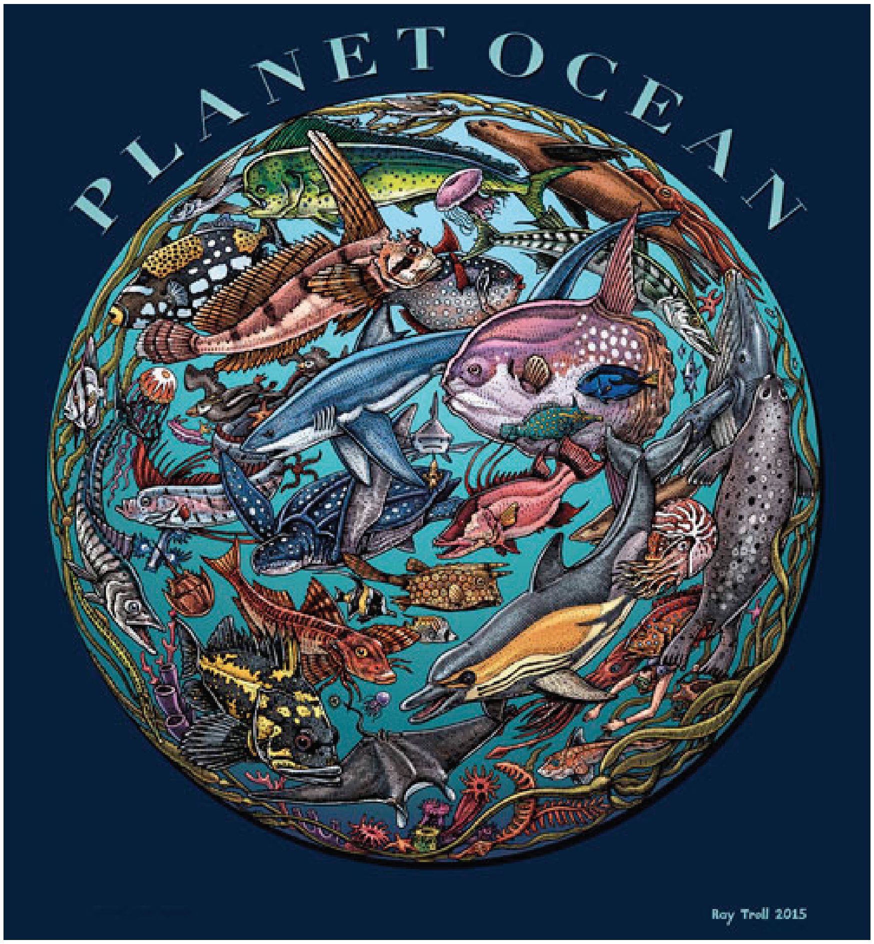
OCS Study
BOEM 2023-030

Demonstrating an Effective Marine Biodiversity Observation Network in the Santa Barbara Channel



U.S. Department of the Interior
Bureau of Ocean Energy Management
Pacific OCS Region, Camarillo, CA

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Demonstrating an Effective Marine Biodiversity Observation Network in the Santa Barbara Channel

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DISCLAIMER

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REPORT AVAILABILITY

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ABOUT THE COVER

Ray Troll's rendition of life in the sea, colorized by Grace Freeman. Originally featured in the 1994 book, Planet Ocean: A Story of Life, the Sea and Dancing to the Fossil Record, by Bradford Matson and Ray Troll, Ten Speed Press, 133 pp. Used with permission of Ray Troll.

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List of Abbreviations and Acronyms

aph	Phytoplankton absorption spectra
AQ	Arroyo Quemado
ASV	Amplicon sequence variant
AUV	Autonomous underwater vehicle
AVIRIS	Airborne Visible/Infrared Imaging Spectrometer
BOEM	Bureau of Ocean Energy Management
CalCOFI	California Cooperative Oceanic Fisheries Investigations
CCE	California Current Ecosystem
CW	Continuous wave
DataONE	Data Observation Network for Earth
DCPP	Diablo Canyon Power Plant
DOI	Department of the Interior
DSMM	Data Set Maturity Model
EBV	Essential biodiversity variables
EDI (edi)	Environmental Data Initiative
EDM	Environmental Data Management
eDNA	Environmental DNA
EOV	Essential Ocean Variables
ESIP	Earth Science Information Partners
ESPIS	Environmental Studies Program Information System
FM	Frequency-modulated
GBIF	Global Biodiversity Information Facility
GHRSST	Group for High Resolution Sea Surface Temperature
GPU	Graphics Processing Unit
HAB	Harmful Algal Bloom
HARP	High-Frequency Acoustic Recording Package
HFM	High-frequency modulated
ICI	Inter-click interval
KFMP	Kelp Forest Monitoring Program
LTER	Long Term Ecological Research
LTSA	Long-term spectral average
MARINe	Multi-Agency Rocky Intertidal Network
MBARI	Monterey Bay Aquarium Research Institute
MBON	Marine Biodiversity Observation Network
MESMA	Multiple Endmember Spectral Mixture Analysis
MFA	Mid-Frequency Active

MR	Mohawk Reef
NASA	National Aeronautics and Space Administration
NCEAS	National Center for Ecological Analysis and Synthesis
NCEI	National Centers for Environmental Information
NIH	National Institutes of Health
NOAA	National Oceanic and Atmospheric Administration
NMFS	National Marine Fisheries Service
NSF	National Science Foundation
OBIS	Ocean Biodiversity Information System
OOS	Ocean Observing Systems
PACE	Plankton, Aerosol, Cloud and Ocean Ecosystem
PnB	Plumes and Blooms
RA	Relative abundances
RCN	Research Collaboration Network
RESON	Regional Ecosystem Services Observation Network
RISE	Redesign Instruction for the Student Experience
SAM	Species archetype models
SBC	Santa Barbara Channel
SBC MBON	Santa Barbara Channel Marine Biodiversity Observation Network
SCCOOS	Southern California Coastal Ocean Observing System
SCCWRP	Southern California Coastal Water Research Project
SEISMIC	Sloan Equity and Inclusion in STEM Introductory Courses
SEM	Structural Equation Model
SOCAL	Southern California Operations Area
SRA	Sequence Read Archive
SST	Sea surface temperature
STEM	Science, technology, engineering, and math
sUAS	Small unmanned aircraft system
TDWG	Taxonomic Databases Working Group (formerly), Biodiversity Information Standards
UCSB	University of California - Santa Barbara
UVC	Underwater visual census

1 Goals and Objectives

Although we spend millions annually monitoring marine resources, these efforts are uncoordinated and have major information gaps. We developed a scalable and transferable Marine Biodiversity Observation Network (MBON) in the Santa Barbara Channel (SBC), one of the most monitored areas of the world, that we are expanding throughout the Southern California Bight. SBC MBON is connecting existing monitoring efforts and continues to fill remaining information gaps. Our overall objective is to provide a complete picture of biodiversity in SBC using a transferable system that integrates and augments existing monitoring programs including the National Science Foundation's (NSF) Long Term Ecological Research (LTER) program, Channel Islands National Park, California Cooperative Oceanic Fisheries Investigations (CalCOFI), and NASA Plumes and Blooms (PnB).

Broad goals were to:

A. Integrate biodiversity data to enable inferences about regional biodiversity: Synthesizing information relevant to biodiversity requires integrating highly heterogeneous data collected at widely different temporal and spatial scales. We employed advanced techniques in spatial statistics for this synthesis and provide multiple biodiversity-related data products, including indices that provide measures of ecosystem diversity and health.
B. Develop advanced methods in optical and acoustic imaging and genomics for monitoring biodiversity in partnership with ongoing monitoring and research programs to begin filling the gaps in our knowledge: A key element we employed was a 'pincer movement' using two classes of methods approaching diversity observation from opposite directions: optical and acoustic imagery from the ecosystem scale down to the species level, and molecular biology from the genetic scale up through community level.
C. Implement a tradeoff framework that optimizes allocation of sampling effort: An effective MBON requires targeted sampling to address key data gaps, while making best use of existing sampling efforts, thereby gaining a complete description of biodiversity while minimizing cost. Optimal decisions about data collection require a framework for balancing costs and benefits of alternative sampling. Such a framework can be used to make recommendations for how resources should be allocated in a full-scale MBON as a function of the program's goals and anticipated funding level.

2 Integrating Biodiversity Data to Enable Inferences About Regional Biodiversity

Our data management system was established and formalized early in the project and operates according to accepted community practices (e.g., Borer et al. 2009). Our dataset design practices use generic composition patterns based on X-MBON and community recommendations for ecological community data. These practices are deliberately flexible to remain appropriate for multiple or unknown uses, i.e., formats that are specifically not tied to any single research question, customer need, or format. SBC MBON data packages resemble an Ecology Society of America data paper, ensuring a high standard of usability. All SBC MBON datasets have an immutable copy archived for citation with a Digital Object Identifier to ensure reliability and consistency per GEO recommendations (2015). The EDI Data Repository (https://EnvironmentalDataInitiative.org, NSF Award #1931143 and #1931174; Gries et al. 2018) supports both provenance and tracking and revision control essential applications and ongoing time series. SBC MBON data are available via DataONE (Data Observation Network for Earth), alongside data from Axiom Research Workspace and NCEI. Spatially explicit data on biodiversity are also available through Marine Cadastre and ESPIS.

During more recent years, the SBC MBON continued major efforts started under the previous SBC MBON grant: adding datasets to its holdings, and continuing work on demonstration applications and workflows. As we work toward appropriate, transferrable pipelines for each type of data, we continue to develop workflows producing Darwin Core Archives for OBIS and connections between archived datasets and the ERDDAP server hosted by SCCOOS. Workflows are collaborations with EDI as part of their effort to prepare hundreds of diverse biological datasets for ingestion by query systems such as GBIF (O'Brien et al. 2021). All demonstration projects and workflows can be repeated but will not be sustainable until all details are formalized. To that end, we are testing a maturity matrix for data systems to assess future needs (Peng et al. 2015) and examining options for developing standardized measurements or inclusion in metadata.

2.1 Data Inventory

Thirty-six distinct datasets spanning a full range of environmental resources were harmonized to enable integration across programs and archived (Table 1). We added five new data packages for the SBC MBON catalog and updated most ongoing time series. The remainder (plus several unanticipated datasets) are staged, pending approval from partners and researchers. SBC MBON datasets include the oceanographic profile, biological and biogeochemical data, as well as microbial genomics from all NASA PnB cruises between August 1996 and December 2018, biodiversity of nudibranchs (sea slugs) and allies from rocky intertidal sites on the mainland coast of the Santa Barbara Channel from 2002 through 2020, and sea urchin microbiome data across three rocky reef habitats in southern California that differed in the types and availability of food resources: kelp forests, urchin barrens, and a hydrocarbon seep; environmental DNA (eDNA) from kelp forest communities in the Santa Barbara Channel with its raw sequence published in the Sequence Read Archive (SRA) database.

Table 1. Inventory of datasets showing their sources and status in the SBC MBON system. Identifiers of archived raw datasets and of SBC MBON final products are given.

Source Project	Description	Source ID[3]	Cleaned	Standardized	Archive ID[4]	Year first published
MBON	Combined species list		x	x	edi.7	2015
MBON	Areal abundance from image analysis		x	x	edi.484	2020
PISCO	Shallow subtidal fish	pisco.19	x	x	edi.5, edi.189, edi.140 (DwC-A)	2016, 2018
SBC LTER	Shallow subtidal fish	knb-lter-sbc.17	x	x		
NPS KFMP	Shallow subtidal fish		x	x		
CINP	Shallow subtidal fish		x	x		
Reef Check	Shallow subtidal fish		x	x	edi.141	2019
PISCO	Shallow subtidal invert/algae cover	pisco.20	x	x	edi.3, edi.490 (DwC-A)	2015, 2020
SBC LTER	Shallow subtidal invert/algae cover	knb-lter-sbc.15	x	x		
CINP	Shallow subtidal invert/algae cover	-	x	x		
NPS KFMP	Shallow subtidal invert/algae cover	-	x	x		

Source Project	Description	Source ID[3]	Cleaned	Standardized	Archive ID[4]	Year first published
PISCO	Shallow subtidal invert/algae abundance & density	pisco.21	x	x	edi.6, edi.491 (DwC-A)	2015, 2020
SBC LTER	Shallow subtidal invert/algae abundance & density	knb-lter-sbc.19	x	x		
CINP	Shallow subtidal invert/algae abundance & density		x	x		
NPS KFMP	Shallow subtidal invert/algae abundance & density		x	x		
Gray Whales Count	Gray whale sightings, UCSB Campus Pt		x	x	edi.257	2018
NWFSC	Groundfish trawl diversity			x	edi.486	2020
Plumes and Blooms	HPLC profiles with chemistry results	knb-lter-sbc.121				2020
Plumes and Blooms	Microbial genomics		x	x	edi.1135	2022
SBC LTER	Sea otter sightings	knb-lter-sbc.61	x	x		
SBC LTER	Landsat kelp biomass	knb-lter-sbc.74	x	x		
Whale Acoustics lab (SIO)	Marine mammals – SO CAL Bight	NA[1]				
SBC LTER	Beach invertebrates	knb-lter-sbc.91	x	x		
SBC LTER	Shore birds	knb-lter-sbc.51	x	x		
Love Lab	Fish/invertebrates, shallow natural reefs		x	x	edi.110, edi.488 (DwC-A)	2017, 2020
Love Lab	Fish/invertebrates, oil/gas platform (shallow)		x	x	edi.111, edi.489 (DwC-A)	2017, 2020
Love Lab	Fish, deep natural reefs		x	x	edi.112, edi.487 (DwC-A)	2017, 2020
Love Lab	Fish/invertebrates, oil/gas platform (deep)		x	x	edi.113	2017
Love Lab	Fish survey, methodology		x	x	edi.470	2020
CalCOFI	Ichthyoplankton abundance		x	x	edi.109	2017
CCE LTER	Pelagic birds	knb-lter-cce.255				NA
Carlson Lab	Microbial genomics, methodology				edi.114	2017
Carlson Lab	Microbial genomics in kelp forest				NCBI	2020

Source Project	Description	Source ID[3]	Cleaned	Standardized	Archive ID[4]	Year first published
SCCWRP	Deep soft bottom benthos		x		edi.485[5]	2020
MBON	eDNA from kelp forest		x	x	NCBI edi.1132	2022
SCCOOS	Harmful Algal Bloom				edi.988	2021

Notes: [1]Anticipated. [2]2009–2012 data from OBIS SEAMOUNT. [3,4]Data from https://dataone.org or https://portal.edirepsitory.org. [5] Dataset archived in staging directory https://portal-s.edirepsitory.org. NA: not applicable; data are managed elsewhere or already available, although further cleaning may be deemed necessary.

2.2 Applications and Workflows

To display the benthic community research using image analysis, we developed an interactive tool to demonstrate the benthic organisms living in the Santa Barbara Channel kelp forest (https://sbc.marinebon.org/data/map/Imagery_BisQue/). The images taken under the water are displayed alongside the documented community. In addition, we also added interactive tools from our collaborators in the region that are based on our data and will be beneficial to our research, including the Ocean Report tool developed by the BOEM/NOAA team and the interactive marine ecosystem for Channel Islands National Marine Sanctuary. The full list of interactive maps and tools are displayed at https://sbc.marinbon.org/data/map/.

This work took advantage of the EDI repository's data harmonization pipeline for community survey data in which similar data are converted to a flexible intermediate to streamline their further use (O'Brien et al. 2021). All datasets (MBON-standard, flexible intermediate, DwC-A) were archived in EDI, and the relationships between them are documented in provenance metadata. EDI initiated a collaboration with GBIF and the US-GBIF nodes for delivering data of interest, including earmarking marine datasets for OBIS. SBC MBON data are highlighted in this process because of its high quality and importance.

2.3 Southern California Coastal Ocean Observing System (SCCOOS) Collaboration

SBC MBON datasets were imported directly from the repository to the ERDDAP maintained by SCCOOS (https://sccoos.org/). In collaboration with the LTER, NOAA developed code to directly ingest EML-described datasets to any ERDDAP system (Simons 2017). We coordinated SBC MBON R-code design and sharing with the larger community of ecosystem science data managers, by participating in community efforts such as Earth Science Information Partners (ESIP) and the MBON DMAC committee, to maintain compatibility with IOOS and NASA practices. SBC MBON installed a test ERDDAP system to test automated ingestion of data tables directly from EDI.

EDI and MBON supported a data fellow (Ian Brunjes) to work with SCCOOS to script the creation of the SCCOOS Harmful Algal Blooms (HABs) data package and archive it at EDI. SCCOOS had already contributed this dataset to OBIS. However, archiving with EDI affords several advantages: the data records are easily discoverable alongside other SBC MBON datasets; the dataset will appear in DataONE; and updated revisions can use the EDI workflow for future contributions. SCCOOS will also have a model for additional contributions to the EDI repository. The HABs data package was added to the EDI repository in September 2021.

2.4 Essential Biodiversity Variables

A large part of data harmonization is the need for measurements with standardized descriptions so that data are understandable across systems. Even for a system as broadly used as Darwin Core, standardized descriptions do not exist for biodiversity data values, because the Darwin Core vocabulary covers only high-level column names. This need is a current focus of TDWG, the parent organization for Darwin Core and its “Distinct values” project (https://github.com/tdwg/dwcqa/tree/master/data), an initiative that tackles biodiversity data quality in a strategic manner. SBC MBON participates in this effort as a TDWG member and in related efforts to describe pertinent measurements using ontology for inclusion in EML metadata (https://github.com/NCEAS/eml/). Data manager O’Brien worked with the NCEAS and the DataONE Network to describe measurement details with ontology, so that these may be harnessed for computer interpretation (https://github.com/DataONEorg/sem-prov-ontologies).

2.5 Sustainability Planning

SBC MBON used evaluation frameworks to evaluate readiness of datasets and management workflows for various uses. A promising system is the Data Set Maturity Model (DSMM, Peng 2015), already in use by several NOAA and NCEI systems (e.g., Casey 2016). Such systems highlight requirements at each stage of a data service’s production and are useful in creating cost models. It will be essential to our ability to connect SBC MBON’s now-established data streams to different types of users. In addition to completing the remaining group of datasets in Table 1, formalizing workflows and sustainability planning is an integral part of our work.

2.6 Data Management Presentations and Products

Presentation: O’Brien M. Data management at EDI and LTER. MBON DMAC meeting, June 8, 2021

2.6.1 Software

https://github.com/lkuiucsb/Excel-to-EML. This tool aims to help labs and small research groups to publish their data on a repository. The tool uses the R programming language to create an EML document from an Excel metadata workbook for ecological data publication.

2.6.2 Datasets

The datasets listed below were initiated, managed, and published by SBC MBON. See catalog for the full dataset list (https://sbc.marinebon.org/data/catalog/):

SBC Marine Biodiversity Observation Network, R. Miller, A. Rassweiler, J. Caselle, D. Kushner, D. Reed, K. Lafferty, L. Kui, and M. O'Brien. 2021. Santa Barbara Channel Marine BON: Nearshore kelp forest integrated fish, 1981-ongoing ver 4. Environmental Data Initiative. https://doi.org/10.6073/pasta/dbf98d8ddc5f48c4bc074ae4293d8d7c

SBC Marine Biodiversity Observation Network, R. Miller, A. Rassweiler, J. Caselle, D. Kushner, D. Reed, K. Lafferty, L. Kui, and M. O'Brien. 2021. Santa Barbara Channel Marine BON: Nearshore kelp forest integrated quad and swath survey, 1980-ongoing ver 4. Environmental Data Initiative. https://doi.org/10.6073/pasta/75ea73fd5ffbe37aeff4aee78cd85581

SBC Marine Biodiversity Observation Network, R. Miller, A. Rassweiler, J. Caselle, D. Kushner, D. Reed, K. Lafferty, L. Kui, and M. O'Brien. 2021. Santa Barbara Channel Marine BON: Nearshore kelp forest integrated benthic cover, 1980-ongoing ver 9. Environmental Data Initiative. https://doi.org/10.6073/pasta/6cf47d3a8310368f62c75e6b6e63076a

SBC Marine Biodiversity Observation Network, R. Miller, A. Rassweiler, J. Caselle, D. Kushner, D. Reed, K. Lafferty, L. Kui, and M. O'Brien. 2021. Santa Barbara Channel Marine BON: Nearshore kelp forest integrated taxa, 1980-ongoing ver 4. Environmental Data Initiative. https://doi.org/10.6073/pasta/eb16a01a1b9b24a3ebb46170745f9e72

SBC Marine Biodiversity Observation Network, R. Miller, S. Sampson, B. Doheny, and F. Puerzer. 2021. MBON: Benthic percent cover in the Santa Barbara Channel ver 4. Environmental Data Initiative. https://doi.org/10.6073/pasta/a976125f2f84c32657efb26276d1953d

Lamy, T., F. Puerzer, and R. Miller. 2022. Kelp forest fish communities environmental DNA samples from Santa Barbara Channel ver 1. Environmental Data Initiative. https://doi.org/10.6073/pasta/9d520a4f132d30dba11d014a41751bce

Love, M., L. Snook, M. Nishimoto, and L. Kui. 2017. Santa Barbara Channel fish surveys at deep reefs: Footprint, Piggy Bank, Anacapa Passage ver 1. Environmental Data Initiative. https://doi.org/10.6073/pasta/59d44ccc0d08bb8735a564aca91e5009

SBC Marine Biodiversity Observation Network, L. Kui, and Northwest Fisheries Science Center. 2020. NWFSC fish and invertebrate diversity derived from west coast groundfish trawl program ver 2. Environmental Data Initiative. https://doi.org/10.6073/pasta/b232c27a13cb02cb3664cc693efecb90

Southern California Bight MBON, D. Catlett, D. Siegel, and N. Guillocheau. 2022. Plumes and Blooms: Curated oceanographic and phytoplankton pigment observations ver 3. Environmental Data Initiative. https://doi.org/10.6073/pasta/29d4ef7976c19d6958e618d1548dcd72

Anderson, C., M. Brzesinski, D. Caron, M. Carter, J. Jones, R. Kudela, J. Largier, K. Negrey, A. Pasulka, H. Ruhl, R. Shipe, G. Smith, J. Smith, J. Tyburczy, R. Walter, and L. Washburn. 2021. California Harmful Algal Bloom Monitoring and Alert Program Data (Darwin Core Archive format) ver 7. Environmental Data Initiative. https://doi.org/10.6073/pasta/bc6bb2963472f34436ea7d83d635a6d5

Miller, P., T. Lamy, H. Page, and R. Miller. 2021. SBC LTER and MBON: Sea urchin microbiomes in Southern California ver 1. Environmental Data Initiative. https://doi.org/10.6073/pasta/201751391a4164340ed9a1c128dbab95

Goddard, J. 2020. Abundance and species composition of benthic heterobranch molluscs from the Santa Barbara Channel mainland ver 1. Environmental Data Initiative. https://doi.org/10.6073/pasta/0f31abcec646cadcbb7f8f95052941ee

Love, M., L. Snook, M. Nishimoto, and L. Kui. 2017. Santa Barbara Channel fish and invertebrate surveys at oil and gas platforms ver 1. Environmental Data Initiative. https://doi.org/10.6073/pasta/2dc1e7a1ce14e0f3f070076fc4a85e43

Love, M., M. Nishimoto, L. Kui, and D. Schroeder. 2017. Santa Barbara Channel fish surveys at shallow regions of oil and gas platforms (SCUBA) ver 1. Environmental Data Initiative. https://doi.org/10.6073/pasta/af1f2c8a402c84b0b6fc17b73a950988

Love, M., M. Nishimoto, L. Kui, and D. Schroeder. 2017. Santa Barbara Channel fish surveys at shallow outcrops ver 1. Environmental Data Initiative. https://doi.org/10.6073/pasta/618ae46a11a00fcd8c220748d54281fc

Love, M., M. Nishimoto, and L. Kui. 2020. Data to support manuscript: A Comparison of Two ROV Survey Methods Used to Estimate Fish Assemblages and Densities Around a California Oil Platform ver 1. Environmental Data Initiative. https://doi.org/10.6073/pasta/5f2d77235388717672ff612cc7fa7d7c

SBC Marine Biodiversity Observation Network, A. Thompson, R. Miller, and L. Kui. 2022. CALCOFI fish larvae at 66 standard stations, 1966-ongoing ver 3. Environmental Data Initiative. https://doi.org/10.6073/pasta/8143504a8c9730750baa806dafe22102

SBC MBON, E. Wear, C. Carlson, E. Wilbanks, and C. Nelson. 2017. Santa Barbara Channel Marine BON: genomics study on 16S primer comparison ver 1. Environmental Data Initiative. https://doi.org/10.6073/pasta/b79f6653c03a9017324f9961adfaaa3b

SBC Marine Biodiversity Observation Network, D. Catlett, D. Siegel, P. Matson, E. Wear, C. Carlson, T. Lankiewicz, and D. Iglesias-Rodriguez. 2022. Plumes and Blooms: Microbial eukaryote diversity and composition ver 1. Environmental Data Initiative. https://doi.org/10.6073/pasta/bbe75db57c4ce7f7842e22923ae6badf

We combined biological data with physical data to allow analysis of the physical drivers of biodiversity. Because our goal was to use these results to develop regional scale inference about patterns of biodiversity, we focused on physical data for which we had continuous coverage within the region, particularly from satellite remote sensing. These data include 1) bathymetry and substrate data derived from sonar surveys, 2) sea surface temperature, chlorophyll, and local kelp canopy derived from satellite imagery, 3) wave disturbance and ocean current data derived from regional models, and 4) data on the shape of the coastline calculated from maps.

2.6.3 Dataset Narratives

MBON postdoc Thomas Lamy developed predictive and spatial models of marine biodiversity using physical and biological data derived from remote sensing. Erin Meyer-Gutbrod joined our group as a postdoc in 2017 and became Assistant Professor at the University of South Carolina. Meyer-Gutbrod received her PhD from Cornell University where she developed climate and prey-driven population models of the critically endangered North Atlantic right whale. For the SBC MBON, she investigated effects of offshore petroleum platform decommissioning alternatives on fish communities in the Southern California Bight, with aid from additional BOEM support, in a project titled Net Environmental Benefits of Decommissioning Scenarios. These results are informing imminent decisions on upcoming decommissioning projects removing all or part of the platform structure as well as future construction of offshore installations and artificial reefs.

Lamy worked with us to complete several MBON papers, including one that he led comparing eDNA data to visual survey data for kelp forest fish communities (Lamy et al. 2021). Lamy et al. (2019, 2020) focused on diverse communities inhabiting productive kelp forests on shallow subtidal rocky reefs in SBC. Lamy (2019) used long-term community surveys of kelp forests to illustrate how species insurance can stabilize aggregate community properties in natural ecosystems where environmental conditions vary over broad spatial scales.

Lamy led a study examining the role of giant kelp in promoting ecosystem stability (Figure 1, Lamy et al. 2020). Giant kelp abundance can be assessed via remote sensing, and the degree to which this marine foundation species is an indicator of ecosystem health and biodiversity is important to us. Foundation species structure communities, promote biodiversity, and stabilize ecosystem processes by creating locally stable environmental conditions. Despite their critical importance, the role of foundation species in stabilizing natural communities has seldom been quantified. In theory, the stability of a foundation species should promote community stability by enhancing species richness, altering the population fluctuations of individual species, or both. We tested the hypothesis that the stability of a marine foundation species, the giant kelp *Macrocystis pyrifera*, increased the stability of the aggregate biomass of a phylogenetically diverse assemblage of understory algae and sessile invertebrates that compete for space beneath the giant kelp canopy. To achieve this goal, we analyzed an 18-year time series of the biomass of giant kelp and its associated benthic community collected from 32 plots distributed among nine shallow reefs in the Santa Barbara Channel. We showed that the stability of understory algae and sessile invertebrates was positively and indirectly related to the stability of giant kelp, which primarily resulted from giant kelp's direct positive association with species richness. The stability of all community types was positively related to species richness via increased species stability and species asynchrony. The stabilizing effects of richness were three to four times stronger when algae and invertebrates were considered separately rather than in combination. Our finding that diversity-stability relationships were stronger in communities consisting of species with similar resource requirements suggests that competition for shared resources rather than differential responses to environmental conditions played a more important role in stabilizing the community. Increasing threats to structure-forming foundation species worldwide necessitates a detailed understanding of how they influence their associated community. This study was among the first to show that dampened temporal fluctuations in the biomass of a foundation species is an important determinant of the stability of the complex communities it supports.

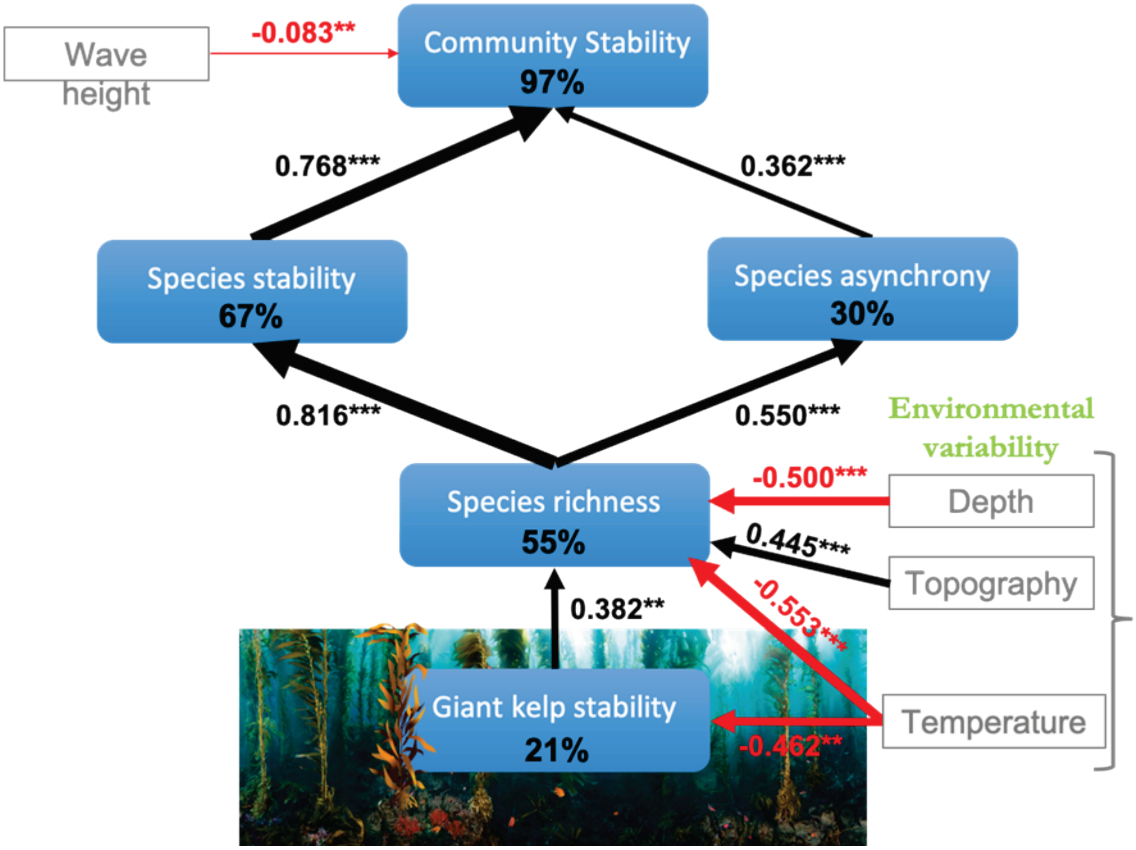


Figure 1. Structural Equation Model (SEM) partitioning the effect of giant kelp stability on biodiversity (species richness), species stability and synchrony, and community stability. Black arrows represent positive effects and red negative; arrow thickness is proportional to effect magnitude. Kelp's positive effect on stability manifests indirectly through its positive effect on biodiversity (Lamy et al. 2020).

Lamy et al. (2021) examined the potential of eDNA to complement or replace traditional methods of measuring biodiversity, particularly beta diversity. Beta diversity, which quantifies species turnover between local and regional scales, is emerging as a key driver of ecosystem function that can inform spatial conservation. Yet measuring biodiversity remains a major challenge, especially in aquatic ecosystems. Decoding eDNA left behind by organisms offers the possibility of detecting species sans direct observation, a Rosetta Stone for biodiversity and a major focus of the MBON endeavor.

While eDNA has proven useful to illuminate diversity in aquatic ecosystems, its utility for measuring beta diversity over spatial scales small enough to be relevant to conservation purposes is poorly known. We paired underwater visual census (UVC) with 12S eDNA metabarcoding and used a spatially structured hierarchical sampling design to assess key spatial metrics of fish communities on temperate rocky reefs in southern California to test how eDNA performs relative to UVC to evaluate beta diversity of marine communities. eDNA provided a more detailed picture of the main sources of spatial variation in both taxonomic richness and community turnover, which primarily arose due to strong species filtering within and among rocky reefs. As expected, eDNA detected more taxa at the regional scale (69 vs. 38) which accumulated quickly with space and plateaued at around 11 samples. Conversely, the discovery rate of new taxa was slower with no sign of saturation for UVC. Based on historical records in the region (2000–2018), we found that 6.9 times more UVC samples would be required to detect 50 taxa compared to eDNA. Our results showed that eDNA metabarcoding can outperform diver counts to capture the spatial patterns in biodiversity at fine scales with less field effort and more power than traditional methods, supporting the notion that eDNA is a critical scientific tool for detecting biodiversity changes in marine ecosystems.

