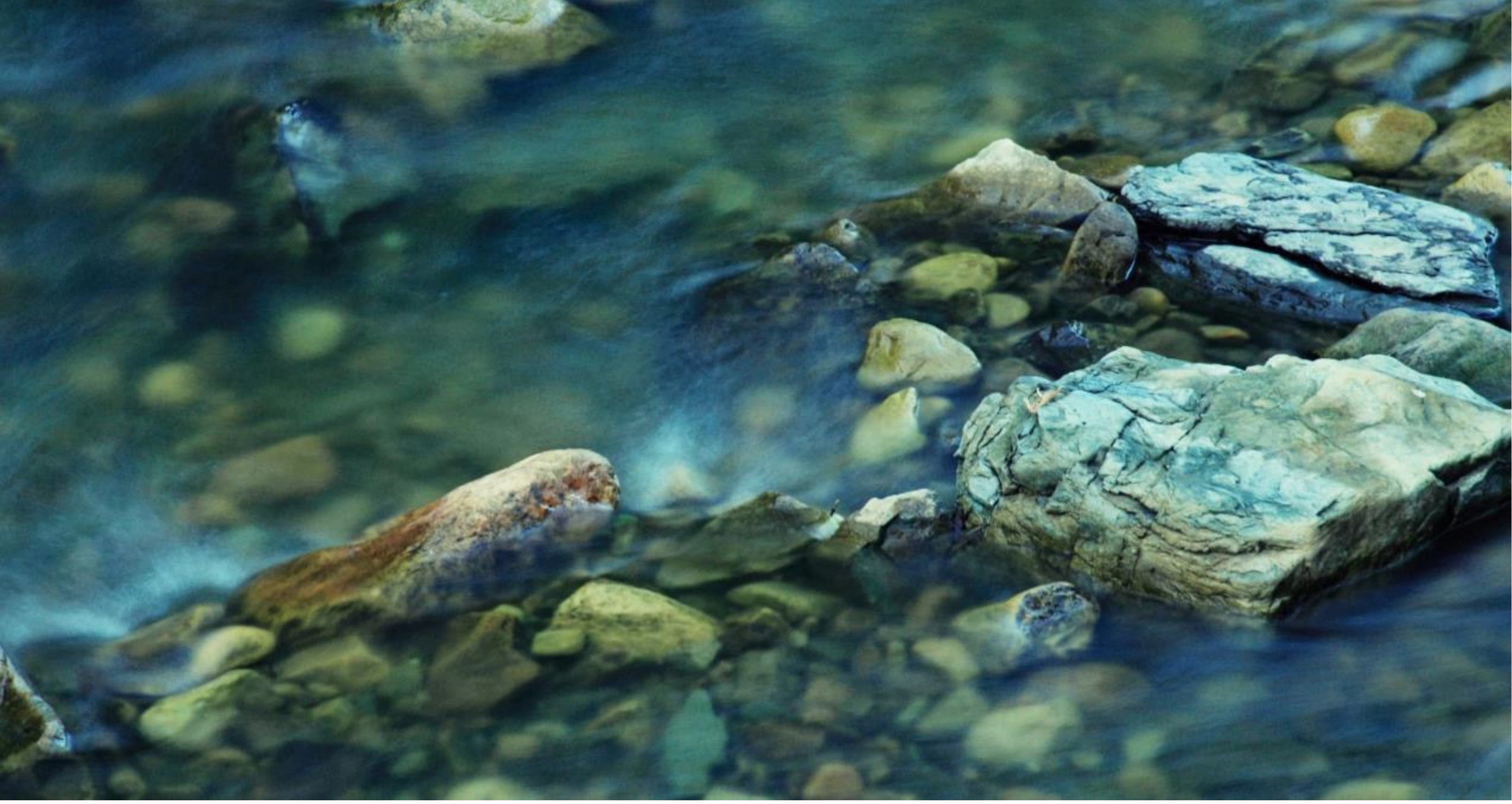

October 2024
Marine Carbon Dioxide Removal Pilot Study

National Pollutant Discharge Elimination System Ecological Safety Methodology

Prepared for Project Macoma, LLC

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ABBREVIATIONS

CO_2	carbon dioxide
Ecology	Washington State Department of Ecology
EPA	U.S. Environmental Protection Agency
ESA	Endangered Species Act
ESM	Ecological Safety Methodology
mCDR	marine carbon dioxide removal
MMAP	Marine Mammal Authorization Program
NMFS	National Marine Fisheries Service
NPDES	National Pollutant Discharge Elimination System
PARIS	Permitting and Reporting Information System
WDFW	Washington State Department of Fish and Wildlife

1 Introduction

This document is an attachment to the Washington State Department of Ecology (Ecology)-issued National Pollutant Discharge Elimination System (NPDES) Permit (Permit No: WA0991051) for Project Macoma. Project Macoma, LLC, a wholly owned subsidiary of Ebb Carbon, is conducting a temporary small-scale pilot project of Ebb Carbon's marine carbon dioxide removal (mCDR) technology at Terminal 7 of the Port of Port Angeles (Port) in Port Angeles, Washington. Ebb Carbon's mCDR technology has been developed to remove carbon dioxide (CO_2) from the atmosphere and address coastal acidification.

This NPDES Ecological Safety Methodology (ESM) documents the monitoring, reporting, and adaptive management protocols to be implemented during project operations. The ESM also outlines voluntary supplemental scientific studies Project Macoma is proposing to gather information to better understand environmental conditions at the project site.

As described in the NPDES Permit and Fact Sheet (Ecology 2024), Project Macoma will initiate operations in a phased approach to assess, monitor, and potentially adaptively manage operations to achieve project objectives in accordance with NPDES requirements (see Table 1).

Table 1
Operational Phases

Phase	Purpose	Discharge Events
Preliminary operations	Validate and update, if needed, the mixing zone model so it better reflects operational realities.	Limited discharges to validate mixing zone model (see Operations and Maintenance Manual for dye test procedures). Maintenance and routine discharge events occur under ebb tide conditions only.
Ongoing operations	Address acidification locally and remove excess CO_2 from the atmosphere.	Routine and maintenance discharge events.
Scientific operations	Understand how elevated pH discharge would equilibrate to standard oceanic conditions over distance from the outflow and within the water column.	Limited scientific discharge events occur under ebb tide conditions only. A submerged camera at a diffuser location will be deployed during scientific operations to capture footage of the discharge event to be reviewed, when necessary, based on visual observations. Recordings will be maintained for 24 hours before being overwritten.

The three discharge events noted in Table 1 are described as follows:

- **Routine discharge events** consist of three process flows: neutralized acidic stream, pretreatment reject and alkaline stream.
- **Maintenance discharge events** consist of one or more process flows including the pretreatment reject (seawater that is filtered out of the treatment process) and the neutralized acidic stream.
- **Scientific discharge events** consist of a single process flow, the alkaline stream.

