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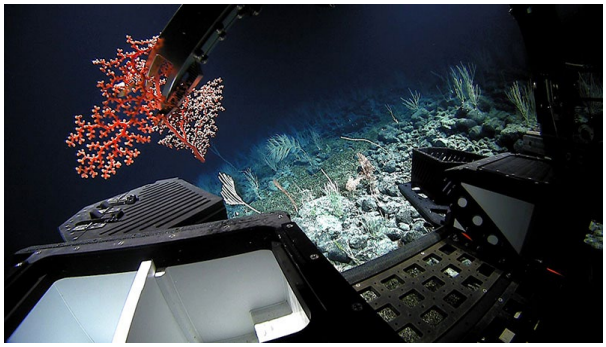
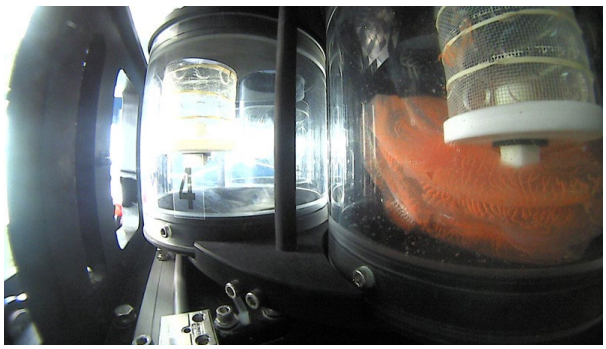
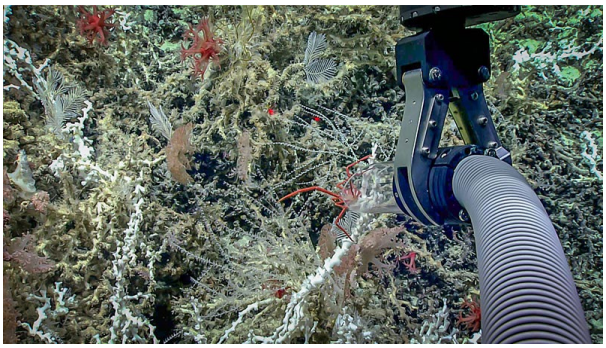
NOAA Ocean Exploration Sampling Procedures Manual



Authors: Christopher Dunn[1], Thomas Morrow[1], Matt Dornback[2], Kasey Cantwell[1], Abigail Reft[4], Kimberly Galvez[2], Rachel Medley[1], Adrienne Copeland[1], Christa Rabenold[3], Bill Moser[4], Courtney Wickel[4], Michael O'Mahoney[4], Karen Reed[4], Mike Vecchione[4], Stephanie Bush[4], Diane Pitassy[4], Dave Pawson[4], Doug Eernisse[5]

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[1] NOAA Ocean Exploration
[2] NOAA Ocean Exploration, FedWriters
[3] NOAA Ocean Exploration, University Corporation for Atmospheric Research
[4] Smithsonian National Museum of Natural History
[5] Department of Biological Science, California State University, Fullerton

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This manual will be updated over time as operations evolve. This version is V.1.0 (2023). The most recent version is available at https://oceanexplorer.noaa.gov/data/publications/welcome.html.

For questions associated with this manual and the processes and reports noted herein, contact ex.expeditioncoordinator@noaa.gov.

Table of Contents

1. Introduction

NOAA Ocean Exploration is dedicated to exploring the unknown ocean, unlocking its potential through scientific discovery, technological advancements, and data delivery. By working closely with partners across public, private, and academic sectors, NOAA Ocean Exploration is filling gaps in our basic understanding of the marine environment. This allows us, collectively, to protect ocean health, sustainably manage our marine resources, accelerate our national economy, better understand our changing environment, and enhance appreciation of the importance of the ocean in our everyday lives.

With priority placed on exploration of deep waters and the waters of the U.S. Exclusive Economic Zone, NOAA Ocean Exploration applies the latest tools and technologies to explore previously unknown areas of the ocean, making discoveries of scientific, economic, and cultural value. By making collected data publicly available in increasingly innovative and accessible ways, NOAA Ocean Exploration provides a unique and centralized national resource of critical ocean information. Through live exploration video, online resources, training and educational opportunities, and public events, NOAA Ocean Exploration shares the excitement of ocean exploration with people around the world and inspires and engages the next generation of ocean scientists, engineers, and leaders.

The purpose of this manual is to document the sampling operations, data archiving procedures, and reporting requirements used by NOAA Ocean Exploration for expeditions on NOAA Ship *Okeanos Explorer*. While this manual is specific to NOAA Ocean Exploration, it may be useful for use on other research vessels as well. Additional guidance, standard operating procedures, and worksheets are available upon request from NOAA Ocean Exploration.

2. Principles of Exploration Sampling

Based on its experience of more than a decade of ocean exploration on *Okeanos Explorer*, NOAA Ocean Exploration developed a unique model of community-driven exploration that serves broad community interests and addresses data gaps while being responsive to specific community, agency, administration, and global priorities and drivers. This “Explorer Model” brings together the ocean science and resource management communities to collaboratively plan expeditions and explore areas of the deep ocean where data are scarce (Cantwell et al., 2020). Explorer Model expeditions use telepresence to engage a broad spectrum of the ocean science and resource management communities in scientific activities and deliver data, including samples, that are publicly accessible and catalyze future deep-ocean research and discovery.

In a deliberate contrast to "research sampling" — sampling operations on conventional research expeditions — the purpose of "exploration sampling" as conducted on *Okeanos Explorer* is to collect a limited number of biological, geological, and water samples to broadly characterize a dive site/area of interest and do so in a way that minimizes negative impacts on the local environment and marine life. The types and sizes of physical samples collected are limited to those that can be safely and efficiently collected by remotely operated vehicle (ROV) or conductivity, temperature, and depth (CTD) rosette and processed and stored on board.

NOAA Ocean Exploration bases its deep-ocean exploration priorities on the exploration variables identified to adequately characterize a site and prioritized in Egan et al. (2021). Currently, priorities include biological sample collection for genetic, morphological, connectivity, and taxonomic analyses, and geological sample collection for assessment of seafloor composition, age, and evolution.

The following principles guide NOAA Ocean Exploration sampling operations and complement the principles of telepresence-enabled exploration using an ROV (Galvez et al., 2024).

- **Meet exploration and science objectives of a diverse oceanographic community**: Samples are not collected to support a specific individual's research. Samples are collected to support as many objectives of the ocean science and management communities as possible. Sampling conducted by NOAA Ocean Exploration incorporates input from partners to support research, resource management, policymaking, and/or applied expedition objectives.
- **Support national and NOAA priorities to understand the largely unknown ocean:** The National Strategy for Ocean Mapping, Exploring and Characterizing the United States Exclusive Economic Zone calls on federal agencies to coordinate efforts with each other and across sectors to increase our knowledge about the nation's resources. Exploration entails collecting data and information to provide a multidisciplinary "first look" or initial assessment of an area's physical, chemical, and biological characteristics. Sampling of seafloor and water column habitats is a fundamental component of exploration and supports site characterization.
- **Balance sampling operations with acquiring other exploration data:** The collection, processing, and storage of physical samples takes time and resources. Sampling operations are balanced against other exploration priorities such as traversing and imaging the seafloor and water column.
- **Adhere to standardized best practices in processing, preserving, and archiving samples:** Physical samples are recorded with robust metadata when collected and processed, preserved using the best methods available with input from subject matter

experts (see **Section 5** and **Appendix A**), and archived using best practices to ensure long-term viability.

- **Embrace findable, accessible, interoperable, and reusable (FAIR) data principles** (Wilkinson et al., 2016)**:** Data are open access and archived in an open architecture framework with robust metadata records to ensure they are reported, archived, and easily discoverable.

3. Planning Sampling Operations

NOAA Ocean Exploration uses a nested approach to expedition planning. This entails packaging individual expeditions into a series of expeditions — and sometimes multiyear campaigns — where exploration priorities, partners, and geographic operating areas overlap.

This section describes how NOAA Ocean Exploration incorporates sampling operations into its expedition planning, including working with the ocean science and management communities, complying with U.S. and international environmental regulations and policies, and partnering with external organizations to archive physical samples collected during expeditions on *Okeanos Explorer*.

More information about NOAA Ocean Exploration's planning process is in Cantwell et al. (2020) and the *NOAA Ocean Exploration ROV and Telepresence Deepwater Exploration Procedures Manual* (Galvez et al., 2024). For each multi-year campaign that NOAA Ocean Exploration has participated in using *Okeanos Explorer,* a specific sampling strategy was developed in order to best address community needs and utilize the sampling capabilities available at the time. A record of these sampling strategies and high priority targets are available in Cantwell et al. (2018), Appendix B (Atlantic Seafloor Partnership for Integrated Research and Exploration — ASPIRE), and Appendix C (Campaign to Address Pacific monument Science, Technology, and Ocean NEeds — CAPSTONE).

3.1 Community-Driven Sampling

Like other *Okeanos Explorer* operations, NOAA Ocean Exploration's sampling operations are community driven. This means that physical sample collections are prioritized based on the needs identified by the ocean science and management communities during the planning process as well as those expressed in real time via telepresence during ROV dives. Opportunities to provide input to sampling operations come in a variety of forms as noted in **Table 1**. Additional information about these opportunities are in Cantwell et al. (2020) and Galvez et al. (2024). **Table 1.** Opportunities for input to sampling operations. Table describes

the tiered levels and timelines on which NOAA Ocean Exploration requests community feedback to inform sample collection.

Exploration Level	Input Timeline (prior to collection)	Medium for Community Input	Example of Sampling Priorities
Campaign	Year(s)	● White papers on regional exploration priorities ● Community workshops ● Calls for community input where geospatial priorities are collected	● Species for ocean basin wide connectivity studies ● Species imaged but not sampled during previous deep submergence work ● Regional geological features or substrates for characterization
Series of Expeditions	Month(s) to Week(s)	● Direct communication with expedition manager/ coordinator ● Input during expedition planning calls	● Regionally important or keystone species ● Species associated with specific habitat types (e.g., chemosynthetic communities at hydrothermal vents) ● Regionally specific geological features (e.g., ridges, fracture zones, seamount chains)
Individual Expedition	Day(s) to Real Time	● Input during daily dive planning calls ● Communication during ROV dives via chat room and teleconference line	● Potential new species ● New or rare species morphologies ● New geographic range or depth extension for a species ● Protruding seamount ridge, oriented slope, or surface; rocks from specific formations or types (i.e., basalt vs. lithified sediment)

3.2 Environmental Compliance

Expeditions on U.S. government vessels are required to comply with U.S. federal, state, and tribal environmental laws, regulations, and policies as well as those that may apply when operating in another country's waters or the high seas. Completing required analyses and securing the proper records of compliance are important parts of the expedition planning process.

All sampling operations conducted during expeditions on *Okeanos Explorer* adhere to conditions stated in the records of compliance (e.g., letters of concurrence, permits). Sampling operations attempt to minimize negative impacts on the local environment and other

organisms. For example, only a subsample is taken of a biological organism (e.g., only a piece of a sponge or a branch of a coral), when possible.

General information about environmental compliance during ROV expeditions is in Galvez et al. (in prep.). More specific information is in annual field season instructions and expedition project instructions.

3.3 Sampling Partnerships

NOAA Ocean Exploration has worked to create a world-class sampling processing, documentation, and archiving process. Recognizing that broad interdisciplinary sampling efforts require input from a collaborative community, NOAA Ocean Exploration relies on partnerships with the Smithsonian National Museum of Natural History, Oregon State University Marine and Geology Repository, other NOAA programs, and the U.S. Geological Survey to provide expert advice about preservation techniques, processing of biological, geological, and water samples (for eDNA analysis), and archiving of the physical samples.

The National Museum of Natural History and Oregon State University Marine and Geology Repository serve as the final repositories for biological and geological samples, respectively. NOAA's National Centers for Environmental Information (NCEI) maintains the Sample Operations Database Application (SODA, see **Section 6.2**; Gottfried et al. Forthcoming) and manages the publicly-accessible archive that contains the digital data associated with NOAA Ocean Exploration's sampling operations. In addition, NOAA Ocean Exploration relies on input from taxonomic and geological subject matter experts to provide input on standard operating procedures.

4. Sampling Operations

NOAA Ocean Exploration's sampling operations were developed over several years of expeditions on *Okeanos Explorer*. However, the procedures, best practices, and protocols in this section can be adopted and adapted for use with other platforms.

