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USING EDNA AND TRADITIONAL METHODS TO ASSESS FISH DIVERSITY IN NARRAGANSETT BAY

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USING EDNA AND TRADITIONAL METHODS TO ASSESS FISH DIVERSITY IN NARRAGANSETT BAY

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ABSTRACT

Coastal ecosystems are degrading rapidly, and molecular analysis of environmental DNA (eDNA) has become an increasingly popular method to quantify changes in biodiversity, and as a powerful and affordable complement to monitor aquatic environments. Yet, side by side comparisons of biodiversity estimates from eDNA and other traditional methods are needed to understand the level of accuracy of the eDNA method. We used eDNA metabarcoding of the 12S mitochondrial gene to generate a multi-species fish biodiversity assessment of Narragansett Bay (NB). Here we estimated biodiversity using eDNA data and compared it to conventional seine surveying data. We then further estimate biodiversity in NB across seasons and finer spatial scales using eDNA, to quantify species composition in NB. When compared to coastal seine surveying, eDNA has similar biodiversity estimates throughout Narragansett Bay, and the eDNA approach detects changes in species composition across seasons. Surprisingly, the number of reads generated through eDNA, and fish density captured with seine surveying are positively correlated, suggesting some value in the eDNA approach to not only capture changes in species composition, but also quantitative estimates per species. We also found substantial differences among seasons in the composition of NB fish communities. Using eDNA, we identified 46 species of fish in winter, 47 species in autumn, 35 species in spring, and 41 species in summer. Historically winter has been considered to have less biodiversity, however; our results suggest including winter in sampling efforts can improve our

understanding of NB. eDNA is a powerful approach to capture community shifts effectively across environmental gradients and seasons, both in compliment and as an alternative approach to traditional methods; especially in areas that are threatened by long term human interference and climate change. The added correlational aspect of eDNA copies with fish quantities is a promising prospect for the management of marine ecosystems.

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PREFACE

The following is the master thesis “Using eDNA and traditional methods to assess fish diversity in Narragansett Bay”. It is written in manuscript format and fulfils the graduate requirements of the Biological and Environmental Sciences program at the University of Rhode Island in Kingston, Rhode Island.

Throughout this process, I have been entirely grateful to be given the opportunity to further my education and career. To create this work, I have learned the skills for operating computer clusters, graphical interfaces such as RStudio and programming language for visualizing biological data, all of which I did not have previous knowledge. Without the guidance of many, this effort would not be possible.

Thank you.

Juliane Mora

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CHAPTER 1

USING EDNA AND TRADITIONAL METHODS TO ASSESS FISH DIVERSITY IN NARRAGANSETT BAY

This chapter is in preparation for submission to Estuaries and Coasts.

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INTRODUCTION

Marine ecosystems face multi-stressor threats which may impact ecosystem health and diversity. In coastal ecosystems with high biodiversity, it is increasingly important to monitor them both time-efficiently and with a cost-effective approach. As new methods emerge for assessing biodiversity, environmental DNA has become a popular method for detecting changes in fish species composition at a low cost (Stoeckle, Soboleva, and Charlop-Powers 2017). Environmental DNA is DNA from cells, organelles, or gametes that are shed into the environment including water, soil, or air (Barnes and Turner 2016). Collection of eDNA is easy and less resource intensive when compared to visual census and other traditional approaches (Bessey et al. 2020; Port, Donnell, and Romero-Maraccini 2015). Depending on the environment where eDNA is shed, it can persist in the environment for a few hours and up to a week in marine environments (Collins et al. 2018; Harrison, Sunday, and Rogers 2019). Detection probabilities of common species with eDNA methods have been correlated with traditional surveying methods (Stoeckle, Soboleva, and Charlop-Powers 2017). The capabilities of eDNA allow for earlier detection of rare species and species that avoid traditional detection methods, allowing for an extension to conventional surveying (Deiner and Bik 2017). In addition, environmental DNA can distinguish spatial variation across locations (Andruszkiewicz et al. 2017), which is important for monitoring populations and ecosystems. As eDNA continues to be implemented for ecosystem monitoring, it has been shown to be useful for

estimating biodiversity and community structure in marine environments. Here we compared eDNA with a traditional method and tested its complementarity to monitor coastal bays.

Traditionally, quantitative PCR of eDNA has been used to provide quantitative data on abundance of single or closely related species. There is interest in testing whether a multispecies approach such as eDNA-metabarcoding can produce quantitative abundance estimates of multiple species simultaneously. Here, we also explored the idea that DNA read counts correlate with species abundance changes. We hypothesize the read count number would increase or decrease according to the abundance or biomass of each species. Even if we are unable to have direct absolute estimates of biomass from metabarcoding read numbers, relative abundance would still be especially useful for managers.

To test these ideas about eDNA monitoring, we chose an ecosystem with an extensive legacy of monitoring using traditional fish surveys. Narragansett Bay in Rhode Island, which has been increasingly impacted by multiple stressors leading to declined health, served as our study site. Over the last 38 years, the Rhode Island Department of Environmental Management (RIDEM) has recorded fish species diversity through their long-standing coastal fish trawling and seine surveys and has compiled data for 132 species (Olszewski and Parkins 2022; Gerber-Williams 2022). Fish species in Narragansett Bay vary throughout the year due to migration and seasonal use of nursery habitats. As these migration patterns are innately

complex, they have previously been difficult to measure and study (Langan 2021). Building on this historical data and yearly monitoring by RIDEM, we initially compared biodiversity estimates from eDNA surveys with the results obtained from traditional seine surveys. To assess if the eDNA approach can be used as a multispecies quantitative approach, we used our eDNA species data to test for a correlation between seine fish counts and eDNA read counts.

Here we test for 1) differences between fish diversity estimates from eDNA and seine data, 2) test for a correlation between read number from eDNA and fish counts from the seine data, and 3) after a validation of the eDNA with the seine data, we also tested the hypothesis that fish species change across space and seasons across NB by systematically sampling across 24 stations for four seasons. eDNA is a robust approach to quantify community shifts, easily and effectively across environmental gradients and across seasons with the added benefit that with some further refinements, quantitative estimates, at least relative, can be obtained simultaneously for multiple species, further increasing the value of the eDNA from the management perspective.

METHODOLOGY

Comparison of eDNA and seine surveys

To compare the eDNA method to a conventional approach, we collected paired samples at 12 locations (Fig. 1a). Seine surveys conducted by the RIDEM were used as a baseline and compared to eDNA samples. All seine survey protocol, gear, and specifications are detailed on RIDEM's website under Seine Surveying (Gerber-Williams 2022). Briefly, the sampling includes 60 m by 3 m, 10 cm mesh beach seine in a total area of 543 square meters and is hauled toward the beach by hand. Our paired sampling scheme includes data from spring, summer, and autumn of 2019, with paired sample locations less than 3.2 km apart. Spring, summer, and autumn sampling locations were visited in late May through June, Autumn, and October, respectively, within one week of each other. In some cases, when sampling schedules did not line up (summer sampling), samples were collected four weeks apart.

Test for spatial and seasonal patterns using eDNA

To further describe spatial and seasonal patterns of biodiversity from eDNA, we collected water samples at 24 locations across Narragansett Bay (Fig. 1b). These 24 sites included the 12 original locations plus 12 additional locations. Sample collection took place during the four seasons of 2019. Locations throughout Narragansett Bay were chosen based on previously existing water monitoring projects such as fixed buoy networks, moored

acoustic doppler current profilers, and water quality monitoring networks. These additional surveys were not benchmarked against traditional monitoring because seine surveys were initially designed to focus on use of nursery habitat by juvenile fishes and so are not conducted in winter.

Sample collection and extraction

One-liter water samples were collected from the shore for three replicates per sample site. Sample filtering was conducted in the lab using 0.45 um Whatman Cellulose Nitrate Membrane Filters with a 47 mm diameter. Water was poured through a Nalgene sterile disposable filter unit and into a large beaker connected to a GAST vacuum pump. Water from the three replicates per site were passed through the filter until 1L of water was successfully filtered, and the unit was cleaned with 10% Clorox after each sample was filtered. One liter of distilled water was filtered as a control. Sample filters were kept at -20℃ until ready to be extracted. Once all the samples had been filtered, we extracted environmental DNA from the filters using the Macherey-Nagel Soil Kit, including the control samples.

PCR and visualization

A two-step PCR was used to prepare the samples for Illumina sequencing. For the first PCR, we amplified each eDNA sample in triplicate using polymerase chain reaction. The primer used was Teleo2-F 5'-AAACTCGTGCCAGCCACC-3' and Teleo2-R 5'-

GGGTATCTAATCCCAGTTTG-3' (Collins et al. 2019). Amplification was performed in 12.5ul reactions containing 2.25ul of water, 6.25ul of Qiagen Hotstart, 1ul each of Teleo2 forward and reverse primers at 10uM concentration, and 2ul of extracted eDNA. The thermocycler was programmed for 10 minutes at 95°C for initial denaturation, followed by 35 cycles of 30 seconds at 95°C for denaturation, 45 seconds at 54℃ for annealing, 30 seconds at 72℃ for extension, and 5 minutes at 72℃ for the final extension.

The second PCR was used to add Illumina adapters for MiSeq Illumina sequencing. The amplification primers used in the second PCR were 5uM Nextera XT Index 1 Primers (N7) and Nextera XT Index 2 Primers (S5) from the Nextera XT Index kit for 12S Metagenomic Sequencing Library Preparation. Each sample was assigned to a different index combination (barcode) to allow for samples to be uniquely identified post sequencing. Each reaction contained 6.5ul of KAPA HiFi Hot Start Ready Mix, 2.25ul of molecular grade water, 2ul of clean DNA from the first PCR amplification, and 1ul of each respective N7 and S5 primer. The thermocycler was programmed for 3 minutes at 98℃ for initial denaturation, followed by 8 cycles of 30 seconds at 98℃ for denaturation, 30 seconds at 55℃ for annealing, 30 seconds at 72℃ for extension, and 5 minutes at 72℃ for the final extension. After each PCR, samples were visualized in 1% agarose gel in 1X TAE buffer with a 1kb DNA and under UV light. Samples were cleaned and resuspended in 25ul of 10mM Tris HCl.

Data sequencing and quality control

Illumina sequencing was used to sequence all eDNA samples. To assess the quality of the samples, raw sequencing data underwent quality control (QC) procedures using the MultiQC tool (version1.9) (Ewels et al. 2016). This tool can provide detailed results for sequencing data quality, G-C content, adapter contamination, sequence length, and duplication rate. The fastp tool was used as a FASTQ preprocessor for trimming and filtering adapter sequences and low-quality bases (Ewels et al. 2016). Sequences that passed the quality control measures were used for subsequent analysis.

Bioinformatic analysis

The Anacapa Toolkit was used to clean, analyze sequence data, and assign taxonomy from the Illumina reads using the DADA module. This toolkit was designed to evaluate metabarcode sequence data and create reference databases, as well as, assign taxonomy after Illumina sequencing (Curd et al. 2019). We used the reference library provided by the ANACAPA drive. Raw demultiplexed Illumina FASTQ data was used to generate amplicon sequence variants (ASV) and conduct standard sequence QC using dada2 (Curd et al. 2019). These ASVs were used to identify unique sequence read counts and easily assign taxonomy. Each ASV read count was used to identify the number of copies present in a sample. All ASVs were aligned to the library using Bowtie2 and received a taxonomy assignment using a BLCA script. Taxonomy was assigned based on the lowest common ancestor. In cases where sequences could not be identified down to the species level, such as

closely related species, these samples were assigned to the same genus. Taxonomic groups that were not detected in all three PCR replicates were removed from the data. This sequence of steps output a taxonomy table that was used for further analysis in R.

Fish Census Data

Fish included in this study were confirmed with previous survey data from RIDEM and historical fisheries records. Capture and observation data has been collected from state, university, and federal sources beginning in the late 1800s (C. Oviatt et al. 2003). Early comprehensive information has been recorded in interviews of fishermen in Rhode Island (Baird 1873; Goode 1887). Since the 1950s, otter trawl survey data has been collected weekly from Fox Island station at the Graduate School of Oceanography at the University of Rhode Island (C. A. Oviatt and Nixon 1973; Jeffries and Terceiro 1985; Collie, Wood, and Jeffries 2008). RIDEM has conducted trawl surveys from 1979 to current (Olszewski and Parkins 2022). The data obtained from these previous surveys allowed us to effectively evaluate and validate eDNA species data in Narragansett Bay. In this study specifically, we used RIDEM seine survey data from 2019.

Data analysis

Environmental DNA and RIDEM Seine Survey data were further analyzed using the RANACAPA and Vegan packages in R version 4.0.3 (Curd

et al. 2019; Oksanen et al. 2022). The rarefy function in RANACAPA was used to confirm if sequencing depth was sufficient to detect the α-diversity in each sample (Fig. S1). Two-dimensional non-metric multidimensional scaling (NMDS) was used to visualize differences in fish community composition. Data were arranged with the total number of species present at each sampling location and grouped by season. For NMDS, the community dissimilarity was calculated based on Jaccard indices with 95% confidence interval. The differences in community structure between sampling seasons and locations were evaluated using permutational multivariate analysis of variance (PERMANOVA). The PERMANOVA analysis was conducted using the adonis2 function and a Jaccard dissimilarity matrix. We calculated *p* values with 9999 permutations. A spatial blocking term for location was included in the model because the same locations were sampled repeatedly across seasons. In these analyses, sampling seasons were defined as spring (June), summer (August), and autumn (October).