This ESM describes monitoring protocols to be applied during all operational discharge events to collect and evaluate data and adaptively manage the system, when necessary, to maintain safe operations and compliance with permit requirements. The ESM is organized into three main components as follows:

- **Section 2:** Water quality compliance monitoring, reporting, and adaptive management (required by the NPDES permit)
- **Section 3:** Ecological monitoring, reporting, and adaptive management (required by the NPDES permit and reflective of reasonable and prudent measures specified in the 2024 Biological Opinion issued for the project by the National Marine Fisheries Service [NMFS])
- **Section 4:** Voluntary supplemental scientific studies and reporting to collect environmental data and document site conditions over time

2 Water Quality Compliance

2.1 Monitoring and Reporting

The U.S. Environmental Protection Agency (EPA) has delegated Clean Water Act NPDES implementation authority to Ecology. Ecology has established state water quality criteria that are consistent or more stringent than EPA criteria that are protective of human health and the environment (including ecological receptors). The NPDES Permit issued for this facility requires monitoring of operational discharge water quality in accordance with state and federal water quality standards. The water quality monitoring (parameters and frequency) and reporting requirements are detailed in Special Condition 2 and 3 of the Permit.

Results of water quality monitoring are publicly accessible via Ecology's Permitting and Reporting Information System (PARIS) database, which can be found online at:

- https://ecology.wa.gov/regulations-permits/guidance-technical-assistance/water-quality-permits-database.

The monitoring results are posted upon submittal of the discharge monitoring reports to Ecology. Other NPDES Permit submittals are also available on the PARIS database.

2.2 Adaptive Management

Adaptive Management with respect to water quality is defined by compliance with the effluent limitations and reporting requirements detailed in the permit. However, qualitative observations such as sheens or visible turbidity are included in the Adaptive Management Decision Framework (Figure 1).

3 Ecological Monitoring, Reporting, and Adaptive Management

Project Macoma is committed to the safe and responsible operation of the mCDR technology proposed as part of this pilot project. Efforts to support safe operation include monitoring and reporting protocols to avoid and minimize potential adverse impacts to the environment. Ecology has outlined supplemental best management practices in the NPDES Permit that will be followed throughout pilot project operations. This section outlines ecological monitoring, reporting, and adaptive management protocols.

3.1 Monitoring

Project Macoma will conduct ecological monitoring to ensure operational discharges proceed safely. Different levels of monitoring would occur based on the operational phases of the project: preliminary operations (i.e., the first runs of routine and maintenance discharge events), ongoing operations (including routine and maintenance), and scientific operations (see Table 2). Monitoring would primarily be conducted by a trained technician and a project biologist. Confirmation of safe start criteria will be met prior to operations, with reporting, and adaptive management if needed.

Table 2
Monitoring Overview

Project Phase	Discharge Event	Occurrence	Responsible Party
Preliminary operations (first runs)	Routine	Continuous; entire discharge event	Project biologist and trained technician
	Maintenance		
Ongoing operations	Routine	Daily; prior to discharge event	Project biologist and trained technician[1]
	Maintenance	Every two weeks, continuous 8-hour maximum)	
Scientific operations	Scientific	Continuous (ebb tide cycle)	Project biologist (first five events) Trained technician (subsequent events)

Note:
1. Training will occur during at least five preliminary and ongoing operation shifts with project biologist on site.

Observational Reporting Checklist (Appendix A)

- The Observational Reporting Checklist guides daily operations by specifying what biological conditions must be present to either begin or continue with operations.
- The Observational Reporting Checklist also specifies under which operational conditions further coordination would be necessary to determine whether adaptive management actions may be needed.

3.2 Reporting

As discussed previously, the Observational Reporting Checklist in Appendix A will be used to observe biological conditions each day and to record observations, including those that might trigger formal reporting requirements. All contact information for reporting will be on-site and available to all personnel. The permit-required reporting requirements that Project Macoma will follow are summarized as follows:

- **NMFS: Incidental take reporting.** NMFS has allowed for take as part of the pilot study implementation in accordance with appropriate ESA regulatory requirements and reporting. Therefore, any incidental take (i.e., capture, harm, harass, or kill) of a protected species will result in applicable reporting to the NOAA NMFS regional office, Northwest Fisheries Science Center. Notes of any incident will be recorded, including date, time, location of the incident, species involved, circumstance of the activity that led to the take, and description of the animals' condition (photographs or videos taken when possible).[1]
- **NMFS: Marine Mammal Authorization Program (MMAP) mortality/injury reporting.** If a marine mammal is harassed, becomes entangled, suffers an injury or death resulting from project activities, Project Macoma will complete the MMAP reporting form for submittal to NMFS. All documentation will be emailed to NMFS and copies will be maintained in Project Macoma's records. Follow-up actions or requests would be subject to NMFS and full cooperation for compliance with the Endangered Species Act (ESA) and the Marine Mammal Protection Act.
- **Washington State Department of Fish and Wildlife (WDFW): Non-ESA-listed fish mortality reporting.** In accordance with the WDFW HPA Permit 2024-6-217+01, if a fish kill occurs or fish are observed in distress, the WDFW will be immediately notified of the problem. If the likely cause of the fish kill or fish distress is related to water quality, Washington Military Department Emergency Management Division will also be notified. The WDFW will advise on next steps and may require additional measures to mitigate impacts.
- **Washington State Department of Ecology and NMFS: ESM compliance.** The Observational Reporting Checklist (Appendix A) will be compiled and retained on site. A final report detailing the timing and adaptive management strategies employed will be provided to both Ecology and NMFS.