4.1 ROV Sampling Equipment

ROVs *Deep Discoverer*[1] and *Seirios* operate together as a two-body ROV system deployed from *Okeanos Explorer*. This system is capable of exploring depths up to 6,000 m. *Deep Discoverer* is

[1] *Deep Discoverer* is maintained and operated by the Global Foundation for Ocean Exploration.

equipped with high- and standard-definition video cameras, LED lights, and a number of tools for collecting physical samples during ROV dives (see **Figures 1-4)**.

Deep Discoverer's sampling tools include:

- A hydraulic force feedback seven-function Kraft Predator manipulator arm (primary, starboard side),
- A hydraulic seven-function Schilling Orion manipulator arm (secondary, port side),
- Four bio boxes (48 x 40 x 33 cm),
- Two rock boxes (port: 51 x 48 x 20 cm with lid; starboard: 48 x 40 x 20 cm),
- A rotary suction sampler with five 2.7-liter sample jars (for fluid, sediment, or biological organisms), and
- Five 1.7-liter individually triggered Niskin bottles (for seawater).

Deep Discoverer is also equipped with a SeaBird SBE9-11 Plus CTD (conductivity, temperature, and depth system) that measures depth, pressure, temperature, salinity, sound velocity, dissolved oxygen, turbidity, and oxidation-reduction potential, as well as a Tracklink TL10000MA ultra-short baseline (USBL) acoustic tracking system that provides ROV positional data in real time.

Real-time imagery of samples collected *in situ* is captured and streamed by *Deep Discoverer*'s video cameras, which include:

- Three high-definition Insite Pacific video cameras: Zeus Plus (tilt/18x optical zoom), its primary ROV camera, labeled ROVHD; Mini Zeus (pan/tilt); and Titan Plus (pan/tilt/zoom) and
- Six high-definition Deep Sea Power and Light HD Multi SeaCam wide-angle video cameras

Deep Discoverer also carries 28 LED lamps (8 on the hydraulic swing arms) that deliver more than 224,000 lumens of light, illuminating the otherwise dark ocean. Two switchable red lasers, projected 10 centimeters apart, are used to determine the scale of objects in *Deep Discoverer*'s view.

More information about the sampling and imaging capabilities of *Deep Discoverer* is in the *NOAA Ocean Exploration ROV and Telepresence Deepwater Exploration Procedures Manual* (Galvez et al., 2024).

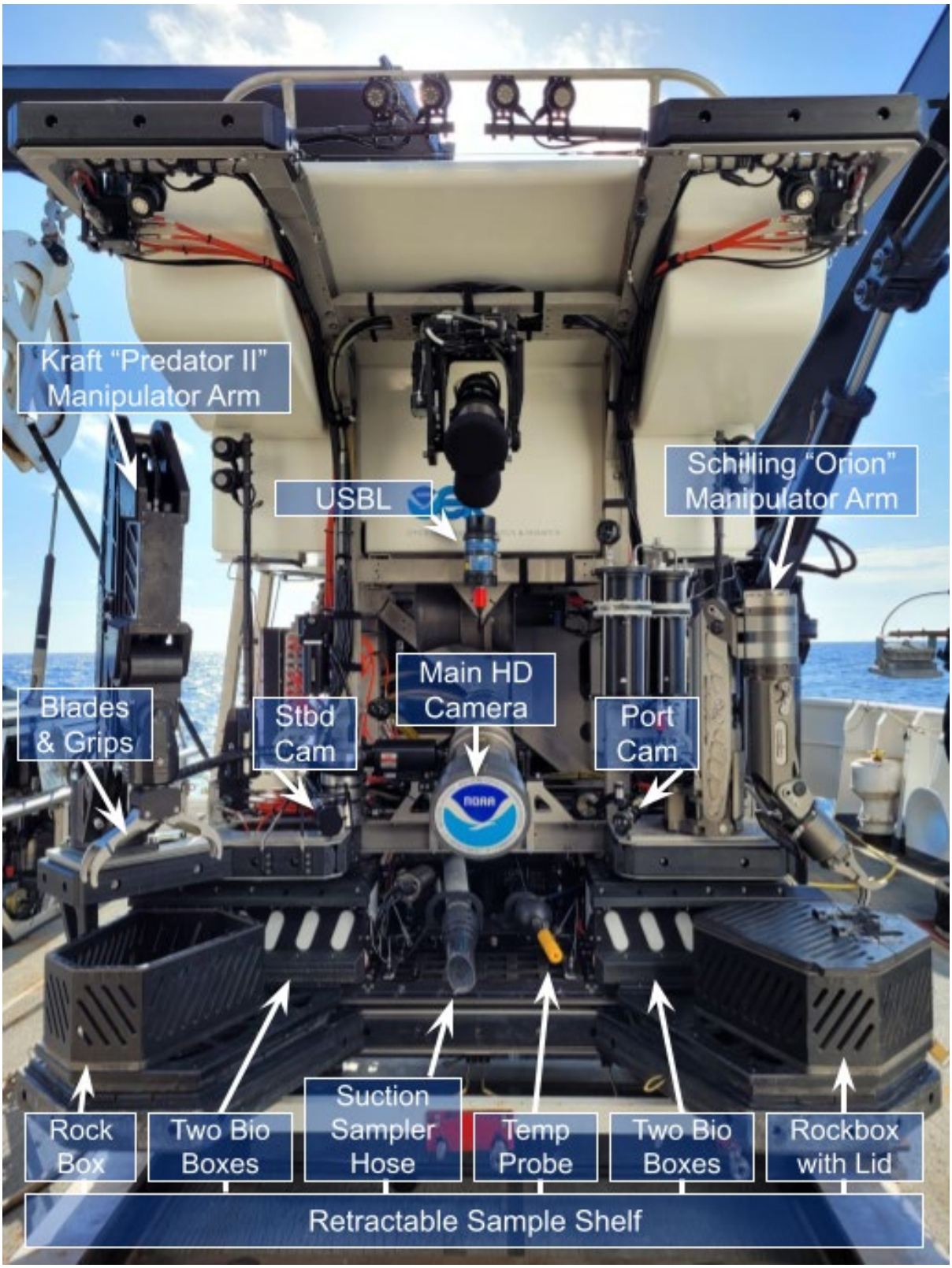

Figure 1. *Deep Discoverer* and select sampling tools.

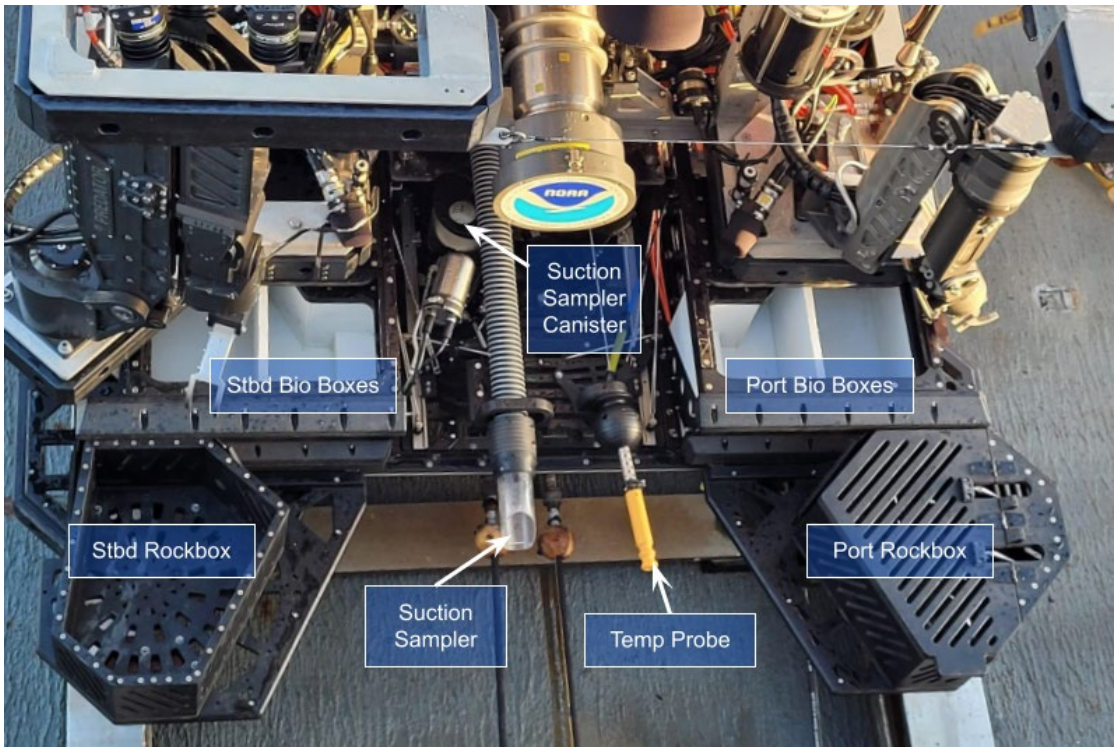

Figure 2. Top down view of *Deep Discoverer*'s extended sampling shelf with sample storage tools.

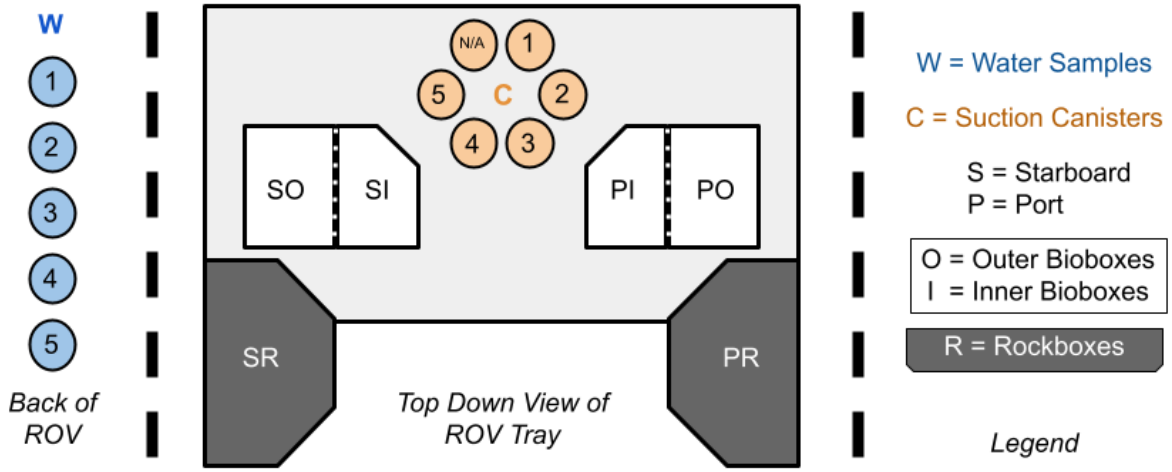

Figure 3. Sample storage diagram on *Deep Discoverer*: Niskin bottles (for water), suction sampler canisters, bio boxes, and rock boxes. This image is used for tracking the location of samples collected during a dive while the ROV is deployed.

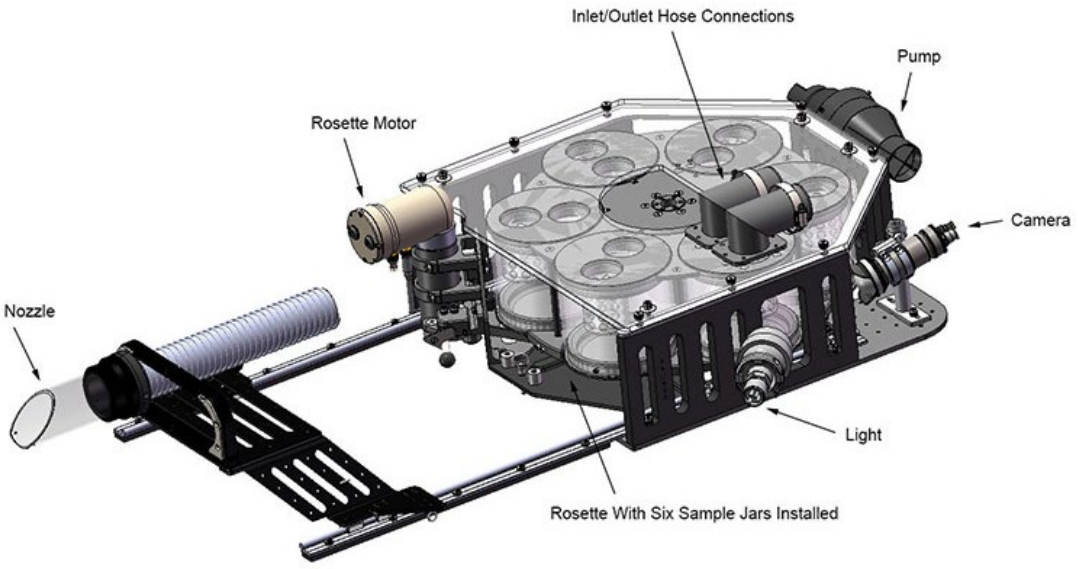

Figure 4. *Deep Discoverer*'s rotary suction sampler with its five 2.7-liter sample jars and one "bypass" jar to flush contaminants from samples. *Image courtesy of the Global Foundation for Ocean Exploration.*

4.2 Sampling Personnel

Based on the current staffing model used on *Okeanos Explorer*, **Table 2** details the roles and responsibilities of expedition personnel and their interaction points with sampling operations or physical samples. Personnel roles may adapt to accommodate berthing limitations or available shoreside augmentation, depending on expedition parameters. More information about roles and responsibilities is in the *NOAA Ocean Exploration ROV and Telepresence Deepwater Exploration Procedures Manual* (Galvez et al., 2024).