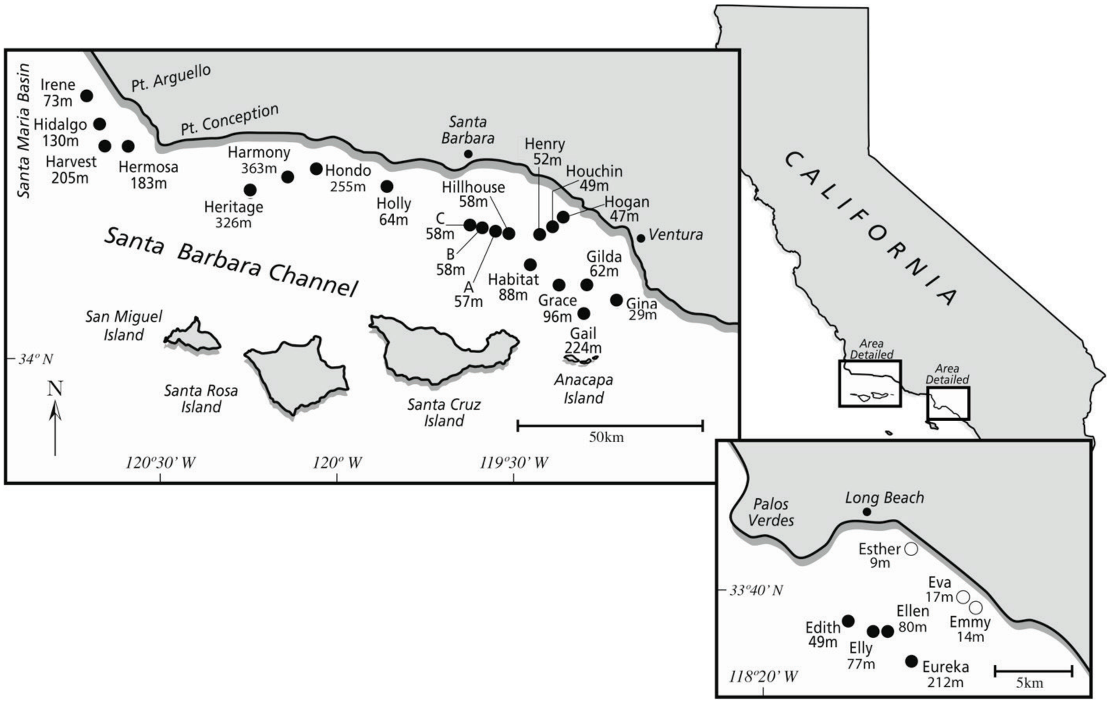


Figure 2. Map with the names, locations, and seafloor depths (in meters) of the 27 southern California offshore oil and gas platforms included in Meyer-Gutbrod et al. (2020).

Meyer-Gutbrod et al. (2020) examined SBC MBON fish community data from 37 oil platforms in southern California (Figure 2). Results indicate that only ~10% of fish biomass in the habitat adjacent to the platforms will be lost in a partial removal scenario at a depth of 26m, whereas a total reversion to soft bottom will result in >90% reduction in fish biomass. Fish densities are highest near crossbeams inside the jacket relative to areas adjacent to and surrounding the rig structure. Meyer-Gutbrod also led studies on the fish communities associated with different platform habitats and the shell mound that forms beneath platforms from sloughed mussel shells. These results inform decisions on upcoming decommissioning projects removing all or part of the platform structure as well as future construction of offshore installations and artificial reefs.

Meyer-Gutbrod et al. 2021 examined SBC MBON fish community data from the Southern California Channel Islands, where visual fish surveys were conducted frequently in a manned submersible at three rocky reefs between 1995 and 2009. This area is characterized by a steep bathymetric gradient, with the surveyed sites Anacapa Passage, Footprint, and Piggy Bank corresponding to depths near 50 m, 150 m and 300 m. Poisson models were developed for each fish species and life stage observed consistently in this network of rocky reefs to determine the impact of depth and year on fish peak distribution. The interaction of depth and year was significant in 23 fish types, with 19 of the modeled peak distributions shifting to a shallower depth over the survey period. Across the 23 fish types, the peak distribution shoaled at an average rate of 8.7 m of vertical depth per decade. Many of the species, including California sheephead, copper rockfish, and blue rockfish, are targeted by commercial and recreational fisheries. CalCOFI hydrographic samples are used to demonstrate significant declines in dissolved oxygen at stations near the survey sites which are forced by a combination of natural multi-decadal oscillations and anthropogenic climate change. This MBON study demonstrated fish depth distribution shifts over a 15-year period were concurrent with oxygen decline. Climate-driven distribution shifts in response to deoxygenation have important implications for fisheries management, including habitat reduction, habitat compression, novel trophic dynamics, and reduced body condition. Continued efforts to predict the formation and severity of hypoxic zones and their impact on fisheries dynamics will be essential to guiding effective placement of protected areas and fisheries regulations.

Predictive modeling of kelp forest communities was also carried out in a collaborative project of SBC MBON and a project funded by NOAA NCCOS using species archetype models (SAM). Sea surface temperature was extracted from the JPL MUR MEaSUREs Project daily GHRSST level 4 MUR dataset (v4.1) and collapsed into annual summary variables. Biweekly surface chlorophyll was extracted from the Kahru et al. California Current merged satellite-derived dataset. This dataset integrates data from multiple satellites and applies a correction factor based on comparisons to local in-situ measurements (from CalCOFI). Significant wave height estimates were generated using the CDIP MOP v1.1 to produce hourly estimates at the biological sampling sites and regional sites used for prediction maps. After compiling the improved environmental covariates, SAMs were fit using a subset of the KFMP biological dataset (82 species identified to the species level, 2004-2014). The resulting model had a high degree of predictive performance, correctly predicting presence/absence in 87% of observations. The SAM was also tested using biological data from the PISCO dataset, which provides a robust assessment because it is a distinct dataset with different sampling methods. SAM predictive performance for the PISCO dataset was also good, with the model correctly assigning presence/absence to 73% of observations. The SAMs were also used to identify indicator species. By identifying one representative species for each archetype and assigning presence/absence of all species within the archetype based on the identified species, presence/absence was correctly assigned in 77% of observations. These results were published in a special issue of Oceanography dedicated to MBON (Rognstad et al. 2021).

2.7 SBC MBON Remote Sensing

Remote sensing data are critical for scaling up local observations of biodiversity and for relating physical and ecological variables to marine biodiversity. The SBC MBON focused on three activities linking remote sensing to observations of marine biodiversity. These built on NSF and NASA-supported projects and spanned subtidal and pelagic environments. Specifically, the remote sensing component of the SBC MBON focused on:

- The remote sensing of giant kelp populations and the application to these products to answer ecological and biodiversity questions
- The analysis of satellite ocean color observations using novel ocean color inversion approaches
- The analysis of planktonic biodiversity indices from the PnB time-series study applied to SBC MBON genomics observations

Both multispectral and hyperspectral remote sensing of giant kelp canopy fronds were used to help answer ecological and biodiversity questions for the SBC MBON. We expanded a Landsat giant kelp (*Macrocystis*) canopy biomass time series from 1984–2021, spanning its dominant range in the northeast Pacific (San Francisco, CA to Punta Eugenia, Baja California Sur, Mexico). The processing of Landsat satellite imagery to kelp canopy biomass was completely automated (Bell et al. 2020a), ensuring the continuity of this time series and expanding its use outside of California and Mexico (Friedlander et al. 2018; 2020) and to additional canopy-forming kelp species (Hamilton et al. 2020). This large dataset was also used to examine the role of the physiological condition of the kelp canopy to regional variations in kelp net primary production (Bell et al. 2018).

Multiple hyperspectral images of the SBC were collected by the Airborne Visible/Infrared Imaging Spectrometer (AVIRIS) as part of the HyspIRI Preparatory Airborne Campaign. Empirical relationships between laboratory reflectance of giant kelp blades and their physiological state (Chl:C) developed in previous work (Bell et al. 2015) were combined with the hyperspectral imagery to show that spatiotemporal patterns of physiological condition, and thus growth and production, are regulated by different processes depending on the scale of observation (Bell et al. 2020a). Nutrient supply was linked to regional-scale physiological condition dynamics, and kelp forest stands in regions with elevated mean nutrient supply were more persistent over decadal timescales (Figure 3). However, on local scales, internal senescence processes, related to canopy age demographics, determined patterns of biomass loss across individual kelp forests, despite uniform nutrient conditions (Figure 4). This work helped to disentangle the roles of the external environment and internal biotic drivers of plant population dynamics across space and time scales.

Due to the large size and complexity of this dataset, we partnered with The Nature Conservancy to build a data visualization and download webtool to make these data more accessible to users with a wide range of computing experience (Figure 5). This tool, called KelpWatch (Bell et al. 2022), is live for the entire coast of California and available from Washington through Baja. Using these Landsat data, we are examining long time regional and local trends and examining the geographic variability in resistance and resilience of kelp to the 2014–2016 marine heat wave events (Figure 6; Bell et al. 2022).

This dataset also led to several important peer-reviewed articles. The catastrophic decline of bull kelp along the northern California coast over the past six years has resulted in several studies examining the drivers of loss (i.e., thermal stress, low seawater nutrients, urchin herbivory, and the decline of the sunflower sea star, an important urchin predator). McPherson et al. (2021) used the Landsat time series to show that the current decline in kelp is much more severe and prolonged than other declines over the past 35 years. They showed that the coupled environmental (low nutrients) and biological shifts (loss of sea stars) resulted in the formation of a persistent, altered ecosystem state with low primary productivity. In another study, Arafeh-Dalmau et al. (2021) used the Landsat time series from central California through Baja California to show that while 7.7% of giant kelp forests are protected by marine reserves, only 4% are protected when accounting for the persistence of kelp forests. They also found that the percentage of kelp protected decreased from 20.9% in central California to less than 1% in Baja California. They go on to recommend a two-fold increase in the protection of kelp to fully protect persistent kelp forest in the northeast Pacific.

We also used the hyperspectral aerial imagery collected by the AVIRIS sensor from 2013–2015 to examine the physiological condition of the kelp canopy in the Santa Barbara Channel. We found that the condition of the kelp canopy was related to the availability of seawater nitrate on the regional scale (1 km) but that patterns on the local scale (20 m) were related to kelp frond senescence and demographic patterns (Figure 7). We deployed a field experiment to empirically show that the concentration of the chlorophyll pigment declines in a consistent nonlinear pattern with age and that this pattern matches the one detected by the aerial imagery (Figure 8). These results were published in PNAS (Bell and Siegel 2022).

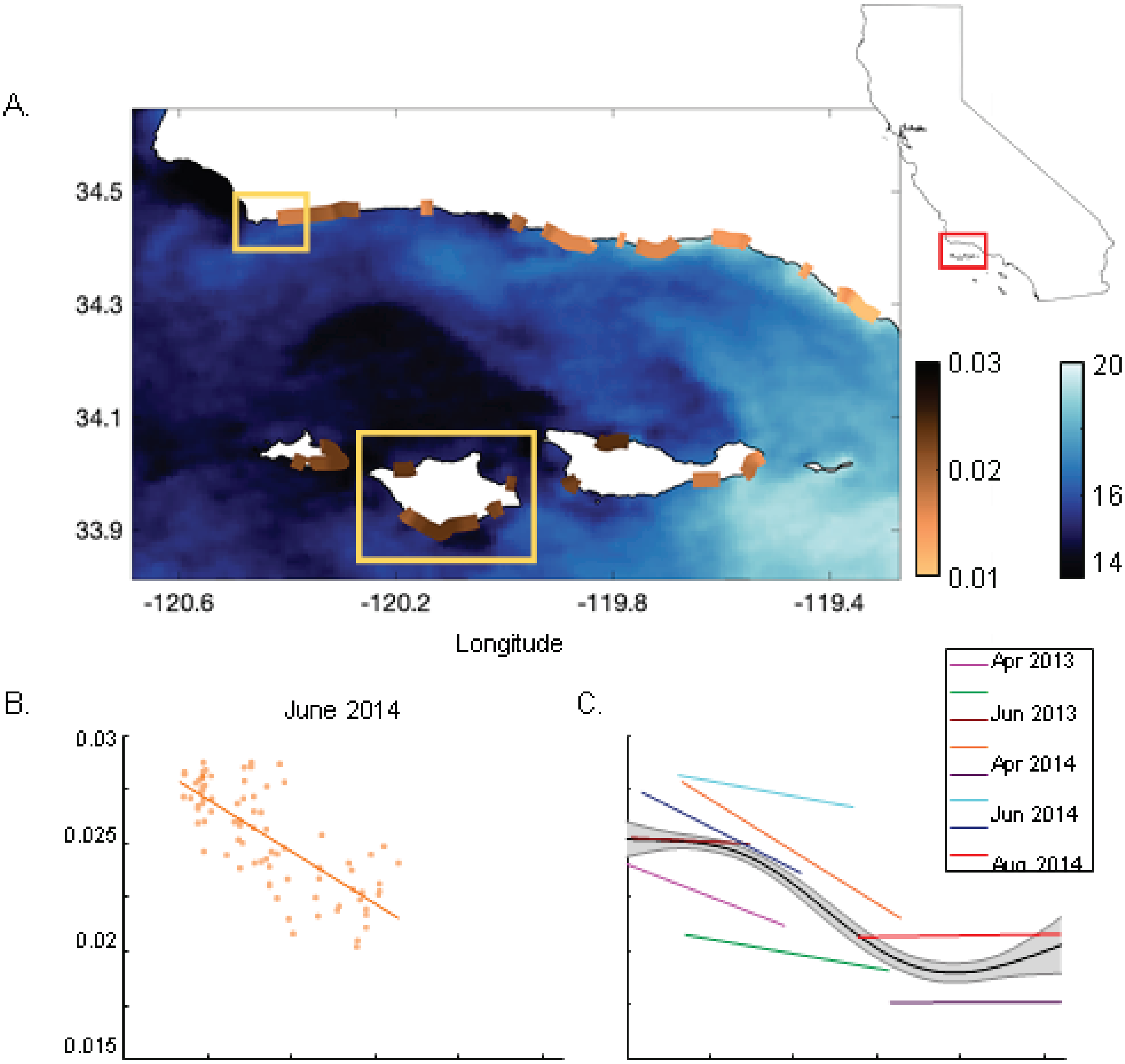


Figure 3. Regional-scale physiological condition is negatively related to seawater temperature. (A) Regional-scale Chl:C and surface temperature in June 2014 where the color of coastline represents the mean value of > 15 remotely sensed Chl:C estimates averaged over a 1 km scale. Yellow box shows the location of the area focused on in Fig. 2. (B) Relationship between surface temperature and Chl:C for the June 2014 image. (C) The mean relationship between surface temperature and Chl:C across all image dates (colored). Black curve represents the mean nonlinear relationship, gray area represents 2x standard error.

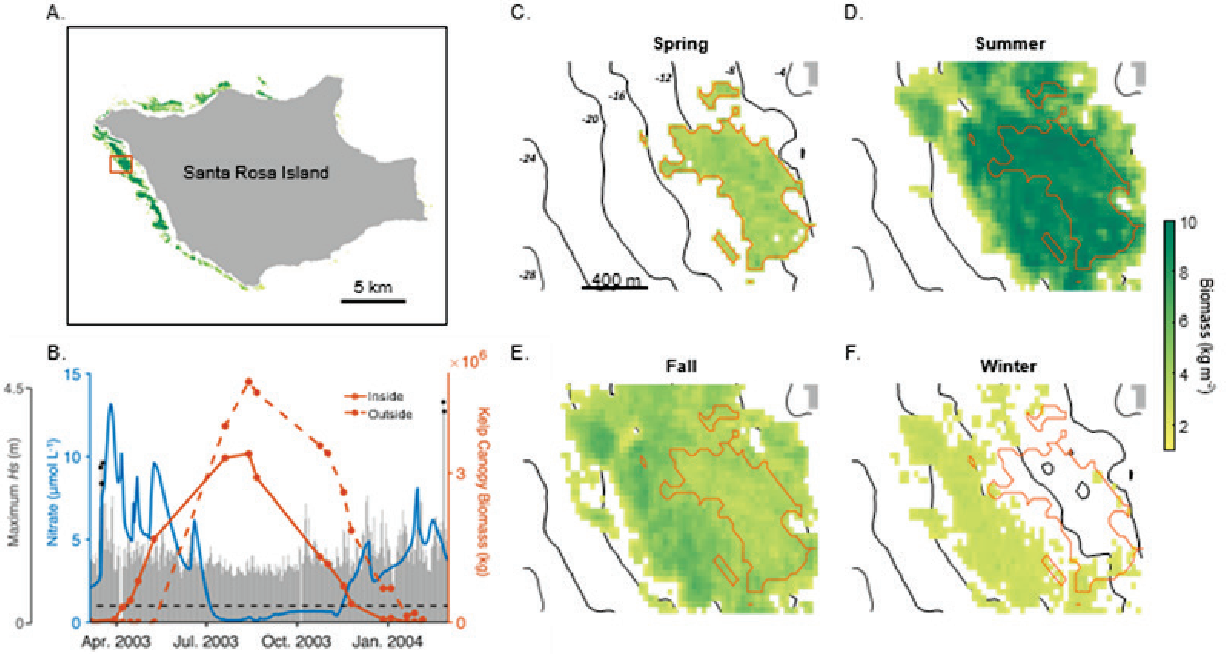
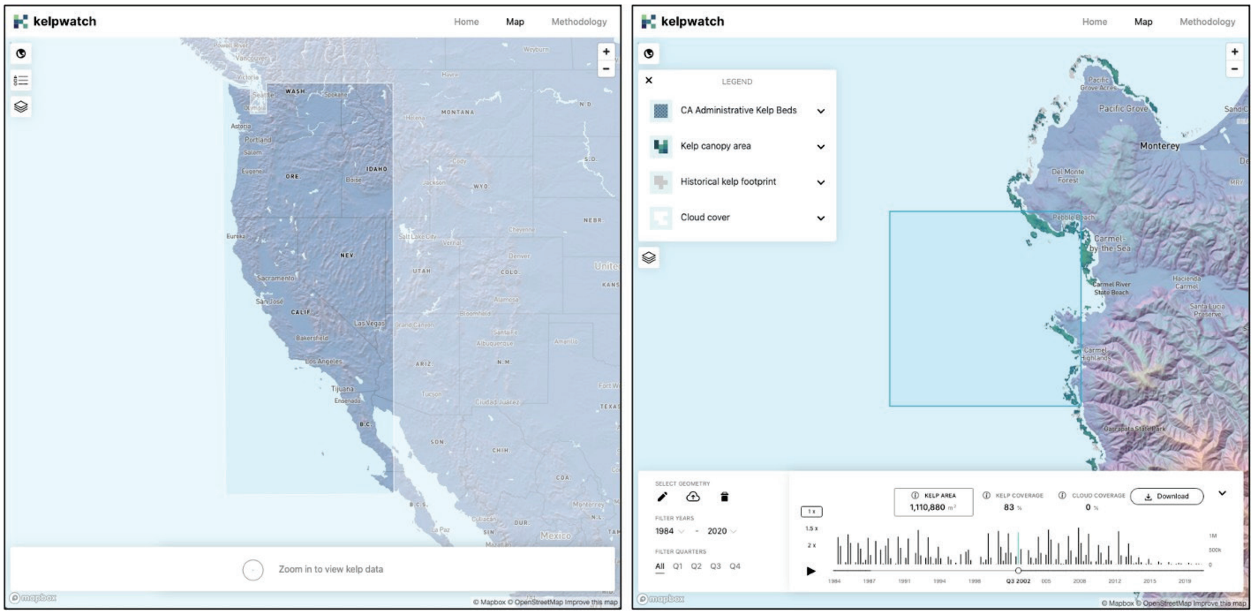


Figure 4. Spatiotemporal and seasonal patterns of kelp biomass dynamics. (A) Map of giant kelp canopy biomass around Santa Rosa Island in summer 2003 with section of kelp forest highlighted in red box. (B) Annual time series of seawater nitrate concentration (blue line), maximum significant wave height (gray bars with large wave events shown with black asterisks), and kelp canopy biomass inside (solid red line) and outside (dashed red line) the red polygon shown in C. Points along the canopy biomass lines show the dates of canopy biomass determinations using Landsat imagery. Dashed black line shows 1 umol L-1 seawater nitrate, the concentration necessary for maximum growth rate. (C–F) Mean seasonal canopy biomass with red polygon showing the area where canopy was present in spring 2003. Black lines represent bathymetry (m).



FILTER YEARS 1984 - 2020

FILTER QUARTERS All Q1 Q2 Q3 Q4

KELP AREA 1,110,880 m²

KELP COVERAGE 83 %

CLOUD COVERAGE 0 %

Figure 5. The KelpWatch webtool (Bell et al. 2022) showing the spatial domain of the Landsat kelp canopy dataset. A 10 x 10 km site (data shown in Figure 6) near the Monterey Peninsula. Kelp canopy area for a particular season is shown as the green and blue pixels along the coast. The seasonal dynamics of kelp canopy area within the box (1984–2020) are shown as the bar graph at the bottom of the figure (note the decline in kelp canopy after the marine heat wave event).

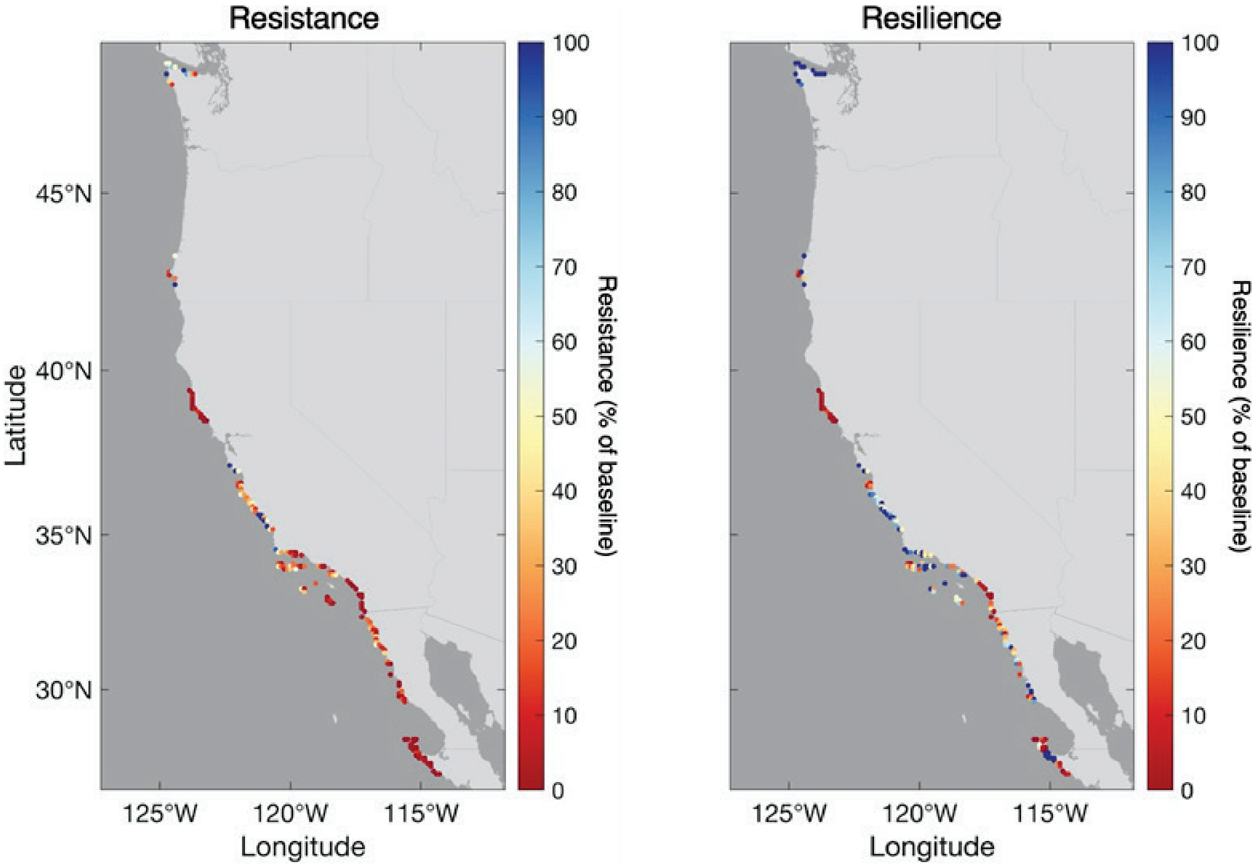


Figure 6. Kelp canopy resistance and resilience to the 2014–2016 marine heat wave events in the NE Pacific (Bell et al. 2022). Sites are 10 x 10 km along the coast (minimum 500 kelp-containing Landsat pixels for each site). Resistance and resilience metrics were calculated as the minimum annual canopy area from 2014–2016 (resistance) and the mean annual canopy area (2017–2020) relative to the mean annual canopy area from the baseline period (2009–2013). High resilience was observed around the Olympic Peninsula Washington, lower half of the central coast and western Santa Barbara Channel California and some areas in Baja California. Low resilience was observed near the U.S./Mexico border, the Monterey Peninsula, and the northern California coast.

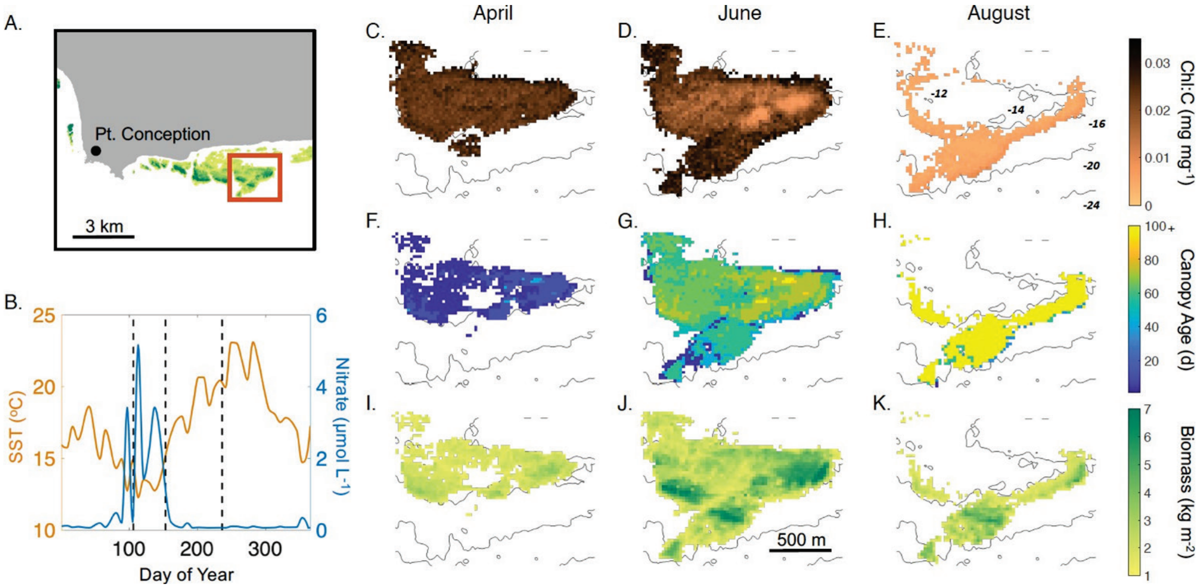


Figure 7. Kelp frond senescence and demographic patterns. (A) Western SBC with large kelp forest highlighted in red box. (B) Time series of sea surface temperature (SST; orange line) and seawater nitrate concentration (blue line) near the kelp forest with hyperspectral image dates in 2015 shown as the dashed black lines. (C–E) Chl:C (AVIRIS, 18m pixels), (F–H) canopy age and (I–K) canopy biomass density (Landsat, 30m pixels) for the kelp forest across the three dates. Black lines represent bathymetry (meters).

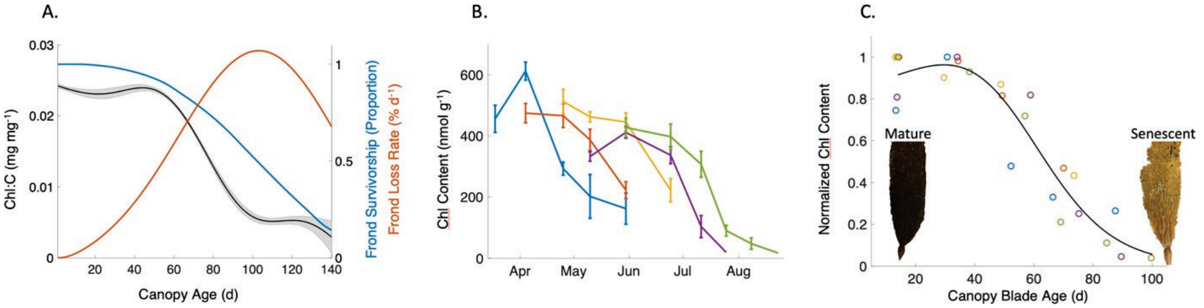


Figure 8. The relationship between chlorophyll pigment and canopy age. (A) The nonlinear relationship between canopy age and Chl:C across all three image dates in 2015 for the large kelp forest in Figure 7 (black curve; gray shaded area represents 2x standard error). The proportional frond survivorship (blue curve) and the loss rate of fronds (red curve) across three kelp forests in the 16 SBC, synthesis of data from (Rodriguez et al. 2013). (B) Canopy chlorophyll a content as a function of age across five frond cohorts sampled in the Santa Barbara Channel in 2019. Error bars show standard error. (C) The relationship between chlorophyll a content, normalized to the maximum of each frond cohort, and age for the five frond cohorts shown in B. Points are slightly jittered for visibility and colored to correspond to their respective frond cohort.

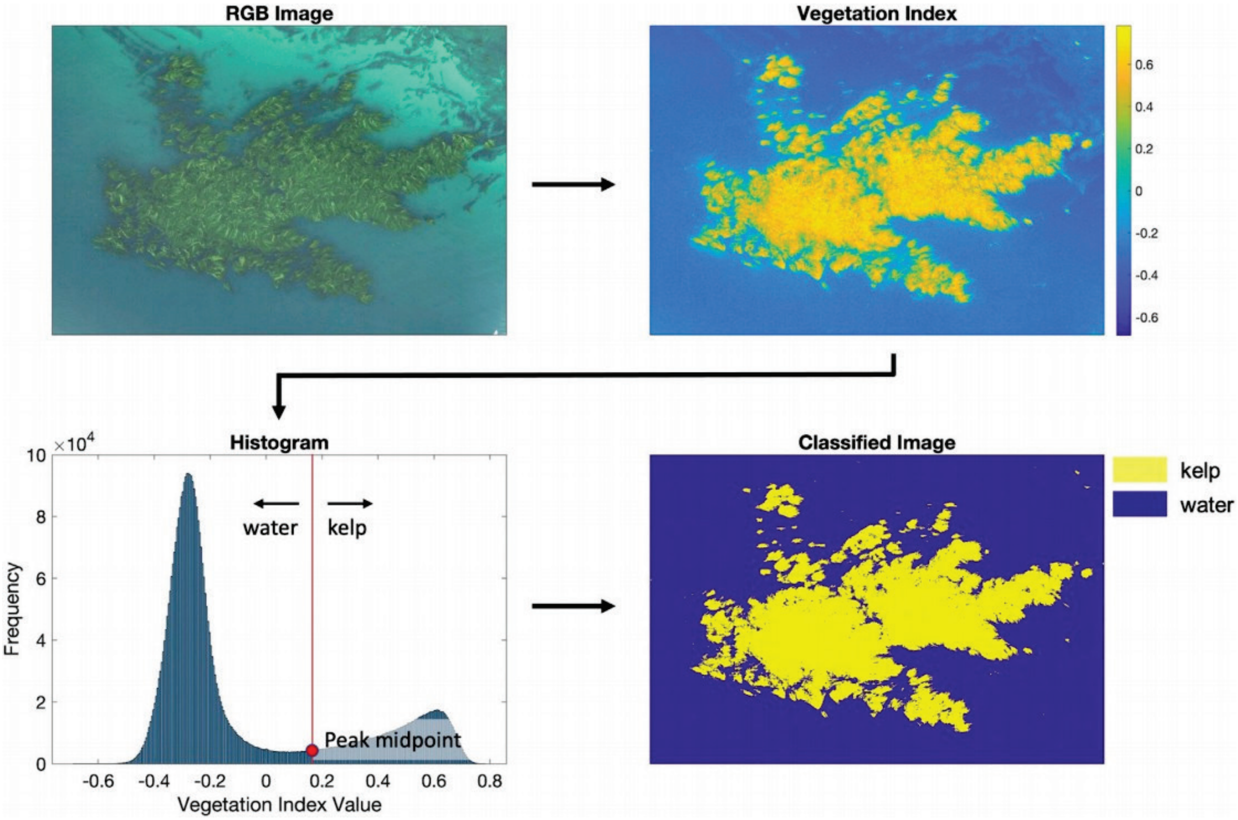


Figure 9. Methodology used in the detection of the presence or absence of kelp in each pixel. For each image, we applied the best vegetation index (based on color or multispectral imagery) and calculated histograms to find unique thresholds for image classifications.

