Project Title: Assessing the impacts of offshore wind development with marine eDNA: an innovative, non-extractive approach for monitoring protected, prohibited, and commercially / recreationally important species

Investigator(s) name(s) and institution(s):

Lead Principal Investigator:
Dr. Jason E. Adolf
Endowed Associate Professor of Marine Science
Monmouth University
400 Cedar Avenue, West Long Branch, NJ
Email: jadolf@monmouth.edu
Phone: (732) 263-5687

Co-PI:
Dr. Keith J. Dunton
Associate Professor
Monmouth University
400 Cedar Avenue, West Long Branch, NJ
email: kdunton@monmouth.edu
Phone: (732) 571-4432

Co-PI:
Dr. Shannon O'Leary
Assistant Professor
St. Anselm College
100 Saint Anslem Drive, Manchester, NH 03102
Email: soleary@anslem.edu
Phone: (603) 641-7000

Project Managers: Colleen Brust (DEP-MRA) and Caitlin McGarigal (DEP-DSR)

Geographic Scope: Primarily focused on the Mid-Atlantic Bight region specifically coastal New Jersey from 0 – 40 miles offshore.

Timeline: 2 yrs. Starting ASAP.

MOTIVATION / OBJECTIVE

The primary objective of this project is to create an ecological and methodological baseline to identify potential impacts of offshore wind development on surrounding marine fish communities and the fisheries that rely on them. Traditionally, trawling and other extractive techniques are used to monitor benthic communities and contribute to long-term data sets comprising community composition and population-specific data including age structure, condition, and life history. However, construction of wind turbines provides unique challenges as sampling at high spatio-temporal scales can be cost prohibitive and safe access to impacted areas with trawling gear in the direct vicinity of these structures will be limited. By contrast, the use environmental DNA (eDNA) is a non-extractive approach that allows us to determine the community composition and relative abundance of species but also increases the likelihood of detecting rare species. In addition, the sample process is less costly (e.g., can utilize less staff, and smaller vessels) and requires little equipment. Therefore, eDNA is uniquely positioned to play a central role in adapting existing trawl surveys to assess impacts of offshore wind during and post-construction. However, as an emerging technique it is necessary to provide continuity with existing techniques which requires coupling eDNA and traditional extractive methods during the pre-construction phase to enable this shift.

This pre-construction project will provide foundational information needed to detect and understand changes in commercial and recreational fish populations and communities that may result from offshore wind development. Collecting eDNA alongside existing New Jersey Department of Environmental Protection Marine Resource Administration (NJDEP MRA) fisheries surveys will further inform calibration of eDNA with capture surveys, while sampling of eDNA from new, key locations will provide data critical for informing causation of changes in fishery metrics, including changes that may result from offshore wind development.

This project directly addresses the NJ Research and Monitoring Initiative Research Priorities:

- 7. Examine the effects of OSW on the distribution/connectivity of fish and invertebrate species and communities.
- 12. Adapt DEP trawl survey design to allow for comparison of biases/limitations in and outside of OSW development areas and calibrate new time series.
- 14. Develop and implement methods to assess impact of OSW on recreational fisheries.

The work proposed in this project addresses two broad goals:

(1) Employ eDNA within an experimental design framework to measure ecological baseline conditions at two permitted offshore wind lease areas (Ocean Wind 1, Atlantic Shores), setting the stage for continued sampling that can detect potential impacts of offshore wind development separate from natural spatial (e.g. cross shelf gradients) and temporal (e.g. seasonal, interannual, climate change) variability.

> *The community analyses inherent to eDNA work will address NJ RMI priority #7. The experimental design within which baseline data collection occurs must be carefully considered so that measurements made during / after construction (in*

subsequent studies) can detect potential changes due to offshore wind development against the backdrop of natural spatial / temporal variability.

(2) Further develop and refine eDNA as a tool for monitoring the impacts of offshore wind development on marine fish communities by pairing eDNA sampling / analyses with ongoing NJDEP capture surveys, and acoustic telemetry arrays (already funded by RMI) to establish a methodological baseline for broader application of the technique.

Collecting eDNA samples concurrently with long-running MRA surveys is the most efficient way to calibrate eDNA with and adapt existing surveys that may be excluded from windfarms, addressing NJ RMI priority #12 and #14 above.

Background

The fish community composition of New Jersey coastal waters and the economic benefits provided by the fisheries they support are the direct result of the current state of the regional continental shelf ecosystem. A concern shared by commercial and recreational fishers, as well as resource managers, is that alterations of the physical habitat of this ecosystem by offshore wind development will change fish community composition, fishing opportunities, and the economy. Well-designed and resilient survey methods that include data collected consistently before, during, and after construction of wind farms are essential to understanding any such impacts of offshore wind development on marine fish community composition.

The marine ichthyofauna of New Jersey consists of at least 336 unique fish species (NJDEP EBS Report 2010) with about 214 species being captured within the nearshore waters less than 30m depth (Levesque 2019). Over 100 species of fish and shellfish are landed commercially within the 6 major fishing ports in NJ, while approximately 20 species are primarily captured by recreationally fisheries throughout the state. Many of these species are of high economical value, with New Jersey commercial/recreational fisheries and aquaculture contributing over $1 billion annually to New Jerseys economy (NJDEP EBS Report 2010).

Temporal and spatial distributions and abundance of fish species within New Jersey coastal waters are largely controlled by environmental conditions, including temperature, salinity, and dissolved oxygen along with other factures such depth, prevailing currents, and day length. Here, many fishes exhibit migratory behavior typically southerly movements in the fall and northerly movements in spring and/or inshore/offshore movement as conditions change (Rothermel et al. 2020). The New Jersey Coast represents a key region in this North-South migratory corridor for many fish populations undergoing seasonal migrations. As it located between the two major river systems (Delaware River and Hudson River), it is particularly important for anadromous fish species, including Atlantic Sturgeon, Striped Bass, and River Herring that undergo extensive migrations along the Mid-Atlantic Bight; northward in the spring, and southward in the fall. Further, this coastline is essential in the migratory routes of coastal sharks (Sandtiger sharks, Sandbar sharks, Dusky sharks, and White sharks) and other elasmobranchs (Atlantic Angel Shark, Roughtail Stingrays, Winter Skate, Little Skate, Smooth Dogfish, and Spiny Dogfish) which are data-limited within this region. Finally, other commercial-recreational species (e.g., summer flounder, monkfish) exhibit seasonal inshore-offshore migrations across the shelf in this region. As a result, this region represents a dynamic

area that continues to experience shifts in species distributions likely due to climate change. A greater "influx" of southern species as well as a northward shift of those species associated with cooler water, is observed in a variety of species and has been documented in both larval (Sciaenidae) and adult life stages (Blacktip sharks). The study design proposed here is intended to enable disentangling various causes of observed shifts in the fish community composition, primarily shifts due to changing environmental conditions compared to the impact of offshore wind turbines.

Like all organisms, marine fishes shed genetic material as cellular and extracellular material through the extraction of bodily fluids and by sloughing off skin cells, scales and other tissue. This environmental DNA (eDNA) can be captured from an environmental sample such as water to detect species' presence in an ecosystem (Barnes and Turner 2016). Interest in exploiting this capability as a new tool in the fisheries monitoring 'toolkit' has increased in recent years. Initially, it was used only for species detection, however, recently studies coupling capture surveys with simultaneous eDNA sampling have additionally yielded comparable results of species composition, both for number of observed species and relative abundance (Thomsen et al. 2016, Stoeckle et al. 2020). Specifically, comparing results from eDNA samples taken during the New Jersey Marine Resources Fisheries Administration (MRA) Ocean Trawl demonstrated that fish diversity measured in 1 L of water was the same as or higher than a single trawl tow and that there was a strong correlation in relative abundance of fish species; these studies are gaining worldwide attention (Stoeckle et al. 2020, Stoeckle et al. 2022).

Overall, the advantages of eDNA include (1) relatively low cost and high throughput compared to capture methods; (2) the fact that it is a non-extractive and non-lethal method of censusing marine fish populations; (3) simple sampling methods that require smaller vessels and allow for better access to habitats that cannot be trawled; and (4) the dispersed nature of eDNA in the ocean compared to the patchy distribution of fishes leading to more detection of rare species (e.g. endangered Atlantic Sturgeon) despite less sampling effort. As a result, eDNA surveys are poised to play a central role monitoring the effects of offshore wind turbines on fish assemblages especially during construction and once turbines are operational and access to certain sites with trawling gear will become increasingly limited. Additionally, the lower cost, more simple and less time-consuming sampling protocols combined with faster processing will enable sampling at higher resolution, which is especially important to understanding temporal and spatial variability and being able to evaluate impacts of offshore wind against a background of other influences of fish community composition including natural spatio-temporal variability and climate change.

As an emerging technique it is important to consider differences in the "gear selectivity" and sampling of eDNA surveys compared to those of more established surveys methods and how this effects the study design and interpretation of the data. For example, analysis of trawling surveys must account for factors such as net avoidance, tow times, and net sizes, while acoustic telemetry detections is highly dependent on the number of acoustic tagged fish in a region, tag power, and array configuration. In both cases, the method creates bias in what the results look like, and we account for this in how we design studies, and we generally do not base policy-decisions on just one method to compensate for this gear bias and how it affects our understanding of community composition and factors affecting it. With eDNA being a more recently developed technique, reviews of the literature indicate that in the last ten years the first five were focused on

developing and optimizing techniques and more recently the number of publications implementing eDNA in biomonitoring has rapidly increased and the ecology of eDNA and best practices are increasingly well understood (Schenekar 2022).

Community assessments using eDNA compared to trawls will give a snapshot of the community that is integrated over more space and time compared to a trawl with the benefit of more species being detected, including more rare and elusive species and species with more patchy distributions. Unlike trawl surveys which only capture individuals in the area towed, experiments suggest that in marine environments eDNA is detectable for ~48h (Collins et al. 2018) and detections of eDNA may result from 1 to 10's of kilometers away from the source, depending on prevailing currents (Shea et al. 2022, Andruskiewicz et al. 2019). As a result, detections represent recent presence of a given fish species over a certain spatial and temporal scale.

To account for and better understand these differences, it is necessary to initially implement coupled surveys that pair eDNA analyses with traditional techniques to further constrain and understand the relationships between these methods (Stoeckle et al. 2020, Hinz et al. 2022). Collecting eDNA samples concurrently with long-running MRA surveys is the most efficient way to calibrate eDNA with existing long-term gear-dependent surveys that may be excluded from turbine areas in the future windfarms to allow for continuous monitoring during and after construction phase. As a result, our proposed study will produce a biological/ecological baseline of fish communities pre-construction (goal #1), while simultaneously laying the methodological foundation for continuity during construction and once turbines are operational in the generated data sets when restricted access forces a shift to eDNA only sampling (goal #2) and away from methods that require larger vessels and more gear.

A distinct shift from developing and optimizing eDNA tools to integrating it as a standard approach in biomonitoring programs has already been observed in freshwater systems (Schenekar 2022). The growing excitement for the same to occur in marine systems is built around the potential impact this tool can have especially in terms of spatial resolution, access to habitats that currently cannot be safely samples used with traditional methods, and limiting habitat impacts. This is underscored both by the growing volume of research articles and reviews focused on critically evaluating how to best implement eDNA in the fisheries toolkit (Hinz et al. 2022), as well as national-level investments such as the establishment of the NOAA 'omics strategic plan[1] which highlights eDNA for marine fisheries applications (Goodwin et al., 2020).