Table 2. Personnel engaged in sampling operations.

Position/Usual Location	Role in Sampling
Expedition Coordinator (EC) On Board (1)	Overall lead for the expedition. Leads dive site planning where samples are collected, distills community input into expedition sampling objectives, and is responsible for ensuring that samples collected are transferred to their final repository. The EC may delegate certain sampling roles as appropriate, but retains responsibility for safe practices by sampling personnel and permit compliance, as applicable.
Expedition Science Leads On Board (2)*	Onboard representatives for the ocean science and management communities. Guide real-time decisions regarding sample collection. Lend their expertise to field identification and sample processing.

Position/Usual Location	Role in Sampling
Sample Data Manager On Board (1)*	Lead for documenting and processing samples. Responsible for documenting collections in the sampling database. Works with the science leads to document, process, and store samples. Additional responsibilities include conducting quality control on sampling data and file names and generating reports.
ROV Pilots/Navigator/Video Engineers On Board (14)	Team of engineers responsible for managing ROV operations and the associated collection of physical and digital data.
Sampling Coordinator Shoreside (1)	Point of contact for sampling operations throughout a field season and for sampling repositories. Provides consistency for sampling operations through standard operating procedures and technical document management and oversight, coordinates an annual evaluation of new operations (when applicable), collaborates with ship personnel regarding lab upgrades and maintenance, and maintains supplies for sampling operations. Orders supplies when needed.
Environmental Compliance Coordinator Shoreside (1)	Lead for compliance with regulations, policy, and guidelines. Ensures NOAA Ocean Exploration meets or exceeds applicable environmental compliance requirements, including standards, international laws, and other permitting conditions.

*In 2021, NOAA Ocean Exploration tested operations with a shoreside science lead and sample data manager. The effectiveness of conducting these roles from shore is being evaluated.

4.3 Types of Samples Collected

NOAA Ocean Exploration collects five types of physical samples during ROV expeditions on *Okeanos Explorer*: primary biological samples, primary geological samples, associate biological and geological samples, subsamples, and water samples.

- **Primary biological samples** are organisms collected for genetic, morphological, connectivity, and taxonomic analyses. Hard-bodied and/or large samples are typically collected with *Deep Discoverer*'s starboard manipulator arm (Kraft Predator), while soft-bodied, small, and/or delicate samples are collected using the suction sampler.
- **Primary geological samples** are rocks, sediment, or other primarily nonbiogenic materials collected for morphological characterization, geochemical composition, and dating analysis if they have the potential to contribute to significant geological discoveries (e.g., provide new insights into the geological history or composition of a feature or help identify potential submarine geohazards). Rocks and rock fragments are typically collected with *Deep Discoverer*'s starboard manipulator arm (Kraft Predator), while sediment is typically collected using the suction sampler.

- **Associate biological and geological samples** are opportunistically collected with primary samples. They are "associates" of the primary sample (e.g., epifauna) and are cataloged and archived separately from the primary sample with which they were collected. Associates may or may not be the same sample type as the primary (e.g., an associate biological sample may be attached to a primary geological sample).
- **Subsamples** are a smaller piece of a primary or associate sample onboard taken for alternate methods of fixation, preparation, or analysis. The most common example of subsampling is the practice of taking a small piece of an organism for later DNA analysis (see **Section 5.2.2**). Subsampling is also done to fix part of a sample in formalin instead of ethanol for morphological analysis, preserve portions of sediment in ethanol for biological analysis, and to conduct immediate destructive or nondestructive microscopic analysis. Note that any subsampling that may occur after samples are deposited in onshore repositories is not tracked by NOAA Ocean Exploration.
- **Water samples** are collected primarily for eDNA analysis using the five 1.7-liter individually triggered Niskin bottles attached to *Deep Discoverer*. Water samples are also collected during CTD casts from the ship using the 12 10-liter Niskin bottles attached to the CTD rosette.

4.4 Sample Naming Convention

NOAA Ocean Exploration uses a standardized convention for naming samples that was developed in partnership with NCEI, the Smithsonian National Museum of Natural History, Oregon State University Marine and Geology Repository, and the Global Foundation for Ocean Exploration. Sample names are based on the metadata, annotation, and imagery associated with a sample. Each sample has a short-form name and a long-form name. The short-form name is most commonly used in publications, while the long-form name includes a date and timestamp and is reserved for database management. Short-form naming conventions for the various sample types collected by NOAA Ocean Exploration are as follows:

- **Primary biological and geological samples and water samples** are named using the expedition identification number (CRUISEID), the dive number (D), the sequential number based on when it was collected during a dive, and whether a sample is a biological (B), geological (G), or a water sample (W).
    - Format: CRUISEID_D##_##(B or G or W)
    - Examples:
        - EX2301_D01_01B was the first primary sample collected during Dive 01 of expedition EX2301. It was a biological sample.
        - EX2301_D05_03G was the third primary sample collected during Dive 05 of expedition EX2301. It was a geological sample.

- **Associate biological and geological samples** (A) are named sequentially based on their primary sample. As of Fiscal Year 2023, associate samples also have a biological (B) or geological (G) designation appended to the sample name. Associate samples from earlier years do not have a B or G designation.
    - Format: CRUISEID_D##_##(B or G)_A##(B or G)
    - Examples:
        - EX2301_D01_01B_A01B was the first biological associate sample taken from the first primary biological sample collected during Dive 01 of expedition EX2301.
        - EX2301_D05_03G_A02B was the second biological associate sample taken from the third primary geological sample, collected during Dive 05 of expedition EX2301.
- **Subsamples** (S) are named sequentially based on their primary sample and the associate they came from (if applicable). The reason for taking a subsample onboard can vary and is noted in the comments of the sample's metadata.
    - Format for a subsample of a primary sample: CRUISEID_D##_##(B or G)_S##
    - Examples:
        - EX2301_D01_01B_S01 is the first subsample taken from the first primary biological sample collected during Dive 01 of expedition EX2301.
        - EX2301_D05_03G_S02 is the second subsample taken from the third primary geological sample, collected during Dive 05 of expedition EX2301.
    - Format for a subsample of an associate sample: CRUISEID_D##_##(B or G)_A##(B or G)_S##
    - Examples:
        - EX2301_D01_01B_A01B_S01 is the first subsample taken from the first biological associate sample, taken from the first primary biological sample collected during Dive 01 of expedition EX2301.
        - EX2301_D05_03G_A04B_S02 is the second subsample taken from the fourth biological associate sample, taken from the third primary geological sample collected during Dive 05 of expedition EX2301.

Occasionally, samples that are not intentionally collected also come to the surface with the ROVs (i.e., outside of the bio and rock boxes and sample jars). When this happens, these "samples of opportunity" are named sequentially as primary samples, and as much information as is known about the sample time, location, and environmental parameters is added to the sample's digital record.

5. Standard Sample Preservation Methods

Following an ROV dive, all samples are recorded in the sampling database (see **Section 6.2**), photographed, and processed in the wet lab on *Okeanos Explorer* (**Figure 5**). They are then prepared for shipping and stored until they are ready to be shipped to their respective repositories for archiving. The following sections describe the standard process. During an expedition, NOAA Ocean Exploration relies on advice from taxonomic and geological subject matter experts for special treatment of unfamiliar samples.

Figure 5. Wet lab onboard *Okeanos Explorer*.

5.1 Onboard Sample Imaging

NOAA Ocean Exploration uses a telepresence-enabled camera setup (**Figure 6**) that enables shoreside support and includes a Canon EOS 70D DSLR camera and a 4k WolfVision VZ-8.UHD camera to collect a set of images for each sample.

- The first image for each sample is of the sample label generated by the sampling database, scale bar, and reference color card for color corrections. This image (with no sample present) identifies the start of a new image set in the saved images.
- The second image is of the whole sample along with the scale bar, label, and, for biological samples, the test tube with the corresponding DNA subsample. The reference color card does not need to be included unless lighting has significantly changed.

- Subsequent images of the sample photographed from different angles (e.g., all sides of a rock) or highlighting specific morphological features include the label and scale bar. The reference color card does not need to be included unless lighting has significantly changed.

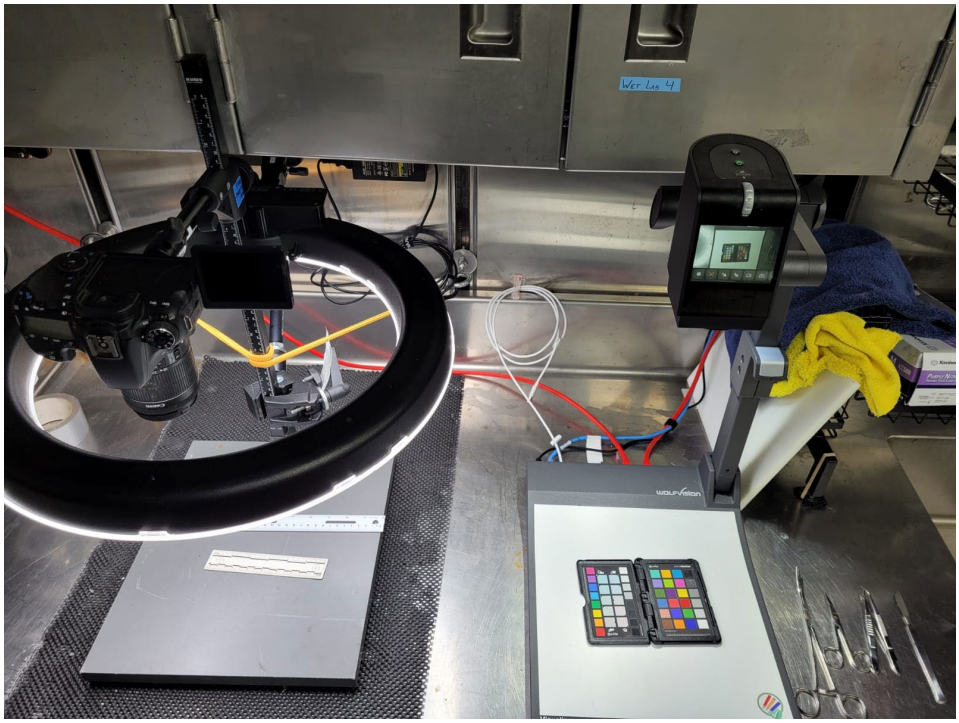

Figure 6. *Okeanos Explorer* wet lab camera systems. Left: Detachable Canon DSLR with full spectrum light ring and stand. Right: 4k WolfVision VZ-8.UHD document photographic system with built-in light source. Also shown: rulers used for scale (left) and the true color card system (right).

Living organisms may be imaged (including video) in water in an acrylic tank. Still images and video of a swimming organism may provide information about soft structures that may deteriorate once an organism is fixed.

A digital microscope is also available to augment imagery at finer scales when requested by the scientific community, when fine scale images are needed to identify an organism prior to preservation, and to measure the texture and grain size of unconsolidated sediment.

5.2 Biological Sampling[2]

After recovery of the ROVs, Science Team members retrieve biological samples from the ROV using gloves and tweezers to protect themselves from sharp edges and irritating compositions. Samples are typically placed in buckets with cold seawater to move them to the wet lab. The biological science lead identifies sensitive samples that must be processed quickly or need additional care and handling when transferring them from the ROV to the wet lab.