The species accumulation curve function in Vegan was used to determine if the two methods yield similar estimates of total species richness. Curves were calculated using expected species richness and first order jackknife species richness estimator with 100 permutations. Rank-abundance curves were generated to measure relative species abundance across seasons. Abundance was calculated using the relative abundance function in Vegan by diving the number of species in one sample by the total number of species found in all samples and values were log-transformed. To test

correlations between eDNA and seine data, a linear regression model was used to test the relationship between total eDNA reads and total seine fish abundance. Data were log-transformed using log(x+1) to normalize the distribution within the data. Species were the replicates in this analysis and data were pooled across locations. A second linear regression model was used to test the correlation of individual species reads and seine counts. Data were log-transformed using log(x+1) and locations were the replicates. The third linear regression model was used to test the relationship between species abundance and location. Data were log-transformed using log(x+1) and all species were pooled across locations.

In addition, the pairwise Wilcox test function in R was used to generate a Wilcoxon rank-sum test between each season for each method to determine differences in Simpson diversity across seasons. The Simpson diversity index was used infer diversity patterns for the number of species present, as well as the relative abundance of each species. All seasons of eDNA data were compared to each other, then each season of seine data were compared. The holm method was used to adjust *p* values for multiple comparisons.

To expand our analysis of Narragansett Bay using eDNA, 24 locations including all four seasons were analyzed using similar methods as mentioned above. The original 12 locations were included in this analysis along with 12 additional locations. This expanded analysis with the addition of winter was important to understand the diversity within Narragansett Bay because winter is not currently included in RIDEM's seine monitoring. To analyze and

visualize species distributions across seasons and space, we used NMDS, PERMANOVA, species accumulation curves, rank-abundance curves, and Wilcoxon rank tests, using previously mentioned functions and packages.

RESULTS

Illumina sequencing results

A total of 61 fish species belonging to 37 families were identified across all samples. Adapter trimmed reads percentage for each sample ranged between 0-%11.8%. The percentage of reads >Q30 per sample after filtering ranged between 73.8%-88.3%. GC content per sample after filtering was between 35.1%-48.7%. The duplication rate in filtered reads was between 0%-43.8%. Average read length per sample was between 200 and 400 base pairs. The number of reads from each sample ranged between 10,000 to 300,000. The average number of reads per sample was 100,000.

Comparing methods

In our paired sampling across 12 locations, there are 21 fish species that overlap between methods, with most being demersal fish (Fig. 2). There are 17 species that are unique to eDNA sampling. In total, eDNA detected 38 fish species within Narragansett Bay. Seine surveying captured 36 species in total, of which 15 species were unique to this method. Both methods showed changes in sample diversity across seasons (Fig. 3).

All seasons for each method reached expected species richness after 10 sample sites, suggesting that differences in species richness between methods were reliable based on the expected species richness in each season (Fig. 4). eDNA and seine data detected approximately 10 to 12 species in each season. Species accumulation curves for pooled and

seasonal data suggests eDNA and seine surveying can capture expected species richness across NB with 10 sampling locations.

We found that Narragansett Bay is home to a diverse population of fish species; both migratory and non-migratory that vary in size, habitat type, and distribution. The most common fish caught across seasons using seining were *Menidia menidia* (Atlantic silverside), *Brevoortia tyrannus* (Atlantic menhaden), *Fundulus majalis* (striped killifish), *Leiostomus xanthurus* (spot croaker), and *Tautoga onitis* (tautog) (Fig. 5). Atlantic silverside and Atlantic menhaden can be found throughout most of the eastern coast of North America (Robins and Ray 1986; Whitehead 1985). Striped killifish are small, non-migratory fish that live within many estuaries, bays, and coastal marshes throughout the western Atlantic (Huber 1996). They are commonly found between New Hampshire and northeastern Florida. Spot croaker ranges between Massachusetts to Mexico; however, they are absent in southern Florida (Robins and Ray 1986). Spot croakers like sandy or muddy bottoms in coastal waters and inhabit river estuaries during summer and fall. Tautogs are commonly found in Rhode Island near the shore in hard-bottom habitats (Leim and Scott 1996). They range from Nova Scotia, Canada to South Carolina in the USA. Although these fish vary in size at maturity, seine surveys traditionally capture juvenile fish that stay close to shore. For this reason, most fish captured during seine surveying are of comparable size. While seine surveying captures various fish that live in different marine zones, demersal fish were the most common.

Similarly, eDNA methods detected high numbers of DNA reads for Atlantic silverside, Atlantic menhaden, and striped killifish. Additionally, the common species detected across seasons via eDNA were *Fundulus heteroclitus* (mummichog), *Pomatomus saltatrix* (bluefish), and *Alosa pseudoharengus* (alewife). Mummichog are small, non-migratory fish found across the eastern coast of North America (Page and Burr 2011). Adult mummichog are found in tidal creeks and saltwater marshes. Bluefish are found around the globe in tropical to subtropical waters. Small adult bluefish can be found in estuaries and shallow coastal waters. They contribute to the largest fishery by volume on the Atlantic coast (Buchheister et al. 2016). Alewife is another species of fish found throughout the North American Atlantic (Whitehead 1985). They migrate up rivers to spawn in lakes, then return to sea shortly after spawning. Alewife fry descend from spawning grounds in summer and autumn or as late as November and December. Like seining, demersal fish were the most common species identified using eDNA. It is likely that bottom-dwelling species were identified in greater numbers using eDNA because our sampling method was employed in shallow water near the shoreline where the bottom of the bay was easily accessible.

Across both methods, spring was the most distinct when compared to the other seasons (Fig. S2). The eDNA rank abundance curve for spring suggests a shift in species composition with the top five ranked species being *Brevoortia tyrannus* (Atlantic menhaden), *Menidia menidia* (Atlantic silverside), *Tautoga onitis* (tautog), *Stenotomus chrysops* (scup), and Tautogolabrus

adspersus (cunner) (Fig. 5). Atlantic menhaden and Atlantic silverside rank in the top five for summer and autumn; however, tautog, scup, and cunner do not. In eDNA, the seasons that were closest in Simpson diversity along with a similar total number of species detected, were summer and autumn. We hypothesize that summer and autumn are similar in diversity due to seasonal migratory patterns of fish. The primary difference between summer and autumn species composition is the presence of *Anguilla rostrata* (American eel). In colder months, American eel travel closer to shore and up coastal rivers, which could explain higher abundance of these fish in autumn (Page and Burr 2011). The summer rank abundance curve is not as steep as autumn suggesting more species evenness in summer than in autumn. The seine rank abundance curve for spring also shows a change in species composition. The top-ranking species for seine data in spring is *Leiostomus xanthurus* (spot croaker) and does not rank in the top five for any other seasons. The other main difference between seasons across methods is the presence of Atlantic menhaden. All seasons of eDNA data ranked Atlantic menhaden in the top five; however Atlantic menhaden was only identified in autumn for seine data. As an overall trend, seine data did not show the same distinction between changes in diversity across seasons, suggesting eDNA is a more sensitive method for detecting small seasonal shifts that may otherwise be overlooked using the traditional seine surveys.

Fish diversity across seasons in Narragansett Bay

When we sampled eDNA across all four seasons, there were 61 species identified in Narragansett Bay with demersal fish being the most abundant across seasons. Out of the 61, there were 25 species present in all seasons (Fig. S3). The five most abundant species within the bay across all seasons were *Brevoortia tyrannus* (Atlantic menhaden), *Menidia menidia* (Atlantic silverside), *Fundulus heteroclitus* (mummichog), *Fundulus majalis* (striped killifish), and *Alosa pseudoharengus* (alewife). Winter is not currently included in seine sampling as most juvenile species of interest for RIDEM are abundant from spring-autumn. Many juvenile fish species leave in the colder months, corresponding to previous knowledge of lower diversity in winter. However, we found both autumn and winter had the highest number of unique and total species identified. Highlighting species that are only found in winter months would expand our current understanding of Narragansett Bay.

Species that were unique to winter include *Hemitripterus americanus* (sea raven), *Hippocampus erectus* (lined seahorse), and *Xiphias gladius* (swordfish). Sea ravens are demersal fish that inhabit rocky, hard seafloor traditionally found from Labrador, Canada to Chesapeake Bay, USA (Robins and Ray 1986). Lined seahorses are small non-migratory, reef associated fish commonly seen in the western and central eastern Atlantic (Lourie, Pollom, and Foster 2016). They are seen in coastal waters but will migrate to deeper water in the winter. Swordfish can be found throughout the globe and average 300 cm in length (Nakamura 1985). These fish are pelagic-oceanic and inhabit

tropical to cold water temperatures. Swordfish migrate toward colder waters in the summer and warm waters in the fall. These three species were identified with less than 200 sequences and demonstrate the eDNA's ability to detect rare fish species.

Across all seasons, eDNA detected variation in diversity (Fig. 6). At least 10 sample sites are required to identify species richness throughout Narragansett Bay, suggesting that our sampling scheme of 24 samples is adequate to accurately estimate fish diversity in NB (Fig. S4). Expected species richness was achieved for all seasons for eDNA data (Fig. S5). The threshold for expected species richness throughout the bay is achieved after 10K reads have been obtained.

eDNA data revealed seasonal differences in species composition. All comparisons across seasons of eDNA show significant differences in Simpson diversity except between summer and autumn (Fig. S6). We hypothesize that summer and autumn are similar in diversity due to seasonal migratory patterns of fish. Previous knowledge of NB suggests lower diversity in colder months, however; winter shows the highest diversity while autumn has the highest number of fish species identified. Out of all the seasons, spring has the lowest diversity and the least number of species identified. In all four seasons of eDNA data, Atlantic menhaden and Atlantic silverside maintain high relative abundance in NB (Fig. 7). Summer and autumn are similar in species composition; the main difference between the two seasons being more species identified in autumn than in summer. The winter rank abundance

curve is not as steep as the other seasons suggesting more species evenness. In total there were 46 species of fish identified in NB during the winter. The most abundant species in winter is *Apeltes quadracus* (fourspine stickleback). These fish are small nearshore species native to the western Atlantic that live along weedy sections of bay and backwaters (Robins et al. 1991). *Alosa pseudoharengus* (alewife) is the second most abundant species in winter. They are small anadromous fish that migrate to coastal rivers during spring spawning season and return to the ocean during the summer and autumn or as late as November/December (Whitehead 1985). Including winter in current sampling efforts of NB can expand our knowledge of species present in the bay throughout the entire year.

Statistical results: correlation between eDNA reads and seine fish counts

When data were pooled across all sites and seasons, there is a significant non-linear relationship between eDNA reads and abundance in the seine surveys bay (Table 1, Fig. 8, $p < 0.01$ and $r^2 = 0.59$). Relative abundance estimated using eDNA thus shows some promise as a method to complement estimates from traditional sampling methods.

For four species with high abundance for both methods, there was also a significant non-linear relationship between eDNA reads and abundance in the seine survey. Site-and time-specific changes in the relative abundance of common species may thus also be predictable using eDNA reads (Fig. 9). These relationships also highlight variable, but non-zero, eDNA reads when

seine counts are zero. This likely reflects higher sensitivity of the eDNA method (Fig. 9).

When data for all species are pooled, so that each point represents the total number per species at each location, there is a positive relationship between eDNA reads across locations, but only in spring; however, it is not a strong relationship (Table 2, Fig. 10, $p < 0.01$, $r^2 = 0.16$). This relationship can be used to estimate the potential number of fish present within the bay by the number of reads eDNA identifies. Lower detection threshold and the association of reads to the number of fish present in an area will make determining population numbers for species of interest easier than traditional methods.

A Wilcoxon rank test was performed to determine how season affects Simpson diversity and if differences in diversify are significant (Table 3). Corresponding with previous rank accumulation curves for eDNA data, diversity in spring is different than both summer and autumn, while summer and autumn remain similar. There were no significant trends between each season of seine data, except for spring and summer. A PERMANOVA test using the adonis2 function was used to assess the model fit for seasonality within the data (Table 4). This test showed seasonality can be used as a good predictor for distinct groups present in both eDNA and seine data ($p < 0.05$).

Statistical results: seasonal variation in fish diversity across Narragansett Bay

The full eDNA dataset includes 24 locations and four seasons. This larger dataset includes a more complete sampling of Narragansett Bay over one year. To test differences in diversity across seasons, we used the Wilcoxon rank and PERMANOVA tests. We found that eDNA can detect changes across time and space throughout one year.

A Wilcoxon Rank Test was used to determine if Simpson diversity changes within NB based on season (Table 5). Each season of eDNA data shows differences in diversity except summer and autumn, which corresponds to previous knowledge of migration patterns of fish in NB. To test for differences among groups within the data, PERMANOVA test with a spatial blocking term for location was used (Table 6). Diversity is shown to seasonally segregate in Narragansett Bay. All four seasons are distinct groups within the data ($p < 0.05$).

