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Integrating eDNA into National Estuarine Monitoring: Successes, Challenges, and Trends

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Abstract

Environmental DNA (eDNA) metabarcoding has strong potential for biodiversity monitoring, but its integration into long-term standardized monitoring networks remains limited. We evaluated the feasibility, value, and challenges of incorporating eDNA into the National Estuarine Research Reserve System System-Wide Monitoring Program, a coordinated estuarine monitoring network spanning the United States. More than 1,300 water samples were collected over a two-year period and analyzed using a 12S rRNA gene targeting fishes and 18S rRNA gene targeting broad eukaryotic communities. Integrating eDNA analyses into an existing water monitoring program supported broad ecosystem assessments and insights into fish assemblages and plankton communities, and supported initial linkages between key species and water quality parameters. The study also identified important operational and interpretive challenges, including costs, uncertain species detections, and the difficulty of balancing methodological standardization with rapidly evolving eDNA protocols. Despite these limitations, integrating eDNA into standardized estuarine monitoring substantially expanded biodiversity characterization while demonstrating that existing national estuarine monitoring infrastructure can support scalable integration of biodiversity assessments across geographically distributed estuaries.

Introduction

Estuaries are dynamic ecosystems that provide critical habitat for a diverse range of species, acting as nurseries for many marine organisms and serving as vital transition zones between freshwater and marine environments (Elliott and Whitfield 2011, Costanza et al. 2017). Biodiversity within estuarine systems promotes ecosystem resilience, fisheries productivity, and water quality regulation (Barbier et al. 2011, Silliman et al. 2019). Long-term water quality monitoring and tracking of biodiversity changes in estuaries are needed to understand anthropogenic and climate-induced stressors that may lead to shifts in species' geographic ranges, phenology, and population sizes, allowing for timely management interventions and conservation efforts (Cloern et al. 2016). Environmental DNA (eDNA) metabarcoding is increasingly used for biodiversity assessment (Ruppert et al. 2019, Gilbey et al. 2021), however, eDNA is still rarely incorporated into long-term standardized comprehensive monitoring programs, particularly at national scales.

The National Estuarine Research Reserve System (NERRS), a network of 30 sites across the United States that supports research, education, training, management, and stewardship, represents a unique opportunity for long-term eDNA-based assessments. All of the sites participate in the System-Wide Monitoring Program (SWMP), a comprehensive program that collects consistent, quality-controlled, high-frequency environmental data for biogeochemical parameters, habitat conditions, and algae. Long-term data sets produced by SWMP have proven valuable for investigating biogeochemical processes at landscape and national scales (e.g. Dix et al. 2013, Baumann and Smith 2018, Jeppesen et al. 2018), but the current SWMP program includes limited biological monitoring; fish and other ecological communities are assessed at many of the sites, but the methods and target species are not always consistent. Because SWMP already provides standardized sampling, metadata collection, long-term environmental

records, and geographically distributed personnel, the NERRS network provides a strong platform for evaluating the implementation of eDNA-based biological monitoring at national scales.

In this study, we added eDNA collection to the existing water quality sampling conducted at 10 NERRs (Fig. 1). By coupling eDNA metabarcoding results with traditional metrics such as species counts, algal bloom composition, and water quality data, we can evaluate the operational and ecological value of coupling eDNA with an existing, multi-site, standardized water quality monitoring program at a national scale.

The objective of this work was to evaluate both the value and the challenges associated with integrating eDNA into an existing multi-site monitoring program. Specifically, we assessed the feasibility of integrating eDNA into SWMP across multiple NERRs, characterized spatial and seasonal biodiversity patterns, compared eDNA-based fish detections with traditional surveys, and identified methodological challenges associated with standardized implementation.

Methods

An overview of our methods is presented here, with more detailed information included in the supplementary materials. The 10 participating NERRs in this study were distributed across the United States, from the Atlantic, Gulf of Mexico, and Pacific coasts and islands (Fig. 1). NERRs were selected to represent a range of geographical, ecological, and anthropogenic conditions. eDNA samples were collected by Reserve staff quarterly, and sent to the University of New Hampshire for laboratory processing and analysis. Our goal was to incorporate eDNA collection as an additional method set in the existing program, and thus samples for eDNA were collected at previously established SWMP sites.

Sampling approach. Samples were collected quarterly (May, August, November and February) over two years (May 2022 to February 2024) to capture seasonal and annual variation. Each Reserve collected triplicate water samples and one control (1L each) at 4–5 of their existing SWMP sampling locations, resulting in over 1,300 total water samples. Water samples were filtered, processed, and amplified using a 12S rRNA gene primer designed to detect fish species (Miya et al. 2015), and an 18S rRNA gene primer for eukaryotes (Amaral-Zettler et al. 2009). 18S data were only available for the second year, May 2023 - February 2024.

Laboratory analyses and sequencing. DNA extraction and handling

Filters collected in year 1 were extracted on a QIAcube Connect system (QIAGEN®, Hilden, Germany). In the second year we moved to a bead-based extraction conducted on a KingFisher Flex System (Thermo Fisher, Waltham, MA). MiFish PCR protocols were changed after year 1 review indicated underrepresentation of species in comparison to expected fish community, particularly at sites with high turbidity. In response we updated our PCR protocols to enhance fish detection. Details and processes are discussed in El Baidouri et al. (2025), and summarized in the methods section. Fig. S1 shows the

change in species with the updated protocol. Changing protocols mid-way through the project enhanced detection, but complicates inter-year comparisons, as elaborated in the discussion section.

18S rRNA gene/Eukaryotic PCR Amplification - For each sample the PCR reaction was prepared using Kapa Master Mix, nuclease-free water, and the 18S primers with Nextera adapters (Table 1), followed by a Zymo clean step. See supplementary materials for the PCR program.

MiFish PCR Amplification. Our initial protocol used KAPA HiFi HotStart (KAPA) and a touchdown thermal profile modified from Pitz et al (2020). In year 2, the PCR reaction was modified to use Platinum SuperFi II Master Mix, and an equimolar primer mix of MiFish-U and MiFish-E. See supplementary materials or El Baidouri et al. (2025) for detailed protocol.

Primer selection. In addition to the 18S-Euk primers, the MiFish-U and MiFish-E primers were used to amplify bony fish and elasmobranch species (sharks and rays). Both types are important in estuarine systems, and multiplexing these primers expands the list of potential target species. Table 1 shows the primer sequences used in this study.

