

Sample Strategy Plan; Outer Continental Shelf (OCS) Genomic Sample Strategy for the Bureau of Ocean Energy Management (BOEM) to Archive OCS Invertebrates



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December, 2018

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Prepared under M14PC00003

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DISCLAIMER

Study concept, oversight, and funding were provided by the US Department of the Interior, Bureau of Ocean Energy Management (BOEM), Environmental Studies Program, Washington, DC, under Contract Number M14PC00003. This report has been technically reviewed by BOEM, and it has been approved for publication. The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the opinions or policies of the US Government, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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CITATION

NMNH, Invertebrate Zoology. 2018. Sample strategy plan; outer continental shelf (OCS) genomic sample strategy for the Bureau of Ocean Energy Management (BOEM) to archive OCS invertebrates. Sterling (VA): US Department of the Interior, Bureau of Ocean Energy Management. OCS Study BOEM 2019-003. 10 p.

ABOUT THE COVER

The NMNH Sant Ocean Hall is a 23,000 square-foot permanent exhibition on the first floor of the Natural History Building that documents and describes the complexity of the earth's oceans. In August, a duplicate voucher specimen (USNM 185419) collected during the BOEM-CABP (Central Atlantic Benchmark Program) study was loaned to the NMNH Department of Exhibitions for display in the Sant Ocean Hall. The specimen from USNM 185419 is a Lady Crab, *Ovalipes ocellatus* (Decapoda, Brachyura, Geryonidae) and will be on display for the duration of the NMNH Sant Ocean Hall exhibit.

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List of Abbreviations and Acronyms

| | |
|---------|--|
| BOEM | Bureau of Ocean Energy Management |
| DNA | deoxyribonucleic acid |
| DOI | US Department of the Interior |
| ESP | Environmental Studies Program |
| GGBM | Global Genome Biodiversity Network |
| GGI | Global Genome Initiative |
| NMNH | National Museum of Natural History |
| NMNH-IZ | National Museum of Natural History, Invertebrate Zoology |
| NOAA | National Oceanic and Atmospheric Administration |
| OCS | outer continental shelf |

1 Introduction

The goal of this National Museum of Natural History – Bureau of Ocean Energy Management (NMNH-BOEM) Genomic Sampling Strategy document is to provide a roadmap for enhancing the global collection of tissue samples for genomic analyses, focusing primarily on marine organisms from BOEM outer continental shelf (OCS) lands. This strategy is conducted in collaboration with the Smithsonian’s Global Genome Initiative (GGI) and in accordance with the standards set forth by the Global Genome Biodiversity Network (GGBN) to ensure the security of derived genomic materials and the fidelity of associated metadata and linked information resources. Herein we review the history of the long-standing partnership between NMNH and BOEM, the purpose of this new genomic archival program, and the steps forward to increase the representation of OCS marine organisms in NMNH’s Biorepository. This plan is intended to be broadly distributed through presentations, conferences, publications, and other outreach efforts in order to successfully implement the proposed strategy.

1.1 Background on NMNH-BOEM Partnership

Under various Department of the Interior organizational structures, the Bureau of Ocean Energy Management Environmental Studies Program (BOEM ESP) has been conducting intensive environmental studies on the OCS for more than 35 years to support information needs for managing oil and gas development on the continental shelf and slope. Acquired information informs decision-making regarding OCS and slope developments. In its partnership of over 35 years with BOEM, the NMNH Department of Invertebrate Zoology provides professional collection management services for the long-term curation of marine invertebrate specimens taken during the environmental baseline surveys of various oil and gas lease sites on the US East and Gulf Coasts, and the US West and Alaskan Coasts. In this time period, more than 383,000 lots of sorted and identified material and 20,000 lots of unprocessed samples or mixed taxa have been received. These specimens represent one of the most extensive collections of marine organisms from the U.S. continental shelf and slope in terms of geographic coverage, sampling density (spatial and temporal), number of phyla represented, and associated data collected concomitantly (other organisms, chemical, hydrographic, geologic). These specimens are useful for a wide variety of taxonomic, evolutionary, and ecological applications. For example, they provide for new species discoveries (e.g., Desbruyères and Toulmond, 1998; Ivanov and Scheltema, 2008; Blake and Maciolek, 2018, etc.), refined taxonomic distribution data (e.g., Kilgour & Shirley, 2008; Ellis et al., 2011; Goddard et al., 2014; Lauth & Conner, 2014; Reuscher & Shirley, 2014, 2017 Chaudhary et al., 2017), and biodiversity baselines for ecological monitoring (e.g., Pie et al., 2015; Hawkins & Popper, 2017).

