volume can be measured after filtering.
- With the vacuum unit on, pour water into the funnel until the sample is completely filtered.

- Use forceps sterilized with 70% ethanol to roll the filter (biomass side facing inward) and place into a pre-labeled 15-ml falcon tube. Transfer into the -80 °C freezer for storage.
- After filtering all samples, wash filter funnels using tap water and place them back on the filtration units. Rinse with about 500 ml of Milli-Q water to remove salt from the vacuum unit.
- Rinse bottles thoroughly with tap water, then Milli-Q water, followed by HCl.
- DNA will be extracted and processed in the Allen Lab following the NCOG protocols described above.

Passive Filtering

Triplicate passive filters were deployed at each station on the CTD/rosette, CalCOBL (Bongo net tow), and Manta net tow. Attachment was done using a fresh pair of nitrile gloves. Upon completion of CTD deployment and net tow, the passive filters were removed from each instrument and placed into a labeled Ziploc® bag using fresh nitrile gloves. Ziploc® bags were placed in the cooler for less than 30 minutes, then transferred into labeled cryovial tubes using sterilized forceps, wrapped in foil, and labeled. Tubes were then placed into a liquid nitrogen dewar. DNA will be extracted and processed in the Allen Lab following the NCOG protocols described above.

Aerosol Filtering

The aerosol sampler was placed on the front of the ship. One aerosol sample was collected per station while CTD, Manta, and CalBOBL (Bongo net) sampling was underway. The aerosol sample started collecting from the time CTD entered the water and stopped when the CalBOBL was removed from the water. Sample filters were collected from the aerosol device, stored in a plastic cup with a lock on top, and sealed in the refrigerator. Sample collection and recovery procedures included:

- Flow rate selection. The CalCOFI cruise used 200 liters per minute (LPM) light-emitting diode (LED).
- Timed run mode rate selection. The CalCOFI cruise used a continuous sampling feature.
- Once the run was completed, the filter was manually recovered from the sampler using gloves.
- Next, the filter cup was placed in a Ziploc® bag, relevant information was recorded on the sample datasheet (end time, latitude, and longitude), and placed into the -80°C freezer.

Additional detailed instructions can be found in InnovaPrep, LLC (n.d.).

eDNA Statistics

Table 4. eDNA sample count.

Method	Average Samples per Station
NCOG (DNA + RNA)	3–7
Passive filter	10
Aerosol	1
GEMCAP	2–7
RREAS-eDNA	9–10 (6 at station 12)

Table 5. eDNA sample depth.

Depth Category	Sample Depth (m)
Shallowest	0–5
Deepest	515
Average	73

Comments and Lessons Learned

- The NCOG method proved more streamlined and easier than RREAS-eDNA (the Goodwin protocol). The RREAS-eDNA method required much more time, personnel effort, and resources.
- The passive filter method was also streamlined and easy to deploy, but has limited literature published on its effectiveness. Unlike NCOG, this approach will not capture all photosynthetic and heterotrophic species due to a larger filter pore size. Assessment of this method's quality is still underway, and the resulting DNA signatures remain to be viewed and interpreted. However, the team appreciated the ease and speed with which the passive filters could be deployed.
- Altogether, five science members worked on eDNA sampling; it would be time intensive to continue all sampling methods concurrently. The NCOG protocol's ease of deployment and history with the CalCOFI program make it ideal for future CalCOFI cruises.
- The high-throughput capabilities for eDNA work in the Allen Lab at the J. Craig Venter Institute are ideal and enable the rapid eDNA sample processing and analysis from the cruise.

Chapter 5:
Marine Mammals and Seabirds

Survey Operations

Marine mammal and bird surveys were typically conducted while in transit to the first sample station of the day. Data were collected while in motion, at a speed of five knots or more. Once sampling was complete, surveying would resume while in transit from one station to the next. Many species of marine mammals and birds were observed throughout the cruise (Table 6).

Observation Overview and Highlights

Fauna was abundant throughout the proposed CHNMS. While crossing Point Conception, wildlife sightings were particularly frequent. Notable observations included killer, humpback, fin, and minke whales; several dolphin species; tuna; black-footed albatrosses; and an abundance of other avifauna. The crew noted the incredible diversity in this area as a perfect reflection of the unique transition zone captured around Point Conception. In the northwest corner of the proposed national marine sanctuary, more fin, minke, and humpback whales were observed in high numbers. A California endemic bird, the ashy storm petrel, was observed, as well as hundreds of Sabine's gulls, pomarine jaegers, and pink-footed shearwaters.