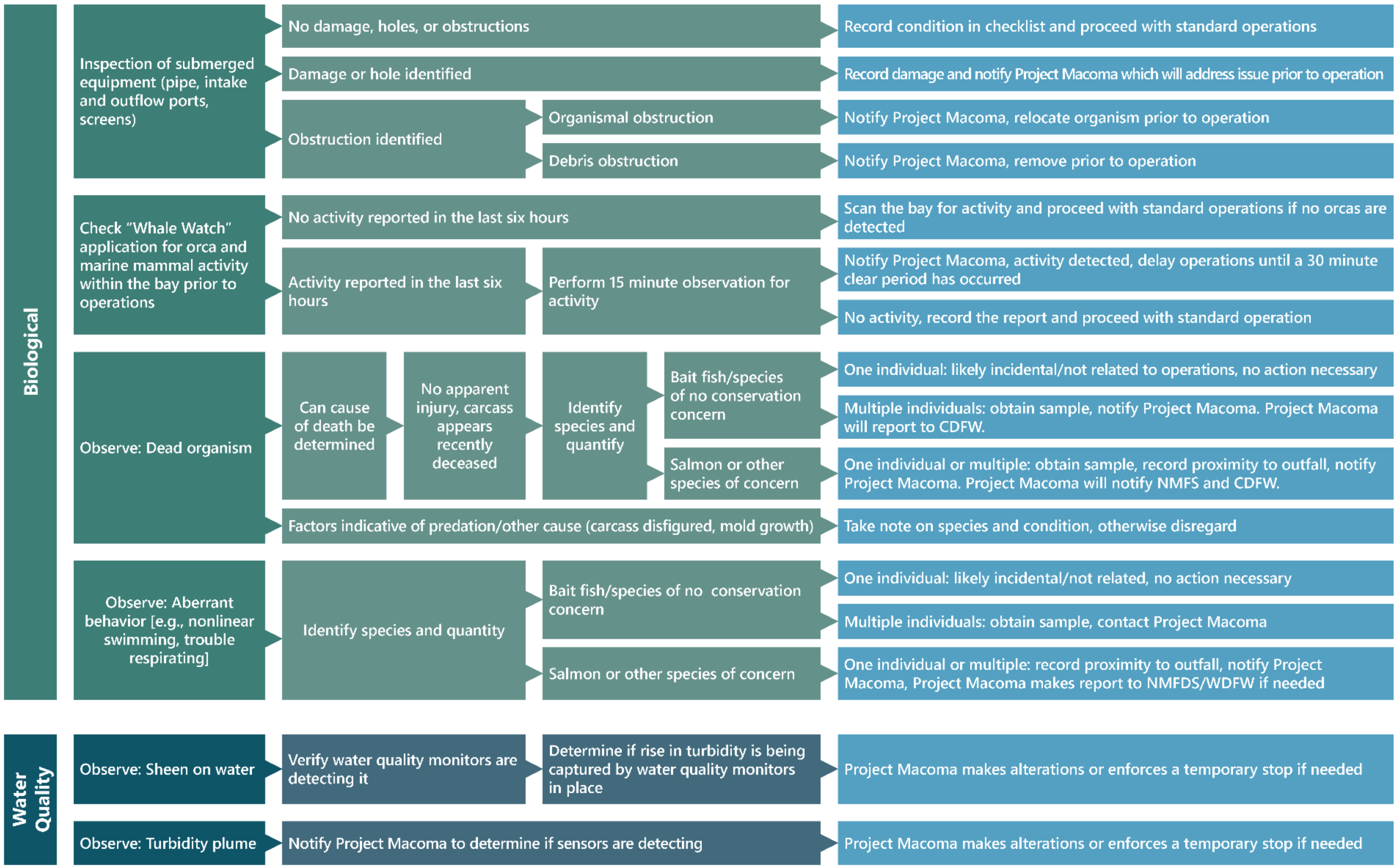
[1] As stated in Special Condition 11 of the NPDES permit, "nothing in this permit authorizes the take for the purpose of a facility's compliance with the Endangered Species Act." For clarity, NPDES permit cannot by definition authorize take which has been authorized for this pilot study by NMFS.

3.3 Adaptive Management

Project Macoma will adaptively manage operations to maintain ecological safety as informed by monitoring, data collection through recorded observations, and collaboration with agencies when reporting occurs. In addition, completed copies of the Observational Reporting Checklist in Appendix A will be maintained on site so Project Macoma and the on-site monitor can review completed checklists to further inform adaptive management over time.

The checklist specifies what biological conditions must be present to either begin or continue with operations. The checklist also specifies under what operational conditions further coordination is necessary to determine whether adaptive management actions may be needed. The decision matrix for the adaptive management framework is shown in Figure 1.

Figure 1
Adaptive Management Decision Framework



4 Voluntary Supplemental Monitoring and Scientific Studies

Project Macoma will also be completing voluntary scientific studies, including those outlined in Table 3. These studies are being performed under the discretion of Project Macoma to collect additional scientific data over the course of operations. The data will be maintained and reported to Ecology at the end of the project or upon request. Ongoing surveys may be completed pending results of the baseline survey and resources available to support future voluntary efforts.

Table 3
Voluntary Supplemental Scientific Studies and Reporting

Study	Baseline Survey	Ongoing Surveys	Reporting Protocol
Ex Situ Salmon Testing	Pre-Operation	May be conducted prior to scientific operations	Report submitted to Ecology 60 days following test completion
Vegetation survey Eelgrass and macroalgae survey	September 2024	May occur and may be iterated upon during and/or after project operations depending on the results of the baseline survey	Interpretative baseline survey report submitted to Ecology within 60 days of field work completion
Sediment Profile Imaging[1] Plan view and cross-sectional view profiles	Pre-Operation	May occur annually in the same month as the baseline operations were conducted	Interpretative report submitted to Ecology within 60 days of field work completion

Notes:

1. Sediment Profile Imaging locations and methods are detailed in Appendix B

5 Reference

Ecology (Washington State Department of Ecology), 2024. Fact Sheet for NPDES Permit WA0991051: Project Macoma, LLC. Draft. July 19, 2024.

Appendix A
Observational Reporting Checklist

<table>
<tr><td colspan="2">Name:</td><td colspan="2">Time start:</td><td colspan="3">Discharge Event:</td></tr>
<tr><td colspan="2">Date:</td><td colspan="2">Time end:</td><td colspan="3">Discharge event #:</td></tr>
<tr><td></td><td>Checklist Item</td><td colspan="2">Recorded Observations</td><td colspan="3">Action</td></tr>
<tr><td rowspan="7">Biological</td><td rowspan="3">Whale Alert Application (www.whalealert.org)</td><td colspan="2" rowspan="2">Orcas observed - previous 6 hours (time observed, species, number) ☐
______
______
______
______
______</td><td rowspan="2">Perform 15-minute observation ☐</td><td>Orcas spotted: wait until there is a 30-minute clear period ☐</td><td>Project Macoma to determine safety to begin/continue operations ☐</td></tr>
<tr><td colspan="2">No Orcas spotted: Begin/Continue Operations ☐</td></tr>
<tr><td colspan="2">No orcas reported - previous 6 hours ☐</td><td colspan="3">Begin/Continue Operations ☐</td></tr>
<tr><td rowspan="4">Sensitive Species (see Biological Assessment)</td><td rowspan="4">Present (Species, number, and behavior) ☐
______
______
______
______</td><td>Normal Behavior ☐</td><td colspan="3">Begin/Continue Operations ☐</td></tr>
<tr><td>Aberrant Behavior ☐</td><td>Immediate notification to project biologist and Ebb on-site manager ☐</td><td colspan="2">Project Macoma to determine mCDR system safety to begin/continue operations ☐</td></tr>
<tr><td>Dead Organism ☐</td><td>Immediate notification to Project Macoma on-site manager and WDFW (Fish)/NMFS ☐</td><td colspan="2">Operation does not begin or is halted until Project Macoma on-site manager confirms mCDR system is safe to operate</td></tr>
</table>

