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Using Environmental DNA to Evaluate the Impact of Tidal Wetland Restoration in the San Francisco Estuary

By

LEIGH SANDERS

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER IN SCIENCE

in

Integrative Genetics and Genomics

in the

OFFICE OF GRADUATE STUDIES

of the

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DAVIS

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Abstract

The San Francisco Estuary historically provided vast tidal wetland habitats for various fishes. However, this habitat has been severely reduced by mining, agricultural development, and climate change, and its hydrology and water quality continue to be altered by water diversions. Gear-based fish capture is commonly used to monitor restoration efforts, but can harm species and are often biased in what they can efficiently catch. Our project aims to enhance and complement existing restoration monitoring using a non-invasive, sensitive, low-cost environmental DNA (eDNA) approach. To isolate eDNA from the environment, replicate water samples were collected and filtered from paired restoration wetlands and reference sites in the San Francisco Estuary from February 2023 to January 2024. Multi-species eDNA metabarcoding was performed to detect community-level species composition using the 16S fish-specific and 12S MiFish-U primers. Metabarcoding taxa assignments were made using a custom reference sequence database and the NCBI nucleotide database. On average, this study found that restored tidal wetlands in the San Francisco Estuary had fish species richness and diversity values similar to those of their paired reference sites. Restored tidal wetlands had comparable or greater native species richness and diversity than their paired reference sites and shared many detections for species of concern, including Longfin Smelt, Chinook Salmon, and White Sturgeon. This provides evidence that tidal wetland restoration projects in the SFE are meeting their goals of increasing habitat and food-web resources that will benefit fishes. The results of this study provide comprehensive, non-invasive, and vital data about which fish are utilizing restoration sites in the San Francisco Estuary. Going forward, this study recommends using multi-species eDNA surveys as an additional monitoring tool for restoration managers to help inform future projects.

Table of Contents

Abstract ii
1 | Funding and Acknowledgements vii
2 | Introduction 1
3 | Materials and methods 5
3.1 | Field collection 5
3.1.1 | Study sites 5
3.1.2 | Paired eDNA sampling and capture surveys 7
3.2 | Filtration 9
3.3 | DNA extraction 10
3.4 | PCR-based library preparation for metabarcoding 11
3.5 | Bioinformatics and sequence processing 14
3.6 | Data analyses 15
3.6.1 | Species detection summaries 15
3.6.2 | Statistical Analyses 16
4 | Results 17
4.1 | Sequencing summary 17
4.1.1 | eDNA water samples 17
4.1.2 | Negative control samples and sequence filtration thresholds 18
4.2 | Species detection summaries 20
4.2.1 | ASV Assignments 20
4.2.2 | Overall sampling effort and sequencing depth 21
4.2.3 | Species Detections 24
4.2.4 | Detections for species-of-concern 30
4.3 | Statistical Analyses 33
4.3.1 | Alpha Diversity 33
4.3.2 | Beta Diversity 39
5 | Discussion 44
5.1 | Species detection and biodiversity assessment 44
5.2 | Key considerations and mitigating limitations 46

5.3 | Implications and future directions 48
6 | References 49
7 | Supplementary Materials (Tables) 59
8 | Supplementary Materials (Figures) 97

Tables

Table 1. Fish eDNA studies in the San Francisco Estuary. 4
Table 2. Paired (restoration/reference) study sites in the Suisun Bay and Sacramento-San Joaquin Delta regions of the San Francisco Estuary. 7
Table 3. eDNA fish monitoring sampling timeline. 8
Table 4. Expected average amplicon sizes for 16S fish-specific and 12S MiFish-U primers. 12
Table 5. eDNA fish species detections after quality sequence filtering. 22

Figures

Figure 1. Map of paired (restoration/reference) study sites in San Francisco Estuary. 6
Figure 2. eDNA filtration setup. 9
Figure 3. Species accumulation curves. 21
Figure 4. 16S relative read abundances for the top 10 species with the highest read counts. 26
Figure 5. 12S relative read abundances for the top 10 species with the highest read counts. 27
Figure 6. 16S relative read abundances for native fishes. 28
Figure 7. 12S relative read abundances for native fishes. 29
Figure 8. Read abundances for Chinook Salmon. 31
Figure 9. 16S read abundances for Longfin Smelt and Delta Smelt. 32
Figure 10. 12S read abundances for White Sturgeon. 33
Figure 11. Alpha diversity comparing fish eDNA from restored and reference sites. 35
Figure 12. Species richness comparing fish eDNA from restored and reference sites. 36
Figure 13. Alpha diversity comparing native fish eDNA from restored and reference sites. 37
Figure 14. Species richness comparing native fish eDNA from restored and reference sites. 38
Figure 15. 16S fish eDNA assemblages at paired restoration and reference sites. 40
Figure 16. 12S fish eDNA assemblages at paired restoration and reference sites. 41
Figure 17. 16S native fish eDNA assemblages at paired restoration and reference sites. 42
Figure 18. 12S native fish eDNA assemblages at paired restoration and reference sites. 43

Supplementary Materials (Tables)

Table S1. eDNA Fish monitoring sampling events—detailed view. 59
Table S2. First-round PCR: 16S fish-specific primers (underlined) with Illumina index adaptor sequences used for eDNA fish monitoring. 61
Table S3. First-round PCR: 12S MiFish-U primers (underlined) with Illumina index adaptor sequences used for eDNA fish monitoring. 61
Table S4. First-round PCR thermocycler conditions: 16S fish-specific primers. Modified from Shaw et al. (2016) to minimize off-target amplification. 62
Table S5. First-round PCR thermocycler conditions: 12S MiFish-U primers. Modified from Miya et al. (2015) to minimize off-target amplification. 62
Table S6. Second-round PCR: Illumina Nextera Index Kit PCR primers used for 16S fish-specific amplicons. 63
Table S7. Second-round PCR: Illumina TruSeq Index Kit PCR primers used for 12S MiFish-U amplicons. 63
Table S8. Second-round PCR thermocycler conditions for indexing samples. 63
Table S9. Sequence filtering read pipeline for 16S fish-specific assay samples. 64
Table S10. Sequence filtering read pipeline for 12S MiFish-U assay samples. 69
Table S11. Sequence filtering read pipeline for 16S assay negative controls. 75
Table S12. Sequence filtering read pipeline for 12S assay negative controls. 77
Table S13. Species removed following quality sequence filtering. 79
Table S14. 16S fish-specific and 12S MiFish-U species detections for individual 1-L eDNA water samples for Ryer Island and Tule Red paired sites. 80
Table S15. 16S fish-specific and 12S MiFish-U species detections for individual 1-L eDNA water samples for Liberty Island and Yolo Flyway Farms paired sites. 82
Table S16. 16S fish-specific and 12S MiFish-U species detections for individual 1-L eDNA water samples for Browns Island and Montezuma Wetlands paired sites. 84
Table S17. 16S fish-specific and 12S MiFish-U species detections for individual 1-L eDNA water samples for Browns Island and Winter Island paired sites. 86
Table S18. 16S fish-specific and 12S MiFish-U species detections for individual 1-L eDNA water samples for Webb Tracts Island and Berms and Decker Island paired sites. 88
Table S19. 16S and 12S species diversity and richness for 1-L eDNA water samples. 89
Table S20. 16S and 12S native species diversity and richness for 1-L eDNA water samples. 93

Supplementary Materials (Figures)

Figure S1. Amplicon and primer visualization of the paired-end library preparation. 97
Figure S2. First-round PCR with 16S fish-specific primers. 98
Figure S3. First-round PCR with 12S MiFish-U primers. 98
Figure S4. Second-round PCR for indexing 16S fish-specific amplicons. 99
Figure S5. Second-round PCR for indexing 12S MiFish-U amplicons. 99
Figure S6. Fish species rarefaction curves. 100
Figure S7. 16S read abundances for the top 10 species with the highest read counts. 101
Figure S8. 12S read abundances for the top 10 species with the highest read counts. 102
Figure S9. 16S sub-site relative read abundances for the top 10 species with the highest read counts. 103
Figure S10. 16S sub-site read abundances for the top 10 species with the highest read counts. 104
Figure S11. 12S sub-site relative read abundances for the top 10 species with the highest read counts. 105
Figure S12. 12S sub-site read abundances for the top 10 species with the highest read counts. 106
Figure S13. Browns, Montezuma, and Winter biological replicate relative read abundances for the top 10 species with the highest read counts. 107
Figure S14. Browns, Montezuma, and Winter biological replicate read abundances for the top 10 species with the highest read counts. 108
Figure S15. Tule and Ryer biological replicate relative read abundances for the top 10 species with the highest read counts. 109
Figure S16. Tule and Ryer biological replicate read abundances for the top 10 species with the highest read counts. 110
Figure S17. Liberty and Flyway biological replicate relative read abundances for the top 10 species with the highest read counts. 111
Figure S18. Liberty and Flyway biological replicate read abundances for the top 10 species with the highest read counts. 112
Figure S19. Decker and Webb biological replicate relative read abundances for the top 10 species with the highest read counts. 113
Figure S20. Decker and Webb Tract biological replicate read abundances for the top 10 species with the highest read counts. 114

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2 | Introduction

The San Francisco Estuary (SFE) is the second-largest estuary along the west coast of North America (Mount and Kimmerer 2022), and is essential to California's freshwater supply and economy (Knowles and Cayan 2002). Due to its wide variety of habitats, the SFE also supports many types of wildlife and plants. Specifically, tidal wetlands in the SFE have historically provided an abundance of essential habitats for fish foraging and reproduction (Sommer and Mejia 2013; Hammock et al. 2019; Colombano et al. 2020; 2021; Aha et al. 2021). Due to mining, climate change, and agricultural development, these tidal wetlands have declined approximately 70-93% in the Bay and 95% in the Delta of the SFE, leading to large, multi-agency habitat restoration projects (Gilbert 1917; Goodwin, Mehtat, and Zedler 2001; Callaway et al. 2011; Cloern and Jassby 2012). In addition to wetland loss, the past few decades have seen a decline in native species, such as Chinook Salmon (*Oncorhynchus tshawytscha*), Longfin Smelt (*Spirinchus thaleichthys*), Delta Smelt (*Hypomesus transpacificus*), and White Sturgeon (*Acipenser transmontanus*) (Yoshiyama, Fisher, and Moyle 1998; Moyle et al. 2016; Blackburn et al. 2019; Lewis et al. 2020). Due to their sensitivity to environmental changes, these species often serve as biological indicators for various ecological functions in the SFE, such as water quality and habitat degradation.

Following the amendments to the Clean Water Act (CWA) in the 1970s-80s, more studies emerged to protect and conserve aquatic habitats, including tidal wetlands (Callaway et al. 2011; U.S. Environmental Protection Agency 1972). For example, the National Estuary Program (NEP) was established under the CWA following the 1987 amendments and is primarily focused on restoring estuaries across the U.S. (NEP 2024). The CWA also catalyzed the

formation of several multi-agency habitat restoration projects in the SFE, along with monitoring studies evaluating their impact (Callaway et al. 2011; Hartman et al. 2024). Over 200 habitat restoration projects are occurring in the SFE, with many focusing on tidal wetlands (CWMW 2025). This includes projects such as the interagency Fish Restoration Program (FRP), tasked with restoring more than 8,000 acres of tidal wetlands in the SFE and monitoring restoration effects on native fishes (FRP 2021), and the Suisun Marsh Fish Study which was formed as a partnership between the University of California Davis (UC Davis) and the California Department of Water Resources (DWR) in 1980 to monitor fishes in Suisun Marsh (O'Rear et al. 2020).

Previous and current methods to monitor fishes at tidal wetland sites in the SFE primarily consist of using traditional gear-based surveys to capture and identify fish (Grimaldo et al. 2012; Colombano, Donovan, et al. 2020; O'Rear et al. 2020; FRP 2021; Stompe and Hobbs 2024). Although these methods can be effective for studies requiring additional life history information, they require expert taxonomic identification, might harm captured individuals, can create physical dangers to the field crew, and can be biased in the sizes and types of fish that are caught (Chopin and Arimoto 1995; Anderson and Davis 2013; Kirsch et al. 2018; Gold et al. 2021). It can also be difficult for programs to provide continuous monitoring due to the intensity and cost of gear-based surveys. This challenge can also limit their ability to detect rare or endangered fishes of interest, such as Delta Smelt (Valentini et al. 2016; Ramírez-Amaro et al. 2022; Andres et al. 2023). In these situations, supplementing surveys with higher sensitivity would be advantageous (Bergman et al. 2016).

In response to these issues, researchers in the US and other countries have begun pairing gear-based surveys with environmental DNA (eDNA) detection to monitor fish (Ogden

2022; Yao et al. 2022; Veron et al. 2023). eDNA is DNA shed by organisms into their surrounding environment and can, therefore, be isolated from environmental samples (i.e., water) without catching or visualizing the organism (Taberlet et al. 2012). It can originate from living and deceased organisms and typically consists of cellular and free-floating extra-cellular DNA. Due to its increased sensitivity, eDNA detection is often more cost-effective than traditional gear-based sampling when sampling rare or difficult-to-catch species (Fediajevaite et al. 2021; Morris et al. 2024), although costs vary depending on project goals and study systems (Smart et al. 2016; Sanches and Schreier 2020). Additionally, eDNA detection has limitations that must be mitigated, such as primer biases, sample inhibition, the spatial and temporal resolution of eDNA molecules, inconsistent interpretations of data analyses, and quantitative estimates for species abundance or biomass (Beng and Corlett 2020). Therefore, when deciding the best monitoring approach, it is critical to consider the feasibility of each design, how they might be altered to balance sensitivity and costs, and how their limitations will be addressed.

While early fish eDNA monitoring studies focused on detecting a couple of species, more recent studies have used metabarcoding with eDNA to detect entire fish assemblages (Jerde et al. 2010; Ruppert, Kline, and Rahman 2019). Metabarcoding combines DNA barcoding, which uses diagnostic barcode markers to make single species identifications, with high-throughput sequencing to identify multiple species from mixed DNA isolated from an environmental sample (Deiner et al. 2017). Similar to gear-based community monitoring, eDNA metabarcoding has limitations, such as its requirement for reference databases and variability in detections across barcode markers (Taberlet et al. 2012). Barcode primers may also differ in amplification efficiency across target species, creating amplification biases (Van Der Loos and Nijland 2021; Kumar et al. 2022; Li et al. 2024). However, as newer studies have begun to focus

on these issues (Zhang, Zhao, and Yao 2020; Schenekar et al. 2020), the limitations of eDNA metabarcoding can be mitigated during the project's design by ensuring a relevant high-quality reference database is set up and the appropriate barcode markers are chosen.

Since eDNA and/or metabarcoding emerged as tools for monitoring fish communities, many studies have explored their use in various water systems (Ruppert, Kline, and Rahman 2019), but studies are limited in estuaries due to their variability and complexity (DiBattista et al. 2022; Nagarajan et al. 2022). Even more limited are studies using eDNA to monitor fishes within the SFE (**Table 1**).

Table 1. Fish eDNA studies in the San Francisco Estuary.

Reference	Title	Study Focus
Sanches and Schreier (2020)	Optimizing an eDNA protocol for estuarine environments: Balancing sensitivity, cost and time	Evaluate various protocols to detect Chinook Salmon with qPCR.
Holmes et al. (2024)	Evaluating environmental DNA detection of a rare fish in turbid water using field and experimental approaches	Detect Delta Smelt with qPCR.
Nagarajan et al. (2024)	CRISPR-based environmental DNA detection for a rare endangered estuarine species	Developed a CRISPR-SHERLOCK assay to detect Delta Smelt.
Bowen et al. (2024)	A comparison of eDNA sampling methods in an estuarine environment on presence of longfin smelt (*Spirinchus thaleichthys*) and fish community composition	Evaluate various protocols to detect Longfin Smelt with qPCR.
Baidouri et al. (2025)	An optimized eDNA protocol for fish tracking in estuarine environments	Detect fish communities across multiple estuaries using metabarcoding.

There is currently no published literature on using eDNA to study the impacts of tidal wetland restoration on fish communities within the SFE. Other studies have successfully implemented eDNA detection to monitor the effects of habitat restoration on fish communities in different estuaries and aquatic environments. One study used eDNA metabarcoding to monitor fish communities in a marine reserve within the Pearl River Estuary of China to study the impacts of habitat degradation on fish biodiversity (Zou et al. 2020), while another study in

England used eDNA metabarcoding to monitor fish communities following the removal of a weir in a river (Muha et al. 2021).

Previous studies have established the importance of combining fish eDNA monitoring with restoration design (Muha et al. 2021; Ito et al. 2023), but there is a limited understanding of how eDNA could serve as a monitoring tool in the SFE and how it might inform adaptive management strategies regarding habitat restoration. The present study aimed to address this gap by focusing on the impact of tidal wetland restoration on fish communities in the SFE. To accomplish this, we explored the potential of a non-invasive eDNA metabarcoding approach to monitor fish at various restored tidal wetlands along the SFE ecocline. Our objective was to determine whether fish species diversity and fish assemblages varied between restored tidal wetlands and paired reference sites, which served as "pristine" benchmarks for whether or not a restored site was meeting its goals (IEP TWM PWT 2017) (criteria for reference sites described in section **3.1.1**). If a restored wetland is performing well, we expect similar fish assemblages between paired sites. A successful restoration will also include positive detections for native biological indicator species, such as Delta Smelt, Longfin Smelt, Chinook Salmon, and/or White Sturgeon.

3 | Materials and methods

3.1 | Field collection

3.1.1 | Study sites

We used an existing framework of paired tidal restoration and reference sites, as described in the Fish Restoration Program (FRP) Study Plan, to perform eDNA sampling (FRP

2021). Gear-based surveys regularly monitor these sites, and we performed eDNA sampling concurrently in collaboration with the FRP and a UC Davis monitoring program, the Suisun Marsh Fish Survey. We selected nine sites within the Suisun Bay and Sacramento-San Joaquin Delta of the SFE for fish eDNA monitoring (**Table 2**, **Figure 1**). Except for Montezuma Wetlands, the study sites were chosen based on the FRP Study Plan. The breach years for each site can be found in **Table 2** (FRP 2021, Vollmar et al. 2022).

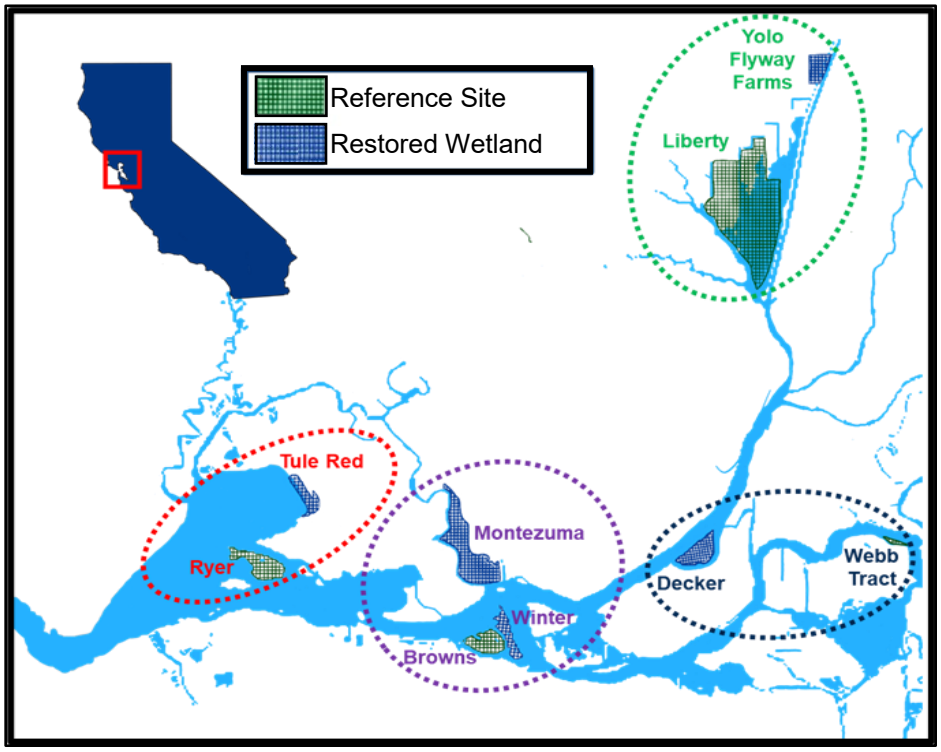


Figure 1. **Map of paired (restoration/reference) study sites in San Francisco Estuary.** Regions: Suisun Bay and Sacramento-San Joaquin Delta. Ellipses = Paired sites; Blue hatching = Restoration sites; Green hatching = Reference sites. (IEP TWM PWT 2017; FRP 2021; Google 2022)

Restoration sites were paired with reference sites with similar species accessibility and were located near each other (IEP TWM PWT 2017; Sherman, Rosemary, and Contreras 2017). To account for the effects of high landscape variability, sites were selected from different areas of SFE's ecocline, considering factors like distance from the ocean, freshwater inputs, tidal effects, and nearby land. Additional criteria for choosing sites as references included having tidal action

restored for multiple decades, such as Liberty Island, or having portions of the site that had not been fully diked or drained in the past 150 years, such as Browns Island, Ryer Island, and Webb Tracts Island and Berms (Webb Tracts).

Table 2. Paired (restoration/reference) study sites in the Suisun Bay and Sacramento-San Joaquin Delta regions of the San Francisco Estuary. Water samples were collected concurrently with capture surveys conducted by the Fish Restoration Program (FRP) or the UC Davis (UCD) Suisun Marsh Fish Survey. Breach years of restored sites in parentheses **(FRP 2021; Vollmar et al. 2022)**.

Restoration sites	Reference sites	Capture-based monitoring
Tule Red (2019)	Ryer Island	FRP
Winter Island (2019)	Browns Island	FRP
Yolo Flyway Farms (2018)	Liberty Island	FRP
Decker Island (2018)	Webb Tracts Island and Berms	FRP
Montezuma Wetlands (2020)	Browns Island	Montezuma (UCD) / Browns (FRP)

3.1.2 | Paired eDNA sampling and capture surveys

We collected 198 water samples for eDNA analysis from the five paired restoration and reference sites (**Table 2**, **Figure 1**). To benchmark fish eDNA detections, capture surveys were performed concurrently by the FRP or UCD Suisun Marsh Fish Survey team using otter trawls, beach seines, and/or lampara nets (**Table 2**, **Table S1**) **(O'Rear et al. 2020; FRP 2021)**. Water quality data, fish counts, and standard lengths of fish were recorded by the capture survey teams. During each sampling season, sites were sampled in three locations, and three 1 L replicate water samples were collected at each location (for a total of nine samples) (**Table 3**, **Table S1**).

Each eDNA sampling event took about 10 minutes, and all sub-sites within a site were sampled within several hours of each other. The eDNA locations sampled with FRP were chosen randomly within the sites by the capture survey crew and, if needed, altered slightly to accommodate the capture survey gear types that followed. The UCD Suisun Marsh Fish Survey chose sites based on their sampling history, which noted spots for safe shore access for beach

seines and adequate waterway depth and length for otter trawls.

Table 3. eDNA fish monitoring sampling timeline. X = The collection of three 1 L water biological replicates from three locations within the site (for a total of nine 1 L water samples). ± = Restoration site; † = Reference site. Dates displayed as MM/YYYY.

Site	02-03/2023	08-09/2023	12/2023 – 01/2024
Tule Red±	X	X	X
Ryer Island†	X	X	X
Winter Island±	X		X
Montezuma Wetlands±	X	X	X
Browns Island†	X	X	X
Yolo Flyway Farms±	X	X	X
Liberty Island†	X	X	X
Decker Island±	X		
Webb Tracts Island and Berms †	X		

On a boat provided by one of the capture survey teams, triplicate eDNA samples were collected from the water's surface at each sampling event using gloved hands to reach over the boat side. When the water's surface could not be reached, the sample bottle was attached to a pole covered in a new trash bag with a pre-sterilized (20% bleach for 20 minutes, rinsed with distilled water) bottle clip. Each sample was double-bagged and stored on wet ice inside a cooler until filtration (< 24 h) (Pilliod et al. 2013; Curtis, Larson, and Davis 2021). Surface water sampling from the boat and off-site filtration were chosen to minimize equipment needs and to decrease sampling time so that all the necessary eDNA and capture surveys could be completed each day.

To minimize contamination, the scientists collecting water samples did not handle fish or boat equipment and sampled upstream of the boat. Additionally, to avoid the introduction of non-contemporaneous eDNA that the capture survey gear might introduce from disturbing the sediment and to avoid cross-site contamination from the gear, all eDNA samples were collected before their paired capture surveys began (Turner, Uy, and Everhart 2015; Díaz et al. 2020). Disposable gloves were changed before each biological replicate sample was collected, and trash

bags (when pole sampling was required) were changed between site replicates. One field negative control (NC) was taken at each of the three sampling events within a site for a total of 66 field NCs across the study's duration (Goldberg et al. 2016). All eDNA samples (including field NCs) were collected in 1 L Nalgene bottles pre-sterilized with 20% bleach for 20 minutes (Kumar et al. 2021; Sanches and Schreier 2020) before rinsing with distilled water. For field NC samples, a 1 L bottle of deionized water was opened at its paired eDNA sampling event and handled in the same manner as field samples (except for dipping it into the water) (Goldberg et al. 2016). The NCs were then processed alongside field samples for filtration, DNA extractions, and all downstream reactions.

3.2 | Filtration

All water samples (including field negative controls) were transported on wet ice to the UC Davis for filtration within 24 hours of sampling (Pilliod et al. 2013; Curtis, Larson, and Davis 2021). The filtrations were performed in a laboratory that was not exposed to fish tissue or concentrated fish DNA to minimize contamination risk. Before filtration and in between new samples, all silicone tubing, filter holders, and forceps used to handle filters were sterilized with 20% bleach for 20 minutes (Sanches and Schreier 2020; Kumar et al. 2021) and rinsed with distilled water. For each water sample (including field negative controls), up to 1 L of water was filtered through a glass fiber filter using silicone tubing and a Series I Geopump™ peristaltic pump with attached Easy-load® II pump heads (Geotech Environmental Equipment, Inc., USA) (**Figure 2**).

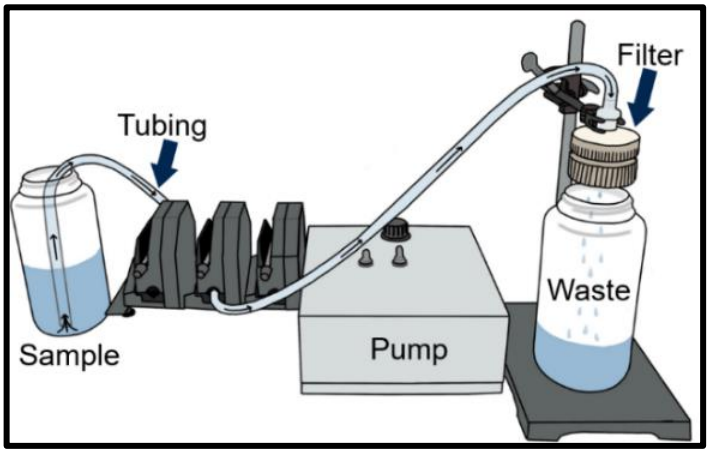


Figure 2. eDNA filtration setup. A peristaltic pump filtered sample water through silicone tubing and a glass fiber filter stored in a filter holder. Wastewater was discarded.

Due to the higher turbidity at some of the sites in this study, we opted for 1.6 µm glass fiber filters (Merck Millipore Ltd, Ireland) to decrease filtration time and limit filter clogging (Sanches and Schreier 2020; Kumar et al. 2021; Holmes et al. 2024). In the event of clogging, multiple filters were used until all 1 L of the water sample was filtered. No samples required more than three filters. Following filtration, filters were folded in half twice, so that any sediment collected on the filter would be folded inside, and then placed in 2 mL microcentrifuge tubes (Sanches and Schreier 2020). Tubes were then immediately bagged and placed on wet ice until they could be transported to a designated clean lab within 4 hours (Goldberg et al. 2016; Taberlet et al. 2018). Filters were stored in a designated clean lab freezer (-20°C) (Jerde et al. 2011; Sanches and Schreier 2020) until DNA extractions were performed within two months of the sampling dates.

3.3 | DNA extraction

All extractions were performed in a designated clean lab (Goldberg et al. 2016; Taberlet et al. 2018) in the Genomic Variation Laboratory in the Department of Animal Science at UC Davis. Workspaces were sanitized with 20% bleach and exposed to UV light for 20 minutes (Sanches and Schreier 2020; Kumar et al. 2021; 2022).

To maximize eDNA yield and minimize the effect of possible inhibitors, all filters were extracted using the DNeasy® PowerWater® Kit (Qiagen, Germany) (Kumar, Eble, and Gaither 2019; Shu, Ludwig, and Peng 2020; Eichmiller, Miller, and Sorensen 2016) following a modified manufacturer protocol. Modifications included: (1) using a separate filtration protocol from the manufacturer's recommendations (See section **3.2**), (2) two cycles of incubating the PowerWater Bead Pro Tubes at 65 °C for 10 minutes and vortexing for 5 minutes, (2) using between 200 and 400 µl of Solution IRS to accommodate the larger volume of supernatant produced by our filters,

(3) adding 150 µl Solution EB, and (4) incubating the filter membrane for 20 minutes at room temperature before centrifuging the final eluate down into low-bind tubes. Additionally, we could not centrifuge our PowerWater Bead Pro Tubes as recommended because we did not have a centrifuge attachment to accommodate the larger 5 mL tubes. An extraction negative control was processed identically in parallel with each batch of eDNA samples extracted.

