EXPLORATION VARIABLES

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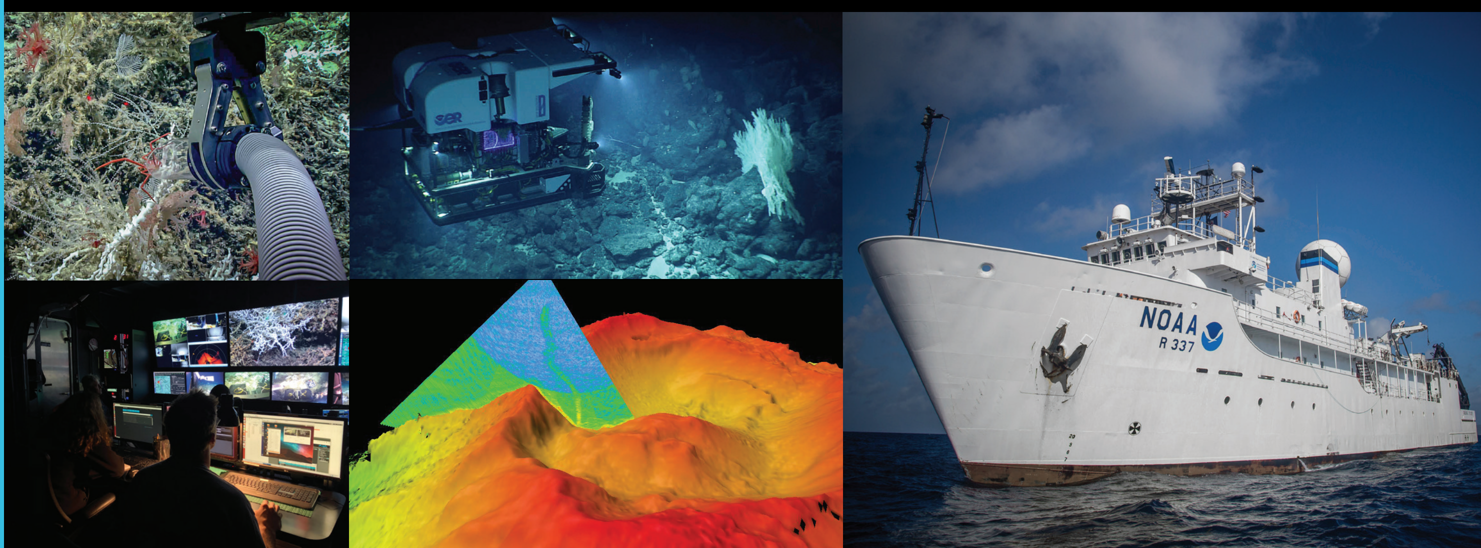

U.S. DEPARTMENT OF COMMERCE
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
OCEANIC AND ATMOSPHERIC RESEARCH
NOAA OCEAN EXPLORATION

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TABLE OF CONTENTS

EXECUTIVE SUMMARY

This report provides the results of a multiyear project to identify deep-ocean exploration variables and evaluate how NOAA Ocean Exploration (the National Oceanic and Atmospheric Administration Office of Ocean Exploration and Research), addresses high-priority exploration variables through its current ocean exploration operations. NOAA Ocean Exploration is the only federal program dedicated to ocean exploration. Through its exploration activities and unique capabilities, NOAA Ocean Exploration reduces unknowns and scientific gaps in deep-ocean areas (greater than 200 m water depth) and provides high-value environmental intelligence required by NOAA and the nation to address current and emerging science and management needs.

To better understand the extent to which NOAA Ocean Exploration is collecting data needed to carry out its exploration mission, the office director tasked a cross-division working group with the review of the oceanographic data recommended for initial exploration of an area (exploration variables). The working group took the following multitiered approach to produce recommendations that address data usability and enhance data collection and presentation:

1. Identify and rank exploration variables required to explore a feature or target area through a literature review that synthesizes deep-ocean exploration and observation needs.
2. Identify the appropriate tools that could be used to address high-priority exploration variables using NOAA Ship *Okeanos Explorer* as an example.
3. Identify high-priority exploration variable data gaps that are not currently addressed by NOAA Ocean Exploration through NOAA Ship *Okeanos Explorer* operations.
4. Develop an approach for incorporating new processes, technologies, and instruments into NOAA Ocean Exploration operations to address high-priority exploration variable data gaps.

The working group identified 91 exploration variables through a literature review of 12 deep-sea publications and reports that synthesize discussions and workshops related to exploration data. Of those 91, 33 exploration variables were identified in three or more reports. Ultimately, the working group deemed 16 exploration variables as high priority for NOAA Ocean Exploration based on the number of mentions in the literature and mission alignment and evaluated if and how we address each of them.

The working group focused on one NOAA Ocean Exploration platform, NOAA Ship *Okeanos Explorer*, because its standard procedures, which are summarized in this report, provide a clear and direct path for evaluating data collection from an ocean exploration perspective. However, it is important to note that NOAA Ocean Exploration also supports ocean exploration through other mechanisms, including grants and partnerships with other government agencies and the academic, private, and philanthropic sectors.

Of the 16 high-priority exploration variables, NOAA Ocean Exploration can address 8 of them with its current *Okeanos Explorer* operations and capabilities. Additionally, it can partially address four others and has the potential to address three more.

The working group identified five high-priority exploration variables as data gaps that NOAA Ocean Exploration could consider in the future. To help address these data gaps, the working group developed a framework to assess the feasibility of incorporating new types of data or data collection procedures into NOAA Ocean Exploration operations. A template and examples of these assessments are included in this report.

As its assets and capabilities change, and as science and technologies continue to advance, NOAA Ocean Exploration continually evaluates and adapts its operations in order to expand the frontiers of ocean exploration.

INTRODUCTION

NOAA Ocean Exploration (the National Oceanic and Atmospheric Administration Office of Ocean Exploration and Research), is the only federal program dedicated to ocean exploration. NOAA Ocean Exploration facilitates deep-sea exploration and discovery by supporting data collection and dissemination in unexplored and underexplored areas, providing initial information about an area for use in further research and decision-making (see **FIGURE 1**). Through its expeditions and unique capabilities, NOAA Ocean Exploration reduces unknowns and scientific gaps in deep-ocean areas (greater than 200 m water depth) and provides high-value environmental intelligence required by NOAA and the nation to address both current and emerging science and management needs.

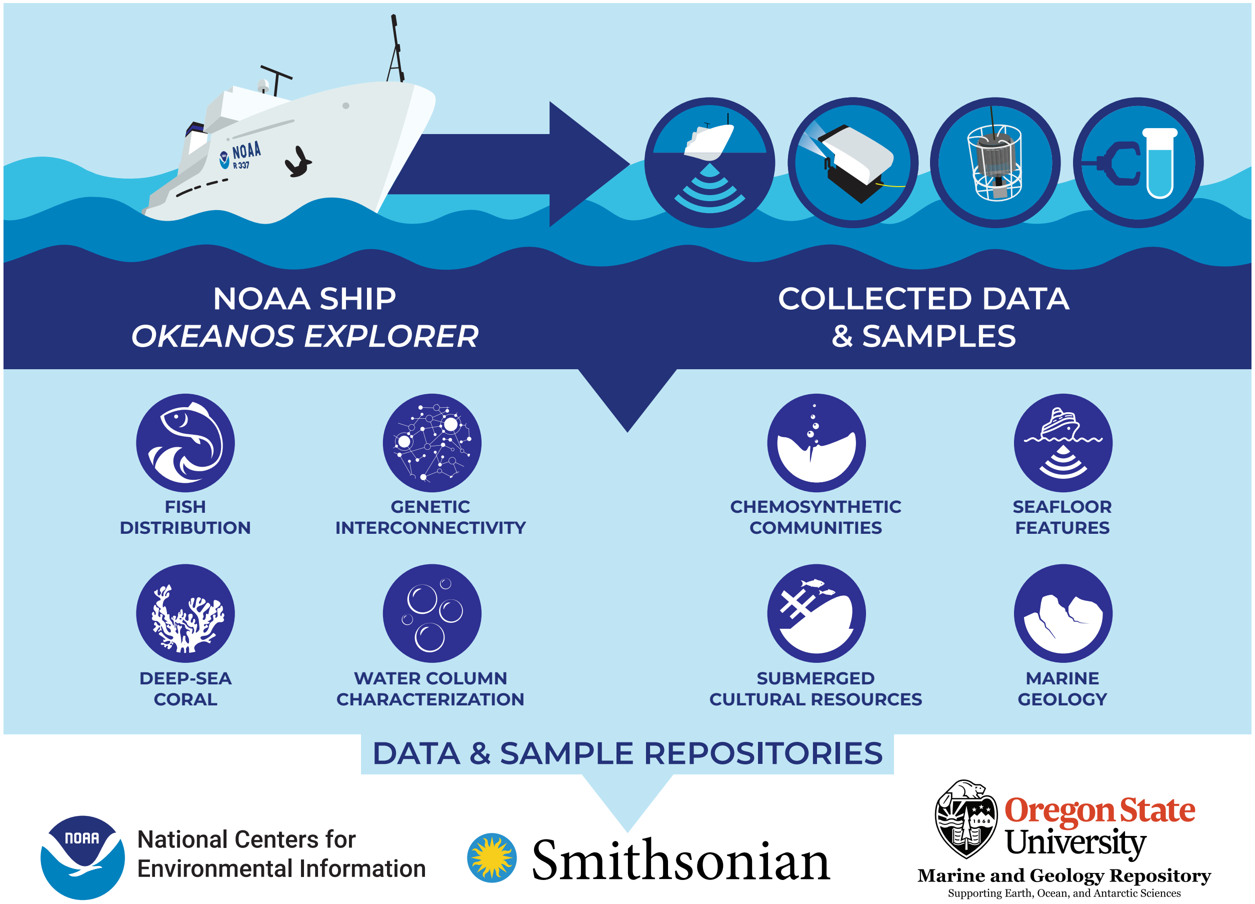

FIGURE 1. The flow of ocean exploration data collected by NOAA Ocean Exploration from NOAA Ship *Okeanos Explorer*. Data and physical samples are made available through three main repositories: NOAA's National Centers for Environmental Information (NCEI), the Oregon State University Marine and Geological Repository, and the Smithsonian Institution's National Museum of Natural History. These data provide baseline information about unexplored and underexplored areas.

NOAA Ocean Exploration is responsible for filling gaps in our basic understanding of U.S. deep waters and the seafloor and providing the critical deep-ocean data needed to strengthen the economy, health, and security of the nation. To do so, we (1) make discoveries of scientific, economic, and cultural value, including mapping ocean basin features of interest; (2) explore geological, physical, chemical, and biological phenomena; (3) explore areas with potential ocean resources; and (4) discover and characterize submerged cultural resources.

This report identifies deep-ocean exploration needs and evaluates the extent to which NOAA Ocean Exploration addresses them. It also serves as an internal audit of our data collection responsibilities as they relate to our mission and identifies improvements we could make to better serve the broad exploration community.

This report was produced by an internal NOAA Ocean Exploration working group tasked with identifying the types of oceanographic data recommended for collection during the initial exploration of an area (exploration variables).[1] The working group consisted of cross-division staff with expert knowledge in NOAA Ocean Exploration data collection through our operations on NOAA Ship *Okeanos Explorer*. They took the following multitiered approach to the task to produce recommendations that address data usability and enhance data collection and presentation:

1. Identify and rank exploration variables required to explore a feature or target area through a literature review that synthesizes deep-ocean exploration and observation needs.
2. Identify the appropriate tools that could be used to address high-priority exploration variables using NOAA Ship *Okeanos Explorer* as an example.
3. Identify high-priority exploration variable data gaps that are not currently addressed by NOAA Ocean Exploration through NOAA Ship *Okeanos Explorer* operations.
4. Develop an approach for incorporating new processes, technologies, and instruments into NOAA Ocean Exploration operations to address high-priority exploration variable data gaps.

Exploration variables contribute directly to the needs of ocean users across academia, industry, nongovernmental organizations, and government. The idea of “exploration variables'' is loosely based on frameworks developed by the Global Ocean Observing System (GOOS) and the Deep Ocean Observing Strategy (DOOS). The GOOS framework of essential ocean variables (EOVs) is designed to advance ocean observations, reduce duplication of efforts, and provide standards

1 This report documents the status of NOAA Ocean Exploration high-priority exploration variables and data gaps as of 2020.

for EOV data collection and utility (IOC n.d.). While similar, the DOOS framework emphasizes observations below 2,000 m and shallow water processes and mechanisms (greater than 200 m) that influence deeper depths (Levin et al. 2019a & 2019b).

Like the GOOS and DOOS, NOAA Ocean Exploration has compiled a list of standard deep-ocean exploration variables to consider when exploring and collecting baseline information of an area for the first time. However, we do not include information about standard data collection. This document describes how NOAA Ocean Exploration collects these data using the capabilities of NOAA Ship *Okeanos Explorer* as an example of how we could address exploration variables that are not part of our current operations.

Ocean data users rely on NOAA Ocean Exploration's exploration activities as the first step toward applications such as baseline characterization, environmental management, and prospecting for natural resources. As a leader in the ocean exploration community, NOAA Ocean Exploration has a responsibility to continually evaluate its performance with community feedback to ensure its data collection is meeting community needs.

EXPLORATION VARIABLES USED TO INFORM DEEP-SEA EXPLORATION NEEDS

The working group conducted a literature review to compile a list of exploration variables recommended for data collection to provide baseline deep-sea information. They reviewed peer-reviewed publications, white papers, and reports that synthesize deep-ocean exploration, observation, and science needs. These materials document comprehensive discussions and workshops involving subject matter experts to capture a multitude of perspectives on data needs.

The working group marked all deep-sea exploration, observation, and science needs mentioned in the literature as exploration variables. They then compiled the exploration variables and preliminarily ranked them based on the number of mentions to determine each exploration variable's relative importance.

The working group reviewed the following 13 papers to compile the list of exploration variables (note: based on their similarity, Levin et al. 2019a and Levin et al. 2019b were combined as one resource). NOAA Ocean Exploration will update the literature review as new workshop reports and relevant literature become available.

LITERATURE ON DEEP-SEA EXPLORATION AND OBSERVATION NEEDS

THE GLOBAL OCEAN OBSERVING SYSTEM ESSENTIAL OCEAN VARIABLES (IOC N.D.)

The Global Ocean Observing System (GOOS) is a program executed by the Intergovernmental Oceanographic Commission of the United Nations Educational, Scientific and Cultural Organization. The GOOS governance model has three tiers: a multinational steering committee to provide oversight, scientific expert panels to guide system requirements, and observation coordination groups to implement global unified network execution. GOOS uses the Framework for Ocean Observing to guide its implementation of an integrated and sustained ocean observing system. Part of the framework addresses ocean observations with a focus on essential ocean variables (EOVs), which are standard variables identified by the GOOS expert panels for collecting ocean observation information.

EXPLORATION OF THE SEAS: VOYAGE INTO THE UNKNOWN (NRC 2003)

In response to a request from the U.S. Congress to examine the feasibility and value of an ocean exploration program, the Ocean Studies Board of the National Academy of Science's National Research Council established the Committee on Exploration of the Seas. The committee convened, along with a public meeting, at the International Global Ocean Exploration Workshop in May 2002 to seek advice from the international community and to discuss the possibilities for, and interest in, a global ocean exploration program. This report documents and provides recommendations on what a new ocean exploration program would look like, how to engage the international community and what kind of funding and governance is needed for such a program. It also identifies science priorities and regions of interest for an ocean exploration program.

NOAA WORKSHOP ON SYSTEMATIC TELEPRESENCE-ENABLED EXPLORATION IN THE ATLANTIC BASIN (OER 2011)

In May 2011, NOAA Ocean Exploration hosted a workshop for members of the scientific community and federal and state partners to identify and discuss potential targets for systematic, telepresence-enabled exploration in the Atlantic Basin, including the Gulf of Mexico and Caribbean Sea. This report summarizes background information, workshop objectives, regions of interest for ocean exploration, and geological, biological, physical, chemical, and archaeological science priorities for those regions.

WORKSHOP ON TELEPRESENCE-ENABLED EXPLORATION OF THE CARIBBEAN REGION (OET 2012)

In November 2012, Ocean Exploration Trust (OET), in partnership with NOAA Ocean Exploration, hosted a workshop for the scientific community and federal and state partners to identify and discuss potential targets for systematic, telepresence-enabled exploration in the Caribbean Region, including the southeastern Gulf of Mexico. This report summarizes background information, workshop objectives, regions of interest for ocean exploration, and geological, biological, physical, chemical, and archaeological science priorities for those regions.

WORKSHOP ON TELEPRESENCE-ENABLED EXPLORATION OF THE EASTERN PACIFIC OCEAN (OET 2014)

In December 2014, OET, in partnership with NOAA Ocean Exploration, hosted a workshop for members of the scientific community to identify and discuss potential targets for telepresence-enabled exploration in the eastern Pacific Ocean. This report summarizes background information, workshop objectives, regions of interest for ocean exploration, and geological, biological, physical, chemical, and archaeological science priorities for those regions.

DEVELOPING SUBMERGENCE SCIENCE FOR THE NEXT DECADE (UNOLS 2016)

In November 1999, marine scientists held a workshop called DEveloping Submergence SCiencE for the Next Decade (DESCEND). The meeting was prompted by a desire to define primary scientific goals of the deep-sea research community and to identify the technologies required for advancing deep-sea studies. The workshop helped set the stage for deep-sea research recommendations. In 2015, the Deep Submergence Science Committee of the University-National Oceanographic Laboratory System (UNOLS) proposed a workshop in response to those recommendations. DESCEND-2016 tasked deep-sea scientists and engineers with (1) identifying the technological and cultural innovations that will enable advancement to understand the deep sea and (2) presenting guidelines that will facilitate government agencies, industry, and philanthropic partners to develop new operational modes and funding opportunities to advance deep-sea research. This report summarizes workshop outcomes and outlines continued deep-sea research needs.

FROM SURFACE TO SEAFLOOR: EXPLORATION OF THE WATER COLUMN (NETBURN 2018)

In 2017, NOAA Ocean Exploration hosted the From Surface to Seafloor: Exploration of the Water Column workshop for scientists, engineers, and program managers to address the following goals related to water column exploration: (1) outline priorities for water column exploration and research; (2) identify best practices for obtaining high-quality, high-resolution data in the water column that address these research priorities; (3) expand the capacity of the "exploration fleet," typically focused on seafloor mapping and remotely operated vehicle surveys, to make water column measurements and observations; (4) collect input on innovation and integration of relevant technologies (e.g., sensors, platforms, instruments) for water column exploration; and (5) encourage collaborations for ongoing and future efforts in water column exploration and research. This report summarizes the outcomes of the workshop and provides guidance to researchers, program managers, foundations, and agencies to mobilize resources to best meet the challenges of fully characterizing the water column.

A THREE-DIMENSIONAL MAPPING OF THE OCEAN BASED ON ENVIRONMENTAL DATA (SAYRE ET AL. 2017)

Sayre et al. (2017) derived a globally comprehensive set of 37 distinct volumetric region units in the ocean called ecological marine units (EMUs). EMUs are constructed on a regularly spaced ocean point-mesh grid, from surface to seafloor, and are attributed with data from the 2013 World Ocean Atlas Version 3. The point data represent the means of decadal averages from 57-years of climatology data for six physical and chemical environmental parameters: temperature, salinity, dissolved oxygen, nitrate, phosphate, and silicate. The authors statistically clustered the point data to define the 37 EMUs, which represent physically and chemically distinct water masses based on spatial variation in those marine environmental characteristics used.

SUMMARY REPORT FOR THE ATLANTIC SEAFLOOR PARTNERSHIP FOR INTEGRATED RESEARCH AND EXPLORATION SCIENCE PLANNING WORKSHOP (OER 2018)

In November 2018, NOAA Ocean Exploration hosted a workshop for experts in deep-sea exploration to discuss North Atlantic Ocean exploration interests and priorities in support of NOAA's Atlantic Seafloor Partnership for Integrated Research and Exploration (ASPIRE) campaign. ASPIRE is a multiyear, multinational collaborative campaign to explore and characterize the North Atlantic Ocean. The objectives of the workshop were to determine Atlantic Ocean-based mapping and characterization needs from a variety of deep-sea exploration interests. This workshop report identifies exploration data types to collect in the North Atlantic Ocean.

A MULTIDISCIPLINARY APPROACH FOR GENERATING GLOBALLY CONSISTENT DATA (WOODALL ET AL. 2018)

In this paper, an interdisciplinary group of marine scientists suggest a formalized, consistent framework of 20 biological, chemical, physical, and socioeconomic parameters that are considered the most important for describing environmental and biological variability. The purpose of the General Ocean Survey and Sampling Iterative Protocol is to establish a consistent approach to data collection. This approach could enable further collaboration among marine scientists from different disciplines to advance ocean knowledge.

DEEP OCEAN OBSERVING STRATEGY 2019 SCIENCE AND IMPLEMENTATION GUIDE (LEVIN ET AL. 2019A)

The Deep Ocean Observing Strategy envisions a globally integrated network of systems to observe the deep ocean in support of strong science, policy, and planning for sustainable oceans. It focuses on ocean depths below the main thermocline (deeper than 2,000 m) with additional attention to poorly sampled, shallower processes and mechanisms below the photic zone (deeper than 200 m) that influence the deeper depths. Three overarching science goals provide the basis for the strategy: (1) understand global deep and bottom water formation rates, their variability, and the time scales of their global property changes while assessing global heat, salt, and freshwater budget dynamics; (2) document deep-ocean tracer transport and ventilation processes and assess their impact on ocean biogeochemical processes, both on the seafloor and in the water column; and (3) understand marine deep-sea biodiversity and ecosystem services in light of human-induced and natural changes. One of the main objectives is to identify EOVs and evolve their specifications to fully consider deep-ocean perspectives across physical, biogeochemical, biological, and ecological variables over the next decade. This includes adding the deep-ocean perspective to the existing GOOS EOVs and adding additional deep-ocean EOVs.

GLOBAL OBSERVING NEEDS IN THE DEEP OCEAN (LEVIN ET AL. 2019B)

In this paper, the authors discuss the scientific need for globally integrated deep-ocean observing, its current status, and the key scientific questions and societal mandates driving observing requirements over the next decade, building off and updating the Deep Ocean Observing Strategy. They identify the EOVs needed to address deep-ocean challenges within the physical, biogeochemical, and biological/ecosystem sciences and map these onto scientific questions. This paper is similar to Levin et al. 2019a, and the same EOVs are mentioned in both papers.

ECOLOGICAL VARIABLES FOR DEVELOPING A GLOBAL DEEP-OCEAN MONITORING AND CONSERVATION STRATEGY (DANOVARO ET AL. 2020)

In this paper, Danovaro et al. identify and rank deep-ocean essential ecological variables for deep-ocean observing and monitoring within five scientific themes: (1) biodiversity; (2) ecosystem functions; (3) impacts and risk assessment; (4) climate change, adaptation, and evolution; and (5) ecosystem conservation. Their results are based on input from 1,155 deep-sea scientists.

EXPLORATION VARIABLES DERIVED FROM DEEP-SEA LITERATURE

Through its literature review, the working group identified 91 exploration variables. Of the 91, 33 are mentioned in three or more of the papers, which is an indicator of importance to the broader community. To further pare down the list, the working group combined similar variables (e.g., biodiversity measures) and dismissed those that serve more specific objectives. The result of this review is a list of 16 high-priority exploration variables for NOAA Ocean Exploration (see **TABLE 1**). This list could be further refined and prioritized based on relevance to the field of study (e.g., biology, geology, etc.) or geographic region.

While the working group recognizes the importance of the other exploration variables, they did not consider them high priority at the time of this report based on the limited number of mentions in the literature (one or two).

TABLE 1. High-priority exploration variables for NOAA Ocean Exploration based on the literature review (see the full list in Appendix A).

High-Priority Exploration Variables	Number of Sources with Mentions	Sources
Species-specific and general biomass, density, distribution, diversity, and abundance: Microbes, plankton, invertebrates, fish, megafauna, marine mammals, meio- and macrofauna	11	GOOS EOVs NRC 2003 OER 2011 OET 2012 OET 2014 UNOLS 2016 Netburn 2018 OER 2018 Woodall et al. 2018 Levin et al. 2019a and 2019b Danovaro et al. 2020
Dissolved oxygen	9	GOOS EOVs NRC 2003 OET 2012 OET 2014 Sayre et al. 2017 Netburn 2018 OER 2018 Woodall et al. 2018 Levin et al. 2019a and 2019b
Distribution and cover of habitats: Mud volcanoes, cold seeps, vent communities, cold water/deep-sea coral communities, general living habitats, unique and sensitive communities	8	GOOS EOVs NRC 2003 OER 2011 OET 2012 OET 2014 OER 2018 Levin et al. 2019a and 2019b Danovaro et al. 2020

High-Priority Exploration Variables	Number of Sources with Mentions	Sources
Specimen collection for genetic and morphological identification, species connectivity analysis, and food web/trophic structure analysis	8	NRC 2003 OET 2012 OET 2014 UNOLS 2016 Netburn 2018 OER 2018 Levin et al. 2019a and 2019b Danovaro et al. 2020
Global seafloor mapping and seafloor composition (substrate)	8	NRC 2003 OER 2011 OET 2012 UNOLS 2016 Netburn 2018 OER 2018 Woodall et al. 2018 Levin et al. 2019a and 2019b
Turbidity, suspended particulates concentration, and flux	8	GOOS EOVs NRC 2003 OET 2012 OET 2014 UNOLS 2016 Netburn 2018 Levin et al. 2019a and 2019b Danovaro et al. 2020
Particulate organic matter, dissolved organic carbon, and heterotrophic and chemoautotrophic carbon	7	GOOS EOVs NRC 2003 OET 2012 OET 2014 Netburn 2018 Levin et al. 2019a and 2019b Danovaro et al. 2020
Inorganic macronutrients, nitrate/nitrite, silicate, and phosphate	7	GOOS EOVs Sayre et al. 2017 OET 2012 Netburn 2018 Woodall et al. 2018 Levin et al. 2019a and 2019b Danovaro et al. 2020

High-Priority Exploration Variables	Number of Sources with Mentions	Sources
Sea surface and subsurface temperature	7	GOOS EOVs UNOLS 2016 Sayre et al. 2017 Netburn 2018 OER 2018 Woodall et al. 2018 Levin et al. 2019a and 2019b
Sea surface and subsurface salinity	7	GOOS EOVs NRC 2003 Sayre et al. 2017 Netburn 2018 OER 2018 Woodall et al. 2018 Levin et al. 2019a and 2019b
Sea surface and subsurface currents	7	GOOS EOVs NRC 2003 OER 2011 OET 2014 Netburn 2018 Woodall et al. 2018 Levin et al. 2019a and 2019b
Carbonate chemistry: Dissolved inorganic carbon, pH, alkalinity, and redox	6	GOOS EOVs OET 2014 Netburn 2018 OER 2018 Woodall et al. 2018 Levin et al. 2019a and 2019b
Biophony, anthrophony, and general ocean sound	4	GOOS EOVs OET 2014 Levin et al. 2019a and 2019b Danovaro et al. 2020
Anthropogenic impacts: Microplastic abundance, size, distribution, and diversity and anthropogenic impacts that may have altered biological communities	4	OET 2014 Netburn 2018 Woodall et al. 2018 Danovaro et al. 2020

High-Priority Exploration Variables	Number of Sources with Mentions	Sources
Bottom pressure	4	GOOS EOVs Netburn 2018 Woodall et al. 2018 Levin et al. 2019a and 2019b
Observations of organisms in their environment, organism behavior	3	UNOLS 2016 Netburn 2018 Danovaro et al. 2020

DATA CURRENTLY COLLECTED BY NOAA OCEAN EXPLORATION ON *OKEANOS EXPLORER*

The second goal of the working group was to identify all data currently collected by NOAA Ocean Exploration during *Okeanos Explorer* expeditions to determine which high-priority exploration variables we are already addressing through this platform and where gaps remain. This also involved inventorying the instruments, protocols, and capabilities associated with NOAA Ocean Exploration's operations on *Okeanos Explorer*.

The information in this section was compiled by the working group, which includes staff from across the office with expertise in NOAA Ocean Exploration's *Okeanos Explorer* operations. Although NOAA Ocean Exploration uses other mechanisms to collect ocean exploration data, such as grants and partnerships with other sectors, the working group chose to focus initially on our *Okeanos Explorer* operations because the standard procedures provide a clear and direct path for evaluating data collection from an ocean exploration perspective.

NOAA Ocean Exploration's *Okeanos Explorer* operations involve the collection of geological, physical, chemical, biological, and archaeological data using acoustic mapping, remotely operated vehicle (ROV) surveys, and more (see **TABLE 2**). These operations follow NOAA Ocean Exploration's Explorer Model, which describes a collaborative effort to identify priorities, pool resources, and address the collective needs of the community beyond the individual (Cantwell et al. 2020).

NOAA Ocean Exploration collects data during two types of expeditions: mapping and ROV. Mapping expeditions involve 24-hour seafloor mapping operations. ROV expeditions involve approximately eight-hour ROV dives during the day with seafloor mapping and other sonar-based operations throughout the night. Both types of expeditions can also include opportunistic CTD (conductivity, temperature, depth) rosette casts and ship-based operations (i.e., ship-mounted meteorological and oceanographic sensors), which are run continuously throughout an expedition.[2]

MAPPING OPERATIONS

As part of its exploration mission, NOAA Ocean Exploration systematically maps waters deeper than 200 m. Our mapping operations target unexplored and underexplored areas with the

2 https://oceanexplorer.noaa.gov/okeanos/collaboration-tools/EX_Capabilities_Overview_2020.pdf

latest tools and technology to expand our knowledge of the deep ocean (Lobecker et al. 2019; Medley et al. 2020). Details on NOAA Ocean Exploration's standard mapping procedures and best practices are in Hoy et al. (2020). Mapping data collected on *Okeanos Explorer* contribute to national directives (see **APPENDIX B**) and provide the information necessary for scientific discovery and research. This section describes the data collected during mapping operations and the tools used to collect them.

BATHYMETRIC AND BACKSCATTER DATA

Bathymetric and backscatter data are collected using multibeam sonar systems and are used to map the seafloor and detect objects in the water column (e.g., biological scattering layers and bubbles from potential seep sites) and on the seafloor.[3] Bathymetric data collection is the first step toward identifying seafloor features of scientific or economic interest. Backscatter data, which is processed from bathymetric data, provide information about the seafloor substrate and objects in the water column based on the amount of acoustic energy returned to the sonar system after it interacts with the seafloor (or object).[4] NOAA Ocean Exploration collects multibeam sonar data continuously on every *Okeanos Explorer* expedition with its Kongsberg EM 304 30 kHz multibeam sonar system.