Over the last 15 years, it has become generally recognized that genomic approaches greatly enhance our ability to document and monitor biodiversity through space and time (e.g., Radulovici et al., 2010; Bucklin et al., 2011). Comparative DNA sequences provide objective, rigorous, and repeatable data for local and regional comparisons (e.g., Jennings et al. 2010), for presence/absence data (e.g., Leray & Knowlton, 2015), and for monitoring patterns of change (e.g., Comtet et al., 2015). Many of these genomic approaches to biodiversity studies and monitoring programs employ DNA barcoding (e.g., Kress et al., 2015; Costa & Antunes, 2012), the use of a short, homologous portion of an organism’s genetic code that provides species-specific identification. Thus, a critical facet for implementing DNA barcoding is to generate a voucher-based comparative reference database. To date, even 15 years after the advent of DNA barcoding, taxonomic coverage across most marine groups remains poor (see Section 2.1). Access to rare species and difficult to sample habitats lead the reasons for such poor representation, as well as general implementation of standardized protocols for specimen preservation and tissue subsampling.

DNA barcoding and taxonomic identification are not the only use of genomic and tissue derived data. If preserved adequately, the organism's genome can reveal exploitable evolutionary novelties (e.g., anti-freeze proteins, nitrogen fixation pathways, unusual enzymes), while their tissues and hard parts can be mined for chemical and isotopic data to record past ocean conditions and ecological relationships. Thus, high quality tissue preservation, curation, and storage are recognized priorities by NMNH's GGI. Appropriate tissue collection, handling, and preservation are critical to the use of these biomaterials for future studies that go beyond the phenotypes of the vouchered specimens.

Recognizing the value of a genomically augmented approach to better manage BOEM assets and enhance their ongoing OCS efforts, BOEM requested an expansion of the NMNH-BOEM partnership. The stated goals of this expanded program are to continue processing and cataloging voucher and archived biological specimens and materials while improving the representation of marine invertebrates in cryogenic and tissue collections. Accordingly, OCS marine organism genomic tissue samples will be acquired, cataloged, barcoded, and curated into NMNH's Biorepository. This will require changes in the handling and processing of specimens collected as part of ongoing BOEM projects. NMNH will provide guidance and best practice documentation to BOEM-funded partners to enhance the genomic utility of their derived biomaterials (see below). In addition, select specimens from historical (i.e., legacy) BOEM-collected material archived in the National Museum of Natural History, Invertebrate Zoology (NMNH-IZ) collections will be subsampled and assessed for genomic utility, following the prioritization strategy presented below.

A barcoding and genomic tissue Sampling Strategy Plan is presented herein. First, we present a review of the taxonomic gap analysis of existing tissues and genomic data for marine groups present in the four major management regions as set forth by BOEM: US East and Gulf Coasts, and US West and Alaskan Coasts. This provides a framework for assessing challenges and opportunities, formulating a strategic way forward, and providing metrics of success. Within this context, we present a strategy for assessing existing legacy collections already cataloged at NMNH or stored in other repositories derived from previous or recently completed BOEM studies or those in BOEM regions. We leverage our existing collections data management systems and prioritize unique collection events, localities, or species. Second, we present a strategy to increase genomic-grade tissues from ongoing BOEM-funded environmental studies and through building new partnerships with stakeholders working in BOEM regions. This involves direct communication with BOEM principle investigators (PIs), a record of their responses and willingness to participate, and a structure for documenting the supply chain. Based on stakeholder feedback and outreach efforts made to date to increase the flow of genomic resources to the NMNH Biorepository, we provide a decision-framework for determining and prioritizing high-value taxa, and propose targeted sampling strategies to augment and gap-fill current tissue holdings. Third, we review and propose a number of case studies that demonstrate the utility of an enhanced genomic approach for more informed decision-making. Finally, we present our strategy for ensuring the long-term security of our genomics resources.

2 Strategic Approach

2.1 Gap Analysis

In partnership with the GGI and using GGI's Biodiversity Data Tools, NMNH researchers produced a gap analysis of available marine invertebrate genomic resources across all GGBN members, including NMNH Biorepository holdings, as one of the earlier deliverables to this BOEM-NMNH program. The analyses culled all available taxonomic groups known to inhabit US coastal waters within the four main regions under BOEM jurisdiction and checked available holdings across all GGBN partners as well as published

genomic sequence data in GenBank. This analysis is meant to provide a state of knowledge document for the availability of global genomic resources across US waters and provide a framework for prioritizing collecting and sampling efforts and conducting genomic analyses. The subsequent analysis demonstrated the lack of coverage across marine groups and the potential of BOEM assets to contribute toward bridging these shortcomings (summarized in Tables 1A and 1B and Figure 1).

