Empire Wind 2023 Baited Underwater Remote Video (BRUV) and Environmental DNA (eDNA) Monitoring Survey

Annual Report

Prepared for:

Empire Offshore Wind LLC
Stamford Office - 600 Washington Blvd. - Suite 800
Stamford, CT 06901

Prepared by:

INSPIRE Environmental
513 Broadway
Newport, RI 02840

and

Monmouth University
West Long Branch, New Jersey

March 2024

REVISION HISTORY

Date	Revision	Note	Prepared	Reviewed	Approved
03/15/2024	0	Draft report for client review.	CC	DW, BG	BG

PROJECT DISTRIBUTION LIST

Name	Project Role	Email	Company
Elizabeth Marchetti	Fisheries Liaison Officer		Equinor
EJ Marohn	Marine Affairs Manager		Equinor
Brian Gervelis	Program and Contract Manager		INSPIRE

TABLE OF CONTENTS

APPENDICES

LIST OF TABLES

Page

LIST OF FIGURES

LIST OF ACRONYMS

ASV	Amplicon Sequence Variant
BAG	Before-After Gradient
BOEM	Bureau of Ocean Energy Management
BRUV	Baited Remote Underwater Video
cm	centimeter(s)
CoC	chain of custody form
CTD	conductivity, temperature, and depth
eDNA	Environmental DNA (deoxyribose nucleic acid)
Empire Wind	Empire Offshore Wind LLC
EW 1	Empire Wind 1
EW 2	Empire Wind 2
FBMP	Fisheries and Benthic Monitoring Plan
F/V	fishing vessel
GB	gigabyte
INSPIRE	INSPIRE Environmental, Inc.
kg	kilogram(s)
km	kilometer(s)
L	liter(s)
m	meter(s)
MaxN	maximum number of individuals observed in a single frame per video
mi	mile(s)
MicroSD	micro secure digital
mm	millimeter(s)
NJDEP	New Jersey Department of Environmental Protection
nm	nautical mile(s)
NYSERDA	New York State Energy Research and Development Authority
PCR	polymerase chain reaction
PERMANOVA	permutational analysis of variance
psu	practical salinity unit
ROSA	Responsible Offshore Science Alliance
SD	standard deviation
SSD	solid-state hard drive
WCSS	within-cluster-sum-of-square
WTG	wind turbine generator

1.0 OVERVIEW

Empire Offshore Wind LLC (Empire Wind) proposes to construct and operate an offshore wind farm located in the designated Renewable Energy Lease Area OCS-A 0512. The Empire Wind Lease Area covers approximately 79,350 acres (32,112 hectares) and is located approximately 14 statute miles (mi) (12 nautical miles [nm], 22 kilometers [km]) south of Long Island, New York and 20 mi (17 nm, 32 km) east of Long Branch, New Jersey (Figure 1-1). The Empire Wind Lease Area will be developed as two wind farms, known as Empire Wind 1 (EW 1) and Empire Wind 2 (EW 2), which will consist of up to 174 wind turbines. Monitoring efforts are combined for the proposed wind farms, covering the entire Empire Wind Lease Area as described in the Fisheries and Benthic Monitoring Plan (FBMP) (INSPIRE 2023). The results provided in this report pertain to samples collected across the entire Empire Wind Lease Area.

The New York Bight supports diverse fish and invertebrate assemblages (Guida et al. 2017; Thorne et al. 2020; NJDEP 2022;). Fisheries monitoring was designed to assess potential impacts of construction and operation activities within the Empire Wind Lease Area on these biological communities. The FBMP was developed in accordance with recommendations made by the Bureau of Ocean Energy Management's (BOEM) *Guidelines for Providing Information on Fisheries for Renewable Energy Development on the Atlantic Outer Continental Shelf* (BOEM 2019) and New York State Energy Research and Development Authority's (NYSERDA) *New York State Offshore Wind Master Plan: Fish and Fisheries Study* (NYSERDA 2017) and was created using an iterative process with the Empire Wind team coordinating with regional fishing organizations, working groups, and individual fishermen. In addition, throughout the permitting and development process the Empire Wind team consulted with state and federal fisheries resource management agencies and solicited feedback directly from stakeholders.

The FBMP (INSPIRE 2023) includes a fisheries monitoring survey that uses a Before-After-Gradient (BAG) design for assessing changes in fish diversity and abundance around wind turbines installed in the Empire Wind Lease Area. A BAG design is a statistically robust approach that samples at varying distances from a turbine both before and after construction and has proven effective for assessing offshore wind farm effects on both marine mammals and birds (Methratta 2020, 2021). In comparison to other survey designs, BAG can evaluate spatial heterogeneity and the spatial scale of effect, it does not require a reference (control) location, and it is capable of comparing post-construction patterns to baseline conditions. However, the utility of this approach for assessing pre- and post-construction changes and spatial effects relies on the ability to sample fish near turbine foundations. Mobile fisheries sampling techniques such as bottom trawl and dredging are problematic due to their broad scale sampling resolution, potential interaction with wind structures, and detrimental impacts to habitat (reviewed by Methratta 2021). When also considering fish mortality associated with traditional sampling techniques, the use of non-destructive and non-extractive techniques is paramount to the accurate and sustainable assessment of marine fauna (Stat et al. 2019; Methratta 2021).

Aquatic Environmental DNA (eDNA) and Baited Remote Underwater Video (BRUV) surveillance systems have emerged in recent years as alternative methods for monitoring marine biodiversity. In comparison to traditional sampling techniques, eDNA and BRUV are inexpensive and non-extractive approaches that can generate accurate, unbiased, and high-resolution data (Stat et al. 2019). These sampling methods are also fixed (relatively stationary) and can sample on or very close to turbine foundations, which is essential to assessing distance-based effects of wind turbine installations (Methratta 2021). Each sampling method, however, possesses its own limitations and biases that can impact monitoring efforts. For instance, BRUV surveillance systems can record a diverse assemblage of mobile fish species, but these systems only record video in a specific field of view where the utility of recorded footage can be influenced by the type of species in the area, the type of bait used, and underwater visibility (Colton and Swearer 2010; Griffin et al. 2016; Jones et al. 2021). Video analysis methods for BRUV data are also underdeveloped, often relying on manual review and annotation which are both time consuming and expensive (Langlois et al. 2020). eDNA metabarcoding can be used to assess fish and other taxa at lower financial and effort costs than BRUV surveillance, but results can be influenced by several factors, including DNA persistence and degradation, sampling strategies, workflows, and availability of taxa markers in reference databases (reviewed by Stat et al. 2019; Kopp et al. 2023).

Given the observed “reef effect” of structure-oriented fish species from wind farms in Europe (e.g., Wilhelmsson et al. 2006; Bergstrom et al. 2013; Reubens et al. 2013) and North America (e.g., Wilber et al. 2022), INSPIRE Environmental (INSPIRE), under contract with Empire Wind, carried out a large-scale BAG survey effort in the Empire Wind Lease Area using eDNA and BRUV with stereo-video camera beginning in July 2023. The survey is designed to occur seasonally (spring, summer, fall, winter) within the Lease Area, with monitoring targeted for two years pre-construction and two years post-construction. Monitoring is also planned during construction, provided the survey will not interfere with construction operations. Two years of sampling are planned prior to the commencement of offshore construction. The eDNA survey will continue during the construction phase and a minimum of two years of eDNA monitoring will be completed following offshore construction. The overall goal of the survey is to assess potential changes in the occurrence, diversity, and abundance of fish species in the Lease Area before, during, and after the installation of wind turbines.

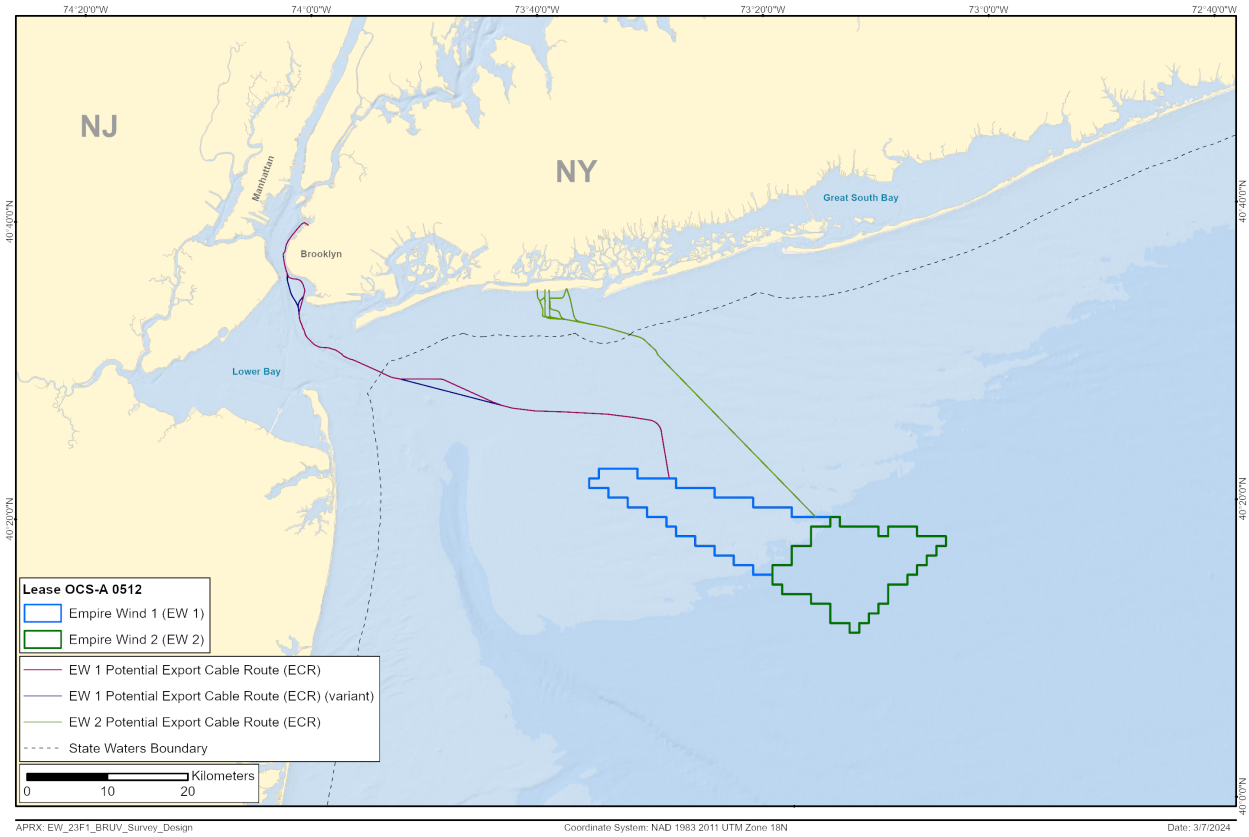


Figure 1-1. Empire Wind Lease Area and Export Cable Route overview map

1.1 Study Objectives

This report provides results of the first year of pre-construction fisheries monitoring via BRUV/eDNA surveys which began July 2023 in the Empire Wind Lease Area. The goals of the pre-construction surveys were to collect baseline information on the community composition and relative abundance of fish species for direct comparison with survey results from the construction and post-construction phases. The Empire Wind fisheries monitoring plan was conducted in accordance with the FBMP as well as guidance from NYSERDA and the Responsible Offshore Science Alliance (ROSA 2021).

2.0 METHODS

2.1 Sampling Design Overview

In-situ water measurements and videos were collected seasonally within the Lease Area aboard the fishing vessel (F/V) *Cailyn and Maren*, a commercial gillnetter and lobster vessel based out of Little Compton, Rhode Island. Samples were strategically collected at eight stations that coincided with proposed wind turbine generator (WTG) locations (Figure 2-1). These WTG stations were chosen to ensure even and full distribution of sampling stations in the Lease Area and divided evenly both above and below the 35-meter (m) depth contour that bisects the proposed wind farm as detailed in the FBMP (INSPIRE 2023). Four bins at distances of 0 m, 50 m, 100 m, and 200 m from the base of each WTG station were designated for collecting stereo BRUV video footage for a period of 1-hour. Water column profiles and eDNA samples were only collected 100 m from each WTG station. Field data collected using a specific technique (i.e., water column profiles, eDNA, stereo BRUV) during a single seasonal excursion will hereafter be referred to as a sampling event.

Collected eDNA water samples were transported to Monmouth University for analysis, and BRUV video footage was analyzed in-house by INSPIRE scientists. Physical variables from water column profile data, including conductivity (i.e., salinity), temperature, and depth, were used by both Monmouth University and INSPIRE to inform and interpret results. A Chain of Custody (CoC) form was established to properly account for data collected by each method and transferred to the respective parties.

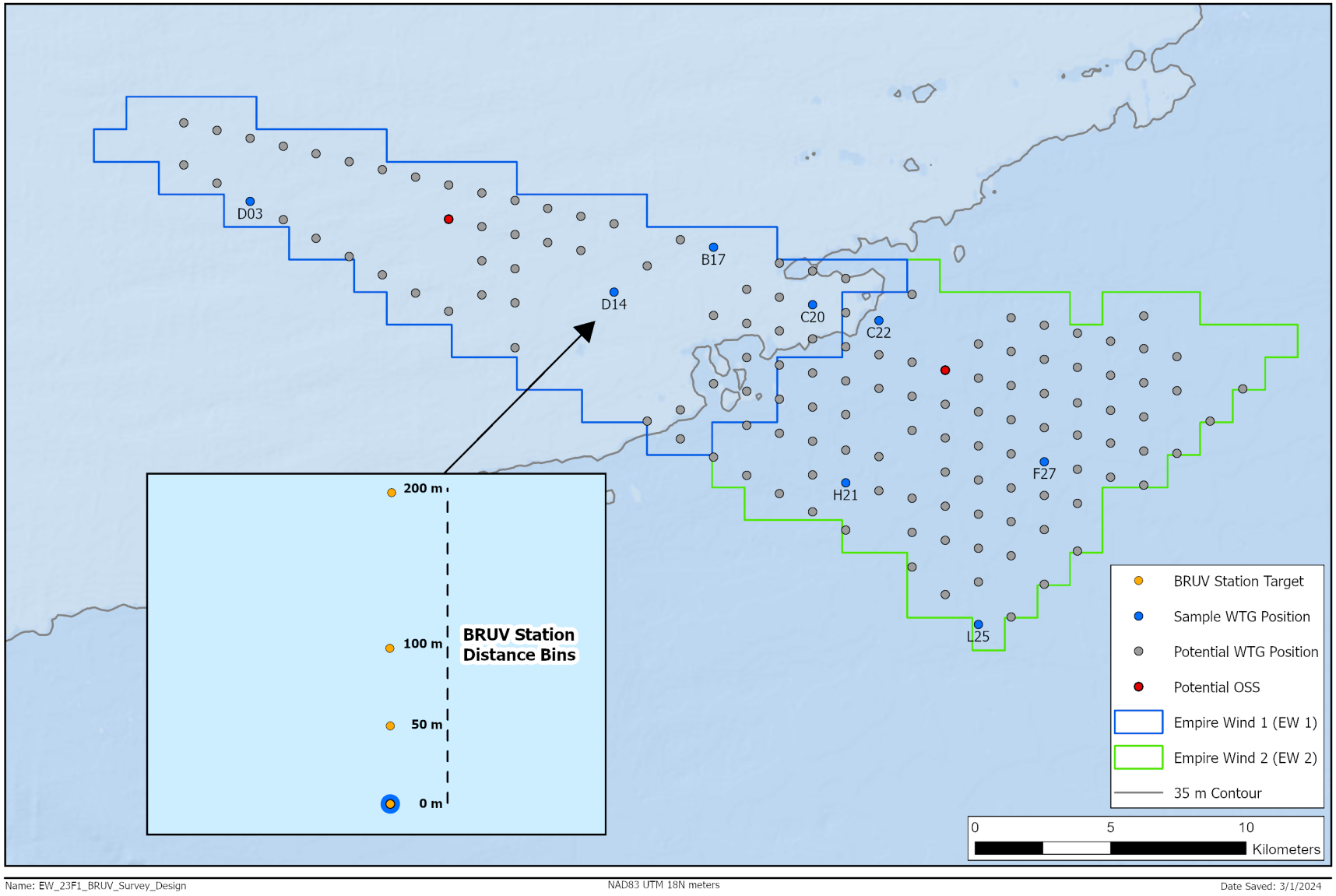


Figure 2-1. Empire Wind Lease Area and WTG sampling stations

2.2 Data Collection

2.2.1 Water Column Profiles

Prior to the deployment of stereo BRUV systems, a single water column profile and eDNA sample were collected from the vessel at 100 m from each WTG station. Conductivity (i.e., salinity), temperature, and depth (CTD) were recorded for each WTG station using a SonTek CastAway CTD (Xylem, Inc., Washington D.C., USA) (Figure 2-2). The CTD was allowed to acclimate at the sea surface briefly before measuring physical parameters throughout the water column and stopping within 2 m of the seafloor to prevent disturbing the benthic environment. Collected water column data were downloaded after each deployment and exported to an external solid-state hard drive (SSD). Data collection activities were recorded on field data sheets, which included WTG station name, time, water depth, and coordinates, as well as CoC forms to document each completed CTD cast.