WATER TEMPERATURE DATA

Water temperature data are essential for calibration for mapping operations and are collected using an expendable bathythermograph (XBT) probe, which is dropped from a ship and measures temperature as a function of depth as it falls through the water. Since temperature influences water density, which influences speed of sound, these temperature data are used to create sound speed profiles to calibrate sonar systems to account for sound speed changes in the water column. NOAA Ocean Exploration uses Lockheed Martin Sippican Deep Blue XBT probes to collect water temperature data from the sea surface to a maximum depth of 750 m. Casts are conducted every two to six hours during mapping operations on every *Okeanos Explorer* expedition.[5]

GEOLOGICAL SUB-BOTTOM PROFILER DATA

Sub-bottom profiler data provide information about the geological environment that can support baseline sediment characterization and identification of geohazards, such as buried gas-charged deposits. Sub-bottom profilers are used to image surficial geological sediment

3 https://oceanexplorer.noaa.gov/technology/sonar/multibeam.html

4 https://oceanexplorer.noaa.gov/okeanos/explorations/ex1104/logs/aug12/welcome.html

5 https://oceanexplorer.noaa.gov/facts/xbt.html

layers to a maximum depth of about 80 meters below the seafloor.[6] They do this by measuring the travel time of the acoustic energy emitted by the profiler, which will vary based on the acoustic impedance of the sediment layer. At each change in impedance (i.e., at the interface of sediment layers), the sound will reflect back upwards. NOAA Ocean Exploration uses a Knudsen Chirp 3260 (3.5 kHz) sonar system to collect sub-bottom profile data about the sedimentary features and bottom topography being mapped simultaneously by the multibeam sonar system on *Okeanos Explorer*.

SPLIT-BEAM ACOUSTIC BACKSCATTER DATA

Acoustic backscatter data measured as a function of depth can be used to estimate distribution, size, and abundance of marine organisms in the water column and can help detect water column anomalies such as gaseous seeps. Split-beam sonar systems (echosounders) are used to collect these data. NOAA Ocean Exploration's Simrad EK60 and EK80 split-beam sonar systems operate at the following frequencies: 18, 38, 70, 120, and 200 kHz. The 70 kHz system is an EK80 that can be operated in wideband mode using multiple frequencies to obtain higher resolution data. All other sonars are EK60s, which operate in narrowband mode only. The EK80 was recently installed on *Okeanos Explorer*, and data collection is expected to start in 2021. NOAA Ocean Exploration collects these data continuously on both mapping and ROV expeditions.

OCEAN CURRENT DATA

Data about the speed and direction of ocean currents provide important insight into the biological, chemical, and physical properties of the ocean. These data can be collected with acoustic Doppler current profilers (ADCPs), which exploit the Doppler Effect by emitting high frequency pulses of sound that scatter due to moving particles in the water.[7] The frequency, or pitch, of the return signal depends on whether particles are moving toward or away from the sound source. NOAA Ocean Exploration uses two ADCPS, a 300 kHz Teledyne Workhorse Mariner and a 38 kHz Teledyne Ocean Surveyor, on mapping and ROV expeditions on *Okeanos Explorer* to collect oceanographic data for scientific purposes and to assess currents near ROV dive locations to inform dive planning and ensure safe ROV deployment and recovery operations.

6 https://oceanexplorer.noaa.gov/okeanos/explorations/ex1404/logs/sept24/sept24.html

7 https://oceanexplorer.noaa.gov/technology/acoust-doppler/acoust-doppler.html

REMOTELY OPERATED VEHICLE OPERATIONS

NOAA Ocean Exploration executes ROV expeditions with a dual-bodied system, ROVs *Deep Discoverer*[8] and *Seirios*[9], and telepresence-enabled technology. Both ROVs are deployed on *Okeanos Explorer* to expand the variety of data collected. These data are often the first collected in an area and are significant contributions to discovery and initial exploration. ROV operations only occur during ROV expeditions.

HIGH-DEFINITION VIDEO

Visual observation of the water column and seafloor has immense exploration and scientific value because of the challenges and expense of deep-sea exploration. Underwater video is used to describe geological, physical, chemical, and biological phenomena and processes and to document archaeological sites. NOAA Ocean Exploration captures high-resolution video from both ROVs continuously during ROV dives. *Deep Discoverer* is equipped with two lasers, spaced 10 cm apart, that are used for scale.

VIDEO ANNOTATIONS

Video annotations provide a record of scientific observations, as documented by experts, made during ROV dives. Types of annotations include physical features, organisms in the water column and on the seafloor, marine debris, and archaeological targets. To capture video annotations during ROV dives, NOAA Ocean Exploration uses collaborative telepresence-enabled exploration,[10] which allows shore-based scientists and students to fully engage in an expedition in real time, and SeaTubeV3, a cloud-based annotation system developed by Ocean Networks Canada and used for NOAA expeditions.[11] NOAA Ocean Exploration partners with scientists to systematically annotate ROV video (both before and after a dive), and annotation effort is dependent on the number of scientists participating in an expedition. To aid annotation and data use, SeaTubeV3 also enables users to search and edit annotations, browse video, and download and manipulate annotation data.

PRIMARY BIOLOGICAL SAMPLES

Physical samples, both biological and geological, can contribute to validation of visual observations (e.g., descriptions of new species) and discovery of novel environmental processes

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8 https://oceanexplorer.noaa.gov/technology/subs/deep-discoverer/deep-discoverer.html

9 https://oceanexplorer.noaa.gov/technology/subs/seirios/seirios.html

10 https://oceanexplorer.noaa.gov/technology/telepresence/telepresence.html

11 https://oceanexplorer.noaa.gov/okeanos/collaboration-tools/science_annotations/welcome.html that are not visible through video (e.g., rock-burrowing organisms). Exploration sampling is intended to acquire a limited number of physical specimens that can provide a general representation of the biological and geological settings for a given dive site or area of interest.[12] During ROV expeditions on *Okeanos Explorer*, NOAA Ocean Exploration uses *Deep Discoverer* to collect primary biological samples. *Deep Discoverer* is equipped with two manipulator arms (with four insulated bioboxes) with custom-built coral cutter jaws with intermeshing fingers to grab objects. The ROV's suction sampler (with five 2.7 L sample jars and one "bypass" jar to flush contaminants from samples) enables the collection of soft, small, or delicate organisms.

PRIMARY GEOLOGICAL SAMPLES

The collection of primary geological samples is similar in purpose to the collection of primary biological samples. Geological samples are acquired to establish the primary geological origin (i.e., volcanic, sedimentary, metamorphic) and characteristic lithology of a site. They can also provide useful information related to the presence or absence of associated biota and processes associated with primary or secondary seafloor mineralization. During ROV expeditions on *Okeanos Explorer*, NOAA Ocean Exploration uses *Deep Discoverer* to collect geological samples. Deep Discover's two manipulator arms are used to grab rocks, which are then stored in the ROV's two rock boxes. Normally, one to two rock samples are collected per dive site (single samples generally weigh approximately 1 kg).

ASSOCIATED BIOLOGICAL AND GEOLOGICAL SAMPLES

Associated samples are opportunistically collected with primary biological and geological samples. They are defined as associates of the primary sample (e.g., epifauna), and are catalogued and archived separately from the primary sample from which they are taken.

CONDUCTIVITY, TEMPERATURE, DEPTH, AND OXYGEN DATA

Conductivity, temperature, depth, and oxygen (CTD-O) data provide important information about the water column as part of an initial assessment of the marine environment. These data can be collected with a CTD (a package of sensors) mounted on an ROV that makes continuous measurements as it travels through water, creating a vertical profile of the water column, which is analogous to a bathymetric map of the seafloor, that illustrates how these variables change relative to depth.[13] NOAA Ocean Exploration collects CTD-O data (and more) during ROV expeditions on *Okeanos Explorer* using Seabird Electronics Model 9/11plus CTDs on *Deep Discoverer* and *Seirios*. These data are collected continuously during ROV dives.

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12 https://oceanexplorer.noaa.gov/okeanos/collaboration-tools/sampling.html

13 https://oceanexplorer.noaa.gov/technology/ctd/ctd.html

TURBIDITY AND OXIDATION REDUCTION POTENTIAL DATA

Turbidity and oxidation reduction potential (ORP) data also provide important details about the water column. Turbidity provides a proxy for resuspended sediments throughout the water column and on the seafloor. ORP (also called redox) is a measure of the tendency of a substance to gain or lose electrons and could, in the case of the latter, signal the presence of chemosynthetic communities. NOAA Ocean Exploration collects turbidity and ORP data during ROV expeditions on *Okeanos Explorer* through light scattering and ORP sensors on Seabird Electronics Model 9/11plus CTDs on *Deep Discoverer* and *Seirios*. These data are collected continuously during ROV dives.

WATER SAMPLES

Water samples can be used to address a variety of exploration variables, such as biodiversity hotspots (e.g., environmental DNA) and nutrient analysis. NOAA Ocean Exploration can collect water samples by request during ROV expeditions on *Okeanos Explorer* with five 1.7 L Niskin bottles that are attached to *Deep Discoverer*.

CTD ROSETTE OPERATIONS

NOAA Ocean Exploration collects oceanographic data and water samples by request during mapping and ROV expeditions on *Okeanos Explorer* with a CTD rosette system that includes a Seabird Electronics Model 9/11plus CTD and a 12-position rosette.

CONDUCTIVITY, TEMPERATURE, DEPTH, AND OXYGEN DATA

Using the same CTD as on *Deep Discoverer*, the CTD rosette contains sensors that measure conductivity, temperature, depth, oxygen, and more. NOAA Ocean Exploration collects CTD-O data continuously during a CTD cast.

TURBIDITY, OXIDATION REDUCTION POTENTIAL, AND FLUOROMETER DATA

The CTD rosette also includes turbidity, ORP, and fluorometer sensors. The fluorometer sensor measures the amount of chlorophyll in the water column as a proxy for phytoplankton concentrations.[14] NOAA Ocean Exploration collects these data continuously during a CTD cast.

14 https://oceanexplorer.noaa.gov/explorations/19gulfofalaska/logs/july26/july26-2.html

WATER SAMPLES

Like ROV water samples, CTD water samples can be used to supplement various data needs. NOAA Ocean Exploration collects water samples opportunistically during a CTD cast with the CTD rosette's twelve 10 L Niskin bottles.

SHIP-BASED DATA COLLECTION

Okeanos Explorer is equipped with several sensors to collect real-time data while the ship is underway. The NOAA Office of Marine and Aviation Operations (OMAO) manages these instruments and the collected data.

METEOROLOGICAL DATA

OMAO manages a suite of meteorological sensors on *Okeanos Explorer*. These sensors measure wind, air temperature, relative humidity, barometric pressure, shortwave radiation, and longwave radiation. OMAO collects these data continuously during all expeditions.

OCEANOGRAPHIC DATA

OMAO manages the onboard Scientific Seawater System on *Okeanos Explorer*, which provides a continuous flow of seawater through a Seabird Electronics SBE 38 remote temperature probe and SBE 45 microthermosalinograph. This system provides temperature, conductivity, salinity, and sound velocity of the sea surface and is used as a backup sea surface sound speed source. OMAO collects these data continuously during all expeditions.

TABLE 2. Data collected during NOAA Ocean Exploration's operations on *Okeanos Explorer*.

Operation Type	Type of Data Collected	Collection Rate
Mapping	Bathymetric and backscatter data	Continuous
Mapping	Water temperature data	2-6 hours
Mapping	Geological sub-bottom profiler data	Continuous
Mapping	Split-beam acoustic backscatter	Continuous (based on sonar frequency)
Mapping	Ocean current data	Continuous
ROV	High-definition video	Continuous
ROV	Video annotations	Variable

Operation Type	Type of Data Collected	Collection Rate
ROV	Primary biological samples	≤9 per dive
ROV	Primary geological samples	≤2 per dive
ROV	Associated biological and geological samples	Variable
ROV	CTD-O data	Continuous
ROV	Turbidity and ORP data	Continuous
ROV	Water samples	≤5 per dive
CTD Rosette	CTD-O data	Continuous
CTD Rosette	Turbidity, ORP, and fluorometer data	Continuous
CTD Rosette	Water samples	≤12 per cast
Ship-based	Meteorological data	Continuous
Ship-based	Oceanographic data	Continuous

ANALYSIS OF NOAA OCEAN EXPLORATION'S CAPABILITIES FOR ADDRESSING HIGH-PRIORITY EXPLORATION VARIABLES

TABLE 1 provides a list of recommended high-priority exploration variables to address in unexplored or underexplored areas of the deep ocean. To identify the data gaps associated with these exploration variables during *Okeanos Explorer* operations, the working group cross-referenced the high-priority exploration variables in **TABLE 1** with the data collected during *Okeanos Explorer* operations from **TABLE 2** as shown in **TABLE 3**. Exploration variables not currently addressed are data gaps (note: that these data gaps could be addressed with a mechanism other than *Okeanos Explorer*). This analysis informs discussions about potential improvements to *Okeanos Explorer* operations and additional assets and mechanisms that may be required for NOAA Ocean Exploration to address these exploration variables.

TABLE 3. Analysis of high-priority exploration variables (Table 1) and data collected during NOAA Ocean Exploration's *Okeanos Explorer* operations (Table 2) showing the exploration variables NOAA Ocean Exploration is already addressing and the data gaps: exploration variables that NOAA Ocean Exploration could address and those we cannot currently address.

High-Priority Exploration Variables	Data Types Collected During Expeditions on *Okeanos Explorer*	High-Priority Exploration Variables: NOAA Ocean Exploration Addresses	High-Priority Exploration Variables: NOAA Ocean Exploration Could Address [1]	High-Priority Exploration Variables: NOAA Ocean Exploration Cannot Address [2]
Species-specific and general biomass, density, distribution, diversity, and abundance: Microbes, plankton, invertebrates, fish, megafauna, marine mammals, meio- and macrofauna	**MAPPING:** Split-beam acoustic backscatter **ROV:** High-definition video, video annotations, water samples* **CTD ROSETTE:** Water samples*, fluorometer data*	Benthic and water column megafauna (fish and invertebrates) biomass, distribution, diversity, and abundance	Bulk biodiversity (microbes, small size classes of benthic and water column fauna, marine mammals) **MECHANISM:** eDNA/'omics techniques with water samples	Species-specific biomass, distribution, and density of small size classes

High-Priority Exploration Variables	Data Types Collected During Expeditions on *Okeanos Explorer*	High-Priority Exploration Variables: NOAA Ocean Exploration Addresses	High-Priority Exploration Variables: NOAA Ocean Exploration Could Address [1]	High-Priority Exploration Variables: NOAA Ocean Exploration Cannot Address [2]
Dissolved oxygen	**ROV:** CTD-O data, ORP data **CTD ROSETTE:** CTD-O data*, ORP data*	Dissolved oxygen	NOAA Ocean Exploration addresses this exploration variable in full	NOAA Ocean Exploration addresses this exploration variable in full
Distribution and cover of habitats: Mud volcanoes, cold seeps, vent communities, cold water/deep-sea coral communities, general living habitats, unique and sensitive communities	**MAPPING:** Bathymetric and backscatter data, geological sub-bottom profiler data, split-beam acoustic backscatter **ROV:** High-definition video, video annotations, CTD-O data, ORP data **CTD ROSETTE:** CTD-O data*, ORP data*	Mud volcanoes, cold seeps, hydrothermal vents, cold water/deep-sea coral communities, general living habitats, unique and sensitive communities	NOAA Ocean Exploration addresses this exploration variable in full	NOAA Ocean Exploration addresses this exploration variable in full
Specimen collection for genetic and morphological identification, species connectivity analysis, and food web/trophic structure analysis	**ROV:** Primary biological samples, associated biological samples	Morphological identification	Genetic identification **MECHANISM:** Barcoding of biological samples	Species connectivity analysis, food web/ trophic structure analysis

High-Priority Exploration Variables	Data Types Collected During Expeditions on *Okeanos Explorer*	High-Priority Exploration Variables: NOAA Ocean Exploration Addresses	High-Priority Exploration Variables: NOAA Ocean Exploration Could Address [1]	High-Priority Exploration Variables: NOAA Ocean Exploration Cannot Address [2]
Global seafloor mapping and seafloor composition (substrate)	**MAPPING:** Bathymetric and backscatter data, geological sub-bottom profiler data **ROV:** Video annotations, primary geological samples	Global seafloor mapping and seafloor composition (substrate)	NOAA Ocean Exploration addresses this exploration variable in full	NOAA Ocean Exploration addresses this exploration variable in full
Turbidity, suspended particulates concentration, and flux	**ROV:** Turbidity data, water samples* **CTD ROSETTE:** Turbidity data*, water samples*	Turbidity	Suspended particulate concentration or mass **MECHANISM:** Filtering water samples	Suspended particulate flux
Particulate organic matter, dissolved organic carbon, heterotrophic and chemoautotrophic carbon	**ROV:** Water samples* **CTD ROSETTE:** Water samples*	NOAA Ocean Exploration does not address this exploration variable	Particulate organic matter, dissolved organic carbon **MECHANISM:** Chemical analysis of water samples, sensors	Heterotrophic and chemoautotrophic carbon

High-Priority Exploration Variables	Data Types Collected During Expeditions on *Okeanos Explorer*	High-Priority Exploration Variables: NOAA Ocean Exploration Addresses	High-Priority Exploration Variables: NOAA Ocean Exploration Could Address [1]	High-Priority Exploration Variables: NOAA Ocean Exploration Cannot Address [2]
Inorganic macronutrients, nitrate/nitrite, silicate, and phosphate	**ROV:** Water samples* **CTD ROSETTE:** Water samples*	NOAA Ocean Exploration does not address this exploration variable	Inorganic macronutrients, nitrate/nitrite, silicate, and phosphate **MECHANISM:** Chemical analysis of water samples, sensors	NOAA Ocean Exploration could address this exploration variable
Sea surface and subsurface temperature	**MAPPING:** Water temperature data **ROV:** CTD-O data **CTD ROSETTE:** CTD-O data* **SHIP-BASED:** Meteorological data, oceanographic data	Sea surface and subsurface temperature	NOAA Ocean Exploration addresses this exploration variable in full	NOAA Ocean Exploration addresses this exploration variable in full
Sea surface and subsurface salinity	**MAPPING:** Water temperature data **ROV:** CTD-O data **CTD ROSETTE:** CTD-O data* **SHIP-BASED:** Meteorological data, oceanographic data	Sea surface and subsurface salinity	NOAA Ocean Exploration addresses this exploration variable in full	NOAA Ocean Exploration addresses this exploration variable in full

High-Priority Exploration Variables	Data Types Collected During Expeditions on *Okeanos Explorer*	High-Priority Exploration Variables: NOAA Ocean Exploration Addresses	High-Priority Exploration Variables: NOAA Ocean Exploration Could Address [1]	High-Priority Exploration Variables: NOAA Ocean Exploration Cannot Address [2]
Surface and subsurface currents	**MAPPING:** Ocean current data	Sea surface and subsurface currents	NOAA Ocean Exploration addresses this exploration variable in full	NOAA Ocean Exploration addresses this exploration variable in full
Carbonate chemistry: Dissolved inorganic carbon, pH, alkalinity, and redox	**ROV:** ORP data, water samples* **CTD ROSETTE:** ORP data*, water samples*	Redox	Dissolved inorganic carbon, pH, alkalinity **MECHANISM:** Chemical analysis of water samples, sensors	NOAA Ocean Exploration partially addresses this exploration variable
Biophony, anthrophony, and general ocean sound	NOAA Ocean Exploration does not address this exploration variable	NOAA Ocean Exploration does not address this exploration variable	NOAA Ocean Exploration does not address this exploration variable	Biophony, anthrophony, and general ocean sound
Anthropogenic impacts: Microplastic abundance, size, distribution, and diversity and anthropogenic impacts that may have altered biological communities	**ROV:** High-definition video, video annotations, water samples*, primary biological specimens, associated biological specimens **CTD ROSETTE:** Water samples*	Macrodebris annotations, interactions between organisms and macrodebris	Water column microplastic abundance, distribution, size, and diversity **MECHANISM:** Chemical analysis of water samples, *in situ* pump	Sediment microplastic, distribution, size, abundance, and diversity; comprehensive quantification of anthropogenic impacts on biological communities

High-Priority Exploration Variables	Data Types Collected During Expeditions on *Okeanos Explorer*	High-Priority Exploration Variables: NOAA Ocean Exploration Addresses	High-Priority Exploration Variables: NOAA Ocean Exploration Could Address [1]	High-Priority Exploration Variables: NOAA Ocean Exploration Cannot Address [2]
Bottom pressure	**MAPPING:** Water temperature data **ROV:** CTD-O data **CTD ROSETTE:** CTD-O data*	Bottom pressure	NOAA Ocean Exploration addresses this exploration variable in full	NOAA Ocean Exploration addresses this exploration variable in full
Observations of organisms in their environment, organism behavior	**ROV:** High-definition video, video annotations	Observations of megafauna in their environment, megafauna behavior	NOAA Ocean Exploration addresses this exploration variable in full	NOAA Ocean Exploration addresses this exploration variable in full

1 This is a data gap because while NOAA Ocean Exploration could collect these data within the context of its current operations, it does not have all the equipment and procedures in place to do so.

2 This is a data gap because the exploration variables require more comprehensive data collection and/or analysis than NOAA Ocean Exploration can address with current operations.

***** *Okeanos Explorer* currently has the capability, but it is not used systematically.

ADDRESSING DATA GAPS

Of the 16 high-priority exploration variables, NOAA Ocean Exploration fully addresses 8 using the current capabilities on *Okeanos Explorer*. The remaining exploration variables are either partially addressed, could potentially be addressed, or are not currently addressed at all (see **TABLE 3**). NOAA Ocean Exploration does not fully address these remaining high-priority exploration variables because:

- NOAA Ocean Exploration does not systematically, or have the capability to, collect required samples or data;
- NOAA Ocean Exploration does not currently have a mechanism for sample analysis; OR
- It is outside of NOAA Ocean Exploration's scope and could be considered characterization rather than exploration.

As noted in **TABLE 3**, the exploration variables that could be fully addressed with available technology or analysis of samples are biogeochemical measurements (i.e., organic matter, inorganic macronutrients, and inorganic carbon) and alkalinity/pH/redox potential. NOAA Ocean Exploration could do this through chemical analysis of water samples or with new sensor integration on the CTD rosette or ROV. If biogeochemical or pH sensor integration onto the CTD rosette is feasible, CTD casts, which are not currently systematically conducted during expeditions on *Okeanos Explorer*, would be required. Sensor integration on the CTD rosette or ROV would allow for the collection of vertical profiles during descent and ascent and throughout a dive.

The ability to systematically sample water could address several data gaps noted in **TABLE 3**. NOAA Ocean Exploration could use systematic CTD casts to address data gaps that require collection of water samples, such as bulk biodiversity through eDNA/'omics techniques and biogeochemical analysis (i.e., suspended particulate concentration, organic matter, inorganic macronutrients, and inorganic carbon), if sensor integration is not feasible. NOAA Ocean Exploration could also use water samples to conduct microplastic analyses in the water column (see **APPENDIX F**).

By collecting sediment samples, NOAA Ocean Exploration could address several of the data gaps or further support current high-priority exploration variables noted in **TABLE 3**. NOAA Ocean Exploration does not currently have the capability to quantitatively sample sediment for infauna biodiversity metrics (i.e., species richness, abundance, distribution, biomass), sediment biogeochemistry, or sediment microplastics.

In addition to water and sediment sample collection, sample processing and analysis would be required to address some of the high-priority exploration variables. NOAA Ocean Exploration is making some progress on this front by partnering with the NOAA Northwest Fisheries Science Center to pilot the collection of water samples for eDNA analysis. Moreover, NOAA Ocean Exploration could leverage its existing partnership with the Smithsonian Institution to barcode collected biological specimens for genetic identification. However, we do not have such partnerships for biogeochemical or microplastic analyses of water or sediment samples.

Additionally, the technology exists for collecting passive acoustics, or collecting information about general ocean sound, but similarly, NOAA Ocean Exploration is not able to collect or analyze these data in a meaningful way.

By adding new instruments (e.g., sensors), systematically collecting data with existing *Okeanos Explorer* capabilities, and establishing new partnerships to analyze samples, NOAA Ocean

Exploration could more fully address many of the data gaps in **TABLE 3**. However, some of the data gaps, while noted as high priority, could be outside the scope of NOAA Ocean Exploration's exploration activities and priorities (i.e., those set by the office or external drivers like those in **APPENDIX B**). For example, further genetic and isotopic analyses of biological samples for species connectivity and trophic dynamics may be considered characterization rather than exploration. Collecting data on biogeochemical fluxes requires continuous or repeated sampling, which necessitates returning to the same area (i.e., exploration through time) and is not how NOAA Ocean Exploration currently operates. Similarly, the comprehensive quantification of anthropogenic impacts on biological communities requires periodic monitoring efforts to which NOAA Ocean Exploration can and does contribute but does not prioritize. In addition, many of the high-priority exploration variables were pulled from documents that are more focused on ocean observing or addressing specific science needs, which are outside of NOAA Ocean Exploration's purview.

FEASIBILITY ASSESSMENT PROCESS

Feasibility assessments are comprehensive evaluations of both science and operations and are part of the due diligence necessary to answer outstanding and arising exploration needs. NOAA Ocean Exploration conducts feasibility assessments to document a priority data gap (or a measurement, instrument, or process) (see **TABLE 3**) and determine how it could be addressed with our current *Okeanos Explorer* operations. The working group developed the framework described here specifically for NOAA Ocean Exploration's *Okeanos Explorer* operations, but it could be adapted for other platforms.

NOAA Ocean Exploration conducts feasibility assessments for data gaps that we identify as exploration priorities. Once identified, staff assess what is needed to address a gap (and possibly others) with new or augmented *Okeanos Explorer* capabilities. This includes researching a data gap itself or the measurement, instrument, or process needed to address it, consulting with subject matter experts throughout the process.

The first phase of a feasibility assessment includes drafting a document that is reviewed internally by NOAA Ocean Exploration staff and then shared with external subject matter experts for review. After making the appropriate edits and corrections, the assessors finalize their determination about the feasibility of addressing the data gap with NOAA Ocean Exploration's *Okeanos Explorer* operations and submit the assessment to NOAA Ocean Exploration leadership with recommendations on how to proceed.

If the tradeoffs identified in the feasibility assessment are determined to be too great, NOAA Ocean Exploration will explore other avenues for addressing the data gap (e.g., grants and partnerships). If it is feasible to be done with *Okeanos Explorer* operations, NOAA Ocean Exploration's Science and Technology and Expeditions and Exploration divisions will work together to develop standard operating protocols, scope workflows, and plan test demonstrations.

The next three subsections provide the feasibility assessment template and summaries of two NOAA Ocean Exploration feasibility assessments as examples: one that was deemed feasible with *Okeanos Explorer* operations and one that was not.

FEASIBILITY ASSESSMENT TEMPLATE

1. NAME AND TYPE OF DATA GAP

- **a.** What is the data gap?
- **b.** What is the type of data gap?
  - **i.** Data are not being collected in general;
  - **ii.** Data are not being collected in a specific location or depth; or
  - **iii.** Data are not being collected at a specific frequency.
- **c.** What high-priority exploration variables could NOAA Ocean Exploration address by filling the gap? (See **TABLE 1**, **TABLE 3**, and **APPENDIX TABLE A1**.)

2. BACKGROUND AND JUSTIFICATION

- **a.** Background information on the data gap, including the exploration/characterization distinction, its importance, and current state of the science.
- **b.** How would the data help address NOAA Ocean Exploration's mission and goals?
- **c.** What big picture scientific questions would the data address?
- **d.** Was the data gap identified by the deep-sea community as important to address? If yes, where was it identified and who identified it?
- **e.** Which stakeholders and partners would these data serve?
- **f.** Have the data been collected during prior NOAA Ocean Exploration-supported expeditions or are there plans to collect the data during upcoming expeditions? If yes, provide details.

3. METHODS, PROTOCOL OPTIONS, AND SAMPLING STRATEGY[15]

- **a.** Where should sampling occur and/or what would be the sampling strategy?
- **b.** Would sampling be part of standard operations or opportunistic?
- **c.** What sampling methods would be used?
- **d.** What would be the protocol? Is there more than one option? Are there ancillary data objectives that are compatible with the protocol?
- **e.** What would an ideal protocol look like? How would it compare to a more feasible option?
- **f.** If physical samples are to be collected, where would they be stored on board?

4. MATERIALS AND COST

- **a.** What materials or equipment would be needed to collect the data?
- **b.** What type of storage and space for samples and supplies would be needed on the ship?
- **c.** How much would it cost to purchase sensors or supplies needed to collect the data?
- **d.** How much would it cost to collect the data?
- **e.** How much would it cost to store the data?
- **f.** How much would it cost to maintain the capability over a five-year period?

5. TIME

- **a.** How much time would it take to collect and/or process the data?

6. PERSONNEL

- **a.** Who would be in charge of collecting the data?
- **b.** Who would be in charge of sample data processing, management, and archiving?
- **c.** Could this work be done with current personnel or would NOAA Ocean Exploration need to hire new personnel?
- **d.** If no new personnel would be needed, how would this work impact other tasks required of current personnel?
- **e.** During what type of expedition could these data be collected (i.e., mapping, ROV)?

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15 Here, "methods" refers to the general process whereas "protocol options" refers to more detailed information on specific steps for collecting the data.

7. NOAA OCEAN EXPLORATION'S CURRENT OPERATIONS ON *OKEANOS EXPLORER*: COMPLEMENTARY AND CONTRASTING DATA COLLECTION

a. Is collecting these data a priority? If so, how does it compare to current data collections?

b. How would collecting the data take away from current operations?

c. How would collecting the data complement current operations and potentially new operations?

8. EXPECTED PRODUCTS

a. What would be the expected scientific products?

b. Would the data be reported in expedition reports?

c. Who would be responsible for producing the expected products?

9. DATA MANAGEMENT, PROCESSING, SUMMARIES, AND QUALITY CONTROL

a. What would be the minimum metadata needed?

b. Would data analysis or processing be needed?

c. How would the data be analyzed or processed?

d. Would a data summary be needed?

e. How would a data summary be formatted and what would it include?

f. What would be the QA/QC process?

g. Would Coastal and Marine Ecological Classification Standard implementation be necessary? If so, how would it be implemented?

10. DATA ACCESSIBILITY, STORAGE, AND ARCHIVING

a. Would (or could) the data be stored at the NCEI? If not, what public repositories could be used for data archiving?

b. What would be the data archiving pipeline?

c. If stored somewhere other than NCEI, how would the information be shared with NCEI and NOAA Ocean Exploration?

d. How would the data stored outside of NCEI be connected to the rest of the collection?

11. PERMITTING AND REGULATIONS

a. Would permits or licenses be needed to collect the data?

12. ENVIRONMENTAL OR TECHNOLOGICAL RISK

- **a.** Given all of the above considerations, could there be environmental or technological risks involved in collecting the data?

13. FEASIBILITY OF COLLECTING THE DATA

- **a.** Given all of the above considerations, is it feasible to collect these data as part of NOAA Ocean Exploration's operations on *Okeanos Explorer*? (yes/maybe/no)
  - **i.** If yes, move forward with implementation.
  - **ii.** If maybe, what modifications to the vessel facilities or exploration assets would be needed to accommodate data collection?
  - **iii.** If no, what would be the most suitable (efficient, effective, economical) way for NOAA Ocean Exploration to obtain the data? Through a new or existing partnership? Through NOAA Ocean Exploration's competitive grant program? How could NOAA Ocean Exploration move forward, if possible?