Table 1A. State of genomic curation for families of marine invertebrates from US regional waters.

| Phylum | # Families | # missing | % missing within phylum | # in hand | % in hand within phylum |
|-----------------|------------|-----------|-------------------------|-----------|-------------------------|
| Arthropoda | 496 | 428 | 86.3% | 78 | 15.7% |
| Mollusca | 435 | 349 | 80.2% | 158 | 36.3% |
| Cnidaria | 175 | 169 | 96.6% | 63 | 36.0% |
| Echinodermata | 110 | 82 | 74.5% | 62 | 56.4% |
| Bryozoa | 109 | 109 | 100.0% | 40 | 36.7% |
| Platyhelminthes | 97 | 96 | 99.0% | 0 | 0.0% |
| Porifera | 94 | 93 | 98.9% | 5 | 5.3% |
| Annelida | 87 | 59 | 67.8% | 27 | 31.0% |
| Nematoda | 73 | 73 | 100.0% | 0 | 0.0% |
| Minor phyla | 133 | 130 | 97.7% | 15 | 11.3% |
| Total | 1,809 | 1,588 | 87.8% | 448 | 24.8% |

Table 1B. State of genomic curation for genera of marine invertebrates from US regional waters.

| Phylum | # Families | # missing | % missing within phylum | # in hand | % in hand within phylum |
|-----------------|------------|-----------|-------------------------|-----------|-------------------------|
| Arthropoda | 1,837 | 1,734 | 94.4% | 131 | 7.1% |
| Mollusca | 1,904 | 1,794 | 94.2% | 341 | 17.9% |
| Cnidaria | 532 | 526 | 98.9% | 133 | 25.0% |
| Echinodermata | 364 | 329 | 90.4% | 135 | 37.1% |
| Bryozoa | 252 | 252 | 100.0% | 51 | 20.2% |
| Platyhelminthes | 213 | 212 | 99.5% | 0 | 0.0% |
| Porifera | 243 | 242 | 99.6% | 5 | 2.1% |
| Annelida | 709 | 656 | 92.5% | 74 | 10.4% |
| Nematoda | 173 | 173 | 100.0% | 0 | 0.0% |
| Minor phyla | 261 | 258 | 98.9% | 17 | 6.5% |
| Total | 6,488 | 6,176 | 95.2% | 887 | 13.7% |