DISCUSSION

Comparison of methods

Progress has been made documenting biodiversity changes in estuarine ecosystems due to nutrient dynamics both spatially and temporally with positive impacts on ecosystem management (Raposa and Schwartz 2009), but two chief barriers remain. The first is that traditional surveys to record biodiversity are expensive, laborious and require taxonomic expertise (Dickens et al. 2011). Given the amount of work to process each sample, sampling is often geographically limited and temporally sparse. The second is that for visual census techniques or quadrats, cryptic variation is often not captured, resulting in lower biodiversity estimates (Yamamoto et al. 2016). Our study builds upon traditional fish biodiversity surveys to compare it with a nondestructive approach based on environmental DNA. We showed that eDNA can be used to monitor fish diversity in nearshore habitats across the Narragansett Bay estuarine system. A comparison between eDNA and seine surveying highlights the ability of the eDNA to identify a more diverse fish community. Additionally, we demonstrated that the eDNA metabarcoding can potentially be used as a method to evaluate seasonal changes in fish communities of coastal wetland ecosystems, and with some additional calibration and fine tuning using read standards, to quantify multi-species fish densities/biomass.

Addressing the limitations of traditional methods, our study combines seine surveying and eDNA metabarcoding. The main limitations of traditional approaches are identifying rare species and the ability to distinguish

morphologically close species and identifying juvenile stages of known species (Danovaro et al. 2016). We suggest using a combination of surveying efforts, such as using eDNA in areas that are difficult or costly to survey. eDNA can provide information regarding wide geographic areas or large temporal scales along with identifying cryptic species (Danovaro et al. 2016). With the advancement of taxonomic identification of species, there is less need for relying on human identification skills.

There is substantial overlap of identified species between eDNA and seine surveying. Our study found that *Brevoortia tyrannus* (Atlantic menhaden), *Stenotomus chrysops* (scup), *Pomatomus saltrix* (bluefish), and *Tautoga onitis* (tautog) are the most common species in coastal areas of NB. This coincides with historical records in the bay since the late 1800s (C. Oviatt et al. 2003). These species range from supporting northeastern Atlantic fisheries to being ecologically important within Rhode Island's coastal waters. Atlantic menhaden supports the largest fishery by volume on the US Atlantic coast (Buchheister et al. 2016). Both scup and tautog are important recreational and commercial fisheries and play a vital role in Narragansett Bay (Terceiro and Northeast Fisheries Science Center (U.S.) 2012; McNamee 20212). Habitat for young bluefish includes coastal bays of the US and may be a bioindicator species (Smalling et al. 2016). The presence of young bluefish during critical life stages in Atlantic coastal estuaries can indicate estuarine health due to their high lipid content and piscivory.

Both eDNA and seine surveying effectively captured species present in the sampled areas after 10 sampling locations. eDNA, however, captured greater seasonal shifts in diversity. The ability for eDNA to target rare species or species that can evade traditional surveying gear makes it a more sensitive detection method. For example, *Salmo salar,* also known as Atlantic salmon, was historically found in Narragansett Bay until the late 1800s (C. Oviatt et al. 2003). Although this species is no longer traditionally found in NB, eDNA identified some Atlantic Salmon in summer, autumn, and winter. It is likely that Atlantic salmon are still present in some areas of Narragansett Bay, at very low densities difficult to detect with traditional surveying methods. As Atlantic salmon are anadromous, it is also possible eDNA detected these fish as they migrate to and from freshwater to breed. RIDEM has released Atlantic salmon along with other freshwater fish throughout Rhode Island lakes and rivers for recreational fishing (Rhode Island Division of Fish and Wildlife 2023). It is possible eDNA can detect Atlantic salmon that have migrated from freshwater rivers into NB. We suspect that eDNA can capture rare fish species that traditional surveying may fail to observe.

Some other species that were unique to eDNA were *Lepomis macrochirus* (bluegill) and *Fundulus diaphanus* (banded killifish). Bluegill is a freshwater fish with some tolerance for brackish waters, found in many streams in North America (Page and Burr 2011). Banded killifish are small brackish fish also found throughout lakes, rivers, and coasts of eastern North

America. These small fish are present in the bay near freshwater outlets that seine efforts are not capturing.

Seasonal and spatial variation

In Narragansett Bay, we determined variation across locations within a 3 km distance. Similarities between eDNA and observational data increase when using data within a <2 km buffer zone (Nakagawa et al. 2018). In some cases, eDNA can distinguish vertebrate community assemblages from habitats separated by as little as ~60 m (Port, Donnell, and Romero-Maraccini 2015). eDNA communities are stable over time and tide, however; consideration should be taken for ecological variables that do not remain geographically consistent (Kelly, Gallego, and Jacobs-Palmer 2018). That said, it will be important to sample close to the ecosystem of interest. Using eDNA and seining in tandem will offer greater insight into NB fish communities over time. For species of interest and larger fish, it will be key to sample the middle of the bay, where they are usually found. The spatial range of eDNA within an aquatic system is important to consider when sampling. In freshwater river systems with lower water velocity, sampling eDNA every 400 m is sufficient to detect a single fish using quantitative PCR methods (Z. T. Wood et al. 2021). eDNA is conserved over short distances in running water but can degrade quickly over distances >1.5km (Tillotson et al. 2018). Studies have found that in freshwater and wetland systems, eDNA reflects species present nearby (Civade et al. 2016; McKee, Spear, and Pierson 2015; Wilcox et al.

2016). In some cases, the observed movement of eDNA from the source can be up to 10 km (Deiner and Altermatt 2014). In nearshore marine environments, the effectively sampled area of individual water samples for eDNA analysis is around <100 m (O'Donnell et al. 2017). However, studies of dynamic coastline habitats with high wave energy have found eDNA transport is limited enough that DNA methods can detect differences among communities separated by 2.5km (O'Donnell et al. 2017).

RIDEM uses seine surveys to estimate juvenile fish species of interest that leave the bay during winter months. Target species include winter flounder (*Pseudopleuronectes americanus*), bluefish, tautog, weakfish (*Cynoscion regalis*), and scup (Meng and Powell 1999). Bluefish, tautog, and scup were present in all seasons of eDNA sampling. Weakfish were identified in spring and summer of eDNA sampling. Winter flounder was not identified; however, summer flounder (*Paralichthys dentatus*) was present in all seasons of eDNA sampling. Using eDNA sampling in the winter may offer a more robust estimate of fish species throughout the year.

eDNA data suggests that in Narragansett Bay, fish abundances peak in mid-September after the maximum temperature in August (A. J. M. Wood, Collie, and Hare 2009). Species that were unique to autumn include *Ambloplites rupestris* (rock bass), *Molva molva* (ling), and *Cichlidae* (cichlid). Rock bass are a freshwater fish native to Missouri but can be found in most northeastern American rivers (Page and Burr 2011). Lings are large demersal fish traditionally found in the northern Atlantic from southern Greenland to

Canada and the Barents Sea and Iceland to Morocco (Cohen et al. 1990). We were also able to identify two sequences of *Cichlidae*, however we were unable to determine the species based on our DNA library. Cichlids can survive in both fresh and salt waters of central and south America. They are also common aquaria fish and seen as an invasive species in Texas, Louisiana, and Florida (Lorenz, O'Connell, and Schofield 2011). Regarding invasive and cryptic species detection, eDNA may prove to be a resource in managing these species. All species uniquely present in autumn and winter were identified with less than 200 reads. Bias may exist for species that amplify poorly and will be rarely observed in the metabarcoding data. However, species rare in the environment with low eDNA concentration should be detected if that taxon has a high amplification rate (Shelton et al. 2023). eDNA offers a sensitive detection method that can distinguish differences across seasons within the bay with a few sequences needed for identification. Differences in seasonal diversity and species richness will need to be further explored to determine species composition versus total abundance of fish.

Metabarcoding of eDNA a tool to estimate fish abundance or biomass

Seining and other traditional methods such as trawling can offer direct biomass estimates, however it is a promising prospect to have quantitative analysis relating eDNA copies with fish quantities. The application of environmental DNA for monitoring coastal ecosystems can inexpensively detect changes in biodiversity in natural systems. Environmental DNA will be

especially useful in areas where conducting traditional surveys are difficult due to rocky shorelines, hazardous weather conditions, or sensitive environments. As eDNA methods are explored for more coastal environments, the applications of using eDNA are likely to expand. Along with traditional surveying methods, eDNA is another tool that can be effectively utilized to gain more insight into the biodiversity of marine ecosystems.

Previously, the capability of eDNA metabarcoding to estimate the total absolute abundance of fish has been demonstrated using teleo-eDNA and traditional methods to estimate fish species abundance (Pont et al. 2023; Boivin-Delisle et al. 2021). Correlation between the teleo-eDNA concentration and fish abundance is comparable to results obtained in species-specific qPCR studies in natural environments (Yates, Fraser, and Derry 2019). However, it will be important to consider the environmental factors that affect the study site and eDNA quality such as temperature, size of study area, and depth (Rourke et al. 2022; Yates, Fraser, and Derry 2019). Understanding these factors will help improve the accuracy of quantification of fish populations using eDNA. For many bio-monitoring purposes, a rough estimation of absolute fish abundance using eDNA metabarcoding is sufficient, as the main objective is to compare fish assemblages on a larger scale (Pont et al. 2023).

To quantify eDNA metabarcoding with fish abundance, it will require contextual information about the ecosystem of interest (Shelton et al. 2023). Studies have shown in both mesocosm and field experiments, eDNA

quantities can be used to infer abundance or biomass (Takahara et al. 2012; Yamamoto et al. 2016; Stoeckle, Soboleva, and Charlop-Powers 2017). eDNA concentration can reflect the potential distribution of fish in the natural environment (Takahara et al. 2012). In most cases, eDNA concentration can be more readily used to assess biomass within 150 m (Yamamoto et al. 2016). In addition, abundant species are often identified more than uncommon fish, inferring that higher abundance of species should correlate to more eDNA reads. In some cases, strong seasonal increase in fish eDNA detection is consistent with the migration patterns of fish populations into regional inshore waters and estuaries in the spring (Stoeckle, Soboleva, and Charlop-Powers 2017). In the future, we expect to compare biomass assessments in NB with read depth in eDNA studies. Mesocosm experiments where there is a known number of fish can be used to create calibration curves for eDNA reads and applied to natural systems. Another key step we want to consider is taking eDNA samples as close in time and space to existing seining efforts. We believe these steps will aid in fine tuning eDNA as a tool for measuring fish abundance.

In conclusion, the integration of eDNA metabarcoding with traditional surveying methods offers a comprehensive approach to studying fish biodiversity in estuarine ecosystems. Moreover, with further calibration and refinement using read standards, eDNA can provide estimates of multi-species fish densities and biomass. By addressing the limitations of traditional methods and leveraging the sensitivity and efficiency of eDNA, managers can

gain deeper insights into fish communities, aiding in effective ecosystem management and conservation efforts.

Table 1. Linear regression model statistics for predicting seine counts based on eDNA sequence reads.

lm(formula = log_total_edna ~ log_total_seine, data = log_seine_edna_rowsums)

Residuals:				
Min	1Q	Median	3Q	Max
-5.180	-0.705	0.278	1.051	4.031
Coefficients:	Estimate Std	Error	t value	Pr (>|t|)
Intercept	5.180	0.700	7.40	2.8e-07
log_total_eDNA	0.824	0.144	5.74	1.1e-5

Residual standard error: 1.93 on 21 degrees of freedom

Multiple R-squared: 0.61, Adjusted R-squared: 0.592

F-statistic: 32.9 on 1 and 21 DF, p-value: 1.08e-05

Table 2. Linear regression model statistics for predicting the number of reads or counts based on sample location.

lm(formula = log_total_seine ~ log_total_edna, data = log_location_totals)

Residuals:				
Min	1Q	Median	3Q	Max
-3.5527	-1.3114	0.2465	1.1214	4.4750
Coefficients:	Estimate Std	Error	t value	Pr (>|t|)
Intercept	-2.7277	2.9103	-0.937	0.3561
log_total_eDNA	0.7634	0.2846	2.683	0.0118

Residual standard error: 1.794 on 30 degrees of freedom

Multiple R-squared: 0.1935, Adjusted R-squared: 0.1666

F-statistic: 7.198 on 1 and 30 DF, p-value: 0.01176

Table 3. Pairwise comparisons of Simpson diversity using Wilcoxon rank test. Each season of eDNA data was compared (spring, summer, autumn). Each season of seine data was compared in the same fashion.

data:eDNA_richness$Simpson and sample_data(eDNA_fish_data)$Season

eDNA	Spring	Summer
Summer	7.5e-05	—
Autumn	1.9e-05	0.33

data:SEINE_richness$Simpson and sample_data(SEINE_fish_data)$Season

Seine	Spring	Summer
Summer	0.0087	–
Autumn	0.4378	0.4378

P value adjustment method: holm

Table 4. PERMANOVA statistics for assessing distinct groups within the data based on season and location ($p<0.05$).