2.2.2 Environmental DNA (eDNA)

Water samples for eDNA analysis were collected alongside CTD casts at a distance of 100 m from each WTG. Each water sample was collected within 2 m from the seafloor using a 1.5-liter (L) water sampler (1520-C20 Kemmerer bottle; Wildco, Yulee, FL, USA) (Figure 2-2). After water samples were collected, sample water was used to rinse the 2-L Nalgene wide-mouth opaque amber storage bottle. The remainder of the sample was then decanted into the same pre-rinsed bottle, labeled with the appropriate sample number, and immediately stored in a chest freezer onboard the vessel in accordance with protocols provided by Monmouth University scientists. A control sample of distilled water underwent the same field processing as the eDNA samples to assess relative exposure to other elements and evaluate any anomalous results. Data collection activities were recorded on field data sheets, which included WTG station name, time, water depth, and coordinates, to document each collected eDNA sample.

After the completion of each survey, the chest freezer and eDNA samples were relocated from the vessel to INSPIRE storage facilities. Collected eDNA water samples remained frozen during packing and were shipped overnight to Monmouth University in an insulated shipping container. The nine frozen sample bottles were received by Monmouth University and then thawed for ~24h at 4°C. Thawed seawater was vacuum filtered onto 0.45 micrometer pore size, 47 millimeters (mm) diameter filters (Cytiva-Whatman NC 45 ST #10401170). The filters were stored with the sample surface folded on the inside, frozen, in sterile tubes until extraction. DNA was extracted from the tubes using the Qiagen DNeasy PowerWater Kit (cat. Nos. 14900-50-NF and 14900-100-NF), with a slight modification to the final DNA elution step to increase yield (Stoeckle et al. 2022). Primers used for amplification were from Riaz et al. (2011) modified to include Illumina adapters for subsequent sequencing (Appendix A).

A portion of the polymerase chain reaction (PCR) product was visualized on a 2.5% agarose gel to confirm the presence of a dominant band at roughly 200 base pairs, indicating successful

amplification of fish (and other vertebrate) sequences. With the samples, a positive (either striped bass DNA or a mixture of elasmobranch species, obtained from verified fin clips) and negative control (molecular grade water) were also run for quality assurance / quality control. Extracted DNA with a band was quantified, and quality checked on a NanoDrop spectrometer (ThermoFisher). Following the acceptance of results from the PCR amplification technique (based on gel electrophoresis, standard quality assessment and control), 20x diluted aliquots of PCR products were shipped to the Bioanalytical Services Lab for sequencing.

2.2.3 Baited Remote Underwater Video (BRUV)

Stereo BRUV systems, hereafter referred to as BRUV systems, typically consist of a frame that supports two convergent video cameras inside waterproof housings with some type of baited container positioned in front of the cameras (Langlois et al. 2020) (Figure 2-2). Aluminum frames, which support the videography hardware and bait, were fabricated locally (The Metal Guy LLC; Newport, Rhode Island) and modeled after BRUV systems produced by commercial businesses (e.g., SeaGIS 2020) and academic research effort (e.g., Langlois et al. 2020). Six steel weights (3.2 kilograms [kg]) were secured to the base of each aluminum frame, providing an additional 19.2 kg of weight for stability on the seafloor. To precisely estimate body size measurements of fish from collected video footage, each BRUV system was fitted with two GoPro HERO11 video cameras. Each GoPro HERO11 video camera was equipped with a 64 gigabyte (GB) MicroSD (secure digital) card, a GoPro Enduro battery, and a silica desiccant pack and sealed inside a SeaGIS photogrammetric underwater camera housing (230 mm diameter; SeaGIS Pty Ltd, Victoria, Australia). Camera housings were secured to a central support bar and set for stereoscopic measurements, i.e., positioned 58.5 centimeters (cm) apart at a 7-degree convergence angle to point at the bait bag, following instructions from SeaGIS (SeaGIS 2020). The bait bag, a Heavy Duty bag (Rainbow Net & Rigging) filled with three to four individual Atlantic mackerel (*Scomber scombrus*), was attached to a 2-m-long bait arm (constructed from schedule-40 PVC) that was marked every 10 cm. A surface marking system was attached to the frame to aid in the safe deployment, identification, and retrieval of each BRUV system. Red Sea Alex buoys were appropriately marked with the project name and INSPIRE contact information. Each buoy was attached to 60 m of 7/16-inch ProFlex Sink Rope and secured to the top of each BRUV system.

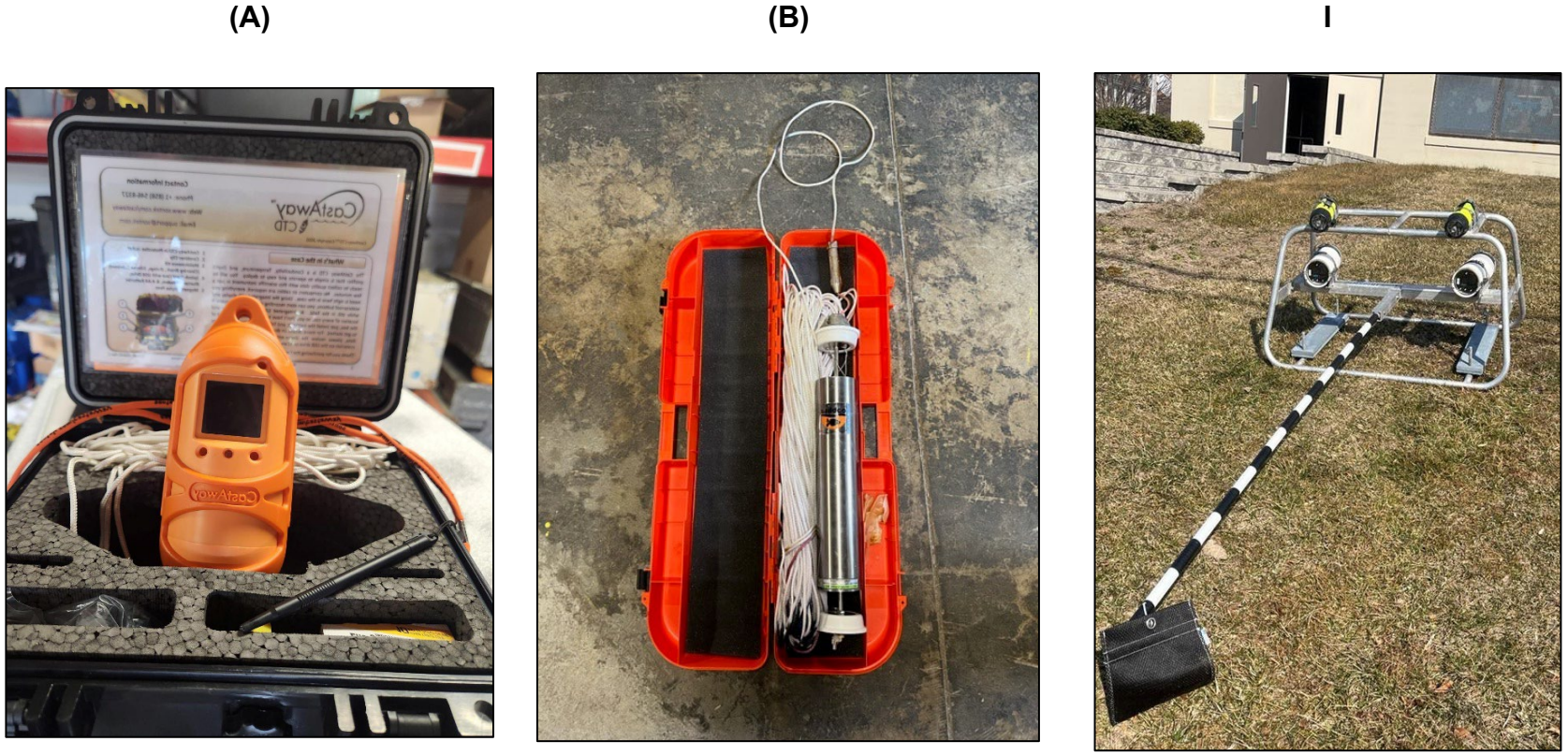


Figure 2-2. Primary field sampling equipment; (A) CastAway CTD; (B) Kemmerer sampling bottle for eDNA collection; (C) stereo BRUV system. Stereo BRUV systems include a subsea lighting setup, two GoPro HERO11 video cameras with SeaGIS camera housings, weights, a bait arm, and a bait bag.

BRUV systems and associated hardware were assembled, calibrated, and baited prior to deployment at the specific distance bins from each WTG station (Figure 2-3). Video camera calibrations were conducted to support stereoscopic measurements of fish body size from collected video footage. A HOBO temperature data logger (MX2203, 120 m depth rating; Onset, Bourne, MA, USA) was also fastened to the BRUV system deployed at the 100-m distance bin to record additional bottom water temperature during deployments. After each system was synchronized using photodiode flash to facilitate video synchronization and future stereo measurements, BRUV systems were deployed on the seafloor in order of increasing distance from the base of the WTG for a period of 1 hour (Figure 2-3). In other words, four BRUV systems were deployed at each previously identified distance from a WTG station in quick succession to ensure video collected at each distance from a WTG station occurred over a similar period. BRUV systems were then hauled onto the vessel after the 1-hour monitoring period whereupon recorded video footage and temperature data were reviewed and transferred to an external SSD; camera batteries and bait were also replaced before deployment at the next station. Field and survey information, including WTG number, BRUV system number, time, coordinates, depth, wind speed, wave height, and Beaufort Sea state, were recorded on field data sheets during the deployment and retrieval of each BRUV system.

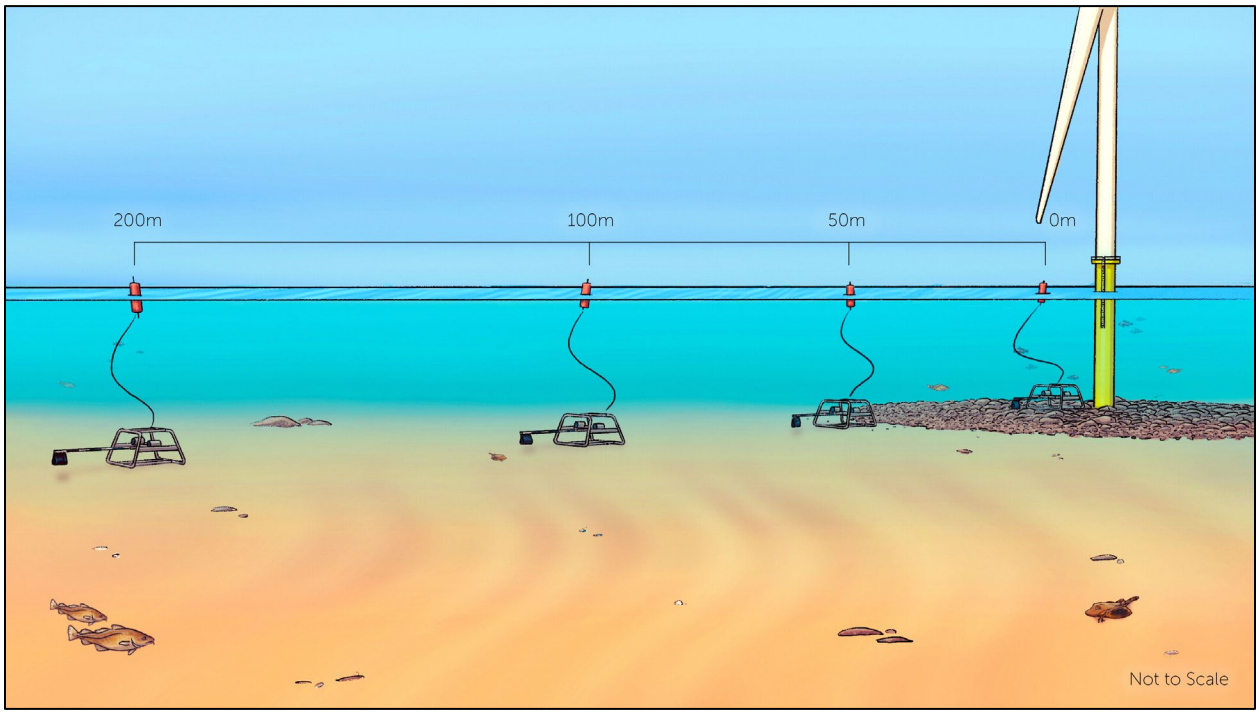


Figure 2-3. BRUV deployments at four distance bins for sampled WTG stations

Based on preliminary observations from the July 2023 survey (see Results), modifications were made to each BRUV system to improve data collection and reduce technical failures. For instance, due to poor ambient light conditions at depth, underwater lighting setups consisting of two dive lights (eLED Light Cannons; Underwater Kinetics USA, CA, USA) were added to BRUV

systems for the September 2023 survey and to improve video quality and species identification following methods from Unsworth et al. (2014). Lighting mounts were then welded to the top of each system directly above the SeaGIS camera housings for the December 2023 survey to prevent light refraction from interfering with video quality (Figure 2-2). Other equipment modifications included the use of internal GoPro Enduro batteries as loose connections between external batteries and GoPro Hero cameras resulted in premature video recordings during the September and December 2023 surveys. The overall number of GoPro Enduro batteries, MicroSD cards and readers, and USB-C cables were also increased to ensure backups existed to support field operations.

2.3 Data Analysis

2.3.1 Water Column Profiles

Collected water column data from each CTD cast were reviewed for inconsistencies and visualized using the statistical computing software R (R Core Team 2023).

2.3.2 Environmental DNA (eDNA)

Sample indexing, normalization, denaturing, and Illumina sequencing were performed at the Biological Services Laboratory according to standard protocols (complete protocols available upon request). For each sample, a set of two FastQ files containing forward and reverse sequences of the amplified DNA was produced. These were delivered via Illumina's data transfer server, BaseSpace, and stored backed up on several different servers when received.

Amplified sequences were then processed by Monmouth University using the DADA2 pipeline (Callahan et al. 2016) in the statistical computing program R (R Core Team 2023) to identify unique sequence variants and match them to a reference of known sequences. The output of DADA2 bioinformatics includes a 'taxa table' that contains detected Amplicon Sequence Variants (ASVs), associated taxa names for known ASVs from a reference database, and the number of times that ASVs were detected for a given sample (i.e., number of reads). ASVs are bits of DNA code that have been ascribed to specific taxa and used to characterize community composition. For instance, the number of unique ASVs detected for a species increases confidence in the identification of the species. It is also common practice to interpret the number of ASV reads of all variants for a given taxa as a relative approximation of a taxa's abundance in a sampled environment (Deagle et al. 2019). However, it should be noted that eDNA methodology assumes DNA release correlates with the abundance of individual present and therefore cannot determine the true number of individuals in an area. The availability of eDNA for inferring relative abundance can also be influenced by various factors, including DNA release and from differences in animal density, degradation from environmental conditions, mechanical force, microbial activity, and chemical reactions (reviewed by Ramirez-Amaro et al. 2022), and masking from overabundant taxa (Skelton et al. 2023) as well as differences in applied methodologies (reviewed by Goldberg et al. 2016).

Multivariate statistical analyses were conducted to examine potential spatial and temporal patterns of teleost taxa from the eDNA community data. A permutational analysis of variance (PERMANOVA) test was conducted on the relative read abundance data using sampling event as a factor and bottom depth as a covariate. A k-means cluster analysis was conducted to determine groups of similar community composition, or clusters, based on relative read abundance. In brief, clustering is a useful tool that assists in finding clusters of samples in a dataset with the greatest similarity in taxonomic composition while also identifying the greatest dissimilarity between other clusters of samples. K-means clustering is the most popular and simple clustering technique for dividing observations into a specific number of groups. Within-cluster-sum-of-square (WCSS) values were first calculated to determine the optimal number of clusters (i.e., *k*) for the eDNA community dataset. A matrix of taxa ASV reads by taxa and sample (i.e., station within each sampling event) were transformed using the Hellinger method (i.e., the square root of the ASV reads divided by the total ASV reads for a sample) and then partitioned into a set of *k* clusters. Fish taxa associated with each cluster were summarized according to the total number of ASV reads across all samples to characterize the identified clusters. Only dominant fish taxa, or those with over 10,000 ASV reads across all samples, were retained when describing fish community composition for identified clusters.

2.3.3 Baited Remote Underwater Video (BRUV)

Collected video footage was uploaded to INSPIRE's network after returning to shore and demobilizing. Original video files were archived on the project-specific external SSD for two months until backups were confirmed. Videos were organized by sampling event, station, distance bin, and camera and then reviewed for completeness and any file corruption. Due to overall visibility conditions observed during video playback and potential analytical implications, uncorrupted video files were ranked in terms of quality similar to Unsworth et al. (2014) using the four-level scale below.