14. RELEVANT LITERATURE

SUMMARY: ASSESSING FEASIBILITY OF COLLECTING ENVIRONMENTAL DNA

See **APPENDIX E** for the full eDNA feasibility assessment.

Environmental DNA (eDNA) is the genetic material shed by organisms into the surrounding environment. By collecting environmental samples that contain organismal mucus, feces, or tissue particles, scientists can process eDNA to make new discoveries about marine life.[16] NOAA Ocean Exploration assessed the feasibility of incorporating eDNA collection into our *Okeanos Explorer* operations as a first test of the feasibility assessment.

For this feasibility assessment, the assessors focused on collecting eDNA to address some of the high-priority data gaps: marine microbial communities (Heidelberg et al. 2010), plankton communities (Suter et al. 2020), and bulk biodiversity (Ruppert et al. 2019; Thomsen and Willerslev 2015. eDNA is also being used to assess deep-sea ecosystems, such as deep-sea coral communities (Everett and Park 2018) and communities around critical mineral locations (Laroche et al. 2020). eDNA could be used to complement data currently collected during NOAA Ocean Exploration's *Okeanos Explorer* operations (e.g., with CTD rosette casts). Specifically, it could be

16 https://oceanexplorer.noaa.gov/technology/edna/edna.html

used to expand the number of taxa that could be included in biodiversity measurements, detect recent presence of organisms not typically imaged by the ROV, inform food web analyses, and detect rare, endemic, or invasive species (Beng and Corlett 2020).

The eDNA feasibility assessment revealed that NOAA Ocean Exploration has the equipment to collect water samples (Niskin bottles on the ROV and CTD rosette), and lab space on *Okeanos Explorer* is adequate for filtering samples for eDNA. Additionally, the time required to collect eDNA samples is relatively small compared to the benefit of addressing several high-priority exploration variables. Nevertheless, challenges to incorporating eDNA sampling into NOAA Ocean Exploration's standard *Okeanos Explorer* operations remain: procuring personnel time; identifying a repository to store the samples; conducting the DNA extraction, sequencing, and bioinformatics; and funding sample analysis.

Through the assessment, NOAA Ocean Exploration identified potential partnerships both within and outside of NOAA to assist with sample analysis and existing partnerships that could be leveraged for long-term storage of filters on which eDNA samples are collected. The assessment also revealed the importance of sampling for eDNA in unexplored areas and continuing to collect primary biological specimens to build up a reference genetic database, activities for which NOAA Ocean Exploration operations are especially well suited.

Based on the assessment, the working group recommends that, at a minimum, NOAA Ocean Exploration systematically collect water samples via the Niskin bottle attachments on *Deep Discoverer* and the CTD rosette, filter the samples, and store the filters in a repository for future analysis. The number of samples and the general sampling strategy should be determined based on the region and its science needs. Long-term partnerships for sample analysis are currently being scoped and identified. As new technologies (e.g., autonomous systems, *in situ* pumps) develop, NOAA Ocean Exploration will assess them to determine the most effective ways to collect eDNA.

SUMMARY: ASSESSING FEASIBILITY OF MICROPLASTIC DISTRIBUTION, ABUNDANCE, SIZE, AND COMPOSITION

See **APPENDIX F** for the full microplastic feasibility assessment.

Microplastics (smaller than 5 mm in length) are a common form of marine debris that are ubiquitous in aquatic environments. They were identified as a data gap as an increasingly significant component of human impact in deep-sea habitats, even those that are considered pristine (Kane and Clare 2019). This feasibility assessment focused on measuring microplastic abundance, distribution, size, and composition using new or existing capabilities during NOAA Ocean Exploration's *Okeanos Explorer* operations.

This assessment explored the microplastics data gap and considered several ways to address it, both in the water column and along the seafloor. It also examined complementary data gaps that could be similarly addressed (e.g., sediment cores could be used for microplastics sampling as well as biodiversity surveys). Because microplastics sampling would require new equipment (e.g., net tows, sediment corers), operations (e.g., towing a net, collection of physical sediment samples), and personnel (e.g., people needed to process samples), microplastic sampling was not recommended for NOAA Ocean Exploration's current *Okeanos Explorer* operations. However, the assessment provided options for collecting and processing samples that could be revisited in the future. If NOAA Ocean Exploration obtains new capabilities, or external drivers prioritize microplastics sampling (e.g., scientific interest significantly increases), this information will help us quickly reevaluate the feasibility of microplastic sampling.

RECOMMENDATIONS AND NEXT STEPS

As described in the introduction, the working group was initially tasked with identifying exploration variables required to explore a feature or target, identifying appropriate tools to address these exploration variables using *Okeanos Explorer* as an example, and developing an approach for addressing exploration variable data gaps. This work is constantly evolving as NOAA Ocean Exploration assets and capabilities change, and as science and technology continue to advance. This report provides a foundation for the ocean exploration community to use to assess where efforts could most effectively expand knowledge of the deep ocean.

Recommendations and next steps for NOAA Ocean Exploration include:

- Periodically review the deep-sea literature for new papers and reports that discuss deep-sea exploration and observation needs to update exploration variable recommendations.
- Continually reevaluate recommended exploration variable priorities based on evolving topical, disciplinary, and geographic needs and in response to internal and external drivers.
  - Develop end-use data products for specific exploration objectives based on NOAA or NOAA Ocean Exploration needs.[17]
  - Align exploration variables with requirements set by the National Strategy for Mapping, Exploring, and Characterizing the United States Exclusive Economic Zone and its implementation plan.
- Continue to conduct feasibility assessments on exploration variables data gaps that have been identified as important by the scientific community.
- Scope other platforms that NOAA Ocean Exploration could use to address exploration variable data gaps.

These recommendations are based on accomplishments and areas of improvement identified in response to changing priorities, capabilities, and needs. As NOAA Ocean Exploration continues to expand the frontiers of ocean exploration, the data we collect will contribute to robust research, well-informed resource management, and discoveries of scientific, economic, and social significance.

17 https://oceanleadership.org/wp-content/uploads/2020/11/OceanExploration_PacificPriorities_WorkshopReport_NOV2020.pdf

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REFERENCES

Beng, K.C. and Corlett, R.T. (2020). Applications of environmental DNA (eDNA) in ecology and conservation: Opportunities, challenges and prospects. Biodiversity and Conservation 29:2089-2121. https://doi.org/10.1007/s10531-020-01980-0

Cantwell, K., Kennedy, B.R.C, Malik, M., Suhre, K.P., Medley, R., Lobecker, E., Hoy, S., Adams, C., Dornback, M., and Cromwell, M. (2020). The Explorer Model: Lessons from 10 years of community led ocean exploration and open data. Journal of Ocean Technology 15(3):77-86.

Danovaro, R., Fanelli, E., Aguzzi, J., Billett, D., Carugati, L., Corinaldesi, C., Dell'Anno, A., Gjerde, K., Jamieson, A.J., Kark, S., McClain, C., Levin, L., Levin, N., Ramirez-Llodra, E., Ruhl, H., Smith, C.R., Snelgrove, P.V.R., Thomsen, L., Van Dover, C.L., and Yasuhara, M. (2020). Ecological variables for developing a global deep-ocean monitoring and conservation strategy. Nature Ecology & Evolution 4:181-192. https://doi.org/10.1038/s41559-019-1091-z

Everett, M.V. and Park, L.K. (2018). Exploring deep-water coral communities using environmental DNA. Deep Sea Research Part II: Topical Studies in Oceanography 150:229-241. https://doi.org/10.1016/j.dsr2.2017.09.008

Heidelberg, K.B., Gilbert, J.A., and Joint, I. (2010). Marine genomics: At the interface of marine microbial ecology and biodiscovery. Microbial Biotechnology 3:531-543. https://doi.org/10.1111/j.1751-7915.2010.00193.x

Hoy, S., Lobecker, E., Candio, S., Sowers, D., Froelich, G., Jerram, K., Medley, R., Malik, M., Copeland, A., Cantwell, K., Wilkins, C., and Maxon, A. (2020). Deepwater Exploration Mapping Procedures Manual. Office of Ocean Exploration and Research. https://doi.org/10.25923/jw71-ga98

Intergovernmental Oceanographic Commission (IOC). (n.d.). Essential Ocean Variables. The Global Ocean Observing System. Retrieved January 15, 2021 from https://www.goosocean.org/index.php?option=com_content&view=article&id=14&Itemid=114

Kane, I.A. and Clare, M.A. (2019). Dispersion, accumulation, and the ultimate fate of microplastics in deep-marine environments: A review and future directions. Frontiers in Earth Science 7:80. https://doi.org/10.3389/feart.2019.00080

Levin, L., Ruhl, H., Heimbach, P., McCurdy, A., and Smith, L. (2019a). Deep Ocean Observing Strategy Science and Implementation Guide. https://deepoceanobserving.org/wp-content/uploads/2019/05/DOOS-2019-Science-and-Implementation-Guide-2019-05-31-V3.pdf

Levin, L.A., Bett, B.J., Gates, A.R., Heimbach, P., Howe, B.M., Janssen, F., McCurdy, A., Ruhl, H.A., Snelgrove, P., Stocks, K.I., Bailey, D., Baumann-Pickering, S., Beaverson, C., Benfield, M.C., Booth, D.J., Carreiro-Silva, M., Colaço, A., Eblé, M.C., Fowler, A.M., Gjerde, K.M., Jones D.O.B., Katsumata, K., Kelley, D., Le Bris, N., Leonardi, A.P., Lejzerowicz, F., Macreadie, P.I., McLean, D., Meitz, F., Morato, T., Netburn, A., Pawlowski, J., Smith, C.R., Sun, S., Uchida, H., Vardaro, M.F., Venkatesan, R., and Weller, R.A. (2019). Global observing needs in the deep ocean. Frontiers in Marine Science 6:241. https://doi.org/10.3389/fmars.2019.00241

Laroche, O., Kersten, O., Smith, C.R., and Goetze, E. (2020). Environmental DNA surveys detect distinct metazoan communities across abyssal plains and seamounts in the western Clarion Clipperton Zone. Molecular Ecology 29:4588-4604. https://doi.org/10.1111/mec.15484

Lobecker, E., Hoy, S., Sowers, D., Copeland, A., Jerram, K., Wilkins, C., and Baechler, N. (2019). NOAA Ship *Okeanos Explorer* Mapping Systems Readiness Report 2019. NOAA Office of Ocean Exploration and Research. https://doi.org/10.25923/kkwz-5t70

Medley, R., Mashkoor, M., and Varner, J. (2020). Identifying and filling gaps in bathymetric coverage deeper than 200m. Hydro International Business Guide 2020. https://www.hydro-international.com/magazines/hydro-international-business-guide-2020.pdf

National Research Council (NRC). (2003). Exploration of the Seas: Voyage into the Unknown. Washington, DC: The National Academies Press. https://doi.org/10.17226/10844

Netburn, A.N., ed. (2018). From Surface to Seafloor: Exploration of the Water Column, Workshop Report, Honolulu, HI, 4-5 March 2017. NOAA Office of Ocean Exploration and Research. NOAA Technical Memorandum OAR OER; 003. https://doi.org/10.25923/rnjx-vn79

NOAA Office of Ocean Exploration and Research (OER). (2011). NOAA Workshop on Systematic Telepresence-Enabled Exploration in the Atlantic Basin, Narragansett, RI, May 10-11, 2011. https://oceanexplorer.noaa.gov/about/what-we-do/media/atl-basin-workshop-2011-summary.pdf

NOAA Office of Ocean Exploration and Research (OER). (2018). Summary Report for the Atlantic Seafloor Partnership for Integrated Research and Exploration Science Planning Workshop, Silver Spring, MD, November 15-16, 2018. https://oceanexplorer.noaa.gov/explorations/aspire/aspire-workshop-report.pdf

Ocean Exploration Trust (OET). (2012). Workshop on Telepresence-Enabled Exploration of the Caribbean Region Report, November 15-18, 2012. https://oeab.noaa.gov/wp-content/uploads/2020/Documents/2012-11-15-11-OET-Caribbean-Priorities-Workshop.pdf

Ocean Exploration Trust (OET). (2014). Workshop on Telepresence-Enabled Exploration of the Eastern Pacific Ocean Report, December 11-13, 2014. https://nautiluslive.org/sites/default/files/documents/2020-05/2014PacifcWorkshopReport.pdf

Ruppert, K.M., Kline, R.J., and Rahman, M.S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. Global Ecology and Conservation 17:e00547. https://doi.org/10.1016/j.gecco.2019.e00547

Sayre, R.G., Wright, D.J., Breyer, S.P., Butler, K.A., Van Graafeiland, K., Costello, M.J., Harris, P.T., Goodin, K.L., Guinotte, J.M., Basher, Z., Kavanaugh, M.T., Halpin, P.N., Monaco, M.E., Cressie, N., Aniello, P., Frye, C.E., and Stephens, D. (2017). A three-dimensional mapping of the ocean based on environmental data. Oceanography 30(1):90-103. https://doi.org/10.5670/oceanog.2017.116

Suter, L., Polanowski, A.M., Clarke, L.J., Kitchener, J.A., and Deagle, B.E. (2020). Capturing open ocean biodiversity: comparing environmental DNA metabarcoding to the continuous plankton recorder. Molecular Ecology 00:1-18. https://doi.org/10.1111/mec.15587

Thomsen, P.F. and Willerslev, E. (2015). Environmental DNA–An emerging tool in conservation for monitoring past and present biodiversity. Biological Conservation 183:4-18. https://doi.org/10.1016/j.biocon.2014.11.019

University-National Oceanographic Laboratory System (UNOLS). (2016). Developing Submergence Science for the Next Decade (DESCEND-2016) Workshop Proceedings, Cambridge, MA, January 14-15, 2016. https://www.unols.org/sites/default/files/DESCEND2%202016%20FINALFINAL_small.pdf

Woodall, L., Andradi-Brown, D., Brierley, A., Clark, M., Connelly, D., Hall, R., Howell, K., Huvenne, V., Linse, K., Ross, R., Snelgrove, P., Stefanoudis, P., Sutton, T., Taylor, M., Thornton, T., and Rogers, A. (2018). A multidisciplinary approach for generating globally consistent data on mesophotic, deep-pelagic, and bathyal biological communities. Oceanography 31(3):76-89. https://doi.org/10.5670/oceanog.2018.301

APPENDIX A: FULL LIST OF EXPLORATION VARIABLES

TABLE A1: Exploration variables mentioned as important to address in the deep-sea literature reviewed. The exploration variables mentioned in three or more reports were narrowed down to the 16 high-priority observations in Table 1.

Exploration Variables	Number of Sources with Mentions	Sources
Dissolved oxygen	9	GOOS EOVs NRC 2003 OET 2012 OET 2014 Sayre et al. 2017 Netburn 2018 OER 2018 Woodall et al. 2018 Levin et al. 2019a and 2019b
Global seafloor mapping (slope, bathyal, abyssal plains, seamounts) and seafloor composition (substrate)	8	NRC 2003 OER 2011 OET 2012 UNOLS 2016 Netburn 2018 OER 2018 Woodall et al. 2018 Levin et al. 2019a and 2019b
Microbial biomass, density, diversity, and distribution	7	GOOS EOVs NRC 2003 OER 2011 UNOLS 2016 Netburn 2018 Woodall et al. 2018 Danovaro et al 2020

Exploration Variables	Number of Sources with Mentions	Sources
Phytoplankton and zooplankton biomass, diversity, distribution, and presence	7	GOOS EOVs NRC 2003 OER 2011 UNOLS 2016 Netburn 2018 Woodall et al. 2018 Danovaro et al 2020
Particulate organic matter, dissolved organic carbon, heterotrophic and chemoautotrophic carbon	7	GOOS EOVs NRC 2003 OET 2012 OET 2014 Netburn 2018 Danovaro et al. 2020 Levin et al. 2019a and 2019b
Inorganic macronutrients, nitrate/nitrite, silicate, and phosphate	7	GOOS EOVs OET 2012 Sayre et al. 2017 Netburn 2018 Woodall et al. 2018 Levin et al. 2019a and 2019b Danovaro et al. 2020
Sea surface and subsurface temperature	7	GOOS EOVs UNOLS 2016 Sayre et al. 2017 Netburn 2018 OER 2018 Woodall et al. 2018 Levin et al. 2019a and 2019b
Sea surface and subsurface salinity	7	GOOS EOVs NRC 2003 Sayre et al. 2017 Netburn 2018 OER 2018 Woodall et al. 2018 Levin et al. 2019a and 2019b

Exploration Variables	Number of Sources with Mentions	Sources
Surface and subsurface currents	7	GOOS EOVs NRC 2003 OER 2011 OET 2014 Netburn 2018 Woodall et al. 2018 Levin et al. 2019a and 2019b
Biodiversity hotspots; distribution of biological "hot spots" and "cool spots"; general biodiversity	6	NRC 2003 OER 2011 OET 2012 OET 2014 Netburn 2018 Danovaro et al. 2020
Species connectivity	6	GOOS EOVs NRC 2003 OET 2012 OET 2014 OER 2018 Danovaro et al. 2020
Turbidity and suspended particulates	6	GOOS EOVs NRC 2003 OET 2012 OET 2014 Netburn 2018 Danovaro et al. 2020
Occurrence and distribution of large marine vertebrates or megafauna	5	OER 2011 Netburn 2018 OER 2018 Woodall et al. 2018 Danovaro et al. 2020
Biophony, anthrophony, and general ocean sound	4	GOOS EOVs OET 2014 Levin et al. 2019a and 2019b Danovaro et al. 2020

Exploration Variables	Number of Sources with Mentions	Sources
Bottom pressure	4	GOOS EOVs Netburn 2018 Woodall et al. 2018 Levin et al. 2019a and 2019b
Bulk biodiversity	4	NRC 2003 OER 2011 Netburn 2018 Woodall et al. 2018
Cold water coral coverage	4	OER 2011 OET 2012 Levin et al. 2019a and 2019b Danovaro et al. 2020
Fluxes: geothermal, bottom boundary, particulate, sediment, nutrients, organic carbon	4	UNOLS 2016 OER 2011 Levin et al. 2019a and 2019b Danovaro et al. 2020
Microplastic abundance and diversity	4	OET 2012 Netburn 2018 Woodall et al. 2018 Danovaro et al. 2020
Fish abundance and distribution	3	GOOS EOVs NRC 2003 Danovaro et al. 2020
Invertebrate abundance and distribution	3	GOOS EOVs NRC 2003 Danovaro et al. 2020
Behavioral characteristics of pelagic biota; Specimens for physiology, morphological identification	3	UNOLS 2016 Netburn 2018 Danovaro et al. 2020
Genetic characteristics of pelagic biota, specimens for genomics identification	3	UNOLS 2016 Netburn 2018 Danovaro et al. 2020

Exploration Variables	Number of Sources with Mentions	Sources
Vent chemistry	3	NRC 2003 OER 2011 OET 2014
Distribution of mud volcanoes, cold seeps, and vent communities	3	NRC 2003 OET 2012 OET 2014
Cover of living habitats; distribution of unique or sensitive communities; habitat heterogeneity	3	Woodall et al. 2018 Levin et al. 2019a and 2019b Danovaro et al. 2020
Species specific density/counts	3	Woodall et al. 2018 Levin et al. 2019a and 2019b Danovaro et al. 2020
Size-specific body size (mass)/specific biomass	3	Woodall et al. 2018 Levin et al. 2019a and 2019b Danovaro et al. 2020
Food web analysis or trophic structure	3	NRC 2003 Netburn 2018 Danovaro et al. 2020
Dissolved inorganic carbon	3	GOOS EOVs Netburn 2018 Woodall et al. 2018
pH, alkalinity, redox	3	OET 2014 Netburn 2018 Woodall et al. 2018
Ocean color	3	GOOS EOVs NRC 2003 Netburn 2018
Quantify the anthropogenic impacts that may have altered the biological communities	3	Netburn 2018 Woodall et al. 2018 Danovaro et al. 2020
Light, irradiance/light scattering, light transmission	2	GOOS EOVs Netburn 2018

Exploration Variables	Number of Sources with Mentions	Sources
Chlorophyll A/fluorescence	2	NRC 2003 Netburn 2018
Records of litter and anthropogenic damage	2	Woodall et al. 2018 Danovaro et al. 2021
Variables to characterize geohazards	2	OET 2012 UNOLS 2016
Sea surface height	2	GOOS EOVs Netburn 2018
Stable carbon isotope, stable isotope analysis	2	GOOS EOVs UNOLS 2016
Mechanisms of sediment transport, deposition, and deformation along margins	2	OET 2014 UNOLS 2016
Ocean turbulence, small scale turbulence	2	UNOLS 2016 Netburn 2018
Bioluminescence	2	OET 2012 Netburn 2018
Oxygen consumption: O2 sediment profile/Sediment Community Oxygen Consumption (SCOC)	2	Levin et al. 2019a and 2019b Danovaro et al. 2020
Pollutants (i.e., crude oil)	2	OET 2012 Danovaro et al. 2020
Species functional traits	2	NRC 2003 Danovaro et al. 2020
Hard coral cover and composition	2	GOOS EOVs NRC 2003
Subseafloor biosphere	1	NRC 2003
Patterns of marine mineral resources - how they relate to tectonic and oceanographic processes	1	OET 2014
Magmatic, tectonic, and morphological characteristics of plate boundary intersections	1	OET 2014

Exploration Variables	Number of Sources with Mentions	Sources
Successional patterns of deep sea communities following perturbation	1	OET 2014
Tectonic/volcanic/sedimentary controls on submarine groundwater flow and discharge	1	OET 2014
Characteristics of submarine geohazards and how they relate to the tectonic setting	1	OET 2014
Origin, distribution, evolution, and eruptive behavior of submarine volcanoes	1	OET 2014
Large scale currents in the upper ocean — interactions with islands, seamounts	1	OET 2014
Eddies and upwelling jets — interactions with islands, seamounts, the abyss	1	OET 2014
Tides, wind-driven waves, and internal waves — interactions with islands, seamounts, continental shelf	1	OET 2014
Direction and magnitude of abyssal flow	1	OET 2014
Composition of oceanic crust	1	UNOLS 2016
Identifying reactive chemical species	1	UNOLS 2016
Composition of the suboceanic and sub-arc mantle	1	UNOLS 2016
Permeability of the oceanic crust and overlying sediments	1	UNOLS 2016
Metals	1	Netburn 2018
Single cell imaging/sorting	1	Netburn 2018
Biological rates (single cell)	1	Netburn 2018
High-resolution biological mapping	1	Netburn 2018
In situ responses	1	Netburn 2018
Methane	1	Netburn 2018
Hydrogen	1	Netburn 2018

Exploration Variables	Number of Sources with Mentions	Sources
Dissolved gases	1	Netburn 2018
Fine and large-scale spatial patterns	1	OER 2018
Migratory/resident species interactions with benthos	1	OER 2018
Bioturbation using Pb-210	1	Levin et al. 2019a and 2019b
Transient tracers for the deep ocean such as CFC-12, CFC-11, 14C, and 39Ar	1	Levin et al. 2019a and 2019b
Species endemicity	1	Danovaro et al. 2020
Species rarity	1	Danovaro et al. 2020
Invasive or alien species	1	Danovaro et al. 2020
Species age	1	Danovaro et al. 2020
Benthic faunal production	1	Danovaro et al. 2020
Meiofauna	1	Danovaro et al. 2020
Species demographic structure	1	Danovaro et al. 2020
Bioaccumulation of xenobiotics	1	Danovaro et al. 2020
Sediment contamination	1	Danovaro et al. 2020
Eco-toxicological markers	1	Danovaro et al. 2020
Species/ecosystem resiliency	1	Danovaro et al. 2020
Cryptic species	1	Danovaro et al. 2020
Local extinctions	1	Danovaro et al. 2020
Species reproduction potential	1	Danovaro et al. 2020
Species reproduction timing	1	Danovaro et al. 2020
Species interactions	1	Danovaro et al. 2020
Phylogenetic analysis	1	Danovaro et al. 2020
Sex ratio	1	Danovaro et al. 2020

APPENDIX B: KEY EXPLORATION DRIVERS

Ocean exploration has been continuously highlighted as an essential step toward (1) making discoveries of scientific, economic, and cultural value; (2) facilitating innovations in exploration tools and capabilities; (3) encouraging the next generation of ocean explorers, scientists, and engineers; and (4) collecting information to identify, understand, and manage ocean resources. This was made especially clear in the release of the National Strategy for Mapping, Exploring, and Characterizing the United States Exclusive Economic Zone was released in June 2020. The national strategy prioritizes ocean exploration and defines it as “a multidisciplinary first look at an unknown or poorly understood area of the seafloor, sub-bottom, and/or water column and an initial assessment of an area’s physical, chemical, and biological characteristics.”[1] Developed by the Ocean Policy Committee of the White House Office of Science and Technology Policy in coordination with NOAA, it calls for coordinating interagency mapping and exploration activities for the U.S. Exclusive Economic Zone (EEZ), developing new and emerging science and mapping technologies, building public and private partnerships, and completing mapping of the deep water of the U.S. EEZ by 2030 and the near shore by 2040.

In addition, as part of its mandate, NOAA Ocean Exploration contributes significantly to other critical initiatives, including the following:

- **SEABED 2030** — This international effort aims to map 100% of the global seafloor by 2030.[2]
- **NOAA SCIENCE AND TECHNOLOGY STRATEGIES** — In 2020, NOAA released strategies for uncrewed systems, artificial intelligence, cloud computing, ’omics, data, and citizen science to guide transformative advancements in NOAA mission areas with an emphasis on data collection and shepherding in support of science and decision-making.[3]
- **FEDERAL DATA STRATEGY 2020 ACTION PLAN** — This plan calls for identifying and addressing data needs to answer priority agency questions.[4]

NOAA Ocean Exploration’s operations on *Okeanos Explorer* and other NOAA Ocean Exploration-supported activities advance and directly support these initiatives as an integral step to understanding unexplored and underexplored areas of the U.S. EEZ for national benefit.

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1 https://oeab.noaa.gov/wp-content/uploads/2021/01/2020-national-strategy.pdf

2 https://seabed2030.org

3 https://nrc.noaa.gov/NOAA-Science-Technology-Focus-Areas

4 https://strategy.data.gov/assets/docs/2020-federal-data-strategy-action-plan.pdf

APPENDIX C: REPORTING OUT ON THE DATA COLLECTION OF EXPLORATION VARIABLES IN NOAA OCEAN EXPLORATION

DIVE SUMMARIES

Following each ROV dive, the mission team produces and archives a summary of dive metadata. The dive summary provides the following information:

- Map of the dive location
- General area descriptor
- Site name where the dive was conducted
- Names and affiliation of the science team leads
- Name and affiliation of the expedition coordinator
- Name and affiliation of the ROV dive supervisor
- Name and affiliation of the mapping lead
- Cruise identifier
- Dive number
- ROV that was used to conduct the dive
    - Camera platform
    - ROV data collected throughout the dive (checklist):
    - CTD
    - Depth
    - Altitude
    - Scanning sonar
    - Ultra-short baseline (USBL) position
    - Heading
    - Pitch
    - Roll
    - High-definition camera 1-2
    - Low-resolution camera 1-5
- Equipment malfunctions

- ROV dive summary data:
  - In water: Coordinates and time
  - On bottom: Coordinates and time
  - Off bottom: Coordinates and time
  - Out of water: Coordinates and time
  - Dive duration
  - Bottom time
  - Maximum depth
- Additional notes
- Name, affiliation, and email addresses for scientists involved with the dive
- Purpose of the dive
- Description of the dive
- Notable observations
- Community presence/absence (checklist):
  - Corals and sponges
  - Chemosynthetic community
  - High-biodiversity community
  - Active seep or vent
  - Extinct seep or vent
  - Hydrates
- Feature type the dive was conducted on
- A link to the dive's SeaTubeV3 annotations
- Overall map of the dive area
- Close-up map of the main dive site and track
- Representative photos of the dive
- Samples collected during the dive
  - Photo of the sample (includes rulers for scale)
  - Sample ID
  - Date and time of collection
  - Depth
  - Temperature
  - If possible, species identification or geological identification of the sample
  - If possible, species identification of organisms associated with the collected sample
  - Comments

CTD ROSETTE SUMMARIES

After conducting a CTD cast, the mission team produces and archives a summary of CTD rosette data. The CTD rosette summary form provides the following information:

- CTD cast name
- Cruise identifier
- CTD number
- Date of the cast
- Name and affiliation of the expedition coordinator
- Name and affiliation of the mapping lead
- Name and affiliation of the science team leads
- General geographic description of cast
- Site name
- Type of CTD operation (checklist):
  - Vertical cast
  - Po-Go
  - Tow-Yo
  - Combination
- Target deployment position (coordinates) and depth
- Deployment date, time, and position
- Time, position (coordinates), and depth at maximum depth
- Recovery time and position (coordinates)
- CTD sensor data acquired and calibration coefficients (checklist):
  - CTD
  - ORP
  - Turbidity (light-scattering spectroscopy)
  - Dissolved oxygen
- Water samples collected and number (if any)
- Sample processing for water samples (checklist):
  - None
  - Room temperature storage
  - -80°C freezer
  - -20°C freezer
  - Refrigerator

- Data archive of the water samples and CTD data
- Equipment malfunctions
- Special notes
- Name, location, affiliation, and email address of the scientists involved
- Purpose of the CTD operation
- Description of the data and results
- Overall map of the CTD cast area
- Screen grab of the sensor data

POST-EXPEDITION REPORTING

Cruise reports are an essential resource for data users that provide an overview of operations (e.g., to identify relevance of the expedition to user needs), detailed information on activities (e.g., to assess how data were collected), and information on how to access the data that were collected.

MAPPING DATA ACQUISITION AND PROCESSING SUMMARY REPORT

After every mapping expedition, the mission team generates a report that describes the acoustic seafloor and water column mapping data collection and processing methods used during the expedition.[1] The report also includes a summary of the overall mapping results and mapping-related expedition activities. These reports are archived with NCEI and the NOAA Central Library. Mapping reports provide the following information:

- Expedition objectives
  - Map showing bathymetry coverage generated during the expedition
- Summary of mapping results
  - Area covered during the expedition
  - Various figures showing areas mapped, backscatter, and 3D images of interesting topographical features
- Mapping statistics
  - Various statistics about the expedition: dates, area surveyed, data volume from each instrument (EM 304 bathymetry/backscatter, EM 304 water column, EK60/EK80 water column, sub-bottom sonar files), number of XBT casts, and number of CTD rosette casts

1 Example: Mapping data and acquisition and processing summary report from Leg 1 of the Windows to the Deep 2019 expedition: https://repository.library.noaa.gov/view/noaa/22037.

