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Advances in ocean exploration

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Published in
Frontiers in Marine Science

ISSN 1664-8714
ISBN 978-2-8325-5432-6
DOI 10.3389/978-2-8325-5432-6

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Advances in ocean exploration

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Citation
Soule, A., Wagner, D., Elmore, A., Hamdan, L. J., eds. (2024). *Advances in ocean exploration*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-5432-6

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frontiers | Frontiers in Marine Science

TYPE Perspective
PUBLISHED 09 August 2023
DOI 10.3389/fmars.2023.1217227

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OPEN ACCESS

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RECEIVED 05 May 2023
ACCEPTED 10 July 2023
PUBLISHED 09 August 2023

CITATION
Bell KLC, Quinzin MC, Amon D, Poulton S, Hope A, Sarti O, Cañete TE, Smith AM, Baldwin HI, Lira DM, Cambronero-Solano S, Chung T-RA and Brady B (2023) Exposing inequities in deep-sea exploration and research: results of the 2022 Global Deep-Sea Capacity Assessment. *Front. Mar. Sci.* 10:1217227. doi: 10.3389/fmars.2023.1217227

Exposing inequities in deep-sea exploration and research: results of the 2022 Global Deep-Sea Capacity Assessment

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The 2022 Global Deep-Sea Capacity Assessment is a baseline assessment of the technical and human capacity for deep-sea exploration and research in every coastal area with deep ocean worldwide. From 200 to nearly 11,000 meters below sea level, the deep sea encompasses the single largest—and arguably the most critical—biosphere on Earth. Globally, two-thirds of all exclusive economic zones combined have water depths between 2,000 and 6,000 meters, making this a particularly critical depth range to access. This study includes information for 186 countries and territories, analyzed by subregional, regional, and income groups. The data were collected through both an online survey and manual research. We found that globally, 52% of respondents agreed that exploration and research were considered important in their community. A third of respondents agreed they had the in-country technology to conduct deep-sea exploration and research, and half agreed they had in-country deep-sea expertise. Survey results revealed that the most important challenges worldwide are funding, access to vessels, and human capacity. The top three global opportunities for deep-sea exploration and research were training opportunities, less expensive data collection technology, and better data access and analysis tools. This assessment provides the baseline information necessary to strategically develop, equitably implement, and quantitatively measure the impact of deep-sea exploration and research capacity development over the coming years. It is now possible to measure the evolution of deep-sea capacity over the next decade, an important indicator of progress during the UN Decade of Ocean Science for Sustainable Development.

KEYWORDS

marine technology, capacity development, small island developing states, UN Ocean Decade, deep submergence vehicles, deep ocean

1 Introduction

Globally, 82% of all countries and territories have jurisdiction over marine areas with depths greater than 200 meters (Bell et al., 2022b). In addition, expense, inefficiency, and inequitable access to existing tools and resources worldwide make it challenging—and often impossible—to explore, understand, and effectively manage one's exclusive economic zones (EEZs) and marine areas beyond national jurisdiction, resulting in only a tiny fraction of the deep sea being studied and characterized (Bell et al., 2022a; Amon et al., 2022d).

The concept for the Global Deep-Sea Capacity Assessment was sparked by a 2018 pilot project, 'My Deep Sea, My Backyard,' which aimed to provide deep-ocean technology and training for scientists, students, and educators in Kiribati and Trinidad and Tobago, two small island developing states (SIDS) without the capacity to explore and research their EEZs (Amon et al., 2022d). Furthermore, the publication of the 2020 Global Ocean Science Report only included the 45 countries responsible for 82% of ocean science publications from 2010 to 2018—in other words, it only included those countries with the most access to oceanographic resources (IOC-UNESCO, 2020). A more comprehensive and equitable assessment, including *all* coastal areas with deep ocean, was necessary to understand where humankind stands today.

At the same time, the imminent United Nations Decade of Ocean Science for Sustainable Development (UN Ocean Decade) and the recently adopted agreement on the conservation and sustainable use of marine biological diversity of areas beyond national jurisdiction (United Nations, 2023a; United Nations, 2023b; the "BBNJ Agreement") included capacity building and the transfer of marine technology as major priorities (Ryabinin et al., 2019; Harden-Davies et al., 2022a; Harden-Davies et al., 2022b; United Nations, 2023a). Additionally, deep-sea exploitation—often without management underpinned by robust science—is rapidly emerging (e.g., Ramirez-Llodra et al., 2011; Amon et al., 2022c; Levin et al., 2023). Thus, the Ocean Discovery League convened a global team to address this challenge via the first Global Deep-Sea Capacity Assessment (Bell et al., 2022b). This assessment aimed to enable ocean stakeholders to strategically develop, equitably implement, and quantitatively measure the progress of deep-sea exploration and research capacity development throughout the UN Ocean Decade and beyond.

2 About the capacity assessment

The 2022 Global Deep-Sea Capacity Assessment is a baseline assessment of the technical and human capacity for deep-sea exploration and research in every coastal area with deep ocean worldwide (Figure 1; Bell et al., 2022b). It was released on September 15, 2022, and includes survey and/or research data for 186 geographical areas, or GeoAreas.[1]

2.1 The survey

Data collected from a 42-question online survey[2] conducted between February and December 2021 formed the foundation of the assessment. The survey included a combination of quantitative and qualitative questions. Respondents took the survey for one GeoArea at a time and as many GeoAreas as they felt qualified to represent. To increase participation in the survey, it was available in English, French, Portuguese, and Spanish, and the team sent personalized invitations to marine professionals in underrepresented GeoAreas.

2.2 The research

A team of eight researchers did manual research on the inhabited GeoAreas in their region with >1% deep sea area within their EEZ. All researchers used the same protocol[3] to identify current and prospective in-country capacity and capacity dependent on foreign partners and capabilities. The researchers' personal and contextual knowledge supported the research and allowed them to consult with professionals in their networks. Online research was conducted mainly in English, French, and

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[1] https://deepseacapacity.oceandiscoveryleague.org/pub/2022-data-collection.

[2] https://deepseacapacity.oceandiscoveryleague.org/pub/2022-survey.

[3] https://deepseacapacity.oceandiscoveryleague.org/pub/2022-research-protocols.

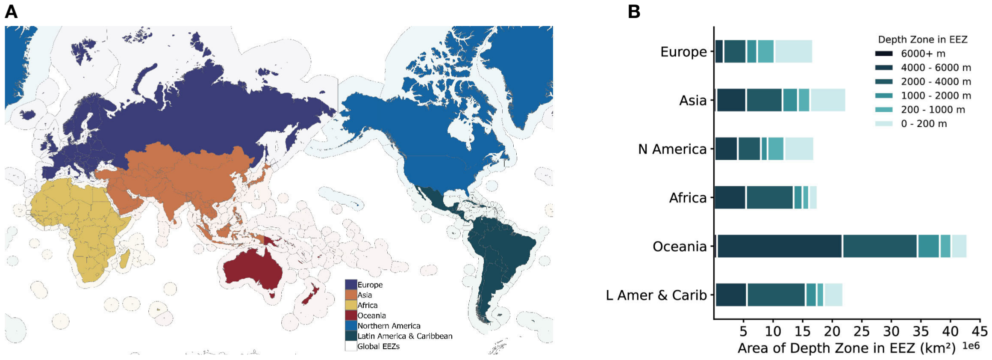

FIGURE 1
Where is the deep sea globally? **(A)** A global map illustrates of the regions included in the assessment: Europe, Northern America, Africa, Asia, Oceania, and Latin America & the Caribbean; **(B)** Area of each depth zone for all EEZs claimed in each region of the world (Bell et al., 2022b).

Spanish, but also in Arabic, Bengali, Georgian, Indonesian, Persian, and Vietnamese when needed.

2.3 The analysis

The assessment includes survey and/or research data for 186 GeoAreas: both survey and research data for 119 GeoAreas, research data only for 62 GeoArea, and survey data only for five GeoAreas[4,5]. Each GeoArea was categorized by region, subregion, SIDS, income group, and EEZ depths and areas (VLIZ, 2019; GEBCO, 2021; ESRI, 2022; UNSD, 2022; World Bank, 2022). The assessment data were analyzed and presented (1) globally[6], (2) by region[7,8,9,10,11] and (3) by SIDS/Non-SIDS and economic groups[12].

We calculated two sets of indices from these data: the Deep-Sea Capacity Status Parameters (SPs) and the Deep-Sea Capacity Indices (DSC Indices)[13]. The Status Parameters were based on survey respondents' level of agreement that they (1) have in-country deep-sea expertise, (2) have in-country deep-sea tools, and (3) that deep-sea exploration and research are considered important in their GeoArea (Figures 2A, B).

Research data documented the presence of organizations, industries, vessels, deep submergence vehicles (DSVs), sensors, and data analysis tools in each GeoArea, and survey responses identified respondents' access to and satisfaction with vessels, DSVs, sensors, and data tools in each subregion or economic group. With these data, we calculated three DSC Indices to compare locations regarding the presence of, access to, and satisfaction with each of the various capacities.

3 Key findings of the 2022 Global Deep-sea Capacity Assessment

3.1 Many who consider deep-sea exploration and research important do not have deep-sea tools and technologies

Survey respondents for several geographic and/or economic groups of GeoAreas indicated that deep-sea exploration and research was considered important in their location but did not have access to the tools and technology needed. Specifically, respondents for geographic subregions like Southeastern Asia, Western Africa, and Melanesia agreed that deep-sea exploration and research were considered important in their GeoArea, but disagreed there were in-country deep-sea tools and technology (Figure 2A). Similarly, respondents for lower-middle income

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[4] https://deepseacapacity.oceandiscoveryleague.org/pub/2022-global-tables/#global-data-sources.

[5] https://deepseacapacity.oceandiscoveryleague.org/data.

[6] https://deepseacapacity.oceandiscoveryleague.org/pub/2022-global-regions-summary.

[7] https://deepseacapacity.oceandiscoveryleague.org/pub/2022-africa-summary.

[8] https://deepseacapacity.oceandiscoveryleague.org/pub/2022-americas-summary.

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[12] https://deepseacapacity.oceandiscoveryleague.org/pub/2022-sids-summary.

[13] https://deepseacapacity.oceandiscoveryleague.org/pub/2022-data-collection.

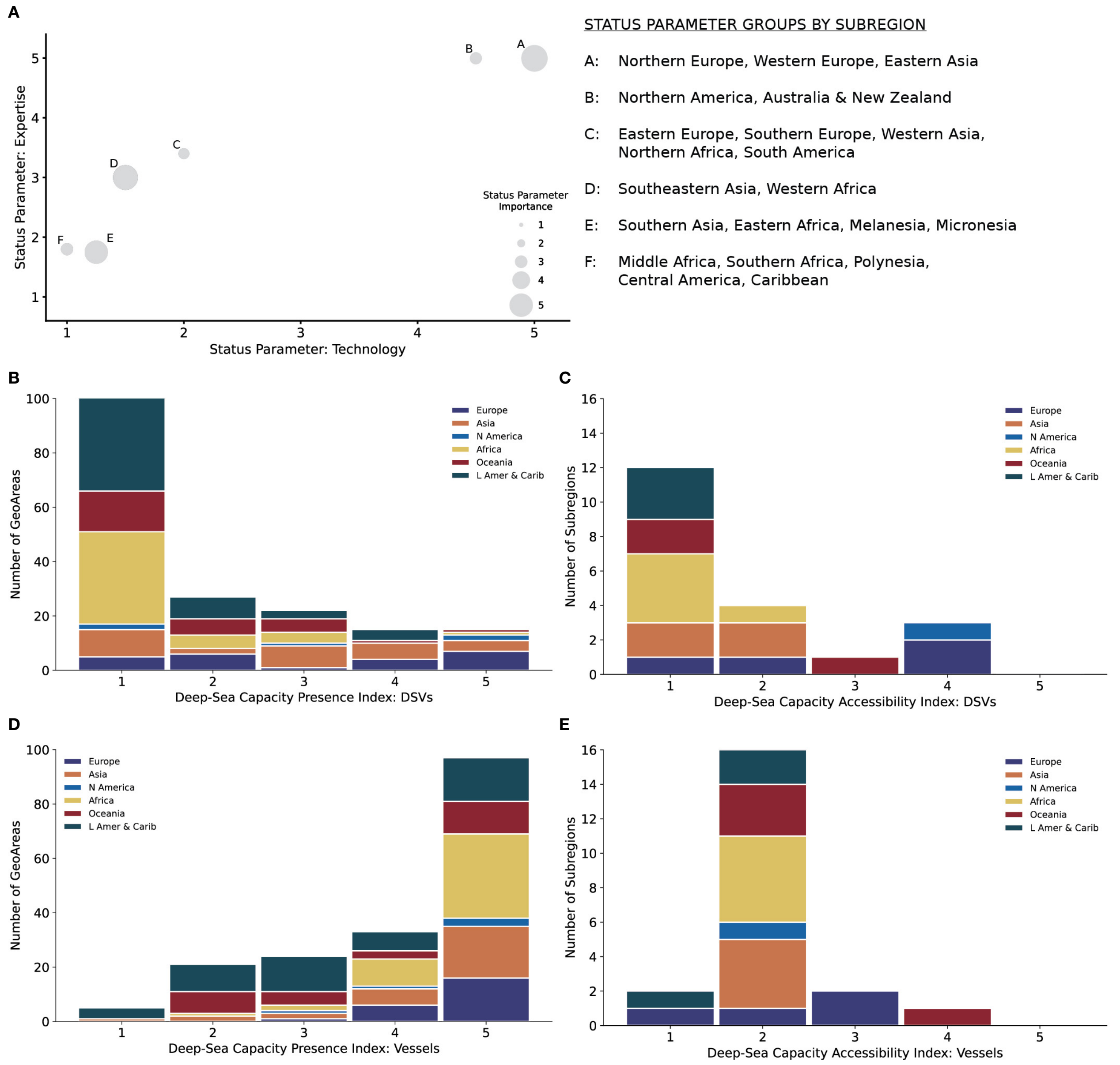

FIGURE 2

(A) Three Status Parameters (SPs) were calculated to compare respondents' perceptions of the existence of in-country deep-sea technology (x-axis) and expertise (y-axis) in their GeoArea, and whether deep-sea exploration and research is considered important in their GeoArea (bubble size). The SPs were calculated for each subregion of the world and can be used to compare similarities between locations. **(B)** Number of GeoAreas worldwide with each DSV Deep-Sea Capacity Presence Index. High DSV DSCPIs indicate higher diversity of vessel types present in each GeoArea. DSVs were the technical capacity with the lowest presence worldwide. **(C)** Number of subregions worldwide with each DSV Deep-Sea Capacity Accessibility Index. High DSV DSCAIs indicate higher respondent-reported access to vessels in their GeoArea. DSVs were the technical capacity to which respondents had the lowest access worldwide. **(D)** Number of GeoAreas worldwide with each Vessel Deep-Sea Capacity Presence Index. High Vessel DSCPIs indicate higher diversity of vessel types present in each GeoArea. Vessels were the technical capacity with the most extensive presence worldwide. **(E)** Number of subregions worldwide with each Vessel Deep-Sea Capacity Accessibility Index. High Vessel DSCAIs indicate higher respondent-reported access to vessels in their GeoArea. Vessels were the technical capacity to which respondents had the second-lowest access. (Bell et al., 2022b).

SIDS, such as Cabo Verde, Haiti, and Papua New Guinea, also agreed that deep-sea exploration and research were considered important in their GeoArea, but disagreed they had in-country deep-sea tools and technologies. More deep-sea tools are needed in locations where they are currently unavailable, particularly those that recognize the importance of deep-sea exploration and research.

3.2 In many places, there is expertise but not technology

In every subregion, respondents indicated that the presence of in-country individuals with deep-sea expertise exceeded the availability of deep-sea tools (Figure 2A). The locations with the highest ratios of in-country deep-sea expertise to technologies were in subregions such as Eastern Europe, South America, and Western Africa. When comparing economic groups of GeoAreas, respondents for high-income, upper-middle income, and lower-middle income SIDS had the highest ratios of in-country expertise to technologies. As such, more access to vessels, deep submergence vehicles, sensors, and data analysis tools could activate available expertise to conduct locally-led deep-sea exploration and research.

3.3 More deep submergence vehicles are needed globally

Deep submergence vehicles (DSVs) were the technical capacity with the lowest presence, access, and satisfaction worldwide. While some DSVs exist worldwide, the majority are concentrated in Europe, Northern America, and Asia (Figure 2B). Most respondents for Africa, Oceania, and Latin America & the Caribbean reported having no access to DSVs (Figure 2C), and half of the DSVs within those regions could not operate deeper than 200 meters, limiting researchers to shallow waters. Nearly two-thirds of respondents for SIDS reported that they had no access to DSVs. In addition, respondents were generally dissatisfied with the DSVs available to them and were least satisfied with DSV cost and availability. Globally, 71% of respondents reported that increased DSV access would have a high impact or be transformative for their work. Greater access to affordable and user-friendly deep submergence technologies capable of operating beyond 200 meters could have a transformative impact on a global scale.

3.4 Non-research assets could be available for deep ocean research

Non-research assets such as commercial vessels or data analysis tools could be used for deep-sea research. For example, while vessels were the technical capacity with the most extensive presence worldwide (Figure 2D), respondents had the second-lowest access (Figure 2E). The most abundant types of vessels found worldwide were fishing and recreational vessels. The most accessible vessel types globally were research vessels, but only in Europe, Northern America, and Asia. Respondents for Africa, Latin America & the Caribbean, and Oceania reported the most access to fishing vessels. Approximately one-third of respondents for these regions reported no access to vessels. Overall, 66% of respondents reported that increased access to vessels would have a high impact or be transformative for their work. New strategies for using non-research assets for research could open up significant opportunities for collecting and analyzing deep-sea data.

3.5 Funding is the top challenge

Increased funding and lower-cost technical and training solutions are key to increasing access to the deep sea globally. Survey respondents identified funding as the greatest challenge regardless of region, geographic group, or income group. In Europe and Northern America, vessel access was the second biggest challenge, followed by DSV access. In Asia and Latin America & the Caribbean, access to vessels and human capacity were equally ranked as the second biggest challenge. Human capacity was the second biggest challenge in Africa and Oceania, followed by access to vessels in Africa and DSVs in Oceania. For SIDS, human capacity was the second-biggest challenge, nearly equal to funding.

3.6 Prioritizing deep-sea exploration is essential

No matter the self-assessed level of in-country expertise and technology, respondents for several subregions thought their communities did not consider deep-sea exploration and research important (Figure 2A, Groups B, C, and F). Below are two excerpts from survey participants' responses on why the prioritization of deep-sea exploration and research would be beneficial for their communities:

"Additional at-sea opportunities for our youth would open jobs at diverse coastal institutions that currently do not engage in oceanographic and deep-sea research or education."– Respondent for Mexico, Central America

"Tonga is one of those countries that gives licenses for exploration in its EEZ. So for all we know from the contracting company, they can give any erroneous data, and we have no way of validating them. Tonga would benefit from an ROV."–Respondent for Tonga, Polynesia

Progress in prioritizing deep-sea exploration could be beneficial in securing long-term support for increased access to tools and training where they are most needed. In addition, a discussion about *why* deep-sea exploration and research are not considered important is also crucial so that stronger cases can be made to prioritize it.

3.7 Tailored strategies are needed for each location

Shallow-water vehicles and sensors are less expensive to manufacture and operate than those needed for deeper waters. A better understanding of the environmental requirements in each location can help ensure the greatest return on technological investments (Figure 1B). For example, over 90% of EEZs in Central America, Eastern Europe, Northern Africa, Northern Europe, and Western Asia are less than 4,000 m in depth. Accessible technologies reaching 4,000 m will unlock the vast majority of EEZs in these subregions. Furthermore, eleven out of twenty-one subregions worldwide have a maximum depth of 6,000 m; deep-sea technology capable of reaching 6,000 m would unlock access to all EEZs within these subregions. Understanding each location's operational needs could help create suitable deep-ocean technologies and strategies.

3.8 Detailed research and inclusion matter

While capacity development and technology transfer have received significant attention in recent years, there is still a need for a comprehensive understanding of global deep-sea technical and human capacity. As of January 2021, the 2020 Global Ocean Science Report was the closest analog but only included the 45 countries responsible for the majority of ocean-science publications from 2010 to 2018 (IOC-UNESCO, 2020). This lack of inclusive global information likely inhibited success. With limited baseline information about deep-sea capacity in most countries worldwide, measuring progress over the UN Ocean Decade would prove challenging, if not impossible.

With more information and perspectives gathered on deep-sea capacity than ever before, the results of this assessment were more nuanced than expected. Reaching out to people in locations often under-resourced and overlooked in many global studies created a community and a sense of inclusion but was also valuable in many other ways. Now that the deep-sea capacity gaps have been documented, it will be possible to strategically develop, equitably implement, and quantitatively measure the progress of deep-sea exploration and research capacity development.

3.9 SIDS and non-SIDS have different priorities for exploration & research

Respondents for SIDS and non-SIDS GeoAreas had different perspectives on the most important deep-sea issues in their GeoAreas. For example, climate change was important for lower-middle and upper-middle income SIDS but was not highlighted as a main issue by non-SIDS GeoAreas. Basic science was an important issue for all five non-SIDS income groups, often the most important one, while it was only highlighted by the non-classified SIDS group. Respondents for non-SIDS GeoAreas appeared to have a greater desire to explore "for the science." In contrast, respondents for SIDS prioritized their subsistence and the protection of their marine environments and communities.

3.10 Training is a critical opportunity

Respondents from different income groups looked forward to seeing different capacity developments in their GeoArea. Training opportunities, for example, were the most exciting for all low, middle, and non-classified income groups of GeoAreas.

"Having the technology is important, but even more important is building capacity and long-term technical training for staff to be able to use these tools, not just to have them."–Respondent for Iraq, Western Asia

Respondents for high-income SIDS and non-SIDS GeoAreas considered less expensive data collection technologies the most exciting opportunity, with training opportunities a close second for high-income SIDS.

4 Toward an equitable deep-sea future

The global inequities in present-day deep-sea exploration and research highlighted by the 2022 Global Deep-Sea Capacity Assessment are deeply problematic (Bell et al., 2022b), presenting significant challenges and opportunities for improvement. They result in exploration, research, and conservation agendas dominated and shaped by those from high-resource countries or regions, which often goes hand-in-hand with parachute science, the norm in deep-sea research (de Vos, 2020; Stefanoudis et al., 2021; Amon et al., 2022d; Bell et al., 2022a; Harden-Davies et al., 2022b). These inequities limit humankind's ability to explore the deep ocean from a scientific perspective, resulting in a rate of research that is too slow to understand and mitigate the pressures we are already placing on this fragile environment. They prevent many nations and regions from advancing sustainable ocean-based economies and ocean-science evidence-based policies that would create jobs, support livelihoods, and promote an equitable deep-sea future for generations to come. They exclude individuals from being inspired by the deep ocean's aesthetic, spiritual, emotional, and historical value so they may become its custodians. Decolonization and equity are at the heart of protecting the wonder and health of the ocean (Bennett et al., 2021; Trisos et al., 2021).

But these inequities present opportunities for transformative change. This capacity assessment is one tool to galvanize action. It sits amidst growing awareness and calls for more equitable research and conservation, where partnerships facilitating change are genuine, durable, equitable, sustainable, and responsive to locally-identified needs (Miloslavich et al., 2018; Woodall et al., 2021; Amon et al., 2022d; Harden-Davies et al., 2022b; Sink et al., 2023; Lopes et al., in prep). From the outset of a project, these partnerships should also be co-designed, co-developed, and co-implemented through meaningful engagement and information-sharing to build a shared understanding of the objectives, aims, and desired outcomes. Sufficient time and resources must be allocated to establish effective, long-term relationships based on mutual trust and respect. These should also be accountable, inclusive, and transparent, with periodic monitoring and evaluation.

Change is already occurring. To date, 2,750 unique users from 112 countries and territories have accessed the report. Examples from this assessment were used to inform countries' and regional groups' positions on capacity building and the transfer of marine technology at the recent negotiations on the BBNJ Agreement (Amon et al., 2022a; Harden-Davies et al., 2022c; United Nations, 2023b; Lopes et al., in prep). There are also emerging technological advancements to help close the gaps in access to tools, training, and infrastructure for deep-sea exploration and research, especially in the many countries where expertise exists. These include less expensive data collection technologies such as low-cost, easy-to-use deep-sea data collection systems (Phillips et al., 2019; Dominguez-Carrió et al., 2021; Amon et al., 2022d; Bell et al., 2022a; Novy et al., 2022), as well as better data access and analysis tools (Katija et al., 2022).

All sectors, from marine scientists and engineers to business leaders, philanthropists, and governments, have a role in transforming and supporting multi-pronged approaches to increasing truly global deep-sea exploration and research efforts. Given the centering of the blue economy with a growing focus on the deep ocean and high seas (Jouffray et al., 2020; Amon et al., 2022b), as well as the continued onset of climate change and associated interventions (IPCC, 2019; Cooley et al., 2022; Levin et al., 2023), a sustainable and equitable global ocean future will certainly depend on it.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://deepseacapacity.oceandiscoveryleague.org/data.

Author contributions

KB, MQ, SP, and AH conceived and designed the study. KB, MQ, DA, and SP drafted the article. All authors executed the study, revised the article critically, gave final approval of the submitted version, and agree to be accountable for all aspects of the work.

Funding

The MIT Media Lab, Slack for Good, and Oceankind provided funds that supported this work.

Acknowledgments

Parts of this manuscript has been released as the 2022 Global Deep-Sea Capacity Assessment Report, Bell, K.L.C., Quinzin, M.C., Poulton, S., Hope, A., & Amon, D. (Eds.). (2022). 2022 Global Deep-Sea Capacity Assessment. Ocean Discovery League, Saunderstown, USA. http://doi.org/10.21428/cbd17b20.48af7fcb. It took a global team to complete this assessment and the report. It would not have been possible without many passionate people who contributed to its evolution and preparation. First, we are greatly indebted to the My Deep Sea, My Backyard, and MIT Media Lab Open Ocean teams, who ignited the spark that grew into this study. We thank our translators who significantly increased the accessibility of the survey and report: M. Primazzi, C. Valdez, S. Al-Sibani, F. Paula, A. Roa-Varon, L. Ponsoni. We thank collaborators and volunteers from DOOS, Slack for Good, Challenger 150, National Geographic Society, and other organizations and networks for sharing the survey with their communities and assisting with background research. We gratefully acknowledge our invited contributors, J. Evans, S. Talma, and Ambassador P. Thomson, for sharing their perspectives on deep-sea capacity. Finally, we owe a debt of gratitude to K. Cantner, J. Ferguson, D. LaScala-Gruenewald, J. Maurer, K. Parker, and K. VanGraafeiland for their contributions to data visualization for the report.

Conflict of interest

Author TC was employed by the company Large Marine Vertebrates Research Institute Philippines Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Marine Science

TYPE Perspective
PUBLISHED 02 October 2023
DOI 10.3389/fmars.2023.1250245

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RECEIVED 29 June 2023
ACCEPTED 30 August 2023
PUBLISHED 02 October 2023

CITATION
Cantwell K, Demopoulos AWJ, Hebner MG, Medley R, Mueller M and Netburn AN (2023) One byte at a time: gathering best practices, guidelines, and resources for data standards to support ocean exploration and characterization. *Front. Mar. Sci.* 10:1250245. doi: 10.3389/fmars.2023.1250245

One byte at a time: gathering best practices, guidelines, and resources for data standards to support ocean exploration and characterization

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Initiated through Presidential direction and now codified, the National Ocean Mapping, Exploration, and Characterization (NOMEC) Council comprises leaders from U.S. federal agencies with a shared goal of mapping all waters of the United States and exploring and characterizing priority areas. The NOMEC Council's two Interagency Working Groups, Ocean and Coastal Mapping (IWG-OCM) and Ocean Exploration and Characterization (IWG-OEC), both achieved major milestones recently with the 2023 release of the Draft Standard Ocean Mapping Protocols (SOMP) and the 2022 publication of the National Priorities for Ocean Exploration and Characterization. Building on this groundwork, the IWG-OEC is now looking to define and share best practices, guidelines, and resources for ocean exploration and characterization with the long-term goal of increasing community wide standardization to help achieve consistent common practices. First, the IWG-OEC plans to compile federal agency resources and share them in a newly developed online resource repository. The next phase is for the IWG-OEC to create opportunities for non-federal sectors to provide input on developing and populating this repository with additional content (existing standards and protocols, best practice and guidelines documents, etc.). After experts representing multiple sectors are identified, a series of results-oriented workshops are planned to provide input on all aspects of the data, products, and services from exploration and characterization. Finally, the IWG-OEC plans to widely share the online repository of best practices and standard operating procedures. A systematic, transparent, and collaborative process to share standards and protocols can help to enhance the interoperability of data and inform new lines of inquiry, discovery, research, and innovation.

KEYWORDS

NOMEC, ocean exploration, ocean characterization, best practices, standard operating procedures, SOPs, interagency coordination

Introduction

In November 2019, a Presidential Memorandum directed U.S. federal agencies to create a national strategy for mapping, exploring, and characterizing the United States Exclusive Economic Zone (EEZ) (EOP, 2019, p.64700). Seven months later in June 2020, the National Ocean Mapping, Exploration, and Characterization (NOMEC) Strategy was released (OSTP, 2020a), setting five ambitious goals and an approach to achieve them: (1) Coordinate interagency efforts and resources to map, explore, and characterize the United States EEZ; (2) Map the United States EEZ; (3) Explore and characterize priority areas of the United States EEZ; (4) Develop and mature new and emerging science and technologies to map, explore, and characterize the United States EEZ; and (5) Build public and private partnerships to map, explore, and characterize the United States EEZ (OSTP, 2020a, p.2). These goals identify some key ocean issues for our national prosperity (e.g., offshore wind and marine energy siting, management of living marine resources, coastal resilience, responding to the climate crisis, preserving marine cultural heritage, etc.) and directly and indirectly impact many federal agencies and a wide range of stakeholders including Tribal nations and Indigenous peoples.

The NOMEC Strategy established the interagency NOMEC Council to lead the execution of the Strategy and increase collaboration and coordination across the federal government, and the Council was further codified into law by the National Defense Authorization Act (NDAA) of 2023. As defined in the NDAA of 2023, "mapping" encompasses activities that provide comprehensive data and information needed to understand seafloor characteristics, such as depth, topography, underlying geologic structure, and benthic flora and fauna. "Exploration" includes activities that provide a multidisciplinary view of an unknown or poorly understood area of the seafloor, sub-bottom, or water column, and an initial assessment of the physical, chemical, geological, biological, archaeological, or other characteristics of such an area. "Characterization" refers to activities that provide comprehensive data and interpretations for a specific area of interest of the seafloor, sub-bottom, water column, or hydrologic features, including water masses and currents, in direct support of specific research, environmental protection, resource management, policy making or applied mission objectives (H.R.7776 – 117th Congress, 2022, p.136. §10302. stat. 3966-3967).

The NOMEC Council is tasked with developing and implementing multidisciplinary, collaborative, and coordinated approaches to mapping, exploration, and characterization of the EEZ (OSTP, 2020a, p.6). The NOMEC Council is currently co-chaired by the National Ocean and Atmospheric Administration (NOAA) and the United States Geological Survey (USGS). Council members include the Bureau of Ocean Energy Management (BOEM), the Bureau of Safety and Environmental Enforcement (BSEE), the Department of Defense (DoD), the Department of Transportation (DoT), the National Aeronautics and Space Administration (NASA), the National Science Foundation (NSF), the Office of the Director of National Intelligence (ODNI), the United States Coast Guard (USCG), the White House Office of Management and Budget (OMB), and the White House Office of Science and Technology Policy (OSTP) (NOAA, 2022c). The NOMEC Council is overseen by the Subcommittee on Ocean Science and Technology which is in turn guided by the Ocean Policy Committee (OPC), co-chaired by the Director of the Office of Science and Technology Policy (OSTP) and the Chair of the Council on Environmental Quality (CEQ) (NOAA, 2023).

The NOMEC Council is supported by two interagency working groups (IWGs). The IWG on Ocean and Coastal Mapping (IWG-OCM) was established in 2006 and facilitates the coordination of ocean and coastal mapping activities, and especially helping to avoid duplicative mapping activities by federal and State governments along with participating industry, academic and non-governmental entities (NOAA, 2022a). The IWG on Ocean Exploration and Characterization (IWG-OEC) was established in 2020 and is tasked with recommending and facilitating exploration and characterization efforts that provide needed information and insights about deep-water environments (water deeper than 40 meters as defined by OSTP, 2020a, p.9) of U.S. oceans, including the seafloor, sub-bottom, and water column (NOAA, 2022b). These two working groups lead efforts to achieve NOMEC Strategy Goal 2 - Map the United States EEZ, and Goal 3 - Explore and characterize priority areas of the United States EEZ. Exploring and characterizing priority areas of the EEZ is particularly challenging due to the broad range of measurements, survey types and methods, and varying mission needs and project objectives that are involved.

NOMEC Strategy Objective 3.2 is to "Establish Exploration and Characterization Standards and Protocols." (Office of Science and Technology Policy (OSTP) (2020a), p.2), noting that common standards for the collection, storage, and control of data and information collected through ocean exploration and characterization activities could maximize their benefits and utility to the nation. Developing more consensus and adopting useful standards and protocols could help to improve data interoperability and our ability to track what has already been done. The IWG-OEC was tasked by the Strategy and Implementation Plan with helping establish such common standards (OSTP, 2020a, p.17). This simply stated requirement is not so simply implemented. The task is akin to the old unattributed proverb, "How do you eat a whale?" The punchline is "One bite at a time." This analogy is useful in identifying a manageable path towards achieving complex and challenging tasks. Ambitious objectives can be achievable with careful planning and full collaboration of relevant experts in implementing a phased, iterative strategy. The NOMEC Strategy (OSTP, 2020a, p.2) defined ambitious "moonshot" types of goals for interdisciplinary ocean science data acquisition approaches, and the subsequent NOMEC Implementation Plan (OSTP, 2020b, pp.8-28) laid out a series of somewhat smaller "bites" (in the form of specific milestones) that the NOMEC Council, the two Interagency Working Groups, federal agencies, and the broader ocean community would need to address in order to accomplish the Goals.

Early NOMEC and IWG accomplishments

Over the past three years, both NOMEC interagency working groups have achieved some of the milestones outlined in the Implementation Plan, meaningfully contributing to the fulfillment of NOMEC Strategy Goals. The IWG-OCM released its "Draft Standard Ocean Mapping Protocols" (SOMP) in 2023 (OCS, 2023) and the IWG-OEC published its "Strategic Priorities for Ocean Exploration and Characterization" report in 2022 (IWG-OEC, 2022). The SOMP is a standardized technical protocol for ocean and coastal mapping data providing national standards and best practices to guide all ocean mappers in data acquisition, processing, and archiving. This protocol can be used to facilitate the widest access to, use of, and integration of data; minimize duplication of effort; and maximize the efficient collection, processing, publishing, preserving, and stewardship of as much ocean and coastal mapping data as possible into publicly accessible archives, repositories, and databases. The SOMP provides an important benchmark and sets the stage for how a community can unify around a single set of protocols and standards.

The Strategic Priorities for Ocean Exploration and Characterization report (2022) was achieved by gathering 92 subject matter experts from over a dozen federal agencies in five disciplinary subgroups: benthic ecology, cultural heritage, marine resources, seafloor hazards, and the water column. Several geographic priority areas were identified, including the Aleutian Arc, the U.S. Caribbean, the California Coastal Region, and the Pacific Islands. Public input was solicited through several listening sessions and formal Federal Register notices. The White House identified three complementary "emerging priorities" for exploration and characterization: climate change, biodiversity, and environmental justice. As early NOMEC products, there were lessons to be learned from the iterative processes for creating both the SOMP and the Strategic Priorities report. For example, there was a strong desire for greater initial participation from the non-federal sectors in developing such products.

Several of the Objectives in the NOMEC Implementation Plan (Office of Science and Technology Policy (OSTP) (2020b), e.g., p.11, p.17) detail the need for improved standardization - including the establishment of standard protocols for data acquisition and processing - of ocean data in order to make data accessible and reusable. While the SOMP provides a standardized technical protocol for acquiring, processing, and archiving ocean and coastal mapping data, no single equivalent exists in the ocean exploration and characterization space. This is partly because the fields of ocean exploration and characterization are inherently multidisciplinary with complex variables (e.g., Heidelberg et al., 2010; Danovaro et al., 2020; Egan et al., 2021; GOOS, 2021; NRC, 2003; Levin et al., 2019). Spanning disciplines from geology, chemical and physical oceanography, microbiology, ecology, and more, it is impossible to define and establish a single standard that addresses all of the variables involved in ocean exploration and characterization. As we scope the best way to build out an ocean exploration and characterization equivalent of the SOMP, it seems an appropriate and useful approach is to compile and share relevant best practices, resources, guidelines, and standard operating procedures that can help improve interoperability and usability of data rather than to attempt to strictly define the only or best ways we think each type of activity or method has to be conducted. This approach better recognizes the more open-ended, and constantly evolving nature of exploration and characterization.

We propose to build a "living library" repository of best (or "good") practices and relevant standards and protocols already in use by some. The hope is that compiling and offering these resources can help to inform data acquisition, processing, and archival and over time and voluntary adoption of the most useful practices naturally lead to more formal standardization which in turn can help improve quality. Throughout the development of this resource repository, we intend to leverage the valuable lessons learned from the SOMP activity, expanding on that approach to include both governmental and non-governmental engagement as well as additional disciplines and variables that are needed to fulfill interdisciplinary ocean exploration and characterization needs (Egan et al., 2021).

The following sections describe the planned workflow to optimize collaboration across federal agencies, academia, industry, Tribal nations and Indigenous peoples, and Non-governmental organizations (NGOs). The goal is for the community-driven development of these standards to systematic and iterative, beginning with existing guidance that can be compiled from the federal space: best practices, guidelines, and authoritative resources for ocean exploration and characterization data variables.

Initial steps

The first step in this process is to compile relevant best practices, guidelines, and resources within the federal government to create a foundational scaffolding for the discussions with the broader community (Figure 1). We plan to begin with soliciting such information from all participating NOMEC agencies. This involves engaging with a variety of subject matter experts (SMEs) across the agencies who acquire, process, and employ these different data types within their specific mission areas. These data types were identified by the discussions of the SMEs that helped guide the creation of the Strategic Priorities for Ocean Exploration and Characterization. The intent of this internal collaborative effort among the participating federal agencies is to present these resources to the community as a starting point for constructive and efficient dialogue.

Once aggregated, we plan to publicly host the federal resources on the NOMEC IWG-OEC website in a searchable and accessible format as the initial framework of the repository. The disciplinary categories identified in the Strategic Priorities for Ocean Exploration and Characterization report (IWG-OEC, 2022, p.20) can be partly used to help guide the development of the initial framework along with the nested subcategories shown in Figure 2 (e.g., acoustics). A government hosted website can serve as an authoritative resource for broader communities, both domestic and

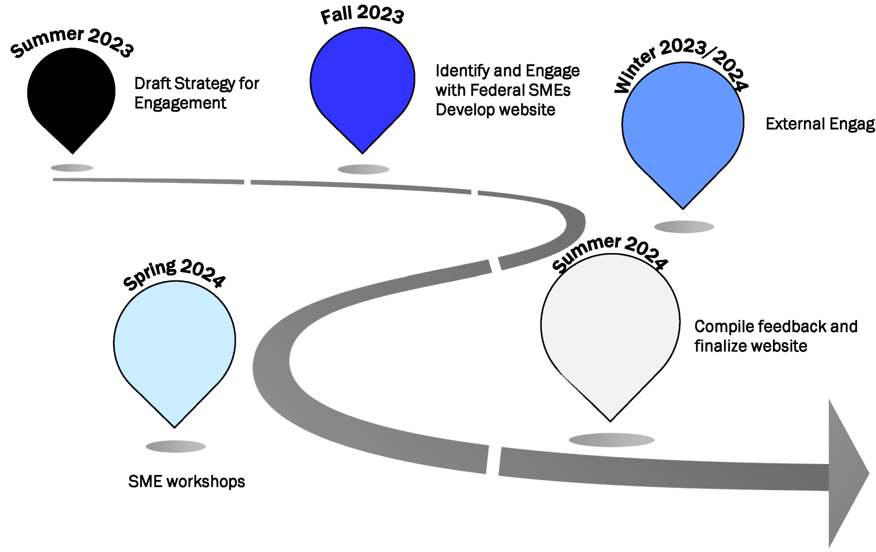

FIGURE 1
Tentative timeline for the goals of the Interagency Working Group - Ocean Exploration and Characterization (IGW-OEC); starting in June 2023 and ending next year in July 2024. Plans for summer of 2023 will focus on the development of a strategy for engagement to develop the resource repository, followed by initial engagement with federal subject matter experts (SMEs) in the Fall of 2023. External engagement begins during the Winter of 2023/2024, casting a broad net to gather input from non-governmental stakeholders. A series of workshops to refine input are planned for the Spring of 2024, culminating in the public launch as an online repository by Summer 2024.

international. The website nesting format (Figure 2) also allows for the flexibility to adapt and add to future exploration and characterization data categories, cross-link relevant documents, and inventory protocols and standards across the diverse fields of ocean exploration and characterization. Once initial input from federal agencies is received and subject matter experts are identified, the IWG-OEC plans to host a series of focused discussions to solicit external (non-federal government) stakeholder contributions to the repository.

External engagement

The objective of the external engagement phase is to build upon the inventoried federal resources by engaging stakeholders in different disciplines and fields that acquire, process, and use exploration and characterization data sets. The goal is for stakeholders to identify missing protocols, best practices, or data types that should be included and provide feedback to improve the utility of the repository website. We plan to solicit external input through science conference sessions and town halls, workshops, and written input solicited in Federal Register Notices (FRN). Awareness of various opportunities for external engagement can be communicated through science publications, NOMEC Newsletter, and posts to relevant listservs and agency social media channels. Additionally, the IWG-OEC intends to convene small working groups of experts to meet, inform, and identify the primary standards and data outputs that have the highest interoperability and utility to meet ocean exploration and characterization objectives across intersecting disciplines, missions, and sectors. The combined efforts of external engagement can help to proactively gather information from personnel who are working directly with the data types to discuss and improve community-wide guidelines and best practices for the repository.

We plan to group multi-sector subject matter experts into teams by data category or type as identified on the NOMEC IWG-OEC best practices website (Figure 2). Drawing on collaborative knowledge and open discussion, teams can begin to shape the collective vernacular around the guidelines, best practices, and eventual implementation of these standards across the community. A major focus for these teams is the end products of data for the repository: what types of data are collected (i.e., data formats) and what is done with data (i.e., data archival), thus ensuring both accessibility and security. The articulation of these parameters regarding the data is the first and most impactful step in informing and establishing community-driven and produced interoperable standards and protocols for ocean exploration and characterization.

Final product and discussion

By the end of the engagement and development process, our goal is to create a living, online repository of best practices and standard operating procedures as a helpful information-sharing resource for federal agencies and interested stakeholders. Due to the diversity of activities and data types covered, we do not intend for this repository to identify any single method as the standard, but to

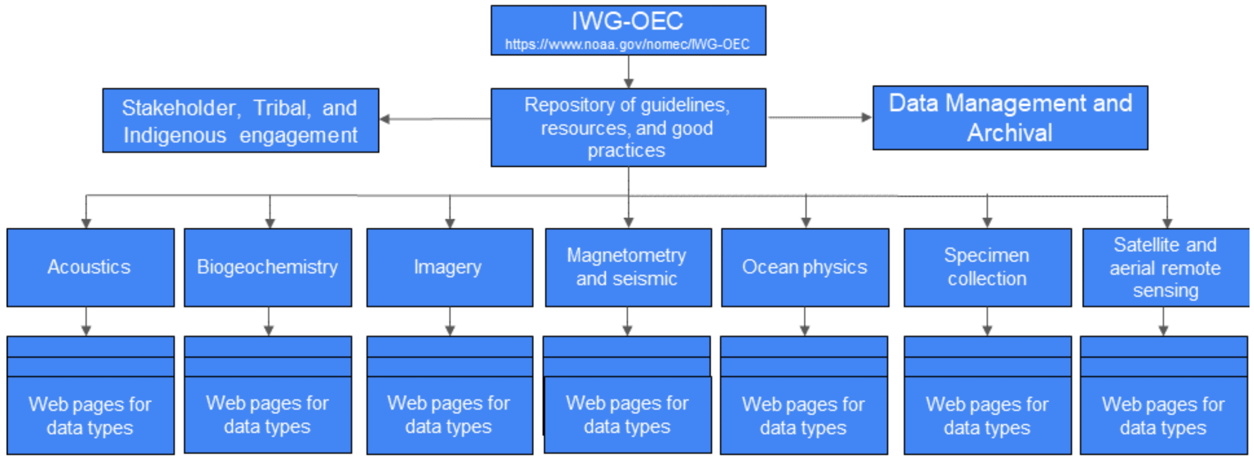

FIGURE 2
Proposed nested structure of the online resource repository to enable easy navigation of the collected best practices, standards, guidelines, and standard operating procedures. This repository is planned to be housed within the Interagency Working Group - Ocean Exploration and Characterization (IGW-OEC) webpage that is part of the greater National Ocean Mapping, Exploration, and Characterization (NOMEC) website. The initial categories proposed reflect those captured in the Strategic Priorities for Ocean Exploration and Characterization of the United States Exclusive Economic Zone (IWG-OEC, 2022).The goal is for the repository to host online resources, link to other publicly available information, and cross-referenced documents with virtual tags when applicable for multidisciplinary activities. The data types within the categories will be created are intended to be based upon feedback from subject matter experts and stakeholders, and over time the repository structure and content can evolve to best suit the needs of users.

initiate sharing of information with an open invitation for contributions and continued refinement to capture feedback from users and stakeholders. We intend for this to be an evolving resource that can avoid duplication of effort across disciplines and organizations, build consensus around best practices, and provide a path forward on standardization and interoperability of data.

Establishing the resource repository can help to enable the ocean community and any interested stakeholders to identify what works best for them and voluntarily adopt the best practices to fit their project needs. Additionally, through the collaborative development of the repository, we hope to establish a robust collection of ocean exploration and characterization best practices and standard operating procedures, creating a legacy of open access knowledge that can grow in the decades to come. As technology evolves, the IWG-OEC recognizes that there needs to be room for amendments and alterations to the associated best practices, standard operating procedures, and data formats. This initial effort to compile the collectively determined best practices and standard operating procedures can help to establish a useful baseline and serve to accelerate advancements across the ocean exploration and characterization space.

There is a strong demand and need for data standards across multidisciplinary ocean exploration and characterization communities in order to enable interoperability, accelerate innovation, and spark new lines of inquiry and discovery. The IWG-OEC is tasked with finding a way to tackle and achieve this goal. This is an ambitious goal that cannot and should not be done in isolation without the collaboration and input from federal and state agencies academia, industry, Tribal nations, Indigenous peoples, and NGOs. Substantial involvement from all interested stakeholders can help to align and maintain standards with the latest innovations and improvements. Government participation can provide authoritative information to the entire ocean community. This process of establishing best practices and standard operating procedures can help us all to achieve this daunting goal. A systematic, transparent, and collaborative process to derive agreed upon standards can enhance the interoperability of data and inform new lines of inquiry, discovery, research, and innovation across the ocean exploration and characterization community. This online repository can help the ocean community to identify and utilize the best practices and standard operating procedures to eat the whale that is ocean exploration and characterization, one byte of data at a time.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

All authors- KC, AD, MH, RM, MM, and AN, equally contributed to manuscript development and iterative edits. RM, MM, AD as the long serving co-chairs of the IWG-OEC initiated the ideas and project described in this manuscript. All authors approved the submitted version.

Funding

All funding for this work associated with this manuscript is supported by the home agencies of the authors- BOEM Office of

Environmental Programs, U.S. Geological Survey and NOAA Ocean Exploration. MGH is funded through the National Sea Grant Dean John A. Knauss Marine Policy Fellowship administered through Washington State Sea Grant at the University of Washington, Seattle, WA. Funding for the ongoing NOMEC actives described throughout this article are supported by agencies represented on IWG-OEC and the NOMEC Council.

Acknowledgments

We would like to acknowledge the past and current members of the Interagency Working Group on Ocean Exploration and Characterization (IWG-OEC) for their collaboration on IWG-OEC activities as well as the NOMEC Council, IWG-OEC, and IWG-OCM co-Chairs and Executive Secretaries, and in particular Ashley Chappel and Amber Butler, for their thoughtful edits and comments during the development of this manuscript. We would also like to thank each of the IWG-OEC participating agencies for their continued support of NOMEC activities and for striving to increase exploration and characterization of the US EEZ.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers | Frontiers in Marine Science

TYPE Community Case Study
PUBLISHED 06 October 2023
DOI 10.3389/fmars.2023.1223197

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RECEIVED 15 May 2023
ACCEPTED 06 September 2023
PUBLISHED 06 October 2023

CITATION
Rotjan RD, Bell KLC, Huber JA, Wheat CG, Fisher AT, Sylvan RL, McManus J, Bigham KT, Cambronero-Solano S, Cordier T, Goode S, Leonard J, Murdock S, Paula FS, Ponsoni L, Roa-Varón A, Seabrook S, Shomberg R, Van Audenhaege L and Orcutt BN (2023) COBRA Master Class: Providing deep-sea expedition leadership training to accelerate early career advancement.
Front. Mar. Sci. 10:1223197.
doi: 10.3389/fmars.2023.1223197

COBRA Master Class: Providing deep-sea expedition leadership training to accelerate early career advancement

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Leading deep-sea research expeditions requires a breadth of training and experience, and the opportunities for Early Career Researchers (ECRs) to obtain focused mentorship on expedition leadership are scarce. To address the need for leadership training in deep-sea expeditionary science, the Crustal Ocean Biosphere Research Accelerator (COBRA) launched a 14-week virtual Master Class with both synchronous and asynchronous components to empower students with the skills and tools to successfully design, propose, and execute deep-sea oceanographic field research. The Master Class offered customized and distributed training approaches and created an open-access syllabus with resources, including reading material, lectures, and on-line resources freely-available on the Master Class website (cobra.pubpub.org). All students were Early Career Researchers (ECRs, defined here as advanced graduate students, postdoctoral scientists, early career faculty, or individuals with substantial industry, government, or NGO experience) and designated throughout as COBRA Fellows. Fellows engaged in topics related to choosing the appropriate deep-sea research asset for their Capstone "dream cruise" project, learning about funding sources and how to tailor proposals to meet those source requirements, and working through an essential checklist of pre-expedition planning and operations. The Master Class covered leading an expedition at sea, at-sea operations, and ship-board etiquette, and the strengths and challenges of telepresence. It also included post-expedition training on data management strategies and report preparation and outputs. Throughout the Master Class, Fellows also discussed education and outreach, international ocean law and policy, and the importance and challenges of team science. Fellows further learned about how to develop concepts respectfully with regard to geographic and cultural considerations of their intended study sites. An assessment of initial outcomes from the first iteration of the COBRA Master Class reinforces the need for such training and shows great promise with one-quarter of the Fellows having submitted a research proposal to national funding agencies within six months of the end of the class. As deep-sea research continues to accelerate in scope and speed, providing equitable access to expedition training is a top priority to enable the next generation of deep-sea science leadership.

KEYWORDS

deep-sea, capacity building, education, ECR, exploration

1 Introduction and context

The deep ocean (greater than 200 m) is the largest ecosystem on planet Earth, yet, only ¼ of the deep ocean has been mapped with state-of-the-art multibeam technology (Mayer et al., 2018; Seabed 2030 Project), and considerably less of the seafloor has been observed by humans. To accelerate the pace of discovery, a multitude of efforts have emerged to bring affordable technology (Phillips et al., 2019; Dominguez-Carrió et al., 2021; Giddens et al., 2021; Bell et al., 2022a; Novy et al., 2022), accessible data (Pesant et al., 2015; Katija et al., 2022), standardized protocols (Pearlman et al., 2019), and more inclusive training (Amon et al., 2022d; Harden-Davies et al., 2022; Smith et al., 2022) to the global ocean community. These efforts are laudable and necessary and work in complement with each other to demystify the deep ocean and address goals of the UN Ocean Decade (Howell et al., 2020). Understanding the deep ocean is critical as the human population (Kramer, 2019) continues to exploit deep-sea fisheries (Watling et al., 2020), interrupt deep-sea planetary processes in terms of carbon sequestration (Teng and Zhang, 2018) and O_2 production (Gao et al., 2019), and now is on the verge of deep-sea mining and other invasive extraction activities (Amon et al., 2022b; Amon et al., 2022c). Yet, the community of deep-sea scientists is small, with relatively few deep submergence assets (Intergovernmental Oceanographic Commission, 2020; Bell et al., 2022b). Though there are Early Career Researchers (ECRs) and other community stakeholders trying and willing to engage in deep-sea research, there is little formal opportunity to access training and preparation to lead such expeditions.

Coordinating and leading a deep-sea expedition requires a wide range of skills and experience, and each individual expedition often takes months-to-years of preparation, and additional months-to-years of post-expedition work and analysis. Therefore, it is difficult for new leaders to enter the field of expedition leadership, and ECRs have limited access to targeted mentorship on leading expeditions. Even for current expedition leaders, few have received formal training, and many have trained "on the job", making unnecessary (and expensive) mistakes that could have been otherwise avoided. Expedition lead scientists often learn from being a participant on other research expeditions; however, this experience is often insufficient to gain comprehensive insight into expedition leadership, and for many ECRs, simply getting a berth and the financial support to participate on a cruise can present challenges. As such, the learning curve towards becoming a lead scientist is steep, and may indeed be too steep to enable inclusive entry for newcomers to the field without formal training in expedition leadership. Ocean sciences are among the least diverse STEM fields (Orcutt and Cetinić, 2014; Bernard and Cooperdock, 2018; Giakoumi et al., 2021; Johri et al., 2021; Legg et al., 2023), and though many countries have deep-sea ecosystems (Amon et al., 2022d; Bell et al., 2022b), few have opportunities to access them for scientific study (Osborne et al., 2022). Recent surveys show a strong interest in gaining deep-sea research capacity, (Bell et al., 2022b), but there are few, if any, training opportunities available. In countries with deep ocean environments, the lack of human capacity and training opportunities were identified as top barriers to pursuing deep-sea research (Amon et al., 2022d). Thus, while the desire to accelerate deep-ocean exploration exists globally, there remains a critical gap in opportunity and access.

To date, many programs have tried to address this gap. The US National Science Foundation (NSF) has supported numerous Chief

Scientist training programs aboard UNOLS research vessels, including those with deep-sea assets like the HOV *Alvin* (Marlow et al., 2017). Such opportunities are of course invaluable, but currently, they are only open to individuals residing in the US, regardless of citizenship, and training material products have been limited. Alternatively, multi-year training programs exist in the form of graduate degrees that include field-based experiences, but PhD programs are long, and few (if any) offer formal training in expedition leadership. Workshops can help with a specific skill, but are often too short to fundamentally alter preparation. To truly accelerate deep-sea research across the globe, the field needs formal expedition leadership training opportunities that are inclusive and equitable, open to all citizens, and that also accomplish the goal of genuine and authentic preparation for career longevity in deep-sea science (Amon et al., 2022d).

The Crustal Ocean Biosphere Research Accelerator (COBRA) is an NSF-funded international network-of-networks with a mission to accelerate research on the structure, function, resilience, and ecosystem services of the crustal ocean biosphere, which includes deep-sea seamounts, hydrothermal vents, and below the seafloor (Huber and Orcutt, 2021). One of the express goals of COBRA is to train future generations in inclusive ocean exploration, policy, research, and data accessibility. To that end, in 2022, COBRA launched its inaugural COBRA Fellowship and accompanying Master Class, which trained 12 early career Fellows in deep-sea expedition leadership (and will do so annually for five years). The goal was to equip Fellows with the skills and tools to successfully design, propose, and execute deep-sea oceanographic field research, with a collaborative, just, equitable, diverse, and inclusive approach. This 13-week virtual class included weekly synchronous, online sessions for two hours, followed by asynchronous interaction throughout the week. The "master class" concept was designed to be a class taught by masters in the field, for the upcoming generation of masters in the field. To do this, Fellows engaged in a variety of topics that included an overview of planning, at-sea, and post-expedition operations to launch their capabilities of leading future expeditions. Fellows also applied these lessons to a "Dream Cruise" project, where they assembled their ideas with resources and methodologies for a proposal of their own. To reflect the time commitment made by the participants to participate in this professional development training, a modest stipend was offered to the Master Class fellows, as is common for other NSF-funded participant support programs.

2 Key programmatic elements

2.1 Cohort selection process

Participation in virtual Master Class was offered to ECRs from around the world, and previous experience with deep-sea research or policy was not required. Early career was defined as senior graduate students, postdoctoral scientists, junior faculty less than 10 years from their terminal degree, and other junior professionals, such as an early career employee of a government, NGO, or industry. Chosen ECRs were provided a stipend for participating in the Master Class. The selection criteria included a) a background in oceanography, marine science, ocean engineering, marine policy, or related field; b) a strong interest in deep-sea exploration and research, at sea and/or from shore; c) an advanced graduate (Ph.D.) student, Postdoctoral scientist/researcher, untenured faculty, or an early career employee of a government, NGO, or industry with sufficient experience to benefit from and contribute to future expeditions; d) a compelling reason to take part in the COBRA Master Class; e) a compelling reason to contribute to COBRA's goal to create a more diverse pool of talent engaged in leading deep-sea research that addresses societal needs. To that end, applicants were asked for a CV and to answer two questions (500 words or fewer): 1) Tell us about your career goals and how becoming a COBRA Fellow will help you achieve those goals, and 2) Tell us about your journey and how you would like to contribute to COBRA's goal to create a larger, more diverse global pool of talent engaged in leading deep-sea research that addresses societal needs. Each application was then read and reviewed by two COBRA CO-PIs and/or partners, and a decision was made as to the eligibility of the applicant, given the selection criteria.