0. Unusable: Cannot see bait bag; unable to identify organisms
1. Poor: Can see bait bag; unable to distinguish fish
2. Good: Clear visualization of bait bag and fish identification
3. Excellent: Clear visualization of bait bag and beyond; species level identification of fish.

Only videos assigned a visibility score of "2 – Good" or "3 – Excellent" were analyzed further to ensure reviewers could confidently identify fish species to the lowest practical identification level for future analyses. Figure 2-4 provides examples of these visibility scores using footage collected from the 2023 sampling events.

Figure 2-4. Examples of BRUV video footage and visibility scores: 0 – Unusable, 1 – Poor, 2 – Good, 3 – Excellent

Videos assigned a score of "2 – Good" or "3 – Excellent" were processed using the video editing software Shotcut (Meltytech, LLC; https://shotcut.org/). Left and right videos were first synchronized using a photodiode flash recorded prior to deployment, ensuring video frames collected from the same BRUV system deployment aligned in space and time, i.e., a fish interacting with the bait bag at 5 minutes time can be seen on the same frame in each video. Synchronized videos were then trimmed to the full length of time the system recorded environmental conditions on the seafloor and exported in a compressed file format (e.g., MPEG-4 Part 14 [.mp4]) for analysis. In cases where visibility could be improved, color correcting filters were applied.

Exported videos were then analyzed for species community assemblage and relative abundance using the Behavioral Observation Research Interactive Software (BORIS, Friard and Gamba 2016). Video footage was reviewed and analyzed for the relative abundance of each species, i.e., the maximum number of individuals observed in a single frame per video (MaxN; Priede et al. 1994). Although BRUV systems recorded video footage using two video cameras, MaxN values were only estimated using video footage from one camera.

3.0 RESULTS

In total, three field sampling events were conducted from July to December of 2023; 5 to 7 July 2023 (Survey ID 23F1), 11 to 13 September 2023 (Survey ID 23F2), and 5 to 7 December 2023 (Survey ID 23F3). Water column profiles, eDNA water samples, and BRUV video footage were collected at each station and sampling event. Vessel operations adhered to the specific vessel strike avoidance measures provided by BOEM as part of the Atlantic Outer Continental Shelf Leases Project Design Criteria and Best Management Practices. Marine mammals documented during survey operations included: one sighting of a common dolphin (*Delphinus delphis*) on 7 July 2023, a pod (>50 individuals) of bottlenose dolphins (*Tursiops truncates*) on 13 September 2023, and four common dolphins on 6 December 2023. Scientific sampling activities did not result in the inadvertent take, or the accidental killing or injuring, of any marine mammals or federally endangered species.

Water column profile results were processed across all three sampling events and are available for review in this report. Due to ongoing data processing and analyses, eDNA and BRUV results are only available for a subset of stations and sampling events. For instance, eDNA results for teleost taxa samples are available for all stations and sampling events whereas results for elasmobranch taxa samples are only available for three stations from the July and September 2023 sampling events. BRUV video results are only available for the July and September 2023 sampling events; relative abundance for observed species were only estimated for video collected in September 2023 due to visibility and video quality issues experienced in July 2023 (see Section 3.3 – Baited Remote Underwater Video (BRUV) below for details).

3.1 Water Column Profiles

Water column profiles were collected at each sampling station and sampling event, resulting in 24 sets of salinity and temperature data. It should be noted that measurements for station D03 in September 2023 were not included in any summaries as the CTD did not record salinity and temperature throughout the full water column. Bottom water salinity was similar in July and September 2023, ranging from 31.2 to 32.5, and decreased to 30.8 to 31.8 psu in December 2023 (Figure 3-1). Bottom water temperature ranges declined from 12.9-16.9ºC in July 2023 to 11.8-12.6ºC in December 2023 (Figure 3-1). Temperature profiles were also observed to invert from September to December 2023 where surface water temperatures were lower than bottom water temperatures at most stations. Salinity and temperature profiles for each station and sampling event are provided in Appendix B.

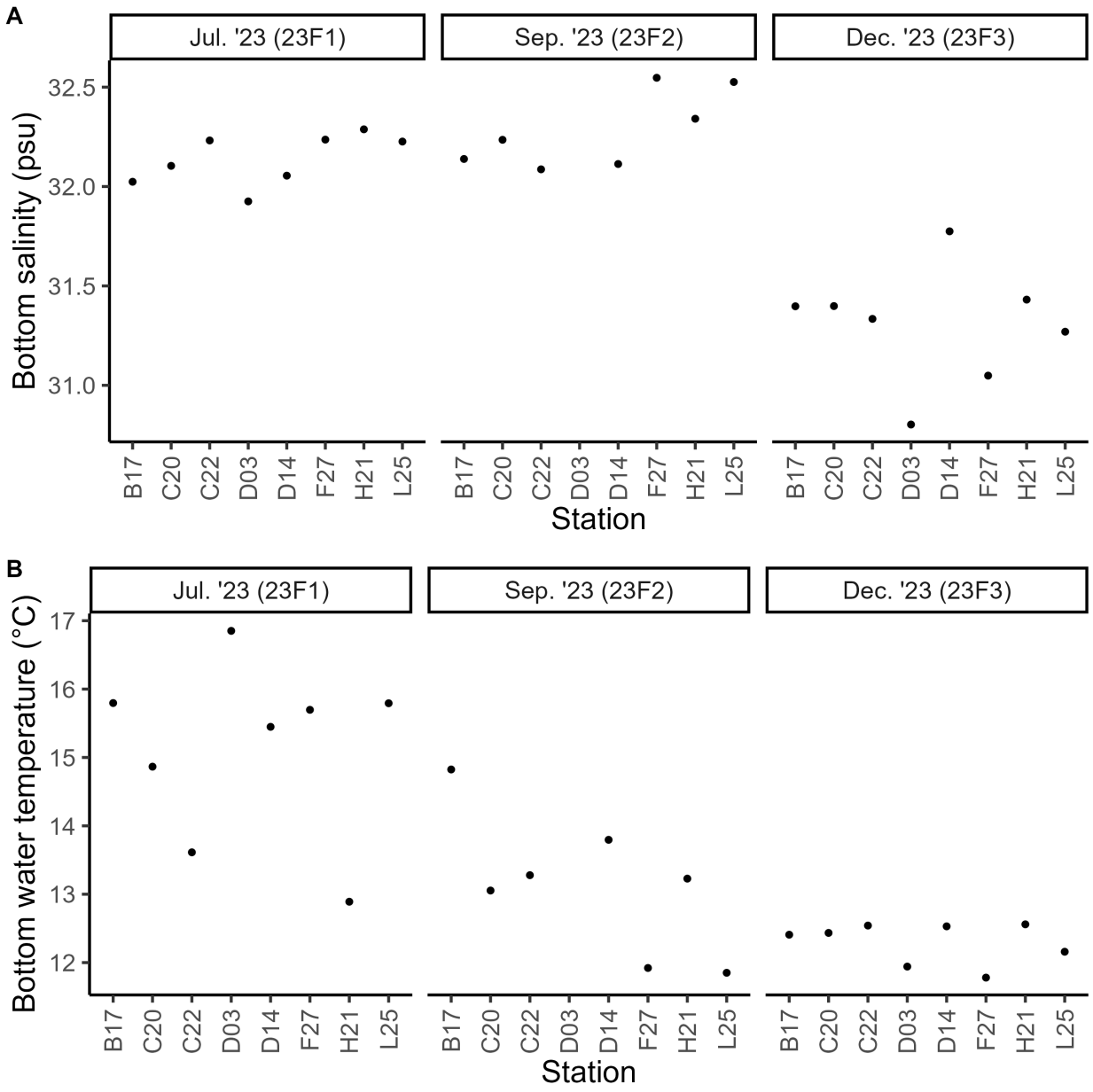


Figure 3-1. Summarized bottom water CTD data, including (A) salinity and (B) temperature, for all stations sampled in July, September, and December 2023. Values are summarized as the average of values between the maximum CTD depth and the depth 5 meters above the maximum recorded depth.

3.2 Environmental DNA (eDNA)

Eight eDNA samples were collected 100 m from each WTG station during each of the three sampling events, resulting in 24 water samples. Freezing and light protection successfully prevented excessive DNA degradation. Teleost taxa samples (n = 24) were processed for all stations sampled during the three sampling events whereas elasmobranch taxa samples (n = 6) were only processed for three stations (B17, D14, D03) from the July and September 2023

sampling events. For the purposes of the eDNA results, “taxa” in this section refers to ASV reads that are associated with taxa (usually species) listed in a DNA library.

Overall, 32 taxa from 35 different teleost ASVs were detected across all three sampling events (Table 3-1; Figure 3-2). The relative abundance (i.e., taxon reads / total fish reads in a sample) for teleost taxa averaged across stations for each sampling event revealed northern sea robin (*Prionotus carolinus*) had the highest relative abundance in July and September 2023 and scup (*Stenotomus chrysops*) had the highest relative abundance in December 2023. Other taxa with high relative abundance estimates included Atlantic herring (*Clupea harengus*), Atlantic mackerel (*Scomber scombrus*), black sea bass (*Centropristis striata*), American butterfish (*Peprilus triacanthus*), and bluefish (*Pomatomus saltatrix*). Seven elasmobranch taxa and ASVs were detected in the samples analyzed for July and September 2023 (Table 3-1; Figure 3-3). Such taxa included spiny dogfish (*Squalus acanthias*), blacktip shark (*Carcharhinus limbatus*), sandbar shark (*Carcharhinus plumbeus*), little skate/winter skate species (*Leucoraja* spp.), roughtail stingray (*Bathytoshia centroura*), Brazilian cownose ray (*Rhinoptera brasiliensis*), and barndoor skate (*Dipturus laevis*). Little skate/winter skate species (*Leucoraja* spp.) had the highest relative abundance for elasmobranchs for both July and September 2023. Additional information on identified taxa and number of reads by station and sampling event can be found in Appendix C.

Table 3-1. Number of taxa detected using eDNA and BRUV sampling methods from July and September 2023. Frequencies represent number of unique taxa and are broadly summarized by teleosts (i.e., bony fish), elasmobranchs (i.e., skates and rays, and sharks), and cephalopods (i.e., squid). eDNA sampling did not attempt to detect cephalopods using molecular methods and thus are not identified (NA). Additional taxa information can be found in Appendix D.

Taxa	**Sampling Method**	
	eDNA	**BRUV**
Bony fish	32	10
Skates and Rays	4	3
Sharks	3	3
Squid	NA	3

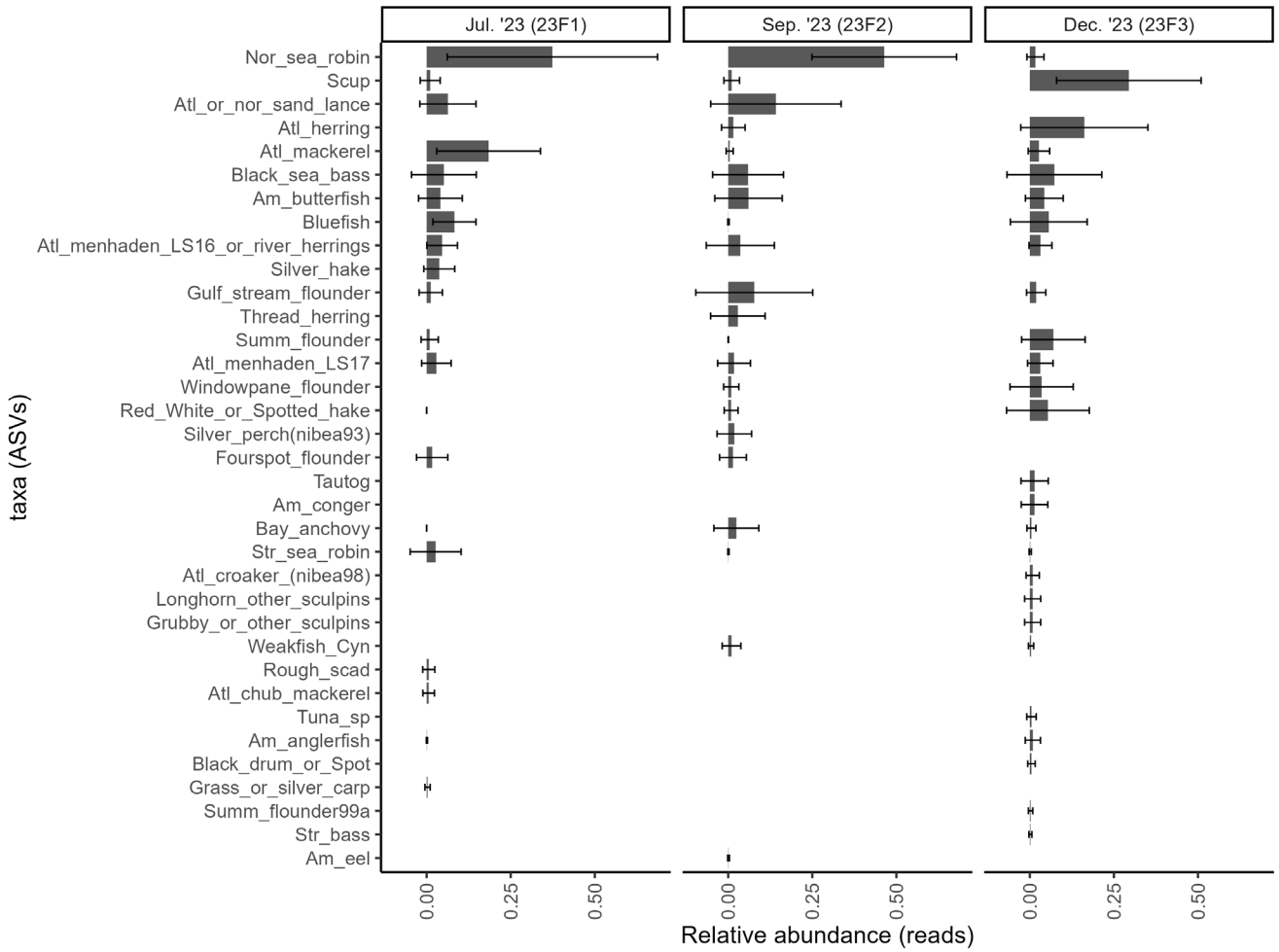


Figure 3-2. Teleost taxa and relative read abundance (+/- standard deviation [SD]) over all stations sampled in July, September, and December 2023. Large SD bars indicate high variability in relative abundance among stations within a sampling event.

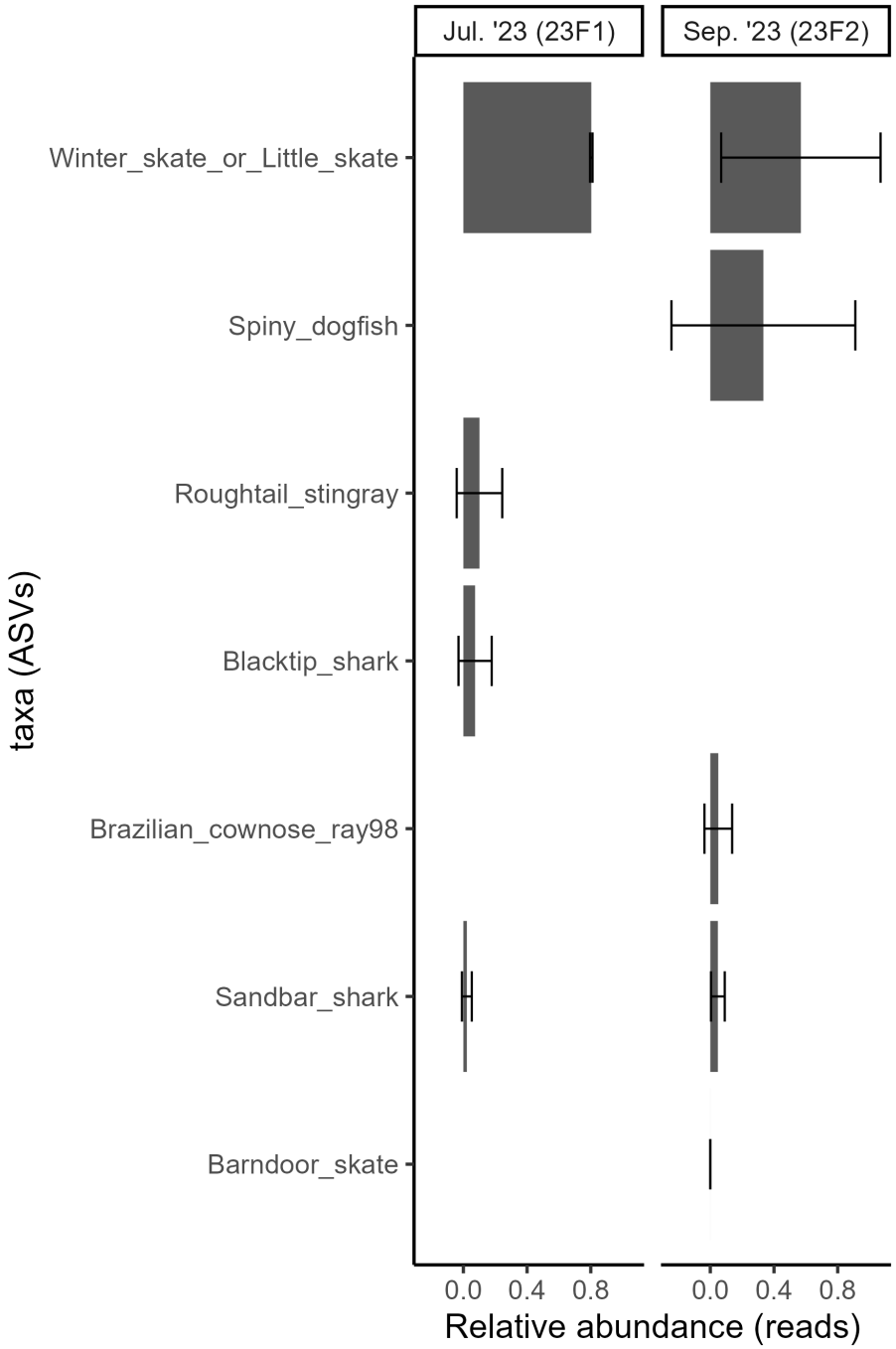


Figure 3-3. Elasmobranch taxa and relative read abundance (+/- standard deviation [SD]) for three stations (B17, D14, D03) sampled in July and September 2023. Large SD bars indicate high variability in relative abundance among stations within a sampling event.