We initiated high-resolution monitoring of select kelp forests along the Santa Barbara coastline using small unoccupied aerial systems (sUAS; quadcopter drones). We developed an automated method for processing these data and examined the effects of tides and currents on sUAS-based estimates of kelp canopy area (Figure 9; Cavanaugh et al. 2021). We found that tidal height can introduce significant variability in high-resolution estimates of canopy area, and that the relationship between tidal height and surface canopy area was site-specific. These results indicate the importance for tidal correction by controlling the timing of image acquisition or using methods such as averaging multiple observations over a given time period (e.g., month or season). sUAS provides the capability for mapping kelp canopy dynamics on scales that align more closely with in-situ monitoring (centimeters to 10's of meters) and differentiating species of canopy-forming kelps (e.g., bull kelp vs. giant kelp).

We acquired sUAS color imagery of two kelp forests in the Santa Barbara Channel near-simultaneous to Landsat overpasses. Using these data, we investigated specific methodological behavior and results from the automated classification and spectral unmixing approach developed for a multi-decade time series of giant kelp canopy with the goal of continued improvement to the dataset, including the validation of areal estimates and the detection of processes influencing pixel scale variability (McPherson & Bell in prep). We focused on specific aspects that influence estimates of kelp canopy at the pixel and kelp-bed scale, including spatiotemporal variability in ocean conditions, and the spectral unmixing and classification of kelp versus seawater (Figure 10).

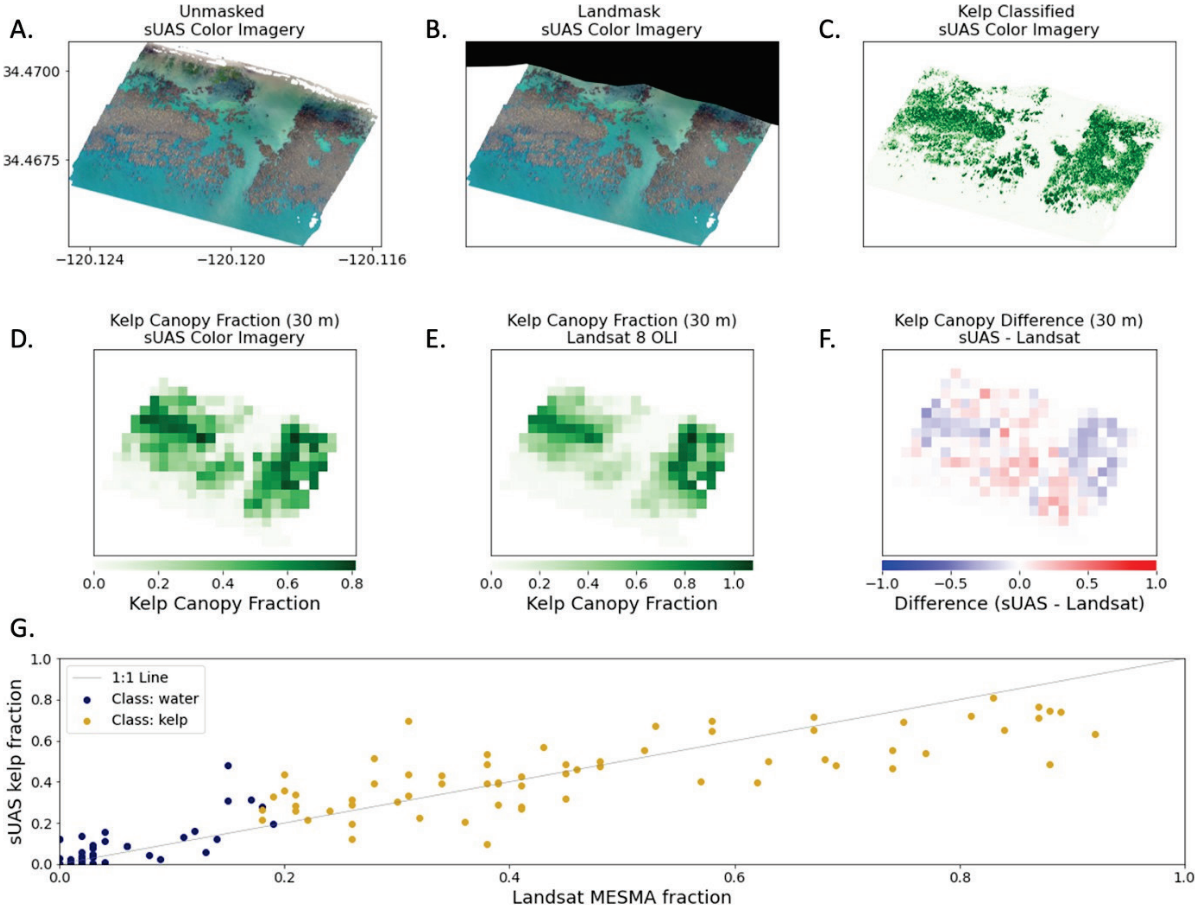


Figure 10. Example processing steps for comparing sUAS and Landsat imagery to kelp canopy fraction from imagery collected on May 10, 2018, at Arroyo Quemado. The unmasked stitched sUAS color imagery (A) is masked for land and waves (B) and classified for kelp canopy (C) using a simple band ratio classification approach (Cavanaugh et al. 2021). The sUAS imagery is degraded to 30 m resolution (D) and compared to 30 m resolution Landsat Multiple Endmember Spectral Mixture Analysis (MESMA) kelp fraction (E). A matchup analysis was conducted at the site and pixel scales using the difference between sUAS and Landsat canopy fractions (F) and direct correlation (G). Orange dots show the pixels classified as kelp canopy and blue dots show pixels classified as seawater using the automated classification algorithm from Bell et al. 2020a.

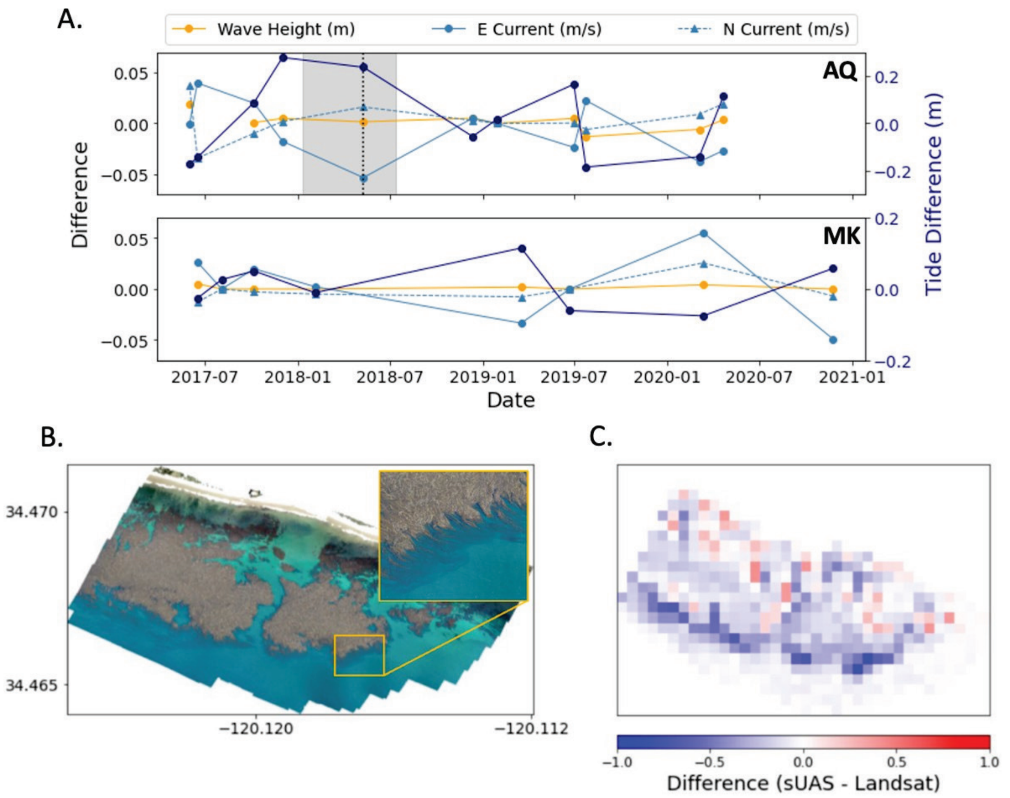


Figure 11. Impacts of environmental conditions on kelp canopy fraction derived from sUAS and Landsat data at Arroyo Quemado (AQ) and Mohawk Reef (MR): (A) Differences between environmental conditions (tidal height, wave height, east current velocity, and north current velocity) between the sUAS and Landsat flight times for AQ and MR on all matchup dates through time. Wave height and current velocity difference are shown on the left y-axis. Tidal difference is shown on the right y-axis. The shaded gray area corresponds to the spring 2018 at AQ, and the vertical dashed blackline shows the difference in environmental conditions between the Landsat imagery and the AQ sUAS flight on May 10, 2018, shown in B and C. (B) Stitched sUAS imagery from AQ on May 10, 2018. (C) Pixel-based difference in kelp canopy fraction between the sUAS and Landsat for the May 10, 2018, matchup. The consistent difference in canopy cover between Landsat and the sUAS classification on the offshore edge is related to the increased westward current velocity dragging canopy fronds below the surface during the drone flight (see inset in B).

Although this study was designed to reduce temporal offsets, and therefore environmental variability, we observed the impacts of current velocity on kelp canopy fraction between matchups and in a seasonal time series of Landsat (Figure 11). Specific kelp bed features, such as seasonal changes in bed size and canopy density/biomass were important for driving matchup performance and error. We found that pre-classification of kelp and seawater is necessary from a quality control perspective, because Multiple Endmember Spectral Mixture Analysis (MESMA) can be noisy across Landsat sensors and can result in underestimation of kelp canopy from small and/or sparse kelp beds. Therefore, we can expect more error in Landsat MESMA estimates when observing low canopy biomass or fringing kelp bed conditions. Overall, this work provides broader context for validation approaches to satellite-derived time series by utilizing a unique multiyear dataset and exploring pixel and patch scale uncertainties.

We also investigated the use of a sUAS mounted hyperspectral imager and 10-band multispectral imager to provide both canopy biomass and physiological condition products for kelp forests in the Santa Barbara Channel (Bell et al. 2020b). To compare the utility of several sUAS sensors, we deployed a color camera, 5-band multispectral sensor, and hyperspectral imager within a three-hour period over the Arroyo

Quemado kelp forest coincident with a Landsat 7 ETM+ overpass. We found that the same MESMA procedure used for the Landsat time series produced similar results for kelp canopy fraction, although some sparse canopy was missed (consistent with results from Cavanaugh et al. 2021). We derived a spectral algorithm for canopy nitrogen content from over three years of laboratory spectral measurements of kelp canopy blades and found that the patterns of nitrogen content in the canopy matched the areas with senescent fronds as seen in the color imagery (Figure 12).

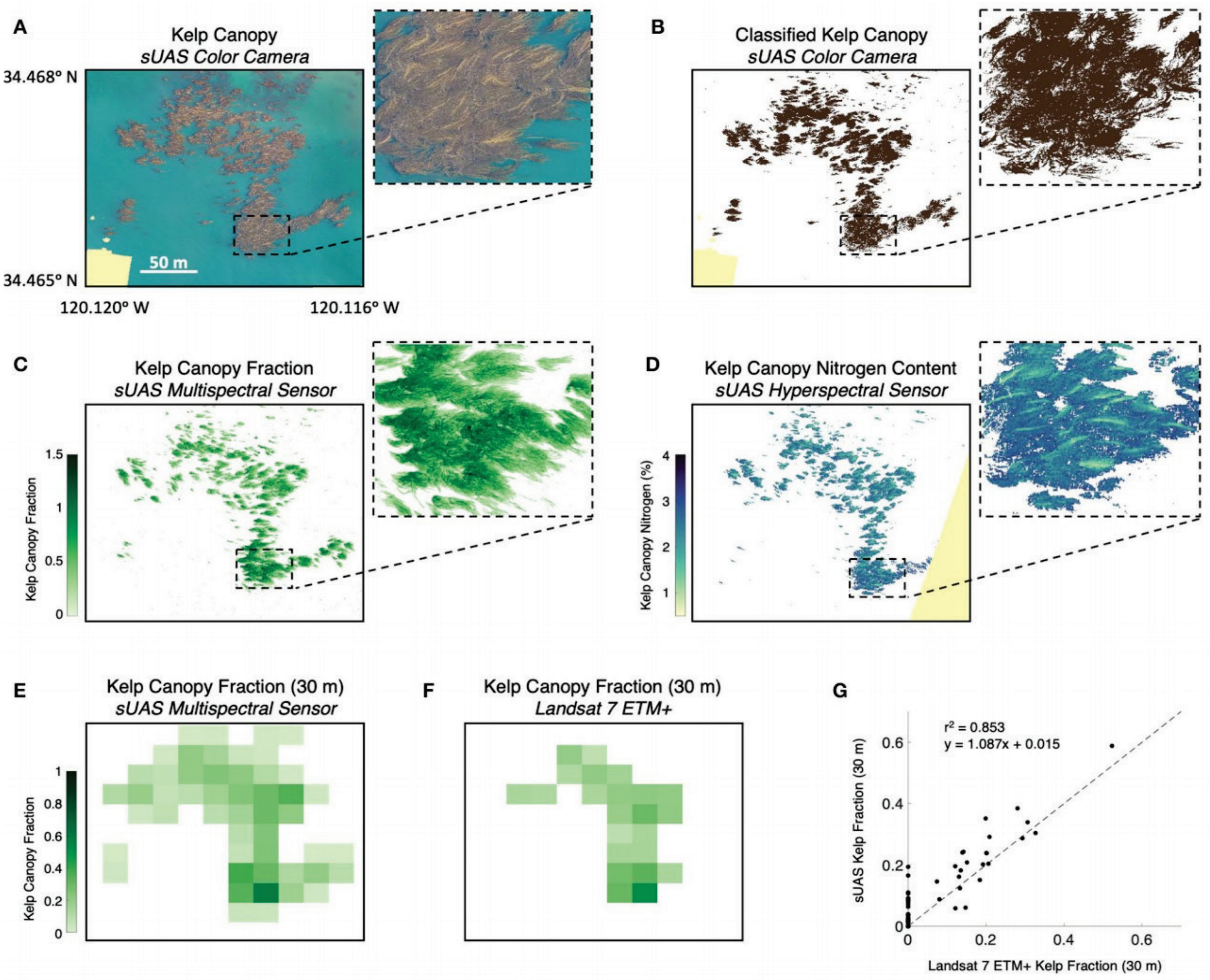


Figure 12. Kelp canopy characteristics derived from imagery. (A) Color orthomosaic of the Arroyo Quemado kelp forest canopy using the color camera on the Phantom 4 Pro sUAS (pixel resolution of 3.2 cm). (B) Kelp canopy classified from the color orthomosaic. (C) Fraction of each pixel covered by kelp canopy determined using Multiple Endmember Spectral Mixture Analysis (MESMA) with imagery from the sUAS multispectral sensor (pixel resolution of 6.5 cm). (D) Kelp canopy nitrogen content determined using the nitrogen content spectral algorithm with imagery from the sUAS hyperspectral sensor (pixel resolution 25 cm). (E) The mean kelp canopy fraction from the multispectral sensor binned into 30 m pixels to compare with (F) kelp canopy fraction determined from the Landsat 7 ETM+ multispectral satellite sensor.) G) Comparison of the kelp canopy fraction from the multispectral sUAS sensor binned into 30 m pixels and the Landsat 7 ETM+ sensor. Pale yellow color shows areas not imaged by the sensor. All imagery acquired between 9:30 AM and 12:00 PM local time on June 30, 2019.

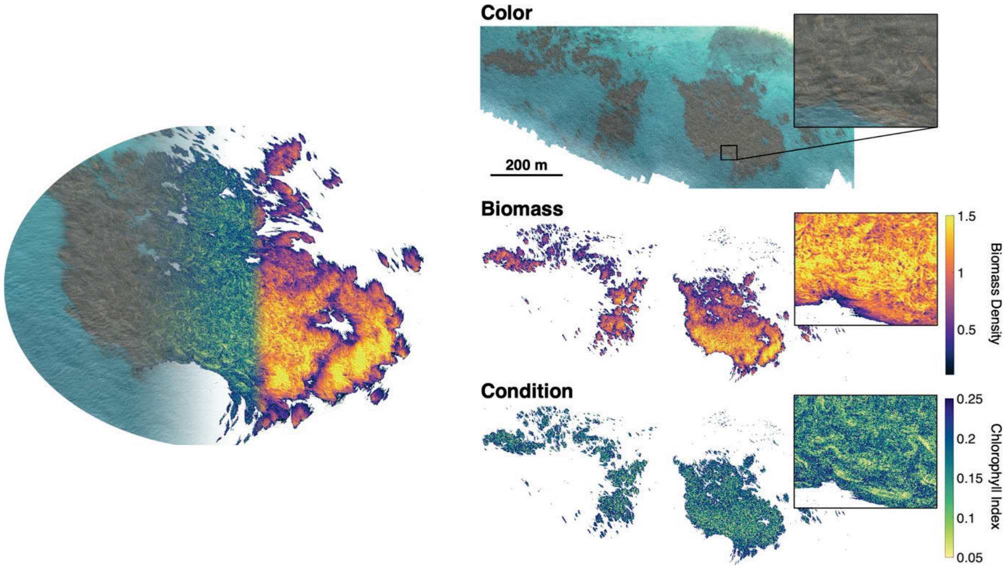


Figure 13. Color orthomosiac and canopy biomass and condition products from the Arroyo Quemado kelp forest on March 17, 2021, using the MicaSense 10-band multispectral sensor. The ability to capture all spectral band images simultaneously allows further-registration of all image products in space.

While the hyperspectral imager acquires imagery with high spectral resolution, the pushbroom sensor requires much postprocessing and georectification to deliver a usable product. A recently released 10-band multispectral imager from MicaSense possesses a band that measures reflectance in the orange region and can be used to estimate the concentration of chlorophyll pigment based on our laboratory reflectance time series and field validation tests. Starting in February 2021, we commenced a monthly time series of 10-band sUAS flights at two focal kelp forests in the Santa Barbara Channel to examine the dynamics of canopy biomass and physiological condition and relate these changes to demographic and environmental processes (Figure 13).

The goal of the second project was to characterize what controls space-time variability of optical properties of the Santa Barbara Channel and, more specifically, to understand how changes in chlorophyll concentrations (chl) and particulate backscattering coefficients (bbp) covary over time and space. Results from this project were published in JGR Oceans (Henderikx Freitas et al. 2017). We collaborated with Maria Kavanaugh in applying these data with her seascape characterization approaches for mapping marine biodiversity and published Kavanaugh et al. in the MBON special issue of Oceanography that was coauthored by Kavanaugh and Siegel.

The last project used data from the long-term PnB time series study supported by the NASA Ocean Biology and Biogeochemistry program to assess and model ocean color changes in a complex coastal site. PnB conducts monthly, day-long cruises at seven stations crossing the Santa Barbara Channel (~45 km long transect). At each station, measurements of ocean color spectra, inherent optical properties, phytoplankton pigment, dissolved and particulate carbon, macronutrient concentrations, and particle size spectra are measured. Samples are also collected for particulate DNA for prokaryotic and eukaryotic organisms. Our goal in the SBC MBON was to statistically relate bio-optical information of planktonic biodiversity from the PnB measurement suite (phytoplankton pigment concentrations, phytoplankton absorption spectra, etc.) with signatures from the SBC MBON genomic work. Dylan Catlett, a recent PhD in the Siegel lab on a NASA Earth System Science Fellowship (now Asstistant Scientist at Woods Hole Oceanographic Institution), led this work in collaboration with Siegel, Carlson, and Iglesias-Rodriguez. Catlett analyzed a 9-year dataset of hyperspectral phytoplankton-specific absorption spectra (aph) and phytoplankton pigment concentrations from PnB (Catlett and Siegel 2018). Catlett found excellent results (often > 80%) in deriving phytoplankton pigment concentrations and suites of pigment communities (determined via EOF 24 analyses) from the aph spectral derivatives. This modeling approach was then applied to the PnB bio-optical archive to extend the phytoplankton biomarker pigment time series from April 1997 to November 2018. This 20+ year monthly biomarker pigment time series was used to assess oceanographic and climate forcings of the dominant phytoplankton groups in the Santa Barbara Channel (Catlett et al. 2021). This study demonstrated that in addition to diatoms, diverse nano phytoplankton groups respond positively to spring upwelling in the Santa Barbara Channel. On multi-decadal time scales, novel associations between anomalous decadal dinoflagellate blooms, regional circulation patterns, and the North Pacific Gyre Oscillation were found.

This study provides novel insights into climate forcings of phytoplankton communities in the coastal ocean. Further, it is a useful proof of concept for modeling phytoplankton community structure from hyperspectral ocean color imagery, such as will be available from NASA's Plankton, Aerosol, Cloud and Ocean Ecosystem (PACE) mission. The data used in this study, including the multi-decadal phytoplankton biomarker pigment time series, were published through the Environmental Data Initiative (Catlett et al. 2020). We compared the marker pigment and bio-optical indices of phytoplankton community structure to those derived from eukaryotic genomic indices to provide a more complete picture of planktonic biodiversity in the SBC. Findings from this study were summarized in an oral presentation at the 2020 Ocean Sciences Meeting and published in Progress in Oceanography (Catlett et al. 2021).

3 Developing Advanced Methods in Optical and Acoustic Imaging and Genomics for Monitoring Biodiversity

3.1 Optical Imagery in the Field

We used an SLR with 14mm lens mounted on a rigid frame (quadrapod) to image shallow benthic and kelp forest communities at 14 reef sites spanning the Santa Barbara Channel, including several Channel Islands National Park monitoring sites, as well as seven offshore oil platforms. We also photographed SBC LTER's annual monitoring transects at the same time as diver surveys for four consecutive years, to examine how image analysis could be used to complement or replace time-consuming in-situ surveys by humans.

In deep water, we obtained still imagery data collected from a SeaBED class autonomous underwater vehicle (AUV) 25 that surveyed the benthos in the Santa Barbara Channel region. The SeaBED AUV is a hover-capable robotic vehicle that is able to work as close as 2m off the seafloor while maintaining precise altitude and navigation control. Its 2,000m depth rating makes it an ideal tool for conducting surveys of reef, shelf, and deep slope habitats. Its small footprint allows it to be operated from platforms ranging from global class oceanographic research ships to small vessels of opportunity. The SeaBED AUV can carry a wide variety of optical, acoustic, and oceanographic sensors for non-extractive surveys of the benthic communities in habitats that are too deep for divers and surface acoustics and too rugose for towed camera sleds and traditional bottom trawling. The area of each image is estimated from the measurement of the AUV altitude off the bottom and specified camera field of view. This allows for density estimates of species abundance, biomass, and diversity. Using these data, we compared the fish species assemblages of two benthic tar volcanoes and the surrounding mud sea floor (Love et al. 2022). Within this region, the hard features are dominated by rockfishes, and the specific species are determined by substrate relief, substrate rugosity, and seafloor depth. Only a few volcano species were habitat generalists and these likely freely moved between the two major habitats.

Transects were conducted on two seamounts known as the “Footprint” and “Piggy Bank.” Elizabeth Clarke, who leads the AUV program at NOAA NMFS NWFSC in Seattle, Washington, provided the data and collaborated with us. The Piggy Bank is about 30 km^2 area, ranging in depth from 275 to 900 meters; the Footprint Bank is about 10 km^2 in area, ranging in depth from 80 to 500 meters. The underwater visual surveys were planned to span from 400m to the top of each seamount. The imagery includes stereo pairs that can be used to estimate size and biomass of organisms.

3.2 Image Processing

We used 54 datasets with over 14,000 high-quality images. Of these, over 3000 were underwater images acquired during the shallow benthic field effort described above and manually annotated for percent coverage of sessile species in the BisQue system. Each image contained 100 annotated locations amounting to 200K data points with over 30 species. We obtained very encouraging results on automated identification of the 11 most abundant classes (over 80% of data points) with classification accuracy of over 85%.

We further explored state of the art deep learning techniques using Convolutional Neural Networks on the same dataset and obtained comparable results. The major advantage of these techniques is that CNNs learn image features automatically (in convolutional layers) and operate directly on image pixels that can be efficiently accessed via the BisQue system. The major disadvantage is the computational complexity that requires use of the latest GPUs (Graphics Processing Units). We have used a very popular CNN library “Caffe” running on an nVidia K20 GPU and observed training times of 14 hours for the aforementioned dataset.

3.3 BisQue Extensions

We integrated the Connoisseur model application component into the BisQue ecosystem for further development and potential user testing in collaboration with ViQi Inc., the startup company further developing BisQue. Furthermore, we containerized the deployments (utilizing the docker container ecosystem) which allows Connoisseur to be hosted on several types of hardware somewhat transparently by abstracting the access to the underlying GPU resources. We tested the system on a set of intertidal imagery collected by a BOEM-funded monitoring program, the Multi-Agency Rocky Intertidal Network (MARINe). We have made many small, but important improvements to the BisQue platform. Examples include fast querying for large datasets and improved graphical annotation tools. We also improved the robustness and uptime of the system with very few outages over the past year. Substantial improvements to the analysis module system were incorporated to support millions of analysis results thereby opening many new avenues for extensions. Finally, continued development of the BisQue nextgen product is entering the alphatest phase. This nextgen product will ensure better scalability and user experience for the next decade.

3.4 BOEM and MARINe Integration

The Multi-Agency Rocky Intertidal Network (MARINe) project, with support from BOEM uploaded over 65 datasets and 70,000 intertidal and shoreline images to ViQi. Metadata mapping from several data sources and quality control were applied to the uploaded data. Furthermore, initial species annotation was provided for over 70 species on 3000 images to generate machine learning models for classifications tasks.

3.5 Acoustics

Using the Channel Islands National Marine Sanctuary vessel R/V Shearwater, John Hildebrand's laboratory team successfully serviced two high-frequency acoustic recording packages (HARPs) in the Santa Barbara Channel. These instruments provided information on the variation of ship noise due to the reduction in commercial shipping related to the COVID-19 pandemic, and potential reactions of marine animals to reduced noise levels. HARP data collected between November and March 2012 near the Diablo Canyon Power Plant were analyzed to test for the presence of marine mammal species. The HARP recorded sound in the frequency band 10 Hz–100 kHz. Data analysis consisted of analyst-scans of long-term spectral average es (LTSAs) and spectrograms. Three frequency bands were analyzed for marine mammal vocalizations: (1) Low-frequency, between 10–500 Hz, (2) Mid- frequency, between 500–5,000 Hz, and (3) High-frequency, between 5–100 kHz.

Ambient sound levels had spectral peaks along with lower frequency harmonics at 45–47 Hz from November to December, related to blue whale B calls. Prominent peaks in sound spectrum levels observed in the frequency band 15–30 Hz during winter are related to seasonally increased presence of fin whale calls.

Three baleen whale species were recorded: blue, fin, and humpback whales. Fin whales were detected throughout the monitoring period with higher activity from November to December 2012. Blue whale B and D calls were found in high numbers from November to January. Humpback whale call types were detected throughout the recording period but began to decrease starting in February.

Several known odontocete signals were detected. Cuvier's beaked whales were detected in low numbers throughout the monitoring period. Baird's beaked whales were detected in low numbers in November and December. Two acoustically identifiable delphinid species were Risso's dolphins and Pacific white-sided dolphins, whose echolocation clicks occurred between early February and March. Sperm whales were detected intermittently throughout the recording period but were highest in December. Unidentified odontocete clicks greater than 20 kHz were detected throughout the monitoring period and peaked in December.

3.6 Genomics

Taxonomic identification of microorganisms has traditionally been challenging because, in addition to limited morphological characteristics, less than 1% of the microbial diversity has been cultured successfully. The introduction of genomic approaches over the past two decades has allowed microbiologists to overcome these limitations and assess microbial diversity in terrestrial and aquatic ecosystems. The use of genetic markers with high-throughput Next Generation (Next Gen) sequencing provides high-resolution taxonomy for phylogenetic analyses, as well as data for biogeographical distributions of marine microbial plankton.

Identification of metazoans has traditionally been based on morphology, which suffers from well-known limitations including phenotypic plasticity, morphologically cryptic taxa and life stages, and the need for taxonomic expertise. In response, the use of DNA 'barcodes' has been increasingly adopted for the study of biodiversity. There are several genetic markers that can be used but the most common is mitochondrial cytochrome oxidase subunit I (COI), which has been successfully used for the identification of fish, crustaceans, protists, and many other organisms. We proposed to build on existing sampling efforts to implement a sustained DNA collection program to be used for metabarcoding and eDNA analyses.

3.6.1 Genomics and Optics: Eukaryotic Phytoplankton and Microzooplankton

We applied our DNA metabarcoding workflow to the archived time series samples from Plumes and Blooms (PnB). We sequenced 218 surface ocean samples and 160 samples from water column profiles across five MiSeq runs. With each sequencing run, we sequenced a mock community positive control in triplicate and four negative controls. Including these controls allowed us to quantify the precision of our eukaryotic diversity estimates and account for the influence of potential contaminants in our downstream analyses. Analysis of this dataset was published in Matson et al. (2019). Our extensive efforts to quality control this dataset will improve the potential to compare genomic diversity estimates with those derived from phytoplankton pigment and bio-optical observations. These results were published in Catlett et al. (2020). Preliminary analysis of the PnB eukaryotic metabarcoding time series revealed that the taxonomic identity of most eukaryotic barcode sequences could not be predicted with standard bioinformatic methods with high enough resolution to distinguish phytoplankton, microzooplankton, and other eukaryotes from one another. This problem was addressed with the development of a novel bioinformatic tool to determine ensemble taxonomic assignments of DNA meta-barcode sequences. The software package is open source and freely available on the Comprehensive R Archive Network (https://cran.r-project.org/web/packages/ensembleTax/ index.html), and a paper detailing the method was published in PeerJ (Catlett et al. 2021). DNA metabarcoding and HPLC phytoplankton pigment observations were integrated to assess phytoplankton composition in the PnB dataset (Catlett et al. 2023). This analysis demonstrated that integrating methods for observing phytoplankton diversity reveals novel insights into phytoplankton physiology and function. We also found that diverse communities of phytoplankton and microzooplankton grazers and parasites covaried with distinct suites of phytoplankton biomarker pigments, demonstrating the potential to monitor ecosystem structure and function via ocean color remote sensing.

3.6.2 Genomics: Microbes

UCSB Grad student, Jacqui Comstock led a study to determine the best practices for collecting and extracting prokaryotic DNA from the marine water column. While there are numerous studies that describe microbial community structure and dynamics in the marine water column, there are few studies that compare biases associated with common practices of prefiltration type, volume of water filtered, filter type used to concentrate cells, and method used to extract DNA. These best-practices comparisons were born out of an Ocean Carbon and Biogeochemistry Workshop “Nucleic Acid Omics” (UNC Chapel Hill, Jan 2020) that C. Carlson was invited to present. Using water from the Santa Barbara Channel, Comstock initially compared four commercially available extractions kits (Qiagen PowerWater, PowerViral, PowerSoil and Blood and Tissue) and traditional phenol/chloroform, isoamyl alcohol methods, with and without bead beating to compare DNA yield and the 16S rDNA community structure using V4 primers. She also compared the extraction with proprietary lysis buffers with homemade sucrose lysis buffer. Her initial results indicated that while many combinations of kit type, bead beating, and lysis buffer revealed similar results with regard to extraction efficiency and community structure there, were several kit combinations that introduced significant bias where major phylum of bacterioplankton failed to amplify (Figure 14).

In addition to investigation of biases in extraction methods, Comstock ran several tests to compare storage conditions, filter type, volume collected, filtration method, and prefiltration type. Filter storage comparisons investigated the potential differences in bacterioplankton community structure when stored dry versus stored with lysis buffer when frozen before extraction. Filtration methods tests compared community structure between samples filtered via vacuum pump, positive pressure pump, and peristaltic pump. For both the storage and filtration method comparisons, no significant differences were found between the treatments used.