Table 1
Primer sequences

MiFish-MIX-F (forward)
MiFish-U-F: 5'- GTCGGTAAAACTCGTGCCAGC-3'
MiFish-E-F: 5'- GTTGGTAAATCTCGTGCCAGC-3'
MiFish-MIX-R (reverse)
MiFish-U-R: 5'-CATAGTGGGGTATCTAATCCCAGTTTG-3'
MiFish-E-R: 5'-CATAGTGGGGTATCTAATCCTAGTTTG-3'
18S Primers
18s-Euk_1391f: 5'-GTACACACCGCCCGTC-3'
18s-EukBr: 5'-TGATCCTTCTGCAGGTTCACCTAC-3'

Library preparation and Sequencing. PCR products were submitted to the University of New Hampshire Hubbard Center for Genome Studies for library preparation and sequencing. Most of the samples were run on an Illumina NovaSeq 6000 instrument, with a subset of the May 2023 samples run on an Illumina iSeq. Briefly, the amplicon products from the 1st PCR were indexed using dual-index barcodes and Illumina sequencing adapters (P5 and P7). The amplified libraries were purified using AMPure XP beads (Beckman Coulter) to selectively remove short fragments and primer dimers.

Sequence Processing and Taxonomic Assignment. Fastp v 0.23.0 was used to trim the poly-g tails and low-quality sequences (mean quality < 25, window size 2 bp from the raw, demultiplexed fastq files (Chen et al. 2018). The demultiplexed fastqs were then imported to QIIME 2 (QIIME 2-2022.8) for adapter

trimming, denoising, and taxonomic classification (Bolyen et al. 2018). The cutadapt plugin in QIIME 2 was used to trim primer sequences and filter out read-pairs that do not contain the priming sequences (Martin 2011). Read-pairs were retained if the primer sequences matched the allowed error rate of 0.1. Retained read-pairs were then denoised, merged, and checked for chimeras with the DADA2 QIIME 2 plugin (Callahan et al. 2016). Forward and reverse reads were truncated to have at least 12 base-pairs (QIIME 2 default) of overlap to merge the forward and reverse reads. To reduce the number of false positive unique amplicon sequence variants (ASVs), we used the 'pseudo' pooling method where samples are initially denoised independently, and then denoised a second time with prior knowledge of the ASVs that are found in at least two samples in the initial denoising step. We classified ASVs using two complementary approaches in QIIME 2: the classify-consensus-vsearch classifier and the scikit-learn naïve Bayes classifier. For 12S sequences, taxonomic assignment was performed against MitoFish, a dereplicated reference database of fish sequences (Lim and Thompson 2021). For 18S sequences, we used the SILVA database (Pruesse et al. 2007) alongside a CRUX-generated reference database from CALeDNA (Curd et al. 2019).

Fish species assignment. The initial species lists generated through the bioinformatics pipeline were reviewed using data in FishBase to identify species unlikely to be present at the collection site. Local resource managers then reviewed species lists and flagged unlikely or misnamed species. The final species lists (Table S1) include the following flags: 1 = Sequence has 100% match, species likely to be present. 2 = Sequence matches multiple species likely to be present; In this case we are not confident we can distinguish between species, so roll back to the genus. 3 = Matches more than one species, but only one likely to be present (e.g matches both Pacific and Atlantic Herring, but only Pacific species likely) and 4 = Species unlikely to be present, no other likely matches.

Water Quality Monitoring. Water quality parameters are measured at each collection event as part of the NERRs SWMP program. Parameters include temperature (ºC), salinity (psu), dissolved oxygen (mg/L), pH, turbidity (FNU), nutrients (phosphorus and nitrogen), chlorophyll a, and weather data. All parameters associated with this sample set are included as metadata in Table S3. SWMP data and details of the methods used are publicly available through the National Estuarine Research Reserve System Centralized Data Management Office (NOAA 2019).

Results

Fish Community results. 297 distinct fish species were detected, with an additional 20 taxa not resolved to the species level. Fish species detected were consistent with expected fish communities at each site, with regional and seasonal variability. Curated fish species lists are included in Tables S1 in the supplementary materials, and the full list of fish ASVs including replicates and blanks can be found in S2. The number of species detected per NERR ranged from 41 (MA) to 87 (GTM). Regional differences in species composition are strongly expressed. The Northeast region (WE, GB, JC) is dominated by estuarine and nearshore Atlantic taxa, including mummichog (*Fundulus heteroclitus*), American eel (*Anguilla rostrata*), and Atlantic menhaden (*Brevoortia tyrannus*). The Southern sites (AB, MA, GTM) are

characterized by species typical of warm, subtropical estuaries, such as mullet (*Mugil sp.*), anchovies (Fam. *Engraulidae*), and croakers (Fam. Sciaenidae). The Pacific mainland sites (SF, SS, PB) are dominated by Pacific taxa, including three-spined stickleback *(Gasterosteus aculeatus*), Pacific herring (*Clupea pallasii*), and Pacific staghorn sculpin (*Leptocottus armatus*), while the Pacific island site (HE) is generally dominated by introduced freshwater catfish (Fam. *Loricariidae*), guppies (Fam. *Poeciliidae*) and gobies (Fam. *Gobiidae*). A Principal Coordinates Analysis (PCoA) dissimilarities analysis shows that fish communities are generally grouped by region, with Hawai□i sharing some species with the other Pacific sites and the southeast and Gulf sites (Fig. 2).

Comparison to traditional sampling methods. Many, but not all, of the participating sites have some form of ongoing fish survey data collected by state or federal agencies in the region, or by NERRs staff, but these surveys do not necessarily align with the eDNA sampling sites. Apalachicola Bay is one of the few NERRs that conducts traditional fish surveys (trawl) near the SWMP sampling locations. A comparison between the eDNA and trawl data shows that both methods, trawl and eDNA, detected a subset of the species expected to be present during the project period (March 2022-Feb 2024). There was relatively low overlap between the results of the two methods, and neither fully captured all of the species likely to be present (Fig. 3). The long-term trawl data set (described in the supplementary materials) for the region spans more than 25 years and contains over 300 species.

The number of species detected increased with sampling events and replicates. The number of samples collected in each estuary ranged from 72 to 126, depending on the number of locations sampled, and whether sampling was conducted in the winter. The number of species detected increased at each location, and did not reach saturation, indicating that more samples, or a longer sampling period, would be needed to fully characterize the local fish community (Fig. 4a). The number of species detected in each sample ranged from 0–28. Species count increased with replication, which is consistent with many studies of this type (Ficetola et al. 2015, Stauffer et al. 2021)(Fig. 4b). However, sample filtering was very time consuming (required 1–4 additional hours) per event, and future work should consider the tradeoffs between effort and value.

Eukaryote species distribution. 18S rRNA gene analysis expanded the scope of the monitoring program beyond fish community assessment by capturing broad eukaryotic biodiversity, including phytoplankton, protists, fungi, plants, and vertebrates. More than 3,000 eukaryotic taxa representing 44 phyla were detected across all sites (Fig. 5). The most commonly detected groups included Bacillariophyta, Ciliophora, and Dinoflagellata, although community composition varied substantially among regions and seasons. Colponemidia and Zoopagomycota, which are relatively rare in marine environments (Tikhonenkov et al. 2020, Thompson et al. 2025) were detected in only a few samples.

These data can be used to understand key biological conditions, focused on the specific management needs for each estuary. Here we use these results to illustrate how integrated eDNA monitoring can support ecosystem-scale assessment by linking biodiversity patterns with environmental gradients and water quality conditions across geographically distributed estuaries.