<table>
<tr><td colspan="2">Name:</td><td colspan="2">Time start:</td><td colspan="2">Discharge Event:</td></tr>
<tr><td colspan="2">Date:</td><td colspan="2">Time end:</td><td colspan="2">Discharge event #:</td></tr>
<tr><td></td><td>Checklist Item</td><td colspan="2">Recorded Observations</td><td colspan="2">Action</td></tr>
<tr><td rowspan="7">Biological</td><td rowspan="2">Sensitive Species (see Biological Assessment)</td><td></td><td></td><td></td><td>☐</td></tr>
<tr><td colspan="2">Absent
☐</td><td colspan="2">Begin/Continue Operations
☐</td></tr>
<tr><td rowspan="4">Non-Sensitive Species</td><td rowspan="3">Present
(Species, number, and behavior)
☐
____
____
____
____________</td><td>Normal Behavior
☐</td><td colspan="2">Begin/Continue Operations
☐</td></tr>
<tr><td>Aberrant Behavior
☐</td><td>Immediate notification to project biologist and Ebb on-site manager
☐</td><td>Project Macoma to determine mCDR system safety to begin/continue operations
☐</td></tr>
<tr><td>Dead Organism
☐</td><td>Immediate notification to Project Macoma on-site manager and WDFW (Fish)
☐</td><td>Project Macoma to determine mCDR system safety to begin/continue operations
☐</td></tr>
<tr><td colspan="2">Absent
☐</td><td colspan="2">Begin/Continue Operations
☐</td></tr>
<tr><td>Inspect ecological safety of equipment</td><td>Condition of submerged equipment pipe/screen inspected
☐</td><td>Damage observed (hole, size, etc.; add notes below)
☐</td><td>Immediate notification to Project Macoma on-site manager who coordinates necessary repairs
☐</td><td>Project Macoma to determine mCDR system safety to begin/continue operations
☐</td></tr>
</table>

<table>
<tr><td colspan="2">Name:</td><td colspan="2">Time start:</td><td colspan="3">Discharge Event:</td></tr>
<tr><td colspan="2">Date:</td><td colspan="2">Time end:</td><td colspan="3">Discharge event #:</td></tr>
<tr><td></td><td>Checklist Item</td><td colspan="2">Recorded Observations</td><td colspan="3">Action</td></tr>
<tr><td rowspan="4">Biological</td><td rowspan="4">Inspect ecological safety of equipment</td><td></td><td>No damage observed
☐</td><td colspan="3">Begin/Continue operations
☐</td></tr>
<tr><td rowspan="2">Species present on equipment (Species, number, and behavior)
☐
____
____
____
____________________</td><td>Sunflower star
☐</td><td>Immediate notification to Project Macoma on-site manager and NMFS
☐</td><td>Relocate animal to suitable habitat outside project area
☐</td><td>Project Macoma to determine mCDR system safety to begin/continue operations
☐</td></tr>
<tr><td>Non-sensitive species
☐</td><td colspan="2">Relocate animal to suitable habitat outside project area
☐</td><td>Project Macoma to determine mCDR system safety to begin/continue operations
☐</td></tr>
<tr><td colspan="2">Species not on equipment
☐</td><td colspan="3">Begin/Continue operations
☐</td></tr>
<tr><td>Water Quality</td><td>Sheen</td><td colspan="2">Present (size/color)
☐
____
____
____________________</td><td colspan="2">Project Macoma on-site manager is immediately notified to determine source of sheen (implements corrective actions if necessary)
☐</td><td>Project Macoma to determine mCDR system safety to begin/continue operations
☐</td></tr>
</table>

Name:		**Time start:**	**Discharge Event:**	
Date:		**Time end:**	**Discharge event #:**	
	Checklist Item	Recorded Observations	Action	
Water Quality		Absent ☐	Begin/Continue Operations ☐	
	Turbidity	Noticeably higher within project site compared to surrounding water ☐	Project Macoma on-site manager is notified to determine source of turbidity (implements corrective actions if necessary) ☐	Project Macoma to determine mCDR system safety to begin/continue operations ☐
		Absent ☐	Begin/Continue Operations ☐	

Name:		Time start:	Discharge Event:
Date:		Time end:	Discharge event #:
	Checklist Item	Recorded Observations	Action

Additional Notes: Include any temporal changes (e.g., reduction in visible submerged aquatic vegetation, algal/bacterial mats forming near project equipment, or perceived visible changes to health of project site encrusting organisms):

___

___

___

___

___

___

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Appendix B
Sediment Profile Imaging Details

LEGEND:

Approximate Chronic Mixing Zone (207 Feet)

Sediment Profile Imaging Target Location

0 100
Feet

Publish Date: 2024/10/04, 2:17 PM | User: joliver
Filepath: \\gstfile01\gis\Jobs\Ebb_Carbon_2331\Maps\reports\EcoSafetyMethodology\EcoSafetyMethodology.aprx\AQ_Macoma_ESM_AppendixB_SedProfileImagingLocation

ANCHOR QEA

Appendix B
Sediment Profile Imaging Locations
Ecological Safety Methodology
Project Macoma

Location ID	X	Y
1	999,827.5	423,098.8
2	999,910.2	423,042.6
3	999,606.0	423,128.4
4	999,688.6	423,072.2
5	999,771.3	423,016.0
6	999,854.1	422,959.9
7	999,936.8	422,903.7
8	999,549.7	423,045.7
9	999,632.4	422,989.5
10	999,715.2	422,933.3
11	999,797.9	422,877.1
12	999,880.6	422,821.0
13	999,493.5	422,963.0
14	999,576.2	422,906.8
15	999,659.0	422,850.6
16	999,741.7	422,794.4
17	999,824.4	422,738.2
18	999,520.1	422,824.1
19	999,602.8	422,767.9
20	999,685.5	422,711.7
21	999,768.2	422,655.5

Notes:

1. Coordinates presented in: NAD 1983 (2011) StatePlane Washington North FIPS 4601 (U.S. Feet)

1.0 SPI Survey Operations

1.1 Sediment Profile Imaging

Sediment Profile Imaging (SPI) will be collected using an Ocean Imaging Systems (OIS) model 3731 digital sediment-profile camera (Figure 1). The sediment-profile camera consists of a wedge-shaped prism with a Plexiglas face plate and a back mirror mounted at a 45-degree angle. Light is provided by an internal strobe. The mirror reflects the image of the profile of the sediment-water interface up to a 35 mm digital camera that is mounted horizontally on top of the prism. The camera can obtain images of up to 20 cm of the upper sediment column in profile.