When possible, biological samples are processed for both genetic and morphological analysis:

- If there is only one sample of a species from a dive, a subsample of the primary sample is taken for genetic purposes while the remainder of the primary sample is prepared as the morphological voucher.
- If there are two samples from a dive that appear to be the same species, one is prepared for genetic analysis and the other is prepared as the morphological voucher.
- When possible, a subsample of 6-10 polyps of a coral colony is preserved for reproduction studies.

The following subsections describe how biological samples are prepared for shipment to the repository. **Appendix A** includes taxa-specific guidance for sample imaging, relaxation, genetic sampling, and fixation/preservation.

5.2.1 Relaxation

To display the full body of an organism for photographing, expose desirable sampling locations, and prepare the samples for archiving, some organisms benefit from a relaxation preparation (see **Appendix A**). NOAA Ocean Exploration uses a relaxation preparation of 7.5% by weight solution of magnesium chloride ($MgCl_2$) diluted with freshwater and cold seawater in a 1:1 ratio. Samples may be put in the freezer for a very short time to reduce motor function of the animal to enable sampling for genetic tissue, imagery, or relaxation prior to fixation. Other methods for relaxation can be prepped and brought aboard prior to an expedition at the recommendation of the biological science lead. Due to the time constraints associated with sampling (e.g., samples decaying or rapidly deteriorating in lab conditions), relaxation is not always possible or advised, and is therefore not required..

[2] This section was written in collaboration with the Smithsonian National Museum for Natural History and is based on guidance developed by the museum and the NOAA National Systematics Lab in conjunction with leading biologists.

5.2.2 Genetic Sampling

Genetic sampling is the process of preserving DNA information for later study. To fit inside of the standard vials used on *Okeanos Explorer*, tissue samples are no more than 1 cm^3, roughly the same size as a lentil or a pea (**Figure 7**). Genetic samples are taken from genetically dense tissue that does not contain degrading enzymes or taxonomically significant features (Prendini et al. 2002) and fixed in 95% ethanol with a ratio of at least 3:1 ethanol to tissue.

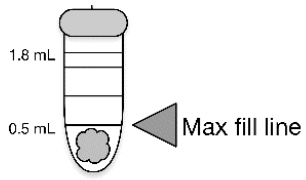

Figure 7. Diagram of tissue sample vial with size and volume considerations.

Generally, muscle tissues are preferred for genetic sampling of invertebrates (see **Appendix A** for preferred locations). The following body parts are avoided:

- Gastric and oral tissues, which may contain enzymes that damage DNA and may also be contaminated with organic matter from food or parasites (Dawson et al. 1998; Prendini et al. 2002).
- Exterior tissues and exoskeleton, which may contain parasitic or symbiotic organisms whose own DNA may contaminate the sample and may have pigments and lipids often found in exterior tissues that may inhibit DNA amplification (Borchiellini et al. 2001; Prendini et al. 2002).
- Forelimb, hind limb, head, tail, mouthpart, and reproductive structures, which tend to be useful for species identification (for some taxa, these body parts are preferred for genetic sampling).

5.2.3 Fixation

When appropriate, a fixative is applied to a sample as soon as possible after collection and relaxation.

Most primary and associate biological samples are fixed in 95% ethanol on the ship. However, formalin may be used as a fixative when requested or as specified for particular taxa (see **Appendix A**), but its use may preclude any further DNA work on a sample.

When used, formalin (a diluted solution of 37.5% formaldehyde) must be diluted to one part formalin and nine parts seawater to make a 10% buffered formalin preservative solution. For most applications, this buffering helps protect a sample from damage. If a sample is to remain

in the solution for a short time (i.e., less than a month), seawater is an adequate buffer. However, if a sample is to sit in the solution for a month or longer, the solution is buffered with 2 grams of borax for every 98 ml of 37.5% formaldehyde formalin solution. When in doubt, buffer with borax.

Many taxa that are fixed in formalin are moved to ethanol at the repository on shore in a stepwise preservation procedure (see **Section 5.2.5**). DNA subsamples are always preserved in 95% ethanol regardless of fixation solution (see **Section 5.2.2**).

5.2.4 Drying

Some taxa can be dried rather than fixed or preserved (see **Appendix A)**. Before a sample is dried, a small tissue sample is taken for the biorepository and is preserved in 95% ethanol. Samples are air-dried in a place with ventilation over a few days.

5.2.5 Storage and Shipping

Fixed and dried samples are stored in sealing plastic bags, heat-sealed bags, sample jars, or other sealed containers in their fixative (if appropriate). Unless specific sampling needs call for it (i.e., a sampling request or species-specific treatment), fixed samples remain in fixative until they are shipped to the repository. Labels for each sample are stored in the container with the corresponding sample. Fixed and dried samples are stored in a controlled indoor environment until they can be shipped to the repository (they do not need to be frozen).

After each expedition, or after a series of expeditions, samples are inventoried, removed from their storage containers and then repacked (e.g., if required to remove excess fixative) and double-bagged in heat-sealed bags for shipping to the repository according to International Air Transport Association (IATA) Dangerous Goods Regulations (DGR). Shipping containers and packaging shall follow the IATA DGR, and at least one member of the onboard team responsible for overseeing packing and shipping must be trained in IATA shipping protocols. Within the IATA DGR constraints, packing materials and containers are situation- and sample-dependent. Formalin-fixed samples and ethanol-fixed samples are packed in separate boxes. Every effort is made to ensure samples arrive intact and undamaged to the archive as quickly as possible after packing, typically overnight.

5.2.6 Preservation

For the purposes of document, preservation refers to chemicals that may be applied to samples for long-term storage after expeditions, as opposed to fixation (see above) which refers to chemicals applied onboard for short-term storage. Preservation is described here and discussed in **Appendix A** for informational purposes. Samples fixed in 95% ethanol are preserved for the

long term in 70-80% ethanol or 50% isopropanol (e.g., cephalopods) once they arrive at the shoreside archive. Upon arrival, some samples fixed in a formalin preservative solution will need to be transferred to ethanol through graded concentrations of ethanol to avoid osmotic imbalances and sample distortion. For example, a sample fixed in a 10% formalin preservative solution that is being transferred to 75% ethanol may be transferred to a solution of water and ethanol, starting with 25% ethanol, then 40% ethanol, then 60% ethanol, and then 75% ethanol with 24 hours at each solution, before it is transferred to a permanent preservative concentration of 75% ethanol. This graded transfer from formalin to ethanol is never performed while at sea.

5.2.7 Archiving

After each expedition on *Okeanos Explorer*, biological samples are sent to the Smithsonian National Museum for Natural History for curation and archiving (see **Sections 6.1.1** and **6.1.2**). Genetic samples are sequenced, bar coded, and archived at Smithsonian National Museum of Natural History Biorepository through a partnership with the Smithsonian and the Bureau of Ocean Energy Management.

5.3 Geological Sampling

After recovery of the ROVs, Science Team members retrieve geological samples using gloves to protect themselves from sharp edges and irritating compositions. To preserve any biological associates, each geological sample is moved to its own bucket with seawater until it is ready to be removed for photographing and drying. All associates (biological and geological) are photographed, identified, removed, and prepared for preservation according to taxa- (see **Section 5.2** and **Appendix A**) or substrate-specific best practices.

5.3.1 Procedures for Rocks

The steps for preparing rocks for shipment to the repository are as follows:

- Gently dry the rock with a paper towel or cloth (this is to avoid glare when taking photographs).
- Photograph the rock as described in **Section 5.1**.
- Weigh and measure (approximate length, width, and height) the rock.
- Allow the rock to dry (amount of time varies, but 24-48 hours is typically sufficient).
- If fissile or fragile (e.g., rock has a fragile ferromanganese crust), wrap the rock in bubble wrap, write the Sample ID on the wrapped rock, place the wrapped rock in a sample bag, and write the Sample ID on the outside of the bag.

- If coherent, wrap the rock in bubble wrap, write the Sample ID on the wrapped rock, place the wrapped in a rock sample bag, and write the Sample ID on the outside of the bag **OR** place the unwrapped sample directly in a rock sample bag with the sample label and write the Sample ID on the outside of the bag.
- Store the rock in the designated large bin on the ship's deck until geological samples can be shipped to the repository.

5.3.2 Procedures for Unconsolidated Sediment

After retrieval from the ROV, unconsolidated sediment is allowed to settle. The steps for preparing unconsolidated sediment for shipment to the repository are as follows:

- Dry the sample in a Petri dish or other suitable shallow container that maximizes surface area and reduces drying time.
- Photograph the sediment as described in Section 5.1.
- Photograph the sample with the digital microscope for texture and grain size.
- Work with the sample data manager and science leads to determine the appropriate storage container for the sediment.
- Place the sample label in the container with the sediment, wrap the container in bubble wrap, place it in a rock sample bag, and write the Sample ID on the outside of the bag.
- Store the sediment sample with other geological samples in the designated large bin on the ship's deck until they can be shipped to the repository. Biological sediment samples are stored and shipped with the biological samples, not the geological samples.

5.3.3 Archiving

After a series of expeditions or the conclusion of the field season on *Okeanos Explorer*, geological samples are sent to Oregon State University's Marine and Geology Repository for curation and archiving (see **Section 6.1.3**).

5.4 Water Sample Preservation

The collection of water samples for eDNA analysis is still relatively new. NOAA Ocean Exploration is developing procedures for water sample collection, processing, and preservation with input from the scientific community. This section describes the current approach to water sampling during expeditions on *Okeanos Explorer*.

After recovery of the ROV or CTD, Science Team members retrieve the water samples. The steps for preparing water samples for shipment to the repository are as follows:

- On deck, transfer the sample from its Niskin bottle to a Whirl-Pak bag for transfer to the eDNA work station in the wet lab.
- In the wet lab, slowly pour the water sample into a filter cartridge while the vacuum pump pulls water through the filter. Discard the water periodically.
- Use plastic forceps to fold and store the filter in a 5 ml centrifuge vial with 3 ml of DNA/RNA shield. The vial is prelabeled with the label generated by the sampling database.
- Wrap the vial with parafilm.
- Store the vial with the filter in refrigerated storage until the end of the expedition when it can be shipped to the repository for archiving. Refrigerated or frozen shipping is not required.

5.4.1 Archiving

After each expedition on *Okeanos Explorer*, water sample filters are sent to the Smithsonian National Museum for Natural History for eDNA processing, genome skimming, and archiving (see **Section 6.1.2**).

6. Sample and Digital Data Access and Reporting

NOAA Ocean Exploration adheres to the federal government's equal and open data policy regarding access to samples collected during expeditions onboard *Okeanos Explorer* and prioritizes sharing data with the public in a timely manner. This section provides information about the repositories for the physical samples and the associated digital data products.

6.1 Sample Repositories

Samples collected during expeditions on *Okeanos Explorer* are shipped to partner repositories for permanent archiving. Access to physical samples is provided by the repositories as quickly as possible and is not subject to proprietary holds, with limited exceptions (e.g., samples collected in foreign waters, specific sample collections funded by NOAA Ocean Exploration partners that may be temporarily withheld from public access for a specified period of time).

Information about how to access these samples is on the NOAA Ocean Exploration website. Questions about accessing samples can be sent to ex.expeditioncoordinator@noaa.gov.

6.1.1 Repository for Biological Samples

Invertebrate and vertebrate primary and associate biological samples are sent to the Smithsonian National Museum of Natural History after an expedition, except in instances where

there are logistical benefits or permitting requirements to keep samples aboard until the conclusion of the field season to send the samples to other repositories.

Invertebrates

Department of Invertebrate Zoology
Smithsonian National Museum of Natural History, Museum Support Center
MRC 534, 4210 Silver Hill Road, Suitland, MD 20746

- Search the Department of Invertebrate Zoology collections (see **Appendix B**)
- Information about physical access to invertebrate samples
- Information about destructive sampling

Vertebrates (Fish)

Division of Fishes of the Vertebrate Zoology Collections
Smithsonian National Museum of Natural History, Museum Support Center
MRC 534, 4210 Silver Hill Road, Suitland, MD 20746

- Search the Division of Fishes Collections
- Information about physical access to vertebrate samples
- Information about destructive analysis

6.1.2 Repository for DNA/Tissue Samples and Water Sample Filters

DNA/tissue samples and water sample filters for eDNA analysis are sent to the Smithsonian National Museum of Natural History Biorepository after an expedition. DNA subsamples are linked to the catalog records in the Department of Invertebrate Zoology and Division of Fishes of Vertebrate Zoology collections and can be accessed via their websites (see above).