Regional and environmental structuring of eukaryotic communities. Overall, 18S-derived communities were less strongly associated with individual estuaries than fish communities detected using 12S metabarcoding. Many taxa identified through the 18S analysis are planktonic or microbial organisms that may be broadly distributed and transported across larger water masses. However, community composition did exhibit large-scale environmental structuring. Unweighted UniFrac analyses showed a strong north-south separation among sites (PERMANOVA, $p = 0.005$), and correlation between community structure and salinity (PERMANOVA, $p = 0.002$) (Fig. 6). Salinity across sites ranged from 0.1 to 36 psu, reflecting the broad environmental gradient encompassed by the participating estuaries. In contrast, dissolved oxygen, temperature, turbidity, and pH were not strongly associated with community structure at the national scale.

Detection of management-relevant taxa. One advantage of broad eukaryotic metabarcoding is the ability to simultaneously monitor multiple species and groups of management interest. Managers participating in the project identified harmful algal blooms as a priority monitoring target. Several potentially harmful genera, including *Alexandrium*, *Dinophysis*, and *Pseudo-nitzschia*, were detected across multiple estuaries. A comparison of HAB species detected through eDNA and concurrent traditional plankton tows at GTM NERR found that eDNA detected a greater number of genera (eDNA: 17, microscopy: 6) and species (eDNA: 18, microscopy: 5) (Fig S2). By correlating the eDNA results to SWMP data we can start to identify the relationship between water quality and potentially toxic blooms. Figure 7 shows *Pseudo-nitzschia* taxa detected in this study and the average salinity measured at the time of sample collection. The nine species detected were generally associated with higher salinity conditions. Because many *Pseudo-nitzschia* species are morphologically difficult to distinguish using traditional microscopy, eDNA approaches provide an additional tool for tracking shifts in potentially harmful taxa across large geographic regions and over time.

Discussion

This paper presents a general overview of the types of information that can be obtained through a national-scale coupled eDNA-water quality monitoring program, as discussed below. More detailed site-specific questions, such as movement of a particular species through an estuary, identification of range-shifting organisms, or focused evaluation of food web dynamics could be generated using this type of data set, particularly if the program is maintained over long time periods.

Value of integrating eDNA into standardized monitoring. Adding eDNA to existing water monitoring programs enhances the value of both applications, but also adds cost and complexity. eDNA is widely recognized as a valuable tool for assessing marine systems, including fish and biodiversity (Lodge et al. 2012, Ruppert et al. 2019), but it is still not commonly included in routine monitoring. Our goal here was to understand both the benefits and the barriers to adding eDNA to the existing, long-term NERRS standardized monitoring program. We demonstrate that eDNA can enhance existing fish survey programs, provide baseline biodiversity information at sites lacking traditional surveys, and expand the ecological value of monitoring when coupled with water quality data and multiple genetic markers. The

broad eukaryotic analyses further demonstrate the value of ecosystem-scale monitoring approaches. In addition to fish community assessment, the 18S analyses captured seasonal shifts in planktonic assemblages, environmental gradients associated with salinity, and potentially harmful algal bloom taxa such as Pseudo-nitzschia. These results highlight the potential for integrated eDNA monitoring programs to provide broader ecosystem context alongside traditional water quality measurements.

Comparison with traditional monitoring approaches. Others have found that traditional and eDNA methods (or any dissimilar monitoring programs) may survey different components of ecological communities, and limited overlap is not uncommon (Alexander et al. 2023, Wang et al. 2024, Knauss et al. 2025). A combination of methods increases the range of species detected, but also increases the cost and complexity of the survey.

Rarefaction curves of cumulative number of species per sample indicate that per-sample richness is consistently higher at some sites, and that species richness saturation was not reached; additional samples would likely increase richness estimates (Fig. 4). This reflects both true species abundance and limitations of the eDNA method; the lowest average species count was detected at Mission Aransas NERR in Texas where high turbidity and inhibition present challenges to both filtering and PCR processes.

Seasonal succession of taxa groups. The biological community in most nearshore environments shifts in response to changing seasons, temperature, migration patterns, and other environmental factors (Grothues. T. M et al. in review). This seasonal turnover in ecological groups was apparent in the 18S rDNA data as relative changes in the distribution of the number of genera within key ecological groups. At Apalachicola Bay NERR, for example, marine eukaryotic communities exhibited seasonal turnover, with dinoflagellates increasing during summer and fall while other algal groups and metazoans peaked earlier in the year (Fig. 8). These shifts suggest seasonal succession within planktonic assemblages and potential trophic coupling between primary producers and higher trophic groups. Relatively stable ciliate proportions across seasons may indicate persistent microbial loop dynamics. The increased presence of land plants in the summer reflects increased land plant biomass entering waterways. These patterns are consistent with seasonal succession within estuarine planktonic communities and demonstrate the ability of eDNA metabarcoding to capture temporal ecological variability within standardized monitoring programs.

Interpretation of species detections and limitations of eDNA. Site-specific species detections are consistent with known communities, with some erroneous species. Lists of species detected with eDNA were reviewed by managers and local experts to flag mis-identified or unlikely fishes. In each estuary we detected some fish species that are very unlikely to be living in, or near, the location they were detected. Some of these detections represent only one sample and/or have very few reads, and could be artifacts from sequencing or bioinformatics protocols.

After discussion with resource managers we elected to include all species, including improbable ones, to a) avoid removing detections that represent real DNA reads, and b) to accurately represent this potential

downside of eDNA analyses. Unlikely species are flagged, but included in all tables and analyses, and range from 2 unlikely species detected at Wells over the 2 year study, to 27 in San Francisco, which represents 25% of all fish detected at the SF site.

San Francisco Bay is a highly urbanized estuary, with extensive shipping, wastewater, and food processing activities, and it is likely that at least some of these detections represent real DNA from species that are not actually living in the area. The estuary also has one of the highest invasive species counts in the world (Cohen and Carlton 1998, Himson et al. 2023), making it difficult to distinguish false detections from very early invasions.

The eDNA survey also missed some species which are known to be present in the systems; for example oyster toadfish (*Opsanus tau*) is common in Jacques Cousteau NERR, but was not detected in our samples. Review of the databases found that sequence information for the 12S rRNA gene region of this species is not available in GenBank, so a taxonomic assignment could not be made. This type of error will become less common over time as databases become more complete. In other cases, however, there is no clear reason a species was not detected; sand seatrout (*Cynoscion arenarius*) is one of the most common species found in trawl surveys in Apalachicola, but was not detected in the eDNA study, and Kanda mullet (*Osteomugil engeli*), which is common in Hawai□i was not detected. These negatives may be related to DNA shedding rates, transport, or simply timing of the surveys.

eDNA-based assessments are subject to inherent uncertainties that are unique to this method. Interpreting eDNA-based species monitoring results therefore requires careful consideration of the limitations and uncertainties associated with the method. It is important to clearly communicate possible sources of error throughout the workflow to allow users to make informed decisions about data interpretation and application. Information should be provided to users both as taxonomic tables, where species identification has been assigned to sequences, and as ASVs. For genus-specific primer sequences, such as MiFish (Miya et al. 2015), taxonomic assignments can be made to the species level for most of the data, although there may be ambiguity in these assignments (Watts et al. 2026). Broader analyses such as 18S rDNA capture a much wider range of organisms, but fewer sequences can confidently be assigned to species-level taxonomy. These approaches therefore provide complementary, but different, types of ecological information.