Applicants were also asked to voluntarily provide some personal identity information to help the evaluation committee to understand the demographics of the applicant pool and assess how different dimensions of diversity are maintained through the selection process, and if efforts to recruit diverse audiences are effective. COBRA aims to increase representation of diverse genders, races and ethnicities, and nations in deep-sea research, with a metric of offering programs that have more demographic diversity than the demographics of recent ocean science graduates in the U.S. (National Center for Science and Engineering Statistics (NCSES), 2022). If provided, demographic data was summarized and reported to the evaluation committee in aggregate, without personally identifying applicants. To avoid conflicts of interest, demographic data was only made available to the COBRA Director and Managing Director, who were not part of the evaluation committee. All eligible, qualified applicants (determined by the evaluation committee) were then entered into a lottery pool that was subjected to a random lottery process, re-sampling from the qualified population as needed to achieve a diverse demographic based on gender identity, race and/or ethnicity, country of citizenship, and country of residence. In this way, the COBRA team actively attempted to remove bias and authentically provide each eligible and qualified applicant with a non-ranked selection opportunity. For the 2022 Master Class, 107 applications were received, 52 were deemed to be eligible based on their qualifications, as determined by the above-mentioned selection criteria, and a randomly-selected cohort of 12 of these eligible applicants was developed to maintain the demographic diversity of the eligibility pool.

2.2 Activities/assignments and the dream cruise project

In addition to weekly synchronous classes, Fellows were assigned pre- and/or post-class reading, activities, and other tasks to support learning and to build toward a Capstone Project known as the "Dream Cruise Project" (https://cobra.pubpub.org/pub/2023-fellow-project-template). The purpose of the Dream Cruise Project was to assign guiding questions over the entire course such that the Fellows would end the class with a draft expedition prospectus for a "Dream Cruise" idea that they could then use as a scaffold for proposals or other expedition planning purposes. They were asked to write a project overview about an expedition that they would like to undertake, including the project title, objectives, team, and timeline (Supplementary Table 1). Fellows then added three objectives to challenge them to think about them in further detail regarding specific hypotheses, samples and analysis required to answer their questions, expected importance, and expertise required to accomplish their objectives. Fellows worked in teams to identify data repositories where they could find information about their areas of interest that may already exist. In addition to the science, Fellows also drafted education, public outreach, and/or communication approaches for their expedition. Fellows completed a table based on the US Department of State's Application for Consent to Conduct Marine Scientific Research table for their area of interest, or another area if theirs was not in another country's jurisdiction. Finally, Fellows were asked to prepare a 3-slide/3-minute overview about their Dream Cruise Project or prepare a presentation on any aspect of the class, which would be delivered to the COBRA instructors and Fellows during the final week.

2.3 Class/schedule/modules/topics

The Master Class met synchronously and virtually weekly, with asynchronous materials provided in advance and with follow-up. To promote asynchronous communications, a Slack channel was devoted to the course. The combination of virtual synchronous and asynchronous delivery was a major factor in successfully engaging a geographically dispersed cohort. In addition, to deliver the Master Class materials, we made use of the open-source publishing platform PubPub to create a course website (https://cobra.pubpub.org/). Class materials from 13 weeks of instruction were made available to Fellows approximately one week prior to the relevant class. Fellows' Dream Cruise Project work remains private for their intellectual use and development.

2.3.1 Week 0: kickoff

The first week of the COBRA Master Class was an asynchronous introduction to COBRA, the Fellows, instructors, and class materials. During this week, Fellows were asked to watch a short introductory video about the COBRA project, record and post an introduction of themselves in the 2022 COBRA Master Class Slack Channel, and watch the introduction videos of other Fellows and instructors to get to know the team. They were also asked to complete two surveys: the first was a pre-class survey to help the instruction team assess their knowledge and feelings about leading deep-sea expeditions, and the second was to help the instruction team identify the Master Class weekly topics that the Fellows were most excited to learn about, and those that they thought would challenge them the most.

2.3.2 Week 1: deep-sea assets

Week 1 was the first synchronous class for all Fellows and instructors. The goals were to: (1) give an overview of deep-sea exploration and research tools, including costs, availability, procurement, etc., (2) introduce the Global Deep-sea Capacity Assessment (Bell et al., 2022b), (3) explore how to access deep-sea assets from different countries and institutions, and (4) discuss the importance of relationship building in finding seagoing opportunities. During this class, COBRA instructors gave an overview of deep-sea research assets, such as ships and deep submergence vehicles. The class covered capacity gaps around the world, and in particular, what kinds of technological capacity exists (or not) in different regions. During class, Fellows worked in teams to investigate the capabilities and availability of assets, and discussed their findings with the class. The class ended with a discussion on relationship-building within the oceanographic community to facilitate access to seagoing assets.

2.3.3 Week 2: funding & proposals

The goals of Week 2 focused on Funding & Proposals were to: (1) provide an overview of proposal structure and important components to consider in writing, (2) explore how to leverage different programs and funding models for getting to sea and doing your work, (3) introduce Fellows to the Schmidt Ocean Institute proposal and funding model, and (4) develop and discuss Fellows' own proposal ideas. COBRA instructors gave an overview of scientific proposal writing, including structure, operational constraints to consider, how to partner or leverage institutions or resources, different funding models, and more. Class discussion highlighted the Schmidt Ocean Institute (SOI) and provided information about SOI's proposal and funding model, which is multinational. Fellows were asked to come to Week 2 prepared with a proposal idea for their "Dream Cruise" that they would use throughout the Master Class to develop a deep-sea proposal. During class, Fellows presented their ideas to small groups in breakout sessions to discuss progress, receive feedback, and think more broadly about their ideas and potential ways to express those ideas to demonstrate need and importance to resolve proposed questions.

2.3.4 Week 3: respectful concept development

Week 3 focused on Respectful Concept Development, with goals to: (1) understand how to build relationships with diverse stakeholders that might be interested in research, and how to engage in equitable co-creation of knowledge before developing proposals; (2) discuss best practices for recruiting and promoting diverse and inclusive teams; (3) acknowledge the history of discrimination in deep-sea science; and (4) begin to unlearn parachute science approaches, with case studies in Trinidad & Tobago and Kiribati. Two guest lecturers from the NOAA Office of Ocean Exploration and Research joined Week 3 to introduce common barriers to entry, persistence, advancement, and success for marginalized and minoritized scholars in STEM, and share findings from a study on supporting marginalized team members at sea (Kelly and Yarincik, 2021; Amon et al., 2022a). Additional resources were also provided to Fellows (and are available on our PubPub site) to provide information on Anti-Harassment, Safety in the Field, Training & Support Services, Reporting & Response. COBRA instructors then discussed two case studies from Trinidad and Tobago and Kiribati under the My Deep-Sea, My Backyard project, which was established in 2018-2019 to develop long-term deep-sea capacity in these Small Island Developing States (SIDS) (Amon et al., 2022d). Presentations led to discussion between Fellows, instructors, and guest lecturers on how to change processes from applications through project execution to ensure equitable access to and support for opportunities in deep-sea exploration and research.

2.3.5 Week 4: pre-expedition planning

Week 4 addressed Pre-expedition Planning and focused on determining: (1) how to assess and prioritize the science needs of the expedition team and to communicate these needs with vessel operators, (2) what a Shiptime/Marine Equipment Request form might include, (3) what to consider when working in various ports regarding permitting and logistics, (4) what types of content are helpful to have in a Cruise Prospectus, and (5) the primary role of the Lead Scientist, which is to set the expectations for preparedness, safety, civility and respect on the expedition. Being a Lead Scientist is a big responsibility, but it is made easier by planning ahead and partitioning tasks into smaller and more manageable goals. COBRA instructors led discussion and activities to help Fellows think through the steps needed to prepare for an expedition as the Lead Scientist, divide responsibilities among the research team, and engage expedition participants for a successful and enjoyable experience at sea. Example documents and guides for pre-expedition preparation were provided, including shiptime requests, expedition prospectuses, and permit applications. The Marine Facilities Portal website (mfp.us) was highlighted as a tool for expedition planning, given many agencies and institutions in Europe (Netherlands, Germany, Spain, Finland, Sweden, Belgium), the UK, and the US are now using this for expedition planning.

2.3.6 Week 5: at-sea operations

During Week 5, which focused on At-Sea Operations, Fellows built on the foundation of Week 4, examining more closely the activities that a lead scientist leads or delegates while at sea. Fellows were introduced to (1) planning and documentation of the expedition (plan-of-the-day, dive plans, reports, contingencies, etc.), (2) how to prepare a general operational plan that considers the time required to complete operational objectives (e.g., bathymetric mapping, transit, ROV/submersible operations, etc.), (3) approaches for assigning shifts, and (4) how to set the tone for maintaining good team relations and a healthy work environment. COBRA instructors reviewed the plan-of-the-day, dive plans and associated documents, daily logs and situation reports, dive reports, sample logs, video and photo archives, and expedition reports. They discussed how to prepare a team for the various tasks that need to happen pre-, during-, and post-dive or operation, and how to help a team succeed while maintaining physical, mental and emotional health. They also reviewed operations for mobilization and demobilization. Fellows and instructors had an open discussion at the end of the class about leading at-sea operations, particularly focused on looking after teammates at sea to ensure good health and a respectful working environment.

2.3.7 Week 6: telepresence

For Week 6, which was focused on Telepresence, the goals were to understand: (1) what telepresence is and (2) the strengths, opportunities, weaknesses and challenges of telepresence with regards to science and outreach. A COBRA instructor and guest lecturer discussed telepresence technology and its power, goals, strengths, and weaknesses. Fellows also participated in a live telepresence interaction with scientists aboard EV *Nautilus*, which supports the ROV *Hercules*. This opportunity enabled Fellows to participate in real-time data streams and use the scientist ashore portal to get a glimpse of the shipboard experience (Wagner, 2023).

2.3.8 Week 7: unwritten rules

Week 7 focused on some of the “unwritten rules” of deep-sea science, which are different from other areas of ocean science that may require less collaboration by design. Goals of this week were to: (1) get an operational view of the shipboard “rules of the road,” (2) discuss the protocol, convention, and guidance for naming new seafloor features, sites, and species, and (3) discuss sometimes contentious subjects such as authorship, fundraising responsibilities, and collaboration expectations. COBRA instructors and guest lecturers discussed Chain of Command and how to interact with the ship’s operation teams as a leader or member of the science party; about life on a ship, including how to be respectful of a ship’s crew, safety, and other issues that are unique to living and working at sea; on guidance for naming discoveries, and collaboration etiquette with regard to data sharing, publication authorship, and funding.

2.3.9 Week 8: introduction to deep data

In Week 8, we focused on Introduction to Deep Data, and the goals were to: (1) familiarize Fellows with existing data repositories, (2) enhance data discoverability by learning to find desired publicly-available data types and datasets for regions of interest, and (3) determine feasibility of at-home data use and what requires pro-software. This class focused on discoverability and use of existing data. COBRA instructors reviewed data repositories, types of data repositories, and how to extract and display data. The class focused on GeoMapApp as a case study of one innovative open-access data repository linked to a data manipulation and display application. In preparation for this class, Fellows researched one or more locations to identify and access data including bathymetric data, but also searched for data types and datasets most relevant to their ‘Dream Cruise’ projects. The class discussed selected examples of physical repositories that contain seafloor and sub-seafloor samples, such as the Smithsonian and IODP Core Repository, and discussed how researchers can discover available sample types and place requests to obtain desired samples. Fellows also discussed how to cross-reference data, and data limitations (including missing data, and QA/QC issues). During the class Fellows produced a bathymetric map.

2.3.10 Week 9: data management plan to cruise report

Week 9 focused on Data Management Plans to Cruise Reports and built upon lectures and work introduced in Week 8. Goals for this week were to: (1) learn about data management plan (DMP) goals, components, and formats for metadata, digital data, sample data, and engineering plans; (2) understand various data storage challenges and strategies; (3) create a data management plan for the Fellows' Dream Cruise; (4) learn about goals for expedition reports, components, and formats; and (5) create an expedition report outline for Fellows' proposed Dream Cruise. COBRA instructors explored the details of DMPs and expedition reports. They reviewed the necessary components of DMPs, as well as a range of styles and resources to help create them. In the first half of this class, Fellows worked in breakout groups to begin drafting their own DMPs. In the second half of the class, discussion focused on different types of expedition reports and the requirements for each. Fellows were then provided the opportunity to revise their DMPs.

2.3.11 Week 10: education & outreach

Week 10 focused on connecting education and public outreach (EPO) with oceanographic expeditions, as this is becoming increasingly common, and is thus an additional layer of planning, execution and assessment for a lead scientist to manage. The goals for Week 10 were for Fellows to: (1) understand the types of common EPO connected with deep-sea oceanographic expeditions, what is required to implement them, and common pitfalls; (2) understand where to get communication training to create effective products; and (3) understand what kinds of assessment tools, processes, and metrics are useful and often required by funders. COBRA instructors reviewed some of the common ways that EPO is incorporated into field work, what is required to implement them (technology and personnel), and some of the common pitfalls of such activities. Two guest lecturers spoke during the first half of the class, about their paths in science communication to help Fellows think creatively about the potential gamut of at-sea and post-expedition EPO opportunities. Finally, COBRA instructors discussed assessment tools, processes, and metrics that are used to gauge the efficacy of EPO activities, and how to describe these for inclusion into proposal planning.

2.3.12 Week 11: international ocean law

Week 11 focused on ocean law and policy, which are critical when conducting oceanographic work within other countries' maritime jurisdiction and, increasingly, in areas beyond national jurisdiction. Goals were for Fellows to: (1) understand maritime jurisdictions and what can and cannot be done within different jurisdictions, (2) understand the process for applying for Marine Scientific Research authorization in another country's exclusive economic zone (EEZ), (3) learn about effective international collaborative research strategies and how they can lead to policy-relevant outcomes, and (4) become familiar with the ongoing negotiations for a new high-seas treaty and understand the pros and cons of such a treaty. In preparation for this class, Fellows were asked to complete an application for consent to conduct Marine Scientific Research in another country's EEZ. Guest lecturers from the US Department of State joined the class to review maritime jurisdictions to understand roles and responsibilities within the different areas, as well as discuss the process by which scientists obtain authorization to conduct work in other countries' waters. Other guest lecturers discussed effective international collaborative research strategies and how they can lead to policy-relevant outcomes and ongoing negotiations for a forthcoming high-seas treaty and its implications for high-seas research.

2.3.13 Week 12: team science

Week 12 focused on Inclusive Team Science with goals for Fellows to (1) understand benefits and challenges of participating in diverse and inclusive science teams, and (2) learn practical strategies for participating in and leading diverse science teams. During this class, guest presenters described the social science research on high-performing teams and provided practical steps Fellows can take to lead and participate in effective collaborations. Fellows then participated in an exercise on team building and inclusive communication to understand how effective teams work together. COBRA instructors discussed factors that predict collective intelligence and the beneficial impacts of diverse teams. Finally, the class discussed how to attract and retain diverse teams, reduce status differences, ensure equitable opportunities, tackle goal differences and knowledge utilization, integrate team members to avoid tokenization, recognition and rewards, build and maintain team trust, resolve conflicts, and start and fund collaborations.

2.3.14 Week 13: on the horizon

The final week of the COBRA Master Class was an opportunity for Fellows to present their work, receive feedback, discuss preparing the open access manual, and share ideas for future engagement as a cohort within the COBRA community. The majority of this class was dedicated to Fellows sharing their class experience. Fellows had three minutes to present a topic of their choice. Many of them discussed the development of their Dream Cruise projects, focusing on their progress from initial concept to expedition prospectus. One Fellow focused on the challenges that they encountered and how they took a different approach to the assignment, preparing them for a future expedition proposal. Prior to the class, Fellows were asked to complete a post-class survey to assess their attitudes on key metrics for the course (described below). The class ended with a presentation of the change in attitudes before and after participating in the Master Class, followed by a discussion on what Fellows thought went well during the class and what could and should change for the future. The final topic of discussion was how Fellows would like COBRA to support sustained engagement following the Master Class, including preparation of an open-access manual based on their learnings that can be used by the following year's cohort.

3 Assessment

3.1 Pre- and post-class surveys

Optional surveys were given to Fellows at the beginning and end of the Master Class to assess readiness and preparation on class topics. All Fellows completed the pre- survey, with one Fellow not completing the post- survey. The following questions were asked in each survey to assess changes in attitudes after participating in the Master Class using a Likert scale (Figure 1):

1. I know where to find information about deep-sea research vessels and assets.
2. I know where to look for funding for deep-sea expeditions.
3. I understand what "co-creation of knowledge" means in the context of working with local and/or Indigenous communities.
4. I know what information should be included in a Cruise Prospectus.
5. I know how to create a Plan-Of-The-Day.
6. I understand how telepresence can be used for scientific purposes and for outreach.
7. I feel confident in my ability to help resolve conflicts with diverse teams.
8. I know how to prepare a Data Management Plan.
9. I know how to archive and report on all data types generated during a deep-sea expedition.
10. I understand the difference between international and national jurisdictions.
11. I understand how to apply for international and national permits.
12. I am ready to submit a proposal to use a deep-sea research ship or asset.
13. I am ready to lead a deep-sea expedition.

In addition, the post-survey asked an additional Likert scale and free-form response questions to assess overall satisfaction with the Master Class (Figure 2):

1. I feel like I was respected by the instructors during my participation in the COBRA Master Class.
2. I feel like I was respected by the other participants in the COBRA Master Class.
3. I think the level of instruction was appropriate.
4. Receiving a stipend was a key factor in my ability to participate in the Master Class.
5. I would recommend the Master Class to a friend.
6. Free-form response: What was the most valuable topic for you?
7. Free-form response: What was the least valuable topic for you?
8. Free-form response: What topic do you wish we had spent more time on?
9. Free-form response: Suggestions for how the Master Class could be improved?

Overall, the assessment indicated that attitudes improved (i.e., more agree/strongly agree compared to disagree/strongly disagree) for all categories (Figure 1). The biggest shifts in opinion were in readiness to propose and lead a deep-sea research expedition, indicating that the Master Class was successful in boosting the confidence of participants to be deep-sea expedition leaders. Some notable anecdotal feedback from fellows included:

Fellow A:

> "More than a topic it was everything as a whole because it made me start thinking as a Chief Scientist! Something just clicked for me, perhaps with the knowledge that I was acquiring every week, my confidence increased."

Fellow B:

> "The course was really helpful to boost my confidence. The learning curve is steep, but here I go … MUCHAS GRACIAS."

When asked to identify the class topic that Fellows deemed most valuable, every topic had at least one person saying it was the most valuable, suggesting that the class successfully offered a wide range of topics to bolster the self-identified priorities across the diversity of the participating Fellows. When asked to identify class topics that were least valuable, there were very few requests to de-emphasize something, and feedback was constructive and minor. Overall, participants requested more time on each topic, suggesting that the two-hour synchronous online sessions were not too lengthy or onerous. Though there was a Slack channel for asynchronous discussion, more infrastructure for asynchronous learning would be helpful to deepen understanding of topics without expanding synchronous session-time.

In addition to the pre- and post-class surveys, Fellows were also asked to select the five topics that they were "most excited to learn about" and the five topics that they thought would "challenge them the most." The most-selected topics that respondents were excited about were Funding & Proposals (100%) and Cruise Preparation (73%). The most-selected topics that respondents thought would challenge them the most were Data Management Plan to Cruise Report (82%), Funding & Proposals (73%), and International Ocean Law (64%).

3.2 Public access to course resources

In addition to supporting the Fellows and the class, the COBRA PubPub site (https://cobra.pubpub.org/) was also intended to be a public resource for deep-sea expedition leadership and planning. All class plans, readings, videos, etc., are publicly available. In the 82 weeks between the creation of the PubPub site (Feb 17 2022) and the page proofs submission for this manuscript (September 17 2023), 1256 users from 69 countries and territories have accessed 22,902 page views on the site (Figure 3).

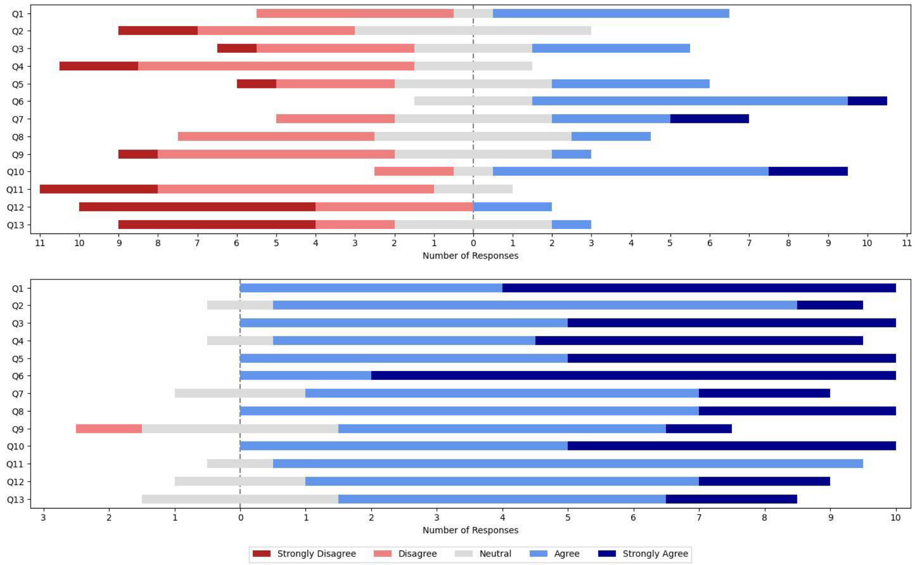

FIGURE 1
Comparison of COBRA Master Class participant attitudes pre- (top panel, N = 12 respondents) and post-class (bottom panel; N = 10 respondents) for 13 assessment questions based on a Likert scale of 1=Strongly Disagree, 2=Disagree, 3=Neutral, 4=Agree, 5=Strongly Agree. Colors correspond to the Likert scale rating; while the X-axis denotes the number of respondents who listed each ranking. Survey questions are found in section 4.1 of the manuscript.

4 Discussion

Expedition leadership in the modern era transcends technical skill alone: good leaders create strong and diverse teams, inclusive, safe and creative spaces, efficient and productive workflows, and enable cross-team competency and growth in addition to the scientific goals and deliverables of a deep-sea expedition (Amon et al., 2022c; Johannesen et al., 2022; Shellock et al., 2022). There is an increased emphasis on team science in the deep-sea, and the skill sets required are essential for future leaders (Bennett et al., 2013; Bennett et al., 2014; Hall et al., 2018). Some of these hard and soft skills can be taught by example, but life on-ship is busy, and a trainee rarely gets the full access to every part of the expedition leadership and decision-making process. Thus, there is a need to more thoughtfully and deliberately advance and accelerate the training of early career deep-ocean leaders to help diversify the leadership pool and advance the stated outcomes of ocean exploration, conservation and science in a team setting.

At the same time, there is a practical need to train early career deep-ocean leaders to maximize productivity and minimize costs that can occur when "learning while leading." Deep-sea shiptime is expensive, ranging from $10,000-100,000+ USD per day, and subpar leadership can waste substantial funds in lost productivity. To reduce that inefficiency, lead scientist training efforts have been developed for US-based deep submergence assets. This training effort is a valuable and critical tool to help burgeoning PIs with developing the at-sea experience and post-award period, however, a comprehensive approach to the lead scientist experience,

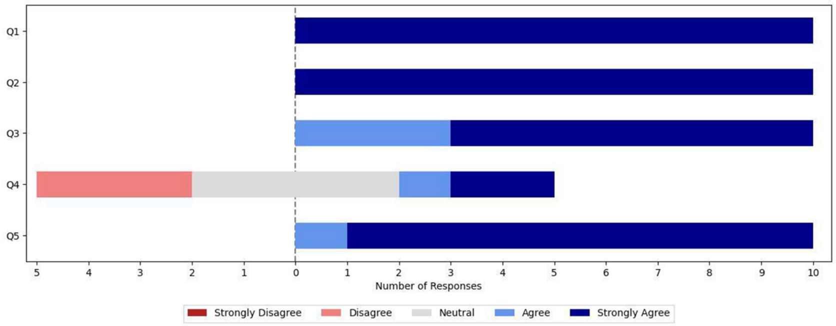

FIGURE 2
Post-class assessment of opinions about whether the COBRA Master Class met expectations. Using a Likert scale of 1=Strongly Disagree, 2=Disagree, 3=Neutral, 4=Agree, 5=Strongly Agree. Colors correspond to the Likert scale rating; while the X-axis denotes the number of respondents (N = 10) who listed each ranking. Of note, all participants strongly agreed that they were respected in class (Q1), there was strong agreement that level of instruction was appropriate (Q2), and all participants agreed that they would recommend the class to a friend (Q5). While receiving a stipend to participate was not important for some, it was important for others (Q4).

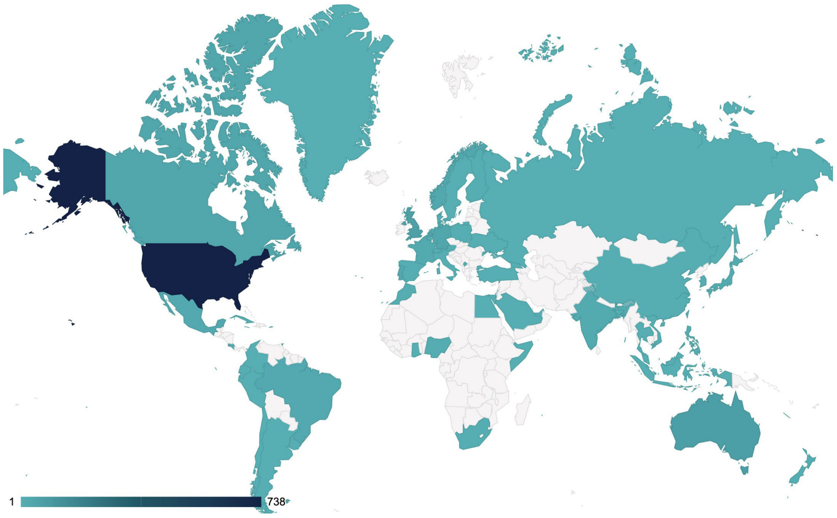

FIGURE 3
Locations of 1256 users from 69 countries and territories (as of 17 September 2023). Color bar denotes number of users per country.

ranging from proposal preparation to final expedition report, is lacking.

For all of the above-mentioned reasons, many potential deep-sea PIs do not achieve lead scientist status as quickly as they are capable of, thus delaying their discoveries for science as well as their own career advancement. In an effort to accelerate scientific understanding of deep-sea ecosystems and their resilience to inform decision making, prevent serious harm, and provide benefit to society, deep-ocean research remains critical and can only be enabled by nurturing and fostering the next generation of deep-ocean scientists and expedition leaders. The COBRA Master Class was the first attempt at an intermediate scale training program. Based on participant feedback and demonstrated outcomes of getting involved in expedition leadership since participating - the inaugural year was a success. Moreover, the COBRA Master Class has created an intermediate time investment model for future training for this (and other) topics of similar complexity.

Specific successes for this inaugural expedition from the Fellow perspective were evident from the pre- and post- class surveys. The biggest shifts in opinion were in readiness to propose and lead a deep-sea research expedition, indicating that the Master Class was successful in boosting the confidence of participants to be deep sea expedition leaders. Fellows also report cohort cohesion and have maintained communication through the COBRA Slack Channel and other COBRA activities. The biggest indicator of success will be demonstrated by the number of Fellows who lead deep-sea expeditions, and to-date, several fellows have submitted proposals to do so (one quarter of the Fellows have submitted their "Dream Cruise" within 6 months of completing the Master Class).

Despite the success of the COBRA Master Class, there were some noted challenges, namely time and balance. Because this class was designed to accommodate working professionals within the context of a normal work week, synchronous time was limited to two hours per week and asynchronous assignments were typically optional. As such, the depth of coverage for each topic was never wholly complete - Fellows commented that each week could have easily been a month of conversation and activity. However, to achieve balance and avoid burnout and fatigue, the content balance provided was sufficient to achieve the COBRA goal of introducing each topic in detail, and providing the follow-on resources, contacts, or tools to enable Fellows to subsequently self-propel. In addition, the balance of time spent on each topic was designed to benefit Fellows regardless of prior preparation. Each topic was covered in sufficient introductory detail for a novice, but also covered in enough advanced detail for a working expert and to glean insights and advance their toolset and skillset. Future iterations of this Master Class will keep the existing balance, but as the class grows, the additional on-line (Pubpub) and written resources will continue to grow as well, thereby providing additional depth of coverage for future COBRA Fellows and the community in general.

One key component of the Master Class model is class size. We found that twelve Fellows was sufficient to generate lively and substantive conversation, but could also ensure space and time for every voice to be heard. In addition, the relatively small cohort size was essential to facilitate contemplative and productive small working groups and partnered activities. The drawback to a small cohort is the number of Fellows trained, but with repeated classes (five total over five years), sixty fellows will be trained over the lifetime of this current program. In addition, online resources can benefit the community at large, and it is hoped and expected that COBRA Fellows will use these materials to help train ECRs under their leadership. Because of the relatively small size of the deep-sea PI community, the contribution of sixty new, capable expedition leaders dramatically increases the existing PI pool. It should be noted that there are many current attempts to broaden and diversify the deep-sea community, and it is hoped that corresponding assets and resources will be amplified to accommodate our growing community.

5 Conclusions

As deep-sea research continues to accelerate in scope and speed, providing equitable access to expedition training is a top priority to enable the next generation of deep-sea science leadership. The COBRA Master Class has generated a successful model for training 12 ECR Fellows at a time, with additional open-access training materials that are freely available to all. While there is no substitute for at-sea training, the Master Class model represents a more modest time investment with a comprehensive approach to expedition leadership, including pre- and post-expedition expectations and responsibilities of a Lead Scientist. This online, 14-week training model involving synchronous and asynchronous activities is an important bridge between targeted workshops and at-sea apprenticeship that will accelerate deep-sea leadership, and therefore deep-sea research and stewardship across the globe.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

All co-authors participated in the co-design, co-development, participation, and execution of the COBRA Master Class. RDR and KLCB co-led the drafting of this manuscript. BNO, JAH, JM, RDR, CGW, ATF secured the funding for this project. BNO, JAH, and RDR led the conceptual design of the Master Class, with curriculum development input from KLCB, CGW, ATF, JM, and RLS. All authors contributed to the article and approved the submitted version.

Funding

The Master Class is an element of the COBRA network-of-networks project funded by the National Science Foundation as part of the AccelNet program (award OISE-2114593 to BNO).

Acknowledgments

COBRA is grateful to many Subject Matter Experts who contributed expertise to the 2022 Master Class, including: Kasey Cantwell, Gabriella David, Allan Doyle, Barbara Endemaño Walker, Dijanna Figueroa, Peter Girguis, Brian Kennedy, Lisa Levin, Catalina Martinez, Leonard Pace, Lu Wang, Amanda Williams, and Karen Young. We are grateful to the deep-sea community for collectively training all of us and providing the inspiration to formally launch this Master Class in expeditionary leadership. The National Science Foundation does not assume responsibility for the findings or the interpretations. Survey data collected is exempt from US federal requirements for human subject research according to the NIH Office of Extramural Research, especially as the subjects are co-authors on this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1223197/full#supplementary-material

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frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 23 October 2023
DOI 10.3389/fmars.2023.1219368

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RECEIVED 09 May 2023
ACCEPTED 03 October 2023
PUBLISHED 23 October 2023

CITATION
Selig GM, Drazen JC, Auster PJ, Mundy BC and Kelley CD (2023) Distribution and structure of deep-sea demersal fish assemblages across the central and western Pacific Ocean using data from undersea imagery.
Front. Mar. Sci. 10:1219368.
doi: 10.3389/fmars.2023.1219368

Distribution and structure of deep-sea demersal fish assemblages across the central and western Pacific Ocean using data from undersea imagery

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Demersal deep-sea fish assemblages from islands and seamounts are poorly described, even in the Hawaiian archipelago. Knowledge across all depths, in similar settings, is even sparser for other archipelagos in the central and western Pacific. However, recent remotely operated vehicle (ROV) explorations and archived video from human-occupied submersible dives conducted by the Hawai`i Undersea Research Laboratory (HURL) provide an opportunity to explore the structure of these assemblages. Here we describe demersal fish assemblages across the central and western Pacific, including in four Marine National Monuments, and examine the relationship of the assemblages to depth and environmental conditions. We used data collected from 227 underwater vehicle dives resulting in the identification of 24,837 individuals belonging to 89 families and 175 genera. The most frequently occurring genera at depths of 250-500 m were *Epigonus*, *Setarches*, *Polymixia*, and *Antigonia*, between 500-1000 m were *Chlorophthalmus*, *Aldrovandia*, and *Neocyttus*, and between 1000-3000 m were *Synaphobranchus*, *Kumba*, *Halosaurus*, *Ilyophis*, and *Ipnops*. There are strong changes in the fish assemblages with depth and region, and assemblages become more similar between regions with greater depth. Depth and region explained the most variance in assemblage structure followed by seafloor particulate organic carbon flux (a food supply proxy), concentrations of dissolved oxygen, and salinity. The Line Islands and Tokelau Ridge had the highest values of seafloor particulate organic carbon flux for all depth zones investigated (250-3000 m) and the highest abundance of fishes at 250-500 m and 500-1000 m, respectively. Taxon accumulation curves indicated that diversity at the genus level within all regions and depth bins (except 1000-2000 m and 2000-3000 m) had not been reached with the existing sampling effort. However, when combining samples from all regions, diversity generally appeared to decrease with depth. Overall, this study demonstrates that there are significant regional differences in the composition of the deep-sea fish fauna as well as differences across depth. Such distribution patterns suggest that the four

Marine National Monuments (Papahānaumokuākea, Marianas Trench, Pacific Remote Islands, and Rose Atoll Marine National Monuments, encompassing an area of 3,063,223 km^2) are not replicates of diversity, but complementary components of the regional fauna.

KEYWORDS

seamount, marine protected area, marine monument, video, remotely operated vehicle (ROV), community ecology, diversity, conservation

1 Introduction

Demersal fish of economic importance are concentrated on the continental shelf and slope to about 1500 m depths (Khedkar et al., 2003; Pitcher et al., 2008), although fisheries for a few deep-sea demersal species also occur on seamounts, guyots, and similar features (Clark et al., 2010). World catches of demersal species increased rapidly during the 20th century and the limits of biological production have been reached in many areas (Khedkar et al., 2003). Therefore, with the potential for changes in surface primary production induced by climate change to alter standing stocks in the food-limited deep-sea (Glover and Smith, 2003; Smith et al., 2008; Brito-Morales et al., 2020), it is important to understand demersal fish assemblage structure as a key characteristic of these ecosystems.

There have been efforts across the globe to characterize demersal fish assemblage structure, and these have been mostly concentrated on continental shelves and upper slopes, with fewer studies off oceanic islands or on seamounts (Clark et al., 2010; Drazen et al., 2021). The focus has been in the southwestern and southeastern Pacific Ocean (e.g., Koslow et al., 1994; Francis et al., 2002; Tracey et al., 2004), off Japan (e.g., Fujita et al., 1995), the Nazca and Sala-y Gomez Ridges (Parin, 1991; Parin et al., 1997; Tapia-Guerra et al., 2021), across the central Pacific Ocean (Drazen et al., 2021), and in several areas of the North Atlantic (Colvocoresses and Musick, 1984; Haedrich and Merrett, 1990; Mahon et al., 1998; Menezes et al., 2006; Bergstad et al., 2008; Menezes et al., 2009; Morato et al., 2009; Amorim et al., 2017; Parra et al., 2017). These studies have found that depth is a strong driver of assemblage structure with major shifts in fauna between the continental shelf (0-200 m), upper slope (200-600 m), mid-slope (600-800 m), and deep mid-slope (800-1200 m). These studies also found that upper bathyal species exhibited more narrow geographic distributions while deeper species were distributed over broader areas.

Gilbert (1905) and Struhsaker (1973) were the first investigators to focus on demersal fishes in the central Pacific region using trawls to sample species in the Main Hawaiian Islands. Chave and Mundy (1994) synthesized a decade of submersible observations on more than 250 demersal fish taxa between depths of 40 and 2000 meters in both the Main Hawaiian Islands and Northwestern Hawaiian Islands, Johnston Atoll, and Cross Seamount from 1982 to 1992. More recent studies have taken place in the Main Hawaiian Islands (Yeh and Drazen, 2009; De Leo et al., 2012; Oyafuso et al., 2017) and on seamounts in the NWHI (Mejía-Mercado et al., 2019). These studies and those from other regions document changes in assemblage structure with depth. Environmental factors that could be driving these differences, while correlated with depth, are changes in water mass properties (i.e., salinity, temperature, and density), substrate type, food, and oxygen availability (e.g., Labropoulou and Papaconstantinou, 2000). Many of these factors covary with depth. Thus, generalizing how these environmental gradients are correlated with the variation in the distribution and assemblage structure of deep-sea fishes could be greatly expanded by surveying large spatial scales and depth gradients in different regions with variable environmental characteristics.

A recent expansion in ROV exploration throughout the central and western Pacific now provides a means to evaluate demersal fish assemblage structure at a broad scale. NOAA's CAPSTONE: Campaign to Address Pacific monument Science, Technology, and Ocean NEeds used the NOAA Ship *Okeanos Explorer* and *Deep Discoverer* ROV to conduct dives between 2015 and 2017 from the Marianas to American Samoa (Kennedy et al., 2019) and produced a unique data set of deep-sea fishes identified from underwater imagery. Other expeditions using *EV Nautilus* and ROV *Hercules* between 2018 and 2019, as well as DSV *Pisces* IV and V submersible surveys by the Hawai`i Undersea Research Laboratory (HURL) in 2005-2013, augmented the CAPSTONE data and filled geographic gaps in coverage. All dives and their respective imagery platforms will be noted as *EV Nautilus*, HURL and CAPSTONE hereafter. These datasets provided an opportunity to explore the relationships between fish assemblages and associated geographies and environmental conditions.

In this study, data from undersea imagery were used to survey demersal fish assemblages across varying oceanographic characteristics in the central and western Pacific. Our objectives were to: 1) determine whether the composition, total abundance, and diversity of demersal fish genera differs between regions, and 2) examine what abiotic factors including temperature, depth, salinity, oxygen, and POC flux explain fish distributions and assemblages. All these factors have been postulated to cause faunal zonation with depth (e.g., Labropoulou and Papaconstantinou, 2000) which also may be related to deep-sea water masses and their specific values of temperature, dissolved oxygen, salinity, and density (Richards et al., 1993; Grothues and Cowen, 1999; Galarza et al., 2009). Water masses have been shown to influence the physiology and distribution of organisms in the water column, however studies on deep-sea fishes have found some variables to have a stronger influence than others (Koslow et al., 1994; Yeh and Drazen, 2009).

2 Methods

2.1 Underwater vehicle surveys

Opportunistic data were acquired from underwater vehicle surveys carried out during cruises from multiple expeditions in the central and western Pacific. We used data from NOAA's three-year Pacific-wide field campaign CAPSTONE that investigated the biodiversity of deep-sea taxa across depths in American Samoa, Johnston Atoll, Line Islands, Main Hawaiian Islands, Musicians Seamounts, Northern Marianas, Northwestern Hawaiian Islands, Southern Marianas, Tokelau Ridge, and Wake Island between July 2015 and September of 2017 (168 dives, 891.5 hours, 0-6000 m depth) (Kennedy et al., 2019).

In addition to the CAPSTONE dives, data from four *EV Nautilus* cruises (NA101, NA110, NA112, and NA114) between 2018 and 2019 were used from multiple areas within the central and western Pacific (36 dives, ~218 hours, 0- 2459 m) (Kelley et al., 2019), and from submersible dives conducted by HURL between 2005 and 2013 in the Main and Northwestern Hawaiian Islands (the Papahānaumokuākea Marine National Monument) (96 dives,~576 hours, 0-2000 m depth), to fill gaps in geographic extent.

Nearly all data from HURL were collected after 2007 when the use of Ultra-short Baseline (USBL) acoustic positioning systems were implemented to increase tracking and positional accuracy of the underwater vehicles. HURL submersibles used a Tracklink 500HA USBL (LinkQuest) system to calculate the position every 10 seconds (Putts et al., 2019). The US Line Islands and Phoenix Island surveys in 2005 used a Sonardyne USBL system. Both had a horizontal tracking accuracy of approximately 30 m at 1000 m depth. The *EV Nautilus* and CAPSTONE ROVs also used USBL navigation and calculated position at ≥1 per second (Quattrini et al., 2017). Data from HURL dives include those conducted for quantitative transects as well as opportunistic transits. The tracking data were unavailable for five of these dives, therefore they were not included in the abundance analysis because dive lengths along the seafloor could not be calculated. The full list of dives used in the analysis is available in Supplementary Table 2. Due to the nature of ocean exploration, the dives from any of the vehicles were occasionally interrupted by stopping the ROV or submersible for sampling or for frequent adjustments of the wide-angle view on the forward-facing, high-definition cameras to zoom in on animals for identification and record other ecological attributes (Quattrini et al., 2017). Putts et al. (2019) found that the fields of view of these different camera systems were comparable. Quattrini et al. (2017) combined dives from similar ocean exploration expeditions to investigate demersal fish in the Caribbean; therefore, it was assumed that all vehicles were comparable in their ability to survey fish. All dives, regardless of vehicle, were annotated by the University of Hawai`i Deep-sea Animal Research Center (DARC), using Video Annotation and Reference System (VARS) annotation software.

All vehicles collected temperature, dissolved oxygen, and salinity data from Seabird CTDs. For every observation, the date, geographic position, depth, temperature, salinity, and dissolved oxygen concentration were recorded. There were 227 dives between 2005 and 2019 included in the analysis and they occurred on a variety of rugged features, predominantly rocky seafloor, but including seamounts, atolls, banks, and islands. Since many of the exploration expeditions focused on exploring high-density deep-sea coral and sponge assemblages around seamounts and islands, much of the data acquired are from hard bottom habitats and the authors note that this limits the interpretation of the results, although such bias is systematic throughout the data set.

2.2 Demersal fish data

Taxa were identified to the lowest taxonomic resolution possible, which ranged from class to species. Identifications were made using a variety of taxonomic keys (Compagno, 1984a; Compagno, 1984b; Böhlke, 1989; Cohen et al., 1990; Nakamura and Parin, 1993; Carpenter and Niem, 1999a; Carpenter and Niem, 1999b; Nielsen et al., 1999; Carpenter and Niem, 2001a; Carpenter and Niem, 2001b) and reference images were sent to taxonomic experts for verification. Due to the challenges of identification, many taxa were not identified to species level. Therefore, analyses were performed on data with identification to family and genus level. This was the highest taxonomic resolution that could provide an adequate number of fishes per sample for assemblage structure patterns to emerge. Midwater taxa were removed, which included all Alepocephalidae, Barbourisiidae, Cetomimidae, Chiasmodontidae, Eurypharyngidae, Gonostomatidae, Myctophidae, Nemichthyidae, Phosichthyidae, Scombridae, Sternoptychidae, Stomiidae, Trichiuridae and the zoarcid, *Melanostigma* spp. Pelagic species were identified based on authors' experience or by reference to the scientific literature (e.g., Mundy, 2005).

The dives spanned ten different regions (Figure 1) and were split into five depth bins for statistical analysis: 250-500 m, 500-750 m, 750-1000 m, 1000-2000 m, and 2000-3000 m (Table 1). The depth bins were chosen to provide higher resolution (250 m) in upper bathyal water (where assemblage change is more rapid) and lower resolution for depths below 1000 m (Carney, 2005; Zintzen et al., 2017). This depth resolution also corresponded with the analysis by Kennedy et al. (2019) for invertebrate fauna from the CAPSTONE program. The full range of data includes deep-sea fishes between depths of 100 and 5877 m; however, there were not enough observations above 250 m or below 3000 m for a robust analysis, so these observations were omitted. After filtering the data using the above criteria, the number of dives available were 138 from CAPSTONE, 33 from *EV Nautilus*, and 56 from the HURL records.

Counts of fishes in depth bins from individual dives will be referred to as samples throughout (Table 1). Samples with five or less fishes observed were removed for the assemblage structure

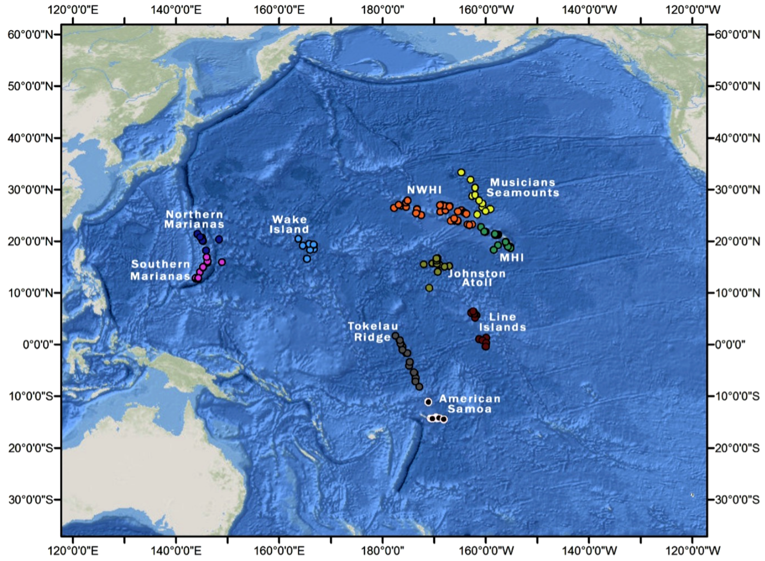

FIGURE 1
Locations of the 241 samples in the ten regions: AS, American Samoa; JA, Johnston Atoll; MHI, Main Hawaiian Islands; MS, Musicians Seamounts; NM, Northern Marianas; NWHI, Northwestern Hawaiian Islands, the Papahānaumokuākea Marine National Monument; SM, Southern Marianas; WI, Wake Island; LI, Line Islands; TR, Tokelau Ridge in the central and western Pacific. Modified from GEBCO Compilation Group (2023).

analyses because there are inherently fewer similarities for samples with few individuals. This threshold was chosen based on iterative non-metric Multidimensional Scaling (NMDS) and cluster analysis in which removing samples with five or less fishes produced the least number of outliers. The number of resulting samples at the genus level (n = 212 samples from 151 dives) and family level (n = 241 from 176 dives) varied between regions (refer to Supplementary Data for genus and family level sample data) ranging from 9 to 52 at the family level. In some cases, no samples were available for a depth bin and region because no dives were conducted there, or no samples were possible due to local bathymetry. For example, summits of the Musicians Seamounts reach no shallower than ~1000 m (Cantwell, 2020).

More data were available at the family level, so these were used to investigate trends in abundance, while genus level data we used to investigate assemblage structure. The length of each ROV dive track was used to standardize abundance (fish/m) for each sample. Track length distances varied from 85 m to 7.1 km (Table S1). Dive track distances were measured in ArcMap 10.8.2, consistent with the methods in Kennedy et al. (2019).

TABLE 1 The number of samples (dive by depth) with fish observations.

Regions	250-500 m	500-750 m	750-1000 m	1000-2000 m	2000-3000 m	Total Samples
American Samoa (AS)	7,7	7,6	3,NA	3,2	1,NA	21,15
Johnston Atoll (JA)	2,2	2,2	NA,NA	9,9	5,3	18,16
Line Islands (LI)	10,10	9,9	9,8	9,9	2,2	39,38
Main Hawaiian Islands (MHI)	20,21	1,2	5,6	11,12	1,NA	38,41
Musicians Seamounts (MS)	NA,NA	NA,NA	NA,NA	3,1	8,6	11,7
Northern Marianas (NM)	6,6	1,1	1,1	NA,NA	2,1	10,9
Northwest Hawaiian Islands (NWHI)	7,7	6,6	5,5	21,15	13,7	52,40
Southern Marianas (SM)	6,5	3,3	NA,NA	2,1	2,NA	13,9
Tokelau Ridge (TR)	5,5	5,5	3,3	12,12	5,4	30,29
Wake Island (WI)	1,1	1,1	1,1	4,3	2,2	9,8
Total Samples in Depth Bin	64,64	35,35	27,24	74,64	41,25	241,212

The number of samples are separated by a comma for identifications at the family and genus level, respectively. NAs indicate where no sampling was available.

2.3 Environmental covariates

There were 24 HURL dives in the Main Hawaiian Islands that did not have salinity or temperature data, and 15 HURL dives in the Northwest Hawaiian Islands that did not have dissolved oxygen data. Therefore, CTD data from dives nearby (within ~50 km) were used to interpolate missing values for the dives that did not have them (Supplementary Table 3).

We used estimates of particulate organic carbon (POC) flux to the seafloor, following methods in Lutz et al. (2007), as a proxy for food supply. Net-Primary-Production (NPP) data were obtained from the Oregon State Ocean Productivity website (http://orca.science.oregonstate.edu/1080.by.2160.monthly.hdf.vgpm.v.chl.v.sst.php), which provided NPP based on the Vertically Generalized Production Model (VGPM). Monthly estimates of NPP data were averaged between 2007 and 2017 at a resolution of 1/6th of a degree. A fixed euphotic zone depth of 100 m (commonly used as noted in Palevsky and Doney, 2018) was used to calculate Lutz POC flux at the depth for each sample (mid-depth of each sample bin).

Temperature (°C), salinity, and depth (m) were used to identify the water mass encountered by each ROV dive sample (Table 2; Figure 2). Kawabe and Fujio (2010) and Emery (2001) were used to identify water masses in the upper waters (0-500 m), intermediate waters (500-1500 m), and deep waters (≥1500 m). Results of the water mass analyses are included in the Supplementary Material.

2.4 Data analysis

Differences in demersal fish assemblage structure between regions and depths were evaluated using PERMANOVA in PRIMER v7 (Clarke and Gorley, 2006) and the program R (Borcard et al., 2011). Assemblage data were visualized using non-metric Multidimensional Scaling (NMDS) ordinations and unconstrained hierarchical clustering. A Mantel test was used to test if differences in assemblage structure between samples covary with the geographic distance between samples. Samples were partitioned into upper bathyal, (250-500 m) intermediate (500-750, 750-1000 m), and deep depths (1000-2000, 2000-3000 m). Genera contributing most to similarity between regions were examined using the Similarity Percentage (SIMPER) analysis with a 70% cutoff for low contributions (Clarke and Gorley, 2006).

We calculated similarities in fish assemblages between all samples using a Bray Curtis similarity matrix. A square-root transformation was used to normalize the variance. As rare genera are expected to be over-represented on geographic features with many samples compared to those with fewer, a Wisconsin double standardization was used to make genera of different abundance equally important (Gauch et al., 1977).

The relationship between assemblage structure and environmental variables was investigated using Canonical Analysis of Principal Coordinates (CAP), a constrained distance-based ordination method, which uses an *a priori* hypothesis to relate a matrix of response variables, Y (genera) with predictor variables, X (quantitative environmental variables). CAP has the advantage of allowing any distance or dissimilarity measure (e.g., Bray-Curtis) to be used. The CAP was performed on the assemblage data using the function capscale in the R package vegan (Oksanen et al., 2016) and the significance of each environmental variable was determined.

To understand how the abundance of fish varies between regions, we used a General Additive Model (GAM) to investigate the response of total fish abundance recorded in each sample (dive by depth bin) to different environmental variables fit with the "mgcv" package (Hastie and Tibshirani, 2023) in R. Average depth, dissolved oxygen, and salinity were calculated for each sample and used as predictors. The GAM used a negative binomial error distribution with a log link function, and the length of the dive track within the depth bin was included as an offset to account for variation in sampling effort across samples. The "deviance explained" is analogous to variance in a linear regression. The effective degrees of freedom (edf), an approximation of how many parameters the smoother (a parameter that controls the smoothness of the curve or estimated predictive accuracy)

TABLE 2 Most frequently observed taxa (genus level) between region and depth bin (m).

Regions	250-500 m	# Fishes	500-750 m	# Fishes	750- 1000 m	# Fishes	1000-2000 m	# Fishes	2000-3000 m	# Fishes
AS	*Epigonus*	287	*Chlorophthalmus*	34	**NA**	**NA**	*Aldrovandia*	4	**NA**	**NA**
JA	*Epigonus*	347	*Epigonus*	27	**NA**	**NA**	*Halosaurus*	28	*Kumba*	4
LI	*Setarches*	6014	*Setarches*	1420	*Neocyttus*	449	*Neocyttus*	83	*Bathypterois*	9
MHI	*Epigonus*	565	*Hymenocephalus*	40	*Aldrovandia*	170	*Aldrovandia*	202	**NA**	**NA**
MS	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	*Kumba*	2	*Ilyophis*	18
NM	*Grammatonotus*	2692	*Chlorophthalmus*	70	*Synaphobranchus*	22	**NA**	**NA**	*Bassozetus*	2
NWHI	*Polymixia*	123	*Synaphobranchus*	4	*Aldrovandia*	16	*Aldrovandia*	54	*Kumba*	23
SM	*Epigonus*	205	*Epigonus*	46	**NA**	**NA**	*Synaphobranchus*	2	**NA**	**NA**
TR	*Antigonia*	189	*Neocyttus*	491	*Neocyttus*	742	*Neocyttus*	86	*Bathypterois*	13
WI	*Epigonus*	11	*Beryx*	12	*Aldrovandia*	10	*Aldrovandia*	14	*Bassozetus*	5

Abbreviations are defined in Figure 1 and Table 1.

represents was calculated. The spread of the data (y axis maxima) change between plots because the plots are showing partial residuals and unexplained variation is added on top of the smoother.

We investigated diversity at the genus level using several metrics. Chao-1 and Chao-2 estimators were used to estimate generic richness for regions and depth bins. Samples were also rarefied using the iNEXT package (Chao et al., 2014) in the R program with the Hill number of order q=0 (genera richness). Lastly, we used Pielou's evenness, which measures the degree of evenness or dominance in each species (genera in our case), to get a thorough description of the assemblage structure (Pielou, 1966) where values range from 0 (no evenness) to 1 (complete evenness).

3 Results

3.1 Summary statistics

Between 250 and 3000 m, 22,162 individual fishes were identified to genus in the 212 samples with six or more fishes (Table 1). The average fish per sample was 104 with the minimum number of fishes in one sample being six and the maximum being 2,689 fishes. Across depths, more samples were available between 250-500 m (n = 64) and 1000-2000 m (n = 64). The 500-750 m depth range had the least number of samples (n = 24). The regions with the most samples were the Main Hawaiian Islands (n = 41), the Northwest Hawaiian Islands (n = 40), and the Line Islands (n = 38). The regions with the least samples were the Musicians Seamounts (n = 7), Wake Island (n = 8), Southern Marianas (n = 9), and Northern Marianas (n = 9).

The most frequently occurring fishes varied between regions with some similarity between depth bins (Table 2; Figure 2). *Epigonus* occurred frequently in the upper and intermediate bathyal depths within American Samoa, Johnston Atoll, the Main Hawaiian Islands, Southern Marianas, and Wake Island. *Neocyttus* occurred frequently in Tokelau Ridge and the Line Islands in intermediate depth bins. At about 1000 m, the taxa transitioned to genera such as *Aldrovandia*, with a deeper depth range, which occurred frequently between 1000 and 3000 m within American Samoa, the Main Hawaiian Islands, and Northwest Hawaiian Islands and Wake Island.

3.2 Regional differences in assemblage structure

Overall, fish assemblage structure at the genus level varied significantly between region (pseudo-F= 2.6, P=0.001), and depth bin (pseudo-F= 6.4, P= 0.001), with a significant region by depth interaction (interaction term pseudo-F= 1.9, P= 0.001). Pairwise tests for each region and depth bin (Table 3) also revealed that assemblage structure differed by depth and region, but differences lessened with depth (assemblages become more similar with depth, 19 of 45 (42%) comparisons significant at 250-500m, 11 of 45 (24%) at 1000-2000 m, 3 of 21 (14%) at 2000-3000 m). There is a horseshoe effect in the NMDS that corresponds with variation along a depth gradient of two clusters that roughly corresponding to depth ranges of 250–750 m and 750–2000 m with some overlap (Figure 3).

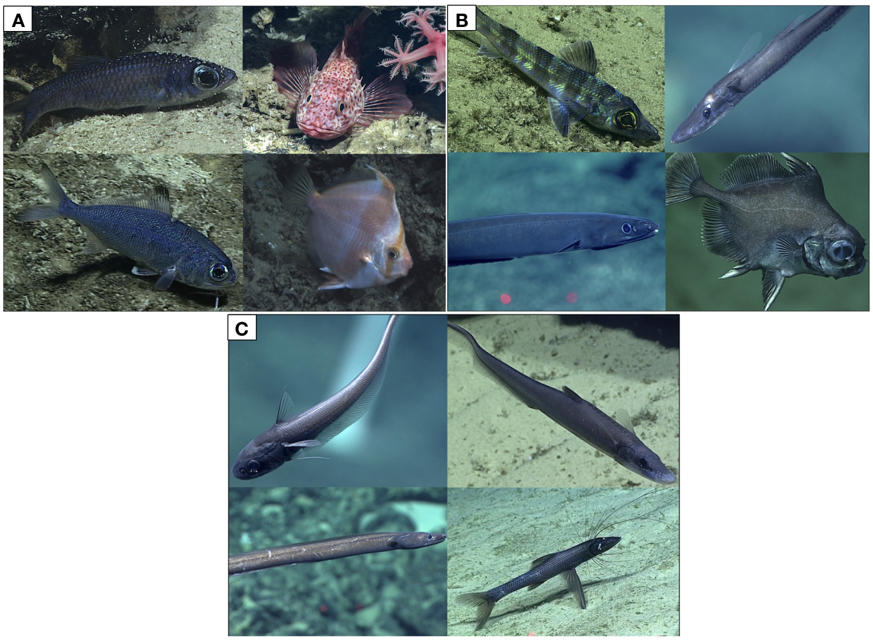

FIGURE 2
The most frequently observed taxa between depth and region. **(A)** Most common genera between 250-500 m. Top to bottom and left to right, *Epigonus, Setarches, Polymixia*, and *Antigonia*. **(B)** Most common genera between 500-1000 m, *Chlorophthalmus*, *Aldrovandia, Synaphobranchus*, and *Neocyttus*. **(C)** Most common genera between 1000 and 3000 m, *Kumba, Halosaurus, Ilyophis* and *Bathypterois*. Depth bins combined due to lack of space for photograph panels. See Table 2 for complete lists of frequently observed fishes by region and depth bin. Images courtesy of the NOAA Office of Ocean Exploration and Research.

TABLE 3 Assemblage structure similarity between regions and depth.