However as is prudent for a relatively new technique, this excitement is tempered by caution and it is understood among eDNA practitioners that transitions from research to application must be carefully managed. In a comprehensive review of the literature, Hinz et al. (2022) identifies three main issues with using eDNA as part of marine Environmental Impact Assessments (EIAs):

1. eDNA is only an indirect representation of the parameter affected by a potential impact ('receptor'), here the fish community.
2. Ensuring a proper sampling strategy to capture eDNA of targeted taxonomic groups.

[1] https://sciencecouncil.noaa.gov/Portals/0/Omics%20Strategic%20Plan_Final%20Signed.pdf?ver=2021-01-19-112404-443

3. Application of proper statistical techniques for eDNA that measures estimated probabilities (of presence) rather than certainties.

In acknowledgement of these concerns, we have incorporated a number of experimental design elements to ensure useful data are collected, in addition to providing a sound strategy based on best practices the results of this proposal will add to our understanding how eDNA should best be implemented as a fisheries monitoring tool.

1. We centered our study around paired eDNA/trawl sampling and a complementary design to acoustic telemetry sampling to better define the link between eDNA and the marine fish community.
2. Our sampling strategy is designed to include randomized replicate sampling of both the 'impact' areas and the areas adjacent to the 'impact' areas in all directions. Coupled with the environmental data such as ADCP profiles taken at each station this will help us account for and understand potential sources of eDNA at each sampling station.
3. A primary component of our hypothesis testing is centered on using a multivariate analysis of metabarcoding data to determine and compare fish community composition, Multivariate analysis is long established in metabarcoding studies in the microbial world and was identified as 'promising' by Hinz et al. (2022) specifically for applications of biomonitoring.

Fig. 1 Project PIs were part of a team with Rockefeller University, Monmouth University, and the NJ DEP Trawl Survey who did a project comparing trawl and eDNA measures of marine fish biomass and community composition (Stoeckle et al. 2020b). Our results showed that eDNA analyses from 1L bottles of water such as is shown on the right could provide similar information on fish communities to the trawl that essential sampled through 66 million liters of water. Methodologies similar to this successful study will be employed in the work proposed here.

Studies of wind farm impacts have primarily utilized either before-after-control-impact (BACI) or before-after-gradient (BAG) experimental designs. As recently reviewed by Methratta (2020) each designed is characterized by specific sets of advantages and disadvantages. For instance, BACI provides a relatively simple and elegant means for detecting an impact if sampling begins before and continues through the construction and operation phases of development. However,

this requires identifying a suitable control site that can be sampled in parallel to the impact site over time. Recently published analyses of the Block Island Wind Farm (Wilber et al. 2022) impacts on fish community composition used a BACI design with two reference sites adjacent to the turbine area to determine effects construction and operation of that small, prototype wind farm. Unfortunately, control site identification can be a problem on the NJ shelf region where development is relatively widespread and conditions, including depth, freshwater inflow, and temperature may vary on a small spatial scale. By contrast, the BAG design alleviates the need for a control site, focusing instead on impacts relative to a gradient extending away from the impact site. This, however, requires *a priori* knowledge of the spatial scale of the impact. Methratta's comparison of studies of wind farm effects on fish determined that indirect effects may be seen 10's of km's from the turbine sites. As a result, sufficient space between wind development areas such that the distal stations from one impact area do not overlap with proximal stations of adjacent impact areas is difficult to achieve due to the number and density of active lease sites. Recently, Rothermel et al. (2020) employed a cross-shelf gridded design for an acoustic telemetry study of striped bass and sturgeon migrations that included areas landward, within, and seaward of a proposed wind development area to relative to broad-scale environmental gradients that exist on the continental shelf. The authors suggest this as robust design to collect baseline data and detect change resulting from wind development on key migratory species in the mid-Atlantic bight. This design combines the most beneficial elements strict BACI and BAG with the goal to be able to differentiate changes due to natural seasonal to interannual variability, and climate change, in fish community composition from potential changes due to windfarm construction, operation and maintenance. The design put forth in Rothermel et al. 2020 will be used as a guide to for the work proposed here as it was in a related acoustic telemetry RMI (Dunton and Adolf).

The goal of this project is to provide critical information to mitigate impacts to fisheries surveys related to offshore wind and to provide pre-construction data for recreationally- and commercially targeted fish. It will (1) collect eDNA samples within an experimental design aimed at generating baseline data that will allow detection of potential changes to fish community composition resulting from offshore wind development, (2) pair eDNA sampling and analyses with MRA surveys over two years to further refine and constrain the relationship between eDNA and current / historical methodologies used by the MRA to describe existing fish communities that support New Jersey's commercial and recreational fisheries.

PROPOSED RESEARCH

With the ultimate mission of monitoring potential impacts of offshore wind development on marine fish community composition on the continental shelf region off the NJ coast, the following hypotheses / objectives are set out:

Hypotheses / objectives

(1) Fish community composition inside vs. outside designated offshore wind development sites will not differ during the 2-yr baseline characterization 'before construction' period.

Understanding the degree to which natural spatial and temporal variability

effect fish community composition is critical to detecting potential effect of offshore wind development.

(2) Fish community composition determined by eDNA will reflect fish community composition determined by capture methods.

Capture methods encompass all survey methods that require the 'take' of marine organisms in contrast to non-extractive methods, including eDNA. Establishing this is critical to the mission of offshore wind monitoring because calibration of eDNA surveys to ongoing capture surveys is necessary to provide continuity with eDNA surveys used in times and places where trawl surveys cannot be used, especially in construction and post-construction phases when access to critical areas close to turbines will be restricted.

(3) Fish presence determined by eDNA will reflect fish presence determined by acoustic telemetry methods.

Establishing this is critical to the mission of offshore wind monitoring because acoustic telemetry will be a critical methodology for monitoring fish in and around wind development areas when trawl access is limited. Acoustic telemetry data will be generated from the related approved RMI project (Dunton and Adolf) occurring in the same area. This is particularly important in pairing with rare and endangered species (e.g. Atlantic sturgeon).

(4) Fish community composition determined by eDNA in the surf zone will not differ from that found in the shelf waters surveyed by the ocean trawl survey; will provide baseline data for important recreational fishing grounds that are not captured elsewhere.

The NJ surf zone represents a huge recreational fishery in NJ with no regular monitoring. With impacts of offshore wind development potentially reaching 10's of km's from turbine sites (Methratta 2020), the surf zone and associated recreational fisheries must be included in the monitoring plan.

Overview of proposed work

The process of using eDNA for fisheries surveys may be broken into three steps detailed below: Water collection, filtration and processing (including sequencing), and bioinformatic analyses.

Water Collection - Collection procedures described in Stoeckle et al. (2021) will be followed. Briefly, water will be collected with a 1.2L stainless steel polypropylene lined Kemerer bottle. The sample bottle will be triple rinsed with sample water before collecting the final sample. The water sample will be stored on ice or in a freezer until transferred to a laboratory for filtering. If the sample cannot be filtered within 24h it will be stored frozen. For the paired capture – eDNA

surveys (apx. 66% of total samples in this study) described below, NJDEP staff will collect water samples in the course of their regular sampling activities.

Filtration and Processing - Filtration and processing procedures similar to Stoeckle et al. (2020) will be followed. Collection bottles will be thawed for ~24h at 4°C and contents poured into a glass filter manifold attached to wall suction with a 47-mm, 0.45μm pore size nitrocellulose filter (Millipore). Filters will be folded to cover retained material and stored in sterile 15-ml tubes at 80°C. As negative controls for each monthly set, several 1-liter samples of laboratory tap water will be filtered using the same equipment and procedures, and on the same day as for field samples. After filtration of contents, collection bottles will decontaminated by washing extensively with tap water, including vigorous shaking of partially filled containers with tops closed, and then air-dried and stored at room temperature- a procedure which relies on mechanical cleansing and dilution, eliminates amplifiable fish DNA from field collection bottles and filtration equipment, while avoiding possible exposure of water samples to residual bleach or other DNA destroying agents (Stoeckle et al. 2017). Frozen filters will be shipped to the Analytical Laboratory at University of MD Institute for Marine and Environmental Biotechnology for DNA extraction, library building, and Illumina sequencing. Products of this service will include de-multiplexed FastQ files and the extracted DNA, which will be archived in a monitored, alarmed –80 °C freezer at Monmouth University.

Bioinformatics - Bioinformatic analyses will use the DADA2 platform run in R statistical computing environment according to procedures and using the internal 12S bony / cartilaginous fish libraries, described in Stoeckle et al. (2017) and Stoeckle et al. (2020). This library currently includes 210 bony fish and 25 cartilaginous unique fish sequences; five species of marine mammal (Humpback, Fin, Pacific Grey, and Northern Right whales; Bottlenose Dolphin); as well as Leatherback and Logerhead Turtles. A full list of taxa is included in the appendix. A 100% sequence match will be used to assign species-level taxonomic identifications. Past sampling indicates that marine mammal and turtle sequences are infrequently found in samples, likely the result of lower eDNA abundance in water for these taxa compared to fish. The results of bioinformatics analyses will be the number of sequence reads per taxonomic unit identified in the 12S reference sequence list. This data will be summarized in tables and graphs by month. Raw and processed data will be archived on secure servers at Monmouth University, as well as on removable media (e.g., external SDD drives).

Sampling for eDNA in this project is divided into several sampling campaigns (Table 1) which are described in more detail below.

Table 1. Sampling campaigns comprising this proposed project. Samples per year includes controls and technical replicates (e.g. negative controls and randomized replicate measurements for quality assurance / quality control of data, respectively).

Sampling Campaign	Objective	Region covered	Frequency	# eDNA samples per year
OSW Development Area.1 (OW1 and Atl. Shores)	Collect data to establish baseline conditions in and around OSW development areas	Area around and includng the OW1 and Atl. Shores OSW areas. Includes impact and four (4) reference areas adjacent to OSW areas. Please see Fig. 2.	Quarterly (seasonal)	216
OSW Development Area.2 (concurrent surf zone sampling)	Collect data to establish baseline conditions in surfzone adjacent to OSW area; establish relationship to offshore sites	Shoreline between north and south cable landing areas adjacent to OSW wind areas. Pleas see Fig. 2.	Quarterly (seasonal)	24
Citizen Scientist Sampling (surf zone)	Collect data to establish baseline conditions in surfzone adjacent to OSW area; establish relationship to offshore sites; test citizen sampling program	Sohreline between Sandy Hook and cape May, including sites within the OSW Development Area	Quarterly (seasonal)	24
Acoustic Telemetry	Opportunistic sampling mainly targeting acoustic telemetry download / maintenance trips; additional data for comparison of eDNA / telemetry	Similar to OSW Development Area but also including other lease areas (Please see red dots on Fig. 2)	Semi-annual to quarterly	68
NJ Ocean Trawl - eDNA Paired Sampling	Calibrate / constrain eDNA - trawl relationship; regional baseline information	NJ neritic zone out to 30m isobath. Depth stratified sampling. Please see Fig. 3.	5 surveys per year	230
Raritan Inventory Project Trawl - eDNA Paired Sampling	Calibrate / constrain eDNA - trawl relationship; regional baseline information	NJ portion of the lower Hudson-Raritan estuary between Raritan and Sandy Hook Bays	8 surveys per year	128
NJ Artificial Reef - eDNA Paired Sampling	Calibrate / contrain eDNA - fish trap relationship; regional baseline information	Sea Girt and Little Egg artificial reef sites (plese see Fig. 3)	3 (months per year)	216

Evaluation of offshore wind development on marine fish community composition

The goal of the experimental design of this portion of the study is to detect potential changes in the marine fish community composition of the continental shelf region where wind development is taking place. While this shelf region has been described as a 'featureless' habitat, there are important environmental gradients occurring cross-shelf, as well as along-shelf, that impact fish distributions. These need to be taken into account when collecting baseline data (e.g., before construction) in and around wind development areas (e.g., Rothermel et al. 2020).