- Mapping sonar setup
  - Description of each instrument used
- Data acquisition summary
- Multibeam sonar data quality assessment and data processing
- Data archival procedures
- Expedition calendar
- Daily expedition log entries
- References

ROV CRUISE REPORTS

After every leg of an ROV expedition, the mission team generates a report that summarizes the exploration activities from a scientific and operational perspective.[2] Similar to mapping reports, cruise reports present an overview of activities and results and are archived with NCEI and the NOAA Central Library. Cruise reports provide the following information:

- Expedition overview
  - Regional description
  - Rationale for exploration
  - Objectives
- List of participants
  - At-sea mission personnel
  - Shore-based science team
- Methodology
  - ROV seafloor surveys
  - Specimen collections
  - Acoustic operations
    - Multibeam sonar
    - Sub-bottom profiler
    - Split-beam sonars
    - Acoustic Doppler current profiler
  - Sun photometer measurements
- Clearance and permits
- Expedition schedule

2 Example: Cruise report from Leg 2 of the Windows to the Deep 2019 expedition: https://repository.library.noaa.gov/view/noaa/22906.

- Expedition map
- Results
  - Sample collections
    - Sample repositories
  - Accessing ROV data
  - Seafloor mapping
    - Mapping data access
  - Sun photometer measurements
  - Education and outreach activities
- Summary
- References
- Appendices
  - Dive summaries
  - Data management plan
  - Categorical exclusion memo under NEPA
  - ESA Section 7 consultation memo
  - NMFS Letter of acknowledgement for operations

APPENDIX D: LIST OF ACRONYMS

ADCP	acoustic Doppler current profiler
ASPIRE	Atlantic Seafloor Partnership for Integrated Research and Exploration
CTD-O	conductivity, temperature, depth, dissolved oxygen
DESCEND	DEveloping Submergence SCiencE for the Next Decade
DOOS	Deep Ocean Observing Strategy
EDNA	environmental DNA
EEZ	Exclusive Economic Zone
EMU	ecological marine unit
EOV	essential ocean variable
FFO	federal funding opportunity
GOOS	Global Ocean Observing System
NCEI	NOAA National Centers for Environmental Information
NOAA	National Oceanic and Atmospheric Administration
OET	Ocean Exploration Trust
OMAO	NOAA Office of Marine and Aviation Operations
ONC	Oceans Network Canada
ORP	oxidation reduction potential
QA/QC	quality assurance, quality control
ROV	remotely operated vehicle
UNOLS	University-National Oceanographic Laboratory System
USBL	ultra-short baseline
XBT	expendable bathythermograph

APPENDIX E: FEASIBILITY ASSESSMENT FOR ENVIRONMENTAL DNA

TABLE OF CONTENTS

NAME AND TYPE OF DATA GAP

WHAT IS THE DATA GAP?

Environmental DNA (eDNA) is the data gap.

WHAT IS THE TYPE OF DATA GAP: (1) DATA ARE NOT BEING COLLECTED IN GENERAL, (2) DATA ARE NOT BEING COLLECTED IN A SPECIFIC LOCATION OR DEPTH, OR (3) DATA ARE NOT BEING COLLECTED AT A SPECIFIC FREQUENCY?

eDNA meets the criteria for all three types of data gaps.

WHAT HIGH-PRIORITY EXPLORATION VARIABLES COULD NOAA OCEAN EXPLORATION ADDRESS BY FILLING THE GAP?[1] (SEE TABLE 1, TABLE 3, AND APPENDIX TABLE A1.)

By incorporating systematic collection of water samples into NOAA Ocean Exploration's standard operations on NOAA Ship *Okeanos Explorer* and applying the eDNA methodology, we could address the following high-priority exploration variables: bulk biodiversity, species-specific occurrence and distribution, species connectivity, and targeting of microbes, plankton, invertebrates, fish, marine mammals, and other fauna of interest.

BACKGROUND AND JUSTIFICATION

BACKGROUND INFORMATION ON THE DATA GAP, INCLUDING THE EXPLORATION/CHARACTERIZATION DISTINCTION, ITS IMPORTANCE, AND CURRENT STATE OF THE SCIENCE.

eDNA is a mixture of genomic DNA from many different organisms found in an environmental sample such as soil, sediment, water, or feces. eDNA has many uses and purposes. One objective is to extract DNA from an environmental sample to obtain comprehensive DNA-based information for the ecosystem under consideration (Taberlet et al. 2018). By collecting these environmental samples, scientists can process eDNA to make inferences about the organisms in certain areas.[2] This feasibility assessment examines the use of eDNA in targeting biodiversity measurements in the water column environment.

1 Gaps are identified in Tables 1, 3, and 1A in the report "Exploration Variables Identified by NOAA Ocean Exploration"

2 https://oceanexplorer.noaa.gov/technology/edna/edna.html

An ’omics white paper written and published by the NOAA ’Omics Task Force[3] outlines a number of eDNA applications used specifically within NOAA (Goodwin et al. 2020). The applications include:

- Establishing a baseline biological inventory of the marine ecosystem;
- Tracking invasive species, harmful algal blooms, aquaculture pathogens and parasites, migratory species, larval dispersal, endangered populations, cryptic species, and targeted species of interest;
- Investigating trophic interactions and improving understanding of food web dynamics; and
- Providing information on biodiversity for monitoring and assessment.

3 The NOAA ’Omics Task Force was established by the NOAA Research Council as a cross-NOAA effort to understand the current portfolio of ’omics related activities across the organization, identify priorities and needs for the future, and work to develop solutions to implementation challenges in this nascent sector of research.

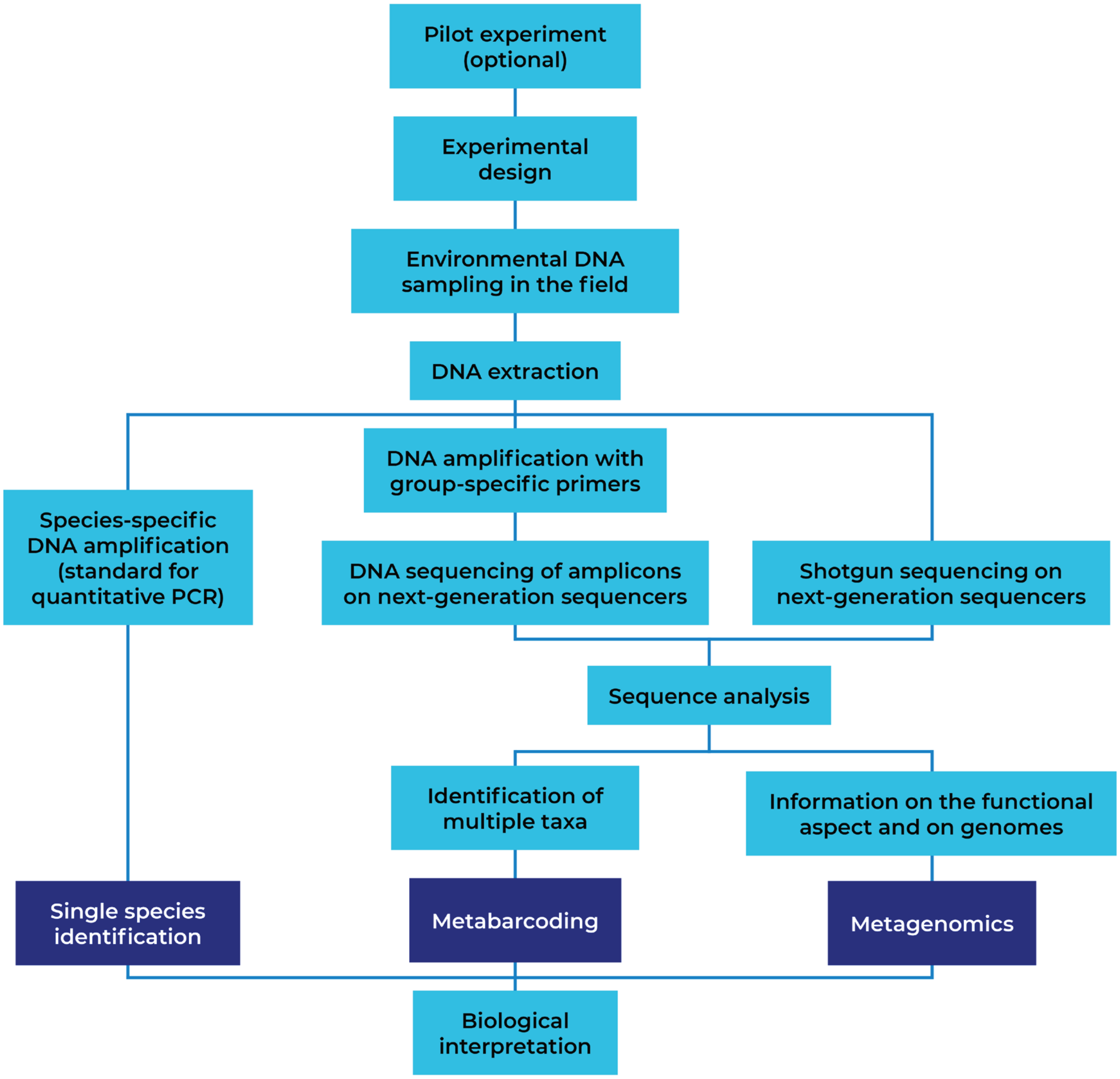

FIGURE E1. The main steps of conducting an eDNA study for single species identification and for biodiversity monitoring. Metabarcoding is the extension of barcoding to provide information on community biodiversity. Metabarcoding extracts DNA from a community or organisms instead of from the tissue of an individual. Amplified and sequenced DNA from tissue is matched to a set of barcodes, which are short pieces of DNA (genetic marker) that identify an organism. Metagenomics is the extension of DNA sequencing to the analysis of communities by using DNA recovered from environmental samples. Targeted sequencing of amplified DNA is typically employed to identify organisms whereas “shotgun” approaches are used to provide information on functional genes. Figure adapted from Taberlet et al. 2018.

Advancements in molecular techniques have improved the scope and scale of technologies with application for exploration and sensing of cryptic species in the deep sea (Jerde et al. 2011; Thomsen and Willerslev 2015). Due to the difficulty of accurately identifying a large number of organisms using remotely operated vehicle (ROV) video, eDNA sampling for identification of species presence could increase the scope of deep-sea organism detection (Kelly et al. 2014; Jerde et al. 2011; Thomsen et al. 2012).

Furthermore, the number of organisms that can be physically sampled during ROV deployments is limited, while eDNA sampling allows for species detection at a far greater scale. eDNA collection also facilitates noninvasive sampling. Once appropriate reference libraries are constructed, physical sampling of organisms will not be required to detect presence (Kelly et al. 2014; Jerde et al. 2011; Thomsen et al. 2012). With the appropriate primers, eDNA can be used to detect organisms across a broad taxonomic range, from bacteria to vertebrates, and be inclusive of both benthic and pelagic organisms. It can also detect organisms that are present but undetected in video (e.g., cryptic species).

For this feasibility assessment, NOAA Ocean Exploration assessed and researched the feasibility of carrying out these four main components of an eDNA study:

- Collecting water samples
- Extracting DNA from collected samples
- Using the appropriate method for DNA amplification/use of primers
- Conducting an analysis to determine family, genus, or species presence in water samples using a bioinformatics pipeline

As water sample collection is required for an eDNA study, this feasibility assessment explores NOAA Ocean Exploration's capacity to collect water samples and store those samples (or filters) in an archive for analysis at a later date.

HOW WOULD THE DATA HELP ADDRESS NOAA OCEAN EXPLORATION'S MISSION AND GOALS?

Incorporating eDNA sampling into standard NOAA Ocean Exploration operations would directly address Goals 1 and 2 of the *Office of Ocean Exploration and Research Strategic Plan for FY 2016-2020*.[4] Goal 1 is to "Explore the ocean to make discoveries of scientific, economic, and cultural value, with priority given to the U.S. Exclusive Economic Zone and extended continental shelf." In particular, incorporating eDNA sampling would help NOAA Ocean Exploration maintain, refine, and expand sampling capabilities needed to explore an area. eDNA data would also contribute to Objective 1.2 "Characterize geological, physical, chemical, and biological phenomena," as the data would help NOAA Ocean Exploration characterize biological phenomena, which would complement standard ROV operations.

Additionally, eDNA sampling would address some objectives of Goal 2 "Promote technological innovation to advance ocean exploration," specifically Objective 2.1 "Stimulate development

4 https://oceanexplorer.noaa.gov/about/oer-strategic-plan-fy16-20.pdf

of ocean exploration sensors, technology, and methods," because eDNA sampling is relatively new, and NOAA Ocean Exploration could develop an eDNA exploration-based protocol to meet this objective. In assessing the feasibility of collecting and analyzing eDNA, NOAA Ocean Exploration is already actively working toward Objective 2.2 "Coordinate development of ocean exploration technology within NOAA." Several researchers across NOAA (from the Pacific Marine Environmental Laboratory, Deep Sea Coral Research and Technology Program, Northwest Fisheries Science Center, etc.) are actively working on implementing eDNA sampling practices and have provided expertise for this feasibility assessment.

WHAT BIG PICTURE SCIENTIFIC QUESTIONS WOULD THE DATA ADDRESS?

What could be addressed with current technology?

- What are the global patterns of marine species distribution?
- Which species are present in a particular location?
- Which mobile fauna or cryptic species are NOAA Ocean Exploration missing with traditional ROV surveys?
- What is the baseline biodiversity, as detected by eDNA, of a location?
- How does biodiversity vary among sites, regions, and depths?

What could be addressed with more advances in eDNA?

- What is the genetic diversity of a species in a location and among regions (i.e., genetic connectivity)?
  - Note: This could be answered with larger DNA fragments, but the primers used for this typically have a much narrower taxonomic scope. However, if NOAA Ocean Exploration is archiving filters and DNA extracts, then this will allow researchers to employ whichever primers they want to use to answer this in depth question.
- Are there invasive species in a particular location?
  - Note: Too little is known about the deep sea to know what is endemic or invasive as we do not have enough observations globally.
- Are there rare species in a particular location?
  - Note: A reference database is needed for this, which involves collecting and barcoding voucher specimens.

WAS THE DATA GAP IDENTIFIED BY THE DEEP-SEA COMMUNITY AS IMPORTANT TO ADDRESS? IF YES, WHERE WAS IT IDENTIFIED AND WHO IDENTIFIED IT?

eDNA sampling was mentioned as a general tool in several reports and identified as important by the deep-sea community.

- Participants of the first U.S. National Conference on Marine Environmental DNA (November 29-30, 2018), including academic, nongovernmental organization, and government officials, identified eDNA sampling as mature enough research-wise for investment (Ausubel et al. 2018). The conference report states that the techniques are low cost and mature enough to be implemented and identified ship time as the biggest expense in implementation. It further identifies the need to focus on eDNA practices in the U.S. Exclusive Economic Zone (EEZ) and underscores the importance of establishing eDNA observational baselines before beginning to address unresolved research questions. The report also recommends using the National Ocean Partnership Program to support implementation of eDNA sampling.
- In Woodall et al. (2018), a group of marine scientists from different disciplines identify a consistent framework of 20 biological, chemical, physical, and socioeconomic parameters that are considered the most important for describing environmental and biological variability. They specifically mention using eDNA sampling to measure biodiversity of epibenthic and hyperbenthic organisms.
- In *From Surface to Seafloor: Exploration of the Water Column* (Netburn 2018), eDNA sampling was classified as a high priority for the purposes of measuring bulk biodiversity in the water column. This workshop report outlines the exploration and scientific needs for water column characterization based on recommendations from water column experts.

Much of the literature used in NOAA Ocean Exploration's exploration variables analysis to identify data collection needs in the deep sea also mentions the need to measure bulk biodiversity and microbial, phytoplankton, and zooplankton communities. Some examples follow.

- *Exploration of the Seas: Voyage into the Unknown* (NRC 2003): This report provides recommendations on what a new ocean exploration program could look like and identifies science priorities and regions of interest. It dedicates a section to justifying the need to better understand the identity, taxonomy, spatial diversity, and function of microbes in an ecosystem and pushes for this area of work to be part of ocean exploration practices.
- *NOAA Workshop on Systematic Telepresence-Enabled Exploration in the Atlantic Basin* (OER 2011): This workshop report summarizes the exploration needs of the Atlantic Basin, including the Gulf of Mexico and Caribbean Sea, based on a workshop synthesizing deep-

sea community input. In particular, it summarizes exploration gaps in particular regions, noting several times the importance of including microbiology and plankton communities in exploration activities.

- *Developing Submergence Science for the Next Decade 2016* (UNOLS 2016): This report synthesizes the workshop recommendations from deep-sea scientists and engineers tasked with (1) identifying the technological and cultural innovations that will enable advancement to understand the deep sea; and (2) presenting guidelines that will facilitate government agencies, industry, and philanthropic partners to develop new operational modes and funding opportunities to advance deep-sea research. The report also outlines continued deep-sea research needs, including several mentions of the importance of conducting long-term monitoring of microbial ecosystems.
- *Deep Ocean Observing Strategy Science and Implementation Guide* (Levin et al. 2019a) and Global Observing Needs in the Deep Ocean (Levin et al. 2019b): These papers discuss the need for a globally integrated network of deep-ocean observing systems, its status, and the key scientific questions and societal mandates driving observing requirements over the next decade. The need for a better understanding of microbial and plankton communities in the deep sea is mentioned several times throughout both papers.
- *Ecological Variables for Developing a Global Deep-Ocean Monitoring and Conservation Strategy* (Danovaro et al. 2020): This paper identifies the need to preserve benthic and pelagic deep-sea ecosystems through ecosystem-based management strategies and monitoring using a set of deep-sea ecological variables. The authors compiled this set of deep-sea ecological variables through expert elicitation. In the paper, they stress the importance of gaining a better understanding of microbial ecology and biology, as well as plankton communities, in the deep sea.

WHICH STAKEHOLDERS AND PARTNERS WOULD THESE DATA SERVE?

- **NOAA DEEP SEA CORAL RESEARCH AND TECHNOLOGY PROGRAM (DSCRTP):** DSCRTP's strategic plan (2010–2019) identifies the need to "locate and characterize deep-sea coral and sponge ecosystems" as part of their exploration and research objectives.[5] NOAA Ocean Exploration is a key partner in cross-NOAA line office DSCRTP regional initiatives, and incorporating eDNA into NOAA Ocean Exploration operations would directly benefit the deep-sea coral community.
- **DEEP-OCEAN STEWARDSHIP INITIATIVE'S MARINE GENETIC RESOURCES WORKING GROUP:** This working group aims to explore and identify options for conserving and sustainably using marine genetic resources, which includes addressing questions related to access to and benefit sharing of these resources.[6] Additional data would contribute to their goals and inform their work.

5 https://www.coris.noaa.gov/activities/deepsea_coral/dsc_strategicplan.pdf

6 https://www.dosi-project.org/topics/deep-sea-genetic-resources/

- **OTHER STAKEHOLDERS AND PARTNERS (E.G., GOVERNMENT AGENCIES, ACADEMIA, AND INDUSTRY):** A number of other stakeholders are interested in identifying species presence and biodiversity through minimally invasive efforts. eDNA sampling can be used in eDNA hotspots in lieu of traditional, capture-based sampling methods, which use nets or traps. Due to its minimally invasive nature, eDNA sampling is being considered as an emerging technology for fisheries stock assessment (Hansen et al. 2018).

HAVE THE DATA BEEN COLLECTED DURING PRIOR NOAA OCEAN EXPLORATION-SUPPORTED EXPEDITIONS OR ARE THERE PLANS TO COLLECT THE DATA DURING UPCOMING EXPEDITIONS? IF YES, PROVIDE DETAILS.

- **OCEAN EXPLORATION TRUST (EXPLORATION VESSEL *NAUTILUS*):** eDNA sampling was successfully conducted on four NOAA Ocean Exploration-supported *Nautilus* expeditions (NA072,[7] NA073,[8] NA074,[9] NA077[10]) between June and August 2016 and reported in a paper about the presence of deepwater octocorals on the west coast of the United States (Everett and Park 2018).
- **NOAA OCEAN EXPLORATION'S OCEAN EXPLORATION FISCAL YEAR 2018 FUNDING OPPORTUNITY SUPPORTED PROJECT:** In September 2019, an expedition supported by NOAA Ocean Exploration through its Fiscal Year 2018 funding opportunity led by Jill McDermott of Lehigh University on Research Vessel *Manta* investigated eDNA as a tool for exploration in deepwater environments.[11] The purpose of the project was to develop a framework to establish eDNA metabarcoding as a standard ocean exploration tool to enable rapid, economical, and comprehensive diversity assessments of deepwater fauna.
- **OTHER 'OMICS APPLICATIONS:** NOAA Ocean Exploration has a history of supporting other 'omics[12] applications (see Appendix 1 in Goodwin et al. 2020). In addition to convening a 2011 NOAA Marine Microbes Workshop, which supported the Marine Biodiversity Observation Network Demo Projects and the NOAA CalCOFI Genomics Project, NOAA Ocean Exploration supports 'omics science and technology projects via its annual funding opportunity and cooperative institute agreements. NOAA Ocean Exploration's 2019 funding opportunity focused on the discovery of microorganisms, sponges, corals, and other organisms with biopharmaceutical or biotechnical potential.

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7 https://nautiluslive.org/cruise/na072

8 https://nautiluslive.org/cruise/na073

9 https://nautiluslive.org/cruise/na074

10 https://nautiluslive.org/cruise/na077

11 https://oceanexplorer.noaa.gov/about/funding-opps/ffo-recipients.html

12 A term for a set of genome-based technologies used to examine DNA, RNA, proteins, or small molecules from a variety of sample types ranging from single cells to communities to identify the organisms present, their metabolic status, and how they might be affected by changing conditions.

Prior opportunities and agreements have enabled such things as:

- The use of next-generation DNA sequencing technologies and metagenomic sequencing to probe the diversity, distribution, and functional roles of microbes in the Arctic;
- Investigation into the abundance of microbes in volcanic rocks of different ages as well as specifics about what microbes are present in the Gulf of Alaska and study of the genetic structure of deep-sea gorgonian corals to determine whether seamount populations are genetically isolated units;
- Study of the various complex networks within Arctic and Antarctic sea ice that intersect to make up these unique, extreme ecosystems: brine channels, food webs, and genetic exchange networks that are all dominated by microbes;
- Exploration in the Gulf of Mexico for novel bioactive compounds from marine organisms that have potential as pharmaceutical products or biomedical research tools; and
- Support for cross-line office ’omics activities. ’Omics was a topic in three bilateral (NOAA Fisheries and NOAA Research) meetings held between 2014 and 2016, for which a white paper and synopses were produced, and the NOAA Marine Microbe Workshop (mentioned briefly above and also sponsored through the NOAA Oceans and Human Health Initiative) was held at the Hollings Marine Laboratory in 2011.

METHODS, PROTOCOL OPTIONS, AND SAMPLING STRATEGY[13]

WHERE SHOULD SAMPLING OCCUR AND/OR WHAT WOULD BE THE SAMPLING STRATEGY?

NOAA Ocean Exploration should define sampling depths prior to dives or sample opportunistically when encountering features or species of interest. If defining depths prior to dives, collecting surface and subsurface samples (e.g., greater than 1 m and 10 m) in addition to samples from certain depths is recommended. NOAA Ocean Exploration should also consider collecting control samples (e.g., water samples taken in areas near sediment or within the water column away from target features or species). Additionally, it is recommended to take replicate samples (two or three) per water depth. There are five 1.7 L Niskin bottles on ROV *Deep Discoverer* and twelve 10 L bottles on the conductivity, temperature, depth (CTD) rosette. The sampling strategy would depend on the instrument used.

13 Here, “methods” refers to the general process whereas “protocol options” refers to more detailed information on specific steps for collecting the data. The feasibility assessment template in the main text of the technical memorandum lays out the most up-to-date version of the feasibility assessment template. The eDNA feasibility assessment was created in 2019, and the template may not entirely be in line with the version used here. The template is meant to serve as a guide for scoping out a measurement, technique, or process for incorporating into NOAA Ocean Exploration’s standard operations.

WOULD SAMPLING BE PART OF STANDARD OPERATIONS OR OPPORTUNISTIC?

Collection of water samples for eDNA analysis could be part of NOAA Ocean Exploration's standard operations on ROV and/or mapping expeditions to help address many of the exploration variables recommended to explore an area. However, if time and personnel are major limitations, eDNA sampling could be conducted opportunistically.

WHAT SAMPLING METHODS WOULD BE USED?

ROV water samples could be collected using clean 1.7 L Niskin bottles on *Deep Discoverer*. If possible, replicate water samples should be collected.

However, it is more feasible to use the CTD rosette for water sample collection because it has twelve 10 L Niskin bottles. This would allow for triplicate samples at four depths (or duplicate samples at six depths). Replicate samples would ensure the robustness of the sample collection and further validate the eDNA results. Tests should be conducted with the Niskin bottles ahead of time to ensure leakage does not occur (e.g., by using dye). One or two liters of water is a common volume for eDNA analyses, but some studies have had success with smaller volumes of water (Singer et al. 2019).

It is imperative that deep-sea sediments are not disturbed before sampling. Not only can sediments contain compounds that inhibit polymerase chain reaction, but DNA from nontarget organisms and old/ancient DNA from organisms that are no longer present in the current environment could contaminate the samples.

Once on board *Okeanos Explorer*, seawater collected during sampling would be transferred to sterile Whirl-Pak Stand-Up Bags and filtered immediately (explained more below) or stored at or below 4°C for up to two hours until filtering can be completed. Samples should be only chilled, not frozen, before processing. To reduce cross-contamination, Niskin bottles should be cleaned following each collection with a 10% bleach rinse (~two minutes) and then rinsed three times with double-distilled water (ddH_2O).

Prior to filtering, all workspaces should be cleaned and wiped down with a 10% bleach solution or DNA Away. It could also be worth having a plexiglass box around the samples to prevent air contamination with portable ultraviolet (UV) sterilizing hoods available on the market.[14] Sample processors also need to ensure UV radiation is kept at a minimum, as UV light accelerates DNA degradation. However, subject matter experts consulted during this process noted that cross-

14 https://biosan.lv/products/uvct-ar/

contamination is more of a concern during the DNA extraction process, which takes place in a lab setting. New, disposable microbiological filter cartridges would be placed on the vacuum filter apparatus (including vacuum pump, manifold, 5 L carboy vacuum trap, tygon tubing), and the samples would be filtered through sterile 47 mm diameter, 0.45 μm cellulose nitrate membrane vacuum filters (contained within the disposable filter apparatus). When filtering samples, a negative ddH_2O control should be run to control for contamination and potential errors experienced during processing (1 L ddH_2O transferred to a Whirl-Pak Stand-Up Bag, filtered, and stored for analysis).

Following filtration, wastewater should be disposed of down the lab sink drain or used as filtered sea water for other experiments/uses. Gloves should then be changed or gloved hands wiped down with a 10% bleach solution or DNA Away. Disposable microbiological filter cartridges should be disposed of and replaced. All materials and tools should be cleaned before continuing the sample processing.

Used filters should be folded in half twice using sterile forceps (sterilized using bleach solution or Fisher HealthCare Bacti-Loop Infrared Micro-Sterilizer or wiped with a new Kimwipe and DNA Away) and then placed in labeled 2 mL cryovials or labeled and wrapped in foil before immediately being frozen at -80°C or -20°C. Alternatively, filters could be stored in Longmire's buffer solution (100 μM Tris (Trizma Base), pH 8.0; 100 μM ethylenedinitrilo tetraacetic acid, disodium salt, pH 8.0; 10 μM NaCl; 0.5% sodium dodecyl sulfate; autoclaved double-distilled water), which can stabilize the DNA at room temperature. Care should be taken when handling filters as they can be cracked.

NOTES: Vacuum filters should not dry during filtration. Care must also be taken when preparing 0.5% sodium dodecyl sulfate (SDS) solution, a component of the Longmire's buffer. Though it is nonhazardous when in solution, powdered SDS is a potent respiratory irritant. As such, Longmire's buffer should be prepared before being brought on board *Okeanos Explorer*.

WHAT WOULD BE THE PROTOCOL?

A protocol was provided for earlier versions of the eDNA feasibility assessment.

IF PHYSICAL SAMPLES ARE TO BE COLLECTED, WHERE WOULD THEY BE STORED ON BOARD?

If samples cannot be filtered immediately after collection, subject matter experts recommend keeping samples cold until they can be. When filtered, filters could be stored in a -80°C or -20°C freezer. However, there is no longer a -80°C freezer on *Okeanos Explorer*. Filters could be stored in a Longmire's buffer solution, which does not require refrigeration (Everett and Park 2018; Williams et al. 2016).

MATERIALS AND COST

WHAT MATERIALS OR EQUIPMENT WOULD BE NEEDED TO COLLECT THE DATA?

TABLE E1: Materials needed to collect the data and associated costs.

Item	Once or for Every Expedition	Quantity	Cost (USD)	Supplier	Purpose
Masterflex Tygon E-Food (B-44-4X) tubing, L/L 15, 50; 50 feet/roll — 4 feet needed per sample	Once	2 per roll	$136.00	Cole Parmer, EW-06418-15	Filtering water samples
Male Luer with lock ring x 3/16 hose barb — 1 needed per filter	Once	25 per pack	$17.00	Cole Parmer, GH-45518-08	Filtering water samples
EMD Millipore Sterivex Sterile Pressure-Driven filters, 0.22 μM pore size* — 1 needed per sample	Every mission	15 per pack	$129.21	Fisher, SVGPL10RC	Filter for capturing DNA
Nasco Whirl-Pak Easy-To-Close Bags, 1.24 L — 1 needed per sample	Every mission (depends)	500 per pack	$276.00	Fisher, 01-812-5Q	Holding the seawater before filtering
Nasco Whirl-Pak Easy-To-Close Bags, 207 mL — 3 needed per sample	Every mission (depends)	500 per pack	$109.00	Fisher, 01-812-120	Storing the replicate filters
Kimberly-Clark Professional Purple Nitrile Exam Gloves	Every mission (depends)	100 per pack	$36.00	Fisher, 19-149-863	Preventing sample contamination
Reusable Glass Low-Form Griffin Beakers, 1,000 mL	Once	Pack of 6	$84.00	Fisher, FB1001000	Measuring the volume of water to be filtered

Item	Once or for Every Expedition	Quantity	Cost (USD)	Supplier	Purpose
Vacuum Pump	Once	1	$570.00	Cole Parmer, EV-07061-40	Filtering water samples
Bemis Parafilm M Laboratory Wrapping Film, 4 in width	Every mission (depends)	250 ft	$138.30	Fisher, 13-374-12	Covering the end of the filters
Fine Point High Precision Forceps	Once	1	$27.25	Fisher, 22-327379	Removing the filters after filtering

NOTE: These materials are used only to filter the water samples on board. There are additional costs associated with sample processing in the lab to obtain information about organism composition. This assessment focuses on the feasibility of the physical collection and filtration of water samples for further eDNA analysis. DNA extraction, primer use, and bioinformatics pipeline would require a partnership (explained further in this feasibility assessment).

***** Pore size of filter varies in collecting DNA. It is dependent on the kind of DNA to be targeted (see Turner et al. 2014).

WHAT TYPE OF STORAGE AND SPACE FOR SAMPLES AND SUPPLIES WOULD BE NEEDED ON THE SHIP?

Space would need to be made to store the vacuum pump with those associated supplies. Other supplies (e.g., gloves, forceps, etc.) are generally available and stored on the ship for other sampling purposes.

Sample storage is covered in the previous section.

HOW MUCH WOULD IT COST TO COLLECT THE DATA? HOW MUCH WOULD IT COST TO STORE THE DATA?