Prefiltration tests compared 1.2 um and 3 um prefiltered communities with whole communities at both the surface and in the mesopelagic. Prefiltration treatments displayed communities that ordinated separately from whole communities at both depths, with the surface showing a greater degree of difference between the treatments than the mesopelagic. The 1.2 um prefiltered community displayed a more dissimilar structure from the whole community than the 3 um prefilter (Figure 15).

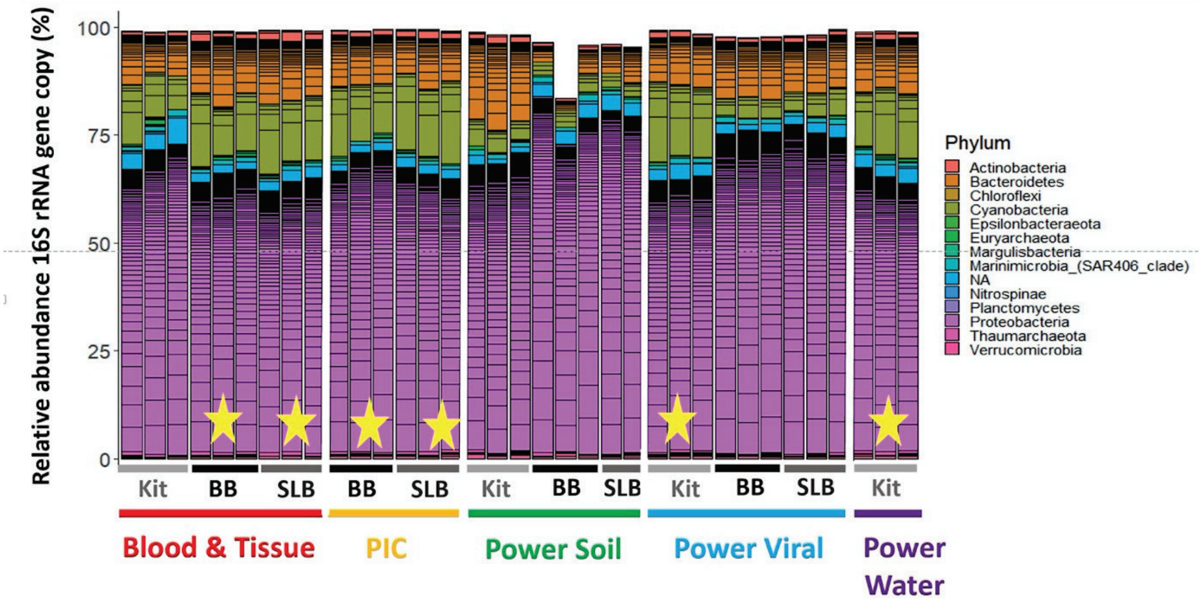


Figure 14. Relative abundance of top 20% of bacterioplankton taxa across extraction methods. Power Soil and Power Viral with modified lysis demonstrates an underrepresentation of Cyanobacteria. Power Soil also shows an underrepresentation of Bacteroidetes and an overrepresentation of Proteobacteria. Yellow stars indicate NMDS ordination of the microbial community were not different from each other (adonis).

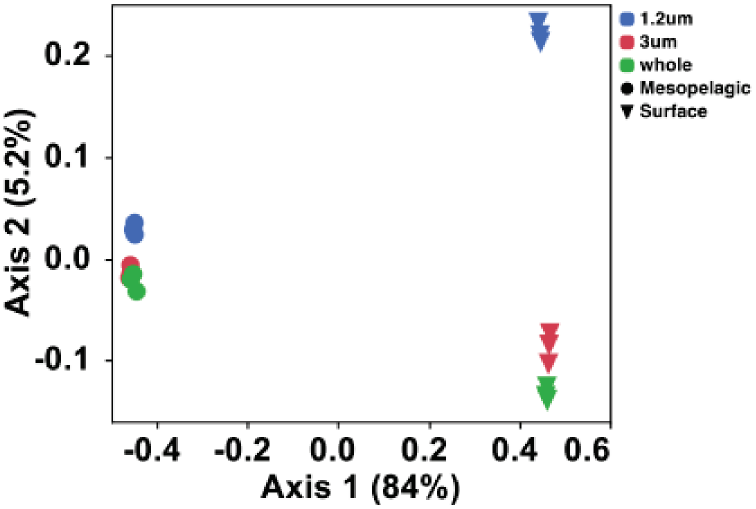


Figure 15. PCoA ordination of prefiltration comparison at the surface and in the mesopelagic.

3.6.3 Presentations

Comstock, J., Santoro, A., Carlson, C. (2020). Comparison of bacterioplankton community structure across extraction methods and filter type. AtlantECO Workshop on standard sampling methods for microbiomes in November 2020.

Comstock J. (2021) Standardization of DNA collection and processing. UCSB's Interdepartmental Graduate Program in Marine Science Seminar Series. March 2021.

3.7 Kelp and Sea Urchin Microbiomes

Anna James, a postdoctoral scholar, worked with Wilbanks and Carlson to study the collection of microbes that live on giant kelp blades (the kelp microbiome). Studies of host microbiomes suggest that these communities are key to maintaining host health, aiding in various processes such as elemental cycling, organic matter transfer, and even pathogen resistance (e.g., Peixoto et al. 2017, Rosenberg et al. 2007, Fan et al. 2013, Singh et al. 2013). To assess the role of the kelp microbiome in maintaining giant kelp health, we conducted a study to evaluate the 30 spatiotemporal patterns in microbial communities associated with kelp blades, as well as the mechanisms enabling giant kelp to harbor microbiomes distinct from the surrounding water column.

During our 2018 sampling campaign, we successfully implemented a sampling regime that relied on stand up paddle boards to navigate Mohawk Reef and Arroyo Quemado kelp beds in Santa Barbara Channel and Supersuckers biofilm sampling syringe (Mark Hatay, SDSU) to dislodge and concentrate kelp microbial communities onto 0.2 µm filters. DNA concentrated onto these filters was extracted and purified using a modified spin-column filtration method (Macherey-Nagel, Dueren, Germany), and gene amplicons of the V4 region of the bacterial 16S rRNA gene were generated using 515F and 806R primers following Wear et al. (2018). Sequencing was conducted at the University of California, Davis DNA Technologies Core on an Illumina MiSeq using PE250 v2 chemistry. Results showed that kelp microbiome communities varied by physiological state of the blade (i.e., uncolonized to fully encrusted blades; Figure 16A). In addition, kelp microbes varied by location and time (Figure 16B). Blades lacking signs of coverage by the epibiont bryozoan *Membranipora serilamella* exhibited distinct communities from blades with $\geq 20\%$ bryozoan coverage (Figure 16A). This pattern has not been described before and suggests the potential for invertebrate epibiont settlement on the blades to alter the kelp microbiome and potentially decrease blade health through settlement of "non-healthy" microbial communities (James et al. 2020).

A. James worked on a project for SBC MBON to better understand the effects of oil spills on microbial communities and carbon cycling in coastal environments. Following the Refugio Oil Spill in May 2015, we collected surface seawater at the site of the oil spill and tracked changes in microbial communities and their consumption of organic matter over the course of a month. Preliminary results demonstrated a significant offset in the overall microbial communities in oil compared to control incubations over the first week of the experiment (Figure 17, p value ≤ 0.01, PERMANOVA). Additionally, during this first week, we observed an enrichment in the relative abundances of bacteria previously shown to play a role in hydrocarbon degradation, including Cycloclasticaceae, Pseudohongiellaceae, and Pseudoalteromonadaceae in the oil treatments compared to controls (Figure 18, p value ≤ 0.01, linear mixed-effects model).

Measurement of dissolved organic carbon over the course of this experiment provided insight as to how these differences in microbial communities between oil and control incubations impacted carbon cycling in the coastal environment during the Refugio Oil Spill. Preliminary results suggested the potential of microbial communities that respond to oil spills to exhibit enhanced bacterial growth efficiencies relative to naturally occurring microbial communities.

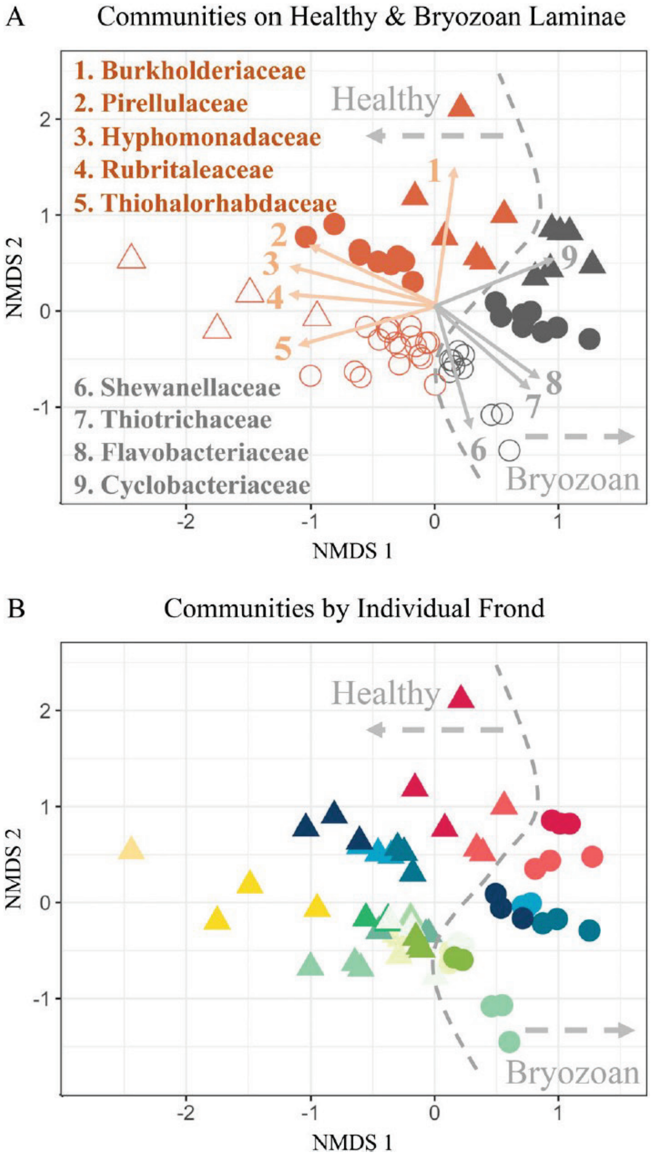


Figure 16. Nonmetric multidimensional scaling ordination plot of the arcsine square root transformed relative abundances (RAs) of amplicon sequence variants (ASVs) observed as a function of bryozoan coverage (A) and frond (B). (A) Shapes indicate sampling at MR in May (open triangles) and August (filled triangles), and AQ in June (open circles) and July (filled circles). Colors indicate healthy (orange) and bryozoan (gray) laminae. Arrows represent ASVs aggregated to family level and fitted to the ordination. Family levels shown are those exhibiting mean RAs ≥ 3% and significant differences between healthy and bryozoan laminae (FDR-adjusted p values ≤0.01). Arrows point in the direction of increasing RAs. (B) Shapes indicate healthy (triangles) and bryozoan (circles) laminae while colors indicate sampling location with MR in May/August in yellow/red and AQ in June/July in green/blue. Hues within each color are indicative of individual fronds. For the majority of fronds, there exist three points of the same hue; these represent the communities from three laminae on a single frond. Dashed line demarcates healthy and bryozoan-covered laminae.

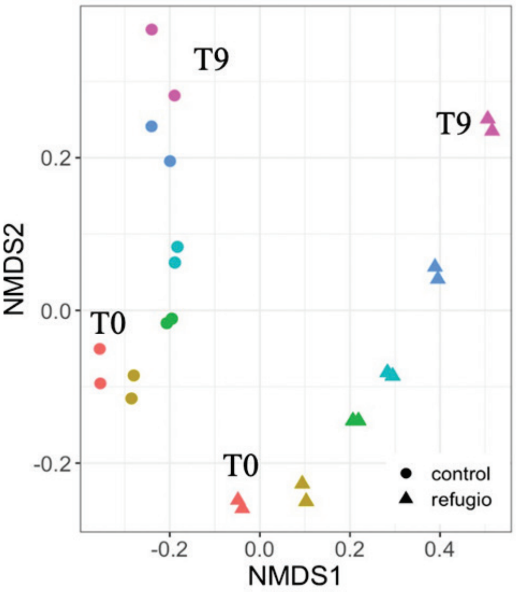


Figure 17. Nonmetric multidimensional scaling ordination plot of the arcsine square root transformed relative abundances of ASVs observed in oil (triangle) and control (circle) incubations. Colors indicate time point from the start of the experiment (T0) to one-week later (T9).

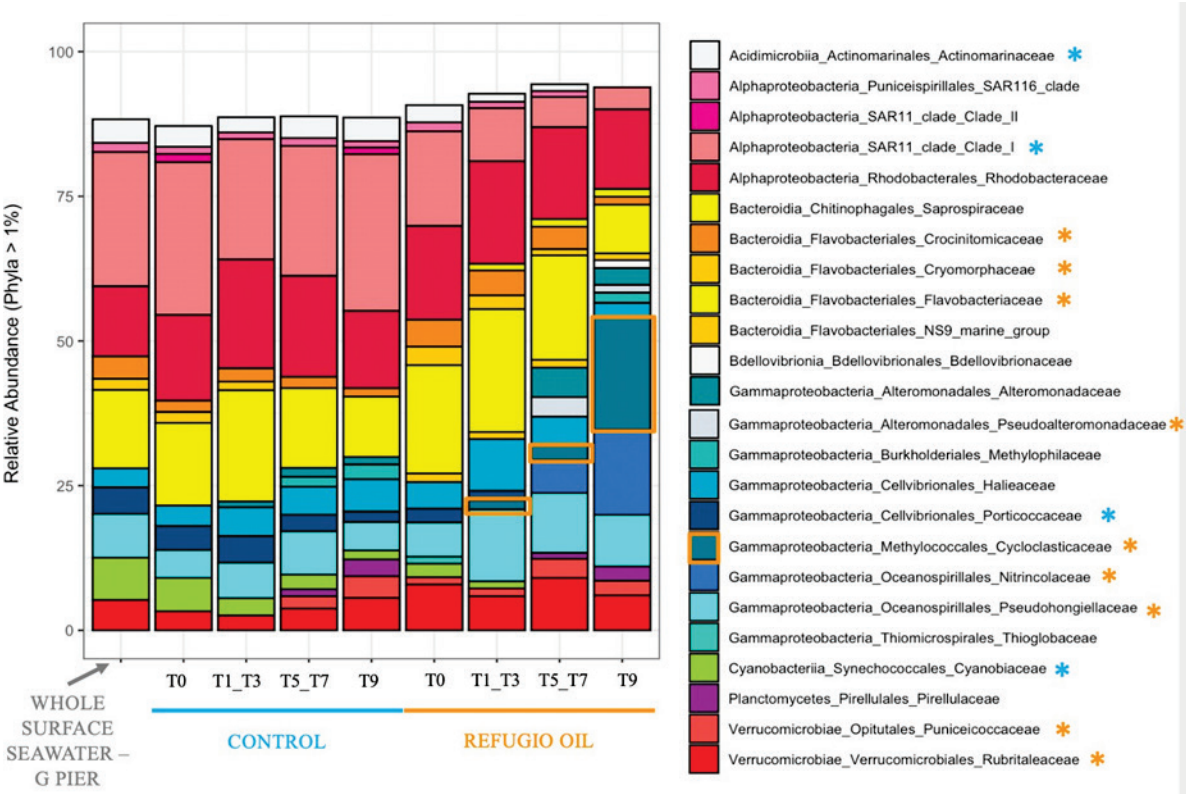


Figure 18. The average relative abundance of top family-level taxa (≥ 1%) grouped by time point for Control and Refugio Oil incubations. Asterisks indicate family-level taxa that demonstrate significant enhancement in Refugio Oil (orange) or Control (blue) incubations over the course of roughly one-week.

Separately, Paige Miller led an MBON study on the gut microbiome of sea urchins. Sea urchins are key grazers in coastal seas, where they can survive a variety of conditions and diets, enhancing their ecological impact on the community structure of kelp forests and other ecosystems. Using 16S rRNA gene sequencing, we characterized bacterial communities associated with guts of the two dominant sea urchin species in southern California, the red urchin *Mesocentrotus franciscanus*, and the purple urchin *Strongylocentrotus purpuratus*. Our results showed that the two urchin species have distinct gut microbiomes that vary with habitat as well as species (Miller et al. 2021). These results highlight the role of microbiomes within macroorganisms as an extended ecological trait and suggest that microbes may be crucial to resource use and partitioning in co-occurring species.

3.8 Ichthyoplankton

SBC MBON made significant progress in elucidating the efficacy of metabarcoding to characterize fish assemblages in the California Current Ecosystem. Our research focused on CalCOFI plankton samples. CalCOFI collects plankton using bongo nets that are composed of paired hoop nets. Plankton from the starboard bongo net is preserved in formalin, and larval fish are manually removed, identified, and counted. SBC MBON co-PI Andrew Thompson is an expert in identifying larval fishes from the CCE and contributed to building a station-specific time series for the abundance of hundreds of larval fishes dating back to 1951.

Plankton from the port net have been preserved in ethanol since 1998 but are not consistently analyzed. SBC MBON collaborator Zack Gold extracted DNA from the ethanol preservative from four samples per year from 1998–2019 and conducted metabarcoding analyses using this DNA. Metabarcoding ultimately provides data on the number of sequence reads per species. However, to assign sequence reads to species, it is necessary to match a given sequence to a reference sequence for each species. Initial screening of metabarcoding sequences generated using primers that amplify a region of the MiFish 12S mitochondrial gene revealed that a high proportion of reads were not delineating species; many reads were only identifying family or genera. To build out the reference library, Gold obtained tissue from 597 species from the Scripps Institution of Oceanography's ichthyology collection, extracted DNA from each individual, and added 252 additional reference species to GenBank's existing 550. As such, reference sequences for almost all common fishes are now available for CCE. This database will allow researchers who collect eDNA to accurately identify fishes in the CCE.

Metabarcoding would be most effective for marine monitoring if it can be used to quantify species abundances. Species-specific amplification efficiency hampers the capacity to utilize the number of sequence reads as a proxy for species abundance. For example, due to interspecific variation, a small amount of template DNA could result in a high number of reads for a species and vice versa. Because bongo nets consist of paired samples, we were able to pair larval counts that were manually identified from the starboard net and sequence reads (DNA extracted from ethanol preservative of the port net) for each species from each station. We were thus able to translate sequence reads per species per station into indices of abundance.

We are in the process of analyzing species-specific trends of larval abundance generated from metabarcoding DNA from ethanol preservative. Preliminary results demonstrate that species composition changed greatly during the 2014–2016 marine heat wave, as there was a marked increase of sequence reads for southern, mesopelagic species. In addition, the metabarcoding results captured known trends in the abundance of ecologically and commercially important species such as sardine and anchovy.

Overall, our metabarcoding work using DNA from ethanol-preserved plankton samples produced important technical advances, revealed ecological patterns, and should help fish-based eDNA sampling moving forward.

3.9 Environmental DNA (eDNA)

In July 2017, we conducted diver surveys with the SBC LTER program of 40 transects off Santa Barbara (two weeks of daily diving by three teams of 4 divers each). We photographed the transects for image analysis, and we collected triplicate eDNA samples on each transect. In collaboration with MBARI (Monterey Bay Aquarium Research Institute) and other MBON programs, these data were analyzed and published in Scientific Reports (Lamy et al. 2021). eDNA metabarcoding outperformed diver data to capture the beta diversity of marine fish communities. Moreover, eDNA resolved spatial variation at finer scales than visual survey data, capturing turnover of taxonomic richness and community structure among rocky reefs with high precision. Our results showed that eDNA can reveal spatial patterns in biodiversity at fine scales with much less field effort than traditional methods. Along with Francisco Chavez and the CenCOOS MBON, we submitted LOIs to SCCOOS and Ocean Protection Council to continue eDNA monitoring in California in collaboration with the State Marine Protected Area monitoring effort. We also began a long-term time series of kelp forest eDNA collections in collaboration with the Santa Barbara Coastal LTER project.

Since 2019, Christopher Jerde, an expert in fish eDNA applications at the Marine Science Institute (UCSB), has been partially supported by the MBON program. His research focuses on data synthesis, modeling, and building the decision-support infrastructure to inform fisheries management using eDNA derived inferences. This research program has contributed a broad understanding about the implications of false positives (Darling et al. 2021) and the maturity of eDNA science applications for invasive species management (Sepulveda et al. 2020) and broadly fisheries management (Jerde 2021).

Under Jerde's advisement, graduate student Terra Dressler led and published a survey of the tidewater goby (*Eucyclogobius newberryi*) in the Andree Clark Bird Refuge in Santa Barbara, CA (Dressler et al. 2020). The survey confirmed the presence of this listed estuarine fish and motivated extra protections to the area during nearby City development and construction. Jerde led a project to evaluate the coverage of eDNA genetic reference libraries in high diversity systems (Jerde et al. 2021). Although the work used the Mekong River as its study subject, showed the importance of using multiple species lists to build the reference library, the added inferential power of using multiple primer pairs in eDNA metabarcoding studies, and the limitations with current reference library evaluation software to consider anadromous and catadromous fish. Research by Gold (discussed previously) addresses many of the reference library knowledge gaps for California.

McElroy et al. (2020) conducted the first fish eDNA metabarcoding calibration to conventional fishing approaches. This research synthesized 37 studies in natural aquatic systems to compare species richness estimates for bony fish between eDNA metabarcoding and conventional methods, such as nets, visual census, and electrofishing. In freshwater systems with fewer than 100 species, we found eDNA metabarcoding detected more species than conventional methods (Figure17A). Using multiple genetic markers further increased species richness estimates with eDNA metabarcoding (Figure 17C compared to D). For more diverse freshwater systems and across marine systems (Figure 17B), eDNA metabarcoding reported similar values of 33 species richness to conventional methods; however, more studies are needed in these environments to better evaluate relative performance. In systems with greater biodiversity, eDNA metabarcoding will require more populated reference databases, increased sampling effort, and multi-marker assays to ensure robust species richness estimates to further validate the approach. eDNA metabarcoding is reliable and provides a path for broader biodiversity assessments that can outperform conventional methods for estimating species richness.

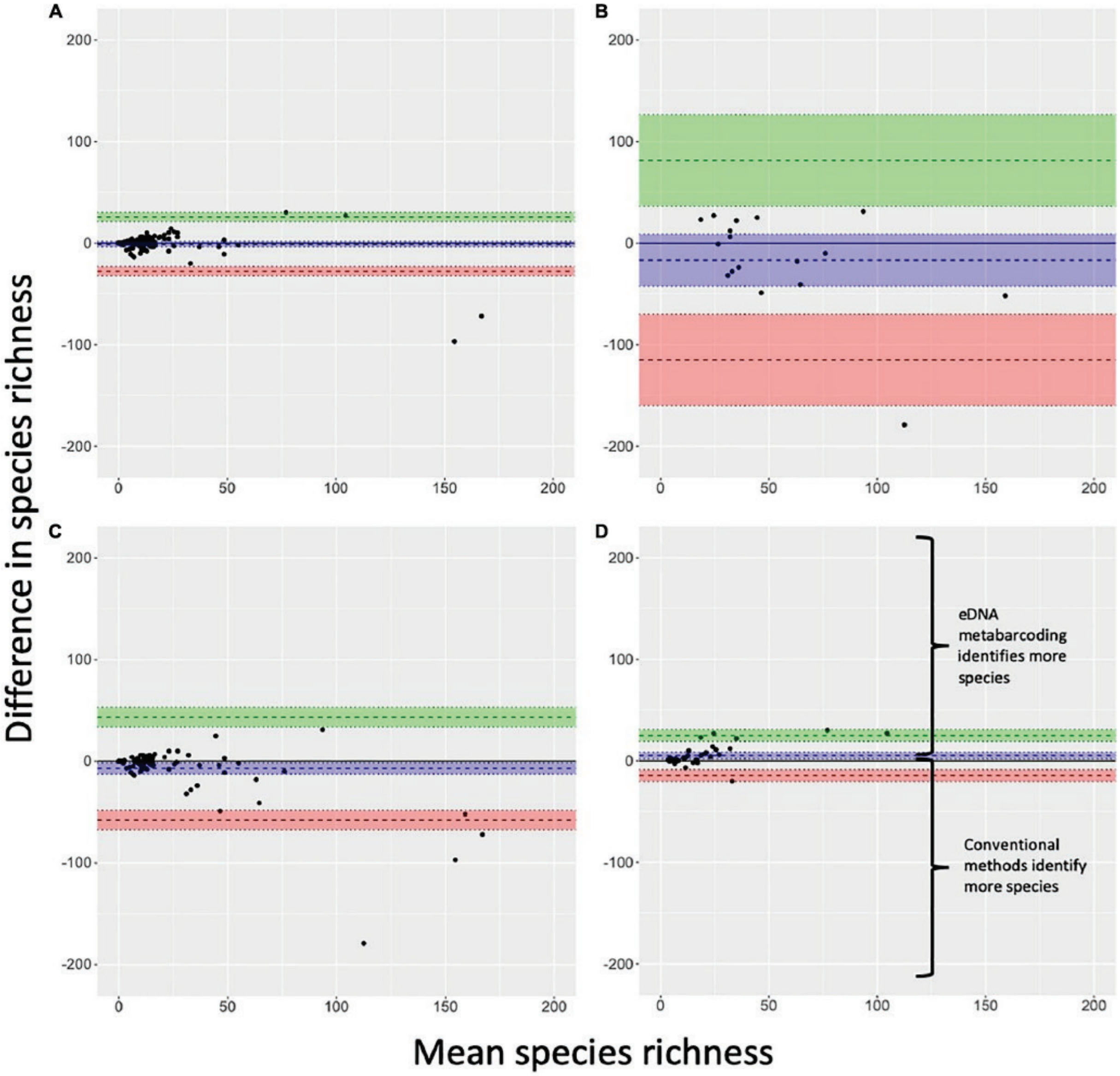


Figure 19. Bland-Altman plots for (A) freshwater and (B) marine systems, (C) single-marker and (D) multi-marker observations for freshwater and marine systems combined. Blue band indicates a mean (wide dashed line) with 95% confidence intervals. Green and red bands indicate two standard deviations (wide dashed lines) from the mean with 95% confidence intervals. Outliers are observations that lie above and below the green and red bands, respectively. Regions of the figure where one method differentially detects more or fewer species than the other method are detailed in Panel D and are consistent across all panels.

4 Implementing a Tradeoff Framework that Optimizes Allocation of Sampling Effort

A goal of SBC MBON was to compare the effectiveness of different methods of biodiversity sampling, exploring how the optimal mix of sampling depends on the goals of monitoring and the resources available. Rassweiler et al. (2020) documented the challenges and potential usefulness of combining data from different fish sampling programs in the Santa Barbara Channel. We showed that the roving divers substantially underestimated fish density relative to density estimates based on transects. Furthermore, the divergence between the two methods varied substantially depending on the fish species, making it difficult to correct for this variation. However, the datasets showed similar patterns of community composition through time and space, supporting their individual value.

We also developed methods that allow us to take pilot data and construct efficiency curves predicting how our confidence in biodiversity estimates at a monitored site will depend on the sampling effort invested in that method. These curves can be compared to evaluate the relative efficacies of different methods for sampling the same taxonomic group (Hernan et al. 2022).

In addition to evaluating methods for sampling biodiversity of a single taxonomic group, we generalized this approach to consider multiple methods sampling different taxonomic groups. We can thus predict our confidence in estimates of total biodiversity that are generated through the application of multiple sampling methods at a single site. We showed that these predictions can be used to evaluate alternative portfolios of sampling and identify the optimal allocation of effort across candidate sampling methods. Importantly, these results do not require pilot data that are balanced across space and time, but instead predictions can be reliably produced from data from disparate sources, such as data from different prior sampling programs. These methods permit analysis of how the optimal mix of sampling will change as technology improves (e.g., lower cost and better accuracy of genetic and computational methods). The methods will provide quantitative tools to guide decisions about design and redesign of marine biodiversity monitoring programs which will allow sampling designs to keep up with advances in technology.

4.1 Outreach and Education

Miller, Bell, and James organized and chaired a session at the 2020 Ocean Sciences meeting in San Diego. Titled “Ecology and Biogeochemistry of Macroalgae”, the session highlighted MBON microbiome and remote sensing work on kelp forests and included both oral presentations and posters.

James mentored Faeben Wossenseged (2019–2020) as part of the California Alliance for Minority Participation which provides research opportunities to underrepresented minority students in science, technology, engineering, and math (STEM) fields. Upon graduating, Wossenseged joined the National Institutes of Health (NIH) as an NIH Academy Enrichment Program Scholar to study the role of systemic racial bias in creating health disparities that disproportionately impact communities of color.

James also mentored an undergraduate in STEM who identifies as an underrepresented minority and is working to dismantle inequities in our education system through cross-university collaborations with groups such as the Sloan Equity and Inclusion in STEM Introductory Courses (SEISMIC). She has participated in numerous meetings aimed at highlighting inequities in education including ‘Delivering on the UC Promise: Providing Equitable STEM Education Opportunities for California’ conference at UC, Santa Barbara, and she was asked by the Center for Innovative Teaching, Research and Learning at UCSB to participate in Redesign Instruction for the Student Experience (RISE). She mentored students at multiple Ocean Sciences Meetings and participated in the Research Education for Undergraduates program at UCSB (2019) to share with students the MBON project designed to study the kelp microbiome. This project highlights the importance of creativity and innovation in conducting science and relies on low-tech transportation (stand up paddle boards) and low-budget sampling tools (altered syringes) to study critical aspects of our coastal ocean. This work was also showcased alongside other projects from UCSB at World Ocean's Day at the Santa Barbara Harbor (2019).

Miller and four other project investigators, including Clarissa Anderson, SCOOS Director, were awarded funding from NSF's Coastlines and People program to support a Research Collaboration Network (RCN) focused on the planning and development of Regional Ecosystem Services Observation Networks. (RESONs) are regionally-focused networks of sensors and data sources necessary to take the pulse of coastal ecosystems, spanning their structure, function, and services provided to people. In our vision, RESONs extend the framework of the regional Ocean Observing Systems (OOSs) to explicitly consider biodiversity, ecosystem function, and species migration in the context of fisheries and coastal infrastructure needs. Our RCN is bringing together marine scientists, ecosystem modelers, engineers, spatial statisticians, and computer scientists from academia and industry in collaboration with stakeholders and managers. RESON is focusing on three data targets: biodiversity, ecosystem function, and species migration, using the California Current Large Marine Ecosystem (CC LME) as an initial model, and is leveraging existing networks including Southern California Coastal OOS, LTER, MBON and Animal Telemetry Network. To expand RESON's reach, the project coordinator gave presentations about RESON and its goals to several groups, including to the California Biodiversity Network, the West Coast Ocean Alliance Stakeholder roundtable, and the West Coast Ocean Tribal Caucus.

RESON held its first virtual workshop over the course of 2 half-days on June 28 and 29, 2021. There were 45 participants. To prepare for the workshop, the PI and project coordinator met with 32 different federal, state, local, tribal, and NGO agencies and conducted one-on-one meetings with them to understand their interests and needs within a new biological monitoring network. A survey was sent out to each party in preparation for the workshop to understand each stakeholder's 36 priorities with regards to the ocean and what information they are lacking to meet those priorities. These answers helped shape the workshop format and questions. A second workshop was held in February 2022 with some of the same stakeholders, plus the technology sector to identify what sensors are feasible to conduct high spatial and temporal monitoring. The results from Workshop 1 shaped the development of the second workshop, which explored various technologies to enhance the monitoring of key ecosystem functional groups to provide insight into ecosystem health. The focus included looking at technology that can fill in knowledge gaps under five key ecosystem functional groups: Primary producers, benthic primary consumers, pelagic primary consumers, nearshore predators, and pelagic predators. The workshop also had participants exploring and refining ways to tie together social and ecological indicators through targeted questions. We are now working on a synthesis paper to develop a roadmap to establishing an operational ESON.

Thomas Turner, a sponge taxonomist working with SBC MBON, described four new species of sponge from our region (Turner 2021). This received quite a bit of press, including an National Public Radio interview that explicitly mentions MBON. https://www.popsci.com/animals/new-sea-sponge-species/. https://timesofsandiego.com/tech/2021/05/17/biologist-discovers-4-newspecies-of-sponge-in-southern-californias-kelp-forests/. https://www.kclu.org/2021-05-18/placeholder.

5 Additional Tasks

5.1 Pacific Seabird Monitoring Network I: Planning and Design

One of the overarching goals of the SBC MBON (identified in the cooperative agreement) was to develop "a scalable and transferable demonstration Biodiversity Observation Network" that "will network existing monitoring efforts and fill the remaining information gaps". During the initial phase of this research seabirds were identified as a notable gap in the suite of monitored species. This gap is significant to BOEM because seabird populations are an important ecosystem health indicator that is sensitive to offshore energy development. An initial step envisioned for this information need was to determine the most efficient monitoring design to distinguish regional population trend modifications resulting from offshore energy projects compared to other factors such as dynamic oceanographic conditions, degraded ocean productivity, or fisheries bycatch.