The sampling design for this portion of the project will focus in and around the Ocean Winds 1 and Atlantic Shores development areas. This region will be broken into five strata for eDNA sampling representing areas 'south', 'landward', 'within', 'seaward', and 'north' of the wind development areas (Fig. 2).

To address broad goal #1 presented above, two sampling campaigns (OSW Development Area.1, OSW Development Area.2) are proposed, wherein eDNA samples will be taken from a grid of eDNA sampling stations established in and around the Ocean Wind 1 and Atlantic Shores OSW development areas, and from adjacent shoreline stations, respectively (Fig. 2).

The following data will be collected during each sampling effort:

- Station number and sample ID
- Latitude and longitude
- Time
- Water depth
- Wind speed
- Wave height
- Weather conditions
- Oceanographic data, as collected using a YSI Castaway and a YSI EXO2 Sonde
- Acoustic Doppler Current Profiles (ADCP) on each boat station

This experimental design builds on the gridded sampling design used by Rothermel et al. (2022) that considers the cross shelf environmental gradient within which the wind development area exists. Additionally, grids of eDNA sampling stations will be stablished that include adjacent areas ('south' and 'north') to account for potential movements of eDNA into and out of the study area and to serve as reference areas for BACI type analyses. Sampling stations for eDNA will be approximately 10 km apart, reflecting current estimates of eDNA dispersal potential within the aquatic environment, although it is understood that these estimates currently remain poorly constrained (Hinz et al. 2022). A total 40 samples will be taken each trip (quarterly), distributed as five from each of the south and north strata, and 10 from each of the landward, 'within', and seaward strata. Five additional samples will be taken as paired surface samples at location where water is deepest on each sampling bout. This will allow us to further elucidate the relationship between surface and deep water eDNA samples in this region (e.g., Stoeckle et al. 2021) and better understand the relationship between fish and eDNA in the ocean. Sampling stations within each of the strata will be randomly selected prior to each sampling bout. Project staff will additionally take five samples along the shoreline adjacent to the OSW development area being sampled. Measurements of hydrographic parameters (Table 2) and ADCP vertical current profiles will be collected on each eDNA boat station and used to support analyses of environmental influences on the eDNA results. An ongoing project led by Dunton and Adolf at Orsted Ocean

Table 2. Summary of hydrographic data collected in vertical profiles while sampling eDNA.

Equipment	Parameters
YSI Exo Sondes / Castaway	Surface to bottom profiles of salinity, temperature, dissolved oxygen, turbidity, pH, and chlorophyll fluor.
Teledyne WH Sentinel 600 kHz ADCP	Surface to bottom profiles of current velocity

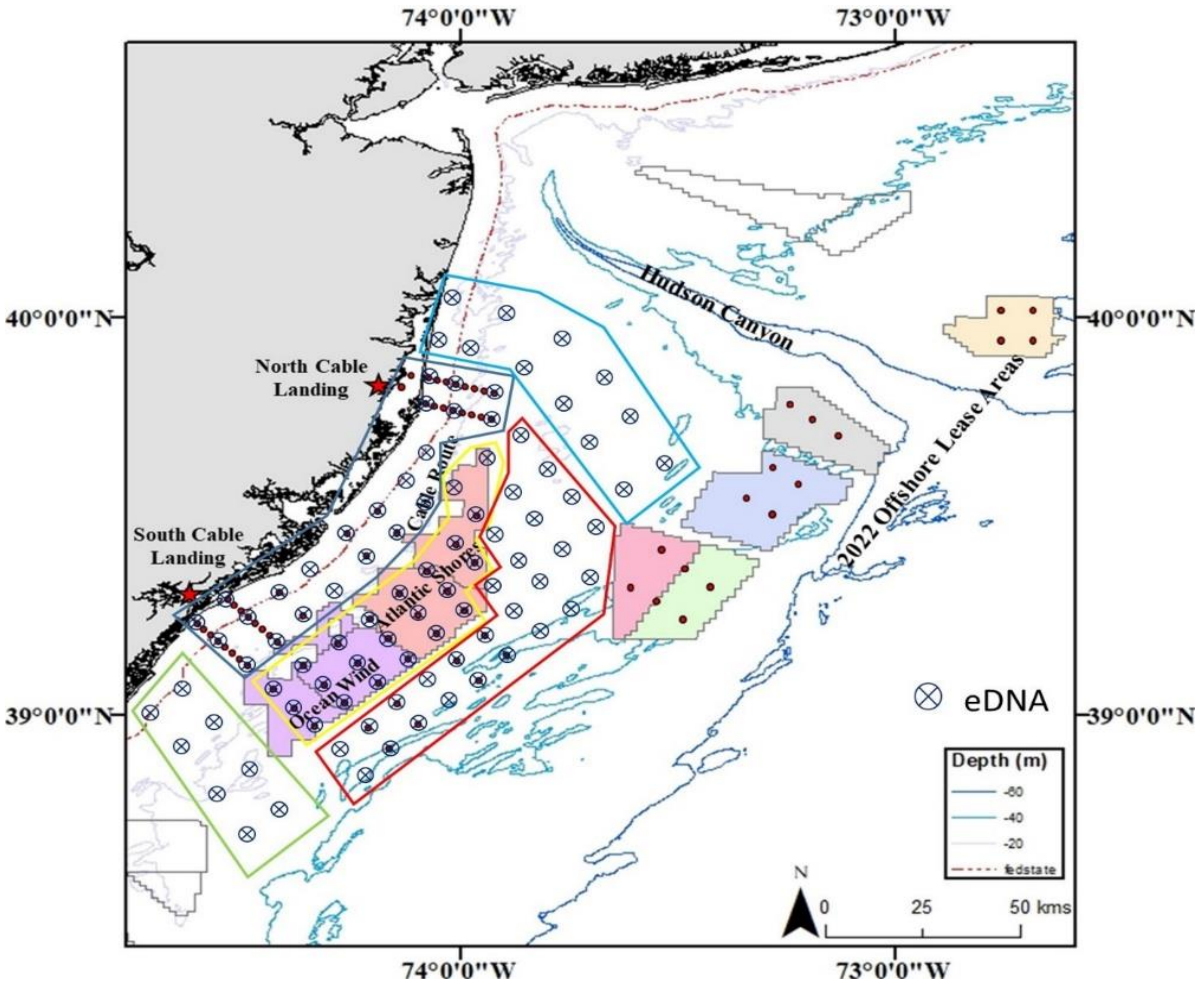

Fig. 2 Proposed sampling design to establish the ecological baseline and detect potential changes in fish community composition resulting from wind energy area development. A total of 40 randomly selected samples will be taken each trip (quarterly), distributed as five from each of the south and north strata, and 10 from each of the landward, 'within', and seaward strata. Additional samples will be taken from the shoreline adjacent to the offshore sampling areas. Acoustic telemetry receivers are shown by red dots, some of which

Wind 1 site includes quarterly sampling at 20 stations within the Ocean Winds 1 area, and if possible, data from that effort will be used in this project. Since acoustic receivers will be deployed in these areas during quarterly sampling on the OSW Development Area campaigns, these data will also be used to compare with acoustic telemetry detections made at the times of sampling. Combining eDNA and telemetry measurements within the same experimental design has advantages including (1) continuous acoustic detections of tagged fish between eDNA sampling periods, and (2) periods of intensive eDNA sampling that can be statistically related to acoustic detections (e.g., Plough et al. 2021), providing further calibration / sea-truthing of the latter method.

Sampling of the surf zone from the shoreline (OSW Development Area.2 sampling campaign) will represent the landward boundary of the proposed eDNA sampling grid design to detect potential impacts of offshore wind development. A lack of good methodological approaches to

comprehensive sampling of surf zone fishes (e.g., Esmaeli et al. 2021) is a recognized problem that has left this important environment severely under sampled despite known connectivity to offshore marine fish communities (e.g., Able et al. 2012, Stoeckle et al. 2020). The lack of a capture survey in the surf zone precludes paired sampling, but similar methodology as is used in the paired eDNA – trawl (ocean and lower HRE) sampling will be used. Sampling the surf zone in the context of offshore wind development is important because of the potential for changes in surf zone fish community composition which could impact recreational anglers. Offshore wind development can have impacts within 10s of km from windfarms (Methratta 2020), so it is possible that activities occurring offshore impact surf zone fish communities at New Jersey beaches.

Sampling of eDNA from the surf zone by citizen scientists (Citizen Scientist Sampling campaign, Table 1) will occur at five (5) locations evenly distributed between Sandy Hook and Cape May. These samples will be taken quarterly, coincidental with offshore surveys, using Smith-Root eDNA samplers provided to the citizen scientists, which we believe will help ensure the quality of citizen samples. Training on the use of these samplers, as well as on best practices for eDNA sampling, will be provided by project staff. Project staff will also coordinate retrieval of samples from citizen scientists.

The Acoustic Telemetry campaign (Table 1) will take advantage of the fact that eDNA sample can be collected at sites of acoustic receiver deployments (e.g., red dots on Fig. 2) during planned downloading / maintenance trips as part of an ongoing RMI project occurring in the same region (Dunton and Adolf, RMI 2022), allowing us to acquire additional data to address hypothesis #3.

NJ Ocean trawl data as a historic reference dataset

The full NJ ocean trawl dataset (1988 – present) will provide an important reference dataset for fish community composition on the NJ shelf, although only one publication (Levesque 2019) has summarized these data. As part of this study, the NJ ocean trawl dataset (including the CTD data collected with each trawl) will be summarized specifically looking for changes over time in species abundance / community composition that might signal a climate change effect (building on work by Levesque 2019), as well as defining the baseline degree spatial, seasonal, and inter-annual variability for this ocean region.

Developing eDNA as a tool for monitoring wind development: Pairing eDNA with select ongoing MRA capture surveys

Further development of eDNA as a tool for assessing marine fish community composition at wind development areas requires calibration of the eDNA method through paired sampling with traditional capture surveys. Three ongoing MRA capture surveys were chosen for paired eDNA sampling (Fig. 3) based on location, survey objectives, and gear used.

NJ Ocean Trawl / eDNA paired sampling campaign

Table 3. NJDEP capture surveys of marine fisheries with proposed number of paired eDNA samples in far-right column to be used in establishing the methodological baseline for eDNA use in monitoring offshore wind development Samples per year includes controls and technical replicates (e.g. negative controls and randomized replicate measurements for quality assurance / quality control of data, respectively).