Teleost taxa richness (i.e., number of taxa) values were also reviewed over all stations for each sampling event (Figure 3-4). Although 35 unique teleost ASVs were detected overall, median taxa richness value across all stations per sampling event were lower, indicating that different taxa were detected among stations and sampling event.

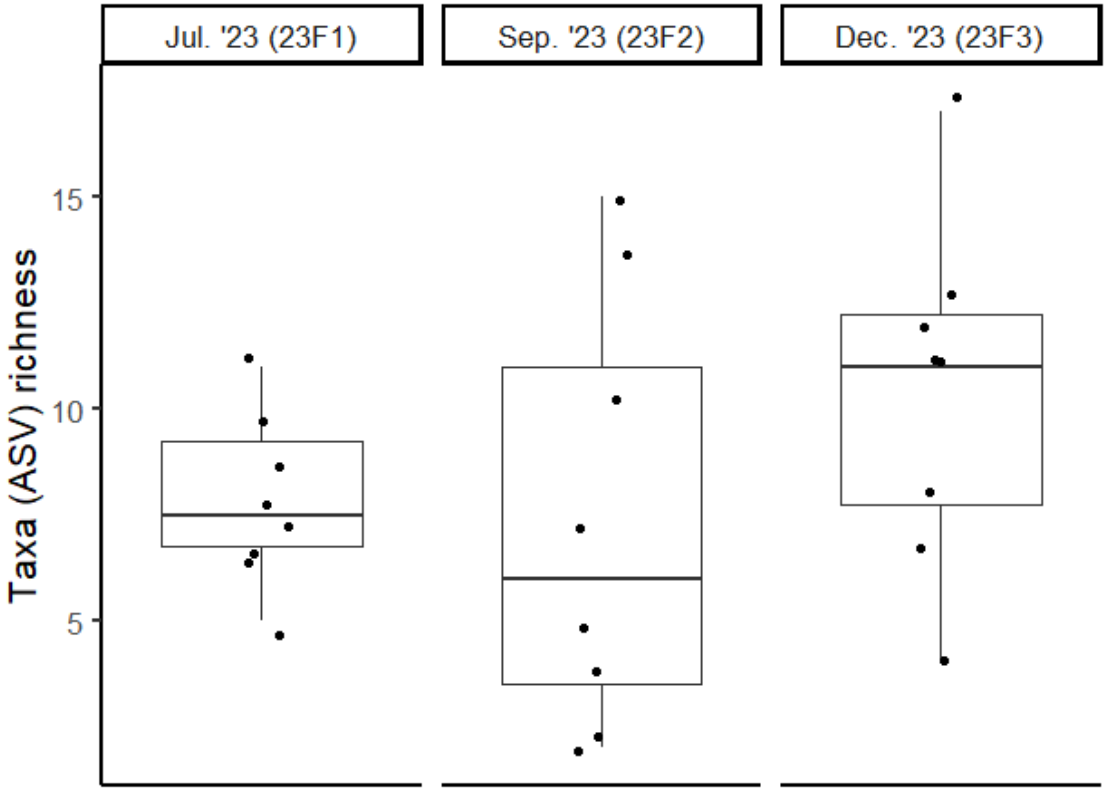


Figure 3-4. Teleost taxa (ASV) richness for all stations in July, September, and December 2023. Jittered points superimposed over each boxplot represent total richness at each station.

Multivariate statistical analyses indicated that teleost taxa composition varied spatially and temporally based on the eDNA methodology. For instance, temporal differences in relative read abundances for teleost taxa ASVs resulted in a dissimilar teleost community detected in December compared to July and September 2023 (PERMANOVA $F = 3.88$, $p < 0.001$). K-means cluster analysis grouped eDNA community data into five clusters of similar taxonomic composition based on the relative read abundances. These clusters were characterized by the following dominant taxa listed in approximate order of relative read abundance (Figure 3-5):

- Cluster 1: Scup, hake, bluefish, and windowpane flounder
- Cluster 2: Atlantic herring, scup, and bluefish
- Cluster 3: Northern sea robin

- Cluster 4: Northern sea robin, Atlantic mackerel, Atlantic menhaden or river herring, black sea bass and Atlantic menhaden

- Cluster 5: Northern sea robin, sand lance, butterfish, and thread herring

A spatial gradient among stations associated with these clusters was most apparent in December 2023, when Cluster 2 (Atlantic herring, scup, and bluefish) dominated three southeastern stations and Cluster 1 (scup, hake, bluefish, and windowpane flounder) was prevalent at the other stations in the Lease Area (Figure 3-5). In September 2023, clusters with high relative read abundances of northern sea robin were dominant at eastern stations In the Lease Area (Figure 3-5).

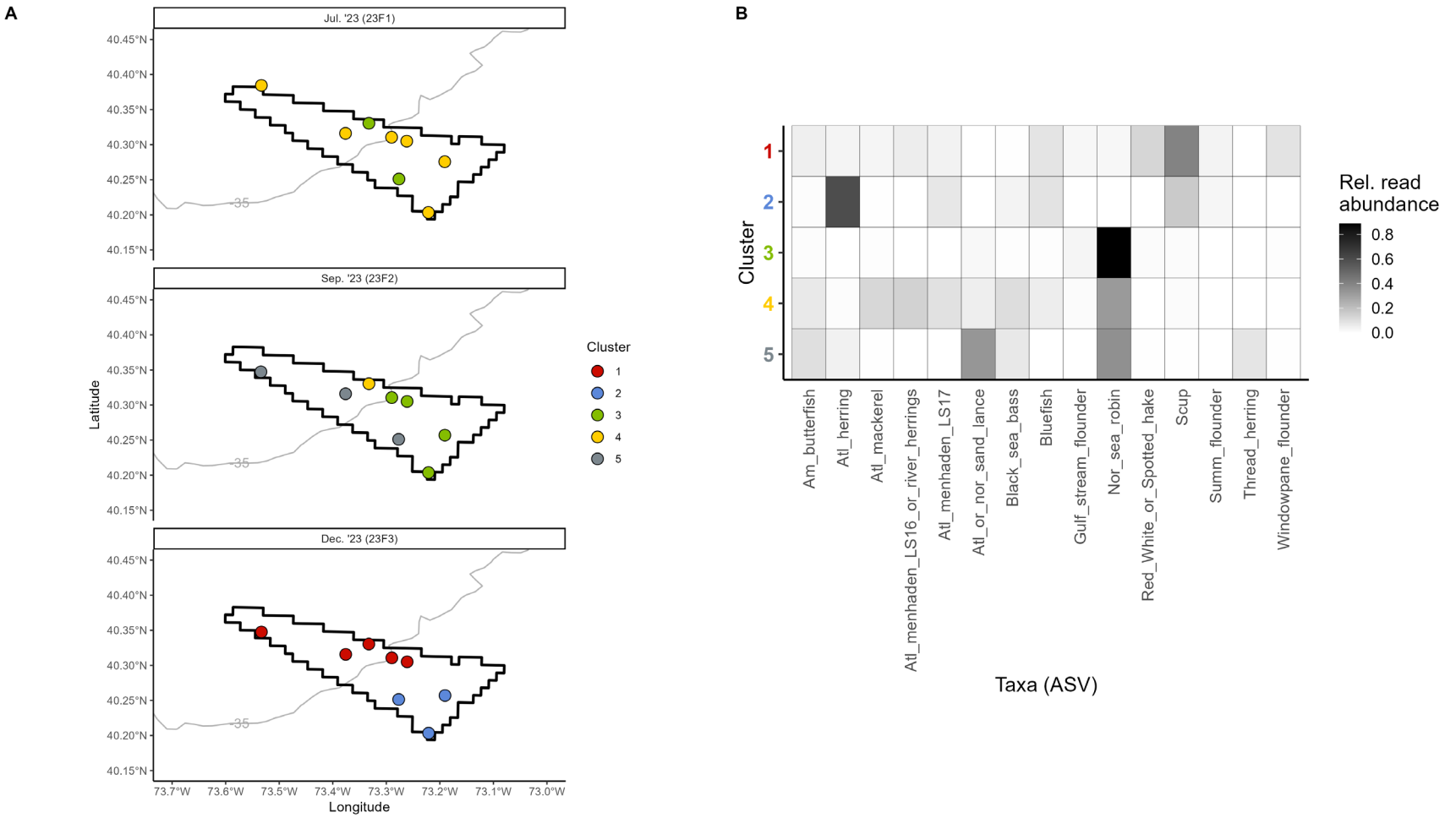


Figure 3-5. K-means cluster analysis of teleost community composition from eDNA methodology, including (A) the distribution of community clusters across stations and sampling events in the Lease Area and (B) teleost taxa represented in each cluster by relative ASV read abundance. Only dominant teleost taxa, or those with at least 10,000 ASV reads overall, were included when characterizing community composition for each cluster.

3.3 Baited Remote Underwater Video (BRUV)

BRUV systems were successfully deployed at each distance bin and WTG station for each sampling event, resulting in 96 BRUV system deployments and 192 video camera recordings for analysis (i.e., two video cameras per system). Overall, at least 92% (n = 177) of the 192 video camera recordings from the three field sampling events were uncorrupted (Table 3-2). Of the 177 uncorrupted videos (which include left and right video camera recordings), 48% (n = 85) of the videos were assigned a visibility score of either "2 – Good" or "3 – Excellent" and suitable for community composition and relative abundance analyses. Visibility and video quality scores were observed to vary by sampling event where the greatest number of videos suitable for analysis were collected in September 2023 (74%) followed by December 2023 (41%) and July 2023 (30%). Only videos collected in July 2023 and September 2023 were analyzed during the current reporting period. However, due to the lack of underwater lighting and visibility conditions observed in the first survey, analysts only evaluated species diversity in videos collected during July 2023, i.e., did not estimate MaxN values for identified taxon groups.

Table 3-2. Metadata summary of video footage collected by BRUV systems in July, September, and December 2023. Presented values represent frequency counts whereas values in parentheses are percentages of for a given variable by survey.

	Frequency (Percent)		
	July 2023 (23F1)	**Sept. 2023 (23F2)**	**Dec. 2023 (23F3)**
Video Files Present			
Yes	62 (96.9)	59 (92.2)	64 (100)
No	2 (3.1)	5 (7.8)	0 (0)
Video Health			
Healthy	56 (90.3)	57 (96.6)	64 (100)
Corrupted	6 (9.7)	2 (3.4)	0 (0)
Video Quality			
0 – Unusable	12 (21.4)	1 (1.8)	22 (34.4)
1 – Poor	27 (48.2)	14 (24.6)	16 (25.0)
2 – Good	17 (30.4)	42 (73.7)	14 (21.9)
3 – Excellent	0 (0)	0 (0)	12 (18.8)

In total, 19 taxa, including teleosts, elasmobranchs, and cephalopods, were identified from "2 – Good" quality videos collected during July 2023 (at 3 stations) and September 2023 (at 7 stations). Four taxa, including unidentified species of flounder, hammerhead shark, sea robin, and skate, were identified during both sampling events (Table 3-3). For the September 2023 sampling event, MaxN values were calculated for 17 different taxa (14 fish taxa; three squid taxa) where MaxN values ranged from one to 19 individuals in a camera frame for a given taxon (Table 3-4). Northern sea robins (*Prionotus carolinus*) were the most common fish taxa as they were observed at 15 deployment locations across five different stations whereas scup (*Stenotomus chrysops*) and summer flounder (*Paralichthys dentatus*) were the least observed

taxon among other unidentified species. American butterfish (*Peprilus triacanthus*) had the greatest relative abundance (i.e., number of individuals observed in a single frame; n = 19 individuals) but were also observed to have high variability (SD = 6.6) in relative abundance among stations within the sampling event.

Table 3-3. Occurrence frequency of taxa observed from videos collected in July and September 2023. Occurrence frequency identifies how many unique stations yielded observations of a given taxon and sampling event. Frequency values were not provided if a taxon was not identified at a station for a sampling event (NA).

Taxa	July 2023 (23F1)	Sept. 2023 (23F2)
American butterfish	NA	4
Atlantic longfin squid	NA	4
Black sea bass	NA	2
Flounder species	1	1
Hammerhead shark species	1	1
Little/Winter skate species	NA	3
Mackerel species	NA	1
Northern sea robin	NA	5
Roughtail stingray	2	NA
Scup	NA	1
Sea robin species	3	2
Shortfin squid	NA	1
Skate species	2	1
Spiny dogfish	1	NA
Spotted hake	NA	1
Squid species	NA	4
Striped sea robin	NA	4
Summer flounder	NA	1
Tiger shark	NA	1

Table 3-4. Occurrence frequency and relative abundance (MaxN) statistics for taxa observed from videos collected in September 2023. Occurrence frequency identifies how many unique stations and deployments yielded observations of a given group. MaxN statistics include minimum and maximum values as well as mean and standard deviation (+/- SD). Standard deviation is not provided for taxa that were only observed during a single deployment (NA).

	Occurrence Frequency		Relative Abundance (MaxN)		
Taxa	**Stations**	**Deployments**	**Minimum**	**Maximum**	**Mean (+/-SD)**
Northern sea robin	5	15	1	3	1.7 (0.7)
Atlantic longfin squid	4	8	1	4	2.1 (1.1)
American butterfish	4	7	1	19	5.1 (6.6)
Squid species	4	5	1	2	1.2 (0.4)
Striped sea robin	4	7	1	2	1.1 (0.4)
Little/Winter skate species	3	4	1	1	1 (0)
Black sea bass	2	2	1	2	1.5 (0.7)
Sea robin species	2	3	2	2	2 (0)
Flounder species	1	1	2	2	2 (NA)
Hammerhead shark species	1	1	1	1	1 (NA)
Mackerel species	1	1	17	17	17 (NA)
Scup	1	1	1	1	1 (NA)
Shortfin squid	1	2	1	1	1 (0)
Skate species	1	1	1	1	1 (NA)
Spotted hake	1	4	1	6	2.3 (2.5)
Summer flounder	1	1	1	1	1.7 (0.7)
Tiger shark	1	2	1	1	1 (0)

4.0 DISCUSSION

Given the number of sampling events and analyses completed during the first year of pre-construction fisheries monitoring, this section highlights overarching trends in analyzed data as well as enhancements to previously discussed methods. CTD casts, for instance, were effective at characterizing temperature and salinity fluctuations throughout the water column both within and between sampling events in the project area. In addition to decreases in bottom water temperature and salinity between September and December 2023, water column turnover was also observed over the same period. As such, water column profiles will be invaluable when evaluating shifts in community composition and relative abundance measured by BRUV and eDNA monitoring techniques.

BRUV and eDNA monitoring techniques detected a diversity of fish species in the project area, which varied by station and sampling event. However, due to the innate biases and intricacies associated with each method, taxa observed by one method were not observed by the other (or to the same taxonomic level) as evidenced with various teleost and elasmobranch groups in this report (Table 3-1; Appendix D). For instance, thirty-two teleost taxa (ASVs) were detected using eDNA metabarcoding whereas only ten of those taxa were identified by BRUV. Seven elasmobranch taxa were detected by eDNA methods and six taxa by BRUV; one taxon (i.e., tiger shark [*Galeocerdo cuvier*]) was only detected by BRUV and not by eDNA. Although eDNA and BRUV analyses are at differing stages of development for monitoring offshore wind impacts, both methods are needed to properly monitor the numerous fish species expected to reside within or visit the Lease Area based on the Empire Offshore Wind Construction and Operations Plan Essential Fish Habitat Assessment (Empire Offshore Wind 2022).