Total eDNA concentrations were calculated using the Qubit® 4 Fluorometer and Qubit® dsDNA HS Assay Kits (Invitrogen, USA). Final DNA extracts were stored in our clean lab freezer (-20 ℃) until further downstream reactions (e.g., metabarcoding, qPCR) could be performed.

3.4 | PCR-based library preparation for metabarcoding

Before library construction, DNA extracts from samples that required multiple filters were combined in volumes equivalent to their filter volume. For example, if a 1L sample had 700 mL go through one filter and 300 mL through another, 70 and 30 µl of their DNA extracts were combined for all downstream reactions.

Illumina libraries for metabarcoding were constructed using the 16S fish-specific (Shaw et al. 2016; Zhang, Zhao, and Yao 2020) and 12S MiFish-U barcode primers (Miya et al. 2015). The first round of PCR was carried out using the locus-specific primers appended with Illumina sequencing primers (**Table S2**; **S3**, **Figure S1**). PCR amplifications were performed using the Bio-Rad T100™ Thermal Cycler (Bio-Rad Laboratories, Inc., USA) in a 25 µl total reaction volume. Each reaction contained 2 µl template eDNA, 9.5 µl DNase/ RNase-free water, 0.5 µl 10 µM forward primer, 0.5 µl 10 µM reverse primer, and 12.5 µl AmpliTaq Gold™ Fast PCR Master Mix (Applied Biosystems, USA) Every PCR plate included a no template control, and all

samples were replicated three times to minimize PCR bias and later pooled to reduce costs (Schmidt et al. 2013; Taberlet et al. 2018; Shirazi, Meyer, and Shapiro 2021). Thermocycler conditions were optimized to minimize off-target products while maximizing on-target primer annealing (**Table S4**; **S5**).

Following PCR amplification, technical replicates were combined and purified using Sera-Mag™ Select beads (Cytiva, USA) following the manufacturer's protocol for a left-side size selection at 1.8x beads to sample volume. Modifications included washing pellets with 80% ethanol and eluting in 10 nM Tris, pH 8.5 buffer to 40 µl. All PCR products were visualized for on-target amplicons with the Bio-Rad Sub-Cell GT Horizontal Electrophoresis System (Bio-Rad Laboratories, Inc., USA). Gels were created with 2% agarose (Denville Scientific, Inc., USA) and 1X GelStar® Nucleic Acid Stain (Lonza, Switzerland), then run in 1X TBE buffer (Bio-Rad Laboratories, Inc., USA) for about 40 minutes to 1 hr at 147V. The expected bands for the 16S fish-specific samples were about 184 bp on average, and for the 12S MiFish-U samples, approximately 291 bp (**Table 4**, **Figure S2**; **S3).**

Table 4. Expected average amplicon sizes for 16S fish-specific and 12S MiFish-U primers.

Locus	Ave. insert size (to be sequenced)	Ave. locus primer size	Ave. total amplicon size	Index primers (excludes overlap)	Ave. total indexed amplicon size
16S	70-Shaw et al. (2016)	114	184	69	253
12S	170-Miya et al. (2015)	121	291	65	356

The second round of PCR was carried out using primers constructed to bind to the first-round PCR products and the Illumina flow cell, as well as to uniquely index samples for downstream identification (**Table S6**; **S7**, **Figure S1**). To increase the number of samples sequenced in a single run, the Illumina index adaptors were changed for the 12S MiFish-U primers to include existing primers in the laboratory, which had been used successfully (Campbell, Harmon, and Narum 2015). However, because the Read 1 and 2 portions of these

index primers are obsolete, all 12S samples in this study required custom sequencing primers (**Table S3**; **S7**).

Second-round PCR amplifications were performed using the MiniAmp™ Thermal Cycler (Applied Biosystems, USA) in a 50 µl reaction volume. Each reaction contained 5 µl first-round purified PCR template DNA, 27 µl DNase/RNase-free water, 3 µl of 5 µM forward primer, 3 µl of 5 µM reverse primer, 10 µl 5X KAPA HiFi Fidelity Buffer, 1.5 µl KAPA dNTP Mix (10 mM each), and 0.5 µl 1 U/µL KAPA HiFi HotStart DNA Polymerase (Roche Sequencing Solutions, Inc., Switzerland). All index PCR runs included a no template control and were amplified per the manufacturer's protocol, with minor cycle length and cycle number modifications (**Table S8**). Following PCR amplification, all samples were bead purified following the same protocol as the first purification but eluted to 50 µl instead. Final PCR products were visualized for on-target indexed amplicons using gel electrophoresis. The expected bands for the 16S samples were about 253 bp, and for the 12S samples, approximately 356 bp (**Table 4**, **Figure S4**; **S5**).

After normalization, the 16S amplicons were pooled into three libraries for sequencing, while the 12S were pooled into one library due to the increase in dual-index combinations. All sequencing was carried out on an Illumina MiSeq by the DNA Technologies and Expression Analysis Core at the UC Davis Genome Center using a MiSeq Reagent Kit v3 (2 x 75 bp cycles) for the 16S and a MiSeq Reagent Kit v2 (2 x 150 bp) for the 12S. All libraries were combined with 10% PhiX control, and an additional purification using AMPure XP Bead-Based Reagent (Beckman Coulter, Inc., USA) was performed on the 12S library by the UC Davis DNA Technologies and Expression Analysis Core to remove excess off-target amplicons.

3.5 | Bioinformatics and sequence processing

All raw sequencing data was demultiplexed by the DNA Technologies and Expression Analysis Core at the UC Davis Genome Center. The demultiplexed data was processed for the 16S fish-specific and 12S MiFish-U libraries separately using *R v4.4.2* (R Core Team 2022). Primers were removed from either end of the sequencing reads using *Cutadapt v4.4* (Martin 2011). *Cutadapt* was also used to trim bases from the 5` and 3` ends of sequence reads when their Phred Quality (Q) score was below 30 (Miya et al. 2015). The percentage of trimmed bases for each sample was then analyzed using *MultiQC v1.25.2* (Ewels et al. 2016), and a subset of the reads were visualized using *FastQC v0.12.1* to determine if additional truncation was required (Andrews 2010). The remaining quality filtering, trimming, denoising, merging, and chimera removal were performed in their listed order with *DADA2 v1.34.0* (Rosen et al. 2012; Callahan et al. 2016). Default parameters were used except for the following modifications. For filtering and trimming, reads with more than two expected sequencing errors [$EE = sum(10^{-Q/10})$] were discarded (Shu, Ludwig, and Peng 2021) and 12S reads had 15 bp trimmed from their 3` end. We did not perform truncation quality trimming here as it was unclear which Illumina encoding system was used by *DADA2*'s *filterAndTrim()* function. Read quality was visualized for a subset of reads using *FastQC* to check filter performance. Following the denoising error rate parameter calculations, samples were pooled for final sequence inferences to help resolve rare ASVs (e.g., contaminant sequencing reads) that may be in samples. Remaining reads outside the specified size range were discarded (16S: 50-90 bp; 12S: 150-190 bp).

The number of contaminating reads for each ASV detected in negative controls (i.e., field, extraction, 1st PCR, and 2nd PCR negative controls) were subtracted from the same ASV in every sample using R. To minimize false positives while also limiting false negatives of species

that may be present at sample sites in extremely low abundances, 0.05% of a sample's total reads were removed from each ASV within that sample (Drake et al. 2022). Additionally, samples with a total read abundance of less than 0.05% of an assay's total read abundance were removed from data analyses. Taxonomic assignments were then performed using *DADA2* and a custom reference database for each barcode marker (R. Nagarajan, Holmes, and Schreier 2023). Unassigned ASVs were then assigned a taxonomic rank using BLAST® *BLASTN* v2.16.1+ (≥ 97% identity; ≤ 10-5 E-value; 100% query coverage) (Camacho et al. 2009; Miya et al. 2015).

3.6 | Data analyses

3.6.1 | Species detection summaries

In line with the scope of this study, if detected, only the taxa in the classes Actinopterygii (ray-finned fishes) or Petromyzontidae (lampreys) were included in analyses, while all other species were only identified and listed in the supplemental data. Overall sampling and sequencing efficiency were assessed using the R package *vegan v2.6.8* by creating species rarefaction (with 10 steps) and accumulation curves (1000 permutations) (Oksanen et al. 2024).

We used quality-filtered reads from every paired restoration/reference site to create presence/absence matrices displaying species detections for each assay and individual sample. The percent agreement between the 16S and 12S barcodes for a particular species detected in a sample was calculated for each species and averaged for an overall percent agreement between barcodes. We created an additional presence/absence matrix of species detections combined for each site to summarize each assay's overall detections.

The read and relative read abundances of the top ten species with the most abundant reads across all samples were visualized with bar plots using *phyloseq*. Multiple plots were made

to visualize read and relative read differences between biological replicates, sub-sites, and sites. Bar plots with biological replicate read data showed detections for every one-liter water sample collected. For the plots displaying sub-site differences, read data from the three biological replicate samples within each sub-site were combined, and for the plots displaying site differences, read data from the three sub-sites within each site were combined.

Additional bar plots were created to display combined relative read abundances of native fish assemblages at each site and sampling season. If detected, bar plots of read abundances were also displayed for Chinook Salmon, Longfin Smelt, Delta Smelt, and White Sturgeon.

3.6.2 | Statistical Analyses

We performed the following statistical analyses to test if eDNA fish assemblages differ between restored tidal wetlands and reference sites in the SFE. All analyses were performed separately for each assay. Following the scope of this study, only fish reads were considered.

Alpha diversity indices were calculated and visualized using the R packages *phyloseq v1.5.0, stats v4.4.2*, and *tidyr v1.3.1* (McMurdie and Holmes 2013; R Core Team 2022; Hadley Wickham, Davis Vaughan, and Maximilian Girlich 2024). For all sampling seasons (Feb-Mar 2023; Aug-Sept 2023; Dec 2023-Jan 2024), we calculated the Shannon diversity index and species richness of every 1-L water sample using the *phyloseq* package for entire fish assemblages and native fish assemblages. The Shannon diversities for each site were also visualized using boxplots. Restoration and reference sites were tested for significant differences in species diversity and richness using Wilcoxon Rank Sum Tests (Wilcoxon 1945; Lahti and Shetty 2017; Huang et al. 2022). The Benjamini-Hochberg correction was used to control the false discovery rate of comparing many species across restoration and reference sites. Non-metric multidimensional scaling (NMDS) plots were performed in *phyloseq* and *ggplot2 v3.5.1*

(Wickham 2016) to further visualize the differences in whole community and native fish compositions between restored and reference sites.

4 | Results

4.1 | Sequencing summary

4.1.1 | eDNA water samples

The 16S assay generated 51,716,593 raw sequencing reads for the eDNA water samples (per sample reads: Average = 263,860; Median = 268,201; Min = 3; Max = 750,008) (**Table S9**), and the 12S assay generated 10,041,094 raw sequencing reads (per sample reads: Average = 50,970; Median = 56,842; Min = 7; Max = 83,140) (**Table S10**). One sample from Ryer in February 2023 was lost during DNA extraction and not submitted for sequencing for either assay. Additionally, for the 16S assay, one sample from Montezuma collected in January 2024 was not assigned a sample ID after demultiplexing by sample indexes and was not included in sequence processing. Following sequence filtering and merging, an additional 21 and 19 samples were removed from data analyses for the 16S and 12S assays, respectively, for having zero reads (denoted with a ▼ symbol in **Table S9**; **S10**). From the original 198 eDNA samples, 175 16S and 178 12S samples remained for data analyses. Around 14% of the 16S reads and 10% of the 12S reads were removed during sequence filtering. This left 44,630,023 quality filtered reads for 16S taxonomic assignments (per sample reads: Average = 227,704; Median = 232,457; Min (with taxonomic assignments) = 28,861 Max = 673,425), and 9,067,519 reads for the 12S (per sample reads: Average = 46,028; Median = 51,342; Min = 9,688; Max = 77,881).

4.1.2 | Negative control samples and sequence filtration thresholds

One 1st PCR negative control (NC) sample from the 16S assay was not assigned a sample ID after demultiplexing and not included in filtration and analysis. Across the remaining 104 NC samples (Field: 66; Extraction: 16; 1st PCR: 14; 2nd PCR: 8), the 16S assay generated 621,019 raw sequencing reads (per NC sample reads: Average = 5,971; Median = 701; Min = 1; Max = 186,243) (**Table S11**). Following sequence filtering and merging, six 16S NC samples (Field: 2; 1st PCR: 2; 2nd PCR: 2) were removed for having zero reads. The NC sample with the highest read count was a field NC from Yolo Flyway Farms restoration site during the December 2023 sampling period. After sequence filtering, 19,037 of this sample's 19,168 reads were assigned to the Menhaden (*Brevoortia*) genus. The NC sample with the second highest read count (8,190 reads) was a 2nd PCR control. After sequence filtering, 5,467 of this sample's reads aligned to Sacramento Splittail (*Pogonichthys macrolepidotus*), and 2,530 reads aligned to Pacific Herring (*Clupea pallasii*). Approximately 94% of the reads across all 16S NC samples were lost during sequence filtering, leaving 39,229 quality filtered control reads (per NC sample reads: Average = 338; Median = 42; Min = 1; Max = 19,168) that were removed from the eDNA water samples (see section **3.5**). After removing the contaminating Menhaden reads from all eDNA samples, this taxon was completely removed from the study. Prior to removal, Menhaden reads were found in 11 eDNA samples (Browns: 1; Flyway: 7; Liberty: 3), with sample read counts ranging from 1 to 735 (Average: 114; Median: 5). Given that the SFE is not within the distribution ranges for *Brevoortia* species (Pozzobon et al. 2021), it is more likely their DNA was contamination from animal feed at the filtering site (A. Holmes, personal communication, Feb 23, 2025). All eDNA filtering was performed in a livestock facility at UC Davis to minimize contamination from other fish species because other available spaces had high-quality fish DNA present.

One 12S field NC sample from Ryer in February 2023 was partially lost during DNA extraction and depleted during 16S sequencing. Therefore, this NC could not be submitted for 12S sequencing. Four 12S NC samples (Extraction: 1; 1st PCR: 1; 2nd PCR: 2) were not assigned a sample ID after demultiplexing and were not included in analyses. Across the remaining 106 12S NC samples (Field: 65; Extraction: 15; 1st PCR: 20; 2nd PCR: 6), the 12S assay generated 62,017 raw sequencing reads (per NC sample reads: Average = 585; Median = 30; Min = 1; Max = 13,849) (**Table S12**). Following sequence filtering and merging, 3 12S NC samples (Field: 1; 1st PCR: 2) were removed due to having zero reads. The NC sample with the highest read count for the 12S assay originated from Decker during the February 2023 sampling period, where 495 of its 546 reads aligned with human DNA. Approximately 96% of reads across all 12S NC samples were removed during data processing and filtering, leaving 2,780 quality filtered control reads (per NC sample reads: Average = 27; Median = 17; Min = 0; Max = 546) that were removed from the eDNA water samples (see section **3.5**).

Other taxa removed from 16S eDNA samples after contaminating reads were subtracted include Wakasagi (*Hypomesus nipponensis*) and White Sturgeon (**Table S13**). Tule Perch (*Hysterocarpus traskii*) and Cattle (*Bos taurus*) were removed after the 0.05% assay total read count threshold was applied. Human eDNA was detected across two extraction NCs (94 reads), and another 24 reads were found across four field NCs and two eDNA water samples. For the 12S assay, Longfin Smelt, North American River Otter (*Lontra canadensis*), and American Bullfrog (*Lithobates catesbeianus*) were removed after the 0.05% assay total read count threshold was applied (**Table S13**). Sixty-seven reads of Human eDNA in the 12S assay were detected across eight eDNA water samples, and 555 reads were detected across seven field NCs.

4.2 | Species detection summaries

4.2.1 | ASV Assignments

For the 16S assay, 112 amplicon sequence variants (ASVs) were identified from the quality-filtered reads across all samples, which were assigned to 44 vertebrate taxa (3 classes, 15 orders, 24 families, 38 genera, and 40 species) (**Table 5; Table S14-S18**). Two Mammalian species were detected: Virginia Opossum (*Didelphis virginiana*) and Elk (*Cervus spp*). Four invertebrates were detected in the Phylactolaemata class (Freshwater bryozoans). The remaining 38 species detected belonged to the Actinopterygii (ray-finned fishes) class. One of these fishes was identified at the genus level (Killifish—*Lucania spp*) and another at the family level (Sculpin—Cottidae *spp*) due to high sequence similarities in the amplified barcode regions. The other 36 fish were resolved down to their species level.

For the 12S assay, a total of 149 ASVs were identified, which were assigned to 45 vertebrates (3 classes, 16 orders, 25 families, 38 genera, and 41 species) (**Table 5; Table S14-S18**). Four mammals were detected: Cattle (*Bos taurus*), North American Beaver (*Castor canadensis*), Wild Boar (*Sus scrofa*), and California Vole (*Microtus californicus*). Additionally, one bird species was detected (Wood Duck—*Aix sponsa*). The remaining 39 species detected belonged to the Actinopterygii class. Like the 16S assay, Killifish were identified at the genus level and Sculpin to the family level. The 12S assay also detected tuna eDNA to the genus level (*Thunnus spp*) in a single sample. The rest of the 36 fish were resolved to their species level.

Using the presence/absence data from **Table S14-S18** columns, for each sample we calculated the percent agreement between the 16S and 12S barcodes for a particular species being detected in that sample. The percent agreement between barcodes was then averaged

across samples to be 43%. Following the scope of this study, only taxa in class Actinopterygii were considered for analysis. After removing non-fish reads, one sample was discarded from the 16S assay (leaving 181 samples) and three from the 12S (182 samples).

4.2.2 | Overall sampling effort and sequencing depth

Species accumulation plots (**Figure 3**) displayed a similar relationship between overall species richness and the number of samples collected. The 16S assay curve reached a clear plateau, indicating that enough water samples were collected throughout the study to detect species present using that barcode. Although the 12S assay appears close to plateauing, there was a full saturation in species richness for that barcode. Sample-based species rarefaction curves also showed clear plateaus across most samples, indicating adequate sequencing depth (**Figure S6**).

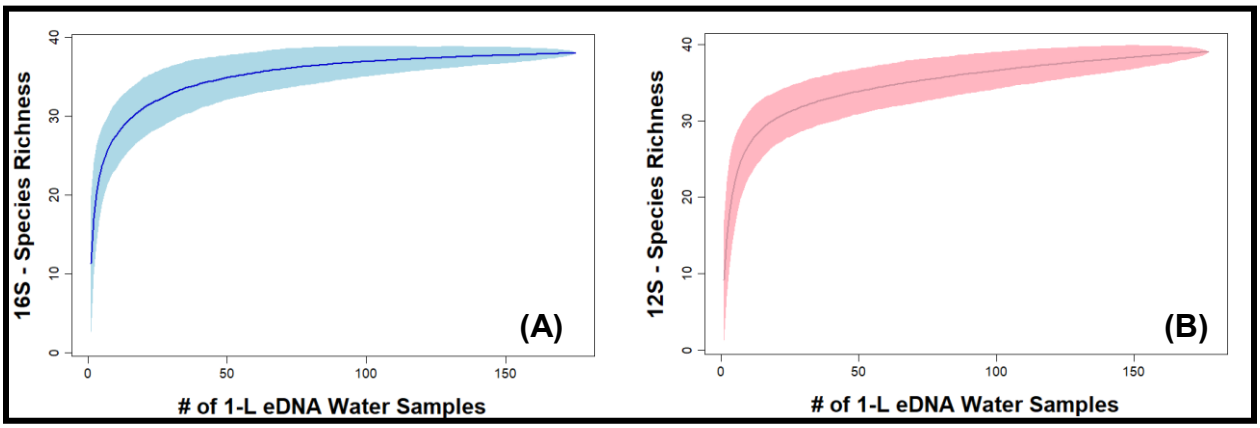


Figure 3. Species accumulation curves. Species richness plotted against overall sampling effort (i.e., the # of 1-L water samples collected). Lighter color = 95% confidence intervals (A) 16S fish-specific barcode, with 175 samples. (B) 12S MiFish-U barcode with 178 samples.

Table 5. eDNA fish species detections after quality sequence filtering. Seasonal data combined for sites. ✓ = Positive detection [Blue ("16S") = 16S only. Pink ("12S") = 12S only. Green ("Both") = both assays.] *Sites*: Browns Island (BRO); Montezuma Wetlands (MON); Winter Island (WIN); Liberty Island (LIB); Yolo Flyway Farms (FLY); Ryer Island (RYE); Tule Red (TUL); Webb Tract Island and Berms (WEB); Decker Island (DEC). ± = Restoration site; † = Reference. Non-fish and individual sample detections in **Table S14-S18**.

Common name (*Species name*)	**16S**	**12S**	**BRO†**	**MON±**	**WIN±**	**RYE†**	**TUL±**	**LIB†**	**FLY±**	**WEB†**	**DEC±**
***California Roach** (*Hesperoleucus symmetricus*)		✓	12S		12S			12S	12S		
***Chinook Salmon** (*Oncorhynchus tshawytscha*)	✓	✓	Both	Both	Both	Both	Both	Both	Both		Both
***Delta Smelt** (*Hypomesus transpacificus*)	✓					16S					
***Hardhead** (*Mylopharodon conocephalus*)	✓	✓		16S				16S	12S		16S
***Hitch** (*Lavinia exilicauda*)	✓	✓	Both	Both	Both	16S		Both	Both	16S	Both
***Jack Silverside** (*Atherinopsis californiensis*)	✓	✓				Both	16S				
***Longfin Smelt** (*Spirinchus thaleichthys*)	✓		16S	16S	16S	16S	16S	16S			16S
***Rainbow Trout** (*Oncorhynchus mykiss*)	✓	✓	12S	Both	Both	12S	Both	Both		Both	Both
***Sacramento Blackfish** (*Orthodon microlepidotus*)	✓	✓						12S	Both		
***Sacramento Pikeminnow** (*Ptychocheilus grandis*)	✓	✓	Both	Both	Both	Both	Both	Both	Both	Both	Both
***Sacramento Splittail** (*Pogonichthys macrolepidotus*)	✓	✓	Both	Both	Both	Both	Both	Both	Both	16S	12S
***Sacramento Sucker** (*Catostomus occidentalis*)	✓	✓	Both	Both	Both		16S	Both	Both	Both	Both
***Sculpin** (Cottidae *spp*)	✓	✓	Both	Both	Both	Both	Both	Both	Both	Both	Both
***Starry Flounder** (*Platichthys stellatus*)	✓	✓		Both	16S		16S	16S			
***Threespine Stickleback** (*Gasterosteus aculeatus*)	✓	✓	Both	Both	Both	Both	Both	16S	12S		Both
***Tule Perch** (*Hysterocarpus traskii*)		✓	12S	12S	12S	12S	12S	12S	12S	12S	12S
***White Sturgeon** (*Acipenser transmontanus*)		✓	12S	12S	12S	12S	12S	12S	12S		
American Shad (*Alosa sapidissima*)	✓	✓	Both	Both	16S	Both	Both	Both	Both		16S
Bigscale Logperch (*Percina macrolepida*)	✓	✓	12S		12S		12S	Both	Both	16S	
Black Bullhead (*Ameiurus melas*)		✓									12S
Black Crappie (*Pomoxis nigromaculatus*)	✓	✓	Both	16S				12S	Both	16S	
Bluegill (*Lepomis macrochirus*)	✓	✓	Both	16S	Both		12S	Both	Both	Both	Both
Channel Catfish (*Ictalurus punctatus*)	✓	✓	16S		16S				Both		
Common Carp (*Cyprinus carpio*)	✓	✓	Both	Both	Both	Both	Both	Both	Both	Both	16S
Fathead Minnow (*Pimephales promezlas*)	✓			16S	16S		16S	16S	16S		

Golden Shiner (*Notemigonus crysoleucas*)	✓	✓	Both	16S	Both			Both	Both	Both	Both
Goldfish (*Carassius auratus*)	✓	✓	Both	Both	Both			Both	Both	16S	Both
Killifish (*Lucania spp*)	✓	✓	Both	16S	Both	16S	16S	Both	Both	16S	Both
Largemouth Bass (*Micropterus salmoides*)	✓	✓	Both	Both	Both			Both	Both	Both	12S
Mississippi Silverside (*Menidia audens*)	✓	✓	Both	Both	Both	Both	Both	Both	Both		Both
Mosquitofish (*Gambusia affinis*)	✓	✓	Both	Both	Both	Both	Both	Both	Both	Both	Both
Northern Anchovy (*Engraulis mordax*)	✓	✓	12S	12S	Both	16S					
Pacific Herring (*Clupea pallasii*)	✓	✓	12S	Both		Both	Both				
Redear Sunfish (*Lepomis microlophus*)	✓	✓	Both	16S	16S	16S		Both	Both	Both	Both
Shimofuri Goby (*Tridentiger bifasciatus*)	✓	✓	Both	Both	Both	Both	Both	Both	Both	16S	Both
Shokihaze Goby (*Tridentiger barbatus*)	✓	✓	Both	Both	Both	Both				16S	Both
Striped Bass (*Morone saxatilis*)	✓	✓	Both	Both	Both	Both	Both	Both	Both	16S	16S
Threadfin Shad (*Dorosoma petenense*)	✓	✓	Both	Both	Both	Both	Both	Both	Both	Both	Both
Tuna (*Thunnus spp*)		✓									12S
Warmouth (*Lepomis gulosus*)	✓	✓						16S	Both	Both	
White Catfish (*Ameiurus catus*)	✓	✓						Both	16S	16S	
White Crappie (*Pomoxis annularis*)	✓								16S		
Yellowfin Goby (*Acanthogobius flavimanus*)	✓	✓	Both	Both	Both	Both	Both	Both	Both		
Total Species	**38**	**39**	**31**	**32**	**31**	**25**	**25**	**33**	**32**	**23**	**27**

4.2.3 | Species Detections

For the 16S assay, the 10 species with the greatest number of quality filtered reads across 175 eDNA samples were [1] Sacramento Splittail (*Pogonichthys macrolepidotus*), [2] Common Carp (*Cyprinus carpio*), [3] Sculpin (Cottidae *spp*), [4] Striped Bass (*Morone saxatilis*), [5] Hitch (*Lavinia exilicauda*), [6] Sacramento Pikeminnow (*Ptychocheilus grandis*), [7] Threespine Stickleback (*Gasterosteus aculeatus*), [8] Yellowfin Goby (*Acanthogobius flavimanus*), [9] Mosquitofish (*Gambusia affinis*), and [10] Shimofuri Goby (*Tridentiger bifasciatus*) (**Figure 4**). These species account for the greatest share of sequencing reads for many sites and seasons. Ryer and Webb Tracts in February 2023 are the only instances where the top ten species with the highest reads account for less than 50% of the reads from those sites.

For the 12S, the 10 species with the greatest number of filtered reads across 178 eDNA samples were [1] Sculpin *spp*, [2] Sacramento Splittail, [3] Yellowfin Goby, [4] Common Carp, [5] Threespine Stickleback, [6] Tule Perch (*Hysterocarpus traskii*), [7] Mississippi Silverside (*Menidia audens*), [8] Largemouth Bass (*Micropterus salmoides*), [9] White Sturgeon, and [10] Hitch (**Figure 5**). Several of these species also account for the greatest share of sequencing reads for the 16S. Similar to the 16S assay, the reads for the top 10 species at Ryer and Webb Tracts in February 2023 accounted for less than 50% of the reads from those sites.

Seven species with the most abundant reads across assays are native to the SFE. For the 16S assay, Sacramento Splittail reads were the most abundant at Browns, Montezuma, Ryer, and Tule across all seasons. However, most Sacramento Splittail reads were detected between August and September 2023. Sculpin reads were most abundant between February and March 2023 at Browns, Montezuma, Winter, Liberty, Webb Tracts, and Decker. Hitch reads were mainly detected at Liberty and Flyway in August and December 2023. Sacramento Pikeminnow reads

were most abundant at Winter Island in December 2023. However, they were also high at Browns and Montezuma in August 2023, and Winter in February 2023. Threespine Stickleback was most abundant at Montezuma and Ryer in February 2023, Winter and Tule in December 2023, and Montezuma in January 2024.

For the 12S assay, the native species that showed a similar trend in relative read abundances across sites were Sculpin, Hitch, Sacramento Splittail, Threespine Stickleback, and Yellowfin Goby. However, in contrast to the 16S data, Sacramento Pikeminnow did not meet the threshold to be included in the top 10% species detected in the 12S assay, although it was frequently detected (**Table 5**). Native species with a high abundance of reads in the 12S assay, but not the 16S, include Tule Perch and White Sturgeon. For the 12S, Tule Perch reads were detected most at Tule in February 2023, Browns and Montezuma in August, Ryer in September, and Browns and Winter in December 2023. White Sturgeon reads were primarily found at Tule in February 2023 and Browns, Winter, Ryer, and Liberty in December 2023.