Survey name	Purpose	Target species	Sample frequency (times per year)	Timing (month)	Samples per trip	Number of samples	Number of eDNA samples per year
Ocean Trawl	Inventory of near/offshore marine finfish and invertebrates	Fish	5	January, April, June, August, October	30 trawls per trip	150	230
Raritan Inventory Project	Trawl survey of fish, schedule to last through 2025	Fish	8 trips per year	March - Oct; monthly	16 trawls	128	128
Artificial Reef	Determine how species utilize different material types and how they use the reefs during different seasons. The data is used to determine the success and productivity of reef sites.	Black sea bass, tautog, summer flounder, American lobster, Jonah crabs, rock crabs	3 months / year	spring, summer, fall	3 reefs per week per month	36	216

The New Jersey Bureau of Marine Fisheries has conducted an ocean trawl survey (NJ OTS) since 1988, focused on the neritic zone between New York Harbor and Delaware Bay, covering the continental shelf from nearshore out to the 30m isobath (Levesque 2019), areas adjacent to and with some overlap of planned wind energy development areas. Our group completed a study comparing eDNA to trawl catch wherein approximately ¼ of all trawls were paired with eDNA sampling in four (4) trawl trips in 2019 (Jan, June, August, and November). Results showed good concordance between trawl and eDNA on a number of important metrics related to fish community composition, including species detected, seasonally dominant species, species richness, and linear regression of relative abundance between trawl capture and eDNA sequence recovery (Stoeckle et al. 2020). Further refining and testing of these relationships is essential to developing eDNA surveys of marine fish community composition as a tool for monitoring offshore wind development.

Sampling proposed here is aimed at extending the dataset of paired trawls / eDNA samples. Specifically, increasing the number of trawls per trip that have a paired eDNA sample taken (only about ¼ trawls were paired with eDNA previously) will provide more data to better constrain the relationship between trawl capture and eDNA. There are 30 trawls in each sampling trip for a total of 150 trawls per year. It is proposed here that each trawl be paired with an eDNA sample taken from water within 2m of the bottom, with ¼ of the trawls also having paired surface samples taken within the upper m of the water column, on every trip. This will allow testing the hypotheses that (1) eDNA and trawl assessments of fish community composition yield similar results, and (2) the relative abundance of species is correlated between eDNA and trawl, and (3) eDNA sampling at the surface vs. deep water column locations yield similar results across a range of habitats and depth profiles. Pairing the surface and bottom

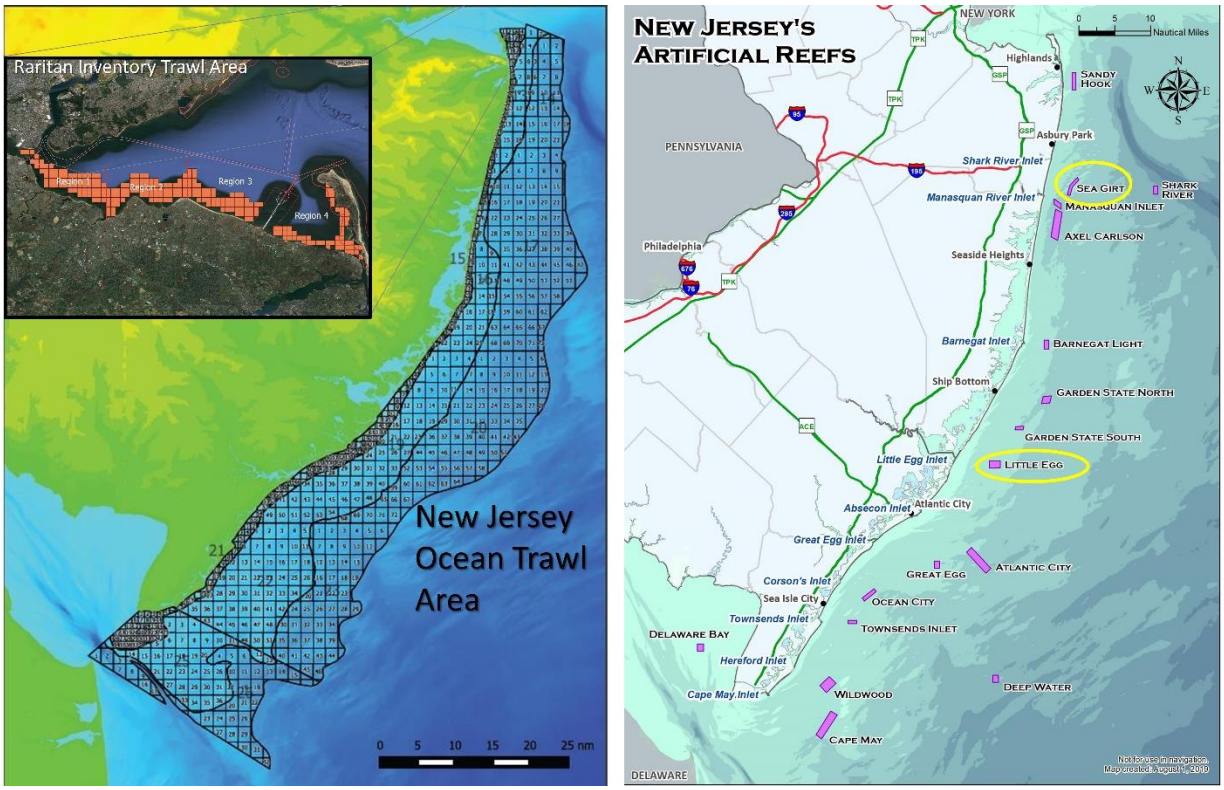

Fig. 3. Maps showing the areas where proposed paired eDNA – capture surveys will occur. For the ocean and Raritan trawl surveys subsets of blocks shown are sampled on each trip. For the artificial reef surveys, Sea Girt and Little Egg (circled) are currently being sampled.

eDNA samples will allow us to further evaluate the relationship of eDNA and trawl assessments as well as water depth in sampling of eDNA. The proposed study would give us the ability to test for differences between surface and bottom samples along a much wider range of oceanographic properties (depth, salinity, temperature). Understanding this relationship is critical to the design of future eDNA sampling programs. For example, can we use ships of opportunity that may not have access to Kemerer or Niskin bottles for deep water sampling? Trawl surveys are widely considered the least biased assessment of fish community composition, although some bias in catch does exist, particularly with the capture of pelagic fishes. However, of all the NJDEP capture surveys, trawl surveys produce data that is most comparable to eDNA estimates of fish community composition.

Raritan Bay Inventory Project / paired eDNA sampling campaign

The Raritan Inventory Project was recently started by NJDEP to investigate fish community composition in Raritan and Sandy Hook Bays. Many important taxa, including Atlantic Sturgeon and Striped Bass are found in the estuary as well as offshore due to migratory behavior – thus there is known connectivity between this estuary and the NJ continental shelf making it important to include in this study of offshore wind impact on marine fish community composition. In a recently completed project by the PIs in which the lower Hudson-Raritan Estuary was sampled by eDNA and trawl surveys, Atlantic Sturgeon and Striped Bass as well as

cobia, cownose ray, sand tiger sharks, smooth dogfish were detected in the estuary as well as in offshore samples.

We will utilize the Raritan Bay inventory project o further calibrate and adapt 'traditional' survey techniques to new methodologies. Enabling the implementation of new biomonitoring tools is a major objective of this project and specifically addresses NJ RMI Research Priority # 12. The Raritan Bay inventory project is essential as an additional calibration dataset because it provides paired eDNA – trawl samples from an environment that is ecologically distinct from habitats in which the NJ Ocean Trawl Survey occurs. A major difference between this trawl survey and the NJ Ocean Trawl survey is the trawl net is significantly smaller (16 ft (Raritan) compared to 30 ft (Ocean Trawl). In short, comparison of the Raritan Bay and NJ Ocean Trawl calibration dataset will provide insight to how robust eDNA is in predicting trawl yields in both different environments and with different gear and thus lead to a more robust understanding of the relationship between the two methods that will better inform use of eDNA as a biomonitoring tool for offshore wind development. Ecologically, sampling in the Raritan Bay Inventory area aligns with our regional view of potential OSW impacts on fisheries and acknowledges the known connectivity between such estuarine environments and offshore fish communities. For instance, many of the fish in the Raritan Bay survey, which also includes Sandy Hook Bay and a good portion of the lower Hudson-Raritan Estuary, transition between the estuarine and offshore environments. Thus, similar to the surf zone fishes changes in estuarine fish communities could accompany changes in offshore fish communities should they occur. Raritan Bay / Sandy Hook is an important staging area for striped bass and likely sturgeon, before they migrate into the Hudson to spawn. Keyport (near Raritan Bay) is a potential cable landing site for offshore wind energy. The impacts of migrating past OSWF and transmission cables could alter the abundance and timing of such populations. Sampling both the estuary and offshore environments during the baseline period will provide important additional calibration data while simultaneously establishing a baseline of connectivity between estuary and offshore that is needed to determine if changes occur as OSW develops.

The Raritan Bay inventory project is led by Stacy van Morter and Gregory Hinks, both of whom were integral partners in the NJ Ocean Trawl survey / eDNA project (Stoeckle et al. 2021). Though this survey uses a combination of trawl and seining methodology to survey fish community composition of the Raritan and Sandy Hook Bays, here, we focus on pairing samples with the trawl survey only. Sixteen (16) stations per month are trawled and we have developed a plan with the survey leaders to pair those with sixteen eDNA water samples per month, or one eDNA sample taken from bottom waters per trawl. This sampling scheme will enable applying the same suite of analyses that was applied to NJ Ocean Trawl / eDNA paired samples.

NJ DEP Artificial Reefs ventless trap / eDNA paired sampling campaign

The NJ Artificial Reef Program conducts ventless trap surveys as part of an effort to examine the ecology of artificial reefs off the NJ coastline. In the context of understanding the impact of wind development on marine fish communities of the NJ continental shelf area, the NJ artificial reef system represents analogous habitat to that which will be introduced by turbine construction. It is reasonable to anticipate that turbine construction will increase the abundance of structure associated fishes by providing additional habitat, and the patterns of fish community composition

around these artificial reefs may provide a glimpse of what fish community composition might look like in constructed wind turbine farms.

According to communications with survey leader, Peter Clarke, two reefs (Little Egg and Sea Girt) are currently being sampled with a total of 22 traps deployed at pre-determined 'on-reef' or 'off-reef' sites during three seasons per year (spring / summer / fall). At each reef, all 22 traps are lifted four times (approximately weekly) per season. After the capture is counted and recorded it is released and the traps redeployed in the same spot. Thus, there are four boat trips to each reef in each of the three seasons sampled, or 15 total boat trips per year per reef (5 trips per reef per year because the first trip is used to deploy the traps). It is recognized that the gear used will produce bias in the fish captured.

We propose, based on discussions with survey leadership, sampling on three trips per reef per season – the first (deployment), the last, and the middle. On each trip to a reef, 5 eDNA samples 'off-reef' and 5 eDNA samples 'on-reef' will be taken from within 2m of the bottom using a Kemerer bottle as traps are being deployed or checked. In order to minimize the risk of contamination, eDNA samples will be taken before a trap is pulled aboard. For any given reef each season, this will yield 30 eDNA samples for each location ('on-reef' and 'off-reef').

This proposed eDNA sampling paired with the ventless trap survey of artificial reefs will target 'on-reef' vs. 'off-reef' locations on each trip, testing the hypotheses that eDNA and trap capture will show similar contrasts in fish community composition in these reef areas within each season. Baseline sampling of eDNA at artificial reefs and wind development areas presents an opportunity compare fish community composition using at two (currently) different environments that we expect to become more similar as turbine construction is completed.