To date, BRUV surveys have primarily occurred in tropical and subtropical marine ecosystems (Whitmarsh et al. 2017) but are becoming more prevalent in temperate ecosystems (e.g., Unsworth et al. 2014; Harrison and Rousseau 2020; Jones et al. 2021; Nyce et al. 2022,). Unfortunately, differences in biological and environmental parameters between geographic areas may not allow a universal set of monitoring guidelines and standardized workflows, potentially impeding comparisons between regional and multi-year studies (Langlois et al. 2020, Jones et al. 2021). For instance, ambient light and visibility conditions in shallow tropical ecosystems are often suitable for the identification and enumeration of fish species using BRUV systems. Ambient light and visibility conditions at depth in the current survey, however, prompted the addition of underwater lighting setups, which permitted diurnal sampling and improved video quality and species identification. Although underwater lighting is known to attract and influence species identified in BRUV surveys (e.g., Harvey et al. 2012; Fitzpatrick et al. 2013; Unsworth et al. 2014), the use of underwater lights in the present effort provided a conservative estimate of fish diversity and abundance in the Lease Area and standardized results for subsequent sampling events. The analysis of suitable video footage has historically relied on manual annotation, which is costly as a single 1-hour video can take several hours to confidently review and analyze. Subsampling routines, such as the review of individual frames at specific intervals over a video's duration (e.g., every 2 minutes), can reduce video processing

times but was observed to bias relative abundance estimates by missing species that only briefly enter the frame (e.g., Bacheler and Shertzer 2015).

Given the manual effort required to process the current volume of video footage and biases associated with other methods, a new analytical protocol is required to analyze all video footage collected from the project area in an efficient and accurate timeframe. To this end, the project team is currently developing a multi-faceted approach that uses semi-supervised machine learning algorithms to automatically identify and enumerate fish species without applying subsampling routines. (Figure 4-1). However, semi-supervised machine learning requires the use of a “training” dataset (i.e., a set of images for each species anticipated to appear in the video footage) to guide the learning process. Because the BRUV technique was pioneered in tropical regions with clear visibility, a set of training images does not exist for more temperate areas such as the northwestern Atlantic Ocean. Project personnel will therefore be amassing an image dataset using existing video footage that will be annotated (i.e., labeled) and used to train an appropriate machine learning model that can identify and categorize fish based on a prediction threshold. In comparison with traditional review methods, semi-supervised machine learning can significantly improve species identification accuracy and reduce processing time of videos collected for estimating relative abundance (reviewed in McGeady et al. 2023).

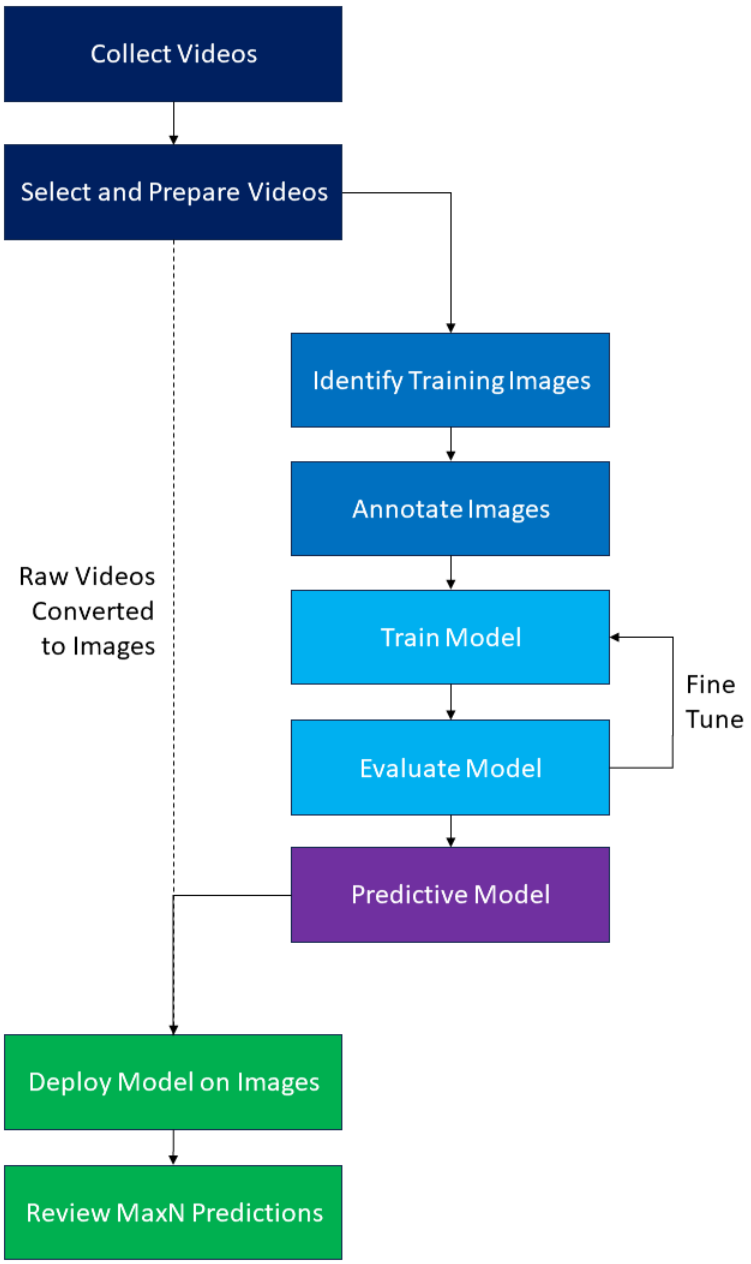


Figure 4-1. Proposed machine learning approach for estimating the relative abundance estimates of fish species from BRUV video footage; the approach uses existing video footage to develop a training image dataset that is used to create a predictive model of all fish identified and annotated in the training dataset. After an appropriate model is trained and evaluated, the predictive model is deployed (i.e., applied) to all the original BRUV videos that are now converted to images. Fish predictions on these images can then be enumerated by species and used to estimate relative abundance per species (i.e., MaxN).

5.0 SUMMARY

The current report presents methodologies and results pertaining to the use of non-extractive sampling techniques to gather baseline fish community composition and relative abundance data in the Empire Wind Lease Area prior to construction activities. Such results include the following:

- Three sampling events were conducted in the Empire Wind Lease Area during July, September, and December 2023, which collected water column profile, eDNA, and BRUV video data at eight stations that coincided with proposed WTG locations; BRUV video data were additionally collected at four distances, including 0 m, 50 m, 100 m, and 200 m, from proposed WTG station locations.
    - Water column profiles were collected at each station per sampling event, resulting in 24 sets of salinity and temperature profiles.
    - eDNA water samples were collected at each station per sampling event, resulting in 24 water samples for analysis.
    - BRUV systems collected 1-hour video clips at four distances from each station per sampling event, resulting in 96 deployments and 192 video camera recordings for analysis (i.e., two video cameras per system).
- For water column profiles, bottom water salinities ranged from 30.8 to 32.5 practical salinity units (psu) and bottom water temperature ranged from 11.8 to 16.9^{o}C across sampling events. Temperature profiles were observed to reverse between September and December 2023, i.e., surface water temperatures were colder than bottom water temperatures in December.
- For eDNA community data, teleost taxa samples were processed for all stations and sampling events (24 samples) whereas elasmobranch taxa samples were only processed for three stations in July and September 2023 (6 samples).
    - Overall, 32 taxa from 35 different teleost ASVs were detected across all three sampling events, with northern sea robin and scup exhibiting the highest relative abundances. Seven taxa from seven elasmobranch ASVs were detected at three stations in July and September 2023, with winter skate/little skate species and spiny dogfish having the highest relative abundances. The use of k-means clustering on taxa ASV reads identified five unique community composition groups, which varied across the Lease Area and by time of year.
- For BRUV systems, 177 videos (including left and right video camera recordings) were uncorrupted, and, of that sample, 85 videos were considered suitable for further

analysis. Only videos collected during the July and September 2023 sampling events were reviewed for species presence while the September videos were analyzed for relative abundance.

    - Nineteen taxa were detected on sampling events in July and September 2023, including four taxa (e.g., unidentified species of flounder, hammerhead shark, sea robin, and skate) that were observed during both sampling events. Relative abundance values were estimated for 17 different taxa observed in September 2023, where values ranged from 1 to 19 individuals in a camera frame for a given taxon. Northen sea robins were the most common fish taxon observed across the Lease Area (observed at five stations) whereas American butterfish had the greatest relative abundance (19 individuals).

- The number of taxa detected using eDNA and BRUV sampling methods from the July and September 2023 sampling events differed despite the incomplete analysis of eDNA samples and BRUV video. The eDNA methodology detected 32 taxa of teleosts in comparison to the ten teleost taxa observed by BRUV analyses. The number of elasmobranchs, including skates, rays, and sharks, was similar between methods though differed in what taxa were detected (e.g., eDNA and BRUV methods both detected three taxa of sharks but only one taxon [spiny dogfish] was detected by both).

Overall, BRUV and eDNA techniques detected a diversity of species in the project area that varied by location and time of year and their combined deployment will provide a holistic view of community composition and relative abundance (Stat et al. 2019). The proposed semi-supervised machine learning approach will continue to be refined for the evaluation of fish community composition and relative abundance estimates from suitable BRUV video footage. Such advances will also permit the project team to isolate specific frames from stereo videos and estimate length frequency of observed individuals. In addition to completing the analysis of BRUV videos and eDNA samples, future research efforts will more effectively compare fish community composition and relative abundances between the two methodologies and with respect to station locations, sampling events, and associated environmental conditions.

6.0 REFERENCES

Bacheler, N. and K. Shertzer. 2015. Estimating relative abundance and species richness from video surveys of reef fishes. Fishery Bulletin, 113,15–26. https://doi.org/10.7755/FB.113.1.2.

Bergström, L., F. Sundqvist, and U. Bergström. 2013. Effects of an offshore wind farm on temporal and spatial patterns in the demersal fish community. Marine Ecology Progress Series, 485, 199-210. https://doi.org/10.3354/meps10344.

Bureau of Ocean Energy Management (BOEM). 2019. Guidelines for providing information on fisheries for renewable energy development on the Atlantic outer continental shelf pursuant to 30 CFR Part 585. Office of Renewable Energy Programs. June 2019.

Callahan, B., P. McMurdie, M. Rosen, et al. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Colton M. A. and S. E. Swearer. 2010. A comparison of two survey methods: differences between underwater visual census and baited remote underwater video. Mar Ecol Prog Ser 400:19-36. https://doi.org/10.3354/meps08377 .

Deagle, B., A. Thomas, J. McInnes, L. Clarke, E. Vesterinen, E. Clare, T. Kartzinel and J. Eveson. 2019. Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? Molecular Ecology, 28, 391–406. https:// doi.org/10.1111/mec.14734.

Empire Offshore Wind. 2022. Essential Fish Habitat Assessment. Appendix U of the Empire Offshore Wind Construction and Operations Plan. Prepared for Equinor by TetraTech for the November 2023 submittal to the Bureau of Ocean Energy Management.

Fitzpatrick, C., D. McLean, and E. Harvey. 2013. Using artificial intelligence to survey nocturnal reef fish, Fisheries Research, 146, 41-50. https://doi.org/10.1016/j.fishres.2013.03.016.

Friard, O., and M. Gamba. 2016. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. Methods in Ecology and Evolution, 7, 1325–1330. https://doi.org/10.1111/2041-210X.12584.

Goldberg, C., C. Turner, K. Deiner, K. Klymus, P. Thomsen, M. Murphy, S. Spear, A. McKee, S. Oyler-McCance, R. Cornman, M. Laramie, A. Mahon, R. Lance, D. Pilliod, K. Strickler, L. Waits, A. Fremier, T. Takahara, J. Herder, and P. Taberlet. 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods Ecol Evol, 7, 1299-1307. https://doi.org/10.1111/2041-210X.12595

Griffin, R., G. Robinson, A. West, I. Gloyne-Phillips, and R. Unsworth. 2016. Assessing fish and motile fauna around offshore windfarms using stereo baited video. PLoS ONE, 11:e0149701. doi:10.1093/icesjms/fsaa026.

Guida, V., A. Drohan, H. Welch, J. McHenry, D. Johnson, V. Kentner, J. Brink, D. Timmons, E. Estela-Gomez. 2017. Habitat Mapping and Assessment of Northeast Wind Energy Areas. Sterling, VA: US Department of the Interior, Bureau of Ocean Energy Management. OCS Study BOEM 2017-088. 312 p.

Harrison, S. and M. Rousseau. 2020.Comparison of Artificial and Natural Reef Productivity in Nantucket Sound, MA, USA. Estuaries and Coasts 43, 2092–2105. https://doi.org/10.1007/s12237-020-00749-6.

Harvey, E., S. Newman, D. McLean, M. Cappo, J. Meeuwig, and C. Skepper. 2012. Comparison of relative efficiencies of stereo-BRUVs and traps for sampling tropical continental shelf demersal fishes, Fisheries Research, 125-126, 108-120. https://doi.org/10.1016/j.fishres.2012.01.026.

INSPIRE Environmental. 2023. Empire Wind Fisheries and Benthic Monitoring Plan. Prepared for Equinor, Empire Offshore Wind LLC. May 2023. 76 pp.

Jones, R., R. Griffin, R. Herbert, and R. Unsworth. 2021. Consistency is critical for the effective use of baited remote video. Oceans, 2, 215-232. https://doi.org/10.3390/oceans2010013.

Kopp, D., R. Faillettaz, A. Le Joncour, J. Simon, F. Morandeau, P. Le Bourdonnec, and S. Méhault. 2023. Assessing without harvesting: Pros and cons of environmental DNA sampling and image analysis for marine biodiversity evaluation. *Marine environmental research*,*188*, 106004.

Langlois T, J. Goetze, T. Bond, J. Monk, R.A. Abesamis, et al. 2020. A field and video annotation guide for baited remote underwater stereo-video surveys of demersal fish assemblages. Methods in Ecology and Evolution 11(11):1401-1409.

McGeady, R., R. Runya, J. Dooley, J. Howe, C. Fox, A. Wheeler, G. Summers, A. Callaway, S. Beck, L. Brown, G. Dooly, and C. Mcgonigle. 2023. A review of new and existing non-extractive techniques for monitoring marine protected areas. Frontiers in Marine Science, 10:1126301. https://doi.org/10.3389/fmars.2023.1126301.

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

Methratta, E. 2021. Distance-based sampling methods for assessing the ecological effects of offshore wind farms: Synthesis and application to fisheries resource studies. Frontiers in Marine Science, 8:674594. doi: 10.3389/fmars.2021.674594.

New Jersey Department of Environmental Protection (NJDEP). 2022. Open Stock Assessment Program. Accessed August 2022. https://dep.nj.gov/njfw/fishing/marine/ocean-stock-assessment-program/.

New York State Energy Research and Development Authority (NYSERDA). 2017. New York State Offshore Wind Master Plan: Fish and Fisheries Study. NYSERDA Report 17-25j. 140 pp.

Nyce, J., W. Koh, K. Wurth, and J. Sulikowski. 2022. A preliminary qualitative study of baited remote underwater video surveys (BRUVS) on fish assemblage in the coastal waters of Maine. Northeastern Naturalist, 29, 1 – 10. doi: 10.1656/045.029.0101.

Priede, I., P. Bagley, A. Smith, S. Creasy, and N. Merrett. 1994. Scavenging deep demersal fishes of the Porcupine Seabight, north-east Atlantic: observations by baited camera, trap and trawl. Journal of the Marine Biological Association of the United Kingdom, 74, 481-498. https://doi.org/10.1017/S0025315400047615.

R Core Team.2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Ramirez-Amaro, S., M. Bassitta, A. Picornell, C. Ramon, and B. Terrasa. 2022. Environmental DNA: State-of-the-art of its application for fisheries assessment in marine environments. Frontiers in Marine Science. 9 :1004674. Doi : 10.3389/fmars.2022.1004674.

Responsible Offshore Science Alliance (ROSA). 2021. Offshore wind project monitoring framework and guidelines. March 2021. Available online at Resources | ROSA 2021 Updated (rosascience.org).

Reubens, J., U. Braeckman, J. Vanaverbeke, C. Van Colen, S. Degraer, and M. Vincx. 2013. Aggregation at windmill artificial reefs: CPUE of Atlantic cod (Gadus morhua) and pouting (Trisopterus luscus) at different habitats in the Belgian part of the North Sea. Fisheries Research, 139, 28-34. https://doi.org/10.1016/j.fishres.2012.10.011.

Riaz T., W. Shehzad ,A. Viari, F. Pompanon, P. Taberlet, E. Coissac. 2011. ecoPrimers: inference of new DNA barcode markers from whole genome sequence analysis. Nucleic Acids Res. 2011 Nov;39(21):e145. Doi: 10.1093/nar/gkr732. Epub 2011 Sep 19. PMID: 21930509; PMCID: PMC3241669.