In total, the 16S assay detected 14 native species (**Figure 6**), and the 12S assay detected 15 (**Figure 7**). Most of these detections were shared between both assays; however, the 16S was the only one to detect Delta Smelt and Longfin Smelt, but unlike the 16S, the 12S assay was able to detect California Roach, Tule Perch, and White Sturgeon. In several instances, the reads belonging to native fish species accounted for more than 50% of that site's relative read abundance. These instances include Browns, Montezuma, and Winter across all three sampling seasons, except for Montezuma in September 2023 for the 12S assay. Additionally, reads from Tule and Decker in February, Liberty in March, and Tule in September and December 2023 were mainly comprised of native species for both assays. For the 12S assay, Tule in December 2023 had more than 50% of reads belonging to native fish species.

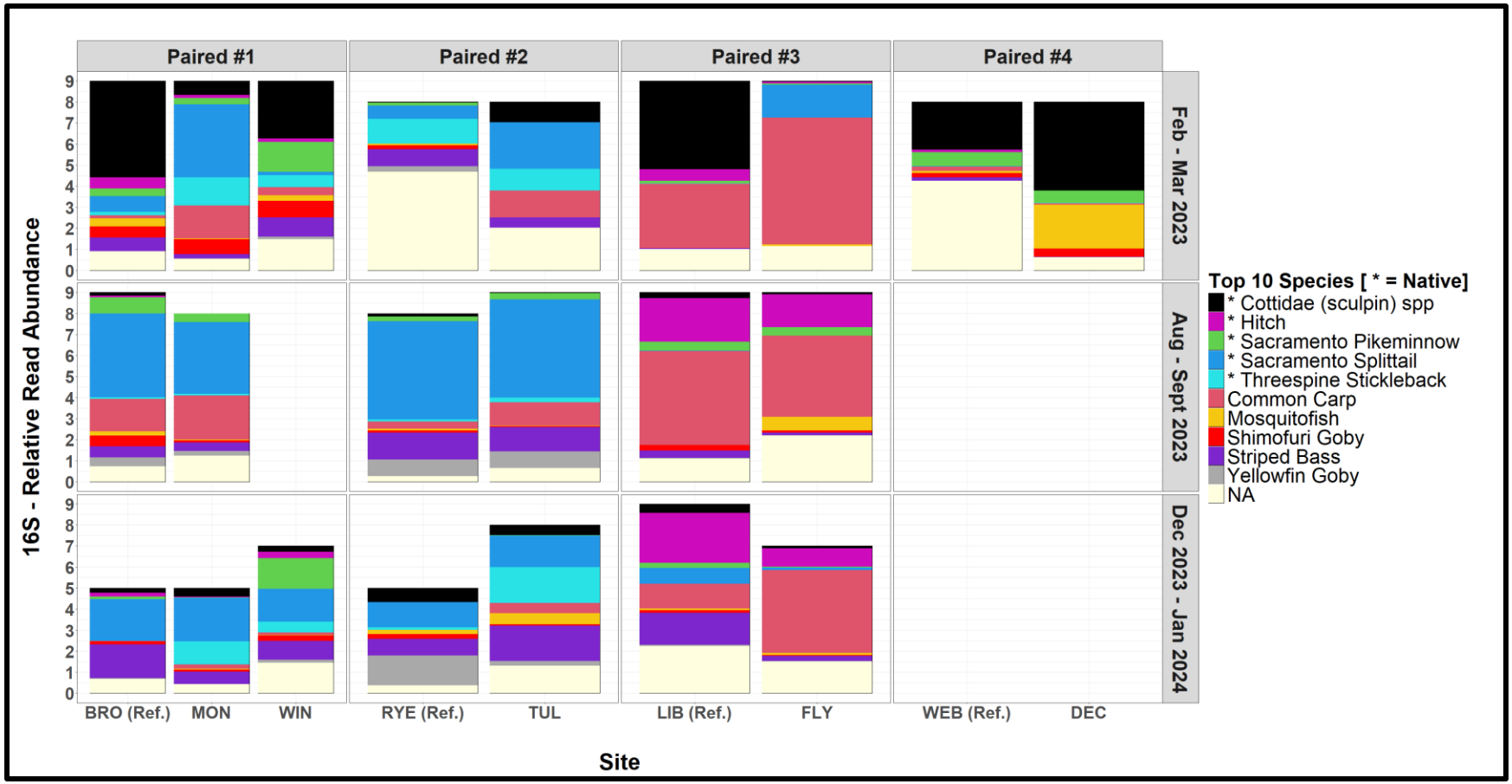


Figure 4. 16S relative read abundances for the top 10 species with the highest read counts. To calculate relative read abundances, we summed across 3 sub-sites and 3 biological replicates from each sub-site for each site. In addition to compounded relative read abundance, the y-axis also corresponds to the number of samples included in each site. There are 9 samples max per site, but some sites had samples removed during sequence filtering process. **NA** = Remaining species. **Ref.** = Reference site. Paired sites include Browns Island (BRO)/Montezuma Wetlands (MON)/Winter Island (WIN); Liberty Island (LIB)/Yolo Flyway Farms (FLY); Ryer Island (RYE)/Tule Red (TUL); Webb Tract Island and Berms (WEB)/Decker Island (DEC).

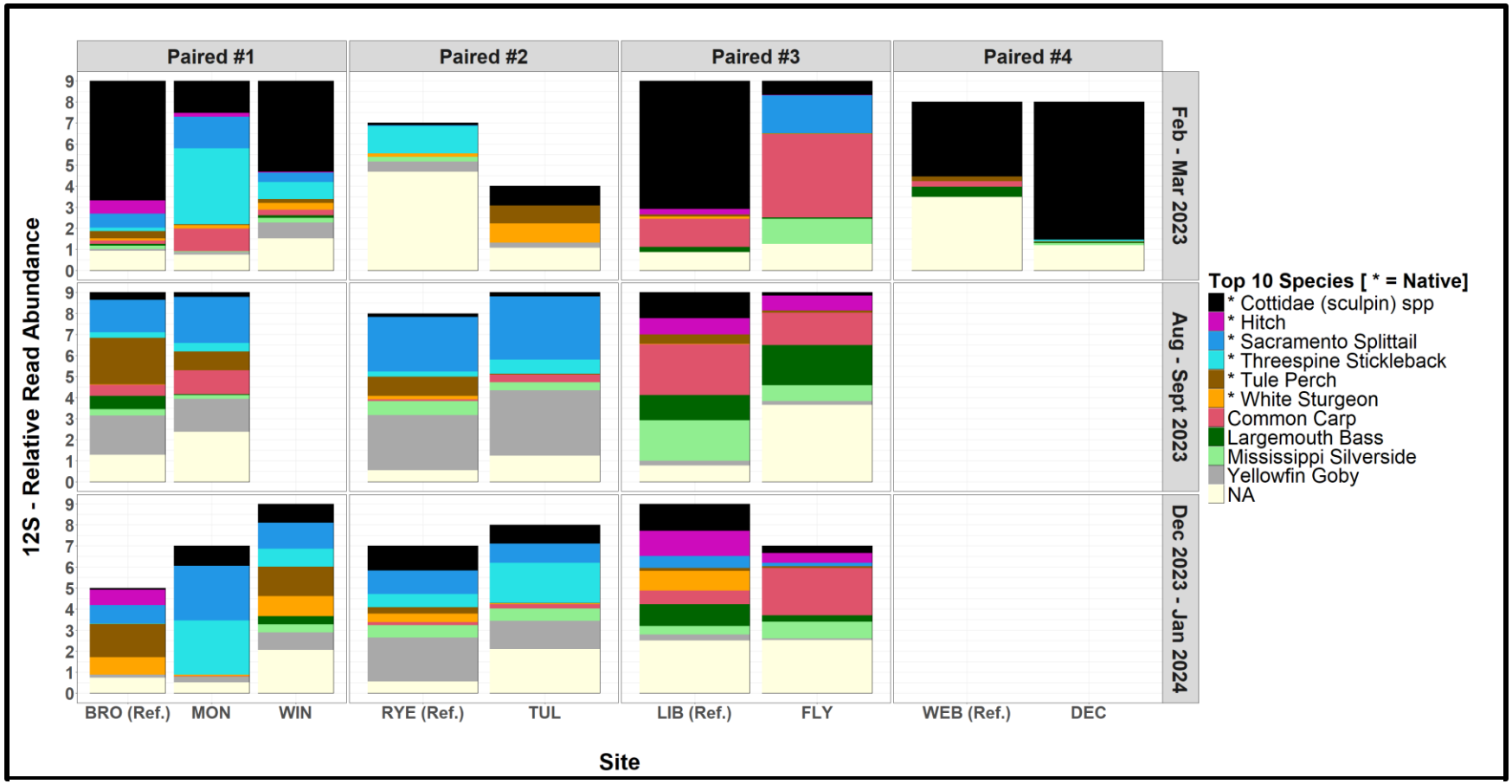


Figure 5. 12S relative read abundances for the top 10 species with the highest read counts. To calculate relative read abundances, we summed across 3 sub-sites and 3 biological replicates from each sub-site for each site. In addition to compounded relative read abundance, the y-axis also corresponds to the number of samples included in each site. There are 9 samples max per site, but some sites had samples removed during sequence filtering process. **NA** = Remaining species. **Ref.** = Reference site. Paired sites include Browns Island (BRO)/Montezuma Wetlands (MON)/Winter Island (WIN); Liberty Island (LIB)/Yolo Flyway Farms (FLY); Ryer Island (RYE)/Tule Red (TUL); Webb Tract Island and Berms (WEB)/Decker Island (DEC).

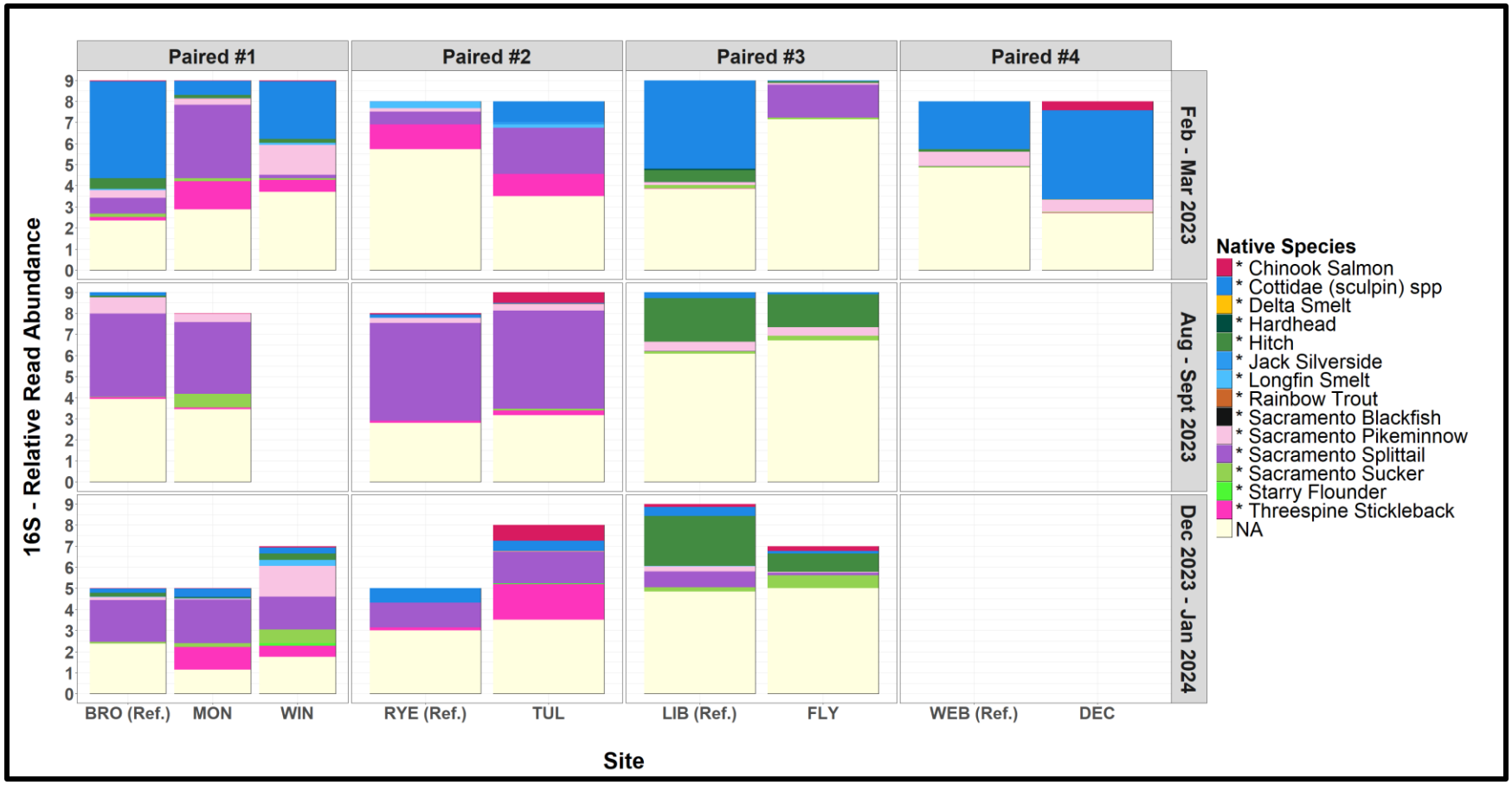


Figure 6. 16S relative read abundances for native fishes. To calculate relative read abundances, we summed across 3 sub-sites and 3 biological replicates from each sub-site for each site. In addition to compounded relative read abundance, the y-axis also corresponds to the number of samples included in each site. There are 9 samples max per site, but some sites had samples removed during sequence filtering process. **NA** = Remaining (non-native) species. **Ref.** = Reference site. Paired sites include Browns Island (BRO)/Montezuma Wetlands (MON)/Winter Island (WIN); Liberty Island (LIB)/Yolo Flyway Farms (FLY); Ryer Island (RYE)/Tule Red (TUL); Webb Tract Island and Berms (WEB)/Decker Island (DEC).

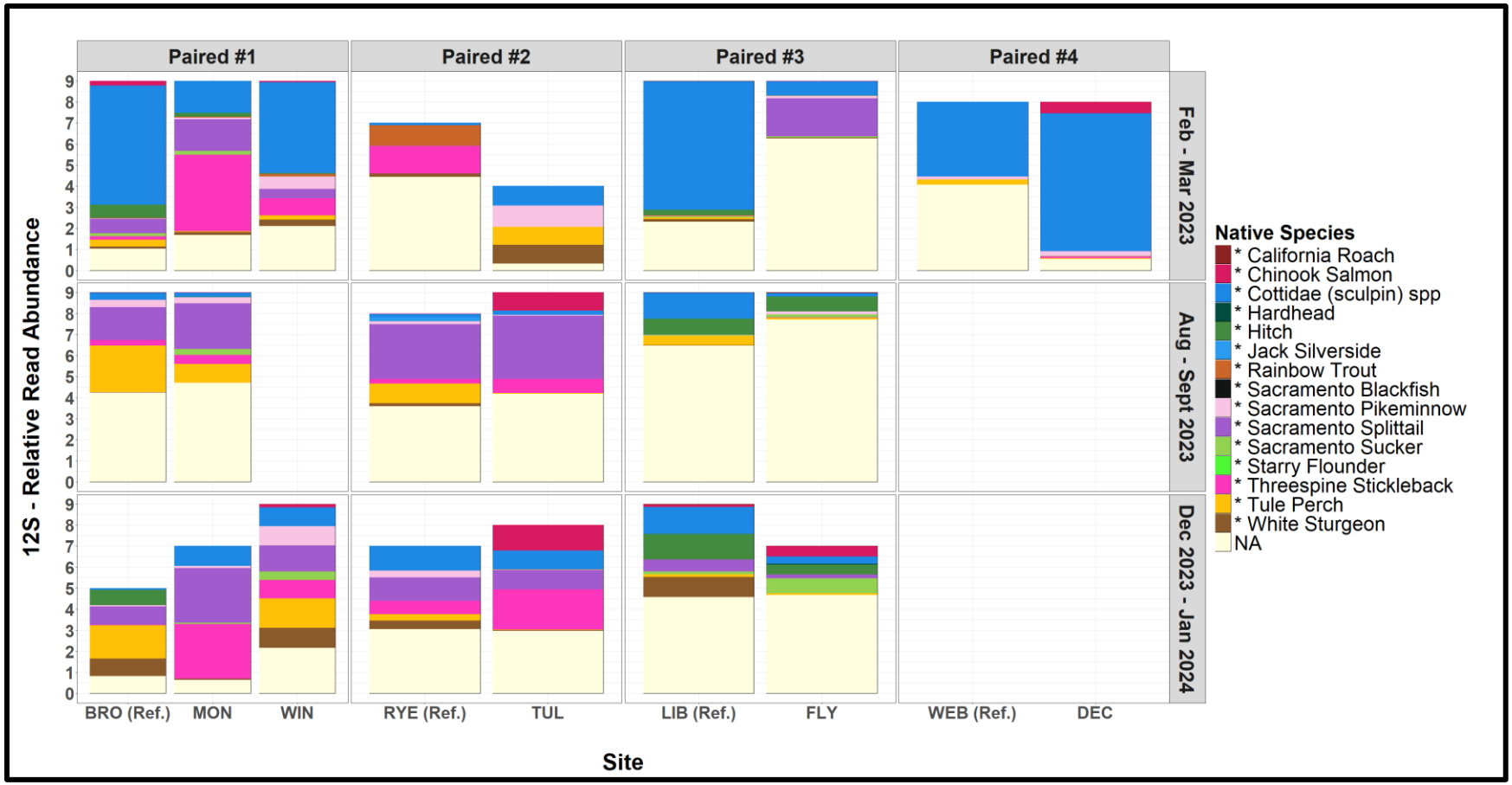


Figure 7. 12S relative read abundances for native fishes. To calculate relative read abundances, we summed across 3 sub-sites and 3 biological replicates from each sub-site for each site. In addition to compounded relative read abundance, the y-axis also corresponds to the number of samples included in each site. There are 9 samples max per site, but some sites had samples removed during sequence filtering process. **NA** = Remaining (non-native) species. **Ref.** = Reference site. Paired sites include Browns Island (BRO)/Montezuma Wetlands (MON)/Winter Island (WIN); Liberty Island (LIB)/Yolo Flyway Farms (FLY); Ryer Island (RYE)/Tule Red (TUL); Webb Tract Island and Berms (WEB)/Decker Island (DEC).

The Common Carp was the non-native species with the highest number of reads assigned for both assays (**Figures 4; 5**). Their reads were the most abundant at Liberty and Flyway throughout all sampling seasons. They were also very abundant at Montezuma and Tule in February, Browns, Montezuma in August and Tule in September 2023. For the 12S assay, Mississippi Silverside reads were found commonly at Flyway for all seasons: Liberty in August and December, Ryer and Tule in September and December, Browns in August, and Winter in December. Largemouth Bass reads were found at Flyway and Liberty in August and December, Browns in August, and Winter in December.

Boxplots of read abundances for species with high sequencing reads can be found in **Figure S7-S8**, along with boxplots of read abundances and relative abundances for sub-sites (**Figure S9-S12**) and biological replicates (**Figure S13-S20**).

4.2.4 | Detections for species-of-concern

Several native species of management concern were identified among sites, including Chinook Salmon, Longfin Smelt, Delta Smelt, and White Sturgeon. The 16S had a higher overall sequencing depth (**Figure S6**), but both assays had similar detections for Chinook Salmon. The 16S detected Chinook at 15 sampling events, with most reads at Tule in September and December 2023 (**Figure 8**). The 12S detected Chinook at 12 events, with most of its reads also at Tule in September and December 2023. The 16S assay detected Longfin Smelt at 11 events and Delta Smelt at one (**Figure 9**). The sites with the most reads for Longfin Smelt were Ryer in February and Winter in December 2023. Delta Smelt was only detected at Ryer in February 2023. The 12S did not detect either smelt. Only the 12S assay detected White Sturgeon (**Figure 10**), with most of its reads at Tule and Winter in February and Browns, Winter, Ryer, and Liberty in December 2023.

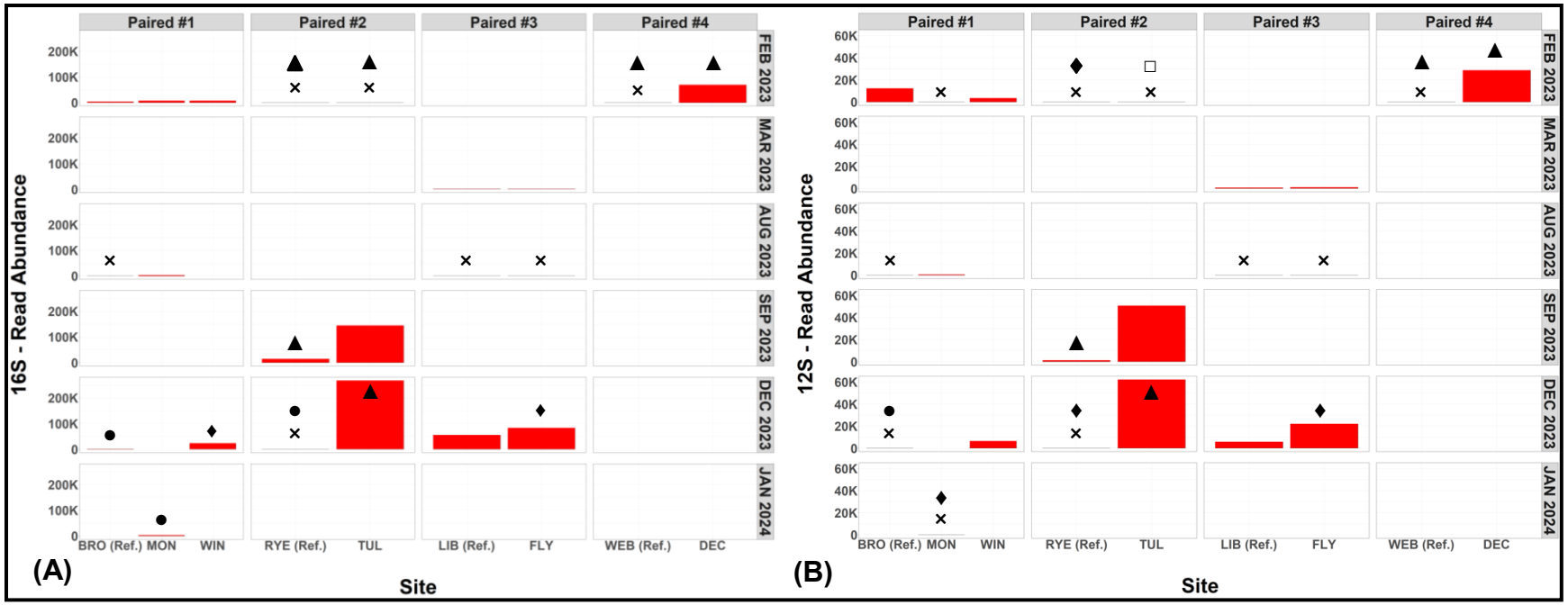


Figure 8. Read abundances for Chinook Salmon. (A) 16S assay (B) 12S assay. Each bar = 3 sub-sites and 3 biological replicates from each sub-site (9 samples max). ▲ = Sites with 8 samples. ♦ = Sites with 7 samples. ● = Sites with 5 samples. □ = Sites with 4 samples. "×" = No detection (this is shown due to the difficulty in visually differentiating the sites with only a few reads to sampled sites with zero reads). **Ref.** = Reference sites. **Paired sites**: Browns Island (BRO)/Montezuma Wetlands (MON)/Winter Island (WIN); Liberty Island (LIB)/Yolo Flyway Farms (FLY); Ryer Island (RYE)/Tule Red (TUL); Webb Tract Island and Berms (WEB)/Decker Island (DEC).

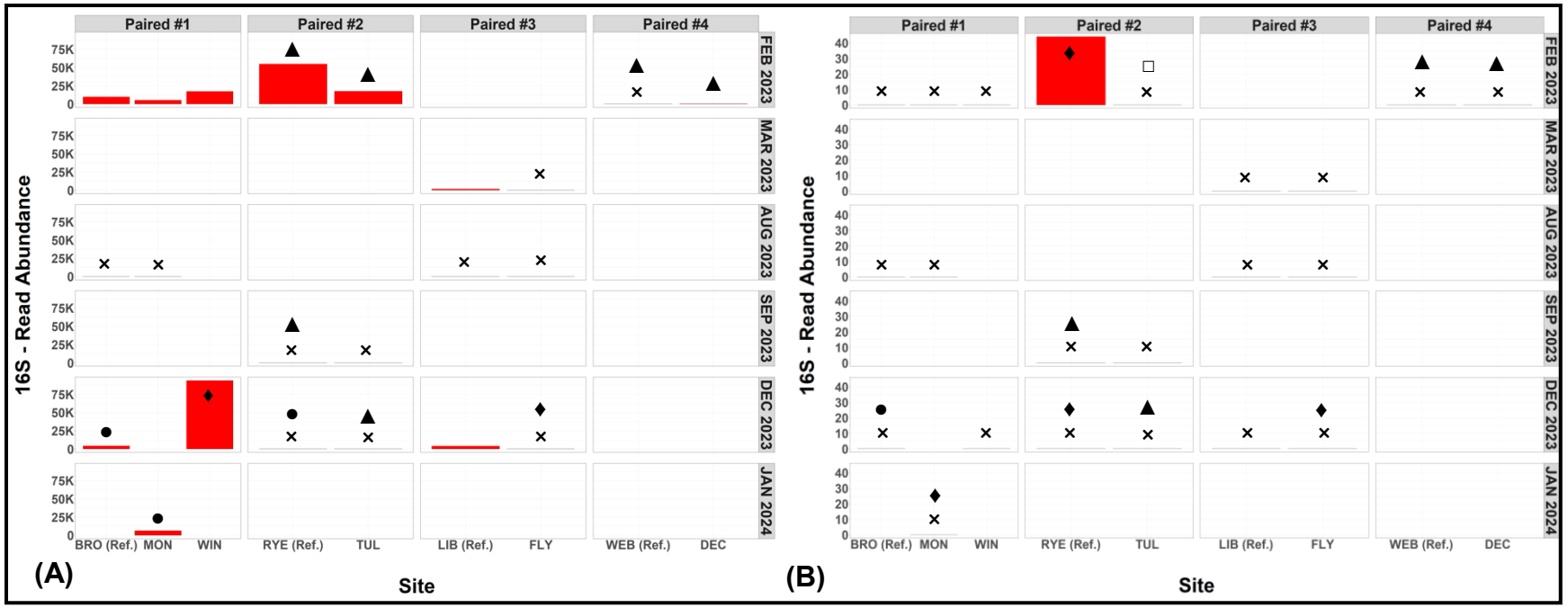


Figure 9. 16S read abundances for Longfin Smelt and Delta Smelt. (A) Longfin Smelt (B) Delta Smelt. Each bar = 3 sub-sites and 3 biological replicates from each sub-site (9 samples max). ▲ = Sites with 8 samples. ♦ = Sites with 7 samples. ● = Sites with 5 samples. □ = Sites with 4 samples. "×" = No detection (this is shown due to the difficulty in visually differentiating the sites with only a few reads to sampled sites with zero reads). **Ref.** = Reference sites. **Paired sites**: Browns Island (BRO)/Montezuma Wetlands (MON)/Winter Island (WIN); Liberty Island (LIB)/Yolo Flyway Farms (FLY); Ryer Island (RYE)/Tule Red (TUL); Webb Tract Island and Berms (WEB)/Decker Island (DEC).

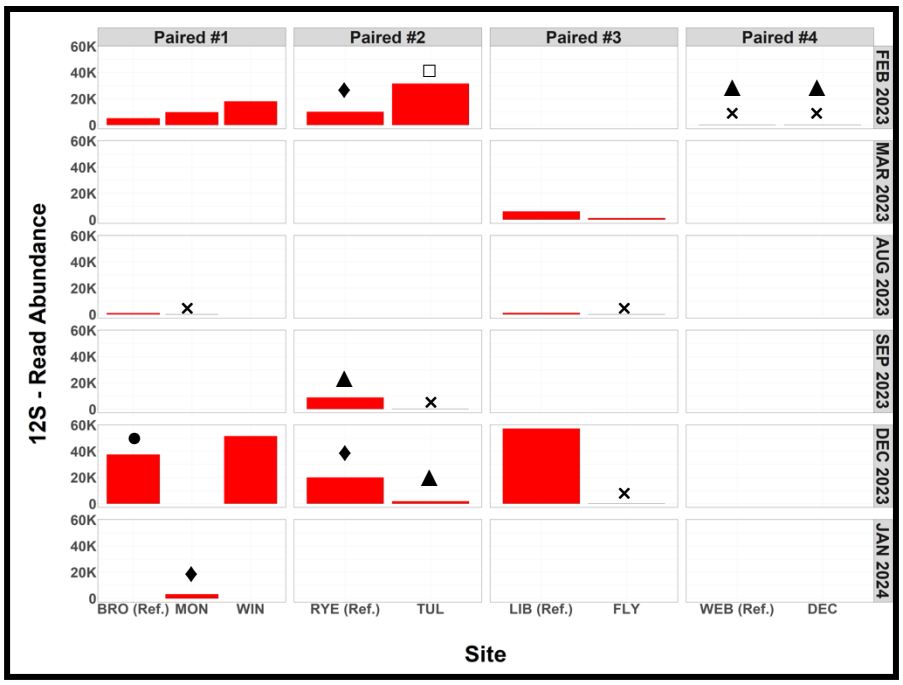


Figure 10. 12S read abundances for White Sturgeon. Each bar = 3 sub-sites and 3 biological replicates from each sub-site (9 samples max). ▲ = Sites with 8 samples. ♦ = Sites with 7 samples. ● = Sites with 5 samples. □ = Sites with 4 samples. "×" = No detection (this is shown due to the difficulty in visually differentiating the sites with only a few reads to sampled sites with zero reads). **Ref.** = Reference sites. **Paired sites**: Browns Island (BRO)/Montezuma Wetlands (MON)/Winter Island (WIN); Liberty Island (LIB)/Yolo Flyway Farms (FLY); Ryer Island (RYE)/Tule Red (TUL); Webb Tract Island and Berms (WEB)/Decker Island (DEC).