Factor in these costs as appropriate:

- Ship time
- Personnel
- Materials purchased for one cruise
- Materials purchased once
- Equipment maintenance

- Partnership(s) to store samples, extract DNA, and analyze (can be expensive)
- Long-term costs of filter and data storage

HOW MUCH WOULD IT COST TO MAINTAIN THE CAPABILITY OVER A FIVE-YEAR PERIOD?

Maintenance of *Deep Discoverer* and the CTD rosette is already part of standard operations. Thus, the additional costs for mechanical operations (specifically upkeep of Niskin bottles) would be minimal. After the one-time expenses have been made (i.e., the vacuum pump with associated materials and equipment — tubing, manifold, etc.), the most costly items would be the filters, chemicals for the Longmire's buffer solution, parafilm, gloves, and Whirl Pak bags. Partnerships with NOAA laboratories or other institutions to archive or analyze samples would likely be the most cost-effective way for NOAA Ocean Exploration to produce useful eDNA data during expeditions on *Okeanos Explorer*. If we decide to move forward with eDNA sampling, then the specifics of such partnerships (e.g., which institutions and agencies, through what mechanisms, costs) would need to be scoped.

TIME

HOW MUCH TIME WOULD IT TAKE TO COLLECT AND/OR PROCESS THE DATA?

Triggering ROV-based Niskin bottles would take about five minutes per sample. Alternatively, a CTD rosette cast would take 1.5-2 hours. It would take approximately 1.5-2 hours to process water samples from five Niskin bottles collected by either the ROV or CTD rosette. While filtering the water samples would not require constant supervision for the full time period, setting up the filtration system, beginning the filtration of each sample, and decontaminating the workspace would take time.

PERSONNEL

WHO WOULD BE IN CHARGE OF COLLECTING THE DATA? WHO WOULD BE IN CHARGE OF SAMPLE AND DATA PROCESSING, MANAGEMENT, AND ARCHIVING?

PROCESSING WATER SAMPLES IN THE WETLAB

During an ROV expedition on *Okeanos Explorer*, the two science leads and the sample data manager process samples on board, and they are already heavily tasked. Thus, unless NOAA Ocean Exploration cuts some component of the other work assignments, processing eDNA samples after every dive would likely require another person on board tasked with filtering water samples, something that is not currently possible given the current berthing plan. This is a challenge, but extra personnel have been brought on board before (e.g., web coordinators), which indicates some degree of flexibility. There is more flexibility during mapping only expeditions since they require a much smaller mission team than ROV expeditions and typically have berths available. Some time could be taken away from mapping operations to conduct a CTD rosette cast (something less feasible on an ROV-specific expedition). An extra person or a mapping watchstander could process water samples after a CTD rosette cast.

ARCHIVING METADATA ON BOARD

Currently, the sample data manager on ROV expeditions is almost exclusively responsible for archiving metadata, and this person would likely be tasked with the extra onboard data archiving duties. This work would need to be balanced with their other assignments as they already perform well over a full day's work while at sea. The extra person on a mapping expedition responsible for filtering water samples could also be in charge of recording metadata for the sample.

PROCESSING FILTERS ON SHORE TO GET DNA

NOAA Ocean Exploration does not have the capacity or equipment to process samples on shore to get DNA extracted, sequenced, and archived. Thus, we would need to contract out these activities to a laboratory with relevant expertise, possibly through a partnership agreement. There are several such laboratories available, both internal and external to NOAA, but their services would need to be scoped for costs. One recommendation is to use Genewiz, a private company that can process and sequence samples.[15] Or, NOAA Ocean Exploration could use the Smithsonian Institution for sample processing and storage. In addition to comparing costs, we would need to identify a contract mechanism to pay for the service, which would require significant administrative support.

ARCHIVING PROCESSED SAMPLES

Since NOAA's National Centers for Environmental Information (NCEI) does not currently archive raw sequence data, NOAA Ocean Exploration would need someone that can submit data to a repository, potentially through the aforementioned partnership. This could be someone from NCEI or NOAA Ocean Exploration (would require training) or someone externally who could be hired or contracted to do the work. The latter would require additional administrative support.

15 https://www.genewiz.com/en

PERMITTING AND REGULATIONS

NOAA Ocean Exploration has a dedicated person in charge of all permitting, regulations, and environmental compliance review.

COULD THIS WORK BE DONE WITH CURRENT PERSONNEL OR WOULD NOAA OCEAN EXPLORATION NEED TO HIRE NEW PERSONNEL? IF NO NEW PERSONNEL WOULD BE NEEDED, HOW WOULD THIS WORK IMPACT OTHER TASKS REQUIRED BY CURRENT PERSONNEL?

NOAA Ocean Exploration would not be able to process the data with current personnel. New personnel would need to be hired specifically for this work. All DNA extraction and processing should be conducted through a cooperative partnership with results, or information about how to access the results, shared with NOAA Ocean Exploration.

DURING WHAT TYPE OF EXPEDITION COULD THESE DATA BE COLLECTED (I.E., MAPPING, ROV)?

Samples can be collected during mapping and ROV expeditions.

NOAA OCEAN EXPLORATION'S CURRENT OPERATIONS ON *OKEANOS EXPLORER*: COMPLEMENTARY AND CONTRASTING DATA COLLECTION

IS COLLECTING THESE DATA A PRIORITY? IF SO, HOW DOES IT COMPARE TO CURRENT DATA COLLECTIONS?

Considering how many exploration variables and subsequent data gaps eDNA can address in exploring the deep sea, yes, systematically collecting water samples for eDNA analyses is a priority.

HOW WOULD COLLECTING THE DATA TAKE AWAY FROM CURRENT OPERATIONS? HOW WOULD COLLECTING THE DATA COMPLEMENT CURRENT OPERATIONS AND POTENTIALLY NEW OPERATIONS?

Water sample collection with the Niskin bottles on the ROV or CTD rosette could take time away from primary operations, but it would not be significant.

- **ROV OPERATIONS:** Niskin bottles would need to be mounted to the ROV, and some basic maintenance and setup would be required that could take away from other operations. But in general, since the ROV is already part of NOAA Ocean Exploration's *Okeanos Explorer* operations, collecting water samples would not take significant time away from other ROV operations.

- **CTD ROSETTE OPERATIONS:** A CTD rosette cast could potentially take away up to two hours (depending on the depth) from mapping or ROV operations.

EXPECTED PRODUCTS

WHAT WOULD BE THE EXPECTED SCIENTIFIC PRODUCTS?

A list of sequencing results processed from the water samples would be expected from this effort. If NOAA Ocean Exploration moves forward with universal primers, information on organisms to at least the family level would be generated. Although, sequences that are not associated with any recorded sequence are possible.

WOULD THE DATA BE REPORTED IN EXPEDITION REPORTS?

Yes, the data would be reported in expedition reports, and would vary depending on the type of expedition during which water samples are collected. The following information is proposed to be included in reports:

- Number of CTD rosette casts
- Number of water samples collected
- What the eDNA samples will be used for (e.g., biodiversity analysis, single species detection, etc.)
- Primers used and bioinformatics analysis conducted
- Information on where to access archived samples or data
- Contact information for person/organization in charge of analysis

DATA MANAGEMENT, PROCESSING, SUMMARIES, AND QUALITY CONTROL

WHAT WOULD BE THE MINIMUM METADATA NEEDED?

- Niskin bottle number
- Date and time the sample was taken
- Volume of sample
- Filter material and pore size (if multiple types are used)
- Latitude/longitude of sample
- Depth
- How the water sample was stored (if the sample is not filtered right away)

- How the filter was stored (if filtering on board, e.g., Longmire's buffer)
- Brief description of site where water sample was taken (site characteristics such as temperature, salinity, etc.)
- Video/imagery accompanying the sample if using the ROV
- Visual identification and abundance estimate of species around water samples if using the ROV

WOULD DATA ANALYSIS OR PROCESSING BE NEEDED?

Water samples would require processing. After DNA extraction and sequencing, a bioinformatics pipeline would need to be established to obtain the expected products (i.e., information on the organisms).

HOW WOULD THE DATA BE ANALYZED OR PROCESSED?

Due to the exploratory nature of eDNA sampling, universal primers would be used to maximize detection of corals, sponges, fishes, microorganisms, etc. with the understanding that these primers may only provide taxonomic resolution to the family level. Downstream DNA sequencing of samples collected from ROV dives and CTD rosette casts could be used to resolve species-level classifications. However, NOAA Ocean Exploration would most likely not analyze or sequence the samples. That work would need to be done through a partnership. Examples of universal primers can be found in Lacoursière-Roussel et al. (2018).

WOULD A DATA SUMMARY BE NEEDED? HOW WOULD A DATA SUMMARY BE FORMATTED AND WHAT WOULD IT INCLUDE?

A data summary of the organisms found in each sample in the form of tabular data (.csv file) is preferred and could be archived with related data at NCEI. The summary could also include the environmental variables associated with each sample (location, salinity, and such). Additionally, it could include the location of archived filters.

WHAT WOULD BE THE QA/QC PROCESS?

Potential avenues for quality assurance and quality control (QA/QC) include processing replicates of the same sample, assessing whether leaving filters open to the air would affect the sample, and conducting the same process with double distilled, UV treated water and comparing the results.

WOULD COASTAL AND MARINE ECOLOGICAL CLASSIFICATION STANDARD (CMECS) IMPLEMENTATION BE NECESSARY? IF SO, HOW WOULD IT BE IMPLEMENTED?

It is unlikely eDNA sampling would lend itself well to CMECS as the classification standard currently exists. The standard's current biotic component emphasizes the dominance of biological community elements on the seafloor or in the water column.[16] Given that eDNA information provides insights primarily into presence/absence and would contain a mix of DNA from benthic and pelagic organisms, application of CMECS does not currently apply. However, strides in this field are being made to derive abundance data from eDNA samples. This is primarily applicable in freshwater ecosystems, and more research is needed (Beng and Corlett 2020).

DATA ACCESSIBILITY, STORAGE, ARCHIVING

WOULD (OR COULD) THE DATA BE STORED AT THE NOAA NATIONAL CENTERS FOR ENVIRONMENTAL INFORMATION (NCEI)? IF NOT, WHAT PUBLIC REPOSITORIES COULD BE USED FOR DATA ARCHIVING?

Physical samples (water and filters) cannot be stored at NCEI. This would most likely involve leveraging NOAA Ocean Exploration's existing partnership with the Smithsonian Institution or establishing a new partnership to store samples long term. If conducting the DNA extraction and bioinformatics analysis, DNA sequences also cannot be stored at NCEI. One common database for stored genetic information is the National Center for Biotechnology Information's (NCBI) Sequence Read Archive,[17] which provides a unique accession number to link to the data. Once the bioinformatics process is conducted, a list of detected families, genera, or species could be generated and stored in a spreadsheet and then archived at NCEI.

WHAT WOULD BE THE DATA ARCHIVING PIPELINE?

If a full eDNA analysis is to be conducted, the entity NOAA Ocean Exploration would partner with would be in charge of archiving raw sequence data with NCBI and ensuring that the appropriate metadata are filled out. Ideally, someone within NOAA Ocean Exploration would be assigned to ensure that the data are made available and would obtain the unique accession number assigned to the dataset. The partner would also be responsible for the bioinformatics pipeline to produce occurrence records of families, genera, or species (depending on primer use). Similarly,

16 https://coast.noaa.gov/data/digitalcoast/pdf/cmecs.pdf

17 https://www.ncbi.nlm.nih.gov/sra

the NOAA Ocean Exploration contact would be responsible for obtaining that data from the partner and ensuring it is appropriately archived with NCEI.

IF STORED SOMEWHERE OTHER THAN NCEI, HOW WOULD THE INFORMATION BE SHARED WITH NCEI AND NOAA OCEAN EXPLORATION?

The externally archived sequence data could be linked to NOAA Ocean Exploration's website. The unique accession number assigned via NCBI and DOI numbers assigned via NCEI could be linked in expedition reports.

PERMITTING AND REGULATIONS

WOULD PERMITS OR LICENSES BE NEEDED TO COLLECT THE DATA?

Collecting water samples in U.S. waters generally does not require a permit. However, in some jurisdictions (e.g., some U.S. marine protected areas, some foreign waters) collecting physical samples, including water samples, does require a collection permit. Since the collection of other samples (i.e., biological and geological) are already part of NOAA Ocean Exploration's *Okeanos Explorer* operations, obtaining permits for water samples should require limited additional work, but would need to be accounted for. Additionally, environmental compliance documents would need to be updated and maintained, but this should also require minimal work.

ENVIRONMENTAL OR TECHNOLOGICAL RISK

GIVEN ALL OF THE ABOVE CONSIDERATIONS, COULD THERE BE ENVIRONMENTAL OR TECHNOLOGICAL RISKS INVOLVED IN COLLECTING THE DATA?

There are no major environmental or technological risks associated with eDNA sampling.

FEASIBILITY ASSESSMENT OF THE MEASUREMENT

GIVEN ALL OF THE ABOVE CONSIDERATIONS, IS IT FEASIBLE TO COLLECT THESE DATA AS PART OF NOAA OCEAN EXPLORATION'S OPERATIONS ON NOAA *SHIP OKEANOS EXPLORER*?

Based on this assessment, it may be feasible for NOAA Ocean Exploration to incorporate eDNA sampling into *Okeanos Explorer* operations considering the existing capabilities to collect water samples. However, there are some considerations:

- **LAB SPACE:** A clean lab would be crucial to avoid contaminating samples. Lab space would also be needed for the filtration setup and equipment storage, and this is limited during ROV expeditions. Lab space could be cleaned and dedicated for eDNA sampling if the CTD rosette were to be used during mapping expeditions.
- **PERSONNEL:** ROV expedition personnel are already heavily tasked during an expedition, which could inhibit their ability to filter and manage the water samples. An additional person might be needed to process samples during ROV expeditions, but berthing space could be a challenge. There is plenty of berthing space on mapping expeditions, so a dedicated person could be tasked with managing collection and processing of the eDNA samples.

RELEVANT LITERATURE

Ausubel, J.H., Stoeckle, M.Y., and Gaffney, P. (2019). 1st US National Conference on Marine Environmental DNA (eDNA) Final Report. Monmouth University.

Beng, K.C. and Corlett, R.T. (2020). Applications of environmental DNA (eDNA) in ecology and conservation: Opportunities, challenges and prospects. Biodiversity and Conservation 1-33. https://doi.org/10.1007/s10531-020-01980-0

Everett, M.V. and Park, L.K. (2018). Exploring deep-water coral communities using environmental DNA. Deep Sea Research Part II: Topical Studies in Oceanography 150:229-241. https://doi.org/10.1016/j.dsr2.2017.09.008

Goodwin, K., Strom, M., Arzayus, F., Bohan, M., Busch, S., Certner, R., Canonico, G., Cross, S., Davis, J., Egan, K., Grieg, T., Kearns, E., Koss, J., Larsen, K., Layton, D., Nichols, K., O'Neil, J., Parks, D., and Werner, F. (2020). NOAA 'Omics White Paper: Informing the NOAA 'Omics Strategy and Implementation Plan (White Paper). National Oceanic and Atmospheric Administration. https://doi.org/10.25923/bd7z-zb37

Hansen, B.K., Bekkevold, D., Clausen, L.W., and Nielsen, E.E. (2018). The sceptical optimist: Challenges and perspectives for the application of environmental DNA in marine fisheries. Fish and Fisheries 19:751-768. https://doi.org/10.1111/faf.12286

Jerde, C.L., Mahon, A.R., Chadderton, W.L., and Lodge, D.M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA: eDNA surveillance of rare aquatic species. Conservation Letters 4:150-157. http://dx.doi.org/10.1111/j.1755-263X.2010.00158.x

Kelly, R.P., Port, J.A., Yamahara, K.M., and Crowder, L.B. (2014). Using environmental DNA to census marine fishes in a large mesocosm. PLoS ONE 9:e86175. https://doi.org/10.1371/journal.pone.0086175

Klymus, K.E., Marshall, N.T., and Stepien, C.A. (2017). Environmental DNA (eDNA) metabarcoding assays to detect invasive invertebrate species in the Great Lakes. PLoS ONE 12:e0177643. https://doi.org/10.1371/journal.pone.0177643

Lacoursière-Roussel, A., Howland, K., Normandeau, E., Grey, E.K., Archambault, P., Deiner, K., Lodge, D.M., Hernandez, C., Leduc, N., and Bernatchez, L. (2018). eDNA metabarcoding as a new surveillance approach for coastal Arctic biodiversity. Ecol Evol 8:7763-7777. https://doi.org/10.1002/ece3.4213

Levin, L., Ruhl, H., Heimbach, P., McCurdy, A., and Smith, L. (2019a). Deep Ocean Observing Strategy Science and Implementation Guide. https://deepoceanobserving.org/wp-content/uploads/2019/05/DOOS-2019-Science-and-Implementation-Guide-2019-05-31-V3.pdf

Levin, L.A., Bett, B.J., Gates, A.R., Heimbach, P., Howe, B.M., Janssen, F., McCurdy, A., Ruhl, H.A., Snelgrove, P., Stocks, K.I., Bailey, D., Baumann-Pickering, S., Beaverson, C., Benfield, M.C., Booth, D.J., Carreiro-Silva, M., Colaço, A., Eblé, M.C., Fowler, A.M., Gjerde, K.M., Jones D.O.B., Katsumata, K., Kelley, D., Le Bris, N., Leonardi, A.P., Lejzerowicz, F., Macreadie, P.I., McLean, D., Meitz, F., Morato, T., Netburn, A., Pawlowski, J., Smith, C.R., Sun, S., Uchida, H., Vardaro, M.F., Venkatesan, R., and Weller, R.A. (2019b). Global observing needs in the deep ocean. Frontiers in Marine Science 6:241. https://doi.org/10.3389/fmars.2019.00241

Netburn, A.N., ed. (2018). From Surface to Seafloor: Exploration of the Water Column. Workshop Report, Honolulu, HI, March 4-5, 2017. NOAA Office of Ocean Exploration and Research, Silver Spring, MD. NOAA Technical Memorandum OAR OER 003. https://doi.org/10.25923/rnjx-vn79

Paul, J.H., Jeffrey, W.H., and DeFlaun, M.F. (1987). Dynamics of extracellular DNA in the marine environment. Applied and Environmental Microbiology 53:170-179.

Schultz, M.T., and Lance, R.F. (2015). Modeling the sensitivity of field surveys for detection of environmental DNA (eDNA). PLoS ONE 10:e0141503. https://doi.org/10.1371/journal.pone.0141503

Singer, G.A.C, Fahner, N.A., Barnes, J.G., McCarthy, A., and Hajibabaei, M. (2019). Comprehensive biodiversity analysis via ultra-deep patterned flow cell technology: a case study of eDNA metabarcoding seawater. Sci Rep 9:5991. https://doi.org/10.1038/s41598-019-42455-9

Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M.D.M., Breiner, H.W., and Richards, T.A. (2010). Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Molecular Ecology 19:21-31. https://doi.org/10.1111/j.1365-294X.2009.04480.x

Taberlet, P., Bonin, A., Coissac, E., and Zinger, L. (2018). Environmental DNA: For Biodiversity Research and Monitoring. Oxford University Press. https://doi.org/10.1093/oso/9780198767220.001.0001

Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., and Kawabata, Z. (2012). Estimation of fish biomass using environmental DNA. PLoS ONE 7:e35868. https://doi.org/10.1371/journal.pone.0035868

Thomas, A.C., Howard, J., Nguyen, P.L., Seimon, T.A., and Goldberg, C.S. (2018). eDNA Sampler: A fully integrated environmental DNA sampling system. Methods Ecol Evol 9:1379-1385. https://doi.org/10.1111/2041-210X.12994

Thomsen, P.F., Kielgast, J., Iversen, L.L., Møller, P.R., Rasmussen, M., and Willerslev, E. (2012). Detection of a diverse marine fish fauna using environmental DNA from seawater samples. PLoS ONE 7:e41732. https://doi.org/10.1371/journal.pone.0041732

Thomsen, P.F. and Willerslev, E. (2015). Environmental DNA - An emerging tool in conservation for monitoring past and present biodiversity. Biological Conservation 183:4-18. https://doi.org/10.1016/j.biocon.2014.11.019

Tsuji, S., Yamanaka, H., and Minamoto, T. (2017). Effects of water pH and proteinase K treatment on the yield of environmental DNA from water samples. Limnology 18:1-7. https://doi.org/10.1007/s10201-016-0483-x

Tucker, A.J., Chadderton, W.L., Jerde, C.L., Renshaw, M.A., Uy, K., Gantz, C., Mahon, A.R., Bowen, A., Strakosh, T., Bossenbroek, J.M., Sieracki, J.L., Beletsky, D., Bergner, J., and Lodge, D.M. (2016). A sensitive environmental DNA (eDNA) assay leads to new insights on Ruffe (Gymnocephalus cernua) spread in North America. Biol Invasions 18:3205-3222. https://doi.org/10.1007/s10530-016-1209-z

Turner, C.R., Barnes, M.A., Xu, C.C.Y., Jones, S.E., Jerde, C.L., and Lodge, D.M. (2014). Particle size distribution and optimal capture of aqueous macrobial eDNA. Methods Ecol Evol 5:676-684. https://doi.org/10.1111/2041-210X.12206

Wilcox, T.M., McKelvey, K.S., Young, M.K., Lowe, W.H., and Schwartz, M.K. (2015). Environmental DNA particle size distribution from brook trout (Salvelinus fontinalis). Conservation Genet Resour 7:639-641. https://doi.org/10.1007/s12686-015-0465-z

Williams, K.E., Huyvaert, K.P., and Piaggio, A.J. (2016). No filters, no fridges: A method for preservation of water samples for eDNA analysis. BMC Research Notes 9:1-5. https://doi.org/10.1186/s13104-016-2104-5

Woodall, L., Andradi-Brown, D., Brierley, A., Clark, M., Connelly, D., Hall, R., Howell, K., Huvenne, V., Linse, K., Ross, R., Snelgrove, P., Stefanoudis, P., Sutton, T., Taylor, M., Thornton, T., and Rogers, A. (2018). A multidisciplinary approach for generating globally consistent data on mesophotic, deep-pelagic, and bathyal biological communities. Oceanography 31. https://doi.org/10.5670/oceanog.2018.301

Yamamoto, S., Minami, K., Fukaya, K., Takahashi, K., Sawada, H., Murakami, H., Tsuji, S., Hashizume, H., Kubonaga, S., Horiuchi, T., Hongo, M., Nishida, J., Okugawa, Y., Fujiwara, A., Fukuda, M., Hidaka, S., Suzuki, K.W., Miya, M., Araki, H., Yamanaka, H., Maruyama, A., Miyashita, K., Masuda, R., Minamoto, T., and Kondoh, M. (2016). Environmental DNA as a 'snapshot' of fish distribution: A case study of Japanese jack mackerel in Maizuru Bay, Sea of Japan. PLoS ONE 11:e0149786. https://doi.org/10.1371/journal.pone.0149786

Yan, X., Tang, X.X., Chen, L., Yi, Z.W., Fang, M.J., Wu, Z., and Qiu, Y.K. (2014). Two new cytotoxic indole alkaloids from a deep-sea sediment derived metagenomic clone. Marine Drugs 12:2156-2163. https://doi.org/10.3390/md12042156

APPENDIX F: FEASIBILITY ASSESSMENT FOR MEASURING MICROPLASTIC DISTRIBUTION, ABUNDANCE, SIZE, AND COMPOSITION

TABLE OF CONTENTS

NAME AND TYPE OF DATA GAP

WHAT IS THE DATA GAP?

The data gap is microplastic occurrence, distribution, abundance, size, and composition.

WHAT IS THE TYPE OF DATA GAP: (1) DATA ARE NOT BEING COLLECTED IN GENERAL, (2) DATA ARE NOT BEING COLLECTED IN A SPECIFIC LOCATION OR DEPTH, OR (3) DATA ARE NOT BEING COLLECTED AT A SPECIFIC FREQUENCY?

Microplastic occurrence, distribution, abundance, size, and composition meet the criteria for all three types of data gaps: Data are not being collected in general, are not being collected in a specific location or depth, and are not being collected at a specific frequency.

WHAT HIGH-PRIORITY EXPLORATION VARIABLES COULD NOAA OCEAN EXPLORATION ADDRESS BY FILLING THE GAP?[1] (SEE TABLE 1, TABLE 3, AND APPENDIX TABLE A1.)

Microplastic sampling addresses the data gap of anthropogenic impacts, which were mentioned in four deep-ocean exploration and observation reports (OET 2014, Netburn 2018, Woodall et al. 2018, and Danovaro et al. 2020). Although anthropogenic impacts encompass more than just microplastics, plastics were specifically mentioned in all four reports.

BACKGROUND AND JUSTIFICATION

BACKGROUND INFORMATION

Plastics are a common form of marine debris that remain in the environment indefinitely. In 2010, it was estimated that 4.8-12.7 million metric tons of plastic, including microplastics, enter the ocean annually, and this is predicted to increase by an order of magnitude by 2025 (Jambeck et al. 2015).

Microplastic diversity is reflective of the different types of plastics commonly used in industrial processes and everyday life (Duis and Coors 2016). Primary microplastics are manufactured in "micro" sizes (e.g., microbeads and plastic preproduction pellets) whereas secondary microplastics result from the fragmentation of larger plastic products (e.g., water bottles, fishing

1 Gaps are identified in Tables 1, 3, and 1A in the report "Exploration Variables Identified by NOAA Ocean Exploration."

gear, plastic bags) through natural weathering processes (Cole et al. 2011). The majority of microplastics in the ocean are secondary microplastics (Gregory and Andrady 2003).

Microplastics also include microfibers, which are synthetic fibers that have a diameter less than 5-10 µm (De Wael and Gason 2008 as cited in Woodall et al. 2015). Microfibers are one of the most prevalent types of microplastics in the marine environment (Browne et al. 2010; Sadri et al. 2014). Additionally, the degradation of microplastics into nanoplastics (1 to 1,000 nm in size) has recently become a concern due to their higher propensity for uptake by aquatic food webs, which can lead to bioaccumulation and human ingestion and thus higher estimated rates of human exposure (Stapleton 2019). This assessment focuses on primary and secondary microplastics and excludes microfibers and nanoplastics because their sample processing requirements differ from those for general microplastics.

Microplastics are ubiquitous in aquatic environments (Auta et al. 2017). They have been found throughout the water column and across the seafloor (Choy et al. 2019; Kane et al. 2020). Microplastics have also been found in the stomachs of many marine organisms, from plankton species to marine mammals. As a result, they are actively assimilated into deep-sea food webs (Lusher et al. 2016; Katija et al. 2017).

Research has shown that plastic debris is a potential vector for the transfer of persistent, bioaccumulative, and toxic pollutants from the water to the food web, potentially creating a risk to marine species (Rochman et al. 2013). However, it should be noted that contamination from microplastic ingestion is limited compared to other sources (Koelmans et al. 2016; 2017) and more research is needed in this area. Chemical additives can also leach out of microplastics into the ocean, and contaminants from the water and surface air (Ogata et al. 2009) may adhere to microplastics that sink to the deep sea (Chen et al. 2019) where microplastic density is greatest (Kane and Clare 2019). Many types of plastics are denser than seawater (1.03 g/cm^3), and even those that are lighter can sink to the bottom of the ocean through various pathways, including physical advection (Enders et al. 2015), biofouling (Auta et al. 2017), and ingestion by organisms (Katija et al. 2017).

STATE OF THE SCIENCE RELEVANT TO THE DEEP SEA

The study of deep-sea microplastics is still in its infancy with relatively few studies (e.g., Kanhai et al. 2019) and best practices (Wang and Wang 2018). The majority of microplastic studies have been conducted at the surface and in relatively shallow waters, albeit there are also data gaps associated with these environments. Furthermore, existing data are difficult to compare due to a lack of standardized methodology for microplastic sampling, processing, analysis, and reporting (Avio et al. 2016).

A few studies have focused on microplastics at depth (Choy and Drazen 2013; Woodall et al. 2014; Kane et al. 2019; Pabortsava and Lampitt 2020). In the deep ocean, there are efforts to report macroplastics and other debris with visual surveys (e.g., through NOAA Ocean Exploration's SeaTubeV3 annotations[2] and the Japan Agency for Marine-Earth Science and Technology's Deep-Sea Debris Database[3]), but a significant proportion of marine plastic (i.e., microplastics), cannot be visually surveyed. This may be why the amount of marine plastics documented does not equal the amount of plastic going into the ocean (van Sebille et al. 2015). The scientific community is actively working on identifying short- and long-term plastic sinks and downstream impacts on human health and the economy.

One pressing scientific question regards the vertical distribution of microplastics in the water column. Egger et al. (2020) found decreasing microplastic concentrations with depth to 2,000 m in the North Pacific using a Multiple Opening and Closing Net with an Environmental Sensing System[4] (90x15 cm, 333 μm filter). Tekman et al. (2020) found similar results in the Arctic, where the highest microplastic concentrations in surface waters decreased with depth to 1,000 m (*in situ* pump, 32 μm filter). However, microplastic concentrations have been shown to increase with depth in the Mariana Trench (0.3 μm filter, Peng et al. 2018) and have peaked within the deep water column elsewhere (100 μm filter, Choy et al. 2019). Pabortsava and Lampitt (2020) found no clear pattern of vertical abundance of different types of plastic polymers. Similar to the distribution of sediment microplastics that can be influenced by physical processes (Kane et al. 2020), water column microplastic distribution is likely influenced by surface microplastic concentrations (Egger et al. 2020), currents and mixing (Choy et al. 2019, Wichmann et al. 2019), and biological processes (Kooi et al. 2017).

HOW WOULD THE DATA HELP ADDRESS NOAA OCEAN EXPLORATION'S MISSION AND GOALS?

Collecting data about deep-sea microplastics and contributing to systematic ocean exploration of anthropogenic impacts would help NOAA Ocean Exploration achieve its mission. The deep ocean is largely unexplored, but humans have profoundly impacted areas that are considered pristine (Nuwer 2014; United Nations 2017), as highlighted by the discovery of marine debris and microplastics in remote areas. Several scientists and subject matter experts, who were consulted during this feasibility assessment, noted the importance of collecting microplastic data as part of systematic exploration. Addressing this data gap could also help NOAA Ocean Exploration

2 https://oceanexplorer.noaa.gov/okeanos/collaboration-tools/science_annotations/welcome.html

3 http://www.godac.jamstec.go.jp/catalog/dsdebris/e/

4 https://www.whoi.edu/what-we-do/explore/instruments/instruments-sensors-samplers/mocness/

expand sampling capabilities for exploring new areas and demonstrating new technologies aboard *Okeanos Explorer*.

OTHER NOAA GOALS

Assuming characterizing an area includes measuring human impacts, collecting data on microplastic abundance, size, and composition is relevant to the *OAR Strategy 2020-2026*, Goal 1, in particular.[5]

GOAL 1: Explore the Marine Environment: Increase knowledge of the oceans, coastal areas, and Great Lakes to support resource management and public awareness.