Thus, SBC MBON undertook this additional task of developing a monitoring framework to support BOEM's need to understand the effectiveness of lease stipulations and mitigation strategies. This important and comprehensive work was published in *Biological Conservation* with the following citation (see also Appendix A):

Croll DA, Ellis AA, Adams J, Cook ASCP, Garthe S, Goodale MW, Hall CS, Hazen E, Keitt BS, Kelsey EC, Leirness JB, Lyons DE, McKown MW, Potiek A, Searle KR, Soudijn FH, Cotton Rockwood R, Tershy BR, Tinker M, VanderWerf EA, Williams KA, Young L, Zilliacus K. 2022. Framework for assessing and mitigating the impacts of offshore wind energy development on marine birds. *Biological Conservation*, Volume 276. ISSN 0006-3207. http://doi.org/10.1016/j.biocon.2022.109795.

5.2 Passive Acoustic Monitoring of Marine Mammals Offshore of Diablo Canyon

One of the primary objectives of the SBC MBON study is to "employ innovative techniques for data discovery and methods that dynamically interrelate datasets and add value to existing monitoring data." During the initial phase of this research, unprocessed data were discovered from one of three acoustic buoys deployed in Morro Bay in 2012 as part of a monitoring program established for a high-energy seismic survey (Pacific Gas and Electric). The seismic surveys were never conducted, and the buoys were recovered about five months after deployment. An additional task was given to the SBC MBON to analyze existing recordings of whale and dolphin vocalizations that were collected from an acoustic buoy near the Morro Bay Wind Energy Area. Appendix B is the report generated from this task.

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Appendix A: Cumulative List of SBC MBON Publications, 2014–2022

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3. Catlett D, Siegel DA, Guillocheau N. 2020. Plumes and Blooms: Curated oceanographic and phytoplankton pigment observations ver 1. Environmental Data Initiative. https://doi.org/10.6073/pasta/f88ee1dc32b8785fe6ce57d80722e78c.

4. Catlett D, Siegel DA, Matson PG, Wear EK, Carlson CA, Lankiewicz TS, Iglesias-Rodriguez MD. 2023. Integrating phytoplankton pigment and DNA meta-barcoding observations to determine phytoplankton composition in the coastal ocean. Limnol Oceanogr. https://doi.org/10.1002/lno.12274.

5. Catlett D, Siegel DA, Simons RD, Guillocheau N, Henderikx-Freitas F, Thomas CS. 2021. Diagnosing seasonal to multi-decadal phytoplankton group dynamics in a highly productive coastal ecosystem. Prog Oceanogr. 102637. https://doi.org/10.1016/j.pocean.2021.102637.

6. Catlett D, Son K, Liang C. 2021. ensembleTax: an R package for determinations of ensemble taxonomic assignments of phylogenetically-informative marker gene sequences. PeerJ 9:e11865 https://doi.org/10.7717/peerj.11865.

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Appendix B: Passive Acoustic Monitoring of Marine Mammals Offshore of Diablo Canyon, November 2012–March 2013

Passive Acoustic Monitoring of Marine Mammals Offshore of Diablo Canyon, November 2012–March 2013

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Baird's Beaked Whale (*Berardius bairdii*) *Photo Credit: Katherine Whitaker*

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Contents

Executive Summary

Two High-Frequency Acoustic Recording Packages (HARPs) were deployed from November 2012 to March 2013 to record marine mammal sounds and study the environmental impacts of offshore geophysical surveys planned near the Diablo Canyon Power Plant. One HARP was deployed on the seafloor at approximately at 100 m in shallow water to collect full-bandwidth recordings of harbor porpoises and coastal bottlenose dolphins. A second HARP was deployed at approximately 1,000 m depth to monitor the full range of marine mammal species present within the vicinity of the Diablo Canyon seismic survey. The analysis conducted in this report focuses on the second HARP deployed for a variety of marine mammal species. The HARP recorded sound in the frequency band 10 Hz–100 kHz. Data analysis consisted of analyst-scans of long-term spectral averages (LTSAs) and spectrograms. Three frequency bands were analyzed for marine mammal vocalizations: (1) Low-frequency, between 10–500 Hz, (2) Mid-frequency, between 500–5,000 Hz, and (3) High-frequency, between 5–100 kHz. The low-frequency ambient soundscape and the presence of Mid-Frequency Active (MFA) sonar were also analyzed.

Ambient sound levels had spectral peaks along with lower frequency harmonics at 45–47 Hz from November to December, related to blue whale B calls. Prominent peaks in sound spectrum levels observed in the frequency band 15–30 Hz during winter are related to seasonally increased presence of fin whale calls.

Three baleen whale species were recorded: blue, fin, and humpback whales. Fin whales were detected throughout the monitoring period with higher activity from November to December 2012.

Blue whale B and D calls were found in high numbers from November 2012 to January 2013. Humpback whale call types were detected throughout the recording period but began to decrease starting in February 2013.

Several known odontocete signals were detected. Cuvier’s beaked whales were detected in low numbers throughout the monitoring period. Baird’s beaked whales were detected in low numbers in November and December 2012. Two acoustically identifiable delphinid species were Risso’s dolphins and Pacific white-sided dolphins, whose echolocation clicks occurred between early February and March 2013. Sperm whales were detected intermittently throughout the recording period but were highest in December 2012. Unidentified odontocete clicks greater than 20 kHz were detected throughout the monitoring period and peaked in December 2012.

Project Background

The Diablo Canyon site is located in the offshore waters of the Pacific Ocean, adjacent to the San Luis Obispo County shoreline. Seismic surveys were planned for the offshore region of the Diablo Canyon Power Plant (DCPP) to help delineate nearby fault zones. A diverse group of marine mammals are known to be present in the proposed project area including species listed under the Endangered Species Act. Stocks of marine mammals are known to use the study area for migration and as their preferred habitat. This report documents the analysis of data recorded by a High-Frequency Acoustic Recording Packages (HARP) that was deployed at Diablo Canyon and collected data from November 2012 to March 2013 (Figure B-1).

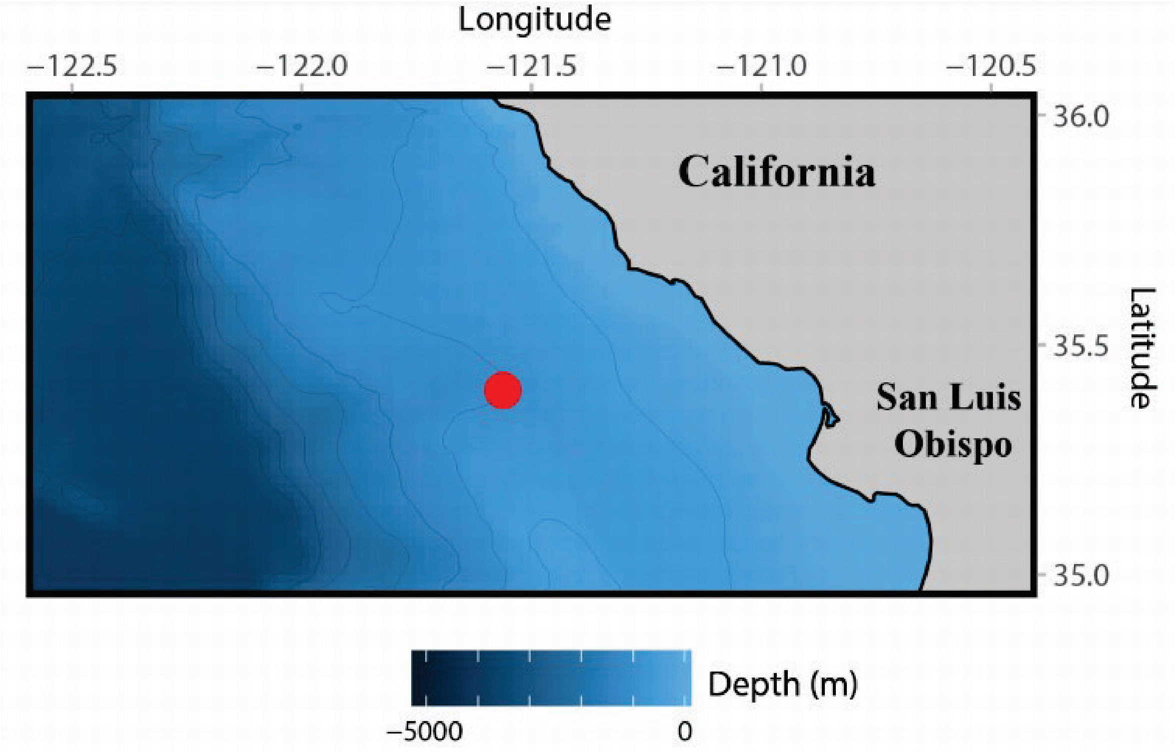

Figure B-1. Location of the High-Frequency Acoustic Recording Packages (HARP) at site DCPP01C located at 35° 24.000 N, 121° 33.750 W, depth 1,000 m deployed in the Diablo Canyon study area from November 2012 to March 2013.

Methods

High-Frequency Acoustic Recording Package (HARP)

HARPs are autonomous underwater acoustic recording packages that can record sounds over a bandwidth from 10 Hz up to 160 kHz and are capable of approximately a year of continuous data storage. The HARPs were deployed in a small mooring configuration with the hydrophone suspended approximately 22 m above the seafloor. Each HARP is calibrated in the laboratory to provide a quantitative analysis of the received sound field. Representative data loggers and hydrophones were also calibrated at the Navy's TRANSDEC facility to verify the laboratory calibrations (Wiggins and Hildebrand, 2007).

Data Collected

One HARP recorded from November 2012 to March 2013 at DCPP at Site C and sampled continuously at 200 kHz to provide 100 kHz of effective bandwidth. The instrument (35° 24.000 N, 121° 33.750 W, depth 1,000 m) recorded for 132 days from November 7, 2012 to March 19, 2013.

Data Analysis

Recording over a broad frequency range of 10 Hz to 100 kHz allows detection of the low-frequency ambient soundscape, baleen whales (mysticetes), and toothed whales (odontocetes). Analysis was focused on the following species: blue whales (*Balaenoptera musculus*), fin whales (*B. physalus*), humpback whales (*Megaptera novaeangliae*), killer whales (*Orcinus orca*), Cuvier's beaked whales (*Ziphius cavirostris*), Baird's beaked whales (*Berardius bairdii*), Risso's dolphins (*Grampus griseus*), Pacific white-sided dolphins (*Lagenorhynchus obliquidens*), and sperm whales (*Physeter macrocephalus*).

Individual blue whale B calls, D calls, and Cuvier's beaked whale echolocation clicks were detected automatically using computer algorithms. Presence of fin whale 20 Hz calls was detected using an energy detection method and is reported as a daily average, termed the 'fin whale acoustic index' (Širović *et al.*, 2015). Detections of Risso's dolphins, Pacific white-sided dolphins, odontocete echolocation clicks, sperm whales, and Baird's beaked whales were detected manually. Details of all detection methods are described below.

We summarize acoustic data collected at DCPP Site C from November 2012 to March 2013. We discuss seasonal occurrence and relative abundance of calls for different species that were consistently identified in the recordings.

Low-Frequency Ambient Soundscape

To determine ambient sound levels, HARP recordings were decimated by a factor of 100 to provide an effective bandwidth of 10 Hz to 1 kHz from which long-term spectral averages (LTSAs) were constructed with 1 Hz frequency and 5 s temporal resolution. Daily spectra were computed by averaging 5 each, 5 s sound pressure spectrum levels calculated from each 75 s acoustic record. System self-noise due to disk drive activation was excluded from these averages.

Low-Frequency Marine Mammals

The Diablo Canyon site is inhabited by a wide array of low-frequency baleen whale species, particularly including blue whales (*Balaenoptera musculus*) and fin whales (*B. physalus*). In addition, North Pacific right whales (*Eubalaena japonica*) may infrequently appear in this region (Josephson *et al.*, 2008, Širović *et al.*, 2014). For the low-frequency data analysis (10–500 Hz), the 200 kHz sampled raw data were decimated by a factor of 100 for an effective bandwidth of 1 kHz. LTSAs were created using a time average of 5 s and frequency bins of 1 Hz.

By examining low-frequency sound, the detection range for marine mammal calls, such as those of blue whales and fin whales, may extend to ranges of ~100 km or more (Širović *et al.*, 2015). The limitation for their detection range is often based on the interaction of the sound with a shallow bathymetric feature. In the case of DCPP Site C, a greater detection range will be experienced for animals located further offshore, where the water depths are generally deep and increasing in depth, versus inshore locations where the water depth is becoming shallow.

Blue whale B and D calls were automatically detected using the spectrogram correlation and general power law methods, respectively, described below. Fin whale 20 Hz calls were detected automatically using an energy detection method and are reported as a daily average termed the 'fin whale acoustic index'. A manual search for North Pacific right whale up calls was undertaken by scrutiny of LTSAs and spectrograms in the custom software program Triton.

● Blue Whales

Blue whales produce a variety of calls worldwide (McDonald *et al.*, 2006). Calls recorded in the eastern North Pacific include the Northeast Pacific blue whale B call (Figure B-2) and the D call (Figure B-3). Northeast Pacific blue whale B calls are geographically distinct and potentially associated with mating functions (McDonald *et al.*, 2006; Oleson *et al.*, 2007). They are low-frequency (fundamental frequency <20 Hz), long duration (> 10 s) calls that are often produced in regularly repeated sequences. D calls are downswept in frequency (approximately 100–40 Hz) with a duration of several seconds. These calls are similar worldwide and are associated with feeding animals; they may be produced as call-counter call between multiple animals (Oleson *et al.*, 2007).

- ***Northeast Pacific Blue Whale B Calls***

Blue whale B calls (Figure B-2) were detected automatically using spectrogram correlation (Mellinger and Clark, 1997). The detection kernel was based on frequency and temporal characteristics measured from 30 calls recorded in the data set, each call separated by at least 24 hours. The kernel was comprised of four segments, three 1.5 s and one 5.5 s long, for a total duration of 10 s.

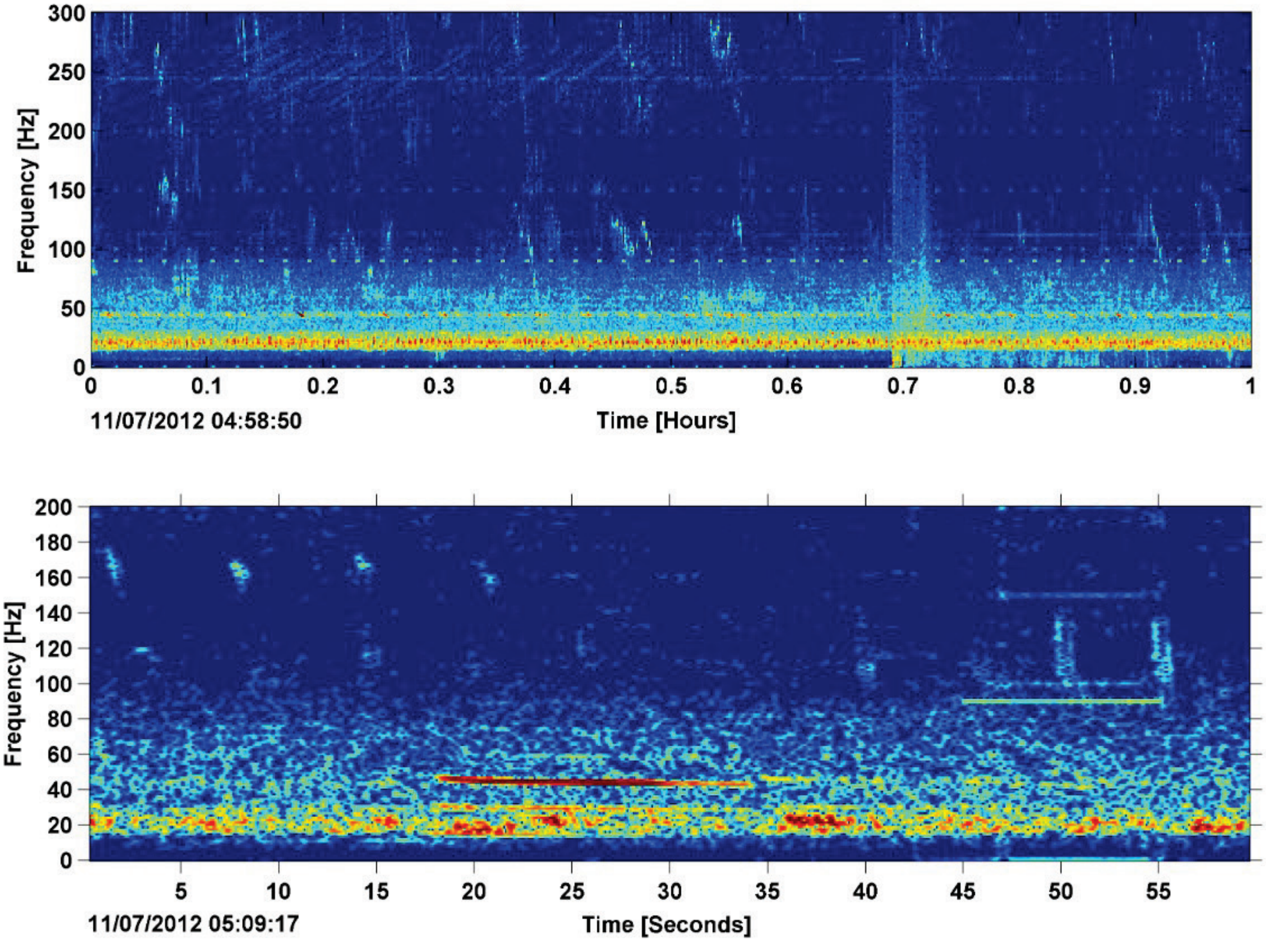

Figure B-2. Northern Pacific blue whale B calls (just below 50 Hz) in Long-Term Spectral Average (LTSA; top) and spectrogram (bottom) at the DCPP Site C, November 2012.

- ***Blue Whale D Calls***

Blue whale D calls were detected using an automatic algorithm based on the generalized power law (Helble *et al.*, 2012). This algorithm was adapted for the detection of D calls by modifying detection parameters that included the frequency space over which the detector operates. A trained analyst subsequently verified the detections (Figure B-3).

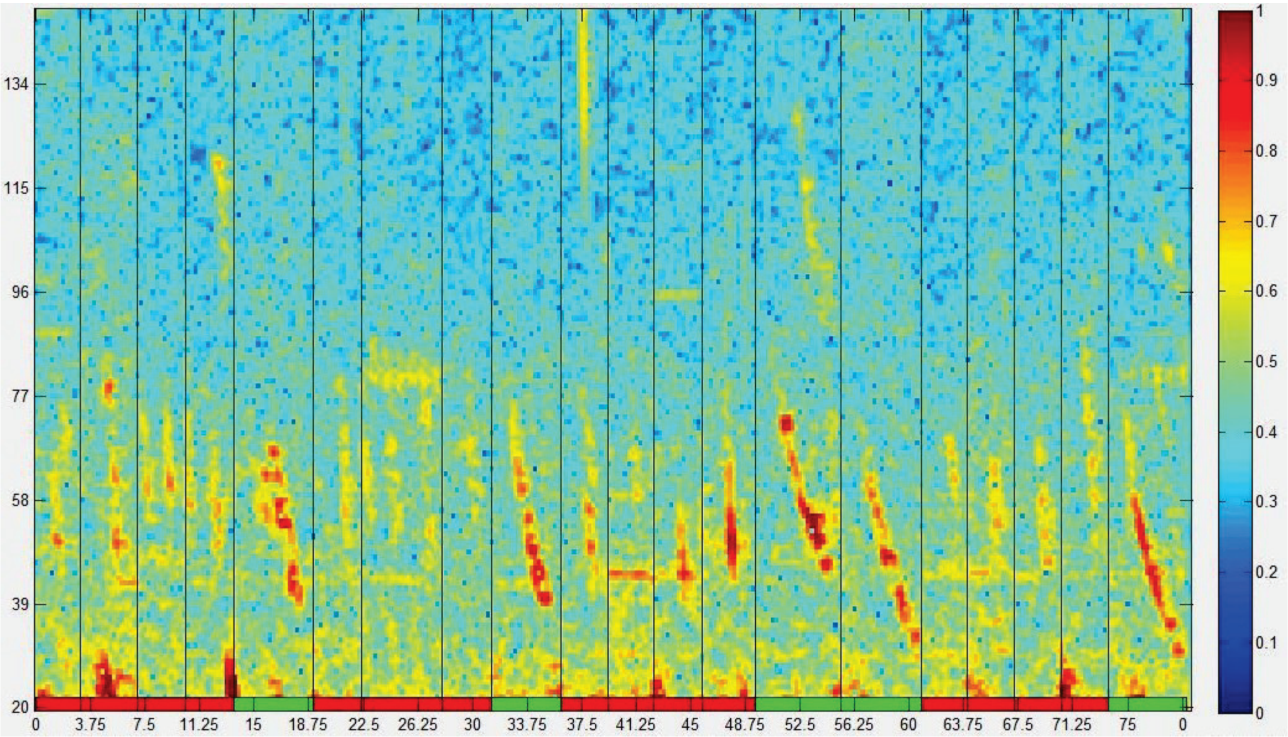

Figure B-3. Blue whale D calls from DCPP Site C in the analyst verification stage of the detector. Green along the bottom evaluation line indicates true detections and red indicates false detections.

- **Fin Whales**

Fin whales produce two types of short (approximately 1 s duration), low-frequency calls. The most common is a frequency downsweep from 30 to 15 Hz, called 20 Hz calls (Watkins, 1981; Figure B-4) The 20 Hz calls can occur at regular intervals as song (Thompson *et al.*, 1992; Oleson *et al.* 2014), or irregularly as call counter-calls among multiple, traveling animals (McDonald *et al.*, 1995). The second call is frequency downswept from 75 to 40 Hz, designated as 40 Hz calls (Širović et al., 2012). The 40 Hz calls most often occur in irregular patterns (Wiggins and Hildebrand, 2020).

- ***Fin Whale 20 Hz Calls***

In the Diablo Canyon study area, fin whale 20 Hz calls are so abundant that it is often impossible to distinguish, and therefore detect, individual calls (Watkins *et al.*, 2000; Širović *et al.*, 2015). Therefore, fin whale 20 Hz calls (Figure B-4) were detected automatically using an energy detection method (Širović *et al.*, 2015). The method uses a difference in acoustic energy between signal and noise, calculated from an LTSA over 5 s with 1 Hz frequency resolution. The frequency at 22 Hz was used as the signal frequency (Nieukirk *et al.*, 2012; Širović *et al.*, 2015), while noise was calculated as the average energy between 10 and 34 Hz. The resulting ratio is termed 'fin whale acoustic index' and is reported as a daily average. All calculations were performed on a logarithmic scale.

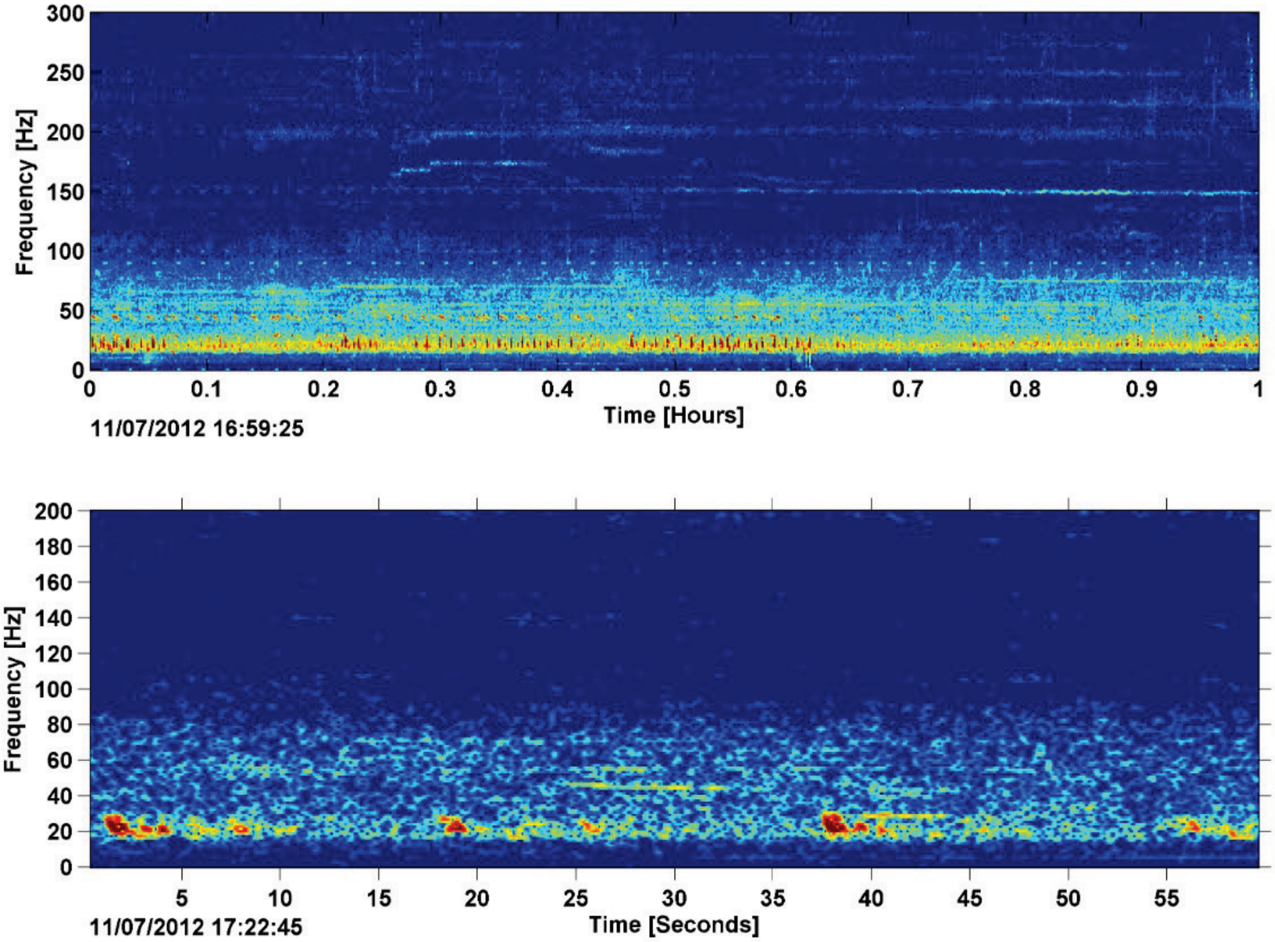

Figure B-4. Fin whale 20 Hz call in LTSA (top) and spectrogram (bottom) at DCPP Site C, November 2012.

● North Pacific Right Whales

Historical whaling activities are responsible for the near extirpation of the eastern population of the North Atlantic right whale, making it difficult to detect in eastern Pacific waters (NMFS 2006). These whales make a variety of sounds, the most common of which is the up call (Figure B-5). The up call typically sweeps from about 90 to 150 Hz or as high as 200 Hz and has a duration of approximately 1 s (McDonald and Moore, 2002).

• *North Pacific Right Whale Up Calls*

For the manual detection of North Pacific right whale up calls, the LTSA frequency was set to display between 1–300 Hz with a 1-hour plot length. To observe individual calls, the spectrogram window was typically set to display 1–200 Hz with a 60 s plot length. The FFT was generally set between 1500 and 2000 data points, yielding about a 1 Hz frequency resolution, with a 90% overlap. When a potential call of interest was identified in the LTSA or spectrogram, its presence during that hour was logged. No North Pacific right whale up calls were detected during this recording period.

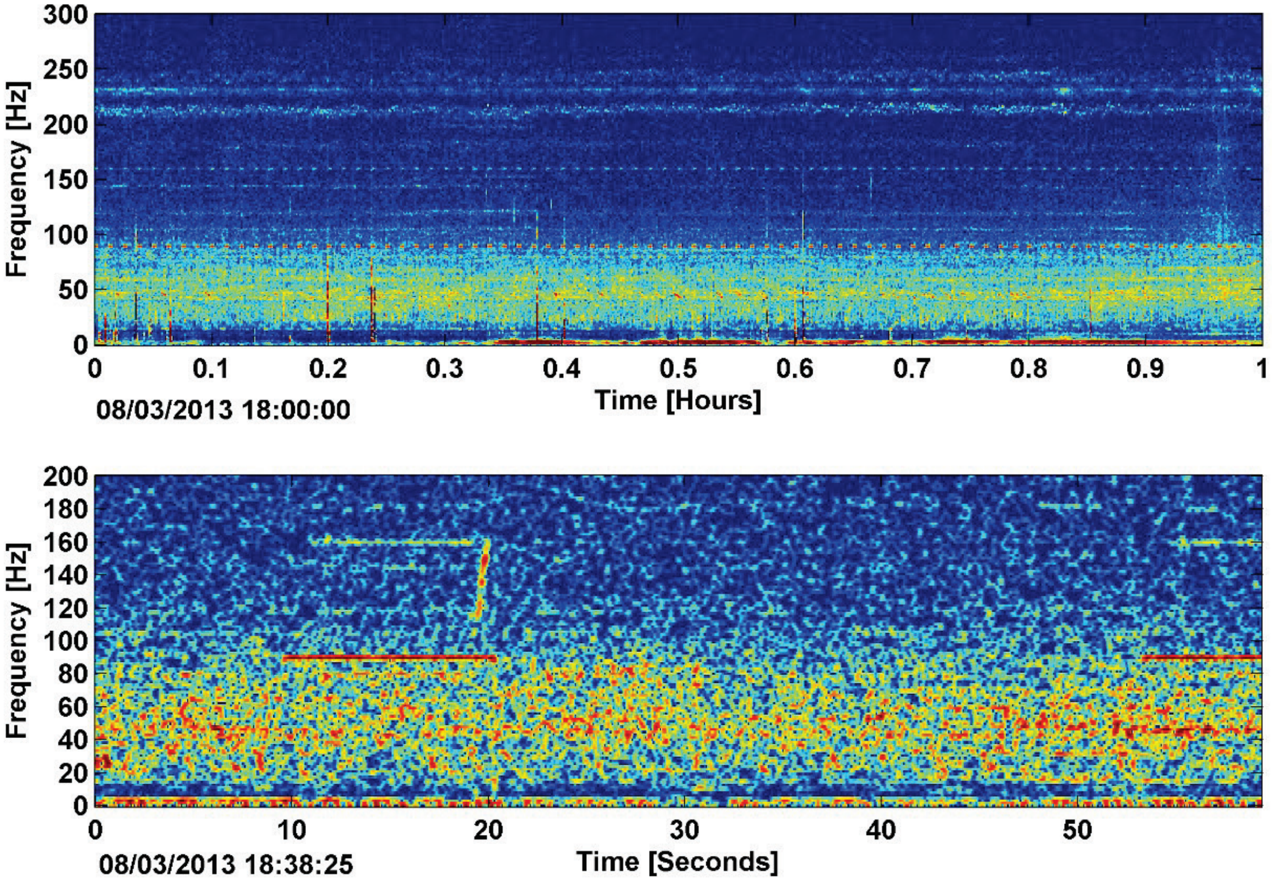

Figure B-5. North Pacific right whale up call in the LTSA (top) and spectrogram (bottom) recorded in the Gulf of Alaska, August 2013.

Mid-Frequency Marine Mammals

Humpback whales *(Megaptera novaeangliae)* and killer whales (*Orcinus orca*) were the only marine mammal species at Diablo Canyon Site C with calls in the mid-frequency range (500–5,000 Hz) monitored for this report. We detected humpback whale calls using an automatic detection algorithm based on the generalized power law (Helble *et al.*, 2012). The detections were subsequently verified for accuracy by a trained analyst. When humpback calls were identified, they were logged according to the start time and end time of the encounter. An encounter was considered to end when there were no calls for 30 min or more. The encounter durations were added to estimate cumulative hourly presence.

● Humpback Whales

Humpback whales produce both song (Figure B-6) and non-song calls. The song is categorized by the repetition of units, phrases, and themes for a variety of calls as defined by Payne & McVay (1971). Non-song vocalizations such as social and feeding sounds consist of individual units that can last from 0.15 to 2.5 seconds (Dunlop *et al.*, 2007; Stimpert *et al.*, 2011). Most humpback whale vocalizations are produced between 100–3,000 Hz. There was no effort to separate song and non-song calls in our analysis.