The camera prism is mounted on an assembly that can be moved up and down within a stainless-steel frame by allowing tension or slack on the winch wire. As the camera is lowered, tension on the winch wire keeps the prism in the up position. Once the camera frame touches the bottom, slack on the winch wire allows the prism to vertically intersect the seafloor. The rate of fall of the prism (6 cm/second) is controlled by an adjustable passive hydraulic piston, which minimizes the disturbance of the sediment-water interface.

A trigger is tripped on impact with the bottom, activating a 13 second time-delay on the shutter release; this gives the prism a chance to obtain maximum penetration before an image is collected. After an image is collected, the camera is raised from the bottom, a wiper blade automatically cleans off any sediment adhering to the prism faceplate, and the strobes are recharged. The camera can then be lowered to collect another replicate image.

When the camera is brought to the surface, optical prism penetration is measured from a penetration indicator, which measures the distance the prism falls relative to the camera base. Two weight racks, each capable of holding 125 lbs. (55 kg) of lead (in 25 lb. [11 kg] increments) can be loaded to increase penetration. If penetration is too great, adjustable stops, which control the distance the prism can descend, can be lowered, and “mud” doors can be attached to each side of the frame to increase the bearing surface of the entire unit.

The SPI camera will be lifted and dropped at each station a minimum of three times to provide three SPI images per station.

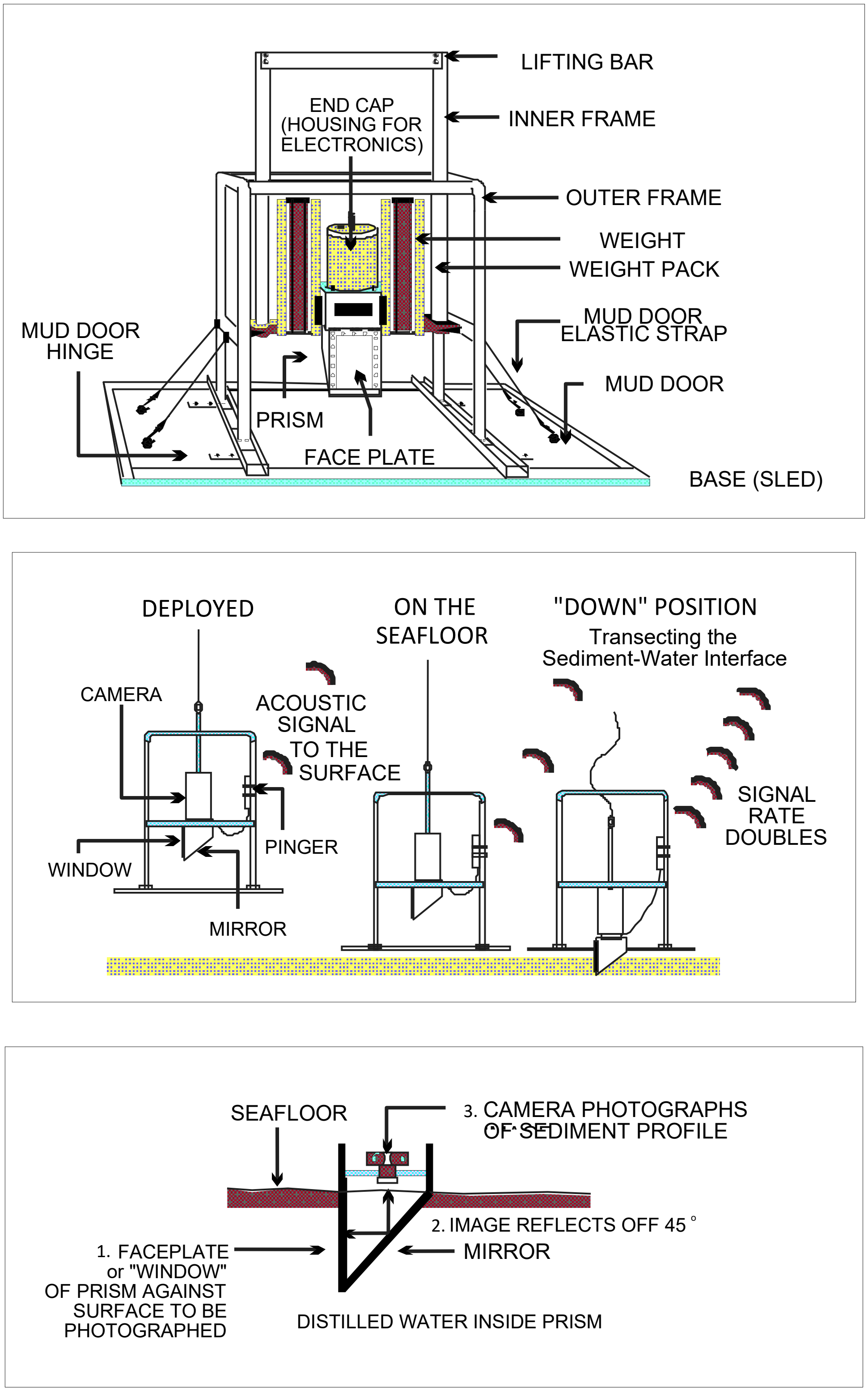


Figure 1. Schematic diagram of the sediment profile camera and sequence of operation on deployment.

1.2 SPI Image Analysis

Computer image analysis of SPI images follows a formal and standardized technique developed by Rhoads and Germano (1982, 1986). Physical and biological parameters are measured directly from the digital SPI images by an SPI image analyst using computer image analysis software. At a minimum the image analysis parameters for this project will include:

- Camera prism penetration depth (cm)
- Grain size major mode and range in phi sizes
- Surface boundary roughness
- Mud clasts
- Depth of the apparent redox potential discontinuity (aRPD) (cm)
- Infaunal successional stage
- Organic Loading - Sediment Oxygen Demand, Sedimentary Methane, and Thiophilic Bacterial Colonies
- Presence and thickness of sedimentary layers (cm)
- Wood debris (estimate of percent coverage)

All data will be edited and verified by a senior-level scientist before final data synthesis, statistical analysis, and interpretation. Specific measurement techniques and interpretive criteria for each parameter are presented below.

1.2.1 Prism Penetration Depth

The prism penetration depth is determined by measuring both the largest and smallest linear distance between the sediment-water interface and the bottom of the film frame. Observations regarding the nature and condition of the sediment-water interface are also recorded. Comparative penetration depths from stations of similar grain-size give an indication of relative sediment water content and shear strength.

