

Project Title: Baseline Characterization and Monitoring of the MPAs along the North Coast: ROV Surveys of the Subtidal (20 – 500 m)

Project Leaders and Key Associated Staff:

Andrew Lauermann – Marine Applied Research & Exploration (MARE) ROV Operation Supervisor/Marine Biologist is an expert in the collection and analysis of imagery using remotely operated vehicles (ROVs). He has conducted MPA baseline assessments using ROVs annually since 2003, including baseline studies at the Northern Channel Islands and within the Central Coast, North Central Coast, and the South Coast MPA Study Regions. Mr. Lauerermann has over 13 years of experience planning and performing fish and invertebrate abundance surveys using ROVs in California, Oregon and Washington State coastal waters. He also has over 13 years of post-survey data processing, analysis and reporting experience. He will lead the experimental design, field science activities, and post-processing analyses and results syntheses.

- Yuko Yokozawa – MARE GIS/Data Management Specialist, has 10 years of experience with ROV field operations in California, Oregon and Washington coastal waters, as well as 10 years of post-survey data processing and GIS analysis. She will assist Mr. Lauerermann with oversight of data collection and will conduct analyses of positional, video and still photographic data.
- MARE Research Team – A team of trained Humboldt State University undergraduates and graduates, along with other trained personnel, will participate in field activities and the post-processing of ROV imagery in the Eureka California MARE Processing Lab.

Dirk Rosen – MARE Executive Director. Mr. Rosen is an expert on ROV technology and operations. He was the co-designer and builder of hundreds of ROVs and several manned submersibles. He has been a co-principle investigator of MPA baseline assessments for the last ten years, including baseline studies at the Northern Channel Islands and within the North Central Coast and South Coast MPA Study Regions. Mr. Rosen has over 27 years of ROV piloting experience and has lead the successful completion of 13 deep-water MPA baseline surveys since 2003. He will lead cruise planning and assist Mr. Lauerermann with field operations of the ROV system and associated technology.

- David Jeffery – MARE Sr. Engineer. Mr. Jeffery has over 30 years of deep-water ROV engineering design, build, and troubleshooting experience; including co-design of the Deep Ocean Engineering Bandit, Phantom and Phoenix ROVs, and Deep Rover, Deep Flight and Challenger manned submersibles. David will oversee all upgrades and maintenance of MARE's ROVs, including at sea troubleshooting and repair.
- Rick Botman – MARE Operations Engineer. Mr. Botman is highly trained in the troubleshooting and repair of marine electronics and has advanced training in electronics and software programming. He will maintain and operate MARE's ROVs while offshore, as well as managing the topside electronic control and data collection systems.

Key Project Partners:

Dr. Rick Starr – California Sea Grant Extension Program and Research Faculty member of Moss Landing Marine Laboratories (MLML). Dr. Starr's research is focused on the biology and ecology of marine species that are harvested as fishery resources. He was co-principal investigator for the Delta submersible and for the California Collaborative Fisheries Research Program baseline assessment of Central Coast MPAs. Dr. Starr will be providing sample design and post-processing guidance, taxonomic quality control and will guide data analysis and assist with the reporting of project results. His research lab at MLML will also assist with annotation of fish and stereographic sizing.

Donna Kline, MS – Contract Researcher for Moss Landing Marine Laboratories (MLML) and The Nature Conservancy (TNC) in Monterey. Donna has a master's degree in marine science with more than 6 years of experience working with ROV's and underwater imagery as well as 15 years studying California marine fishes. She was previously the science lead for collecting and analyzing video and still photographic imagery to support marine ecological studies, including MPA baseline assessment of the North Central Coast and Central Coast Trawl Impacts at California State University, Monterey Bay. Ms. Kline will provide assistance with QA/QC development and implementation, annotation of fish and stereographic sizing, and provide analysis and reporting support.

Dr. Bob Lea (CDFG retired) – Dr. Bob Lea is a retired CDFG biologist and an expert on California fishes. He will provide consultation for fish identifications.

Commercial fishermen – We will collaborate with local fishermen who can provide vessel time for ROV operations and who will provide local knowledge of study areas during the planning of transects inside and outside of proposed MPAs.

Project Goals and Objectives

Three *primary* project goals are proposed and include 1) baseline characterization of selected MPAs across the North Coast Study Region, 2) assessment of initial ecological changes in fishes, macrofaunal invertebrates, and associated seafloor habitats in those MPAs during the first two years following designation, and 3) recommendations for future monitoring efforts in the region. We will survey three of the ecosystem features identified as priorities in the North Coast RFP: Mid-depth Rock Ecosystems, Soft-bottom Subtidal Ecosystems, and Deep Ecosystems including canyons within MPAs and adjacent reference study sites. The objectives for each primary goal are described below:

GOAL 1: Baseline Characterization

Prior to field data collection, we will review available historical information on subtidal communities represented in North Coast MPAs. This will include an evaluation of remote operated vehicle (ROV) data collected by the Project Leaders in partnership with California State University Monterey Bay and the California Department of Fish and Wildlife in the North Coast Study Region during 1999, 2001, and 2004 and the North-Central Coast Study Region from 2009-2011, focusing primarily on the fishes and invertebrates found in the rocky habitats sampled at those locations. Using available multibeam mapping data, we will analyze benthic habitat distribution within each proposed site and identify transect locations for data collection.

Following the review of historical information, we will collect both video and still photographic imagery of mid-depth (20-100m) and deep (>100 m) subtidal, soft and hard bottom habitats, inside and out of (from north to south) Pt. St. George Offshore SMCA, Reading Rock SMR, Mattole Canyon SMR, and Ten Mile SMR to provide a permanent record of seafloor communities occurring at the time of MPA implementation. Sampling will include imagery of 1) substrate and seafloor structure, 2) epifaunal macro-invertebrates (both sessile and mobile), and 3) associated fishes (including selected exploited and non-exploited species identified as priorities (Table 1)). Using video and still imagery collected (over both sampling years), we will provide a summary description and assessment of ecological conditions inside SMRs and/or SMCAs and comparable reference sites outside, as initial data for future comparison of trends over long-term monitoring timeframes. The description will include benthic ecosystem structure, habitat characteristics and species assemblages in the context of the key attributes, indicators and focal species defined by the monitoring framework and local State and Federal marine managers.