> **1.1. MAP AND CHARACTERIZE THE DEEP OCEAN FRONTIER:** Explore the deep portions of the U.S. Exclusive Economic Zone (EEZ) and beyond to discover and inform decision-making regarding resources. Coordinate and partner with others using a variety of characterization methods and techniques to acquire data for environmental, physical, and biological parameters. Develop a catalog of standard measurements that aid understanding of the resource potential of a location and create high-resolution maps of the seafloor. Provide critical information to evaluate the economic potential within the U.S. EEZ.

In addition, the *NOAA Marine Debris Program Strategic Plan 2016-2020* is relevant to this feasibility assessment.[6] One of the goals is to focus on coordination, with a specific action item to “increase coordination at the federal level with an efficient and engaged Interagency Marine Debris Coordinating Committee by collaborating on at least three projects with other federal agencies.” Another goal focuses on research with a specific objective to “identify, analyze, and increase understanding of the environmental and societal impacts of marine debris by assessing impacts and risks to targeted species and sectors.” NOAA Ocean Exploration could partner with the Marine Debris Program to help them achieve the goals of their strategic plan.

WHAT BIG PICTURE SCIENTIFIC QUESTIONS WOULD THE DATA ADDRESS?

- What is the spatial distribution of microplastics throughout a region of interest?
- How does the abundance, size, and composition of microplastics compare spatially and temporally through data contribution to baseline establishment?
- How does abundance, size, and composition of microplastics vary with depth, location, topography, and habitat?

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5 https://research.noaa.gov/Portals/0/Files/OAR%20Strategy%202020-2026.pdf

6 https://marinedebris.noaa.gov/sites/default/files/Strategic%20Plan%202016.pdf

- What is the behavior of microplastics in the marine environment?
- What is the vertical distribution of microplastics throughout the water column?
- What is the fate and transport of microplastics? Where are the microplastic sinks located?

WAS THE DATA GAP IDENTIFIED BY THE DEEP-SEA COMMUNITY AS IMPORTANT TO ADDRESS? IF YES, WHERE WAS IT IDENTIFIED AND WHO IDENTIFIED IT?

The collection of microplastic abundance and composition data was identified as a need in a number of publications, white papers, and stakeholder papers related to deep-sea observations. Some examples follow.

- *Workshop on Telepresence-Enabled Exploration of the Eastern Pacific Ocean* (OET 2014): The lateral and vertical extent of plastics in the water column is identified in the report of a workshop to identify ocean exploration knowledge and data gaps in the eastern Pacific Ocean. It is noted as a key data gap in the Physical and Chemical Oceanography section and as important for the Line Islands, Hawaii, and the U.S./Mexico Margin.
- *From Surface to Seafloor: Exploration of the Water Column* (Netburn 2018): Participants in an NOAA Ocean Exploration-sponsored water column workshop identified plastic as important to measure in the water column and posed the following questions: What mechanisms govern the distribution of plastics in the upper ocean and the deep sea? How does marine litter impact biota? This report provides a framework for addressing key knowledge gaps in the water column environment.
- *A Multidisciplinary Approach for Generating Globally Consistent Data on Mesophotic, Deep-Pelagic, and Bathyal Biological Communities* (Woodall et al. 2018): In this paper, the authors provide 20 biological, chemical, physical, and socioeconomic parameters they consider important for describing biological and environmental variability in the deep sea. One of the socioeconomic parameters includes measuring microplastic abundance and diversity.
- *Global Observing Needs in the Deep Ocean* (Levin et al. (2019a) and Levin et al. (2019b)): Both of these papers discuss the need for a globally integrated network of deep-ocean observing systems, its status, and the key scientific questions and societal mandates driving observing requirements over the next decade. They note that quantifying and measuring pollution, contamination, and litter in the deep sea should be considered for a deep-ocean observing strategy. Both papers ask: What are the sources, pathways, fates and consequences of deep-ocean contaminants (including plastics) introduced by humans from land and ocean activities?
- *Ecological Variables for Developing a Global Deep-Ocean Monitoring and Conservation Strategy* (Danovaro et al. 2020): This paper identifies the need to preserve benthic and pelagic deep-sea ecosystems through ecosystem-based management strategies and monitoring using a set of deep-sea ecological variables. The authors compiled this set

of deep-sea ecological variables through expert elicitation. In the paper, they stress the importance of measuring deep-sea ecosystem health and impacts, including plastics, microplastics, and other chemical contaminants.

WHICH STAKEHOLDERS AND PARTNERS WOULD THESE DATA SERVE?

In general, the availability of extensive, long-term microplastic data will help the research community better understand the impacts of microplastic pollution on aquatic biota, human health, and the environment. Some specific examples of stakeholders and partners that would be served by these data follow.

- **DEEP OCEAN OBSERVING STRATEGY:** One of the essential ocean variables (biogeochemistry) under consideration is litter, including microplastics (Levin et al. 2019a, Levin et al. 2019b).
- **NOAA MARINE DEBRIS PROGRAM:** The Save Our Seas Act 2.0 (P.L. 116-224) was signed into law in December 2020 and stresses the growing concern of the global marine debris problem.[7] It reauthorizes the NOAA Marine Debris Program through Fiscal Year 2022 and encourages the program to engage with the U.S. State Department to address the global marine debris problem on an international level. NOAA Ocean Exploration could help the program investigate and prevent the adverse impacts of marine debris by providing baseline information of microplastics in the deep sea.
- **OCEANS NETWORK CANADA (ONC) AND THE JAPAN AGENCY FOR MARINE-EARTH SCIENCE AND TECHNOLOGY (JAMSTEC):** In their recent call for expressions of interest, ONC received a submission for microplastic data collection. They are in the preliminary process of testing compatibility of sampling sediments for microplastics with their current operations, specifically during the servicing of their underwater observatories in the eastern Pacific. Additionally, JAMSTEC is currently making similar measurements in the western Pacific. With NOAA Ocean Exploration returning to the Pacific with *Okeanos Explorer* in upcoming years, there may be the potential to partner for basin-wide microplastic data collection.
- **DEEP-OCEAN STEWARDSHIP INITIATIVE (DOSI):** DOSI's Pollution and Debris Working Group aims to increase knowledge about deep-sea pollution and debris, to advocate for their consideration in decision-making, and to provide expert opinion on the topic. Additional data will contribute to their goals and inform their work.
- **OTHER PARTNERS:** Partners with research and exploration vessels that do similar work as NOAA Ocean Exploration have plans for microplastic sampling (e.g., Schmidt Ocean Institute,[8] Ocean Exploration Trust, N. Raineault, pers. comm.).

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7 https://www.congress.gov/bill/116th-congress/senate-bill/1982/text

8 https://schmidtocean.org/cruise/microplastics-of-the-alaskan-gulf/

HAVE THE DATA BEEN COLLECTED DURING PRIOR NOAA OCEAN EXPLORATION-SUPPORTED EXPEDITIONS OR ARE THERE PLANS TO COLLECT THE DATA DURING FUTURE EXPEDITIONS?

- NOAA Ocean Exploration used a manta trawl on *Okeanos Explorer* during its transit across the Pacific Ocean at the conclusion of the Indonesia-USA Deep-Sea Exploration of the Sangihe Talaud Region expedition in 2010,[9,10] to collect plankton and microplastic samples (Goldstein et al. 2013).
- Although not specific to microplastics, scientists note the occurrence of marine debris, litter, and macroplastics during regular video annotations (using SeaTubeV3) of remotely operated vehicle (ROV) dives from *Okeanos Explorer*.

METHODS, PROTOCOL OPTIONS, AND SAMPLING STRATEGY[11]

WHERE SHOULD SAMPLING OCCUR AND/OR WHAT WOULD BE THE SAMPLING STRATEGY? WOULD SAMPLING BE PART OF STANDARD OPERATIONS OR OPPORTUNISTIC?

Ideally, NOAA Ocean Exploration would conduct microplastic sampling as part of standard operations, at specified water column depths and on the seafloor along with measurements of associated environmental variables (e.g., temperature, salinity, dissolved oxygen). This would be especially useful to the scientific community as it would contribute to an almost nonexistent dataset, identify potential microplastic sinks for further study, and improve understanding of anthropogenic impacts on the deep ocean. However, given NOAA Ocean Exploration's limited capacity, a more realistic sampling strategy would be opportunistic sampling with priority given to areas that have no baseline data on microplastics.

WHAT SAMPLING METHODS WOULD BE USED?

Overall, methods for collecting microplastic data in the deep ocean fall into three categories: water column sampling, sediment sampling, and biological sampling. Below are generalized steps for data collection synthesized from several references (Van Cauwenberghe et al. 2013; Masura et al. 2015; Rocha-Santos and Duarte 2015; Van Cauwenberghe et al. 2015; Barrows et al. 2017; Bergmann et al. 2017; Coppock et al. 2017; Shim et al. 2017; Mai et al. 2018; Rivers et

9 https://oceanexplorer.noaa.gov/okeanos/explorations/10index/welcome.html

10 https://oceanexplorer.noaa.gov/ex10years/stories/plankton.html

11 Here, "methods" refers to the general process whereas "protocol options" refers to more detailed information on specific steps for collecting the data.

al. 2019; Compa et al. 2020). Further details (e.g., pros and cons, equipment specifications, and ancillary measurements) can be found in the response to the next question about protocol.

WATER COLUMN SAMPLING

Net tow sampling is the most common water column sampling method noted in the published literature and is likely feasible since NOAA Ocean Exploration has conducted such sampling operations on *Okeanos Explorer* in the past (see the Background and Justification section). Neuston or manta trawl nets[12] could be used at the surface while bongo nets could be used below the surface. The latter would require an open-close system and depth meter to sample accurate depth horizons (one at a time). Storage space required for the nets is minimal, an estimated 1 m^2. Net setup is done at the beginning of an expedition, and breakdown is done at the end, each takes approximately 30 minutes.

Steps for water column sampling net tows:

1. Check the net for tears and holes that require maintenance.
2. Rinse the net thoroughly to avoid risk of contamination from storage.
3. Hook up the net onto the J-frame and lower it into the water. This should be done from the side of the ship to avoid the wake, which could disturb the surface water and result in an underestimate of microplastics.
4. Tow the net for 20-30 minutes at a constant speed of 1-2 knots before retrieving the net and sample.
5. Rinse the outside of the net with water to gather the contents at the cod-end of the net for collection.
6. Preserve the samples by freezing them or with formalin or ethanol.

The amount of water needed at varying depths for an accurate measurement is open for debate. Even at the better-studied surface, the range of sample volumes varies: net tows can filter thousands of liters of water whereas bulk samples are generally orders of magnitude smaller. Karlsson et al. (2020) found that net tows collect a higher concentration of microplastics ideal for both quantitative and compositional analysis when compared with bulk samples collected using *in situ* pumps at the surface. Deeper in the water column, *in situ* pumps (e.g., outfitted to an ROV or conductivity, temperature, depth (CTD) rosette) need to be deployed for longer periods of time. This issue will not be resolved until researchers and other stakeholders can collect samples across a range of volumes, depths, and ocean basins.

12 https://www.fisheries.noaa.gov/about/fisheries-resources-division-southwest-fisheries-science-center

Another way to sample for microplastics in surface water or the water column is to collect and filter water (bulk sampling), for example using the ship's flow-through system, Niskin bottles, an *in situ* pump, or an ROV sampler.

- **SHIP'S FLOW-THROUGH SYSTEM:** The flow-through system could be used to sample surface water, but there is a high risk of plastic contamination given that these systems are often largely composed of plastic.[13] *This assessment will not explore this option further due to these contamination concerns.*
- **NISKIN BOTTLES:** Niskin bottles could be useful in the upper water column, but could not collect the water volume necessary to yield a microplastic signal at depth, which could be hundreds to thousands of liters (Liu et al. 2019; Choy et al. 2019; Tekman et al. 2020). *This assessment will not explore this option further due to the inability to collect large volumes of water.*
- ***IN SITU* PUMP (E.G., MCLANE LARGE VOLUME WATER TRANSFER SYSTEM[14]):** Tekman et al. (2020) deployed *in situ* pumps attached to a CTD rosette for one hour of filtration for microplastic sampling at discrete depths ranging from 0-5,569 m. Karlsson et al. (2020) found that, if deployed in a known area of high microplastic concentration, the *in situ* pump was sufficient for collecting microplastic measurements. They collected six replicate pump samples at the surface and filtered 20 m^3 of water per sample. They concluded that a higher sample volume would be needed for more comprehensive quantitative and compositional data. It should be noted that this research was based on samples collected from one location.
- **ROV SAMPLER:** Choy et al. (2019) adapted ROV *Ventana* to accommodate existing detritus samplers to pump and filter water specifically for microplastics in Monterey Bay, California. To collect water samples, ROV pilots opened the sampling chamber at a target depth, moved forward along the depth horizon at 0.1-0.3 knots, and then closed the water-tight chamber when sampling was completed. The study took place in one geographic area, so may not be representative of the volume necessary in other places.

Regardless of the method used to collect the samples, once the water samples are on board, the risk of contamination could be reduced by using nonplastic equipment, closed sample containers to minimize open air exposure, and cotton or wool clothing; covering working spaces

13 Before any plastic equipment is used for microplastic sampling, it would be ideal to determine the composition of any plastic components (e.g., via Fourier transform infrared spectroscopy or Raman spectrometry) that the sample encounters during the workflow (i.e., the ship's flow-through system). This would allow for identification of potential contamination and biases in the resulting data. The incorporation of blank samples would also be ideal. Using bulk samples, this would entail filtering deionized water and then processing the blanks using the same methods as used for the collected water samples. Additionally, letting a few empty filters sit out in ambient air would provide information about ambient air microplastics.

14 https://mclanelabs.com/wts-lv-large-volume-pump/

during active processing; covering samples with aluminum foil; and working under a laminar flow bench (a fume hood could be the source of contamination, e.g., dust). These precautions are considered best practices to employ if possible (e.g., Pabortsava and Lampitt 2020), but are not necessary.

Steps for processing water samples on board:

1. Filter bulk water samples through a water filtration system (2x3 ft footprint, glass flask, ceramic Buchner funnel, rubber or silicon tubing, and a vacuum pump) onto a preweighed filter (1-20 µm).
2. Record the volume of water filtered, which will likely increase with sampling depth.
3. Fold the filters in half with metal forceps and wrap the filters with labeled aluminum foil.
4. Clean the forceps with ethanol after every sample to prevent contamination.
5. Place wrapped filters into nonplastic containers or bags and freeze at -20°C for preservation for onshore processing. (Filtered water samples can remain frozen indefinitely. Unfiltered water samples can be preserved in formalin or ethanol and kept indefinitely.)

Any further processing would likely need to be done on shore by external partners. Shipping methods would depend on how the samples are preserved:

- Frozen filters can be shipped with dry ice, which is possible with UPS, FedEx, and DHL.
- Samples preserved in less than 10% formalin (4% formaldehyde), which is the common standard, can be shipped with traditional methods since the preservative is unregulated for transport.
- Samples preserved in ethanol (Hazard Class 3, Packing Group II or III) require hazmat shipping, which is possible with UPS, FedEx, and DHL.

These are domestic shipping guidelines and also apply to sediment samples (see next section).

To further process the samples once they are on shore:

1. Place the filters into individual glass dishes and cover loosely with aluminum foil to avoid contamination in the oven.
2. Dry the filters at 60-90°C for 24-48 hours.
3. Cool dried filters in a desiccator and weigh them to calculate the total mass of organic and inorganic material.
4. Using a microscope, extract identified microplastic particles from the filter into a capped, preweighed glass vial.

5. Once all targeted particles are removed, weigh the vial to calculate and record the total mass of microplastics. Other characteristics could also be recorded, such as color, size, surface area (Rivers et al. 2019), and shape.
6. Conduct additional chemical analysis (as wanted), such as (micro) Fourier transform infrared spectroscopy (FTIR) or Raman spectrometry, to confirm particle identity and to determine composition. This is standard for microplastic studies and is essential for identifying the type of plastic found.

SEDIMENT SAMPLING

The incorporation of sediment sampling could also help address other data gaps, such as sediment properties (grain size, mineralogical composition, porosity, organic material, critical minerals), epifauna and infauna diversity and distribution, and environmental DNA (eDNA). Sediment microplastic data are of great interest to the scientific community because they could inform estimates of deposition and accumulation over time (e.g., Brandon et al. 2019), but additional equipment would need to be acquired if NOAA Ocean Exploration were to undertake this approach.

Options for collecting sediment samples include using an ROV-deployed push corer, a multicorer, or a box corer. Multicorer and box corer deployments are similar to each other and involve hooking the instrument onto the winch and deploying it using the A-frame or the J-frame. If using a box corer, subsamples of known surface area and volume could be collected for microplastic purposes while the rest of the sample could be used to address other data needs.

Steps for processing sediment samples on board:

1. Section the sediment core into vertical fractions (e.g., 0-0.5 cm, 0.5-1 cm, 1-2 cm, etc.) and place into covered, nonplastic containers.
2. Sieve sediment samples through multiple, nested wire mesh sizes (Martin et al. 2018). This could be done on shore, but if not sieved on board, the sample volume and weight would be much greater. A >5 mm sieve could be used to separate large particles that are not of interest for microplastic studies.
3. Preserve particles retained on the mesh, or whole sediment samples if sieving later, using formalin or ethanol or via vacuum sealing (Brandon et al. 2019). (Freezing sediment microplastics, which tend to be smaller than those found in the water column (R. Nakajima, pers. comm.), could lead to fragmentation and an overestimate of abundance.)
4. Store samples in a cool, dry, dark place.

Any further processing would be done on shore by external partners (see notes about shipping in the water column sampling section above). To further process the samples once they are on shore:

1. Sieve sediment samples (if not sieved on board) and rinse with deionized water.
2. Separate microplastics from the remaining sediment by stirring a concentrated salt mixture into the sample to float particles of interest into the supernatant. Use of a special filtration system (e.g., Nakajima et al. 2019) could be used during this step.
3. If necessary, rinse the particles in the supernatant into a glass beaker for organics digestion.
4. After digestion, rinse the supernatant onto a filter.
5. Optional: Dry all sediment samples, dry-sieve them, and dry-sort them under a microscope before chemical analysis. This would allow for the use of the same sample for multiple purposes, e.g., to collect fish otoliths or foraminifera.
6. Extract particles of interest using a microscope[15] and place them into a preweighed glass vial. Weigh the glass vial to obtain the mass of microplastics per surface area or volume of sediment and record this information. Other characteristics, such as color, size (surface area; Rivers et al. 2019), and shape, could also be recorded.
7. Conduct additional chemical analysis to determine microplastic composition (this is standard in microplastic studies).

BIOLOGICAL SAMPLING

NOAA Ocean Exploration is currently able to collect biological samples (using the manipulator arms and suction sampler on ROV *Deep Discoverer*), such as fish or invertebrates, which could be used for microplastic analysis as well as for other scientific objectives, including detailed gut content analysis (e.g., stable isotopes, microbiome, molecular analysis).

Once collected and brought on board, preserve samples by freezing them at -20°C or -80°C or storing them in ethanol. Freezing at -20°C allows for stable isotope analysis whereas preservation in ethanol allows for molecular analysis. Freezing at -80°C allows for both.

Any further processing would be done on shore by external partners. Frozen tissue samples are considered UN3373 Biological Substance Category B, which require watertight primary and secondary receptacles, absorbent material, sturdy outer packaging, and clear labeling to ship (see notes about shipping formalin and ethanol in the water column sampling section above). The onshore workflow would include the following steps:

15 Microplastics in deep-sea sediments tend to be on the smaller end of the size spectrum and likely need micro-FTIR or micro-Raman spectrometry to accurately identify particles.

1. Remove stomach and intestinal tracts.
2. Add reagents for organics digestion.
3. Separate particles of interest using density flotation.
4. Identify microplastics by eye (e.g., microscope) and chemical analysis (e.g., FTIR or Raman spectrometry).

WHAT WOULD BE THE PROTOCOL? IS THERE MORE THAN ONE OPTION? ARE THERE ANCILLARY DATA OBJECTIVES THAT ARE COMPATIBLE WITH THE PROTOCOL?

Protocols are often selected based on specific scientific objectives and there is not yet a universal, gold standard for microplastic sampling. Surface (generally within the top 5 m of the water column) and shallow water samples have been better constrained (e.g., NOAA Marine Debris Program Laboratory Methods (Masura et al. 2015), JPI Oceans Standardised Protocol (Frias et al. 2018), and the Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean (GESAMP 2019)), but sampling at-depth is less common. Below are options for water column, sediment, and biological sampling synthesized from the same references as noted in response to the previous question.

WATER COLUMN SAMPLING OPTIONS

- **NEUSTON, MANTA, OR BONGO NET TOWS:** Net tow surface and subsurface sampling is the most common water column sampling method noted in the published literature and is likely feasible since NOAA Ocean Exploration has conducted such sampling operations on *Okeanos Explorer* in the past (see the Background and Justification section). Nets should be deployed on the J-frame, to avoid the ship's wake, for 20-30 minutes. Mouth sizes range from 0.03-2.0 m2 (Hidalgo-Ruz et al. 2012). Ancillary objectives that could be met with collected samples include (micro)zooplankton and neuston diversity and distribution.
- ***IN SITU* PUMP:** An *in situ* pump could be deployed on the CTD rosette (Tekman et al. 2020) or outfitted to an ROV for bulk sampling (Choy et al. 2019). Of the options, an *in situ* pump would filter the largest volume of water. It would also reduce contamination and capture the entire microplastic size spectrum without need for onboard processing. However, it is likely the most expensive of the options and could be time-consuming (Tekman et al. (2020) cites one hour per specific depth). If microplastics emerge as a high-scientific priority for NOAA Ocean Exploration, this option could be revisited. Ancillary objectives that could be met with collected samples include (micro)zooplankton diversity and distribution.

SEDIMENT SAMPLING OPTIONS

Note: As of this assessment, NOAA Ocean Exploration cannot quantitatively sample deep-sea sediments using *Okeanos Explorer* or *Deep Discoverer*. Any increase in benthic sampling capabilities would be beneficial to the scientific community. Ancillary objectives that could be met with samples collected with the following options include sediment properties (grain size, mineralogical composition, organic matter, critical minerals), epifauna and infauna diversity and distribution, eDNA, and pore water chemistry.

- **ROV PUSH CORER:** Push corers are standardly used on ROVs (e.g., ROV *Hercules*, ROV *SuBastian*) and could be incorporated onto the platform on which *Deep Discoverer* places its samples (dependent on the Global Foundation for Ocean Exploration, which operates *Deep Discoverer*). Although generally made with plastic (as are multicorers), adjustments could be made to reduce contamination (Tsuchiya et al. 2019). These adjustments would not impact other science objectives related to sediment cores but due to the resulting loss of transparency, it would be more difficult to confirm whether an intact sample had been collected. At least one transparent core could be collected first to ensure the sediment is soft enough to be collected at the inserted depth.
- **MULTICORER OR BOX CORER:** These coring instruments require a winch for deployment. A camera could be mounted on them to ensure collection of viable samples. A sample from a box corer would need to be subsampled for microplastics but could then be used for other exploration objectives.

BIOLOGICAL SAMPLING OPTION

NOAA Ocean Exploration currently collects biological samples using *Deep Discoverer*'s manipulator arms, suction sampler, and scoop. Ancillary objectives that could be met with collected samples include gut content analysis (e.g., stable isotopes, microbiome, molecular analyses) and morphological and demographic information (size, sex, age).

MATERIALS OPTIONS

- **NETS FOR WATER COLUMN SAMPLING:** These typically come in 100-500 μm sizes (Mai et al. 2018). A nested approach could be used with one 500 μm and one 300-335 μm net with the latter being the most commonly used net size (e.g., Barrows et al. 2017; Rivers et al. 2019; Compa et al. 2020; Egger et al. 2020). Smaller mesh sizes could require reinforcement or regular maintenance to ensure there are no holes.
- **SIEVES FOR SEDIMENT PROCESSING:** Sieves could also be nested but vary more widely in size in the literature than nets. In benthic infauna sampling, macrofauna are retained on a 300 μm sieve (de Smet et al. 2017) whereas meiofauna are retained on a 20-63 μm sieve (Rosli et al. 2018). The larger mesh size could be replaced by a 5.6 mm sieve to

discard larger particles. (Note: Several steps within the protocol require discussion of mesh sizes. Smaller mesh sizes would be more inclusive but samples would take longer to process because of the filtering process, the greater number of particles of interest, and the greater potential for clogging.)

- **FILTERS FOR MICROPLASTIC PARTICLE COLLECTION:** Several types of filters are noted in the literature, including stainless steel (e.g., Bergmann et al. 2017), glass fiber (e.g., Kanhai et al. 2019), and cellulose nitrate filters (e.g., Barrows et al. 2017; Dai et al. 2018). Filter sizes range from 1 to 20 μm, but a 20 μm filter is likely sufficient for general measurements.
- **ENCOUNTERED NET OR SIEVE SIZES:** 5.6 mm, 1 mm, 500 μm (Courtene-Jones et al. 2017a), 300-335 μm (Compa et al. 2020), 200 μm (Lushner et al. 2015), 100 μm (Choy et al. 2019), 63 μm, 45 μm (Barrows et al. 2017), 35 μm (van Cauwenberghe et al. 2013)
- **ENCOUNTERED FILTER SIZES:** 20 μm (Bergmann et al. 2017), 10 μm, 5 μm (Dai et al. 2018), 1-2 μm (Kanhai et al. 2019)

PRESERVATION OPTIONS

- **FORMALIN OR ETHANOL:** Water, sediment, and biological samples could be preserved in formalin or ethanol. Formalin is cheaper, but samples preserved in ethanol could potentially be used for molecular analysis. However, ethanol is highly flammable and can be difficult to ship (requires hazmat shipping). Both of these methods can preserve microplastic samples indefinitely, but care should be taken to ensure avoid microplastic degradation (e.g., 95% ethanol has been shown to break down plastics (J. Lynch, unpublished).)
- **FREEZING:** Water (filtered or bulk) and biological samples could be frozen at -20°C indefinitely. Sediment samples should not be frozen due to risk of fragmentation.
- **VACUUM SEALING:** Deep-sea sediment cores, if fully intact, could be vacuum sealed and stored at 4°C.

NOTE: NOAA Ocean Exploration would need to work with external partners for the following activities.

ORGANICS DIGESTION OPTIONS

- **OXIDATION WITH H_2O_2:** A solution of 30% H_2O_2 is often used. While a solution of 35% H_2O_2 may remove organic material better, it could cause degradation of microplastics.
- **FENTON'S REAGENT WITH $FeSO_4$ AND H_2O_2 (I.E., WET PEROXIDE OXIDATION):** This reagent has been validated and is commonly used (Masura et al. 2015; Hurley et al. 2018).
- **ENZYME SUCH AS PROTEINASE (COLE ET AL. 2014) OR TRYPSIN (COURTENE-JONES ET AL. 2017B):** This approach could be time-consuming.

- **ACID OR ALKALINE:** Both acid (Rocha-Santos and Duarte 2015) and alkaline (Dehaut et al. 2016) digestions have been shown to degrade microplastics, especially those on the smaller end of the size spectrum.

OPTIONS FOR DENSITY FLOTATION

Note: Microplastic densities typically range from 1.10 to 1.40 g/cm^3.

- **NaCl (1.20 g/cm^3):** Sodium chloride is cheap, but is low density and therefore may not recover more dense microplastics.
- **ZnCl$_2$ (1.50-1.70 g/cm^3):** Zinc chloride has the potential to recover the majority of microplastics, but it is toxic.
- **NaI (1.80 g/cm^3):** Sodium iodide is high density, but it is expensive. However, it is recyclable, which could reduce costs (Kedzierski et al. 2017).

OPTIONS FOR COMPOSITION ANALYSIS

- **FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR):** This method has been recommended by two subject matter experts (J. Brandon, R. Nakajima, pers. comm.). Additionally, many science and academic institutions have the machine and dedicated technicians who know how to use it (e.g., NOAA Global Monitoring Laboratory,[16] institutions affiliated with the Ocean Exploration Cooperative Institute). For deep-sea sediment samples, micro-FTIR, which is more expensive, would likely be necessary.
- **RAMAN SPECTROMETRY:** This capability likely exists within a NOAA laboratory and can be cheaper than FTIR. Micro-Raman spectrometry can examine smaller particles that can be important to measure, although at a more expensive cost than both FTIR and regular Raman spectrometry. However, results are sensitive to additives and pigments within plastics.
- **PYROLYSIS-GAS CHROMATOGRAPHY, MASS SPECTROMETRY, AND THERMAL DECOMPOSITION-GC/MS:** These methods could be used on bulk water and sediment samples. However, they are destructive, so samples could not be used for subsequent analysis. Other emerging options include time-of-flight secondary ion mass spectrometry and atomic force microscopy.

WHAT WOULD AN IDEAL PROTOCOL LOOK LIKE? HOW WOULD IT COMPARE TO A MORE FEASIBLE OPTION?

An ideal protocol would include specialized sampling equipment to avoid contamination (e.g., ROV water sampler, aluminum corers (Tsuchiya et al. 2019)), filtration through the smallest mesh

16 https://www.esrl.noaa.gov/gmd/obop/mlo/programs/ndacc/ftir/ftir.html

sizes to capture the full size spectrum of microplastics, and some form of micro composition analysis. However, a more feasible protocol for NOAA Ocean Exploration on *Okeanos Explorer* would focus on sampling surface waters with a net tow and preserving samples for composition analysis by an external partner (e.g., a NOAA laboratory or academic institution). The interest in and lack of deep-sea sediment microplastic samples highlights the need for NOAA Ocean Exploration to develop these capabilities for *Okeanos Explorer* or other platforms, such as autonomous sensors (e.g., MantaRay (Edson and Patterson 2015)) or systems (e.g., Draper[17]).

IF PHYSICAL SAMPLES ARE TO BE COLLECTED, WHERE WOULD THEY BE STORED ON BOARD?

Physical samples are required for collecting microplastic data, and their storage on board is dependent on the preservation method: frozen in the -20°C freezer (the -80°C freezer has been removed from *Okeanos Explorer*); or formalin- or ethanol-preserved and stored in a dry, dark, cool area. The former could be applied to water samples, filtered or bulk, and biological samples. Frozen samples could be stored indefinitely until ready to process. Sediment samples cannot be frozen due to concerns about particle size and fragmentation. All samples could be preserved and stored in formalin or ethanol indefinitely.

MATERIALS AND COSTS

WHAT MATERIALS OR EQUIPMENT WOULD BE NEEDED TO COLLECT THE DATA?

The materials and equipment needed would depend on the method chosen. A detailed list of materials can be found in the Laboratory Methods for the Analysis of Microplastics in the Marine Environment: Recommendations for Quantifying Synthetic Particles in Waters and Sediments (Masura et al. 2015). There are also materials listed in the Methods, Protocol Options, and Sampling Strategy section and costs table.

General lab supplies necessary on board include gloves, jars with lids, funnels, Milli-Q or deionized water, Kimwipes, aluminum foil, forceps, ethanol, and filters. (If possible, all materials should be nonplastic.)

WHAT TYPE OF STORAGE AND SPACE FOR SAMPLES AND SUPPLIES WOULD BE NEEDED ON BOARD THE SHIP?