adonis2_eDNAresult<-adonis2(eDNA_fish_dist~season+location, data=data.frame(sample_data(eDNA_fish_data)), permutations = 9999)

eDNA	Df	SumOfSqs	R2	F	Pr(>F)
Season	2	0.0112374	0.30595	28.9001	1e-04
Location	11	0.0095499	0.26001	4.4655	1e-04
Residual	82	0.0159422	0.43404	*NA*	*NA*
Total	95	0.0367295	1.00000	*NA*	*NA*

adonis2_seine_result <- adonis2(SEINE_fish_dist ~ season + station_name, data=data.frame(sample_data(SEINE_fish_data)), permutations = 9999)

Seine	Df	SumOfSqs	R2	F	Pr(>F)
Season	2	0.0048688	0.28007	8.1223	0.0001
Location	11	0.0059218	0.34064	1.7962	0.0058
Residual	22	0.0065938	0.37930	*NA*	*NA*
Total	35	0.0173844	1.00000	*NA*	*NA*

Table 5. Pairwise comparisons of Simpson diversity using Wilcoxon rank test with continuity correction for 24 eDNA locations across four seasons.

data:eDNA_richness$Simpson and sample_data(eDNA_fish_data)$season

	Spring	Summer	Autumn
Summer	0.0033	-	-
Autumn	0.0094	0.1934	-
Winter	2.4e-06	0.0041	0.0001

P value adjustment method: holm

Table 6. PERMANOVA statistics for assessing seasonality and location as distinct groups within the data across 24 locations and four seasons.

adonis2(eDNA_fish_dist ~ season + location, data=data.frame(sample_data(eDNA_fish_data)))

	Df	SumOfSqs	R2	F	Pr(>F)
Season	**3**	**0.038088**	**0.23792**	**39.4515**	**0.001**
Location	**24**	**0.038328**	**0.23942**	**4.9625**	**0.001**
Residual	**260**	**0.083671**	**0.52266**	*NA*	*NA*
Total	**287**	**0.160087**	**1.00000**	*NA*	*NA*

(a) eDNA vs. Seine (b) eDNA

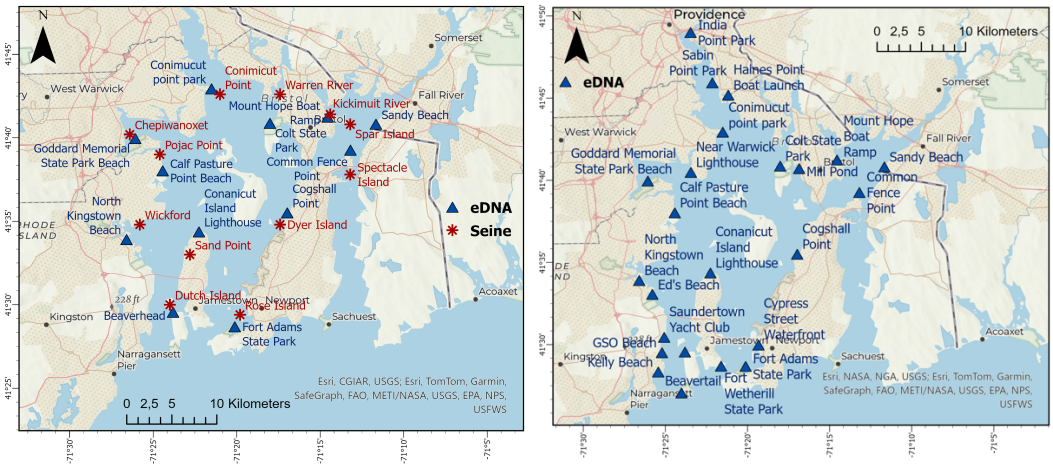


Figure 1. Sample locations. (a) Locations for eDNA and seine surveying comparison. Red symbols represent seine survey locations while blue represents eDNA sampling sites. (b) The map on the right shows our expanded eDNA sampling scheme of 12 additional locations along with the 12 original locations.

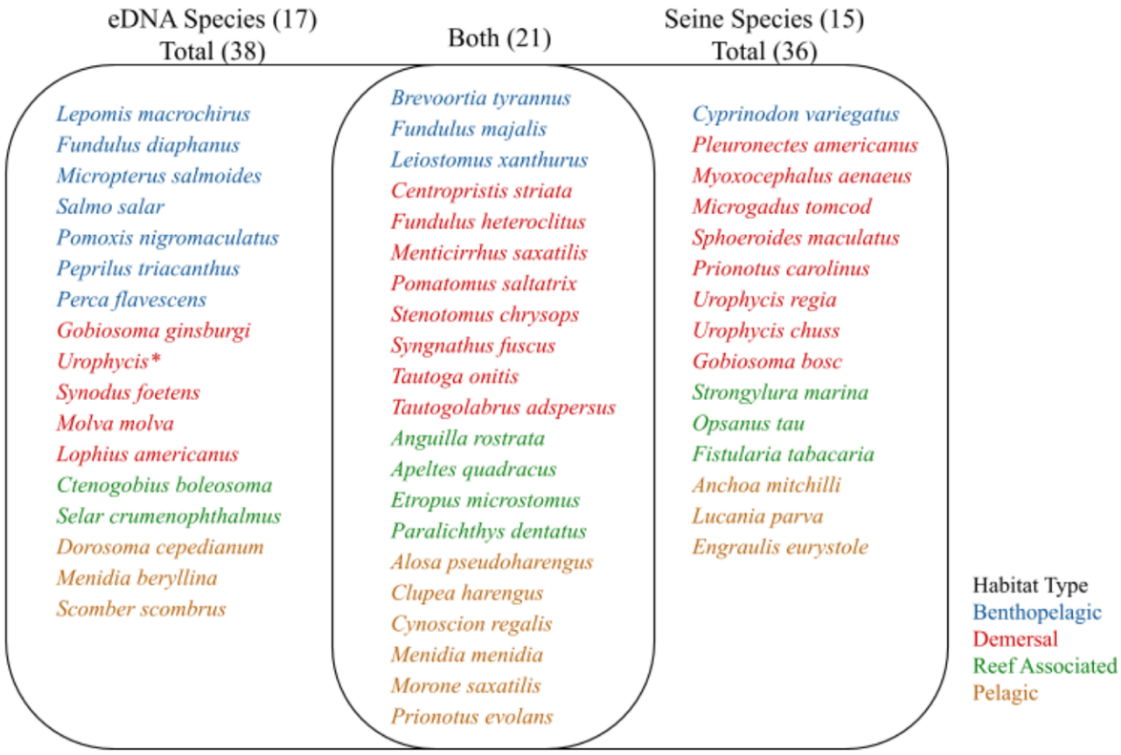


Figure 2. Venn diagram of the species identified by each method. Species detected only with eDNA are on the left, species collected only using the seines are on the right, and shared species are in the middle. Each color represents a different habitat type.

*Sequence data unable to distinguish to the species level, closely related species assigned to the same genus

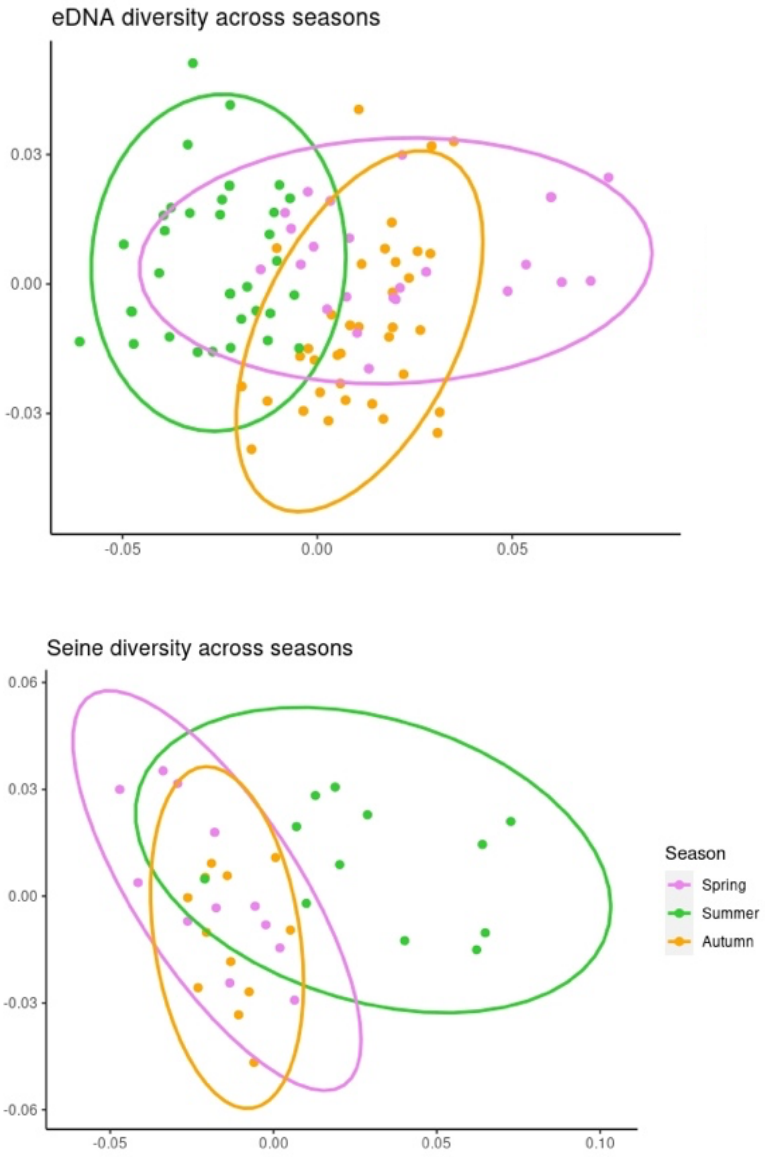
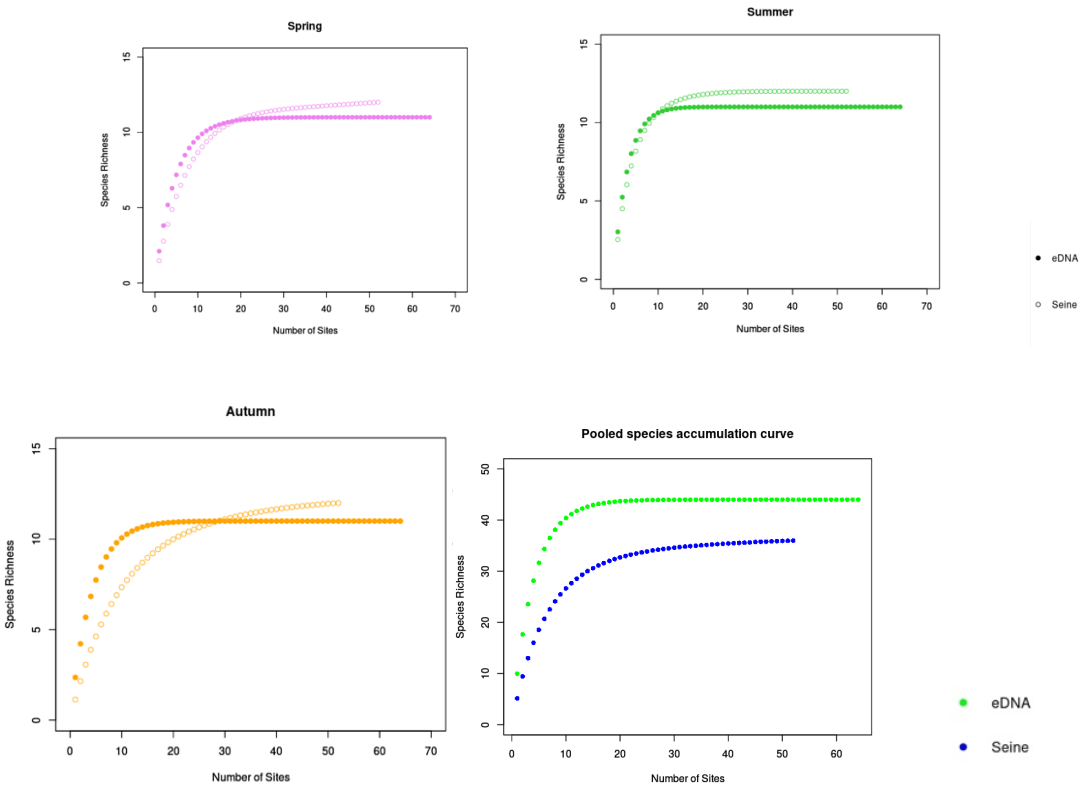


Figure 3. Two dimensional NMDS plot of specific composition across seasons with 95% confidence ellipses by method. The three sampling seasons were spring, summer, and autumn.