GOAL 2: Assessment of Initial Ecological Changes

One year after the initial ROV survey, we will collect a second year of video and still photographic imagery from the same treatment sites sampled in Year 1. Using both years' data, we will describe any ecological changes to benthic community composition and structure in sites sampled, including all fishes and macro-invertebrates (species composition, abundance and density, as well as size distributions) and seafloor habitats (percent cover and relief of biogenic and physical habitat attributes identified as priorities). We will also identify data elements considered sensitive and/or rapid to respond to MPA implementation. Potential explanations for any changes observed, specifically whether they can be attributed to MPA establishment, will be provided.

GOAL 3: Recommendations for Future Monitoring

Using data collected, we will evaluate the spatial and temporal scale at which ROV imagery should be sampled to produce robust data on species assemblages and habitat composition. We will recommend a set of indicator species (based on quantitative analysis), technology, and methods to assess long-term status and trends for subtidal ecosystems surveyed.

Table 1. Organisms, features and metrics proposed for collection at proposed North Coast ROV baseline sites based on the Project Leaders experience using ROVs to collect similar data in the study region and elsewhere.

| Ecosystem Feature | | Metric |
|--|--|--|
| Mid-depth Rock Ecosystems (30-100 m depth) | | |
| Community | Multiple species Multiple species Dominant habitat (50% coverage) Subdominant habitat (20%) | Species diversity Species richness Composition and relief Composition and relief |
| Biogenic habitat | Biogenic habitat composition Structure-forming invertebrates Encrusting invertebrates | Height and complexity Density and cover Cover |
| Fishes | Rockfishes (<i>Sebastes spp.</i>) Perches (Embiotocidae) Other fishes - including lingcod, greenlings, | Density and Size Structure Density and Size Structure Density and Size Structure |
| Invertebrates | Crabs Corals Sea urchin Sea cucumbers Other invertebrates - including anemones, sea stars, and sponges | Density and Size Structure Density and Size Structure Density and Size Structure Density and Size Structure Density and Size Structure |
| Soft-bottom Subtidal Ecosystems (0-100 m depth) | | |
| Community | Multiple species Multiple species Dominant habitat (50% coverage) Subdominant habitat (20%) | Species diversity Species richness Composition and relief Composition and relief |
| Biogenic habitat | Biogenic habitat composition Multiple species | Height and complexity Cover and diversity |
| Fishes | Rockfishes (<i>Sebastes spp.</i>) Perches (Embiotocidae) Flat fishes (Parlichthyidae and Pleuronectidae) Other fishes | Density and Size Structure Density and Size Structure Density and Size Structure Density and Size Structure |
| Invertebrates | Shrimps Crabs Sea cucumbers Other invertebrates - including anemones, sea stars and sea whips | Density and Size Structure Density and Size Structure Density and Size Structure |
| Deep Ecosystems (>100 m) | | |
| Community | Multiple species Multiple species Dominant habitat (50% coverage) Subdominant habitat (20%) | Species diversity Species richness Composition and relief Composition and relief |
| Biogenic habitat | Biogenic habitat composition Multiple species | Height and complexity Cover and diversity |
| Fishes | Rockfishes (<i>Sebastes spp.</i>) Flat fishes Other fishes | Density and Size Structure Density and Size Structure Density and Size Structure |
| Invertebrates | Shrimps Crabs Corals Other invertebrates - including anemones, sea stars and sponges | Density and Size Structure Cover and diversity Density and Size Structure |

Project Rationale

Data collected by the Project Leaders and key project partners thus far, in all California regions combined, exceeds 1,100 km of visual transects and provides a comprehensive and permanent/archival visual snapshot of baseline conditions within newly formed MPA networks (Table 2). Non-invasive survey platforms have been used for the collection of video and still photographic imagery throughout the state's MPAs. The use of ROVs for the collection of imagery is now well-established, including major sampling efforts by the Project Leaders as part of the baseline characterization and monitoring of the MPA's in the Central Coast, the North Central Coast and the South Coast Study Regions, including the Northern Channel Islands.

In the present proposal, ecosystem characterization and monitoring of mid-depth (20-100 m) rock and soft-bottom communities and selected deep (>100 m) ecosystems will be undertaken by Marine Applied Research and Exploration (MARE), in partnership with Dr. Rick Starr and Donna Kline at Moss Landing Marine Laboratories and local commercial fishermen (TBD). Baseline data collection and analyses will occur over the first two of the three project years, focusing on North Coast draft MPA monitoring metrics, as well as metrics derived from ROV surveys conducted in other MLPA study regions, including historical ROV surveys conducted by the Project Leaders in the North Coast Study Region. The third year will be focused on data analysis and interpretation, including development of recommendations for future monitoring.

The proposed approach and partnerships are uniquely positioned to address monitoring priorities in the region:

- The proposed ROVs are configured specifically for biological data collection and are suitable for this region and depth ranges, including the Vector M4 "Beagle" ROV and the Phantom S2 "Hammerhead" ROV. The proposed sampling depths are generally below the depth at which divers can operate safely, but well within the limits of the ROVs. The camera configuration will be similar on each ROV and includes forward-facing stereo video, high-definition video and digital still cameras, as well as a downward-facing video camera. This camera configuration provides comprehensive habitat coverage, high-definition imagery for difficult species identifications and high-precision stereographic measurements of size.
- Both of the Project Leaders and key project partners have prior experience in MPA monitoring and assessment, including ten consecutive years of ROV surveys in support of California's baseline MPA assessments. The experience gained in the Northern Channel Islands, Central Coast, North-Central Coast and South Coast MPAs can be leveraged to help conduct this research and help maintain continuity in data production methods.
- In addition to MPA baseline assessments, the Project Leaders have prior experience working in the harsh marine environment of the North Coast Region, including ROV surveys off the former Punta Gorda Marine Reserve and select sites off Fort Bragg California, as well as surveys conducted in Oregon and Washington coastal waters.
- Data collection and post-processing protocols used by the Project Leaders for baseline characterization in previously implemented MPA study regions will be maintained for consistency, allowing for direct comparison across the entire statewide network of MPAs.
- Stereographic imaging analysis will provide highly accurate estimates of size frequency distribution, accurate to approximately 5 mm (Bower et al. 2011).
- The study design provides data that can be calibrated with other technologies such as SCUBA diver and collaborative fisheries research already been used extensively in other regions and depths in California waters. ROV data collected by the Project Leaders at the Northern Channel Islands has already shown similarities to data collected by PISCO SCUBA divers in nearby shallow water.
- Video files and still photographs will provide a permanent archival record of baseline conditions that can be used for a variety of purposes in addition to baseline analyses such as undergraduate and graduate student projects, public education or future re-analysis using refined techniques.