Operational and methodological challenges The total cost of a sampling program, including sample collection, analysis, and interpretation, can be a barrier to implementation. Site personnel estimated that preparing for, collecting, and filtering samples in conjunction with existing water monitoring required an additional time commitment of approximately 0.25–1 hour per sample. Additional effort was needed at locations where eDNA samples could not be collected concurrently with other sampling efforts, and at sites with high turbidity where filtering was slow. However, integrating eDNA collection into an existing long term program such as SWMP substantially reduces the logistical burden compared to developing independent biodiversity monitoring programs.

The cost of collection materials such as filter funnels, gloves, and bleach ranged from approximately $5–10 per sample, while commercial laboratory analysis currently ranges from approximately $100–200 per sample. Sample collection, particularly filtering, is time consuming and requires dedicated space, equipment, and training to reduce contamination risk. Data analysis and interpretation can also be challenging. Ambiguity in species identification, complexity of bioinformatics analyses, and handling of large data sets may limit accessibility for managers without additional training or technical support.

Standardization is important - but challenging. Standardization of all elements of the eDNA workflow is important for ecosystem monitoring because it ensures that data collected across different sites, times, and researchers are consistent, comparable, and reliable. This consistency is essential for detecting real ecological changes rather than artifacts of varying methods or interpretations (Lindenmayer and Likens 2010, Borja et al. 2016).

eDNA-based monitoring is particularly sensitive to methodological variation because variations in the workflows spanning sample collection, filtration, extraction, amplification, and bioinformatics can all influence results (Theroux et al. 2025). Without consistent methods, results can vary substantially, making it challenging to compare data across studies or build robust long-term data sets.

However, as eDNA methods are evolving rapidly, adhering to static methods may result in suboptimal results. In this study, we intended to maintain consistent protocols throughout the project, but feedback from managers after year 1 indicated that key fish species were not being detected. This presented a quandary; a primary goal of the study was to understand the value of a standardized eDNA sampling program across a network, but adhering to the original protocol would have reduced the overall value of the data set. We decided, in this case, to modify specific steps in the lab protocol, retain consistency in all other aspects and clearly document when and what changes were made (El Baidouri et al. 2025). The balance between optimization and standardization will likely remain an ongoing challenge as eDNA technologies continue to improve. Managers and researchers must weigh the benefits of adopting evolving methodologies against the importance of maintaining temporal comparability within long-term monitoring programs. Clear documentation of protocol changes and continued communication among stakeholders are essential.

Conclusion

This study demonstrates that integrating eDNA metabarcoding into an established, standardized monitoring framework can substantially enhance our ability to assess estuarine biodiversity across broad spatial and temporal scales. Programs like the NERRs, which already maintains standardized field protocols, long-term environmental datasets, and distributed technical staff, provide a strong foundation for scalable implementation of biodiversity monitoring at a national scale. By capturing hundreds of fish species and thousands of eukaryotic taxa, eDNA provides a broad, complementary view of community composition, while also enabling detection of ecologically important groups such as harmful algal bloom species and early-stage invasions. At the same time, there are challenges—including

methodological sensitivity, interpretation of uncertain detections, cost/effort, and tradeoffs between standardization and protocol optimization—that must be addressed to ensure reliable long-term data sets. For resource managers, these findings are particularly important: eDNA offers a scalable, flexible tool that can complement existing monitoring programs, but managers must also be aware of limitations and challenges associated with the technology.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and material: Processed data is included in supplementary information, raw reads are available on NCBI (in process)

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Authors' contributions:

Conceiving the ideas and design of the experiment: AW, EB, ND, MF, JSG, TG, CP, YR, SS

Participating in the active execution of the study: AW, EB, LC, ND, FEB, MF, JSG, HG, TG, ML, SL, JM, CP, YR, NS, SS, SY

Analyzing and interpreting the data: AW, LC, ND, FEB, MF, JSG, TG, ML, SL, JM, JM, CP, YR, NS, SS, SY

Writing the manuscript: AW, EB, LC, ND, FEB, MF, HG, JSG, TG, ML, SL, JM, CP, YR, NS, SS, SY

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Figures

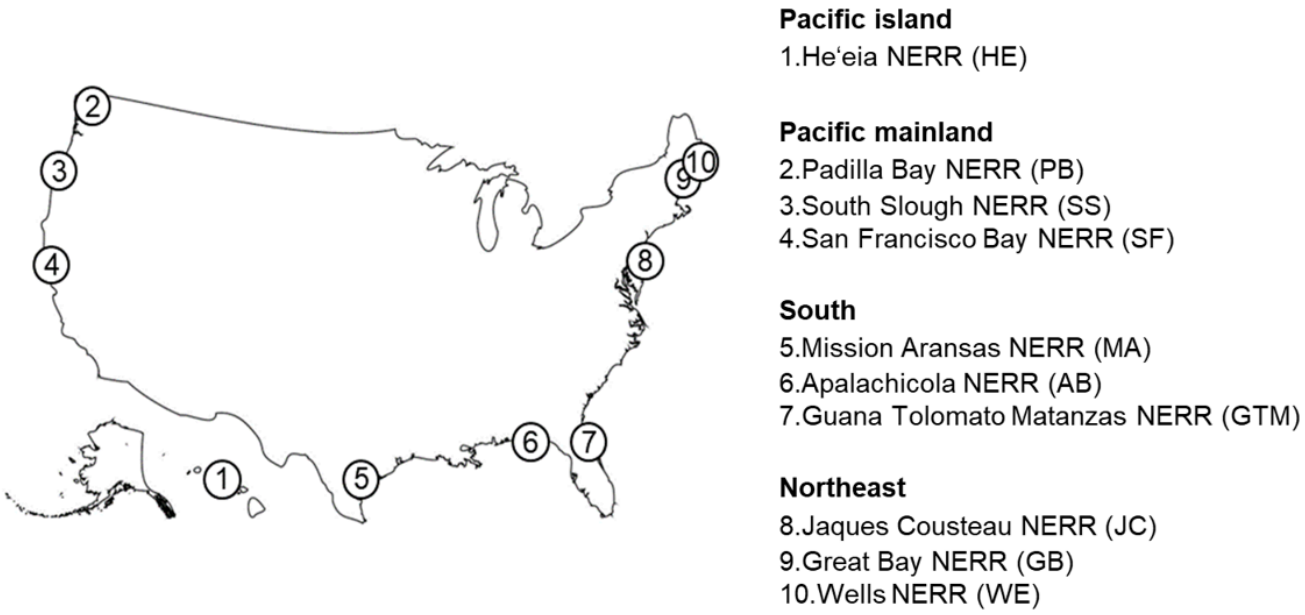


Figure 1

Ten participating NERRs across the United States, with 4-5 sampling locations within each estuary. The NERRs were selected to represent geographically and ecologically different locations