1.2.2 Grain Size Major Mode

The sediment grain-size major mode and range, in phi units, are visually determined from the SPI images by overlaying a grain-size comparator at the same scale. This comparator was prepared by photographing a series of Udden-Wentworth size classes (equal to or less than coarse silt up to granule and larger sizes) through the SPI optical system. Seven grain-size classes are on this comparator: ≥ 4 phi (silt/clay), 4 to 3 phi (very fine sand), 3 to 2 phi (fine sand), 2 to 1 phi (medium sand), 1 to 0 phi (coarse sand), 0 to -1 phi (very coarse sand), and < -1 phi (gravels). The lower limit of optical resolution is approximately 62 micrometers (μm), allowing recognition of grain sizes equal to or greater than coarse silt. The accuracy of this method has been documented by comparing SPI estimates with grain-size statistics determined from laboratory sieve analyses (SAIC 1986).

1.2.3 Surface Boundary Roughness

Surface boundary roughness is determined by measuring the vertical distance (parallel to the image border) between the highest and lowest points of the sediment-water interface. In addition, the origin (physical or biogenic) of this small-scale topographic relief is sometimes evident and can be recorded. In sandy sediments, boundary roughness can be a measure of sand-wave height. On silt-clay bottoms, boundary roughness values often reflect biogenic features such as fecal mounds or surface burrows. These features are abundant only in areas where boundary shear stresses are low enough that such delicate features are preserved. Other surface features are noted when evident, including shell fragments/lag deposits, mud-clay clasts, and wood debris.

1.2.4 Mud Clasts

When fine-grained, cohesive sediments are disturbed, either by physical bottom scour or faunal activity (e.g., decapod foraging), intact clumps of sediment are often scattered about the seafloor. Recent physical disturbance such as dredged material disposal can also result in the presence of relict sediment clumps on the seafloor. These mud or clay clasts can be seen at the sediment-water interface in SPI images and their abundance, distribution, oxidation state, and appearance of mud clasts may be used to make inferences about the recent pattern of seafloor disturbance.

1.2.5 Apparent Redox Potential Discontinuity (aRPD) Depth

The depth of the aRPD, which is the change from oxidized to reduced sediment, can be measured using SPI imaging and computer image analysis. The upper surface of aerobic fine-grained sediments has a higher light reflectance value than underlying hypoxic or anoxic sediments. This is readily apparent in SPI images and is due to oxidized surface sediment that contains minerals in an oxidized state (typically an olive brown color), while the reduced sediments below this oxygenated layer are generally green, gray, blue, or black. The boundary between the colored ferric hydroxide surface sediment and underlying sediment is called the aRPD. The aRPD is a sensitive indicator of infaunal succession, sediment bioturbation activity, and sediment oxygen demand. The depth of the aRPD has proven to be a useful parameter for mapping gradients of enrichment on the seafloor (Rhoads and Germano 1982, Lyle 1983).

The actual RPD is the boundary that separates the positive Eh region (presence of free oxygen) of the sediment column from the underlying negative Eh region (absence of free oxygen). The exact location of the Eh boundary (where Eh = 0) can only be determined with microelectrodes. Therefore, the reflectance boundary observed in the SPI images is termed the apparent RPD. In general, the depth of the actual RPD will be shallower than the depth of the apparent RPD, because organisms cause bioturbation of ferric hydroxide-coated particles downward below the Eh = 0 horizon. As a result, the apparent RPD depth provides an estimate of the degree of biogenic sediment mixing. This variable is important in evaluating the effect of colonizing benthos on disposed materials. Bioturbation vertically transports buried reduced compounds to the sediment surface and exposes them to an oxidizing water column (Aller 1982). Bioturbation also affects sediment transport by changing the physical properties of sediments and their mechanical behavior (Rhoads and Boyer 1982).

Another important characteristic of the aRPD is the contrast in reflectance values at this boundary. This contrast is related to the interactions among the degree of organic-loading in the sediment, bioturbation, and bottom-water dissolved oxygen levels. High inputs of labile organic material increase sediment oxygen demand, stimulate sulfate reduction rate, and result in sulfitic products. This results in more highly reduced (lower reflectance) sediments at depth and higher aRPD contrasts. In a region where generally low aRPD contrasts exist, images with high aRPD contrasts indicate localized sites of relatively high inputs of organic-rich material.

1.2.6 Infaunal Successional Stage

The mapping of infaunal successional stages from SPI images is based on the theory that organism-sediment interactions follow a predictable sequence after a disturbance. This theory states that primary succession results in "the predictable appearance of macrobenthic invertebrates belonging to specific functional types following a benthic disturbance and these invertebrates interact with sediments in specific ways. Moreover, functional types are the biological units of interest, and by definition do not demand a sequential appearance of particular invertebrate species or genera" (Rhoads and Boyer 1982).

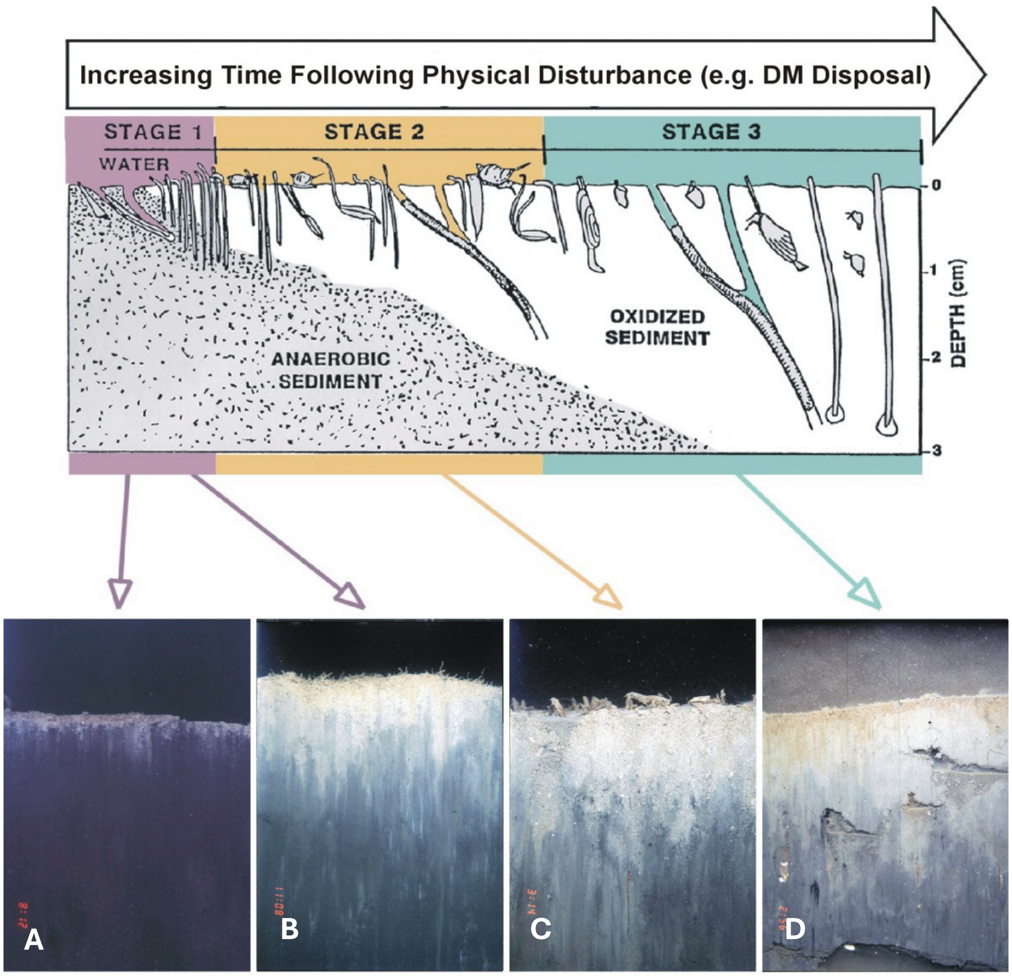
Benthic disturbance can result from natural processes, such as seafloor erosion, changes in seafloor chemistry, and predator foraging, as well as from human activities like dredging, dredged material disposal, bottom trawling, pollution from industrial discharge, and excessive organic loading. Evaluation of successional stages involves deducing dynamics from structure, a technique pioneered by R. G. Johnson (1972) for marine soft-bottom habitats. The application of this approach to benthic monitoring requires in situ measurements of salient structural features of organism-sediment relationships as imaged through SPI.