- The Project Leaders and partners will produce a variety of compelling visualizations directed at disseminating the results of the research to wider audiences, including policy makers, managers and the general public, including posters describing the project placed in Harbor Masters' offices near proposed study sites.
- MARE will utilize its local Eureka office and personnel to conduct survey operations, post-processing and base analysis and reporting.
- The Project Leaders have been in communication with other north coast baseline projects about survey locations and will continue to seek additional ways to integrate sampling locations, data products and analysis.

Table 2. ROV surveys conducted in support of MPA baseline assessments by the Project Leaders and key project partners. MARE/CDFW surveys conducted as part of baseline biological MPA assessments and MARE/CSUMB ROV surveys conducted as part of the Monitoring Enterprise's MPA baseline characterization.

| Study Area | Year | MARE / CDFW ROV (km) | MARE / CSUMB ROV (km) | No. of Transects (size varies) |
|-----------------------------------|------|----------------------|-----------------------|--------------------------------|
| Northern Channel Islands | | | | |
| | 2003 | 18 | | 95 |
| | 2004 | 75 | | 485 |
| | 2005 | 117 | | 1,014 |
| | 2006 | 85 | | 1,120 |
| | 2007 | 71 | | 933 |
| | 2008 | 77 | | 981 |
| | 2009 | 81 | | 737 |
| Central Coast Region | | | | |
| | 2007 | 26 | | 486 |
| | 2008 | 53 | | 971 |
| | 2009 | 24 | | 308 |
| | 2012 | 33 | | 611 |
| North Central Coast Region | | | | |
| | 2009 | 18 | | 220 |
| | 2010 | | 80 | 35 |
| | 2011 | 33 | 94 | 682 |
| South Coast Region | | | | |
| | 2011 | | 80 | 57 |
| | 2012 | | 80 | 51 |
| Totals | | 711 | 334 | 8,735 |

Approach

In collaboration with partners, MARE will collect both structural and biological community data in the deeper subtidal across the full extent of the North Coast Study Region. Our four proposed study sites include areas that have been affected by higher levels of human use, including fishing, and are expected to show changes related to MPA establishment. They are (listed from north to south): 1) Pt. St. George Offshore SMCA, 2) Reading Rock SMR 3) Mattole Canyon SMR, and 4) Ten Mile SMR (Figure 1).

Our study design replicates sampling conducted by the project Leaders, and key project partners, in previous MPA regions and combines two techniques. The first technique requires the ROV to be flown very near the bottom and somewhat slowly to most effectively sample the benthic structure and species strongly associated with the benthos. Transects (totaling 0.5 to 1 km in length) will be distributed throughout each study area to encompass both sedimentary and hard substrate environments, as well as the transitional areas between the two environments. These slower and lower transects will allow for consistent baseline characterization of the entire MPA and outside reference areas at the time of implementation. The second technique will be used within defined areas determined to be predominantly rocky habitat to collect additional data on size distribution and density of demersal and epibenthic finfishes and invertebrates associated with hard bottom. This technique requires the ROV to be flown more quickly and higher above the bottom to more effectively sample mobile epibenthic fishes and larger structure-forming macro-invertebrates. This intensive sampling will help decrease sampling variability, while increasing the statistical power to detect changes over time (Karpov et al. 2010) as well as maintaining consistency with previous baseline surveys in other MPA regions.

Up to three treatments will be sampled within each study site (depending on the MPA configuration) — inside the SMR, inside the SMCA, and a site relatively distant from both, but of similar bottom habitat composition that will be considered a control/reference site for each. Each of the selected sites has a range of habitats available from at least two of the three protection levels (SMR, SMCA, and no protection) so that we will be able to collect data for soft substrate, hard substrate, and transitional habitats under one or two levels of protection and at unprotected adjacent locations at comparable water depths. Data collection and analysis will span three project years and are as follows:

Project Year 1

In Year 1, we will conduct extensive sampling both inside and outside of MPAs at each of the four sites. Transects will be co-located where possible to maximize continuous ROV dive time and encompass rock, soft sediment, and transitional substrates, as well as deep sites (including canyons and canyon heads) at multiple water depths. Continuous imagery will be collected and sub-sampled at different spatial scales, which will depend on the scale of analysis to be performed.

Characterization based on this imagery will include collection and analysis of data on 1) seafloor substrate and structure, 2) macrofaunal invertebrates (both sessile and mobile), and 3) associated fishes (including selected exploited and non-exploited species identified as priorities). The selection of species targeted for monitoring (Table 1) was developed from the RFP and augmented based on the experience of the Project Leaders with similar surveys. Data post-processing and analysis will be coordinated by Andrew Lauermann and will utilize local personnel, which will include Humboldt State University graduates and undergraduates, at MARE's Eureka California office. In addition, a subset (approximately 20%) of the video data will be processed at Moss Landing Marine Laboratories by Donna Kline under the guidance of Dr. Rick Starr.

Project Year 2

In Year 2, we will replicate surveys at sites sampled in Year 1 to not only increase the baseline sampling effort, but also to compare community composition, abundance, and density of selected fish and invertebrate species with Year 1 data. The sampling levels and prioritization for Year 2 will be based on a review of the post-processing results of Year 1 imagery.