250-500 m										
	AS	JA	LI	MHI	MS	NM	NWHI	SM	TR	WI
AS	16.3									
JA	15.1	41.5								
LI	**10.9**	**16.3**	32.2							
MHI	**8.6**	**12.5**	**14.8**	13.5						
MS	NA	NA	NA	NA	NA					
NM	13.4	13.0	**12.1**	**7.2**	NA	15.5				
NWHI	**8.1**	**8.6**	**9.0**	**12.2**	NA	**4.0**	23.4			
SM	19.1	17.3	19.2	**10.8**	NA	23.8	**9.3**	30.7		
TR	**11.7**	**6.5**	**19.0**	**10.2**	NA	**9.4**	**10.0**	**14.0**	16.1	
WI	13.5	43.2	11.0	7.0	NA	19.0	4.9	25.3	7.6	0.0
500-750 m										
	AS	JA	LI	MHI	MS	NM	NWHI	SM	TR	WI
AS	12.8									
JA	5.1	11.5								
LI	**5.3**	**4.1**	30.2							
MHI	7.9	8.4	**6.0**	10.0						
MS	NA	NA	NA	NA	NA					
NM	13.7	3.0	0.2	7.1	NA	0.0				
NWHI	**3.9**	**0.7**	**7.2**	5.1	NA	6.1	13.9			
SM	10.5	10.9	**13.2**	6.0	NA	18.1	**5.7**	13.0		
TR	**9.4**	**3.1**	**20.6**	**7.4**	NA	2.2	**7.5**	**12.6**	30.8	
WI	9.5	2.3	0.6	8.2	NA	34.6	19.3	21.3	3.7	0.0
750-1000 m										
	AS	JA	LI	MHI	MS	NM	NWHI	SM	TR	WI
AS	NA									
JA	NA	NA								
LI	NA	NA	24.9							
MHI	NA	NA	**4.8**	25.0						
MS	NA	NA	NA	NA	NA					
NM	NA	NA	6.6	6.7	NA	0.0				
NWHI	NA	NA	**5.7**	17.9	NA	29.5	20.4			
SM	NA	NA	NA	NA	NA	NA	NA	NA		
TR	NA	NA	17.4	**6.7**	NA	9.6	9.8	NA	18.2	
WI	NA	NA	12.0	8.9	NA	14.0	8.7	NA	14.1	0.0

(Continued)

TABLE 3 Continued

1000-2000 m										
	AS	JA	LI	MHI	MS	NM	NWHI	SM	TR	WI
AS	0.0									
JA	32.0	21.2								
LI	11.5	**14.0**	24.8							
MHI	20.1	**21.9**	**5.1**	41.1						
MS	53.4	33.3	14.7	11.6	0.0					
NM	NA	NA	NA	NA	NA	NA				
NWHI	30.6	21.4	**9.2**	**20.8**	29.2	NA	22.5			
SM	12.1	22.6	7.3	16.2	33.9	NA	17.3	0.0		
TR	14.3	**16.6**	21.6	**10.2**	24.6	NA	**12.2**	14.5	21.4	
WI	**43.1**	23.6	**6.9**	22.6	35.1	NA	25.6	14.3	**12.1**	22.5
2000-3000 m										
	AS	JA	LI	MHI	MS	NM	NWHI	SM	TR	WI
AS	NA									
JA	NA	16.3								
LI	NA	16.7	13.5							
MHI	NA	NA	NA	NA						
MS	NA	14.8	14.3	NA	24.2					
NM	NA	14.6	8.3	NA	4.4	0.0				
NWHI	NA	**15.6**	**12.4**	NA	31.2	5.2	39.0			
SM	NA	NA	NA	NA	NA	NA	NA			
TR	NA	21.1	22.1	NA	13.6	9.9	**8.1**	NA	19.8	
WI	NA	24.8	15.6	NA	21.4	33.0	32.0	NA	8.0	57.9

Numbers indicate the average similarity with bolded values indicating a significant difference between regions (PERMANOVA, P<0.05). NA: too few samples were available for a test to be conducted. Abbreviations are defined in Figure 1 and Table 1.

Regional variation was also apparent as some regions clustered together in the upper and intermediate depth bins. The Line Islands and Tokelau Ridge between 250–1000 m and the Main Hawaiian Islands and Northwest Hawaiian Islands between 250–500 m had distinctly different assemblages compared to the rest of the regions (Figure 3).

Across all regions, there were more significantly different assemblages in the upper bathyal and intermediate depth bins indicating that there was more assemblage structure in the upper bathyal depths compared to the deep. The 250-500 m depth zone had the most regions that were statistically different and had a few regions that were different from one another despite being in relatively close geographic proximity. For instance, American Samoa was different from Tokelau Ridge just to its north and Johnston Atoll was different from the Main Hawaiian Islands and Northwest Hawaiian Islands despite being less than 1000 km away. Between 500-750 m, the number of regions that were different compared to the upper bathyal (250-500 m) was nearly cut in half. However, there were still differences between regions that were relatively close. American Samoa was still different from the Tokelau Ridge and Johnston Atoll was different from the Northwest Hawaiian Islands. The intermediate 750-1000 m depth bin has limitations for interpretation due to gaps in sampling; however, the Main Hawaiian Islands was different from the Line Islands and Tokelau Ridge. The deeper depth bins (1000-3000 m) had the least number of different regions. However, the statistical power available for samples from 2000-3000 m is hampered by no samples available in American Samoa and the Main Hawaiian Islands. Despite this limitation, Johnston Atoll was different from the Northwest Hawaiian Islands in the 2000-3000 m depth bin but not in the 1000-2000 m. Overall, the differences in assemblage structure covaried significantly with geographic distance in upper bathyal (250-500 m), intermediate (500-750, 750-1000 m) and deep (1000-2000, 2000-3000 m) depth strata (Mantel statistic: 250-500 m: 0.17, P = <0.001, 500-750 m: 0.27, P = <0.001, 750-1000 m: 0.23, P = <0.001, 1000-2000 m: 0.19, P= 0.001, 2000-3000 m: 0.13, P =0.001).

The regional differences in assemblage structure may be related to deep-sea water masses and their specific values of temperature, dissolved oxygen, salinity, and density. We identified six water individual masses across the ten sampling regions (WNPCW,

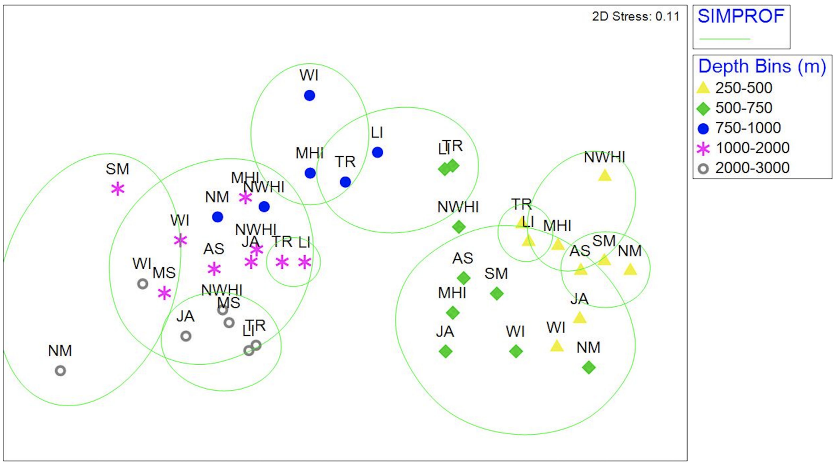

FIGURE 3
NMDS ordination based on Bray Curtis similarities calculated on square-root transformed and Wisconsin double standardized averaged abundances. Combined factors of region and depth bin (stress =0.11). Green ovals correspond to similar assemblages determined by SIMPROF analysis (SIMPROF, $P<0.05$). AS, American Samoa; JA, Johnston Atoll; LI, Line Islands; MHI, Main Hawaiian Islands; MS, Musicians Seamounts; NM, Northern Marianas; NWHI, Northwest Hawaiian Islands; SM, Southern Marianas; TR, Tokelau Ridge; WI, Wake Island.

NPIW, AAIW, NPDW, UCDW, LCDW, Supplementary Table 1) occurring in eight different combinations as some samples occurred at the nexus of two water masses and could not be differentiated (i.e., AAIW/NPIW, NPDW/LCDW, AAIW, UCDW). The intermediate depths encompassing American Samoa, Tokelau Ridge, Line Islands and Johnston Atoll, and the Main Hawaiian Islands are mainly occupied by Antarctic Intermediate Water (AAIW) whereas the Northwest Hawaiian Islands, Musicians Seamounts, Wake Island, and the Mariana Islands are mainly occupied by North Pacific Intermediate Water (NPIW/AAIW). Communities at sites that included WNPCW in the Main Hawaiian Islands, Northern Marianas, Southern Marianas, and Johnston Atoll were similar within 250-500 m. There were moderate similarities between assemblages in the NPIW/AAIW and AAIW within 500-1000 m. Overall, water mass generally followed depth strata with shallow samples on the far right, intermediate in the middle and deep samples on the far left (Supplementary Figure 3B).

3.3 Assemblage structure

In general, there were more upper bathyal assemblages compared to deep ones and a wider range in depths of assemblages in the upper bathyal regions compared to the deep (Table 4). Group average similarities ranged from 16% to 81%. The group that occurred in the most regions was group d (750-1000 m) which included samples from Johnston Atoll, Line Islands, Northern Marianas, Northwest Hawaiian Islands and Tokelau Ridge (21% similarity). The group with the highest similarity was group n (250-750 m) at 81% similarity which just included the Main Hawaiian Islands. Groups (250-500 m) at 16% just included American Samoa and had the least similar assemblages of all the assemblages. The large range in group average similarity may indicate limitations in the sample effort and or low spatial resolution. There were four assemblages that encompassed wide depth ranges between 250 and 1000 m and 13 assemblages that occurred between 250 and 750 m. There were only three assemblages that occurred strictly between 1000 and 3000 m. There were six assemblages in the upper bathyal and intermediate depths that occurred in only one region. These included the 250-500 m Line Island assemblage which were dominated by *Chrionema* (14.7% contribution), 250-500 m Main Hawaiian Islands assemblage (*Owstonia*, 31% contribution), 250-750 m Main Hawaiian Islands assemblage (*Chascanopsetta*, 22% contribution), 250-500 m Northern Marianas assemblage (*Plectranthias*, 23% contribution), 250-500 m American Samoa assemblage (*Etelis*, 70% contribution), and 500-750 m American Samoa assemblage (*Phenacoscorpius*, 90% contribution).

3.4 The influence of environmental variables on assemblage structure

The environments of seamounts and oceanic islands are not homogenous as each have different physical, chemical, and geological characteristics with varying influence on fish assemblage structure. This pattern was observed as depth, dissolved oxygen, POC flux, and salinity together explained a total of 24% of the variation in assemblage structure (Figure 4, constrained proportion = 0.24). The first axis, CAP1, explained ~10% of the constrained variation (proportion explained = 0.09) and was strongly correlated with depth, moderately correlated with POC flux, and weakly correlated with concentrations of dissolved oxygen and salinity. POC flux varies in the opposite direction along this axis which was expected as POC flux generally declines with depth. The second axis, CAP2, explained ~4% of the constrained variation (proportion explained = 0.03) and has dissolved oxygen

TABLE 4 List of genera that contribute most (70%; SIMPER) to similarity within the 24 fish assemblages identified by hierarchical analysis. .

Depth m	Group	Sim%	Region	Genera	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
250-500	o	39.29	LI, MHI, NWHI	*Grammicolepis*	0.15	15.16	2.83	38.57	38.57
				Hollardia	0.12	7.65	1.07	19.47	58.04
				Randallichthys	0.13	7.56	1.15	19.24	77.29
250-500	r	62.71	LI	*Chrionema*	0.11	9.21	5.45	14.68	14.68
				Hoplostethus	0.09	8.28	5.1	13.2	27.88
				Grammicolepis	0.09	7.81	5.43	12.45	40.33
				Setarches	0.1	7.57	2.74	12.07	52.4
				Synagrops	0.09	6.53	2.02	10.41	62.81
				Coelorinchus	0.06	5.66	18.21	9.02	71.83
250-500	u	36.15	LI, SM	*Synchiropus*	0.13	10.39	SD=0!	28.75	28.75
				Myroconger	0.08	7.35	SD=0!	20.33	49.08
				Plectranthias	0.08	4.37	SD=0!	12.08	61.16
				Hoplostethus	0.04	4.24	SD=0!	11.74	72.89
250-500	p	39.5	MHI	*Owstonia*	0.13	12.4	7.21	31.38	31.38
				Hollardia	0.08	8.29	12.5	20.97	52.35
				Lophiodes	0.07	5.44	3.53	13.78	66.13
				Epigonus	0.06	4.41	1.16	11.17	77.3
250-750	n	81.13	MHI	*Chascanopsetta*	0.17	18.05	SD=0!	22.25	22.25
				Malacocephalus	0.16	17.42	SD=0!	21.47	43.73
				Hymenocephalus	0.15	15.09	SD=0!	18.6	62.32
				Satyrichthys	0.16	14.74	SD=0!	18.17	80.49
250-500	w	47.4	NM, SM	*Hoplostethus*	0.14	14.77	6.27	31.16	31.16
				Plectranthias	0.14	13.66	2.56	28.81	59.97
				Antigonia	0.06	4.74	2.32	10	69.98
				Pontinus	0.05	3.9	1.08	8.22	78.2
250-500	t	37.02	NM	*Plectranthias*	0.08	8.52	SD=0!	23.02	23.02
				Pristilepis	0.11	7.38	SD=0!	19.94	42.96
				Gymnothorax	0.07	7.25	SD=0!	19.58	62.54
				Odontanthias	0.06	4.76	SD=0!	12.85	75.39
				Synaphobranchus	0.05	2.36	0.48	7.61	76.44
250-500	x	28.17	AS, SM	*Chlorophthalmus*	0.07	5.36	1.56	19.03	19.03
				Plectranthias	0.11	5.02	1.82	17.81	36.84
				Odontanthias	0.06	4.4	2.13	15.64	52.47
				Etelis	0.06	2.87	0.58	10.18	62.65
				Parascombrops	0.06	2.27	0.58	8.04	70.69
250-500	s	16.14	AS	*Etelis*	0.15	11.24	SD=0!	69.63	69.63
				Epigonus	0.04	2.57	SD=0!	15.93	85.56
250-750	k	23.31	AS, JA, MHI, NM, NWHI, SM, WI	*Beryx*	0.15	9.48	0.69	40.67	40.67

(Continued)

TABLE 4 Continued

Depth m	Group	Sim%	Region	Genera	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
				Chlorophthalmus	0.14	7.7	0.57	33.05	73.72
250-500	y	24.77	AS, MHI, NM, TR	*Pontinus*	0.07	5.99	3.32	24.18	24.18
				Chrionema	0.06	3.61	2.16	14.58	38.76
				Symphysanodon	0.06	3.11	0.83	12.56	51.31
				Antigonia	0.06	3.02	0.78	12.2	63.51
				Etelis	0.06	2.37	0.56	9.58	73.1
250-750	j	18.55	AS, JA, MHI, TR	*Chlorophthalmus*	0.07	4.95	1.35	26.68	26.68
				Cyttomimus	0.06	3.88	0.91	20.92	47.6
				Hymenocephalus	0.08	3.17	0.54	17.1	64.7
				Plesiobatis	0.11	1.84	0.22	9.94	74.64
250-750	l	23.01	LI, MHI, NWHI, TR	*Chaunax*	0.09	5.33	0.8	23.17	23.17
				Squalus	0.08	4.61	0.62	20.05	43.22
				Hollardia	0.05	3.43	0.89	14.89	58.11
				Polymixia	0.04	2.17	0.83	9.42	67.53
				Laemonema	0.03	1	0.42	4.34	71.87
250-1000	ab	24.55	AS, LI, MHI	*Bathycongrus*	0.21	14.57	0.8	59.35	59.35
				Synagrops	0.2	7.93	0.41	32.32	91.67
250-1000	aa	21.87	LI, SM, TR, WI	*Coelorinchus*	0.12	7.13	0.9	32.61	32.61
				Hoplostethus	0.08	4.42	0.76	20.21	52.82
				Neocyttus	0.05	3.4	1.09	15.55	68.37
				Bathycongrus	0.04	1.04	0.35	4.74	73.11
500-750	z	26.51	AS	*Phenacoscorpius*	0.25	23.99	SD=0!	90.48	90.48
500-750	e	30.96	MHI, NWHI	*Ectreposebastes*	0.25	15.46	0.86	49.93	49.93
				Neoscopelus	0.12	7.99	0.9	25.79	75.72
500-1000		31.98	MHI, NWHI	*Nettastoma*	0.16	18.23	15.1	57.01	57.01
				Synaphobranchus	0.11	7.29	0.58	22.8	79.81
750-2000	i	45.7	JA, MHI, NWHI, TR	*Synaphobranchus*	0.39	38.48	1.78	84.21	84.21
750-2000	d	20.66	JA, LI, NM, NWHI, TR	*Diplacanthopoma*	0.09	4.37	0.63	21.13	21.13
				Coryphaenoides	0.07	4.24	0.91	20.53	41.66
				Nezumia	0.04	2.27	0.56	10.99	52.66
				Bassozetus	0.04	1.6	0.42	7.75	60.4
				Halosaurus	0.04	0.99	0.33	4.77	65.17
				Lepidion	0.04	0.93	0.27	4.5	69.68
				Spectrunculus	0.03	0.71	0.23	3.42	73.09
750-2000	h	34.5	MHI, NWHI	*Aldrovandia*	0.14	11.06	1.59	32.07	32.07
				Synaphobranchus	0.07	5.75	1.08	16.65	48.72
				Nettastoma	0.12	5.57	0.74	16.15	64.87
				Apristurus	0.09	4.87	0.64	14.1	78.98
1000-2000	g	30.26	JA, NWHI, TR, WI	*Synaphobranchus*	0.12	10.07	3.17	33.28	33.28

(Continued)

TABLE 4 Continued

Depth m	Group	Sim%	Region	Genera	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
				Venefica	0.19	9.96	0.62	32.92	66.2
				Monomitopus	0.11	3.47	0.32	11.47	77.67
1000-3000	b	24.83	JA, LI, MS, NM, TR	*Bassozetus*	0.23	15.78	1.56	63.55	63.55
				Acanthonus	0.09	3.15	0.34	12.68	76.23
1000-3000	c	30.96	AS, JA, MS, NWHI, SM, WI	*Kumba*	0.22	17.6	1.24	56.84	56.84
				Ilyophis	0.09	3.71	0.42	11.99	68.83

Abbreviations are defined in Figure 1 and Table 1.

and POC flux occurring in opposite directions and little contribution from depth and salinity. The Line Islands and Tokelau Ridge had the highest POC flux values and lowest concentrations of dissolved oxygen and therefore may be driving this pattern on the CAP 2 axis. The influence of the environmental variables observed may also be driven by the structure of the different water masses found in the regions (Supplementary Table 1). Full ranges of environmental variables analyzed are provided in Supplementary Figure 1.

3.5 Total abundance

Total fish abundance generally decreased with depth across the regions. Samples with the lowest abundances (<0.01) occur mostly in the intermediate to deep depths, however the Main Hawaiian Islands and Tokelau Ridge had very high abundances between 750-1000 m (Figure 5), which were driven by the high abundance of Epigonidae and Setarchidae, respectively. Total abundance was greatest in the Line Islands between 250-500 m (6.05 fish/m) followed by the Northern Marianas between 250-500 m (4.42 fish/m). Changes in fish abundance with depth were significant in all regions (P = 0.001) except for the Musicians Seamounts (P = 0.9), with 75.1% of the deviance explained in the model.

3.6 Relationship between total abundance and environmental variables

Depth, concentrations of dissolved oxygen, POC flux, and salinity all had a significant relationship with fish abundance (fish/km) (P=0.001) and explained a little over 50% of the variation in total abundance (deviance explained =73.6%, generalized cross-validation score= 900). Total abundance was highest at depths of ~250 and ~725 m but then declined at ~1500 m (P=0.001). Dissolved oxygen was strongly related to abundance

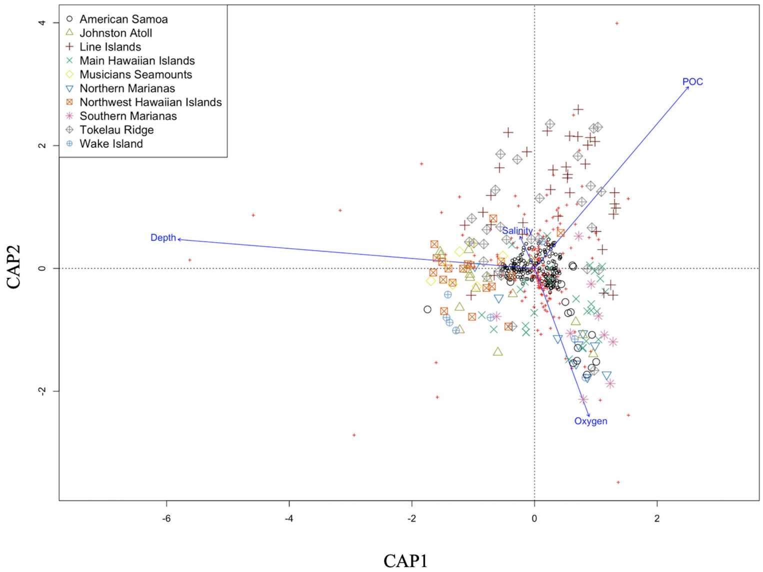

FIGURE 4
Canonical analysis of principle coordinates (CAP) based on Bray Curtis similarities calculated on Wisconsin transformed data (n = 212 samples). CAP1 explains 10% of the total variation while CAP2 explains 4%. The total constrained variation explained by all axes is 24%. CAP statistics generated by capscale in R. Colors=regions. Temperature was removed from the analysis as it was highly correlated to depth.

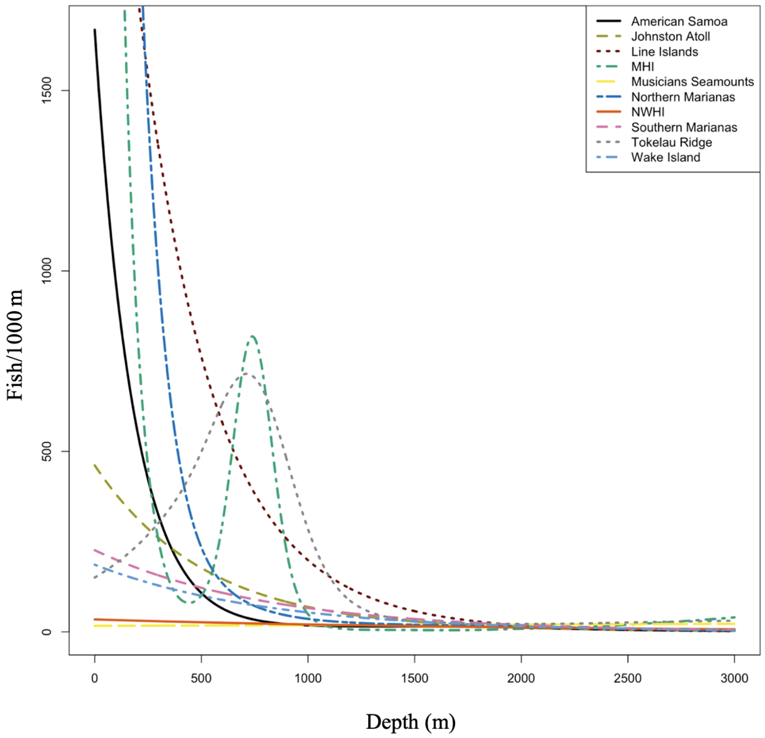

FIGURE 5
Smoothers (a parameter that controls the estimated predictive accuracy) in Generalized Additive Model (GAM) for total abundance (fish/km) by depth plotted by all regions. Changes in fish abundance with depth were significant in all regions (*P*= 0.001) except for the Musicians Seamounts (P = 0.9). MHI, Main Hawaiian Islands; NWHI, Northwest Hawaiian Islands.

(P=0.001) where total abundance appeared highest between dissolved oxygen concentrations of 1.5 and 3.5 mg/L. Abundance increased with salinity, possibly due to water mass differences (Supplementary Figure 3); however, confidence intervals >34.5 are very large so this trend could be driven by a few observations with high salinity and high total abundance. For instance, the Line Islands 250-500 m sample bin had the highest salinity and abundance values. Fish abundance increased with increasing POC flux. In summary, fish abundances were predicted to be highest at the upper bathyal depths (250-500 m), with concentrations of dissolved oxygen between 1.5-3.5 mg/L, salinity values ~ 34.5, and POC flux values > 4 mg C/m^2/day (Figure 6).

3.7 Diversity

There were not enough samples to generate curves based on region by depth bin, therefore, samples were parsed separately as region and depth. Rarefaction curves (Hill, q=0) were used to compare samples at the same sampling intensity (the same number of individuals) to determine whether generic richness differed between regions and depths. Extrapolation was included in the curves for reference but not used in comparisons. There were four regions with enough individuals (n = 2500) to compare at the same sampling intensity (the same number of individuals). These regions included the Northern Marianas, Line Islands, the Main Hawaiian Islands and Tokelau Ridge (Figure 7A). Out of these, Tokelau Ridge and the Main Hawaiian Islands had the highest estimated generic richness as these curves are well above the Line Islands and Northern Marianas curves. Results for samples parsed by depth bin indicated that deeper depths were far less sampled than upper bathyal locations (Figure 7B). The only depth bins with enough individuals (n = 2500) to compare at the same sampling intensity were 250-500 m and 500-750 m, which were closely aligned.

Chao 1 and Chao 2 were used to estimate generic richness based on samples rather than pooled individuals (limit of the rarefaction curves). Chao1 and 2 richness estimators predict higher richness for all regions and depths bins than the rarefaction extrapolation even for curves that are near asymptotes (LI and 250-500 m). In all Chao 1 and 2 cases, estimates exceed the number of genera collected indicating that there are still genera that remain uncollected. For example, Chao 1 and Chao 2 both estimate over 120 genera in the Northern Marianas, whereas only ~40 genera were collected (Supplementary Figures 2A, B). Similarly, Chao 1 and Chao 2 both estimate ~140 genera in 250-500 m, whereas only ~100 genera were collected, indicating that this depth bin is estimated to have the highest regional richness with many genera yet to be collected (Supplementary Figures 2C, D). There were six regions with enough samples (n = 10) to compare estimated generic richness at the same sampling intensity: Johnston Atoll, Northwest Hawaiian Islands, American Samoa, Main Hawaiian Islands, Line Islands, and Northern Marianas (Supplementary Figure 2A). Out of these, the Northern Marianas, followed by the Line Islands and Main

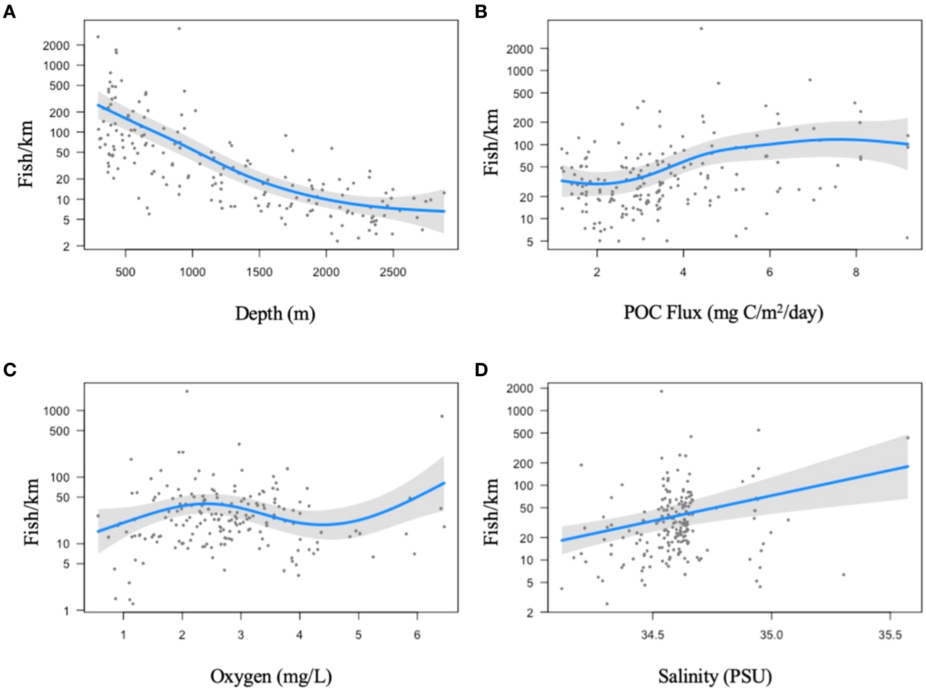

FIGURE 6
Generalized additive models (GAM) for total abundance (fish/km) in relation to **(A)** depth (m), **(b)** POC flux (mg C/m^2/day), **(C)** dissolved oxygen (mg/l), and **(D)** Salinity (PSU) to the seafloor. Back transformed GAM functions with residuals. The spread of the data (y axis maxima) changed between plots because the plots show partial residuals and unexplained variation is added on top of the smoother. The shaded grey bands indicate confidence intervals on the standard deviation scale.

Hawaiian Islands had the highest estimated generic richness (Supplementary Figure 2B). However, Chao 2 estimated the Main Hawaiian Islands to have the second highest generic richness. Results of Chao1 and 2 by depth bin indicated that there were enough samples to compare all depth bins at n = 10. Estimated generic richness generally was highest in the upper bathyal depth bins and decreased with depth. However, estimates of richness in the 500-750 m and 750-1000 m depth bins were more closely aligned in Chao 1 compared to Chao 2 (Supplementary Figures 2C, D).

The Line Islands, Main Hawaiian Islands, and Northern Marianas, and Tokelau Ridge had the lowest generic evenness (Pielou), with median index values under 0.75. The regions with the highest evenness were the Musicians Seamounts and the Northwest Hawaiian Islands (Figure 8A). When separating the data by depth bin, it becomes apparent that overall, evenness increased with depth. Although 750-1000 m has the lowest evenness value, 250-750 m are not far off, and the 2000-3000 m depth bin has the highest evenness value (Figure 8B).

4 Discussion

4.1 Patterns in assemblage structure

Comparisons of demersal fishes between regions in the central and western Pacific indicate that depth (and its correlates, temperature, and pressure) may play the most influential role in structuring assemblages. This was expected as depth zonation of demersal fishes is a common phenomenon in the deep-sea globally (Carney, 2005) and has been observed in other studies in the Pacific Ocean within the Hawaiian archipelago (Yeh and Drazen, 2009; De Leo et al., 2012). The reduction of light, temperature, dissolved oxygen, and food supply along with increasing pressure has been found to strongly influence the spatial distribution of species along with their functions and morphologies (Gallo and Levin, 2016). Despite some limited sampling at intermediate depths, assemblages became more similar with increasing depth. Deeper living fishes are also known to have wider distributions (resulting in increased similarities in assemblages) than upper bathyal fishes due to the increase in homogeneity and stabilization of environmental variables (Clark et al., 2010).

Regional separation was also apparent between assemblages suggesting that there are significant regional differences in the composition of demersal fauna across archipelagos in the central and western Pacific. The Main Hawaiian Islands and Northwest Hawaiian Islands had an upper bathyal assemblage (250-750 m) that was different from all other regions (Figure 5A). Shallow reef studies show that the Main Hawaiian Islands are characterized by a large number of endemic species (30% of inshore fishes) due to its geographic isolation (Hourigan and Reese, 1987). We suggest that Hawaii`s isolation may be causing its distinct upper bathyal fish assemblage. The genera that may be contributing to these differences in the upper bathyal zone include *Owstonia*, a

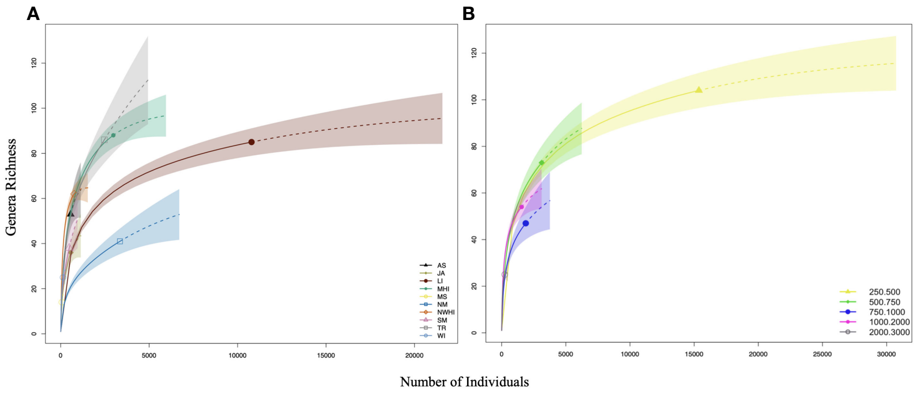

FIGURE 7
Rarefaction curves (by number of individuals) parsed by region and depth bin for estimating sampling effectiveness. **(A, B)** The top curves represent all regions and depths pooled, respectively. Extrapolations are indicated by dashed lines. Abbreviations are defined in Figure 1 and Table 1.

bandfish genus that is found in the deep waters of the Indian and Pacific Ocean (Smith-Vaniz and Johnson, 2016) and *Chascanopsetta*, lefteye flounders with a tropical distribution from the Western Atlantic to the central Pacific (Hensley and Smale, 1997). Although we were unable to identify the majority of fishes beyond genera in the present analysis, our patterns could derive from patterns of endemic species similar to shallow reef environments. The Line Islands and Tokelau Ridge had an upper bathyal and intermediate assemblage (250-1000 m) that was different from all other regions (Figure 3) which align with studies that have found that neighboring regions harbor more similar fish assemblages (Clark et al., 2010). Also, these regions are within the equatorial upwelling zone with high productivity due to the dynamic seasonal changes in sea surface temperature (SST) and water column thermal and oxygen structure. Cold SST that is prevalent in equatorial waters during ENSO events like La *Niña* and shoaling of the thermocline and oxycline from enhanced upwelling may be creating a physiological barrier in these regions (Carlisle et al., 2017). The Line Islands and Tokelau Ridge had some of the lowest concentrations of dissolved oxygen and highest POC flux values, suggesting that these regions may be a biogeographic zone of faunal change. The center of the Pacific provinces as described in Watling et al. (2013) are within oligotrophic central gyres congruent with a zone of change near the equator. Further, a distinct change in mesopelagic fish fauna has been observed between two Equatorial components in the Pacific between a 14.5°N and 7°N (“north”) and 7°N and 3°S (“south”). Across these components, species adapted to low oxygen were observed in the north component and species adapted to high productivity were adapted to high productivity (Sutton et al., 2017). Faunal studies conducted across the postulated transition zone of the Equatorial and North Central Pacific provinces have observed significant changes in the diversity of mesopelagic fishes (Barnett, 1984; Clarke, 1987) as well as macrofaunal polychaetes and sediment-dwelling foraminiferans matching food availability at abyssal depths (Smith et al., 2008). Therefore, it’s possible that a similar transition zone may exist across the Equatorial and South Pacific, contributing to the changes observed in the demersal fish assemblage.

4.2 Relationship between assemblage structure and environmental gradients

Oxygen played a role in structuring central and western Pacific fish assemblages with the presence of low-oxygen zones from 250-1000 m in some regions. Persistent OMZs are likely to be important boundaries to species distributions and are hypothesized to be barriers to gene flow within populations in the deep sea (Rogers, 2000). Regional variation in the thickness and intensity of OMZs can be attributed to the differences in oceanographic currents, productivity, and aerobic respiration in the water column (Stramma et al., 2009). In the Hawaiian archipelago, there is a relatively weak OMZ between depths of 600 and 700 m with minimum oxygen concentrations of 0.84 mg/L at ~650 m (Yeh and Drazen, 2009; De Leo et al., 2012). Reduced fish abundances have been found there (De Leo et al., 2012). In the present study, very low concentrations of dissolved oxygen were found in several regions with the lowest concentrations of dissolved oxygen found in the Line Islands (minimum dissolved oxygen value of 0.56 mg/L), Johnston Atoll (minimum value of 0.75 mg/L), and Tokelau Ridge (minimum value of 1.3 mg/L, lowest values given rather than lowest sample averages). Although dissolved oxygen in the last two regions is higher than the threshold considered for oxygen minimum zones around the globe (0.7 mg/L, Gibson and Atkinson, 2003), and more characteristic of an oxygen minimum layer characterized as hypoxic (dissolved oxygen (DO < 2 mg/L) (Jaker et al., 2020), the low oxygen in all three regions could be acting as a physiological barrier to some genera.

Part of the variance in the assemblage structure of demersal fishes in the central and western Pacific can be attributed to POC flux, a proxy for food availability. Generally, POC flux decreases with depth and the distance from shore, the source of coastal nutrients, and vascular plant and macroalgae material. However, depth-related decrease in POC flux becomes more complicated with more complex bathymetry and where OMZs intersect continental margins and seamounts (Levin et al., 2001; De Leo et al., 2014). Both indirect and direct changes in food quantity and quality in deep-sea assemblages can alter food web structure, abundance, and diversity;

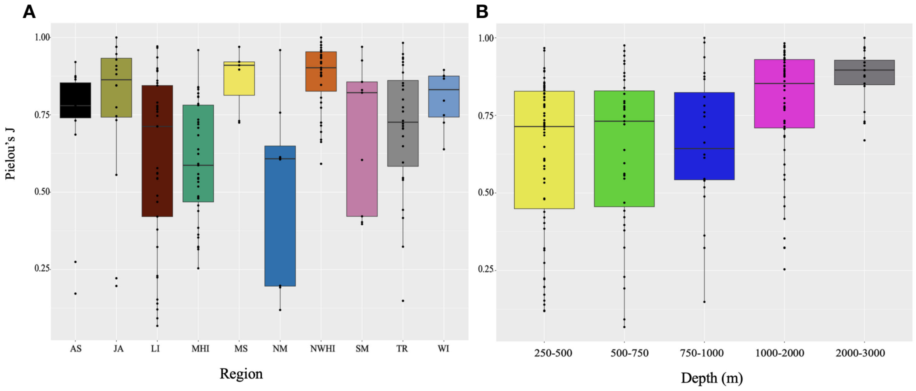

FIGURE 8
Boxplot of evenness values (Pielou's J) for the genera observed parsed by **(A)** regions and **(B)** depth bins. Five summary statistics are displayed (median, two hinges, two whiskers, and all outlying points). Whiskers = minimum and maximum, box= lower and upper quartile, horizontal bold line = median. Abbreviations are defined in Figure 1 and Table 1.

therefore, it's not surprising that POC flux values were correlated with the variation in assemblage structure across all regions. Deep-sea habitats generally have low biomass due to the low food availability, and reproduction and growth of fishes are all reduced with increasing depth which is likely related to both food supply and temperature (Levin et al., 2001). POC flux was highest in the Line Islands and Tokelau Ridge for all depths which also had some of the lowest oxygen levels. It's likely that the low-oxygen environments in these OMZs are influencing material cycling in the region and the transfer of organic matter to deep waters (Ma et al., 2021). Further, surface waters in these regions may have higher biological productivity due to equatorial upwelling processes (Chavez and Messié, 2009).

Care is needed when interpreting the overall importance of environmental variables in governing assemblage structure. The first two axes in the CAP analysis combined accounted for ~14% of the variation, indicating that there are other predictors that explain the variation not included in the present study. Small-scale habitat variability has been found to contribute to the spatial variation of deep-sea fish assemblages (Auster et al., 2005) and notably among different slopes of the same seamount in the Northwest Hawaiian Islands (Mejía-Mercado et al., 2019). Other abiotic factors such as mesoscale oceanography, light intensity, and hydrostatic pressure along with biotic factors like competition, food web linkages, and parasitism may be contributing to the unexplained variation (Levin et al., 2001).

4.3 Relationship between total abundance and environmental gradients

Overall, total abundance was found to decrease with depth which is in alignment with the decrease in food input available for organisms inhabiting deeper depths and the physiological adaptations fishes have acquired for dealing with low food quantity and quality at depth (Cocker, 1978). An exponential decrease in fish abundance with depth has also been observed in oligotrophic areas of the Atlantic (Merrett et al., 1991). However, there were two exceptions to this general pattern in the Main Hawaiian Islands and Tokelau Ridge where there was a high abundance of fishes at ~750 m. This was driven primarily by two genera, *Epigonus* and *Setarches.* In a 2015 dive conducted during CAPSTONE (D2-EX1504L4-01), which took place just south of O`ahu (Main Hawaiian Islands), there was a high abundance of *Epigonus*. Similarly, in a 2017 dive conducted during CAPSTONE (D2-EX1703-08) which also took place on an island flank near Howland Island (Tokelau Ridge), there were a high abundance of *Setarches.* There is evidence of enhanced primary productivity within the Line Islands (Howland, Baker, and Jarvis Island) in relation to the central Pacific Ocean gyres to the north and south of the equator. Evidence suggests that enhancement and concentration of phytoplankton from equatorial upwelling occur in fronts at the leading and trailing edges of tropical waves in which chlorophyll concentrations have been found to be an order of magnitude greater than background levels (Maragos et al., 2008; Mundy et al., 2010). It's possible that these high abundances are also driven by the enhancement of phytoplankton in near-island ecosystems known as the Island Mass Effect (IME). Although much yet remains unknown about the exact mechanisms causing this phenomenon, the increase in phytoplankton biomass in close proximities to island ecosystems has been documented for over half a century. Across the central and western Pacific, islands and atolls exposed to elevated levels of nearshore phytoplankton support higher fish biomass. However, IME strength can vary depending on the geomorphic type, bathymetric slope, and local human-derived nutrient input (Gove et al., 2016).

The relationship between total abundance and all environmental predictors (depth, concentrations of dissolved oxygen, POC flux, and salinity) was significant. Total abundance was predicted to be highest at upper bathyal depths between 250-500 m which is in accordance with the strong depth zonation patterns in fish assemblages that are linked with both biotic (competition, predation, and nutritional resource availability) and abiotic variables (substrate, temperature, light) that vary with depth

(Scott et al., 2022). Total abundance increased with higher POC flux values which were generally highest in upper bathyal water as expected. However, it's important to note that seafloor POC flux values were averaged across varying water column characteristics, therefore there may be regional differences that are unable to be captured by the POC flux model (Leitner et al., 2020). Further, organic matter may be underestimated, especially from human-derived runoff near islands (Gove et al., 2016). In addition to the positive relationship with POC flux, total abundance was found to be highest between dissolved oxygen values of 1.5 to 3.5 mg/L, however, sampling was limited where values were >4 mg/L. Many studies have found a general decrease in demersal fish density, biomass, or CPUE with decreasing oxygen levels, but the effect is nonlinear and there are greater reductions below certain oxygen thresholds which are region-specific and influenced by depth, temperature, and the demersal fauna inhabiting them (Gallo and Levin, 2016). In Hawai`i, where OMZ conditions are weak, there is a reduction in demersal fish abundances where oxygen conditions are lowest. In contrast, the regions with the lowest dissolved oxygen and highest values of benthic POC flux (Line Islands and Tokelau Ridge) had some of the highest average fish abundance values. Since there are limitations in the POC flux model's ability to capture regional habitat variation, this relationship (and potential correlation with POC flux) is hard to disentangle. Total abundance was highest at salinity values ~34.6, however, the dynamic range of salinity is very small (34.1-36.2), therefore this pattern may not be ecologically relevant.

4.4 Diversity patterns

Although none of the regions were sampled adequately for a comprehensive comparison of diversity patterns, there were a few major trends that can be identified and linked to ecological theory. Overall, genera richness was found to decrease with depth and evenness to increase with depth. Trends in richness may be explained by the kinetic energy hypothesis which states that warmer temperatures in the upper bathyal zone may support higher diversity than cooler, deeper zones (Woolley et al., 2016). The decrease in richness with depth may also be explained by the more-individuals hypothesis which theorizes that higher energy availability promotes a higher number of individuals in an assemblage allowing more species to persist (Storch et al., 2018). Bottom-water oxygen availability could also be contributing to this phenomenon because the Line Islands, Tokelau Ridge, and Main Hawaiian Islands had some of the lowest oxygen concentrations and the highest richness. Habitat diversity may be added in these regions due to the weak, yet thick OMZ that is not present in the other regions. OMZs have been found to provide hypoxia-tolerant species refuge from non-tolerant species leading to changes in assemblage structure (Gallo and Levin, 2016). However, the high richness in these regions may also be due to the disproportionate sampling that was conducted in the upper bathyal depths, which is especially relevant for the Main Hawaiian Islands and Tokelau Ridge. The trend in evenness increasing with depth may be explained by fish species in deeper habitats being more uniformly abundant due to a reduced input of energy (Zintzen et al., 2017). Some studies on Atlantic seamounts (Perez et al., 2018; Victorero et al., 2018) find a similar pattern and have suggested that a few specialized species dominate shallow waters with high food availability, then evenness increases with depth as their dominance subsides. Also, it is easier to estimate evenness when there are more samples, so the greater sampling effort in the upper bathyal depths may be contributing to this trend. However, as Pielou's evenness is not independent of richness, it's important to consider that the upper bathyal samples were generally genera rich with more dominant species whereas deep samples had lower general richness but were more evenly distributed.

The present study includes the first published central and western Pacific records of a number of taxa. A prominent example is the first record of the family Oreosomatidae based on observations of a species of *Neocyttus* which resembles or may be the same as *N. acanthorhynchus*, otherwise known only from the western Indian ocean (Yearsley and Last, 1998). There were also the first observations of *Halosaurus* species in the central Pacific. The ROVs and submersibles did not have the capabilities to collect fish specimens during these surveys. Thus, physical specimens of *Neocyttus* were not collected to confirm the sightings despite compelling videographic evidence. However, *Neocyttus* and *Halosaurus* are known to be distributed in the New Zealand EEZ and Australia (McMillan et al., 2011). These new records and others emphasize the importance of exploration and the need for detailed follow-up studies in the central and western Pacific, especially at intermediate depths of 750–1000 m and past 3000 m.

4.5 Management implications and future considerations

This study has direct management implications as it demonstrates that there is clear regional variation in the demersal fish assemblages in the central and western Pacific. Our results clearly show that existing Marine National Monuments are complementary components of the regional diversity and harbor unique assemblages which highlights the need to maintain this broad network of protection. Nonetheless, there is still much to learn about the deep-sea and as our understanding of these habitats improves, many more threats to these environments are recognized. Therefore, the effectiveness of the Monuments will depend on the spatial distribution and depths of human-caused disturbances such as climate change, deep-sea mining, and fishing. For instance, there has already been an increase in the frequency of extreme El Niño and La Niña events which could lead to more physiological barriers and decreases in habitat availability (Carlisle et al., 2017). To get a better understanding of how these systems will be influenced by anthropogenic effects, we first need to get a complete characterization of the assemblages inhabiting the regions and gain greater clarity of the boundaries and gradients of faunal change.

Due to the sample resolution and study design, we did not investigate the relationships between assemblage structure and smaller scale habitat structure such as boulder fields, and larger seafloor features such as seamount summits, and submarine canyons. However, all of these have been found to influence deep-sea fish assemblage structure (Auster et al., 1995; Auster et al., 2005; Quattrini and Ross, 2006; Ross and Quattrini, 2007; Milligan et al., 2016; Leitner et al., 2020; Leitner et al., 2021). The present study provides a first look at these assemblages at a broader regional scale, but it is important to note that further studies should investigate assemblage structure at a finer scale to fully understand the ecological patterns.

5 Conclusions

This basin-wide analysis provides the first insight into the assemblage structure and distribution of deep-sea demersal fish fauna inhabiting the diverse island and seamount groups across the central and western Pacific. Depth was found to be important for structuring assemblages, which become more similar with depth. Fish assemblages of the Hawaiian archipelago and the Equatorial regions (Line Islands and Tokelau Ridge) were unique and are likely influenced by the presence of an OMZ, with high fish abundance likely caused by regionally high food availability (seafloor POC flux). The present analysis was made possible by significant exploratory survey results. Future studies could use the present work to inform sampling designs and increase sampling effort (especially at depths of 750–1000 m) to more systematically advance our knowledge of the variables (and spatial scales) driving the assemblage structure of fishes in the central and western Pacific. Additionally, studies need to collect specimens and identify taxa to the species level for greater insights into biogeographic patterns, especially in light of increasing anthropogenic activity in the deep sea (Glover and Smith, 2003).

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: NOAA Deep Sea Coral Research and Technology Program (https://deepseacoraldata.noaa.gov/) and Hawaii Undersea Research Laboratory Archive (http://www.soest.hawaii.edu/HURL/HURLarchive/index.php).

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because this study uses video recordings and no handling of animals was required.

Author contributions

GS analyzed all data and wrote manuscript. JD, BM, and PA contributed to methodological design, statistical advice, and writing. CK provided edits and critical information. All authors discussed the results and agree to be accountable for all aspects of the work. All authors contributed to the article and approved the submitted version.

Funding

This material is based upon work supported by the National Science Foundation Graduate Research. Fellowship Program under Grant No. (1842402 and 2236415).

Acknowledgments

The authors thank Kyle Edwards and Craig Smith for commenting on drafts and providing research advice. We also thank Meagan Putts, Sarah Bingo, Tiffany Bachtel, and Virginia Moriwake (University of Hawai`i at Manoa) for their video annotations, data compilation and assistance. John Caruso (emeritus, Tulane University, LA; TU); Dominique Didier (Millersville University, PA; no abbreviation); David Ebert (Moss Landing Marine Laboratories, CA; MLML); Mackenzie Gerringer (State University of New York Geneseo; no abbreviation); Brian Greene (Bernice P. Bishop Museum, HI; BPBM); Hsuan Ching (Hans) Ho (National Museum of Marine Biology & Aquarium, Taiwan; NMMBA); Tomio Iwamoto (emeritus, California Academy of Sciences, CA; CAS); John McCosker (California Academy of Sciences, CA, emeritus; CAS); Astrid Leitner (Monterey Bay Aquarium Research Institute, CA; MBARI); Douglas Markle (emeritus, Oregon State University, OR; OSU); Peter Rask Møller (Natural History Museum of Denmark, University of Copenhagen; NHMD); Jørgen Nielsen (emeritus, Natural History Museum of Denmark, University of Copenhagen; NHMD); Werner Schwarzhans (Natural History Museum of Denmark, University of Copenhagen; NHMD); Shinpei Ohashi (Hokkaido University, Japan; HUMZ); Andrea Quattrini (US National Museum of Natural History, DC; USNM); David G. Smith (emeritus, US National Museum of Natural History, DC; USNM); Kenneth Tighe (US National Museum of Natural History, DC; USNM); and Kenneth Sulak (emeritus, US Geological Survey, FL; USGS) assisted with identifications of fishes seen during the CAPSTONE expeditions. We thank David Riser (University of Connecticut) for his initial analysis of the CAPSTONE meta-data, Andrew Yool (National Oceanography Centre, UK), Les Watling, Mahany Lindquist (University of Hawai`i at Manoa), and Travis Washburn (Geological Survey of Japan, National Institute of Advanced Industrial Science and Technology, Japan) for their help with the POC flux modeling, and Marti Anderson (Massey University, New Zealand) for their statistical expertise. Finally, we thank the Ocean Exploration Trust (OET), NOAA Deep Sea Coral Research and Technology Program (DSCRTP), NMFS Pacific Islands Fisheries Science Center, and the Deep-sea Animal Research Center (DARC) for making this data available. This is Ocean Research Explorations contribution ORE-014, Hawaii Biological

Survey contribution 2023-007, Pacific Biological Survey contribution 2023-003, and SOEST Contribution # 11733.

Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1219368/full#supplementary-material

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frontiers | Frontiers in Marine Science

TYPE Brief Research Report
PUBLISHED 25 October 2023
DOI 10.3389/fmars.2023.1251562

OPEN ACCESS

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RECEIVED 01 July 2023
ACCEPTED 06 October 2023
PUBLISHED 25 October 2023

CITATION
Johannes KN, Kennedy BRC and Bell KLC (2023) Seafloor Observation Scenario Exploration Tool: enabling representative exploration of the global deep seafloor. *Front. Mar. Sci.* 10:1251562.
doi: 10.3389/fmars.2023.1251562

Seafloor Observation Scenario Exploration Tool: enabling representative exploration of the global deep seafloor

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Visual observation of the seafloor is invaluable in understanding deep-sea biogeography, uncovering and expanding key global geological, oceanographic, and climate processes. And yet, due to the expense, inefficiency, and inequitable distribution of deep-sea tools, we have barely explored a small, biased fraction of it. Systematic and efficient exploration of the entire deep sea will require a concerted global effort. The current study uses global-coverage geospatial data to develop an interactive online tool for deep seafloor observation planning (i.e., depths below 200 m). The *Seafloor Observation Scenario Exploration Tool* integrates stakeholder goals and constraints into a dynamic modeling process that combines information about seafloor depth, seafloor geomorphology features, sites of past seafloor observation, Exclusive Economic Zones, and vessel traffic density. With the tool, users can identify maximally suitable areas for seafloor observation, which we envision supporting seafloor expedition planning and marine conservation action.

KEYWORDS

seafloor observation, marine spatial planning, web tool, capacity development, deep submergence vehicles, seafloor geomorphology

1 Introduction

The deep ocean – with depths between 200 and almost 11,000 meters below sea level – covers two-thirds of Earth's surface, encompassing the largest biosphere on the planet (Danovaro et al., 2020). Current and historical observation of deep-ocean ecosystems suggests that this environment supports some of the highest levels of biodiversity on the planet (Stuart et al., 2003; Rabone et al., 2023). And yet, less than 0.01% of the deep seafloor has been sampled, observed, and studied in detail (Ramirez-Llodra et al., 2010; Bell et al., 2022; Tyler et al., 2002).

Direct observation of deep seafloor environments, in particular, is critical for building an understanding of biogeographical and ecological patterns across and within large areas of the seafloor. There is a pressing need to understand these patterns ahead of accelerating resource exploration and extraction efforts, as many ecologically rich and biologically diverse regions are also abundant in mineral resources (IOC-UNESCO, 2017). For example, Amon et al. (2016) surveyed megafauna at multiple candidate mining sites in the abyssal Clarion-Clipperton Zone (CCZ). Regions of the 4.5 million km2 CCZ are under natural resource exploration contracts by multiple countries interested in mining polymetallic nodules on the abyssal seabed (International Seabed Authority, 2023). While we still know little of the biological and ecological characteristics of the CCZ and other areas of the abyssal seabed, baseline surveys of megafauna, conducted to advance scientific knowledge and inform conservation, revealed high levels of species richness, including species and genera that were both new to science and collocated with polymetallic nodules (Amon et al., 2016; Rabone et al., 2023; see also Gooday et al., 2015).

Deep-seabed mining and other resource extraction efforts, such as deep-sea fisheries, cause a wide range of impacts, both proximal and distal, on deep ocean environments (Ramirez-Llodra et al., 2011; Clark et al., 2016; Levin et al., 2016; Levin et al., 2020). While the sum of these anthropogenic impacts is yet unquantified, these activities minimally include the removal of habitat and critical substrate (Vanreusel et al., 2016), changes to the physical and geochemical properties of the deep seafloor (Van Dover, 2014), perturbation and contamination of the surrounding water column, disruptive changes in vibration and light (Levin et al., 2016; Levin et al., 2020), and impacts on ocean mitigation of climate change (Levin et al., 2023). The effects of anthropogenic impacts are especially critical for small island developing states (SIDS), where much less is known about what resources are present in their deep ocean (Amon, Rotjan, et al., 2022). Taken together with our nascent understanding of deep-sea ecosystems and organisms, it is imperative that we accelerate the exploration and research of these areas to stay ahead of the projected harms of potential extraction efforts (Amon et al., 2022).

With so little known and much at stake (Jamieson et al., 2021), why isn't deep ocean exploration – understood here to be the "initial multidisciplinary view or assessment of unknown or poorly understood areas" (Interagency Working Group on Ocean and Coastal Mapping, 2022 p.4) – and particularly visual deep seafloor observation, a more urgent global priority? One answer lies in disparities in global capacity and inequities in access to expertise and resources. Many countries that are geographically well-positioned to carry out observation activities lack access to the resources required for seafloor exploration (Bell et al., 2022). Visual observation, especially at abyssal and hadal depths, is costly and requires deep submergence vehicles and sensors that can tolerate the high-pressure, low-light environments in the deep sea. This technology necessitates further access to crewed vessels, relevant expertise, and developed systems for processing, interpreting, and sharing observational data. The cost of a properly outfitted expedition is tens of thousands of dollars (USD) per day (Kintisch, 2013; Brandt et al., 2016; IOC-UNESCO, 2017), resulting in exploration, research, and conservation agendas shaped by those from high-resource areas or regions (Bell and Amon, 2022).

A recent Global Deep-Sea Capacity Assessment (Bell et al., 2022) quantified these global disparities in access to the resources, technology, and expertise required to conduct deep-ocean research. The assessment included survey and/or manual research data on 186 geographical areas, documenting the presence of and access to various technical and human capacities for deep-sea exploration and research in every coastal area with deep ocean worldwide. Technical capacities assessed included vessels, deep submergence vehicles (DSVs), deep-sea sensor systems, and data tools. As DSVs and related equipment are typically necessary for deep seafloor exploration, we specifically examined the deep-sea presence of DSVs across geographical areas, noting that the highest presence of this equipment is concentrated in high-income countries, primarily in Northern America, Europe, and Eastern Asia (Figure 1; Bell et al., 2022).

The Global Deep-Sea Capacity Assessment (Bell et al., 2022) revealed that participation in deep-sea exploration is not representative of the range of global stakeholders and that countries vary widely in the specific combinations of expertise, technology access, conservation goals, and collaborative partnerships needed to support deep-sea exploration. Examining the global metadata for past deep submergence activities also underscores a lack of representation in the distribution of activities across geographical regions, depth ranges, and geomorphological features observed in deep-sea exploration to date. Global participation and coordination are needed to develop representative plans for observing the global seafloor in the future.

Real-time marine spatial planning tools such as *SeaSketch* (Berger et al., 2023) and *OceanReports*, (Moore, 2022) have proven instrumental in facilitating collaborative ocean use, management, and conservation planning activities. However, these tools have been used primarily and in service of coastal planning and analysis and, in the case of *OceanReports*, developed specifically for US waters. Both platforms serve as examples of how planning-relevant data can be made available through accessible online interfaces. We extend this approach to the global deep ocean and seafloor and present a prototype *Seafloor Observation Scenario Exploration Tool* that allows users to engage multiple global datasets to plan exploration under various scenarios. Specifically, based on user-specified priorities, the *Seafloor Observation Scenario Exploration Tool* generates global and regional heatmaps of seafloor areas, reflecting areas best aligned with the user's priorities. The tool integrates critical geospatial planning information, such as vessel traffic routes and Exclusive Economic Zones (EEZs), with past seafloor exploration activity records, global bathymetry, and seafloor geomorphology, facilitating real-time coordination and deep-sea exploration planning via an open-access web tool.