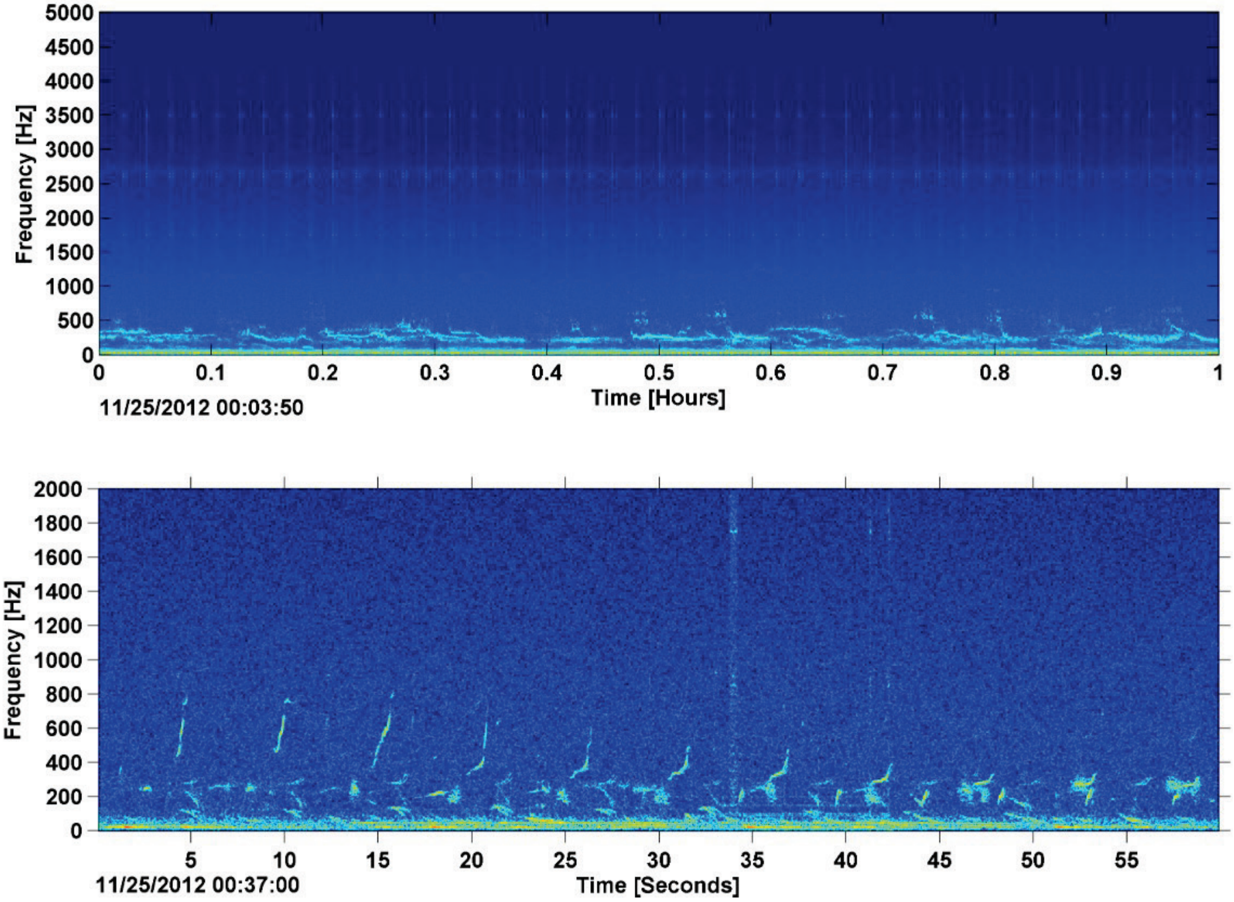

Figure B-6. Humpback whale song calls in the LTSA (top) and spectrogram (bottom) recorded at DCPP Site C, November 2012.

- **Killer Whales**

Killer whales are known to produce four call types: pulsed calls, low-frequency whistles, echolocation clicks, and high-frequency modulated (HFM) signals (Ford 1989, Samarra *et al.* 2010, Simonis *et al.* 2012). Killer whale pulsed calls are well documented and are the best described of all killer whale call types (Figure B-7). The primary energy of pulsed calls is between 1 and 6 kHz, with high-frequency components occasionally >30 kHz and duration primarily between 0.5 and 1.5 seconds (Ford & Fisher 1983; Ford 1989). HFM signals have only recently been attributed to killer whales in both the Northeast Atlantic (Samarra *et al.* 2010) and the North Pacific (Filatova *et al.* 2012, Simonis *et al.* 2012), and have fundamental frequencies between 17 and 75 kHz, the highest of any known delphinid tonal calls. Killer whale whistles and pulsed calls were detected via manual scanning of the recordings.

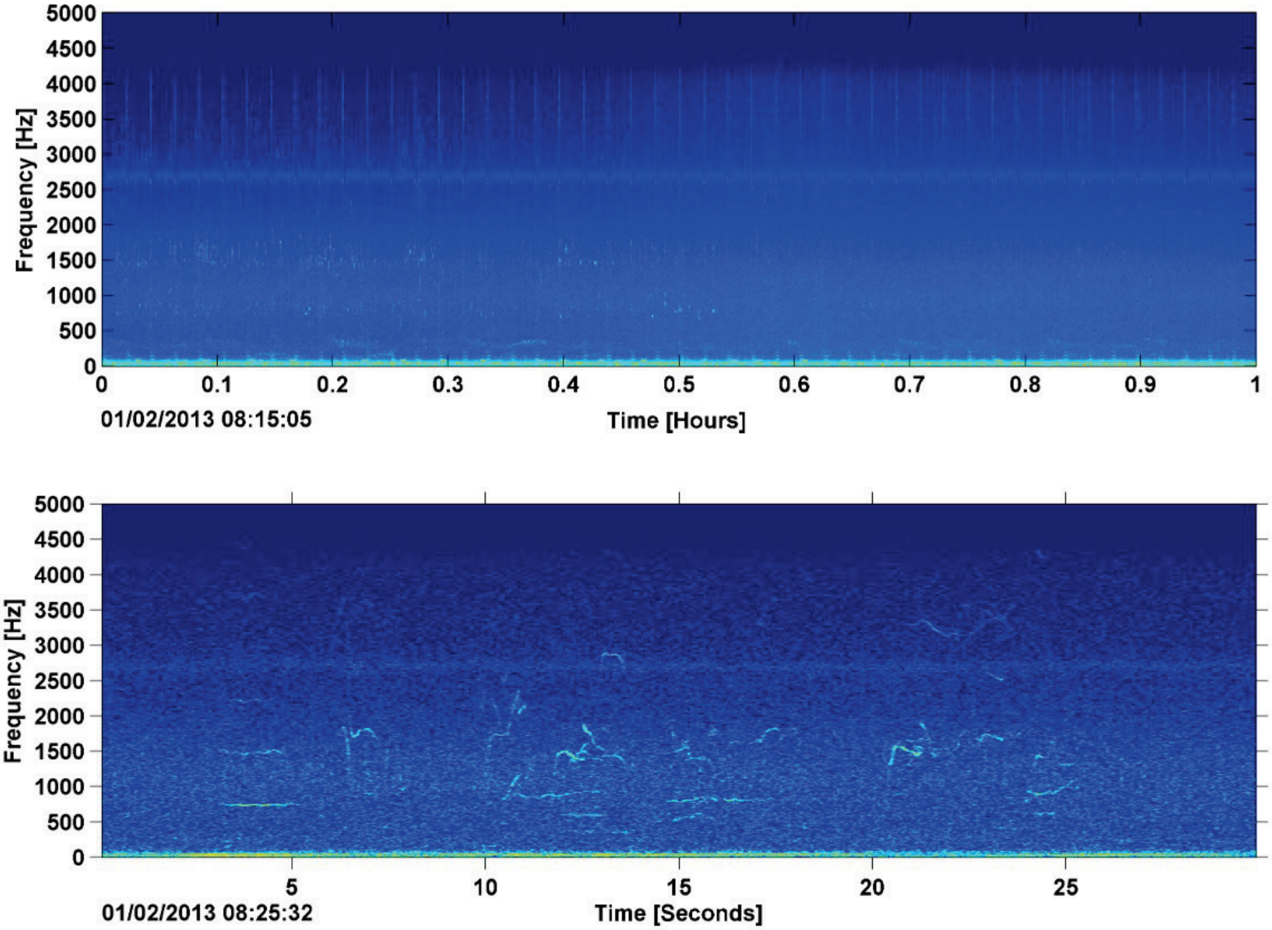

Figure B-7. Killer whale calls in the LTSA (top) and spectrogram (bottom) recorded at DCPP Site C, January 2013.

High-Frequency Marine Mammals

Marine mammal species with sounds in the high-frequency range (5–100 kHz) and possibly found in the Diablo Canyon include harbor porpoises (*Phocoena phocoena*), Dall's porpoise (*Phocoenoides dalli*), Pacific white-sided dolphins (*Lagenorhynchus obliquidens*), Risso's dolphins (*Grampus griseus*), Northern right whale dolphin (*Lissodelphis borealis*), bottlenose dolphins (*Tursiops truncatus*), short-beaked common dolphins (*Delphinus delphis*), long-beaked common dolphin (*Delphinus capensis*), Striped dolphin (*Stenella coeruleoalba*), short-finned pilot whale (*Globicephala macrorhynchu*), pygmy killer whales (*Feresa attenuata*), melon-headed whales (*Peponocephala electra*), sperm whales (*Physeter macrocephalus*), dwarf sperm whales (*Kogia sima*), Cuvier's beaked whales (*Ziphius cavirostris*), Baird's beaked whales (*Berardius bairdii),* Blainville's (*Mesoplodon densirostris*), Stejneger's (*Mesoplodon stejnegeri*), Hubbs' (*Mesoplodon carlhubbsi*), Perrin's (*Mesoplodon perrini*), and pygmy (*Mesoplodon peruvianus*) beaked whales

● Beaked Whales

Beaked whale species potentially found in the California Current include Baird's, Cuvier's, Blainville's, Stejneger's, Hubbs', Perrin's, and pygmy beaked whales (Baumann-Pickering *et al.*, 2014; Jefferson *et al.*, 2015). Beaked whales can be identified acoustically by their echolocation signals (Baumann-Pickering *et al.*, 2013a). These signals are frequency-modulated (FM) upsweep pulses, which appear to be species specific and distinguishable by their spectral and temporal features. Identifiable signals are known for Baird's, Blainville's, Cuvier's, and Stejneger's beaked whales (Baumann-Pickering *et al.*, 2013b).

Other beaked whale signals detected in the California Current include FM pulses known as BW35, BW43, and BW70, which may belong to Hubbs', Perrin's, and pygmy beaked whales, respectively (Baumann-Pickering *et al.*, 2013a; Baumann-Pickering *et al.*, 2014; Griffiths *et al.*, 2018). Only Cuvier's and Baird's beaked whales were detected during this recording period, and their signals are described below in more detail.

Beaked whale FM pulses were detected with an automated method. This automated effort was applied for all identifiable beaked whale signals found in the California Current except for those produced by Baird's beaked whales because they produce a signal with a lower frequency content than that typical of other beaked whales, and therefore are not reliably identified by the detector. After all echolocation signals were identified with a Teager Kaiser energy detector (Soldevilla *et al.*, 2008; Roch *et al.*, 2011), an expert system discriminated between delphinid clicks and beaked whale FM pulses.

A decision about presence or absence of beaked whale signals was based on detections within a 75 second segment. Only segments with more than seven detections were used in further analysis. All echolocation signals with a peak and center frequency below 32 and 25 kHz, respectively, a duration less than 355 μs, and a sweep rate of less than 23 kHz/ms were deleted. If more than 13% of all initially detected echolocation signals remained after applying these criteria, the segment was classified to have beaked whale FM pulses. This threshold was chosen to obtain the best balance between missed and false detections. A third classification step, based on computer-assisted manual decisions by a trained analyst, was used to label the automatically detected segments to pulse type level and reject false detections (Baumann-Pickering *et al.*, 2013). The rate of missed segments is generally approximately 5%, varying slightly across deployments. The start and end of each segment containing beaked whale signals was logged and their durations were added to estimate cumulative weekly presence.

- ***Cuvier's Beaked Whales***

Cuvier's echolocation signals are polycyclic, with a characteristic FM pulse upsweep, peak frequency around 40 kHz (Figure B-8) and uniform inter-pulse interval of about 0.4–0.5 s (Johnson *et al.*, 2004; Zimmer *et al.*, 2005). An additional feature that helps with the identification of Cuvier's FM pulses is that they have two characteristic spectral peaks around 17 and 23 kHz.

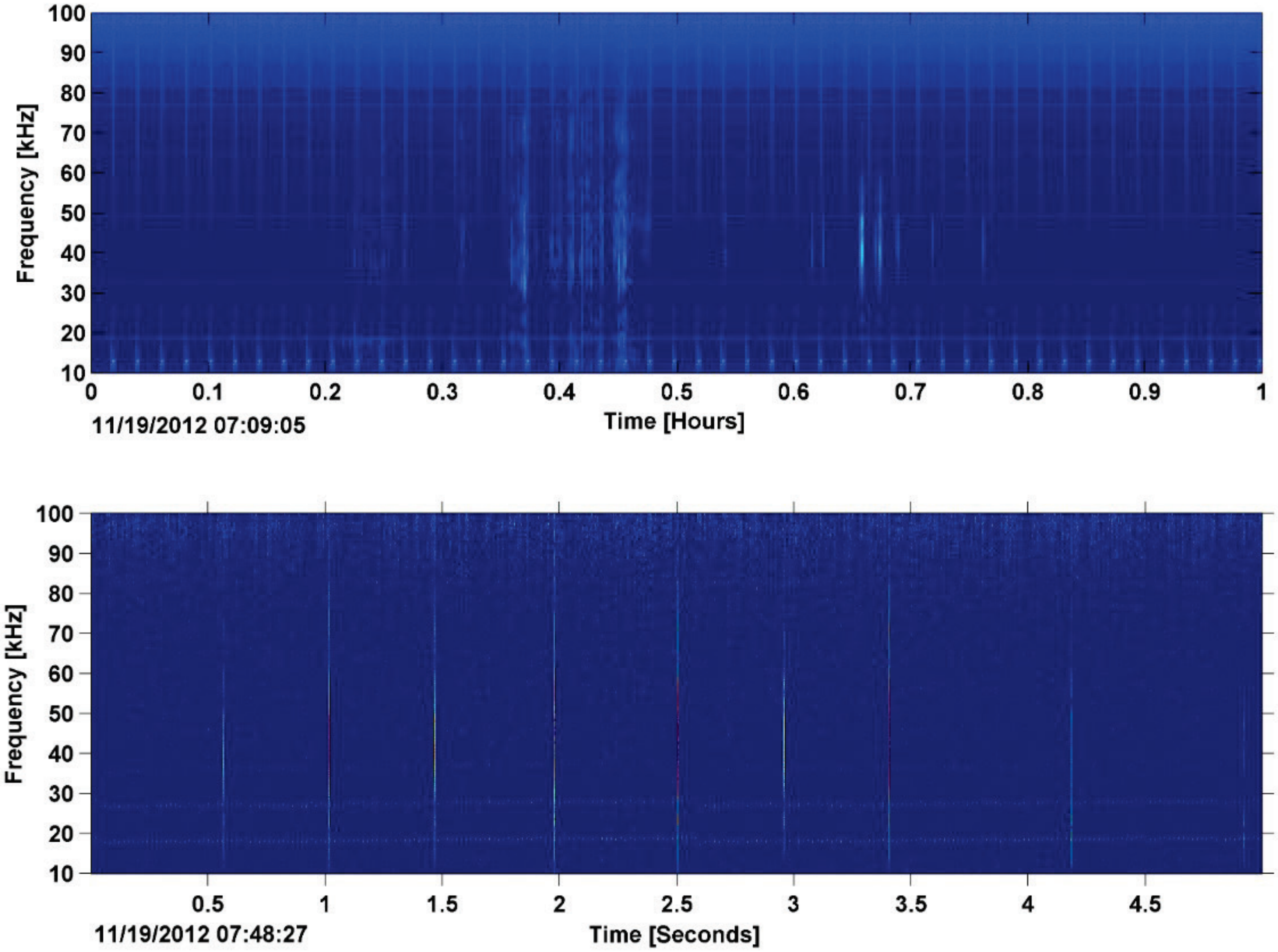

Figure B-8. Cuvier's beaked whale signals in LTSA (top) and spectrogram (bottom) from HARP recording at DCPP Site C, November 2012.

- ***Baird's Beaked Whales***

Baird's beaked whale echolocation signals have a multiple peak structure with the largest spectral peak between 22 and 25 kHz, and the second largest spectral peak between 35 and 45 kHz (Figure B-9). Baird's beaked whales use two distinctly different echolocation signals: an FM pulse and a broadband click. Both signal types showed a consistent multi-peak structure in their spectra with peaks at approximately 9, 16, 25, and 40 kHz (Baumann-Pickering *et al.*, 2013).

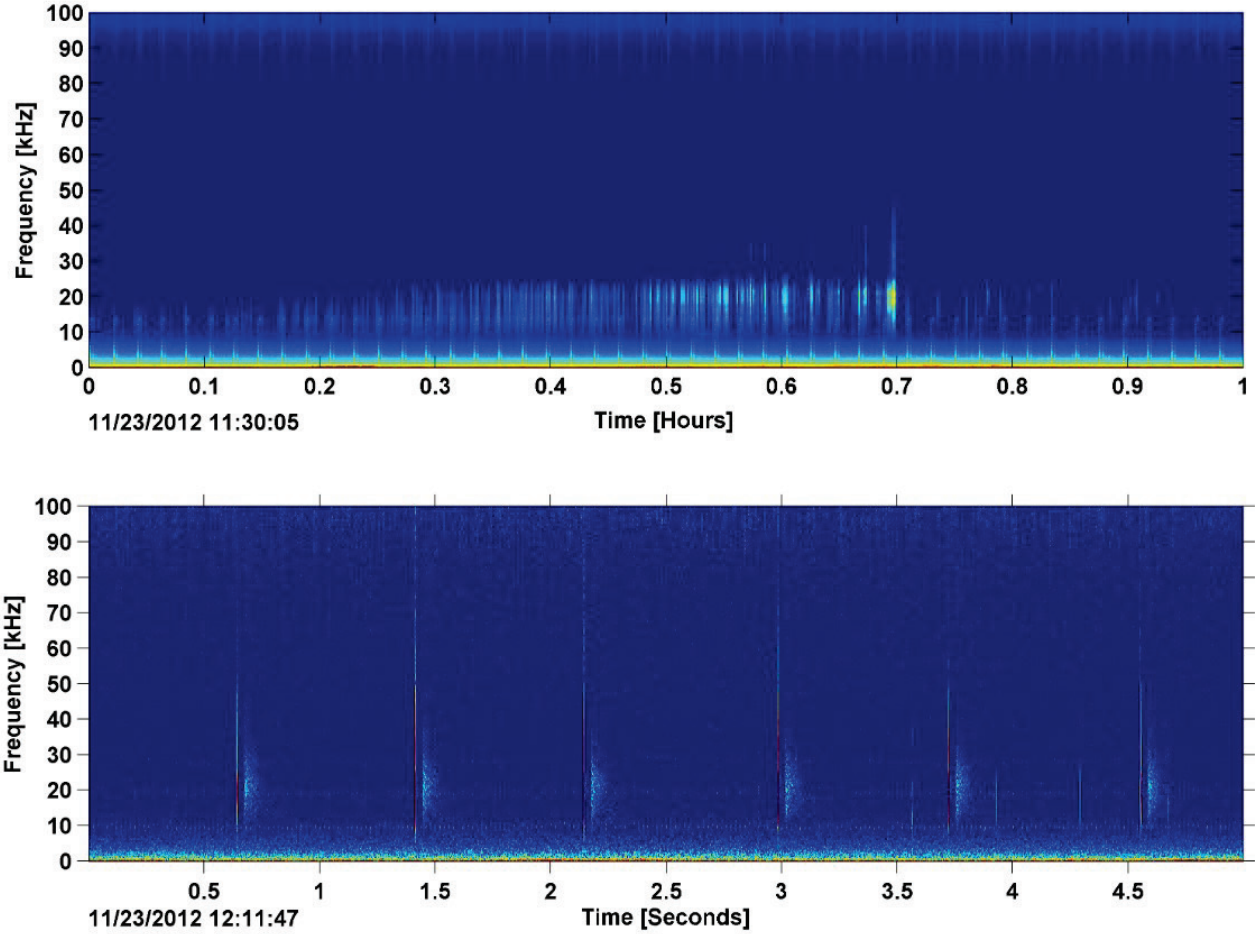

Figure B-9. Baird's beaked whale echolocation clicks in LTSA (top) and spectrogram (bottom) from HARP recording at DCPP Site C, November 2012.

● Dolphins

• *Echolocation Clicks*

Delphinid echolocation click analysis effort consisted of manual scans and review of the LTSA with a 1-hour display duration and a frequency range of 10–10,000 Hz.

• *Risso's Dolphins*

Risso's dolphin echolocation clicks can be identified to species by their distinctive banding patterns observable in the LTSA (Figure B-10). Studies show that spectral properties of Risso's dolphin echolocation clicks vary based on geographic region (Soldevilla *et al.,* 2017). Risso's dolphin clicks detected in this recording period had peaks at 23, 26, and 33 kHz. Modal inter-click interval (ICI) was 165 ms.

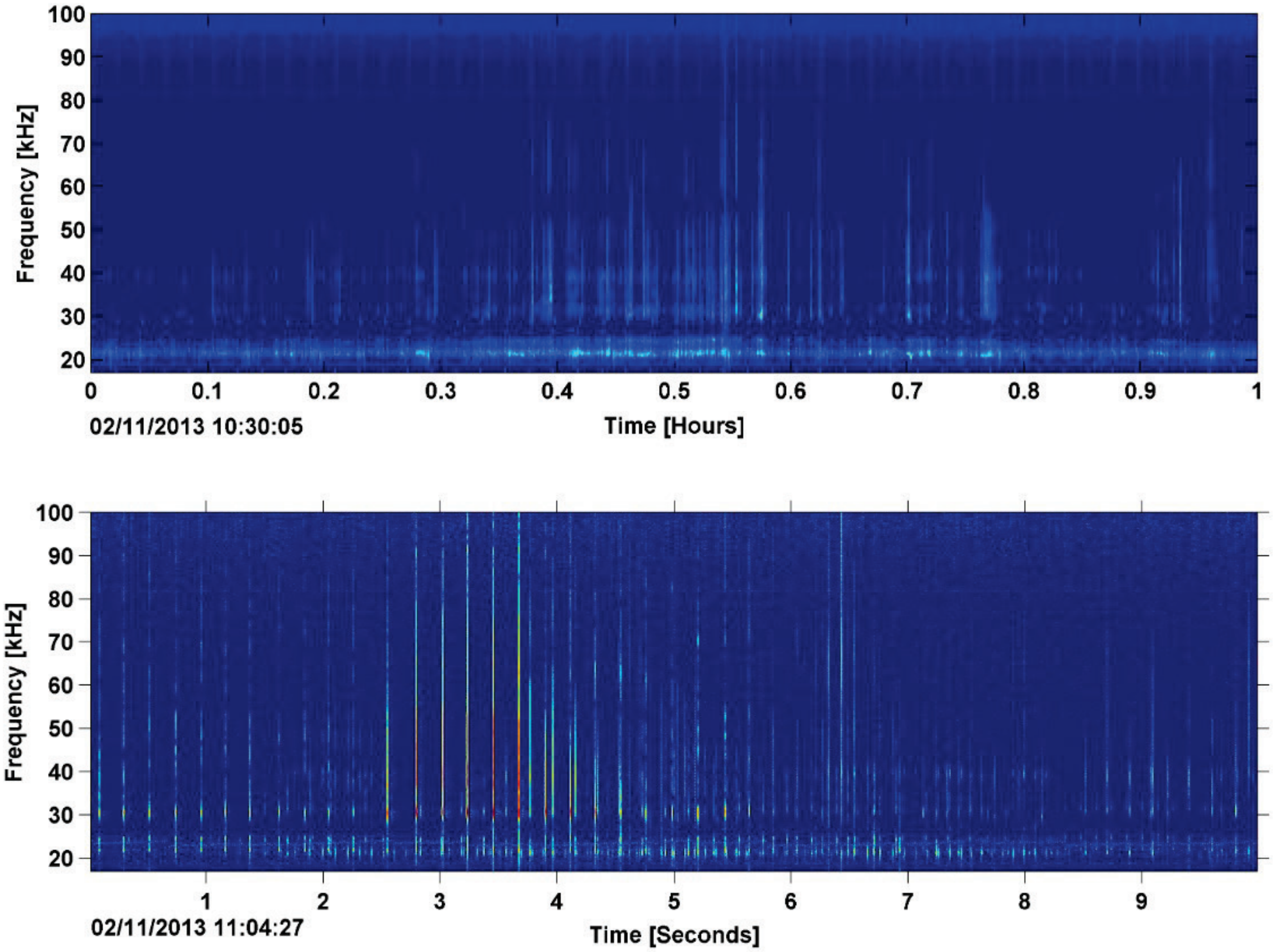

Figure B-10. Risso's dolphin acoustic encounter in LTSA (top) and spectrogram (bottom) from HARP recording at DCPP Site C, February 2013.

- ***Pacific White-Sided Dolphins***

Pacific white-sided dolphin clicks can be separated into two click types, designated type A and B. The significance of these two click types remains unknown and for this recording period both click types were logged. In combination with the other peaks and notches, a frequency peak near either 27.4 or 26.1 kHz can distinguish these clicks as type A or B, respectively (Soldevilla *et al.*, 2008).

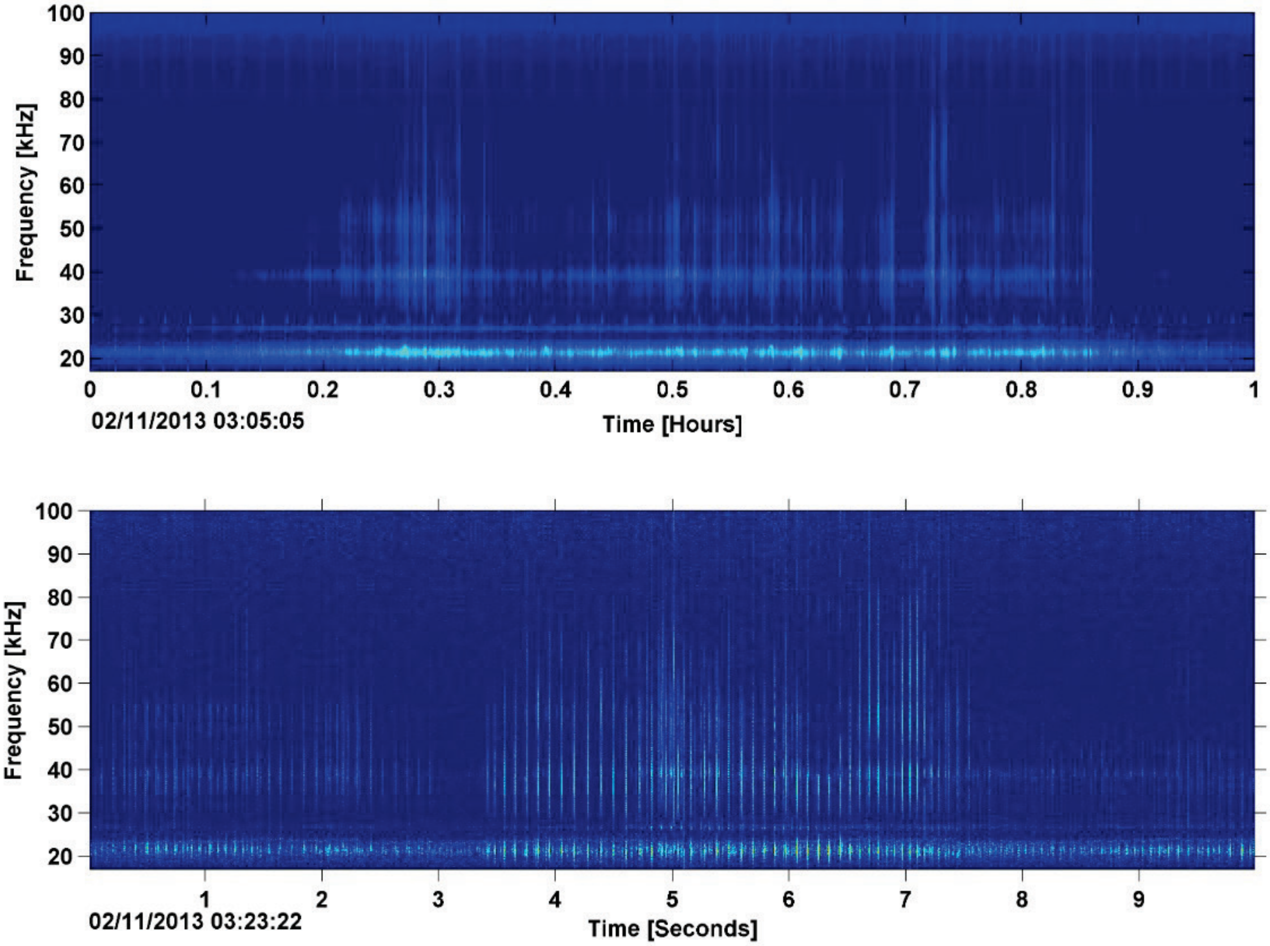

Figure B-11. Pacific white-sided dolphin type B echolocation clicks in LTSA (top) and spectrogram (bottom) recorded at DCPP Site C, February 2013.

- ***Sperm Whales***

Sperm whale clicks contain energy from 2 to 20 kHz, with most energy between 10 and 15 kHz (Møhl *et al.*, 2003) (Figure B-12). Regular clicks, observed during foraging dives, demonstrate an ICI from 0.25 to 1 s (Goold and Jones, 1995; Madsen *et al.*, 2002a). Short bursts of closely spaced clicks called creaks are observed during foraging dives and are believed to indicate a predation attempt (Wysocki *et al.*, 2006). Slow clicks (> 1 s ICI) are used only by males and are more intense than regular clicks (Madsen *et al.*, 2002b). Codas are stereotyped sequences of clicks which are less intense and contain lower peak frequencies than regular clicks (Watkins and Schevill, 1977). Effort was not expended to denote whether sperm whale detections were codas, regular or slow clicks.

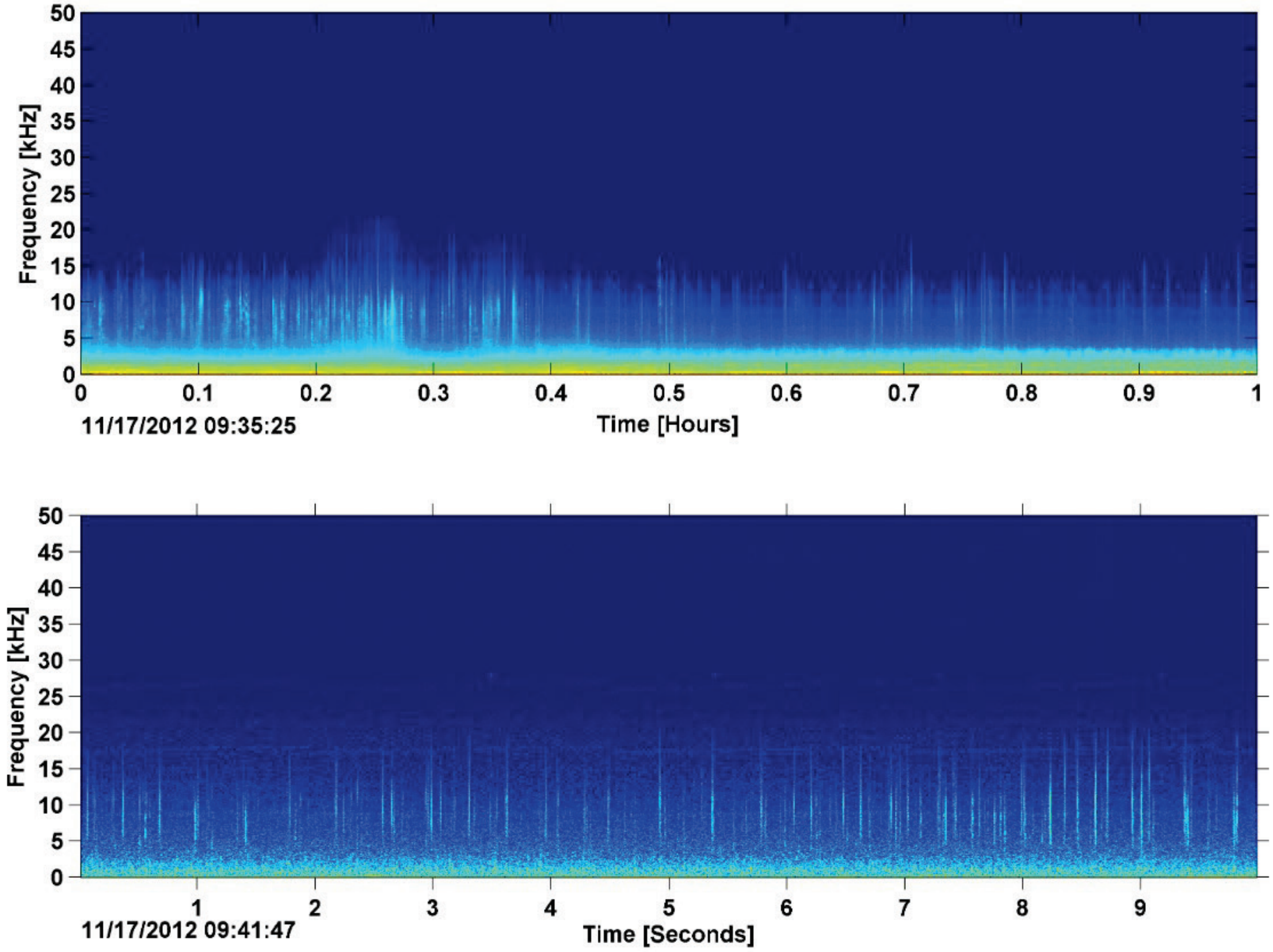

Figure B-12. Sperm whale echolocation clicks in LTSA (top) and spectrogram (bottom) recorded at DCPP Site C, November 2012.

