

Environmental Studies Program: Ongoing Study

Field	Study Information
Title	Continued Partnership with National Museum of Natural History, Department of Invertebrate Zoology (NMNH-IZ) for a Voucher-based, Genomic Reference Facility for Ocean Biodiversity (NT-20-06)
Administered by	Office of Environmental Programs
BOEM Contact(s)	Jonathan Blythe (jonathan.blythe@boem.gov)
Procurement Type(s)	Interagency Agreement
Conducting Organization(s)	Smithsonian, National Museum of Natural History, Invertebrate Zoology
Total BOEM Cost	\$ 1,700,000
Performance Period	FY 2020–2024
Final Report Due	N/A
Date Revised	October 23, 2023
Problem	Marine invertebrates from benthic and pelagic habitats that are impacted by Outer Continental Shelf (OCS) energy development and federally permitted marine minerals mining are an important economic resource for OCS fishing communities and play a significant role in the ecological function of these habitats.
Intervention	Marine invertebrates have been used for over 100 years as a proxy to assess environmental conditions and cumulative effects, and detect natural and anthropogenic impacts. BOEM's expanding OCS development activities across all of its program areas will need this rigorous collections based research to facilitate better understanding of biological diversity and help characterize ecological impacts from these OCS activities.
Comparison	BOEM's OCS regions are largely unique for their marine fauna. Some marine invertebrates that were previously thought to be pandemic throughout more than one OCS region may in fact include a cryptic species in part of this range. Marine animal distributions are shifting in response to global climate change. Invasive species are a growing component of OCS invertebrate communities. These comparisons of OCS marine fauna can only be deciphered if researchers are able to accurately classify biological samples.
Outcome	Cumulative knowledge of marine invertebrate taxa developed through collections based research will improve the rigor of BOEM's studies of ecosystem functions and assessments of species diversity.
Context	All OCS regions.

BOEM Information Need(s): This study leverages capabilities and resources provided by NMNH-IZ to improve the rigor of biological specimen identification on Environmental Studies Program (ESP) studies. This occurs directly through NMNH-IZ staff assisted curation of biological specimens from ongoing studies. Expanded acquisitions of OCS specimens from these and other sources will improve the baseline of biological materials and enable innovation in OCS research and monitoring approaches. NMNH-IZ will

champion NMNH-BOEM OCS Genomic Sample Strategy that will specifically target key OCS invertebrate taxa that are invasive species, species critical to seafloor impact assessment, and to fill important taxonomic knowledge gaps that have been identified about the OCS environment (NMNH-IZ, 2019).

Background: Over the past 40 years, while the ESP has been conducting intensive environmental studies on the OCS, the NMNH-IZ has partnered with the ESP to provide professional collection management services for the long-term curation of marine invertebrate specimens. For example, the NMNH-IZ houses biological specimens collected on many baseline surveys that ESP conducted on the U.S. Atlantic and Gulf of Mexico OCS, and the U.S. Pacific and Alaskan OCS. 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).

NMNH-IZ specimens collected under this partnership 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). However, over the last 15 years, genomic analyses have become an increasingly important use of biological samples in this collection. Genomic approaches can greatly enhance our ability to document and monitor biodiversity through space and time (e.g., Radulovici et al., 2010; Bucklin et al., 2011). Comparative deoxyribonucleic acid (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).

NMNH-IZ provides a voucher-based comparative reference facility for biodiversity studies and monitoring programs that employ DNA barcoding (e.g., Kress et al., 2015; Costa & Antunes, 2012). This type of research analyzes a short, segment of genetic code that is homologous across a wide variety of biological specimens to provide more robust species-specific identification. This genetic technique enables researchers to sample biological diversity in a variety of ways that does not depend on the organism's phenotype (e.g., gut contents analysis, tissue traps, morphologically indistinct specimens, or environmental DNA [eDNA]). However, future research with this collection will not be limited to this approach, as other research may focus on genetic loci to enhance the resolution of population or individual units, or analysis of functional genes, such as anti-freeze and nitrogen fixation proteins, to answer more targeted ecological questions. Therefore, genomic analyses of the NMNH-IZ collections are becoming an increasingly important contribution of ESP's investment in biological specimen archiving.

Despite the growing importance of genomic techniques in the marine sciences, DNA barcoding coverage of most marine taxa remains poor for a variety of reasons. First, invertebrates are very specious, and they represent a disproportionately large component of OCS biodiversity. Second, it is generally recognized that biodiversity has a long tail, and it is not typically possible to represent species biodiversity except for concerted efforts targeting specimens from habitats or from taxonomic groups that are of particular interest. Finally, in many cases, it can be logistically difficult to collect specimens and tissue samples from benthic and pelagic OCS environments (Templado et al., 2010). Dedicated expertise and facilities for tissue collection, handling, and preservation will help ensure the viability of

biomaterials from ESP field research. Further, continued stewardship and strategic expansion of the OCS collection will ensure that the ESP has the framework to effectively leverage innovative research and monitoring techniques in the future.