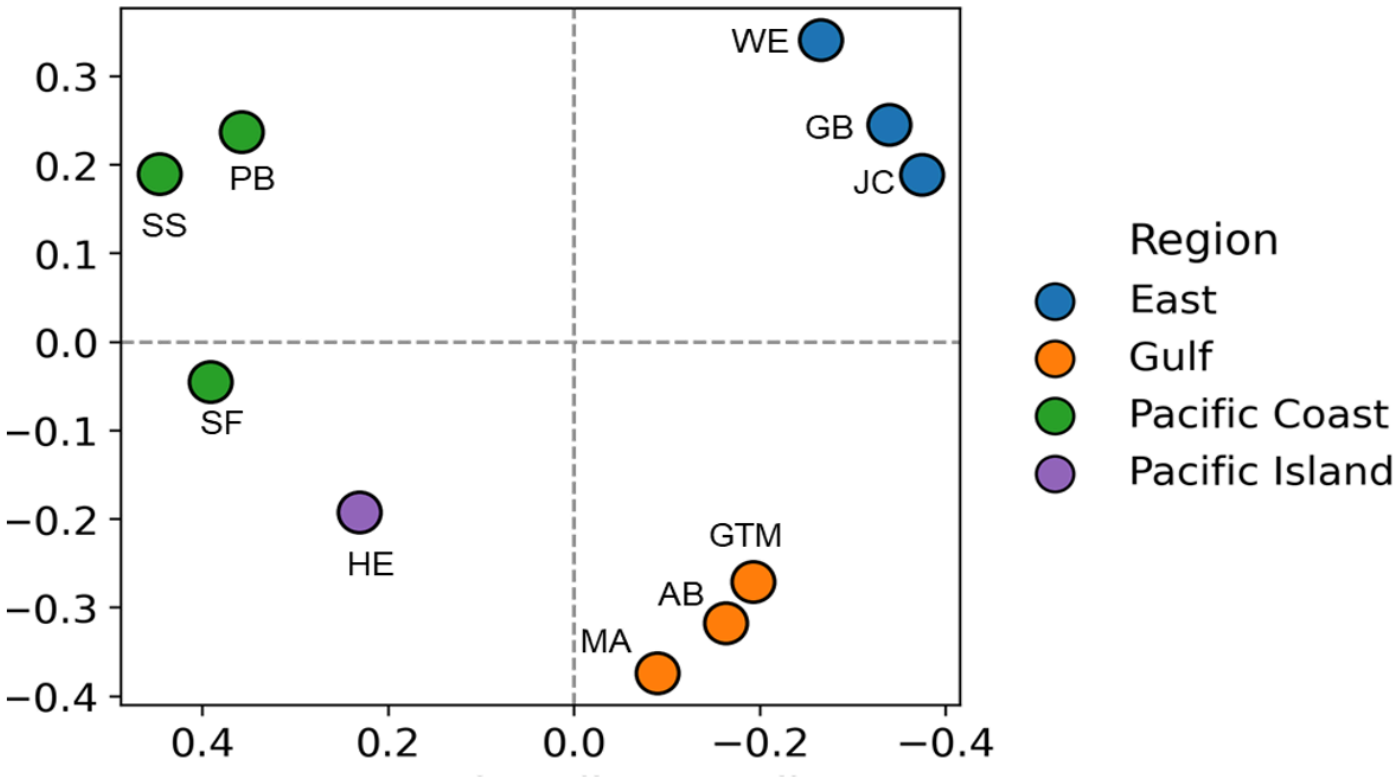


Figure 2

Principal Coordinates Analysis (PCoA) dissimilarities for all 10 sampling locations based on species detection frequencies. Sites cluster strongly by coastline, with the Pacific mainland locations (SF, SS, PB) separated along the primary axis from Northeast and South sites. Northeast locations (Wells, GB, JC) and South (GTM, AB, MA) form distinct subclusters along the secondary axis