4.3 | Statistical Analyses

4.3.1 | Alpha Diversity

For each assay, Wilcoxon rank-sum tests ($\alpha = 0.05$) with Benjamini-Hochberg correction were used to determine if differences in species alpha diversity (Shannon Diversity Index) between paired sites were significant (**Figure 11**). Diversity values considered the richness and evenness of sequencing reads belonging to each species. For the 16S and 12S assays, 4 of the 12 paired site comparisons had significantly different species alpha diversity. The assays agreed that the following paired sites had significant differences in species diversity: Webb Tracts/Decker in Feb-Mar (16S: $p<0.01$, 12S: $p<0.05$) and Liberty/Flyway in Aug-Sept (16S: $p<0.05$, 12S:

$p<0.01$). Between these paired sites, Webb Tracts and Flyway had a higher overall diversity compared to their paired sites. The 16S assay showed significantly higher diversity in Winter over Brown in Feb-Mar ($p<0.05$) and Dec-Jan 2023 ($p<0.01$). The 12S assay found significantly higher diversity in Flyway over Liberty in Feb-Mar ($p<0.01$) and Tule over Ryer in Dec-Jan ($p<0.05$).

Fewer significant differences were found in species richness between paired sites than for species alpha diversity (**Figure 12**). Using Wilcoxon rank-tests to compare paired sites, both assays agreed that Flyway had significantly higher species richness over Liberty in Aug-Sept 2023 ($p<0.01$). The 16S additionally found that Tule had significantly higher species richness over Ryer in Dec-Jan ($p<0.05$), and the 12S indicated significantly higher species richness in Flyway over Liberty and Decker over Webb Tracts in Feb-Mar ($p<0.05$), and Ryer over Tule in Aug-Sept ($p<0.05$).

Native fish alpha diversity was also compared for each assay (**Figure 13**). The assays agreed that the following sites had significantly higher diversity among native species than their paired site: Winter over Browns (16S: $p<0.01$, 12S: $p<0.05$) and Tule over Ryer in Dec-Jan (16S/12S: $p<0.05$). For 16S, Tule had a significantly higher species diversity than Ryer in Aug-Sept. The 12S assay detected significantly higher native species richness in Montezuma over Browns ($p<0.05$) and Flyway over Liberty in Feb-Mar ($p<0.05$).

Native species richness was also compared between paired sites (**Figure 14**). For the 16S, Tule in Dec-Jan 2023 and Decker in Feb-Mar 2023 showed significantly higher ($p<0.05$ & 0.01, respectively) native species richness than their paired reference counterparts, Ryer and Webb Tracts. For the 12S, native species richness was significantly higher for Montezuma over Browns in Aug-Sept ($p<0.05$) and Decker over Webb Tracts in Feb-Mar ($p<0.01$).

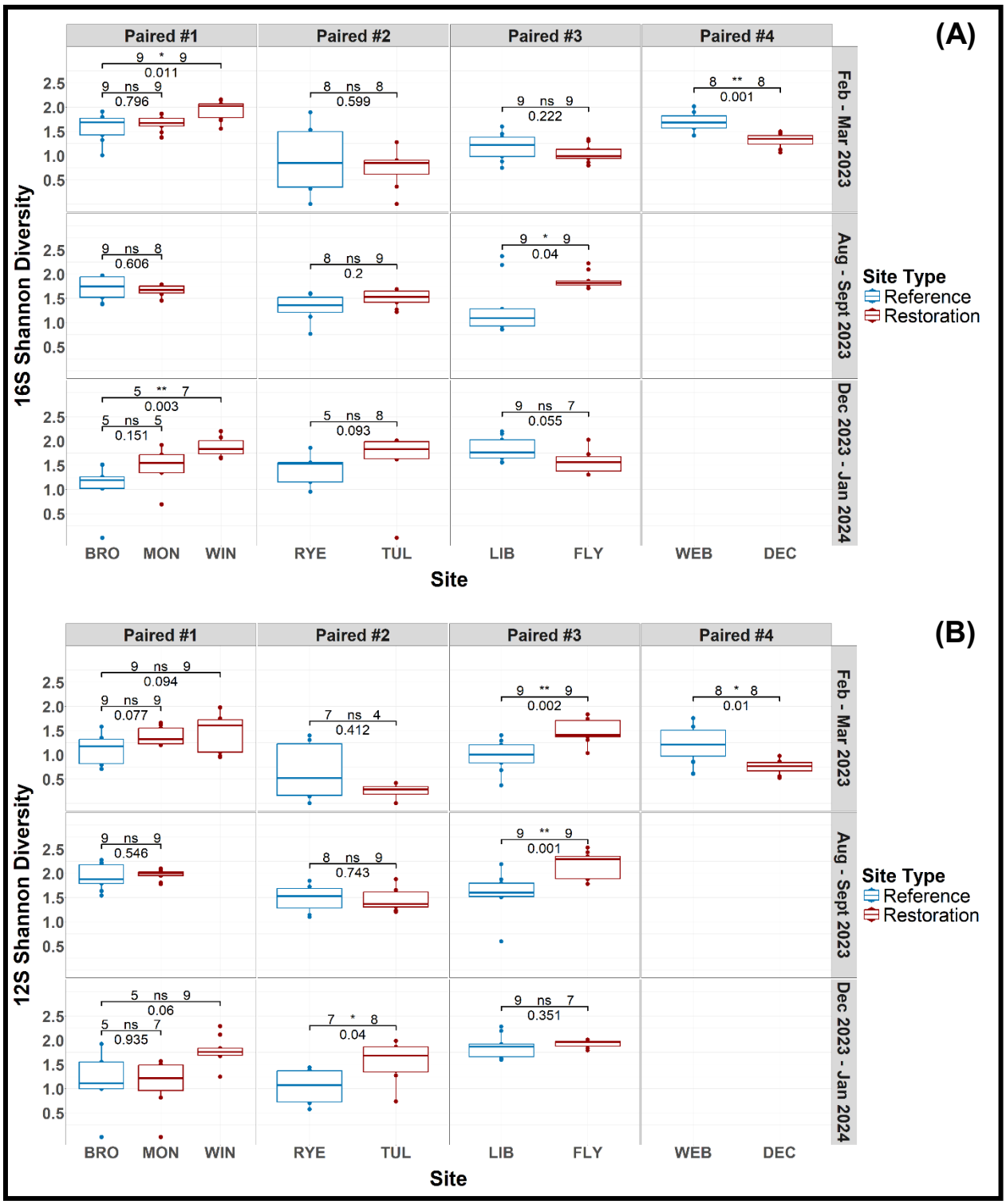


Figure 11. Alpha diversity comparing fish eDNA from restored and reference sites. Data points are biological replicates. Sample number shown on either side of significance notation above brackets. (A) 16S. (B) 12S. Wilcoxon Rank Sum Test p-values with Benjamini-Hochberg correction shown beneath brackets. See **Table S19** for numerical diversity values. P-value not significant: "ns" ($p > 0.05$) | significant: * ($p \leq 0.05$), ** ($p \leq 0.01$)

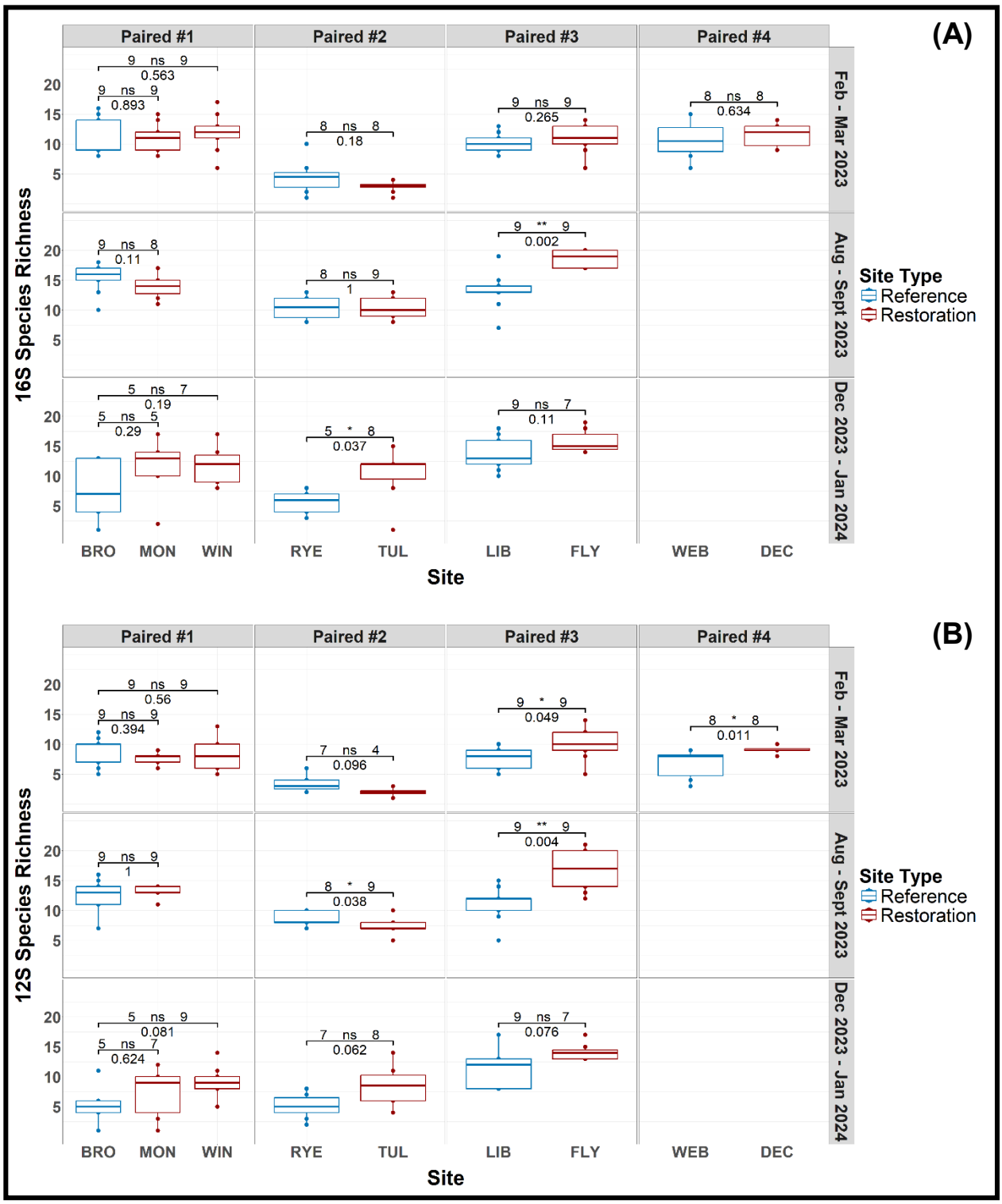


Figure 12. Species richness comparing fish eDNA from restored and reference sites. Data points are biological replicates. Sample number shown on either side of significance notation above brackets. (A) 16S. (B) 12S. Wilcoxon Rank Sum Test p-values with Benjamini-Hochberg correction shown beneath brackets. See **Table S19** for numerical richness values. P-value not significant: "ns" ($p > 0.05$) | significant: * ($p \leq 0.05$), ** ($p \leq 0.01$)

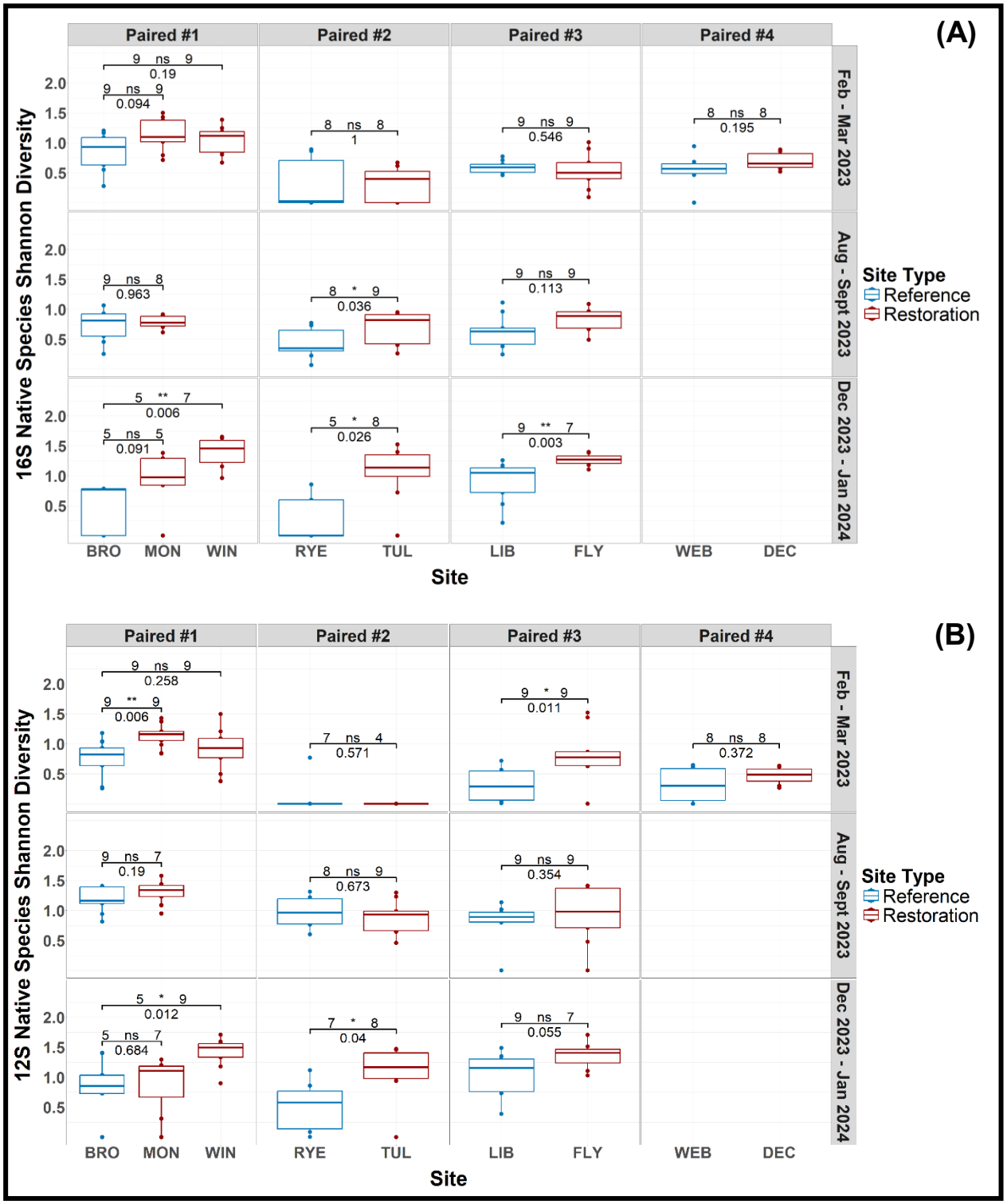


Figure 13. Alpha diversity comparing native fish eDNA from restored and reference sites. Data points are biological replicates. Sample number shown on either side of significance notation above brackets. (A) 16S. (B) 12S. Wilcoxon Rank Sum Test p-values with Benjamini-Hochberg correction beneath brackets. See **Table S20** for numerical diversity values. P-value not significant: "ns" ($p > 0.05$) | significant: * ($p \leq 0.05$), ** ($p \leq 0.01$)

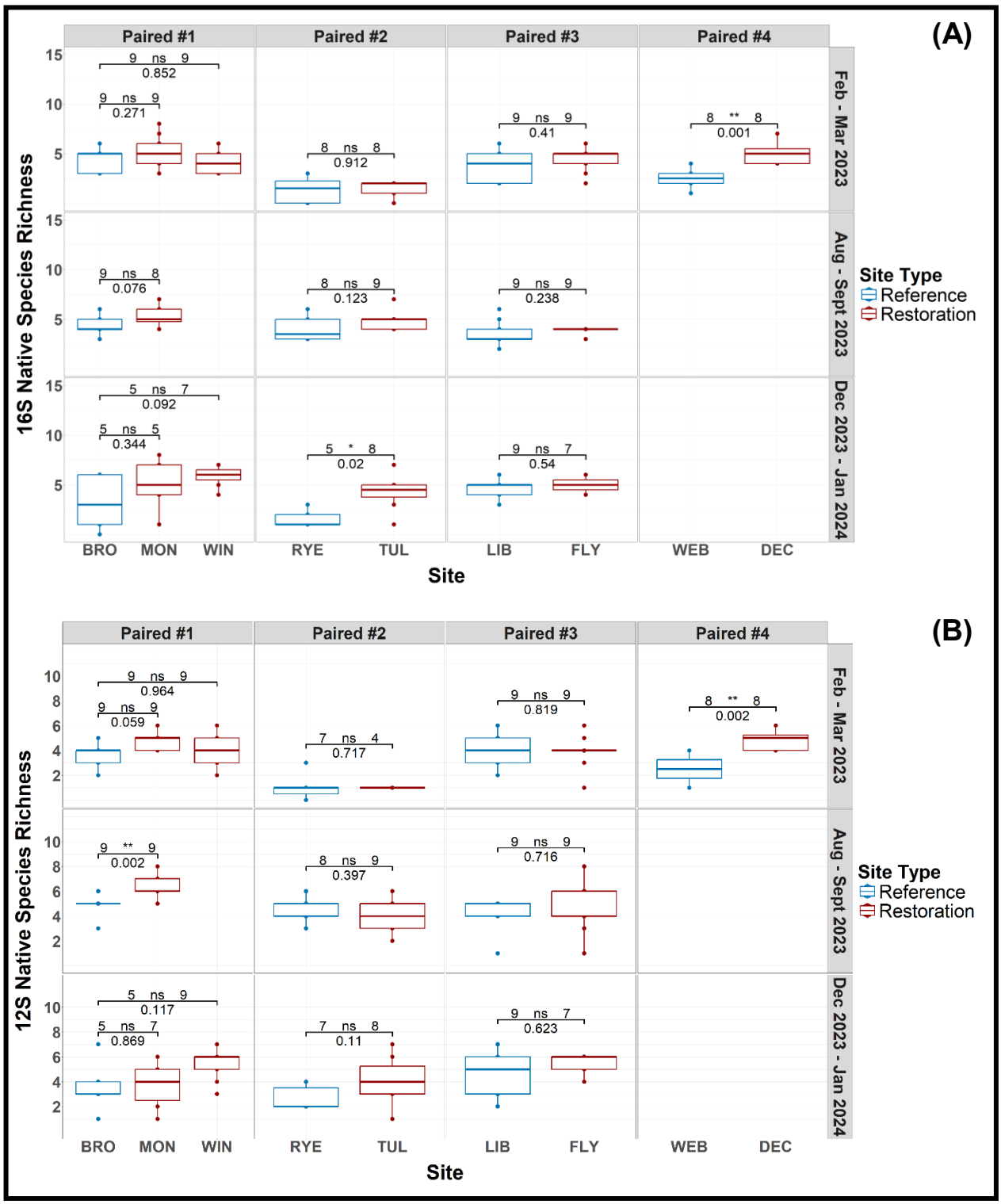


Figure 14. Species richness comparing native fish eDNA from restored and reference sites Data points are biological replicates. Sample number shown on either side of significance notation above brackets. (A) 16S. (B) 12S. Wilcoxon Rank Sum Test p-values with Benjamini-Hochberg correction beneath brackets. See **Table S20** for numerical richness values. P-value not significant: "ns" ($p > 0.05$) | significant: * ($p \leq 0.05$), ** ($p \leq 0.01$)

4.3.2 | Beta Diversity

Bray-Curtis coefficients were used to calculate the dissimilarity in fish species eDNA assemblages between paired restoration tidal wetlands and reference sites and were visualized using NMDS plots (**Figures 15**; **16**). Both assays agreed that fish assemblages were the most dissimilar between Decker and Webb Tracts, as there was no overlap between the paired sites on either axis of the NMDS plot. In contrast, Browns and Winter Island had the most overlap, and their fish assemblages were the most similar. Both assays also agreed that fish assemblages at Ryer and Tule Red were mostly similar; however, the 16S showed more overlap between paired sites. The 16S assay found slight dissimilarity between Browns and Montezuma, but the sites did not show as much overlap for the 12S assay on axis 1. Liberty and Flyway also showed partial overlap for both assays; however, Flyway was more dispersed across axis 1, and Montezuma was more uniform across both axes.

Native fish eDNA assemblages were also visualized using NMDS plots for both assays (**Figures 17**; **18**). Similar to whole assemblages, the NMDS plots supported that Decker and Webb Tracts were the most dissimilar in their native fish assemblages. Both assays also agreed that the native fish assemblages between Ryer and Tule, and Browns and Winter were the least dissimilar. Browns and Montezuma exhibited slightly more dissimilarity in their native fish assemblages compared to their whole fish assemblages, however there was still overlap between the paired sites. NMDS plots showed a similar dispersion in data points and overlap for Flyway and Liberty for their native fish assemblages.

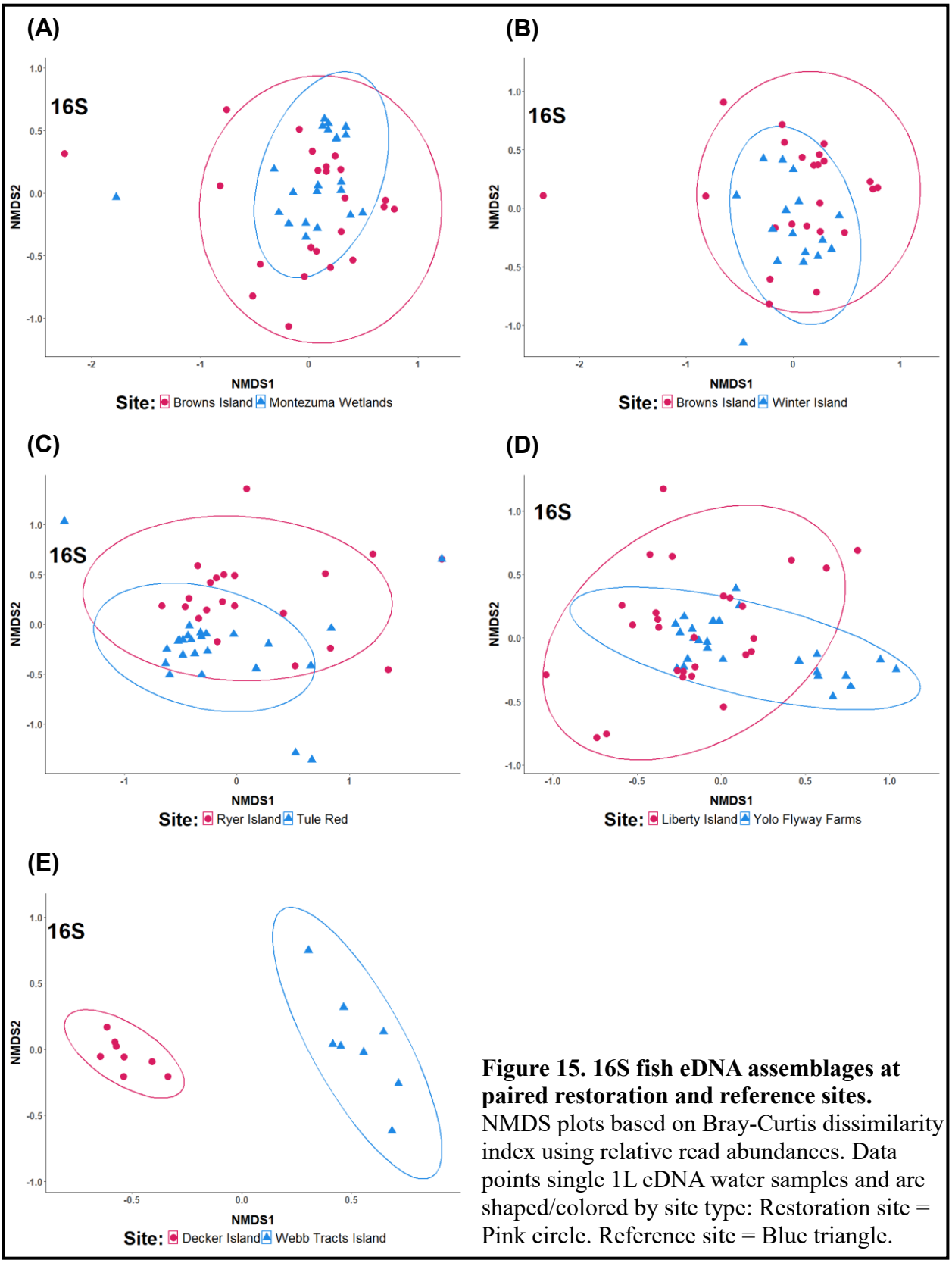


Figure 15. 16S fish eDNA assemblages at paired restoration and reference sites. NMDS plots based on Bray-Curtis dissimilarity index using relative read abundances. Data points single 1L eDNA water samples and are shaped/colored by site type: Restoration site = Pink circle. Reference site = Blue triangle.

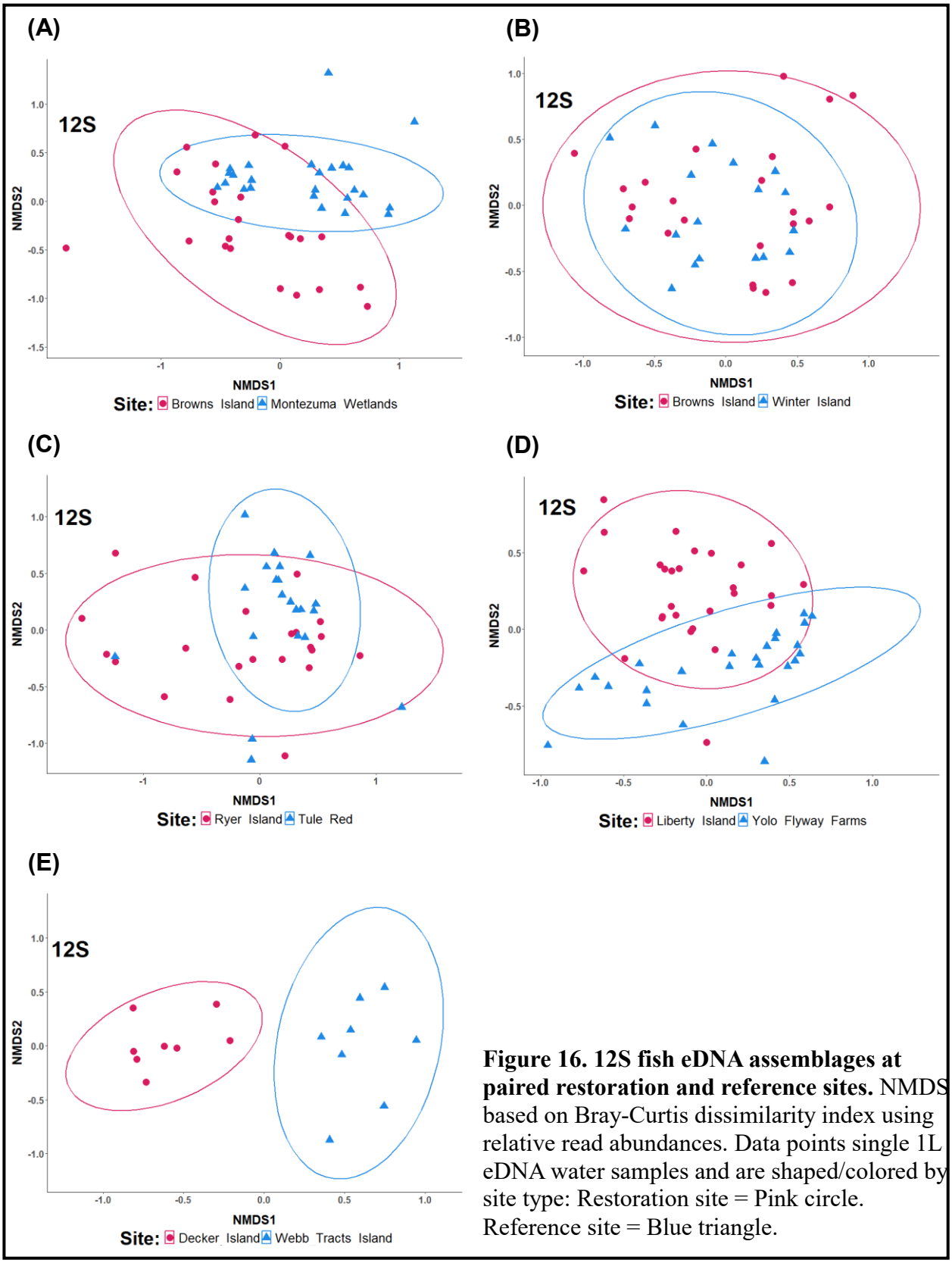


Figure 16. 12S fish eDNA assemblages at paired restoration and reference sites. NMDS based on Bray-Curtis dissimilarity index using relative read abundances. Data points single 1L eDNA water samples and are shaped/colored by site type: Restoration site = Pink circle. Reference site = Blue triangle.