SeaGIS. 2020. https://www.seagis.com.au/hardware.html. Accessed Feb. 2024.

Skelton, J., A, Cauvin, and M. Hunter. 2023. Environmental DNA metabarcoding read numbers and their variability predict species abundance, but weakly in non-dominant species. Environmental DNA, 5, 1092–1104. https://doi.org/10.1002/edn3.355.

Stat M, J. John, J. D. DiBattista, S. J. Newman, M. Bunce, E. S. Harvey. 2019. Combined use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity. Conserv Biol. 2019 Feb;33(1):196-205. Doi: 10.1111/cobi.13183. Epub 2018 Sep 12. PMID: 30004598; PMCID: PMC7379492.

Stoeckle, M., J. Adolf, J. H. Ausubel, Z. Charlop-Powers, K. J. Dunton, G. Hinks. 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, Volume 79, Issue 2, March 2022, Pages 403–412, https://doi.org/10.1093/icesjms/fsab273.

Thorne, L., J. Nye, J. Warren, and C. Flagg. 2020. Development and implementation of an ocean ecosystem monitoring program for New York Bight. Annual Report, MOU #AM10560 NYS DEC & SUNY Stony Brook for the period January 1, 2020 – December 31, 2020. New York State Environmental Protection Fund Ocean and Great Lakes Program and Stony Brook University School of Marine and Atmospheric Sciences. https://www.dec.ny.gov/docs/fish_marine_pdf/dmrsomasmonitoring.pdf.

Unsworth, R., J. Peters, R. McCloskey, and S. Hinder. 2014. Optimising stereo baited underwater video for sampling fish and invertebrates in temperate coastal habitats, Estuarine. Coastal and Shelf Science, 150, 281-287. https://doi.org/10.1016/j.ecss.2014.03.020.

Whitmarsh, S., P. Fairweather, and C. Huveneers. 2017. What is Big BRUVver up to? Methods and uses of baited underwater video. Reviews in Fish Biology and Fisheries, 27, 53–73 https://doi.org/10.1007/s11160-016-9450-1.

Wilber, D., L. Brown, M. Griffin, G. DeCelles, and D. Carey. 2022. Demersal fish and invertebrate catches relative to construction and operation of North America's first offshore wind farm. ICES Journal of Marine Science 79:1274-1288.

Wilhelmsson, D., M. Torleif, and M. Öhman. 2006. The influence of offshore windpower on demersal fish. ICES Journal of Marine Science, 63, 775–784. https://doi.org/10.1016/j.icesjms.2006.02.001.

Empire Wind 2023 Baited Underwater Remote Video (BRUV) and Environmental DNA (eDNA) Monitoring Survey

Annual Report

APPENDICES

Prepared for:

Empire Offshore Wind LLC
Stamford Office - 600 Washington Blvd. - Suite 800
Stamford, CT 06901

Prepared by:

INSPIRE Environmental
513 Broadway
Newport, RI 02840

and

Monmouth University
West Long Branch, New Jersey

March 2024

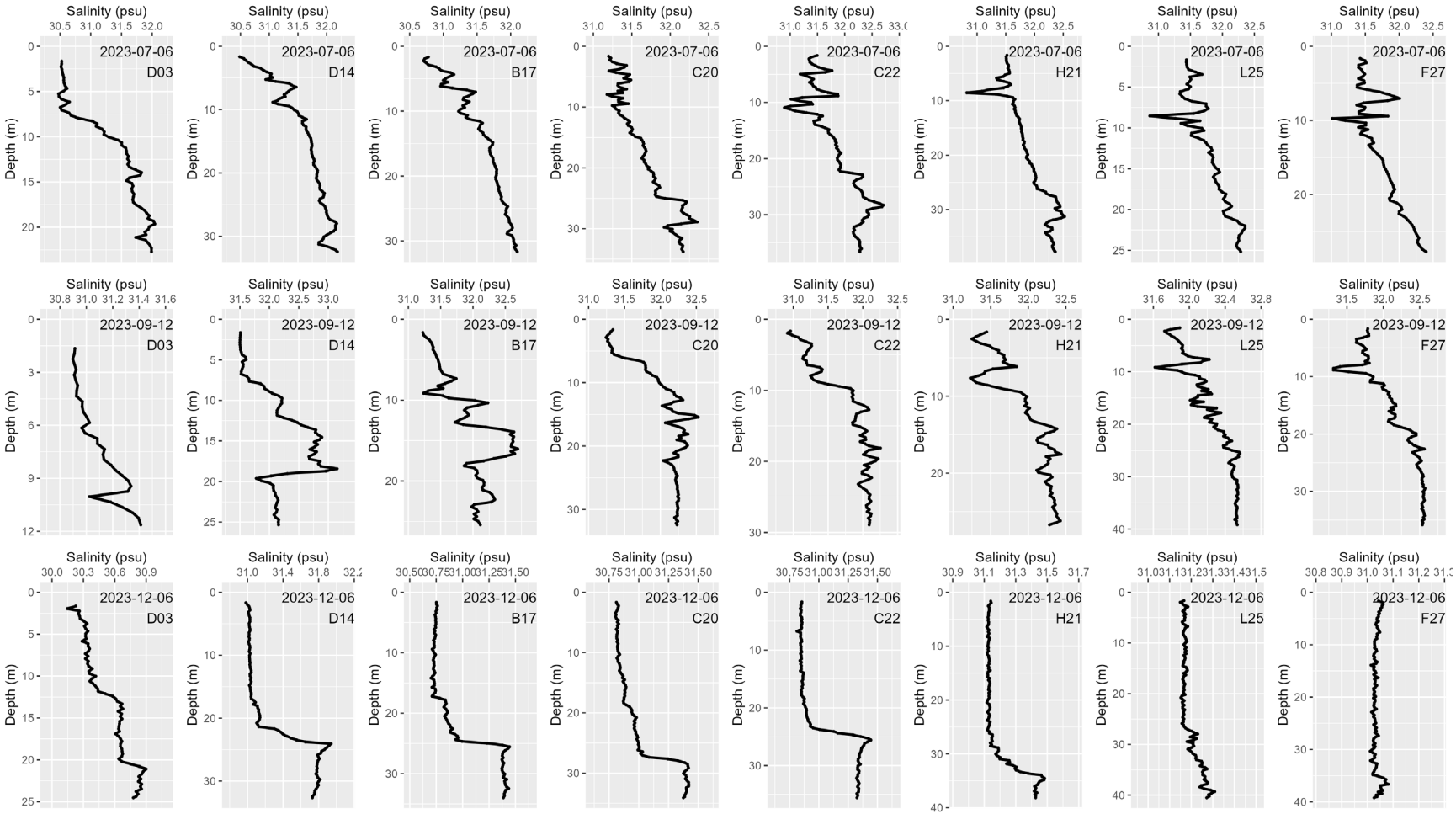
LIST OF APPENDICES

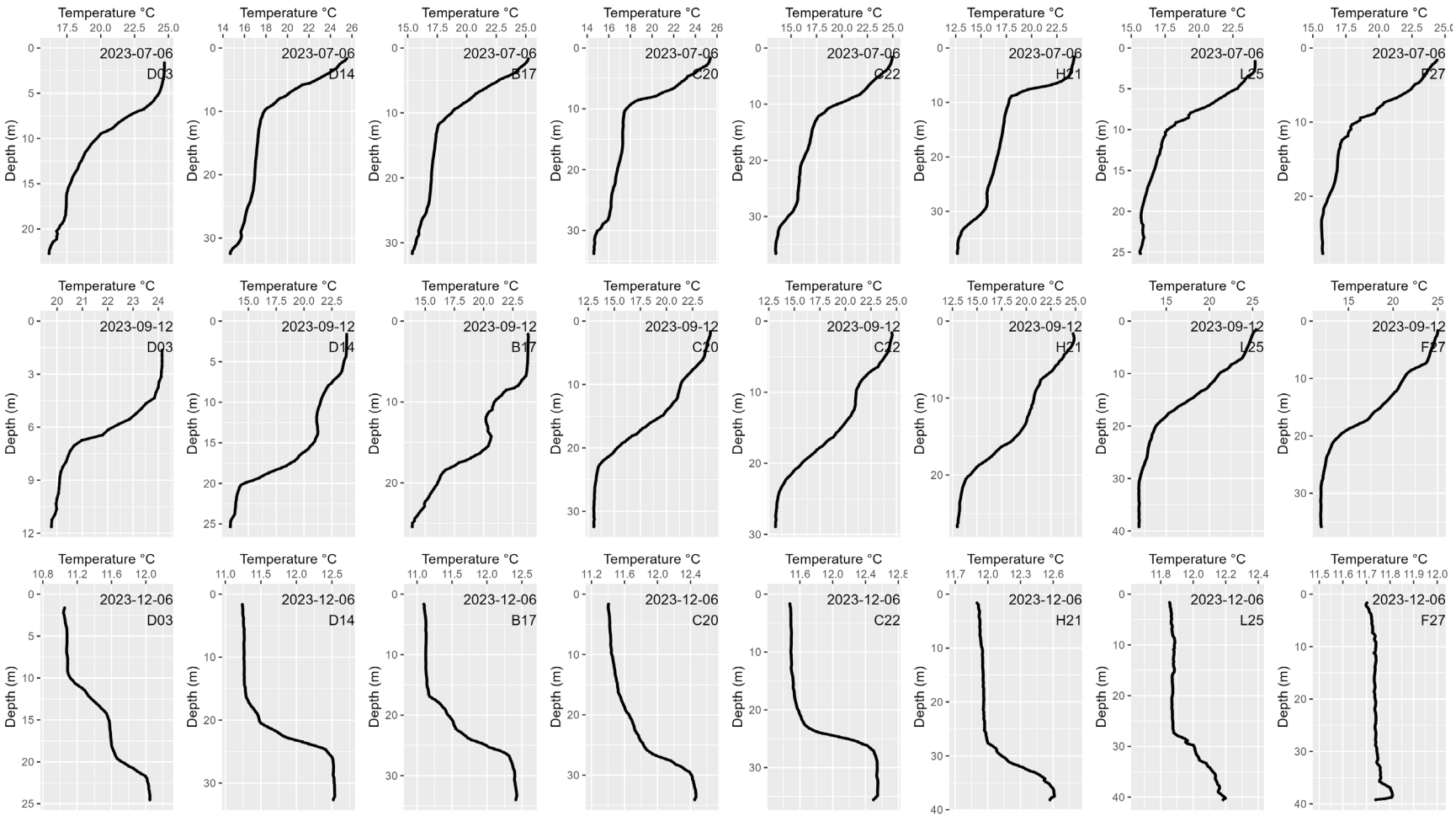
Appendix A - PCR Amplification of Fish Sequences from eDNA Samples

- Primers used for amplification are from Riaz Nucl Acids Res 2011 39: e145, modified to include Illumina adapters (bold) for subsequent sequencing
    - For Teleost fishes:
        - Forward: 5'- **TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG** ACT GGG ATT AGA TAC CCC -3'
        - Reverse: 5'- **GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G**TA GAA CAG GCT CCT CTA G -3'
    - For Elasmobranch fishes
        - Forward_elasmo: 5'- **TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG** ACT GGG ATT AGA TAC CCT -3'
        - Same as Teleosts
    - 25 microliter reaction volume (5 microliter template DNA) with Takara High Yield PCR EcoDry Premix for mastermix with the following thermocycler protocol
        - 95ºC x 5 min
        - 40 cycles: 95ºC x 20s, 58ºC x 30s, 73ºC x 20s
        - 72ºC x 1 min
        - Hold at 4ºC

Appendix B – CTD Salinity and Temperature Profiles for all Stations

Note:
The annotation from each graph comes from the Castaway file header.
Station D03 in September 2023 did not reach the seafloor; results were not included in the report.


Salinity (psu)
Depth (m)
2023-07-06 D03
2023-07-06 D14
2023-07-06 B17
2023-07-06 C20
2023-07-06 C22
2023-07-06 H21
2023-07-06 L25
2023-07-06 F27
2023-09-12 D03
2023-09-12 D14
2023-09-12 B17
2023-09-12 C20
2023-09-12 C22
2023-09-12 H21
2023-09-12 L25
2023-09-12 F27
2023-12-06 D03
2023-12-06 D14
2023-12-06 B17
2023-12-06 C20
2023-12-06 C22
2023-12-06 H21
2023-12-06 L25
2023-12-06 F27


Temperature °C
Depth (m)
2023-07-06 D03
2023-07-06 D14
2023-07-06 B17
2023-07-06 C20
2023-07-06 C22
2023-07-06 H21
2023-07-06 L25
2023-07-06 F27
2023-09-12 D03
2023-09-12 D14
2023-09-12 B17
2023-09-12 C20
2023-09-12 C22
2023-09-12 H21
2023-09-12 L25
2023-09-12 F27
2023-12-06 D03
2023-12-06 D14
2023-12-06 B17
2023-12-06 C20
2023-12-06 C22
2023-12-06 H21
2023-12-06 L25
2023-12-06 F27

Appendix C – Teleost and Elasmobranch Taxa Tables from eDNA Sampling by Station and Sampling Event

Survey	Date and Time	Station	Bottom Salinity (psu)	Bottom Temperature (°C)	sample_id	Bottom Depth (m)
23F1	7/5/2023 20:00	B17	32.02401578	15.79600807	emp-060123-G-B17-B-1	33
23F1	7/5/2023 20:00	B17	32.02401578	15.79600807	emp-060123-G-B17-B-1	33
23F1	7/5/2023 20:00	B17	32.02401578	15.79600807	emp-060123-G-B17-B-1	33
23F1	7/5/2023 20:00	B17	32.02401578	15.79600807	emp-060123-G-B17-B-1	33
23F1	7/5/2023 20:00	B17	32.02401578	15.79600807	emp-060123-G-B17-B-1	33
23F1	7/5/2023 20:00	B17	32.02401578	15.79600807	emp-060123-G-B17-B-1	33
23F1	7/5/2023 20:00	B17	32.02401578	15.79600807	emp-060123-G-B17-B-1	33
23F1	7/5/2023 20:00	B17	32.02401578	15.79600807	emp-060123-G-B17-B-1	33
23F1	7/5/2023 20:00	B17	32.02401578	15.79600807	emp-060123-G-B17-B-1	33
23F1	7/5/2023 20:00	B17	32.02401578	15.79600807	emp-060123-G-B17-B-1	33
23F1	7/5/2023 20:00	B17	32.02401578	15.79600807	emp-060123-G-B17-B-1	33
23F1	7/5/2023 20:00	C20	32.10407908	14.86535222	emp-060123-G-C21-B-1	34
23F1	7/5/2023 20:00	C20	32.10407908	14.86535222	emp-060123-G-C21-B-1	34
23F1	7/5/2023 20:00	C20	32.10407908	14.86535222	emp-060123-G-C21-B-1	34
23F1	7/5/2023 20:00	C20	32.10407908	14.86535222	emp-060123-G-C21-B-1	34
23F1	7/5/2023 20:00	C20	32.10407908	14.86535222	emp-060123-G-C21-B-1	34
23F1	7/5/2023 20:00	C20	32.10407908	14.86535222	emp-060123-G-C21-B-1	34
23F1	7/5/2023 20:00	C20	32.10407908	14.86535222	emp-060123-G-C21-B-1	34
23F1	7/5/2023 20:00	D03	31.92526948	16.85144391	emp-060123-G-D03-B-1	25
23F1	7/5/2023 20:00	D03	31.92526948	16.85144391	emp-060123-G-D03-B-1	25
23F1	7/5/2023 20:00	D03	31.92526948	16.85144391	emp-060123-G-D03-B-1	25
23F1	7/5/2023 20:00	D03	31.92526948	16.85144391	emp-060123-G-D03-B-1	25
23F1	7/5/2023 20:00	D03	31.92526948	16.85144391	emp-060123-G-D03-B-1	25
23F1	7/5/2023 20:00	D03	31.92526948	16.85144391	emp-060123-G-D03-B-1	25
23F1	7/5/2023 20:00	D14	32.0547521	15.44786176	emp-060123-G-D14-B-1	32
23F1	7/5/2023 20:00	D14	32.0547521	15.44786176	emp-060123-G-D14-B-1	32
23F1	7/5/2023 20:00	D14	32.0547521	15.44786176	emp-060123-G-D14-B-1	32
23F1	7/5/2023 20:00	D14	32.0547521	15.44786176	emp-060123-G-D14-B-1	32
23F1	7/5/2023 20:00	D14	32.0547521	15.44786176	emp-060123-G-D14-B-1	32
23F1	7/5/2023 20:00	D14	32.0547521	15.44786176	emp-060123-G-D14-B-1	32
23F1	7/5/2023 20:00	D14	32.0547521	15.44786176	emp-060123-G-D14-B-1	32
23F1	7/5/2023 20:00	D14	32.0547521	15.44786176	emp-060123-G-D14-B-1	32
23F1	7/5/2023 20:00	D14	32.0547521	15.44786176	emp-060123-G-D14-B-1	32
23F1	7/5/2023 20:00	D14	32.0547521	15.44786176	emp-060123-G-D14-B-1	32