Biorepository
Smithsonian National Museum of Natural History Institution, Museum Support Center
4210 Silver Hill Road, Suitland, MD 20746

6.1.3 Repository for Geological Samples

Geological samples are shipped to Oregon State University's Marine and Geology Repository and archived in the NOAA Collection (NOAA Rocks) after a series of expeditions or the conclusion of a field season. At the repository, rocks are sectioned for microscopy, photographed, and described petrographically (e.g., mineral content, texture, alteration, rock type). The repository's publicly available online database provides sample metadata, images (including thin sections), and details.

Marine and Geology Repository
Oregon State University

Burt 346, Corvallis, OR 97331-5503

- Search NOAA Rocks
- Information about how to request samples

6.2 Sample Operations Database Application

The Sample Operations Database Application (SODA) is a custom Microsoft Access database input application used to collect sample metadata, print labels, and generate reports and documentation. SODA ingests metadata (location, time, depth, environmental sensor readings, and preliminary identification) from each sampling event and can quickly export labels for archival printing. The sample data manager is responsible for adding additional information about each sample to SODA to complete the record of the sample with notes about the sample identification and condition, subsamples, fixation and preservation methods, sample associates, subsamples, and associated images and videos.

More information is in the *Sampling Operations Database Application (SODA) Manual 2023* (Gottfried et al., 2024).

6.3 Digital Sample Data

During an expedition, operational data and products are available to Science Team members through a suite of internet-based collaboration tools. Digital sample data products available to shoreside participants during an expedition are noted in **Table 3**.

Table 3. Digital sample data products available during an expedition to Science Team members.

Product	Description
Daily Sample Report	Daily output from SODA that includes information about every sample, including lab preparation and associated video and images
Daily List of Sampling Images and Video	List of samples and associated dive video segments and screen grab images by dive
In Situ Images	Screen grab images of samples taken *in situ* and during collection
In Situ Video	Video of samples taken *in situ* and during collection
Lab Images	Images of samples taken with a color pallet and sample label
Microscope Images	Images taken using a digital microscope (when applicable)
Microscope Video	Video taken using digital microscope (when applicable)

Table 4 contains select metadata recorded for each sample collection. These data are captured in SeaTube (online annotation system, additional details available in Galvez et al., 2024) and are recorded in the respective ROV dive summary and SODA. Information about metadata recording procedures is in the *NOAA Ocean Exploration Remotely Operated Vehicle (ROV) and Telepresence Deepwater Exploration Procedures Manual* (Galvez et al., 2024).

Table 4. Metadata fields collected for each sample.

Metadata Field	Units | Format
Date	UTC | yyyymmdd
Time	UTC | hhmmss
Latitude	+/- Decimal Degrees | 00.000000
Longitude	+/- Decimal Degrees | 000.000000
Depth	Meters |#.00000
Salinity	Parts Per Thousand | #.00
Temperature	Celsius | #.00
Dissolved Oxygen	ml/l | #.00

Digital data and products are typically submitted to the NOAA archives within 120 days of the end of an expedition. The primary tools for accessing digital data collected during expeditions on *Okeanos Explorer* are the NOAA Ocean Exploration Data Atlas, the NOAA Ship *Okeanos Explorer* data landing pages, and the NOAA Ocean Exploration Video Portal. More information about how to access NOAA Ocean Exploration data and products is on the Data Access page of NOAA Ocean Exploration's website and in the *NOAA Ocean Exploration ROV and Telepresence Deepwater Exploration Procedures Manual* (Galvez et al., 2024).

Questions about accessing digital sample data can be sent to NOAA Ocean Exploration's Data Management Team by submitting a data request form or emailing oer.info.mgmt@noaa.gov.

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Appendix A. Sample Processing and Preservation Taxa Guide

Technical experts from the Smithsonian National Museum of Natural History, the NOAA National Systematics Lab, and collaborating biologists have developed extensive guidance on the processing and preservation of common taxa which is represented below in sections A.1 through A.12 in summary tables with imagery guides. This guidance includes how to photograph a sample, how to relax it (if needed and possible), where to extract tissue samples for genetic sampling, and how to fix and preserve a sample. NOAA Ocean Exploration has adapted this guidance here for expeditions on NOAA Ship *Okeanos Explorer*.

Unless noted otherwise, the genetic subsamples are preserved in 95% ethanol for DNA analysis.

"n/a" in this appendix indicates that there is no guidance for a processing step.

A.1 Phylum Brachiopoda

Imaging:	n/a
Relaxation:	Magnesium chloride solution or propylene phenoxitol may be needed to open the shell halves (Templado et al. 2010)
Genetic Sampling:	• Preferred: The muscular tissues holding the shell halves together • Alternative: Gonad tissue (Stechmann and Schlegel 1999)
Fixation/Preservation:	70-80% ethanol

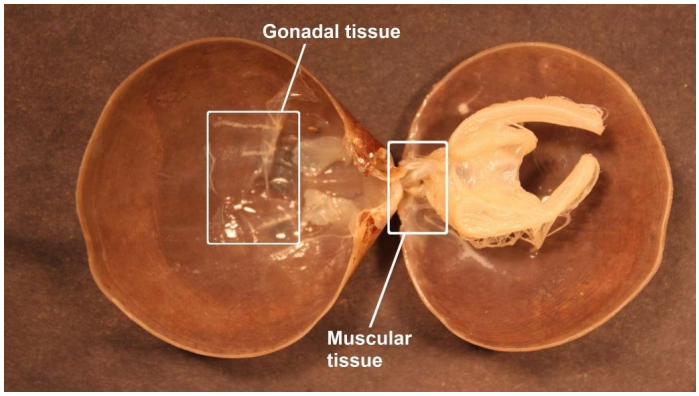

Figure A1. A dissected brachiopod. *Image courtesy of Smithsonian National Museum of Natural History.*

A.2 Phylum Bryozoa

Imaging:	n/a
Relaxation:	n/a
Genetic Sampling:	Cluster of polyps
Fixation/Preservation:	70-80% ethanol; can be dried

Figure A2. A bryozoan *in situ* from the Mountains in the Deep: Exploring the Central Pacific Basin expedition in 2017.

A.3 Phylum Chordata

This section includes fish and tunicates.

A.3.1 Fish

This subsection applies to:

- Subphylum Craniata (Fish)

Imaging:	• Capture the coloration of the fish • Use a color card in the photograph if possible • Photograph as soon as possible to avoid discoloration as the fish expires • Photograph the left side of the fish (unless the fish is compressed like a flatfish, then a dorsal image is preferred)
Relaxation:	n/a

Genetic Sampling:	• Preferred: Tissue from the right side (unless it is more intact than the left side) • Alternatives: Gill filaments, pectoral fin clips, eyeballs
Fixation/Preservation:	• Fixation (on ship): 10% formalin • Preservation (on shore): Step-wise transfer at the museum to water, then 75% ethanol, starting with 25% ethanol and then 40%, 60%, and 75% ethanol, spending 24 hours in each stepwise solution. After the final 75% ethanol solution, repeat the transfer to 75% ethanol for final preservation.

Figure A3. A bottom dwelling fish *ex situ* from Islands in the Stream 2002.

A.3.2 Tunicates (Larvaceans, Salps, and Doliolids)

This subsection applies to:

- Subphylum Tunicata (tunicates, sea squirts)
  - Class Appendicularia (larvaceans)
  - Class Thaliacea (salps and doliolids)

Imaging:	Image in the lab acrylic tank if organism is still alive on recovery
Relaxation:	n/a
Genetic Sampling:	n/a
Fixation/Preservation:	10% formalin mixed slowly with seawater at a ratio of 1:1 (results in a 5% formalin solution)

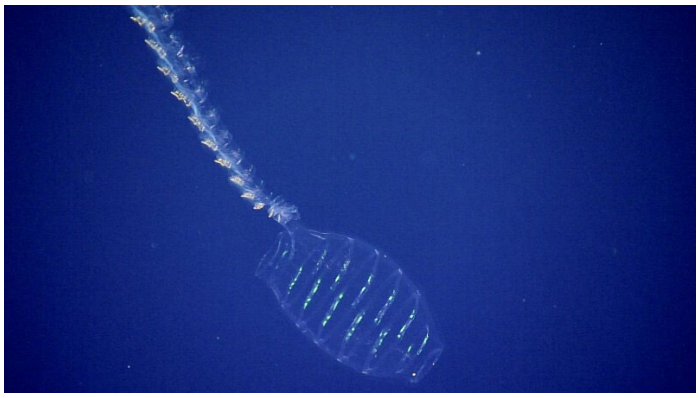

Figure A4. A doliolid *in situ* from 2017's Mountains in the Deep: Exploring the Central Pacific Basin.

A.3.3 All Other Tunicates

This subsection applies to tunicates other than larvaceans, salps, and doliolids.

Imaging:	n/a
Relaxation:	Menthol (not kept aboard *Okeanos Explorer*)
Genetic Sampling:	n/a
Fixation/Preservation:	10% formalin (generally preferred)

Figure A5. A tunicate *in situ* from the 2016 Deepwater Exploration of the Marianas.

A.4 Phylum Cnidaria

This section includes anemones, antipatharians, octocorals, scleractinians, and medusozoans.

A.4.1 Anemones

This subsection applies to:

- Class Anthozoa
    - Subclass Hexacorallia
        - Order Actinaria (actinarians, true/sea anemones)
        - Order Zoantharia (zoanthids, encrusting anemones, colonial anemones)
    - Subclass Ceriantharia (cerianthids, tube-dwelling anemones)

Imaging:	n/a
Relaxation:	Menthol (not kept aboard *Okeanos Explorer*) — as the animal will contract their tentacles if agitated while active (Templado et al. 2010)
Genetic Sampling:	• Preferred: Tentacles • Alternative: A chunk of column
Fixation/Preservation:	• Fixation (on ship): 10% formalin • Preservation (on shore): Transfer to ethanol • Mid-sized and large anemones should have fixative/preservative injected into the center, e.g., through the mouth, to preserve internal structures; if injection is not possible, the anemone should be sliced open on one side

Figure A6. A sea anemone *in situ* from the 2017 Laulima O Ka Moana expedition.

A.4.2 Antipatharians

This subsection applies to:

- Class Anthozoa
    - Subclass Hexacorallia
        - Order Antipatharia (antipatharians, black corals)

Imaging:	n/a
Relaxation:	n/a
Genetic Sampling:	A short branch fragment with several polyps
Fixation/Preservation:	• 70-80% ethanol; can be dried • 6-10 polyps should be preserved in 10% formalin preservation when possible

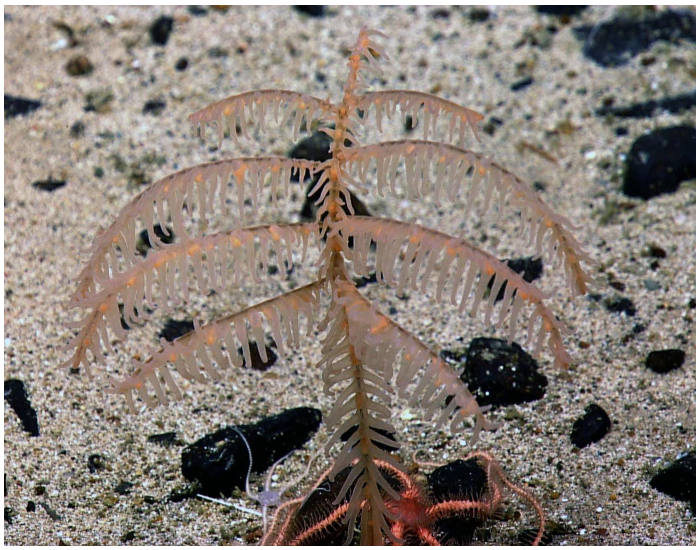

Figure A7. A deepwater black coral *in situ* from exploring the Atlantic Canyons and Seamounts 2014 expedition.