Ideally, the filtration setup and processing materials would be kept covered during active use to avoid contamination by ambient microplastic particles. A laminar flow bench would be

17 https://www.draper.com/explore-solutions/microplastics-sensor

ideal for both sample processing and supplies storage. Glass and ceramic supplies are often used for microplastic sampling, so care should be taken to ensure their security (e.g., nonslip mats, cushions).

HOW MUCH WOULD IT COST TO PURCHASE SENSORS OR SUPPLIES NEEDED TO COLLECT THE DATA? HOW MUCH WOULD IT COST TO COLLECT THE DATA? HOW MUCH WOULD IT COST TO STORE THE DATA?

The total direct cost to add net tow capabilities onto *Okeanos Explorer* is approximately $5,200-5,300, depending on the net. The cost of sediment capabilities range varies, with a multicorer on the low end and an ROV-deployed push corer on the high end. An additional $8,200 would be necessary for a laminar flow bench, which would be ideal for reducing contamination. These costs do not include labor and partnership costs associated with a new operation or maintenance. Consumable costs per expedition would be approximately $450. These are only the costs associated with collecting the samples and do not include sample processing.

TABLE F1: Materials needed to conduct microplastic sampling and associated costs.

Item	Once or for Every Expedition	Quantity	Cost (USD)	Supplier	Purpose
Manta net	Once	1	$2,277.00	Aquatic BioTech, ABT-ERM-6025	Water sampling at the surface*
Bongo net	Once	1	$2,373.00	Aquatic BioTech, ABT-ERB-60	Water sampling at depth*
Flow meter	Once	1	$586.00	Aquatic Biotech, ABT-FM-01	Net tow sampling
In situ water pump	Once	1	Contact company for price	McLane, WTS-LV[1]	Water sampling at depth*
Box corer	Once	1	$49,300.00	Ocean Instruments, BX-750	Sediment sampling*

Item	Once or for Every Expedition	Quantity	Cost (USD)	Supplier	Purpose
Multicorer	Once	1	$42,785.00	Ocean Instruments, MC-800	Sediment sampling*
ROV push corer with quivers	Once	1 set	Variable	Variable	Sediment sampling*
Core extruder	Once	1	Requires further scoping	Requires further scoping	Sediment processing
Slicing rings	Once	10 ft with 2.5 in ID/2.85" OD	$100.00	PVC Pipe Supplies, PL-025-10	Sediment processing
Slicing knife	Once	12x12 ft aluminum sheet	$10.98	Home Depot, #100248617	Sediment processing
Sieve	Once	5.6 mm, 8 in diameter	$149.10	Fisher, 04-881-10A	Sediment processing
Sieve	Once	300 µm, 8 in diameter	$149.10	Fisher, 04-881-10T	Sediment processing
Sieve	Once	45 µm, 8 in diameter	$282.00	Fisher, 04-881-10EE	Sediment processing
Density flotation device[2]	Once	1	Offer of gift	JAMSTEC	Sediment processing
NaCl	Every mission	2.5 kg	$240.95	Fisher, 18-606-419	Density flotation*
ZnCl2	Every mission	3 kg	$978.00	Fisher, Z33-3	Density flotation*
NaI	Every mission	2.5 kg	$1,391.25	Fisher, 18-606-598	Density flotation*

Item	Once or for Every Expedition	Quantity	Cost (USD)	Supplier	Purpose
Filters	Every mission	100 pack, 1.5 µm mesh, 110 mm diameter	$161.00	Fisher, 09-873H	Sample processing
20L carboy	Once	1	$368.50	Fisher, 23200050	Filtering
Vacuum pump	Once	1	$507.85	Fisher, 13-310-900	Filtering
Buchner funnel	Once	1 550 ml ceramic	$105.00	Fisher, FB966G	Filtering
Glass flask	Once	1 1,000 ml flask	$93.41	Fisher, K953760-0000	Filtering
Silicone tubing	Once	15.24 m	$241.50	Fisher, 14-176-332D	Filtering
Ethanol 70%	Every mission	1 gallon	$82.00	Fisher, P82031GAL	Preservation*
Formaldehyde 37%	Every mission	1 L	$84.40	Fisher, RSOF00101A	Preservation*
Aluminum foil	Every mission	25 ft	$8.25	Fisher, 01-213-100	Prevent contamination
Latex gloves	Every mission	50 pairs	$25.75	Fisher, 19-169-063	Prevent contamination
Metal forceps	Once	1 pair	$25.50	Fisher, 12-000-122	Sample processing
Kimwipes	Every mission	280 wipes	$10.75	Fisher, 06-666A	Sample processing
Glass jars with foil caps	Every mission	24 jars	$127.19	Fisher, 13-756-755	Sample containment

Item	Once or for Every Expedition	Quantity	Cost (USD)	Supplier	Purpose
Electrical tape	Every mission	20.11 m	$15.49	Fisher, 19-047-279	Sample containment
Water filtration system	Once	1	$1,092.00	Fisher, ZFDI00001	Sample processing
Laminar flow bench	Once	1	$8,200.00	Fisher, AC4000HLF	Sample processing

NOTE: This list only includes materials necessary for sampling and on board processing, and does not include supplies necessary for visual or chemical analyses, which would need to be done by an external partner.

***** One of several options for this purpose.

1 https://mclanelabs.com/wp-content/uploads/2018/08/McLane-WTS-LV-Standard-Datasheet.pdf

2 Nakajima et al. 2019

HOW MUCH WOULD IT COST TO MAINTAIN THE CAPABILITY OVER A FIVE-YEAR PERIOD?

Maintenance of *Okeanos Explorer* (i.e., the winch) and *Deep Discoverer* are already part of standard operations. Thus, additional maintenance costs would be minimal relative to the mechanical operations.

Nets and push corers get worn with use, and sometimes lost, so they might need to be repaired and/or replaced occasionally. There would also be maintenance costs associated with maintaining push corers on the ROV. After the one-time expenses have been made (i.e., sampling equipment, sieves, and water filtration system), the most costly item would be the salt for density flotation, depending on which salt is chosen, and this would only be necessary for sediment samples if at all. Partnerships with NOAA labs and academic institutions to visually and chemically analyze collected samples would likely be the most cost-effective way for NOAA Ocean Exploration to produce useful microplastic data during expeditions on *Okeanos Explorer*. If we decide to move forward with microplastic sampling, then the specifics of such partnerships (e.g., which institutions and agencies, through what mechanisms, costs) would need to be scoped.

TIME

HOW MUCH TIME WOULD IT TAKE TO COLLECT AND/OR PROCESS THE DATA?

WATER COLUMN SAMPLING

The time it would take for net tow operations on *Okeanos Explorer* would depend on the duration of the tow, which could vary widely. Setting up and breaking down the net only needs to be done once (usually at the beginning and then the end of an expedition) and takes around 30 minutes each. It takes 10 minutes to deploy a net. To minimize impact on other operations, 20 to 30 minutes is recommended for the length of each tow. It would then take about 30 minutes to preserve the samples collected in the cod-end for post-expedition processing.

CTD casts typically take 1.5-2 hours, but could take longer depending on the depth. Collecting water with an *in situ* pump attached to the CTD rosette would add approximately one hour per depth to that time. Similar to a CTD cast, an *in situ* pump outfitted to the ROV would require long periods of time to pump water at a constant depth horizon. The amount of time it would take to filter the water would depend on the volume of water being filtered and the pore size of the filter (or filters, if nested). Relative to surface samples, water collected at depth will likely have less microplastics in them and, therefore, a larger volume would need to be filtered to yield a signal. Smaller filter pore sizes would capture a larger range of microplastic sizes but would take longer to filter water, so a slightly larger pore size may be more suitable for current operations.

SEDIMENT SAMPLING

Collecting sediment samples with a box corer or multicorer could take two-three hours depending on the bottom sampling depth. The time it would take with an ROV-deployed push corer would depend on the ability to find a sampling site and the sediment being sampled and could range from 10 to 40 minutes. Sediment sampling with a multicorer or box corer would decrease the time available for seafloor and water column mapping while sampling with a push corer would cut into time for collecting imagery. Processing sediment samples collected with a multicorer or push corer entails extruding the sediment, slicing it into specific horizontal layers, sieving (optional), and preserving it. This would take about 15 to 30 minutes per sample. A multicorer generally produces 8 samples whereas a push corer produces 8 to 20 samples, depending on basket availability. For microplastics, a box corer sample could be subsampled with a push corer and processed in a similar manner.

BIOLOGICAL SAMPLING

NOAA Ocean Exploration currently collects biological samples during ROV dives on *Okeanos Explorer*, so sample collection for microplastic analysis would not significantly alter time available for other operations.

PERSONNEL

WHO WOULD BE IN CHARGE OF COLLECTING THE DATA? WHO WOULD BE IN CHARGE OF SAMPLE DATA PROCESSING, MANAGEMENT, AND ARCHIVING?

WATER COLUMN SAMPLING: NET TOW

- **SETTING UP THE NET TOW:** This would require time, *Okeanos Explorer* crew, and use of the J-frame. One dedicated person should oversee net tow operations (or existing science personnel could be tasked with the additional responsibility).
- **CONDUCTING THE TRAWL:** This would require time (ship speed of approximately 1-2 knots) and assistance from *Okeanos Explorer* crew.
- **COLLECTING AND STORING SAMPLES ON *OKEANOS EXPLORER*:** One dedicated scientist should be responsible for collecting the samples from the cod-end of the net, storing them in jars for post-expedition analysis, and recording metadata.
- **PROCESSING, MANAGING, AND ARCHIVING DATA:** This could potentially be the sample data manager, who commonly sails on ROV cruises.

WATER COLUMN SAMPLING: *IN SITU* PUMP

- **COLLECTING THE *IN SITU* PUMP SAMPLE:** The ROV pilots (ROV sampler) or senior survey technician (CTD rosette) would be in charge of remotely turning the pump on and off and ensuring that the instruments stay at the correct depth while sampling.
- **STORING THE FILTERS IN THE WETLAB ON *OKEANOS EXPLORER*:** One dedicated scientist should be responsible for extracting the filters from the *in situ* pump, storing the filters, and recording pertinent metadata. If workloads permit, these duties could be relegated to existing personnel.
- **PROCESSING, MANAGING, AND ARCHIVING DATA:** This could potentially be the sample data manager, who commonly sails on ROV cruises.

SEDIMENT SAMPLING

- **COLLECTING THE SEDIMENT:**
  - **ROV-DEPLOYED PUSH CORER:** The ROV pilots would be in charge of sediment collection and the engineers would be responsible for ensuring the push corer works properly.
  - **MULTICORER OR BOX CORER:** If sediment is collected during a mapping expedition, significant help would be needed to deploy these instruments, specifically the J-frame, from the crew of *Okeanos Explorer*.
- **PROCESSING OR STORING THE SEDIMENT CORES IN THE WETLAB ON *OKEANOS EXPLORER*:** One dedicated scientist should be responsible for processing or storing the sediment samples and recording pertinent metadata.
- **PROCESSING, MANAGING, AND ARCHIVING DATA:** This could potentially be the sample data manager, who commonly sails on ROV cruises.

BIOLOGICAL SAMPLING

- **COLLECTING THE BIOLOGICAL SAMPLE:** The ROV pilots would be in charge of biological sample collection as they are currently.
- **PROCESSING OR STORING THE BIOLOGICAL SAMPLES:** One dedicated scientist should be responsible for processing or storing the biological samples and recording pertinent metadata. Microplastics would likely be one of several purposes for collecting biological samples.
- **PROCESSING, MANAGING, AND ARCHIVING DATA:** This could potentially be the sample data manager, who commonly sails on ROV cruises.

RECORDING METADATA ON *OKEANOS EXPLORER*

On ROV expeditions, the sample data manager is responsible for recording metadata in conjunction with the science leads, the Global Foundation for Ocean Exploration data management team, and the expedition coordinator. The sample data manager would likely pick up the extra on board data archiving duties associated with the water samples, net tow samples, and sediment core samples unless there is berth space for a dedicated person who could take on these responsibilities. Without additional personnel, this work would need to be balanced with other assignments performed by the sample data manager, such as recording metadata for the biological and geological samples that are routinely collected during ROV expeditions. On mapping expeditions, a dedicated person would need to be on board to process samples and record metadata.

PERMITTING AND REGULATIONS

NOAA Ocean Exploration has a dedicated person in charge of all permitting, regulations, and environmental compliance review.

COULD THIS WORK BE DONE WITH CURRENT PERSONNEL OR WOULD NOAA OCEAN EXPLORATION NEED TO HIRE NEW PERSONNEL?

One dedicated person from NOAA Ocean Exploration would need to be in charge of coordinating all activities associated with microplastic sampling trial runs (most likely someone from the Science and Technology Division). This person would work closely with the Expeditions and Exploration Division to conduct the trial runs, securing necessary equipment, developing the sampling standard operating procedures, ensuring the samples are stored properly and shipped to partner institutions for archive or analysis, and ensuring that the data from the analysis are made available to NOAA Ocean Exploration and appropriately archived. NOAA Ocean Exploration should learn a lot from the forthcoming eDNA sampling trial runs that could be applied to microplastic sampling trial runs.

Some degree of processing could be done on board *Okeanos Explorer* (see the Methods, Protocol Options, and Sampling Strategy section). Water samples could be filtered, and the filters could be preserved for analysis post-expedition. Water samples themselves could also be preserved for later filtration and analysis. Similarly, sediment samples could be prepared and preserved for post-expedition for analysis.

Until they could be processed and analyzed, filters and water samples could potentially be archived with the Smithsonian Institution's National Museum of Natural History and sediment samples could potentially be archived with Oregon State University, leveraging existing partnerships. For processing and analysis, NOAA Ocean Exploration could work with the Ocean Exploration Cooperative Institute (OECI) or form a partnership with a NOAA laboratory (e.g., the NOAA Global Monitoring Laboratory[18]) or other institution that is equipped to analyze filters and sediment samples for microplastics. The University of Rhode Island has both micro-FTIR[19] and Raman spectrometry[20] capabilities (although they are outside the Graduate School of Oceanography), and Scott Gallagher's lab at the Woods Hole Oceanographic Institution also has Raman spectrometry capabilities. Both of these institutions are OECI partners. Other science and

18 https://www.esrl.noaa.gov/gmd/obop/mlo/programs/ndacc/ftir/ftir.html

19 https://web.uri.edu/nano/fourier-transform-infrared-microscope/

20 https://web.uri.edu/nano/raman-microscope/

academic institutions likely have spectrometry machines and dedicated personnel, representing partnership opportunities for NOAA Ocean Exploration.

After analysis, a spreadsheet of processed data (including size distribution of microplastics, composition of the plastics per sample, and abundance) could be made available for archiving. Staff from NOAA's National Centers for Environmental Information (NCEI) could archive the data from the filters and water and sediment samples (see the Data Accessibility, Storage, and Archiving section).

IF NO NEW PERSONNEL WOULD BE NEEDED, HOW WOULD THIS WORK IMPACT OTHER TASKS REQUIRED OF CURRENT PERSONNEL?

Water column and biological sampling would not need new personnel but would likely require one dedicated person to oversee sample collection and conduct sample preservation. Sediment sampling would require one-two new personnel.

DURING WHAT TYPE OF EXPEDITION COULD THESE DATA BE COLLECTED (I.E., MAPPING, ROV)?

- Water column sampling and sediment sampling (via multicorer or box corer deployed from the J-frame) could be collected on an ROV or mapping expedition.
- Sediment sampling via ROV-deployed push corer and biological sampling could only be collected on an ROV expedition.

NOAA OCEAN EXPLORATION'S CURRENT OPERATIONS ON *OKEANOS EXPLORER*: COMPLEMENTARY AND CONTRASTING DATA COLLECTION

IS COLLECTING THESE DATA A PRIORITY? IF SO, HOW DOES IT COMPARE TO CURRENT COLLECTIONS?

Anthropogenic impacts have been identified as an important measurement by the deep-ocean exploration and observation community. Marine microplastics comprise only one component of anthropogenic impact, but they are a growing area of environmental concern. Samples collected for microplastic analysis can be used for multiple purposes. However, it is likely that until those additional uses are defined and protocols are developed, sampling for microplastics alone would not be prioritized over current collections.

HOW WOULD COLLECTING THE DATA TAKE AWAY FROM CURRENT OPERATIONS? HOW WOULD COLLECTING THE DATA COMPLEMENT CURRENT OPERATIONS AND POTENTIALLY NEW OPERATIONS?

WATER COLUMN SAMPLING

Net tows would require the ship to operate at a slower speed than usual for mapping operations (1-2 knots). The duration of the tow and tow-related activities would be determined by the science objectives for that particular cruise, but in general would be approximately one hour.

Currently, NOAA Ocean Exploration collects water samples with the ROV opportunistically. Although CTD rosette operations are part of our standard *Okeanos Explorer* operations, they are done opportunistically, as well, and depend on the objectives of the expedition. Conducting a CTD rosette cast with a mounted *in situ* pump to collect water samples could take a few hours.

Incorporating systematic collection of water samples (by net tow or *in situ* pump) into NOAA Ocean Exploration's standard *Okeanos Explorer* operations could also address the following data gaps identified as important by the deep-sea community (see OET (2012, 2014), UNOLS (2016), OER (2011), Sayre et al. (2017), Netburn (2018), Woodall et al. (2018), Levin et al. (2019)): bulk biodiversity, microbial community characteristics, plankton community characteristics, particulate organic matter, and suspended particulate concentration.

eDNA and microplastic sampling could complement each other if an *in situ* pump is used to collect water samples. The McLane Standard Large Volume Water Transfer System[21] could also be used for eDNA analysis and to measure suspended particles, particulate metals, and plankton (Morrison et al. 2000). This system has been used by researchers to measure microbial community structure in the water column (Acherger et al. 2016), trace elements (Twining et al. 2015), and archaea and bacteria in the water column (Pitcher et al. 2011). There is no research at this time indicating whether the same filter used with the pump could be used for both microplastics and eDNA analysis. This would need to be explored further.

SEDIMENT SAMPLING

Use of an ROV-deployed push corer would not take away from main ROV operations (i.e., imaging the seafloor). However, extra sampling time during an ROV dive would be required to collect the sediment. NOAA Ocean Exploration could consider reconfiguring *Deep Discoverer* to swap out one of the rock boxes for storing sediment samples. Use of a multicorer or box corer

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21 https://mclanelabs.com/wp-content/uploads/2018/08/McLane-WTS-LV-Standard-Datasheet.pdf could significantly take away from other operations (ROV and mapping) as they involve long deployment times.

By incorporating systematic collection of sediment samples into standard *Okeanos Explorer* operations, NOAA Ocean Exploration could also address the following data gaps identified as important by the deep-sea community (see OET (2012, 2014), UNOLS (2016), OER (2011), Sayre et al. (2017), Netburn (2018), Woodall et al. (2018), Levin et al. (2019)): inorganic macronutrients, nitrate/nitrite, silicate, phosphate, microbial biomass and density, particulate organic matter, dissolved organic carbon, dissolved inorganic carbon, bulk biodiversity, stable isotope analysis, bioturbation, metals, and other pollutants. Further assessments would be needed to determine the feasibility of processing sediment samples to address these additional data gaps.

BIOLOGICAL SAMPLING

Okeanos Explorer currently collects biological samples during ROV dives and doing so for microplastic analysis would not impact current ship operations.

EXPECTED PRODUCTS

WHAT WOULD BE THE EXPECTED SCIENTIFIC PRODUCTS?

Through these operations, NOAA Ocean Exploration could provide samples for microplastic analysis to evaluate human impact in the deep sea. Analysis of these samples from external partners and researchers could reveal information about abundances of microplastics per unit of volume or area, size and weight distribution of microplastics, microplastic color and composition, microplastic accumulation rates, identification of microplastic sources and sinks, microplastic distribution (if systematically collecting samples over an area), and other areas of interest for the scientific community.

WOULD THE DATA BE REPORTED IN EXPEDITION REPORTS?

See the Data Management, Processing, Summaries, and Quality Control section (*What would be the minimum metadata needed?*).

WHO WOULD BE RESPONSIBLE FOR PRODUCING THE EXPECTED PRODUCTS?

The sample data manager would be responsible for metadata in the expedition reports, but data results from the samples would be the responsibility of external partners.

DATA MANAGEMENT, PROCESSING, SUMMARIES, AND QUALITY CONTROL

In their June 2020 paper, Cowger et al. provide reporting guidelines to follow when conducting microplastic research, from sampling in the environment to laboratory analyses. The paper includes a checklist that details information that needs to be reported for best practices, quality assurance/quality control, data, field sampling, sample preparation, microplastic identification, microplastic categorization, and microplastic quantification, and considerations for toxicology studies. The data section in this feasibility assessment closely aligns with this paper's microplastic checklist. If NOAA Ocean Exploration decides to move forward with microplastic sampling, following this checklist as closely as possible is recommended.

WHAT WOULD BE THE MINIMUM METADATA NEEDED?

WATER COLUMN SAMPLING: NET TOW

- Tow number or ID
- Name of the individual who processed the sample or is in charge of the operation
- Type of tow
- Duration of tow
- Speed of ship during the tow
- Start and end latitude/longitude of tow
- Date and time the sample was collected
- Volume of water filtered
- Number of flowmeter rotations
- Depth of the tow (can determine based on angle of the wire and how much wire was used or by using a depth gauge)
- Sea surface and atmospheric conditions
- Additional purposes for the sample (plankton, collection of other organisms, etc.)
- Fixative used to preserve the sample
- Field and equipment blanks, including all information necessary for a field sample (above) and a brief description about how the blank was collected

WATER COLUMN SAMPLING: *IN SITU* PUMP

- Sample number or ID
- Name of the individual who processed the sample

- Cartridge number
- Date and time the sample was collected
- Volume of water filtered (*in situ* samplers have devices on them to measure this)
- Filter material and pore size (if multiple types are used)
- Latitude/longitude of sample
- Depth of sample site
- Brief description of site where water sample was taken (site characteristics such as temperature, salinity, etc.) if collected with the ROV
- Video/imagery accompanying the sample if collected with the ROV
- Information about how the water or filter is stored (stored in solution, frozen, etc.)
- Associated CTD data
- Field and equipment blanks, including all information necessary for a field sample (above) and a brief description about how the blank was collected
- Storage and archiving information

SEDIMENT SAMPLING

- Sample number or ID
- Name of the individual who processed the sample
- ROV dive if collected with an ROV-deployed push corer
- Push core number if collected with an ROV-deployed push corer
- Multicore number if collected with a multicorer
- Date and time the sample was collected
- Depth of the sediment core (approximation of how much sediment)
- Height of vertical fractions
- Number of vertical fractions
- Latitude/longitude of sample
- Depth of sample site
- Brief description of site where sediment sample was taken (site characteristics such as temperature, salinity, etc.)
- Video/imagery accompanying the sample if collected with the ROV
- Information about how the sediment sample is preserved (stored in solution, frozen, etc.)
- Associated CTD data
- Additional purposes for the sample (meiofauna, eDNA, etc.)

- Field and equipment blanks, including all information necessary for a field sample (above) and a brief description about how the blank was collected
- Storage and archiving information

BIOLOGICAL SAMPLING

- Sample number or ID
- Name of the individual who processed the sample
- ROV dive
- Biobox number
- Date and time the sample was collected
- Latitude/longitude of sample
- Depth of sample site
- Brief description of site where biological sample was taken (site characteristics such as temperature, salinity, etc.)
- Brief description of the biological sample (taxa, color, size, associates, etc.)
- Video/imagery accompanying the sample
- Information about how the biological sample is preserved (stored in solution, frozen, etc.)
- Associated CTD data
- Additional purposes for the sample (morphological analysis, genetic analysis, etc.)
- Storage and archiving information

WOULD DATA ANALYSIS OR PROCESSING BE NEEDED?

Data processing is necessary for microplastic sampling to determine microplastic distribution, abundance, size, and composition. However, filters and water and sediment samples could be stored long term until analyses could be conducted. NOAA Ocean Exploration would need to scope out long-term storage options with the Smithsonian Institution's National Museum of Natural History and Oregon State University.

HOW WOULD THE DATA BE ANALYZED OR PROCESSED?

See the Methods, Protocol Options, and Sampling Strategy section.

WOULD A DATA SUMMARY BE NEEDED? HOW WOULD A DATA SUMMARY BE FORMATTED AND WHAT WOULD IT INCLUDE?

A data summary of the identifications from each sample in the form of tabular data (.csv file) would be preferred and could be archived with related data NCEI. The summary could also include the environmental variables associated with each sample (location, salinity, and such). Some information resulting from the analysis could also be included as part of the working group effort to create a data synthesis product that summarizes information on the data collected after each expedition. Here are some examples from the literature that show how the data could be visualized:

- **CHOY ET AL. 2019 FIGURE 1:** Microplastic concentration with depth
- **CHOY ET AL. 2019 FIGURE 2:** Chemical composition of plastic as a proportion of total sample
- **GOLDSTEIN ET AL. 2013 FIGURE 1:** Microplastic abundance per sample superimposed on sea surface temperature per season
- **GOLDSTEIN ET AL. 2013 FIGURE 3:** Microplastic abundance per net tow sample
- **KANE AND CLARE 2019 FIGURE 4:** Microplastic density per deep-sea environment type
- **LAW 2017 FIGURE 3:** Map showing the distribution of particle count and particle mass from net tow samples
- **LUSHER ET AL. 2016 FIGURE 4:** Length of microplastics and microfibers based on abundance
- **WOODALL ET AL. 2014 FIGURE 2:** Quantity and type of microfibers found in sediment samples

WHAT WOULD BE THE QA/QC PROCESS?

Potential avenues for quality assurance/quality control (QA/QC) include processing replicates of the same water and sediment samples. For water samples, it is important to assess whether leaving filters open to the air would affect the sample as microplastics and microfibers occur in the air and could contaminate samples. A blank water sample could also be processed through the filtration system. It is also crucial to know the chemical composition of any plastic instruments, equipment, or items that are used to process both the net tow, water, and sediment samples.

WOULD COASTAL AND MARINE ECOLOGICAL CLASSIFICATION STANDARD (CMECS) IMPLEMENTATION BE NECESSARY? IF SO, HOW WOULD IT BE IMPLEMENTED?

CMECS classifications do not apply to microplastic abundance, size, and composition. However, CMECS classifications could be used if using net tow, water, and sediment samples to further define and characterize a location.

DATA ACCESSIBILITY, STORAGE, ARCHIVING

WOULD (OR COULD) THE DATA BE STORED AT THE NOAA NATIONAL CENTERS FOR ENVIRONMENTAL INFORMATION (NCEI)? IF NOT, WHAT PUBLIC REPOSITORIES COULD BE USED FOR DATA ARCHIVING?

The data could be stored at NCEI. Ebenezer S. Nyadjro of the Northern Gulf Institute and NCEI, along with his colleagues, is creating a global database specifically for information on microplastics to be made freely accessible and maintained by NCEI indefinitely. The database is expected to be a one-stop repository where microplastic data and other data related to microplastics (e.g., ocean currents and winds) are aggregated, archived, and served in a consistent and reliable manner.

WHAT WOULD BE THE DATA ARCHIVING PIPELINE?

Like other data collected by NOAA Ocean Exploration on *Okeanos Explorer*, after analysis of the samples, the tabular data set containing information about the microplastic analysis (including abundance, size, and chemical composition) could be archived at NCEI. If archiving in Nyadjro's microplastic database, the information could be emailed directly to him. The information could also be sent through NCEI's data receiving portal.[22] One dedicated person (most likely someone from NOAA Ocean Exploration's Science and Technology Division) would need to be responsible for shepherding the data through this process. This person would also need to be the point of contact if microplastic samples are to be processed further and stored by other institutions. Once received by NCEI, the data would be put through QA/QC procedures to ensure reliability, among other things. The data would then be added to the global microplastic database and a GIS map that would show the occurrence, distribution, and abundance of microplastics in the sampling area.

IF STORED SOMEWHERE OTHER THAN NCEI, HOW WOULD THE INFORMATION BE SHARED WITH NCEI AND NOAA OCEAN EXPLORATION?

The data can be stored easily at NCEI.

HOW WOULD THE DATA STORED OUTSIDE OF NCEI BE CONNECTED TO THE REST OF THE COLLECTION?

Once established, links could be included on NOAA Ocean Exploration's website and included in the subsequent expedition reports.

22 https://www.nodc.noaa.gov/s2n/

PERMITTING AND REGULATIONS

WOULD PERMITS OR LICENSES BE NEEDED TO COLLECT THE DATA?

Collecting water samples in U.S. federal waters generally does not require a permit. However, in some jurisdictions (e.g., some U.S. marine protected areas, sanctuaries, some foreign waters, and state government waters) collecting physical samples, including water and sediment samples, does require a collection permit. Since the collection of other samples (i.e., biological and geological) are already part of NOAA Ocean Exploration's *Okeanos Explorer* operations, obtaining permits for water and sediment samples should require limited additional work, but would need to be accounted for. Additionally, environmental compliance documents would need to be updated and maintained, but this should also require minimal work.

ENVIRONMENTAL OR TECHNOLOGICAL RISK

GIVEN ALL OF THE ABOVE CONSIDERATIONS, COULD THERE BE ENVIRONMENTAL OR TECHNOLOGICAL RISKS INVOLVED IN COLLECTING THE DATA?

Sediment sampling is invasive and could minimally disturb critical habitat. NOAA Ocean Exploration could reduce this risk by using an ROV-deployed push corer, rather than a multicorer or box corer, which would allow for more precise sampling. There are no major risks associated with water column sampling.

FEASIBILITY ASSESSMENT OF COLLECTING THE DATA

GIVEN ALL OF THE ABOVE CONSIDERATIONS, IS IT FEASIBLE TO COLLECT THESE DATA AS PART OF NOAA OCEAN EXPLORATION'S OPERATIONS ON NOAA SHIP *OKEANOS EXPLORER*?

It is not currently feasible for NOAA Ocean Exploration to systematically conduct microplastic sampling using *Okeanos Explorer*. However, some sampling methods could be easier to operationalize than others as exploration priorities change and technology develops.

WATER COLUMN SAMPLING

- **NET TOW:** NOAA Ocean Exploration could conduct net tows to collect water samples on *Okeanos Explorer*. The upfront costs of the net tow and the need for help from the ship's crew would need to be considered. The time it would take to conduct a net tow would be minimal (the equivalent of conducting a CTD rosette cast, if not less) and preserving and storing the samples on board would require minimal effort. However, processing the samples from net tows would require a partnership. Using a net tow could also provide information about plankton. Net tows are more commonly used in microplastic sampling than sediment collection, and the methods for collection and analysis are more standard.

- ***IN SITU* PUMP:** *In situ* water filtration is also possible, but there would be a high cost up front for the equipment and associated costs for maintenance. Deployment is straightforward. An *in situ* pump could be attached to a CTD rosette or ROV and towed at a specific depth interval for a specified period of time (a longer cast would result in a larger volume of water filtered). After collection, the filter would be removed from the pump and preserved for later analysis.

SEDIMENT SAMPLING

An ROV-deployed push corer is not feasible for use on *Okeanos Explorer* with NOAA Ocean Exploration's current assets. It would be expensive to install a push corer on *Deep Discoverer*. Multicorers and box corers are also not feasible due to upfront equipment costs, the potential need to use the ship's A-frame, the need for significant help from the ship's crew, and the time it would take to conduct the sampling. If sediment sampling were feasible, identifying an external partner to analyze the sediment cores would be crucial.