- ***Unidentified Odontocete Clicks Greater Than 20 kHz***

Unidentified odontocete clicks greater than 20 kHz are not currently identified to species but were used as a general category for manual analysis. An example encounter is shown in Figure B-13.

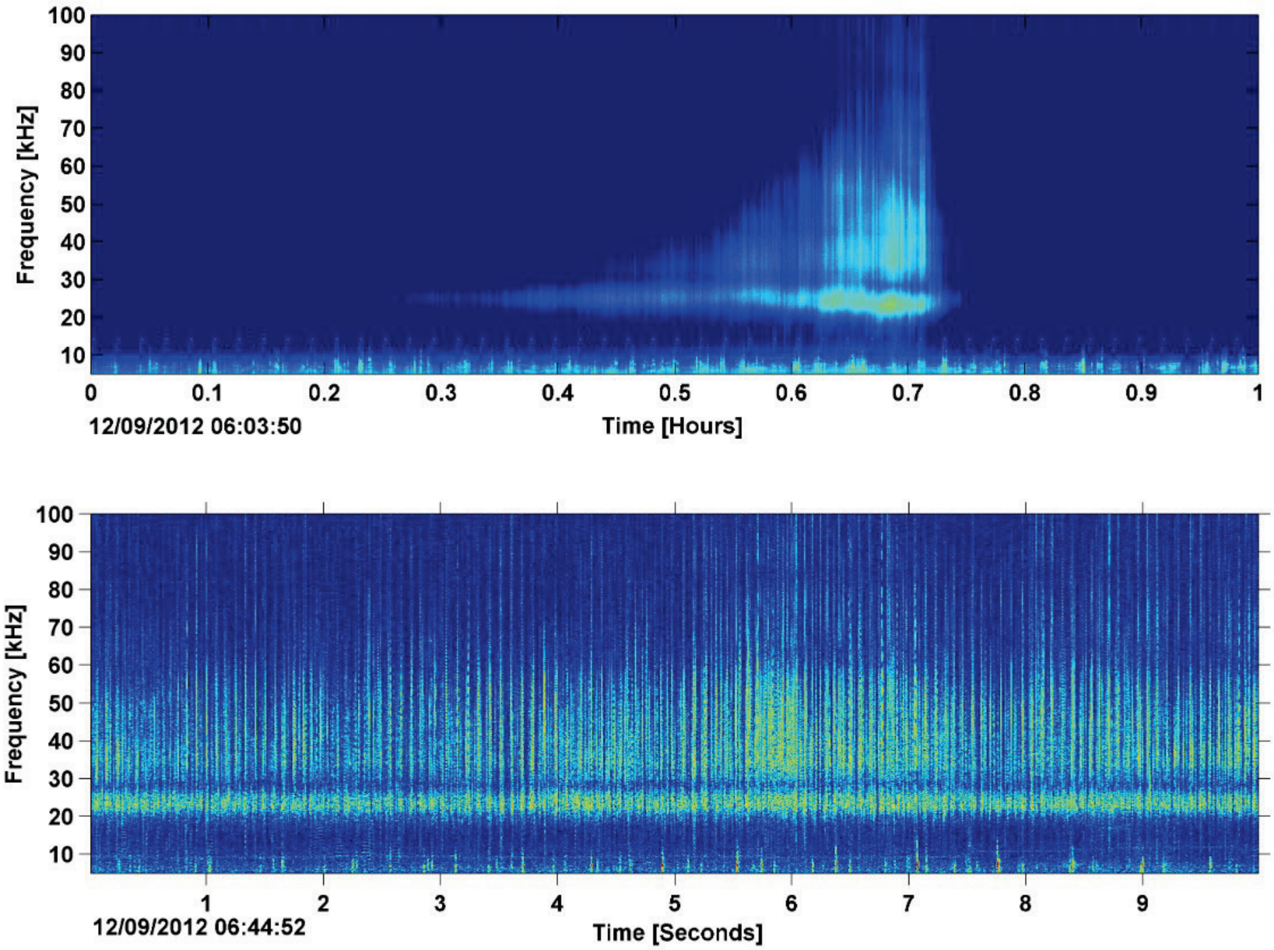

Figure B-13. UO CT > 20 kHz acoustic encounter in LTSA (top) and spectrogram (bottom) recorded at DCPP Site C, December 2012.

Anthropogenic Sounds

One anthropogenic sound was monitored for this report: Mid-Frequency Active (MFA) sonar. These sounds were detected by a computer algorithm. The start and end of each sonar session was logged and their durations were added to estimate cumulative weekly presence.

● Mid-Frequency Active Sonar

Sounds from MFA sonar vary in frequency (1–10 kHz) and are composed of pulses of both FM sweeps and continuous wave (CW) tones grouped in packets with durations ranging from less than 1 s to greater than 5 s. Packets can be composed of single or multiple pulses and are transmitted repetitively as wave trains with inter-packet-intervals typically greater than 20 s (Figure B-14). In the SOCAL Range Complex, off San Clemente Island, the most common MFA sonar signals are between 2 and 5 kHz and are more generically known as '3.5 kHz' sonar.

MFA sonar was detected using a modified version of the *Silbido* detection system (Roch *et al.*, 2011a) originally designed for characterizing toothed whale whistles. The algorithm identifies peaks in time-frequency distributions (e.g., spectrogram) and determines which peaks should be linked into a graph structure based on heuristic rules that include examining the trajectory of existing peaks, tracking intersections between time-frequency trajectories, and allowing for brief signal dropouts or interfering signals. Detection graphs are then examined to identify individual tonal contours looking at trajectories from both sides of time-frequency intersection points. For MFA detection, parameters were adjusted to detect tonal contours at or above 2 kHz in data decimated to a 10 kHz sample rate with signal to noise ratios of 5 dB or above, and contour durations of at least 200 ms with a frequency resolution of 100 Hz. The detector frequently triggered on noise produced by instrumental disk writes that occurred at 75 s intervals, but these times were identified and removed from the analysis.

Over periods of several months, disk write detections dominated the number of detections and could be eliminated using an outlier detection test. Histograms of the detection start times modulo the disk write period were constructed and outliers were discarded. This removed some valid detections that occurred during disk writes, but as the disk writes and sonar signals are uncorrelated this is expected to only have a minor impact on analysis. As the detector did not distinguish between sonar and nonanthropogenic tonal signals within the operating band (e.g., humpback whales), human analysts examined detection output and accepted or rejected contiguous sets of detections. No MFA detections were found during the recording period.

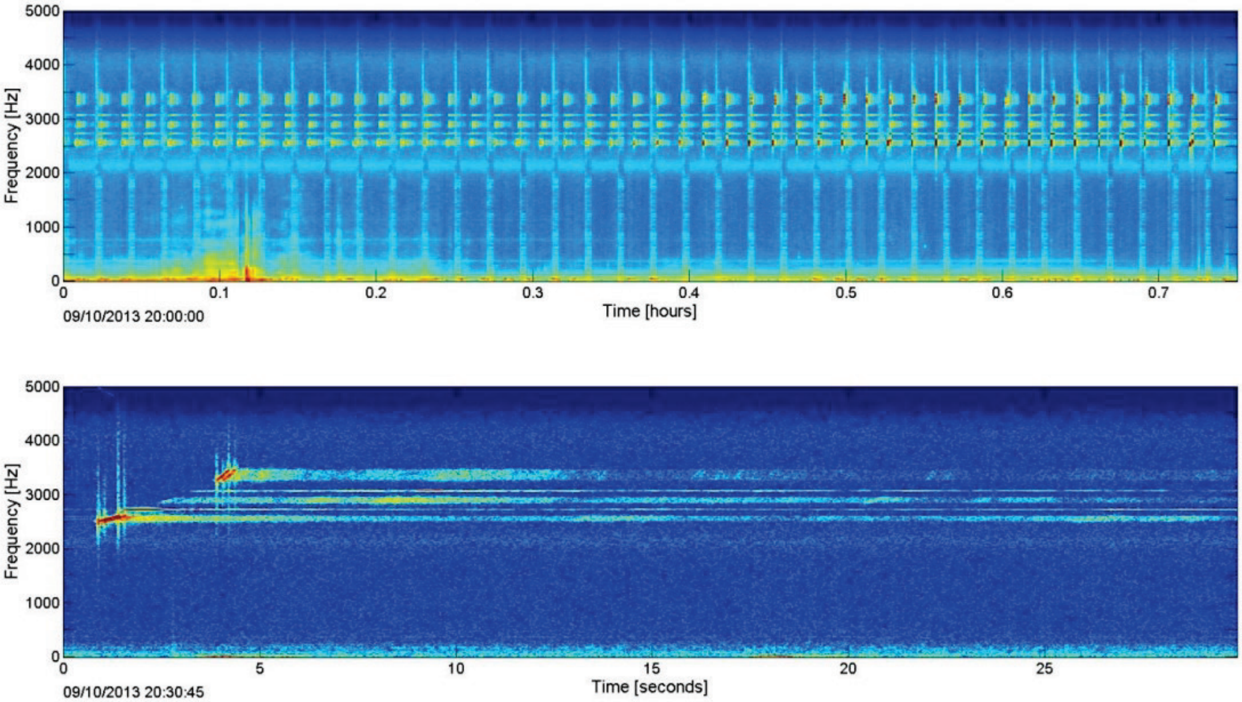

Figure B-14. MFA sonar recorded in southern California at Site H and shown as a wave train event in a 45 minute LTSA (top) and as a single packet with multiple pulses in a 30 second spectrogram (bottom).

Results

The results of acoustic data analysis at DCPP Site C from November 2012 to March 2013 are summarized, and the seasonal occurrence and relative abundance of marine mammal acoustic signals and anthropogenic sounds are documented.

Low-Frequency Ambient Soundscape

The monthly average low-frequency sound spectrum levels at DCPP Site C are shown in Figure B-15. These spectra are generally more energetic at low frequencies (< 100 Hz). Various peaks appear in the spectra that correspond to the presence of blue and fin whales as follows.

- November–December spectral peaks at 45–47 Hz, along with lower frequency harmonics are related to blue whale B calls (Figure B-15).
- Prominent peaks in sound spectrum levels observed in the frequency band 15–30 Hz during winter are related to seasonally increased presence of fin whale calls (Figure B-15).

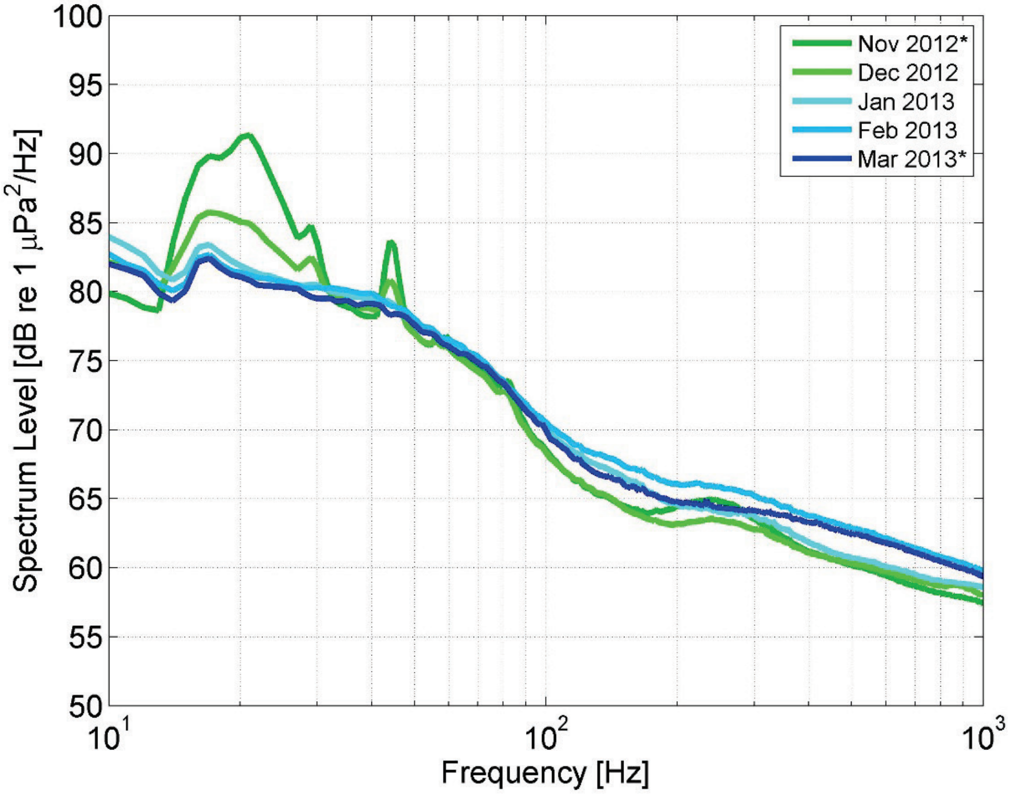

Figure B-15. Monthly averages of sound spectrum levels at DCPP Site C from November 2012 to March 2013. Legend gives color-coding by month. * Denotes months with partial effort.

Mysticetes

Three known baleen whale species were recorded between November 2012 and March 2013: blue whales, fin whales, and humpback whales. More details of each species' presence are given below.

● Blue Whales

Blue whale B and D calls were detected from the beginning of the recording in November, but greatly diminished in January.

- ***Northern Pacific Blue Whale B Calls***
  - Northern Pacific blue whale B calls were detected in very high numbers from November 2012 to January 2013, drastically dropping off after January 2013 (Figure B-16).
  - There was no discernible diel pattern for Northern Pacific blue whale B calls (Figure B-17).

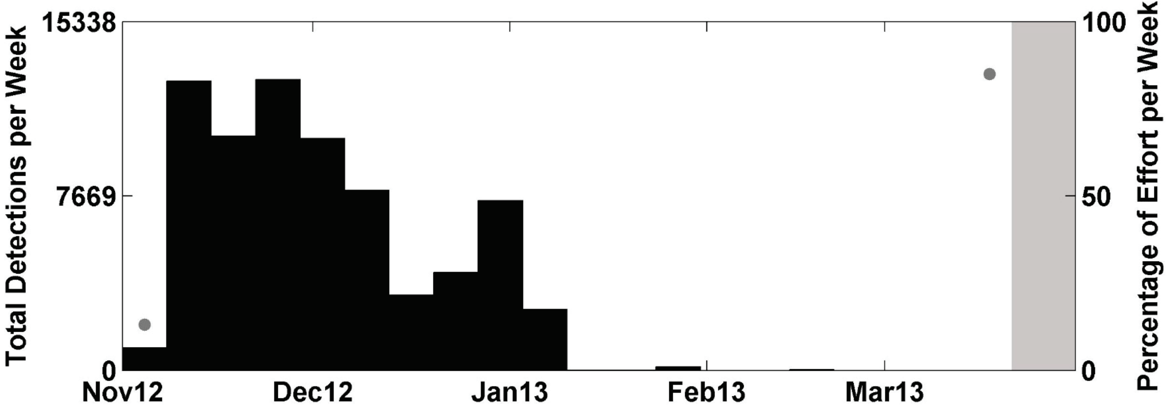

Figure B-16. Weekly presence of Northern Pacific blue whale tonal calls detected from November 2012 to March 2013 at DCPP Site C. Gray dots represent percent of effort per week in weeks with less than 100% recording effort. Where gray dots are absent, full recording effort occurred for the entire week. X-axis labels refer to month and year of recording.

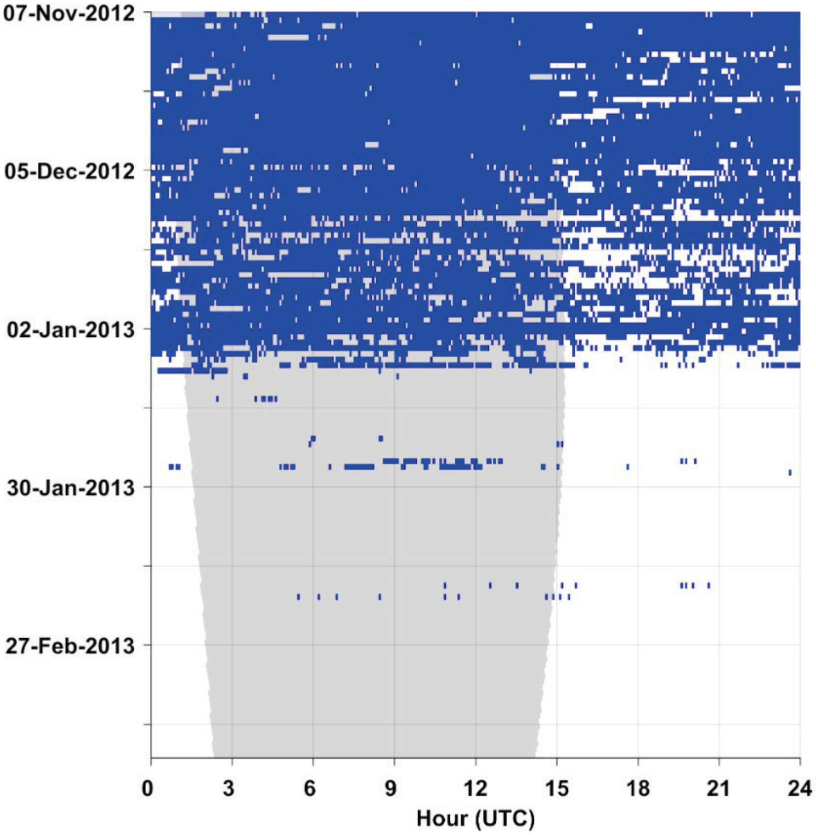

Figure B-17. Northern Pacific blue whale tonal calls in hourly bins at DCPP Site C from November 2012 to March 2013. Gray vertical shading denotes nighttime.

- ***Northern Pacific Blue Whale D Calls***
  - Northern Pacific blue whale D calls were detected in high numbers from November 2012 to January 2013, drastically dropping off after January 2013 (Figure B-18).
  - There was no discernible diel pattern for Northern Pacific blue whale D calls (Figure B-19).

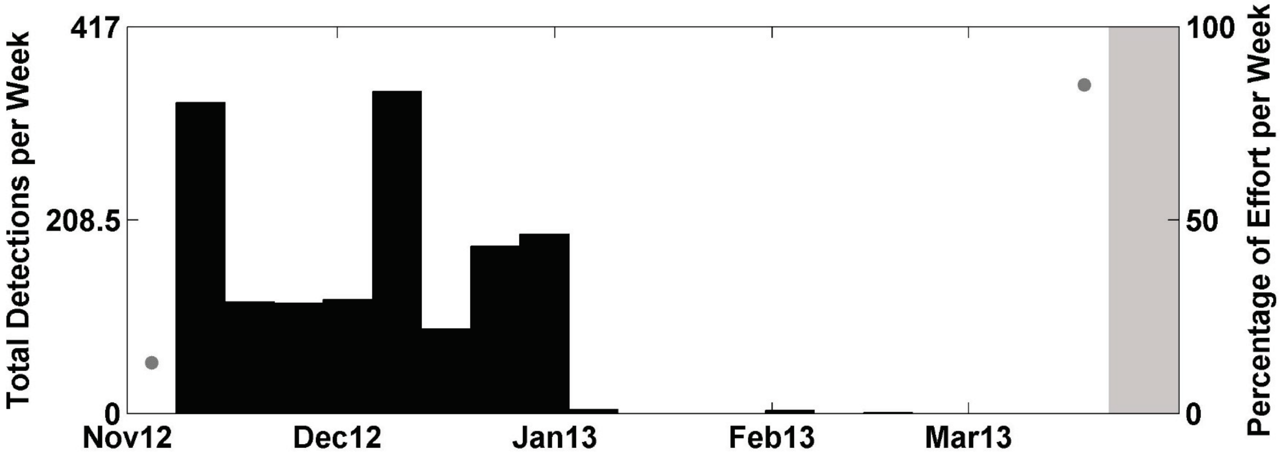

Figure B-18. Weekly presence of Northern Pacific blue whale D calls detected from November 2012 to March 2013 at DCPP Site C. Effort markings are described in Figure B-6.

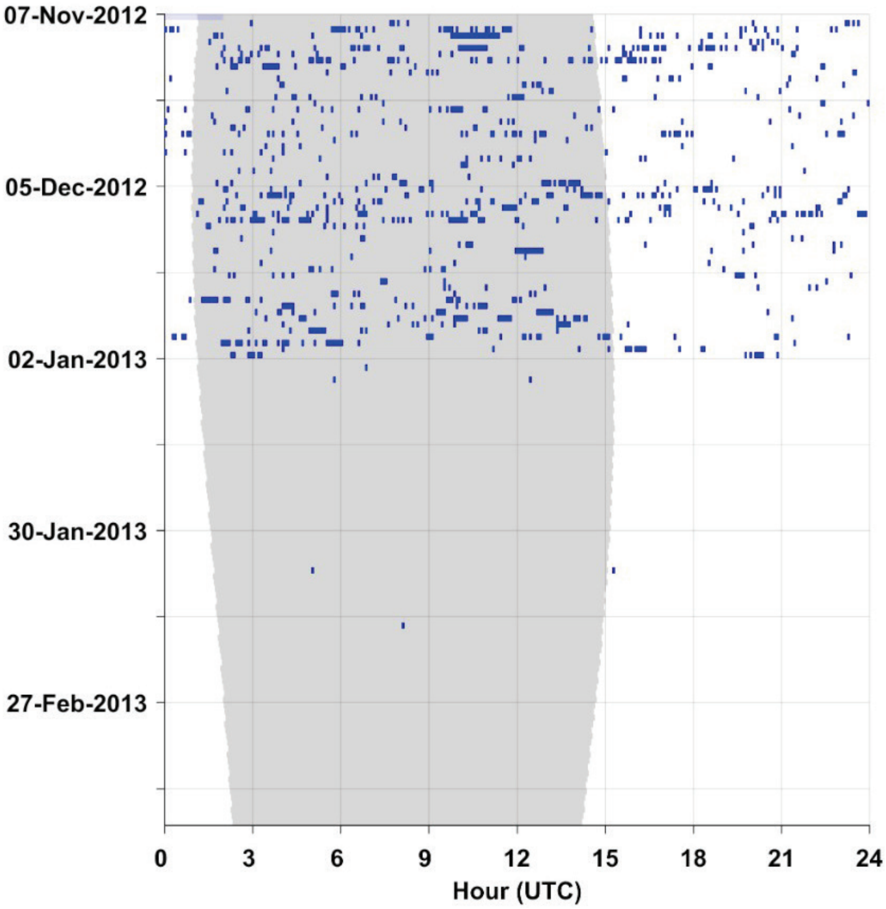

Figure B-19. Northern Pacific blue whale D calls in hourly bins at DCPP Site C from November 2012 to March 2013. Gray vertical shading denotes nighttime.

● Fin Whales

The fin whale acoustic index, a proxy for 20 Hz calls, was high from November 2012 and the beginning of December 2012 but decreased from mid-December 2012 to January 2013 (Figure B-20).

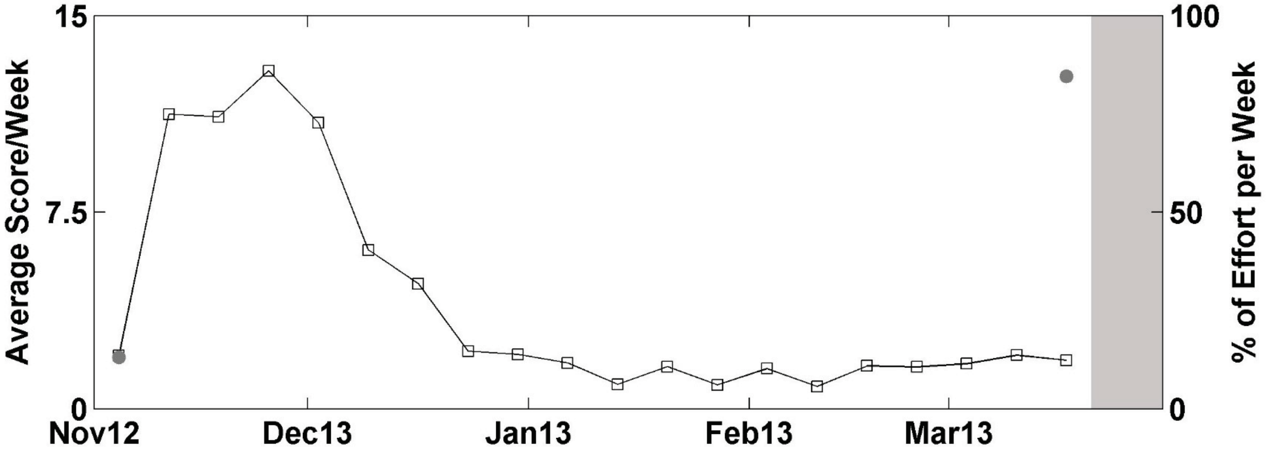

Figure B-20. Weekly value of fin whale acoustic index (proxy for 20 Hz calls) detected from May 2017 to 2018 at HAT Site B. Effort markings are described in Figure B-16.

● Humpback Whales

Humpback whale calls were detected at high numbers from the beginning of the recording in November and decreased throughout the recording period.

- Humpback whale call types were observed in high numbers in November and December 2012 then gradually decreased (Figure B-21).
- There was no discernible diel pattern for humpback whale call types (Figure B-22).

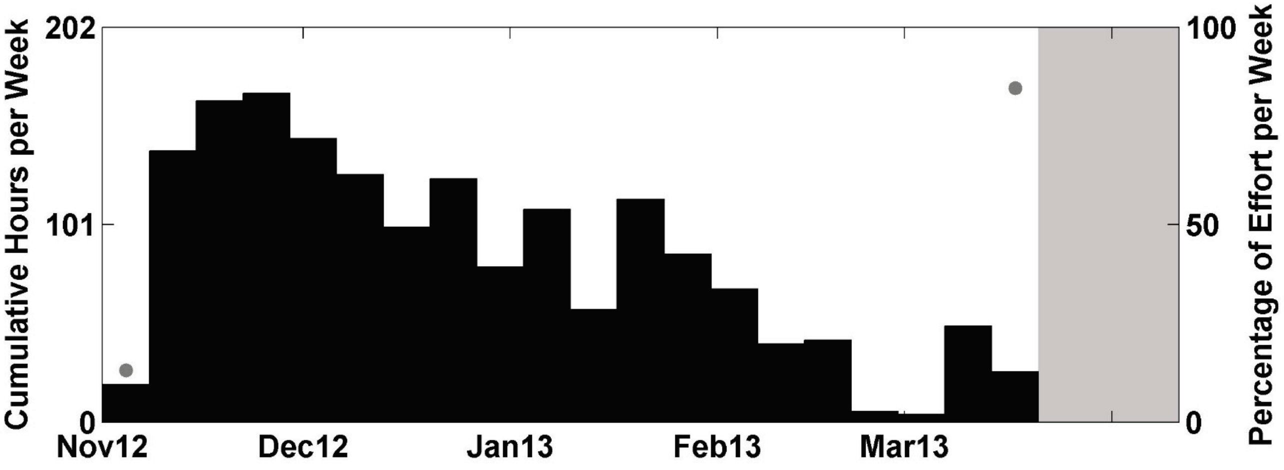

Figure B-21. Weekly presence of humpback whale calls from November 2012 to March 2013 at DCPP Site C. Effort markings are described in Figure B-16.

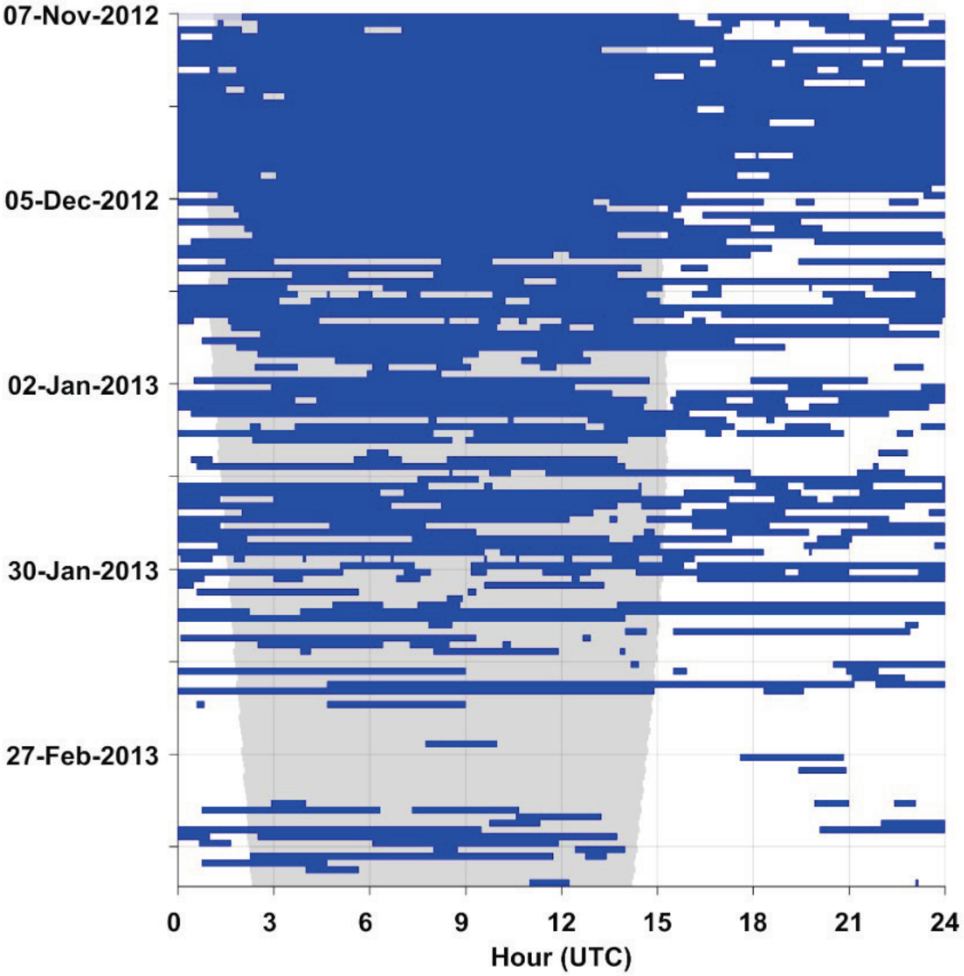

Figure B-22. Humpback whale calls in hourly bins at DCPP Site C from November 2012 to March 2013. Gray vertical shading denotes nighttime.

Odontocetes

Killer whale call types, Cuvier's beaked whale, Baird's beaked whale, Risso's dolphins, Pacific white-sided dolphins, sperm whales, and clicks of unidentified odontocetes were discriminated in the DCPP Site C data. Details of each species' presence are given below.

- **Killer Whales**
  - Killer whale call types were detected in low numbers throughout the recording period.
  - Detections occurred in November 2012 and from February to March 2012 (Figure B-23).
  - There was no discernible diel pattern for killer whale call types (Figure B-24).

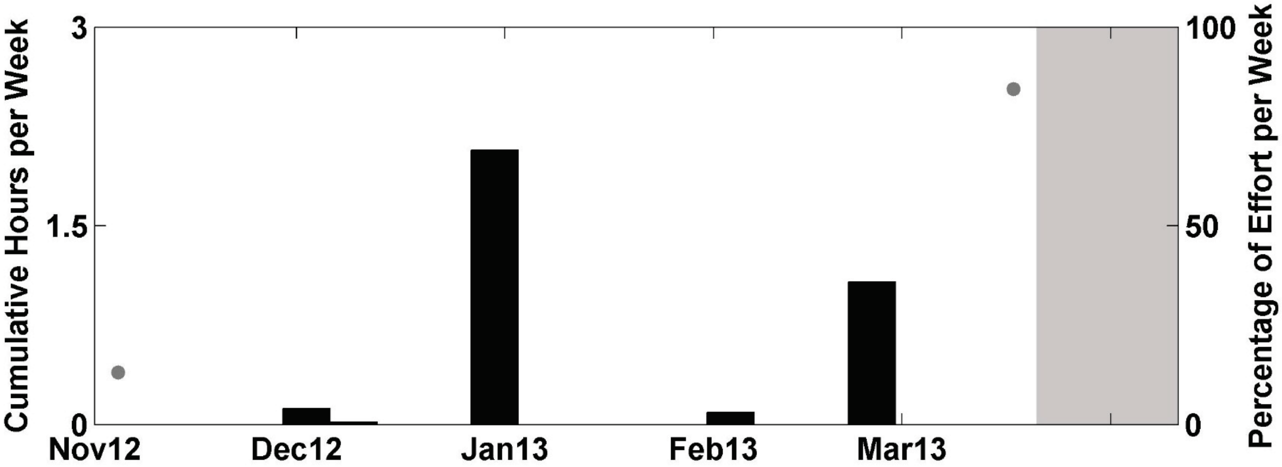

Figure B-23. Weekly presence of killer whale call types detected from November 2012 to March 2013 at DCPP Site C. Effort markings are described in Figure B-16.