A.4.3 Octocorals

This subsection applies to:

- Class Anthozoa
    - Subclass Octocorallia (octocorals)

Imaging:	n/a

Relaxation:	n/a
Genetic Sampling:	A short branch fragment with several polyps
Fixation/Preservation:	● 70-80% ethanol; if possible, fix a second sample in 10% formalin (minimum 6-10 polyps) ● Large samples can be dried

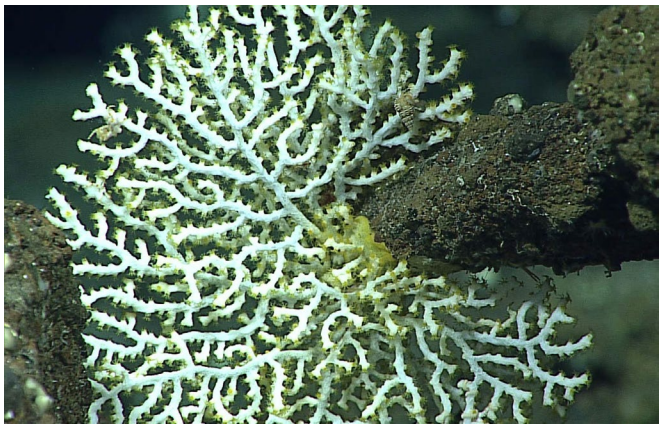

Figure A8. An octocoral *in situ* from the 2016 Deepwater Exploration of the Marianas.

A.4.4 Scleractinians and Stylasterids

This subsection applies to:

- Class Anthozoa
    - Subclass Hexacorallia
        - Order Scleractinia (scleractinians, stony corals, hard corals)
- Class: Hydrozoa
    - Subclass Hydroidolina
        - Order Anthoathecata
            - Family Stylasteridae (stylasterids, hydrocorals, lace corals)

Imaging:	n/a
Relaxation:	n/a
Genetic Sampling:	Scraping of surface or small chunk of colony bearing live tissue
Fixation/Preservation:	● Preferred: Dried; prior to drying, preserve a small portion of the colony in 95% ethanol in addition to the genetic sample ● Alternative: 70-80% ethanol

Figure A9. A scleractinian *in situ* from the Lophelia II 2009: Deepwater Coral Expedition: Reefs, Rigs, and Wrecks expedition.

A.4.5 Medusozoa (Clade), Medusa Stage

This subsection applies to:

- Class Hydrozoa (hydrozoans, e.g., hydroids, siphonophores)
- Class Cubozoa (cubozoans, box jellyfish)
- Class Scyphozoa (scyphozoans, jellyfish)

Imaging:	• Whole body photos, ideally live once acclimated in a tank • High-definition video footage of the animal *in situ* prior to collection, particularly if captured from different angles • Strongly side-lit photos, which may reveal interior structures such as canals • Animals imaged from their side and along their oral-aboral axis • Close-ups of medusa rims and mouths, if time allows • Strong side lighting with black background, felt is good, relatively far behind the sample
Relaxation:	Magnesium chloride or menthol (latter not kept aboard *Okeanos Explorer*)
Genetic Sampling:	• Tentacles or a bit of bell rim or margin for medusae, after relaxation (a bit of stem or loose nectophore from a siphonophore works well)

	• If the individual sample is too small for genetic sampling, consult with taxonomic experts to identify preferred sampling procedures
Fixation/Preservation:	10% buffered formalin mixed slowly with seawater at a ratio of 1:1 (results in a 5% formalin solution)

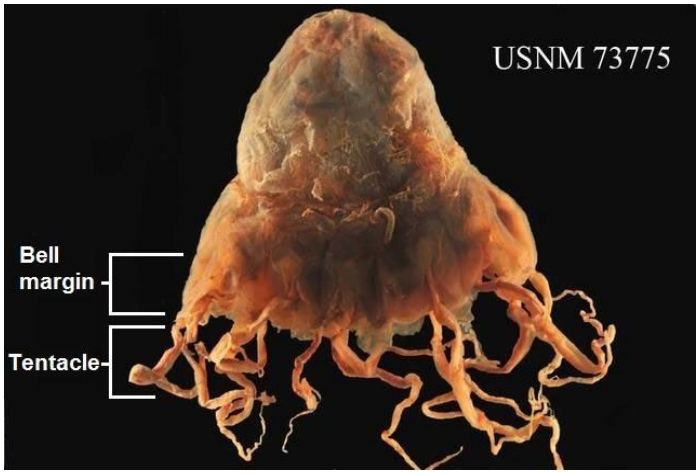

Figure A10. A medusozoa in a medusa stage *ex situ*. *Image courtesy of Smithsonian National Museum of Natural History.*

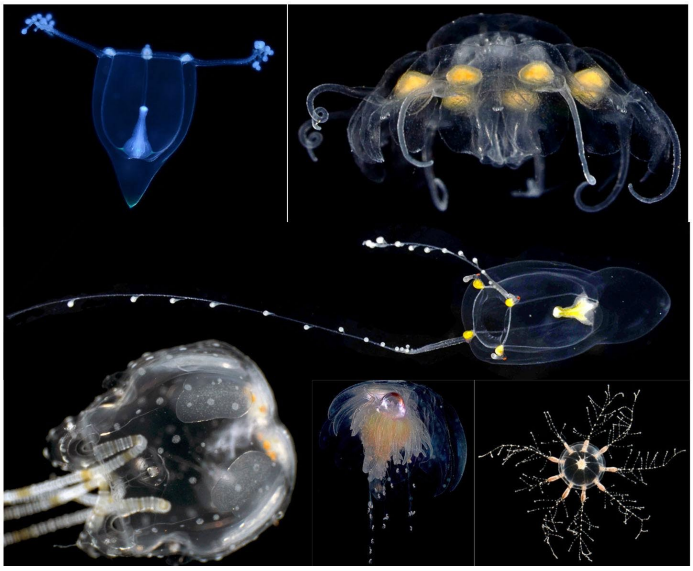

Figure A11. Six different medusozoa *in situ*. *Images courtesy of Smithsonian National Museum of Natural History*.

A.4.6 Medusozoa (Clade), Polyp Stage

This subsection applies to:

- Class: Hydrozoa (hydrozoans, e.g., hydroids, siphonophores)
- Class: Cubozoa (cubozoans, box jellyfish)
- Class: Scyphozoa (scyphozoans, jellyfish)

Imaging:	n/a
Relaxation:	n/a
Genetic Sampling:	• General: Polyps (individually or a cluster) • Large solitary hydroids: A tentacle or two
Fixation/Preservation:	• Fixation (on ship): 10% formalin (colony or individual polyps) • Preservation (on shore): Step-wise transfer at the museum to water, then 75% ethanol, starting with 25% ethanol and then 40%, 60%, and 75% ethanol, spending 24 hours in each stepwise solution. After the final 75% ethanol solution, repeat the transfer to 75% ethanol for final preservation.

Figure A12. A giant solitary hydroid, a hydrozoa polyp, from the Exploring Atlantic Canyons and Seamounts 2014 expedition.

A.5 Phylum Arthropoda/Subphylum Crustacea (Crustaceans)

Imaging:	n/a
Relaxation:	Clove oil, initially (not kept on *Okeanos Explorer*)
Genetic Sampling:	• Preferred: Legs; middle leg, as the fore and hind limbs may be useful for species identification (Radulovici et al. 2009)

	○ In large samples, if terminal leg segments exceed the size of the vial, the terminal leg segment may be clipped, the shell removed, and a portion of the leg tissue stored in the vial ○ If terminal leg segments fit in the vial, the terminal leg segment may be put in a vial whole if removal of the shell is difficult or impossible ○ If terminal leg segments are relatively miniscule, several leg segments or an entire leg may be put in the vial ● Alternatives: ○ Several millimeters of abdominal tissue; may be used for any sized sample where leg samples alone do not provide an adequate sample size or are difficult or impossible to obtain ○ Tissue from the gonads (Geller et al. 1997) ○ For the smallest samples, the entire animal may need to be put in a vial ● Barnacles: ○ Preferred: The muscular peduncle at the base of the barnacle (may require dissection not feasible in the field) ○ Alternative: A general sample from the barnacle's soft inner tissues (Van Syoc 1994)
Fixation/Preservation:	70-80% ethanol

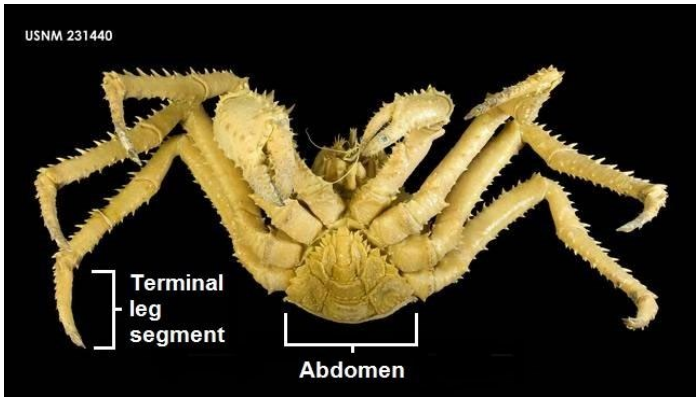

Figure A13. A crustacean *ex situ*. *Image courtesy of Smithsonian National Museum of Natural History*.

A.6 Phylum Ctenophora (Ctenophores, Comb Jellies)

Imaging:	● Whole body photos, ideally live once acclimated in a tank ● Photos and/or video from different angles lit so that interior canals and branching patterns can be seen

	• Strong side lighting with black background (felt is good), relatively far behind the sample
Relaxation:	n/a
Genetic Sampling:	• Preferred: Comb-row tissues • Alternative: Body tissues; avoid tissues from or near the stomach, as these may contain a high concentration of DNA-degrading enzymes (Dumont et al. 2004) or prey items • If the individual sample is too small for genetic sampling, consult with taxonomic experts to identify preferred sampling procedures
Fixation/Preservation:	• Difficult to preserve • Preferred: 5% formalin • Alternative: 70-80% ethanol if no formalin is available

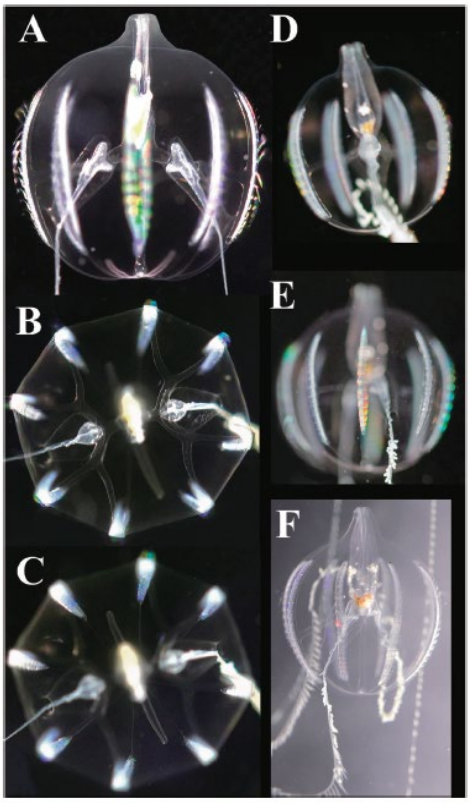

Figure A14. Multiple *in situ* photos of the same ctenophore from six different angles all *in situ*. Image courtesy of D.J. Lindsey (2017).

A.7 Phylum Echinodermata

This section includes asteroids, crinoids, echinoids, holothuroids, and ophiuroids.

A.7.1 Asteroids

This subsection applies to:

- Subphylum Asterozoa
    - Class Asteroidea (asteroids, sea stars)

Imaging:	n/a
Relaxation:	Magnesium chloride solution
Genetic Sampling:	Tissue from tube feet, arms, or gonads, which may be sampled by inserting forceps through a cut in the peristomial membrane (Ward et al. 2008; Templado et al. 2010)
Fixation/Preservation:	• General: 70-80% ethanol (formalin only if requested) • Large individuals can be dried

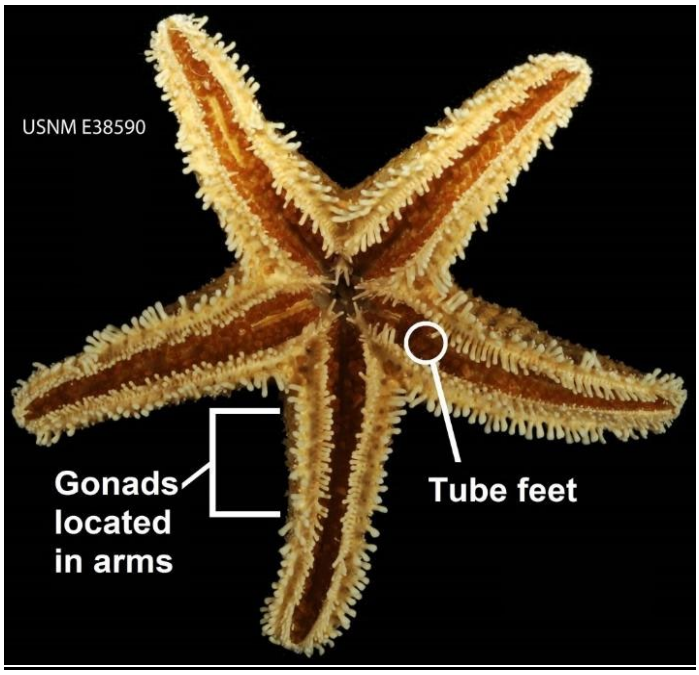

Figure A15. A sea star *ex situ*. *Image courtesy of Smithsonian National Museum of Natural History*.