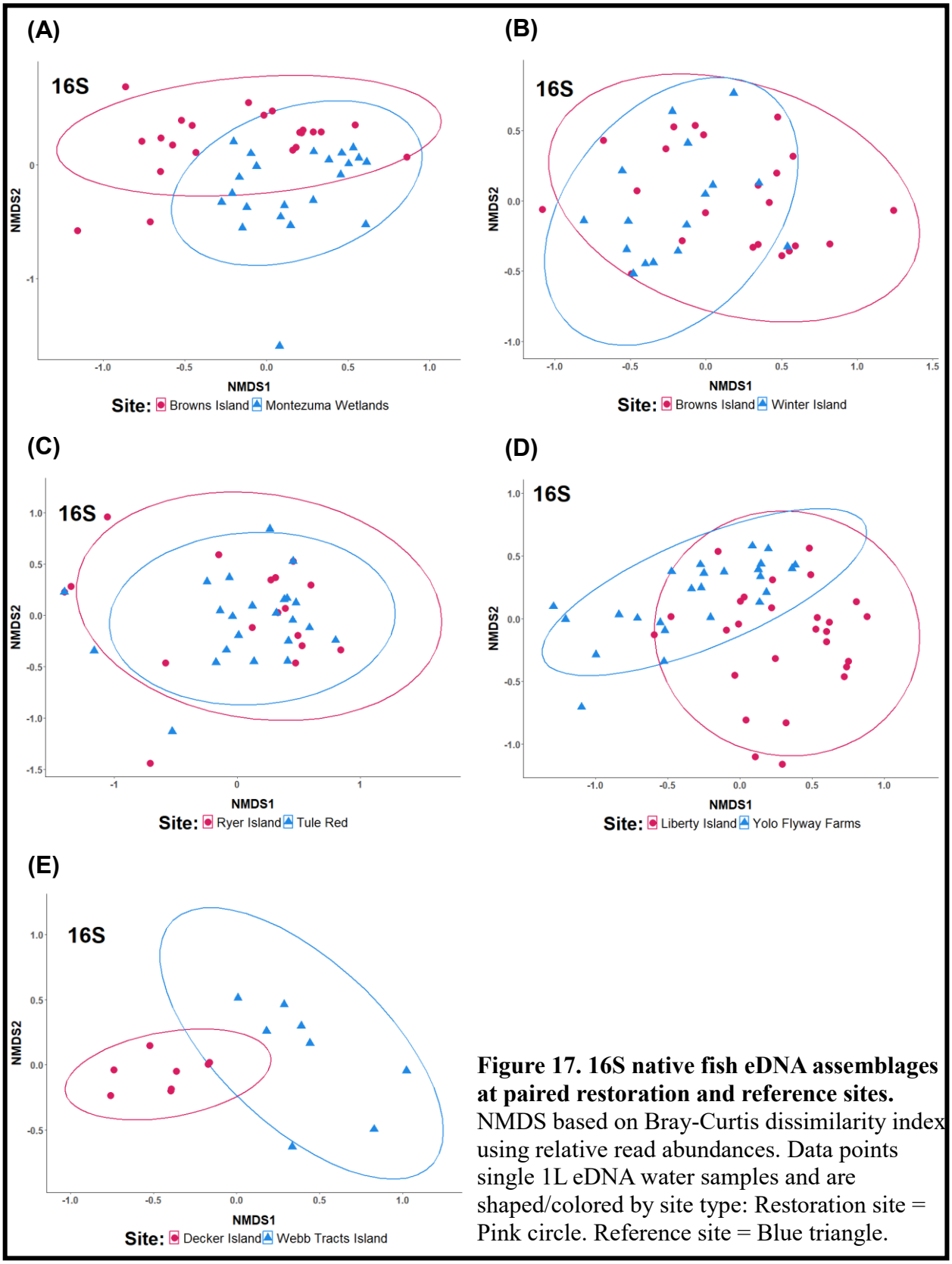


Figure 17. 16S native fish eDNA assemblages at paired restoration and reference sites. NMDS based on Bray-Curtis dissimilarity index using relative read abundances. Data points single 1L eDNA water samples and are shaped/colored by site type: Restoration site = Pink circle. Reference site = Blue triangle.

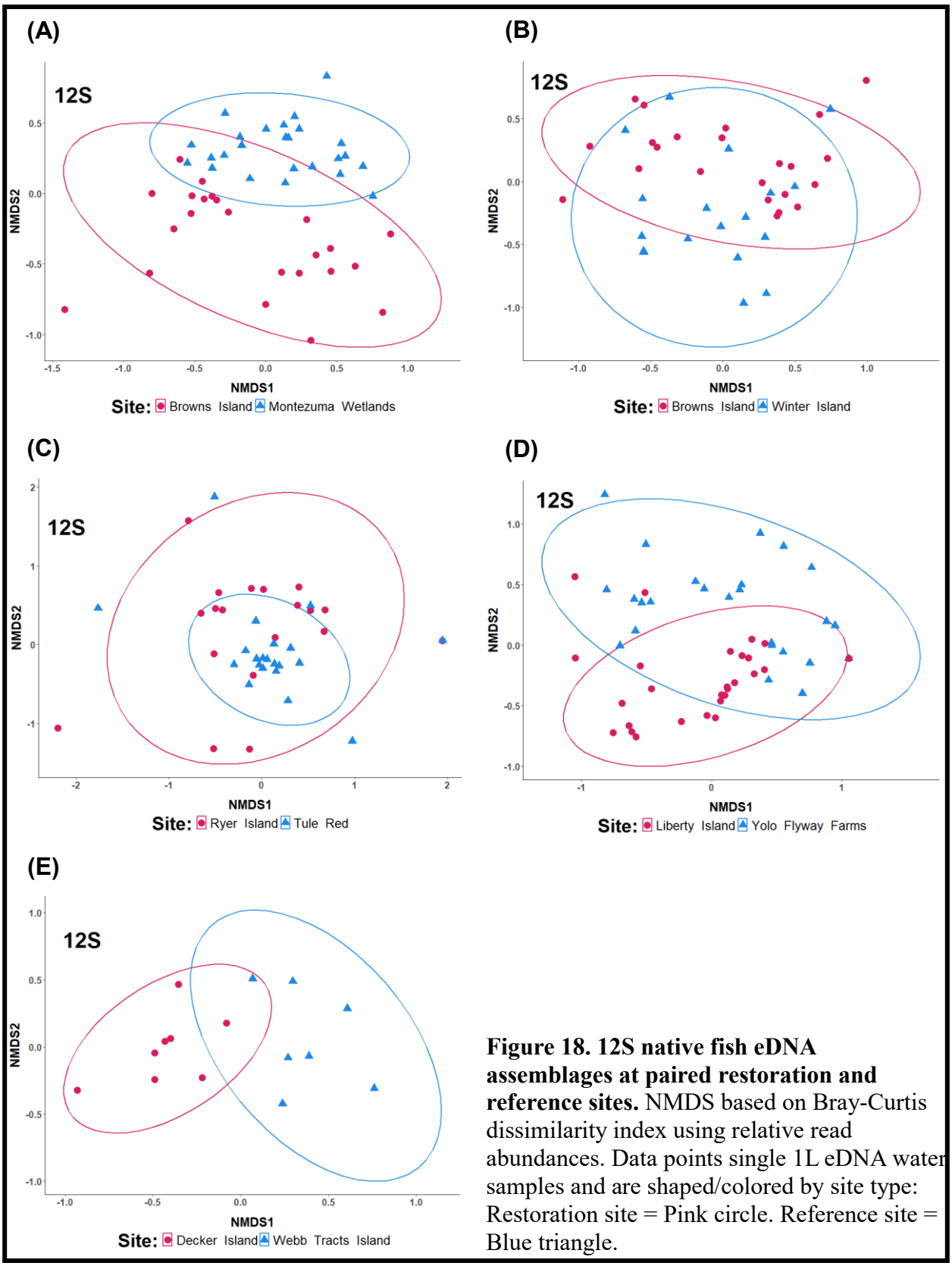


Figure 18. 12S native fish eDNA assemblages at paired restoration and reference sites. NMDS based on Bray-Curtis dissimilarity index using relative read abundances. Data points single 1L eDNA water samples and are shaped/colored by site type: Restoration site = Pink circle. Reference site = Blue triangle.

5 | Discussion

This study monitored fish species diversity and richness at restored tidal wetlands and reference sites in the SFE using a non-invasive eDNA metabarcoding approach to determine if restored wetlands in the SFE are meeting fish conservation and management goals (FRP 2021). If restoration had a positive impact on fish communities, the expectation is that a restored wetland will have similar fish assemblages to its paired reference site (IEP TWM PWT 2017) along SFE's ecocline. Successful restoration sites will also show positive detections for native species, including biological indicator species, like Chinook Salmon, Longfin Smelt, Delta Smelt, and White Sturgeon.

5.1 | Species detection and biodiversity assessment

Across the 16S fish-specific and 12S MiFish-U assay, this study detected 43 fish species in the SFE between February 2023 and January 2024. As expected, based on previous gear-based surveys from the Suisun Marsh Fish Survey and FRP, we observed native and non-native fish detections for both assays with eDNA metabarcoding (CDFW et al. 2018; O'Rear et al. 2020). The proportion of native versus non-native fish reads varied across paired sites. While some paired sites were dominated by reads from non-native fishes, such as Yolo Flyway Farms and Liberty Island, others demonstrated a greater proportion of reads from native species, such as Winter Island, Montezuma Wetlands, and Browns Island (except for the summer sampling events). The higher prevalence of reads from non-native fish species at the Liberty Island and Yolo Flyway Farms paired sites is consistent with previous capture surveys, which estimated that nearly two-thirds of the fish species in the Yolo Bypass and North Delta were non-native (ICF 2017). Potential factors contributing to the higher metabarcoding reads from introduced fishes at

these sites could be the proliferation of invasive submerged aquatic vegetation (SAV) in the North Delta and increased water temperature, which favors non-native fishes (Conrad et al. 2020; 2023). Although some of the variability in fish assemblages between paired sites may be the result of seasonal influences, the results clearly demonstrate an abundance of eDNA from native and non-native fishes at restoration sites.

Comparing paired sites within each sampling season, the restored sites, with the exception of Decker Island, generally displayed fish assemblages similar to those of their paired reference counterpart. While the same number of non-native species were detected at Decker Island and Webb Tract, a higher proportion of Webb Tract's metabarcoding reads assigned to non-native fish species. Decker Island also had a higher native species richness compared to Webb Tract. The locations of each site could explain differences in fish assemblages. Decker Island is located in the Confluence of the Delta supplied by freshwater from the Sacramento River, and Webb Tract is situated in the central Delta, supplied with freshwater diverted from the Sacramento River and the San Joaquin River. The Sacramento River drainage is the main source of freshwater to the Delta, while water from the San Joaquin River drainage is largely diverted for agriculture (Brown and Moyle 2005).

Several native species of management concern were also identified at sites in the SFE, including Chinook Salmon, Longfin Smelt, and White Sturgeon. Delta Smelt was only detected at the reference site, Ryer Island. Given the substantial decline of this species since the 1980s and its endangered classification under the California Endangered Species Act (CESA), it is not surprising that the reads of this species were rarely observed compared to other native species (Federal Register 1994; Moyle et al. 2016; LaCava et al. 2024). Chinook Salmon were detected at most sites, likely corresponding to their rearing stage and out-migration from the Delta (DOI

2020). However, their sequencing reads were predominately detected at restored tidal wetlands compared to reference sites. Longfin Smelt seemed to be more heavily influenced by environmental variables, such as salinity levels, as they were detected primarily in the Suisun Bay and Confluence of the SFE, including the sites Ryer Island, Tule Red, Browns Island, Montezuma Wetlands, and Winter Island. Although Longfin Smelt spawning adults have been found in the upper regions of the SFE Delta, where salinity tends to be higher, they preferentially spawn in lower salinity environments (Hobbs, Bennett, and Burton 2006; Kimmerer 2009). As most of their sequencing reads were detected in the winter sampling months at sites with lower salinity levels, the sequencing reads detected likely correspond to their spawning season (Merz et al. 2013). White Sturgeon were commonly detected by the 12S assay at all paired sites, except for Webb Tract and Decker Island. We predict that because White Sturgeon spawn from February to June in rivers (Doroshov, Moberg, and Van Eenennaam 1997), their numbers were likely too low in the Sacramento-San Joaquin rivers for eDNA detection in February, when Webb Tract and Decker were sampled. The low read abundances of White Sturgeon detected at Liberty and Flyway in March further support this hypothesis.

5.2 | Key considerations and mitigating limitations

A key consideration in aqueous eDNA monitoring is the spatial and temporal variability of eDNA in the water system (De Souza et al. 2016; Bedwell and Goldberg 2020). Furthermore, different species shed DNA at various rates and times due to differences in physiology, life history, and behaviors. Some other factors that influence the persistence of eDNA in aquatic systems include light, pH levels, microbial activity, salinity, and type of eDNA (e.g., cellular, free-floating, length) (Barnes et al. 2014). To mitigate the eDNA fluctuations of fishes due to the

above physiological, behavioral, and environmental factors, this study sampled during multiple seasons and across the ecocline of the SFE.

Sample collection challenges included limited on-site storage for field collection bottles. To accommodate the research crew members and capture survey gear, only a maximum of 24 eDNA 1 L bottles could be collected on a single sampling day, meaning that only two wetlands could be sampled per day. For a system as complicated as the SFE, it is ideal to collect extra water samples outside wetlands to assess for false positives caused by fishes outside the wetlands that may contribute eDNA into the wetlands during flood tides. To address this limitation in future analyses, eDNA fish assemblage data can be compared to fish caught by the CDFW FRP and the UC Davis Suisun Marsh Fish Survey teams just outside the wetlands on the same day as this study's eDNA sample collections.

When incorporating abundance estimates into a study, it is important to recognize that the quantitative analysis of eDNA requires an in-depth understanding of its distribution throughout the water system, as well as the use of complex statistical models and mock communities to calibrate the data (Lamb et al. 2019; Di Muri et al. 2020; Van Der Loos and Nijland 2021; Nakagawa et al. 2022; URycki et al. 2024). Therefore, this study focused on presence/absence data, diversity and richness estimates, and how relative read abundances differed between restoration and reference sites.

Highlighting the importance of using multiple primer sets in eDNA metabarcoding to mitigate primer biases, the fish assemblages detected with eDNA in this study were generally aligned for both assays; however, some native species, such as Delta Smelt and White Sturgeon, were detected by only one barcode (Schenekar et al. 2020; Van Der Loos and Nijland 2021; Marinchel et al. 2023). To further address primer biases, more studies are beginning to create and

sequence mock communities to benchmark their actual eDNA samples. For studies in which developing a mock community was not feasible, rarefaction has been used to minimize sequencing biases by subsampling all eDNA samples to the same sequencing depth (Schloss 2024). Moving forward, this study recommends benchmarking eDNA diversity estimates to capture fish surveys or a mock community of known DNA concentration from target species in the study system. Additional rarefaction can also provide more accurate diversity estimates.

5.3 | Implications and future directions

Like previous eDNA monitoring studies in estuaries, the data from this study was able to detect distinct fish assemblages at each tidal wetland across the SFE ecocline (Ahn et al. 2020; Gibson et al. 2024). On average, this study found that restored tidal wetlands had similar fish species richness and diversity values to their paired reference sites. Additionally, restored tidal wetlands had high native species richness and diversity, and aside from Delta Smelt, restored and reference wetlands shared many detections for species of concern, including Longfin Smelt, Chinook Salmon, and White Sturgeon. To maximize detections and decrease false positives, future eDNA tidal wetland monitoring projects should utilize more than one metabarcoding primer, perform sampling only during ebb tides, sample at varying depths, and collect additional water samples outside target sites. Together, these improvements will increase the power of species detections made.

Although eDNA metabarcoding data does not provide life history information and has limited quantitative power (Lamb et al. 2019; Ruppert, Kline, and Rahman 2019), this study has proven it to be a cost-effective and sensitive tool for non-invasively monitoring fishes in restored wetlands in the SFE. It has also provided evidence for continued restoration efforts by showing that these restored sites provide suitable habitats for key native fishes and fish assemblages

similar to paired reference sites. Going forward, we recommend that adaptive restoration programs integrate eDNA detection into their monitoring framework to achieve a deeper and broader understanding of the species utilizing their study sites.

6 | References

Aha, Nicole M., Peter B. Moyle, Nann A. Fangue, Andrew L. Rypel, and John R. Durand. 2021. "Managed Wetlands Can Benefit Juvenile Chinook Salmon in a Tidal Marsh." *Estuaries and Coasts* 44 (January):1440–53. https://doi.org/10.1007/s12237-020-00880-4.

Anderson, James T., and Craig A. Davis, eds. 2013. *Wetland Techniques: Volume 2: Organisms*. Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-94-007-6931-1.

Andres, Kara J., Timothy D. Lambert, David M. Lodge, Jose Andrés, and James R. Jackson. 2023. "Combining Sampling Gear to Optimally Inventory Species Highlights the Efficiency of eDNA Metabarcoding." *Environmental DNA* 5 (1): 146–57. https://doi.org/10.1002/edn3.366.

Andrews, Simon. 2010. "FastQC." Java. A Quality Control Tool for High Throughput Sequence Data. Babraham Bioinformatics: Babraham Institute. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Baidouri, Fouad El, Alison W. Watts, Jeffrey T. Miller, Muriel Kelly, Joseph L. Sevigny, Heather Gilbert, and W. Kelley Thomas. 2025. "An Optimized eDNA Protocol for Fish Tracking in Estuarine Environments." *Scientific Reports* 15 (January):1175. https://doi.org/10.1038/s41598-025-85176-y.

Barnes, Matthew A., Cameron R. Turner, Christopher L. Jerde, Mark A. Renshaw, W. Lindsay Chadderton, and David M. Lodge. 2014. "Environmental Conditions Influence eDNA Persistence in Aquatic Systems." *Environmental Science & Technology* 48 (3): 1819–27. https://doi.org/10.1021/es404734p.

Bedwell, Mallory E., and Caren S. Goldberg. 2020. "Spatial and Temporal Patterns of Environmental DNA Detection to Inform Sampling Protocols in Lentic and Lotic Systems." *Ecology and Evolution* 10 (3): 1602–12. https://doi.org/10.1002/ece3.6014.

Beng, Kingsly C., and Richard T. Corlett. 2020. "Applications of Environmental DNA (eDNA) in Ecology and Conservation: Opportunities, Challenges and Prospects." *Biodiversity and Conservation* 29 (7): 2089–2121. https://doi.org/10.1007/s10531-020-01980-0.

Bergman, Paul S., Gregg Schumer, Scott Blankenship, and Elizabeth Campbell. 2016. "Detection of Adult Green Sturgeon Using Environmental DNA Analysis." Edited by Peng Xu. *PLOS ONE* 11 (4): e0153500. https://doi.org/10.1371/journal.pone.0153500.

Blackburn, Shannon E., Marty L. Gingras, Jason DuBois, Zachary J. Jackson, and Michael C. Quist. 2019. "Population Dynamics and Evaluation of Management Scenarios for White Sturgeon in the Sacramento–San Joaquin River Basin." *North American Journal of Fisheries Management* 39 (5): 896–912. https://doi.org/10.1002/nafm.10316.

Bowen, Lizabeth, Shannon Waters, Lyndsay Rankin, Karen Thorne, Daphne Gille, Susan De La Cruz, Isa Woo, et al. 2024. "A Comparison of eDNA Sampling Methods in an Estuarine Environment on Presence of Longfin Smelt (Spirinchus Thaleichthys) and Fish Community Composition." *Environmental DNA* 6 (3): e560. https://doi.org/10.1002/edn3.560.

Brown, Larry R, and Peter B Moyle. 2005. "Native Fishes of the Sacramento–San Joaquin Drainage, California: A History of Decline." *American Fisheries Society*, American Fisheries Society Symposium, 45:19.

California Department of Fish and Wildlife (CDFW), Rosemary Hartman, Stacy Sherman, Dave Contreras, and Daniel Ellis. 2018. "Fish Catch, Invertebrate Catch, and Water Quality Data from the Sacramento-San Joaquin Delta Collected by the Fish Restoration Monitoring Program, 2015-2017." Environmental Data Initiative. https://doi.org/10.6073/pasta/86810e72766ad19fccb1b9dd3955bdf8.

California Wetlands Monitoring Workgroup (CWMW). 2025. "Habitat Projects." EcoAtlas. https://www.ecoatlas.org.

Callahan, Benjamin J, Paul J McMurdie, Michael J Rosen, Andrew W Han, Amy Jo A Johnson, and Susan P Holmes. 2016. "DADA2: High-Resolution Sample Inference from Illumina Amplicon Data." *Nature Methods* 13 (7): 581–83. https://doi.org/10.1038/nmeth.3869.

Callaway, John C., V. Thomas Parker, Michael C. Vasey, Lisa M. Schile, and Ellen R. Herbert. 2011. "Tidal Wetland Restoration in San Francisco Bay: History and Current Issues." *San Francisco Estuary and Watershed Science* 9 (3). https://doi.org/10.15447/sfews.2011v9iss3art2.

Camacho, Christiam, George Coulouris, Vahram Avagyan, Ning Ma, Jason Papadopoulos, Kevin Bealer, and Thomas L Madden. 2009. "BLAST+: Architecture and Applications." *BMC Bioinformatics* 10 (1): 421. https://doi.org/10.1186/1471-2105-10-421.

Campbell, Nathan R., Stephanie A. Harmon, and Shawn R. Narum. 2015. "Genotyping-in-Thousands by Sequencing (GT-seq): A Cost Effective SNP Genotyping Method Based on Custom Amplicon Sequencing." *Molecular Ecology Resources* 15 (4): 855–67. https://doi.org/10.1111/1755-0998.12357.

Chopin, F.S., and T. Arimoto. 1995. "The Condition of Fish Escaping from Fishing Gears—a Review." *Fisheries Research* 21 (3–4): 315–27. https://doi.org/10.1016/0165-7836(94)00301-C.

Cloern, James E., and Alan D. Jassby. 2012. "Drivers of Change in Estuarine-coastal Ecosystems: Discoveries from Four Decades of Study in San Francisco Bay." *Reviews of Geophysics* 50 (4): 2012RG000397. https://doi.org/10.1029/2012RG000397.

Colombano, Denise D., John M. Donovan, David E. Ayers, Teejay A. O'Rear, and Peter B. Moyle. 2020. "Tidal Effects on Marsh Habitat Use by Three Fishes in the San Francisco Estuary." *Environmental Biology of Fishes* 103 (5): 605–23. https://doi.org/10.1007/s10641-020-00973-w.

Colombano, Denise D., Thomas B. Handley, Teejay A. O'Rear, John R. Durand, and Peter B. Moyle. 2021. "Complex Tidal Marsh Dynamics Structure Fish Foraging Patterns in the San Francisco Estuary." *Estuaries and Coasts* 44 (6): 1604–18. https://doi.org/10.1007/s12237-021-00896-4.

Colombano, Denise D, Amber D Manfree, Teejay A O'Rear, John R Durand, and Peter B Moyle. 2020. "Estuarine-Terrestrial Habitat Gradients Enhance Nursery Function for Resident and Transient Fishes in the San Francisco Estuary." *Marine Ecology Progress Series* 637 (March):141–57. https://doi.org/10.3354/meps13238.

Conrad, J. Louise, Dylan Chapple, Eva Bush, Edward Hard, Jeffry Caudill, John D. Madsen, Wendy Pratt, et al. 2020. "Critical Needs for Control of Invasive Aquatic Vegetation in the Sacramento-San Joaquin Delta." Report to the Delta Plan Interagency Implementation Committee.

Conrad, J. Louise, Madison Thomas, Karen Jetter, John Madsen, Paul Pratt, Patrick Moran, John Takekawa, Gina Darin, and Lydia Kenison. 2023. "Invasive Aquatic Vegetation in the Sacramento–San Joaquin Delta and Suisun Marsh: The History and Science of Control Efforts and Recommendations for the Path Forward." *San Francisco Estuary and Watershed Science* 20 (4). https://doi.org/10.15447/sfews.2023v20iss4art4.

Curtis, Amanda N., Eric R. Larson, and Mark A. Davis. 2021. "Field Storage of Water Samples Affects Measured Environmental DNA Concentration and Detection." *Limnology* 22 (1): 1–4. https://doi.org/10.1007/s10201-020-00634-y.

De Souza, Lesley S., James C. Godwin, Mark A. Renshaw, and Eric Larson. 2016. "Environmental DNA (eDNA) Detection Probability Is Influenced by Seasonal Activity of Organisms." Edited by Hideyuki Doi. *PLOS ONE* 11 (10): e0165273. https://doi.org/10.1371/journal.pone.0165273.

Deiner, Kristy, Holly M. Bik, Elvira Mächler, Mathew Seymour, Anaïs Lacoursière-Roussel, Florian Altermatt, Simon Creer, et al. 2017. "Environmental DNA Metabarcoding: Transforming How We Survey Animal and Plant Communities." *Molecular Ecology* 26 (21): 5872–95. https://doi.org/10.1111/mec.14350.

Di Muri, Cristina, Lori Lawson Handley, Colin W. Bean, Jianlong Li, Graeme Peirson, Graham S. Sellers, Kerry Walsh, Hayley V. Watson, Ian J. Winfield, and Bernd Hänfling. 2020. "Read Counts from Environmental DNA (eDNA) Metabarcoding Reflect Fish Abundance and Biomass in Drained Ponds." *Metabarcoding and Metagenomics* 4 (October):e56959. https://doi.org/10.3897/mbmg.4.56959.

Díaz, Cecilia, Franziska-Frederike Wege, Cuong Q. Tang, Alexandra Crampton-Platt, Heinz Rüdel, Elke Eilebrecht, and Jan Koschorreck. 2020. "Aquatic Suspended Particulate Matter as Source of eDNA for Fish Metabarcoding." *Scientific Reports* 10 (1): 14352. https://doi.org/10.1038/s41598-020-71238-w.

DiBattista, Joseph D., Ashley M. Fowler, Indiana J. Riley, Sally Reader, Amanda Hay, Kerryn Parkinson, and Jean-Paul A. Hobbs. 2022. "The Use of Environmental DNA to Monitor Impacted Coastal Estuaries." *Marine Pollution Bulletin* 181 (August):113860. https://doi.org/10.1016/j.marpolbul.2022.113860.

Doroshov, Serge I., Gary P. Moberg, and Joel P. Van Eenennaam. 1997. "Observations on the Reproductive Cycle of Cultures White Sturgeon, Acipenser Transmontanus." *Environmental Biology of Fishes* 48 (1–4): 265–78. https://doi.org/10.1023/A:1007336802423.

Drake, Lorna E., Jordan P. Cuff, Rebecca E. Young, Angela Marchbank, Elizabeth A. Chadwick, and William O. C. Symondson. 2022. "An Assessment of Minimum Sequence Copy Thresholds for Identifying and Reducing the Prevalence of Artefacts in Dietary Metabarcoding Data." *Methods in Ecology and Evolution* 13 (3): 694–710. https://doi.org/10.1111/2041-210X.13780.

Eichmiller, Jessica J., Loren M. Miller, and Peter W. Sorensen. 2016. “Optimizing Techniques to Capture and Extract Environmental DNA for Detection and Quantification of Fish.” *Molecular Ecology Resources* 16 (1): 56–68. https://doi.org/10.1111/1755-0998.12421.

Ewels, Philip, Måns Magnusson, Sverker Lundin, and Max Käller. 2016. “MultiQC: Summarize Analysis Results for Multiple Tools and Samples in a Single Report.” *Bioinformatics* 32 (19): 3047–48. https://doi.org/10.1093/bioinformatics/btw354.

Federal Register. 1994. *Endangered and Threatened Wildlife and Plants; Critical Habitat Determination for the Delta Smelt*. *F.R.* Vol. 59.

Fediajevaite, Julija, Victoria Priestley, Richard Arnold, and Vincent Savolainen. 2021. “Meta-analysis Shows That Environmental DNA Outperforms Traditional Surveys, but Warrants Better Reporting Standards.” *Ecology and Evolution* 11 (9): 4803–15. https://doi.org/10.1002/ece3.7382.

Fish Restoration Program (FRP). 2021. “Sampling Fish and Food-Web Resources in Tidal Wetlands: Restoration Monitoring in 2022.” Technical Report. California Department of Fish and Wildlife and California Department of Water Resources: Interagency Ecological Program.

Gilbert, Grove Karl. 1917. *Hydraulic-Mining Debris in the Sierra Nevada*. Professional Paper 105. US Government Printing Office.

Gold, Zachary, Joshua Sprague, David J. Kushner, Erick Zerecero Marin, and Paul H. Barber. 2021. “eDNA Metabarcoding as a Biomonitoring Tool for Marine Protected Areas.” Edited by Andrea Belgrano. *PLOS ONE* 16 (2): e0238557. https://doi.org/10.1371/journal.pone.0238557.