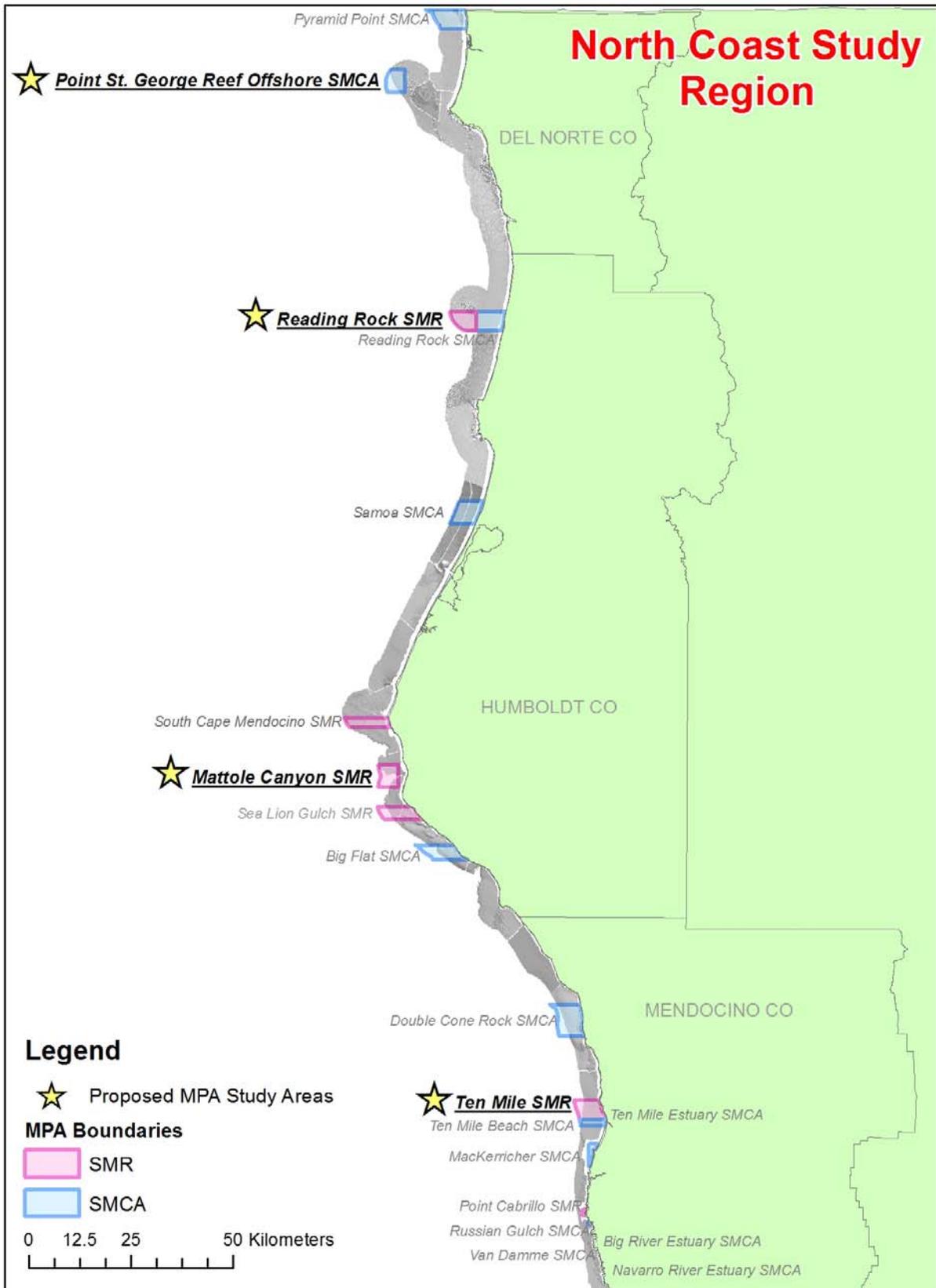


Figure 1. Map of the North Coast Study Region including the four study areas selected for sampling in this proposal.

Project Year 3

Our efforts in Year 3 will focus entirely on data summarization, analysis and reporting. We will produce a comprehensive project report describing the characterization of the seafloor communities inside and outside selected MPAs across the North Coast Study Region. This will include density, size distribution and habitat associations for selected species, as well as recommendations for future monitoring for use by managers and policy-makers. MARE will include data from the North Coast Baseline Characterization in their ongoing outreach and education efforts on California MPAs. Outreach to the general public will be conducted through a variety of compelling data visualizations. Outreach opportunities to be considered include yacht clubs, scuba clubs, community service clubs and schools. Imagery libraries will be made available via the web through California Ocean Spaces, MARE website and Facebook pages, and provided to online taxonomic resources. Our experience in sampling within the other MPA regions has shown us that these localized cruises are also excellent opportunities for outreach, with stakeholder and concerned citizens. We will continue to meet and talk with locally interested parties when and where practical.

Experimental Design

Our proposed study areas (Figure 1) were selected to represent the full latitudinal extent of the North Coast Study Region and were selected as a cross-section of three ecosystems prioritized for monitoring in the RFP, including Mid-depth Rock Ecosystems (30-100 m), Soft-Bottom Subtidal Ecosystems (0-100 m), and Deep Ecosystems (> 100 m) including canyons. The study sites (from north to south) are: 1) Pt. St. George Offshore SMCA, 2) Reading Rock SMR 3) Mattole Canyon SMR, and 4) Ten Mile SMR. Selected sites have a range of habitats available including soft bottom, hard bottom, transitional, and canyons under each level of protection and at comparable water depths (Figure 2). Planned transects will be positioned to collect data within all three habitat types (with a focus on hard-bottom in the areas in which it occurs to provide more in-depth data for CA fisheries resources), which will be identified using GIS analyses of multibeam data.