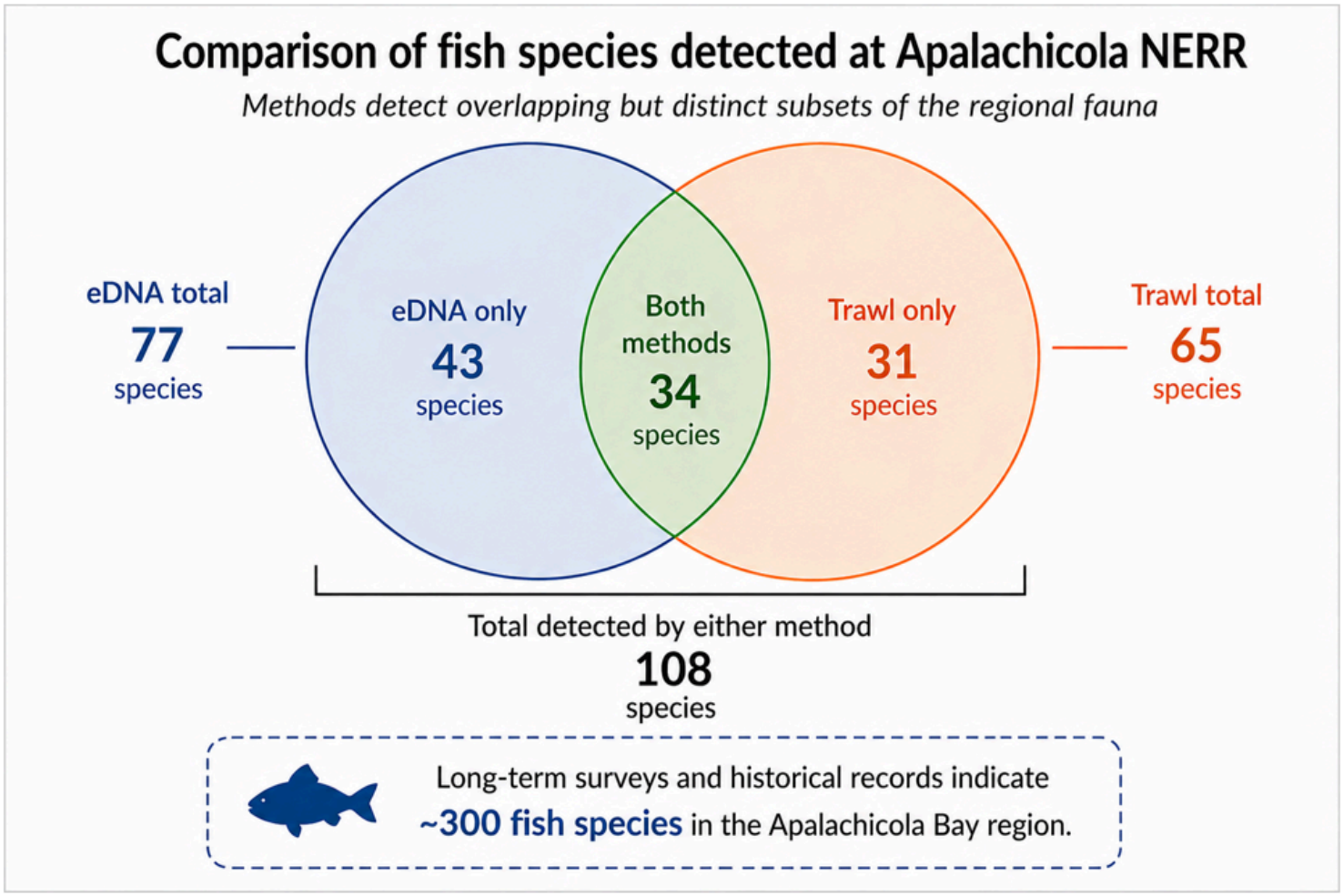


Figure 3

Comparison of fish species detected at Apalachicola NERR by eDNA and trawl surveys. 87 species were detected with eDNA, 65 in the trawl survey, and 114 in the combined data set. The long-term trawl data set for the region contains over 300 species

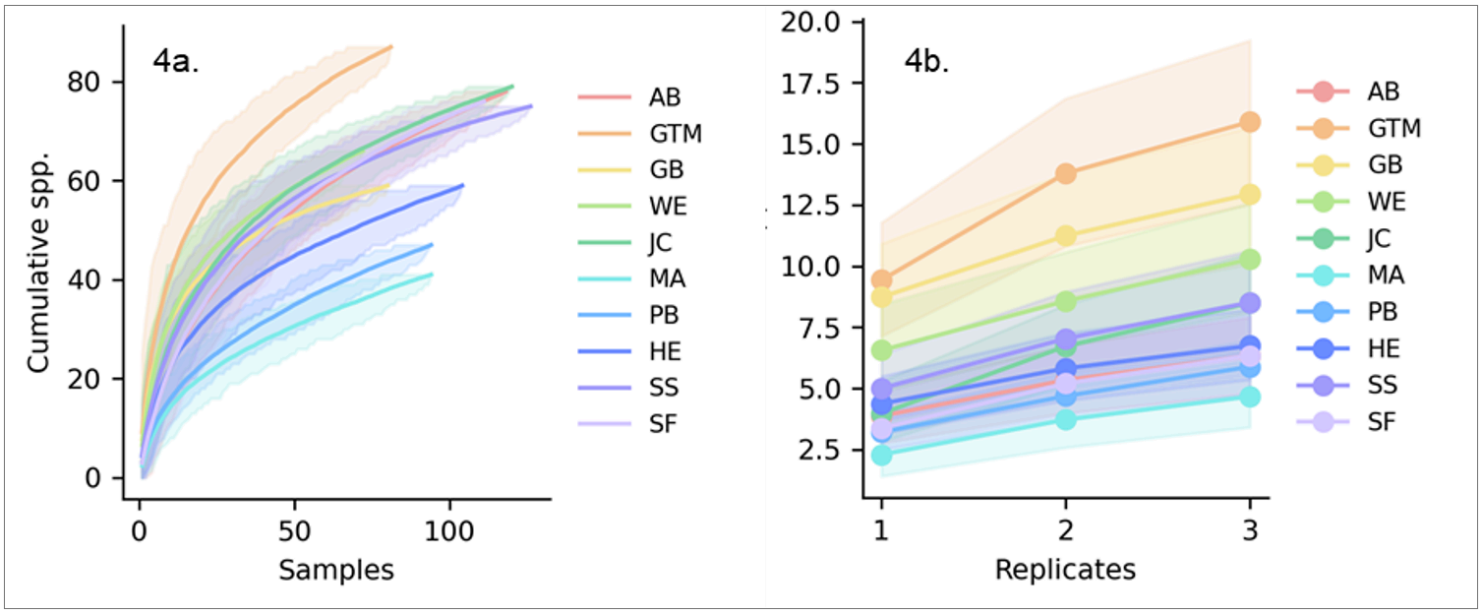


Figure 4

a) *Species rarefaction curves for each site show that the highest increase in species counts occurs in the first samples, with the slope generally decreasing after 10 samples. Note that the chart includes samples from different locations and seasons within a NERR so variability within samples is expected. Although the slope decreases with more samples, saturation is not reached. b) Increase in species detected per replicate sample. Greater number of replicates increases species detection. Shading indicates 95% confidence*