2 Methods

We use a series of global-coverage Geographic Information System data layers, described in detail below, as input to an interactive *Seafloor Observation Scenario Exploration Tool*. Currently the tool is built with five input data layers, which can be combined and visualized based on user goals. Specifically, users can weight these different layers through an interactive web interface, yielding a heatmap of seafloor areas that reflect their combined priorities. We illustrate this process with an example in our Results.

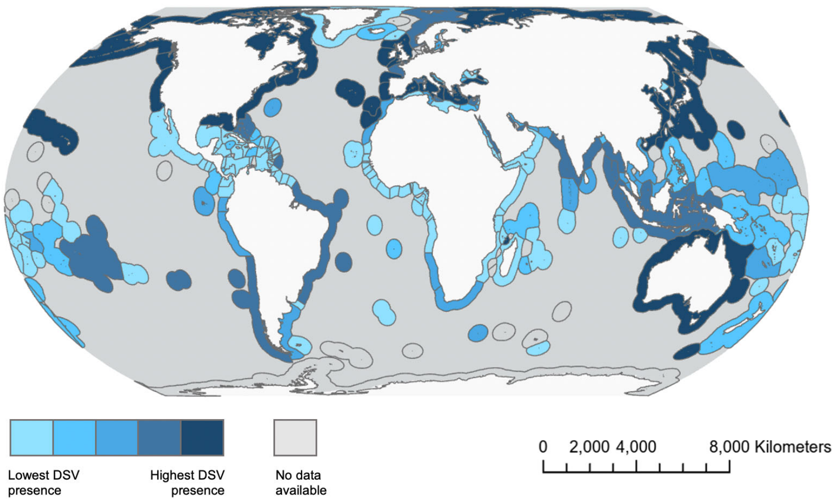

FIGURE 1
Global Exclusive Economic Zones are colored according to their deep submergence vehicle (DSV) presence index, a relative index based on the presence of types of DSVs in each country and territory; higher values indicate higher diversity of DSV types present in each (Bell et al., 2022). Types of DSVs assessed in the 2022 Global Deep-Sea Capacity Assessment include remotely operated vehicles (ROVs), autonomous underwater vehicles (AUVs), human-occupied vehicles (HOVs), benthic landers, drifters, or tow sleds (Bell et al., 2022).

2.1 Input layers

We selected geospatial data layers to include in the *Seafloor Observation Scenario Exploration Tool* based on their relevance to deep-seafloor planning, and further refined our selection based on several conditions. Each layer needed to provide global and appropriately high-resolution coverage for the nature of the data being displayed in that layer. For example, seafloor slope data were not included, as this information could not be accurately computed from available global bathymetry at a resolution that would be useful for planning. Layers compiled from geospatial data files (e.g.,.shp files,.csv files with geographical coordinates) were sourced from published datasets. Layers sourced from the ESRI *Living Atlas of the World* collection (https://livingatlas.arcgis.com) were selected for use based on their status as "Authoritative" layers with the most complete and accurate information. The resulting five layers, reviewed individually below, included: deep submergence metadata for seafloor observations, global Exclusive Economic Zone (EEZ) areas, global ocean bathymetry, global seafloor geomorphology, and global vessel traffic density. Planned data layer additions for future iterations are included in the Discussion.

2.1.1 Deep seafloor observation metadata

We adapted a dataset of deep submergence vehicle metadata collected by Kennedy and Rotjan (in review for this special issue), which included 35,346 records from 13 institutions across 6 countries, and added approximately 13,000 additional deep submergence vehicle deployment records from newly available sources not sampled by Kennedy and Rotjan. Metadata were sourced by country and institution through a combination of public and internal databases, data requests, and published research findings. A complete set of sources are listed in the Supplemental Materials. The dataset represents point-based locations of remotely operated vehicles (ROVs), autonomous underwater vehicles (AUVs), human-occupied vehicles (HOVs), benthic landers, and camera sled tows that generate images of the deep seafloor. Key metadata variables included point-based latitude-longitude coordinates, maximum depth, date of observation, institution and country responsible for data collection, platform name, and type of equipment (Supplemental Materials). Much of the metadata were shared with either single latitude/longitude coordinates, or start-end coordinates for transects and the data were displayed as a point-based layer with 48,053 individual sites (Figure 2A) in ArcGIS Pro and converted to a raster layer for analysis using counts per cell on a global grid of 270 km^2 cells.

2.1.2 Global exclusive economic zone area

Global EEZ areas and boundaries were accessed through the ESRI Living Atlas (Flanders Marine Institute, 2019). EEZ areas were processed in ArcGIS Pro to remove boundaries between countries and territories to reflect the total global EEZ area (Figure 2A). This global layer was converted to a raster layer for use within the *Seafloor Observation Scenario Exploration Tool*.

2.1.3 Global ocean bathymetry

Gridded bathymetry data, accessed through the General Bathymetric Chart of the Oceans (GEBCO, 2021; Figure 2B), was used to generate depth estimates across the entire global ocean.

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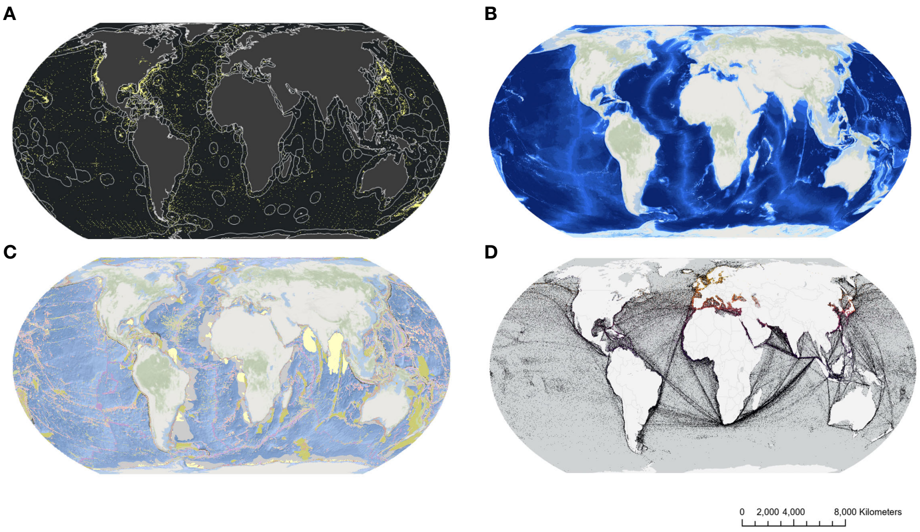

FIGURE 2
(A) Locations of ROV, AUV, HOV dives, benthic landers, and tows used in seafloor observation metadata set (adapted from Kennedy & Rotjan, in review) overlaid with Global Exclusive Economic Zone boundaries (Flanders Marine Institute, 2019). Individual yellow points represent individual dive sites; white lines represent EEZ boundaries. **(B)** Global deep ocean bathymetry data is derived from the General Bathymetric Chart of the Oceans (GEBCO, 2021). **(C)** World Seafloor Geomorphology features (Harris et al., 2014) are displayed as a series of polygon layers. **(D)** The density of global vessel traffic from 2015-2021 (Cerdeiro et al., 2020). Brighter colors represent a higher density of vessel traffic. Additional map images with detailed color scale legends are included in the Supplemental Materials.

Bathymetry estimates were displayed and analyzed as a raster layer, with a resolution of 15 arc seconds.

2.1.4 Global seafloor geomorphology

World Seafloor Geomorphology layers, available through the ESRI Living Atlas of the World, were used to capture the distribution of geomorphological features on the global seafloor. The layers were developed using mapping data from GRID-Arendal (Harris et al., 2014) and characterize physical features on the seafloor and the global ocean zones in which they occur with a 30-arc second resolution. Geomorphic features were displayed as polygon layers (Figure 2C) and converted to a raster layer for analysis and use in the tool. The dataset includes 17 geomorphic features (e.g., seamounts, ridges, guyots, rifts), which are sparsely distributed across the global seafloor. In addition to these geomorphic features of interest, the World Seafloor Geomorphology dataset includes individually identified ocean basins, and seafloor terrain for slope, shelf, hadal and abyssal zones, which were not used in the preliminary version of the web-app tool.

2.1.5 Global vessel traffic density

Global vessel traffic was represented as density patterns across the global ocean, based on hourly AIS positions, for all types of ship traffic, including shipping, leisure, fishing, passenger, and oil and gas (Figure 2D). These datasets were obtained for positions from January 2015 to February 2021 and synthesized by the World Bank through a partnership with the International Monetary Fund (IMF) as part of the IMF World Seaborne Trade Monitoring System (Cerdeiro et al., 2020). This information was used as a grid-based raster layer, with a 500m^2 cell size (at the Equator).

2.2 Seafloor Observation Scenario Exploration Tool development

The *Seafloor Observation Scenario Exploration Tool* was developed using the Suitability Modeler toolkit in Esri ArcGIS Pro (Stauder, 2014) and made available to users through the ArcGIS Experience Builder web-app interface. As input into the web-app interface, we combined the raster layers for each global dataset—bathymetry, seafloor geomorphology, previous deep submergence activity, EEZ areas, and global vessel traffic—into a mosaic raster file using the *Create Mosaic* tool (Esri, Redlands California). This mosaic created a weighted raster overlay, which encoded the factors, levels, and structure needed for users to dynamically adjust their model priorities when interacting with the scenario exploration tool. The weighted raster overlay was shared to ArcGIS Experience Builder as a server-hosted imagery layer, which allows users to interact with the model layers through the web-app.

3 Results

3.1 Historical observation coverage

Access to resources and human capacity have historically driven exploration areas and priorities (Bell, Chow, et al., 2022). Consistent with capacity assessment findings (Bell et al., 2022), countries with

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developed deep-ocean observation programs and access to deep submergence technology generated the greatest proportion of visual observation data in our sample. Most observation activities in our sample were undertaken within countries' EEZs, with approximately 78% of all activities in the sample located within EEZs (Figure 2A). Furthermore, 98% of the total observation activities within global EEZ boundaries, regardless of EEZ sovereign, were conducted by just 5 countries: United States, Japan, New Zealand, France, and Germany.

Globally, only a tiny fraction of the deep seafloor has been observed through image-generating deep submergence technologies, and the geospatial distribution of these observations is not representative of seafloor bathymetry or the distribution of seafloor geomorphological features by area. The distribution of seafloor observations across bathymetric zones is roughly inverse to the distribution of seafloor area across these depth zones (Figure 3A). Half of the global seafloor lies at depths of 4,000 meters or deeper, but only about 6.5% of observations occurred at these depths. Similarly, while 42.7% of deep seafloor observations in our deep submergence records were made between 200 and 1000 meters, this depth range accounts for only 6.2% of the deep seafloor by area.

Similarly, the density of deep submergence activity across different geomorphological features of the seafloor is neither uniform nor representative of the global seafloor area characterized by these features (Figure 3B). Rift valleys and canyons, which characterize a relatively small area of the global seafloor, have been observed at roughly 5 and 75 times, respectively, the rate of geomorphological features with much greater global presence.

The geographical distribution, bathymetric coverage, and geomorphological coverage of past seafloor exploration activities provide an essential snapshot of the areas and environments that are over- and under-represented in current seafloor observation. These patterns guide where to prioritize future exploration efforts and highlight existing inequities in global deep-sea presence, especially when considering information about global capacity and distribution of seafloor exploration resources such as deep submergence vehicles (Figure 1).

3.2 Seafloor Observation Scenario Exploration Tool

The *Seafloor Observation Scenario Exploration Tool* is a dynamic web-based planning tool that allows users to adjust the importance of different considerations based on their seafloor exploration goals, priorities, and access to resources, vessels, and technology. The tool weights these considerations to generate a suitability heat map of regions that best fit the user-specified priorities. Users can engage or ignore each of five global information layers: EEZ areas, seafloor depth, seafloor geomorphology features, sites of past seafloor observation, and vessel traffic density.

Once users have chosen which information layers are relevant to their priorities, they can adjust how important each is by assigning a percent value, balanced so that all assigned values add to 100% (Figure 4A). For example, suppose a stakeholder is limited by depth-specific technology. In this case, they can give greater importance to the "Seafloor Depth" layer by assigning it a higher percent-based weight in the tool.

Users can further adjust how specific levels of a factor are important in their planning. For a factor like "seafloor depth", levels reflect specific depth ranges (Figure 4B). Priority is assigned to levels via a numeric scale of 0-9, controlled by a simple slider interface, and reflect relative importance within a single factor; all levels can be set to be equally important, or specific levels can be given a higher value to reflect greater priority within that factor. If, for example, a stakeholder has access to depth-limited tools and technology, they can place more weight on specific depth ranges – levels of the depth factor – using higher numbers on the slider and place less weight on other ranges using lower numbers on the slider.

To illustrate, consider a stakeholder group in Mexico with the following priorities:

- Vessel access to the seafloor within the country's EEZ;
- Access to deep submergence technology with a 4,000 m depth rating;

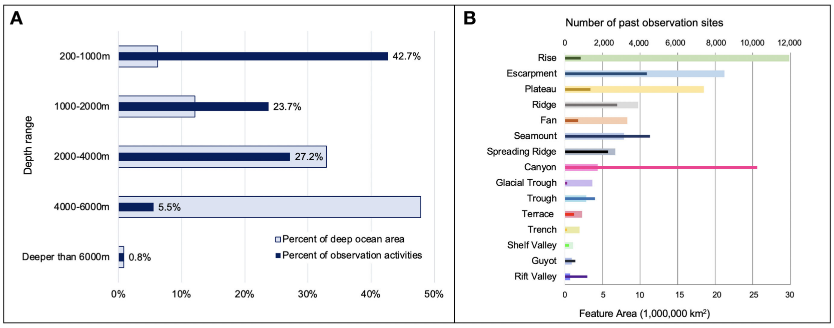
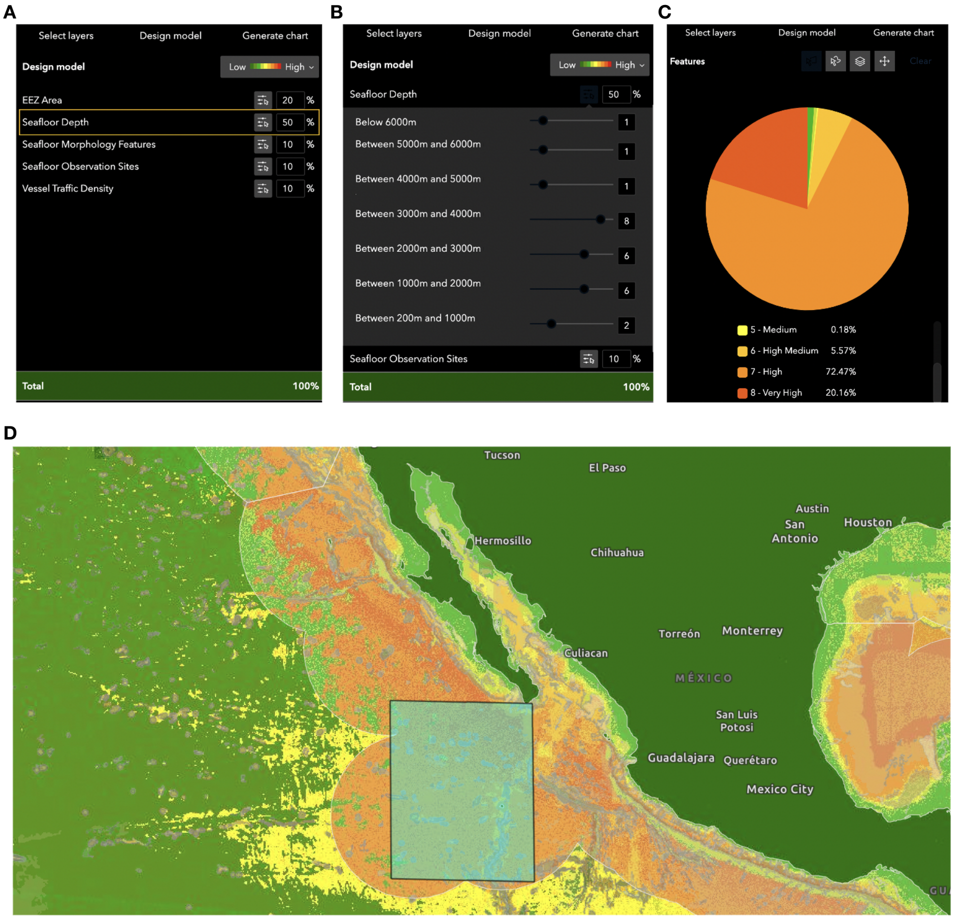


FIGURE 3
(A) Distribution of past seafloor observation activities by depth (dark narrow bars) superimposed on the global distribution of deep seafloor area by depth (light blue wide bars). **(B)** Distribution of past observation sites across different seafloor geomorphological features. Dark bars represent the number of historical and proposed sites collocated with different seafloor geomorphological features and are overlaid on light bars representing the total area occupied by each feature (Harris et al., 2014). Bridge and Sill features were both unobserved in our record (i.e., 0 historical observations).



A

EEZ Area 20 %
Seafloor Depth 50 %
Seafloor Morphology Features 10 %
Seafloor Observation Sites 10 %
Vessel Traffic Density 10 %
Total 100%

B

Seafloor Depth 50 %
Below 6000m 1
Between 5000m and 6000m 1
Between 4000m and 5000m 1
Between 3000m and 4000m 8
Between 2000m and 3000m 6
Between 1000m and 2000m 6
Between 200m and 1000m 2
Seafloor Observation Sites 10 %
Total 100%

FIGURE 4
(A) After choosing global information layers of interest, users can assign different levels of importance to each layer by assigning a greater or lesser percentage contribution to the model. **(B)** Within the Seafloor Depth layer, users can use the slider to increase (larger values) or decrease (smaller values) the weight of each depth range in the model. **(C)** Suitability breakdown pie chart for the user-drawn region of interest within the EEZ of Mexico. **(D)** Heatmap of suitability for a user-drawn region of interest within the EEZ of Mexico.

- Aims to capture a specific distribution of seafloor geomorphic features;
- Aims to observe unexplored areas of the seafloor; and,
- A need to avoid other vessel traffic while conducting observations.

Once all layers of interest have been specified and weighted to sum to 100% total model coverage, users can run the tool to reflect their model priorities across the entire global ocean (Figure 4D). To accommodate their region-specific planning needs, users can zoom into different areas of the resulting interactive web map and use drawing tools to create a custom polygon around regions of interest of any size (Figure 4D). In the pie chart visualization generated for the region of interest, given the stakeholder priorities listed above (Figure 4C), over 92% of the area is designated with a "High" or "Very-High" degree of suitability – reflected in shades of orange in the chart and on the map. In this way, stakeholders receive an immediate snapshot of their regions of interest with information about how suitable the seafloor in their region is, given the priorities and constraints they specified in the tool's model design.

4 Discussion

Although sparse, historical visual observation of the seafloor has proved invaluable in understanding unique deep-sea ecosystems (Danovaro et al., 2014), discovering novel organisms, and uncovering and expanding key global geological, oceanographic, and climate processes (Ramirez-Llodra et al., 2010; Gasparin et al., 2020; Levin et al., 2023). Systematic and efficient exploration of these regions requires a sustained collective effort (Danovaro et al., 2017), undergirded by global observation observing programs such as the Global Ocean Observing Strategy and Deep Ocean Observing Strategy (Levin et al., 2019; Danovaro et al., 2020; Satterthwaite et al., 2021). Collaborative planning and exploration efforts are increasingly necessary to fulfill global conservation goals, such as the Global Biodiversity Framework "30 by 30" agreement (Convention on Biological Diversity, 2022), and to realize the deep-sea conservation opportunities enabled by ratification of the Agreement for conservation of biodiversity in areas beyond national jurisdictions (BBNJ; UN General Assembly, 2023).

This need is also reflected in the challenges put forth by the UN Decade of Ocean Science for Sustainable Development (UNESCO-

IOC, 2023), which directly engages with deep ocean research and stewardship through a series of challenges (Howell et al., 2020). With these challenges top of mind, data collected through collaborative seafloor observation can contribute to "protection and restoration of ecosystems and biodiversity" (Challenge 2), "expand the Global Ocean Observing System" (Challenge 7), "create a digital representation of the ocean: (Challenge 8), develop "skills, knowledge, and technology for all" (Challenge 9), and "change humanity's relationship with the ocean" (Challenge 10).

Our *Seafloor Observation Scenario Exploration Tool* contributes to a multifaceted effort to accelerate deep ocean observation on a global scale. These resources also function alongside efforts to develop low-cost, easy-to-use technology innovations to lower barriers to deep-sea data collection, global collaboration to promote and streamline high-quality data processing and sharing, and movements towards increasing global capacity and building partnerships. We envision the tool supporting current and future chief scientists and expedition leaders through programs like the Crustal Ocean Biosphere Research Accelerator (COBRA; Huber & Orcutt, 2021; Rotjan et al., 2023) and contributing to a growing set of tools for real-time marine spatial planning (e.g., SeaSketch; Berger et al., 2023). To this end, we intend to continue the development of the *Seafloor Observation Scenario Exploration Tool* through iterative user and community feedback, including the addition of GIS layers encoding the location of Marine Protected Areas and deep-sea fishing areas across the global ocean as well as layers containing planning-relevant data about benthic substrate, seafloor slope, environmental variables (e.g., dissolved O_2, salinity, particulate organic carbon) and topography.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author. The prototype Seafloor Observation Scenario Exploration Tool can be accessed at http://bit.ly/seafloor-tool.

Author contributions

KJ and KB conceived and designed the study. KJ and BK collected data. KJ drafted the article. All authors contributed to the article and approved the submitted version and agree to be accountable for all aspects of the work.

Acknowledgments

The authors would like to thank formative feedback and encouragement from Greg Rouse, Judith Gobin, Randi Rotjan, Karen Stocks, Leslie Smith, Helen Pilar, Susan Poulton and the Ocean Discovery League team. We would also like to acknowledge all the individual researchers who have shared relevant exploration data, improving the global coverage of our datasets. Continued work on this project is made possible by a generous fellowship to KJ from the Edna Bailey Sussman Fund.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1251562/full#supplementary-material

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frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 23 November 2023
DOI 10.3389/fmars.2023.1219799

OPEN ACCESS

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RECEIVED 09 May 2023
ACCEPTED 16 October 2023
PUBLISHED 23 November 2023

CITATION
Kennedy BRC and Rotjan RD (2023) Mind the gap: comparing exploration effort with global biodiversity patterns and climate projections to determine ocean areas with greatest exploration needs.
Front. Mar. Sci. 10:1219799.
doi: 10.3389/fmars.2023.1219799

Mind the gap: comparing exploration effort with global biodiversity patterns and climate projections to determine ocean areas with greatest exploration needs

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The oceans contain 1,335 million km^3 of water covering 361.9 million km^2 of seafloor across 71% of the planet. In the past few decades, there has been substantial effort put into mapping and exploring the ocean fueled by the advent of new technologies that more easily enable deepwater access. However, we are still far from achieving our shared goals of a well characterized and documented ocean. In 2010, Webb et al. documented the paucity of deep-sea data in general, with a specific focus on the lack of pelagic records in the Ocean Biogeographic Information System OBIS, which is the largest of the ocean biodiversity archives. While significant exploration progress has been made, the rate of change in the ocean is outstripping the rate of characterization and research. Given the limited resources available, future work needs to be prioritized to focus on areas of greatest need. Here, we investigated several lines of inquiry to determine priority areas for future exploration. We accumulated the largest database of global deep submergence dive records ever compiled and used it, plus OBIS biodiversity records, to assess the level of exploration in different ocean regions. Then, we compared these measures of exploration effort with different biogeographic province schemas and estimates of climate change velocity projections to identify the largest remaining gaps in exploration and research sampling. Given that marine science has only explored between 5 and 20% of the ocean (depending on estimates) in the last hundred and fifty years, future exploration needs to be more targeted to attempt to keep pace with the rate and impact of environmental and biodiversity change in the ocean.

KEYWORDS

ocean exploration, biodiversity, biogeography, deep-sea, global change

Introduction

We are facing a global crisis of biodiversity (Ceballos et al., 2015; Boyce et al., 2022). More than a million species are known to be at risk for extinction globally (IPBES, 2022), the majority of which reside in marine environments (Mora et al., 2011). Because the ocean is still so underexplored, the need to value and conserve taxa and habitats that we know so little about has been termed a 'paradox of marine conservation' (Webb, 2009). A 2006 Science editorial highlighted that biodiversity research was 'grounded' in terrestrial environments (Hendriks et al., 2006) with only about 10% of the research published or presented at international biodiversity conferences focused on marine biodiversity, with a similar terrestrial-focus found in related disciplines including conservation biology and macroecology (Raffaelli et al., 2005; Richardson and Poloczanska, 2008). This terrestrial focus logically extends from ease of access; however, it is not representative of our planet. The ocean covers 71% of the planet's surface and represents nearly 90% of the habitable space (over a billion km^3) for multicellular life (Angel, 1993; Kunzig, 2003; Robison, 2004; Robison, 2009). Several efforts have been made to address the knowledge gap in ocean biodiversity research. In the early 2000s, the Census of Marine Life (Ausubel et al., 2010; Ramirez-Llodra et al., 2010) and other projects such as the Marine Biodiversity and Ecosystem Functioning EU Network of Excellence (Heip et al., 2009) made major strides in documenting new species in the ocean. More recently, projects such as the UN Ocean Decade (Ryabinin et al., 2019) and Challenger 150 (Baker et al., 2021) continue to cast a spotlight on gaps, with the goal of filling them. While this mismatch of effort (terrestrial) and habitable area (marine) is a well-known paradox, marine scientists still struggle to close the gap between ocean area and terrestrial research because the ocean is disproportionately large, expensive to study, and historically under characterized.

As the planet faces a rapidly shifting climate (IPBES, 2022) and a biodiversity crisis (Boyce et al., 2022), the lack of knowledge about the biodiversity of our oceans is becoming a problem that is increasingly an issue of global relevance for food security, national security, and international peacekeeping (e.g. Grabarz, 2009; Fedotova et al., 2021; Talukder et al., 2022). The ocean provides a range of globally important ecosystem services (Thurber et al., 2014) including support of fisheries, natural products for medical and industrial chemical processes, regulation of climate and ocean chemistry, providing approximately 50 percent of atmospheric oxygen (Riser and Johnson, 2008) and sequestering 37,000 Gigatonnes of carbon that includes the absorption of ~25% of the carbon aggregately released from the sum total of human activities (Canadell et al., 2007; Sabine and Feely, 2007; Gruber et al., 2019). The biological mechanisms that regulate these processes come from a diversity of organisms, many of which are as-of-yet undescribed or unknown (Mora et al., 2011). These new taxa are not just cryptic invertebrates, but also include large megafauna such as whales and sharks (Weigmann et al., 2020; Rosel et al., 2021).

The ocean is not a monolith. It hosts a wide variety of ecosystems and different habitats that are governed by a range of abiotic and biotic factors such as light input, ocean currents, depth, upwelling, migrations, etc. (Tittensor et al., 2010), all of which contribute to biodiversity. To account for the complexity, numerous attempts have been made to understand the structure of biodiversity of the ocean by defining eco-regions or biogeographic provinces, which continues to be an area of active research (Longhurst, 1985; Longhurst, 2006; Watling et al., 2013; Sutton et al., 2017). The ocean community has not yet reached consensus on a single geodescriptive system, because each has different strengths in characterizing biodiversity patterns by specific depth range or taxa (Longhurst, 1985; Longhurst, 2006; Watling et al., 2013; Costello et al., 2017; Sutton et al., 2017). As such, it remains challenging to point to a specific ecoregion or biogeographic province and infer associated biodiversity estimates, which hinders ocean practitioners from being able to manage, conserve, restore, or predict biodiversity loss/gain at scale.

Webb et al. (2010) quantified the gaps in biodiversity information in the ocean broadly, with a specific focus on pelagic waters using the Ocean Biodiversity System (OBIS) and found significant gaps in biodiversity records across distance from shore, and across depths. Here, we build upon that to document progress (e.g., the change in distribution of OBIS records) in the last decade and to identify remaining areas of priority exploration needs. To accomplish this, we have integrated biogeographic provinces of the ocean with OBIS data to identify the least-characterized areas. Additionally, we compiled a global dataset of deep submergence dive records to build upon the OBIS records to identify the areas of the world's oceans that are least explored by deep submergence vehicles. Finally, we integrated this information with climate change projections to determine the areas of greatest expected change with lowest number of biodiversity records.

Methods

As a proxy for ocean exploration effort, deep submergence dive locations and depths were collected from 12 institutions globally including: The National Oceanic and Atmospheric Administration's Office of Ocean Exploration (NOAA-OE), the Hawaii Undersea Research Laboratory (HURL), Ocean Exploration Trust (OET), Harbor Branch Oceanographic Institute (HBOI), Monterey Bay Aquarium Research Institute (MBARI), National Deep-Submergence Facility (NDSF) and the Schmidt Ocean Institute (SOI) from the United States. Outside the United States, records were collected from the Japan Agency for Marine-Earth Science and Technology (JAMSTEC), the French National Institute for Ocean Science (IFREMER), the United Kingdom's Natural Environment Research Council (NERC), Russian Academy of Sciences, and GEOMAR Helmholtz Centre for Ocean Research Kiel. Attempts to gather additional records from other institutions were made but the authors were unable to obtain them. Records for human-occupied submersibles, remotely operated vehicles, autonomous underwater vehicles, and towed cameras were included in the dataset. Dive locations were accessed through publicly available data portals when possible (NOAA-OE, HURL, OET, NDSF, NERC) or though data requests made to the institutions (MBARI, HBOI, SOI, GEOMAR, IFREMER, JAMSTEC). Records without position data were eliminated from the dataset and records for sites that are not publicly available were rounded to the nearest 0.01 of a degree to protect sensitive site locations.

Biogeographic provinces for different depths were overlaid from the literature as follows: Longhurst provinces were used to describe the surface waters (Longhurst, 2006), accessed from https://www.marineregions.org/ (Flanders Marine Institute, 2019); Sutton et al. (2017) proposed biogeographic provinces for the mesopelagic was accessed via the supplementary information; and Watling et al. (2013) proposal for the lower bathyal and abyssal seafloor was accessed through personal communications with the authors. Ocean biodiversity records were downloaded from the Ocean Biodiversity Information System (OBIS.org database up to date as of 2022-10-22). Detailed definitions of the zones we used can be found in Table 1. It is important to note that we took an inclusive definition of the provinces proposed by Watling et al., 2013 to include the pelagic waters of the same depth ranges because there is so little work on biogeography of the pelagic communities at depth. Additionally, we also combined the Abyssal and Hadal zone for this analysis because of a lack to hadal records/OBIS records that were flagged as terrestrial and those without depth information were excluded from the data set. The total number of OBIS records is 107,390,009. Reduced by nonsensical geographic positioning and negative depths left us with 94,053,712 records. We then removed all records with no depth information, which left us with a total of 58,442,819 records that were used in this study. OBIS records, as well as all four of the biogeographic province schemes noted above, were spatially joined with a global 100 x 100 km grid (Equal Earth Projection) for surface waters (0-200 m), mesopelagic (200-1,000), Bathypelagic (1,000-4,000m), Abyssopelagic (>4,000) using the R package 'sf' (Pebesma, 2018). OBIS records for each grid or province were tallied for total number of records, and the number of unique families, genera, and species found in each area. A quartile rank for the number of records was assigned for each 100 km x 100 km grid.

TABLE 1 Table of Global Marine Biogeographic Provinces used in this study.

Depth Zone	Depths	Description	Base reference
Surface	0-200 Meters	Used as proposed by Longhurt, though initially based on plankton records focused on the biogeography of the surface waters.	Longhurst, 1985
Mesopelagic	200-1000	Used as proposed by Sutton et al, though initially created to describe communities of mesopelagic fish.	Sutton et al., 2017
Bathypelagic	1000-4000	We added the bathypelagic to Watling et al's 2013 proposed benthic provinces	Watling et al., 2013
Abyss and Hadal Pelagic	>4000	We added the abyssal and hadal pelegic to Watling et al's 2013 proposed benthic provinces	Watling et al., 2013

Includes depth, zones, original reference, and a brief description of the organizing framework for each biogeographic province schema.

Following the methods outlined in (Webb et al., 2010), we used the ETOPO2 30 arcs-second relief model (NOAA National Centers for Environmental Information, 2022) to get ocean depths for each occurrence record. Together, sample depth and bottom depth describe the position in the water column of each record. Prior to analysis, we removed any record with a negative depth. For any record with unreconciled depths (e.g., where the sample depth was greater than the reported depth from ETOPO2), we assumed the record was a benthic one, and thus replaced the bathymetry maximum depth with the sample depth assuming the sample depth is more accurate for that location. We then used global seafloor bathymetry to determine the proportional area of the oceans occurring in each zone, represented by cells (Figure 1). We corrected the number of OBIS records based on the proportional volume of ocean meeting the appropriate criteria for sample depth and ocean depth. A more detailed description of this method can be found in (Webb et al., 2010).

Climate change velocity estimates were harvested from the supplementary information of Brito-Morales et al. (2020). They used data from Coupled Model Intercomparison Project Phase 5 (CMIP5) climate models averaged annually with a one degree resolution to estimated climate velocity for different depths of the ocean based on three scenarios published by the Intergovernmental Panel on Climate Change (IPCC). Climate change velocity illustrate the distance north an organism would have to move north to find the same temperature conditions based on different warming scenarios. We took the average value of Brito-Morales et al. change velocity estimates contained within each of the same 100 kmx100 km grid cells populated with OBIS data. We then assigned a rank-ordered value for the mean change velocity across all of the grid cells. To determine the areas of highest priority for exploration, we multiplied the climate change velocity rank (indicative of the likelihood of species movement under various IPCC scenarios) by the inverse OBIS rank (indicative of the areas with the fewest records available). This data manipulation yielded cells, translating to spatial coordinates, demonstrating areas with the lowest number of biodiversity records and the highest value for climate change velocity. Data for the IPCC RCP 8.5 "very high baseline emission scenario", which representing the 90th percentile of the policy baseline scenario from the IPCC are presented here; visualizations using IPCC RCP 2.6 and 4.5 are located in the Supplementary Information.

Results

In total, we were able to collect location information for 30,733 dives made by deep-submergence platforms (Figure 1B). While the majority of the ocean has no dives, there are three grid cells that dominate the dive records (Figures 1B, C): Monterey Canyon, California where MBARI focuses most of its work with 4237 dives, and offshore of Yokohama, Japan with two cells containing 1,715 dives combined where JAMSTEC has conducted much of their work (Figures 1B, D). These three cells alone account for 16.8% of the dives recorded in this dataset, even though they only account for ~0.001%

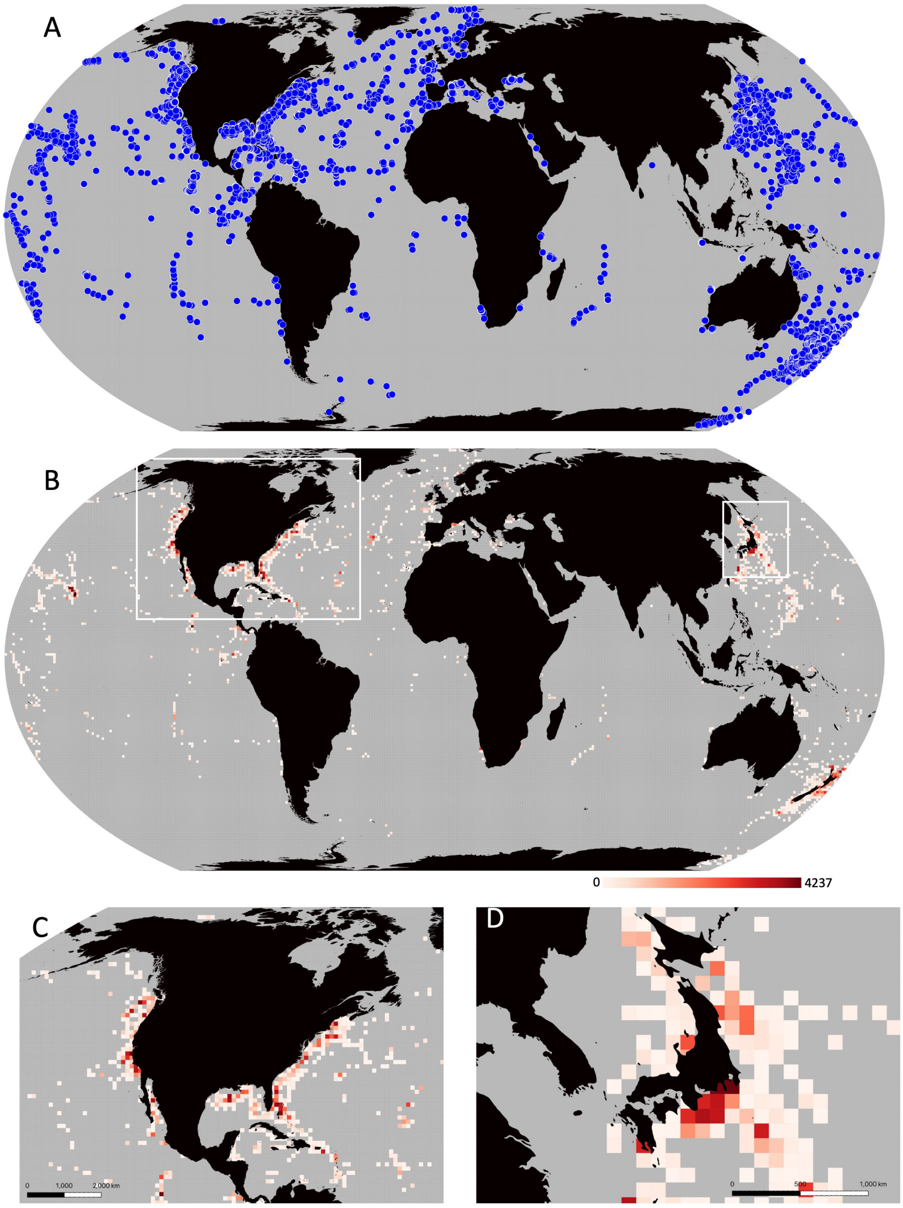

FIGURE 1
(A) All 35,346 deployment records collected including HOV, ROV, AUV, and towed imaging vehicle deployments from SOI, MBARI, NOAA, HURL, OET, SOI, GEOMAR, JAMSTEC, HBOI, IFREMER, and cruise locations from the Russian Academy of Sciences MIR 1 and 2 expeditions. **(B)** Number of dives per 100x100 km grid, Grey cells denote areas with no dive records. **(C, D)** Zoomed in subset highlighting the high number of dives around North America and Japan respectively.

of the surface area of the ocean. Notably, 82.8% of the dives were conducted in the Northern Hemisphere, with only 6,172 dives (17.2%) conducted in the Southern Hemisphere.

The number of OBIS biodiversity records per cell varies by seven orders of magnitude across the globe (Figure 2). With highest numbers of records per cell topping out at 2,813,641 records, corresponding with 1,985 species, however the vast majority of cells have a significantly lower number of records. The median number of records per cell ranged between 5 in the abyss to 55 in the surface waters. All the depth zones examined had more cells without any OBIS records than cells with OBIS records. In surface waters, 49% of cells had at least one record, and in the abyss only 5% did.

The global distribution through the water column of recorded marine biodiversity is shown in Figure 3. Even on the logarithmic scale of number of records, the dominance of shallower and coastal waters within the OBIS database is clear. The deep pelagic ocean in particular reflects the paucity of records from this habitat with less than one OBIS record per 3,500,000 km^3. When we consider that each cell of 200m depth over the abyssal plain and hadal depths represents a volume of c. 3.5 million km^3 and that the color scale of Figure 3 exceeds 7 orders of magnitude, the global mean number of OBIS records per cell is only 585 and the median is 11 records per 3.5 million km^3 of ocean; in other words, over half the ocean has less than 11 records total.

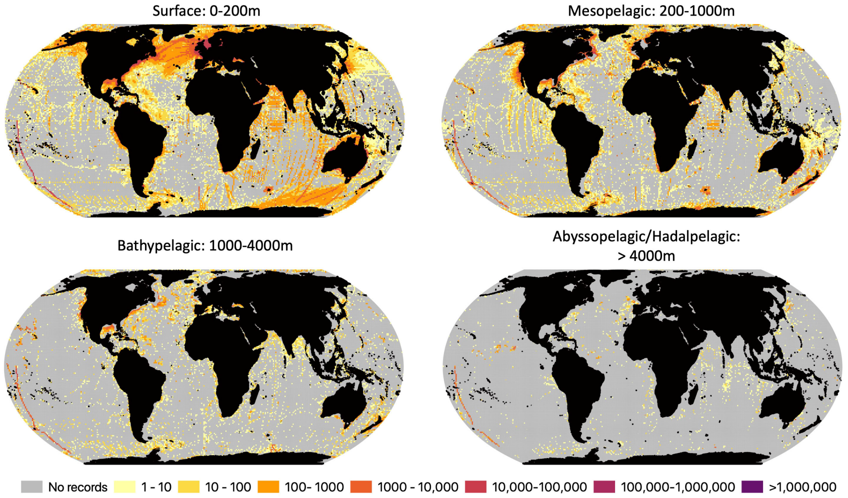

FIGURE 2
Number of OBIS records with depth information per 100 x 100 km grid for surface (0-200m), mesopelagic (200-1,000), bathypelagic (1,000-4,000m) and abyssopelagic/hadalpelagic (>4,000m) waters. Grey cells denote areas with no OBIS records.

Ocean biodiversity records for different proposed geodescriptive schema were examined for total number of records as well as number of unique species, genera and families. For surface waters, we used the Longhurst (1985) proposed provinces (Figure 4), which include 53 provinces across 4 'biomes'. The number of records per square kilometer of each zone varied widely. For example, there were 12.5 records/km^2 in the Coastal - NE Atlantic shelves province, compared to only 0.009 records/km^2 in the Trades - South Atlantic Gyral Province (Supplementary Table 1). Across the Longhurst provinces, there was substantial variation in the proportion of records that were identified to the species level. The Guinea Current Coastal Province had over 95% of its records contain a species level identification, while the Subtropical Convergence province had less than 18% of records identified to the species level. The Sutton et al. (2017) provinces represented the mesopelagic, with 33 provinces for this depth range. The biodiversity records for this schema were much sparser than the surface waters, with the most records in the Tasman Sea (0.8 records/km^2; Figure 5; Supplementary Table 2). The highest species level identification for the mesophotic provinces was 86%, which is lower than surface waters. Watling et al. (2013) provinces for the lower bathyal and abyssal/hadal depth were likewise assessed Figure 6 (Supplementary Tables 3, 4): the South East Pacific Ridges province had the lowest number of records (0.0004 records per km^2). Consistent with global trends, abyssal/hadal depths displayed the fewest records (0.00000204 records/km^2, only 14 total records) which were found in the Brazil Basin province. The Brazil Basin from Watling et al.'s abyssal/hadal province (>4000m) only had 6 different species recorded, while in the surface waters, the Longhurst Trades - Archipelagic Deep Basins province had 23,357 unique species records in surface waters (0-200m). In the abyssal South Pacific province, 34.7% of records contained a family level identification, but only 3.6% at the species level.

To help prioritize future research and exploration, we coupled climate change velocity estimates with OBIS biodiversity records to find the ocean areas with the fewest biological records coupled with the highest intensity climate change projections at the end of the century (2050-2100; Figure 7). In surface waters, the equatorial east Pacific was the largest tract of highest priority area for future exploration. There were also high priority bands located in the South Atlantic and central Indian Ocean. In mesopelagic waters, there were two high priority bands flanking the Equator. Additionally, mesopelagic waters near the Maldives, the Bay of Bengal, and the Coral Triangle all had some of the highest values. In the Bathypelagic, the highest priority waters are found in both the Atlantic and Indian oceans, flanking the mid-ocean ridges. In the abyssopelagic, there are so few OBIS records that the priority ranks are nearly completely driven by the climate velocity projections, and it is clear that there is a need for increased exploration at these depths on the whole.

Discussion

The ocean is chronically underexplored compared to terrestrial environments, especially corrected for the ocean's size and depth (Rowden et al., 2010; Webb et al., 2010; Kennedy et al., 2019). Though previous attempts have been made to assess the distribution of OBIS records globally (Kot et al., 2010; Webb et al., 2010), gaps still remain, creating the opportunity for the ocean exploration community to strategically assess these remaining gaps and prioritize targeted exploration efforts. There is a global need for accurate and comprehensive biodiversity data, given the increasing emphasis on ocean protection targets and exploitation limits that use biodiversity as their key indicator (Hughes et al., 2021). However, the current disparity of biodiversity data spans from no data available to areas with over

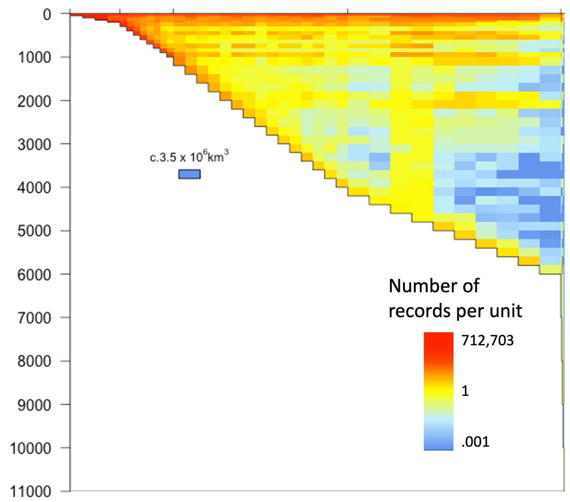

FIGURE 3
Global distribution within the water column of recorded marine biodiversity. The horizontal axis is nondimensional but show the ocean grouped as water column over a specific seafloor depth range. The vertical axis is ocean depth, on a linear scale. This means that the area on the graph is proportional to the volume of the ocean. For instance, in the deep sea, each cell of 200 m depth represents c. 3.5M km^3. The number of records in each cell is standardized to the volume of water represented by that cell, and then log10-transformed.

1M records. One of the key contributions of this paper is a comprehensive visualization of existing biodiversity data parsed by biogeographic provinces by depth, generating a biology-centered approach to prioritizing effort for the future.

There are areas in the ocean that are notably well-explored with repeated effort, and these areas provide some of the best insights into biodiversity dynamics. However, sites with high data density are extremely rare, and only target a small geographic area. We found that the three areas with the highest density of dives were in close proximity to some of the largest deep submergence research institutions (MBARI and JAMSTEC). Of all the deep submergence dives that we compiled globally, 19% of them were located in less than ~0.03% of the surface area of the ocean (Figures 1B–D). We also found a strong exploration effort in the Northern Hemisphere (94.6% of deep submergence dives), with only 5.4% of dives (1,644 deep submergence dives) in the Southern Hemisphere. This finding is consistent with a recent Global Capacity Survey that showed that the majority of deep submergence platforms are based in North America and Europe (Bell et al., 2023). Similarly, a recent study by Amon et al. (2022) showed a strong bias toward the Northern Hemisphere in terms of the capacity to conduct deep-sea science and exploration. This northern bias in terms of research and effort is exacerbated in terms of understanding global patterns of biodiversity by the fact that the majority (81%) of oceanic waters are contained in the southern hemisphere (Webb, 2021). These spatial biases are likely driven by the practical constraints of fuel and time costs, but they must be considered when researchers use this spatially biased data in OBIS to extrapolate larger spatial scale patterns.

Disproportionate exploration effort, as noted above, is one of the contributors to uneven distribution of biodiversity data across the globe (Hughes et al., 2021). Even OBIS, which is a comprehensive, community driven, user-input marine biodiversity database (Klein et al., 2019) cannot adequately catalog or index every taxon on the planet because there is uneven distribution of records, many taxa are extremely difficult to identify even by experts, and there is continued interest in database improvement (Klein et al., 2019). The uneven distribution of OBIS records makes the description of marine biogeographic provinces difficult, and prohibits comprehensive biodiversity hypothesis-testing in different regions. For example, in

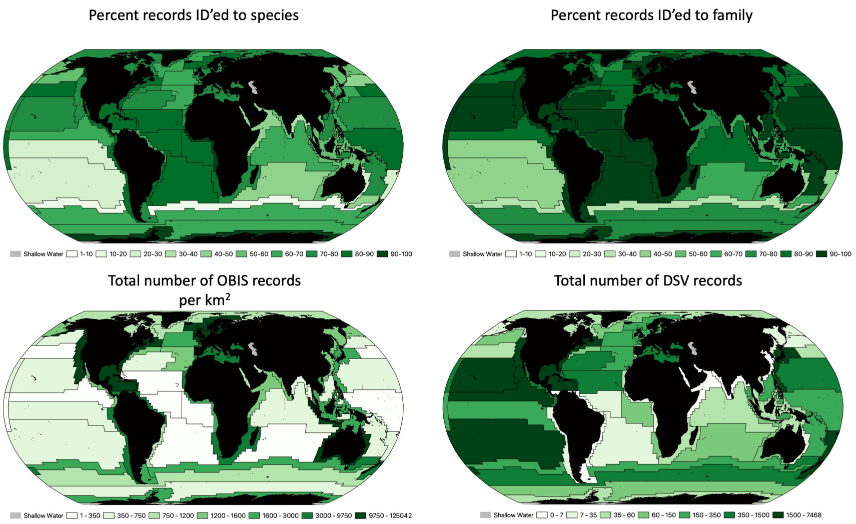

FIGURE 4
Longhurst (2006) proposed biogeographic provinces for surface waters. Top left is the percentage of records that were identified to the species level. Top right is the percentage of records that were identified to the family level. bottom left represents the total number of OBIS records per km^2 for each proposed province (range of records goes from 0.009 to 12.5). Bottom right is the is the total number of deep submergence dive records for each province.

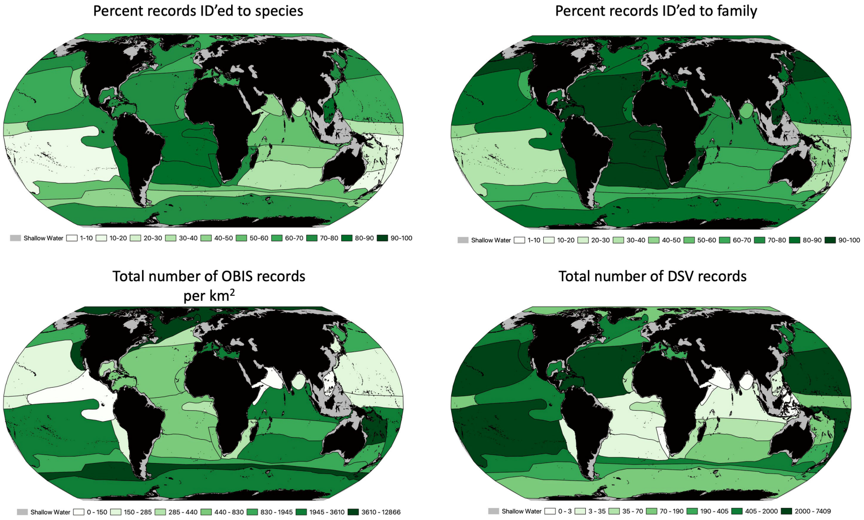

FIGURE 5
Sutton et al. (2017) proposed biogeographic provinces for mesopelagic waters (200-1,000 m). Top left is the percentage of records that were identified to the species level. Top right is the percentage of records that were identified to the family level. Bottom left represents the total number of OBIS records per km^2 for each proposed province (range of records goes from 0.006 to 1.287). Bottom right is the is the total number of deep submergence dive records for each province.

abyssal waters, Watling et al.2013's Brazil Basin province is described using only 14 total records (0.00000204 records km^{-2}) in OBIS representing only six species, while in contrast, Longhursts 1996's Coastal - Alaska Downwelling Coastal Province is represented by 2,175,924 records (3.32 records/km^2). This issue is further exacerbated by the level of taxonomic identification provided within the OBIS records; while higher classification is typical, family, genus, and/or species classifications are not guaranteed. For example, in the abyssal South Pacific province, 34.7% of records contained a family level identification, but only 3.6% at the species level. Indeed, when examining across the entirety of OBIS records with depth information, only 53.8% are identified to species level (31,448,375 out of 58,442,819 records), and even at the family level, only 69.9% (40,872,702 records). Because biogeographic research

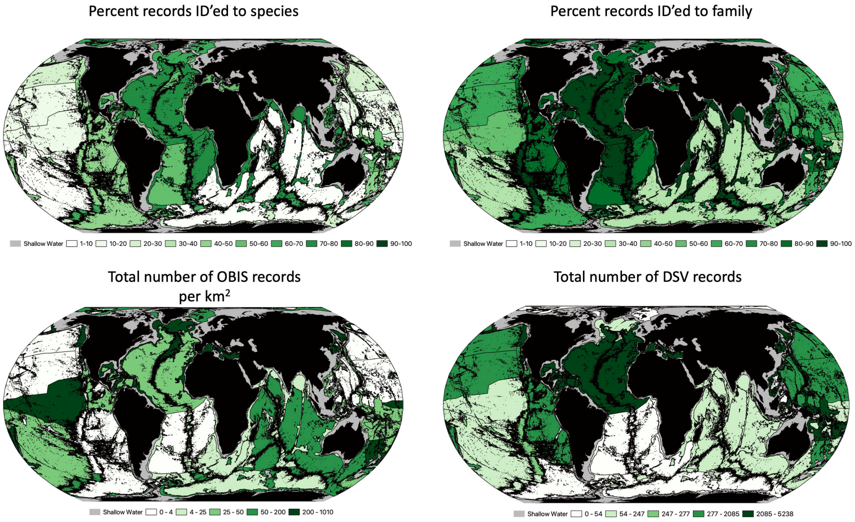

FIGURE 6
Watling et al. (2013) proposed biogeographic provinces for the lower bathyal and abyssal seafloor (in this project hadal depths are also included). Top left is the percentage of records that were identified to the species level. Top right is the percentage of records that were identified to the family level. Bottom left represents the total number of OBIS records per km^2 for each proposed province (range of records goes from 0.00001 to.0458). Bottom right is the names of each province. Bottom right is the is the total number of deep submergence dive records for each province.

most typically requires genus or species level identification (Costello and Chaudhary, 2017), OBIS records with only order or higher level identifications are of limited utility.

In addition to geographic disparities in data density, we also found data density to be strongly influenced by depth, as evidenced by examining records by biogeographic province (surface, mesophotic, and deepwater). We found that - in places with records with known depths - there was a spread of records ranging from 0.00116 to 172,702 per 3.5M km^3, with the fewest records per cell below 4,000 m (Figure 5). It should be noted that this disparity is not fully due to lack of deep submergence dives: even in places where deep submergence dives have been done, not all ROV image data gets annotated or incorporated into OBIS records. Annotating visual data from ROV video is particularly time intensive to process and has a large volume of observations, which makes it challenging to ingest into OBIS (Tippett et al., 2022). Several efforts have been launched to help streamline this process from improving annotation software (Gomes-Pereira et al., 2016) through metadata standardization (Tippett et al., 2022), but there is still significant work to be done in this regard.

Though deep-sea records in OBIS are sparse compared to surface records, there is additional bias within deepwater records towards benthically-associated species. We found that at all ocean depths, there are increased OBIS occurrence records near the seafloor when compared to the water column below 200 meters. This finding is consistent with other works pointing out that the water column is particularly undersampled (Netburn et al., 2018; Drazen et al., 2020). However, the data paucity at depth and in certain regions provides a clear directive and justification to prioritize future exploration efforts in service of global marine biodiversity records. Such baseline records are urgently needed, especially in the Anthropocene where species migrations and other climate change impacts are expected to have dramatic impacts on biodiversity, globally (Burrows et al., 2011).

As the planet experiences an unprecedented rate of change (Pörtner et al., 2022), the oceans are changing faster than the pace of exploration (Halpern et al., 2019). To determine the priority areas for future exploration in different climate change scenarios, we determined the regions with the lowest data densities that were simultaneously the most likely to be impacted by climate change. In the mesopelagic, one of the priority areas includes the Coral Triangle, which is well known to be the center of marine biodiversity on the planet (Veron et al., 2009), suggesting that even well-studied areas require additional effort at certain depths in preparation for inevitable climate change consequences. The bathypelagic unsurprisingly has a need for additional data throughout, but one of the priority regions we identified are the areas flanking mid-ocean ridges, which should help to set targets for future exploration in a post-high seas treaty era (Gjerde et al., 2021). However, on the whole, predicting climate velocities is an area of active research and therefore these priorities should all be re-visited and updated as new IPCC projections are released in future years.

Future efforts for exploration need to be thoughtfully targeted given limited resources (Bell et al., 2022). Future prioritization will focus new fieldwork efforts and new data collection in areas with the fewest records and effort to-date, although this is not the only path to addressing existing data gaps. Large quantities of biodiversity data remain hidden and unpublished within institutions, or linger behind when only subsets of the data (e.g., for specific taxa) get published (Chavan and Ingwersen, 2009). These non-public or underutilized data can be rescued to help fill some of these gaps comparatively inexpensively and on an accelerated timeline because no field work is required. Although these types of rescue efforts are labor intensive and require new injection of funding into old projects, they are a valuable source of baseline data and insight into the past. In short, data gaps can be filled via a) new effort, and/or via b) rescue efforts of hidden or forgotten raw data that can be revived, mined, or utilized for a new

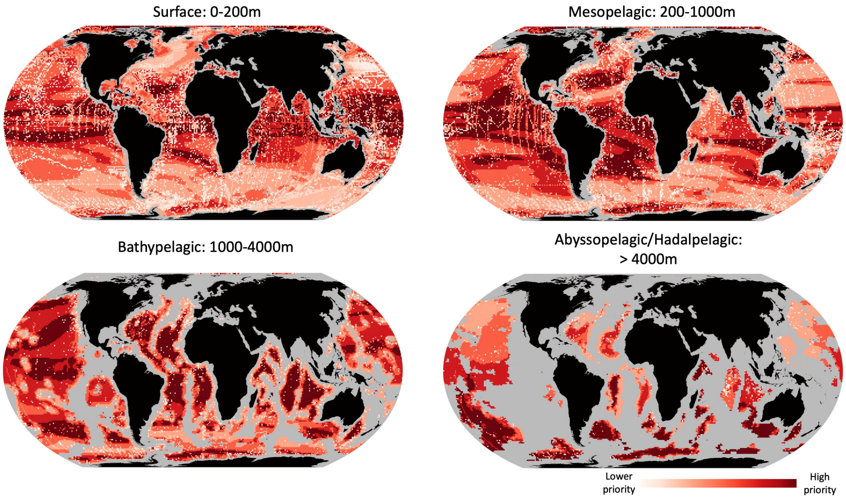

FIGURE 7
Areas of highest priority for exploration in an era of global change. Combining climate velocity estimates from Brito-Morales et al. (2020) 8.5 degree scenario with the lowest number of OBIS records for surface (0-200m), mesopelagic (200-1,000), bathypelagic (1,000-4,000m) and abyssopelagic/hadalpelagic (>4,000m) waters. Redder colors denote areas of lower number of biodiversity records and higher estimated climate change velocities.

purpose. However, at the same time, existing data quality in explored regions is still sufficient to start enabling ocean protection, management, and insight into biological processes even though these data sets are incomplete. Given this caveat, inference and the precautionary principle can be used in order to make progress. Achieving truly comprehensive data and a fully explored ocean is unattainable in a short time frame, but focused and prioritized efforts can make disproportionately large contributions to our understanding of the ocean if we take into account data scarcity and data need.