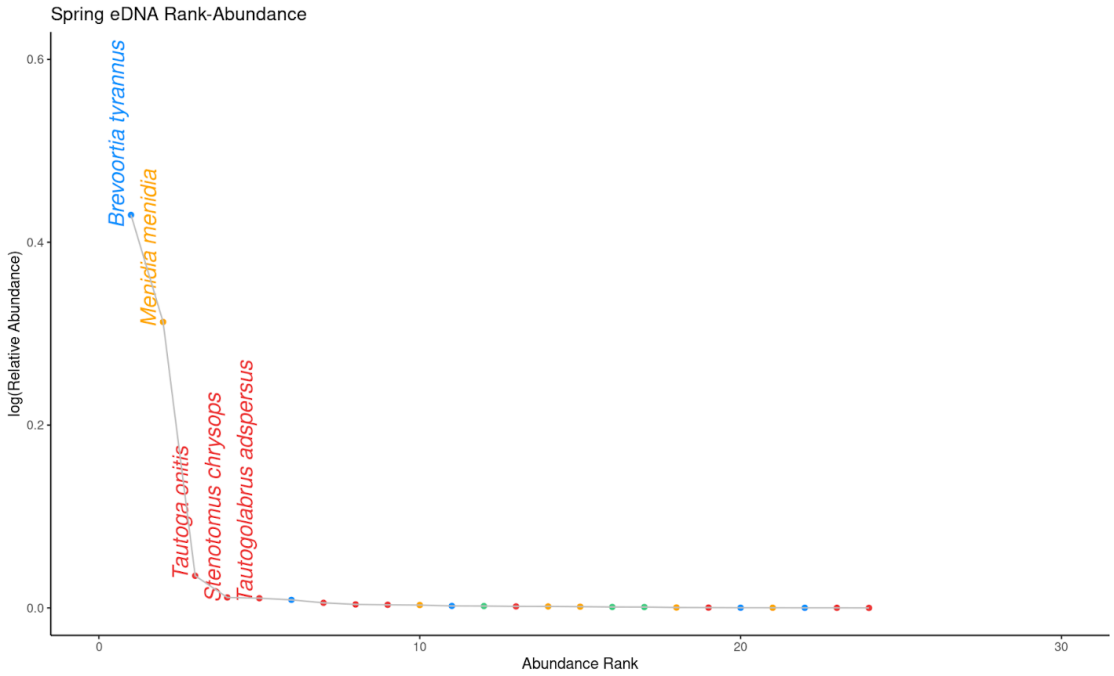
(a) (b)



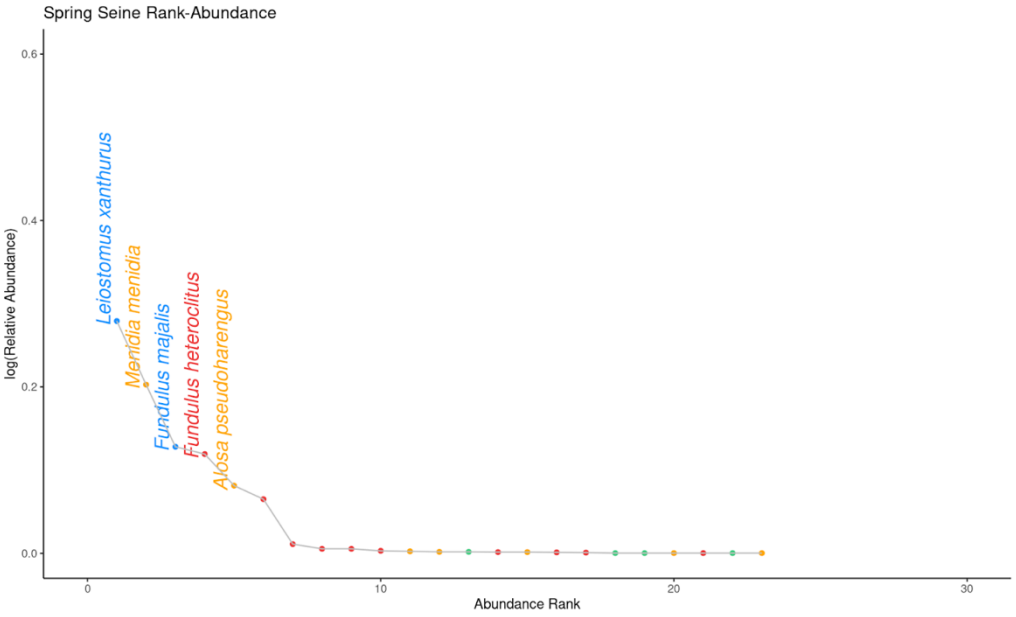
(c) (d)

Figure 4. Species accumulation curves for each season of data separated by method (a, b, c). eDNA samples are represented with solid points while seine samples are the open points. Species accumulation curve of pooled data for all seasons separated by sampling method (d). eDNA data is in green and seine data is in blue. The relationship between species richness and number of sites sampled indicates that 10 samples sites is adequate to reach expected species richness across methods.

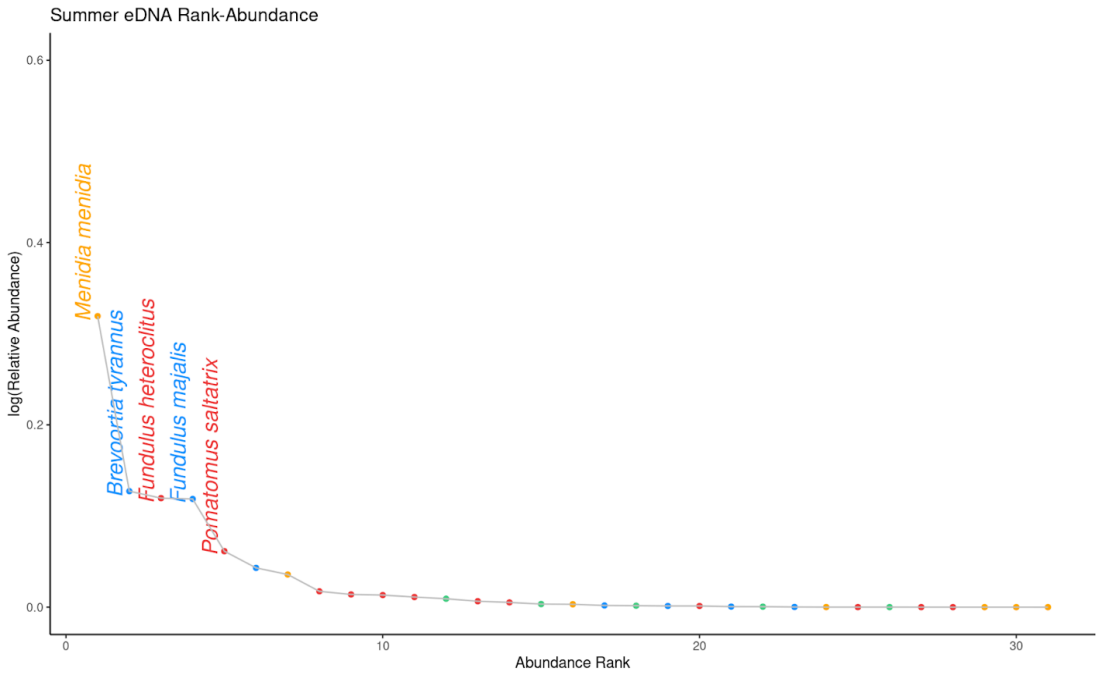
(a)


Spring eDNA Rank-Abundance
log(Relative Abundance)
0.6
0.4
0.2
0.0
Brevoortia tyrannus
Menidia menidia
Tautoga onitis
Stenotomus chrysops
Tautogolabrus adspersus
0
10
20
30
Abundance Rank

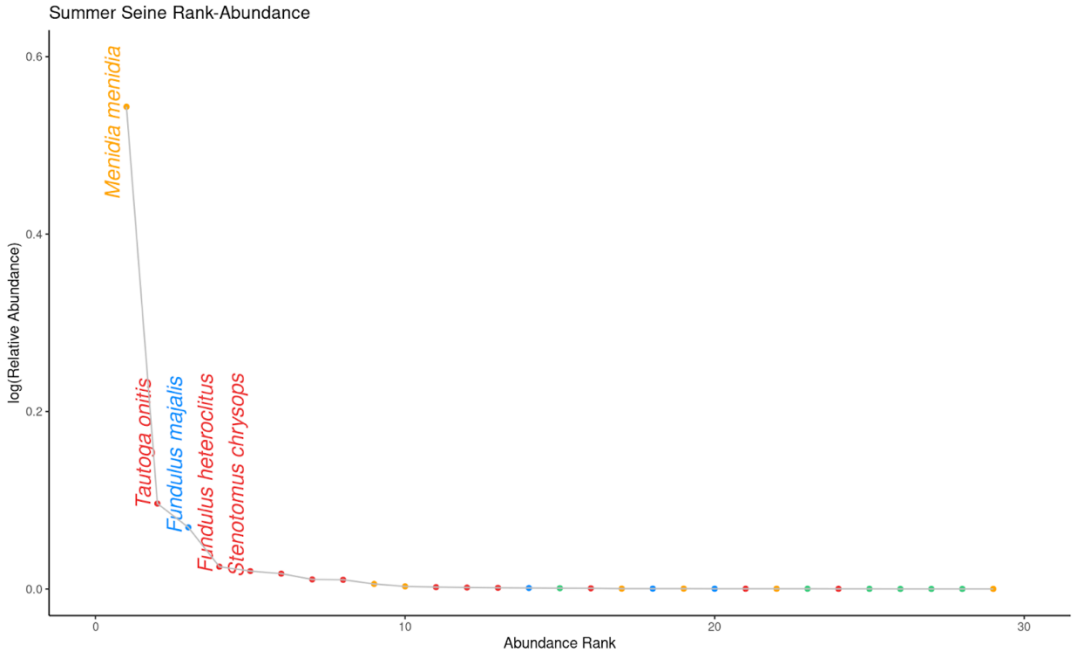
(b)


Spring Seine Rank-Abundance
log(Relative Abundance)
0.6
0.4
0.2
0.0
Leiostomus xanthurus
Menidia menidia
Fundulus majalis
Fundulus heteroclitus
Alosa pseudoharengus
0
10
20
30
Abundance Rank

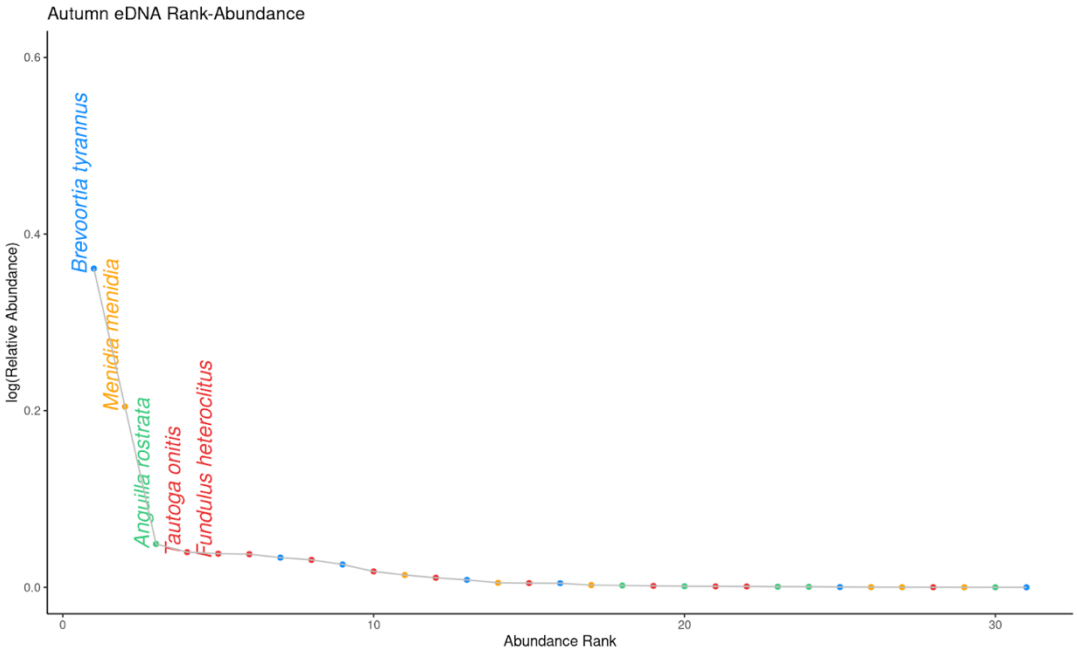
(c)


Summer eDNA Rank-Abundance
log(Relative Abundance)
0.6
0.4
0.2
0.0
Menidia menidia
Brevoortia tyrannus
Fundulus heteroclitus
Fundulus majalis
Pomatomus saltatrix
0
10
20
30
Abundance Rank

(d)


Summer Seine Rank-Abundance
log(Relative Abundance)
0.6
0.4
0.2
0.0
Menidia menidia
Tautoga onitis
Fundulus majalis
Fundulus heteroclitus
Stenotomus chrysops
0
10
20
30
Abundance Rank

(e)



(f)

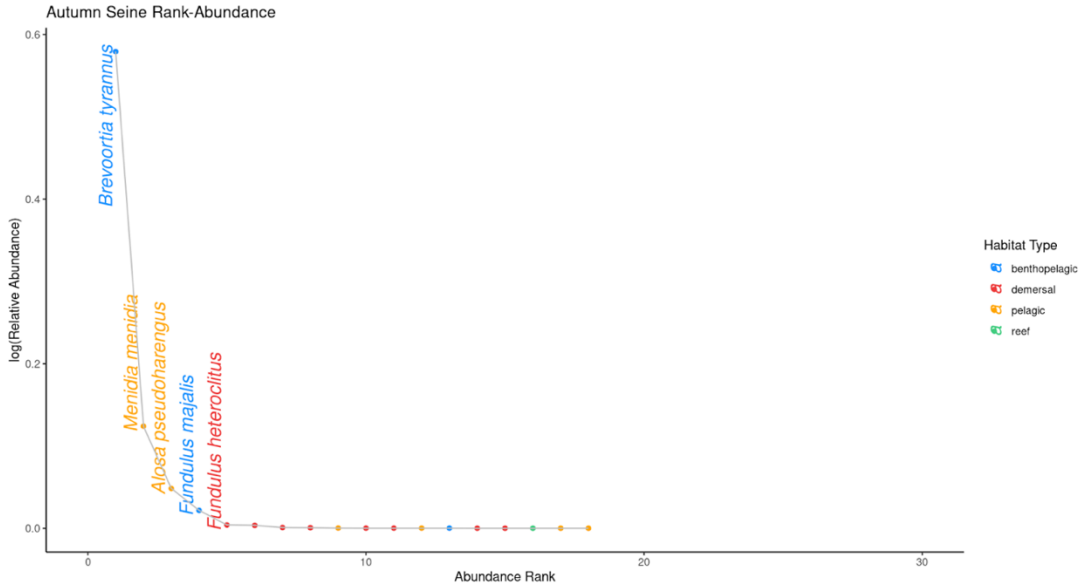


Figure 5. Rank abundance curves for each season, showing relative abundance and species composition across seasons and methods. Each color represents a different habitat type.