Goldberg, Caren S., Cameron R. Turner, Kristy Deiner, Katy E. Klymus, Philip Francis Thomsen, Melanie A. Murphy, Stephen F. Spear, et al. 2016. “Critical Considerations for the Application of Environmental DNA Methods to Detect Aquatic Species.” Edited by M. Gilbert. *Methods in Ecology and Evolution* 7 (11): 1299–1307. https://doi.org/10.1111/2041-210X.12595.

Goodwin, Peter, Ashish J Mehtat, and Joy B Zedler. 2001. “Tidal Wetland Restoration: An Introduction.”

Grimaldo, Lenny, Robert Miller, Chris Peregrin, and Zachary Hymanson. 2012. “Fish Assemblages in Reference and Restored Tidal Freshwater Marshes of the San Francisco Estuary.” *San Francisco Estuary and Watershed Science* 10 (1). https://doi.org/10.15447/sfews.2012v10iss1art2.

Hadley Wickham, Davis Vaughan, and Maximilian Girlich. 2024. “Tidyr: Tidy Messy Data.” R Package. https://tidyr.tidyverse.org.

Hammock, Bruce G., Rosemary Hartman, Steven B. Slater, April Hennessy, and Swee J. Teh. 2019. “Tidal Wetlands Associated with Foraging Success of Delta Smelt.” *Estuaries and Coasts* 42 (3): 857–67. https://doi.org/10.1007/s12237-019-00521-5.

Hartman, Rosemary, Matthew Young, Stacey Sherman, David Ayers, Elizabeth Brusati, Dylan Chapple, Emma Mendonsa, Edward Hard, and J. Louise Conrad. 2024. “Management of Tidal Wetland Restoration and Fish in the Upper San Francisco Estuary: Where Are We Now and How Do We Move Forward? A Summary of the 2023 Wetland Science Symposium.” *San Francisco Estuary and Watershed Science* 22 (4). https://doi.org/10.15447/sfews.2024v22iss4art3.

Hobbs, J. A., W. A. Bennett, and J. E. Burton. 2006. “Assessing Nursery Habitat Quality for Native Smelts (Osmeridae) in the Low-salinity Zone of the San Francisco Estuary.” *Journal of Fish Biology* 69 (3): 907–22. https://doi.org/10.1111/j.1095-8649.2006.01176.x.

Holmes, Ann E., Melinda R. Baerwald, Jeff Rodzen, Brian M. Schreier, Brian Mahardja, and Amanda J. Finger. 2024. “Evaluating Environmental DNA Detection of a Rare Fish in Turbid Water Using Field and Experimental Approaches.” *PeerJ* 11 (January):e16453. https://doi.org/10.7717/peerj.16453.

Huang, Pei-Yu, Emily Shui Kei Poon, Lai Ying Chan, David Tsz Chung Chan, Stella Huynh, Ivy Wai Yan So, Yik-Hei Sung, and Simon Yung Wa Sin. 2022. “Dietary Diversity of Multiple Shorebird Species in an Asian Subtropical Wetland Unveiled by DNA Metabarcoding.” *Environmental DNA* 4 (6): 1381–96. https://doi.org/10.1002/edn3.350.

ICF. 2017. “Adaptive Management and Monitoring Plan Yolo Flyway Farms Restoration Project.” Adaptive Management and Monitoring Plan. Yolo Flyway Farms Restoration Project. Reynier Fund, LLC.

Interagency Ecological Program Tidal Wetlands Monitoring Project Work Team (IEP TWM PWT). 2017. “Tidal Wetland Monitoring Framework for the Upper San Francisco Estuary.” Technical Report Version 1.0. http://www.water.ca.gov/iep/about/tidal_wetland_monitoring.cfm.

Ito, Gen, Hiroshi Yamauchi, Miwa Shigeyoshi, Kousuke Ashino, Chie Yonashiro, Maki Asami, Yuko Goto, Jeffrey J. Duda, and Hiroki Yamanaka. 2023. “Using eDNA Metabarcoding to Establish Targets for Freshwater Fish Composition Following River Restoration.” *Global Ecology and Conservation* 43 (June):e02448. https://doi.org/10.1016/j.gecco.2023.e02448.

Jerde, Christopher L., Andrew R. Mahon, W. Lindsay Chadderton, and David M. Lodge. 2010. “‘Sight-Unseen’ Detection of Rare Aquatic Species Using Environmental DNA: eDNA Surveillance of Rare Aquatic Species.” *Conservation Letters* 4 (2): 150–57. https://doi.org/10.1111/j.1755-263X.2010.00158.x.

———. 2011. “‘Sight-Unseen’ Detection of Rare Aquatic Species Using Environmental DNA: eDNA Surveillance of Rare Aquatic Species.” *Conservation Letters* 4 (2): 150–57. https://doi.org/10.1111/j.1755-263X.2010.00158.x.

Kimmerer, Wim. 2009. “Modeling the Delta Smelt Population of the San Francisco Estuary.” Presentation to National Center for Ecological Analysis and Synthesis presented at the Pelagic Organism Decline Workshop, Santa Barbara, California, USA, September 9. http://www. cwemf.org/Asilomar/WimKimmererDeltaSmeltModel.pdf.

Kirsch, Joseph E., Julie L. Day, James T. Peterson, and David K. Fullerton. 2018. “Fish Misidentification and Potential Implications to Monitoring Within the San Francisco Estuary, California.” *Journal of Fish and Wildlife Management* 9 (2): 467–85. https://doi.org/10.3996/032018-JFWM-020.

Knowles, Noah, and Daniel R. Cayan. 2002. “Potential Effects of Global Warming on the Sacramento/San Joaquin Watershed and the San Francisco Estuary.” *Geophysical Research Letters* 29 (18). https://doi.org/10.1029/2001GL014339.

Kumar, Girish, Jeff E. Eble, and Michelle R. Gaither. 2019. “A Practical Guide to Sample Preservation and pre-PCR Processing of Aquatic Environmental DNA.” *Molecular Ecology Resources* 20 (1): 29–39. https://doi.org/10.1111/1755-0998.13107.

Kumar, Girish, Emily Farrell, Ashley M. Reaume, Jeff A. Eble, and Michelle R. Gaither. 2021. "One Size Does Not Fit All: Tuning eDNA Protocols for High- and Low-turbidity Water Sampling." *Environmental DNA* 4 (1): 167–80. https://doi.org/10.1002/edn3.235.

Kumar, Girish, Ashley M. Reaume, Emily Farrell, and Michelle R. Gaither. 2022. "Comparing eDNA Metabarcoding Primers for Assessing Fish Communities in a Biodiverse Estuary." Edited by Arnar Palsson. *PLOS ONE* 17 (6): e0266720. https://doi.org/10.1371/journal.pone.0266720.

LaCava, Melanie E. F., Isoline M. Donohue, Mary E. Badger, Tien-Chieh Hung, Luke Ellison, Md Moshiur Rahman, Kerry Kelvas, Amanda J. Finger, and Evan W. Carson. 2024. "Assessing Captive Spawning Strategies for Supplementation Production of Delta Smelt." *Transactions of the American Fisheries Society* 153 (1): 129–38. https://doi.org/10.1002/tafs.10450.

Lahti, Leo, and Sudarshan Shetty. 2017. "Tools for Microbiome Analysis in R." http://microbiome.github.io/microbiome.

Lamb, Philip D., Ewan Hunter, John K. Pinnegar, Simon Creer, Richard G. Davies, and Martin I. Taylor. 2019. "How Quantitative Is Metabarcoding: A Meta-analytical Approach." *Molecular Ecology* 28 (2): 420–30. https://doi.org/10.1111/mec.14920.

Lewis, Levi S., Malte Willmes, Arthur Barros, Patrick K. Crain, and James A. Hobbs. 2020. "Newly Discovered Spawning and Recruitment of Threatened Longfin Smelt in Restored and Underexplored Tidal Wetlands." *Ecology* 101 (1): e02868. https://doi.org/10.1002/ecy.2868.

Li, Yiwen, Minzhe Tang, Suxiang Lu, Xiaochun Zhang, Chengchi Fang, Li Tan, Fan Xiong, Honghui Zeng, and Shunping He. 2024. "A Comparative Evaluation of eDNA Metabarcoding Primers in Fish Community Monitoring in the East Lake." *Water* 16 (5): 631. https://doi.org/10.3390/w16050631.

Marinchel, Nadia, Alexis Marchesini, Davide Nardi, Matteo Girardi, Silvia Casabianca, Cristiano Vernesi, and Antonella Penna. 2023. "Mock Community Experiments Can Inform on the Reliability of eDNA Metabarcoding Data: A Case Study on Marine Phytoplankton." *Scientific Reports* 13 (1): 20164. https://doi.org/10.1038/s41598-023-47462-5.

Martin, Marcel. 2011. "Cutadapt Removes Adapter Sequences from High-Throughput Sequencing Reads." *EMBnet.Journal* 17 (1): 10. https://doi.org/10.14806/ej.17.1.200.

McMurdie, Paul J., and Susan Holmes. 2013. "Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data." Edited by Michael Watson. *PLoS ONE* 8 (4): e61217. https://doi.org/10.1371/journal.pone.0061217.

Merz, Joseph E., Paul S. Bergman, Jenny F. Melgo, and Scott Hamilton. 2013. "Longfin Smelt: Spatial Dynamics and Ontogeny in the San Francisco Estuary, California." *California Fish and Game* 99 (3): 122–48.

Miya, M., Y. Sato, T. Fukunaga, T. Sado, J. Y. Poulsen, K. Sato, T. Minamoto, et al. 2015. "MiFish, a Set of Universal PCR Primers for Metabarcoding Environmental DNA from Fishes: Detection of More than 230 Subtropical Marine Species." *Royal Society Open Science* 2 (7): 150088. https://doi.org/10.1098/rsos.150088.

Morris, Lenore, Leah S. Beesley, Emma R. Stevens, Daniel C. Gwinn, Josephine Hyde, Suzanne Thompson, Deirdre B. Gleeson, and Michael M. Douglas. 2024. "Active eDNA Is More Cost-Effective Than Fyke Nets or Passive eDNA Collection When Monitoring the Invasion of an Alien Freshwater Fish." *Environmental DNA* 6 (5): e70010. https://doi.org/10.1002/edn3.70010.

Mount, Jeffrey, and Wim Kimmerer. 2022. “The Largest Estuary on the West Coast of North America.” Blog. *California WaterBlog* (blog). December 18, 2022. https://californiawaterblog.com/2022/12/18/the-largest-estuary-on-the-west-coast-of-north-america/.

Moyle, Peter, Larry Brown, John Durand, and James Hobbs. 2016. “Delta Smelt: Life History and Decline of a Once-Abundant Species in the San Francisco Estuary.” *San Francisco Estuary and Watershed Science* 14 (2). https://doi.org/10.15447/sfews.2016v14iss2art6.

Muha, Teja P., Deiene Rodriguez-Barreto, Richard O’Rorke, Carlos Garcia De Leaniz, and Sofia Consuegra. 2021. “Using eDNA Metabarcoding to Monitor Changes in Fish Community Composition After Barrier Removal.” *Frontiers in Ecology and Evolution* 9 (February):629217. https://doi.org/10.3389/fevo.2021.629217.

Nagarajan, Raman, Ann Holmes, and Andrea Schreier. 2023. “Reference Sequence Database for eDNA Metabarcoding of San Francisco Estuary Fishes and Invertebrates.” Dryad. https://doi.org/10.5061/dryad.0p2ngf25z.

Nagarajan, Raman P., Mallory Bedwell, Ann E. Holmes, Thiago Sanches, Shawn Acuña, Melinda Baerwald, Matthew A. Barnes, et al. 2022. “Environmental DNA Methods for Ecological Monitoring and Biodiversity Assessment in Estuaries.” *Estuaries and Coasts* 45 (7): 2254–73. https://doi.org/10.1007/s12237-022-01080-y.

Nagarajan, Raman P., Leigh Sanders, Natalie Kolm, Alejandro Perez, Taylor Senegal, Brian Mahardja, Melinda R. Baerwald, and Andrea D. Schreier. 2024. “CRISPR-based Environmental DNA Detection for a Rare Endangered Estuarine Species.” *Environmental DNA* 6 (1): e506. https://doi.org/10.1002/edn3.506.

Nakagawa, Hikaru, Keitaro Fukushima, Masaru Sakai, Luhan Wu, and Toshifumi Minamoto. 2022. “Relationships between the eDNA Concentration Obtained from Metabarcoding and Stream Fish Abundance Estimated by the Removal Method under Field Conditions.” *Environmental DNA* 4 (6): 1369–80. https://doi.org/10.1002/edn3.346.

National Estuary Program (NEP). 2024. “Overview of the National Estuary Program.” *Unites State Environmental Protection Agency* (blog). June 12, 2024. https://www.epa.gov/nep/overview-national-estuary-program.

Ogden, Lesley Evans. 2022. “The Emergence of eDNA.” *BioScience* 72 (1): 5–12. https://doi.org/10.1093/biosci/biab120.

O’Rear, Teejay A, Jacob Montgomery, Peter B Moyle, and John R Durand. 2020. “Trends in Fish and Invertebrate Populations of Suisun Marsh (January 2020 - December 2020).” Technical Report. University of California, Davis and CalTrout: California Department of Water Resources.

Pilliod, David S., Caren S. Goldberg, Robert S. Arkle, and Lisette P. Waits. 2013. “Estimating Occupancy and Abundance of Stream Amphibians Using Environmental DNA from Filtered Water Samples.” Edited by John Richardson. *Canadian Journal of Fisheries and Aquatic Sciences* 70 (8): 1123–30. https://doi.org/10.1139/cjfas-2013-0047.

Pozzobon, Allan Pierre Bonetti, Pablo Rodrigues Gonçalves, Joel D. Anderson, Luiz A. Rocha, Juan M. Díaz De Astarloa, and Fabio Di Dario. 2021. “Phylogenetic Relationships, Genetic Diversity and Biogeography of Menhadens, Genus Brevoortia (Clupeiformes, Clupeidae).” *Molecular Phylogenetics and Evolution* 160 (July):107108. https://doi.org/10.1016/j.ympev.2021.107108.

R Core Team. 2022. “R: A Language and Environment for Statistical Computing.” R Foundation for Statistical Computing. Vienna, Austria. https://www.R-project.org/.

Ramírez-Amaro, Sergio, Marta Bassitta, Antònia Picornell, Cori Ramon, and Bàrbara Terrasa. 2022. “Environmental DNA: State-of-the-Art of Its Application for Fisheries Assessment in Marine Environments.” *Frontiers in Marine Science* 9 (December):1004674. https://doi.org/10.3389/fmars.2022.1004674.

Rosen, Michael J, Benjamin J Callahan, Daniel S Fisher, and Susan P Holmes. 2012. “Denoising PCR-Amplified Metagenome Data.” *BMC Bioinformatics* 13 (1): 283. https://doi.org/10.1186/1471-2105-13-283.

Ruppert, Krista M., Richard J. Kline, and Md Saydur Rahman. 2019. “Past, Present, and Future Perspectives of Environmental DNA (eDNA) Metabarcoding: A Systematic Review in Methods, Monitoring, and Applications of Global eDNA.” *Global Ecology and Conservation* 17 (January):e00547. https://doi.org/10.1016/j.gecco.2019.e00547.

Sanches, Thiago M., and Andrea D. Schreier. 2020. “Optimizing an eDNA Protocol for Estuarine Environments: Balancing Sensitivity, Cost and Time.” Edited by Hideyuki Doi. *PLOS ONE* 15 (5): e0233522. https://doi.org/10.1371/journal.pone.0233522.

Schenekar, Tamara, Martin Schletterer, Laurène A. Lecaudey, and Steven J. Weiss. 2020. “Reference Databases, Primer Choice, and Assay Sensitivity for Environmental Metabarcoding: Lessons Learnt from a Re-evaluation of an eDNA Fish Assessment in the Volga Headwaters.” *River Research and Applications* 36 (7): 1004–13. https://doi.org/10.1002/rra.3610.

Schloss, Patrick D. 2024. “Rarefaction Is Currently the Best Approach to Control for Uneven Sequencing Effort in Amplicon Sequence Analyses.” Edited by Katherine McMahon. *mSphere* 9 (2): e00354-23. https://doi.org/10.1128/msphere.00354-23.

Schmidt, Philipp-André, Miklós Bálint, Bastian Greshake, Cornelia Bandow, Jörg Römbke, and Imke Schmitt. 2013. “Illumina Metabarcoding of a Soil Fungal Community.” *Soil Biology and Biochemistry* 65 (October):128–32. https://doi.org/10.1016/j.soilbio.2013.05.014.

Shaw, Jennifer L.A., Laurence J. Clarke, Scotte D. Wedderburn, Thomas C. Barnes, Laura S. Weyrich, and Alan Cooper. 2016. “Comparison of Environmental DNA Metabarcoding and Conventional Fish Survey Methods in a River System.” *Biological Conservation* 197 (May):131–38. https://doi.org/10.1016/j.biocon.2016.03.010.

Sherman, Stacy, Hartman Rosemary, and Dave Contreras. 2017. “Effects of Tidal Wetland Restoration on Fish: A Suite of Conceptual Models.” IEP Technical Report 91. CA Department of Fish & Wildlife: Department of Water Resources, Sacramento, California.

Shirazi, Sabrina, Rachel S. Meyer, and Beth Shapiro. 2021. “Revisiting the Effect of PCR Replication and Sequencing Depth on Biodiversity Metrics in Environmental DNA Metabarcoding.” *Ecology and Evolution* 11 (22): 15766–79. https://doi.org/10.1002/ece3.8239.

Shu, Lu, Arne Ludwig, and Zuogang Peng. 2020. “Standards for Methods Utilizing Environmental DNA for Detection of Fish Species.” *Genes* 11 (3): 296. https://doi.org/10.3390/genes11030296.

———. 2021. “Environmental DNA Metabarcoding Primers for Freshwater Fish Detection and Quantification: In Silico and in Tanks.” *Ecology and Evolution* 11 (12): 8281–94. https://doi.org/10.1002/ece3.7658.

Smart, Adam S., Andrew R. Weeks, Anthony R. Van Rooyen, Alana Moore, Michael A. McCarthy, and Reid Tingley. 2016. “Assessing the Cost-efficiency of Environmental DNA Sampling.” Edited by Nigel Yoccoz. *Methods in Ecology and Evolution* 7 (11): 1291–98. https://doi.org/10.1111/2041-210X.12598.

Sommer, Ted, and Francine Mejia. 2013. “A Place to Call Home: A Synthesis of Delta Smelt Habitat in the Upper San Francisco Estuary.” *San Francisco Estuary and Watershed Science* 11 (2). https://doi.org/10.15447/sfews.2013v11iss2art4.

Stompe, Dylan Keating, and James A. Hobbs. 2024. “Experimental Gillnet Study to Inform Future Large Fish Monitoring in the San Francisco Estuary.” *California Fish and Wildlife Journal* 110 (4). https://doi.org/10.51492/cfwj.110.16.

Taberlet, Pierre, Aurélie Bonin, Lucie Zinger, and Eric Coissac. 2018. *Environmental DNA: For Biodiversity Research and Monitoring*. Oxford University Press. https://doi.org/10.1093/oso/9780198767220.001.0001.

Taberlet, Pierre, Eric Coissac, Mehrdad Hajibabaei, and Loren H. Rieseberg. 2012. “Environmental DNA.” *Molecular Ecology* 21 (8): 1789–93. https://doi.org/10.1111/j.1365-294X.2012.05542.x.

Taberlet, Pierre, Eric Coissac, François Pompanon, Christian Brochmann, and Eske Willerslev. 2012. “Towards Next-generation Biodiversity Assessment Using DNA Metabarcoding.” *Molecular Ecology* 21 (8): 2045–50. https://doi.org/10.1111/j.1365-294X.2012.05470.x.

Turner, Cameron R., Karen L. Uy, and Robert C. Everhart. 2015. “Fish Environmental DNA Is More Concentrated in Aquatic Sediments than Surface Water.” *Biological Conservation* 183 (March):93–102. https://doi.org/10.1016/j.biocon.2014.11.017.

URycki, Dawn R., Anish A. Kirtane, Rachel Aronoff, Colton C. Avila, Rosetta C. Blackman, Luca Carraro, Olivier Evrard, et al. 2024. “A New Flow Path: eDNA Connecting Hydrology and Biology.” *WIREs Water* 11 (6): e1749. https://doi.org/10.1002/wat2.1749.

U.S. Department of the Interior (DOI). 2020. “North-of-the-Delta Offstream Storage Investigation.” Final Feasibility Report Appendix F. Prepared for Reclamation by AECOM under Contract/Order No. GS-00F-188CA, 140R2018F0052. California-Great Basin: Bureau of Reclamation.

U.S. Environmental Protection Agency. 1972. *Clean Water Act*. *§ 1251 et Seq*.

Valentini, Alice, Pierre Taberlet, Claude Miaud, Raphaël Civade, Jelger Herder, Philip Francis Thomsen, Eva Bellemain, et al. 2016. “Next-generation Monitoring of Aquatic Biodiversity Using Environmental DNA Metabarcoding.” *Molecular Ecology* 25 (4): 929–42. https://doi.org/10.1111/mec.13428.

Van Der Loos, Luna M., and Reindert Nijland. 2021. “Biases in Bulk: DNA Metabarcoding of Marine Communities and the Methodology Involved.” *Molecular Ecology* 30 (13): 3270–88. https://doi.org/10.1111/mec.15592.

Veron, Pierre, Romane Rozanski, Virginie Marques, Stéphane Joost, Marie Emilie Deschez, Verena M Trenkel, Pascal Lorance, et al. 2023. “Environmental DNA Complements Scientific Trawling in Surveys of Marine Fish Biodiversity.” Edited by Sarah Helyar. *ICES Journal of Marine Science* 80 (8): 2150–65. https://doi.org/10.1093/icesjms/fsad139.

Vollmar Natural Lands Consulting, Lipton Environmental Group LLC, and Far West Engineering. 2022. “Post Breach Monitoring, Phase I (Fall 2020 - Summer 2022).” Technical Report. Solano County, California: Montezuma Wetlands LLC.

Wickham, Hadley. 2016. *Ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org.

Wilcoxon, Frank. 1945. “Individual Comparisons by Ranking Methods.” *Biometrics Bulletin* 1 (6): 80–83.

Yao, Meng, Shan Zhang, Qi Lu, Xiaoyu Chen, Si-Yu Zhang, Yueqiao Kong, and Jindong Zhao. 2022. “Fishing for Fish Environmental DNA: Ecological Applications, Methodological Considerations, Surveying Designs, and Ways Forward.” *Molecular Ecology* 31 (20): 5132–64. https://doi.org/10.1111/mec.16659.

Yoshiyama, Ronald M., Frank W. Fisher, and Peter B. Moyle. 1998. “Historical Abundance and Decline of Chinook Salmon in the Central Valley Region of California.” *North American Journal of Fisheries Management* 18 (3): 487–521. https://doi.org/10.1577/1548-8675(1998)018<0487:HAADOC>2.0.CO;2.

Zhang, Shan, Jindong Zhao, and Meng Yao. 2020. “A Comprehensive and Comparative Evaluation of Primers for Metabarcoding eDNA from Fish.” Edited by M. Gilbert. *Methods in Ecology and Evolution* 11 (12): 1609–25. https://doi.org/10.1111/2041-210X.13485.

Zou, Keshu, Jianwei Chen, Huiting Ruan, Zhenhai Li, Wenjie Guo, Min Li, and Li Liu. 2020. “eDNA Metabarcoding as a Promising Conservation Tool for Monitoring Fish Diversity in a Coastal Wetland of the Pearl River Estuary Compared to Bottom Trawling.” *Science of The Total Environment* 702 (February):134704. https://doi.org/10.1016/j.scitotenv.2019.134704.

7 | Supplementary Materials (Tables)

Table S1. eDNA Fish monitoring sampling events—detailed view. Coordinates and tide flow for each sampling event are listed. eDNA sampling events were paired with fish capture surveys conducted by the Fish Restoration Program (FRP), except for Montezuma Wetlands, which was surveyed by the UC Davis Suisun Marsh Fish Survey (UCD). Capture survey methods included lampara nets, otter trawls, and beach seines. ± = Restoration wetland; † = Reference site.

Site	Sampling Event Date	Number of 1-L eDNA water samples collected	eDNA latitude	eDNA longitude	Tide	Paired capture survey
Tule Red[±]	02/28/2023	3	38.13399697	-121.99554651	Flood	FRP—lampara
		3	38.12796484	-121.98705895	Flood	FRP—lampara
		3	38.12216920	-121.98310353	Flood	FRP—lampara
	09/05/2023	3	38.126051	-121.985978	Ebb	-
		3	38.131157	-121.990273	Ebb	-
		3	38.133896	-121.995366	Ebb	-
	12/22/2023	3	38.12367	-121.97998	Flood	FRP—lampara
		3	38.12341	-121.98142	Flood	FRP—lampara
		3	38.12881	-121.98834	High slack	FRP—lampara
Ryer Island[†]	2/28/2023	3	38.08294897	-122.00661673	Flood	FRP—lampara
		3	38.08857016	-122.01948027	Flood	FRP—lampara
		3	38.08623817	-122.01889857	Flood	FRP—lampara
	09/05/2023	3	38.08640843	-122.02391239	Ebb	-
		3	38.08543134	-122.01522450	Ebb	-
		3	38.08065350	-122.01710420	Ebb	-
	12/22/2023	3	38.08430	-122.00908	Flood	FRP—lampara
		3	38.08882	-122.01969	Flood	FRP—lampara
		3	38.088285	-122.01804	High slack	FRP—lampara
Winter Island[±]	02/01/2023	3	38.05235	-121.85458	Flood	-
		3	38.05179155	-121.85132497	Flood	-
		3	38.04775888	-121.85155899	Flood	-
	12/11/2023	3	38.04397	-121.84656	Flood	FRP—lampara
		3	38.03315	-121.84340	Flood	FRP—lampara
		3	38.03069	-121.84201	Flood	FRP—lampara

Montezuma Wetlands[±]	2/20/2023	3	38.09885294	-121.88946948	Flood	UCD
		3	38.09679767	-121.88220917	Flood	UCD
		3	38.10668446	-121.88591260	Flood	UCD
	8/14/2023	3	38.10453873	-121.88321957	Flood	UCD
		3	38.10477081	-121.87855470	Flood	UCD
		3	38.09738077	-121.88248010	Flood	UCD
	01/19/2024	3	38.09635320	-121.88216270	Flood	UCD
		3	38.10473211	-121.87717593	Flood	UCD
		3	38.09907240	-121.88955157	Flood	UCD
Browns Island[†]	02/08/2023	3	38.03252717	-121.85728965	Flood	FRP—lampara
		3	38.03252717	-121.85728965	Flood	FRP—lampara
		3	38.03904249	-121.85708751	Flood	FRP—lampara
	08/22/2023	3	38.042482	-121.870429	Ebb	-
		3	38.040015	-121.870301	Ebb	-
		3	38.038301	-121.860333	Ebb	-
	12/11/2023	3	38.03281	-121.85745	Flood	FRP—lampara
		3	38.04222	-121.86880	Flood	FRP—lampara
		3	38.03837	-121.86559	Flood	FRP—lampara
Yolo Flyway Farms[±]	03/08/2023	3	38.36507934	-121.64290056	Ebb	FRP—beach seine
		3	38.36193686	-121.64282627	Ebb	FRP—lampara
		3	38.36102280	-121.64301292	Ebb	-
	08/17/2023	3	38.36058965	-121.64022538	Ebb	-
		3	38.36058965	-121.64022538	Ebb	-
		3	38.36427412	-121.64308183	Ebb	-
	12/20/2023	3	38.36062	-121.64041	Flood	FRP—lampara
		3	38.36087	-121.64614	Flood	FRP—lampara
		3	38.36207	-121.64277	High slack	FRP—lampara
Liberty Island[†]	03/08/2023	3	38.24202447	-121.68217864	Ebb	FRP—beach seine
		3	38.27219899	-121.66895627	Ebb	FRP—lampara
		3	38.26631027	-121.69124477	Flood	FRP—beach seine
	08/17/2023	3	38.30369382	-121.67601572	Ebb	-
		3	38.30367297	-121.67601722	Ebb	-
		3	38.24355299	-121.68278006	Ebb	-

	12/20/2023	3	38.25042	-121.68583	Flood	FRP—lampara
		3	38.27137	-121.66970	Flood	FRP—lampara
		3	38.30038	-121.68041	Flood	FRP—lampara
Decker Island[±]	02/08/2023	3	38.08420	-121.72403	Ebb	FRP—lampara
		3	38.08453494	-121.72392770	Ebb	-
		3	38.08432071	-121.72402670	Ebb	-
Webb Tracts Island and Berms [†]	02/08/2023	3	38.09491322	-121.59107184	Ebb	FRP—lampara
		3	38.09610466	-121.58972332	Ebb	FRP—lampara
		3	38.09617833	-121.58798746	Ebb	FRP—lampara

Table S2. First-round PCR: 16S fish-specific primers (underlined) with Illumina index adaptor sequences used for eDNA fish monitoring. The location of diversity spacer sequences (none, TA, CATA, CGCATA) were shown as 'xxx' (Fadrosh et al. 2014; Wu et al. 2015). *PCR primer (bold/underline):* (Shaw et al. 2016). *Index adaptors:* Oligonucleotide sequences © 2015 Illumina, Inc. All rights reserved. (Kumar et al. 2021). *N used in this study instead of base I.