Data availability statement

Several publicly available datasets were analyzed in this study. The biodiversity datasets analyzed for this study can be found in at OBIS.org. The climate velocity analysis data is hosted on Zenodo by the original authors https://doi.org/10.5281/Zenodo.3596584. Previous dive locations are available on Zenodo https://zenodo.org/records/10038353.

Author contributions

BK and RR jointly conceived of the project. BK did the primary analysis. Both authors contributed to the drafting and editing of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was made possible with funding from NOAA's Office of Ocean Exploration through a partnership with the University Corporation for Atmospheric Research through grant numbers SUBAWD002610 and SUBAWD00248.

Acknowledgments

The authors would like to thank the OBIS team for the tireless work to integrate disparate datasets keep OBIS up and running. We would also like to acknowledge all the individual researchers who have gone the extra mile to share their data with the rest of us. We also like to thank the following individuals for their assistance in collecting metadata for the deep submergence asset deployments; Corinne Bassin and Allison Miller (SOI), Bruce Robinson (MBARI), Olivier Soubigou (IFREMER), Malcom Clark, David Bowden and Kevin Mackay (NIWA), Dhugal Lindsay and Yamakita Takehisa (JAMSTEC), Kerry Howell (NERC) and Karen HIssmann and Inken Suck (GEOMAR).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1219799/full#supplementary-material

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Frontiers in Marine Science

TYPE Original Research
PUBLISHED 07 December 2023
DOI 10.3389/fmars.2023.1250150

OPEN ACCESS

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RECEIVED 29 June 2023
ACCEPTED 03 October 2023
PUBLISHED 07 December 2023

CITATION
Pries A, Netburn AN, Batchelor H and Hermanson VR (2023) A little bit of *Sargassum* goes a long way: seafloor observations of *Sargassum fluitans* and *Sargassum natans* in the Western Atlantic Ocean.
Front. Mar. Sci. 10:1250150.
doi: 10.3389/fmars.2023.1250150

A little bit of *Sargassum* goes a long way: seafloor observations of *Sargassum fluitans* and *Sargassum natans* in the Western Atlantic Ocean

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The North Atlantic Ocean features high seasonal productivity of the brown seaweed *Sargassum*, which floats on the ocean surface and accumulates in large numbers in the Sargasso Sea. *Sargassum* blooms can stretch from the west coast of Africa to the Gulf of Mexico, and have created the largest seaweed blooms ever observed. *Sargassum* blooms have increased in intensity in recent years, and can negatively impact coastal communities when they wash up onshore in large quantities and decay. While seaweed sinking from surface waters to the seafloor may be an important carbon sink by removing carbon from the atmosphere, the magnitude of carbon sequestration by *Sargassum* and other macroalgae remains poorly understood. Given the magnitude of *Sargassum* blooms in the North Atlantic, they may pose a significant mechanism for carbon sequestration in the deep sea, though direct observations are rare. In this study, we documented the presence and distribution of *Sargassum* seaweed on the seafloor using video from ten remotely operated vehicle dives conducted on NOAA Ship *Okeanos Explorer*. Locations included sites in the Gulf of Mexico, in the Caribbean Sea, and off the Southeastern United States. *Sargassum* was observed in numbers ranging from 0 to over 112 per dive, and a frequency of between 0-11.23 observations for every 100 meters of horizontal distance. These observations suggest that *Sargassum* does make its way to the deep sea in potentially significant amounts. Natural systems like *Sargassum* sinking could serve as natural laboratories for understanding and managing seaweed burial as a climate mitigation strategy. Long-term monitoring of the fate of sunken *Sargassum* on the seabed is needed in order to determine how much is ultimately sequestered rather than recycled back into the system. Such observations would inform the feasibility of *Sargassum* farming and/or facilitated sinking as potential carbon dioxide removal strategies.

KEYWORDS

***Sargassum*, macroalgae, ocean exploration, carbon sequestration, algae blooms, *Okeanos Explorer*, deep seafloor**

1 Introduction

Macroalgae (commonly referred to as seaweed) provide many important ecological, environmental, and economic services. Seaweed is important food and habitat to a range of animals, including commercially-important fisheries species. Seaweed has market value for direct human consumption, and compounds derived from seaweed are commonly used in a range of commercial products like cosmetics, supplements, and fertilizers (Sugumaran et al., 2022). Through photosynthesis, algae fix carbon dioxide and can ultimately sequester organic carbon in coastal sediments and in the deep ocean for hundreds to thousands of years (Raven, 2017). Protection and restoration of macroalgae habitats can help preserve these blue carbon ecosystems. Seaweed cultivation and sinking is being proposed as a possible strategy to reduce atmospheric carbon dioxide (Duarte et al., 2017; National Academies of Sciences, Engineering, and Medicine, 2022). Seaweeds are also being studied for potential use as a biofuel (Godvin et al., 2021). However, when they occur in excess, seaweeds can outcompete, smother, or block sunlight from reaching other photosynthetic organisms and can wash ashore, accumulating in noxious heaps (Rodríguez-Martínez et al., 2019).

Sargassum is a genus of brown seaweed that is widespread in temperate, subtropical, and tropical waters. *Sargassum* morphology includes a holdfast, a cylindrical main axis, small leaflike blades, and gas-filled bladders, or pneumatocysts (Graham et al., 2009). A flexible body helps it withstand strong currents. There are two species of *Sargassum* that occur in the Atlantic Ocean off the coast of the United States and in the Gulf of Mexico: *Sargassum fluitans* and *Sargassum natans.* These forms are unique in that they are free-floating and do not have a holdfast or attach to a substrate at any stage in their life cycle. They are the only species of *Sargassum* that are holopelagic, meaning that they remain pelagic drifters throughout their entire life cycle, and free-floating *Sargassum* is only found in the Atlantic Ocean (Doyle and Franks, 2015; Stiger-Pouvreau et al., 2023). These pelagic species of *Sargassum* can double in size every 9-13 days (Hanisak and Samuel, 1987).

The two free-floating *Sargassum* species can occur in extensive, highly productive rafts on the ocean's surface that harbor distinctive communities of organisms adapted to the buoyant *Sargassum* habitat, including juvenile fish species such as amberjack and triggerfish (Wells and Rooker, 2004). *Sargassum natans* and *Sargassum fluitans* only reproduce asexually through fragmentation, a type of vegetative asexual reproduction where an individual *Sargassum* breaks into two or more parts, each of which continues to live and grow (Lee, 2008). *Sargassum*'s fast growth rate contributes to its rapid spread. Hereafter in this paper both *Sargassum fluitans* and *Sargassum natans* will be referred to as *"Sargassum"*.

1.1 *Sargassum* blooms

The North Atlantic Gyre is a circular system of ocean currents in the Atlantic Ocean that, through the rotating pattern of currents and effects of wind and weather, amasses *Sargassum* in an area known as the Sargasso Sea. The Sargasso Sea is the only sea bounded by currents rather than by land (NOAA, 2019). The Gulf Stream acts as a conduit that transports *Sargassum* through the Caribbean, into the Gulf of Mexico, and off the coast of the southeastern United States, and evidence suggests that much of the *Sargassum* transported through the Gulf of Mexico originates from the North Equatorial Recirculation Region, a complex system of currents around western Africa and South America, in addition to the Sargasso Sea (Franks et al., 2016).

Since 2011, giant floating *Sargassum* mats in the Atlantic have increased in density and range to form an 8,850 kilometer-long belt, called the Great Atlantic *Sargassum* Belt, that can extend from West Africa to the Gulf of Mexico (Wang et al., 2019). High quantities of *Sargassum* have washed up on beaches and inundated coastlines in the Southeast U.S., Gulf of Mexico, and the Caribbean in recent years, presenting a logistical challenge for coastal communities to manage the tons of seaweed piling up on their shores (Lamb, 2018; Conley and Oliver, 2019). Satellite measurements suggest that *Sargassum* blooms start to develop in the Central Western Atlantic in February-March and are transported by winds and currents to the Caribbean as massive blooms from May-August (Wang and Hu, 2017). A changing climate may have unexpected impacts on *Sargassum* productivity and sinking as well as its tendency to be transported, aggregated, and scattered due to climate influences on algal productivity, weather patterns, and ocean currents (Sanchez-Rubio et al., 2018).

There have been recent efforts to quantify the amount of *Sargassum* on the sea surface through methods such as satellite imagery analysis (Wang and Hu, 2016), numerical models (Schamberger et al., 2022), and field measurements (Ody et al., 2019). A 2019 study that analyzed satellite remote sensing data from 2000 to 2018 found a significant increase in estimated *Sargassum* biomass in the Great Atlantic *Sargassum* Belt beginning in 2011, with the highest biomass estimated at more than 20 million metric tons in June 2018, the last year of the study (Wang et al., 2019). Given the economic impacts of these *Sargassum* blooms, new monitoring systems (Duffy et al., 2019; Valentini and Balouin, 2020) have been developed to better plan for and respond to *Sargassum* accumulation on coasts.

1.2 *Sargassum* ecology

Sargassum is abundant in the Atlantic Ocean and forms an essential surface habitat that supports a diversity of marine organisms, including fish, invertebrates, sea turtles, marine birds, and marine mammals. Floating *Sargassum* mats serve as a primary nursery area for many fish species, some of which are commercially important (dolphinfishes, jacks, and amberjacks), and they provide a source of energy in an otherwise nutrient-poor area of the Atlantic (Casazza and Ross, 2010). These *Sargassum* mats provide essential habitat for approximately 120 species of fish and more than 120 species of invertebrates (Doyle and Franks, 2015).

Carbon sequestration is the process of storing carbon dioxide and other forms of carbon out of the atmosphere for long periods of time. It has been suggested that marine primary producers such as phytoplankton, macroalgae, mangroves, and seagrasses are more efficient at sequestering carbon than their terrestrial counterparts due to their high productivity and efficiency in trapping sediments and associated organic carbon (Mcleod et al., 2011; Arenas and Vaz-Pinto, 2015). Through photosynthesis, *Sargassum* converts sunlight, carbon dioxide, and ocean nutrients into sugars and other carbon compounds. This organic material, and the carbon it contains, can then end on a number of different pathways, such as washing up on beaches, being eaten by herbivores, or sinking to the bottom of the ocean. Once it ends up in deep ocean currents or seafloor sediments hundreds of meters below the surface, the carbon is prevented from being exchanged with the atmosphere over several hundred to several thousand years (Volk and Hoffert, 2013). Traditionally, seagrasses and mangroves have been considered the dominant form of oceanic carbon sequestration (Duarte and Cebrián, 1996); however, in recent years researchers have been looking at seaweeds like *Sargassum*'s role as important carbon sinks (N'Yeurt et al., 2012; Raven, 2017; Kokubu et al., 2019).

The gravitational sinking of *Sargassum* from the surface to the seafloor, and degradation of *Sargassum* within the water column and at the seafloor, are poorly understood processes. The mechanisms that deliver drifting seaweed to marine sediments include wind-induced Langmuir circulation that can transport floating seaweed fragments to a depth where pressure collapses its air bladders, rendering the seaweed negatively buoyant and removing it from the surface (Krause-Jensen and Duarte, 2016). Additionally, *Sargassum* living in the shaded understory of floating *Sargassum* may be prevented from photosynthesizing and lose buoyancy and die and start to degrade. It will also lose buoyancy with age and from encrustation of epizoans (Stoner, 1983). Once it achieves negative buoyancy, the *Sargassum* sinks at an estimated rate of 3.5 cm per second (Johnson and Richardson, 1977). At this rate, *Sargassum* would reach the seafloor at 1000 meters in just 8 hours. This is relatively fast for sinking particles and does not allow much time for remineralization, consumption, or degradation (Giering et al., 2020; Omand et al., 2020).

Sargassum sinking has been studied before using Autonomous Underwater Vehicle (AUV)-collected seafloor photographs in the southern part of the North Atlantic ocean (three stations between ~10-12° N and ~36-50° W), within the North Atlantic Subtropical Gyre where the Sargasso Sea is located. The biomass density of *Sargassum* deposited on the seafloor was estimated at 0.07 to 3.75 g/m^2, which is greater than the surface biomass as estimated through literature review at 0.024-0.84 g/m^2 (Baker et al., 2017). Krause-Jensen and Duarte (2016) estimated that 11 percent of macroalgal particulate organic carbon (POC) export, or 35 TgC/yr, reaches the deep sea globally, serving as an important carbon sink. Start-up companies and financial investors have noted the potential for macroalgae aquaculture for commercial products like bioplastics and food or to sink and bury on the deep seafloor to sequester carbon from the atmosphere (López Miranda et al., 2021; Oxenford et al., 2021). *Sargassum* in particular has been identified as a target species for such efforts because of its naturally high abundance and reproductive rate.

The factors that contribute to organic carbon export in the open ocean and its subsequent carbon sequestration are driven by a complex combination of ecological, biogeochemical, and physical oceanographic processes. Developing a predictive understanding of carbon export pathways for seaweeds including *Sargassum* is critical for understanding present and future rates of ocean carbon sequestration and informing the emerging marine carbon dioxide removal industry. This study provides an initial characterization of *Sargassum* on the seafloor by describing the number of *Sargassum* observed, its relationship to benthic composition, any interactions with benthic organisms, and geological features of the dive sites explored. We revisited a series of archived deep ocean exploration visual surveys that took place on seabed areas underlying known and likely *Sargassum* surface blooms as one step toward understanding the fate of sinking *Sargassum* in the marine system.

2 Methods

2.1 NOAA Ship *Okeanos Explorer*

The National Oceanic and Atmospheric Administration (NOAA)'s Office of Ocean Exploration and Research is a U.S. federal program dedicated to exploring the unknown ocean for public benefit, filling in knowledge gaps about the marine environment through scientific discovery, technological advancements, and access to data. It conducts interdisciplinary ocean exploration expeditions on NOAA Ship *Okeanos Explorer* and other research vessels. *Okeanos Explorer* is a 68-meter research ship outfitted with an array of mapping sonars and a dual body Remotely Operated Vehicle (ROV) system: ROV *Deep Discoverer (D2)* and ROV *Seirios*. A unique and advantageous aspect of *Okeanos Explorer* operations is its ability to facilitate real-time communication and collaboration with shore-based scientists (Peters et al., 2019). Scientists on the ship and on land collaborate through a live video feed, text chatroom, shared conference phone line, and video annotation system, allowing participants not only to follow along live but to provide feedback and scientific expertise in real-time during the ROV dives (Kennedy et al., 2016; Selig et al., 2019).

2.2 *Deep Discoverer* and *Seirios* ROVs

Kennedy et al. (2019) describe the technical details on the *Okeanos Explorer*'s seafloor mapping and ROV systems and operations. All ROV dives examined in this study were conducted with NOAA's dual body ROV system *Deep Discover (D2)* and *Seirios*, although only video from *D2* was reviewed. The main capability of *D2* is the ability to capture high-definition video, with its primary camera able to zoom in and provide close-up video of relatively small organisms (Kennedy et al., 2019). *Seirios* is directly tethered to the *Okeanos Explorer* via a fiber-optic cable and is also tethered to *D2*, a configuration that allows *Seirios* to absorb the sway of the ship while keeping *D2* stable. The cable provides the ROVs with power and allows for data transfer between the ROVs and the ship. Both *Seirios* and *D2* are equipped with a suite of sensors to measure environmental parameters like temperature, dissolved oxygen, salinity, and depth. Surveys conducted with this two-body system are exploratory in nature, providing baseline information on the broader biological, geological, and physical context of poorly explored regions of the ocean (Selig et al., 2019; Cantwell et al., 2020). Because the ROV time is maximized for a variety of objectives, true quantitative analyses of the imagery are not always possible, yet important insights may still be gleaned.

2.3 ASPIRE campaign

From 2018-2022, *Okeanos Explorer* conducted a series of expeditions as part of the Atlantic Seafloor Partnership for Integrated Research and Exploration (ASPIRE), a multi-national collaborative ocean exploration campaign to raise the collective knowledge and understanding of the North Atlantic Ocean. Three of the four expeditions reviewed – Océano Profundo 2018: Exploring Deep-sea Habitats off Puerto Rico and the U.S. Virgin Islands (EX1811), Windows to the Deep 2019: Southeast and Mid-Atlantic U.S Continental Margin, Port Canaveral, FL to Norfolk, VA (EX1903L2), and 2019 Southeastern U.S. Deep-sea Exploration (EX1907) – were part of this ASPIRE campaign.

2.4 Site selection

A subset of expeditions and ROV dive sites were selected for this study based on the following considerations:

- Expedition took place in region with known seasonal occurrences of *Sargassum (*Wang and Hu, 2017*).*
- "*Sargassum*" or "seaweed" was noted in SeaTube annotations at some point during the dive.
- High algal density on the sea surface was observed through the University of South Florida's Satellite-based *Sargassum* Watch System (SaWS) (Trinanes et al., 2023) - this informed selection of EX1811 Dives 7 and 8 and EX1907 Dives 11 and 12.
- Dives were selected to represent different types of bathymetric and ecological features (for instance a canyon versus coral mounds) and depths to sample a variety of site characterizations.

Details on bathymetric and ecological features are available in Supplementary Information Table 1.

2.5 ROV video footage review, *Sargassum* annotation, and site characterization

Okeanos Explorer ROV video footage is streamed live through SeaTube V3, a platform developed by Ocean Networks Canada (ONC) for scientists to view and annotate ROV footage both in real time and afterwards (Selig et al., 2019). SeaTube V3 provides open access and archives of dive videos, dive imagery, dive logs, navigational data, and metadata for deep ocean exploration (Jenkyns et al., 2013). An informal science chatroom developed by the Global Foundation for Ocean Exploration is also used for sidebar conversations between scientists and captures some of the annotations as well; the messages of the chat are then archived for future access[1,2].

Annotations of *Sargassum* were done manually, by reviewing the selected footage from the *Deep Discoverer* ROV. Each of the dives selected for review was viewed in SeaTube V3 from the start of the ROV's descent from the surface to the end of its ascent back to the surface to account for the possibility of *Sargassum* being observed in the water column. An object was identified as *Sargassum* based on its shape, color, texture, and movement. Figure 1 shows screen grabs of the ROV video to show the variability of what the *Sargassum* looks like when it reaches the seafloor.

When there was an object that was potentially *Sargassum* but identification was questionable, higher-resolution footage of that part of the dive was accessed and viewed in NOAA's Ocean Exploration Video Portal[3]. If after reviewing the high-resolution footage the object could not with absolute certainty be identified as *Sargassum*, the annotation included the note "likely *Sargassum*" to indicate a degree of uncertainty. Annotations with "likely *Sargassum*" designation were counted as *Sargassum* in this report. All other observations were identified to the *Sargassum* genus referencing the World Register of Marine Species (WoRMS) system of classification and annotated in SeaTube V3.

For dives with abundant *Sargassum*, a new *Sargassum* annotation was made for every new frame of view that contained *Sargassum*. For instance, even if there were five clumps of *Sargassum* in the frame, this was counted as one *Sargassum* annotation. As soon as the ROV had *Sargassum* in its frame of view that was not clearly visible in the previous annotation, this was entered as a new annotation. Each *Sargassum* annotation was classified as "low," "medium," or "high" biomass based on visual estimation of the approximate amount of *Sargassum* within the frame of view on a scale from 1-8 (Supplementary Information Table 2). Additionally, when mobile animals were observed on or in the near vicinity of the *Sargassum* detritus, this was noted, as well as whether the animal appeared to be grazing on the *Sargassum*. Bathymetric features (rocks, sediment, etc.) and other observations (high marine snow, presence of anthropogenic debris) were also noted in Supplementary Information Table 2.

In addition to reviewing the ROV footage and accompanying audio commentary, the archived chatroom logs that include expert perspectives, and post-dive summaries, accessed through NOAA's

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[1] https://exdata.tgfoe.org/OkeanosCruises/.

[2] https://oceanexplorer.noaa.gov/okeanos/collaboration-tools/im-eventlog/participating-eventlog.html.

[3] https://www.nodc.noaa.gov/oer/video/.

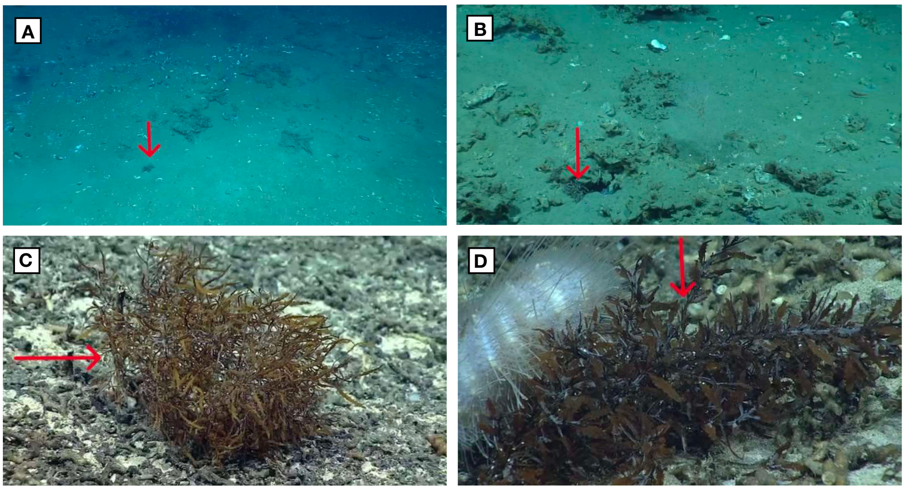

FIGURE 1
Images annotated as: "Likely *Sargassum*" **(A)** and "*Sargassum*" **(B)** from EX1803 Dive 6. Close-up photo of *Sargassum* **(C)** and an urchin appearing to graze on *Sargassum* **(D)** from EX1903 L2 Dive 2.

Institutional Repository[4], were reviewed to help characterize each dive site.

Distance transited along the seafloor was calculated by importing spatial coordinates from the ROV recorded while at depth and plotting them in ESRI's ArcGIS Pro 2.9 software. A line was then generalized based on the points and the length in meters was calculated for each dive. Distance transited varied from approximately 250 meters to over 1,000 meters per dive.

3 Results

Video from a total of 10 NOAA Ship *Okeanos Explorer* ROV dive sites across four expeditions was reviewed for this study, totaling 55 hours, 54 minutes of time on bottom (Table 1). A total of approximately 7,350 linear meters were traveled horizontally. *Sargassum* was observed at 9 of the 10 dive sites, with a total of 237 instances of *Sargassum* annotated and between 0 to over 112 *Sargassum* observed per dive. Although we reviewed video from descent to ascent, no *Sargassum* was observed in the water column. Figure 2 shows location and *Sargassum* observations for all ROV dives included in the study.

3.1 Dive site characterizations

3.1.1 Gulf of Mexico 2018 (EX1803), April 11 - May 3, 2018 Pascagoula, Mississipi to Key West, Florida

Gulf of Mexico 2018 was a 23-day expedition in the Gulf of Mexico Basin to identify, map, and explore the diversity and distribution of deep-sea habitats in the region. The 15 ROV dives of this expedition, which ranged from 305 to 3,010 meters in bottom depth, focused on benthic habitats, including fish habitats, deep-sea coral and sponge communities, chemosynthetic communities (brine pools, gas seeps, mud volcanoes), and biological communities around shipwrecks (Maxon et al., 2018).

3.1.1.1 Hidalgo Basin (Dive 6)

This dive targeted Hidalgo Basin (Figure 3A), an area that was being considered for expansion of the Flower Garden Banks National Marine Sanctuary but was not ultimately included in the expansion[5]. Specifically, this dive explored a mound feature for hard-bottom communities, particularly deep-sea corals, sponges, and associated fauna. Approximately 250 kilometers south of central Louisiana, this is a relatively unexplored area that was first observed in 2014 by *Okeanos Explorer*. A high abundance of bivalve shells and carbonate rocks were seen on this dive. Anthropogenic debris from the surface – fishing line, canvas, metal container – was observed at this site. Parts of this site had signs of past seepage, including bacterial mats and a high abundance of bivalves, mostly empty shells but some living. A gastropod appeared to be grazing on *Sargassum*. The seafloor was heavily sedimented, with large depressions and pockmarks. There was a slight current on the bottom with high turbidity in the water column. This dive transited 932 linear meters along the seafloor. Thirty *Sargassum* observations were made over this distance, for an average 3.22 *Sargassum* observations per 100 meters (Table 1). The average depth for *Sargassum* observations was 1,077 meters.

3.1.1.2 DeSoto Canyon (Dive 8)

This dive targeted the northern end of the West Florida Escarpment in the DeSoto Canyon region (Figure 3B). At the time

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[4] https://repository.library.noaa.gov/.

[5] https://flowergarden.noaa.gov/management/sanctuaryexpansion.html.

TABLE 1 Summary of *Sargassum* observations during the selected ROV dives.

Expedition Name	Dive Site Name (Dive Number)	Date	Depth Range (m)	Bottom Time (hh:min)	# *Sargassum* Observations	# *Sargassum* Observations per 100m
Gulf of Mexico 2018 (EX1803)	Hidalgo Basin, Gulf of Mexico (Dive 6)	4/19/2018	1050 - 1104	5:59	30	3.22
Gulf of Mexico 2018 (EX1803)	DeSoto Canyon, Gulf of Mexico (Dive 8)	4/25/2018	2315 - 2635	5:25	7	1.49
Océano Profundo 2018 (EX1811)	Punta Yeguas, Puerto Rico (Dive 6)	11/6/2018	636 - 877	7:10	16	4.11
Océano Profundo 2018 (EX1811)	Caja de Muertos Island, Puerto Rico (Dive 7)	11/7/2018	401 - 535	4:35	>19*	3.32
Océano Profundo 2018 (EX1811)	South of La Parguera, Puerto Rico (Dive 8)	11/8/2018	804 - 1101	6:57	>112*	11.23
Océano Profundo 2018 (EX1811)	Mona Canyon, Puerto Rico (Dive 10)	11/10/2018	2536 - 2766	3:36	>25*	3.79
Windows to the Deep 2019 (EX1903L2)	Stetson Mesa South Mounds, Eastern Florida (Dive 2)	6/22/2019	728 -784	5:44	16	1.54
Windows to the Deep 2019 (EX1903L2)	"Dodge" Canyon, North Carolina (Dive 11)	7/3/2019	1209 - 1348	3:29	6	2.39
2019 Southeastern U.S. Deep-sea Exploration (EX1907)	Key West Deep (Dive 11)	11/18/2019	1168 - 1208	6:08	6	0.65
2019 Southeastern U.S. Deep-sea Exploration (EX1907)	"Berg Bits", Southwestern Florida (Dive 12)	11/19/2019	927 - 973	6:51	0	0.00

Full dive names are: Gulf of Mexico 2018 (EX1803), Océano Profundo 2018: Exploring Deep-sea Habitats off Puerto Rico and the U.S. Virgin Islands (EX1811), Windows to the Deep 2019: Southeast and Mid-Atlantic U.S Continental Margin, Port Canaveral, FL to Norfolk, VA (EX1903L2), 2019 Southeastern U.S. Deep-sea Exploration (EX1907).

*For dives with abundant *Sargassum*, a new *Sargassum* annotation was made for every new frame of view that contained any *Sargassum*. For instance, even if there were 5 clumps of *Sargassum* in frame, this was counted as one *Sargassum* annotation.

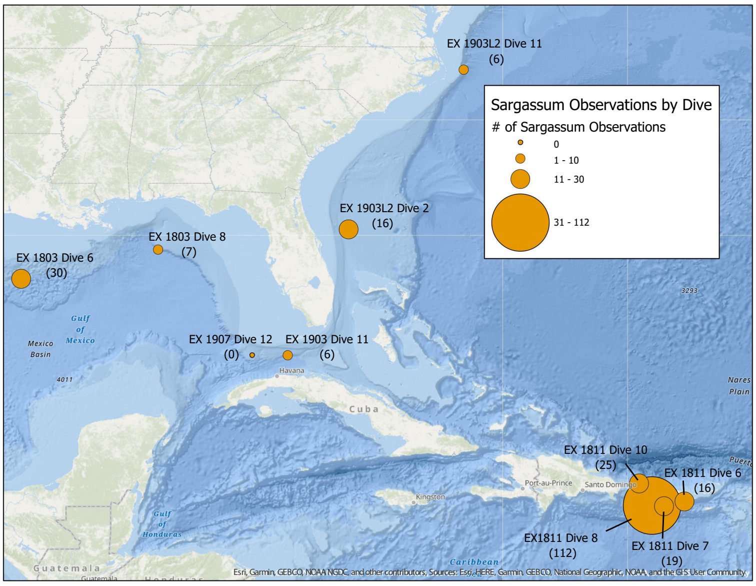

FIGURE 2

Location of ROV dive sites that were included in this study. The size of the circles indicates the range of the number of *Sargassum* observations. The number of *Sargassum* observations are shown in parentheses. (Gulf of Mexico 2018 (EX1803) - Dive 6: Hidalgo Basin and Dive 8: DeSoto Canyon, Océano Profundo 2018: Exploring Deep-sea Habitats off Puerto Rico and the U.S. Virgin Islands (EX1811) - Dive 6: Punta Yeguas, Puerto Rico, Dive 7: Caja de Muertos Island, Puerto Rico, Dive 8: South of La Parguera, Puerto Rico, and Dive 10: Mona Canyon, Puerto Rico, Windows to the Deep 2019: Southeast and Mid-Atlantic U.S Continental Margin, Port Canaveral, FL to Norfolk, VA (EX1903L2) - Dive 2: Stetson Mesa South Mounds, Eastern Florida and Dive 11: "Dodge" Canyon, North Carolina, 2019 Southeastern U.S. Deep-sea Exploration (EX1907) - Dive 11: Key West Deep and Dive 12: "Berg Bits", Southwestern Florida).

of the expedition this area was being considered for expansion of the Flower Garden Banks National Marine Sanctuary but was not ultimately included in the expansion. Dive 8 explored the escarpment feature at depths between 2,200-2,600 meters for hard-bottom communities, particularly deep-sea corals, sponges, and associated fauna. There have been five previous scientific ROV dives in this general area, all of which documented extensive and diverse deep-sea coral communities, which are the deepest high-density communities known in the Gulf of Mexico (McLetchie et al., 2018). Approximately 220 kilometers off Alabama, this site was characterized by a hard substrate. It also featured a steep carbonate rock wall with highly fractured detached boulders at the base of the wall. Terrace features with relatively gentle slopes had a blanket of sediment cover. This dive transited 469 linear meters along the seafloor. Seven *Sargassum* observations were made over this distance, for an average 1.49 *Sargassum* observations per 100 meters (Table 1). The average depth for *Sargassum* observations was 2,534 meters.

3.1.2 Océano Profundo 2018: Exploring deep-sea habitats off Puerto Rico and the U.S. Virgin Islands (EX1811), October 30 - November 20, 2018 San Juan, Puerto Rico to San Juan, Puerto Rico

Océano Profundo 2018 was a 22-day expedition to explore the unknown and poorly understood deep-water areas surrounding Puerto Rico and the U.S. Virgin Islands. The 19 ROV dives of this expedition, which ranged from 250 to 5,000 meters in depth, surveyed a diversity of habitats and geological features, including midwater habitats, deep-sea coral and sponge communities, deep-sea fish habitats, submarine canyons, and submarine landslides. In combination with deep-sea mapping operations, information and data were collected to increase understanding of deep-sea ecosystems of this poorly studied area, as well as to provide publicly-accessible data to spur further research, exploration, and management (Wagner et al., 2018).

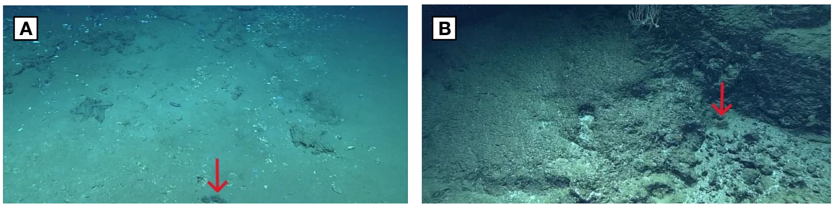

FIGURE 3
Dive sites reviewed from Gulf of Mexico 2018 (EX1803). **(A)** Hidalgo Basin (Dive6) **(B)** DeSoto Canyon (Dive 8).

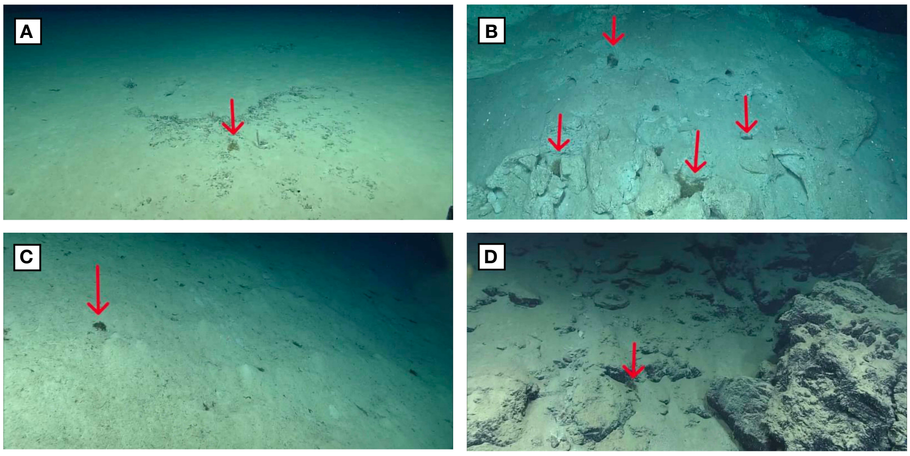

FIGURE 4
Dive sites reviewed from EX1811 Océano Profundo 2018: Exploring Deep-sea Habitats off Puerto Rico and the U.S. Virgin Islands. **(A)** Dive 6: Punta Yeguas, Puerto Rico, **(B)** Dive 7: Caja de Muertos Island, Puerto Rico, **(C)** Dive 8: South of La Parguera, Puerto Rico, **(D)** Dive 10: Mona Canyon, Puerto Rico.

3.1.2.1 Punta Yeguas, Puerto Rico (Dive 6)

The site was located within the Inés María Mendoza Nature Reserve, also known as Punta Yeguas (Figure 4A). This dive targeted the potential habitats of deep-water fish species, including snappers and groupers. Approximately 8 kilometers off the southeastern coast of Puerto Rico, this dive took place at approximately 860 meters depth up a steep mound, which has a prominent ridge on the crest of the mound. Flat areas were fairly heavily sedimented, and there was a slight current on the bottom. High turbidity was observed in the water column, and there was an abundance of benthic organisms swimming in the water column. This dive transited 389 linear meters along the seafloor. Sixteen *Sargassum* observations were made over this distance, for an average 4.11 *Sargassum* observations per 100 meters (Table 1). The average depth for *Sargassum* observations was 757 meters.

3.1.2.2 Caja de Muertos Island, Puerto Rico (Dive 7)

This site was located to the south of the Caja de Muertos Island, south of Ponce, Puerto Rico (Figure 4B). The dive started on a steep slope (40 degrees), traversed northeast along a gentle slope area, and then moved up a mound. The habitat at the start of the dive was dominated by soft sediment with a few scattered boulders, with transitions between larger boulders to smaller boulders to soft sediment. At the beginning of the dive, small clumps of *Sargassum* were observed slowly tumbling along a steep, sedimented slope. This tumbling movement was not seen at any other sites included in this study. An urchin appeared to be grazing on a piece of *Sargassum* towards the beginning of the dive. The second half of the dive along the ridge was dominated by carbonate boulders with intermittent soft-bottom expanses. In addition to *Sargassum*, turtlegrass and anthropogenic debris were often observed on this dive. The ROV pilots mentioned that there had been high amounts of *Sargassum* observed on the surface in the area. Since *Sargassum* was so abundant on this dive, it is worth noting that a new *Sargassum* annotation was made for every new frame of view that contained *Sargassum*, not for every piece of *Sargassum* observed. Thus, while 19 *Sargassum* observations were recorded on this dive, the number of individual *Sargassum* was higher. This dive transited 572 linear meters along the seafloor. Nineteen *Sargassum* observations were made over this distance, for an average 3.32 *Sargassum* observations per 100 meters (Table 1). The average depth for *Sargassum* observations was 470 meters.

3.1.2.3 South of La Parguera, Puerto Rico (Dive 8)

This dive explored an unexplored ridge feature (Figure 4C) off La Parguera in southwest Puerto Rico. Beginning near the bottom of a steep slope (average 30 degree incline), this dive continued eastward toward a more moderate ridge. With a gentle slope dominated by soft sediment, the seafloor was largely homogeneous in composition, and no hard substrate was observed. Scours and burrows were common in this area, and *Sargassum* was often seen gathered in pits and burrows in the sediment. There were quite a few pieces of wood and other organic debris, like seagrass blades and *Sargassum*. *Sargassum* was observed in abundance at the beginning of the dive where the seafloor was relatively flat. *Sargassum* continued to be present on the slope, but in lesser quantities than on the flat surfaces. Many really small pieces of *Sargassum* were observed throughout the dive that were able to be positively identified in zoomed-in shots. During this dive, the science watch lead noted that the *Sargassum* on the surface had been a bit of a hazard for the ship for the past few days. Since *Sargassum* was so abundant on this dive, it is worth noting that a new *Sargassum* annotation was made for every new frame of view that contained *Sargassum*, not for every piece of Sargassum observed. Thus, while 112 *Sargassum* observations were recorded on this dive, the number of individual *Sargassum* was higher. This dive transited 997 linear meters along the seafloor. 112 *Sargassum* observations were made over this distance, for an average 11.23 *Sargassum* observations per 100 meters (Table 1). The average depth for *Sargassum* observations was 936 meters.

3.1.2.4 Mona Canyon, Puerto Rico (Dive 10)

This dive took place along the north side of the Mona Passage in the Mona Canyon (Figure 4D). Approximately 50 kilometers off the northwest side of Puerto Rico, this site is more exposed to the greater North Atlantic Ocean than the other sites from this expedition that we included in this study. This dive traversed up the western wall of a giant landslide scarp. Seafloor bathymetry and imagery in this area may provide evidence of historical landslide activity that could pose a potential geohazard to this region of the Atlantic Ocean. The dive started with high quantities of *Sargassum* detritus in large clumps, much larger than were observed on the other dives. At steeper features, such as large rocks on a steep canyon wall face, or on a rock avalanche geologic feature, there were many fewer *Sargassum* observations. Several instances of anthropogenic debris (metal cans, etc.) were also observed. Few other organisms were encountered throughout the dive. Since *Sargassum* was so abundant on this dive, it is worth noting that a new *Sargassum* annotation was made for every new frame of view that contained *Sargassum*, not for every piece of *Sargassum* observed. Thus, while 25 *Sargassum* observations were recorded on this dive, the number of individual *Sargassum* was higher. This dive transited 659 linear meters along the seafloor. Twenty-five *Sargassum* observations were made over this distance, for an average 3.79 *Sargassum* observations per 100 meters (Table 1). The average depth for *Sargassum* observations was 2,725 meters.

3.1.3 Windows to the Deep 2019: Southeast and Midatlantic U.S continental margin (EX1903L2), June 20 - July 12, 2019

Port Canaveral, Florida to Norfolk, Virginia

The second leg of Windows to the Deep 2019 was a 23-day expedition to explore the deepwater areas offshore Florida, Georgia, South Carolina, and North Carolina. This expedition mapped and characterized these areas, which are some of the least-explored off the U.S. East Coast, with the goal of providing baseline information to support science needs and management of sensitive habitats, maritime heritage sites, and potential resources. The 19 ROV dives of this expedition, which ranged from 298 to 3,490 meters in depth, focused on improving the knowledge of unexplored areas within the U.S.

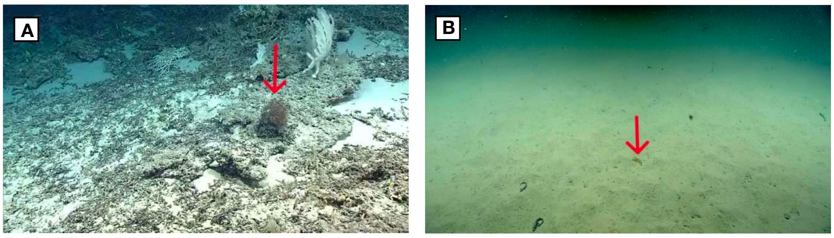

FIGURE 5
Dive sites reviewed from EX1903L2, Windows to the Deep 2019: Southeast and Mid-Atlantic U.S Continental Margin, Port Canaveral, FL to Norfolk, VA. **(A)** Dive 2: Stetson Mesa South Mounds, Eastern Florida, **(B)** Dive 11: "Dodge" Canyon, North Carolina.

Exclusive Economic Zone (EEZ), particularly the deep-sea habitats of the U.S. continental margin and the connections between marine communities throughout the Atlantic Basin (Cantwell et al., 2019).

3.1.3.1 Stetson Mesa South Mounds, Eastern Florida (Dive 2)

This dive explored several deep-water coral mounds (Figure 5A) in the Stetson Miami Terrace Deep Water Coral Habitat Area of Particular Concern (HAPC), approximately 150 kilometers off the east coast of Florida. This dive also included several hours of water column exploration. The area explored was just inside the Gulf Stream, and it started out with a high quantity of marine snow at 500-700 meters. The general trend observed throughout the dive was a large abundance of coral rubble at the bottom of each mound with increasing abundance of live coral coverage on the east to southeastern side of each mound. A pancake urchin appeared to be grazing on a piece of *Sargassum*. This dive transited 1,041 linear meters along the seafloor. Sixteen *Sargassum* observations were made over this distance, for an average 1.54 *Sargassum* observations per 100 meters (Table 1). The average depth for *Sargassum* observations was 761 meters.

3.1.3.2 "Dodge" Canyon, North Carolina (Dive 11)

This dive explored the Deep "Dodge" Canyon, specifically the mouth of an inner canyon/minor canyon area (Figure 5B) approximately 65 kilometers offshore of the Outer Banks, North Carolina. This site was characterized by a soft, heavily sedimented silty bottom, a lot of marine snow, and poor visibility. This is likely a result of organic material produced at the surface and in the midwater sinking and washing down the slope to accumulate on the bottom. The seafloor continued to be heavily sedimented, even while traversing up a relatively steep slope (>30 degrees), and almost no organisms were actually attached and growing on the benthos. This was a canyon dive and closer to shore than most of the other dives in this expedition, which had more elevated mounds with clearer water and exposed rocky substrate. Six *Sargassum* were observed on this dive, with two of the *Sargassum* under a light layer of silt. This dive transited 251 linear meters along the seafloor. Six *Sargassum* observations were made over this distance, for an average 2.39 *Sargassum* observations per 100 meters (Table 1). The average depth for *Sargassum* observations was 1,288 meters.

3.1.4 2019 Southeastern U.S. Deep-sea Exploration (EX1907), October 31-November 20, 2019

Miami, Florida to Key West, Florida

2019 Southeastern U.S. Deep-sea Exploration was a 21-day expedition to explore a diversity of poorly known deep seafloor and midwater habitats, as well as unique geological features, in areas off the U.S. Southeast. The 12 ROV dives of this expedition, which ranged from 404 to 1,218 meters in maximum depth, explored a diversity of poorly explored deepwater habitats and geological features, such as biogenic mounds, deep-sea coral and sponge habitats, and fish habitats, that are of interest to resource managers and scientists (White et al., 2019). In combination with deep-sea mapping operations, critical information and data were collected to characterize unknown and poorly known areas of the southern U.S. continental margin, with the goal of increasing our understanding of deep-sea ecosystems and supporting ecosystem-based management of marine resources (White et al., 2019).

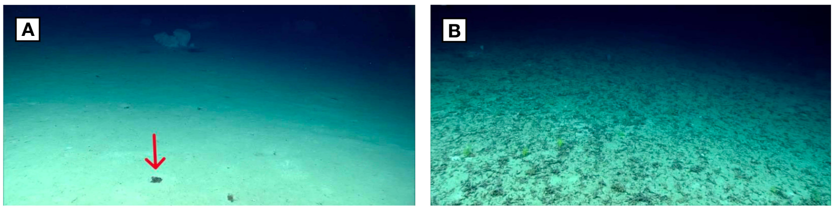

FIGURE 6
Dive sites reviewed from EX1907, 2019 Southeastern U.S. Deep-sea Exploration **(A)** Dive 11: Key West Deep, **(B)** Dive 12: "Berg Bits", Southwestern Florida.

3.1.4.1 Dive 11: Key West Deep

The primary objective of this dive was to explore and characterize a small canyon (Figure 6A) that has the potential to be suitable habitat for deep-water coral, sponges, and associated fauna. This dive explored two small mounds that rise about 5 meters from the surrounding seafloor as well as an escarpment feature and a potential seep site with authigenic carbonate and bacterial mats. The current was swift on the bottom. The soft, silty seafloor continued to be sedimented even when traversing up a relatively steep slope (>30 degrees), and there were many little mounds from bioturbation. There were large terraces on the escarpment with unconsolidated sediment cover. The vast majority of this dive was exploring near vertical inclines on the boulders and escarpment feature. *Sargassum* was observed on the little horizontal sandy surface that was explored. Six *Sargassum* were observed on this dive, including one patch of *Sargassum* at 19:25 that was more degraded than the other *Sargassum* observed in this study. This dive transited 927 linear meters along the seafloor. Six *Sargassum* observations were made over this distance, for an average 0.65 *Sargassum* observations per 100 meters (Table 1). The average depth for *Sargassum* observations was 1,208 meters.

3.1.4.2 Dive 12: "Berg Bits", Southwestern Florida

Fifty nautical miles southwest of the Dry Tortugas, this dive explored two mounds and an escarpment at the base of the "Antarctica mound" (Figure 6B) - a plateau shaped like Antarctica with "bergie bits," or iceberg-like carved out features, surrounding the plateau. The plateau at the top of the escarpment and the seafloor in between mounds were sedimented. While approaching both mounds, small blocks from the escarpment decorated the sediment. Live coral was observed on the mounds, while standing dead coral and coral rubble was also prevalent on the mounds, along with seagrass. Most of the horizontal movement was done in transit from one feature to the next, so the ROV was moving fairly quickly and did not stop and zoom on features to allow a positive identification of *Sargassum*. No *Sargassum* were positively identified on this dive. Given the speed and distance from the ROV, it was not possible to distinguish between *Sargassum* and coral rubble and it is possible that there was *Sargassum* present. This dive transited 932 linear meters along the seafloor. The maximum depth reached was 973 meters.

4 Discussion

Because the deep sea is so under-studied, any new observations can lend valuable insight into the dynamics of deep-sea communities and how the deep sea is connected to the surface and to global environmental processes. *Sargassum* has been directly observed at local scales on the sea surface for centuries. The use of satellite-technologies has scaled up the spatial and temporal resolutions at which *Sargassum* can be observed, helping to better understand the species trajectory at the surface. However, once *Sargassum* dies and loses buoyancy, its fate is poorly understood after it sinks below the sea surface.

Through the use of deep submersible technologies and archived video, this study documented significant quantities of intact *Sargassum* on the seafloor in areas with known surface blooms. *Sargassum* was observed on 9 of the 10 dives reviewed for this project, in numbers ranging from 0 to more than 112 observations per dive. Given the limited spatial scale of our observations, we can surmise that *Sargassum* makes its way to the deep sea in likely significant amounts. Though we reviewed the descents and ascents for all the ROV dives, no *Sargassum* was observed within the water column, indicating that *Sargassum* might sink quickly once it loses its buoyancy, consistent with the relatively fast sinking rate estimated by Johnson and Richardson (1977). The relatively low volume of water imaged by the ROV may limit the ability to observe *Sargassum* sinking through the water column. It is also possible that some portion of the sinking *Sargassum* is consumed by pelagic herbivores.

Though we do not have quantitative estimates of *Sargassum* at the sea surface for each of the dives in this study, we do note two instances where expedition participants noted the heavy presence of *Sargassum* at the surface (e.g., Caja de Muertos Island and South of La Parguera). These dives correspond with some of the highest *Sargassum* observation numbers (more than 112 and more than 19), supporting the expectation that where *Sargassum* blooms are thick at the surface, large numbers sink below. *Sargassum* blooms have continued since this study time period, with March 2023 setting records for the most *Sargassum* observed in the month of March (University of South Florida (USF) and Optical Oceanography Lab, 2023). *Sargassum* has continued to wreak havoc in the Southeastern U.S. and Caribbean coastal communities, even interfering with ocean exploration operations[6].

For this study, we considered the general environmental and ecological context of a dive site to explore whether there are areas that may be aggregators of *Sargassum*. There were no obvious patterns, though a few things to consider. "Dodge" Canyon off North Carolina was characterized by thick sediment and poor visibility, likely as a result of the high amount of marine snow that was observed both in the water column and on the seafloor. This marine snow indicates that there is likely high productivity on the surface to produce so much organic material. However, this large amount of marine snow may have quickly buried any *Sargassum* on the bottom, as two of the *Sargassum* that were observed there were partially covered in a fine layer of silt. Although only 6 specimens of *Sargassum* were observed on this dive, the second lowest out of the 10 dives surveyed, there may have been more there just buried from view. If the *Sargassum* is in fact being buried under silt at high surface productivity sites, this could remove the availability of the *Sargassum* to feeding by abyssal omnivores and scavengers such as sea urchins and gastropods, thus increasing the likelihood of sequestration. The high number of anthropogenic debris observed relatively far offshore (approximately 250 kilometers) at the Hidalgo Basin dive site indicates there might be some factor leading to accumulation here, such as currents or seafloor depressions also aggregating *Sargassum*. The bathymetry of the Mona Canyon dive

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[6] https://oceanexplorer.noaa.gov/okeanos/explorations/22voyage-to-the-ridge/features/sargassum/sargassum.html.

site may have resulted in the larger piles of *Sargassum* observed at this dive, with the steep walls of the underwater canyon helping to funnel the *Sargassum* into large aggregations. The shallowest of the dives studied (Caja de Muertos, 470 meters) also included the only observation of *Sargassum* slowly tumbling down a slope. It is possible that, being closer to the surface, the *Sargassum* was more intact and thus more likely to be transported in this way.

There were three instances of invertebrates (two sea urchins and one gastropod) appearing to graze on *Sargassum*, observed on separate dives. This provides evidence that *Sargassum* is a source of food for benthic animals, with the further implication that some carbon is not being sequestered but instead is cycled back into the marine system. Bacterial degradation of the *Sargassum* would additionally diminish the carbon removal potential.

Part of the inherent value of exploration-driven study of the ocean is that some of the most interesting and impactful insights that may be gleaned from the research are typically unknown at the outset and unplanned for (e.g., Selig et al., 2019; Ford et al., 2020; Simon-Lledo et al., 2023). Although the *Okeanos Explorer* expeditions did not explicitly seek to study *Sargassum* on the seabed, through review of these 10 exploratory video surveys, we were able to significantly increase the number of confirmed direct observations of *Sargassum* on the deep seabed and contribute to broader understanding of the fate of sinking *Sargassum*. While observations of the Southeast U.S. and Gulf of Mexico remain sparse, they are comparatively well explored areas, with a decades-long history of submersible surveys (e.g., Harbor Branch Oceanographic Institute's Johnson Sea-Link submersible). Further investigation could delve into available archived video footage to investigate trends in the quantity of *Sargassum* on the seafloor over time as blooms have increased in quantity. Additionally, we acknowledge that our study has inherent bias in that sites were intentionally selected where *Sargassum* were known to be observed. Further studies could use a random sampling design to provide an unbiased and quantitative assessment of *Sargassum* sinking at the seafloor.

Macroalgal ecosystems like those harboring *Sargassum* may sequester and store significant amounts of carbon from the atmosphere and ocean and hence are now recognized for their role in mitigating climate change. Developing a predictive understanding of carbon export pathways such as gravitational sinking is thus critical to diagnosing present and future rates of ocean carbon sequestration. Using ROV dive surveys to study the fate of *Sargassum* confirmed that relatively large amounts of *Sargassum* complete the first step in the process toward potential carbon sequestration on the seabed – sinking to the seafloor. Long-term monitoring of the fate of sunken *Sargassum* on the seabed is needed in order to determine how much is ultimately sequestered rather than returned back into the system. Such observations would inform the feasibility of *Sargassum* farming and/or facilitated sinking as potential carbon dioxide removal strategies.

Further observations throughout the global ocean are required to fill in the gaps in the distribution of *Sargassum* in the deep sea. Further observation of the fate of *Sargassum* blooms on the deep seabed could serve as a natural model to inform research and development for emerging seaweed sequestration programs for climate mitigation.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

AP conceived of this project, conducted the video analysis, created the plots and figures, and led the drafting of the manuscript. AN advised on deep-sea video analysis and NOAA Ship *Okeanos Explorer* operations and wrote sections of the manuscript. HB provided guidance on plotting the dive and *Sargassum* observations and CTD data analysis and conducted analysis of distance traveled. VH advised on potential machine learning applications. AN, HB, and VH all provided feedback and guidance on the project design. All authors contributed to the article and approved the submitted version.

Funding

Funding for field work was provided by NOAA Office of Ocean Exploration and Research.

Acknowledgments

This study used publicly-available data provided through NOAA Ocean Exploration and Research operations on NOAA Ship *Okeanos Explorer*. We thank the expedition coordinators, onboard science leads, ship crew, Global Foundation for Ocean Exploration ROV pilots, and other personnel who supported the expeditions considered in this study. We additionally thank Dr. Christopher Kelley for advising on the processing of CTD data. An early version of this study, Capstone paper "A Little Bit of Sargassum Goes A Long Way: Observations and Mapping of *Sargassum fluitans* and *Sargassum natans* from NOAA Ship *Okeanos Explorer*'s ROV *Deep Discoverer*" was published online in 2020 through escholarship.org as part of a Master of Advanced Studies Capstone project at Scripps Institution of Oceanography (Pries, 2020). This study updates and expands upon the Capstone paper by reviewing and characterizing four additional dives.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1250150/full#supplementary-material

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frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 11 December 2023
DOI 10.3389/fmars.2023.1219993

OPEN ACCESS

EDITED BY
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RECEIVED 11 May 2023
ACCEPTED 10 November 2023
PUBLISHED 11 December 2023

CITATION
Govindarajan AF, Llopiz JK, Caiger PE, Jech JM, Lavery AC, McMonagle H, Wiebe PH and Zhang W(G) (2023) Assessing mesopelagic fish diversity and diel vertical migration with environmental DNA.
Front. Mar. Sci. 10:1219993.
doi: 10.3389/fmars.2023.1219993

Assessing mesopelagic fish diversity and diel vertical migration with environmental DNA

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Mesopelagic fishes are an important component of the world's oceans in terms of their abundance, biomass, and ecosystem function. These fishes are important contributors to the biological carbon pump via their feeding and behaviors, whereby they facilitate the transfer of carbon from shallow waters to the deep sea. Several species undertake diel vertical migration, feeding in shallower waters at night and moving to deeper waters during the day. This process actively expedites the downward flux of carbon. However, carbon budgets and climate models require accurate information regarding the depth distributions and migration patterns of these fishes, and environmental DNA (eDNA) analyses can provide this information. Here, we utilize eDNA approaches, generating taxonomically-informative COI and 12S reference barcodes for 80 species of mesopelagic fishes, which can be used for species-level identification of eDNA sequences. Using these, along with a publicly available barcodes database, we compare results from eDNA analysis with traditional net sampling, and explore the ability of eDNA techniques to detect diel vertical migration in fishes from samples collected in Northwest Atlantic Slope Water. We found that eDNA and net samples often resulted in different species identifications, demonstrating that eDNA can detect species that would otherwise be missed with traditional methods. In our eDNA samples, we also detected more species (12) in our shallowest depth category (0 - 100 m) from night samples than from day samples (3). This is consistent with increased diversity in shallow waters at night due to diel vertical migration. Based on the variability observed in sample duplicates, we suggest that future mesopelagic eDNA studies incorporate larger sample volumes and scaled-up sampling efforts. We also note the potential applications of eDNA analysis in addressing ecological questions related to predator-prey relationships identification of foraging hotspots, and carbon flow through the ocean's midwaters.

KEYWORDS

mesopelagic, diel vertical migration, fish, eDNA, biodiversity, barcoding

1 Introduction

Mesopelagic fishes are a critical component of the global ecosystem in terms of their biomass, abundance and ecological function. They comprise the majority of the world's fish biomass, with estimates ranging from 2-16 billion tons (Proud et al., 2019). Mesopelagic bristlemouth fishes (family Gonostomatidae) are thought to be the most abundant vertebrates on the planet (Nelson, 2006). Many mesopelagic fish actively transport carbon from the surface to the deep sea through their vertical migration behaviors, moving up to productive surface waters to feed at night, and back down to depth during the day (Cavan et al., 2019; Saba et al., 2021). Non-migrating fish remineralize carbon through their metabolism (Sarmiento-Lezcano et al., 2022), but they may also contribute to downward carbon transport by consuming migrating zooplankton that would otherwise return to surface waters (Davison et al., 2013). The depth distributions, biomass, and behaviors of mesopelagic fishes, particularly of those that migrate, represent important knowledge gaps in our understanding of the biological carbon pump (Henson et al., 2022).