Infaunal succession following a major seafloor disturbance initially involves pioneering populations (Primary or Stage I succession) of very small organisms that live at or near the sediment/water interface (Pearson and Rosenberg 1978, Rhoads and Germano 1986). In the absence of further disturbance, infaunal deposit feeders eventually replace these early successional assemblages. The start of this "infaunalization" process is designated as Stage II. Large, deep-burrowing infauna (Stage III taxa) represents a high order successional stage typically found in areas of low disturbance. Pioneering assemblages (Stage I assemblages) usually consist of dense aggregations of near-surface living, tube-dwelling polychaetes; alternately, opportunistic bivalves may colonize in dense aggregations after a disturbance (Rhoads and Germano 1982, Santos and Simon 1980). These functional types are usually associated with a shallow redox boundary; and bioturbation depths are shallow, particularly in the earliest stages of colonization (Figure 2).

Many deep-burrowing infauna feed at depth in a head-down orientation. This localized feeding activity results in distinctive excavations called feeding voids. Diagnostic features of these feeding structures include a generally semicircular shape with a flat bottom and arched roof, and a distinct granulometric change in the sediment particles overlying the floor of the structure. The relatively coarse-grained material represents particles rejected by head-down deposit-feeders, as this deep-dwelling infauna preferentially ingest the finer sediment particles. Other subsurface structures, including burrows or methane bubbles, do not exhibit these characteristics. The bioturbation activities of these deposit-feeders are responsible for aerating the sediment and causing the redox horizon to be located several centimeters below the sediment-water interface.

The presence of Stage III feeding voids indicates the presence of Stage III organisms. The mapped distribution of deep infaunal assemblages may be useful in identifying undisturbed sites in both shallow and deep-water environments. In sediment environments rich in labile organic material, it is not uncommon to observe Stage I communities coexisting with Stage II or III communities (Rhoads and Germano 1986). These mixed infaunal stages are classified as Stage I on II, or Stage I on III.

In sandy, dynamic environments, the climax communities may consist primarily of surface dwellers that reside in the upper cm of the sediment surface and have few if any naturally burrowing community members. These type communities are classified as Stage I communities by SPI image analysis reflective of an area influenced by physical factors (e.g., higher energy) and the presence of a sandy substrate, rather than a higher order successional stage that would typically be assigned a climax community (as described above) in a depositional environment dominated by a silt/clay substrate.



The development of infaunal successional stages over time following a physical disturbance or with distance from an organic loading source (from Rhoads and Germano 1986). The SPI images below the drawing provide examples of the different successional stages.

Image A: Image A shows highly reduced sediment with a very shallow redox layer (contrast between light colored surface sediments and dark underlying sediments) and little evidence of infauna.

Image B: Numerous small polychaete tubes are visible at the sediment surface in image B (Stage I), and the redox depth is deeper than in image A.

Image C: A mixture of polychaete and amphipod tubes occurs at the sediment surface in image C (Stage II).

Image D: Image D shows numerous burrow openings and feeding pockets (voids) at depth within the sediment; these are evidence of deposit-feeding, Stage III infauna. Note the RPD is relatively deep in this image, as bioturbation by the Stage III organisms has resulted in increased sediment aeration and causing the redox horizon to be located several centimeters below the sediment-water interface.

Figure 2. Successional stage assemblages and relationship to SPI

1.2.7 Organic Loading - Sediment Oxygen Demand, Sedimentary Methane, and Thiophilic Bacterial Colonies

Sediment oxygen demand (SOD) represents the overall rate of oxygen consumption in surface sediments from chemical and biological processes. The relative amount of organic enrichment is indicated by sediment color; darker coloration indicates more reduced sediments with greater organic loading and higher SOD (Fenchel 1969, Lyle 1983).

Under high organic matter loading and subsequently high SOD, microbial sulfate reduction proceeds and may completely deplete porewater sulfate concentrations. Under these conditions, methanogenesis can occur, leading to methane bubbles in the sediment column. In SPI, methane appears as gas-filled voids with a glassy texture (due to the reflection of the strobe off the gas bubble).

Similarly, under highly reduced anoxic conditions, thiophilic sulfate-reducing bacterial colonies (*Beggiatoa*) can exist on the sediment surface (at the interface between oxic and anoxic conditions). Bacterial colonies typically live within sediments and their presence on the surface indicates the lack of oxygen in underlying sediments. Bacterial mats can have the appearance of layers of white fibrous material overlying sediments that may appear black and anoxic (Nilsson and Rosenberg 1997, Germano et al. 2011).

1.2.8 Sedimentary Layering

The ability to detect sedimentary layering in SPI is dependent on observed differences in grain size, color, and other sedimentary characteristics such as organic material and or/debris. Layering can be the result of natural processes such as seasonal variation in sedimentation as well as anthropogenic disturbances such as dredged material disposal.

1.2.9 Wood Debris and Percent Coverage

Presence of wood debris relies on the visual identification of wood-like particles in surface sediments that contrast with the surrounding sedimentary texture. Wood debris can consist of a range of particle sizes, may be slower to decay, and may be visible and identifiable (e.g., dark or brown particles, shards, bark or large wood pieces). The wood debris may be mixed with fine-grained, sulfide-rich, and organic-rich sediments that are dark gray or black in color. These layers may or may not be greater than the camera prism penetration depth. If less than penetration depth, the thickness of the layer is measured. The estimate of percent wood debris in SPI images will then be determined visually using Munsell charts for estimation proportions of mottled and coarse fragments (GretagMacbeth 2000).