A.7.2 Crinoids

This subsection applies to:

- Subphylum Crinozoa
    - Class Crinoidea (crinoids, sea lilies and feather stars)

Imaging:	n/a
Relaxation:	n/a
Genetic Sampling:	Gonad tissue, rinsed in ethanol to remove organic detritus and excess mucus, if possible
Fixation/Preservation:	• 70-80% ethanol; push animal oral surface down into a pan of ethanol to fix the arms in a spread position • Formalin should not be used for crinoids

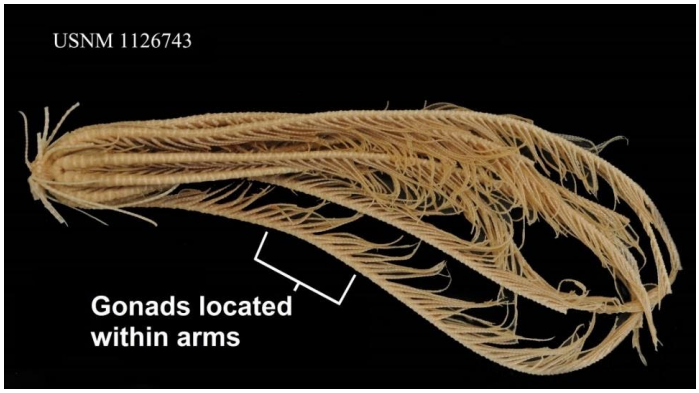

Figure A16. A crinoid *ex situ*. *Image courtesy of Smithsonian National Museum of Natural History*.

A.7.3 Echinoids

This subsection applies to:

- Subphylum Echinozoa
    - Class Echinoidea (echinoids, sea urchins, and sand dollars)

Imaging:	n/a
Relaxation:	Magnesium chloride solution
Genetic Sampling:	Tissue from tube feet or gonads or the muscular tissue surrounding Aristotle's lantern (Edmands et al. 1996)
Fixation/Preservation:	• 70-80% ethanol (formalin only if requested) • Large individuals can be dried

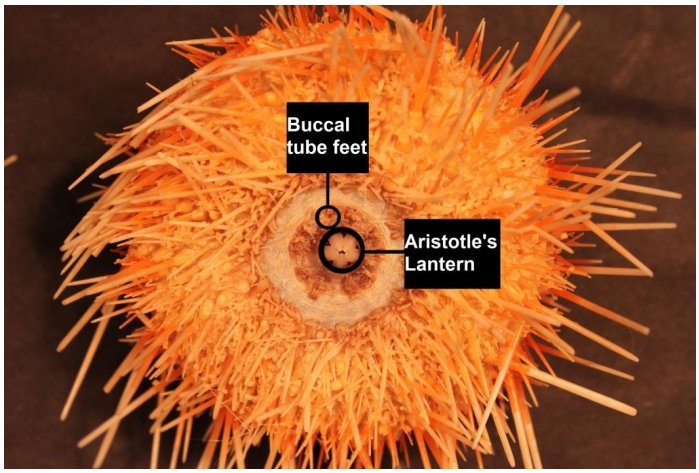

Figure A17. A sea urchin *ex situ*. *Image courtesy of Smithsonian National Museum of Natural History*.

A.7.4 Holothurians

This subsection applies to:

- Subphylum Echinozoa
    - Class Holothuroidea (holothurians, sea cucumbers)

Imaging:	n/a
Relaxation:	Magnesium chloride or chloretone solution
Genetic Sampling:	● Preferred: Tissue from gonads, gut, or inner body wall muscle (Templado et al. 2010) ● Alternative: Sample of the hemolymph (blood equivalent); slit animal open to extract (Xu and Doolittle 1990) ● Avoid tentacle samples if possible, as they may be useful for species identification
Fixation/Preservation:	● 70-80% ethanol; inject animal if possible, particularly large samples ● Formalin should not be used for holothurians

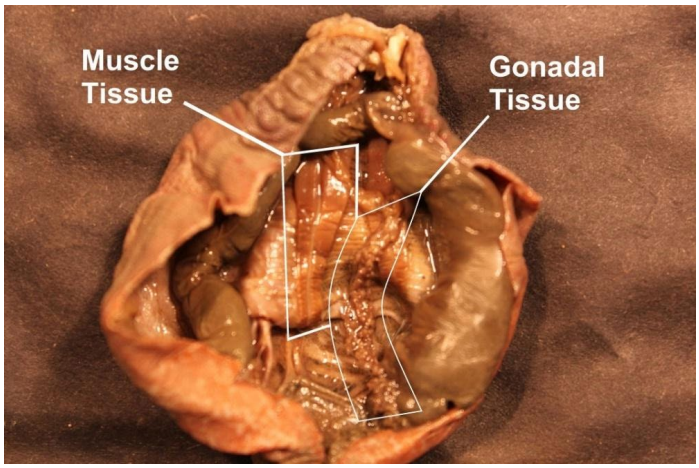

Figure A18. A dissected sea cucumber. *Image courtesy of Smithsonian National Museum of Natural History*.

A.7.5 Ophiuroids

This subsection applies to:

- Subphylum Asterozoa
    - Class Ophiuroidea (ophiuroids, brittle stars)

Imaging:	n/a
Relaxation:	Magnesium chloride solution
Genetic Sampling:	Tissue from arm tips (Sponer and Roy 2002)
Fixation/Preservation:	● 70-80% ethanol (formalin only if requested) ● Large individuals can be dried

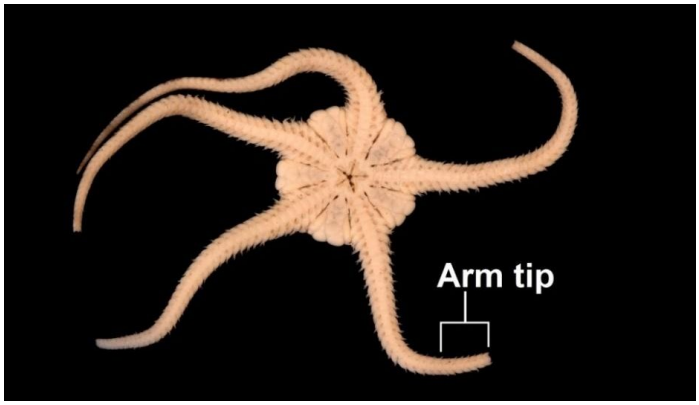

Figure A19. A brittle star *ex situ*. *Image courtesy of Smithsonian National Museum of Natural History*.

A.8 Phylum Mollusca

This section includes bivalves, cephalopods, gastropods, and polyplacophorans.

A.8.1 Bivalves

This subsection applies to:

- Class Bivalvia (bivalves)

Imaging:	n/a
Relaxation:	Magnesium chloride solution or propylene phenoxetol may be needed to open the shell halves (Templado et al. 2010)
Genetic Sampling:	Tissue from the muscular margin of the left and/or right mantle (Fisher and Skibinski 1990)
Fixation/Preservation:	70-80% ethanol

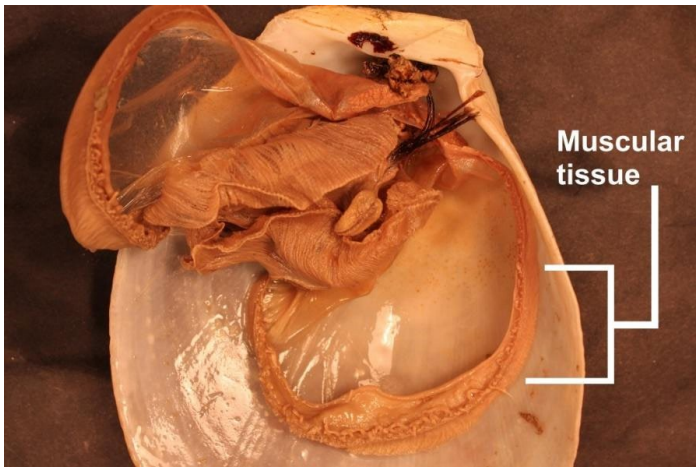

Figure A20. A dissected bivalve. *Image courtesy of Smithsonian National Museum of Natural History*.

A.8.2 Cephalopods

This subsection applies to:

- Class Cephalopoda (cephalopods)

Imaging:	Ventral and dorsal view (if only one is possible, ventral is more useful); if still alive on recovery, tank photos in addition to bench sampling photos

Relaxation:	n/a
Genetic Sampling:	• Preferred: Tissue from either the edge of the mantle, but not where it contacts the funnel, or the edge of the fin (keep most of the fin intact) • Alternative (last resort): Arm tips (not the tentacle tips — arms are the shorter appendages, tentacles are the two longer ones) (Söller et al. 2000) • Minimize the presence of colored skin tissue, as it may contain pigments that inhibit PCR (Prendini et al. 2002)
Fixation/Preservation:	• Fixation (at sea): 10% formalin (whole sample) • Preservation (on shore): Transfer to 50% isopropanol at the museum (isopropanol is not kept on *Okeanos Explorer*) • Cephalopods should not be put in ethanol

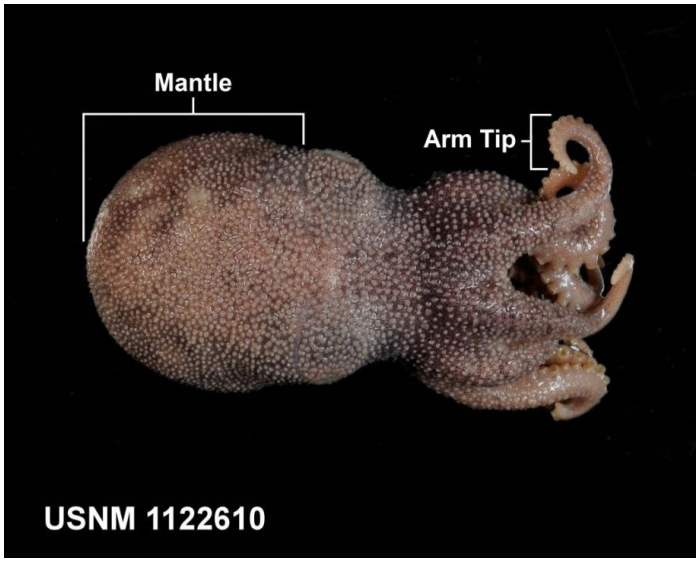

Figure A21. A cephalopod *ex situ*. *Image courtesy of Smithsonian National Museum of Natural History*.

A.8.3 Gastropods, Benthic

This subsection applies to:

- Class: Gastropoda (gastropods, snails and slugs)

Imaging:	n/a
Relaxation:	n/a

Genetic Sampling:	• Preferred: Tissue from the muscular foot tissue (Sokolov et al. 2002)
	• Alternatives:
	  ○ If the animal has retracted into its shell, allow the sample to crawl along a sterile glass surface, i.e., a petri dish lid and then cut a tissue sample from the trailing foot with a scalpel
	  ○ If the shell is unimportant, drill it with a Dremel tool or other device and extract a tissue sample

Fixation/Preservation:	70-80% ethanol

Figure A22. A gastropod on a stalked crinoid with the muscular foot extending outside of the shell from the Mountains in the Deep 2017 expedition.