Other MRA surveys considered for paired eDNA / capture surveys include DE River Seine Survey, River Herring seine and gillnet surveys, and the Eel pot survey. After discussions with survey leaders at MRA, and with consideration to the location and gear bias associated with these surveys, it is recommended that pairing eDNA with these surveys would not benefit the development of eDNA as a tool for monitoring offshore wind development.

Data analysis

Data analyses in this project will be focused on enabling the detection of any potential changes in fish community composition that may result from offshore wind development.

Analyses planned for these data fall into two categories related to the two broad objectives of this project. Since widespread eDNA sampling will be used in and around offshore wind development areas, eDNA results will be analyzed in the context of the experimental design described in Fig. 2 to define ecological baseline conditions against which potential impacts of offshore wind development can be compared in future studies. Specifically, we will apply methods that allow us to estimate the amount of variance in fish community composition explained by environmental vs. spatial (e.g., inside vs. outside proposed wind development areas) factors during the baseline characterization period. Further, statistical analyses will be applied to the paired eDNA – capture data to better understand the relationship between these

two methods and establish a strong methodological baseline for broader application of eDNA in monitoring offshore wind development.

Baseline conditions of offshore wind development areas data analysis

Data analysis for the ecological baseline study will focus on comparing fish community composition and environmental conditions among the five strata (including and surrounding the proposed offshore wind development areas) illustrated in Fig. 2. These strata capture the cross-shelf and north-south environmental variability of the region that may have an impact on where species are found (Rothermel et al. 2020), and our sampling includes measurement of a suite of environmental variables in addition to eDNA. Sampling these areas before construction will give us a snapshot of the spatial and temporal (e.g., seasonal) variability inherent to the area and the data needed in future studies to separate environmental effects from potential effects of offshore wind development on our response variable of interest, fish community composition.

An important consideration in attempting to determine potential effects of offshore wind development of fish community composition is separating that potential effect from the effects of natural spatial / temporal variability. There are several approaches to doing that. As described above, nMDS ordinations will be performed on fish community composition data, and PERMANOVA will be used to determine if clusters (e.g., groups of stations with similar fish community composition) are significantly different based on fixed categorical factors (strata, season) following the approach of Liu et al. (2019). Results of these analyses will confirm whether observed 'clusters' of stations with similar fish community composition correlate to being 'inside' vs. 'outside' a wind development area. The analysis of ordinated data can be extended to include consideration of environmental variables. Redundancy analysis (RDA) will be used to examine relationships between fish community composition and major spatial / temporal trends in environmental parameters. Specifically, an RDA of fish community composition constrained by environmental factors will allow us to determine which environmental parameters, such as SST, D.O., station depth and 'location' (e.g., inside vs. outside the wind development area) have explanatory power for the observed variance in fish communities and will indicate the proportion of variation in fish community composition observed during the baseline period is explained by these factors. In the 'during' and 'after' constructions periods this analysis can be expanded using variance partitioning to identify change in the proportion of variance explained by environmental factors vs. 'site' (e.g., inside vs. outside a turbine farm).

The eDNA sampling in Fig. 2 is primarily set up as a BACI design with multiple control (C) sites adjacent to the defined 'Impact' (I) sites. Methratta (2020) cites BACI as the most used experimental design in windfarm studies but identifies and discusses three assumptions of BACI designs that may impact their usefulness with regard to fisheries monitoring plans. (1) that a suitable control may be found; (2) that the area (i.e., fish habitat) within the windfarm and control areas is homogenous; and (3) that he spatial extent of the 'effect' is known (or may be reasonably guessed).

The way in which we plan to execute the BACI design allows for testing of each of these assumptions:

- Assumption (1) will be tested by comparing C and I sites during the baseline period.
- Assumption (2) will be tested by randomly subsampling within each of the C and I regions and explicitly examining how within site variability in fish community composition relates to within site physical / chemical variability.
- Assumption (3) will be tested by using analytical approaches such as an Analysis of Covariance (ANCOVA) or general linearized models (GLM) with continuous predictor variables. Incorporating distance from turbine as a continuous covariate will allow us to address distance from turbines as an explanatory variable and extend comparisons of site differences using an analysis of variance

Our sampling stations are ~10 km apart from each other, extending in four directions from the 'Impact' sites. This is similar to the approach used by Wilber et al. (2022) at the Block Island Wind Farm which implemented GLMs with categorical 'site' predictors as well as continuous covariates (water depth, bottom temperature, and dissolved oxygen in that case). Similarly, our analyses will include consideration of categorical predictor variables (e.g., site) as well as covariates such as distance from turbines and environmental parameters.

As discussed above, several approaches exist to effectively use our data to address the fundamental question of whether offshore wind development has an impact on marine fish community composition. However, the current project is aimed at baseline characterization. The descriptions of various techniques above should therefore be viewed as a set of potential analyses that can be done based on the experimental design chosen for the baseline characterization – all of which are based on a BACI design with multiple control sites but also including consideration of covariates such as environmental parameters and distance from turbines.

Sample sizes required for these analyses were estimated in a power analysis ("pwr" package in R, pwr.anova.test function). With a significance level of p =0.05, power = 0.8, and anticipated 'high' effect size resulting from being 'inside' vs. 'outside' a turbine field, an estimated 15 samples (group *n*) are necessary. Our planned 2-year sampling will achieve this sample size.

Sampling of eDNA during scheduled trips to deploy, recover, and / or download data from acoustic receivers will result in a dataset of paired acoustic telemetry and eDNA observations, which may be analyzed following the technique of Plough et al. (2021), wherein the probability of eDNA detection is modeled as a function of cumulative acoustic detections occurring near the time / place of sample eDNA collection. Specifically, Plough et al. (2021) used a combination of linear and logistic regression analyses with eDNA detection (using a qPCR assay for Atlantic Sturgeon) modelled as a function of cumulative 2 or 5-day acoustic detections, finding statistically significant relationships in each case. We will adapt these methods to examine the relationship between eDNA detection in out metabarcoding datasets and time-dependent (e.g., 2-day, 5-day) cumulative acoustic detections in the acoustic telemetry dataset generated in related, funded RMI project (Dunton and Adolf) occurring in the same region.

Paired eDNA – capture data analyses

Analyses of paired eDNA – trawl data used to establish the methodological baseline will largely follow methods employed by Thompsen et al. 2016 and Stoeckle et al. 2020. Each trawl sample

results in a 'taxa table' wherein species caught are listed and quantified as # of individuals and total mass (kg) per tow. Relative trawl abundance can be calculated using the # of individuals or total mass of a taxon divided by the total # or mass caught in the trawl. Relative trawl biomass may also be expressed as % surface area, accounting for suspected allometric relationships between body size and eDNA shedding rates (Stoeckle et al. 2020). Each eDNA sample analyzed results in a taxa table that contains the name of every taxon detected by DNA sequence in that sample as well as the number of times that DNA sequence was detected (e.g., # of reads). Implicit in the eDNA taxa table is the total number of fish reads detected in a sample, making it possible to express the abundance of each taxon as a proportion of total fish reads obtained for that sample, referred to here as that fish's 'relative eDNA abundance'. The combination of species presence and relative abundance data from paired eDNA – trawl samples will be used to perform analyses such as were employed by Stoeckle et al. (2020). Specifically, species richness (# taxa per sample), dominant taxa per season, and diversity indices (e.g., Shannon-Weiner, based on presence and relative abundance) determined by eDNA and trawl will be compared within each seasonal sampling trip. Further, we will examine correlation-regression relationships between eDNA relative abundance and trawl relative abundance (based on biomass or surface area), building on the good correspondence between these parameters reported by Stoeckle et al. (2021).

Sample sizes required for these analyses were estimated in a power analysis ("pwr" package in R, pwr.f2.test function). With a significance level of p =0.001 and power = 0.8 and anticipated 'high' r2 between trawl and eDNA data, 30-40 samples (*n*) are necessary, which matches the amount of trawls per season somewhat closely. However, the goal of this work is to better define the relationship between trawl and eDNA data, so estimating the strength of that relationship is difficult and may not be useful. If we anticipate that the eDNA – trawl relationship is inherently weaker (e.g., lower r^2), the number of samples required to detect a relationship increases. Thus, 30-40 samples may be optimistic to detect the relationship we are looking for, but as our *n* increases through the year our ability to detect a relationship between trawl and eDNA data will also increase. With these data in hand, we will be able to better estimate 'how much eDNA is enough' but using randomly selected subset of eDNA results (e.g., as if we sampled 25%, 50%, 75%, etc… of all trawls) and looking at potential changes in the regression outcomes.

In addition to taxa-specific correlation analysis, multivariate representations of fish community composition, such as non-metric multi-dimensional scaling (nMDS) based on eDNA or trawl data (e.g., Liu et al. 2019), will also be used to compare eDNA and trawl representations of fish community composition. In brief, ordination methods such as nMDS are used to reduce highly dimensional data to fewer dimension for visualization and analyses. Analyses start with a matrix of taxa observed at different locations and or times (e.g., stations inside vs. outside a wind development area or in different seasons), expressed either as presence / absence, relative abundance, or absolute abundance, and these are used to compute a dissimilarity matrix (e.g., Bray-Curtis) among samples. Samples having similar fish community composition (e.g., low dissimilarity) form clusters that are distinct from other samples with different fish community composition. In the context of comparing eDNA and trawl, ordination will be used to ask whether these two methods produce similar representations of fish community composition and how it differs among important factors such as seasons and locations.

Each of the ongoing MRA capture surveys collects conductivity, temperature, depth (CTD), pH and dissolved oxygen data during sampling, and these data will be employed as covariates in analyses of the relationship between eDNA and capture methods. Specifically, we will test whether or not the nature of the relationship between trawl catches and eDNA results is related to differing environmental conditions encountered between sampling trips.

Community outreach

To address community interest and concerns in offshore wind development, project staff will host bi-annual 'town hall' discussion to (1) initially present the nature and the purpose of monitoring being done on this project, and (2) present results as they become available. This will include making data available through interact maps on the internet using the R statistical programming language and Leaflet package. Additionally, we will provide opportunities for citizen scientists to become trained / equipped in collecting eDNA samples from shoreline locations. Our experience shows that engaging community members in participatory citizen science that is part of a bigger, professionally led effort increases awareness and engagement. We will continue to engage the industry throughout the project as well as reposting in "Notice to All Mariners" when planned sample collection trips occur.

Data management, transparency, and sharing plan

Sequencing data generated by the contracted laboratory is staged on the Illumina platform. The raw data is downloaded and stored redundantly on the cloud (currently Box) and on a RAID-configured NAS Synology DiskStation. For analysis, data is transferred to a high-performance cluster (currently Dartmouth) and demultiplexed for bioinformatics processing and analysis. Data analysis is completed in a full reproducible fashion using a research compendium that organizes raw and processed data, results while simultaneously documenting each processing step using digital notebooks containing the necessary code to execute each step (primary coding languages are R and bash) and documenting any parameter settings and versions of R packages and other programs used. Any specific programs used for processing are open source and executed on the command line, thus making it straightforward to document any parameter settings. After completion of the project, data associated with reports and peer-reviewed projects will be made available using standard practices to make the underlying sequence and a fully reproducible analysis available using publicly available databases such as Genbank and GitHub.

All 'downstream' manipulation of eDNA sequence data, including indexing and statistical analysis with environmental data, will be done in the R statistical programming language. The code documenting these analyses will be made available along with the primary data sources to ensure transparency and repeatability of all steps of the process.