In Year 1 we will target a minimum of two (2) transect (at least 0.5 km each) within each habitat type (hard bottom, soft bottom and transitional) at each treatment site (SMR or SMCA and adjacent reference site of similar bottom habitat composition). In addition, six (6) transects (0.5 km each) will be collected within each treatment site that focuses on a defined area of hard bottom habitat. Figure 3 shows a hypothetical distribution of survey lines; actual line placement at each site will be determined based on analysis of individual site characteristics. Adjacent reference sites will also be selected based on analysis of site characteristics, with the goal of most closely matching depths and habitats with those found inside the nearby MPA study site. Sampling within the Reading Rock SMR and the adjacent reference site will be reduced (one half day at each site) due to the remote location and lack of suitable anchorage.

The sampling effort and prioritization for Year 2 sampling will be based on our accomplishments in Year 1 and from preliminary analyses of the resultant data.

ROV Operations

The MARE owned Vector L4 ROV “Beagle” will be used. This ROV is rated to a depth of 1,000 m and will be used for video and still photographic sample collection. The Vector L4 is equipped with five geo-referenced cameras (forward-looking stereo video, high-definition video and digital still, downward video and digital still), and associated lights, sensors, scaling lasers and strobe. In addition to using the Vector L4, the MARE owned Phantom S2+2 will be on standby as a backup vehicle. The 300 meter rated Phantom S2+2 has similar cameras, lighting, and ranging altimeter configuration as the Vector L4, but does not have imaging sonar. The depth range and diversity of the study area, as well as the desire to cover both hard and soft bottom habitats and the transitional area between the two, fits the depth and versatile performance capabilities of these two vehicles. The ROV controllability, neutral buoyancy and navigational sensors allow the ROV to be flown along straight line transects that closely follow the terrain. By applying sampling protocols previously used in other MPA study regions, we will collect data that is compatible and complementary to existing deep-water datasets of the Northern Channel Islands, Central Coast, North-Central Coast and South Coast MPA networks.

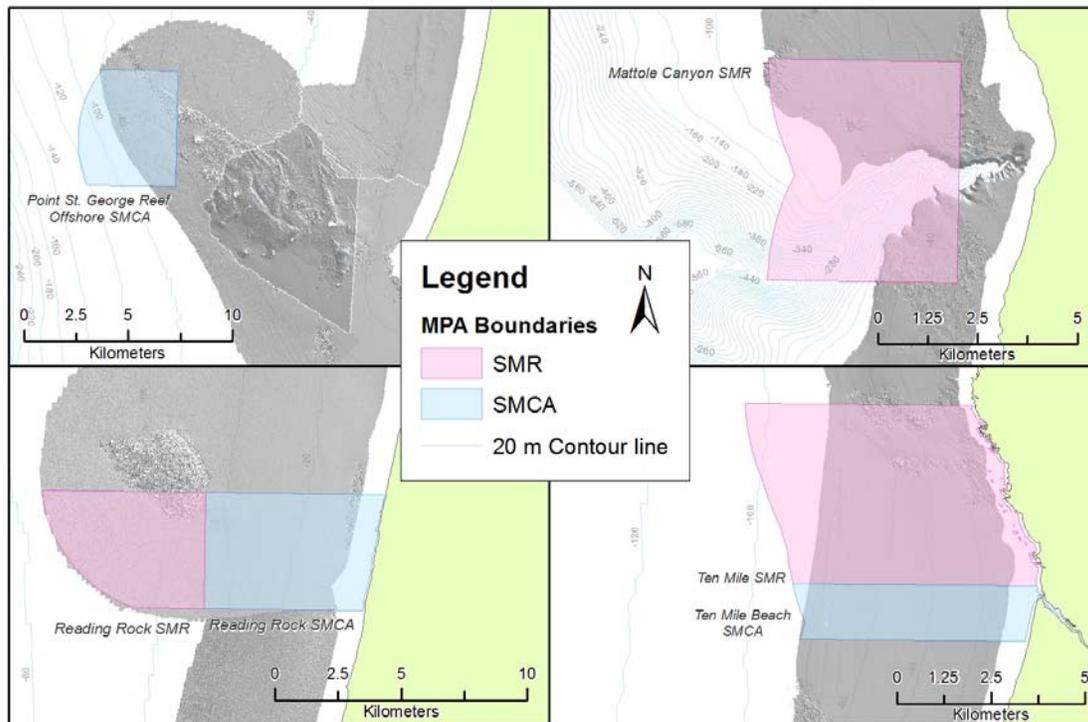


Figure 2. Maps of each of the four study areas including MPA boundaries, depth strata for each ecosystem type, and multibeam bathymetry (where available).

Piloting for both vehicles is assisted by auto heading, auto depth and vertical ranging altimeter to maintain a straight course (± 1 degree) and altitude above substrate (± 0.3 m) with minimal corrections. In addition, a thruster “cruise control” will help the pilot maintain a constant forward velocity. A Blueview multi-beam sonar will provide a real time wide area view to 15m. ROV acoustic tracking will yield distance and visual area surveyed. A Trackpoint III acoustic positioning system will be used to reference the ROV position relative to the ship’s GPS position. The resulting ROV GPS coordinates will be logged by Hypack navigational software. Hypack will also record GPS time, ROV heading, depth, speed, altitude, forward-facing camera tilt angle, and forward-facing camera distance to seafloor with associated viewing screen width. Area surveyed will be calculated by multiplying the distance traveled by viewing screen width for each second of video captured.

One color video camera will be positioned forward facing and angled approximately 30° below horizontal with another pointing straight downwards. These two-cameras will provide a near continuous view forward and downward. In addition, a stereographic video camera system will be mounted facing forward, for use in highly accurate measurements of size (to 0.5 mm or less). Video from the stereo camera system will be recorded sub-sea along with GPS time for subsequent geo-referencing. The camera images will be well illuminated by two highly-efficient dimmable Nuytco 200 Watt HMI lights and two dimmable 250 watt Deep Sea Power and Light tungsten halogen lights. Paired 10 cm wide red lasers will be used for image scaling and will be centered in the viewing area of the forward and down cameras. Digital video for both the forward and down facing cameras will be captured on digital video cassettes using SONY DSR 45 digital tape recorders and redundantly on DVD by Pioneer DVR550h DVD recorders. The two digital still cameras will be co-positioned with forward and downward video cameras to make use of forward and downward facing red lasers, spaced 10 cm apart, for image scaling. In addition to capturing biological and habitat observations, the forward video will capture redundant onscreen overlay recordings of GPS time code, depth, heading, altitude, screen width and temperature.