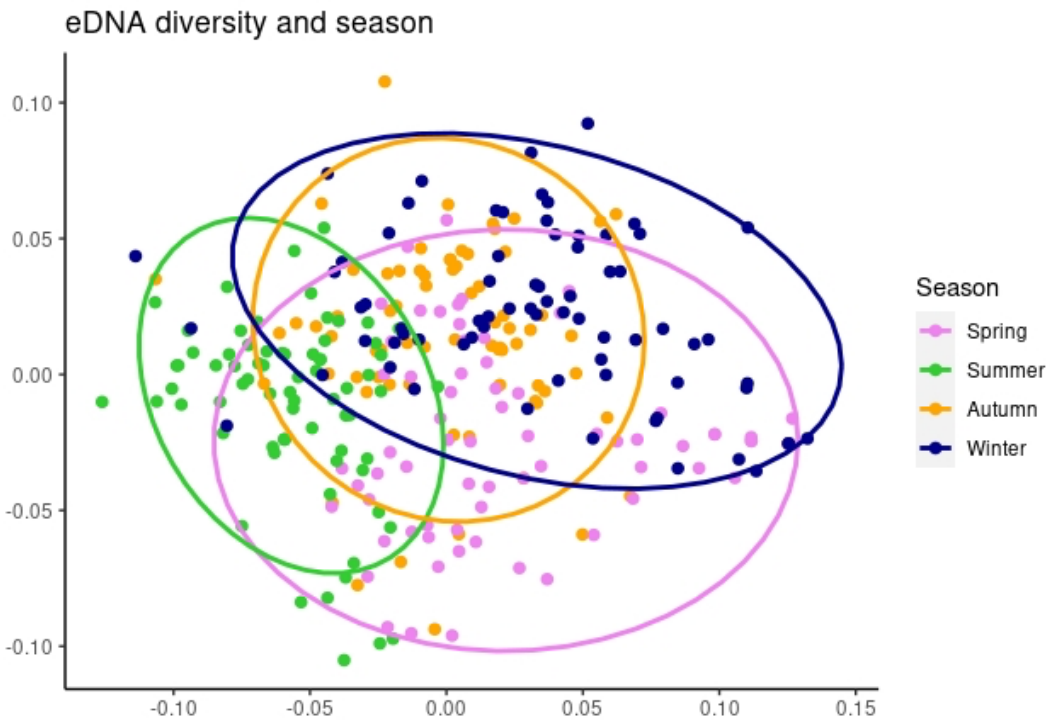
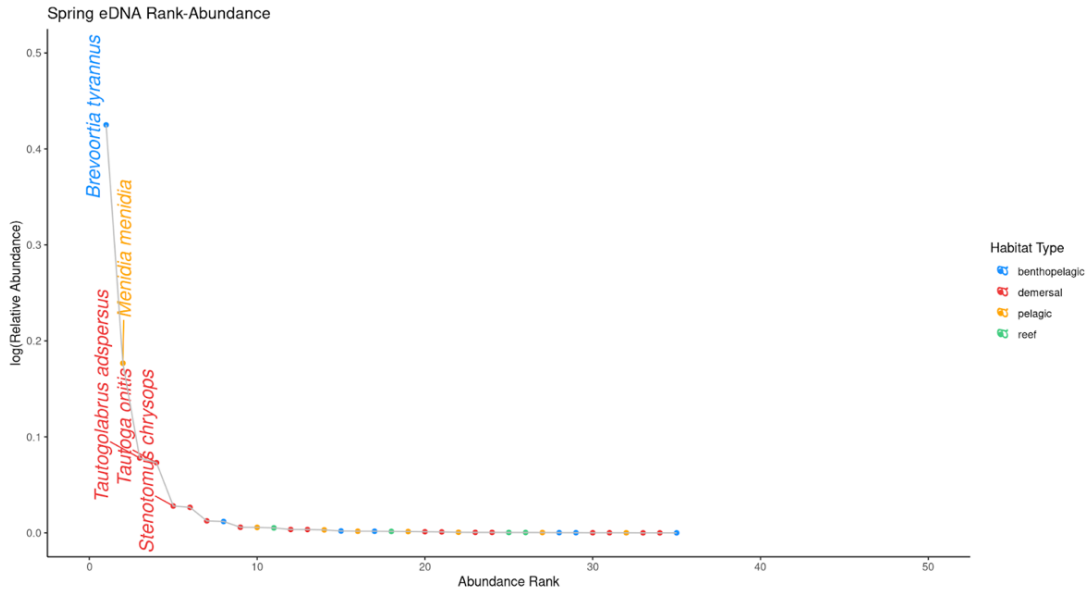
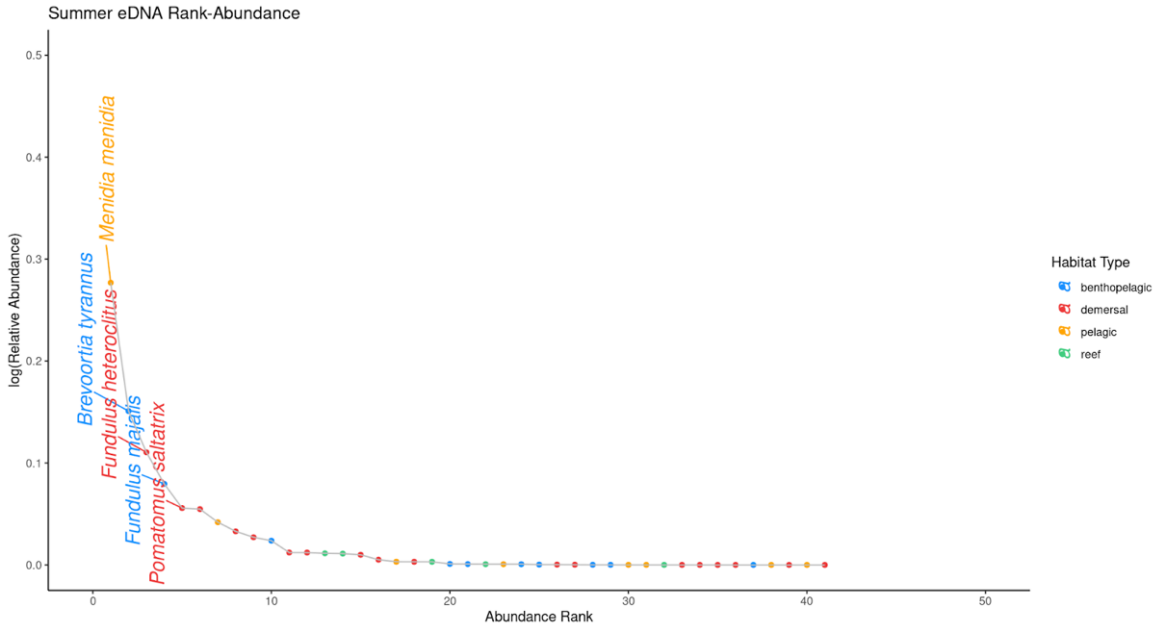


Figure 6. Two dimensional NMDS plot of specific composition across seasons with 95% confidence ellipses. Samples are plotted in a two-dimensional space to represent the relationship between sample diversity and season. Sampling seasons include spring, summer, autumn and winter.

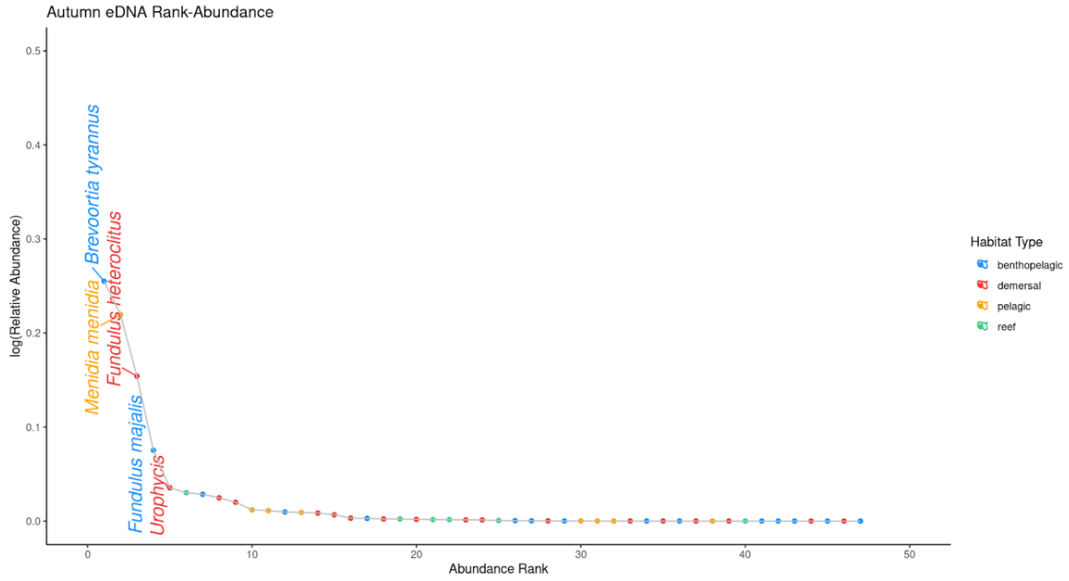
(a)


Spring eDNA Rank-Abundance
log(Relative Abundance)
Abundance Rank
Brevoortia tyrannus
Menidia menidia
Tautogolabrus adspersus
Tautoga onitis
Stenotomus chrysops
Habitat Type
benthopelagic
demersal
pelagic
reef

(b)


Summer eDNA Rank-Abundance
log(Relative Abundance)
Abundance Rank
Brevoortia tyrannus
Menidia menidia
Fundulus heteroclitus
Fundulus majalis
Pomatomus saltatrix
Habitat Type
benthopelagic
demersal
pelagic
reef

(c)



(d)

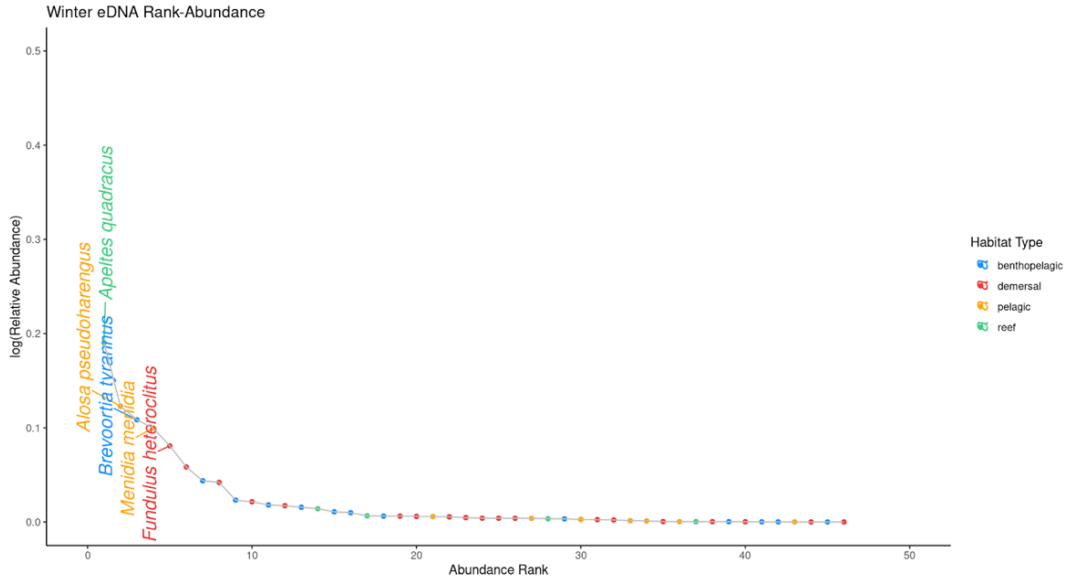


Figure 7. Rank abundance curve for each season, depicting the relative abundance of each species present in eDNA data. Each color represents a different habitat type.