Ranging from 200 to 1000 m water depth, the mesopelagic environment is exceptionally vast and challenging to access, and consequently, it is poorly explored. Environmental DNA (eDNA) analysis could enable more efficient study of the region's biodiversity to provide insights for understanding the biological carbon pump and to enable sustainable use of mesopelagic resources (Cavan et al., 2019; Saba et al., 2021). Specifically, metabarcoding analysis of eDNA samples provides "snapshots" of the diversity associated with the sampled water (e.g., Easson et al., 2020; Laroche et al., 2020; Canals et al., 2021; Govindarajan et al., 2021). Environmental DNA sampling is rapidly being incorporated into ecosystem monitoring programs (Mirimin et al., 2021; Ray et al., 2022; Stefanni et al., 2022), and metabarcoding sequence analysis of eDNA samples will provide important records of how biological communities are impacted by climate change and other anthropogenic stressors (Leduc et al., 2019; Lanzén et al., 2021; Miya, 2022).

Metabarcoding analyses require barcode libraries consisting of reference barcode sequences originating from specimens that have been previously identified for taxonomic assignment of the eDNA sequences (Duhamet et al., 2023). However, reference libraries are incomplete and may potentially contain sequences from specimens that have been misidentified (Lindsay et al., 2017; Bucklin et al., 2021). For fishes, the mitochondrial COI gene has been widely used for species identification (Bucklin et al., 2011; Bucklin et al., 2021). As a result of COI barcoding efforts, reference sequences for common mesopelagic fishes in the North Atlantic are publicly available (Kenchington et al., 2017). Indeed, in many cases, reference sequences from multiple specimens and geographic locations have been obtained, enabling detection of population-level variation and cryptic speciation (Kenchington et al., 2017; Christiansen et al., 2018).

With the introduction of high-throughput sequencing and metabarcoding, shorter barcode markers have become necessary. In particular for fishes, a short, hypervariable region of the 12S gene has become the barcode marker of choice. Miya et al. (2015) greatly enabled this approach by designing a "universal" primer pair (MiFish-U-F/R) that amplifies a wide variety of fish species and is able to resolve taxonomy to the species level in most cases, when reference sequences are available. However, there are fewer reference sequences for 12S than for COI (Stoeckle et al., 2021). Thus, it is critical that 12S reference libraries are developed in order for eDNA metabarcoding analyses to be useful for understanding mesopelagic phenomena such as diel vertical migration.

There are relatively few eDNA studies to date that focus on the mesopelagic environment and address diel vertical migration (Easson et al., 2020; Canals et al., 2021; Govindarajan et al., 2021; Feng et al., 2022). Environmental DNA studies have great potential to improve the detection of migrating fish species, many of whom may be missed by traditional net tows (Skjoldal et al., 2013) and cannot be identified from acoustic analyses (e.g., Wiebe et al., 2023). A recent modeling study evaluating the dispersal and fate of mesopelagic eDNA found that eDNA remains close to its source of origin in the vertical dimension, indicating that it should be able to detect migration patterns (Allan et al., 2021). However, while eDNA field data demonstrating vertical structure in biological communities are encouraging, observations are extremely limited (Easson et al., 2020; Canals et al., 2021; Govindarajan et al., 2021).

The goals of this study are to: 1) enable eDNA metabarcoding analyses of mesopelagic fish by obtaining new 12S reference barcode sequences for mesopelagic fish species; 2) assess the ability of eDNA analysis to detect mesopelagic fish species using both previously available reference barcodes and our new barcode sequences; and 3) explore the ability of eDNA analyses to detect the presence of diel vertical migration. For the first goal, we sequenced and analyzed mesopelagic fish specimens collected over the course of several cruises in the North Atlantic Ocean. For the second and third goals, we analyzed eDNA samples obtained from depths between the surface and 1000 m during day and night Conductivity Temperature Depth (CTD) casts on a 2018 cruise in the North Atlantic Slope Water. These samples were previously sequenced with the 18S V9 barcode marker, which, while useful for detecting a broad range of metazoan taxa, may not be suitable for fish (Govindarajan et al., 2021). Here, we applied 12S metabarcoding using the MiFish primer set on these samples and incorporated the new reference barcodes into the taxonomic assignment step. We compared fish species detected from the eDNA samples with those from net tows. We then compared fish species detected from eDNA samples from comparable depth categories during day and night casts. Lastly, to explore the representativeness of the CTD results, we compared fish species detected from duplicate samples taken at four depths during a single CTD cast.

2 Materials and methods

2.1 Reference library generation

Reference library generation consisted of collecting fish specimens, obtaining accurate identifications for those specimens through a combination of morphological analysis and COI barcoding, and then generating 12S barcode sequences. Fish specimens were collected using trawls and net tows from a series of cruises in the northwest Atlantic Ocean over the course of several years (Supplementary Table S1). Fish were identified based on morphological criteria (Carpenter, 2002; Sutton et al., 2020) and photographed. The photographs were deposited in Dryad (doi: 10.5061/dryad.cc2fqz6bp). A small amount of muscle tissue was dissected from each specimen and stored at -80°C until it was used for DNA extraction.

DNA was extracted using DNEasy Blood and Tissue kits (Qiagen, Germantown, MD, USA) following the manufacturer's protocol. The COI barcode marker was PCR amplified using either the Folmer et al. (1994) HCO-LCO, or the Ward et al. (2005) Fish F1/R1 or Fish F2/R2 primer sets. Fifty µl PCR reactions were run consisting of 10 µl of buffer, 5 µl of Mg, and 0.3 µl Taq polymerase (all from the Promega GoTaq Flex kit), 2 µl of each primer (10 µM), 1-2 µl genomic DNA, and 23.7-24.7 µl water. The following PCR cycle was used for all primer sets: 95°C for 3 minutes, 35 cycles of 95°C for 30 seconds, 48°C for 30 seconds, and 72°C for 1 minute, and 72°C for 5 minutes. PCR products were visualized on a 1% agarose gel under UV light using GelRed (Biotium, Hayward, CA, USA). Successful amplifications were purified using the Qiaquick PCR Purification kits (Qiagen, Germantown, MD, USA) and quantified with a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Purified products were sent to Eurofins Genomics (eurofins.com) for sequencing in both directions.

Sequence chromatograms were analyzed and consensus sequences generated using the Geneious version 9.0.5 (Biomatters, Inc) software platform. DNA sequences were blasted against the GenBank database for comparison with publicly available sequences. Matches with 98% identity or greater for at least 90% of the read length were considered species-level identifications (e.g., Kenchington et al., 2017; Teramura et al., 2022). Barcode identifications were compared with morphological identifications. In all cases, they either provided the same species-level identification, or they provided species-level identification to specimens identified to only the genus or family level.

For unique species that were identified, we additionally obtained the 12S barcode sequence. This sequence was amplified in 50 µl PCR reactions (with same reagents and concentrations as for the COI PCRs) using the MiFish-U-F/R primer set (Miya et al., 2015) with the following thermal cycler conditions: 95°C for 3 minutes, 35 cycles of 95°C for 20 seconds, 47°C for 20 seconds, and 72°C for 20 seconds, and 72°C for 5 minutes. Successful amplifications were purified, quantified, sequenced, and compared with Genbank in the same way as the COI barcodes. All COI and 12S sequences were deposited on GenBank (Supplementary Table S1). The specimen-voucher ID provided in the GenBank metadata can be used to cross-reference the sequences with the photographic archive in Dryad.

2.2 eDNA and MOCNESS sample collection and genomic DNA extraction

This study uses eDNA and 1-m^2 MOCNESS samples that were previously analyzed with the 18S V9 barcode marker (Govindarajan et al., 2021), and detailed sample collection and DNA extraction methods are provided there. To summarize, samples were collected from our study area during a cruise on the NOAA Ship Henry B Bigelow during the summer of 2018. The study area was located in the slope water off the shelf break south of the island of Martha's Vineyard, Massachusetts (Figure 1). Environmental DNA samples were collected from eight 5-liter Niskin bottles mounted on a Seabird 911 plus CTD rosette. Casts were conducted during either day or night, avoiding migrations. Dawn and dusk migration were observed on a shipboard Simrad EK60 echosounder and occurred approximately between 1800 – 2100 (dusk) and 0400 - 0700 (dawn) local time. There were two night casts, collecting a single sample at each of 8 depths; two day casts, collecting a single sample at each of 8 depths, and one day cast collecting duplicate samples at each of four depths (Supplementary Table S2). Overlapping temperature and salinity profiles indicated that these casts, and the MOCNESS tow, all of which were closely spaced, were from the same water mass (Govindarajan et al., 2021). Sample bottles were triggered at depths based on acoustic backscatter detected by the EK60 echosounder and so were not consistent between casts. Once on board, the seawater samples were filtered on to 0.2 µm PES Sterivex filters using peristaltic pumps, and immediately stored at -80°C. Filtration blanks using sterile water were generated with each cast, and rigorous precautions were taken to avoid contamination. Genomic DNA was extracted using DNEasy extraction kits (Qiagen) as described in Govindarajan et al. (2021).

For the MOCNESS sampling, a depth-stratified tow (8 nets sampling discrete depth bins of 0-24 m, 24-50 m, 50-100 m, 100-200 m, 200-400 m, 400-600m 600-800 m, and 800-1000 m) was conducted during the night. The contents of each net were split into four equal portions using a Folsom plankton splitter, one of which was preserved in ethanol for metabarcoding analysis. Upon return to the laboratory, the samples were run through 1000 µm sieve and homogenized using a homogenizer with a 10 mm sawtooth probe (Benchmark Scientific). Genomic DNA from the homogenates was extracted using DNEasy extraction kits (Qiagen) as described in Govindarajan et al. (2021).

2.3 12S amplicon library preparation and sequencing

Aliquots of genomic DNA from the eDNA samples and the >1000 m MOCNESS samples were sent to the University of Connecticut Center for Genome Innovation for amplicon library preparation and sequencing. A 2-step PCR protocol adapted from Pitz et al. (2020) was used to amplify the 12S barcode marker region. The first PCR utilized a custom fusion design using the MiFish primers as the target-specific region (Miya et al., 2015) and Illumina-compatible Fluidigm adaptors CS1 and CS2 to add on sequence necessary for sequencing. Each PCR reaction consisted of 2.5 µl template, 12.5 µl NEB Phusion High Fidelity PCR Master Mix, 1 µl forward primer (1 µM), 1 µl reverse primer (1 µM), and 8 µl molecular grade water. The PCR followed a touchdown protocol that consisted of 95°C for 15 minutes, 13 cycles of 94°C 30s, 69.5°C 30s, 72°C 90s (with the annealing temperature decreasing by 1.5°C

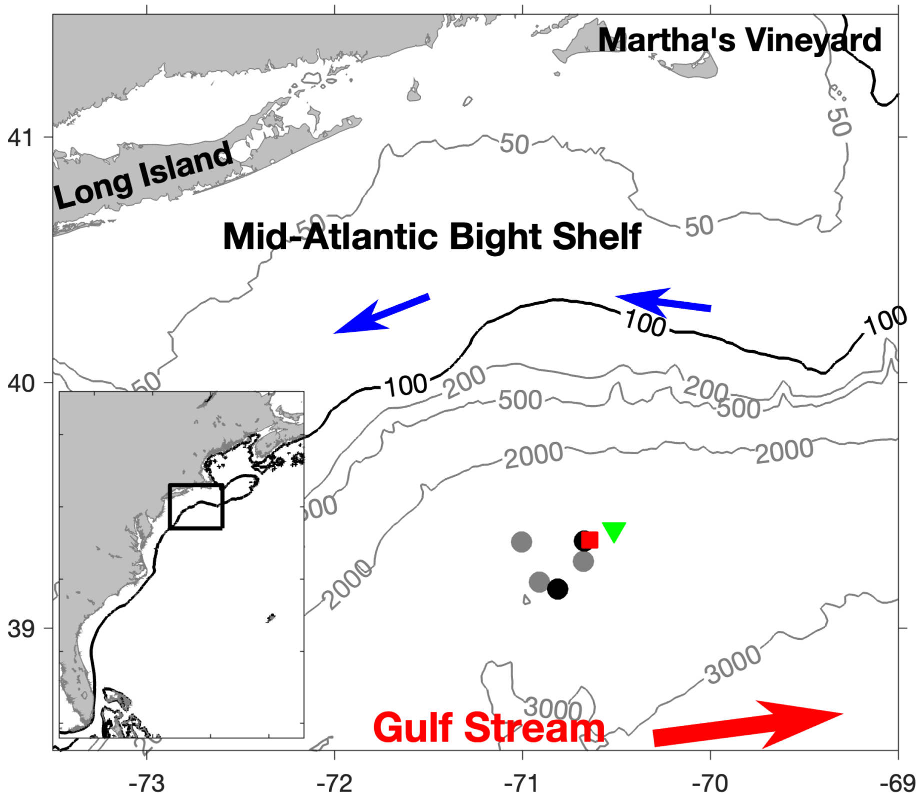

FIGURE 1
Map of study site. Black circles indicate locations of night CTD casts and gray circles indicate loctions of day CTD casts. The green triangle and red square indicate the starting and ending locations of the MOCNESS tow, respectively. Contour lines connect area of equal depth (m).

for each cycle), 25 cycles of 94°C 30°Cs, 50°C 30s, 72°C 45s, and 72°C for 10 minutes. Duplicate PCRs for each sample were run and pooled after initial amplification. Amplicons from the pooled PCRs were visualized with the Agilent 4200 TapeStation electrophoresis system using the High Sensitivity DNA D1000 assay (Agilent Technologies, Santa Clara, CA). Expected – sized amplicons were selected using the Pippin Prep HT gel cassette (2% Agarose, PippinHT, 100-600 bp). The second PCR incorporated index primers that targeted the CS1/CS2 Fluidigm adapters from the primary PCR products. PCR reactions consisted of 5 µl size-selected and purified PCR product, 25 µl NEB Phusion High Fidelity PCR Master Mix, 15 µl molecular grade water, and 5 µl of a unique dual index 10bp barcode (IDT for Illumina Nextera DNA UD barcode plate). The PCR cycle conditions were 95°C for 3 minutes, 12 cycles of 95°C 15s, 60°C 30s, 72°C 60s; and 72°C for 3 minutes. Amplicon libraries were purified using AMPure XP Beads (Agencourt) and assessed with an Agilent 4200 TapeStation electrophoresis system (High Sensitivity DNA D1000 assay). Libraries were quantified using a Qubit 3.0 fluorometer (Life Technologies, Carlsbad, CA), normalized, pooled, and denatured according to the Illumina MiSeq sample preparation guide. 20–30% PhiX (Illumina, San Diego, CA) was added to the amplicons, which were run on the Illumina MiSeq using the 500 cycle v2 reagent kit (250 bp paired-end). The field negative control samples and the PCR blank were also sequenced. These did not contain detectable amounts of DNA so equimolar pooling was not possible; therefore, the maximum volume that was used for the samples was used for library preparation.

2.4 Analysis

Metabarcoding sequence data was processed using Quantitative Insights Into Microbial Ecology 2 (QIIME2) version 2020.11 (Bolyen et al., 2019), following the general approach described in (Govindarajan et al., 2021; Govindarajan et al., 2022). After examining sequence quality plots, sequence primers and read-through (sequence data extending beyond the reverse primer at the far ends of the forward and reverse amplicons) were trimmed using the Cutadapt QIIME2 plugin (Martin, 2011). Sequences were quality filtered, truncated to 150 base pairs in length, denoised, and merged to create amplicon sequence variants (ASVs) using DADA2 (Callahan et al., 2016) within the QIIME2 platform. After this initial quality control, samples contained 172,325 ± 83,257 reads. Our expected amplicon size was ~170 base pairs (Miya et al., 2015), and so sequences less than 150 bp and greater than 190 bp were eliminated from the dataset. Amplicon sequence variants (ASVs) present in any of the control samples were also removed (these came to 198,513 reads belonging to 11 ASVs, or 2.15% of the dataset). Rare ASVs, defined as having a summed frequency of <100 were also removed.

Three hundred and ninety-five ASVs remained at this stage, and these were classified with GenBank using batch Blast searches within the Geneious software platform followed by manual curation of the results. Blast searches are the most common approach for identifying fish eDNA reads (Xing et al., 2022) and manual curation reduces errors associated with GenBank misidentifications (Claver et al., 2023; although note this approach may not be feasible for larger datasets). Amplicon sequence variants that were identified as non-fish or did not have a match with at least 90% query coverage were excluded from subsequent analyses, except for a small number of ASVs that matched marine mammals which were kept, for a total of 170 remaining ASVs. Fish and mammal ASVs were identified to species if they had a ≥ 98% identity with at least 90% coverage with the reference sequence If multiple Genbank species met this criterion, the ASV was identified to the highest common level (e.g., if the ASV matched two different species belonging to the same genus, the ASV was only identified to genus). Fish ASVs were also compared to our new 12S sequences using the Blast function in Geneious and the same criteria for species identification. These results were integrated with the GenBank results to produce final ASV taxonomic assignments. Ninety-eight percent identity is commonly set as the threshold for species-level identification with ASVs generated by the MiFish primers (Díaz et al., 2020; Andres et al., 2023; He et al., 2023).

The final annotated dataset was analyzed using R (R Studio Team, 2020; R Core Team, 2021). Sequence data were categorized by sampling method (CTD or MOCNESS) and the two day CTD profiles and the two night CTD profiles were combined and further categorized into depth categories (0 – 100 m, 100-200 m, 200-400 m, and 400-800 m). Temperature and salinity data from this cruise showed that all samples were collected from the same water mass (Govindarajan et al., 2021). There were the same number of samples in the day and night casts in each depth category, so we compared fish diversity (number of species and relative read abundances) between day and night for each depth category (but not among depth categories). We also compared fish diversity from the night casts to the diversity in the MOCNESS samples (sequences were combined into corresponding depth categories), which were also collected at night. In our fifth cast (cast 10), we assessed the consistency of duplicate samples taken at 4 depths ranging from 375 - 800 m). To provide context for all of our results, identified fish species were further assessed to determine which are associated with mesopelagic depths and vertical migration behavior based on species information provided in Fishbase (https://www.fishbase.se).

3 Results

3.1 DNA barcoding

We present COI and 12S barcode sequences from 80 specimens, each representing a unique species of mesopelagic fish and comprising 30 families (Supplementary Table S1). Thirty-five of the COI sequences originate from Quigley et al., 2023, and 44 are newly presented here. We were unable to obtain a COI sequence for *Nesserohamphus ingolfianus*. For the 44 newly presented sequences, in all cases, the COI barcoding returned 99%-100% matches from our GenBank Blast searches that were consistent with our morphological identifications (Supplementary Table S3) and confirmed taxonomic assignments. However, in nine of our searches, we found close (99-100%) matches with other species, in addition to those with the expected species name annotation. Because intraspecific differences are typically 99-100% in the COI marker in fishes and interspecific distances much larger (Ward et al., 2009; Kenchington et al., 2017), and because our specimens were also identified morphologically, these results indicate probable misidentifications on GenBank. In four of our searches, we found additional matches with the same species name but with lesser percent identity, suggesting either cryptic lineages or misidentifications.

For each of our 80 fish species, we also obtained 12S barcode sequences. The GenBank Blast search results for this dataset were variable (Supplementary Table S3). Eighteen species did not have close matches and our sequences appear to be the first 12S record on GenBank for these species. Seven species had close matches to other species that are likely misidentified. Fifty-two species had close (>98%) matches on Genbank to sequences with the expected species identifications, although eight of these also produced close matches to other species as well. Two species had lesser matches (94.6% and 97%) to sequences with their same identifications; one of these also had a 97% match to a different species. One species (*Physiculus fulvus*) had an identical (100%) match to a sequence with the same identification, but with a low query cover (77.65%). One species (*Scopelogadus mizolepis)* had a 100% match to a closely related species (*S. beanii*).

3.2 Metabarcode taxonomic assignment

Of the 395 ASVs remaining after our sequence processing and quality control steps, a total of 114 ASVs were identified to species from GenBank, and another 56 ASVs were considered as unidentified vertebrates (i.e., fish or marine mammal) (Supplementary Table S4). When these ASVs were searched against our new sequence library, 114 ASVs yielded the same identifications (69 ASVs representing 21 species, and the remaining ASVs "unidentified"); 37 ASVs representing 24 species were able to be identified by species from GenBank only, and 11 ASVs representing four species were identified from our new sequence library only, for a total of 49 species. The identification of five ASVs, identified as *Scopelogadus beanii* on GenBank was qualified to *Scopelogadus* sp., based on a > 98% identity with *S. mizolepis* from our reference library. Three ASVs representing one species yielded conflicting identifications. These were identified and *Nemichthys curvirostris* on GenBank and *N. scolopaceus* with our sequence library. The discrepancy between our reference sequence and GenBank was noted in our Blast search results (Supplementary Table S3), and we considered *N. scolopaceus* to be the correct identification based on our morphological analysis and COI barcode from our reference specimen. Four of the species identified from GenBank were marine mammals (*Tursiops* *truncatus, Ziphius cavirostris, Stenella* sp., and *Mesoplodon miris*). Thirty-five out of the 45 fish species that we identified are associated with mesopelagic depths, and 22 of these are reported on Fishbase to engage in vertical migration behavior (Supplementary Table S5). Fishbase also reported that one species identified from the MOCNESS, *Ostorhinchus apogonoides*, is a tropical reef fish and so is unlikely to be in our study area and its identification may be erroneous.

3.3 Results from MOCNESS samples

After the sequence quality control steps, there were 1,206,750 sequence reads in the MOCNESS dataset, with 150,844 ± 74,219 reads per sample (Supplementary Table S6). Twenty-three fish species and several unidentified ASVs were identified (Figure 2; Supplementary Figure S1). Most of the identified species are associated with mesopelagic environments (Supplementary Table S5). All nets except for 50 – 100 m contained unidentified sequence reads (Figure 2; Table 1). In terms of relative read abundance, the surface net (0-24 m) was dominated by *Diogenichthys atlanticus*, the longfin lanternfish, which is a vertically migrating mesopelagic species typically found between 0 and 100 m at night (which is when the MOCNESS sampling conducted), and *Bothias robins*, the two-spot flounder, which is generally found at depths 0-100 m (Supplementary Table S5). Sequence reads from *Benthosema glaciale*, the Glacier lanternfish, dominated the 24 – 50 m net and comprised about half of the reads in the 50 – 100 m net. *Benthosema glaciale* is also a vertically migrating fish typically found in the top 200 m at night (Supplementary Table S5). Sequence reads identified as *Arctozenus risso*, the spotted barracudina which is not reported on Fishbase to undertake DVM, were also relatively abundant in the 50 – 100 m and 100 – 200 m nets. Sequence reads from *Cyclothone pallida*, the non-migratory tan bristlemouth, and *Stomias boa*, the migratory Boa dragonfish, were also prominent in the 50 – 100 m net. In addition to *Arctozenus risso*, the 100 m net also contained *Astronesthes niger* (Barbed dragonfish, migratory), the bristlemouths *Cyclothone microdon*, *Cyclothone pseudopallida* and *Cyclothone braueri* (all non-migratory), *Hygophum benoiti* (Benoit's lanternfish; migratory), *Scopeloberyx opisthopterus* (bigscale; non-migratory), and *Sternoptyx diaphana* (diaphanous hatchetfish, non-migratory). The 200 – 400 m contained sequence reads from *Argyropelecus hemigymnus* (the half-naked hatchetfish, most abundant; migratory), *Benthosema suborbitale* (smallfin lanternfish, migratory), and *Cyclothone microdon*. The 400 - 600m contained sequence reads primarily from *Cyclothone microdon* and *Hygophum benoiti*, but also *Cyclothone pseudopallida*. The 600 – 800 m net was dominated by *Cyclothone microdon* reads, and the 800 – 1000 m net contained sequence reads from *Boreogadus saida*, *Cyclothone microdon*, *Hygophum benoiti*, *Lobiancha dolfeini*, and *Scopelogadus* sp.

Hippoglossina oblonga, the American four-spot flounder, and *Merluccias bilinearis* can be found in waters deeper than 200 m but are classified as demersal. *Boreogadus saida*, Arctic cod, can occur throughout the epipelagic and mesopelagic zones but are often classified as cryopelagic (occurring near sea ice). All of the rest (18 identified species) are mesopelagic or bathypelagic, and many (7 species) are known vertical migrators (Supplementary Table S5; Figure 2). Several of these "deep sea" species were detected exclusively or primarily in samples from >200 m. Three species were found primarily in deeper waters, with a small number of reads from the shallowest net.

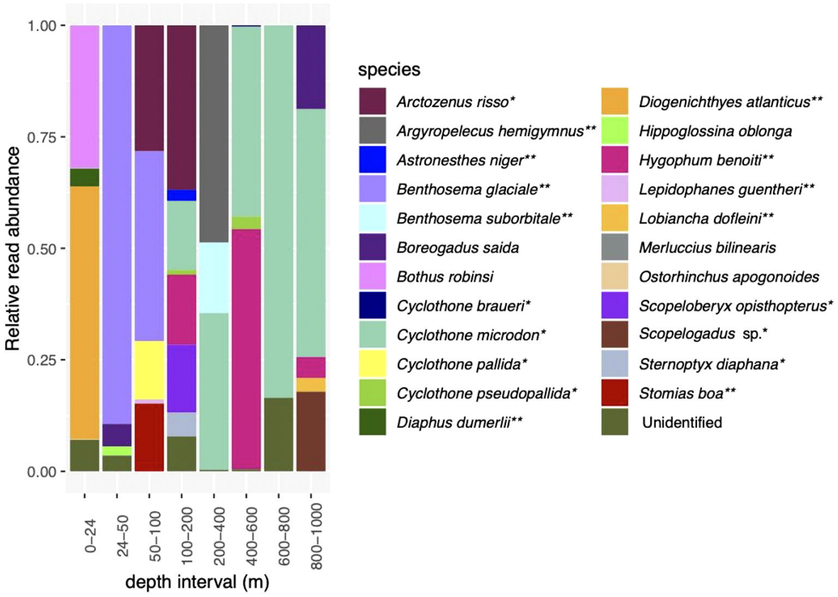

FIGURE 2
Relative read abundances of fish species detected in the MOCNESS. See Supplementary Figure 1 for depth categories that correspond with eDNA depth categories. *indicates species associated with a mesopelagic habitat; **indicates mesopelagic species thought to undertake DVM.

TABLE 1 Comparison of species from corresponding depth categories in A) day and night CTD samples, and B) night CTD and MOCNESS samples.

A.					
Depth category (m)	# species unique to CTD-day	# species unique to CTD-night	# species shared	CTD - day unidentified ASVs	CTD - night unidentified ASVs
0-100	2	11	1	yes	yes
100-200	3	3	0	no	no
200-400	6	2	0	yes	yes
400-800	5	5	9	yes	yes
B.					
Depth category (m)	# species unique to CTD-day	# species unique to CTD-night	# species shared	CTD - day unidentified ASVs	CTD - night unidentified ASVs
0-100	9	11	3	yes	yes
100-200	1	6	2	no	yes
200-400	2	4	0	yes	yes
400-800	13	3	1	yes	yes

Species composition and relative abundances are found in Figure 3; Supplementary Figure S1 for the CTD and MOCNESS datasets, respectively. Samples collected from 801-1000 m depth by the MOCNESS are not included, as the CTD samples do not include this depth interval. The last two columns indicate the presence or absence of unidentified ASVs (not included in the species counts).

3.4 Results from CTD water samples

There were 6,037,388 sequence reads after the quality control steps in the CTD dataset, with 150,933 ± 88,097 reads per sample (Supplementary Table S6). This includes five samples which had zero reads and one sample that had only two reads. Altogether, there were a total of 32 identified species and several unidentified reads in the CTD dataset (Figures 3, 4). As with the MOCNESS dataset, most species that were identified are associated with the mesopelagic (26 out of 32), and of these, 15 species are known vertical migrators (Supplementary Table S5; Figure 3).

The combined two day vertical profiles (casts 5 and 7) and two night vertical profiles (casts 6 and 9) each had four samples in the 0-100 m category, one sample in the 101 - 200 m category, two samples in the 201-400 m category, and 9 samples in the 401 - 800 m category. Thus, sample sizes between depth categories were

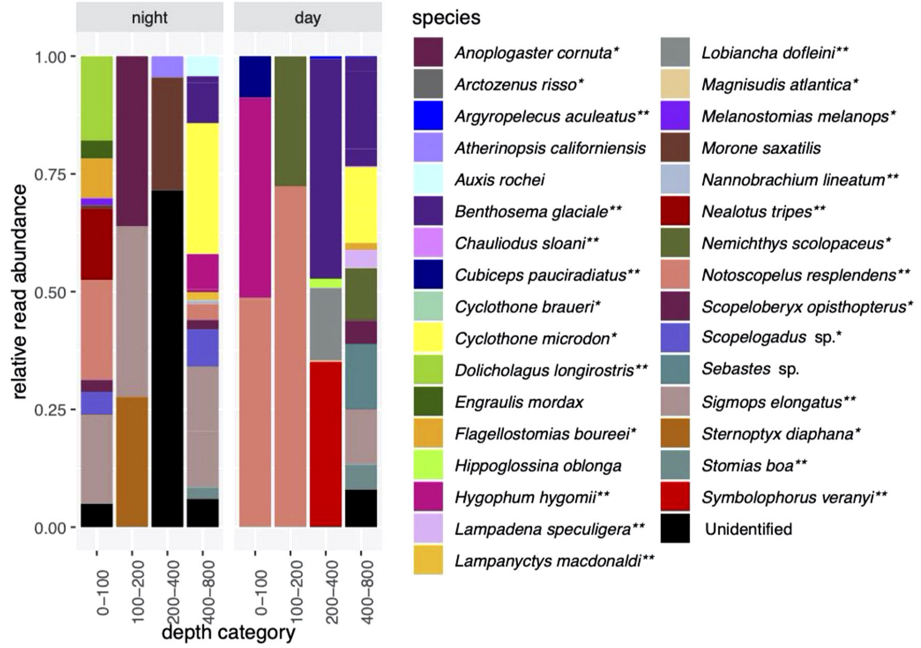

FIGURE 3
Relative read abundances of fish species detected in night (casts 6 and 9) and day (casts 5 and 7) eDNA samples. Four mammal species were also detected (not shown). *indicates species associated with a mesopolagic habitat; **indicates mesopelagic species thought to undertake DVM.

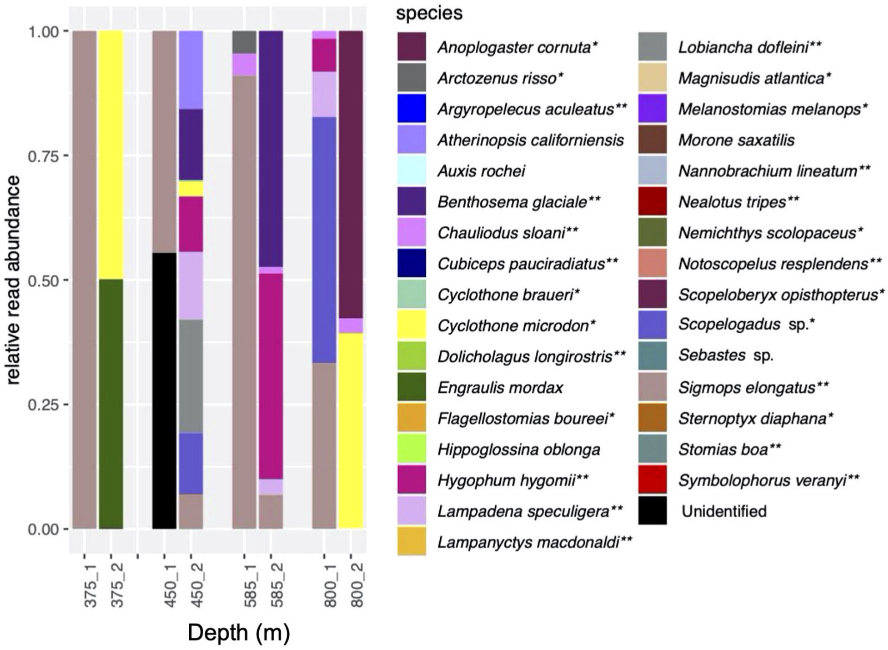

FIGURE 4
Relative read abundances in duplicate eDNA samples (cast 10). Cast was taken during the daytime. Colors represent same species as in Figure 3. *indicates species associated with a mesopelagic habitat; **indicates mesopelagic species thought to undertake DVM.

unequal, so day - night diversity comparisons were made within depth categories only (Table 1). Notably, many more species were found in the night 0-100 m category than in the day 0-100 m category (13 species vs 4 species).

Species that were prominent in terms of their relative abundance and presence in multiple samples include *Notoscopelus resplendens* (prominent from 0-200 m in the day and night samples), *Hygophum hygomii* (0-100 *m* in the day samples), *Benthosema glaciale* (400-800 m in the day samples), and *Sigmops elongatus* (in several day and night sample categories). Species that are not typically mesopelagic (Supplementary Table S5) that were found include *Morone saxatalis* (striped bass), detected in the 200-400 m depth category at night and the bullet tuna (*Auxis rochei*), detected in the 400-800 m depth category also at night. Overall, there was very little overlap in species composition between the night CTD samples and the MOCNESS samples (also taken at night; (Figure 3; Supplementary Figure S1; Table 1). The non-migrating bristlemouth *Cyclothone microdon*, however, was found in deeper depth categories in the day CTD, night CTD, and MOCNESS sample sets (Figures 2, 3). In addition to fishes, four marine mammals, which prey on fish and zooplankton, were detected at various depths (*Mesoplodon mirus*, *Stenella longirostris*, *Tursiops truncatus*, and *Ziphius cavirostris*; Table 2). As expected, no marine mammals were detected in the CTD duplicate or MOCNESS sample sets.

Species composition in the duplicate samples from four depths ranging from 375 to 800 m taken during Cast 10 was highly variable, with generally little overlap between the duplicates (Figure 4). Prominent species were *Sigmops elongatus* (found in all but one sample), *Cyclothone microdon*, and *Hygophum hygomii*, all of which were also found in the day and night CTD sample sets.

4 Discussion

4.1 DNA barcoding and reference library

Our DNA barcoding provided several new insights and resources for the barcoding community. We provided new 12S sequences for 80 mesopelagic fish species which will further improve taxonomic accuracy of eDNA studies in this ocean region. Many of our sequences will be first records on GenBank for those species. Even for species where other 12S sequences are available, our sequences will help shed light on intraspecific

TABLE 2 Marine mammals detected in the CTD dataset.

Species	Common name	Sample	Time of day
Mesoplodon mirus	True’s beaked whale	Cast 9, 220 m	night
Stenella longirostris	Spinner dolphin	Cast 9, 600 m	night
Tursiops truncatus	Bottlenose dolphin	Cast 5, 370 m	day
Ziphius cavirostris	Cuvier’s beaked whale	Cast 9, 50 m	night

variation and expand the geographic representation for this marker on GenBank. Our COI barcodes provided validation for our species identification and will also add to knowledge on intraspecific variation and geographic differentiation, which can facilitate the discovery of cryptic species (Kenchington et al., 2017; Teramura et al., 2022).

Importantly, because we barcoded both the COI and 12S markers from the same specimens, we were able to detect species identification discrepancies on GenBank. While our COI barcoding was consistent with our morphological identifications in all cases, we discovered several GenBank records from species that appear to be misidentified. We suggest that the identifications for these are erroneous based both on the discrepancy with our morphological identifications, which were made by individuals with expertise in fish taxonomy based on published meristic characters and dichotomous keys (Carpenter, 2002; Sutton et al., 2020) and the fact that the vast majority of GenBank records matching our sequences in these cases were identified consistently with our specimens. Moving forward, new approaches such as genome skimming (Hoban et al., 2022) that can sequence several markers from multiple species simultaneously, may be a valuable approach for improving both efficiency and consistency in generating barcodes.

4.2 eDNA and MOCNESS comparison

Consistent with other water column studies (Govindarajan et al., 2021; Merten et al., 2021), the combined MOCNESS and eDNA datasets revealed more taxa than either method individually. Both approaches detected several fish species that the other approach did not, and the eDNA dataset included signals from large fish (bullet tuna) and marine mammals, which would not be sampled by the MOCNESS. The presence of unique species in each dataset can be explained by several factors, but the overarching issue is that the two approaches are fundamentally sampling different entities. The MOCNESS targets organisms that are in the sampled area at the time of sampling, while the eDNA signal represents particles, which may have originated elsewhere or from animals that were present at an earlier time. Also, the volume of water sampled is vastly larger with the MOCNESS (Govindarajan et al., 2021) and the sampling is integrated over time and space – potentially obscuring smaller-scale patchiness and temporal changes, unlike small volume Niskin bottle sampling, which represents a point source (Govindarajan et al., 2023) and signatures of many taxa in the local area may fall outside that volume. Furthermore, MOCNESS sampling may miss species that can avoid net capture (Potter and Lough, 1987; Skjoldal et al., 2013). Given these differences in the nature of sampling, the detection of unique taxa with each method is expected and neither approach is "better" or "worse" than the other. Rather, each approach contributes independent insights that can add to the overall understanding of the ecosystem (Govindarajan et al., 2023). Additionally, our observation that there was little overlap in the CTD and MOCNESS samples in the species detected suggests that reliance on a single approach undoubtedly underestimates biodiversity.

A challenge for detecting ecologically meaningful patterns and making sampling approach comparisons is the ability to obtain multiple, replicated samples over small spatiotemporal scales, which is limited by logistical and resource constraints associated with deep sea research. With Niskin bottle sampling, there is a tradeoff between depth resolution and replication, due to the limited number of bottles. Our CTD rosette accommodated 8 Niskin bottles, and with this resource we conducted 4 CTD sampling casts that sampled without replication at 8 depths, and one CTD sampling cast that sampled duplicates at 4 depths. Other CTD rosettes may have up to 24 bottles (e.g., Laroche et al., 2020), but this is still inadequate for highly resolved, replicated surveys. In the future, autonomous samplers and sampling platforms – including autonomous or uncrewed vehicles that are independent of ships - could enable more rigorous and resolved experimental designs (Truelove et al., 2022; Govindarajan et al., 2023; Preston et al., 2023).

4.3 Detecting diel vertical migration with eDNA

In typical diel vertical migration patterns, some mesopelagic species migrate to shallow waters to feed during the night, while spending day hours at depth. Our finding of greater mesopelagic species richness in the 0-100 m depth bins during the night is strongly indicative of that pattern. Similarly, Canals et al. (2021) found evidence of mesopelagic fish vertical migration in a 12S metabarcode analysis of eDNA. Easson et al. (2020) also found evidence of DVM from eDNA metabarcoding data for a variety of animal taxa. However, they did not detect a substantial fish signal in their data, possibly due to their use of a broadly-amplifying 18S barcode marker ("V4"), which is not fish-specific. Govindarajan et al. (2021), also using a broadly-amplifying 18S marker ("V9") on the same sample set analyzed in this work, did not detect DVM or a significant fish signal. The different findings of this study and Canals et al. (2021) compared to Easson et al. (2020) and Govindarajan et al. (2021) are likely due to the choice of barcode marker. Based on these results, we suggest that future eDNA studies focused on fish DVM use a fish-specific marker such as 12S.

The process of DVM has significant implications for the biological pump by expediting the movement of carbon from the surface to the deep sea. A better understanding of this process, including the species-specific distribution patterns of migrators as well as variations on migration behaviors and migrations beyond mesopelagic depths (van Haren, 2007; van Haren and Compton, 2013; Kaartvedt et al., 2020), is essential for understanding biogeochemical cycling and perturbations that may occur with climate change (Henson et al., 2022). Environmental DNA analysis has great potential to efficiently enable this research. Observed eDNA signals are the product of several physiological and environmental factors, including eDNA shedding, transport and dispersal, and decay processes (Govindarajan et al., 2023). In the mesopelagic zone, the movement of vertical migrators relative to eDNA shedding and decay processes could potentially make it difficult to understand whether eDNA signals originate from the sampling location or elsewhere in the water column. In a modeling study that accounted for the movement of migrators as well as eDNA shedding and decay in typical temperate ocean conditions, Allan et al. (2021) found that eDNA signals remain close to their point of origin (on the scale of 20 meters) in the vertical dimension, indicating that eDNA can be useful to elucidate vertical distribution and migration. The results presented here and elsewhere (Easson et al., 2020; Canals et al., 2021) support this finding.

4.4 Predator detection and inferring trophic relationships

Environmental DNA analyses can provide valuable ecological insights beyond species detection (D'Alessandro and Mariani, 2021; Merten et al., 2021) and has applications in food web ecology. In addition to detecting the presence of mesopelagic fish species, our results identified signals from pelagic fish and marine mammals that are potentially preying on these species. In particular, the tuna and three out of the four marine mammal species that were detected were all from Cast 9. All of these predator species are known to feed on mesopelagic fishes to some extent, as well as cephalopods and crustaceans (Santos et al., 2001; Benoit-Bird and Au, 2003; Mostarda et al., 2007; Hernandez-Milian et al., 2017). While these eDNA signatures originated from different depths, the concentration of signals from this cast suggests that this particular cast location was a foraging hotspot. For example, foraging hotspots may form inside mesoscale eddies (Braun et al., 2019; Della Penna and Gaube, 2020) and be associated with other oceanographic features such as seamounts (Romagosa et al., 2020). While these features were outside the scope of this study, future research could incorporate eDNA analyses into studies involving fish tag and satellite data, to test hypotheses on the prey fields associated with mesoscale oceanographic features and predator presence.

A caveat to our observations is that in some cases, the eDNA signal was found at depths deeper than what the species is known to inhabit - specifically, the bullet tuna *Auxis rochei* is typically found in epipelagic waters (Sabatés and Recasens, 2001), but the eDNA signature was found at 775 m. We also observed eDNA from the spinner dolphin *Stenella longirsotris* at 600 m, although these dolphins take advantage of the evening migration to feed on mesopelagic prey in shallower water (Benoit-Bird and Au, 2003). These observations suggest that at least some of the eDNA signatures originate from shallower water sources, or that these species forage deeper than expected. However, this caveat is still consistent with the possibility that a foraging hotspot in shallower water exists at this location.

Other studies have used eDNA metabarcode analyses in combination with other methods to infer deep sea and oceanic trophic relationships. Visser et al. (2021) combined marine mammal predator biologging with eDNA analysis of potential cephalopod prey and found that while the predators occupied distinct niches, their distributions were unexpectedly controlled by factors other than cephalopod community composition. In another approach, Satterthwaite et al. (2023) combined eDNA data with larval fish sampling to create an ecological co-occurrence network that revealed both potential larval fish predators and prey. D'Allesandro and Mariani (2021) combined eDNA metabarcoding datasets and species inventory data from the literature and generated consumer-resource interaction matrices to identify trophic linkages. Integrating eDNA analyses with other data types, particularly acoustic approaches that can identify migrating layers and tagging methods to characterize predator behaviors, has great potential to elucidate mesopelagic food webs.

4.5 eDNA origin

As noted above, there were some detections of species at depth ranges where we would not expect them to occur (e.g., we found non-migratory *Cyclothone* species in the 100 m depth category; the demersal American four-spot flounder in the midwater; and the shallow water tuna at depth). It is possible that the source of these eDNA signals originated from larvae or eggs, which may be found at shallower depths than the adults (Sabatés and Masó, 1990). Environmental DNA analyses cannot distinguish the form of the eDNA signal (e.g., sloughed cells, gametes, fecal pellets) or the ontogenetic stage of the source individuals. Therefore, other types of sampling and sensing data, such as net tows and video that can provide complementary organismal data, will continue to be important as eDNA analyses become integrated into ocean observing workflows.

4.6 Sampling effort

We observed significant variability in our dataset between duplicate Niskin bottle samples taken at the same depth and time. This is very likely a consequence of small sample volumes, which may fail to capture the full biodiversity present at the sampling location (McClenaghan et al., 2020; Govindarajan et al., 2022). Indeed, some of our samples did not contain any fish reads at all. Our samples were obtained from filtering ~5 liters of water each, which was the volume captured by the Niskin bottles. While this volume is more than the liter or less samples often used in coastal or freshwater studies, it is miniscule relative to the scale of the midwater environment. Autonomous sampling from AUVs with *in situ* filtration and large-area filters that can capture very large volumes (several 10's of liters or more per sample; (Govindarajan et al., 2015; Govindarajan et al., 2022) are a promising approach for maximizing species detection in the future. That said, despite the small volumes sampled, the overall number of species detected was comparable to the MOCNESS dataset, which sampled animals over several orders of magnitude greater volume (Govindarajan et al., 2021). This observation highlights the power of eDNA to more efficiently detect taxa compared to conventional methods, in terms of sampling effort. Stochasticity associated with the PCR process may be another contributing factor to the observed variability between our duplicate samples (Shirazi et al., 2021). To minimize this, we ran duplicate PCRs for each sample (that were subsequently pooled), but additional replication (e.g., PCR triplicates, Cananzi et al., 2022) could potentially improve consistency.

5 Conclusions and future directions

We present new 12S barcode sequences for 80 unique mesopelagic species that were identified using an integrative taxonomic approach that included morphological assessment and COI barcode analysis. These sequences will facilitate future metabarcoding efforts from mesopelagic eDNA samples. By sequencing both the COI and 12S sequences from the same specimens, we discovered likely identification errors from sequences on GenBank, and also provided a pathway to link 12S-based identifications to potential cryptic lineages identified by COI.

We applied our library in combination with sequences from GenBank to identify species from our MOCNESS and eDNA 12S metabarcoding datasets. We found that each approach detected species that the other did not, consistent with expectations based on the different natures of the two approaches. Intriguingly, the eDNA results also included the detection of tuna and mammal species which would not be caught in net sampling. We suggest that eDNA and net tow approaches are complementary and that using both approaches can bring a more complete understanding of the ecosystem. To identify evidence of DVM, we compared eDNA samples taken during the day and night. We found that night mesopelagic fish diversity was greater than day diversity in the shallowest depth bins, consistent with the typical DVM pattern.

Future research should focus on continued reference sequence generation to improve the accuracy and completeness of reference databases. We note that many ASVs in our MOCNESS and eDNA samples were not able to be identified. Additionally, our results show the potential for eDNA to address ecological questions relating to diel vertical migration and food web interactions. We expect that future studies (especially those with expanded sampling, integration with different sensing approaches, and inclusive of a broad range of animal groups) will provide exciting new insights into these phenomena, contributing to a greater understanding of the biological carbon pump, and climate change impacts.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/, OQ883774-OQ883853; https://www.ncbi.nlm.nih.gov/genbank/, OQ870399-OQ870442; https://datadryad.org/stash, DOI: 10.5061/dryad.sj3tx9694. The photographic library can be found in https://datadryad.org/stash, DOI: 10.5061/dryad.cc2fqz6bp.

Author contributions

AG conceived the study. AG, PC, JMJ, AL, JL, HM, PW, and WZ planned, prepared for, and/or conducted the shipboard sampling. HM and PC conducted the morphological identifications and photographed and dissected the fish. AG conducted the DNA barcoding and sequence analyses. AG drafted the manuscript. All authors contributed to the article and approved the submitted version.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research is part of the Woods Hole Oceanographic Institution's Ocean Twilight Zone Project, funded as part of The Audacious Project housed at TED. Funding for the NOAA Ship Henry B Bigelow was provided by NOAA's Office of Marine and Aviation Operations (OMAO).

Acknowledgments

We thank the crew and scientists of the NOAA Ship Henry B. Bigelow, Glenn McDonald (WHOI) for assistance with eDNA sampling; Jessica Barrow, Rene Francolini, Erin Frates, Katie Pell, John Selby, and Sarah Stover (WHOI), for assistance with DNA barcoding; Lyndsey Lefebre (WHOI) for assistance with compiling fish metadata; Bo Reese (UConn Center for Genomic Innovation) for assistance with library preparation and DNA sequencing, and Martha Hauff (Stonehill College) for providing helpful insights on the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1219993/full#supplementary-material

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Frontiers in Marine Science

TYPE Original Research
PUBLISHED 12 December 2023
DOI 10.3389/fmars.2023.1211337

OPEN ACCESS

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RECEIVED 24 April 2023
ACCEPTED 10 August 2023
PUBLISHED 12 December 2023

CITATION
Wei Y, Ma G, Zhang H, Zhang X and Quan X (2023) Optimization design of optical windows for deep-sea pressure-resistant structures based on transition materials. *Front. Mar. Sci.* 10:1211337.
doi: 10.3389/fmars.2023.1211337

Optimization design of optical windows for deep-sea pressure-resistant structures based on transition materials

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Introduction: The design of high-pressure optical windows is one of the key tasks in deep-sea exploration, as it directly determines the reliability and maximum operating depth of underwater optical equipment.

Method: In this paper, mechanical modeling and finite element analysis methods are employed to study optical windows.

Results and discussion: Results show that changes in boundary conditions during loading significantly affect the stress distribution of the window. Based on this conclusion, an optimization design method is proposed, which involves the use of transition materials to reduce the impact of base deformation on the window. The study further analyzes the effects of the transition material's Young's modulus, Poisson's ratio, thickness, and friction coefficient on the stress of the optical window. A calculation method for material selection criteria is proposed to select appropriate transition materials, and actual materials are used for verification. Finally, the reliability of the optimization design scheme based on transition materials is confirmed through high-pressure experiments.

KEYWORDS

pressure-resistant structure, optical window design, finite element analysis, mechanical model analysis, optimized design scheme

1 Introduction

1.1 Research background of optical windows in deep-sea pressure-resistant structures

As one of the important means of human understanding of the world, optical sensing has been widely used in deep-sea exploration, such as marine archaeology, marine biology observation, and seabed geological exploration (Bingham et al., 2010; Ahn et al., 2018; Du et al., 2021; Peng et al., 2023). Unlike the terrestrial environment, the deep-sea environment has a higher pressure, so such equipment requires pressure-resistant structures with optical windows to ensure stable operation at the seabed (Rajput et al., 2020; Vlachos and Skarlatos, 2021). The larger the size of the optical window, the more information the equipment can obtain and the higher the efficiency of deep-sea exploration (Boffety et al., 2012; Zhao et al., 2015). However, at the same time, it will also cause a decrease in the mechanical performance of the pressure-resistant structure. Therefore, in the past few decades, researchers worldwide have conducted a large amount of research on the performance and design methods of pressure-resistant window components, hoping to find a more simple and effective optimization design method while ensuring the reliability of the pressure-resistant structure in improving the efficiency of deep-sea exploration.

1.2 Related work

At present, optical windows commonly come in three structural forms: flat disc, conical, and spherical shell sector. The flat disc optical window has a simple structure and low manufacturing and installation difficulties; it was the main form of underwater equipment optical windows in the early days. However, with the increasing depth of human diving, this form of optical window gradually cannot meet people's requirements for the mechanical and visual range of pressure-resistant structures (Zhu, 1992). Due to the fact that the internal stress of the conical window under the external pressure of seawater is mainly compressive stress, its pressure resistance performance has been greatly improved compared to the flat disc form. Stachiw (1967) proposed the use of organic glass material to manufacture conical optical windows and analyzed the bearing capacity of this scheme under short-term loads with different angles and thickness-to-diameter ratios, which greatly improved the pressure resistance performance of the window. Additionally, Stachiw (Stachiw, 1970a; Stachiw, 1970b; Stachiw, 1972) conducted subsequent long-term load tests under different hydrostatic pressures to obtain complete data results. These data provided a large number of numerical references for the American Society of Mechanical Engineers (ASME) to establish design standards for deep-sea pressure-resistant equipment. Since the properties of organic glass materials are greatly affected by the load situation and environmental factors, Luo et al (Luo et al., 2007; Wang et al., 2019). explored the optimal design parameters from the perspectives of material creep and environmental temperature. In comparison to the conical optical window, the spherical shell sector optical window makes it easier to control the volume of the window. In 1972, Stachiw et al (Stachiw and Sletten, 1976). replaced the optical window of the "Alvin" manned submersible with a spherical shell sector window, obtaining better mechanical performance within a limited volume. They also analyzed the optimal parameters of the spherical shell sector optical window made of organic glass and proposed a method of impregnating epoxy resin or chlorobutyl rubber-coated cloth at the bottom of the optical window to avoid point contact (Stachiw, 1975). Xu and Pei (Junhou, 1984; Binghan, 1987) simplified the mechanical model of this structure and applied the boundary coefficient method to give the theoretical stress distribution results. Du et al. (2011) analyzed the coordination between the optical window and the base and applied the contact finite element method to analyze the optimal cone angle of the flanged spherical shell sector optical window. SB, et al. (2018) optimized the design of the local part using the biological growth method to obtain a better design scheme for the stress concentration problem of the spherical shell sector optical window.

1.3 Our work and paper structure

The above research work primarily focuses on improving the mechanical performance of pressure-resistant structures by identifying optimal design parameters for optical windows. However, the potential improvement achieved through adjustments to the window's design parameters alone is often limited. Previous studies have also overlooked the mutual influence between components during compression. To address these limitations, this paper proposes an optimized design approach for deep-sea pressure-resistant structural optical windows using transition materials and conducts relevant research in this area. Specifically, we investigate the impact of base deformation on the optical window by selecting the spherical sector optical window as our research subject. We aim to calculate and analyze the actual effects of base deformation on the optical window. To mitigate this impact, we incorporate transitional materials into the design. The experimental results obtained validate the effectiveness of this approach.

The organization of this paper is as follows: In Theoretical conceptualizations, we introduce the theoretical foundations, relevant mathematical models, and simulation methods employed in our calculations. Optimization methods presents the optimization design method and analyzes the influencing factors. Subsequently, in Experiments and results, we outline the experimental design and present the obtained results. The Discussion talks about the outcomes of the optimization approach. Finally, in the Conclusion, we provide a comprehensive summary and draw conclusions based on our findings.

2 Theoretical conceptualizations

Compared with other configurations, spherical shell sector optical windows have the advantages of high-pressure resistance, small size, and a large field of view. They are also the most commonly used design for deep pressure-resistant structures at present. Therefore, this paper selects this form of the optical window as the research object and conducts related optimization design research.

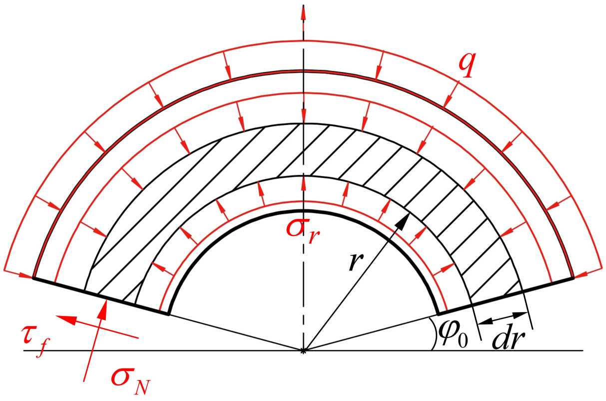

FIGURE 1
Theoretical analysis of spherical shell sector optical window.

2.1 Theoretical analysis of spherical shell sector optical windows

As shown in Figure 1, a thin spherical surface of radius r and thickness dr is taken as the spherical shell sector optical window. The symmetry axis of the optical window is defined as the z-axis, with the center of the sphere as the origin. A spherical coordinate system (r, θ, φ) is established, with the positive direction being away from the center of the sphere. σ_N is the normal stress on the boundary, τ_f is the shear stress on the boundary, σ_r is the normal stress on the inner side of the thin spherical surface, and $\sigma_r + d\sigma_r$ is the normal stress on the outer side.

Thus, the forces acting on the positive and negative directions of the z-axis of a thin spherical shell at a radius of r are expressed by Eqs. (1) and (2), respectively.

$$\sigma_r \sin\varphi \cdot S_1 + (\tau_f \sin\varphi_0 + \sigma_N \cos\varphi_0) dS_0 \tag{1}$$

$$\int_{\varphi_0}^{90^\circ} 2\pi (r + dr)^2 (\sigma_r + d\sigma_r) \sin\varphi \cos\varphi d\varphi \tag{2}$$

$$S_1 = \int_{\varphi_0}^{90^\circ} 2\pi r^2 \cos\varphi d\varphi \tag{3}$$

$$dS_0 = 2\pi r \cos\varphi_0 dr \tag{4}$$

One can see that the first term of Eq. 1 represents the z-component of the internal surface stress on the thin spherical shell, and the second term represents the z-component of the boundary stress on the shell. S_1 denotes the total surface area of the thin spherical shell at the given radius, and dS_0 represents the area of contact with the base.

By combining Eqs. (1) and (2), we can establish the equilibrium equation of the z-axis and neglect higher-order terms:

$$2\sigma_r + r\frac{d\sigma_r}{dr} = \sigma_N + \tau_f \tan\varphi_0 \tag{5}$$

When the boundary is in sliding contact, we can establish a relationship between σ_N and τ_f:

$$\tau_f = f \cdot \sigma_N \tag{6}$$

Substituting Eq. (6) into Eq. (5), we can obtain:

$$2\sigma_r + r\frac{d\sigma_r}{dr} = (1 + f \cdot \tan\varphi_0)\sigma_N \tag{7}$$

The general solution of a first-order linear nonhomogeneous differential equation is the sum of the general solution of the corresponding homogeneous equation and a particular solution of the nonhomogeneous equation. Thus, the general solution of Eq. (7) can be expressed as:

$$\sigma_r(r) = C \cdot r^{-2} + \sigma_r^* \tag{8}$$

However, as the boundary normal stress σ_N is a function of radius r, the particular solution σ_r^* of the equation is also dependent on radius r, making it an indeterminate value. The physical interpretation of this conclusion is that changes in the boundary conditions of a spherical shell sector optical window may cause a change in its stress distribution. Therefore, the deformation of the base may affect the stress distribution of the optical window.

2.2 Finite element analysis of spherical shell sector optical window

The base material of the window selected for this study is TC4 titanium alloy, and the transparent material of the window is high-borosilicate glass (HBG), with material properties shown in Table 1. Compared to other traditional metal materials, TC4 titanium alloy has advantages such as higher specific strength, corrosion resistance, and rust resistance, and is commonly used in the field of deep-sea engineering. As a new type of glass material, high-boron silicon glass has the advantages of high strength, a small thermal expansion coefficient, low deformation, and high transmittance. Compared to traditional organic glass, high-boron silicon glass has a smaller impact on light transmission and is more suitable for application in the pressure-resistant structures of optical equipment with small volumes, high precision, and diving depths exceeding 3,000 m (Quan et al., 2022).