Deliverables and regional cooperation

Deliverables will include (1) species tables with relative abundance based on eDNA, (2) analyses of the relationship between survey catch and eDNA detections, done at the level of relative abundance of individual species (dominant taxa, linear regression) and community composition (e.g. multivariate metrics (e.g. PCA, NMDS) of community composition, (3) Analysis of the

relationship between eDNA and MRA survey fish composition and environmental parameters (e.g., does the relationship between eDNA – trawl vary as a function of environmental conditions such as water temperature, salinity, dissolved oxygen, etc.). We will develop relationships and work with academic, state and federal partners to coordinate regional sampling efforts with the associated goal of detecting potential responses of marine communities to offshore wind development.

Schedule of Activities and Key Project tasks

Please see proposed project schedule in Appendix.

Expected Outcomes

Success of the project relies on the successful collection, processing, and analyses of eDNA and associated ancillary (CTD, water quality conditions) data. In the broadest sense, we expect outcomes to include (1) a dataset that characterizes ecological baseline conditions (fish community composition and environmental conditions) of offshore wind development areas to be used to address potential changes to the fish community composition as a result of offshore wind development; (2) establishment of a methodological baseline that further validates eDNA as an additional tool for monitoring potential effects of offshore wind on marine fish communities through collection and analysis of a comprehensive dataset from paired eDNA – capture sampling that furthers our understanding between eDNA and more commonly employed extractive techniques used by the NJDEP to assess marine fish communities; and (3) improved community engagement and understanding of the scientific monitoring supported by NJDEP of offshore wind development activities.

Total Project Budget $1,161,583

EXPERTISE

Dr. Jason Adolf: PI., has 20 years' experience working in the field of biological oceanography since obtaining his PhD. He has successfully managed or co-managed several large projects from state and federal funding agencies including National Science Foundation, NOAA, EPA, and NJ DEP. Most recently, he was part of a team of scientists working together with the NJ DEP bi-monthly trawl survey to investigate the relationship between eDNA and trawl measurements of marine fish abundance and community composition. This resulted in a peer-reviewed publication in ICES J Marine Science (Stoeckle et al. 2020b). Dr. Adolf currently oversees a participatory citizen science program, the Coastal Lakes Observing Network, in which community members assist with water quality and biological monitoring of Monmouth County Coastal Lakes (www.monmouth.edu/clonet).

Dr. Keith Dunton: Co-PI. Dr. Dunton is a fisheries ecologist with 17 years' experience working on fisheries, community ecology, and ecosystem dynamics and is an expert in acoustic telemetry. He has authored 17 peer-reviewed papers and contributed to over 40 professional talks at professional conferences related to fisheries biology, ecology, and management. Over the last ten years he maintained several large acoustic telemetry arrays (NY, NJ, and DE) and has surgically implanted over 700 fish on a variety of species including Atlantic sturgeon, Coastal sharks, winter skates, striped bass, monkfish, flounders, horseshoe crabs and dogfish including wind specific projects (see summary below). Current research focuses on monitoring and acoustic tagging Atlantic sturgeon at Naval Weapons Station Earle, working with recreational anglers to acoustic and Satellite tag sharks to monitor post-release effects and mortality, evaluating species relative abundance and richness within Orsted Ocean Wind using eDNA samples, and evaluating the effects of EMF from wind energy cable landings on marine fish migrations in New York. Currently, he serves on the ACT_MATOS Network Steering committee for offshore wind and acoustic telemetry as well as serving at a Research Advisor for the Responsible Offshore Science Alliance.

Dr. Shannon O'Leary: Dr. O'Leary has worked on a wide range of projects using genetic markers to characterize and identify individuals, populations, and species to better understand evolutionary processes and ecological patterns. Dr. O'Leary is currently involved in projects using eDNA to monitor the effects of climate change on temperature, water level changes, and species assemblages in headwater streams in New Hampshire and the impact of offshore windfarms on benthic fishes in New Jersey. Dr. O'Leary is a founding member of iCatch, which focuses on combining predictive AI technology with precision genomic testing to make rapid, accurate, in-the-field species identification possible. Previously, she worked as a research scientist for the CIMAGE consortium contributing to understanding the population-level effects of the 2010 Deepwater Horizon oil spill on the genomic diversity of demersal fishes. Overall, Dr. O'Leary has produced and analyzed large-scale genomic data sets for almost 10 fish species and a large focus of her work has been creating efficient workflows for working with large data sets and improving the efficiency of bioinformatics pipelines to produce robust data sets for downstream analysis.

Postdoc / technician. This is a new hire for the project, targeting MS / PhD level individuals with experience in molecular ecology of fisheries.

Community coordinator. This is a new hire for the project targeting individuals with project coordination experience, as well as familiarity of collaborative research between university, federal, state, and citizen participants.

RESOURCES

Monmouth University has (or has budgeted for herein) all the resources necessary to carry out the tasks described in this document.

Facilities available

The research lab space of JEA includes apx. 200 sq. ft. with two sinks, a six-place vacuum manifold system for eDNA filtrations, and bench space for DNA extractions and genetic analyses of seawater samples. Additionally the lab contains a Nikon Diaphot inverted microscope with camera, a Nikon TE300 inverted epifluoresence microscope with camera, cell counting chambers, a BD-Accuri flow cytometer, Turner Trilogy fluorometer, (2) YSI Pro multiparameter water quality probes on 10m cable, (2) YSI EXO2 multiparameter sondes, YSI Castaway CTD, LaMotte turbidimeter, pH meter, Secchi disks, YSI 9500 photometer for nutrient measurements, various plankton nets, illuminated plant growth incubator, multiple freezers refrigerator, laminar flow hood for handling cultures, clinical centrifuge, equipment for IDEXX assays of fecal indicator bacteria. All freezers are attached to uninterrupted power in case of blackouts. Several alarmed -80 °C freezers are available for storage of samples.

Monmouth University's Marine and Environmental Biology (MEBP) teaching lab apx. 792 sq. Ft. With 4 stainless steel sinks, a fume hood, a full set of compound and dissecting microscopes, a PC, projector, and screen, and a white board. There are 5 x 4-seat resin benchtops each with storage cabinets beneath and a moveable snorkel-style fume hood above.

All PIs have offices (apx 100 sq. ft.) equipped with computers, printers and standard office equipment. Additionally, Adolf and Dunton have student spaces where research-active students can use desk space, computer and printers for research activities.

Monmouth University's Urban Coast Institute (UCI) will be critical to this project. UCI's mission is to serve Monmouth University and the public as a forum for research, education, and collaboration in the development and implementation of science-based policies and programs that support stewardship of healthy, productive and resilient coastal ecosystems and communities. UCI is responsible for the operation and maintenance of Monmouth University's vessels that will be used in this project.

Saint Anselm College is part of the New Hampshire INBRE-network of institutions that allows direct access to Dartmouth's high-performance clusters and Linux servers. Both Drs. Dunton and Adolf and Dr. O'Leary have NAS synology systems in place to allow for redundancy of data storage and back-up. Dr. O'Leary has an additional back-up system on the cloud creating additional redundancy.

Equipment available for this project

Monmouth University's *R/V* Heidi Lynn Sculthorpe is a 49 foot ex-U.S. Coast Guard buoy tender fully equipped to operate up to 20 n.m. off shore with twin 8V-71 Detroit diesel engines @ 305 HP each; 225 sq. ft. of deck space; a 16.8 ft beam. The *R/V* Heidi Lynn Sculthorpe can accommodate 22 passengers in addition to the captain and has an endurance of up to 4 days at sea. Electric power includes 22 kW, 120 / 240 V, 60 Hz, 120 amps service. Equipment on the boat includes a windlass, two main winches, 18 foot A-frame and a 72" x 36" diameter net reel. Outfitting allows scientific operations including deployment of hydrographic survey equipment; water quality sampling; trawling, dredging, and deployment of fixed gear for near shore fisheries stock assessments; scientific diving operations; sediment sampling with benthic grabs and gravity corers; deployment and recovery of oceanographic buoys and moorings. A variety of sampling gear is also available for use from this boat. This boat may be operated by individuals with a USCG Captain's license.

Monmouth University's *R/V* Seahawk is available to support this project. The Seahawk is a versatile, trailerable, 27-foot fiberglass hulled survey vessel (Maycraft). It's cabin is equipped with heat and air condition and lighted for nighttime operations. The Seahawk is rigged to support a variety of scientific work including single and multibeam hydrography, subbottom profiling, side-scan sonar, ROV support, ADCP surveys, and benthic and water column sampling. This vessel can supply 120v AC power for instrument operation. This boat may be operated by individuals with a USCG Captain's license.

Monmouth University's *R/V* Little Hawk is available for this project. This 18' foot Parker has a 115 HP outboard, is trailerable, but is kept at the Atlantic Highlands Municipal Marina.

Reporting Requirements:

1. Applicant(s) must deliver:
   a) Final Project Plan, Detailed Quality Assurance Project Plan, and Health and Safety Plan. These Plans, described below, should be submitted for review at least sixty days prior to commencement of data collection.
      i. *Final Project Plan.* The project plan should provide a comprehensive overview of the work after any required revisions to the submitted Proposal from the RMI team. This may include a request for additional information about methods, staffing, schedule, budget, etc.
      ii. *Quality Assurance Project Plan.* A Quality Assurance Project Plan (QAPP) to cover all aspects of the project, from project design to final report. The QAPP should include how the success of the proposed work will be evaluated, sources of error and potential effects on results, and should include field work and data collection (*e.g.*, standard operating procedures, data recording, instrument calibration, etc.), data analysis activities (*e.g.*, data quality objectives, modeling, statistical procedures).
      iii. *Health and Safety.* Projects involving laboratory or field research shall prepare a Health and Safety Plan that describes hazards of the work, how risks will be reduced to ensure the continued health and safety of all personnel, how personnel will be informed and prepared, communication in the field, emergency response, and required personal protective equipment.

   b) Quarterly Performance and Financial Reports. Performance and financial reports are required to be submitted to the DEP on a quarterly basis to provide an update and explanation of the project status. These reports are vital to the success of the project and shall be submitted complete and on time for payments to be made under this agreement. Failure to submit timely and complete reports may result in non-payment. Quarterly Performance Reports are required to be submitted in digital format. All interim work products, deliverables, as well as the Quarterly Financial Reports with documentation (receipts, vouchers, etc.) are required to be submitted with the appropriate Quarterly Performance Report.

   c) Draft Final Report. An electronic copy of the draft final report shall be submitted to the State Contract Manager. The Draft will be reviewed by the RMI steering committee and comments will be provided to the Project Manager.

   d) Final Report. An electronic copy of the final report shall be submitted by the Project Manager upon the completion of the project. The final report will include resolution of all comments made to upon the draft final report.

   e) Data. See Data Management, Transparency, and Sharing Plan above. Additionally, a database consisting of all qualitative and quantitative information recorded as part of the

study shall be submitted with the final report. In addition to the data tables, the database should include a codebook that describes the data [e.g., variable names, descriptions, format (number, data, text), units], metadata (e.g., personnel, any relevant site conditions not included as variables), and GIS files.

f) Regional Coordination. The PIs will provide the Final Report and Data to offshore wind regional coordination entities (NYSERDA, ROSA, RWSE) and/or any relevant offshore wind data sharing platforms that are developed and accepting submissions.