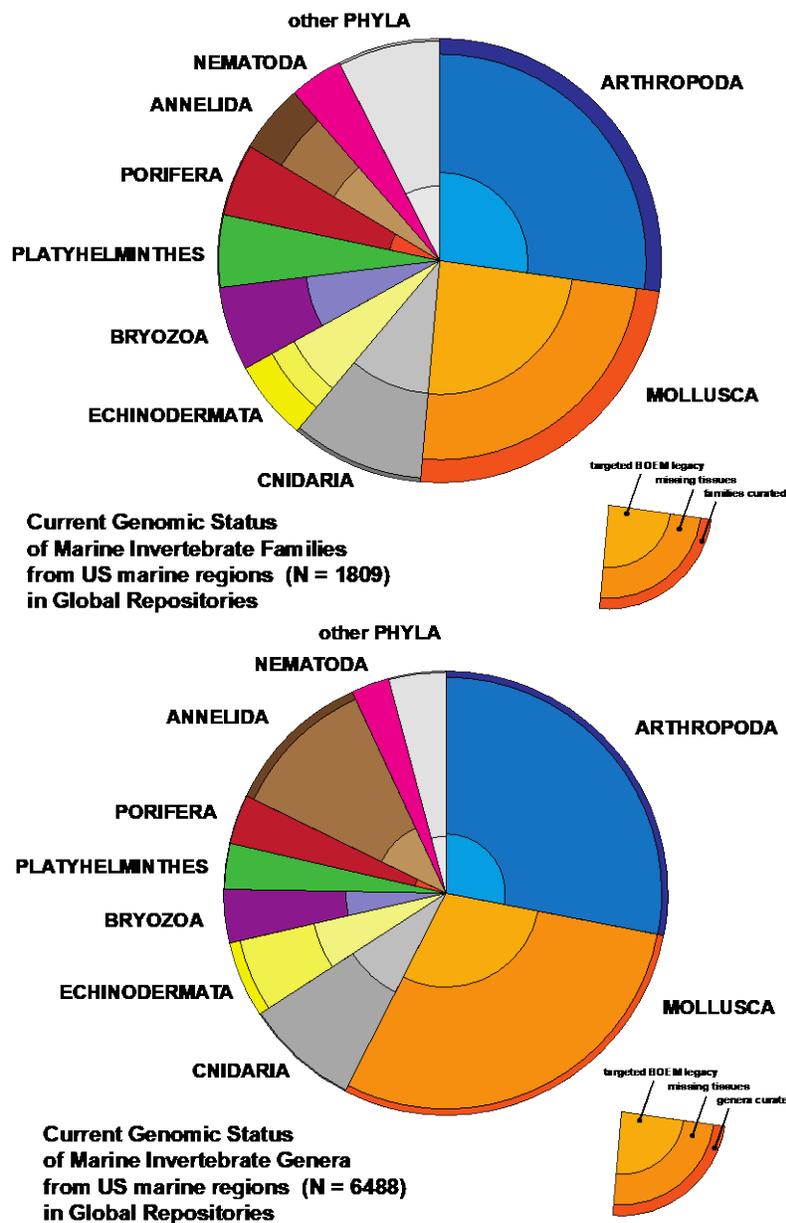


Figure 1. Gap analysis.

Current genomic status of marine invertebrate families and genera from US marine regions in global repositories.

Currently, 1,809 marine invertebrate families have been found in BOEM jurisdictional waters. Of these, only 221 families are represented in global tissue collections with available sequence data in GenBank. These families and genera are represented by the outer darker rings within each phylum slice on Figure 1. In other words, 88% (almost 9 out of 10 marine invertebrate families) have no genomic representation in public repositories. Nearly half of these missing families are either Arthropoda or Mollusca (49%), highlighting the potential emphasis on those two phyla for better taxonomic coverage across marine lineages. At the genus level, the unrepresented proportion is even larger, with 95% (6,176 of 6,488) of all marine invertebrate genera having no genomic representation in global repositories. Similarly, 57% of all missing genera are from the Phyla Arthropoda and Mollusca.

These analyses expose the poor taxon sampling across a broad swath of marine invertebrate diversity found in US waters and highlight the added value of increased sampling for tissue curation of marine invertebrates in order to reach GGI's goal of having curated tissues for all families on the tree of life.

2.2 Legacy Collections

The inner lighter ring for each phylum in Figure 1 represents specimens from high-value legacy collections from BOEM projects over the last ten years that currently exist in NMNH collections. These vouchered specimens were acquired as part of the previous non-genomic prioritized BOEM-NMNH partnership but are more likely to yield genomic data because they are recently collected (within the last 10 years) and are either dried or preserved in ethanol. We call these legacy BOEM collections because they were acquired prior to emphasis on genomic tissue collections and as such represent fortuitous opportunities to fill existing gaps in tissue curation and sequence coverage without recollection. Because these legacy materials were collected without special attention toward preserving genomic assets, we cannot count on them providing viable or high-grade DNA, but they provide a prioritization strategy for sampling our existing BOEM assets. The goal of this portion of the sampling strategy is to use these cross-referenced analyses to fill the uncurated wedges in Figure 1 from the inside out, based on these existing BOEM biomaterials and their ability to fill unrepresented portions of existing knowledge. Over the next year, BOEM-funded staff will pull tissues from these prioritized legacy specimens, extract and quantify the DNA (e.g., Mulcahy et al., 2016), and attempt to generate DNA barcodes for these vouchered, high-value target specimens. These BOEM legacy collections have the potential to fill almost one-quarter of all missing families (24.8%) and 13.7% of all missing genera (Table 1B). The strategic challenge ahead to meet the mission of the contract is to fill the gap strategically between these two rings—"mind the gap".