Amplicon Size (bp) [This study]	**Primer Sequence (5`→ 3`)**
Average: 67 Min: 62 Max: 80	F – TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGxxx**CGAGAAGACCCTWTGGAGCTTI*AG** R – GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGxxx**GGTCGCCCCAACCRAAG**

Table S3. First-round PCR: 12S MiFish-U primers (underlined) with Illumina index adaptor sequences used for eDNA fish monitoring. The location of diversity spacer sequences (none, TA, CATA, CGCATA) are shown as 'xxx' (Fadrosh et al. 2014; Wu et al. 2015). *PCR primer (bold/underline):* (Miya et al. 2015). *Index adaptors:* Oligonucleotide sequences © 2007-2013 Illumina, Inc. All rights reserved. (Campbell, Harmon, and Narum 2015). NOTE: These are obsolete, and this study required custom sequencing primers. We recommend using the current Illumina index adaptors.

Amplicon Size (bp) [This study]	**Primer Sequence (5`→ 3`)**
Average: 171 Min: 159 Max: 189	F – TCTACACGTTCAGAGTTCTACAGTCCGACGATCxxx**GTCGGTAAAACTCGTGCCAGC** R – GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTxxx**CATAGTGGGGTATCTAATCCCAGTTTG**
Custom sequencing primers: CTACACGTTCAGAGTTCTACAGTCCGACGATC; GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	

Table S4. First-round PCR thermocycler conditions: 16S fish-specific primers. Modified from Shaw et al. (2016) to minimize off-target amplification.

Step	# Cycles		Temperature (°C)	Time
Activation	1x		95	10 min
Touchdown	8x	*Denaturation*	96	10 s
		Annealing	62	10 s
		Extension	72	30 s
Amplification	30x	*Denaturation*	96	10 s
		Annealing	57	10 s
		Extension	72	30 s
Final Extension	1x		72	5 min
Hold	1x		12	∞

Table S5. First-round PCR thermocycler conditions: 12S MiFish-U primers. Modified from Miya et al. (2015) to minimize off-target amplification.

Step	# Cycles		Temperature (°C)	Time
Activation	1x		95	10 min
Amplification #1	10x	*Denaturation*	96	10 s
		Annealing	68	10 s
		Extension	72	30 s
Touchdown	10x	*Denaturation*	96	10 s
		Annealing	68-1°C per cycle	10 s
		Extension	72	30 s
Amplification #2	18x	*Denaturation*	96	10 s
		Annealing	58	10 s
		Extension	72	30 s
Final Extension	1x		72	5 min
Hold	1x		12	∞

Table S6. Second-round PCR: Illumina Nextera Index Kit PCR primers used for 16S fish-specific amplicons. [i7] and [i5] represent unique 8bp dual-index sequences. Index 1 (i7) adapters used in this study include N701-707, N710-712, N714-16, and N718-720. Index 2 (i5) adapters used include S502-503, S505-508, S510-511, S513, and S515-517**.** The 3' sequence (Read 1 & 2) will bind to the first-round PCR products and the Illumina sequencing MiSeq primers. The 5' sequence (P5 & P7) will allow binding to the Illumina flow cell.

Primer Name	Primer Sequence (5`→ 3`)
Index 1 Read	F – CAAGCAGAAGACGGCATACGAGAT [i7] GTCTCGTGGGCTCGG
Index 2 Read	R – AATGATACGGCGACCACCGAGATCTACAC [i5] TCGTCGGCAGCGTC

Table S7. Second-round PCR: Illumina TruSeq Index Kit PCR primers used for 12S MiFish-U amplicons. [i7] and [i5] represent unique 6bp Illumina dual-index sequences. Index i7 adapters used in this study can be found in (Campbell et al. 2015 Supplementary). The 3' sequence (Read 1 & 2) will bind to the first-round PCR products and the Illumina sequencing MiSeq primers. The 5' sequence (P5 & P7) will allow binding to the Illumina flow cell.
NOTE: Read 1 and Read 2 are obsolete, and this study required custom sequencing primers. We recommend using the current Illumina index adaptors.
Custom sequencing primers used:
5-CTACACGTTCAGAGTTCTACAGTCCGACGATC-3
5-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3.

Primer name	Primer Sequence (5`→ 3`)
Index 1 Read	F – AATGATACGGCGACCACCGAGATCTACAC [i5] TCTACACGTTCAGAGTTCTACAGTCCGACGATC
Index 2 Read	R – CAAGCAGAAGACGGCATACGAGAT [i7] GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

Table S8. Second-round PCR thermocycler conditions for indexing samples. Follows manufacturer PCR recommendations for KAPA HiFi HotStart PCR Kit (Roche Sequencing Solutions, Inc., Switzerland; Product #: 07958897001). Modifications made in this study are denoted by an asterisk (*).

Step	# Cycles		Temperature (°C)	Time
Activation	1x		95	3 min
Amplification	*10x	*Denaturation*	98	*30 s
		Annealing	55	*30 s
		Extension	72	30 s
Final Extension	1x		72	5 min
Hold	1x		12	∞

Table S9. Sequence filtering read pipeline for 16S fish-specific assay samples. Total read abundance for eDNA samples remaining after each step of quality filtration. Steps occurred in listed left→ right order. Total read abundance and total % of reads remaining after each filtration step are displayed at the bottom of the table. ± = Restoration wetland; † = Reference site. ▼ = Represent the 23 samples excluded from data analyses.

Site	Sample Event	Sample ID	Raw	Cutadapt Filter	DADA2 Filter	Denoised Forward	Denoised Reverse	Merged	Chimera Removal	Min/Max Length	Controls Removed	Sample Threshold	Assay Threshold
Tule Red±	**FEB 2023**	**TUL_A1_02▼**	374	135	99	99	99	75	75	75	0	0	0
		TUL_A2_02	169,603	156,957	153,887	153,837	153,791	145,727	145,341	141,397	138,976	138,339	138,339
		TUL_A3_02	52,875	48,921	47,221	47,180	47,139	45,863	45,804	44,193	42,895	42,764	42,764
		TUL_B1_02	217,040	202,016	198,492	198,398	198,395	187,789	187,733	185,848	172,844	171,997	171,997
		TUL_B2_02	138,653	129,321	127,413	127,383	127,365	126,080	126,074	125,984	113,600	113,111	113,111
		TUL_B3_02	59,882	55,438	54,449	54,421	54,409	52,693	52,675	52,410	50,280	50,081	50,081
		TUL_C1_02	159,667	146,214	143,371	143,276	143,274	140,184	140,091	139,685	128,924	128,346	128,346
		TUL_C2_02	125,229	113,736	111,753	111,662	111,632	104,711	104,690	103,883	93,783	93,400	93,400
		TUL_C3_02	96,463	88,499	85,390	85,286	85,265	68,060	68,009	65,098	64,434	64,133	64,133
	SEP 2023	**TUL_A1_09**	377,486	355,813	353,030	352,950	352,928	346,600	339,849	339,795	325,687	322,596	322,596
		TUL_A2_09	224,829	215,453	214,059	214,026	214,019	211,978	211,835	211,576	198,239	196,549	196,549
		TUL_A3_09	345,960	330,814	328,776	328,705	328,720	323,397	323,072	323,027	309,714	307,177	307,177
		TUL_B1_09	319,274	306,105	304,287	304,241	304,228	300,070	299,683	299,623	285,304	282,855	282,855
		TUL_B2_09	364,822	342,372	339,448	339,363	339,324	333,454	333,151	333,092	319,364	316,939	316,939
		TUL_B3_09	329,742	316,001	313,927	313,845	313,858	310,714	310,380	310,347	296,378	294,176	294,176
		TUL_C1_09	365,945	348,260	345,190	345,118	345,117	339,675	339,357	339,323	324,385	320,911	320,911
		TUL_C2_09	177,037	167,796	166,363	166,343	166,337	163,351	163,265	163,217	151,540	150,625	150,625
		TUL_C3_09	361,172	343,141	340,721	340,677	340,671	337,801	337,549	337,528	322,307	319,300	319,300
	DEC 2023	**TUL_A1_12**	419,115	394,131	389,172	389,108	389,086	385,409	384,362	384,307	369,049	365,071	365,071
		TUL_A2_12	367,655	345,772	341,424	341,374	341,319	337,838	337,446	337,399	321,910	319,182	319,182
		TUL_A3_12	477,655	448,170	441,853	441,796	441,753	436,464	433,667	433,641	419,316	415,129	415,129
		TUL_B1_12	445,511	418,328	412,685	412,546	412,472	404,392	399,604	399,231	385,364	381,247	381,247
		TUL_B2_12	496,651	466,100	459,995	459,893	459,806	454,252	454,158	453,400	440,959	437,264	437,264
		TUL_B3_12▼	26	20	20	20	20	19	19	19	0	0	0
		TUL_C1_12	256,817	238,192	231,872	231,782	231,755	226,213	226,163	225,739	213,970	212,268	212,268
		TUL_C2_12	71,845	67,357	65,852	65,841	65,834	65,159	65,148	65,019	64,512	64,414	64,414
		TUL_C3_12	487,583	450,772	442,869	442,805	442,723	434,426	434,231	433,720	421,607	418,519	418,519
Ryer Island†	**FEB 2023**	**RYE_A1_02**	159,203	149,382	146,956	146,873	146,863	144,316	144,279	144,208	129,889	128,995	128,995
		RYE_A2_02	243,998	228,622	223,723	223,627	223,586	217,282	217,225	217,137	212,398	211,064	211,064
		RYE_A3_02	205,401	192,763	189,231	189,168	189,116	186,477	186,457	186,313	182,105	181,158	181,158

		RYE_B1_02	254,539	234,705	226,153	225,888	225,745	207,831	206,944	205,543	201,941	199,537	199,537
		RYE_B2_02	112,241	103,984	100,714	100,591	100,604	94,389	94,338	93,731	90,421	89,555	89,555
		RYE_B3_02	48,006	43,861	41,388	41,291	41,317	33,642	33,620	30,404	27,070	26,861	26,861
		RYE_C1_02	135,441	126,389	123,388	123,309	123,249	119,338	119,302	118,983	115,012	114,332	114,332
		RYE_C2_02	345,965	321,859	315,664	315,492	315,456	304,186	303,137	302,879	287,906	285,039	285,039
		RYE_C3_02▼	Sample lost during DNA extractions.										
	SEP	**RYE_A1_09**	345,020	328,981	324,703	324,658	324,665	320,206	319,976	319,915	305,613	302,541	302,541
	2023	**RYE_A2_09**	281,334	267,125	264,428	264,391	264,393	262,014	261,712	261,571	249,177	247,425	247,425
		RYE_A3_09	346,183	328,597	324,648	324,599	324,597	322,050	321,792	321,772	308,220	305,633	305,633
		RYE_B1_09	338,985	323,411	321,218	321,151	321,155	316,808	316,395	316,348	301,062	297,751	297,751
		RYE_B2_09	249,520	238,017	236,625	236,597	236,590	234,762	234,608	234,595	221,556	219,793	219,793
		RYE_B3_09	263,391	250,693	249,207	249,179	249,164	247,633	246,342	246,292	233,429	231,586	231,586
		RYE_C1_09▼	9,106	8,285	6,439	6,436	6,436	6,368	6,365	6,254	5,320	5,317	0
		RYE_C2_09	328,675	313,010	310,952	310,901	310,902	305,939	303,832	303,785	288,972	286,153	286,153
		RYE_C3_09	278,844	266,982	265,029	264,993	265,005	262,244	262,000	261,981	248,427	246,286	246,286
	DEC	**RYE_A1_12**▼	2,257	898	368	368	366	282	282	208	1	1	0
	2023	**RYE_A2_12**	426,857	397,892	389,731	389,591	389,555	382,671	382,117	378,687	366,869	364,411	364,411
		RYE_A3_12▼	14	11	11	11	11	10	10	10	0	0	0
		RYE_B1_12	300,476	280,515	274,913	274,795	274,712	266,210	265,670	265,431	261,019	259,313	259,313
		RYE_B2_12	140,566	132,336	128,680	128,633	128,625	127,148	127,002	126,822	115,986	115,561	115,561
		RYE_B3_12	386,540	360,542	354,180	354,094	353,969	345,696	345,597	345,194	333,735	331,435	331,435
		RYE_C1_12	409,701	382,646	376,611	376,536	376,469	370,819	370,743	370,561	359,740	357,675	357,675
		RYE_C2_12▼	1,204	366	222	222	222	169	169	163	0	0	0
		RYE_C3_12▼	20	14	14	14	14	13	13	13	0	0	0
Winter	**FEB**	**WIN_A1_02**	187,123	173,698	170,701	170,586	170,544	166,713	165,466	165,392	160,550	159,251	159,251
Island±	**2023**	**WIN_A2_02**	53,701	49,689	48,969	48,931	48,933	48,019	47,992	47,919	43,435	43,196	43,196
		WIN_A3_02	164,281	152,000	149,425	149,337	149,230	145,101	145,003	144,978	131,872	130,923	130,923
		WIN_B1_02	182,187	170,478	168,057	167,938	167,949	165,711	165,594	165,259	154,975	153,539	153,539
		WIN_B2_02	231,353	215,306	211,907	211,843	211,783	207,253	206,952	206,903	201,222	199,180	199,180
		WIN_B3_02	150,524	139,244	136,865	136,806	136,771	135,179	135,055	135,036	122,511	121,329	121,329
		WIN_C1_02	222,701	206,902	202,772	202,708	202,644	194,711	194,552	194,445	182,832	181,381	181,381
		WIN_C2_02	71,644	66,763	65,466	65,417	65,395	64,325	64,265	64,213	59,745	59,247	59,247
		WIN_C3_02	181,023	167,046	164,548	164,418	164,382	159,428	159,345	159,146	156,380	155,772	155,772
	DEC	**WIN_A1_12**	409,117	380,337	374,352	374,314	374,286	366,373	366,128	366,092	352,980	350,087	350,087
	2023	**WIN_A2_12**	319,713	299,095	295,524	295,491	295,490	291,642	291,443	291,406	276,815	274,094	274,094
		WIN_A3_12	411,956	382,778	377,617	377,575	377,551	372,883	372,792	372,334	359,233	356,465	356,465
		WIN_B1_12	386,142	360,270	355,361	355,326	355,278	349,742	349,551	349,480	333,558	329,557	329,557
		WIN_B2_12	338,880	316,662	310,624	310,553	310,531	305,981	305,857	305,749	292,597	289,749	289,749
		WIN_B3_12	372,823	346,536	340,654	340,599	340,586	337,430	337,195	337,016	323,429	321,823	321,823
		WIN_C1_12▼	77	50	49	49	48	46	46	46	0	0	0

		WIN_C2_12▼	3	1	1	1	1	1	1	1	0	0	0
		WIN_C3_12	364,654	340,268	334,999	334,920	334,901	330,620	330,135	329,986	320,310	318,533	318,533
Montezuma Wetlands±	**FEB 2023**	**MON_A1_02**	440,947	413,698	409,125	408,987	408,901	401,945	401,200	400,778	386,075	382,704	382,704
		MON_A2_02	394,319	367,730	362,117	361,944	361,871	355,573	354,826	354,502	341,474	338,227	338,227
		MON_A3_02	385,500	360,319	354,827	354,720	354,601	344,931	344,300	343,875	329,728	326,495	326,495
		MON_B1_02	389,732	364,444	360,000	359,829	359,818	354,054	353,353	353,098	339,805	337,172	337,172
		MON_B2_02	223,885	209,938	207,276	207,196	207,167	202,397	202,045	202,006	187,591	185,971	185,971
		MON_B3_02	434,570	402,983	397,835	397,652	397,587	385,862	374,076	373,896	359,131	356,039	356,039
		MON_C1_02	410,468	381,449	376,041	375,892	375,813	366,236	365,035	364,795	349,288	344,767	344,767
		MON_C2_02	448,289	415,594	408,491	408,317	408,244	398,752	383,386	382,792	368,847	364,533	364,533
		MON_C3_02	413,976	386,459	380,959	380,752	380,795	367,645	367,140	366,659	351,079	346,687	346,687
	AUG 2023	**MON_A1_08**	414,020	392,462	389,727	389,673	389,686	385,577	382,893	382,867	367,483	363,165	363,165
		MON_A2_08	339,901	322,040	319,817	319,754	319,752	316,501	316,074	316,053	300,498	296,442	296,442
		MON_A3_08	421,812	400,484	397,887	397,800	397,825	393,908	393,288	393,243	377,513	371,944	371,944
		MON_B1_08▼	976	315	249	249	249	196	194	194	0	0	0
		MON_B2_08	348,213	330,223	327,976	327,929	327,928	323,843	323,355	323,341	307,823	303,979	303,979
		MON_B3_08	750,008	710,026	704,705	704,595	704,607	695,914	695,042	695,005	680,047	673,425	673,425
		MON_C1_08	428,955	406,855	403,793	403,705	403,732	398,453	397,607	397,579	382,466	377,588	377,588
		MON_C2_08	364,214	346,420	344,111	344,052	344,067	340,234	339,194	339,182	323,486	319,816	319,816
		MON_C3_08	397,042	376,896	374,407	374,345	374,368	370,876	370,165	370,067	354,671	350,115	350,115
	JAN 2024	**MON_A1_01▼**	1,122	464	322	321	321	276	276	231	0	0	0
		MON_A2_01▼	207	187	184	184	184	183	182	182	0	0	0
		MON_A3_01▼	Sample was not assigned a sample ID following demultiplexing.										
		MON_B1_01	424,769	396,003	391,266	391,227	391,198	387,393	387,059	387,036	371,629	368,118	368,118
		MON_B2_01▼	22,424	20,469	402	398	401	327	325	323	2	2	0
		MON_B3_01	381,412	353,877	349,762	349,707	349,679	341,491	341,067	340,957	325,360	321,757	321,757
		MON_C1_01	84,517	78,832	77,548	77,528	77,523	75,074	74,939	74,861	73,380	73,270	73,270
		MON_C2_01	381,013	354,920	350,527	350,476	350,395	347,278	346,953	346,869	332,619	330,005	330,005
		MON_C3_01	411,248	382,934	378,133	378,064	378,009	370,317	369,813	369,737	353,573	349,659	349,659
Browns Island†	**FEB 2023**	**BRO_A1_02**	226,577	210,693	207,006	206,929	206,900	204,737	204,410	204,291	200,092	198,646	198,646
		BRO_A2_02	139,978	129,145	126,776	126,723	126,692	122,487	122,355	122,162	118,832	118,132	118,132
		BRO_A3_02	84,352	78,712	77,236	77,171	77,149	74,711	74,622	74,559	71,135	70,704	70,704
		BRO_B1_02	78,858	73,398	71,869	71,804	71,813	70,969	70,851	70,851	67,767	67,459	67,459
		BRO_B2_02	264,154	245,840	241,886	241,848	241,796	238,314	238,040	237,989	224,511	222,332	222,332
		BRO_B3_02	101,694	92,400	90,211	90,191	90,162	88,829	88,693	88,686	77,252	76,809	76,809
		BRO_C1_02	263,151	246,662	243,583	243,545	243,504	240,880	240,500	240,481	225,451	223,645	223,645
		BRO_C2_02	185,647	173,640	171,311	171,268	171,251	168,621	167,088	167,067	151,652	150,089	150,089
		BRO_C3_02	182,368	170,518	168,357	168,306	168,283	165,029	164,740	164,722	148,767	147,334	147,334
	AUG 2023	**BRO_A1_08**	379,934	358,723	355,852	355,806	355,797	352,004	347,591	347,569	331,671	327,394	327,394
		BRO_A2_08	345,473	327,418	324,794	324,747	324,752	320,858	320,417	320,383	305,098	301,516	301,516

		BRO_A3_08	344,733	325,918	323,147	323,090	323,084	319,494	316,926	316,880	302,038	299,172	299,172
		BRO_B1_08	451,080	431,848	428,761	428,705	428,697	423,986	423,546	423,512	408,381	403,622	403,622
		BRO_B2_08	319,706	305,483	303,413	303,372	303,370	300,317	300,001	299,771	284,589	280,816	280,816
		BRO_B3_08	379,915	363,588	361,122	361,090	361,074	356,924	356,558	356,548	341,501	336,903	336,903
		BRO_C1_08	351,135	336,561	334,799	334,760	334,775	327,634	326,891	326,879	311,206	306,294	306,294
		BRO_C2_08	400,890	383,676	381,595	381,544	381,543	376,383	375,650	375,623	360,551	355,687	355,687
		BRO_C3_08	318,748	305,905	304,157	304,095	304,112	300,552	300,082	300,074	285,010	280,982	280,982
	DEC 2023	**BRO_A1_12**	134,275	126,555	124,738	124,722	124,717	121,326	121,285	121,130	109,746	109,130	109,130
		BRO_A2_12	382,701	356,794	351,936	351,920	351,888	348,769	348,747	348,725	337,967	336,480	336,480
		BRO_A3_12	364,355	343,690	339,406	339,375	339,367	336,122	335,202	335,104	320,417	317,317	317,317
		BRO_B1_12	168,714	154,084	111,213	111,193	111,192	110,341	110,333	110,319	109,421	109,330	109,330
		BRO_B2_12▼	59	47	46	45	46	45	45	44	0	0	0
		BRO_B3_12▼	4	1	1	1	1	1	1	1	0	0	0
		BRO_C1_12▼	5,680	5,012	243	243	241	200	200	172	0	0	0
		BRO_C2_12▼	1,709	1,095	795	793	793	740	738	738	0	0	0
		BRO_C3_12	217,350	200,088	197,093	197,069	197,071	193,926	193,456	193,444	179,140	177,328	177,328
Yolo Flyway Farms±	**MAR 2023**	**FLY_A1_03**	267,919	243,695	240,981	240,898	240,869	236,780	236,482	236,433	221,861	219,446	219,446
		FLY_A2_03	117,619	110,460	109,529	109,480	109,436	108,446	108,383	108,310	95,269	94,414	94,414
		FLY_A3_03	275,390	257,180	255,066	255,000	254,991	251,748	251,382	251,304	236,415	233,328	233,328
		FLY_B1_03	240,039	226,840	224,997	224,915	224,912	222,159	221,857	221,822	206,573	204,253	204,253
		FLY_B2_03	259,303	234,955	232,563	232,490	232,472	230,005	229,813	229,767	217,580	216,093	216,093
		FLY_B3_03	278,139	261,211	258,778	258,656	258,622	255,895	253,676	248,816	233,666	230,997	230,997
		FLY_C1_03	252,575	236,168	233,349	233,293	233,298	230,706	230,450	230,369	215,215	212,567	212,567
		FLY_C2_03	187,354	175,278	173,509	173,474	173,470	171,374	171,275	171,249	157,720	156,073	156,073
		FLY_C3_03	291,301	269,991	267,305	267,255	267,251	264,371	263,954	263,882	248,807	246,130	246,130
	AUG 2023	**FLY_A1_08**	485,210	463,970	460,277	460,161	460,166	453,168	450,081	449,983	444,166	437,303	437,303
		FLY_A2_08	329,703	314,554	312,412	312,340	312,331	308,846	307,860	307,824	302,271	297,564	297,564
		FLY_A3_08	410,132	391,858	389,243	389,159	389,175	381,262	379,785	379,770	374,118	368,726	368,726
		FLY_B1_08	357,912	343,086	340,822	340,764	340,770	332,739	330,835	330,816	325,241	320,549	320,549
		FLY_B2_08	345,187	329,633	327,077	327,024	327,031	316,389	314,354	314,347	308,744	304,805	304,805
		FLY_B3_08	353,613	339,534	336,658	336,598	336,627	330,039	328,386	328,357	322,627	318,374	318,374
		FLY_C1_08	396,910	377,902	374,120	374,045	374,076	366,331	365,271	365,143	360,476	355,530	355,530
		FLY_C2_08	398,179	379,162	376,264	376,203	376,228	368,182	367,095	367,080	362,441	357,721	357,721
		FLY_C3_08	263,104	248,459	246,376	246,324	246,331	240,463	238,668	238,662	233,790	230,794	230,794
	DEC 2023	**FLY_A1_12**	379,536	354,043	348,338	348,274	348,278	343,735	343,145	343,058	330,500	325,470	325,470
		FLY_A2_12	263,861	248,166	245,140	245,105	245,095	242,575	242,266	242,229	237,109	234,071	234,071
		FLY_A3_12	432,480	401,709	396,626	396,564	396,489	393,682	392,914	392,784	381,924	376,351	376,351
		FLY_B1_12▼	3,567	1,662	652	647	642	476	474	237	0	0	0
		FLY_B2_12	437,398	407,772	402,194	402,134	402,114	396,988	394,198	394,118	379,493	374,900	374,900
		FLY_B3_12	502,251	468,574	462,445	462,356	462,295	458,255	457,555	457,477	445,194	440,170	440,170

		FLY_C1_12	423,022	394,141	387,707	387,576	387,542	383,590	383,235	383,043	369,660	365,319	365,319
		FLY_C2_12	404,162	378,966	373,976	373,905	373,884	369,629	369,239	369,127	357,836	353,064	353,064
		FLY_C3_12▼	2,565	1,022	416	411	416	298	296	245	1	1	0
Liberty Island†	**MAR 2023**	**LIB_A1_03**	268,483	251,871	248,604	248,566	248,508	244,941	244,533	244,391	239,896	237,659	237,659
		LIB_A2_03	240,922	225,074	221,590	221,536	221,492	218,830	218,484	218,461	213,121	211,418	211,418
		LIB_A3_03	192,957	179,501	176,525	176,491	176,449	174,310	174,066	173,977	170,682	169,625	169,625
		LIB_B1_03	255,487	238,959	236,097	236,037	236,007	232,418	232,039	231,998	228,046	226,031	226,031
		LIB_B2_03	133,786	124,710	123,346	123,324	123,296	121,767	120,862	120,845	115,635	114,666	114,666
		LIB_B3_03	280,537	260,267	257,324	257,284	257,251	254,338	253,890	253,837	248,251	245,905	245,905
		LIB_C1_03	194,341	179,208	176,129	176,060	176,041	172,890	172,589	172,532	167,704	166,395	166,395
		LIB_C2_03	199,436	186,071	183,713	183,636	183,616	181,425	181,164	181,112	177,170	175,870	175,870
		LIB_C3_03	250,253	234,575	231,795	231,641	231,641	227,716	227,473	227,192	223,704	222,185	222,185
	AUG 2023	**LIB_A1_08**	330,825	314,838	312,605	312,583	312,599	310,438	308,096	308,083	302,698	299,977	299,977
		LIB_A2_08	317,179	301,071	298,267	298,245	298,255	295,747	293,689	293,680	288,366	285,269	285,269
		LIB_A3_08	317,471	301,481	299,006	298,975	298,989	296,730	294,400	294,382	289,336	286,569	286,569
		LIB_B1_08	414,163	394,818	392,196	392,143	392,134	389,950	388,290	388,267	383,494	379,908	379,908
		LIB_B2_08	312,638	297,923	296,077	296,041	296,039	294,474	292,320	292,307	288,002	285,186	285,186
		LIB_B3_08	351,593	335,221	333,324	333,269	333,291	331,283	329,613	329,577	324,680	321,364	321,364
		LIB_C1_08	385,685	368,256	364,331	364,220	364,230	361,762	360,368	360,342	346,354	341,053	341,053
		LIB_C2_08	240,604	228,809	225,956	225,898	225,900	221,420	221,129	221,117	219,220	217,325	217,325
		LIB_C3_08	305,169	290,513	287,122	287,004	287,029	284,011	282,063	281,912	275,960	272,262	272,262
	DEC 2023	**LIB_A1_12**	457,461	426,203	420,291	420,232	420,230	412,840	411,959	411,888	405,458	399,806	399,806
		LIB_A2_12	460,147	429,091	422,608	422,540	422,524	416,999	416,304	416,144	409,793	404,191	404,191
		LIB_A3_12	513,451	479,678	472,804	472,747	472,709	464,471	458,649	458,468	452,937	447,359	447,359
		LIB_B1_12	396,886	371,987	367,242	367,197	367,191	362,012	360,766	360,733	356,076	351,985	351,985
		LIB_B2_12	354,511	334,555	330,964	330,931	330,920	326,207	325,406	325,378	312,486	308,855	308,855
		LIB_B3_12	431,379	403,061	398,514	398,454	398,436	391,155	390,113	390,054	384,797	379,959	379,959
		LIB_C1_12	445,157	415,172	409,250	409,159	409,128	400,900	400,240	400,032	386,396	381,720	381,720
		LIB_C2_12	483,885	452,386	446,069	445,992	445,953	440,357	439,955	438,469	424,800	420,100	420,100
		LIB_C3_12	508,337	476,425	469,914	469,792	469,731	455,977	454,169	454,063	439,430	433,634	433,634
Webb Tracts Island and Berms†	**FEB 2023**	**WEB_A1_02**	219,693	205,495	202,136	202,078	202,066	191,165	191,043	191,013	186,179	184,136	184,136
		WEB_A2_02	230,310	213,433	209,422	209,335	209,307	200,975	200,738	200,690	193,766	191,468	191,468
		WEB_A3_02	179,494	165,952	162,691	162,644	162,590	158,757	158,639	158,620	153,799	152,086	152,086
		WEB_B1_02	200,094	186,349	183,392	183,322	183,285	179,224	179,083	179,005	175,058	173,305	173,305
		WEB_B2_02	43,969	40,830	40,162	40,141	40,127	39,314	39,298	39,141	35,544	35,255	35,255
		WEB_B3_02	218,606	202,208	199,081	199,028	198,964	195,879	194,670	193,783	190,522	189,178	189,178
		WEB_C1_02	137,303	129,554	127,595	127,550	127,528	124,759	124,616	124,557	122,057	121,093	121,093
		WEB_C2_02	180,933	168,198	165,625	165,594	165,549	161,791	161,636	161,600	159,277	158,254	158,254
		WEB_C3_02▼	22,626	20,248	19,806	19,797	19,800	19,497	19,471	19,471	18,041	18,013	0
		DEC_A1_02▼	1,461	909	535	535	533	456	452	225	2	2	0

Decker Island±	**FEB 2023**	**DEC_A2_02**	117,197	109,053	107,363	107,342	107,308	106,597	106,446	106,383	99,482	98,909	98,909
		DEC_A3_02	230,642	213,976	210,508	210,486	210,420	208,389	207,974	207,947	200,662	199,400	199,400
		DEC_B1_02	191,005	174,955	171,813	171,784	171,705	170,439	169,861	169,671	164,032	163,183	163,183
		DEC_B2_02	230,838	216,286	213,011	212,963	212,919	211,161	210,803	210,725	206,094	204,538	204,538
		DEC_B3_02	174,007	162,840	160,272	160,239	160,204	158,587	158,330	158,247	151,126	149,691	149,691
		DEC_C1_02	204,821	190,189	185,832	185,793	185,746	182,796	182,465	182,442	176,134	174,552	174,552
		DEC_C2_02	121,818	113,281	111,156	111,136	111,115	110,097	109,906	109,869	105,794	105,069	105,069
		DEC_C3_02	233,523	213,857	209,943	209,899	209,849	207,363	207,013	207,003	199,793	197,996	197,996
		TOTAL	**51,716,593**	**48,557,686**	**47,884,364**	**47,872,560**	**47,868,634**	**47,062,235**	**46,911,487**	**46,866,514**	**45,109,967**	**44,653,359**	**44,630,023**
		Remaining	**100.00 %**	**93.89 %**	**92.59 %**	**92.57 %**	**92.56 %**	**91.00 %**	**90.71 %**	**90.62 %**	**87.23 %**	**86.34 %**	**86.30 %**

Table S10. Sequence filtering read pipeline for 12S MiFish-U assay samples. Total read abundance for eDNA samples remaining after each step of quality filtration. Steps occurred in listed left→ right order. Total read abundance and total % of reads remaining after each filtration step are displayed at the bottom of the table. ± = Restoration wetland; † = Reference site. ▼ = Represent the 20 samples excluded from data analyses.