LITERATURE CITED

Able, K. W., Wuenschel, M. J., Grothues, T. M., Vasslides, J. M. and Rowe, P \.M. (2013) Do surf zones in New Jersey provide "nursery" habitat for southern fishes? Environ Biol Fish 96:661-675.

Andruszkiewicz, E. A., Koseff, J. R., Fringer, O. B., Ouellette, N. T., Lowe, A. B., Edwards, C. A., & Boehm, A. B. (2019). Modeling environmental DNA transport in the coastal ocean using Lagrangian particle tracking. *Frontiers in Marine Science*, *6*(JUL). https://doi.org/10.3389/fmars.2019.00477

Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. In *Conservation Genetics* (Vol. 17, Issue 1, pp. 1–17). Springer Netherlands. https://doi.org/10.1007/s10592-015-0775-5

BOEM. (2019). Guidelines for Providing Information on Fisheries for Renewable Energy Development on the Atlantic Outer Continental Shelf Pursuant to 30 CFR Part 585.

Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. Conservation Genetics, 17(1), 1–17. https://doi.org/10.1007/s10592-015-0775-4

Collins, R. A., Wangensteen, O. S., O'Gorman, E. J., Mariani, S., Sims, D. W., & Genner, M. J. (2018). Persistence of environmental DNA in marine systems. Communications Biology, 1(1), 1–11. https://doi.org/10.1038/s42003-018-0192-6

Esmaelili, Y.M.L., Corte, G., Checon, H.H., Gomes, T.R.C., Lefcheck, J.S., Amaral, A.C.Z, and Turra, A. (2021) Comprehensive assessment of shallow surf zone fish biodiversity requires a combination of sampling methods. Mar Ecol Prog Ser, 667, DOI: 10.3354/meps13711

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

Hinz, S., Coston-Guarini, J., Marnane, M., & Guarini, J. M. (2022). Evaluating eDNA for Use within Marine Environmental Impact Assessments. In *Journal of Marine Science and Engineering* (Vol. 10, Issue 3). MDPI. https://doi.org/10.3390/jmse10030375

Levesque, J.C., (2019). Spatio-temporal patterns of the oceanic conditions and nearshore marine community in the Mid-Atlantic Bight (New Jersey, USA). PeerJ, 7, p.e7927.

Liu, Y., Wikfors, G. H., Rose, J. M., McBride, R. S., Milke, L. M., & Mercaldo-Allen, R. (2019) Application of Environmental DNA Metabarcoding to Spatiotemporal Finfish Community Assessment in a Temperate Embayment. *Frontiers in Marine Science*, *6*(November). https://doi.org/10.3389/fmars.2019.00674

Methratta, E. T. (2020). Monitoring fisheries resources at offshore wind farms: BACI vs. BAG designs. *ICES Journal of Marine Science*, *77*(3), 890–900. https://doi.org/10.1093/icesjms/fsaa026

New Jersey Department of Environmental Protection Ecological Baseline Studies (NJDEP EBS). (2010). Volume IV: Fish and Fisheries. https://www.nj.gov/dep/dsr/ocean-wind/.

Plough, L. V., Bunch, A. J., Lee, B. B., Fitzgerald, C. L., Stence, C. P., & Richardson, B. (2021). Development and testing of an environmental DNA (eDNA) assay for endangered Atlantic sturgeon to assess its potential as a monitoring and management tool. *Environmental DNA*, *3*(4), 800–814. https://doi.org/10.1002/edn3.186

Rothermel, E.R., Balazik, M.T., Best, J.E., Breece, M.W., Fox, D.A., Gahagan, B.I., Haulsee, D.E., Higgs, A.L., O'Brien, M.H., Oliver, M.J. and Park, I.A., (2020) Comparative migration ecology of striped bass and Atlantic sturgeon in the US Southern mid-Atlantic bight flyway. PloS one, 15(6), p.e0234442.

Schenekar, T. (2022). The current state of eDNA research in freshwater ecosystems: are we shifting from the developmental phase to standard application in biomonitoring? *Hydrobiologia*, *0123456789*. https://doi.org/10.1007/s10750-022-04891-z

Shea, D., Frazer, N., Wadhawan, K., Bateman, A., Li, S., Short, S., & Krkosek, M. (2022) *Environmental DNA dispersal from Atlantic salmon farms*.

Stoeckle, M. Y., Adolf, J., Ausubel, J. H., Charlop-Powers, Z., Dunton, K. J., & Hinks, G. (2022). Current laboratory protocols for detecting fish species with environmental DNA optimize sensitivity and reproducibility, especially for more abundant populations. *ICES Journal of Marine Science*, *79*(2), 403–412. https://doi.org/10.1093/icesjms/fsab273

Stoeckle, M. Y., Soboleva, L., & Charlop-Powers, Z. (2017). Aquatic environmental DNA detects seasonal fish abundance and habitat preference in an urban estuary. PLoS ONE, 12(4), 1–16. https://doi.org/10.1371/journal.pone.0175186

Stoeckle, M. Y., Das Mishu, M., & Charlop-Powers, Z. (2020). Improved Environmental DNA Reference Library Detects Overlooked Marine Fishes in New Jersey, United States. Frontiers in Marine Science, 7(May). https://doi.org/10.3389/fmars.2020.00226

Stoeckle, M.Y., Adolf, J., Charlop-Powers, Z., Dunton, K.J., Hinks, G., and VanMorter, S. 2021. Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USA. *ICES Journal of Marine Science*, Volume 78, Issue 1, January-February 2021, Pages 293–304, https://doi.org/10.1093/icesjms/fsaa225

Thomsen, P. F., Møller, P. R., Sigsgaard, E. E., Knudsen, S. W., Jørgensen, O. A., & Willerslev, E. (2016). Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater fishes. PLoS ONE, 11(11), 1–22. https://doi.org/10.1371/journal.pone.0165252

Wilber, D. H., Brown, L., Griffin, M., DeCelles, G. R., & Carey, D. A. (2022). Demersal fish and invertebrate catches relative to construction and operation of North America's first offshore wind farm. *ICES Journal of Marine Science*. https://doi.org/10.1093/icesjms/fsac051

Appendix A: Project milestone and timeline

Project Month	1	2	3	4	5	6	7	8	9	9	10	11
Month and Year	Sept 2022	Oct 2022	Nov 2022	Dec 2022	Jan 2023	Feb 2023	Mar 2023	Apr 2023	May 2023	Jun 2023	Jul 2023	Aug 2023
Goals	Initiate grant and finalize forms. Begin hiring process of staff members Begin purchases of required equipment for project Begin coordination with NJDEP capture surveys	Continue initiating project Develop Health and Safety Plans Develop QAPP Continue coordination with eDNA capture surveys Sample NJ Ocean Trawl	Begin (autumn) eDNA sampling at offshore wind development sites Begin training sessions for community scientists	Process eDNA samples and work on analyses Construct analyses of environmental data	Continue processing of paired eDNA – capture data Continue processing of eDNA wind development area data Winter eDNA sampling at offshore wind development sites NJ Ocean Trawl	Winter eDNA sampling at offshore wind development sites (alternate dates) Community outreach meeting #1 (winter)	Continue processing of paired eDNA – capture data Continue processing of eDNA wind development area data	Spring eDNA sampling at offshore wind development sites Continue processing of paired eDNA – capture data Continue processing of eDNA wind development area data NJ Ocean Trawl	Spring eDNA sampling at offshore wind development sites (alt dates)	Community outreach meeting #2 (summer) NJ Ocean Trawl	Summer eDNA sampling at offshore wind development sites	Summer eDNA sampling at offshore wind development sites (alt dates) NJ Ocean Trawl
12	13	14	15	16	17	18	19	20	21	22	23	24
Sept 2023	Oct 2023	Nov 2023	Dec 2023	Jan 2024	Feb 2024	Mar 2024	Apr 2024	May 2024	Jun 2024	Jul 2024	Aug 2024	Sept 2024
Continue processing of paired eDNA – capture data Continue processing of eDNA wind development area data	Continue coordination with eDNA capture surveys NJ Ocean Trawl	(autumn) eDNA sampling at offshore wind development sites Continue training sessions for community scientists	Process eDNA samples and work on analyses Construct analyses of environmental data	Continue processing of paired eDNA – capture data Continue processing of eDNA wind development area data Winter eDNA sampling at offshore wind development sites NJ Ocean Trawl	Winter eDNA sampling at offshore wind development sites (alternate dates) Community outreach meeting #3 (winter)	Continue processing of paired eDNA – capture data Continue processing of eDNA wind development area data	Spring eDNA sampling at offshore wind development sites Continue processing of paired eDNA – capture data Continue processing of eDNA wind development area data NJ Ocean Trawl	Spring eDNA sampling at offshore wind development sites (alt dates)	Community outreach meeting #4 (summer) NJ Ocean Trawl	Summer eDNA sampling at offshore wind development sites	Summer eDNA sampling at offshore wind development sites (alt dates) NJ Ocean Trawl	Continue processing of paired eDNA – capture data Continue processing of eDNA wind development area data
25	26	27	28									
Oct 2024	Nov 2024	Dec 2024	Jan 2025									

Prepare final report(s) Plan for following 2-4 yrs continued sampling NJ Ocean Trawl	Prepare final report(s) Plan for following 2-4 yrs continued sampling	Draft Final Report submitted to NJDEP/BPU	Submit Final report after incorporation of comments from Project Managers RMI steering committee									

Appendix B. Taxa list in the current reference database, including fish, marine mammals, and sea turtles

Animal Group	Taxa (ASV)
Bony Fish	Agujon_needlefish_Tylosurus_acus
	American_anglerfish_Lophius_americanus
	American_butterfish_Peprilus_triacanthus
	American_conger_Conger_oceanicus
	American_eel_Anguilla_rostrata
	American_gizzard_shad_Dorosoma_cepedianum
	American_plaice_Hippoglossoides_platessoides_also_matches_Winter_flounder_refseq
	Arctic_char_others_Salvelinus_sp
	Armored_searobin_Peristedion_miniatum
	Atlantic_chub_mackerel_Scomber_colias
	Atlantic_cod98_Gadidae98
	Atlantic_cod_and_other_gadidae
	Atlantic_croaker_(nibea98)
	Atlantic_halibut_Hippoglossus_sp
	Atlantic_herring_Clupea_harengus
	Atlantic_mackerel_Scomber_scombrus
	Atlantic_menhaden_Brevoortia_tyrannus_LS17
	Atlantic_menhaden_LS16_or_river_herrings_Clupeidae_sp
	Atlantic_moonfish_Selene_setapinnis
	Atlantic_needlefish_Strongylura_marina
	Atlantic_salmon_Salmo_salar
	Atlantic_salmon_Salmo_salar(2)
	Atlantic_or_northern_sand_lance_Ammodytes_americanus_or_dubius
	Atlantic_silverside2_Menidia_menidia2
	Atlantic_silverside_Menidia_menidia
	Atlantic_spadefish_Chaetodipterus_faber
	Banded_killifish_Fundulus_diaphanus
	Barramundi_Lates_calcarifer
	Bay_anchovy_Anchoa_mitchilli
	Blackbelly_rosefish_Helicolenus_dactylopterus
	Blacknose_dace_Rhinichthys_atratulus
	Black_crappie98_Pomoxis_nigromaculatus98
	Black_crappie_Pomoxis_nigromaculatus
	Black_drum_or_Spot_Pogonias_cromis_or_Leiostomus_xanthurus
	Black_sea_bass_Centropristis_striata
	Bluefish_Pomatomus_saltatrix
	Bluegill_Lepomis_macrochirus
	Blue_catfish_Ictalurus_furcatus
	Broad_striped_anchovy_Anchoa_hepsetus
	Brook_trout_Salmo_trutta
	Brown_bullhead99_Ameiurus_nebulosus99