Other high-value legacy collections that will be targeted in this phase include:

1. The Ocean Genome Legacy samples from the National Antipatharia (Cnidaria, black coral) collection, including BOEM-collected specimens and material from BOEM regions
2. Marine invertebrates from the Natural History Museum of Los Angeles with special emphasis on subsampling and barcoding their legacy BOEM-collected specimens
3. A recently acquired large Crustacean collection from University of Louisiana at Lafayette that includes BOEM-derived specimens
4. Cnidaria and Porifera collected by Robert Stone (National Oceanic and Atmospheric Administration [NOAA]) from the Alaska Aleutians
5. Vent and seep fauna collected by Robert Vrijenhoek (Monterey Bay Aquarium Research Institute) and Charles Fisher (Penn State University)

2.3 Building Partnerships and Expanding Acquisitions

In order to grow the collection of genomic-grade tissue and fill the missing portions of taxonomic space revealed through the gap analysis, we have set up an aggressive public outreach strategy with existing BOEM ESP studies. BOEM-funded environmental studies represent unique opportunities to catalog rare and difficult to access habitats and biotas. As mentioned previously, BOEM specimens collected over the last 10 years might provide up to one-quarter of all missing families from US waters. With enhanced and focused effort, and our proposed outreach and sampling strategy, we expect to push those numbers to increase genomic representation to at least half of the missing marine families over the course of the next five years. Over the course of the next year, we will contact the PIs of all of the current and recently completed BOEM ESP studies in the four OCS regions (Alaska, Atlantic, Gulf of Mexico, and Pacific) that may generate specimen vouchers. We will record all efforts to garner specimens and genomic-grade

material, including, but not limited to, the date of first communication, the person to whom the request was sent, the date of a response or the date of a follow up email if no response was received, the nature of the response, and the characterization of the engagement. To take advantage of genomic and barcoding viability of voucher specimens and tissues, it is important to engage BOEM partners prior to the collecting events, if possible, in order to assure appropriate handling and preservation of the vouchers and biomaterials. Working early and directly with BOEM studies represents a cost-effective strategy, where NMNH-IZ provides direct logistical support to assist with any genomic augmentation of BOEM ESP studies. Progress on these inquiries will be presented in the NMNH-IZ BOEM project Quarterly Reports.

In addition to the specific pursuit of all BOEM ESP studies, we will collaborate with taxonomic specialists, available networks (including already ongoing partnerships with the Western Association of Marine Laboratories and National Association of Marine Laboratories), and “vessels of opportunity” (e.g., NOAA, institute, and university cruises) to gather available genomic-grade tissue. Although BOEM-funded projects will be prioritized, we will continue to accept genomic-grade tissues for missing taxa and regions through this federal effort to genomically characterize our nation’s marine invertebrate realm.

2.4 Case Studies

The genomic data generated under this project will allow more informed decision-making for BOEM OCS management activities. The following are several case studies that demonstrate the utility of an enhanced genomic approach under the NMNH-BOEM partnership.

2.4.1 Commercially Important Species

Utilizing the checklists from NOAA Fisheries and the US Food & Drug Administration Seafood list, we will prioritize tissue sampling and barcoding for all commercially important taxa. Where available, we will partner with existing taxonomic or regional centers to collect these high-value targets. For instance, we have partnered with an US Food & Drug Administration scientist to tissue sample and barcode economically important Crustacean taxa. Tissue samples and barcodes will be deposited in the NMNH Biorepository, and voucher specimens will be deposited in the NMNH-IZ collections. During the project year, we will collaborate and network via national and international taxonomic meetings with various sources to tissue sample and barcode the economically important Mollusca (mostly squid, bivalves, and snails).

2.4.2 Invasive Species Monitoring

Understanding the extent and patterns of invasive species is an important component of managing the OCS and securing our national waters. We are working with Dr. Greg Ruiz (Smithsonian Environmental Research Center) and other coastal partners to tissue sample and barcode the invertebrate species on the National Exotic Marine and Estuarine Species Information System database. In addition, NMNH, Department of Invertebrate Zoology partnered with Smithsonian Environmental Research Center and the Smithsonian GGI to collect, tissue sample, and barcode marine invertebrates from Alaska’s largest fishing port of Dutch Harbor/Unalaska, located at the east end of the Alaska Aleutians. The Alaska Aleutians are an area of high marine biodiversity and endemism, but are also at high risk of change and introductions from non-native species. Endemic, invasive, and underrepresented groups have been collected, tissue sampled, and are being barcoded as part of the NMNH, Department of Invertebrate Zoology, Smithsonian Environmental Research Center, and GGI partnership in Dutch Harbor, Alaska.