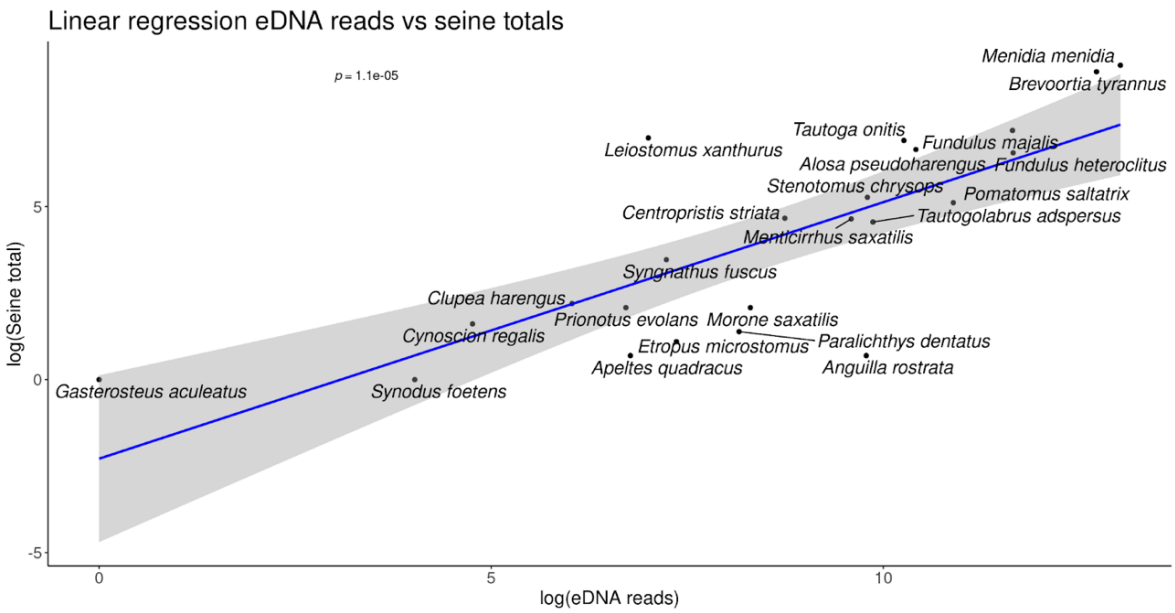


Figure 8. The number of fish captured using seine methods is related to the number of sequence reads identified using eDNA. Points are defined by one species total across all sampling locations for eDNA plotted with the species total across all sampling locations for seine surveying.

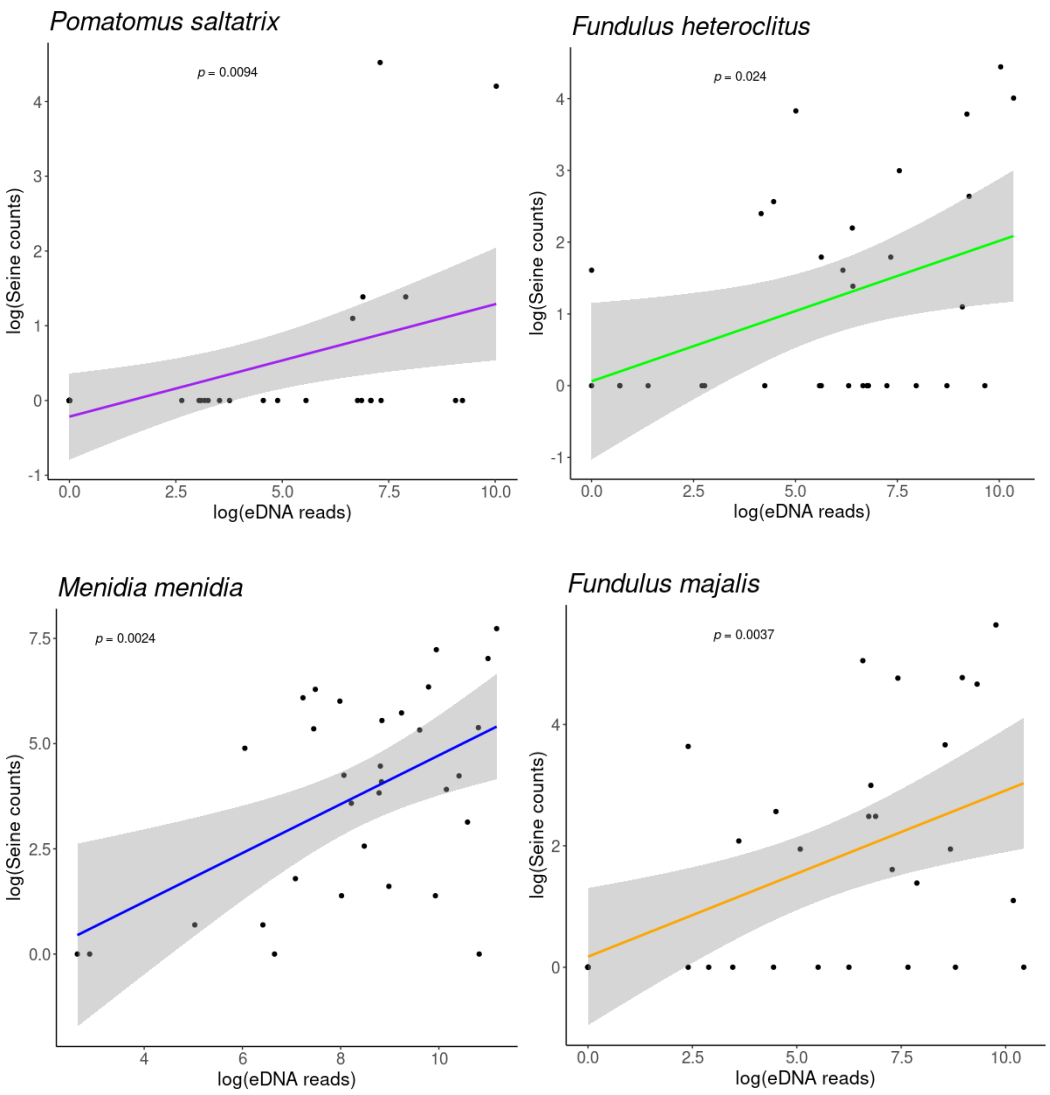


Figure 9. Linear regression of individual species with high abundance, showing the relationship between eDNA reads and seine fish count totals. Each point represents the number of eDNA reads and seine fish counts at each sample location.

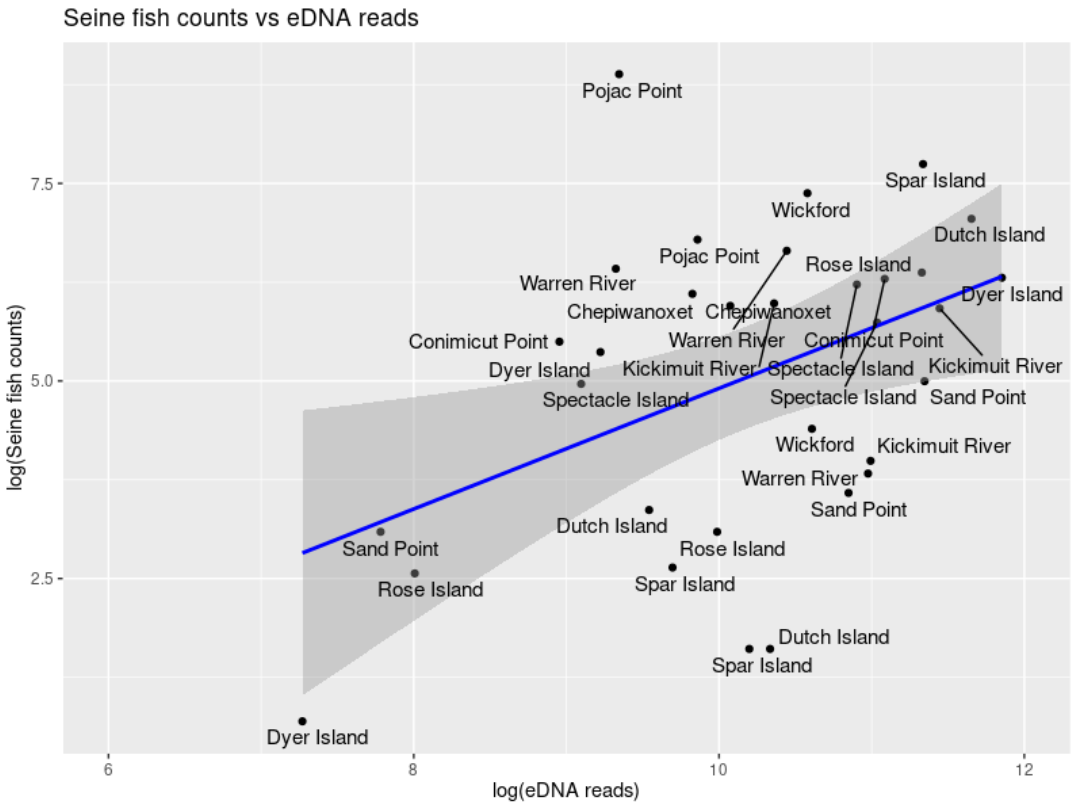


Figure 10. Linear regression showing the relationship between eDNA reads and seine fish count totals by location. Each point represents the total number of sequences (eDNA)/fish (seine) recorded at each sampling location. There is one point for each sampling location per season.

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SUPPLIMENTAL MATERIALS

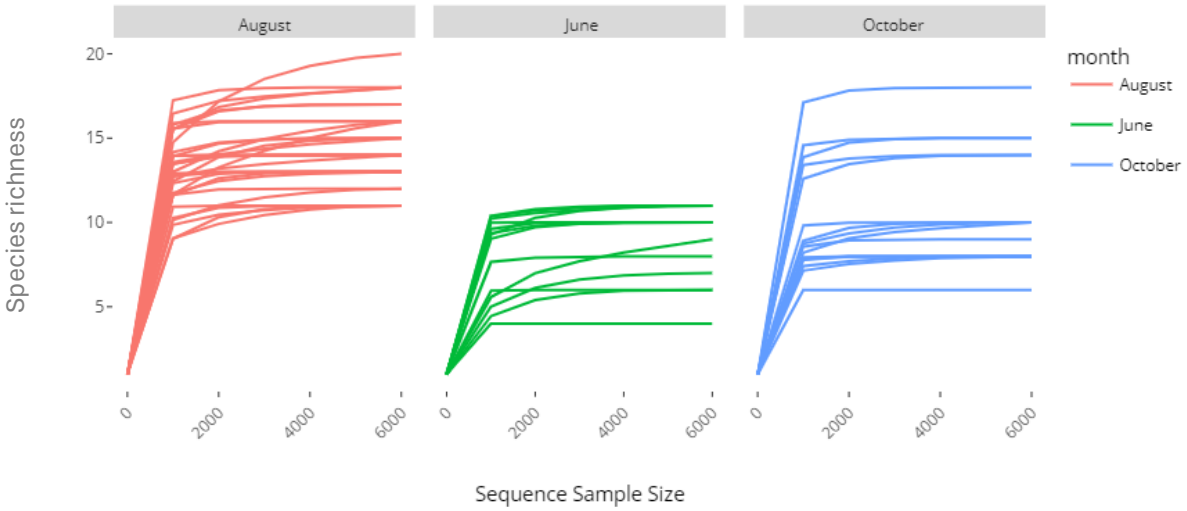

Figure S1. The relationship between species richness and sequencing depth for eDNA data. It represents the number of sequences that is expected per sample to properly assess sample diversity. Each line represents an individual sample location and the number of sequences needed to predict the diversity of that sample. The number of sequences required to reach expected species richness is where the curve levels out.