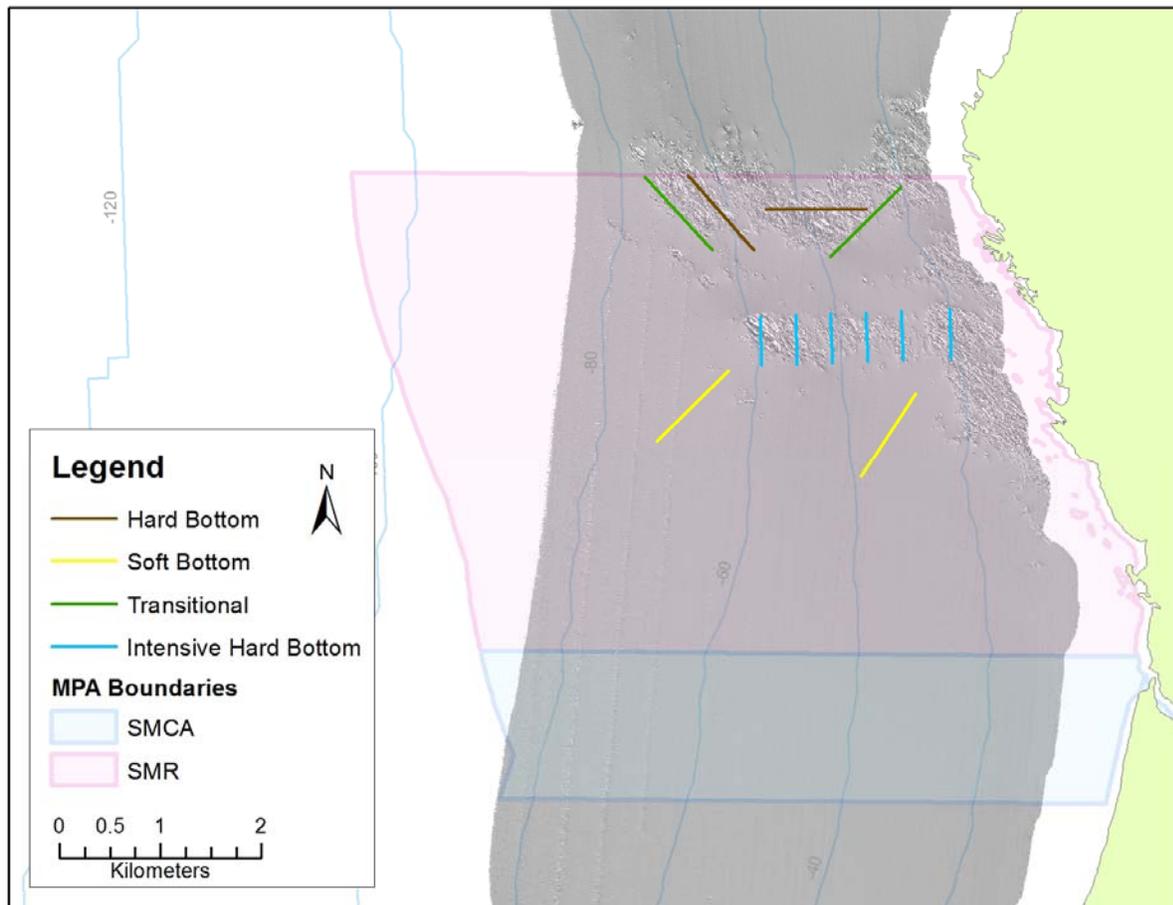


Figure 3. Hypothetical distribution of planned survey lines at Ten Mile SMR.

Data Collection & Sampling Strategy

Prior to field sampling, a thorough review of existing regional ROV data and publications related to subtidal communities within the study region will be made to inform our selection of transect locations and targeted species. This review will include in-depth geospatial analyses of existing multibeam maps to identify locations within each study area where habitat conditions can be duplicated most closely inside and out of MPAs.

In years 1 and 2 we will target 10 days of sampling per year, which will yield about 60 km a year (120 km total) of video and still photographic imagery. We will collect both forward and downward looking imagery of 1) seafloor structure, 2) epifaunal macro-invertebrates (both sessile and mobile), and 3) associated fishes (including selected exploited and non-exploited species identified by in the RFP (Table 1). We will use protocols for data collection, post-processing, and analysis derived from our existing ROV research programs, which have been used throughout the statewide network of MPAs.

Comprehensive video data compilation, recording all structural and biological elements resolvable, combined with high-resolution still for difficult species identification, will provide a complete geo-referenced dataset for use in a broad range of subsequent analyses. We will identify all fauna to the lowest possible taxonomic level; provide size frequencies using stereographic camera techniques and document habitat and depth associations. The archived video and still photographic records will provide a comprehensive dataset that will allow for the evaluation of community components that will be most appropriate for long-term monitoring, which will help to achieve the goals identified by the MPA Monitoring Enterprise.

Sampling levels

The collection of continuous imagery will allow for subsampling of that imagery at a variety of spatial scales. Based on similar data collected by the Project Leaders and others (e.g., Bergen et al. 2006; Karpov et al. 2006; Lindholm et al. 2004) it is possible to specify a sampling strategy that involves sub-samples of the larger transects in 300 m or smaller increments based on species and habitat accumulation curves plotted in both soft sediment and rocky subtidal communities. As such, ROV transects will be configured to include a target of three (with a minimum of two) transects per habitat type in each treatment per sampling year, subject to weather and other uncontrollable factors. In addition, increased ROV sampling will occur within areas of predominantly rocky habitat for comparison to historical data collected similarly in other MPA study regions.