A.8.4 Gastropods, Pelagic

This subsection applies to:

- Class Gastropoda (gastropods, snails and slugs)

Imaging:	One dorsal and one ventral image, preferably after relaxed
Relaxation:	Magnesium chloride solution
Genetic Sampling:	• Bilaterally symmetric fins or wings: Small amount of tissue from one of the muscular wings or a fin; store in 95% ethanol, extraction buffer, or freeze • Single fin: Small filet of tissue off one of the sides of the body (not the fin)

Fixation/Preservation:	• Shelled (thecosome) pteropods or heteropods (or anything with a calcareous shell): 70-75% ethanol; if possible, use 70% buffered ethanol (0.1 ml ammonium hydroxide in 1 l 70% ethanol, Oakes et al. 2019)
	• Non-shelled (gymnosome) pteropods, non-shelled heteropods, and pseudothecosomes: 10% formalin for one+ days, then dilute with seawater to achieve 5% buffered formalin
	• If unsure of the taxa:
	  ○ If it has a hard shell, put it in ethanol
	  ○ If it does not have a shell or has a cartilaginous shell, put it in formalin

Figure A23. A pelagic pteropod from Arctic Exploration 2002.

A.8.5 Chitons

This subsection applies to:

- Class Polyplacophora (chitons)

Imaging:	n/a
Relaxation:	• Magnesium chloride (chilled works best); go slowly to prevent animal from rolling up
Genetic Sampling:	• Preferred: Piece of foot • Alternative: Tissue from the girdle; keep the chunk small and try to take it from an inconspicuous area, as structures on the girdle that may be useful for species identification

Fixation/Preservation:	70-80% ethanol

Figure A24. A dorsal view of a chiton from the 2016 Hohonu Moana expedition.

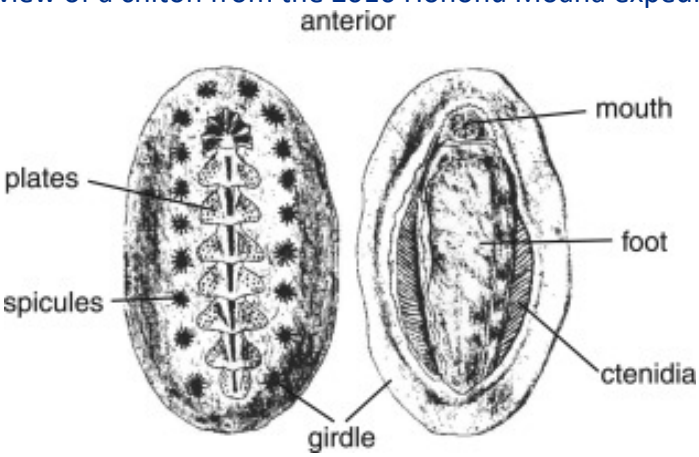

Figure A25. Diagram of a chiton (modified from Gray 1857).

A.9 Phylum Platyhelminthes (Flatworms)

Imaging:	n/a
Relaxation:	n/a
Genetic Sampling:	A small piece of the tail end (Templado et al. 2010)
Fixation/Preservation:	• Fixation (on ship): ○ Preferred: Allow animal to crawl on a piece of moistened paper, then place paper and animal gently onto frozen formalin (Templado et al. 2010)

	○ Alternative: Use regular 10% formalin if frozen formalin is not available ● Preservation (on shore): Move to ethanol at the museum

Figure A26. A flatworm *in situ* from the Mountains in the Deep 2017 expedition.

A.10 Phylum Annelida/Class Polychaeta (Polychaetes, Polychaete Worms)

Imaging:	● Whole body photos of dorsal and ventral sides ● Details of the anterior and posterior ends
Relaxation:	Magnesium chloride solution
Genetic Sampling:	● Tissue from the middle body segment ● A few parapodia from one side ● Avoid anterior and posterior ends as they contain features useful for species identification (Patti and Gambi 2001)
Fixation/Preservation:	● 10% formalin (to be moved to ethanol later) or 70-80% ethanol

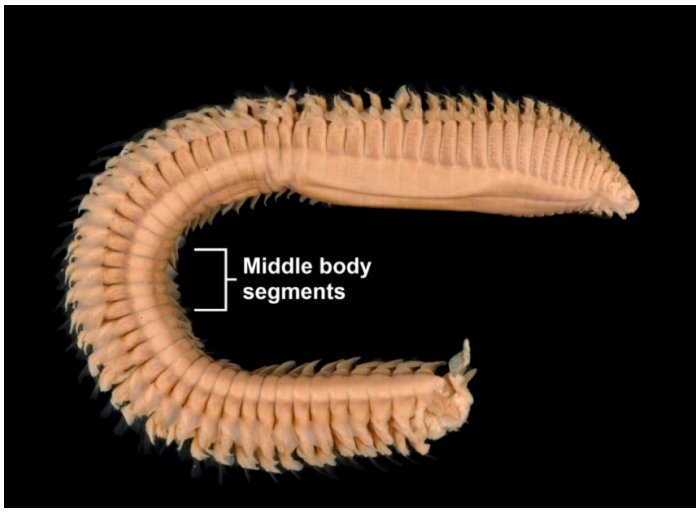

Figure A27. A polychaete worm *ex situ*. *Image courtesy of Smithsonian National Museum of Natural History*.

A.11 Phylum Porifera (Sponges)

Imaging:	n/a
Relaxation:	n/a
Genetic Sampling:	Tissue from the interior of the sponge to lessen the chance of contamination by algae, bacteria, and other animals living within the surface (Borchiellini et al. 2001); should be large enough to allow for further subsampling using a stereomicroscope
Fixation/Preservation:	• 95% ethanol; transfer to a new solution after a few days (the water inside the sponge will dilute the initial ethanol) • Large sponges can be dried if there is not a suitable container for storage in ethanol; to dry, leave in a sealed chemical hood with the fan running or put in 95% ethanol overnight, then remove and air dry in an open location

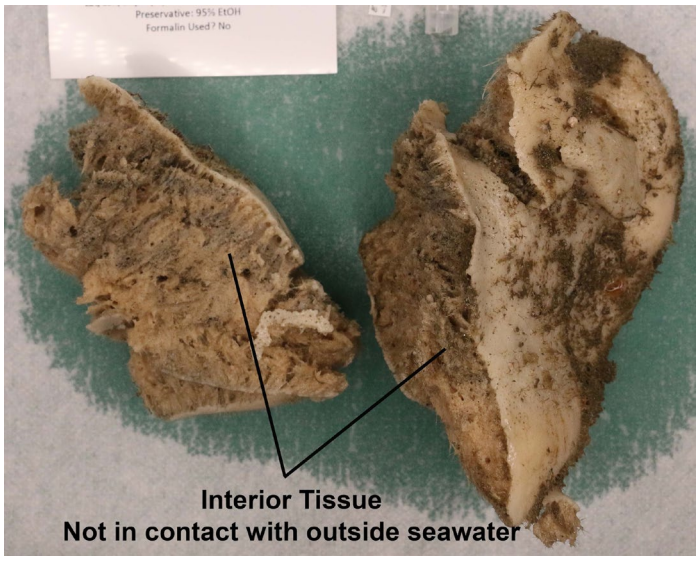

Figure A28. A dissected sponge. *Image courtesy of Smithsonian National Museum of Natural History*.

A.12 Sediment/Sand

Sediment/sand can be a biological or geological sample, depending on the collection objectives. For biological purposes, fix the sample in 95% ethanol. It may be possible to extract meiofauna or perform barcoding using a soil extraction kit.

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Appendix B. Atlantic Seafloor Partnership for Integrated Research and Exploration Target Species Sampling Priorities

The Atlantic Seafloor Partnership for Integrated Research and Exploration (ASPIRE) was a major multiyear, multinational collaborative ocean exploration field program focused on raising collective knowledge and understanding of the North Atlantic Ocean. Between 2017 and 2022, the campaign provided data to inform and support research planning and management decisions in the region. ASPIRE's goals included locating and characterizing deep-sea coral, sponge, and chemosynthetic communities and increasing understanding of deep-sea ecosystem connectivity across the Atlantic basin. In developing sampling priorities, NOAA Ocean Exploration worked with the deep-sea science communities to develop a list of species used in broad, multidisciplinary and ongoing connectivity studies.

Table B1 is based on expert input from Scott France, Shirley Pomponi (in consultation with the SponGES project team), Murray Roberts (in consultation with the ATLAS and iAtlantic Project Steering Committee), Tina Molodtsova, and Andrea Quattrini, and edited to account for feasibility (likelihood of finding the organisms in multiple locations, ability to collect using current capabilities on remotely operated vehicle *Deep Discoverer*, etc.) and likely value of the samples to contributing new scientific knowledge about dispersal dynamics in the North Atlantic Ocean. A field guide version of this appendix can be made available upon request.

Table B1. Target species for the ASPIRE campaign.

Type	Species
High Priority Coral	*Acanella arbuscula* (octocoral) Image 1 | Image 2
High Priority Coral	*Paramuricea* sp. With *Asteroschema* sp. (octocoral and ophiuroid) Image 1 | Image 2
High Priority Coral	*Leiopathes* spp. (black coral) Image
High Priority Coral	*Bathypathes alternata* (black coral) Image 1 | Image 2 | Image 3
High Priority Coral	*Lophelia pertusa** plus *Eunice norvegica* polychaetes (scleractinian) Image 1 | Image 2
High Priority Coral	*Madrepora oculata* plus *Eunice norvegica* polychaetes (scleractinian) Image 1 | Image 2

Type	Species
Secondary Priority Coral	*Primnoa resedaeformis* (octocoral) Image
Secondary Priority Coral	*Desmophyllum dianthus* (scleractinian) Image 1 | Image 2
Secondary Priority Coral	*Paragorgia arborea* (octocoral) Image
Secondary Priority Coral	*Dendrophyllia cornigera* (scleractinian) Image
Tertiary Priority Coral	*Anthomastus* spp. (octocoral) Image 1 | Image 2
Sponge	*Geodia pachydermata* (demosponge) No image available
Sponge	*Aphrocallistes beatrix* (hexactinellid) Image
Sponge	*Vazella pourtalesii* (hexactinellid) Image
Sponge	*Pheronema carpenteri* (hexactinellid) Image
Chemosynthetic Organism	*Bathymodiolus* spp. (mussel) Image
Chemosynthetic Organism	*Abyssogena* spp. (clam) Images

*There has been a name/taxonomic change to *Desmophyllum pertusum*. Samples collected and annotations/digital records created during ASPIRE (2016-2022) refer to this coral as *Lophelia pertusa*.

Appendix C. Campaign to Address Pacific monument Science Technology and Ocean Needs Sampling Priorities

Initiated in July 2015 and ending in 2017, the Campaign to Address Pacific monument Science Technology and Ocean Needs (CAPSTONE) was a major multiyear foundational science effort focused on deepwater areas of U.S. marine protected areas in the central and western Pacific (Kennedy et al. 2019). The investment provides timely, actionable information to support decision-making based on reliable and authoritative science.

During CAPSTONE, 767 biological samples were collected because they were suspected of being new species, new records, and range extensions, as well as morphological variations and diversity assessments. The 278 rocks collected during the multiyear campaign are available to the community to provide information on the geological history of Pacific seamounts and characterize ferromanganese content on seamounts in and around the Prime Crust Zone, an area of the Pacific with the highest levels of commercially valuable deep-ocean mineral deposits (Cantwell et al. 2018).

CAPSTONE sampling was limited to the minimum number of samples needed to provide general representation of the biological and geological setting of each dive site. Biological collections were generally limited to dominant fauna or morphotype in a habitat, undescribed organisms, organisms that appeared to be outside their known habitats, or other samples' with significant discovery potential. Priorities for geological sampling included obtaining rocks for geochemical analysis and age-dating, analysis of ferromanganese content, and meeting other expedition-specific objectives.

All biological samples are accessible through the Invertebrate Zoology collection at the Smithsonian National Museum of Natural History. When possible, selected coral and sponge samples were split, and subsamples were sent to the Bishop Museum's Invertebrate Zoology Collection to facilitate access by scientists in the Pacific Islands region. Prior to preservation, a small aliquot of tissue was also removed and preserved for genetic analysis when doing so did not effectively destroy the sample. These tissue samples were sent to the Ocean Genome Legacy Center at Northeastern University.

Geological samples were sent to the Marine Geology Repository at Oregon State University, where the rocks were curated and described from a petrology perspective (e.g., mineral content, texture, alteration, rock name). Thin and polished sections were cut,

microphotographed, and entered into the repository's sample library. The repository provides online metadata and images of the samples as well as images of thin and polished sections.

Appendix C References

Smithsonian National Museum of Natural History

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