Survey	Date and Time	Station	Bottom Salinity (psu)	Bottom Temperature (°C)	sample_id	Bottom Depth (m)
23F1	7/6/2023 2:17	F27	32.23608154	15.69661905	emp-060123-G-F27-B-1	39
23F1	7/6/2023 2:17	F27	32.23608154	15.69661905	emp-060123-G-F27-B-1	39
23F1	7/6/2023 2:17	F27	32.23608154	15.69661905	emp-060123-G-F27-B-1	39
23F1	7/6/2023 2:17	F27	32.23608154	15.69661905	emp-060123-G-F27-B-1	39
23F1	7/6/2023 2:17	F27	32.23608154	15.69661905	emp-060123-G-F27-B-1	39
23F1	7/6/2023 2:17	F27	32.23608154	15.69661905	emp-060123-G-F27-B-1	39
23F1	7/6/2023 2:17	F27	32.23608154	15.69661905	emp-060123-G-F27-B-1	39
23F1	7/6/2023 4:17	L25	32.22632681	15.79285619	emp-060123-G-L23-B-1	42
23F1	7/6/2023 4:17	L25	32.22632681	15.79285619	emp-060123-G-L23-B-1	42
23F1	7/6/2023 4:17	L25	32.22632681	15.79285619	emp-060123-G-L23-B-1	42
23F1	7/6/2023 4:17	L25	32.22632681	15.79285619	emp-060123-G-L23-B-1	42
23F1	7/6/2023 4:17	L25	32.22632681	15.79285619	emp-060123-G-L23-B-1	42
23F1	7/6/2023 4:17	L25	32.22632681	15.79285619	emp-060123-G-L23-B-1	42
23F1	7/6/2023 4:17	L25	32.22632681	15.79285619	emp-060123-G-L23-B-1	42
23F1	7/6/2023 4:17	L25	32.22632681	15.79285619	emp-060123-G-L23-B-1	42
23F1	7/6/2023 6:21	H21	32.28779924	12.89040459	emp-060123-G-H21-B-1	39
23F1	7/6/2023 6:21	H21	32.28779924	12.89040459	emp-060123-G-H21-B-1	39
23F1	7/6/2023 6:21	H21	32.28779924	12.89040459	emp-060123-G-H21-B-1	39
23F1	7/6/2023 6:21	H21	32.28779924	12.89040459	emp-060123-G-H21-B-1	39
23F1	7/6/2023 6:21	H21	32.28779924	12.89040459	emp-060123-G-H21-B-1	39
23F1	7/6/2023 8:01	C22	32.23189809	13.61270294	emp-060123-G-C22-B-1	36
23F1	7/6/2023 8:01	C22	32.23189809	13.61270294	emp-060123-G-C22-B-1	36
23F1	7/6/2023 8:01	C22	32.23189809	13.61270294	emp-060123-G-C22-B-1	36
23F1	7/6/2023 8:01	C22	32.23189809	13.61270294	emp-060123-G-C22-B-1	36
23F1	7/6/2023 8:01	C22	32.23189809	13.61270294	emp-060123-G-C22-B-1	36
23F1	7/6/2023 8:01	C22	32.23189809	13.61270294	emp-060123-G-C22-B-1	36
23F1	7/6/2023 8:01	C22	32.23189809	13.61270294	emp-060123-G-C22-B-1	36
23F1	7/6/2023 8:01	C22	32.23189809	13.61270294	emp-060123-G-C22-B-1	36
23F1	7/6/2023 8:01	C22	32.23189809	13.61270294	emp-060123-G-C22-B-1	36
23F2	9/12/2023 2:30	D03	31.22634891	20.18293732	emp-090123-G-D03-B-1	25
23F2	9/12/2023 2:30	D03	31.22634891	20.18293732	emp-090123-G-D03-B-1	25
23F2	9/12/2023 2:30	D03	31.22634891	20.18293732	emp-090123-G-D03-B-1	25
23F2	9/12/2023 2:30	D03	31.22634891	20.18293732	emp-090123-G-D03-B-1	25
23F2	9/12/2023 2:30	D03	31.22634891	20.18293732	emp-090123-G-D03-B-1	25

Survey	Date and Time	Station	Bottom Salinity (psu)	Bottom Temperature (°C)	sample_id	Bottom Depth (m)
23F2	9/12/2023 2:30	D03	31.22634891	20.18293732	emp-090123-G-D03-B-1	25
23F2	9/12/2023 2:30	D03	31.22634891	20.18293732	emp-090123-G-D03-B-1	25
23F2	9/12/2023 2:30	D03	31.22634891	20.18293732	emp-090123-G-D03-B-1	25
23F2	9/12/2023 2:30	D03	31.22634891	20.18293732	emp-090123-G-D03-B-1	25
23F2	9/12/2023 2:30	D03	31.22634891	20.18293732	emp-090123-G-D03-B-1	25
23F2	9/12/2023 2:30	D03	31.22634891	20.18293732	emp-090123-G-D03-B-1	25
23F2	9/12/2023 2:30	D03	31.22634891	20.18293732	emp-090123-G-D03-B-1	25
23F2	9/12/2023 2:30	D03	31.22634891	20.18293732	emp-090123-G-D03-B-1	25
23F2	9/12/2023 2:30	D03	31.22634891	20.18293732	emp-090123-G-D03-B-1	25
23F2	9/12/2023 2:30	D03	31.22634891	20.18293732	emp-090123-G-D03-B-1	25
23F2	9/12/2023 5:13	D14	32.11337995	13.7956233	emp-090123-G-D14-B-1	33
23F2	9/12/2023 5:13	D14	32.11337995	13.7956233	emp-090123-G-D14-B-1	33
23F2	9/12/2023 5:13	D14	32.11337995	13.7956233	emp-090123-G-D14-B-1	33
23F2	9/12/2023 5:13	D14	32.11337995	13.7956233	emp-090123-G-D14-B-1	33
23F2	9/12/2023 5:13	D14	32.11337995	13.7956233	emp-090123-G-D14-B-1	33
23F2	9/12/2023 5:13	D14	32.11337995	13.7956233	emp-090123-G-D14-B-1	33
23F2	9/12/2023 5:13	D14	32.11337995	13.7956233	emp-090123-G-D14-B-1	33
23F2	9/12/2023 5:13	D14	32.11337995	13.7956233	emp-090123-G-D14-B-1	33
23F2	9/12/2023 5:13	D14	32.11337995	13.7956233	emp-090123-G-D14-B-1	33
23F2	9/12/2023 5:13	D14	32.11337995	13.7956233	emp-090123-G-D14-B-1	33
23F2	9/12/2023 6:55	B17	32.1386844	14.82420863	emp-090123-G-B17-B-1	34
23F2	9/12/2023 6:55	B17	32.1386844	14.82420863	emp-090123-G-B17-B-1	34
23F2	9/12/2023 6:55	B17	32.1386844	14.82420863	emp-090123-G-B17-B-1	34
23F2	9/12/2023 6:55	B17	32.1386844	14.82420863	emp-090123-G-B17-B-1	34
23F2	9/12/2023 6:55	B17	32.1386844	14.82420863	emp-090123-G-B17-B-1	34
23F2	9/12/2023 6:55	B17	32.1386844	14.82420863	emp-090123-G-B17-B-1	34
23F2	9/12/2023 6:55	B17	32.1386844	14.82420863	emp-090123-G-B17-B-1	34
23F2	9/12/2023 6:55	B17	32.1386844	14.82420863	emp-090123-G-B17-B-1	34
23F2	9/12/2023 6:55	B17	32.1386844	14.82420863	emp-090123-G-B17-B-1	34
23F2	9/12/2023 6:55	B17	32.1386844	14.82420863	emp-090123-G-B17-B-1	34
23F2	9/12/2023 6:55	B17	32.1386844	14.82420863	emp-090123-G-B17-B-1	34
23F2	9/12/2023 6:55	B17	32.1386844	14.82420863	emp-090123-G-B17-B-1	34
23F2	9/12/2023 6:55	B17	32.1386844	14.82420863	emp-090123-G-B17-B-1	34

Survey	Date and Time	Station	Bottom Salinity (psu)	Bottom Temperature (°C)	sample_id	Bottom Depth (m)
23F2	9/12/2023 8:35	C20	32.23512672	13.05391862	emp-090123-G-C20-B-1	34
23F2	9/12/2023 8:35	C20	32.23512672	13.05391862	emp-090123-G-C20-B-1	34
23F2	9/12/2023 8:35	C20	32.23512672	13.05391862	emp-090123-G-C20-B-1	34
23F2	9/12/2023 8:35	C20	32.23512672	13.05391862	emp-090123-G-C20-B-1	34
23F2	9/12/2023 10:07	C22	32.08656731	13.27846319	emp-090123-G-C22-B-1	36
23F2	9/12/2023 10:07	C22	32.08656731	13.27846319	emp-090123-G-C22-B-1	36
23F2	9/12/2023 10:07	C22	32.08656731	13.27846319	emp-090123-G-C22-B-1	36
23F2	9/12/2023 10:07	C22	32.08656731	13.27846319	emp-090123-G-C22-B-1	36
23F2	9/12/2023 10:07	C22	32.08656731	13.27846319	emp-090123-G-C22-B-1	36
23F2	9/12/2023 10:07	C22	32.08656731	13.27846319	emp-090123-G-C22-B-1	36
23F2	9/12/2023 10:07	C22	32.08656731	13.27846319	emp-090123-G-C22-B-1	36
23F2	9/12/2023 11:51	H21	32.34105416	13.22798351	emp-090123-G-H21-B-1	38
23F2	9/12/2023 11:51	H21	32.34105416	13.22798351	emp-090123-G-H21-B-1	38
23F2	9/12/2023 11:51	H21	32.34105416	13.22798351	emp-090123-G-H21-B-1	38
23F2	9/12/2023 11:51	H21	32.34105416	13.22798351	emp-090123-G-H21-B-1	38
23F2	9/12/2023 11:51	H21	32.34105416	13.22798351	emp-090123-G-H21-B-1	38
23F2	9/12/2023 13:45	L25	32.52589357	11.85106942	emp-090123-G-L25-B-1	41
23F2	9/12/2023 13:45	L25	32.52589357	11.85106942	emp-090123-G-L25-B-1	41
23F2	9/12/2023 15:32	F27	32.54718436	11.92134109	emp-090123-G-F27-B-1	40
23F2	9/12/2023 15:32	F27	32.54718436	11.92134109	emp-090123-G-F27-B-1	40
23F3	12/6/2023 0:25	D03	30.80270379	11.94163318	emp-120123-G-D03-B-1	24
23F3	12/6/2023 0:25	D03	30.80270379	11.94163318	emp-120123-G-D03-B-1	24
23F3	12/6/2023 0:25	D03	30.80270379	11.94163318	emp-120123-G-D03-B-1	24
23F3	12/6/2023 0:25	D03	30.80270379	11.94163318	emp-120123-G-D03-B-1	24
23F3	12/6/2023 0:25	D03	30.80270379	11.94163318	emp-120123-G-D03-B-1	24
23F3	12/6/2023 0:25	D03	30.80270379	11.94163318	emp-120123-G-D03-B-1	24
23F3	12/6/2023 0:25	D03	30.80270379	11.94163318	emp-120123-G-D03-B-1	24
23F3	12/6/2023 0:25	D03	30.80270379	11.94163318	emp-120123-G-D03-B-1	24
23F3	12/6/2023 0:25	D03	30.80270379	11.94163318	emp-120123-G-D03-B-1	24
23F3	12/6/2023 2:58	D14	31.77469194	12.53026158	emp-120123-G-D14-B-1	33
23F3	12/6/2023 2:58	D14	31.77469194	12.53026158	emp-120123-G-D14-B-1	33
23F3	12/6/2023 2:58	D14	31.77469194	12.53026158	emp-120123-G-D14-B-1	33
23F3	12/6/2023 2:58	D14	31.77469194	12.53026158	emp-120123-G-D14-B-1	33
23F3	12/6/2023 2:58	D14	31.77469194	12.53026158	emp-120123-G-D14-B-1	33

Survey	Date and Time	Station	Bottom Salinity (psu)	Bottom Temperature (°C)	sample_id	Bottom Depth (m)
23F3	12/6/2023 2:58	D14	31.77469194	12.53026158	emp-120123-G-D14-B-1	33
23F3	12/6/2023 2:58	D14	31.77469194	12.53026158	emp-120123-G-D14-B-1	33
23F3	12/6/2023 2:58	D14	31.77469194	12.53026158	emp-120123-G-D14-B-1	33
23F3	12/6/2023 2:58	D14	31.77469194	12.53026158	emp-120123-G-D14-B-1	33
23F3	12/6/2023 2:58	D14	31.77469194	12.53026158	emp-120123-G-D14-B-1	33
23F3	12/6/2023 2:58	D14	31.77469194	12.53026158	emp-120123-G-D14-B-1	33
23F3	12/6/2023 4:38	B17	31.39759937	12.40795224	emp-120123-G-B17-B-1	34
23F3	12/6/2023 4:38	B17	31.39759937	12.40795224	emp-120123-G-B17-B-1	34
23F3	12/6/2023 4:38	B17	31.39759937	12.40795224	emp-120123-G-B17-B-1	34
23F3	12/6/2023 4:38	B17	31.39759937	12.40795224	emp-120123-G-B17-B-1	34
23F3	12/6/2023 4:38	B17	31.39759937	12.40795224	emp-120123-G-B17-B-1	34
23F3	12/6/2023 4:38	B17	31.39759937	12.40795224	emp-120123-G-B17-B-1	34
23F3	12/6/2023 4:38	B17	31.39759937	12.40795224	emp-120123-G-B17-B-1	34
23F3	12/6/2023 4:38	B17	31.39759937	12.40795224	emp-120123-G-B17-B-1	34
23F3	12/6/2023 4:38	B17	31.39759937	12.40795224	emp-120123-G-B17-B-1	34
23F3	12/6/2023 4:38	B17	31.39759937	12.40795224	emp-120123-G-B17-B-1	34
23F3	12/6/2023 4:38	B17	31.39759937	12.40795224	emp-120123-G-B17-B-1	34
23F3	12/6/2023 4:38	B17	31.39759937	12.40795224	emp-120123-G-B17-B-1	34
23F3	12/6/2023 6:15	C20	31.39865426	12.43425817	emp-120123-G-C20-B-1	34
23F3	12/6/2023 6:15	C20	31.39865426	12.43425817	emp-120123-G-C20-B-1	34
23F3	12/6/2023 6:15	C20	31.39865426	12.43425817	emp-120123-G-C20-B-1	34
23F3	12/6/2023 6:15	C20	31.39865426	12.43425817	emp-120123-G-C20-B-1	34
23F3	12/6/2023 6:15	C20	31.39865426	12.43425817	emp-120123-G-C20-B-1	34
23F3	12/6/2023 6:15	C20	31.39865426	12.43425817	emp-120123-G-C20-B-1	34
23F3	12/6/2023 6:15	C20	31.39865426	12.43425817	emp-120123-G-C20-B-1	34
23F3	12/6/2023 6:15	C20	31.39865426	12.43425817	emp-120123-G-C20-B-1	34
23F3	12/6/2023 6:15	C20	31.39865426	12.43425817	emp-120123-G-C20-B-1	34
23F3	12/6/2023 6:15	C20	31.39865426	12.43425817	emp-120123-G-C20-B-1	34
23F3	12/6/2023 6:15	C20	31.39865426	12.43425817	emp-120123-G-C20-B-1	34
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36

Survey	Date and Time	Station	Bottom Salinity (psu)	Bottom Temperature (°C)	sample_id	Bottom Depth (m)
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36
23F3	12/6/2023 7:44	C22	31.33422919	12.54178407	emp-120123-G-C22-B-1	36
23F3	12/6/2023 9:28	H21	31.43109462	12.55967976	emp-120123-G-H21-B-1	38
23F3	12/6/2023 9:28	H21	31.43109462	12.55967976	emp-120123-G-H21-B-1	38
23F3	12/6/2023 9:28	H21	31.43109462	12.55967976	emp-120123-G-H21-B-1	38
23F3	12/6/2023 9:28	H21	31.43109462	12.55967976	emp-120123-G-H21-B-1	38
23F3	12/6/2023 9:28	H21	31.43109462	12.55967976	emp-120123-G-H21-B-1	38
23F3	12/6/2023 9:28	H21	31.43109462	12.55967976	emp-120123-G-H21-B-1	38
23F3	12/6/2023 11:18	L25	31.26957668	12.1588265	emp-120123-G-L25-B-1	40
23F3	12/6/2023 11:18	L25	31.26957668	12.1588265	emp-120123-G-L25-B-1	40
23F3	12/6/2023 11:18	L25	31.26957668	12.1588265	emp-120123-G-L25-B-1	40
23F3	12/6/2023 11:18	L25	31.26957668	12.1588265	emp-120123-G-L25-B-1	40
23F3	12/6/2023 13:13	F27	31.04873385	11.78031344	emp-120123-G-F27-B-1	40
23F3	12/6/2023 13:13	F27	31.04873385	11.78031344	emp-120123-G-F27-B-1	40
23F3	12/6/2023 13:13	F27	31.04873385	11.78031344	emp-120123-G-F27-B-1	40
23F3	12/6/2023 13:13	F27	31.04873385	11.78031344	emp-120123-G-F27-B-1	40
23F3	12/6/2023 13:13	F27	31.04873385	11.78031344	emp-120123-G-F27-B-1	40
23F3	12/6/2023 13:13	F27	31.04873385	11.78031344	emp-120123-G-F27-B-1	40
23F3	12/6/2023 13:13	F27	31.04873385	11.78031344	emp-120123-G-F27-B-1	40
23F3	12/6/2023 13:13	F27	31.04873385	11.78031344	emp-120123-G-F27-B-1	40