Transect Calculations

Positional information, in the form of XY metric coordinates recorded for each one second of video transect, will be filtered for outliers and smoothed using a 21-point running mean, which averages each position by using 10 positions on either side of that position. Planar length of smoothed positions tracked will be calculated for each second and combined with width to calculate area surveyed per second. The one-second intervals will be combined to produce quantitative sampling units, the size of which will depend on the scale of analysis to be performed. Gaps in the positional data that occur due to deviations from quantitative protocols, such as pulls (ROV pulled off transect or off the bottom by the ship), stops (ROV stops to let the ship catch up) or loss of target altitude caused by traveling over backsides of high relief structures (visual loss of 4 m target distance for more than 6 seconds which typically occurs on the downward slope of high relief habitat) will be removed from the data prior to generating quantitative sampling units from each survey line. Sampling unit size will vary and will be determined post-hoc based on the spatial scales at which substrate and associated biological communities occur.

A pair of Tritech 500 kHz ranging sonars, which measure distance across a range of 0.1–10 m using a 6° conical transducer, will be used as the primary method for measuring transect width. Readings from these sonars will be averaged five times per second and recorded at a one-second interval with all other sensor data. Measurements of transect width using ranging sonar is accurate to ± 0.1 m (Karpov et al. 2006). The area surveyed will then be calculated for each second of video using distance traveled multiplied by transect line width.

Seafloor Habitats

Seafloor habitat characteristics will be determined and recorded comprehensively for all substrate types including physical substrata (such as boulders and sand waves), and biogenic structure (such as erect sea whips, sponges, corals or anemones) that extend more than 10 cm above the surrounding seafloor. Quantitative approaches will be used to evaluate benthic habitat composition and characteristics. Habitat characteristics within each transect will be categorized and delineated from the videotape during post-processing using definitions adapted from Greene et al. (1999).

Fish and Macro-Invertebrates

All geo-referenced video records will be reviewed during post-processing. Each video transect will be treated as a series of non-overlapping video quadrats (Auster et al. 1991, Lindholm et al. 2004) with contents recorded comprehensively. Organism counts will be derived from the forward facing video by sampling continuously as individuals occur. Select megafaunal individuals will be identified to the lowest taxonomic level possible, counted and recorded. Paired lasers projected into the cameras' field of view, combined with forward facing ranging sonar projected in line with the forward lasers, will be used as reference for distance and to establish an upper limit for the data collection field. Forward digital still photographs will be used to verify species identifications where high resolution of species characteristics is required.

Encrusting and structure-forming invertebrate coverage will be estimated during video review and entered as patches with discrete start and stop points along transects. Each invertebrate patch will be given an estimated percent cover between the forward projecting lasers and lower edge of the field of view. Using

this approach, colonial patches will be estimated by total area coverage for each transect (total area of transect with invertebrate patch x percent patch coverage).

Size Measurements

Accurate size measurements will be made using stereographic video cameras mounted on the ROV combined with SeaGIS software (<http://www.seagis.com.au/>). Individual video frames from the two camera system, with a calibrated stereographic spatial relationship, will be post-processed following protocols refined at Moss Landing Marine Laboratories. Estimates of object size derived using stereographic camera systems are accurate to 5 mm or less (Bower et al. 2011). For select species, a random sampling of the data will be used to produce estimates of size frequency distribution for each survey site.

Detailed Post-Processing Methods

We will utilize data processing protocols developed by MARE and its partners during MPA surveys at the Northern Channel Islands and in the central coast, north central coast and south coast MPA regions. In addition, stereographic sizing will be employed to allow for increased size frequency resolution not previously attained from ROV survey data collected in other MPA regions. Video annotation techniques, combined with local trained personnel with prior ROV data collection experience will ensure that data extraction occurs in a timely manner and with a high degree of precision. Quality control protocols will be used during post-processing to ensure accuracy in species identification and enumeration.

A protocol to characterize substrates observed in video along survey lines will be used and is designed to be compatible to a hierarchical classification system developed by Green et al. (1999). The video record will be reviewed and substrate types will be classified independently as rock, boulder, cobble, sand or mud. In addition, substratum composition will be categorized by using the dominant (primary >50%) and subdominant (secondary >20%) percentages of substratum cover. In addition, three types of slope (<30°, 30 – 60°, >60°) and three rugosity levels (Low, Medium, High) will be identified to describe the size of crevices in the substrate that can hide fishes (e.g., no room, can hide small fishes, crevices large enough to hide large fishes).

During habitat video review, a transparency film overlay with guidelines approximating a 1.5 m wide swath will be placed over a video monitor screen. Each of the substrate types listed will be identified by the processor independent of each other and will be recorded as discrete segments with a beginning and ending time code, thus the segments of substrate types may overlap each other along the survey line, creating segments of mixed substrate combinations (e.g. rock/sand, sand/cobble). A substrate segment will be considered continuous until a break of 2 m or greater occurs along the survey line or the substrate drops below 20% of the total combined substrates for a distance of at least 3 m. During the review, each segment will be defined as the dominant or subdominant substratum. We will also combine substrates to create three independent habitat types: hard (rock and/or boulder), mixed (rock and/or boulder with either cobble and/or sand) or soft (cobble and/or sand).

Fish density will be calculated using various habitat and depth combinations. Sampling unit size will vary and will be determined post-hoc based on the spatial scales at which substrate and associated biological communities occur. Our primary sampling unit will be the ROV dive, and each dive will have multiple transects. Multiple ROV transects at each sampling site will provide a robust estimate of mean and variance associated with each sampling variable. Within each MPA, there will be multiple sampling locations (locations are based on habitat types) that are randomly chosen, so we avoid problems associated with auto correlation of samples. A secondary sampling unit will be the collection of similar-habitats on a transect. For example, by providing a density of species A in mixed rock and sand habitats, we can more closely compare habitat-specific densities of species A in each MPA. Also, if desired, estimates of total abundance can be made by extrapolating fish or invertebrate densities by habitat area obtained by GIS analysis.