Objectives:

- Strategically reinvest in and modernize ESP's biological specimen collecting to provide a reference facility for future OCS research and monitoring efforts.
- Ensure accurate and consistent scientific information from the biological sciences through contribution of specimens from ESP studies and strategic acquisition of specimens from other sources in the marine science community in order to address key information/knowledge gaps regarding OCS biodiversity.
- Provide the baseline of biological information needed to consider cumulative impacts of offshore energy and mineral resources decision making from issues of increasing environmental concern, such as climate change and invasive species.

Methods: Partner with Smithsonian to leverage expertise and facilities of the NMNH-IZ. Specimens archived through this study will be curated, using NMNH-IZ's taxonomic expertise in OCS invertebrate species; and preserved, using NMNH's world class collection facilities at the Museum Support Center in Suitland, Maryland (NMNH-IZ, 2014). Further, tissue samples will be subsampled from OCS invertebrate specimens, sequenced, and cryogenically preserved in the NMNH biorepository. NMNH-IZ will work with regional institutions who have specific expertise in OCS invertebrates and establish memoranda of understanding (MOUs) to work effectively towards shared goals.

The ESP will strategically enhance OCS biological specimen collections at the NMNH-IZ, following the NMNH-BOEM OCS Genomic Sample Strategy (NMNH-IZ, 2019), including: 1) working with legacy collections at the NMNH and other museum repositories to subsample tissue for genomic sample preservation; and 2) pursuing targeted specimen acquisitions through collaboration with BOEM ESP researchers and other marine scientists.

This study will assure the quality of biological samples by preserving, curating and storing biological specimens and associated viable genomic tissue, following standards approved by the Global Genome Biodiversity Network (GGBN) and protocols required by the NMNH-IZ for biological sample curation and database documentation. Information about phenotypes and DNA barcodes of OCS invertebrates will be disseminated through NMNH-IZ and GGBN websites and scientific publication outlets. The ESP will contribute to oversight, study Principal Investigator collaboration, information dissemination, and the strategic focus of NMNH-IZ's OCS collection, particularly with respect to National Environmental Policy Act (NEPA) decision analysis support, to best leverage this facility for ESP's biological sample archive.

Specific Research Question(s):

1. How can ESP improve the quality of biological information used in environmental assessments of benthic and pelagic OCS habitats affected by agency activities by strategically collaborating on fieldwork campaigns from ongoing studies and quality assuring the identification of invertebrates?
2. How can the NMNH provide a baseline of information to facilitate the broader adoption of genomic techniques and enable faster and more effective use of taxonomic information in BOEM studies and assessments?

Current Status: In the first year, the BOEM specimen archiving project produced 893 cytochrome oxidase I (COI) sequences and cataloged 656 invertebrate specimens. Over the past year, it produced an additional 1,725 COI sequences and cataloged 356 invertebrate specimens.

The period of performance was extended for six months because of setbacks to the project due to COVID. The Museum Support Facility subsequently reopened and became fully operational.

This year, the NMNH-IZ began implementing and refining a new sequencing technique called genome skimming, which will capture the sequences for the 13 mitochondrial protein-coding genes (COX1, COX2, COX3, CYTB, ND1, ND2, ND3, ND4, ND4L, ND5, ND6, ATP6, and ATP8) and two ribosomal genes 16S (rrnL) and 12S (rrnS). The idea in implementing this new method is that the NMNH-IZ can generate sequences of multiple loci simultaneously, and this technique may work better for DNA extracted from preserved specimens that have sheared DNA fragments rather than intact DNA that are more suitable substrate for traditional DNA barcoding methods. Genome skimming is more expensive than COI gene barcoding, but a pilot effort is permissible given the remaining budget and staff availability under the existing agreement.

Genome skimming was performed on 33 anthozoans from the Deep Sea Exploration and Research of Coral/Canyon/Cold seep Habitats (DEEP SEARCH) expeditions, and 135 invertebrate taxa from the NOAA Fisheries and Smithsonian's Illuminating Biodiversity in Deep Water of Puerto Rico expedition. Another 235 samples were comprised of traditionally understudied meiofauna. The final batch of 144 samples consisted of morphologically verified invasive species. The NMNH-IZ has made progress towards optimizing this method and seeks to improve library preparation methods and analysis pipelines in the coming year.

The NMNH-IZ also seeks to enhance its efforts to bring in new samples from ongoing environmental study PIs, and the effort to apply genome skimming to historical specimens that have degraded DNA will be balanced with BOEM's priority to sequence high quality, viable genetic material from recently collected and properly preserved samples. BOEM will take a closer look at these results at the end of 2024 and evaluate whether to continue the collections research under a planned new agreement.

Publications Completed: None

Affiliated WWW Sites:

<https://naturalhistory.si.edu/research/invertebrate-zoology/boem>

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