2.4.3 Cryptic Species

Resolving multispecies complexes and assisting with identifications of taxa is another goal of our partnership, especially for problematic taxonomic groups used frequently in impact assessment studies and surveys. In consultation with local stakeholders, NMNH, along with Western Alliance of Marine Laboratories, Southern California Coastal Water Research Project, and the Natural History Museum of Los Angeles, has launched a collaborative project as a case study to tissue sampling and barcoding Ampeliscid amphipods, Tellinid bivalves, and Sphaeromatid isopods. These three groups of marine invertebrates were identified and prioritized by our west coast (California to Alaska) partners because they are often encountered and used in benthic assessment surveys and environmental programs but are difficult to identify and have potential invasive lineages. The addition of molecular data will clarify species delimitations and geographic ranges and highlight specific morphological features to assist with field identifications in the future. Such partnerships with ongoing environmental programs will be prioritized as part of this strategic plan in order to better serve stakeholders and maximize the return on investment for genomic tissue sample acquisition funded through this BOEM task.

2.5 Ensuring the Security of Our Genomics Resources

Existing NMNH infrastructure, resources, collections management information systems and institutional policies ensure the security of existing genomic tissue samples and the fidelity of associated information, or metadata. NMNH's Biorepository was inaugurated in 2011 and is currently the largest museum-based biorepository in the world. Its mission is to provide excellent stewardship of frozen collections housed in the Biorepository and to facilitate their use. The Biorepository primarily holds NMNH non-human animal, bacterial, and plant genetic resources, including both archived tissues and extracted genomic DNA; it has been augmented to store environmental samples as well. It is operated under the auspices of NMNH according to the terms of Smithsonian Institution and NMNH policies on collections, safety and security.

NMNH collections policies were revised in April 2012 and can be found at https://naturalhistory.si.edu/rc/biorepository/docs/NMNH_CMP_final_2017.pdf. Policies specific to

NMNH's Biorepository can be found at https://naturalhistory.si.edu/rc/biorepository/docs/NMNH%20Biorepositoy%20Standards%20and%20Services%20May%202012_5_29.pdf. Both mechanical and liquid nitrogen freezers can store up to 5 million cryovials.

All freezers in the Biorepository are continuously monitored by an electronic system that calls responders during an outage or other emergency. This monitoring system is also accessible via a secure web application. As with previous voucher specimens, all BOEM tissues and genomic samples (DNA extractions) will be catalogued using NMNH's collections database management system (Axiell-EMu), and biorepository samples will be tracked using our customized FreezerPro management system that is linked to Emu. These collections standards are unparalleled and guarantee the long-term security and utility of the curated biomaterials.

Moreover, NMNH is the seat of the GGI with a mission to preserve and understand the genomic diversity of life on earth. All biomaterials associated with this NMNH-BOEM program will be part of this broader endeavor to capture the earth's genomic biodiversity, professionally cryo-preserve representative samples, and make them accessible through the GGBN. GGBN, GGI, and NMNH work together to ensure that all biomaterials are acquired legally, exchanged under appropriate Material Transfer Agreements, transported without degradation, and made available to the research community. For example, NMNH has recently

signed a wide-ranging Memorandum of Understanding with Natural History Museum of Los Angeles to exchange tissues for extraction and deposition in NMNH's Biorepository. These materials include marine invertebrates from BOEM-funded programs. Because NMNH has been at the forefront of these global efforts, BOEM materials will have instant exposure and be immediately available to the research community. Existing workflows and informatics pipelines submit unembargoed sequence data monthly to GenBank following required metadata contextual standards for the global user community.

3 Conclusion

In summary, this genomic sample strategy plan provides a roadmap to increase the representation of OCS marine organisms in GGBN by filling existing taxonomic gaps in global repositories. BOEM-funded ESP studies represent significant, and often unique, opportunities to provide missing tissues and genomic representation in our national genomic collections and reference databases. Better taxonomic scope and genomic coverage will enhance agency and research capacity. We will employ a multi-tiered strategy, targeting missing, high-value legacy collections, increasing the supply chain through increased interactions with ongoing BOEM environmental programs, and prioritizing taxa of specific interest based on stakeholder requests for commercial relevance, augmented impact assessment, or taxonomic refinement. We have an established system of record and partnership with GGI and the GGBN to monitor progress toward these goals. Moreover, NMNH maintains the premiere national biodiversity tissue repository and policies in place ensure the long-term security and accessibility of these national resources.

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Department of the Interior (DOI)

The Department of the Interior protects and manages the Nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors the Nation's trust responsibilities or special commitments to American Indians, Alaska Natives, and affiliated island communities.



Bureau of Ocean Energy Management (BOEM)

The mission of the Bureau of Ocean Energy Management is to manage development of U.S. Outer Continental Shelf energy and mineral resources in an environmentally and economically responsible way.

BOEM Environmental Studies Program

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