Finfish video review and enumeration will classify finfish to the lowest taxonomic level possible. Finfish that are not able to be classified to the species level will be grouped into a complex of species, or recorded as unidentified. All finfish species and groupings will be selected after a preliminary review of video prior to the formal enumeration processing.

A screen overlay representing a diminishing perspective will be used during fish review to approximate the transect width across the viewing screen, calculated by the ranging sonar, at mid-screen. The overlay serves as a guide for determining if a fish is in or out of the ROV transect. Finfish enumeration will be limited to a maximum distance of four meters, as historically performed by the Project Lead in other MPA regions statewide. Using the sonar range value depicted on the screen as a gauge, the processor will determine if a fish is within four meters as it enters the viewing area. Fish that enter the viewing area will be counted if more than half of the fish crosses the overlay guidelines.

In order to accurately correlate the location of the fish with the habitat, time code entry will be made when the fish crosses the mid-screen line. For finfish that are within four meters, but swim away before they cross the mid-screen line, time code entry will be made when the location where the finfish had been observed reaches the mid-screen point. All data entries will be recorded in a Microsoft Access® database linked with the time code.

Fish size (total length) will be measured using two methods: 1) stereo camera measurements and 2) coarse visual size estimates. Stereo camera size measurement will utilize two cameras mounted to the ROV in a fixed relationship to each other. Using specialized stereographic software, measurements will be made from each camera's field of view, which are precisely linked using GPS time. Measurements using stereographic camera techniques are precise to the nearest one centimeter.

In addition to stereographic camera sizing techniques, fish size will be estimated by the video observer with the use of two parallel lasers placed 10 cm apart aimed to hit the seafloor in the center of the video viewing screen of the forward facing camera. Fish will be placed into bin size groups (coarse length) by estimating how many units of laser widths would fit along the total length of a fish when it passed the plane of the laser marks projected on the sea floor and then selecting the appropriate 10 cm bin. Fish sizes will be entered into the data entry form as single integer codes as follows: 0 = 0-9cm, 1 = 10-19, 2 = 20-29 etc. The use of this technique will allow for the direct comparison of historical data sets collected without stereographic cameras in other MPA regions.

Invertebrate video review and enumeration will identify macro invertebrates to the lowest taxonomic classification level possible, or group them into a complex of species. All invertebrate species and groupings will be based on review of video prior to enumeration. Only macro invertebrates with body forms and colors that are uniformly identifiable on video will be selected for enumeration. Macro invertebrate species that form large colonial mats or cover large areas, will not be enumerated as individuals, but rather identified as patches with discrete start and stop points and assigned a percent cover.

A screen overlay will also be used during invertebrate review and enumeration to approximate the transect width, calculated by the ranging sonar, at the bottom of the screen. The diminishing perspective overlay lines serve as a guide for determining if an invertebrate is in or out of the ROV transect. The overlay used in invertebrate enumeration will be the same as the overlay used in habitat classification, allowing for direct correlation of habitat to each invertebrate observation. All data entries will be recorded in a Microsoft Access® database linked with time code. Invertebrates that enter the viewing area will only be counted if more than half the animal crosses the overlay guidelines at the bottom of the screen.

To obtain estimates of percent cover of smaller ("turf-forming") invertebrates, we will subsample the video transect by selecting a random starting point and then analyze individual video frames at a set time interval. This will provide a stratified random quadrat approach to sampling invertebrate cover. For each of the still frames analyzed from transects, we will assign a percent cover value for the invertebrate taxa evaluated. The percent cover value will be a categorical value, i.e., 0 = not present, 1 = < 33%, 2 = 34 –

66%, 3 = 67 – 99%, and 4 = 100% invertebrate cover. A percent cover index for each dive site will be obtained by averaging the categorical numbers assigned to each video frame analyzed.

All raw and post-processed data, including data summaries will be stored in a Microsoft Access® database with metadata describing all tables, fields and steps used during processing. ROV navigational data, sensor data, biological and habitat observations will be time liked using Greenwich Mean Time (GMT) time code and sequential dive number and dive date. All database products will adhere to standardized historic ROV database standards.

Analytical Methods

Our study design adheres to previously implemented ROV surveys and thus allows for direct statistical comparison to data collected in other MPA regions and also is similar to the submersible surveys conducted in the central coast region. Our study design is focused on conducting visual surveys using a stratified random sampling strategy to collect information to allow a comparison of north coast MPAs and associated reference sites. The first stratum contains the MPAs and reference sites. Then we will stratify by depth and habitat type, using GIS and bottom habitat type maps to identify candidate sampling locations that we will randomly (or haphazardly based on logistical constraints) select to be sampled using the ROV.

Our analytical objectives will be to (a) characterize habitats and associated fish and invertebrate communities in the north coast, (b) provide a quantitative baseline of fish and macroinvertebrate densities and percent invertebrate cover for future comparison of MPA changes, and (c) compare similarities and differences in fish and invertebrate community compositions among study sites in the selected MPAs and reference sites. We will address objective (a) with descriptive statistics and non-parametric tests. For example, habitats will be described by a combination of substrate type, and slope and rugosity of the seafloor. Combining habitat and species densities, we will describe species- habitat associations using cluster analysis and principal component analyses, and non-metric multi-dimensional scaling (NMDS) plots based on Bray-Curtis similarity indices.

We will address objective (b) by describing quantitative metrics of species composition, diversity, richness, size, density, and percent cover. Conducting multiple transects at each sampling site will provide a robust estimate of mean and variance associated with each sampling variable, for a given site. Within each MPA, there will be multiple sampling locations (locations are based on habitat types) that are randomly chosen, so we avoid problems associated with auto correlation of samples.

We will address objective (c) by examining the similarities of fish and invertebrate assemblages among the four MPA and associated reference areas by comparing metrics of species composition, diversity, richness, size, density, and percent cover. For these comparisons, we will use a combination of parametric and non-parametric statistics, including ANOVAs, t-tests, regression analyses, K-S tests, MDS plots, Analyses of Similarities (Primer 6), etc. These analyses can also be used to compare fish and invertebrate communities in different MPAs in northern California with communities in other parts of the West Coast.

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