1.3 Plan View Imaging

Plan view (PV) images will be captured using a downward-facing underwater Chimaera MKII camera with external flash, manufactured by SubC Control, Newfoundland, Canada (Figure 3). The plan view camera and external flash are mounted on the frame of the SPI camera in a downward-looking orientation. Images are collected just before the SPI camera touches the seafloor, using a lead ball and cable attached to a bounce trigger. When the SPI camera is raised from the bottom, the bounce trigger is reset, and the plan view camera is ready to take another image. The total surface area captured in the plan view camera field of view can increase or decrease depending on water clarity at the site. Initial settings for the plan view camera will capture a 6 square foot (ft^2) area (0.6 square meter [m^2] area) of the seafloor and may be modified depending on water clarity. The Chimaera MKII is equipped with dual lasers located 62.5 millimeter (mm) apart allowing an accurate scale to be applied to each image. Triplicate plan view images will be attempted in conjunction with the triplicate SPI camera drops. On some occasions, the second or third replicate plan view images can be cloudy or turbid if fine grained sediments are disturbed after the first SPI camera drop. A minimum of one acceptable plan view image will be collected at each station.

1.4 Plan View Image Analysis

PV imaging provides a larger view of surface sediment conditions and can provide valuable information on landscape ecology and sediment topography. For example, irregular surface distribution of dredged material (e.g., clumps of silts/clays) may be observed in PV imaging but may not be evident in the co-located SPI images. The underwater PV lasers allow for an accurate scale to be applied to each image and can allow for measurement of bedform wavelengths and provide density counts of biological features (e.g., burrows, epifaunal colonies, large macrofauna or fish).

PV analysis will consist of evaluating the images for the following physical and biological features:

- Surface sediment type and grain size characteristics (e.g., sand and silt, silt, sand, depositional, biogenic, physical surface)
- Bedforms (presence: Yes/No)
- Mud clasts (presence: Yes/No; count)
- Presence of wood debris (presence: Yes/No; description)
- Presence of man-made debris (presence: Yes/No; description)
- Burrows, mounds (presence: Yes/No; count/density)
- Tubes (presence: Yes/No; count/density)
- *Lebenspurren* (i.e., biologically formed, sedimentary structures found in sediments, including tracks, trails, burrows, and fecal casts) (presence: Yes/No; count and/or description)
- *Beggiatoa* (presence: Yes/No; description)
- Flora/fauna present on the seafloor (presence: Yes/No; count and/or description)

2.0 Reporting

A data summary report will be prepared and will include data tables and Geographic Information System (GIS) generated figures of the key data parameters:

a. Geographic coordinates for collected and analyzed SPI and PV images
b. Depth of SPI prism penetration for each station location
c. Grain-size major mode and range
d. Boundary roughness at each station location
e. Evidence of erosional or depositional environments
f. Depth of the apparent RPD at each station location
g. Infaunal successional stage at each station location
h. Evidence of organic loading and high sediment oxygen demand
i. Presence of epifauna at each station
j. Sedimentary layers, if present
k. Wood debris percent coverage, if present

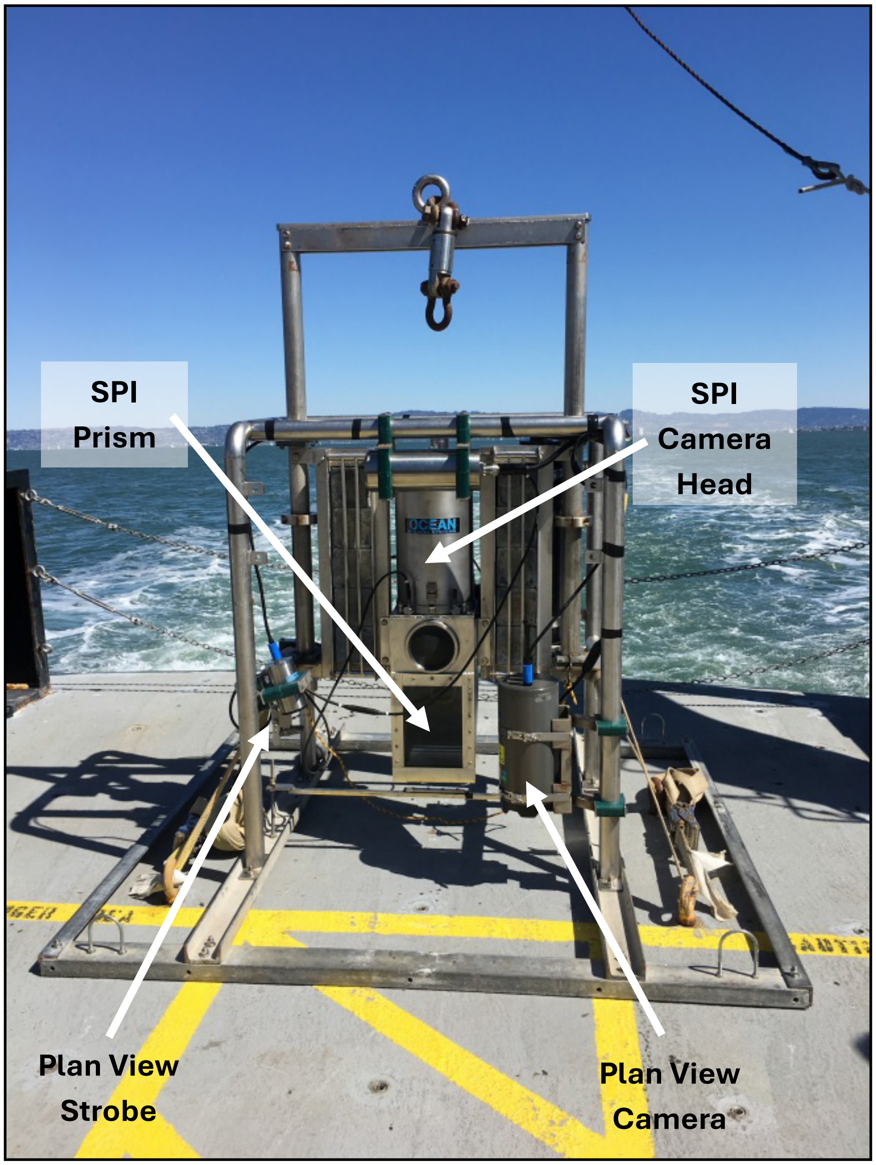


Figure 3. Chimaera MKII plan view camera and strobe attached to OIS 3731 SPI camera

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