The structural parameters of the spherical shell sector optical window and pressure-resistant structure are shown in Figure 2. Here, R_1 denotes the internal radius of the optical window, R_2

TABLE 1 Material properties of TC4 titanium alloy and high-borosilicate glass.

Material	Young's modulus (MPa)	Poisson's ratio	Bulk modulus (MPa)	Shear modulus (MPa)
HBG	82,000	0.206	46,485	33,997
TC4	96,000	0.36	114,290	35,294

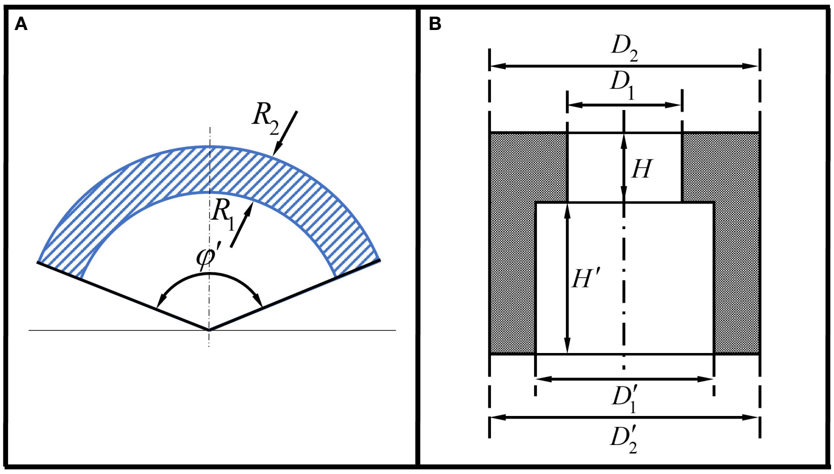

FIGURE 2
(A) Parameters of spherical shell sector optical window. **(B)** Parameters of base and cylinder.

denotes the external radius of the optical window, and φ' denotes the opening angle of the window. Additionally, D_1 denotes the internal diameter of the optical window's base, D_2 denotes the external diameter of the optical window's base, and H_1 denotes the thickness of the base. Finally, D_1' denotes the internal diameter of the cylinder, D_2' denotes the external diameter of the cylinder, and H' denotes the length of the cylinder.

For the spherical shell optical window with $\varphi' = 180°$ and subjected to a working pressure of 115 MPa, the relevant design parameters were calculated based on the ASME (2016) design standards. The calculated design parameters are presented in Tables 2, 3.

Establish a model, divide the mesh, and control the element size to 1 mm, as shown in Figure 3.

As shown in Figure 4, in order to eliminate the influence of frictional force on the results, a frictionless contact is set between the window and the base. The base part with high stiffness and a large area is selected as the target body, and the window part is selected as the contact body.

The boundary conditions and load situation are shown in Figure 5. In order to avoid the difference between the displacement boundary conditions and the actual situation, which may lead to inaccurate finite element calculation results near the contact surface between the window and the base, setting $H' = 100$ mm, and the displacement constraint is set at the far end A surface (bottom of the cylinder), with axial displacement restricted to 0 mm and radial displacement free. The static water pressure is simulated by applying a normal pressure of 115 MPa on the B surface (outside of the optical window, the top surface of the base, and outside of the cylinder).

The base and cylinder of the suppression preprocessing model were removed, and the displacement boundary condition was set at the bottom of the optical window (restricting axial displacement to 0mm and allowing radial freedom). The load conditions remained unchanged, and the analysis results were obtained for the stress distribution of the spherical shell optical window under an external pressure of 115 MPa, assuming no deformation due to the absence of a base. In order to ensure the convergence of the solution and avoid the occurrence of mutual infiltration between materials, the Normal Lagrange method was chosen for the solution.

The stress and strain distribution of the optical window are presented in Figure 6, and the maximum values of data in two scenarios are presented in Table 4. Compared to the situation where the base has no deformation, the maximum equivalent elastic strain and maximum equivalent stress inside the optical window increase by 69% when the base deforms. Additionally, the equivalent elastic

TABLE 2 Design parameters of spherical shell sector optical window.

Parameters	R_1 (mm)	R_2 (mm)	φ' (°)
Value	38	50	180

TABLE 3 Design parameters of optical window base and cylinder.

Parameters	D_1 (mm)	D_2 (mm)	H_1 (mm)	$D_1'(mm)$	$D_2'(mm)$	H' (mm)
Value	60	134	45	94	134	100

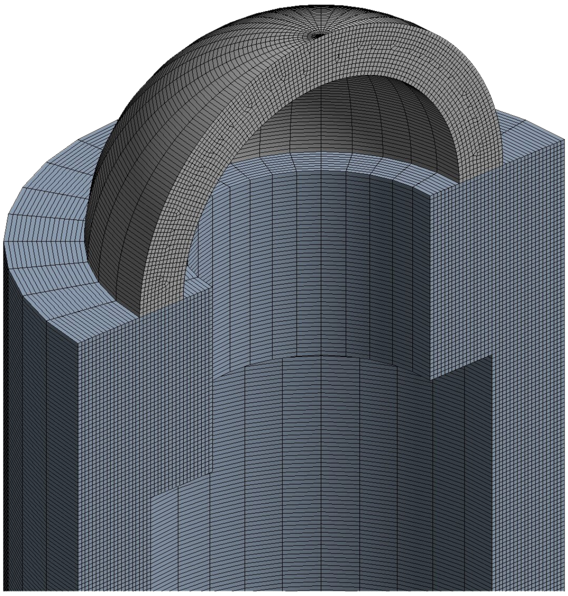

FIGURE 3
Finite element model of optical window and base.

strain and equivalent stress distribution inside the optical window are uniform when the base has no deformation, as it is a part of the regular spherical shell. However, a significant stress concentration phenomenon appears at the inner diameter of the optical window when the base deforms. Based on the aforementioned data, it can be concluded that the deformation of the base during the loading process of the pressure-resistant structure has a significant impact on the stress distribution of the optical window. It aligns with the conclusion of the theoretical analysis in the Theoretical analysis of spherical shell sector optical windows section. Moreover, since stress concentration appears at the inner diameter of the optical window, it can be inferred that this area will be the first to fail during the loading process. The failure mode will manifest in the form of circular layer peeling.

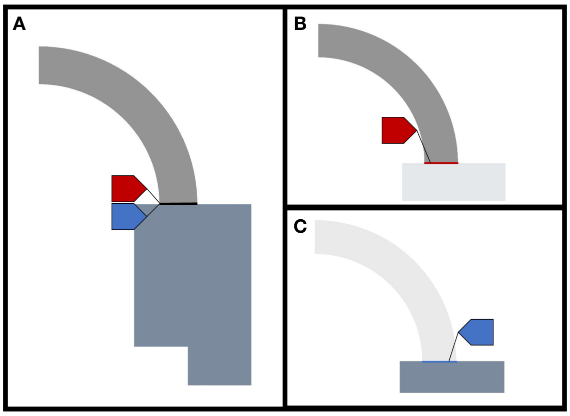

FIGURE 4
Setting of contact surface. **(A)** Contact surface between window and base. **(B)** Contact body view. **(C)** Target body view.

3 Optimization methods

The total deformation of the base and cylinder is shown in Figure 7. There is a significant depression at the contact surface between the base and optical window, and the overall deformation pattern of the base is similar to that of a cantilever beam, where the deformation increases as the radius of the contact end decreases. Based on the calculation results and conclusions obtained in the previous section, the main cause of failure of the deep-sea pressure-resistant structure's optical window is due to the increased stress concentration inside the window caused by the deformation of the base, which exceeds the ultimate compressive strength of the component, resulting in the failure of the pressure-resistant structure. Therefore, it is suggested to fill the transition between the optical window and the base with other materials with reinforced gaskets to prevent the direct impact of base deformation on the optical window, thereby reducing stress concentration in the optical window itself.

3.1 Optimization design scheme

To fully utilize the role of the transition material, the inner and outer diameters of the transition material should completely cover the end face of the optical window. Therefore, we established a model with an inner diameter of 66 mm and an outer diameter of 110 mm for the transition material gasket, as shown in Figure 8. The binding contact was set between the gasket and the base, and frictionless contact was set between the optical window and the reinforced gasket. We selected the Normal Lagrange method for solving and ensured that the boundary conditions and loads were consistent with those in the Finite element analysis of spherical shell sector optical window section.

The maximum stress value on the window was calculated when the filling thickness was 1–4 mm, the Young's modulus range was 1–300 GPa, and the Poisson's ratio range was 0–0.45 for the transition material. The data were linearly interpolated and expanded, and the results were represented in three-dimensional space, as shown in Figure 9. The analysis of the results shows that, when the thickness of the transition material is constant, the maximum stress value on the window decreases as the Young's modulus of the transition material increases and decreases as the Poisson's ratio of the transition material increases. Increasing the filling thickness makes the stress more sensitive to changes in the Young's modulus and Poisson's ratio of the transition material.

When a transition material with a thickness of 4 mm, a Young's modulus of 1 GPa, and a Poisson's ratio of 0 is used, the equivalent stress on the window is the highest, at 1,191.596 MPa, which is an increase of approximately 129% compared to the maximum stress when no transition material is used. However, when a transition material with a thickness of 4 mm, a Young's modulus of 300 GPa, and a Poisson's ratio of 0.45 is used, the equivalent stress on the window is the lowest, at 438.5262 MPa, which is a decrease of approximately 15.7% compared to the maximum stress when no transition material is used.

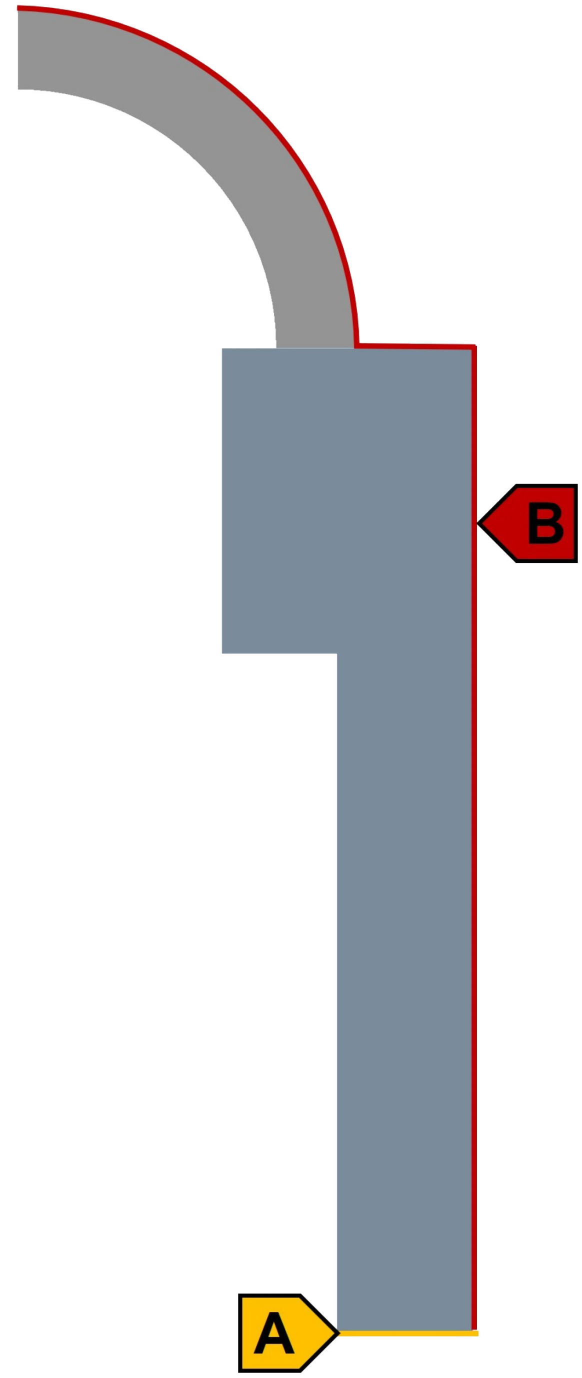

FIGURE 5
The part A (yellow) is the location of boundary, and the part B (red) is the location of load condition.

The maximum stress value on the window before reinforcement was inserted into the results after reinforcement, i.e., plane σ-max = 520.27 MPa, as shown in Figure 10. The before-reinforcement results were made to intersect with the after-reinforcement results, and a third-degree polynomial was used to fit the intersection line, represented as $y = Ax^3 + Bx^2 + Cx + D$. The x value is the Young's modulus E of the transition material, with units of 10^3 GPA, and the y value is the Poisson's ratio ν of the material. The A, B, C, and D values under 1–4-mm transition material thickness were calculated and shown in Table 5. A judgment value Q was defined, and when the thickness is constant, if the judgment value $Q< 0$ for the transition material, it indicates that using the material of that size can alleviate stress concentration on the optical window in the pressure-resistant structure.

According to the data presented in Table 5, it is evident that all four parameters are correlated with the thickness of the transition material. Overall, as the thickness increases, the odd-order parameters A and C decrease, while the even-order parameters B and D increase. Furthermore, within the same expression, coefficients of terms with lower orders are smaller. For each individual parameter, the trend remains the same with increasing material thickness, although it is not a simple linear relationship. It can be observed that when the material thickness increases from 2 mm to 3 mm, the gradients of the four parameters exhibit a rapid increase, which is not observed in other thickness ranges. In terms of their physical implications, these findings suggest that as the material thickness increases, there is a reduced requirement for Young's modulus and an increased requirement for Poisson's ratio of the transition material itself. However, it is important to note that these conclusions are derived from quantitative analysis and need to be verified through further calculations in practical applications.

3.2 Real material simulation for validation

As some of the data in the Optimization design scheme section was obtained through linear interpolation, it is necessary to validate the results by inputting actual data. For this purpose, we selected three materials: Nylon 66, POM, and silicon nitride ceramics (SNC). Their material properties are shown in Table 6. Nylon 66 and POM have similar Young's modulus, but POM has a higher Poisson's ratio. Silicon nitride ceramics have a Poisson's ratio similar to that of Nylon 66 but a higher Young's modulus.

According to the finite element analysis model in the Optimization design scheme section, the maximum stress and strain values of the optical window, when reinforced with 1–4 mm thicknesses of the three materials, were calculated. The calculation results are shown in Figure 11.

Based on the analysis of the calculation results, it can be concluded that when the gasket thickness is between 1 mm and 4 mm, the maximum stress of the optical window using silicon nitride ceramics as the transition material is always smaller than that of using Nylon 66 or POM. Specifically, when the thickness is 1 mm,

TABLE 4 Optical window solution results.

Results	Base has no deformation	Base has deformation
Maximum equivalent elastic strain $\varepsilon_{\text{max-0 mm}}$	0.003749	0.0063448
Maximum equivalent stress $\sigma_{\text{max-0 mm}}$/MPa	307.42	520.27

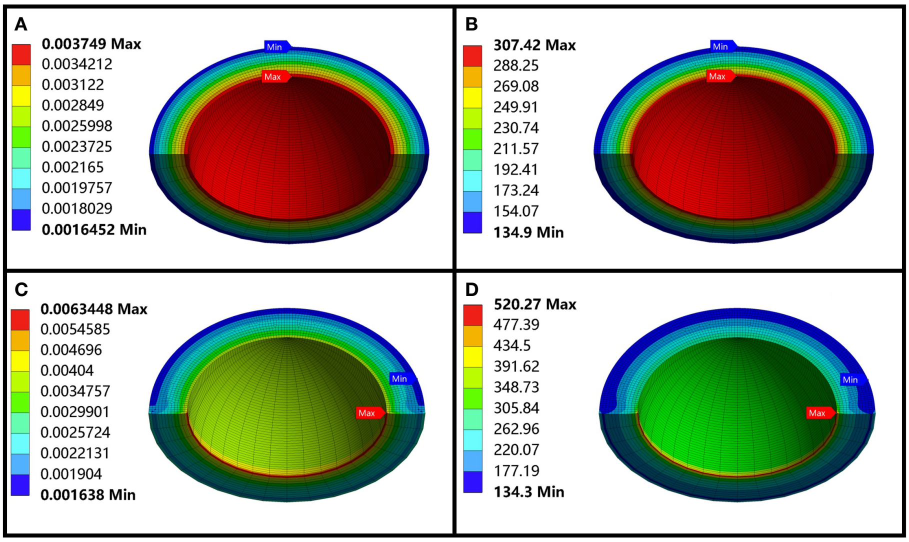

FIGURE 6
The calculation results of the optical window. **(A)** The equivalent elastic strain of the optical window when the base is not deformed. **(B)** The equivalent stress of the optical window when the base is not deformed. **(C)** The equivalent elastic strain of the optical window when the base is deformed. **(D)** The equivalent stress of the optical window when the base is deformed.

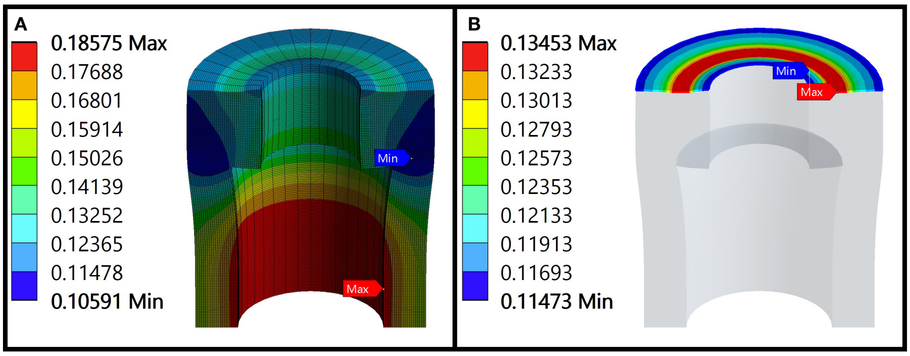

FIGURE 7
Total deformation of base, cylinder, and contact surface. **(A)** Total deformation of base and cylinder. **(B)** Total deformation of contact surface.

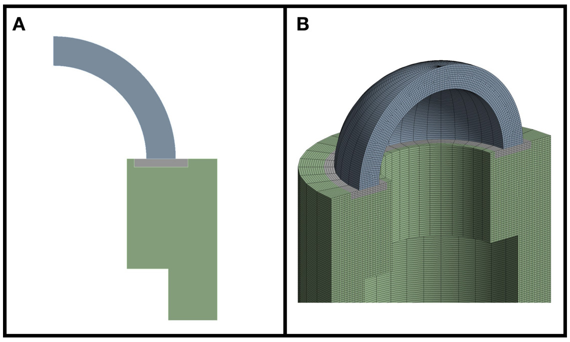

FIGURE 8
The model after adding the gasket. **(A)** Two-dimensional cross-sectional image. **(B)** Three-dimensional cross-sectional image.

the maximum stress of the optical window using silicon nitride ceramics as the transition material is 496.21 MPa, which is about 2% lower than that without using the transition material. In contrast, the maximum stress of the optical window using Nylon 66 as the transition material is 656.97 MPa, which is about 26% higher than that without using the transition material. The maximum stress of the optical window using POM as the transition material is 576.48 MPa, which is about 11% higher than that without using the transition material. Moreover, as the thickness of the transition material increases, the maximum stress of the optical window using silicon nitride ceramics gradually decreases, while the maximum stress of using the other two materials gradually increases.

When the gasket thickness is between 1 mm and 4 mm, the judgment values $Q_{SNC} < 0$, $Q_{Nylon\ 66} > 0$, $Q_{POM} > 0$, and $Q_{Nylon\ 66} > Q_{POM}$. According to the conclusion in the Optimization

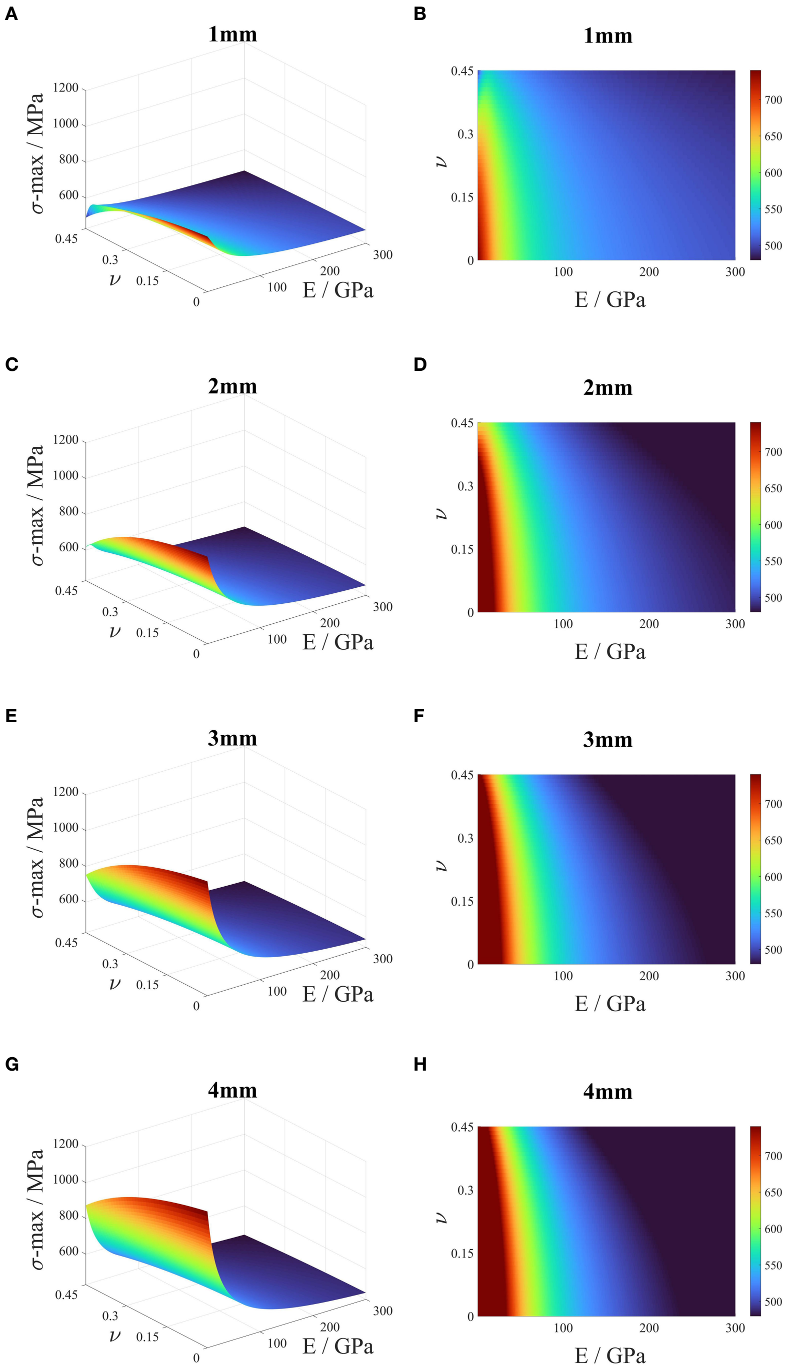

FIGURE 9
Maximum stress of optical window under different thickness, Young modulus and Poisson's ratio of transition material. **(A)** The 3D image of stress of optical window with 1mm transition material thickness. **(B)** The 2D image of stress of optical window with 1mm transition material thickness. **(C)** The 3D image of stress of optical window with 2mm transition material thickness. **(D)** The 2D image of stress of optical window with 2mm transition material thickness. **(E)** The 3D image of stress of optical window with 3mm transition material thickness. **(F)** The 2D image of stress of optical window with 3mm transition material thickness. **(G)** The 3D image of stress of optical window with 4mm transition material thickness. **(H)** The 2D image of stress of optical window with 4mm transition material thickness.

design scheme section, using POM or Nylon 66 will increase the maximum stress value of the optical window in this model, while using silicon nitride ceramics will decrease the maximum stress value of the optical window in this model. Moreover, as the thickness of the transition material increases, the change in stress becomes more significant. Therefore, the conclusion from the verification of the real material simulation is consistent with the conclusion in the Optimization design scheme section.

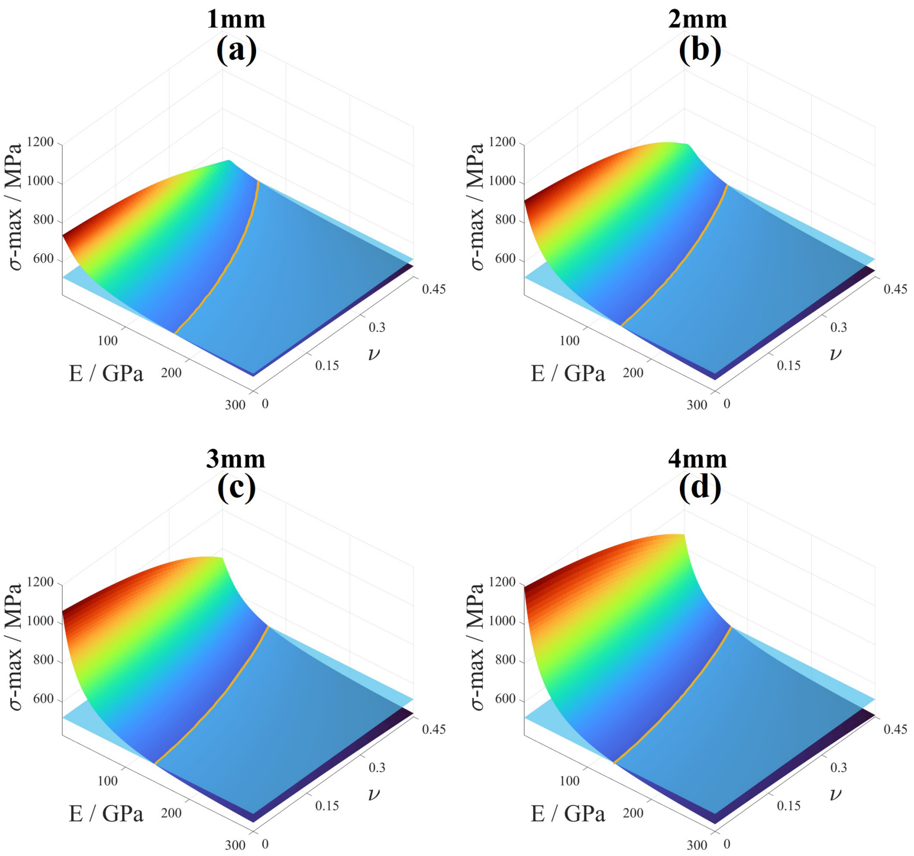

FIGURE 10
Maximum stress fitting before and after reinforcement. **(A)** The fitting result of optical window with 1mm transition material thickness. **(B)** The fitting result of optical window with 2mm transition material thickness. **(C)** The fitting result of optical window with 3mm transition material thickness. **(D)** The fitting result of optical window with 4mm transition material thickness.

TABLE 5 Values of parameters A, B, C, and D at the thickness of 1–4 mm transition material.

Item	A	B	C	D
1 mm	−145.3055	32.8845	−4.8803	0.6524
2 mm	−181.1302	26.5056	−3.8488	0.6472
3 mm	−832.9385	223.8558	−24.0365	1.3397
4 mm	−960.8872	257.8747	−27.4415	1.4666

3.3 Analysis of the impact of friction

To investigate the impact of the friction coefficient on the stress distribution of the optical windows when the transition material remains unchanged, a finite element analysis model in the Optimization design scheme section was used. Silicon nitride ceramic was selected as the transition material. The contact mode between the optical window and the base was changed from nonfrictional contact to frictional contact, and the friction coefficient was increased from 0 to 0.8 in increments of 0.2. The maximum equivalent elastic strain and maximum equivalent stress of the optical window were calculated and statistically analyzed for

TABLE 6 Nylon 66, POM, and Silicon Nitride Ceramics are three material properties.

Material	Young's modulus (MPa)	Poisson's ratio	Bulk modulus (MPa)	Shear modulus (MPa)
Nylon 66	3,190	0.28	2,416.7	1,246.1
POM	2,700	0.4	4,500	964.3
SNC	290,000	0.26	201,390	115,080

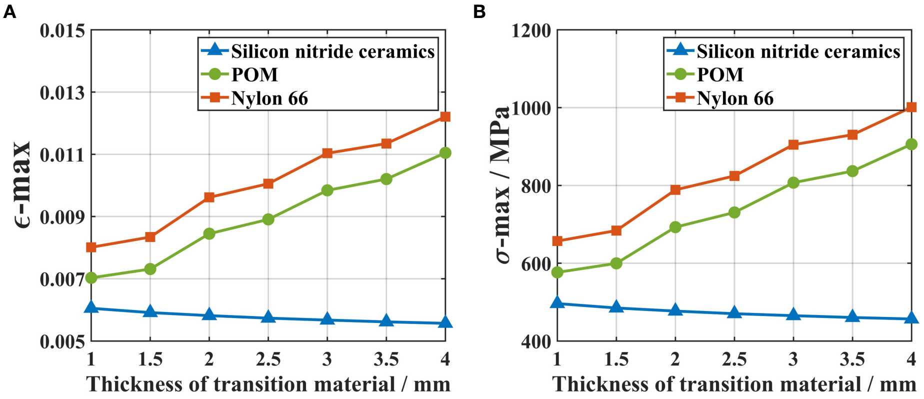

FIGURE 11
Effect of adding three kinds of spacers with different thicknesses on the spherical shell sector optical window. **(A)** The maximum equivalent elastic strain of the spherical shell sector optical window. **(B)** The maximum equivalent stress of the spherical shell sector optical window.

different gasket thicknesses and friction coefficients. The calculation results are shown in Figure 12.

Based on the analysis of the calculation results, it can be concluded that the friction coefficient has a significant impact on the maximum equivalent elastic strain and equivalent stress of the optical window reinforced using silicon nitride ceramic gaskets. As the friction coefficient increases, the maximum equivalent stress of the optical window also increases, and the thickness of the gasket used has a greater impact. When the friction coefficient is less than 0.2, the impact of the friction coefficient on the stress distribution of the optical window is particularly significant. This is mainly due to the fact that when the friction coefficient is small, the contact between the optical window and the base is mainly sliding contact. When the friction coefficient is greater than 0.2, the contact between the optical window and the base changes to adhesive contact, and the effect of the increasing friction coefficient on the stress of the optical window gradually decreases.

Furthermore, based on the analysis of the results from both the friction coefficient-stress curve and the gasket thickness-stress curve, it can be concluded that thicker gaskets have a more significant reinforcement effect on the optical window and are also more sensitive to the increase in friction coefficient. However, this effect does not follow a linear pattern, and when the gasket thickness is around 2.5 mm, the overall effect is more optimal.

4 Experiments and results

To test the pressure resistance of the structure, we conducted a high-pressure simulation test using a deep-sea ultra-high-pressure simulation

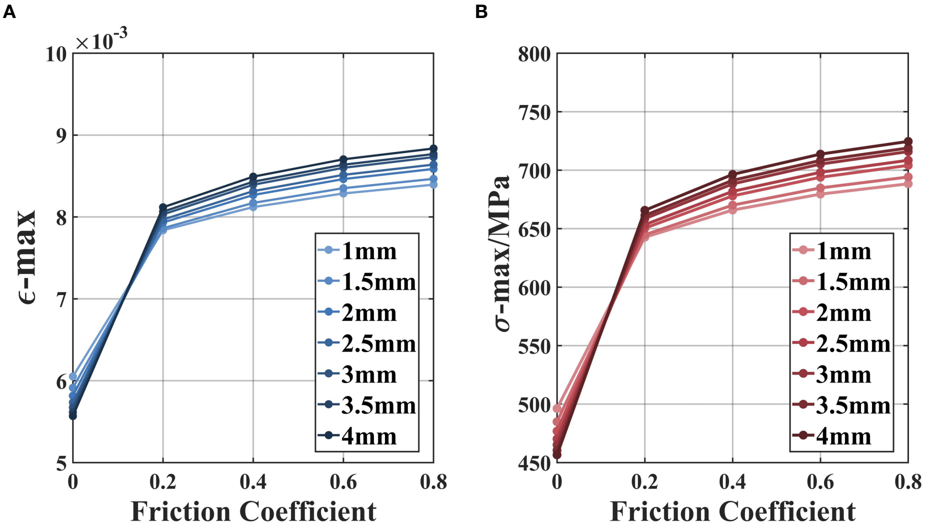

FIGURE 12
The impact of different friction coefficients. **(A)** The maximum equivalent elastic strain of the windows. **(B)** The maximum equivalent stress of the windows.

FIGURE 13
Experimental environment and object. **(A)** Deep-sea ultra-high-pressure simulation experimental equipment. **(B)** Silicon nitride ceramic gasket-reinforced pressure-resistant structure. **(C)** Pressure-resistant structure fixed.

test device. To ensure safety, a safety factor of 1.05 was applied. The internal pressure of the pressure vessel was increased from 0 MPa to 121 MPa and maintained for 120 min before being depressurized to 0 MPa. The pressurization rate was set at 2 MPa/min, and the depressurization rate was set at –2 MPa/min, based on the actual diving and rising rates of deep-sea equipment in engineering applications. The experimental device and settings are shown in Figure 13.

The first transition material tested was silicon nitride ceramic with a thickness of 2.4 mm. The experimental results are shown in Figure 14. After the high-pressure simulation test, the pressure-resistant structure reinforced using silicon nitride ceramic remained visually intact, with no signs of water leakage inside. Additionally, the optical window's inner and outer surfaces and supporting surfaces were free from any damage, demonstrating its ability to meet the requirements for the working pressure of 115 MPa.

The second transition material tested was POM, with a thickness of 2.3 mm. The experimental results are shown in Figure 15. After the high-pressure simulation test, we observed obvious plastic deformation of the POM gasket, with a large number of silver streaks appearing on the outer ring, indicating that it was on the verge of failure. The pressure-resistant structure reinforced using POM as the transition material may not be reliable for use, and the deformed gasket may block the optical window, affecting the normal operation of internal equipment.

The two sets of experiments described above demonstrated the reliability of using silicon nitride ceramic as a transition material, while POM was found to be unsuitable due to its inability to effectively reduce stress concentration on the optical window and its lack of potential for multiple uses. In summary, the selection of transition materials is crucial for the success of pressure-resistant structure design with optical windows for deep-sea applications. The optimized design method and concept proposed in this study have significant implications for the field of deep-sea equipment.

5 Discussion

Based on the calculations and discussions presented in the previous sections, it can be concluded that the stress distribution and concentration of optical windows are important considerations in

FIGURE 14
Optical window using silicon nitride ceramics as transition materials after a high-pressure test.

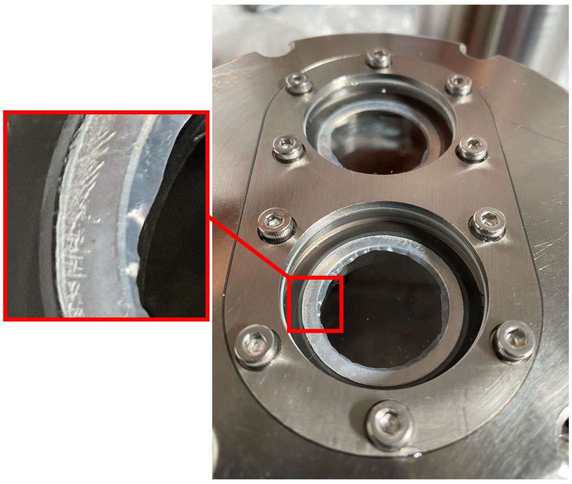

FIGURE 15
POM gasket after a high-pressure simulation test.

TABLE 7 Window calculation results for each scheme.

Scheme	Maximum window stress (MPa)	Maximum equivalent elastic strain
Unreinforced	520.27	0.0063448
Nylon 66 coating	1,001.1	0.012208
POM coating	905.83	0.011047
Epoxy resin coating	618.77	0.0075459
SNC transition	456.63	0.0055686

TABLE 8 Judgment value *Q* calculation results.

Scheme	Young's modulus (MPa)	Poisson's ratio	Judgment value *Q*
Nylon 66 coating	3,190	0.28	1.102
POM coating	2,700	0.4	0.994
Epoxy resin coating	5,000	0.35	0.279
SNC transition	290,000	0.26	−8.499

designing underwater pressure-resistant structures. In this study, we investigated the stress of optical windows with end-face reinforcement using computational and finite element analysis models. To compare the results with relevant research work, we summarized the data in Table 7.

Considering the actual physical conditions, we set the epoxy resin thickness to 1 mm and the thickness of the remaining reinforcing materials to 4 mm. According to our data results, when using SNC for the transition, the maximum stress and equivalent elastic strain decreased by 12.2% compared to the unreinforced case. This indicates that our proposed transition material optimization design can effectively reduce stress concentration in optical windows. On the other hand, when using materials such as nylon 66, POM, and epoxy resin, although point contact effects can be avoided in practical situations, they also lead to more severe stress concentration in the window. This may be due to differences in the characteristics, stiffness, and strength of these materials.

Regarding the judgment value Q of the materials, the Q values for each scheme were calculated as shown in Table 8.

By comparing the data in Tables 7, 8, it can be observed that we found the material to effectively reduce stress concentration in optical windows only when the judgment value Q is less than 0. This validates the rationality of the judgment value calculation method proposed in the Optimization methods section.

In conclusion, our proposed transition material optimization design scheme can significantly reduce stress concentration in optical windows. Compared to previous relevant works, our solution demonstrates superior performance in terms of practical effectiveness.

6 Conclusion

This study focuses on the optimization design of spherical sector optical windows in pressure-resistant structures. The negative impact of base deformation on the pressure resistinlance limit is analyzed using both theoretical calculations and simulation analysis. Based on this, an optimization design method using transition materials filled between the base and the optical window is proposed, and relevant calculations and experiments are conducted. The results indicate the following: (1) The deformation of the cylindrical end face significantly alters the stress distribution of the spherical sector optical window. A notable stress concentration occurs at the bottom corner of the inner diameter, which is one of the main reasons for the window's inability to reach the theoretical pressure resistance limit. (2) After filling the transition material between the cylindrical end face and the optical window, significant changes in the mechanical performance of the window are observed. The maximum stress in the window is influenced by the properties of the transition material and physical conditions. Specifically, the maximum stress is negatively correlated with the elastic modulus and Poisson's ratio of the transition material, and an increase in the filling thickness makes the stress more sensitive to these factors. (3) The proposed criterion value Q for material selection is effective. However, it should be noted that when using materials with $Q > 0$ for transition, the maximum stress in the window is negatively correlated with the thickness of the transition material, while it is positively correlated when $Q< 0$. An increase in the friction coefficient leads to an increase in the maximum stress in the window; especially when the friction coefficient is less than 0.2, this effect becomes more pronounced.

In future research, we suggest further considering the following aspects: (1) Whether selecting anisotropic materials for transition is superior to isotropic materials; (2) It is necessary to study the effects of fatigue and creep of transitional materials on optical windows and estimate the maximum service life; (3) Theoretically, selecting nonlinear materials for transition can reduce the thickness of the base while ensuring the same mechanical performance, but feasibility needs to be further validated.

The contribution of this study lies in the introduction of a new approach for the design of optical windows in deep-sea pressure-resistant structures, along with the validation of its feasibility and effectiveness. The reliability of the research results has been thoroughly verified, and they hold a certain level of generality. Furthermore, the innovative aspect of this study is the proposal of an evaluation method based on transition materials, which provides a novel approach and methodology for the design and optimization of optical windows. Overall, this research holds potential value for the future development of deep-sea equipment and carries profound implications for the advancement of deep-sea scientific progress.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

Conceptualization: YW, GM, and XQ. Methodology: YW, GM, and HZ. Analysis: YW and XZ. Manuscript writing: YW, XZ, and XQ. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the Youth Innovation Promotion Association CAS (No. 2020361), the Scientific Instrument Developing Project of the Chinese Academy of Sciences (No.YJKYYQ20190053), and National Key R&D Program of China (NO.2021YFC2800300).

Acknowledgments

The authors thank Kelin Sun, Jingchuan Yang, Bing Zhang, and Chen Li (Institute of Deep-sea Science and Engineering, Chinese Academy of Science) for their assistance during the experiment and test.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 05 April 2024
DOI 10.3389/fmars.2024.1357879

OPEN ACCESS

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RECEIVED 18 December 2023
ACCEPTED 19 March 2024
PUBLISHED 05 April 2024

CITATION
Barnard K, Daniels J, Roberts PLD, Orenstein EC, Masmitja I, Takahashi J, Woodward B and Katija K (2024) *DeepSTARia*: enabling autonomous, targeted observations of ocean life in the deep sea.
Front. Mar. Sci. 11:1357879.
doi: 10.3389/fmars.2024.1357879

DeepSTARia: enabling autonomous, targeted observations of ocean life in the deep sea

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The ocean remains one of the least explored places on our planet, containing myriad life that are either unknown to science or poorly understood. Given the technological challenges and limited resources available for exploring this vast space, more targeted approaches are required to scale spatiotemporal observations and monitoring of ocean life. The promise of autonomous underwater vehicles to fulfill these needs has largely been hindered by their inability to adapt their behavior in real-time based on what they are observing. To overcome this challenge, we developed Deep Search and Tracking Autonomously with Robotics (*DeepSTARia*), a class of tracking-by-detection algorithms that integrate machine learning models with imaging and vehicle controllers to enable autonomous underwater vehicles to make targeted visual observations of ocean life. We show that these algorithms enable new, scalable sampling strategies that build on traditional operational modes, permitting more detailed (e.g., sharper imagery, temporal resolution) autonomous observations of underwater concepts without supervision and robust long-duration object tracking to observe animal behavior. This integration is critical to scale undersea exploration and represents a significant advance toward more intelligent approaches to understanding the ocean and its inhabitants.

KEYWORDS

ocean, autonomy, machine learning, computer vision, robotics, tracking

1 Introduction

The world's ocean, particularly the deep ocean, is one of the least accessible places on the planet, and represents nearly 98% of the habitable living space by volume (Haddock et al., 2017). Due to its importance in regulating climate (Smith et al., 2018), support of ecosystems that sustain sources of food (Pikitch et al., 2014; Vigo et al., 2021), and other ecological services (Thurber et al., 2014), understanding the ocean and how it changes with time is vitally important. However, conducting observations at spatiotemporal scales that meaningfully characterize a changing ocean is no small feat (Capotondi et al., 2019). The chemical and physical oceanography communities are beginning to meet this challenge by successfully implementing programs that rely on large-scale autonomy, robotics, and data sharing to achieve their goals (McKinna, 2015; Claustre et al., 2020). For a number of reasons, biological observations have fallen behind, where long-term observations cover only 7% of the ocean's surface waters, and are focused largely in coastal regions (Hughes et al., 2021; Satterthwaite et al., 2021). This lack of observational capacity creates large knowledge gaps in our accounting for and understanding of marine biodiversity, creating challenges for regulation and monitoring of human activities in the ocean (Hughes et al., 2021). Ocean scientists and stakeholders must improve our ability to observe the ocean as the Blue Economy (Bennett et al., 2019) — ocean-related industries and resources from renewable energy generation to food harvesting and culturing — grows and the marine environment continues to shift as the climate changes (Danovaro et al., 2020).

Relying on fully manual, labor-intensive approaches to exploration and monitoring in the ocean are too costly to execute at the necessary scale; established ship-based protocols require hours of highly trained human effort on specialized vessels. Expanding our biological observational capacity requires new autonomous sampling strategies that respond to the environment by adapting behavior or opportunistically targeting organisms (Costello et al., 2018; Ford et al., 2020). Here we present *DeepSTARia* (Searching and Tracking Autonomously with Robotics), a class of algorithms that enables autonomous underwater vehicles to execute targeted sampling tasks based on real-time visual signals, a strategy previously only available to human operators. *DeepSTARia* represents a significant advance in deep sea autonomy, illustrating the potential for autonomous underwater vehicles to effectively scale up our ability to study marine organisms by reducing the need for costly ship time and limiting reliance on manual operation.

Non-extractive biological observations can be conducted in many ways using various modalities, including imaging, environmental DNA (or eDNA), and acoustics (Benoit-Bird and Lawson, 2016; Masmitja et al., 2020; Chavez et al., 2021). Of these modalities, imaging is the most direct approach, and its use has grown with various platforms, imaging systems, and sampling missions (Durden et al., 2016; Lombard et al., 2019). Benthic landers, cabled observatories, and drop cameras for example can provide temporal data of animal distributions at a fixed location (Danovaro et al., 2017; Giddens et al., 2020). Other approaches using remotely operated vehicles (ROVs) and autonomous underwater vehicles (AUVs) have the benefit of mobility to provide varying views in time and space of biological communities in the ocean (Robison et al., 2017). While AUVs have the benefit of autonomy (Schoening et al., 2015; Ohki et al., 2019), most of these platforms do not have adaptive and targeted sampling capabilities when compared with manually controlled ROVs (Durden et al., 2021).

Biological observations using ROVs and AUVs traditionally involve quantitative transects (Howell et al., 2010). *Transects* are missions where imaging parameters and vehicle behavior are kept constant (e.g., position relative to the seafloor for benthic missions, observation depth for missions in the water column, vehicle speed, vehicle heading, sampling duration, imaging field of view, camera exposure, illumination power) while sampling a particular location in the ocean. *Transects* can be conducted at different locations or time intervals to address a number of ecological questions (Robison et al., 2017). At the conclusion of transect missions, researchers download and review the collected visual data to identify animals, quantify species occurrence and counts, and denote the physical environment to characterize the biological community (Howell et al., 2010; Aguzzi et al., 2021). Such missions are often conducted for marine biodiversity monitoring but are not sufficient to properly account for all organisms, especially those that are small in body size, relatively rare, or patchily distributed (Brandt et al., 2014). Adaptive sampling strategies are necessary to properly account for marine biodiversity, especially in the difficult-to-access deep sea (Costello et al., 2018).

Oftentimes, research goals dictate a more opportunistic approach, seeking out and capitalizing on rare encounters. This necessitates a very different sampling strategy, usually requiring a closer look to identify animals or observe their behavior (Ford et al., 2020). These *Discovery* missions involve pausing a *Transect* to collect close-up or extended recordings of an animal to facilitate identification (Figure 1). These missions are usually directed by scientists, viewing the *in situ* video feed on a topside monitor, and adjusting vehicle behavior when they see an animal or phenomenon of interest and need more time or additional perspective views for study and evaluation. More recently, researchers have been interested in understanding not only presence and absence of animal systems, but also their fine-scale behavior to understand their ecomechanics (Katija et al., 2020). These studies require *Follow* missions to keep the target in view for longer periods of time. Both *Discovery* and *Follow* operations are typically run on an ROV flown by a skilled human pilot, which we define here as an individual with many hours of experience and who operates ROVs in a professional capacity.

Thanks to recent improvements in AUV capabilities and performance (e.g., power, control, and on-board computational resources), the research community has begun developing targeted and adaptive biological observation capabilities for these autonomous robotic platforms (Zhang et al., 2021). By switching from ROVs – which require significant physical infrastructure and personnel that cost on the order of tens of thousands of dollars per day to operate – to vehicles like AUVs, we could enable large-scale, global surveys of ocean life capable of meeting the endurance, depth range, and maneuverability requirements for such missions (Reisenbichler et al., 2016). Making these sampling strategies entirely autonomous involves leveraging vehicle sensor data (imaging, acoustics, or both) to locate animals of interest and

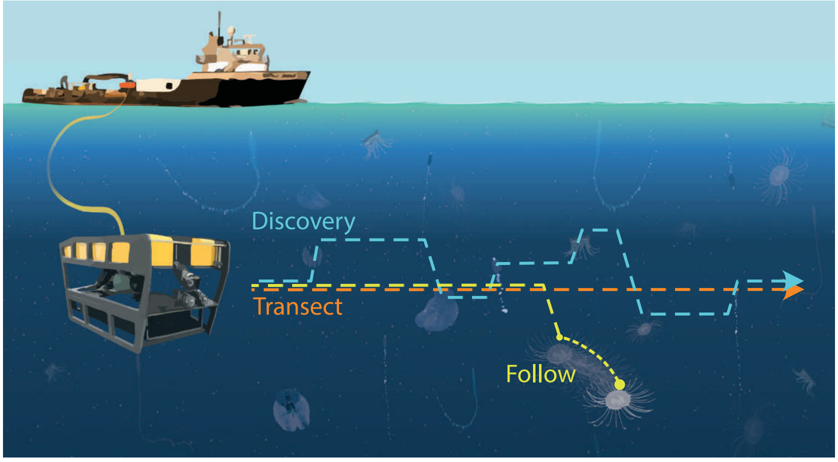

FIGURE 1
Integrating machine learning (ML) algorithms into vehicle controllers (or *DeepSTARia*) enables a suite of underwater observational missions. By varying the duration of various modes (*search*, *acquire*, *track*) of *DeepSTARia*, an autonomous underwater vehicle can conduct a variety of underwater observational missions: (Orange) *Transect*, where the vehicle moves at a constant speed and depth at specified time intervals; (Blue) *Discovery*, where the vehicle moves at a prescribed depth and changes vehicle behavior (e.g., range, bearing, depth) to slow down and observe a detected object for a specified duration before continuing on its sampling mission; and (Yellow) *Follow*, where the vehicle again moves at a prescribed depth, and slows down and continues to shadow a detected object for as long as needed for the specified mission. The ML algorithms enable selection of detected objects, enabling targeted sampling during *Discovery* and *Follow* missions.

maintain the position of the vehicle relative to the target for as long as possible (Yoerger et al., 2021). Using these signals for visual tracking and control (or visual servoing) has a long history (Wu et al., 2022), and more modern algorithms (Girdhar and Dudek, 2016; Katija et al., 2021) show promise in enabling the entire range of vehicle missions described here. However, *Discovery* and *Follow* behaviors remain challenging to implement in the open ocean and require significant algorithmic improvements before they can be conducted without humans-in-the-loop.

To address this challenge, we developed *DeepSTARia* to expand the opportunistic and adaptive sampling capabilities of remote and autonomous vehicles in the deep ocean. *DeepSTARia* consists of four modules: an object detection and classification model, a 3D stereo tracker, a vehicle controller, and a Supervisor module. By integrating real-time machine learning models operating on visual data into vehicle controllers, *DeepSTARia* has achieved a range of biological observation missions (e.g., *Transect*, *Discovery*, and *Follow*) completely autonomously for the first time. We demonstrate that vehicles using *DeepSTARia* can conduct traditional and adaptive biological observation missions without human intervention. Field tests were conducted in Monterey Bay, California, USA with a flyaway ROV as a proxy for any AUV carrying a stereo camera system. An object detection model, trained on 15 taxonomic groups, enabled near-real-time iterative improvements to the *DeepSTARia* algorithm and timely human intervention if required. Minimal user input to the algorithm enabled a suite of autonomous observations that either match or improve our biological observation capabilities during fully remote missions. Our results demonstrate the potential for *DeepSTARia* and similar tracking-by-detection algorithms to enable future autonomous missions to ply the ocean for known and unknown life. These approaches are an important step toward scaling biological observations in the ocean by reducing the human, fiscal, and environmental costs of fully manual operations. The valuable resulting data could inform intelligent, sustainable management of our shared ocean resources and inspire the future of large-scale ocean exploration.

2 Materials and methods

In order to evaluate the effectiveness of *DeepSTARia*, we conducted field trials using a deep sea robotic platform in Monterey Bay. After field trials, data were reviewed to compare various water column exploration missions using the metrics described below.

2.1 Robotic platform used to demonstrate *DeepSTARia*

Field trials of *DeepSTARia* were conducted in the Monterey Bay National Marine Sanctuary at Midwater Station 0.5 (latitude: 36.781 N, longitude: 122.012 W) with bottom depths exceeding 500 m. We used a tethered remotely operated vehicle (ROV) for our field trials as a proxy for an autonomous vehicle so as to enable real-time iterative improvements to the algorithm during trials and utilize human intervention if the need arose. Five dives were made with the 1500 m-rated ROV *MiniROV* (Figure 2) as part of these trials; results reported here were all obtained within a 6-hour window during a single dive on May 24th, 2021 to a maximum depth of 293 m. In these trials, the science/pilot camera (Insite Pacific Inc. Mini Zeus II) and white lights were complemented by a fixed stereo imaging system (based on Yoerger et al., 2021) to provide repeatable position measurements and red lights to reduce interference with animal behavior for these trials (Allied Vision

G-319B monochrome cameras and Marine Imaging Technologies underwater housings with glass dome ports, and Deep Sea Power and Light MultiRay LED Sealite 2025 at 650–670 nm). The stereo imaging system (baseline approximately 190 mm) was mounted such that the port (left) side camera was aligned with the vertical plane of the science camera, and the center of the vehicle. The center of this camera view was chosen as the origin of the vehicle's orthogonal reference frame for the purposes of *DeepSTARia* (Figure 2A). The machine learning models and vehicle control algorithms (Figure 2B) were operated on a shipboard (or topside) Tensorbook laptop (Lambda Labs, Inc.) outfitted with an Nvidia RTX 2070 GPU to allow for rapid switching between pilot control and autonomous operation.

2.2 Overview of *DeepSTARia*

Deep Search and Tracking Autonomously with Robotics (*DeepSTARia*) enables robust autonomous *Transect*, *Discovery*, and *Follow* missions in the ocean based on visual signals by combining machine learning models with vehicle control algorithms. *DeepSTARia* integrates a multi-class RetinaNet object detection model (Lin et al., 2017), a 3D Stereo Tracker, and a Supervisor module that makes vehicle control decisions to be actuated by the vehicle controller (Figure 2). The object detector is run on each of the stereo cameras, and bounding boxes of target classes are then matched within the Tracker module to estimate their position in 3D space. The object class, location, and track are passed to the Supervisor module (Figure 3), which can adjust behavior of the vehicle based on current and past 3D Stereo Tracker information. Lightweight Communications and Marshaling [LCM; Huang et al. (2010)] is used to share data between modules and save all information for later analysis.

The implementation of DeepSTARia is under active research and development and as such is not intended as a plug-and-play solution. Researchers interested in utilizing the subsequent methods of DeepSTARia should be aware that significant adaptation from the current research implementation may be required to support its deployment. Further details about the initial development of DeepSTARia (known as ML-Tracking), including the challenges encountered, are described in Katija et al. (2021).

2.2.1 Multi-class object detector and 3D stereo tracker modules

Still images from past ROV deployments were used to train the multi-class detector; (Katija et al., 2021) this included both typical color images from the science cameras of several ROVs (drawn from the underwater image database *FathomNet* (Katija et al., 2022), and monochrome images obtained with the stereo camera setup described here. Images of animals commonly observed in the Monterey Bay area were used to form 17 classes (15 taxonomic and 2 semantic categories) using visually distinct taxonomic groups of varying taxonomic levels (e.g., *Aegina*, *Atolla*, *Bathochordaeus*, *Bathocyroe*, *Beroe*, *Calycophorae*, *Cydippida*, *Lobata*, *Mitrocoma*, *Physonectae*, *Poeobius*, *Prayidae*, *Solmissus*, *Thalassocalyce*, *Tomopteridae*; see Figure 4). In addition, parts or associated elements were defined in some cases to enable more precise tracking objectives [e.g., *Bathochordaeus* inner filter, *Bathochordaeus* outer filter, *Calycophorae* (nectosome), *Physonectae* (nectosome), and *Prayidae* (nectosome)]. Labeled images were annotated and localized by experts using a variety of tools (VARS Annotation (Schlining and Stout, 2006), VARS Localize (Barnard, 2020), GridView (Roberts, 2020), RectLabel (Kawamura, 2017), and Tator (CVision AI, Inc, 2019)).

We obtained between 205 and 6,927 images per class in the labeled set, for a total of 28,485 images. This annotated image set was used to fine-tune a RetinaNet model with a ResNet50 (He et al., 2016) backbone pre-trained on ImageNet (Deng et al., 2009). Labeled training data and the MBARI Midwater Object Detector can be accessed via FathomNet at www.fathomnet.org (Katija et al., 2022) and www.github.com/fathomnet/models (Woodward et al., 2022).

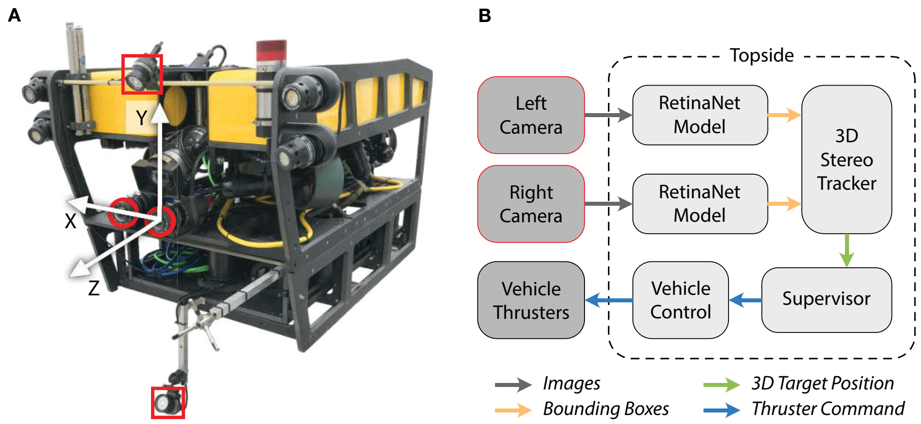

FIGURE 2
Demonstration vehicle and high-level diagram describing the *DeepSTARia* algorithm. **(A)** The ROV *MiniROV* was used as a proxy for an autonomous vehicle, with lighting (red squares) and imaging (red circles) integrated for the field trials. The vehicle reference frame is indicated by the white arrows. **(B)** Images from the left and right camera were transferred up the ROV tether for processing topside. The *DeepSTARia* algorithm involves processing images with a RetinaNet detection model Woodward et al. (2022), where detected object positions in the vehicle reference frame were computed in the 3D Stereo Tracker module. The Supervisor module then uses these inputs and prescribed logic to issue commands to the vehicle controller, which is visualized in real-time during vehicle operations. Modifications to the Supervisor module can then elicit a spectrum of vehicle missions as described here.