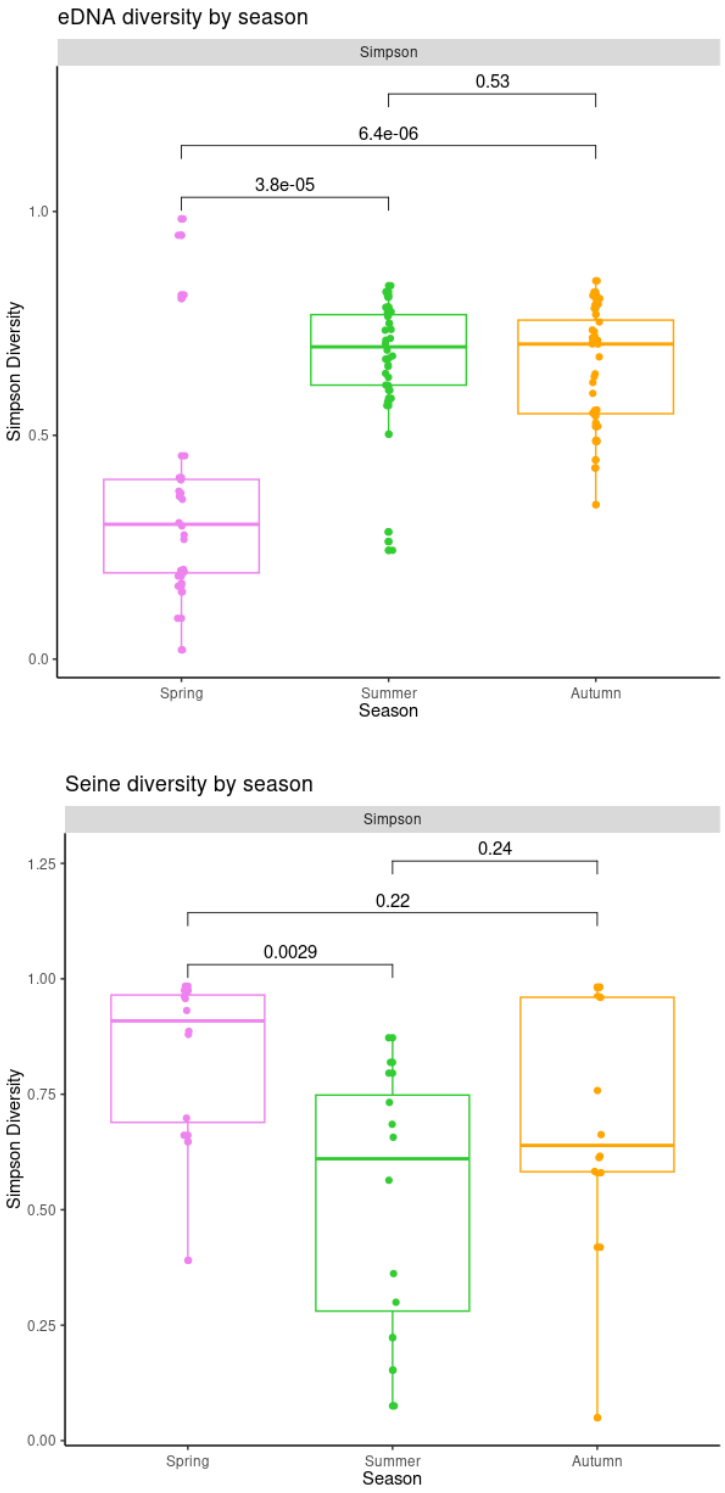

Figure S2. Simpson diversity of species across seasons and methods.

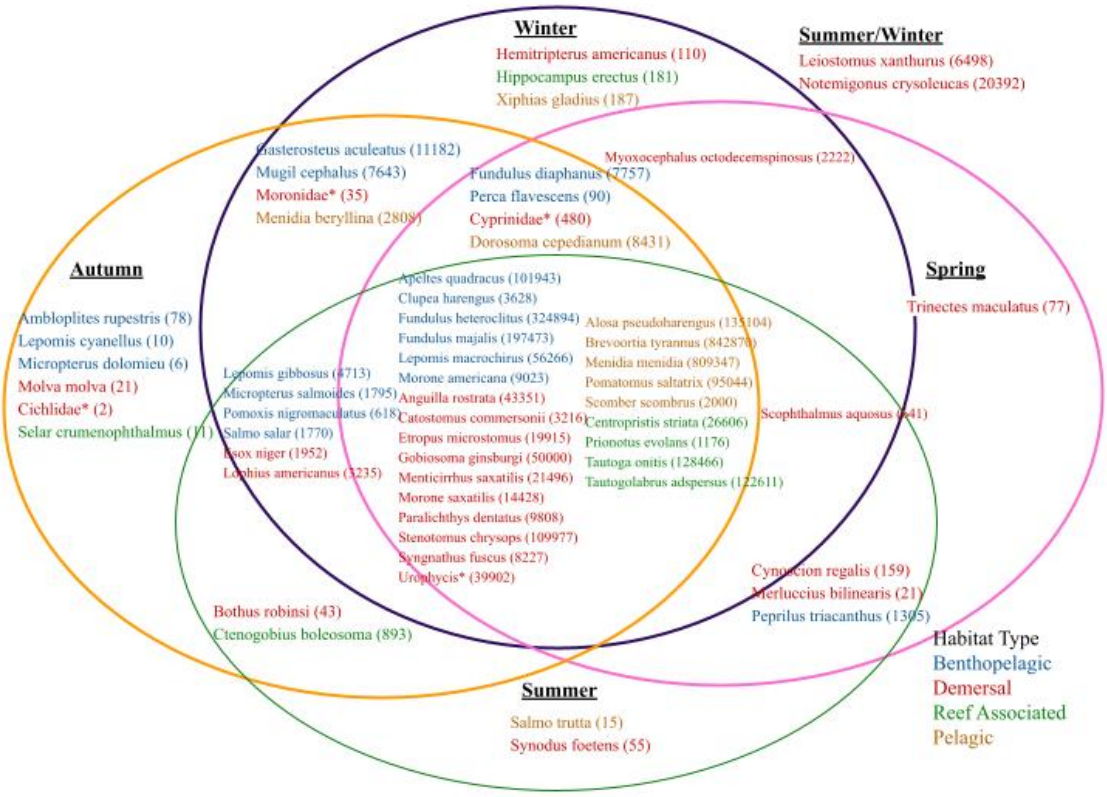

Figure S3. Venn diagram showing the species overlap found in Narragansett Bay during each season. Each color represents a different habitat type. The number of sequences assigned to each species is shown in parentheses. *Limited data unable to distinguish sequences down to the species level

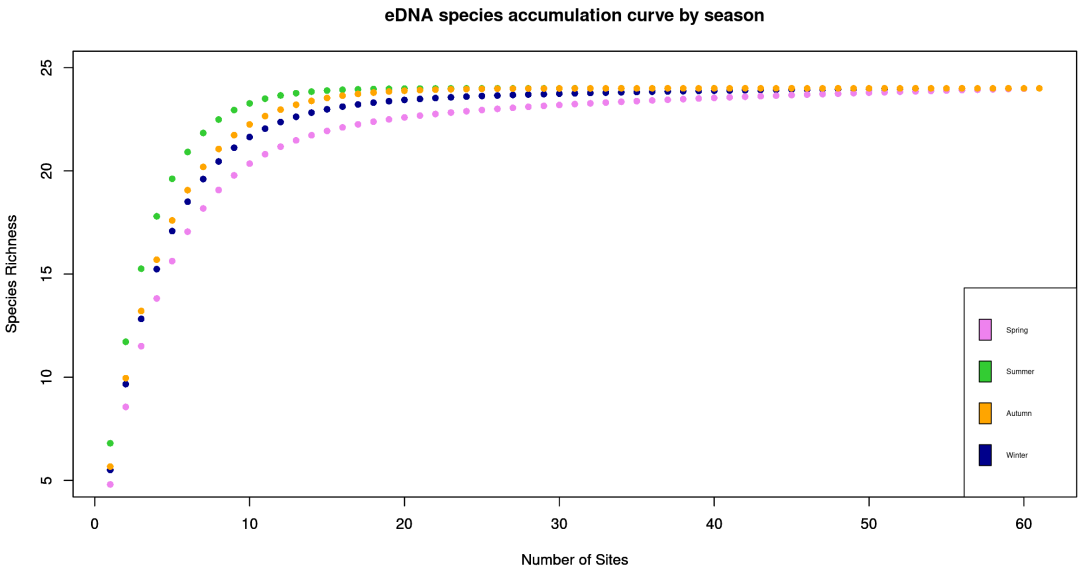

Figure S4. Species accumulation curves for each season including 24 locations and four seasons of eDNA data.

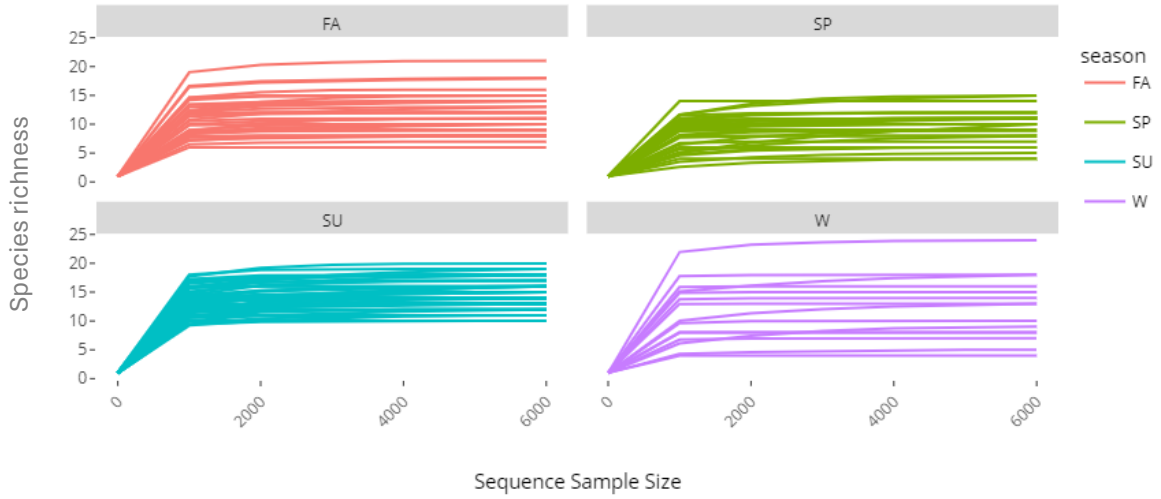

Figure S5. The relationship between species richness and sequencing depth. It represents the number of sequences that is expected per sample to properly assess sample diversity. Each line represents an individual sample location and the number of sequences needed to predict the diversity of that sample. The number of sequences required to reach expected species richness is where the curve levels out.

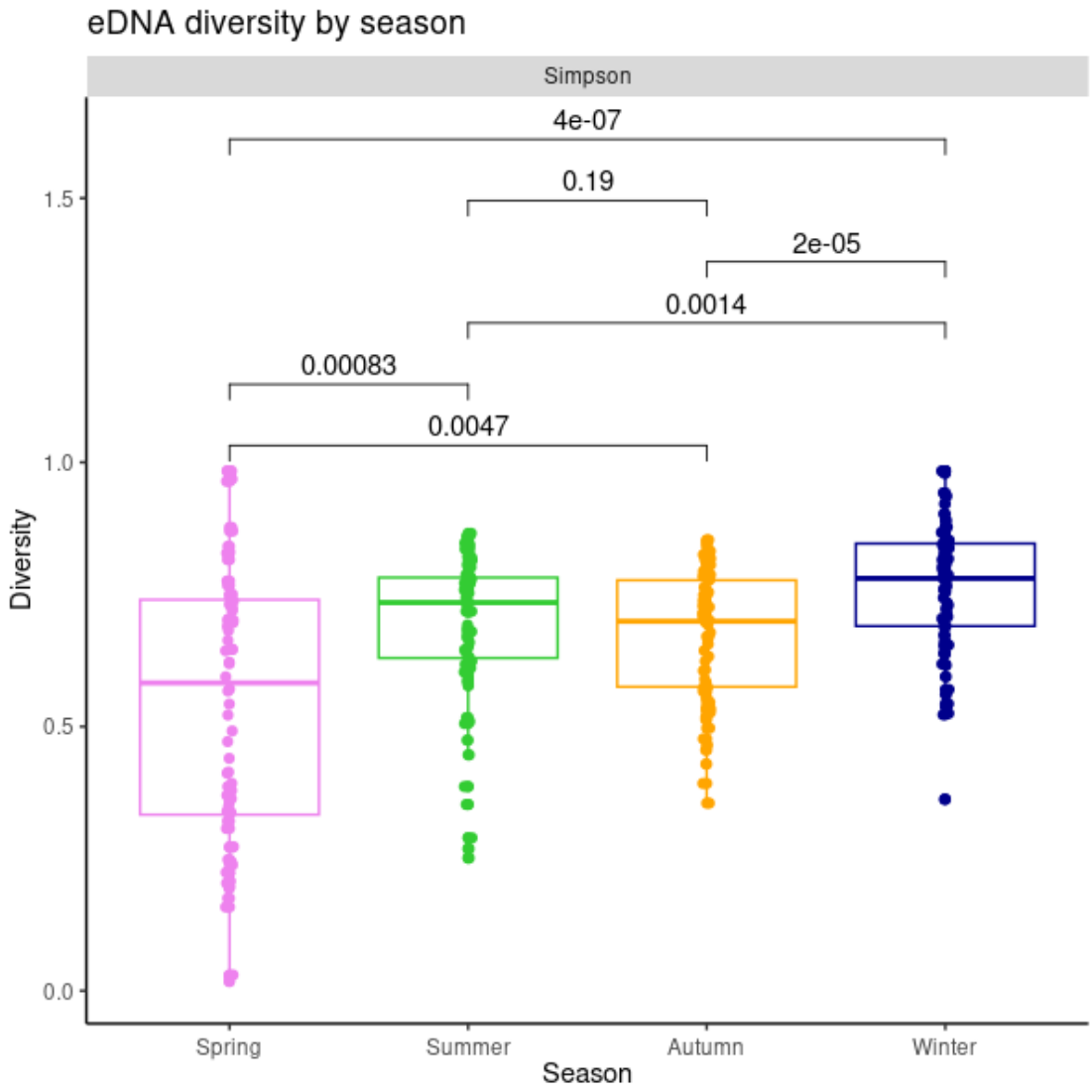

Figure S6. Seasonal variation of fish diversity (Simpson) including 24 locations and four seasons of eDNA data.