BIOLOGICAL SAMPLING

Okeanos Explorer currently collects biological samples during ROV dives, so sample collection and onboard processing (i.e., preservation) could be done. However, external partners would be required in order to analyze the samples for microplastics.

MOVING FORWARD

Considering the justification and need from the deep-sea community for microplastic sampling, it is recommended that NOAA Ocean Exploration use either *Okeanos Explorer* or other exploration mechanisms to collect data about microplastic distribution, abundance, size, and composition. It is recommended that NOAA Ocean Exploration conduct net tow trial runs on *Okeanos Explorer*. As stated, this method is common in microplastic research. However it would limit collection of microplastics to surface waters. While there are still gaps in our understanding of microplastics in surface waters, there remains a need to collect baseline information on microplastics in the deep sea. To meet this gap, NOAA Ocean Exploration could explore the use and advancement of *in situ* pumps. According to the research conducted for this assessment, *in situ* pumps are an emerging technology, especially for use in the microplastic field.

It is also recommended that NOAA Ocean Exploration explore working with the OECI to conduct microplastic sampling. Ocean Exploration Trust is interested in using push corers for microplastic sampling (N. Raineault, pers. comm.), and some of the other OECI institutions, in particular University of Rhode Island and Woods Hole Oceanographic Institution, are able to measure microplastic abundance, size, and composition. It is also recommended that NOAA Ocean Exploration consider microplastic assessments in the deep sea as a special topic for our annual funding opportunity or a joint funding opportunity with the NOAA Marine Debris Program.

RELEVANT LITERATURE

Achberger, A.M., Christner, B.C., Michaud, A.B., Priscu, J.C., Skidmore, M.L., Vick-Majors, T.J., and the WISSARD Science Team. (2016). Microbial community structure of subglacial Lake Whillans, West Antarctica. Frontiers in Microbiology 7:1457. https://doi.org/10.3389/fmicb.2016.01457

Arthur, C., Baker, J., and Bamford, H, eds. (2009). Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris. Sept 9-11, 2008. NOAA Technical Memorandum NOS-OR&R-30.

Auta, H.S., Emenike, C.U., and Fauziah, S.H. (2017). Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. Environment International 102:165-176. https://doi.org/10.1016/j.envint.2017.02.013

Avio, C.G., Gorbi, S., and Regoli, F. (2017). Plastics and microplastics in the oceans: From emerging pollutants to emerged threat. Marine Environmental Research 128:2-11. https://doi.org/10.1016/j.marenvres.2016.05.012

Baechler, B., Stienbarger, C.D., Horn, D.A., Joseph, J., Taylor, A.R., Granek, E.F., and Brander, S.M. (2019). Microplastic occurrence and effects in commercially harvested North American finfish and shellfish: Current knowledge and future directions. Limnology and Oceanography Letters 5. https://doi.org/10.1002/lol2.10122

Ballent, A., Pando, S., Purser, A., Juliano, M.F., and Thomsen, L. (2013). Modelled transport of benthic marine microplastic pollution in the Nazaré Canyon. Biogeosciences 10:7957-7970. https://doi.org/10.5194/bg-10-7957-2013

Barboza, L.G.A., Dick Vethaak, A., Lavorante, B.R.B.O, Lundebye, A.K., and Guilhermino, L. (2018). Marine microplastic debris: An emerging issue for food security, food safety and human health. Marine Pollution Bulletin 133:336-348. https://doi.org/10.1016/j.marpolbul.2018.05.047

Barrows, A.P.W., Neumann, C.A., Berger, M.L., and Shaw, S.D. (2017). Grab vs. neuston tow net: A microplastic sampling performance comparison and possible advances in the field. Analytical Methods 9:1446-1453. https://doi.org/10.1039/C6AY02387H

Bergmann, M., Wirzberger, V., Krumpen, T., Lorenz, C., Primpke, S., Tekman, M.B., and Gerdts, G. (2017). High quantities of microplastic in Arctic deep-sea sediments from the HAUSGARTEN Observatory. Environmental Science & Technology 51:11000-11010. https://doi.org/10.1021/acs.est.7b03331

Brandon, J.A., Jones, W., and Ohman, M.D. (2019). Multidecadal increase in plastic particles in coastal ocean sediments. Science Advances 5:eaax0587. https://doi.org/10.1126/sciadv.aax0587

Chen, Q., Allgeier, A., Yin, D., and Hollert, H. (2019). Leaching of endocrine disrupting chemicals from marine microplastics and mesoplastics under common life stress conditions. Environment International 130:104938. https://doi.org/10.1016/j.envint.2019.104938

Choy, C.A. and Drazen, J.C. (2013). Plastic for dinner? Observations of frequent debris ingestion by pelagic predatory fishes from the central North Pacific. Marine Ecology Progress Series 485:155-163. https://doi.org/10.3354/meps10342

Choy, C.A., Robison, B.H., Gagne, T.O., Erwin, B., Firl, E., Halden, R.U., Hamilton, J.A., Katija, K., Lisin, S.E., Rolsky, C., and Van Houtan, K. (2019). The vertical distribution and biological transport of marine microplastics across the epipelagic and mesopelagic water column. Scientific Reports 9. https://doi.org/10.1038/s41598-019-44117-2

Cole, M., Lindeque, P., Halsband, C., and Galloway, T. (2011). Microplastics as contaminants in the marine environment: A review. Marine Pollution Bulletin 62:2588-2597. https://doi.org/10.1016/j.marpolbul.2011.09.025

Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., and Galloway, T.S. (2014). Isolation of microplastics in biota-rich seawater samples and marine organisms. Scientific Reports 4:4528. https://doi.org/10.1038/srep04528

Collignon, A., Hecq, J., Glagani, F., Voisin, P., Collard, F., and Goffart, A. (2012). Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. Marine Pollution Bulletin 64:861-864. https://doi.org/10.1016/j.marpolbul.2012.01.011

Compa, M., Alomar, C., Mourre, B., March, D., Tintoré, J., and Deudero, S. (2020). Nearshore spatio-temporal sea surface trawls of plastic debris in the Balearic Islands. Marine Environmental Research 158:104945. https://doi.org/10.1016/j.marenvres.2020.104945

Coppock, R.L., Cole, M., Lindeque, P.K., Queirós, A.M., and Galloway, T.S. (2017). A small-scale, portable method for extracting microplastics from marine sediments. Environmental Pollution 230:829-837. https://doi.org/10.1016/j.envpol.2017.07.017

Cordova, M.R. and Wahyudi, A.J. (2016). Microplastic in the deep-sea sediment of southwestern Sumatran waters. Marine Research in Indonesia 41:27. https://doi.org/10.14203/mri.v41i1.99 DOI: 10.14203/mri.v41i1.99

Courtene-Jones, W., Quinn, B., Ewins, C., Gary, S.F., and Narayanaswamy, B.E. (2019). Consistent microplastic ingestion by deep-sea invertebrates over the last four decades (1976-2015), a study from the North East Atlantic. Environmental Pollution 244:503-512. https://doi.org/10.1016/j.envpol.2018.10.090

Courtene-Jones, W., Quinn, B., Gary, S.F., Mogg, A.O.M., and Narayanaswamy, B.E. (2017a). Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean. Environmental Pollution 231:271-280. https://doi.org/10.1016/j.envpol.2017.08.026

Courtene-Jones, W., Quinn, B., Murphy, F., Gary, S.F., and Narayanaswamy, B.E. (2017b). Optimisation of enzymatic digestion and validation of specimen preservation methods for analysis of ingested microplastics. Analytical Methods 9:1437. https://doi.org/10.1039/C6AY02343F

Cowger, W., Booth, A., Hamilton, B., Primpke, S., Munno, K., Lusher, A., and Hermabessiere, L. (2020). EXPRESS: Reporting guidelines to increase the reproducibility and comparability of research on microplastics. Applied Spectroscopy, 0003702820930292. https://doi.org/10.1177/0003702820930292

Danovaro, R., Fanelli, E., Aguzzi, J., Billett, D., Carugati, L., Corinaldesi, C., Dell'Anno, A., Gjerde, K., Jamieson, A.J., Kark, S., McClain, C., Levin, L., Levin, N., Ramirez-Llodra, E., Ruhl, H., Smith, C.R., Snelgrove, P.V.R., Thomsen, L., Van Dover, C.L., and Yasuhara, M. (2020). Ecological variables for developing a global deep-ocean monitoring and conservation strategy. Nature Ecology & Evolution 4:181-192. https://doi.org/10.1038/s41559-019-1091-z

De Smet, B., Pape, E., Riehl, T., Bonifacio, P., Colson, L., and Vanreusel, A. (2017). The community structure of deep-sea macrofauna associated with polymetallic nodules in the eastern part of the Clarion-Clipperton Fracture Zone. Frontiers in Marine Science 4. https://doi.org/10.3389/fmars.2017.00103

De Wael, K. and Gason, F. (2008). Microfibre transfer experiments. Global Forensic Sci. Today 3:31-37. https://doi.org/10.1016/j.marpolbul.2015.04.044

Dehaut A, Cassone, A, Frere L, Hermabessiere L, Himber C, Rinnert E, et al. (2016). Microplastics in seafood: Benchmark protocol for their extraction and characterization. Environmental Pollution 215:223-233. https://doi.org/10.1016/j.envpol.2016.05.018

Duis, K. and Coors, A. (2016). Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environmental Sciences Europe 28. https://doi.org/10.1186/s12302-015-0069-y

Edson, E.C. and Patterson, M.R. (2015). MantaRay: a novel autonomous sampling instrument for *in situ* measurements of environmental microplastic particle concentrations. OCEANS 2015-MTS/IEEE Washington (pp. 1-6). IEEE. https://doi.org/10.23919/OCEANS.2015.7404541

Egger, M., Sulu-Gambari, F., and Lebreton, L. (2020). First evidence of plastic fallout from the North Pacific Garbage Patch. Scientific Reports 10:7495. https://doi.org/10.1038/s41598-020-64465-8

Enders, K., Lenz, R., Stedmon, C.A., and Nielson, T.G. (2015). Abundance, size and polymer composition of marine microplastics ≥ 10 μm in the Atlantic Ocean and their modelled vertical distribution. Marine Pollution Bulletin 100:70-81. https://doi.org/10.1016/j.marpolbul.2015.09.027

Frias, J.P.G.L., Pagter, E., Nash, R., O'Connor, I., Carretero, O., Filgueiras, A., Viñas, L., Gago, J., Antunes, J.C., Bessa, F., Sobral, P., Goruppi, A., Tirelli, V., Pedrotti, M.L., Suaria, G., Aliani, S., Lopes, C., Raimundo, J., Caetano, M., Palazzo, L., Lucia, G.A.D., Camedda, A., Muniategui, S., Grueiro, G., Fernandez, V., Andrade, J., Dris, R., Laforsch, C., Scholtz-Bottcher, B., and Gerdts, G. (2018). Standardised protocol for monitoring microplastics in sediments. JPI-Oceans BASEMAN project. https://doi.org/10.13140/RG.2.2.36256.89601/1

GESAMP. (2019). Guidelines or the monitoring and assessment of plastic litter and microplastics in the ocean (Kershaw PJ, Turra A, Galgani F. eds), (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP/ISA Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). Rep. Stud. GESAMP No. 99.

Gigault, J., ter Halle, A., Baudrimont, M., Pascal, P.Y., Gauffre, F., Phi, T.L., El Hadri, H., Grassl, B., and Reynaud, S. (2018). Current opinion: What is a nanoplastic? Environmental Pollution 235:1030-1034. https://doi.org/10.1016/j.envpol.2018.01.024

Goldstein, M.C., Titmus, A.J., and Ford, M. (2013). Scales of spatial heterogeneity of plastic marine debris in the northeast Pacific Ocean. PLoS ONE 8:e80020. https://doi.org/10.1371/journal.pone.0080020

Hanvey, J.S., Lewis, P.J., Lavers, J.L., Crosbie, N.D., Pozo, K., and Clarke, B.O. (2017). A review of analytical techniques for quantifying microplastics in sediments. Analytical Methods 9:1369-1383. https://doi.org/10.1039/C6AY02707E

Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A.E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N.P., Lusher, A.L., and Wagner, M. (2019). Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. Environmental Science & Technology 53:1039-1047. https://doi.org/10.1021/acs.est.8b05297

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., and Thiel, M. (2012). Microplastics in the marine environment: A review of the methods used for identification and quantification. Environmental Science & Technology 46:3060-3075. https://doi.org/10.1021/es2031505

Hurley, R.R., Lusher, A.L., Olsen, M., and Nizzetto, L. (2018). Validation of a method for extracting microplastics from complex, organic-rich, environmental matrices. Environmental Science and Technology 52:7409-7417. https://doi.org/10.1021/acs.est.8b01517

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan R., and Law, K.L. (2015). Plastic waste inputs from land into the ocean. Science 347:768-771. https://doi.org/10.1126/science.1260352

Jamieson, A.J., Brooks, L., and Reid, W.D.K. (2019). Microplastics and synthetic particles ingested by deep-sea amphipods in six of the deepest marine ecosystems on Earth. Royal Society Open Science 6:180667. https://doi.org/10.1098/rsos.180667

Kane, I.A. and Clare, M.A. (2019). Dispersion, Accumulation, and the ultimate fate of microplastics in deep-marine environments: A review and future directions. Frontiers in Earth Science 7. https://doi.org/10.3389/feart.2019.00080

Kane, I.A., Clare, M.A., Miramontes, E., Wogelius, R., Rothwell, J.J., Garreau, P., and Pohl, F. (2020). Seafloor microplastic hotspots controlled by deep-sea circulation. Science:eaba5899. https://doi.org/10.1126/science.aba5899

Kanhai, L.D.K., Johansson, C., Frias, J.P.G.L., Gardfeldt, K., Thompson, R.C., and O'Connor, I. (2019). Deep sea sediments of the Arctic Central Basin: A potential sink for microplastics. Deep Sea Research Part I: Oceanographic Research Papers 145:137-142. https://doi.org/10.1016/j.dsr.2019.03.003

Karlsson, T.M., Kärrman, A., Rotander, A., and Hassellöv, M. (2020). Comparison between manta trawl and *in situ* pump filtration methods, and guidance for visual identification of microplastics in surface waters. Environmental Science and Pollution Research 27(5):5559-5571. https://doi.org/10.1007/s11356-019-07274-5

Katija, K., Choy, C.A., Sherlock, R.E., Sherman, A.D., and Robison, B.H. (2017). From the surface to the seafloor: How giant larvaceans transport microplastics into the deep sea. Science Advances 3:e1700715. https://doi.org/10.1126/sciadv.1700715

Kezierski, M., Le Tilly, V., Cesar, G., Sire, O., and Bruzard, S. (2017). Efficient microplastics extraction from sand. A cost effective methodology based on sodium iodide recycling. Marine Pollution Bulletin 115:120-129. https://doi.org/10.1016/j.marpolbul.2016.12.002

Koelmans, A.A., Bakir, A., Burton, G.A., and Janssen, C.R. (2016). Microplastic as a vector for chemicals in the aquatic environment: Critical review and model-supported reinterpretation of empirical studies. Environmental Science and Technology 50(7):3315-3326. https://doi.org/10.1021/acs.est.5b06069

Koelmans, A.A., Besseling, E., Foekema, E., Kooi, M., Mintenig, S., Ossendorp, B.C., Redondo-Hasselerharm, P.E., Verschoor, A., van Wezel, A.P., and Scheffer, M. (2017). Risks of plastic debris: Unravelling fact, opinion, perception, and belief. Environmental Science and Technology 51(20):11513-11519. https://doi.org/10.1021/acs.est.7b02219

Kooi, M., Van Nes, E.H., Scheffer, M., and Koehlmans, A.A. (2017). Ups and downs in the ocean: Effects of biofouling on vertical transport of microplastics. Environmental Science and Technology 51:7963-7971. https://doi.org/10.1021/acs.est.6b04702

Levin, L.A., Bett, B.J., Gates, A.R., Heimbach, P., Howe, B.M., Janssen, F., McCurdy, A., Ruhl, H.A., Snelgrove, P., Stocks, K.I., Bailey, D., Baumann-Pickering, S., Beaverson, C., Benfield, M.C., Booth, D.J., Carreiro-Silva, M., Colaço, A., Eblé, M.C., Fowler, A.M., Gjerde, K.M., Jones D.O.B., Katsumata, K., Kelley, D., Le Bris, N., Leonardi, A.P., Lejzerowicz, F., Macreadie, P.I., McLean, D., Meitz, F., Morato, T., Netburn, A., Pawlowski, J., Smith, C.R., Sun, S., Uchida, H., Vardaro, M.F., Venkatesan, R., and Weller, R.A. (2019a). Global observing needs in the deep ocean. Frontiers in Marine Science 6. https://doi.org/10.3389/fmars.2019.00241

Levin, L.A., Ruhl, H., Heimbach, P., McCurdy, A., Smith, L., Baumann-Pickering, S., Carreiro-Silva, M., Gjerde, K., Howe, B., Janssen, F., Katsumata, K., Kelley, D., Le Bris, N., Smith, C., Snelgrove, P., Song, S., Soule, A., Stocks, K., Rome, N., Venkatesan, R., and Weller, R. (2019b). DOOS 2019 Science and Implementation Guide. https://deepoceanobserving.org/wp-content/uploads/2019/05/DOOS-2019-Science-and-Implementation-Guide-2019-05-31-V3.pdf

Liu, K., Zhang, F., Song, Z., Zong, C., Wei, N., and Li, D. (2019). A novel method enabling the accurate quantification of microplastics in the water column of the deep ocean. Marine Pollution Bulletin 146:462-465. https://doi.org/10.1016/j.marpolbul.2019.07.008

Lusher, A.L., O'Donnell, C., Officer, R., and O'Connor, I. (2016). Microplastic interactions with North Atlantic mesopelagic fish. ICES Journal of Marine Science 73:1214-1225. https://doi.org/10.1093/icesjms/fsv241

Lusher, A.L., Tirelli, V., O'Connor, I., and Officer, R. (2015). Microplastics in Arctic polar waters: The first reported values of particles in surface and sub-surface samples. Scientific Reports 5. https://doi.org/10.1038/srep14947

Mai, L., Bao, L.J., Shi, L., Wong, C.S., and Zeng, E.Y. (2018). A review of methods for measuring microplastics in aquatic environments. Environmental Science and Pollution Research 25:11319-11332. https://doi.org/10.1007/s11356-018-1692-0

Martin, J., Lusher, A., Thompson, R.C., Morley, and A. (2017). The deposition and accumulation of microplastics in marine sediments and bottom water from the Irish continental shelf. Scientific Reports 7. https://doi.org/10.1038/s41598-017-11079-2

Martin, K.M., Hasenmueller, E.A., White, J.R., Chambers, L.G., and Conkle, J.L. (2018). Sampling, sorting, and characterizing microplastics in aquatic environments with high suspended sediment loads and large floating debris. J Vis Exp 137. https://doi.org/10.3791/57969

Masura, J., Baker, J.E., Foster, G.D., Arthur, C., and Herring, C. (2015). Laboratory methods for the analysis of microplastics in the marine environment: Recommendations for quantifying synthetic particles in waters and sediments. NOAA Technical Memorandum NOS-OR&R-48.

Maximenko, N., Corradi, P., Law, K.L., Van Sebille, E., Garaba, S.P., Lampitt, R.S., Galgani, F., Martinez-Vicente, V., Goddijn-Murphy, L., ,Veiga, J.M., Thompson, R.C., Maes, C., Moller, D., Löscher, C.R., Addamo, A.M., Lamson, M.R., Centurioni, L.R., Posth, N.R., Lumpkin, R., Vinci, M., Martins, A.M., Pieper, C.D., Isobe, A., Hanke, G., Edwards, M., Chubarenko, I.P., Rodriguez, E., Aliani, S., Arias, M., Asner, G.P., Brosich, A., Carlton, J.T., Chao, Y., Cook, A.M., Cundy, A.B., Galloway, T.S., Giorgetti, A., Goni, G.J., Guichoux, Y., Haram, L.E., Hardesty, B.D., Holdsworth, N., Lebreton, L., Leslie, H.A., Macadam-Somer, I., Mace, T., Manuel, M., Marsh, R., Martinez, E., Mayor, D.J, Le Moigne, M., Molina Jack, M.E., Mowlem, M.C., Obbard, R.W., Pabortsava, K., Robberson, B., Rotaru, A.E., Ruiz, G.M., Spedicato, M.T., Thiel, M., Turra, A., and Wilcox, C. (2019). Toward the Integrated Marine Debris Observing System. Frontiers in Marine Science 6. https://doi.org/10.3389/fmars.2019.00447

Morrison, A.T., Billings, J.D., and Doherty, K.W. (2000, September). The McLane WTS-LV: a large volume, high accuracy, oceanographic sampling pump. In OCEANS 2000 MTS/IEEE Conference and Exhibition. Conference Proceedings (Cat. No. 00CH37158) (Vol. 2, pp. 847-852). IEEE. https://doi.org/10.1109/OCEANS.2000.881365

Nakajima, R., Tsuchiya, M., Lindsay, D.J., Kitahashi, T., Fujikura, K., and Fukushima, T. (2019). A new small device made of glass for separating microplastics from marine and freshwater sediments. PeerJ 7:e7915. https://doi.org/10.7717/peerj.7915

Netburn, A.N., ed. (2018). From Surface to Seafloor: Exploration of the Water Column. Workshop Report, Honolulu, HI, March 4-5, 2017. NOAA Office of Ocean Exploration and Research, Silver Spring, MD. NOAA Technical Memorandum OAR OER 003. https://doi.org/10.25923/rnjx-vn79

NOAA Office of Ocean Exploration and Research (OER). (2011). NOAA Workshop on Systematic Telepresence-Enabled Exploration in the Atlantic Basin, Narragansett, RI, 10-11 May 2011. https://oceanexplorer.noaa.gov/about/what-we-do/media/atl-basin-workshop-2011-summary.pdf

Nuwer, R. (2014). Are there any pollution-free places left on Earth? BBC News. https://www.bbc.com/future/article/20141104-is-anywhere-free-from-pollution

Ocean Exploration Trust (OET). (2012). Workshop on Telepresence-Enabled Exploration of the Caribbean Region Report, 15-18 November 2012. https://nautiluslive.org/sites/default/files/documents/2020-05/2012CaribbeanWorkshopSummary.pdf

Ocean Exploration Trust (OET). (2014). Workshop on Telepresence-Enabled Exploration of the Eastern Pacific Ocean Report, 11-13 December 2014. https://nautiluslive.org/sites/default/files/documents/2020-05/2014PacifcWorkshopReport.pdf

Ogata, Y., Takada, H., Mizukawa, K., Hirai, H., Iwaasa, S., Endo, S., Mato, Y., Saha, M., Okuda, K., Nakashima, A., Murakami, M., Zurcher, N., Booyatumanondo, R., Zakaria., Dung, L.Q., Gordon, M., Miguez, C., Suzuki, S., Moore, C., Karapanagioti, H.K., Weerts, S., McClurg, T., Burres, E., Smith, W., Van Valkenburg, M., Lang, J.S., Lang, R.C., Laursen, D., Danner, B., Stewardson, N., and Thompson, R.C. (2009). International pellet watch: Global monitoring of persistent organic pollutants (POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs. Marine Pollution Bulletin 58:1437-1446. https://doi.org/10.1016/j.marpolbul.2009.06.014

Pabortsava, K. and Lampitt, R.S. (2020). High concentrations of plastic hidden beneath the surface of the Atlantic Ocean. Nature Communications 11:4073. https://doi.org/10.1038/s41467-020-17932-9

Peng, X., Chen, M., Chen, S., Dasgupta, S., Xu, H., Ta, K., Du, M., Li, J., Guo, Z., and Bai, S. (2018). Microplastics contaminate the deepest part of the world's ocean. Geochemical Perspectives Letters 9:1-5. https://doi.org/10.7185/geochemlet.1829

Pitcher, A., Villanueva, L., Hopmans, E.C., Schouten, S., Reichart, G.J., and Damsté, J.S.S. (2011). Niche segregation of ammonia-oxidizing archaea and anammox bacteria in the Arabian Sea oxygen minimum zone. The ISME Journal 5(12):1896-1904. https://doi.org/10.1038/ismej.2011.60

Riley, T., Rowley, K., Cheever, E., and Roberts, J. (n.d.) Ship-Based Marine Plastic Litter. https://repository.library.noaa.gov/view/noaa/20223

Rivers, M.L, Gwinnett, C., and Woodall, L.C. (2019). Quantification is more than counting: Actions required to accurately quantify and report isolated marine microplastics. Marine Pollution Bulletin 139:100-104. https://doi.org/10.1016/j.marpolbul.2018.12.024

Rocha-Santos, T. and Duarte, A.C. (2015). A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. TrAC Trends in Analytical Chemistry 65:47-53. https://doi.org/10.1016/j.trac.2014.10.011

Rochman, C.M., Hoh, E., Kurobe, T., and The, S.J. (2013). Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Scientific Reports 3:3263. https://doi.org/10.1038/srep03263

Rosli, N., Leduc, D., Rowden, A.A., and Probert, P.K. (2018). Review of recent trends in ecological studies of deep-sea meiofauna, with focus on patterns and processes at small to regional spatial scales. Meio Extreme 48:13-34. https://doi.org/10.1007/s12526-017-0801-5

Sanchez-Vidal, A., Thompson, R.C., Canals, M., and de Haan. W.P. (2018). The imprint of microfibres in southern European deep seas. PLOS ONE 13:e0207033. https://doi.org/10.1371/journal.pone.0207033

Sayre, R.G., Wright, D.J., Breyer, S.P., Butler, K.A., Van Graafeiland, K., Costello, M.J., Harris, P.T., Goodin, K.L., Guinotte, J.M., Basher, Z., Kavanaugh, M.T., Halpin, P.N., Monaco, M.E., Cressie, N., Aniello, P., Frye, C.E., and Stephens, D. 2017. A three-dimensional mapping of the ocean based on environmental data. Oceanography 30(1):90-103. https://doi.org/10.5670/oceanog.2017.116

Shim, W.J., Hong, S.H., and Eo, S.E. (2017). Identification methods in microplastic analysis: a review. Analytical Methods 9:1384-1391. https://doi.org/10.1039/c6ay02558g

Shim, W.J., and Thompson, R.C. (2015). Microplastics in the ocean. Archives of Environmental Contamination and Toxicology 69:265-268. https://doi.org/10.1007/s00244-015-0216-x

Stapleton, P.A. (2019). Toxicological considerations of nano-sized plastics. AIMS Environmental Science 6:367-378. https://doi.org/10.3934/environsci.2019.5.367

Tekman, M.B., Wekerle, C., Lorenz, C., Primpke, S., Hasemann, C., Gerdts, G., and Bergmann, M. (2020). Tying up loose ends of microplastic pollution in the Arctic: Distribution from the sea surface through the water column to deep-sea sediments at the HAUSGARTEN Observatory. Environmental Science and Technology 54:4079-4090. https://doi.org/10.1021/acs.est.9b06981

Tsuchiya, M., Nomaki, H., Kitahashi, T., Nakajima, R., and Fujikura, K. (2019). Sediment sampling with a core sampler equipped with aluminum tubes and an on board processing protocol to avoid plastic contamination. MethodsX 6:2662-2668. https://doi.org/10.1016/j.mex.2019.10.027

Twining, B.S., Rauschenberg, S., Morton, P.L., Ohnemus, D.C., and Lam, P.J. (2015). Comparison of particulate trace element concentrations in the North Atlantic Ocean as determined with discrete bottle sampling and *in situ* pumping. Deep Sea Research Part II: Topical Studies in Oceanography 116:273-282. https://doi.org/10.1016/j.dsr2.2014.11.005

United Nations (2017). The conversation and sustainable use of marine biological diversity of areas beyond national jurisdiction: A technical abstract of the first global integrated marine assessment. New York.

University-National Oceanographic Laboratory System (UNOLS). (2016). Developing Submergence Science for the Next Decade (DESCEND-2016) Workshop Proceedings, Cambridge, MA, 14-15 January 2016. https://www.unols.org/sites/default/files/DESCEND2 %202016%20FINALFINAL_small.pdf

Van Cauwenberghe, L., Devriese, L., Galgani, F., Robbens, J., and Janssen, C.R. (2015). Microplastics in sediments: A review of techniques, occurrence and effects. Marine Environmental Research 111:5-17. https://doi.org/10.1016/j.marenvres.2015.06.007

Van Cauwenberghe, L., Vanreusel, A., Mees, J., and Janssen, C.R. (2013). Microplastic pollution in deep-sea sediments. Environmental Pollution 182:495-499. https://doi.org/10.1016/j.envpol.2013.08.013

Van Sebille, E., Wilcox, C., Lebreton, L., Maximenko, N., Hardesty, B.D., van Franeker, J.A., Eriksen, M., Siegel, D., Galgani, F., and Law, K.L. (2015). A global inventory of small floating plastic debris. Environmental Research Letters 10. https://doi.org/10.1088/1748-9326/10/12/124006

Wang, W. and Wang, J. (2018). Investigation of microplastics in aquatic environments: An overview of the methods used, from field sampling to laboratory analysis. Trends in Analytical Chemistry 108:195-202. https://doi.org/10.1016/j.trac.2018.08.026

Wichmann, D., Delandmeter, P., and van Sebille, E. (2019). Influence of near-surface currents on the global dispersal of marine microplastics. Journal of Geophysical Research: Oceans 124. https://doi.org/10.1029/2019JC015328

Woodall, L., Andradi-Brown, D., Brierley, A., Clark, M., Connelly, D., Hall, R., Howell, K., Huvenne, V., Linse, K., Ross, R., Snelgrove, P., Stefanoudis, P., Sutton, T., Taylor, M., Thornton, T., and Rogers, A. (2018). A multidisciplinary approach for generating globally consistent data on mesophotic, deep-pelagic, and bathyal biological communities. Oceanography 31. https://doi.org/10.5670/oceanog.2018.301

Woodall, L.C., Gwinnett, C., Packer, M., Thompson, R.C., Robinson, L.F., and Paterson, G.L.J. (2015a). Using a forensic science approach to minimize environmental contamination and to identify microfibres in marine sediments. Marine Pollution Bulletin 95:40-46. https://doi.org/10.1016/j.marpolbul.2015.04.044

Woodall, L.C., Robinson, L.F., Rogers, A.D., Narayanaswamy, B.E., and Paterson, G.L.J. (2015b). Deep-sea litter: A comparison of seamounts, banks and a ridge in the Atlantic and Indian oceans reveals both environmental and anthropogenic factors impact accumulation and composition. Frontiers in Marine Science 2. https://doi.org/10.3389/fmars.2015.00003

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., and Thompson, R.C. (2014). The deep sea is a major sink for microplastic debris. Royal Society Open Science 1:140317. https://doi.org/10.1098/rsos.140317

Zobkov, M. and Esiukova, E. (2017). Microplastics in Baltic bottom sediments: Quantification procedures and first results. Marine Pollution Bulletin 114:724-732. https://doi.org/10.1016/j.marpolbul.2016.10.060