Survey	Taxa Common Name	Common Name	No. of reads
23F1	Am_butterfish	American butterfish	227
23F1	Atl_mackerel	Atlantic mackerel	44
23F1	Atl_or_nor_sand_lance	Sand lance	5
23F1	Bay_anchovy	Bay anchovy	8
23F1	Blacktip_shark	Blacktip shark	360
23F1	Bluefish	Bluefish	113
23F1	Nor_sea_robin	Northern sea robin	199027
23F1	Red_White_or_Spotted_hake	Hake (Urophycis spp)	10
23F1	Sandbar_shark	Sandbar shark	107
23F1	Summ_flounder	Summer flounder	12
23F1	Winter_skate_or_Little_skate	Little skate/ Winter skate species	1975
23F1	Atl_mackerel	Atlantic mackerel	1738
23F1	Atl_menhaden_LS16_or_river_herrings	Atlantic menhaden or river herrings	584
23F1	Atl_menhaden_LS17	Atlantic menhaden	509
23F1	Black_sea_bass	Black sea bass	1999
23F1	Bluefish	Bluefish	1163
23F1	Nor_sea_robin	Northern sea robin	4352
23F1	Silver_hake	Silver Hake	1049
23F1	Am_butterfish	American butterfish	255
23F1	Atl_mackerel	Atlantic mackerel	831
23F1	Atl_menhaden_LS16_or_river_herrings	Atlantic menhaden or river herrings	401
23F1	Bluefish	Bluefish	302
23F1	Nor_sea_robin	Northern sea robin	1187
23F1	Str_sea_robin	Striped sea robin	811
23F1	Am_anglerfish	Goosefish	10
23F1	Atl_mackerel	Atlantic mackerel	1377
23F1	Atl_menhaden_LS16_or_river_herrings	Atlantic menhaden or river herrings	318
23F1	Atl_menhaden_LS17	Atlantic menhaden	746
23F1	Atl_or_nor_sand_lance	Sand lance	382
23F1	Fourspot_flounder	Fourspot flounder	879
23F1	Nor_sea_robin	Northern sea robin	2971
23F1	Roughtail_stingray	Roughtail stingray	1271
23F1	Scup	Scup	5
23F1	Winter_skate_or_Little_skate	Little skate/ Winter skate species	5016

Survey	Taxa Common Name	Common Name	No. of reads
23F1	Am_butterfish	American butterfish	283
23F1	Atl_mackerel	Atlantic mackerel	438
23F1	Atl_menhaden_LS16_or_river_herrings	Atlantic menhaden or river herrings	81
23F1	Atl_or_nor_sand_lance	Sand lance	367
23F1	Bluefish	Bluefish	212
23F1	Silver_hake	Silver Hake	90
23F1	Summ_flounder	Summer flounder	115
23F1	Atl_mackerel	Atlantic mackerel	5419
23F1	Atl_menhaden_LS16_or_river_herrings	Atlantic menhaden or river herrings	1284
23F1	Bluefish	Bluefish	779
23F1	Grass_or_silver_carp	Grass or Silver carp	249
23F1	Gulf_stream_flounder	Gulf Stream flounder	1111
23F1	Nor_sea_robin	Northern sea robin	1131
23F1	Scup	Scup	954
23F1	Silver_hake	Silver Hake	430
23F1	Am_anglerfish	Goosefish	55
23F1	Atl_mackerel	Atlantic mackerel	1586
23F1	Atl_or_nor_sand_lance	Sand lance	1064
23F1	Bluefish	Bluefish	2142
23F1	Nor_sea_robin	Northern sea robin	6318
23F1	Am_butterfish	American butterfish	2315
23F1	Atl_chub_mackerel	Atlantic chub mackerel	1387
23F1	Atl_menhaden_LS17	Atlantic menhaden	2159
23F1	Atl_or_nor_sand_lance	Sand lance	3480
23F1	Black_sea_bass	Black sea bass	6696
23F1	Bluefish	Bluefish	2416
23F1	Nor_sea_robin	Northern sea robin	5360
23F1	Rough_scad	Rough scad	1447
23F1	Silver_hake	Silver Hake	3249
23F2	Am_butterfish	American butterfish	46
23F2	Am_eel	American eel	274
23F2	Atl_menhaden_LS16_or_river_herrings	Atlantic menhaden or river herrings	30
23F2	Atl_or_nor_sand_lance	Sand lance	20797
23F2	Bay_anchovy	Bay anchovy	133

Survey	Taxa Common Name	Common Name	No. of reads
23F2	Black_sea_bass	Black sea bass	621
23F2	Bluefish	Bluefish	212
23F2	Nor_sea_robin	Northern sea robin	14650
23F2	Red_White_or_Spotted_hake	Hake (Urophycis spp)	357
23F2	Scup	Scup	380
23F2	Spiny_dogfish	Spiny dogfish	1232
23F2	Str_sea_robin	Striped sea robin	192
23F2	Summ_flounder	Summer flounder	64
23F2	Windowpane_flounder	Windowpane flounder	284
23F2	Winter_skate_or_Little_skate	Little skate/ Winter skate species	148952
23F2	Am_butterfish	American butterfish	247
23F2	Atl_herring	Atlantic herring	6133
23F2	Atl_mackerel	Atlantic mackerel	292
23F2	Atl_or_nor_sand_lance	Sand lance	15141
23F2	Bay_anchovy	Bay anchovy	5
23F2	Black_sea_bass	Black sea bass	9256
23F2	Nor_sea_robin	Northern sea robin	30386
23F2	Sandbar_shark	Sandbar shark	2427
23F2	Scup	Scup	5
23F2	Winter_skate_or_Little_skate	Little skate/ Winter skate species	39150
23F2	Am_butterfish	American butterfish	3049
23F2	Atl_herring	Atlantic herring	712
23F2	Atl_mackerel	Atlantic mackerel	993
23F2	Atl_menhaden_LS16_or_river_herrings	Atlantic menhaden or river herrings	9533
23F2	Atl_menhaden_LS17	Atlantic menhaden	4591
23F2	Atl_or_nor_sand_lance	Sand lance	433
23F2	Barndoor_skate	Barndoor skate	29
23F2	Bay_anchovy	Bay anchovy	5
23F2	Black_sea_bass	Black sea bass	598
23F2	Brazilian_cownose_ray98	Brazilian cownose ray	7461
23F2	Nor_sea_robin	Northern sea robin	13174
23F2	Sandbar_shark	Sandbar shark	4177
23F2	Scup	Scup	176
23F2	Winter_skate_or_Little_skate	Little skate/ Winter skate species	37878

Survey	Taxa Common Name	Common Name	No. of reads
23F2	Atl_or_nor_sand_lance	Sand lance	5323
23F2	Nor_sea_robin	Northern sea robin	45133
23F2	Red_White_or_Spotted_hake	Hake (Urophycis spp)	3407
23F2	Scup	Scup	3870
23F2	Am_butterfish	American butterfish	2922
23F2	Bay_anchovy	Bay anchovy	5469
23F2	Fourspot_flounder	Fourspot flounder	3252
23F2	Gulf_stream_flounder	Gulf Stream flounder	3621
23F2	Nor_sea_robin	Northern sea robin	9488
23F2	Weakfish_Cyn	Weakfish	2271
23F2	Windowpane_flounder	Windowpane flounder	1862
23F2	Am_butterfish	American butterfish	13884
23F2	Atl_or_nor_sand_lance	Sand lance	11537
23F2	Nor_sea_robin	Northern sea robin	5153
23F2	Silver_perch(nibea93)	Silver perch	7105
23F2	Thread_herring	Thread herring	11203
23F2	Gulf_stream_flounder	Gulf Stream flounder	6040
23F2	Nor_sea_robin	Northern sea robin	6195
23F2	Black_sea_bass	Black sea bass	2058
23F2	Nor_sea_robin	Northern sea robin	5117
23F3	Am_butterfish	American butterfish	4907
23F3	Am_conger	American conger eel	9250
23F3	Atl_menhaden_LS16_or_river_herrings	Atlantic menhaden or river herrings	2290
23F3	Bay_anchovy	Bay anchovy	10
23F3	Nor_sea_robin	Northern sea robin	1879
23F3	Red_White_or_Spotted_hake	Hake (Urophycis spp)	29481
23F3	Scup	Scup	7251
23F3	Summ_flounder	Summer flounder	5563
23F3	Windowpane_flounder	Windowpane flounder	22125
23F3	Am_anglerfish	Goosefish	96
23F3	Atl_croaker_(nibea98)	Atlantic croaker	3513
23F3	Atl_herring	Atlantic herring	2198
23F3	Atl_mackerel	Atlantic mackerel	3871
23F3	Atl_menhaden_LS16_or_river_herrings	Atlantic menhaden or river herrings	1594

Survey	Taxa Common Name	Common Name	No. of reads
23F3	Black_drum_or_Spot	Black drum or Spot	1973
23F3	Bluefish	Bluefish	20679
23F3	Nor_sea_robin	Northern sea robin	3852
23F3	Red_White_or_Spotted_hake	Hake (Urophycis spp)	970
23F3	Scup	Scup	22672
23F3	Summ_flounder	Summer flounder	1401
23F3	Am_anglerfish	Goosefish	82
23F3	Am_butterfish	American butterfish	5620
23F3	Atl_herring	Atlantic herring	5502
23F3	Atl_mackerel	Atlantic mackerel	3130
23F3	Atl_menhaden_LS16_or_river_herrings	Atlantic menhaden or river herrings	2505
23F3	Atl_menhaden_LS17	Atlantic menhaden	2185
23F3	Bay_anchovy	Bay anchovy	13
23F3	Black_sea_bass	Black sea bass	170
23F3	Gulf_stream_flounder	Gulf Stream flounder	1133
23F3	Scup	Scup	17953
23F3	Str_bass	Striped bass	502
23F3	Weakfish_Cyn	Weakfish	864
23F3	Am_butterfish	American butterfish	2508
23F3	Atl_herring	Atlantic herring	1039
23F3	Atl_mackerel	Atlantic mackerel	132
23F3	Atl_menhaden_LS16_or_river_herrings	Atlantic menhaden or river herrings	7076
23F3	Atl_menhaden_LS17	Atlantic menhaden	7207
23F3	Black_drum_or_Spot	Black drum or Spot	571
23F3	Gulf_stream_flounder	Gulf Stream flounder	3697
23F3	Red_White_or_Spotted_hake	Hake (Urophycis spp)	1391
23F3	Scup	Scup	49300
23F3	Summ_flounder	Summer flounder	1511
23F3	Weakfish_Cyn	Weakfish	574
23F3	Atl_croaker_(nibea98)	Atlantic croaker	254
23F3	Atl_mackerel	Atlantic mackerel	938
23F3	Atl_menhaden_LS16_or_river_herrings	Atlantic menhaden or river herrings	855
23F3	Atl_menhaden_LS17	Atlantic menhaden	611
23F3	Black_sea_bass	Black sea bass	999

Survey	Taxa Common Name	Common Name	No. of reads
23F3	Bluefish	Bluefish	825
23F3	Grubby_or_other_sculpins	Grubby or other sculpins	1351
23F3	Gulf_stream_flounder	Gulf Stream flounder	1462
23F3	Longhorn_other_sculpins	Longhorn or other sculpins	1351
23F3	Nor_sea_robin	Northern sea robin	987
23F3	Red_White_or_Spotted_hake	Hake (Urophycis spp)	808
23F3	Scup	Scup	6629
23F3	Str_sea_robin	Striped sea robin	185
23F3	Summ_flounder	Summer flounder	1279
23F3	Summ_flounder99a	Summer flounder	371
23F3	Tuna_sp	Tuna species	792
23F3	Windowpane_flounder	Windowpane flounder	270
23F3	Am_butterfish	American butterfish	513
23F3	Atl_herring	Atlantic herring	1368
23F3	Atl_mackerel	Atlantic mackerel	128
23F3	Black_sea_bass	Black sea bass	583
23F3	Scup	Scup	1581
23F3	Summ_flounder	Summer flounder	463
23F3	Am_anglerfish	Goosefish	14
23F3	Atl_herring	Atlantic herring	998
23F3	Black_sea_bass	Black sea bass	1324
23F3	Summ_flounder	Summer flounder	939
23F3	Am_anglerfish	Goosefish	3627
23F3	Atl_herring	Atlantic herring	28478
23F3	Atl_menhaden_LS17	Atlantic menhaden	3787
23F3	Bay_anchovy	Bay anchovy	2114
23F3	Bluefish	Bluefish	4497
23F3	Scup	Scup	6933
23F3	Str_bass	Striped bass	47
23F3	Tautog	Tautog	6401

Appendix D – Comparison of Taxa Detected by eDNA and BRUV Monitoring Techniques in the Lease Area

Notes:
*Managed fishery in the Project Area as identified by the Empire Wind Construction and Operation Plan (Appendix U - Essential Fish Habitat Assessment).
NA – Detection of this taxa was not attempted by molecular methods.
Dash (-) identifies eDNA was able to detect the taxon group at a lower practical identification level than BRUV technique.

Taxon Group	Sampling Method	
	eDNA	BRUV
Bony Fish		
American Eel* (*Anguilla rostrata*)	X	
Atlantic Butterfish* (*Peprilus triacanthus*)	X	X
Atlantic Chub Mackerel (*Scomber colias*)	X	
Atlantic Croaker (*Scomber colias*)	X	
Atlantic Herring* (*Clupea harengus*)	X	
Atlantic Mackerel* (*Scomber scombrus*)	X	
Atlantic Menhaden* (*Brevoortia tyrannus*)	X	
Bay Anchovy (*Anchoa mitchilli*)	X	
Black Drum (*Pogonias cromis*)	X	
Black Sea Bass* (*Centropristis striata*)	X	X
Bluefish* (*Pomatomus saltatrix*)	X	
Conger Eel (*Conger spp.*)	X	
Fourspot Flounder (*Paralichthys oblongus*)	X	
Gulf Stream Flounder (*Citharichthys arctifrons*)	X	
Northern Sea Robin (*Iprionotus carolinus*)	X	X
Rough Scad (*Trachurus lathami*)	X	
Sand Lance (*Ammodytes spp*)	X	
Scup* (*Stenotomus chrysops*)	X	X
Silver Hake* (*Merluccius bilinearies*)	X	
Silver Perch (*Bairdiella chrysoura*)	X	
Spotted Hake (*Urophycis regia*)	X	X
Striped Bass* (*Morone saxatilis*)	X	
Striped Sea Robin (*Prionotus evolans*)	X	X
Summer Flounder* (*Paralichthys dentatus*)	X	X
Tautog* (*Tautoga onitis*)	X	
Thread Herring (*Opisthonema oglinum*)	X	
Weakfish* (*Cynoscion regalis*)	X	
Windowpane Flounder (*Scophthalmus aquosus*)	X	
Carp spp. (*Ctenopharyngodon spp.*)	X	
Flounder spp. (*Pleuronectoidei* spp.)	-	X
Goosefish* spp. (*Lophius spp.*)	X	
Mackerel spp. (*Scomber spp.*)	-	X
Sculpin spp. (*Rhamphocottidae spp.*)	X	
Sea Robin spp. (*Triglidae spp.*)	-	X
Tuna spp.* (Thunnini)	X	
Skates and Rays		
Barndoor Skate (*Dipturus laevis*)	X	
Brazilian Cownose Ray (*Rhinoptera brasiliensis*)	X	
Little Skate/ Winter Skate* (*Leucoraja spp.*)	X	X
Roughtail Stingray (*Bathytoshia centroura*)	X	X
Skate spp.	-	X
Sharks		
Blacktip Shark (*Carcharhinus limbatus*)	X	
Sandbar Shark* (*Carcharhinus plumbeus*)	X	
Spiny Dogfish* (*Squalus acanthias*)	X	X
Tiger Shark* (*Galeocerdo cuvier*)		X
Hammerhead Shark spp. (*Sphyrna spp.*)		X
Squid		
Atlantic Longfin Squid* (*Doryteuthis pealeii*)	NA	X
Shortfin Squid (*Illex illecebrosus*)	NA	X
Squid spp.	NA	X