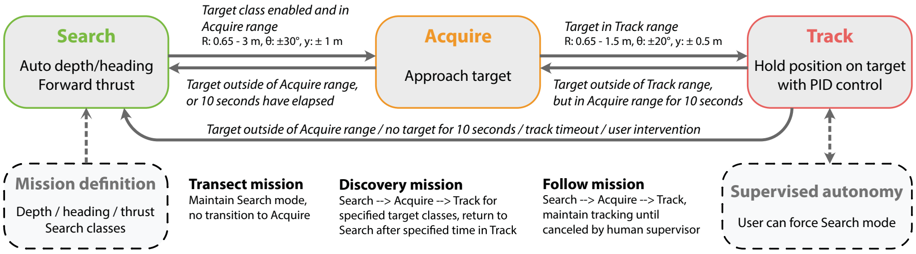

FIGURE 3
Supervisor module within *DeepSTARia*. The *DeepSTARia* Supervisor handles transition between three modes, each associated with different vehicle behavior: *search*, *acquire*, and *track*. Thresholds for transition between modes include values for range (*R*), bearing (*θ*), and vertical position (*y*, defined to be consistent with the *y*-axis in Figure 2). Once initial thresholds and set points were set, only minimal input was required to perform any of three mission types (*Transect*, *Discovery*, *Follow*).

The object detector provides information from each camera to the 3D Stereo Tracker, designed to distinguish individual objects (e.g., organisms), by including their positional history (or trajectory) relative to the vehicle. The 3D Stereo Tracker module is a multi-target tracking algorithm based on the unscented Kalman filter [UKF; Wan and Van Der Merwe (2000); Katija et al. (2021)]. It provides the best estimate of the target location relative to the left-camera on the vehicle based on stereo video, target state estimation, and vehicle inertial measurements. It uses stereo intersection over union (IOU) to solve for correspondence between pairs of bounding boxes from the object detector. If a pair has a valid stereo IOU, the Tracker searches for an existing trajectory to update with a measurement using Mahalanobis Distance (Mahalanobis, 2018) as the matching criterion. If no matching trajectory is found, the Tracker starts a new trajectory. At each iteration, the Tracker updates each trajectory with a score based on whether or not a new measurement was assigned to the trajectory. The trajectory with the highest score is used to estimate the target location and output to the Supervisor module (Figure 3). When the tracked object leaves the field of view or is no longer detected by the object detector, the Tracker will “coast” the trajectory for a given number of iterations before deleting the trajectory. In these cases, the Supervisor module will defer to the raw object detector output or Kernelized Correlation Filter (Henriques et al., 2015) Tracker initialized on the latest object detector bounding box.

2.2.2 Supervisor module

Given the positions and classifications of detected objects in the vehicle stereo cameras, a series of commands can be issued to the vehicle controller to progress through various mission modes, a process handled by the *DeepSTARia* Supervisor module (Figure 3). The Supervisor consists of three modes – *search*, *acquire*, and *track* – that loop continuously until transitions are initiated by input from the object detectors, 3D Stereo Tracker, mode timeouts, and external communications (user intervention or Supervised Autonomy). The Supervisor interfaces with the vehicle controller to adjust the vehicle behavior. The vehicle controller has been adapted from (Rife and Rock, 2006; Yoerger et al., 2021) for our system.

In the *search* mode, the vehicle closes the loop on heading (as measured by a compass) and depth (as measured by a pressure sensor) using proportional, integral, and derivative (PID) controllers on all vehicle axes. The user can then specify a desired forward speed in the form of percent thruster effort, with a default of 20% used in our field trials (Figure 3). This mode is analogous to how an ROV pilot would typically fly a vehicle during a midwater or benthic transect mission. For *Transect* missions with *DeepSTARia*, the Supervisor never leaves the *search* mode. For *Discovery* and *Follow* missions, the Supervisor remains in the *search* mode until a particular object or list of objects is detected within the predefined acquisition range of 0.65 m to 3.0 m (Figure 3), and thereby triggering a transition to *acquire* mode. Note that this range can be adjusted depending on your mission requirements.

Upon entering *acquire* mode, the vehicle’s behavior is changed, slowing down and centering the detected object in the cameras’ field of view. The Supervisor achieves this by slewing the heading and depth setpoints towards the estimated target bearing and vertical offset from the vehicle origin. The same PID control and gains are used in this mode as in *search*. The vehicle forward effort is set proportionally to the range of the object such that as the vehicle approaches the object the forward effort decreases until it becomes zero when the object is within the tracking range (defined below). The Supervisor will remain in the *acquire* mode until the target enters the tracking range (and transitions to *track*) or the target remains outside of the acquisition range for more than 10 seconds (and transitions to *search*).

In *track* mode, the vehicle will attempt to hold its position relative to the target object constant. This is done by enabling the target tracking controller, which closes the loop on range, bearing, and vertical offset of the target with a defined range setpoint (typically set between 0.65 m and 1.5 m; Figure 3) and bearing and vertical offset of 0 (i.e., centered on the left stereo camera). A different set of gains is used in this mode (compared to *search* and *acquire*) to enable more precise tracking of the target with the faster response time to target movement. The Supervisor will remain in the *track* mode until one of four conditions is met: (1) The target drifts outside of the tracking range but remains in the acquisition range for more than 10 seconds (and returns to *acquire*); (2) the Supervisor receives an external command to end the tracking (and returns to *search*, ‘supervised autonomy’); (3) The target remains outside the acquisition range for more than 10 seconds (and returns to *search*; ‘target lost’); or (4) the track duration exceeds a predefined time limit (and returns to *search*). In *Discovery* missions, this time limit was set to 15 seconds in our field trials, forcing the vehicle to move on in search of new animals that matched the selected classes. In order to prevent reacquiring the previous target in this case, the *search* mode is locked for 1 second after leaving *track* mode. In *Follow* missions, the system can be set to remain in *track* mode indefinitely (until interruption by human intervention); for the purposes of our field demonstrations, this duration was limited to 15 minutes. Note that we distinguish human intervention as an emergency precaution during our field trials to ensure the safety of the vehicle and its operators, whereas human supervision is done during normal operations of the vehicle in an autonomous mode only when prompted by the vehicle.

The Supervisor module implements a list of target classes to track out of the total set of classes the object detector was trained on (Figure 4). During the supervisor loop, detected targets are compared to the list of selected classes and mode transitions occur only when the detected target is in the list of classes of interest, or when the Supervisor is set to ignore class label during target acquisition. This final mode enables the Supervisor to acquire any detected target, but only track targets that belong to a subset of all possible targets.

2.3 Metrics for evaluating *DeepSTARia* field trials

The raw trajectories produced by the 3D stereo Tracker module were subject to several errors common to tracking-by-detection algorithms; due to erroneous detections (false positives and false negatives), misclassifications, and false associations of new detections with existing object tracks, these raw trajectories needed correction. Two post-processing steps were performed for the sake of more meaningful quantitative analysis. The first step aimed to resolve the issue of falsely-joined trajectories comprised of several distinct objects. As these trajectories corresponded to significant time gaps between detections of the distinct objects, all trajectories with gaps of more than 2 seconds between successive detections were split accordingly. Once split, all resulting trajectories with at least 4 frames were maintained. The values reported in Table 1 are representative of the post-processed trajectories. Each trajectory was included in a mission if the timestamp of its first detection fell within the mission time bounds. The duration represents the time between the first and last detections of a trajectory. We report the number of trajectories meeting or exceeding a duration of 15 seconds as a point of comparison with the 15-second tracking timeout for *Discovery* missions.

A trajectory T can be represented as a sequence of n detections, where each detection d_i consists of a timestamp in seconds $t_i \in \mathbb{R}$ and 3D position in the vehicle frame $\mathbf{p}_i \in \mathbb{R}^3$:

$$T = (d_1, d_2, \ldots, d_n)$$

$$d_i = (t_i, p_i)$$

$$\mathbf{p}_i = [\, x_i \; y_i \; z_i \,]^\top$$

The number of detections per second is computed as the frequency of detection events within a 1-second window around each point in the trajectory. As detections occur at a maximum of 10 Hz, these values may range from 1 to 11. The average vehicle-relative target speed is estimated as the sum of point-to-point Euclidean distances (in the vehicle coordinate frame), likewise within a 1-second window around each point.

At each point p_k, the time window is defined by detections d_l and d_r, with d_l minimizing t_l where $t_l \geq t_k - 1$ and d_r maximizing t_r where $t_r \leq t_k + 1$. Within these bounds, we arrive at the windowed subsequence $W = (d_l, \ldots, d_k, \ldots, d_r)$.

The detection frequency f is simply the size of the subsequence divided by the true window duration:

$$f = \frac{r - l}{t_r - t_l},$$

and the average vehicle-relative speed $\bar{v}$ is

$$\bar{v} = \frac{\sum_{i=l+1}^{r} \| \mathbf{p}_i - \mathbf{p}_{i-1} \|_2}{t_r - t_l}.$$

3 Results

Field trials of *DeepSTARia* were performed on ROV *MiniROV* (Figure 1) in the Monterey Bay National Marine Sanctuary over five days in May 2021. The first three days focused on iterative improvements of settings and operational interfaces, while the final two days prioritized testing and performing consecutive *Transect* (Video S1) and *Discovery* (Video S2) missions with a wide array of midwater animal targets (Figure 4). Here, we present only data from our fourth experimental day, to ensure consistency across our tests in terms of vehicle configuration, algorithm settings, and staffing. While the object detection model and 3D stereo Tracker (Figure 2) operated continuously through the entire ROV deployment, we present the results of distinct missions where the ROV pilot relinquished control of the vehicle, and no human supervisor input was provided (Figure 3). Via the Supervisor module, *DeepSTARia* cycled between three modes – *search*, *acquire*, and *track* – that dictate vehicle behavior based on input from the object detector, 3D Stereo Tracker, user-defined settings (e.g. mission type, mode timeouts), and user intervention. Table 1 summarizes the 4 *Discovery* missions performed at different depths, lasting at least 17 minutes each, and the 6 *Follow* missions exceeding 5 minutes that we conducted. Additionally, 3 *Transect* missions are also reported for comparison. We note that a human supervisor did tune the target vertical offset of the 3D Stereo Tracker in small increments over the course of 30 seconds in mission H (Table 1), but no changes to the model parameters or vehicle controller were made.

All but one of the *Follow* missions listed were purposely terminated by human intervention; mission M concluded due to a tracking failure (Table 1). In that case, the tracked object (*Physonectae* nectosome) was particularly low in contrast due to the high level of transparency in this species (*Resomia ornicephala*),

TABLE 1 Data summary of missions conducted during *DeepSTARia* field trials.

Mission		Duration [min:s]	Genus	Mean depth [m]	# of trajectories	Mean trajectory duration [s]	# of trajectories ≥ 15 s
ID	Type						
A	Discovery	22:27		252	111	7.1	21
B	Transect	12:32		252	104	3.2	1
C	Discovery	19:40		201	110	3.7	5
D	Transect	11:28		201	52	2.5	0
E	Discovery	17:22		151	27	5.8	4
F	Transect	10:25		151	16	1.2	0
G*	Discovery	21:11		101	62	5.6	9
H	Follow	11:16	*Solmissus*	247	77	27.8	14
I	Follow	05:00	*Solmissus*	251	27	16.4	2
J	Follow	35:50	*Bolinopsis*	267	152	19.5	11
K[†]	Follow	08:20	*Bathochordaeus*	250	32	19.4	3
L[†]	Follow	08:13	*Bathochordaeus*	246	44	19.0	6
M*	Follow	09:10	*Resomia*	111	31	19.0	1

The mission duration is defined as the time between enabling the search behavior and the next human intervention (canceling the mission), with the exception of the Follow missions: here the duration in track mode (without any human input) is reported. While each Follow mission tracked one individual animal (identified to the genus level by expert annotators), other objects entered the field of view, and the associated trajectories are included here. The number of recorded trajectories and their mean duration takes into account all observations with a minimum number of 4 stereo detections. The number of trajectories greater than 15 seconds indicates the number of times track mode was successful. Missions visualized in Figures 5 and 6 are highlighted in grey.

*Exposure settings of the stereo cameras were different from the other missions, creating a brighter image and affecting object detection rates.

[†]Follow missions K and L tracked the same individual.

even after tuning the camera parameters specifically for this individual. We subsequently focus our analysis on *Follow* mission H (Table 1), where a *Solmissus* jellyfish was tracked for more than 11 minutes. We chose this mission because it showcases several challenges for the algorithm, including: (i) another object of the same class passing by; (ii) total occlusion; and (iii) physical interference by another object (Video S3).

Differences in animal community composition and abundance caused large variations in object detections and trajectories (sequences of 3D positions of a single object derived from detections multiple video frames) between missions at different depths. On average, between A-F (Table 1), the mean trajectory duration (e.g., number of recorded image frames per individual) increased by 187% in *Discovery* missions, and yielded 5% fewer trajectories per unit time than *Transect* missions at the same target depth. As a result, the distance covered per minute was on average 24% less in *Discovery* missions. One of the model classes, (*Physonectae* nectosome), was excluded from triggering the *acquire* and *track* modes due to the high abundance of this class, so that vehicle behavior did not change when this class was detected. However, trajectories were still being recorded, and accounted for ~ 18% of trajectories in *Discovery* missions.

Transect missions are characterized by the Supervisor remaining in *search* mode throughout the mission (i.e., not stopping to track). By comparison, *Follow* missions represent a continuous span of time spent in *track* mode following a single target organism. *Discovery* missions represent a balance between these extremes, where the vehicle repeatedly stops to acquire and track targets of interest for fixed durations before returning to *search* mode. The Supervisor modes over time for the three representative missions A, B, and H is shown in Figure 5, along with the proportion of time spent in each mode. In the *Discovery* mission A, transitions from *search* to *acquire* represent attempts to stop, and transitions to *track* represent successful acquisitions. Whenever the timeout of 15 seconds during each stop was reached, the Supervisor transitioned back to *search* mode. Unsuccessful acquisitions can be seen around 350 and 850 seconds into the mission, where the Supervisor returned back to *search* after a short time in *acquire*.

The *Discovery* and *Follow* missions were conceived to increase the amount of time and number of views per observation of an organism, allowing for a more detailed look for identification by moving the vehicle such that the animal enters the most well-resolved and illuminated area in front of the vehicle with minimal motion blur. This also provides the opportunity to observe the animal's behavior by keeping it centered in the field of view, which is rare during the relatively fast fly-by speeds associated with transects (Figure 5). During *Transect* missions, the vehicle does not respond to object detections, which therefore move radially past and out of view as the vehicle moves forward. *Discovery* and *Follow* mission instead actively align objects with respect to the image center, increasing the number of recorded views. In *Discovery*, the vehicle aligns briefly for a pre-specified duration (15 seconds) with each animal, showing a much larger fraction of bounding box observations near the image center and offering more image frames of each individual.

FIGURE 4
Highlight images of midwater animals that served as target objects during *DeepSTARia* field trials. Each image represents one of the 15 taxonomic groups that formed 17 separate classes in the RetinaNet model used in this work. Three classes were defined for *Bathochordaeus*: the animal, house, and outer filter, to address the different size scales of the outer structures and the small animal of interest inside, allowing initial detection of the larger structure and subsequent tracking of the animal inside.

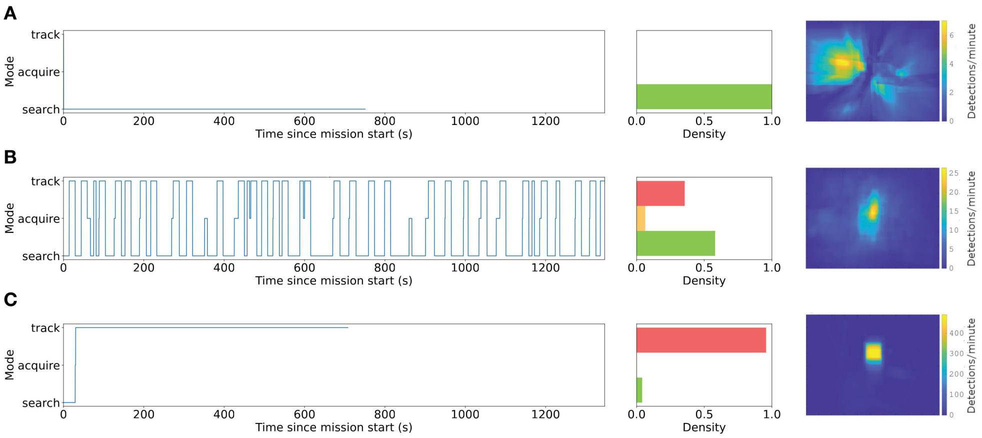

FIGURE 5
Changing modes (e.g., search, acquire, track) and cumulative distributions of detected bounding boxes from **(A)** *Transect*, **(B)** *Discovery*, and **(C)** *Follow* underwater vehicle missions during *DeepSTARia* field trials. Left column shows the mode switching over time for a representative vehicle mission and the middle illustrates the cumulative time within each mode (red = *track*, orange = *acquire*, green = *search*) for the corresponding mission. Heatmaps are based on bounding box locations in the left camera image during each mission type, where *Transect* missions B, D, and F, and *Discovery* missions A, C, and E have been combined, respectively. Note that the range of the color scale increases panels.

Finally, during *Follow*, a single object is kept in place with respect to the vehicle, resulting in higher rates of detections centered in the imaging field of view.

The 3D position of detected objects relative to the vehicle frame can be seen in more detail in Figure 6. The *Transect* mission (Figures 6A, B) sees objects passing by in nearly straight lines at constant velocity, with their detection rate increasing as the vehicle approaches (i.e., the Z position decreases; Figure 6A), until they are lost from the field of view. In *Discovery* missions, the trajectories converge as the vehicle positions itself to center the object of interest within the field of view at a fixed distance, and is associated with a reduction in relative object speeds. The *Follow* mission takes this one step further, maintaining a high rate of detection and very low relative speed throughout once the vehicle is centered on the animal. The proportion of time spent in each mode across *Discovery* missions A, C, E, and G can be seen in Figure 7.

4 Discussion

DeepSTARia enables a range of underwater vehicle missions for biological observations that are otherwise considered impossible to execute autonomously. In addition to traditional transects (where vehicle depth and heading are kept constant) (Howell et al., 2010; Robison et al., 2017), *DeepSTARia* allows for fully autonomous *Discovery* and *Follow* missions that typically require scientists and researchers to monitor and direct underwater vehicle operations (Figure 5). *Discovery* missions enable collection of more images and views – nearly 2.5 times as many on average – of individual targets (Table 1) and higher rates of detections (Figures 6A, C, E) than *Transects*. These improvements enhance the quality and composition of the imagery obtained (Figure 5), enabling extended duration animal behavior observations and more precise and accurate animal identification. *Follow* missions expand our ability to capture long duration observations of an animal in its environment as prescribed by the Supervisor *track* mode timeout setting (Table 1; missions H-M), which was defined to be 15 minutes for our field trials.

Our approach distinguishes itself from other real-time object tracking and visual servoing approaches by integrating a multi-class object detector that includes visually complex classes and the Supervisor module functionality. For *Discovery* and *Follow* missions, the multi-class approach is very effective at reducing undesired changes in vehicle behavior when compared to traditional shape-based approaches [e.g., blob detection (Yoerger et al., 2021)]. A human operator can adapt the observational focus by actively selecting or ignoring certain classes, either for research interests or to account for target abundance. For example, *Discovery* missions that continuously slow on very common species, such as the physonect siphonophore [*Nanomia bijuga*; a member within the same family (Physonectae) is shown in Figure 3] in Monterey Bay, would take a significant amount of survey time. Selective targeted vehicle behaviors like this are generally difficult to specify and control with other unsupervised methods (Girdhar and Dudek, 2016). Here, we manually defined these rejected and target classes prior to the start of a mission. Future work could involve augmenting the Supervisor module to enable the vehicle to dynamically adjust its focus, either disregarding or prioritizing classes surpassing a specified abundance threshold. Furthermore, the object detector used in *DeepSTARia* included three nested classes for the giant larvacean *Bathochordaeus* [animal, house, and outer filter (Katija et al., 2020)], which allows for initial

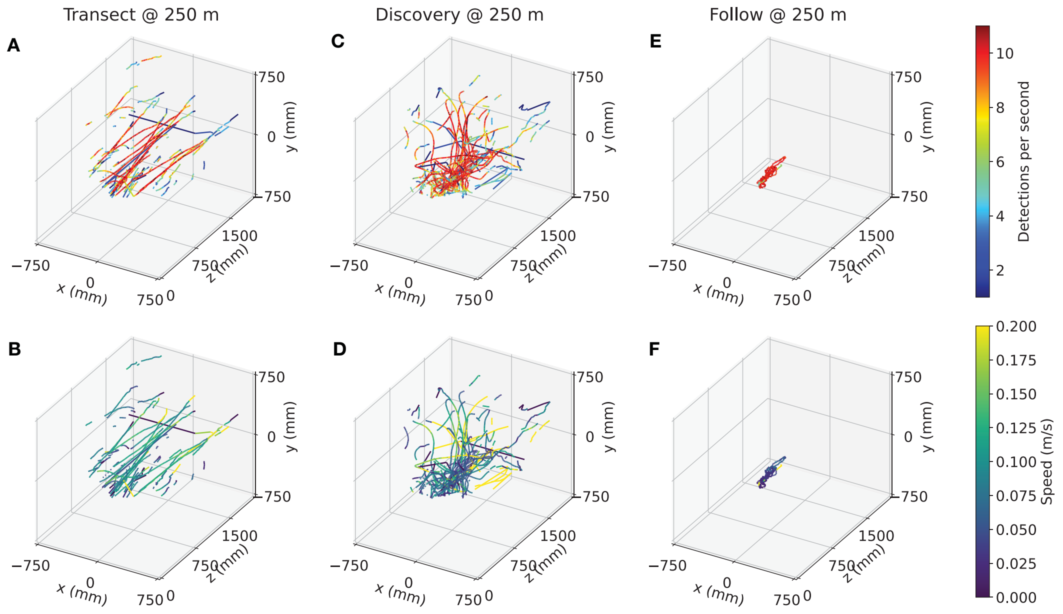

FIGURE 6
Trajectories of observed detections during representative **(A, B)** *Transect*, **(C, D)** *Discovery*, and **(E, F)** *Follow* missions. Top row shows the trajectories colored by the detection rate within a 1-second window, and bottom row shows the average vehicle-relative speed within a 1-second window, for *Transect* mission B, *Discovery* mission A, and *Follow* mission H, respectively.

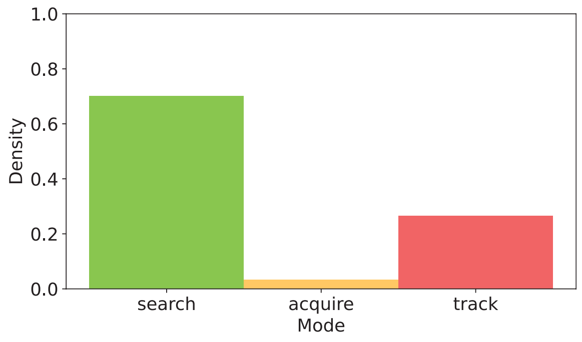

FIGURE 7
Cumulative duration within the three vehicle modes. *search* = green, *acquire* = orange, *track* = red) over four *Discovery* missions (total duration of 80:40 for missions A, C, E, G; Table 1) performed during *DeepSTARia* field trials.

detection of the large outer filter at an extended range, and tracking of the animal itself once the vehicle has approached and slowed. Not only do nested classes like these help to increase the likelihood of successful detection and subsequent tracking of smaller objects that associate with larger ones, changing vehicle behavior farther afield helps to minimize vehicle disturbance of the fragile outer mucus structures as demonstrated in (Katija et al., 2020, Katija et al., 2021) and potential changes in animal behavior.

Both the *Discovery* and *Follow* missions effectively enhance our ability to densely sample organisms of interest either with images, video, or auxiliary sensors. These sorts of long duration observations are invaluable for assessing interactions between an organism, other individuals, and the environment (Norouzzadeh et al., 2018). The missions yield data suitable for novel studies of trait-based (Orenstein et al., 2022) and movement ecology (Abrahms et al., 2021) that fundamentally rely on studying how an organism moves through space. Without bursts of images or videos, ecologists are limited to studying count data in particular spatiotemporal regions (Kennedy et al., 2019). Studying these facets of animal behavior are particularly challenging in the deep sea, where tracking individuals has historically been a labor intensive task requiring the careful attention and precise movements of a skilled ROV pilot. With consistent access to such data, scientists will be able to better assess individual biological fitness, study cryptic predator-prey interactions, and better understand migratory behavior to name a few. These missions can also generate valuable machine learning training data on new objects and animals (Katija et al., 2022), by providing a variety of perspective views on a single organism during *Track* modes that cannot be similarly achieved at the same temporal resolutions during *Transect* missions (Table 1).

Besides enabling unique ecological studies, the *Follow* mission could be used to update the behavior of an individual fully autonomous robot, inform vehicle behavior in multi-vehicle missions (Zhang et al., 2021), or coordinate robot swarms observing collections of targets (Connor et al., 2020). One potential scenario might entail a system of two vehicles: an AUV carrying an imaging system communicating acoustically with an Autonomous Surface Vehicle (ASV) tracking the subsea asset (Masmitjà Rusiñol et al., 2019). Based on the onboard *DeepSTARia* state, imagery can periodically pass between the AUV to the ASV, and be transmitted onwards via cellular or satellite networks to an onshore AUV operator. The AUV operator would monitor the AUV's behavioral changes during deployment using the Supervised Autonomy mode (Figure 2), which can be used to override the *track* mode and have the vehicle resume *search*. The workflow would function akin to our ROV-based work on a single AUV, but could be extended to trigger behavioral changes on additional vehicles carrying other sampling equipment like genomic or acoustics payloads (Zhang et al., 2021). In practice, one could expect that the energy expense of on-board computation for DeepSTARia would limit the deployment time of an AUV; however, our estimates suggest that the power budget would be more heavily impacted by the demands for illuminating the scene rather than the recording or processing of visual data. The Supervised Autonomy framework enables autonomous vehicle behavior adjustments while retaining low-latency guardrails by keeping a human in the loop.

We advocate for the selective automation of ship-borne activities, emphasizing that tasks amenable to automation, such as tedious and repetitive activities like biological monitoring via midwater transects, should be targeted for autonomous execution. Ultimately, the long-term goal is for AUVs to sample biological targets fully autonomously. However, classical supervised ML algorithms trained off-line for real-time detection and identification are unlikely to work in all scenarios in dynamic environments like the ocean: models often struggle when deployed in real world settings due to changing relative proportions of the target classes, the introduction of previously unseen concepts, or discrepancies in the pixel-level image statistics (Recht et al., 2019; Koh et al., 2021). This typically manifests in ecological applications as distribution shifts – where the statistics of the target data differ from that of the training – as a function of time or space (Koh et al., 2021). These challenges are inherent in ocean sampling and limit the ability of fully autonomous systems to adjust their behavior based on visual signals. There are several bleedingedge, pure ML solutions that are well-worth experimentation: Open World Object Detection frameworks to identify novel classes in a new domain (Joseph et al., 2021); contrastive learning to identify out-of-distribution samples and study areas (Yamada et al., 2021); and uncertainty quantification to compute robust confidence thresholds around ML outputs for hypothesis testing (Angelopoulos et al., 2022). Additionally, the promise of reinforcement learning holds potential for addressing the control problem associated with handling more complex animal behavior (e.g., swimming): an area where the current implementation of simple PID thruster-effort-based control struggles. While these approaches are promising, they are experimental, and implementation in the field will benefit from the use of Supervised Autonomy to ensure the routines are effectively acquiring the desired data and evoking the appropriate vehicle behavior.

5 Conclusion

DeepSTARia is a significant stride toward expanding the capabilities of underwater robots by actively adjusting behavior in response to real-time visual observations. Our experiments demonstrated the approach's efficacy on an ROV, allowing a human operator to completely step away from the controls. Deploying *DeepSTARia* on AUVs would fundamentally alter our approach to studying organisms in the deep sea, speeding the discovery of ocean life and processes unknown to the research community. Such a step change in observational capacity is desperately needed: estimates suggest that between 30 and 60% of marine life have yet to be described (Appeltans et al., 2012) and current methods for marine species description can take more than 21 years on average per species (Fontaine et al., 2012). The future of species discovery must someday leverage algorithms like *DeepSTARia* to autonomously run *Discovery* and *Follow* missions to continuously monitor an ocean region or explore a new one (Aguzzi et al., 2020). As algorithms and embedded hardware continue to improve on autonomous vehicles, data collected during these missions may someday lead to onboard learning of features of animals and objects without loss of performance on existing classes, identification of unknown classes (Joseph et al., 2021), and verification by human observers via Supervised Autonomy. These advances, enabled by algorithms like *DeepSTARia*, are critical to scale our ability to discover, study, and monitor the diverse animals that inhabit our ocean.

Data availability statement

The datasets presented in this study can be found in online repositories. Labeled data can be accessed through FathomNet at www.fathomnet.org, and the midwater object detector can be accessed at www.github.com/fathomnet/models. All software related to the DeepSTARia project is open-source. Code used for the May 2021 deployment of DeepSTARia is available at https://bitbucket.org/mbari/ml-boxview.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

KB: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. JD: Data curation, Investigation, Validation, Visualization, Writing – original draft, Writing – review & editing. PR: Conceptualization, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. EO: Investigation, Writing – original draft, Writing – review & editing. IM: Investigation, Writing – original draft, Writing – review & editing. JT: Methodology, Software, Writing – review & editing. BW: Conceptualization, Methodology, Software, Writing – review & editing. KK: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This effort was supported by NSF-GEO-OTIC grant #1812535 (to KK), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant #893089 (to IM), and the David and Lucile Packard Foundation (to KK), the Spanish National Program Ramón y Cajal grant #RYC2022-038056-I (to IM).

Acknowledgments

The authors would like to thank Brett Hobson, Dale Graves, Alana Sherman, Frank Flores, and the crew of RV *Rachel Carson* (MBARI) for their engineering and operational contributions to this effort, and Steve Rock (Stanford Univ.) for his invaluable input and guidance. Additional thanks goes to Alexandra Lapides (MLML) for her assistance generating labeled data using our stereo imaging system.

Conflict of interest

Authors JT and BW were employed by company CVision AI.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1357879/full#supplementary-material

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Frontiers in Marine Science

TYPE Perspective
PUBLISHED 13 June 2024
DOI 10.3389/fmars.2024.1387204

OPEN ACCESS

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RECEIVED 16 February 2024
ACCEPTED 08 May 2024
PUBLISHED 13 June 2024

CITATION
Wang L, Adams C, Fundis A, Hsiao J, Machado C, Malik M, Quadara R, Rodriguez C, Soule A, Suhre K, Wu L and Elmore AC (2024) Broadening inclusivity at sea.
Front. Mar. Sci. 11:1387204.
doi: 10.3389/fmars.2024.1387204

Broadening inclusivity at sea

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Ocean sciences in the U.S. remains a field with one of the lowest rates of diversity, having disproportionately low representation from marginalized groups, including Black, Asian, LatinX, Indigenous, and other people of color; LGBTQIA + individuals; disabled persons; women; those with neurological differences; and those from low-income groups. With equity and inclusion in mind, recent efforts have been made to increase the number of ocean science professionals from marginalized groups through multiple entry points, including internships. However, there still exists a large gap between the diversity found in the general population and the diversity within ocean sciences. Perhaps one reason why this field continues to have lower diversity owes to the unique component of many oceanographic careers, which continues to present an especially high barrier for marginalized groups: participating in sea-going research expeditions. Herein, we have synthesized possible ways to prioritize the physical and emotional safety of marginalized ocean science professionals participating in a research expedition, including guidance on preparation, implementation, and providing support post-cruise. These suggestions are intended to be useful for the broader oceanographic research community to consider the safety and well-being of individuals from marginalized groups at sea, since the field of ocean sciences - like all fields - would greatly benefit from increased representation and diversity.

KEYWORDS

marine science, diversity & inclusion, ocean science, field work, oceanography

Importance of research expeditions in oceanographic career development

Careers in the ocean sciences can span a variety of focus areas, from scientific research to national security, the energy sector, supply chains, food availability, and much more. These careers are generally well-paid, and the sector is expected to grow in the US by 7% between 2020-2030 as more and more focus turns to the growing Blue Economy (Bureau of Labor Statistics, 2022). One component of many ocean science careers that differs from most other science disciplines is the necessity of sea-going expeditions - field campaigns that are frequently set on isolated vessels, far from shore. While research expeditions are often described as exciting and rewarding (e.g., LaCapra, 2018; Windom, 2019; Theising, 2021), they can also be a time of unexpected challenges, isolation, and - unfortunately - harassment and/or abuse (Harris, 2022). For those who are about to undertake their first oceanographic expedition, some resources are available to assist with personal preparation (e.g., Glessmer, 2019); however, most of these resources do not specifically address the additional struggles faced by sea-going individuals from marginalized backgrounds aboard research vessels (Ackerman et al., 2023). For the purposes of this manuscript, we are referring to ocean science professionals generally to mean those who are working at sea but who are not necessarily professional mariners nor PhD-level researchers, including hydrographers, marine biologists, marine geologists, physical oceanographers, vehicle and systems engineers, marine technicians, sea-going data scientists, and more. Our authorship represents multiple perspectives though all are currently working in the U.S., so our recommendations are likely most suitable for U.S.-based activities. We focus our guidance on research expeditions, much of which should apply across ocean domains and sectors, but note that some seagoing work (e.g., ocean shipping, offshore energy) may differ significantly.

Many efforts to diversify the ocean science field include sending students and early-career professionals to sea as part of research expeditions (e.g., Ocean Exploration Cooperative Institute (OECI), 2023; American Geophysical Union (AGU), 2023; NSF's STEMSEAS program; Ocean Exploration Trust's ONR-funded internship program). In fact, going to sea is largely considered a critical component of many ocean science careers (e.g., EnvironmentalSciences.org, 2023), with career planning information suggesting field experiences for individuals could start as early as high school (American Geosciences Institute (AGI), 2023). Broadly, undergraduate participation in fieldwork has been shown to lead to greater STEM retention and graduation rates (Beltran et al., 2020), improved understanding of theory, and acquisition of hands-on experience for future career positions (Roberts, 2020). Importantly, in one survey of students participating in a National Science Foundation Research Experience for Undergraduates (REU) program as part of a collaboration between Historically Black Colleges and Universities (HBCUs) and marine laboratories, at-sea research experience of all professional development experiences in the program, was determined to be the most important contributor to personal and professional growth (Gilligan et al., 2007). Positive experiences in the field can lead students to pursue a major or career in a related STEM field (Stokes et al., 2015; Cook et al., 2016), while negative experiences, including experiences related to racial bias, drove students away from further STEM fieldwork and careers (Park et al., 2020). Thus, the efforts to diversify the marine sciences should carefully consider factors of inclusion specifically related to experiences at sea.

Demographics of ocean science professionals

Ocean sciences suffer from a lack of diversity (National Center for Science and Engineering Statistics, 2021). Researchers from minoritized communities - BIPOC (Black, Indigenous, People of Color), as well as other protected classes on the basis of gender, sexual orientation, religion, age, disability, national origin, and other factors such as socioeconomic status - have been historically excluded and continue to be marginalized in these fields (e.g., Valenzuela-Toro and Viglino, 2021; Chen et al., 2022). The history of gender disparity in ocean sciences has been discussed previously, in part due to the fact that women were largely excluded from at-sea research expeditions until the second half of the twentieth century (Day, 1999; Thompson et al., 2011; Duncombe, 2019; Hendry et al., 2020; Legg et al., 2022). This exclusion has in part led to a lack of female representation in marine scientist positions (Kappel, 2014), geoscience faculty positions (Ranganathan et al., 2021), and senior leadership positions in marine science and conservation (Giakoumi et al., 2021). There is also a lack of racial and ethnic diversity in the ocean sciences (Roberts, 2020) at both the student and faculty level (Cook et al., 2016), particularly for women of color (Bernard and Cooperdock, 2018). These researchers often experience persistent systemic and individual bias, microaggressions, and/or exclusion (Marin-Spiotta et al., 2020; Morris, 2021; Marin-Spiotta et al., 2023), in part resulting in a "hostile obstacle course" that decreases retention throughout the ranks and by career stage (Berhe et al., 2022). These are just two examples - sexism and racism - of the many "-isms" that exist in this and many other homogeneous fields.

Efforts to diversify

In recent years, there have been numerous efforts and discussions related to promotion of diversity, equity, inclusion, and justice in STEM, including in the geosciences, oceanography, and other field sciences (e.g., Cooper & Lewis, 2017; Greene et al., 2021; Wilson et al., 2021; Barabino et al., 2023). This includes discussion regarding the general workforce (Johnson et al., 2016), federal workforce (National Science and Technology Council, 2021), academic faculty (Ormand et al., 2021), federal research funding (Chen et al., 2022), conference speaking opportunities (Ford et al., 2018), and student opportunities (Karsten, 2019; Garza, 2021), as well as discussions on promoting safety and inclusion at institutional and program levels (Kelly and Yarincik, 2021; Ali et al., 2021). While some gains have been made in increasing the number of early career oceanographers from marginalized backgrounds, many efforts to diversify and recruit students in the ocean sciences continue to have problems with retention (Bernard and Cooperdock, 2018; Behl et al., 2021). In part, retention issues are linked to the lack of representation in faculty and leadership positions, positions of authority, which can have ripple effects on recruitment and retention of the future blue economy workforce. Mentorship from those with similar lived experiences and backgrounds (Hernandez et al., 2020; Olcott and Downen, 2020; Behl et al., 2021, Orcutt and Cetinić, 2014, Coles et al., 2011; Lozier, 2015; Aikens et al., 2017) is particularly important for students and early career researchers from minoritized backgrounds in cultivating self-efficacy and building a professional support network.

Obstacles/limitations preventing early career professionals from going on expeditions

A myriad of barriers may prevent ocean science professionals at all career stages from participating in at-sea research expeditions, including; historical exclusionary factors, lack of representation, lack of appropriate accommodations, and potentially hostile work environments, which can all contribute to hesitancy and feeling unsafe at the prospect of going to sea. One barrier that was frequently discussed during the writing of this manuscript is the personal financial costs associated with going to sea (Giles et al., 2020; Roberts, 2020). It was noted that personal protective equipment and specialty clothing is sometimes not supported by funding, leaving especially early career oceanographers with financial hardship. Additionally, some programs that do provide funding for equipment, travel, supplies or materials, or other necessary items, do so through reimbursements, which may take long periods of time to process, creating a situation unfeasible for many students, especially those from economically depressed communities. Another example of this is the potential for high cost medications, which can be excluded by insurance companies if they are requested for longer periods of time, which might be required for longer cruises. Finally, the prospect of being away from home for a longer period can also create financial and logistical hardships, especially for those from lower socioeconomic backgrounds, for paying bills at home, pets, dependent care, and more.

Additionally, lack of accommodations for researchers with disabilities (Gilley et al., 2015; Bower, 2018; Giles et al., 2020) as well as the historical portrayal of oceanographers as able-bodied (Garza, 2021) can prevent those with disabilities and other health issues from going to sea. Researchers with disabilities also face additional obstacles and barriers during fieldwork at sea, including that many research vessels are not accessible for wheelchairs or other required support (Hall and Healey, 2005). The current lack of representation of ocean science professionals with disabilities is likely directly tied to the lack of accessibility on research vessels in addition to other systemic barriers. Other situations which may preclude researchers from going to sea include those with caretaker responsibilities or religious commitments, who cannot be away for weeks or months at a time. Over the last decade, efforts to incorporate and improve telepresence operations that enable scientists on shore to participate in cruises in-real-time from their home or office have increased participation of those who are not able to sail on the cruise, including if they are pregnant, wheelchair-bound, or unwell (Marlow et al., 2017; Gallaudet et al., 2020; Xia et al., 2022).

Furthermore, sea-going research expeditions can take an emotional toll and present new situations and customs, long hours, isolation from support systems, concentrated time with small groups of people, distance away from medical attention, and the prospect of being far from shore or getting seasick. These situations can create and exacerbate feelings of anxiety (Tucker and Horton, 2018; Lawrence and Dowey, 2021). Sea-going expeditions can also involve travel to new and foreign places depending on the port of call, many of which will have different laws, rules, and customs. For the large number of STEM students and researchers in the US who are not U.S. citizens but wish to go to sea, they may need to go through extra security checks and medical screening. These procedures not only 'other' them in the process and restrict their expedition opportunities, but also subject them to additional stressors. In a survey of LGBTQ+ geoscientists by Olcott and Downen (2020), over half of respondents also indicated that they have felt unsafe in a field work location due to their gender identity or expression. Additionally, isolated, hierarchical groups and power dynamics at sea can embolden harassment, particularly sexual harassment (Clancy et al., 2014) and racial harassment (Dowey et al., 2021).

Current efforts to promote safety and belonging at sea/in the field

Many sea-going expeditions now require participants to watch orientation videos and/or undergo harassment training prior to setting foot on the ship. UNOLS (University-National Oceanographic Laboratory System), an organization that coordinates U.S. research vessels, provides resources for harassment, reporting, and field safety. Congress has passed existing law (PL114-328 Subtitle C), and introduced current bills (e.g. H.R. 2865) requiring the establishment and/or strengthening of sexual harassment and assault prevention and response within NOAA and other maritime groups (e.g. MARAD, US Merchant Marine Academy). Other organizations have been founded as a response to the rampant harassment associated with field work (e.g., The Fieldwork Initiative) and a community-derived approach to preventing sexual harassment at sea has been proposed (Ackerman et al., 2023). However, there is still a need for large scale improvement in programs and systems with regard to safety and inclusion for at-sea researchers, particularly as more programs recruit students and researchers from marginalized backgrounds for programs that include sea going experiences (Amon et al., 2022).

Opportunities for improvements to benefit marginalized oceanographers

The premise of this publication initially arose from a personal conversation between colleagues seeking to provide helpful advice to an early career researcher who was heading out on their first oceanographic expedition. Subsequently, a collection of resources was gathered to share with that researcher to help them prepare for the expedition, including important things to know and pack. Conversations were then held between the coauthors to centralize these resources for onward cruise participants, with a focus on how ocean science professionals can better create a culture of inclusivity while at sea. A decision was then made that we should gather helpful information from as many ocean science professionals as we can, in order to make a list of considerations that can be used widely in support of diversifying the ocean science community. Onward informal discussions were then held with colleagues from a variety of backgrounds in order to centralize their recommendations herein. While attempts were made to gather experiences and information from ocean science professionals from diverse backgrounds with diverse experiences, certainly many unique perspectives may not have been included below, since the whole community of ocean science professionals was not officially surveyed.

Below, we list some considerations for improving safety and inclusion on at-sea expeditions, broken down into time periods of: 1) before the expedition, 2) during the expedition, and 3) after the expedition. This list has been compiled by the authors, many of whom have minoritized identities, from the literature, from speaking with our broad networks, gathering feedback on earlier iterations of the below list of suggestions at the American Geophysical Union Fall Meeting in New Orleans, Louisiana in December 2021, and at the virtual Ocean Sciences Meeting in February, 2022. Our target audience encompasses expedition planners, program managers, principal investigators, vessel operators, funding agencies, and anyone who will be planning expeditions and bringing on researchers from marginalized backgrounds. This resource may also be useful for anyone participating in at-sea research expeditions to consider, particularly for individuals going to sea for the first time, as many may be overwhelmed and may not know what questions to ask nor which measures to consider. Ultimately, the onus should be upon the programs and leaders sending researchers to sea to ensure safety and inclusion for all participants.

This manuscript serves as a useful resource for expedition planners to review and consider, while acknowledging that it may not be feasible to implement all recommendations simultaneously. We also acknowledge that this list is not all encompassing, and we will inevitably miss important topics and suggestions for consideration. Though many of these recommendations can be broken up chronologically throughout the at-sea expedition experience, many changes can be incorporated throughout the expedition process and at all times. Additionally, while this paper highlights specific diversity needs and challenges of scientists going to sea, it is acknowledged that ocean-based careers extend beyond only scientists including artists, culinary experts, firefighters, rope handlers, boat operators, etc. Lastly, we hope that this resource initiates future conversations about ways to further improve safety and inclusion at sea, fully inclusive of all identities.

TABLE 1 Considerations and recommendations prior to the cruise and during cruise planning at both the organizational and expedition level.

Organization, institution, or funder
In order to include minoritized participants, programs can collaborate with Minority Serving Institutions as equal partners (Gilligan et al., 2007; National Science and Technology Council, 2021) to support cohort-based at-sea research experience for students; one example of this is the Tuskegee University Internship program of the Ocean Exploration Cooperative Institute (OECI) by the University of Southern Mississippi.
For application-based positions aboard, make clear from the application process what expenses will be covered, and what funding opportunities are available (Giles et al., 2020).
Provide upfront travel, lodging, per diem, and registration funds for cruise participants, rather than reimbursement, to alleviate financial stress.
Supplement additional costs for those with caretaker responsibilities when at sea (Hendry et al., 2020), such as support for women returning to the field soon after having children (Orcutt and Cetinić, 2014, Vila-Concejo et al., 2018). Some funders have recently provided additional resources for dependent care (e.g., National Geographic Society, 2023; National Science Foundation, 2023; Schmidt Ocean, 2023).
Provide and account for accommodations, to the extent possible, for researchers with disabilities to safely and fully participate in research expeditions. Have open communication about potential barriers and possible accommodations or mitigations the research team can provide (Stokes et al., 2019).
Provide as needed support for the paperwork and travel logistics required for participants to sail, including visa paperwork for foreign national researchers.
Provide a list of resources for participants in an orientation packet, which will be helpful for those sailing for the first time, and act as a refresher for more experienced sea-goers. This can include information on “unwritten rules” of living on a ship, recommended packing list, ship safety, etc.
Conduct targeted outreach through clubs, conferences, organizations, particularly conferences focusing on supporting minoritized groups (e.g.,SACNAS) (Dutt, 2019; National Science and Technology Council, 2021).
Provide training in mental health, unconscious bias, sexual harassment, and bystander behavior for all expedition team members (including the science party and crew) (see, Anadu et al., 2020; Hendry et al., 2020; Hill et al., 2021).
Provide bystander intervention training for all expedition team members (including the science party and crew). As an example, ADVANCEGeo has provided this during its four year tenure (Hill et al., 2021). Acknowledge that although bystander intervention may be helpful in certain situations, intervening may be dangerous or even backfire at other times. Emphasize the goal of a clear and reliable reporting system and a shift in culture in which we no longer need to rely on bystanders to intervene.
Establish a code of conduct and expectations for all parties.
Dispel notions that seemingly minor transgressions or microaggressions are not worth reporting. These experiences add up to create larger effects.
Compile practical resources for cruise planners (expedition leaders, chief scientist, PIs, etc.) on ways to address potential issues (e.g. best practices for responding to harassment or assault allegations; clear guidance for reporting incidents to the appropriate authority) as the first step planners could take proactively if they anticipate complexities in planning their cruise (Appendix A).

(Continued)

TABLE 1 Continued

Organization, institution, or funder
Fund and promote bursaries for minoritized group members to go to sea, particularly in leadership positions (e.g. Hendry et al., 2020).
Expedition coordinators, planners, or leaders
Avoid placing a single, minoritized individual within a larger, homogenous group. This can be accomplished by implementing cohort based at-sea research expedition experiences when recruiting students and early career researchers, and include cohort-building opportunities. Examples of cohort-based experiences include the OceanX Young Explorers Program, NOAA Ocean Exploration's Explorer-In-Training Program, Ocean Exploration Trust's Science & Engineering Internship Program and Science Communication Fellowship program, and the STEM Student Experiences Aboard Ships (Cooper and Lewis, 2017) program.
Assign multiple mentors, peer mentors, and points of contact for new participants to discuss questions and concerns. Peer mentorship can increase retention (Coles et al., 2011; Jin et al., 2019), create a supportive community and network (Behl et al., 2021; Stofer et al., 2021), and enhance researchers' science identity.
Be transparent about expedition logistics and mitigation planning with all participants (Lawrence and Dowey, 2021). Mitigation planning might mean hosting team meals and happy hours only at publicly inclusive restaurants, or clearly communicating why a certain action is being taken to increase safety and inclusivity. Acknowledge that expedition logistics are always subject to change, and communicate itineraries and updates in a timely manner.
Train staff to identify potential dangers to minoritized groups as part of risk assessments (Anadu et al., 2020; Olcott and Downen, 2020; Lawrence and Dowey, 2021). Expedition planners need to be aware of potential safety issues associated with traveling to certain locations, in certain ports of call, and during travel. This can be particularly dangerous for certain racial and ethnic groups and LGBTQ+ scientists.
Create a dress code focused on safety that is non-sexist and gender-inclusive
Conduct an orientation meeting before the expedition to discuss what to expect and answer any additional questions.
Make introductions for all expedition participants prior to departure, and share travel itineraries and contact details in case any participants need support during travel.
Assign a travel buddy, if possible, and coordinate travel to and from airport, lodging, and port. Consider that members of minoritized groups may not feel safe or comfortable traveling alone to and from certain port locations.
Include preferred name and space for pronouns in the participant list.
If cabin rooms are being shared, inquire what gender berthing participants require, and never suggest to a participant that they should compromise their berthing requirements for the sake of the mission. Provide single occupancy berthing if possible to accommodate.
Clearly describe and promote reporting protocols for concerns about the work environment and how cases and conflicts will be handled, including multiple points of contact for reporting. Enforce accountability and consequences.
Consider breaking up expeditions into shorter segments (when possible), or allow switching of personnel, to accommodate those with caretaker responsibilities and other factors which may preclude one from being away for long periods of time (Orcutt and Cetinić, 2014; Vila-Concejo et al., 2018; Hendry et al., 2020).
Accommodate religious calendars and specific hours of prayer when scheduling expeditions (Lawrence and Dowey, 2021) and provide appropriate space for religious activities.
Provide meals suitable for religion- or heath-based specialty diets.

TABLE 2 Recommendations for inclusivity during an overnight expedition at sea.

Recommendations during an overnight expedition at sea
Be clear about daily schedules and be upfront about potential changes to the schedule. Schedule regular breaks (Greene et al., 2020; Lawrence and Dowey, 2021), including prayer breaks (Giles et al., 2020).
Emphasize that there are codes of conduct and expectations for behavior, and clearly post reporting protocols, and remind participants of reporting protocols with multiple points of contact for reporting.
Enforce accountability and consequences, with no acceptance of intolerance and microaggressions.
Continually evaluate working conditions and regularly meet during the expedition to discuss potential issues or concerns (Kelly and Yarincik, 2021).
Provide adequate personal protective equipment for all persons, regardless of size, ability, or gender.
Encourage frequent restroom and mental health breaks and establish gender neutral restrooms (Greene et al., 2020).
Make menstruation products available, provide clear directions and identify locations for their disposal.
Accommodate and account for dietary restrictions of participants, including religious restrictions and fasting periods (Giles et al., 2020).
Ensure that participants can communicate externally at all times with support systems on shore. Consider expanding internet and communication capabilities for participant communication with family or children at home.
Consider using or expanding ship-based telepresence capabilities to allow for full remote participation in the expedition, particularly for those with care responsibilities, those who are pregnant, and those with disabilities.
Avoid potential biases when assigning or distributing tasks.
Refrain from making comments on other participants' appearance, attractiveness, or your intentions to pursue them sexually/romantically.
While socializing is an integral part of the at-sea experience, discourage overt flirting or sexual activity during the trip. Sexual behavior, even between a pair of consenting cruise participants, can make others (such as roommates) uncomfortable.
Always use correct pronouns.
Actively dispel the pervasive at-sea culture that participants or observers should be willing to accept minor discrimination, sexism, harassment, etc. for the sake of a successful mission.
Be cognizant of the alcohol culture in the geosciences (e.g., Guertin, 2019), particularly during and after fieldwork (Miller, 2018), which can be a barrier to inclusivity to those who do not drink (Fernando and Antell, 2020; Dowey et al., 2021), and can lead to reduced inhibitions that could be dangerous for all participants, especially including members of minoritized groups (Forrester, 2021).
Specific expectations should be put in place for participants to consent to being filmed beyond simply a blanket consent that lasts the duration of a cruise. While at sea operations can occur at all hours of the day, media collection or interactions (videography, photography, etc), should be planned in advance, whenever possible. Certain marginalized groups may be sensitive to being filmed and potentially publicly shown in conditions when they feel they won't be represented in a way they are comfortable with.
Ensure that minoritized groups do not become overly highlighted or 'tokenized' in promotional efforts through careful/deliberate messaging via cruise related media efforts.

Based on the information that we have gathered and experienced, we propose a lengthy list of considerations and recommendations for institutions and individuals to strongly consider actioning in order to ensure the emotional and physical safety of participants both prior to an expedition (Table 1) and during an expedition (Table 2). However, we additionally recommend that a range of actions would also be strongly beneficial to the entire community following an expedition, including;

- Request feedback from all participants relevant to personal safety and comfort, and use this feedback to address needs prior to the next expedition.
- Continue mentor relationships and peer-mentor circles after the expedition.
- Provide support forward – guidance for early career participants on how to add at-sea experience to their resume/CV, LinkedIn, and job interviews.
- Ensure resulting abstracts and publications include an inclusive authorship that appropriately reflects contributions to the research.
- Provide funds to register for, travel to, and participate in major conferences.
- Allow team members to control what information is publicly shared. For example, many cruises feature participant websites including photos and descriptions which remain online indefinitely after the cruise. If a participant chooses to transition gender, presentation, change pronouns, names, etc., an outdated website could create an uncomfortable reference to a past presentation that an individual may be uncomfortable having online and searchable, and they could be hesitant to undertake the process of trying to get it removed or changed. Making these sorts of pages easy for participants to update, having a set expiration, or having a longer term “opt-in” could alleviate this concern.

Summary

Much of the research regarding demographics in the ocean sciences, particularly at the faculty level, are broken down by gender and are not further disaggregated by racial or ethnic identity. Even so, these data follow the gender binary, and many surveys do not include information on LGBTQ+ scientists (Olcott and Downen, 2020). More research is needed to understand the demographics of ocean science professionals at all levels, sectors, and needs. Programs need to acknowledge that much more work is needed to address issues of racism, sexism, ableism, safety, and belonging in the field (Dutt, 2019; García-Gonzales et al., 2019; Marin-Spiotta et al., 2020), and work towards diversity, equity, inclusion and justice, not just to “check a box”. Furthermore, research programs and researchers need to confront and address the phenomenon of “parachute science” (Stefanoudis et al., 2021), and work in partnership with and with full inclusion and participation of members of Indigenous communities when conducting geographically or culturally relevant research (Ali et al., 2021). At all times, geoscience professionals, researchers, and students should raise the visibility of past and present ocean researchers from a variety of backgrounds and perspectives (Núñez et al., 2019; Olcott and Downen, 2020). Additionally, conversations regarding both visible and invisible disabilities should be initiated and sustained to bring the topic of accessibility in at-sea research expeditions to the forefront. This representation is needed for students and early career researchers to see themselves in this field. Throughout all this, there will need to be buy-in at all levels - from leadership, to chief scientists, to expedition coordinators, funding agencies, and those in positions of authority.

These recommendations, which are certainly not all encompassing, serve as a catalyst for onward conversations, and that further recommendations can be employed continually to ensure that oceanographers from minoritized groups are not pushed out of oceanography careers due to negative experiences at sea. Every person and every situation is unique. Only once our community fully embraces the breadth of cultures and experiences of all potential oceanographers can our field really be inclusive and exceptional. Additionally, while this manuscript relates to ship-based missions, many of the recommendations above could also apply for long-term, shore-based scientific missions including; travel to remote locations, shared living quarters, and back-to-back day-long cruises, all of which can yield harmful work environments, especially for people from minoritized groups (e.g., Langin, 2022; Woolston, 2022).

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

LuW: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. CA: Writing – review & editing. AF: Writing – review & editing. JH: Writing – review & editing. CM: Writing – review & editing. MM: Writing – review & editing. RQ: Writing – review & editing. CR: Writing – review & editing. AS: Writing – review & editing. KS: Writing – review & editing. LiW: Writing – review & editing. ACE: Conceptualization, Resources, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. Coauthor staff time was provided by NOAA Ocean Exploration directly and through the Ocean Exploration Cooperative Institute (OECI).

Acknowledgments

Thank you to the 2022 COBRA Master Class for insightful discussions regarding this topic. Thank you also to attendees of the AGU 2021 Fall Meeting and OSM 2022 for suggestions that have contributed to the manuscript and for many thoughtful discussions. We thank Dr. Genene Fisher, Dr. Jay Grove, and others for helpful comments on earlier versions of this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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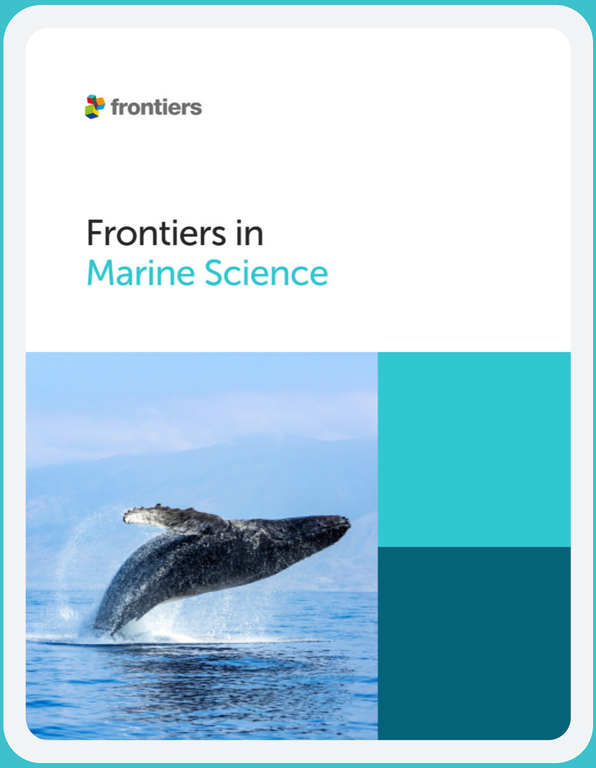
Appendix 1- Helpful resources

- ○ https://scripps.ucsd.edu/ships/preventing-harassment-and-discrimination
- ○ UNOLS Shipboard Civility Training: https://www.unols.org/shipboard-civility
- ○ RAINN National Sexual Assault Hotline and Chat: https://www.rainn.org/ Free, Confidential, Available 24/7, via Phone or Chat
- ○ NOAA SASH Prevention and Response Guidance
- ○ Sailing Guide for NOAA Ship *Okeanos Explorer* seagong participants. This includes information on shipboard culture, safety, expectations, key things to know about life at sea, and a suggested packing list.
- ○ How to find inclusive “Open to All“ businesses and restuarants via Yelp: https://blog.yelp.com/news/yelp-makes-it-easier-than-ever-to-support-inclusive-businesses-with-new-open-to-all-search-filter/



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