Brown_bullhead_Ameiurus_nebulosus
Buckler_dory_Zenopsis_conchifera
Butterfly_mackerel_Gasterochisma_melampus
Capelin_Mallotus_villosus
Catfish_sp_Ictalurus_sp
Chinook_salmon_Onchorhynchus_tshawytscha
Cichlid_Maylandia_zebra_others
Climbing_perch_Anabas_testudineus
Cobia_Rachycentron_canadum
Common_carp_Cyprinus_carpio
Common_guppy_Poecile_reticulata
Coney_Cephalopholus_fulva
Creek_chubsucker_Erimyzon_oblongus
Crested_blenny_Hypleurochilus_germinatus_refseq_not_full_length
Cunner_Tetragolabrus_adspersus
Gulf_kingfish98_formerly_Drum_family_nibea94b
Gulf_kingfish99_formerly_Drum_family_nibea95b
Dwarf_goatfish_Upeneus_parvus
Eastern_mudminnow_Umbra_pygmea
European_pilchard_Sardina_pichardus
European_sea_bass_Dicentrarchus_labrax
Fathead_minnow_Pimephales_promelas
Fawn_cuskeel_Lepophidium_profundorum
Feather_blenny_Hypsoblennius_hentz
Flagfin_mojarra_Eucinostomus_melanopterus
Flathead_grey_mullet_Mugil_cephalus
Florida_pompano99_Trachinotus_carolinus99
Florida_pompano_Trachinotus_carolinus
Fourspine_stickleback_Apelties_quadraacus
Fourspot_flounder_Hippoglossina_oblonga
Frigate_or_bullet_tuna_Auxis_thazard_or_rochei
Giant_trevally99_Caranx_ignobilis99
Gilt_head_seabream_Sparus_aurata
Golden_shiner_Notemigonus_chrysoleucas
Golden_tilefish_Lopholatilus_chamaeleonticeps
Goldfish_Carassius_auratus
Grass_or_chain_pickerel_Esox_americanus_or_niger
Grass_or_silver_carp_Cyprinidae_sp
Gray_angelfish_Pomacanthus_arcuatus
Gray_angelfish_Pomacanthus_arcuatus99
Gray_snapper_Lutjanus_griseus
Greenland_halibut_Reinhardtius_hippoglossoides
Green_sunfish_Lepomis_cyanellus
Grey_triggerfish_Balistes_capriscus
Grubby_or_other_sculpins_Cottidae_sp

Gulf_kingfish_Menticirrhus_littoralis_formerly_Drum_family_nibea97
Gulf_stream_flounder_Citharichthys_arctifrons_MM02
Halfbeak_sp98_Hemiramphus_sp98
Hilsa_shad__Tenuolosa_ilisa
Hogchoker_trinectes_maculatus
Inland_silverside_Menidia_beryllina
Inshore_lizardfish_Synodus_foetens_partial
Iridescent_shark_catfish_Pangasianodon_hypopthalmus
Jack_family_caranx_ignobilis99
Johnny_darter99_Ethiostomum_nigrum99
King_mackerel_Scomberomorus_cavalla
Ladyfish98_Elops_saurus98
Ladyfish99_Elops_saurus99
Largemouth_bass_Micropterus_salmoides
Large_yellow_croaker_Larimichthys_crocea
Lebranche_mullet_Mugil_liza
Lined_seahorse_Hippocampus_erectus
Little_tunny_or_skipjack_tuna_Euthynnus_alletteratus_or_Katsuwonus_pelamis
Longhorn_other_sculpins_M_octodecemspinosus_others
Longnose_sucker_or_Buffalo_fish_Catostomus_catostomus_or_Ictiobus_sp
Mahi_mahi_Coryphaena_hippurus
Mosquitofish_Gambusia_affinis
Mummichog_Fundulus_heteroclitus
Naked_goby_Gobiosoma_bosc
Nile_tilapia_Oreochromis_niloticus
Noodlefish_sp_Salangidae_sp
Northern_kingfish_Menticirrhus_saxatilis
Northern_pipefish96_Syngnathus_fuscus96
Northern_pipefish_Syngnathus_fuscus
Northern_puffer_Sphoeroides_maculatus
Northern_sea_robin_Prionotus_carolinus
Northern_sennet_Sphyraena_borealis_(Sphyraena95)
Northern_snakehead_Channa_argus
Northern_stargazer
Ocean_pout_Zoarces_americanus
Ocean_sunfish_Mola_mola
Offshore_hake_Merluccius_albidus
Olive_flounder_Paralichthys_olivaceus
Orange_filefish_Aluterus_schoepfii
Oyster_toadfish95
Oyster_toadfish_Opsanus_tau
Pacific_creolefish_Paranthias_colonus
Pacific_red_snapper_Lutjanus_peru
Pacific_sand_lance_Ammodytes_hexapterus
Pacific_worm_eel97_Myrophis_wafer97

Pinfish_Lagodon_rhomoboides
Planehead_filefish_Stephanolepis_hispidus
Pollock_Polachius_virens_or_Whiting_Merlangus_merlangus
Porgy_sp_Pagrus_major99
Pumpkinseed_Lepomis_gibbosus
Radiated_shanny_Ulvaria_subbifurcata
Rainbow_trout_or_Salmon_sp_Oncorhynchus_sp
Rainwater_killifish_Lucania_parva_(Banded_killifish97_cluster1)
Redbelly_tilapia_Coptodon_zillii
Red_drum98_Sciaenops_ocellatus98
Red_drum_Sciaenops_ocellatus
Red_eye_mullet_Mugil_rubrioculus
Red_eye_round_herring_Etrumeus_teres
Red_goatfish_Mullus_auratus
Red_grouper_Epinephelus_morio
Red_White_or_Spotted_hake_sp_Urophycis_sp
Rock_gunnel_Pholis_gunnelus
Rough_scad_Trachurus_lathami
Rough_silverside_Membras_martinica_formerly_Odontesthes94
Scup_Stenotomus_chrysops
Seaboard_goby_Gobiosoma_ginsburgi
Sea_lamprey_Petromyzon_marinus
Sheepshead_minnow_Cyprindon_variegatus
Silver_anchovy_Engraulis_eurystole
Silver_hake_Merluccius_bilinearis
Silver_perch_Bardiella_chrysoura_(nibea93)
Skilletfish99_Gobiesox_strumosus99
Smallmouth_bass_Micropterus_dolomieu
Smallmouth_flounder_Etropus_microstomus
Southern_kingfish_Menticirrhus_americanus_(nibea95)
Spanish_mackerel_Scomberomorus_maculatus
Spotfin_butterfly_fish_Chaeton_ocellatus
Spotfin_killifish_Fundulus_luciae_formerly_Fundulus_grandis97
Spotfin_mojarra_Eucinostomus_argentatus
Spotted_goatfish_Pseudupeneus_maculatus
Spotted_rose_snapper97_Lutjanus_guttatus97
Spotted_rose_snapper98_Lutjanus_guttatus98
Spotted_rose_snapper_Lutjanus_guttatus
Spotted_sea_trout_Cynoscion_nebulosus
Striped_bass_Morone_saxatilis
Striped_blenny_Chasmodes_bosquianus
Striped_burrfish_Chilomycterus_schoepfi
Striped_cusk_eel_Ophidon_marginatum
Striped_killifish_Fundulus_majalis
Striped_sea_robin_Prionotus_evolans

	Sturgeon_sp_Acipenser_sp
	Summer_flounder99a
	Summer_flounder_Paralichthys_dentatus
	Swordfish_Xiphias_gladius
	Tautog_Tautoga_onitis
	Thread_herring_Opisthonema_oglinum
	Threespined_stickleback_Gasterosteus_aculeatus
	Tidewater_or_slender_mojarra_Eucinstomus_harengulus_jonesii
	Tilapia_Oreochromis_sp
	Tomcod_Microgadus_tomcod
	Tuna_sp_Thunnus_sp
	Unknown_fish_meadow_lake
	Unknown_fish_Oplegnathus_fasciatus93
	Unknown_fish_Ostracion_rhinorhynchos93
	Unknown_fish_Psenopsis97
	Unknown_fish_Stiphodon_alcedo93
	Unknown_fish_tautog90
	Walking_catfish_Clarias_batrachus
	Walleye_Sander_vitreus
	Weakfish_Cynoscion_regalis
	Whitefish_Coregonus_sp
	White_bass_Morone_chrysops
	White_catfish_Ameiurus_catus
	White_mullet_Mugil_curema
	White_perch_Morone_americana
	White_sucker_Catastomus_commersoni
	Windowpane_flounder_Scophthalmos_aquosus
	Winter_or_Yellowtail_flounder_Pseudopleuronectes_americanus_or_Pleuronectes_ferrugineus
	Witch_flounder_Glyptocephalus_cynoglossus
	Yellowfin_goatfish_Mulloidichthys_vanicolensis
	Yellow_bullhead_Ameiurus_natalis
	Yellow_jack_Carangoides_bartholomaei
	Yellow_perch_Perca_flavescens
	Zebrafish_Danio_rerio
	Atlantic_menhaden_LS15_Brevoortia_tyrannus
	Atlantic_silverside_cluster2
	Atlantic_silverside_cluster3
	Mummichog_Fundulus_heteroclitus_cluster2
	Mummichog_Fundulus_heteroclitus_cluster3
	Tautog_cluster4
	Tautog_Tautoga_onitis_cluster2
	Tautog_Tautoga_onitis_cluster3
Cartilaginous fish	Atlantic_angel_shark_Squatina_dumeril
	Atlantic_sharpnose_shark_Rhizoprionodon_terraenovae

	Atlantic_stingray_Dasyatis_sabina
	Barndoor_skate_Dipturus_laevis
	Blacktip_shark_Carcharhinus_limbatus
	Bluntnose_stingray_Dasyatis_say_formerly_unknown_ray94B
	cart_Brazilian_cownose_ray_Rhinoptera_brasiliensis_formerly_Cownose_ray98
	Bullnose_ray_Myliobatis_freminvillii
	Chain_dogfish_Scyliohinus_retifer
	Clearnose_skate_Raja_eglanteria
	Cownose_ray_Rhinoptera_bonasus
	Nurse_shark_Ginglymostoma_cirratum
	Roughtail_stingray_Dasyatis_centroura
	Sandbar_shark_Carcharhinus_plumbeus
	Sand_tiger_shark_Carcharias_taurus
	Smooth_dogfish_Mustelus_canis
	Southern_stingray_Dasyatis_americana
	Spiny_butterfly_ray_Gynmura_altavela
	Spiny_dogfish_Squaalus_acanthias
	Thresher_shark_Alopias_vulpinus
	Unknown_ray94c
	Unknown_ray_sp95
	Unknown_shark_sp_shark96B
	White_shark_Carcharhinus_leucas
	Winter_skate_or_Little_skate_Leucoraja_ocellata_or_erinacea
Marine Mammals	Bottlenose_dolphin_Tursiops_truncatus
	Fin_whale_Balaenoptera_physalus
	Humpback_whale
	Northern_right_whale
	Pacific_gray_whale
Marine Turtles	Leatherback_turtle
	Loggerhead_sea_turtle