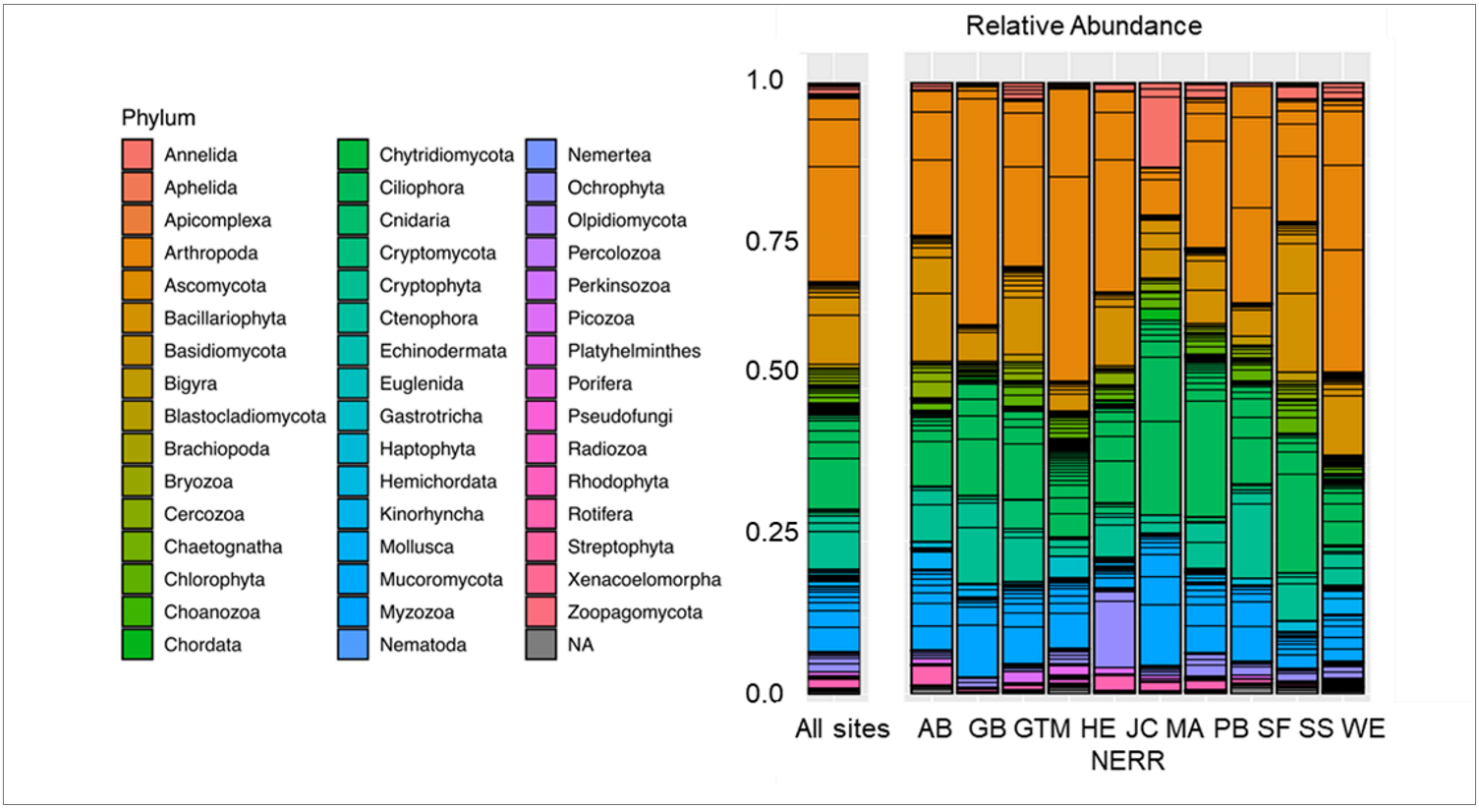


Figure 5

Over 18,000 eukaryotic sequences, representing thousands of species, were detected across sites

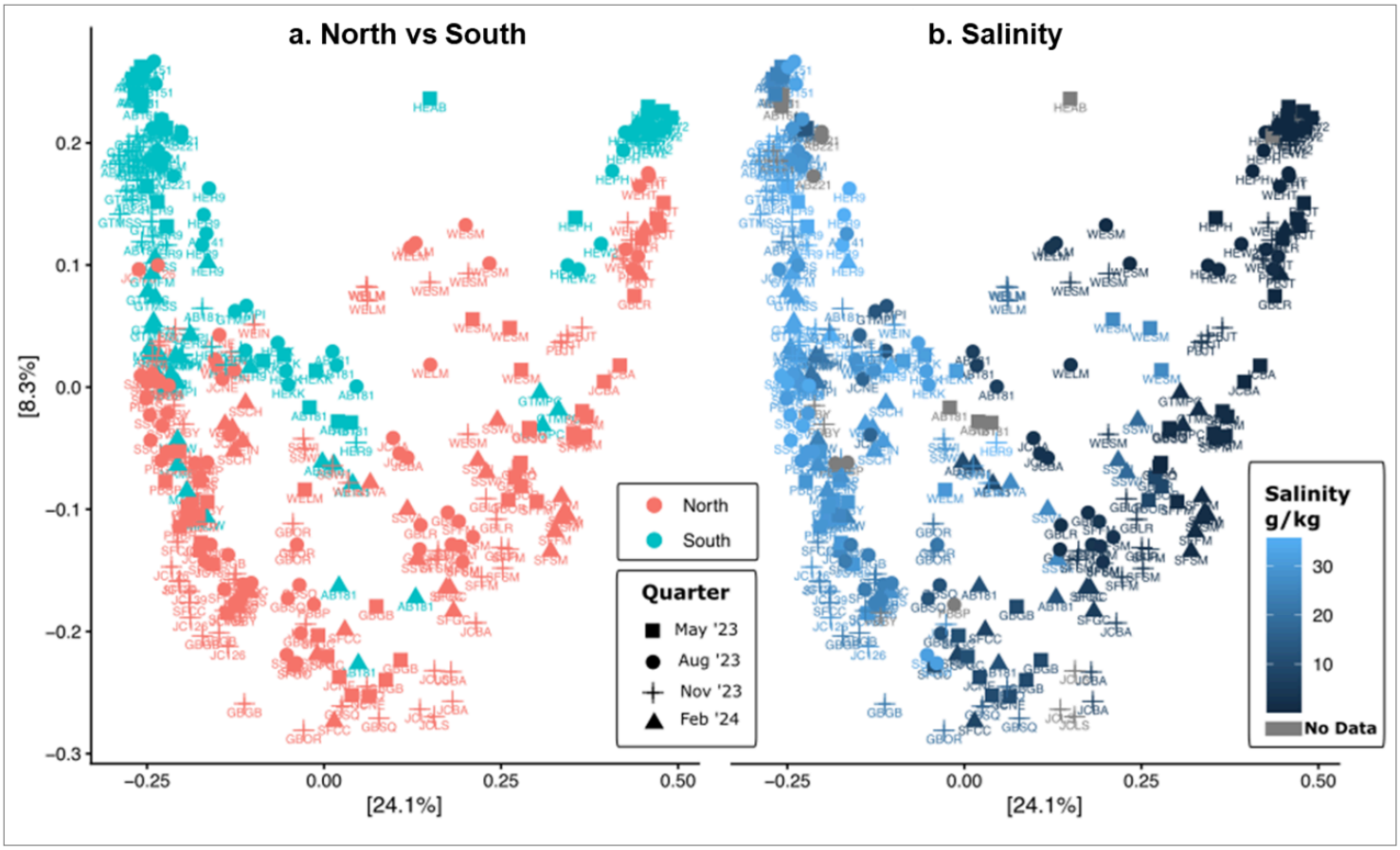


Figure 6

18s rDNA metabarcoding ASVs by unweighted Beta diversity (Unifrac PCoA). Unweighted PCoA reflects community composition (presence/absence). In each plot, points are colored from A) North/South, and B) Salinity. PC1 (x-axis) shows that community composition varied between high and low salinity sites