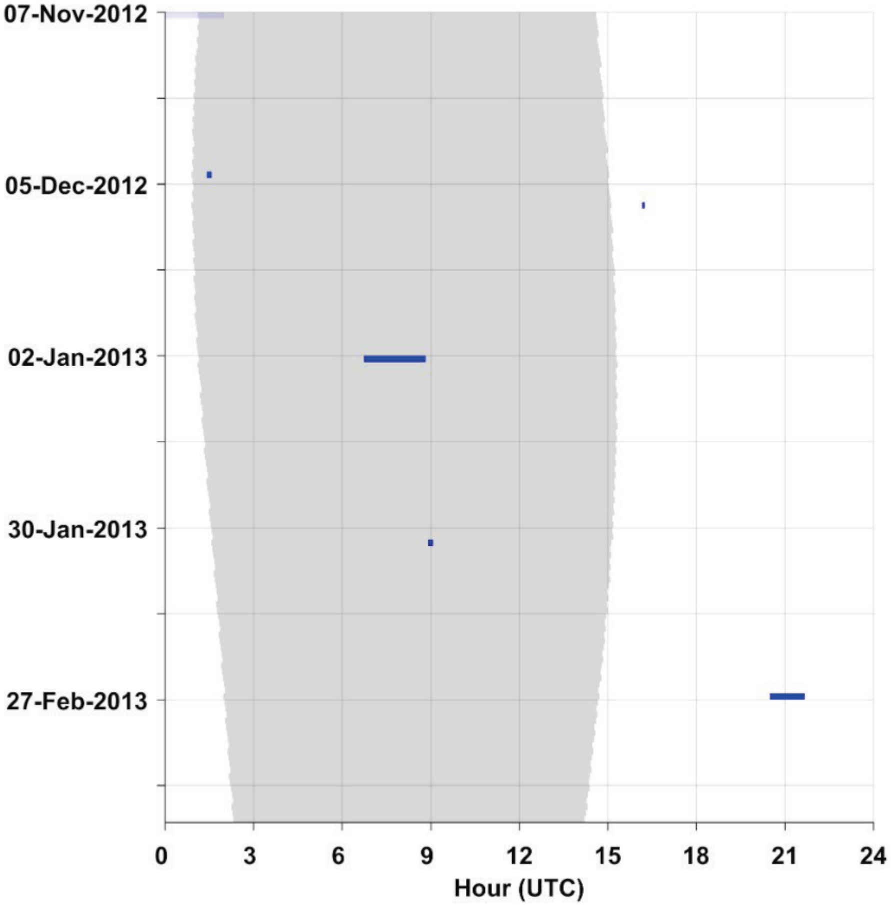

Figure B-24. Killer whale call types in one-minute bins at DCPP Site C from November 2012 to March 2013. Gray vertical shading denotes nighttime

- **Cuvier's Beaked Whale**
  - Cuvier's beaked whale echolocation clicks were detected in low numbers throughout the recording period. Detections occurred in November 2012 and February to March 2013 (Figure B-25).
  - There were not enough encounters to discern a diel pattern (Figure B-26).

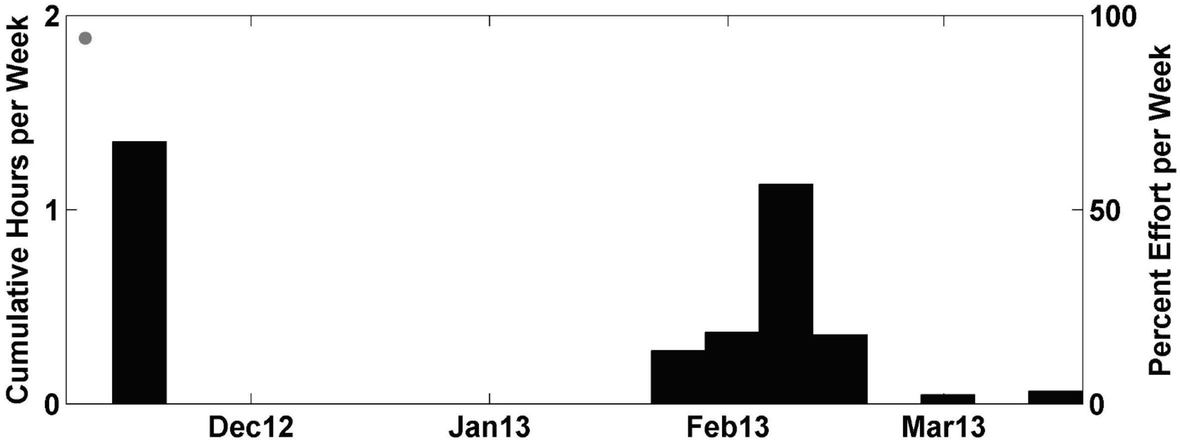

Figure B-25. Weekly presence of Cuvier's beaked whale echolocation clicks detected from November 2012 to March 2013 at DCPP Site C. Effort markings are described in Figure B-16.

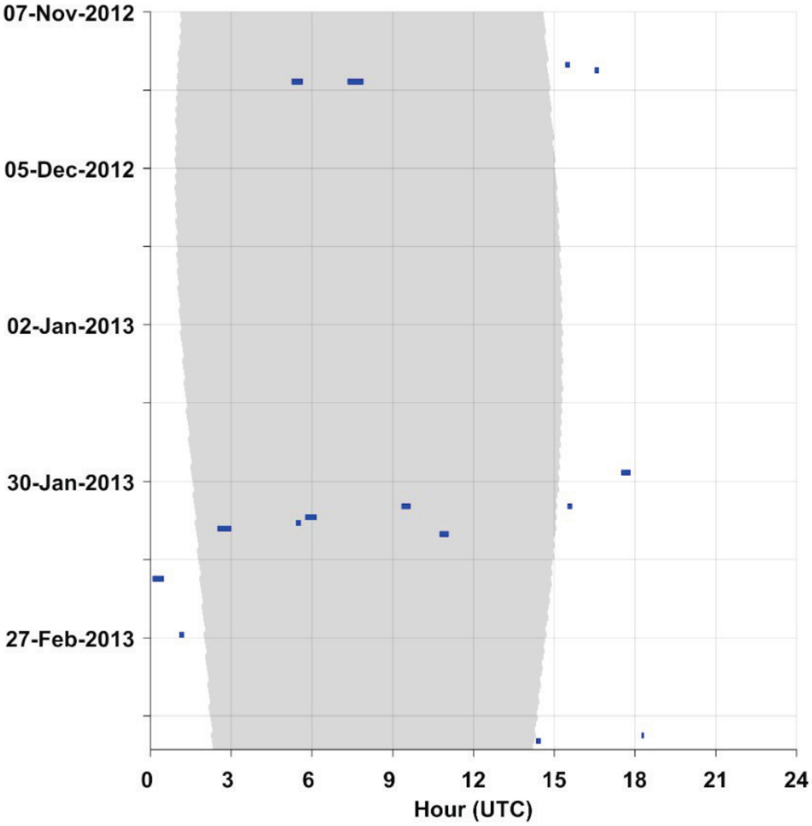

Figure B-26. Cuvier's beaked whale echolocation clicks in one-minute bins at DCPP Site C from November 2012 to March 2013. Gray vertical shading denotes nighttime.

- **Baird's Beaked Whale**
  - Baird's beaked whale echolocation clicks were detected in low numbers in November and December 2012 (Figure B-27).
  - There were not enough encounters to discern a diel pattern (Figure B-28).

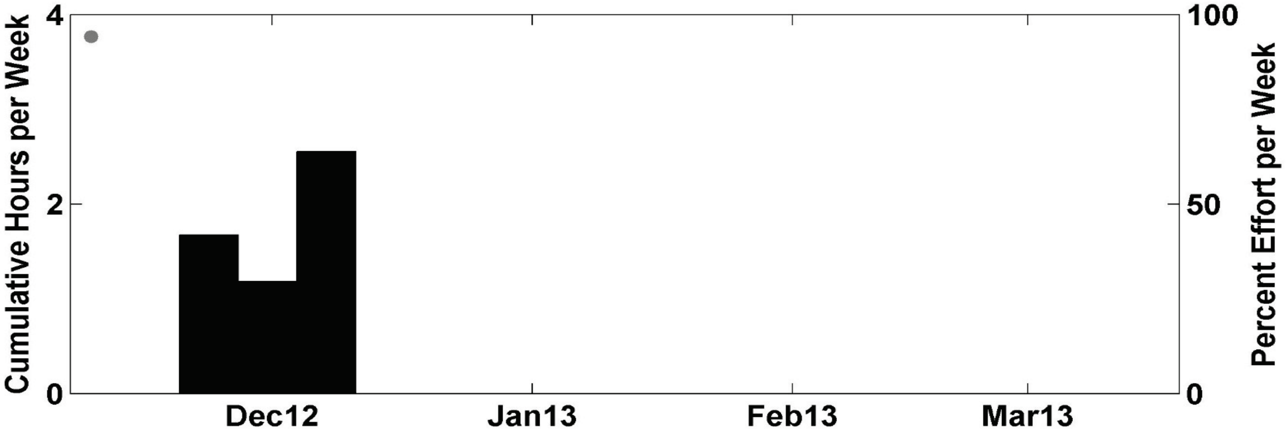

Figure B-27. Weekly presence of Baird's beaked whale echolocation clicks from November 2012 to March 2013 at DCPP Site C. Effort markings are described in Figure B-16.

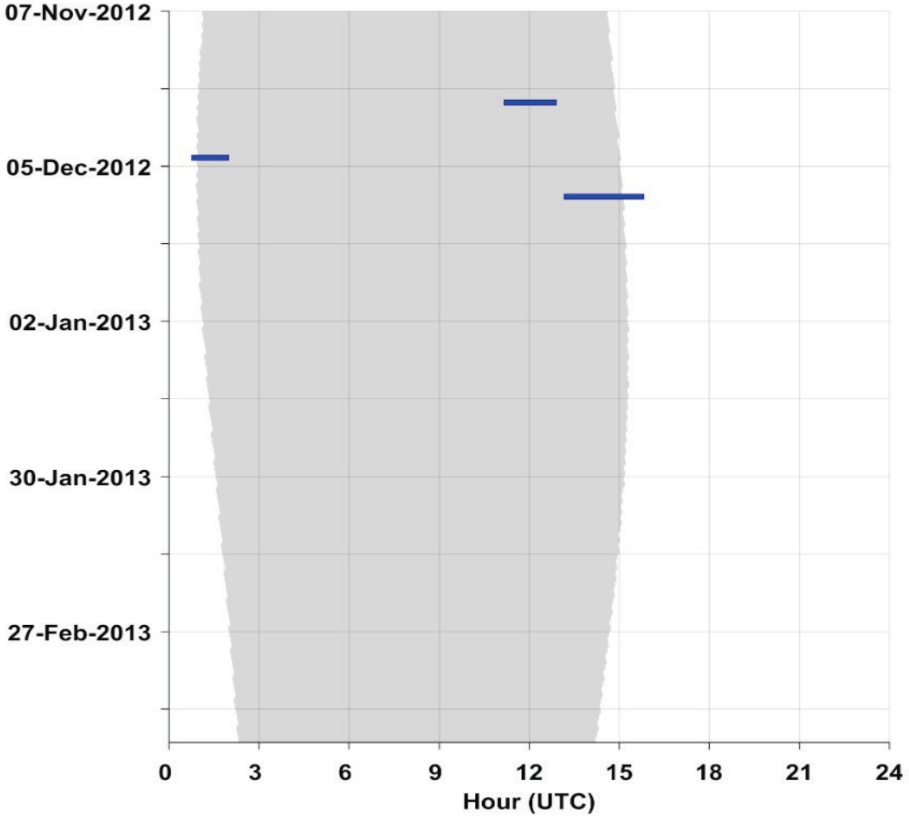

Figure B-28. Baird's beaked whale echolocation clicks in one-minute bins at DCPP Site C from November 2012 to March 2013. Gray vertical shading denotes nighttime.

- **Risso's Dolphins**
  - Risso's dolphin echolocation clicks began in January 2013 and increased from February to March 2013 (Figure B-29).
  - Risso's dolphin echolocation clicks were primarily detected during nighttime (Figure B-30).

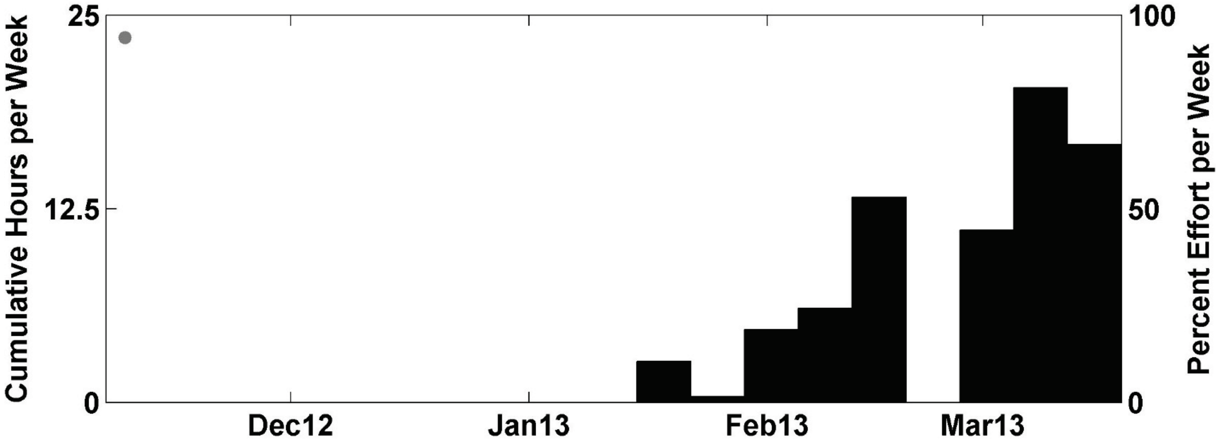

Figure B-29. Weekly presence of Risso's dolphin echolocation clicks detected from November 2012 to March 2013 at DCPP Site C. Effort markings are described in Figure B-16.

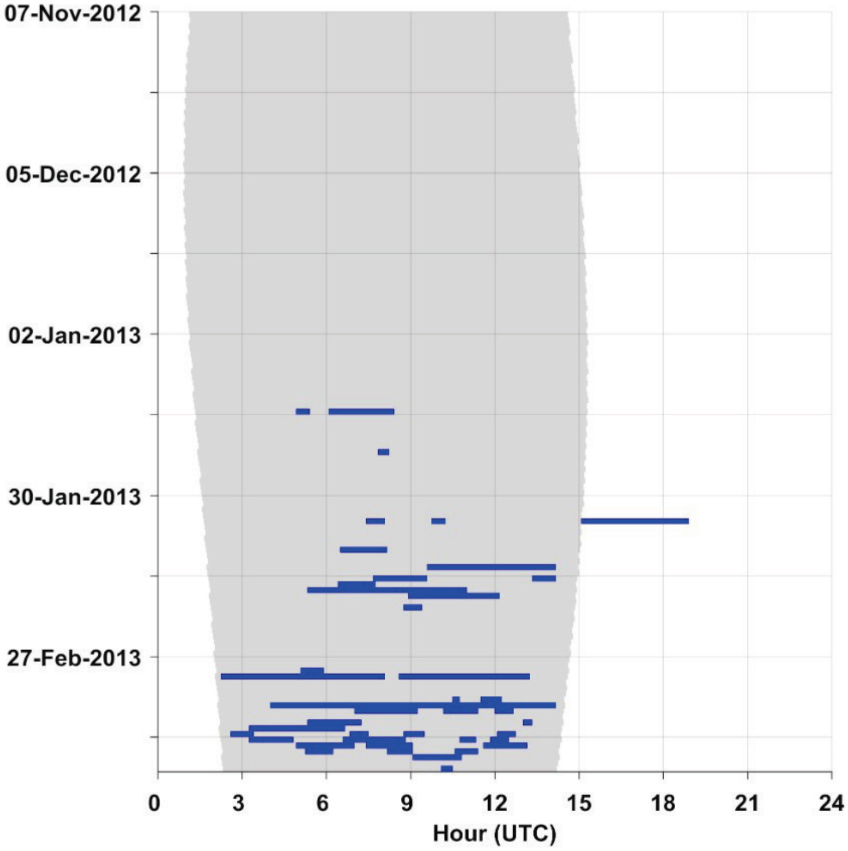

Figure B-30. Risso's dolphin echolocation clicks in five-minute bins at DCPP Site C from November 2012 to March 2013. Gray vertical shading denotes nighttime.

- **Pacific White-Sided Dolphins**
  - Pacific white-sided dolphin type B echolocation clicks were first detected in low numbers in November 2012 and increased during February and March 2013 (Figure B-31). No type A echolocation clicks were logged.
  - Type B echolocation clicks were primarily detected during nighttime (Figure B-32).

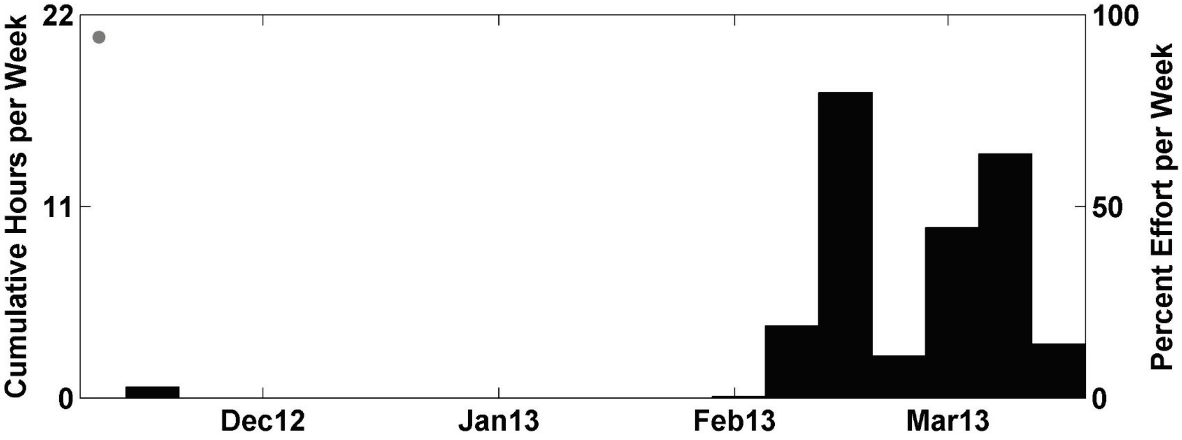

Figure B-31. Weekly presence of Pacific white-sided dolphin echolocation clicks detected from November 2012 to March 2013 at DCPP Site C. Effort markings are described in Figure B-16.

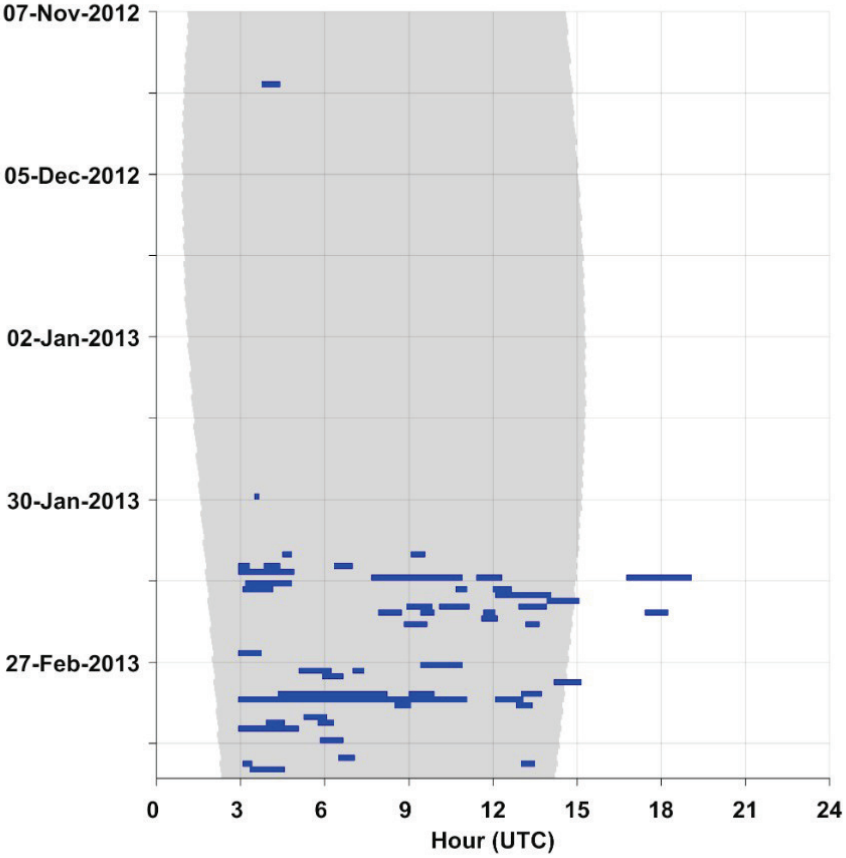

Figure B-32. Pacific white-sided dolphin echolocation clicks in five-minute bins at DCPP Site C from November 2012 to March 2013. Gray vertical shading denotes nighttime.

- **Sperm Whales**
  - Sperm whale clicks were detected throughout the recording, although with some fluctuation in their numbers (Figure B-33).
  - There was no discernible diel pattern for sperm whale clicks (Figure B-34).

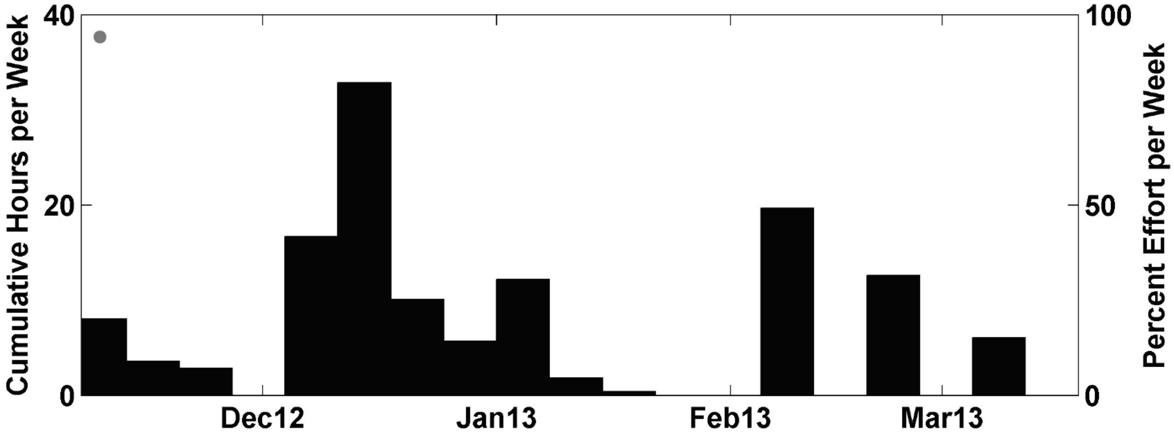

Figure B-33. Weekly presence of sperm whale clicks detected from November 2012 to March 2013 at DC PP Site C. Effort markings are described in Figure B-16.

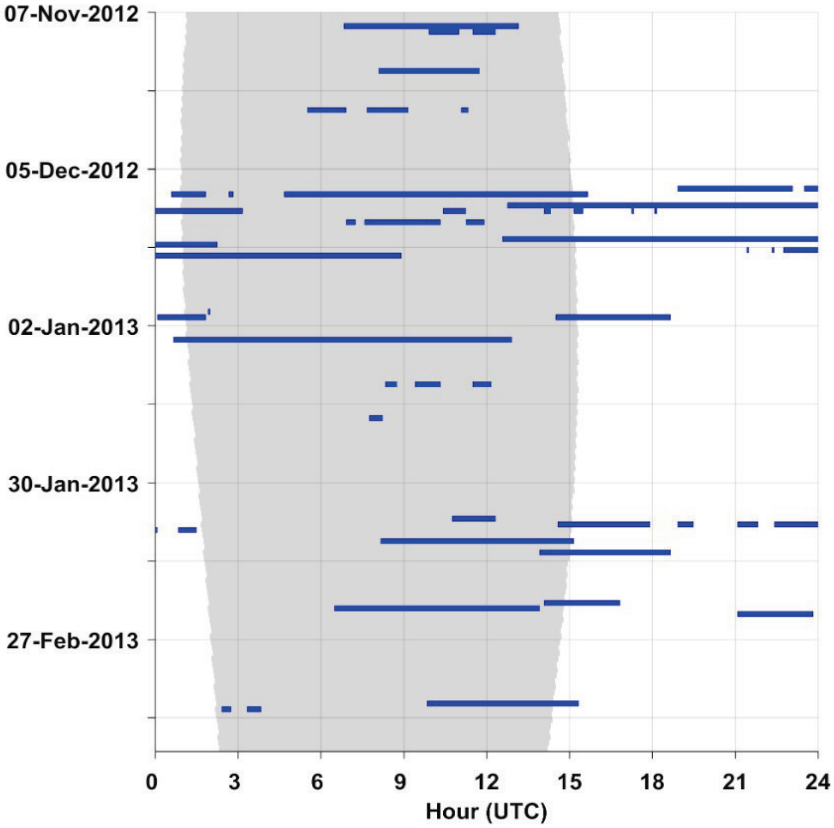

Figure B-34. Sperm whale clicks in one-minute bins at DCPP Site C from November 2012 to March 2013. Gray vertical shading denotes nighttime.

- **Unidentified Odontocete Clicks Greater Than 20 kHz**
  - UO CT > 20 kHz was detected throughout the deployment with a peak during December (Figure B-35).
  - UO CT > 20 kHz was more often detected during nighttime (Figure B-36).

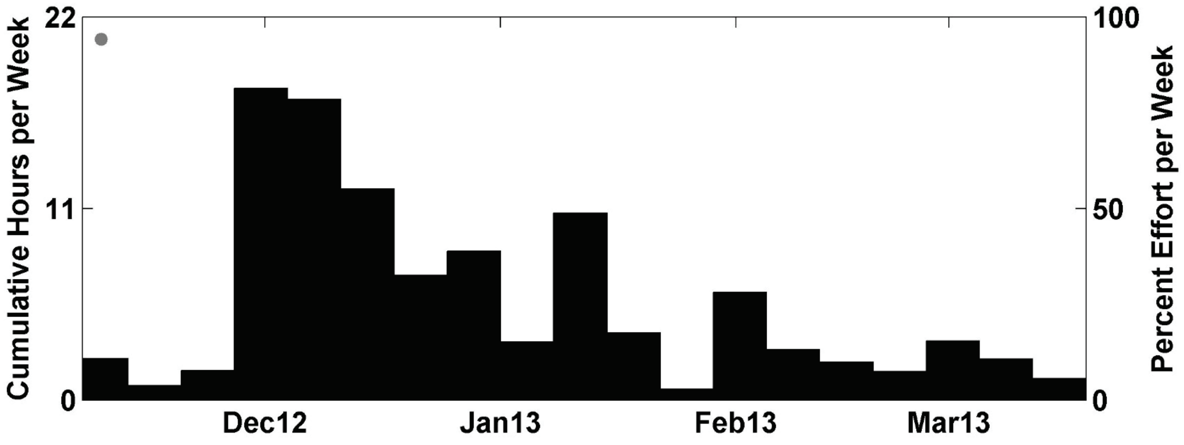

Figure B-35. Weekly presence of UO CT > 20 kHz detected from November 2012 to March 2013 at DCPP Site C.Effort markings are described in Figure B-16.

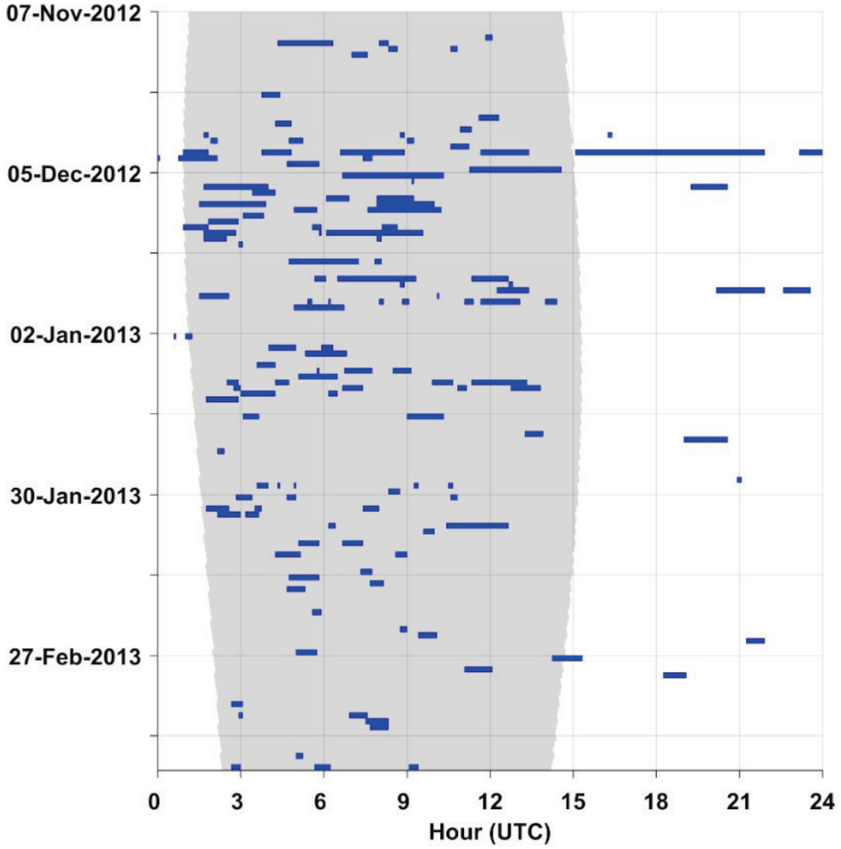

Figure B-36. UO CT > 20 kHz in five-minute bins at DCPP Site C from November 2012 to March 2013. Gray vertical shading denotes nighttime.

Conclusions

Passive acoustic monitoring was conducted at the Diablo Canyon Site C from November 2012 to March 2013 to test for the presence of marine mammal signals and anthropogenic sonar. The initial goal of monitoring was to provide insight into the impact of seismic airguns on endangered baleen whales (blue, fin and humpback), and other marine mammals in the region, although the planned seismic survey was canceled, and no airgun sounds are present in the dataset.

Three baleen whale species were recorded: blue, fin, and humpback whales. Blue whales B and D calls were recorded in high numbers from November 2012 to January 2013, dropping off after January 2013. Fin whales were seen in high numbers in November 2012 but decrease starting in mid-December 2012. Humpback whales were observed in high numbers throughout the monitoring period but began to drop off in late February 2012. No North Pacific Right Whale sounds were detected in this recording.

Seven odontocete species were recorded: killer whales, Cuvier's beaked whales, Baird's beaked whales, Risso's dolphins, Pacific white-sided dolphins, sperm whales, and unidentified odontocete clicks. Killer whale pulsed calls were detected in low numbers throughout the monitoring period. Cuvier's and Baird's beaked whale echolocation clicks were the lowest detected species within the monitoring period but are consistent with other recordings in the area (Keating et al., 2018). Risso's and Pacific white-sided dolphin echolocation occurred in high numbers in January through March 2013. Sperm whales were detected throughout the monitoring period, with highest numbers occurring in December 2012. Unidentified odontocete clicks greater than 20 kHz were found throughout the monitoring period. No MFA was found during the monitoring period.

The Diablo Canyon site is specifically of interest for monitoring because species under the Endangered Species Act inhabit the area during different periods of the year. Anthropogenic noise at low frequencies, primarily due to commercial shipping, overlaps with blue whale and fin whale calls at low frequencies (< 100 Hz). All three of the detected baleen whales (blue, fin and humpback) had song type calls in these data which suggests some use of the area for breeding.

Future work in the Diablo Canyon area using passive acoustic monitoring over a full annual cycle would enable documentation of the seasonal presence of marine mammal species, as well as the presence of anthropogenic signals and possible study of their potential impacts.

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