Conditions were calm and clear while passing through the MBNMS area. Sightings were dominated by marine mammals, and included: blue and other large sharks; swordfish; Pacific white-sided dolphins; large groups of northern right whale dolphins; fur seals; fin, minke, and mating humpback whales; and a large blue whale. Notable bird sightings in MBNMS included Guadalupe murrelets, south polar skua, abundant Cassin's auklets, rhinoceros auklets, gulls, and fulmars.

Survey Statistics

Table 6. The top five most observed marine mammals and seabirds. A total of 15 species of marine mammals and 54 species of seabirds were observed.

Category	Species	Number of Sightings	Number of Individuals
Marine mammal	Common dolphin	21	900
Marine mammal	Humpback whale	16	43
Marine mammal	Unidentified pinniped	16	29
Marine mammal	Fin whale	15	38
Marine mammal	Minke whale	13	16
Seabird	Black-vented shearwater	121	634
Seabird	Western gull	118	178
Seabird	Pink-footed shearwater	85	156

Category	Species	Number of Sightings	Number of Individuals
Seabird	Cassin's auklet	77	147
Seabird	Pomarine jaeger	60	76

Chapter 6:
Operations Overview

Introduction

In general, operations were very smooth during the 2210RL Enhanced CalCOFI cruise aboard the NOAA Ship *Reuben Lasker*, especially considering the limited time available to plan and prepare for the project and the challenges in ensuring that the ship was both ready and compliant with OMAO regulations to sail.

Technical Issues

Issues with supplying uncontaminated seawater were encountered. Best practices for underway IFCB and eDNA collection suggest that the centrifugal pumps currently used to provide uncontaminated seawater are too harsh on cells, which can result in a loss of approximately 40% of cells. On University-National Oceanographic Laboratory System vessels, the CalCOFI team has successfully used an electrically driven diaphragm pump to bypass the centrifugal pumps and provide seawater to the lab space. The feasibility of this bypass remains unclear on the vessels used for CalCOFI surveys (R/V *Reuben Lasker* and R/V *Bell M. Shimada*). Additionally, there are concerns that the diaphragm pump will create inaccuracies in the thermosalinometer and fluorometer data, as this equipment also requires uncontaminated seawater. Additional investigation and testing could assess the feasibility and effects of installing a different pump.

Emergency Rescue

A medical emergency also occurred while underway. A crew member had a fall that resulted in a helicopter evacuation with the United States Coast Guard. Underway operations (transiting to the last station) were halted, and the science party assisted where feasible in clearing all loose articles and equipment from the weather deck. Following that, the science party stayed clear while crew and officers prepared the ship for the Coast Guard Helicopter MH-65. The MH-65 arrived with two pilots and two crew members. Upon arrival, the Coast Guard crew identified the bow of the ship as the best evacuation spot. After deploying a rescue swimmer, a basket was dropped and the injured crew member was hoisted into the MH-65. The Coast Guard crew took the injured crew member to a hospital 40 nm east in San Luis Obisbo. The crew member remained in stable condition overnight before leaving the next morning in good health. The mission team is grateful to the Coast Guard for their assistance.

Weather Conditions

Weather and sea conditions were very calm during the mission, which allowed for straightforward data collection at all stations and safe marine mammal and seabird operations.

Acknowledgements

Many groups and individuals provided invaluable support to make this research effort successful, including Scripps Institution of Oceanography, California Department of Fish and Wildlife, J. Craig Venter Institute, Channel Islands National Marine Sanctuary, NOAA's Pacific Marine Environmental Laboratory, and NOAA's Southwest Fisheries Science Center. In particular, special thanks to the United States Coast Guard for executing an emergency rescue.

Glossary of Acronyms

CalCOFI	California Cooperative Oceanic Fisheries Investigations
CalBOBL	CalCOFI Bongo Oblique
CDFW	California Department of Fish and Wildlife
CINMS	Channel Islands National Marine Sanctuary
CHNMS	Chumash Heritage National Marine Sanctuary
CTD	conductivity, temperature, depth
eDNA	environmental DNA
GEMCAP	Global eDNA Marine Collection and Analysis Program
IFCB	Imaging FlowCytobot
MBNMS	Monterey Bay National Marine Sanctuary
NCOG	NOAA-CalCOFI Ocean Genomics
NOAA	National Ocean and Atmospheric Administration
RREAS-eDNA	Rockfish Recruitment and Ecosystem Assessment Survey eDNA
SIO	Scripps Institution of Oceanography
SWFSC	Southwest Fisheries Science Center

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