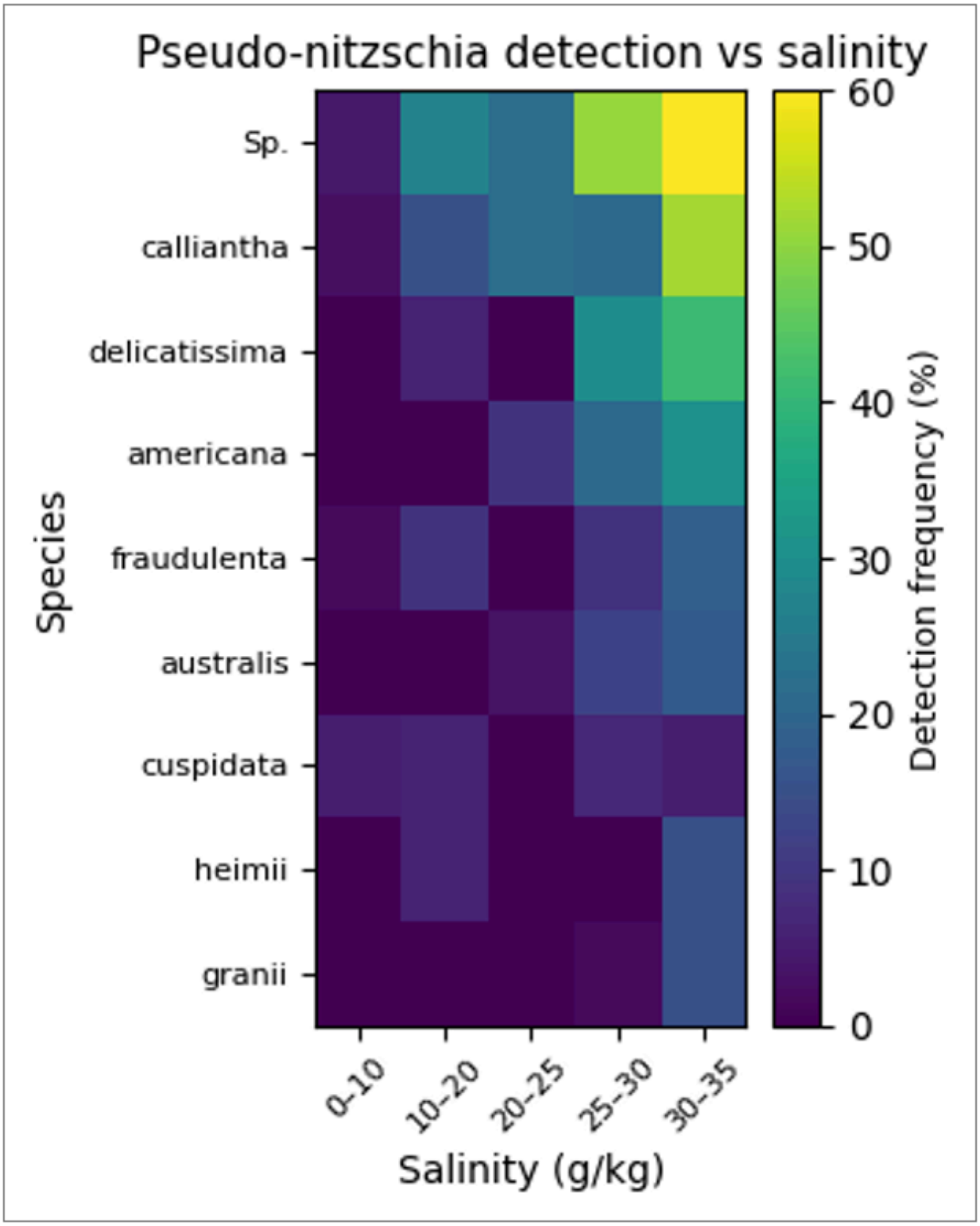


Figure 7

Pseudo-nitzschia detections are associated with higher salinity. The 18S rDNA analysis provided sufficient resolution to differentiate many species, and the associated water chemistry data allows us to start to build correlations with water quality

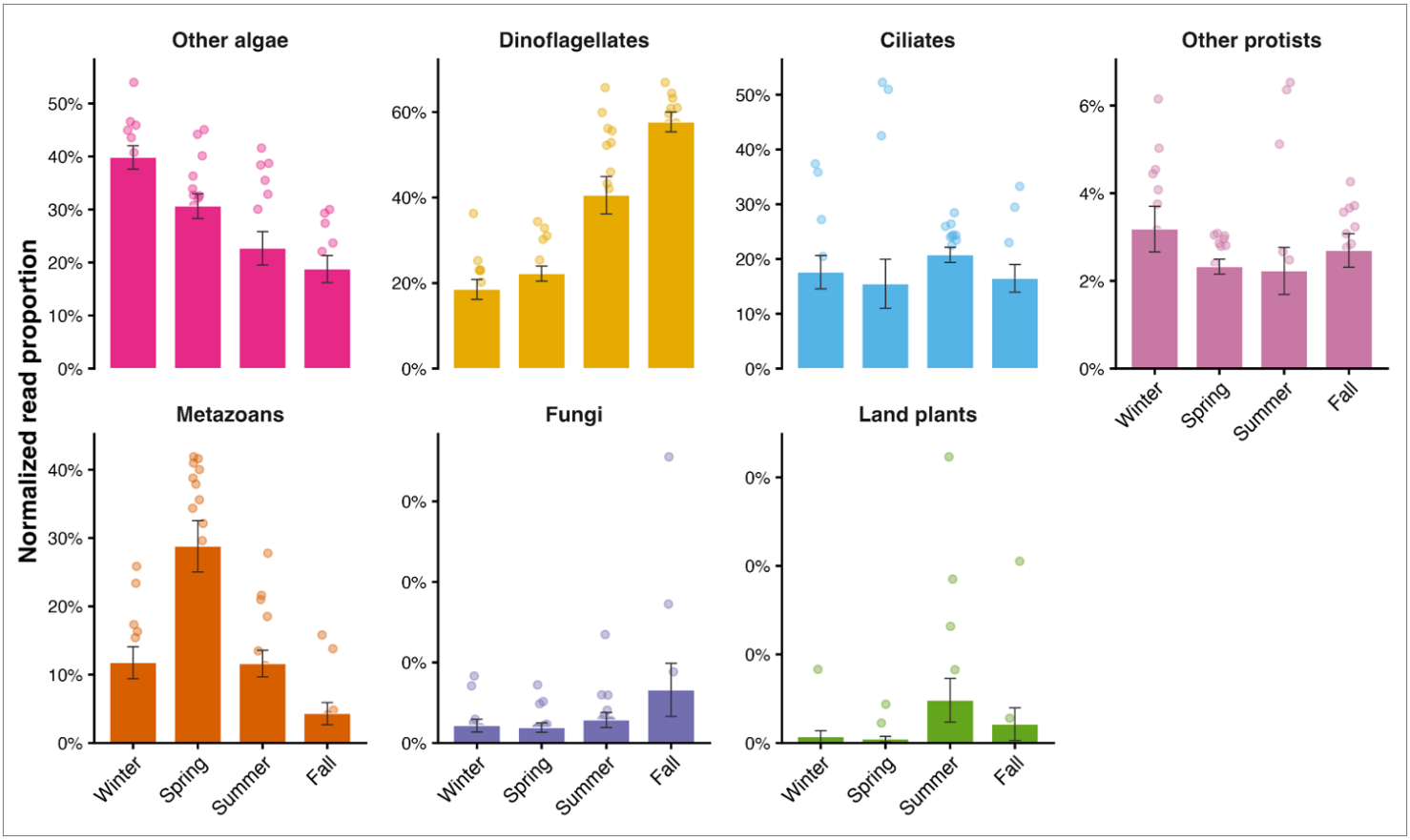


Figure 8

Seasonal change in ecological groups at AB NERR

Supplementary Files

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- SupplementaryInformation.docx