Site	Sample Event	Sample ID	Raw	Cutadapt Filter	DADA2 Filter	Denoised Forward	Denoised Reverse	Merged	Chimera Removal	Min/Max Length	Controls Removed	Sample Threshold	Assay Threshold
Tule Red±	**FEB 2023**	**TUL_A1_02▼**	96	72	67	67	67	37	35	35	2	2	0
		TUL_A2_02	10,921	10,555	10,421	10,420	10,417	10,376	10,357	10,357	10,273	10,254	10,254
		TUL_A3_02	34,807	33,887	33,722	33,714	33,716	33,662	33,633	33,632	33,198	33,117	33,117
		TUL_B1_02	37,313	35,941	35,492	35,486	35,466	35,392	35,341	35,341	35,065	35,003	35,003
		TUL_B2_02	15,283	14,796	14,675	14,675	14,674	14,656	14,628	14,628	14,083	14,034	14,034
		TUL_B3_02▼	11,281	10,837	10,718	10,715	10,712	45	43	43	11	11	0
		TUL_C1_02▼	92	81	78	74	75	30	30	30	3	3	0
		TUL_C2_02▼	890	848	833	831	833	811	809	809	389	389	0
		TUL_C3_02▼	186	156	144	141	143	53	52	52	0	0	0
	SEP 2023	**TUL_A1_09**	68,647	66,205	65,513	65,507	65,488	65,126	64,199	64,194	63,167	62,505	62,505
		TUL_A2_09	63,211	60,789	60,062	60,047	60,035	59,695	59,136	59,132	58,063	57,565	57,565
		TUL_A3_09	65,961	63,666	63,013	63,002	62,941	62,224	61,330	61,328	60,395	59,753	59,753
		TUL_B1_09	55,262	53,415	52,913	52,910	52,891	52,711	51,908	51,901	50,864	50,484	50,484
		TUL_B2_09	62,204	60,025	59,321	59,307	59,295	58,969	58,240	58,238	57,087	56,519	56,519
		TUL_B3_09	61,013	58,948	58,360	58,346	58,346	58,150	57,202	57,197	55,660	54,904	54,904
		TUL_C1_09	59,987	57,910	57,309	57,185	57,284	56,924	56,153	56,148	55,001	54,415	54,415
		TUL_C2_09	64,882	62,651	62,013	62,005	61,985	61,225	60,923	60,922	60,415	59,979	59,979
		TUL_C3_09	70,107	67,794	67,108	67,098	67,079	66,878	66,314	66,314	64,982	64,404	64,404
		TUL_A1_12	56,749	54,846	54,339	54,331	54,310	54,038	52,526	52,522	50,928	50,188	50,188

	DEC 2023	**TUL_A2_12**	52,502	50,676	50,225	50,218	50,205	50,038	48,723	48,723	47,096	46,325	46,325
		TUL_A3_12	54,361	52,603	52,159	52,141	52,139	51,337	50,134	50,133	48,759	48,251	48,251
		TUL_B1_12	54,007	52,511	52,128	52,116	52,116	51,626	51,321	51,321	50,282	49,906	49,906
		TUL_B2_12	53,264	51,513	51,005	50,995	50,984	49,845	49,584	49,584	48,460	47,931	47,931
		TUL_B3_12	56,557	54,660	54,019	53,996	53,994	51,110	50,704	50,703	50,042	49,667	49,667
		TUL_C1_12	63,606	61,337	60,671	60,653	60,647	59,171	58,762	58,175	57,381	56,981	56,981
		TUL_C2_12▼	46	42	42	42	42	31	31	31	0	0	0
		TUL_C3_12	69,038	66,759	66,122	66,089	66,085	65,290	65,193	65,190	64,826	64,532	64,532
Ryer Island†	**FEB 2023**	**RYE_A1_02**	44,018	42,330	41,821	41,817	41,812	41,650	41,547	41,547	40,637	40,439	40,439
		RYE_A2_02	61,530	59,319	58,586	58,581	58,574	56,921	56,869	56,868	56,272	56,007	56,007
		RYE_A3_02	30,106	28,955	28,681	28,680	28,675	28,622	28,616	28,616	28,337	28,234	28,234
		RYE_B1_02	62,933	60,510	59,691	59,681	59,660	59,418	59,360	59,358	58,963	58,779	58,779
		RYE_B2_02	46,139	44,148	43,255	43,248	43,233	42,948	42,923	42,923	42,807	42,683	42,683
		RYE_B3_02▼	27	20	18	18	18	15	15	15	0	0	0
		RYE_C1_02	47,658	45,454	44,536	44,536	44,521	44,245	44,230	44,230	43,946	43,837	43,837
		RYE_C2_02	34,945	33,734	33,269	33,266	33,261	33,180	33,180	33,180	32,810	32,730	32,730
		RYE_C3_02	Sample lost during DNA extractions.										
	SEP 2023	**RYE_A1_09**	71,523	69,223	68,335	68,330	68,316	68,113	67,627	67,625	66,562	66,048	66,048
		RYE_A2_09	60,138	58,102	57,499	57,454	57,472	57,280	56,893	56,890	56,099	55,563	55,563
		RYE_A3_09	62,498	60,267	59,625	59,621	59,597	58,924	58,364	58,360	56,846	56,340	56,340
		RYE_B1_09	56,603	54,808	54,252	54,249	54,236	54,075	52,864	52,861	51,596	51,053	51,053
		RYE_B2_09	60,435	58,289	57,671	57,662	57,640	57,462	56,621	56,618	55,345	54,819	54,819
		RYE_B3_09	68,590	66,142	65,432	65,418	65,411	65,167	64,218	64,210	62,980	62,465	62,465
		RYE_C1_09▼	42	35	35	35	35	34	34	34	0	0	0
		RYE_C2_09	49,157	47,520	47,114	47,102	47,097	46,968	45,929	45,927	44,549	44,182	44,182
		RYE_C3_09	59,637	57,469	56,952	56,947	56,918	56,741	55,642	55,641	54,050	53,632	53,632
	DEC 2023	**RYE_A1_12**▼	47	39	35	35	35	33	32	32	0	0	0
		RYE_A2_12	51,527	49,860	49,453	49,447	49,436	49,286	48,927	48,925	47,863	47,506	47,506
		RYE_A3_12	64,476	62,375	61,872	61,838	61,846	60,713	60,457	60,451	60,024	59,786	59,786
		RYE_B1_12	58,921	57,040	56,456	56,444	56,433	55,975	55,432	55,428	54,829	54,593	54,593
		RYE_B2_12	53,003	51,137	50,526	50,521	50,508	50,368	50,281	50,281	49,901	49,739	49,739
		RYE_B3_12	72,963	70,311	69,693	69,676	69,668	69,411	69,173	69,172	68,423	67,964	67,964
		RYE_C1_12	70,888	68,537	67,988	67,977	67,973	67,360	67,036	67,034	66,284	65,845	65,845
		RYE_C2_12▼	14	12	11	11	11	10	9	9	0	0	0
		RYE_C3_12	69,937	67,855	67,277	67,270	67,254	66,462	65,758	65,758	64,852	64,531	64,531
Winter Island±	**FEB 2023**	**WIN_A1_02**	61,930	59,869	59,421	59,408	59,413	59,241	58,585	58,582	58,009	57,636	57,636
		WIN_A2_02	72,494	69,701	68,927	68,915	68,888	68,633	68,227	68,227	67,122	66,591	66,591
		WIN_A3_02	63,048	60,848	60,307	60,294	60,296	60,175	59,781	59,780	58,893	58,504	58,504

		WIN_B1_02	60,984	59,032	58,574	58,559	58,521	58,296	56,997	56,996	55,864	55,122	55,122
		WIN_B2_02	63,694	61,343	60,825	60,817	60,799	59,952	58,774	58,773	57,815	57,232	57,232
		WIN_B3_02	60,891	58,766	58,318	58,310	58,304	58,118	57,203	57,201	56,085	55,498	55,498
		WIN_C1_02	64,573	62,459	61,947	61,940	61,929	59,541	59,206	59,206	58,477	57,940	57,940
		WIN_C2_02	59,873	57,899	57,449	57,440	57,430	57,226	56,568	56,565	55,457	54,855	54,855
		WIN_C3_02	74,692	72,424	71,859	71,852	71,844	71,726	71,120	71,119	70,191	69,605	69,605
	DEC 2023	**WIN_A1_12**	59,815	57,741	57,155	57,147	57,140	56,965	56,247	56,245	55,231	54,791	54,791
		WIN_A2_12	63,447	61,138	60,558	60,546	60,519	60,339	59,578	59,574	58,278	57,696	57,696
		WIN_A3_12	58,778	56,738	56,068	56,057	56,049	55,825	54,874	54,874	53,747	53,269	53,269
		WIN_B1_12	52,668	50,798	50,259	50,248	50,242	49,721	49,030	49,029	48,072	47,575	47,575
		WIN_B2_12	53,681	51,851	51,358	51,346	51,340	51,148	50,006	50,006	48,500	47,900	47,900
		WIN_B3_12	56,842	54,801	54,326	54,312	54,311	54,072	53,539	53,538	52,694	52,279	52,279
		WIN_C1_12	66,595	64,050	63,451	63,435	63,420	63,177	62,467	62,465	61,734	61,278	61,278
		WIN_C2_12	60,090	58,259	57,732	57,726	57,700	57,399	55,965	55,963	55,052	54,487	54,487
		WIN_C3_12	62,784	60,718	60,137	60,125	60,124	59,977	59,506	59,506	58,624	58,235	58,235
Montezuma Wetlands±	**FEB 2023**	**MON_A1_02**	69,440	67,016	66,228	66,213	66,202	64,146	63,112	63,112	61,827	61,282	61,282
		MON_A2_02	72,039	69,245	68,504	68,496	68,483	68,314	67,288	67,287	66,342	65,753	65,753
		MON_A3_02	72,378	69,725	69,146	69,131	69,131	68,962	68,517	68,517	67,265	66,803	66,803
		MON_B1_02	70,174	68,031	67,524	67,517	67,511	67,336	66,674	66,674	65,446	64,990	64,990
		MON_B2_02	54,773	53,068	52,489	52,476	52,460	52,228	51,365	51,365	50,123	49,668	49,668
		MON_B3_02	66,440	63,998	63,442	63,430	63,423	63,117	62,327	62,327	61,224	60,726	60,726
		MON_C1_02	60,529	58,392	57,894	57,882	57,874	57,580	56,712	56,712	55,749	55,250	55,250
		MON_C2_02	67,821	65,090	64,578	64,478	64,559	64,120	63,292	63,292	62,290	61,656	61,656
		MON_C3_02	71,407	68,844	68,245	68,238	68,230	68,074	67,474	67,474	66,257	65,773	65,773
	AUG 2023	**MON_A1_08**	49,226	47,436	46,882	46,867	46,858	46,636	45,165	45,162	43,616	43,021	43,021
		MON_A2_08	53,162	51,186	50,580	50,565	50,550	50,325	48,825	48,822	47,173	46,491	46,491
		MON_A3_08	59,240	56,905	56,360	56,347	56,335	55,883	54,599	54,595	52,927	52,202	52,202
		MON_B1_08	60,655	58,395	57,768	57,754	57,736	57,475	56,040	56,038	54,375	53,709	53,709
		MON_B2_08	58,067	56,344	55,899	55,894	55,881	55,705	54,314	54,313	52,944	52,329	52,329
		MON_B3_08	52,797	50,847	50,257	50,249	50,243	48,224	47,129	47,128	45,496	44,984	44,984
		MON_C1_08	53,361	51,363	50,789	50,780	50,778	50,547	48,162	48,157	46,456	45,806	45,806
		MON_C2_08	64,021	61,620	60,899	60,883	60,878	60,623	59,166	59,165	57,569	56,921	56,921
		MON_C3_08	53,349	51,784	51,294	51,282	51,275	51,012	50,097	50,095	48,465	47,764	47,764
	JAN 2024	**MON_A1_01**▼	23	16	15	15	15	15	15	15	0	0	0
		MON_A2_01	63,202	60,953	60,309	60,293	60,293	60,086	57,771	57,770	56,384	55,801	55,801
		MON_A3_01	60,152	58,383	57,918	57,914	57,908	57,809	57,027	57,027	56,617	56,375	56,375
		MON_B1_01	60,374	58,088	57,385	57,367	57,359	57,168	55,930	55,930	54,462	53,847	53,847
		MON_B2_01▼	31	26	24	24	24	23	22	22	0	0	0

		MON_B3_01	57,189	55,115	54,650	54,632	54,628	54,476	53,444	53,443	51,924	51,377	51,377
		MON_C1_01	30,772	29,735	29,355	29,354	29,348	28,960	28,947	28,947	28,701	28,625	28,625
		MON_C2_01	61,537	59,273	58,657	58,649	58,637	58,478	57,431	57,431	56,624	56,193	56,193
		MON_C3_01	55,773	54,036	53,652	53,645	53,637	53,473	52,345	52,345	51,148	50,662	50,662
Browns Island[†]	**FEB 2023**	**BRO_A1_02**	57,800	56,100	55,689	55,684	55,675	55,594	55,034	55,034	54,131	53,834	53,834
		BRO_A2_02	70,312	67,932	67,263	67,255	67,243	66,908	66,295	66,294	65,402	64,733	64,733
		BRO_A3_02	56,642	54,673	54,216	54,210	54,194	54,088	53,448	53,447	52,654	52,224	52,224
		BRO_B1_02	65,419	63,161	62,633	62,631	62,622	62,398	61,957	61,957	61,368	60,882	60,882
		BRO_B2_02	63,652	61,450	60,955	60,939	60,941	60,803	60,299	60,295	59,461	58,970	58,970
		BRO_B3_02	61,520	59,540	59,093	59,089	59,089	59,002	58,630	58,630	58,102	57,670	57,670
		BRO_C1_02	60,112	58,036	57,543	57,535	57,532	57,416	56,662	56,658	55,317	54,673	54,673
		BRO_C2_02	64,712	62,577	62,061	62,047	61,784	61,515	60,200	60,195	58,919	58,018	58,018
		BRO_C3_02	63,828	61,658	61,113	61,085	61,097	60,313	59,119	59,116	57,962	57,222	57,222
	AUG 2023	**BRO_A1_08**	52,214	50,436	49,922	49,912	49,901	49,503	49,020	49,016	47,953	47,522	47,522
		BRO_A2_08	50,666	48,798	48,324	48,320	48,304	48,166	47,308	47,306	45,759	45,263	45,263
		BRO_A3_08	49,192	47,335	46,835	46,825	46,814	46,510	45,690	45,689	44,313	43,821	43,821
		BRO_B1_08	60,284	58,362	57,904	57,902	57,884	57,613	56,676	56,674	55,112	54,408	54,408
		BRO_B2_08	59,974	57,913	57,380	57,376	57,368	57,136	56,113	56,109	54,426	53,809	53,809
		BRO_B3_08	50,968	49,066	48,610	48,598	48,589	48,276	47,310	47,306	45,790	45,124	45,124
		BRO_C1_08	53,613	51,624	51,074	51,055	51,055	50,752	49,202	49,200	47,472	46,718	46,718
		BRO_C2_08	52,405	50,498	50,068	50,062	50,043	49,683	48,505	48,503	46,718	45,957	45,957
		BRO_C3_08	47,168	45,697	45,352	45,336	45,328	45,054	43,694	43,686	41,974	41,276	41,276
	DEC 2023	**BRO_A1_12**	40,836	39,406	38,962	38,961	38,953	38,805	38,730	38,729	38,358	38,199	38,199
		BRO_A2_12	66,087	63,859	63,379	63,377	63,362	63,287	63,271	63,271	63,081	63,020	63,020
		BRO_A3_12	61,327	58,917	58,212	58,202	58,165	57,990	56,951	56,951	55,359	54,775	54,775
		BRO_B1_12▼	67	54	52	52	52	36	36	36	7	7	0
		BRO_B2_12	57,230	54,995	54,456	54,452	54,414	54,277	53,952	53,951	53,485	53,293	53,293
		BRO_B3_12▼	24	22	19	19	19	19	19	19	0	0	0
		BRO_C1_12▼	7	7	7	7	7	7	7	7	0	0	0
		BRO_C2_12	10,149	9,810	9,722	9,721	9,718	9,701	9,701	9,701	9,693	9,688	9,688
		BRO_C3_12	13,789	13,369	13,231	13,225	13,227	13,186	12,947	12,947	12,012	11,901	11,901
Yolo Flyway Farms[±]	**MAR 2023**	**FLY_A1_03**	52,627	50,590	50,019	49,979	49,972	49,533	47,188	47,186	46,021	45,415	45,415
		FLY_A2_03	52,566	50,804	50,295	50,267	50,276	49,800	48,106	48,106	47,052	46,644	46,644
		FLY_A3_03	56,238	54,102	53,496	53,466	53,476	53,169	51,247	51,246	50,094	49,502	49,502
		FLY_B1_03	54,705	52,875	52,278	52,255	52,251	51,988	50,136	50,135	49,021	48,559	48,559
		FLY_B2_03	52,197	50,424	46,413	46,402	46,404	46,149	45,371	45,371	44,691	44,355	44,355
		FLY_B3_03	58,689	56,841	54,607	54,587	54,580	54,318	52,218	52,218	50,967	50,530	50,530
		FLY_C1_03	63,609	62,176	61,724	61,676	61,710	61,284	58,869	58,868	57,632	56,944	56,944

		FLY_C2_03	37,655	31,261	29,574	29,551	29,474	29,105	28,071	28,070	26,970	26,761	26,761
		FLY_C3_03	59,402	57,462	56,918	56,888	56,895	56,609	54,626	54,622	53,496	52,929	52,929
	AUG 2023	**FLY_A1_08**	38,132	36,805	36,492	36,470	36,465	35,960	34,310	34,310	32,919	32,244	32,244
		FLY_A2_08	42,534	40,900	40,502	40,484	40,471	40,076	38,607	38,606	37,172	36,443	36,443
		FLY_A3_08	38,353	36,916	36,591	36,579	36,562	36,274	34,963	34,953	33,568	33,004	33,004
		FLY_B1_08	37,380	36,107	35,761	35,742	35,735	35,319	33,943	33,943	32,569	31,958	31,958
		FLY_B2_08	43,003	41,541	41,114	41,094	41,098	40,671	39,347	39,347	38,307	37,693	37,693
		FLY_B3_08	45,711	44,111	43,681	43,648	43,663	43,172	42,002	42,002	40,817	40,257	40,257
		FLY_C1_08	46,678	44,991	44,598	44,569	44,567	43,848	41,222	41,222	40,078	39,343	39,343
		FLY_C2_08	39,991	39,013	38,774	38,754	38,762	38,228	36,074	36,074	34,933	34,454	34,454
		FLY_C3_08	23,197	18,808	17,885	17,880	17,827	17,493	16,819	16,819	15,987	15,794	15,794
	DEC 2023	**FLY_A1_12**	49,498	48,062	47,587	47,564	47,558	47,291	46,311	46,311	44,770	44,234	44,234
		FLY_A2_12	46,476	45,010	44,504	44,486	44,489	44,234	43,321	43,321	42,146	41,621	41,621
		FLY_A3_12	55,889	54,166	53,554	53,529	53,532	53,188	51,701	51,701	50,288	49,608	49,608
		FLY_B1_12▼	38	29	26	26	26	24	24	24	1	1	0
		FLY_B2_12	57,198	55,376	54,767	54,734	54,677	54,154	53,051	53,051	51,757	51,054	51,054
		FLY_B3_12	56,986	55,141	54,536	54,497	54,510	54,136	52,989	52,989	51,576	50,886	50,886
		FLY_C1_12	54,708	52,944	52,405	52,373	52,384	52,136	51,137	51,137	50,375	49,870	49,870
		FLY_C2_12	48,453	46,930	46,438	46,425	46,419	46,180	45,268	45,268	44,130	43,559	43,559
		FLY_C3_12▼	31	20	19	19	18	18	18	18	0	0	0
Liberty Island†	**MAR 2023**	**LIB_A1_03**	55,615	53,778	53,347	53,347	53,337	53,203	52,674	52,674	51,814	51,342	51,342
		LIB_A2_03	63,958	61,704	61,191	61,191	61,185	61,108	60,625	60,625	59,601	59,210	59,210
		LIB_A3_03	62,129	60,028	59,566	59,565	59,559	59,445	58,817	58,817	57,896	57,395	57,395
		LIB_B1_03	63,694	61,527	59,380	59,369	59,372	59,218	57,603	57,603	56,729	56,224	56,224
		LIB_B2_03	62,820	60,903	60,404	60,389	60,386	60,184	58,333	58,333	57,395	56,830	56,830
		LIB_B3_03	50,140	48,544	48,176	48,162	48,162	48,013	46,605	46,602	45,653	45,171	45,171
		LIB_C1_03	58,048	56,243	55,802	55,792	55,787	55,520	54,718	54,716	54,007	53,683	53,683
		LIB_C2_03	61,370	59,343	58,882	58,878	58,876	58,693	57,824	57,824	56,945	56,486	56,486
		LIB_C3_03	59,211	57,323	56,600	56,577	56,583	56,432	55,485	55,485	54,735	54,388	54,388
	AUG 2023	**LIB_A1_08**	44,963	43,387	42,957	42,926	42,940	42,671	40,475	40,474	39,147	38,542	38,542
		LIB_A2_08	50,080	48,352	47,900	47,881	47,886	47,646	45,546	45,545	44,237	43,656	43,656
		LIB_A3_08	51,894	50,045	49,488	49,461	49,476	49,241	47,073	47,073	45,736	45,032	45,032
		LIB_B1_08	41,470	40,156	39,783	39,744	39,764	39,546	37,996	37,996	36,845	36,459	36,459
		LIB_B2_08	45,468	43,869	43,365	43,345	43,349	43,097	41,399	41,397	40,106	39,647	39,647
		LIB_B3_08	36,882	35,711	35,434	35,416	35,412	35,206	33,804	33,803	32,625	32,234	32,234
		LIB_C1_08	38,449	37,108	36,729	36,714	36,705	36,384	35,429	35,429	33,903	33,440	33,440
		LIB_C2_08	52,286	50,390	49,871	49,865	49,847	49,420	49,266	49,266	48,995	48,856	48,856
		LIB_C3_08	51,238	49,479	49,015	49,009	49,003	48,830	48,441	48,441	47,337	46,979	46,979

	DEC 2023	**LIB_A1_12**	60,856	58,924	58,427	58,411	58,408	57,825	56,434	56,433	55,056	54,193	54,193
		LIB_A2_12	59,078	56,997	56,451	56,434	56,431	56,151	54,758	54,757	53,554	52,858	52,858
		LIB_A3_12	60,182	58,182	57,606	57,588	57,581	57,302	56,129	56,129	54,774	54,001	54,001
		LIB_B1_12	80,801	78,369	77,712	77,627	77,697	77,209	74,786	74,786	73,439	72,395	72,395
		LIB_B2_12	81,051	78,463	77,727	77,697	77,700	77,231	74,364	74,361	73,355	72,576	72,576
		LIB_B3_12	54,954	53,185	52,706	52,688	52,690	52,284	50,577	50,577	49,528	48,876	48,876
		LIB_C1_12	62,639	60,531	59,885	59,872	59,857	59,656	58,904	58,899	58,052	57,476	57,476
		LIB_C2_12	67,172	65,051	64,292	64,268	64,252	63,880	62,141	62,139	61,104	60,467	60,467
		LIB_C3_12	73,816	71,486	70,829	70,807	70,790	70,122	69,626	69,626	68,617	68,020	68,020
Webb Tracts Island and Berms[†]	**FEB 2023**	**WEB_A1_02**	58,064	56,036	55,591	55,584	55,574	55,320	54,452	54,451	53,783	53,165	53,165
		WEB_A2_02	53,417	51,899	51,552	51,540	51,536	51,244	50,493	50,493	49,837	49,233	49,233
		WEB_A3_02	66,473	64,172	63,702	63,695	63,674	61,620	60,502	60,502	59,920	59,270	59,270
		WEB_B1_02	54,102	52,207	51,854	51,839	51,826	51,524	50,670	50,670	49,935	49,359	49,359
		WEB_B2_02	57,073	55,186	54,868	54,867	54,856	54,654	54,269	54,269	53,769	53,480	53,480
		WEB_B3_02	63,095	60,848	60,304	60,284	60,278	59,817	59,117	59,116	58,239	57,718	57,718
		WEB_C1_02	83,140	80,468	79,886	79,873	79,873	79,726	79,278	79,278	78,392	77,881	77,881
		WEB_C2_02	70,270	67,747	67,237	67,224	67,230	67,124	66,871	66,871	66,389	66,068	66,068
		WEB_C3_02▼	2,993	2,862	2,833	2,833	2,832	2,155	2,154	2,154	2,044	2,041	0
Decker Island[±]	**FEB 2023**	**DEC_A1_02**▼	37	19	18	18	17	16	16	16	0	0	0
		DEC_A2_02	55,436	53,661	53,267	53,258	53,253	52,933	52,431	52,431	51,615	51,068	51,068
		DEC_A3_02	61,469	59,427	57,173	57,161	57,162	56,521	56,255	56,254	55,759	55,304	55,304
		DEC_B1_02	64,504	62,318	61,866	61,863	61,853	61,723	60,938	60,933	60,112	59,407	59,407
		DEC_B2_02	56,664	54,730	54,322	54,319	54,318	54,184	53,588	53,585	52,637	52,011	52,011
		DEC_B3_02	57,043	55,245	54,861	54,859	54,844	54,276	53,573	53,568	52,464	51,823	51,823
		DEC_C1_02	53,291	51,841	51,549	51,548	51,546	51,496	51,015	51,013	50,297	49,816	49,816
		DEC_C2_02	49,036	47,416	47,023	47,008	47,010	46,849	46,373	46,372	45,576	45,002	45,002
		DEC_C3_02	67,943	65,714	64,761	64,756	64,747	64,199	63,497	63,494	62,553	61,824	61,824
		TOTAL	**10,041,094**	**9,689,937**	**9,586,794**	**9,584,200**	**9,582,779**	**9,506,450**	**9,343,250**	**9,342,415**	**9,158,327**	**9,069,973**	**9,067,519**
		Remaining	**100.00 %**	**96.50 %**	**95.48 %**	**95.45 %**	**95.44 %**	**94.68 %**	**93.05 %**	**93.04 %**	**91.21 %**	**90.33 %**	**90.30 %**

Table S11. Sequence filtering read pipeline for 16S assay negative controls. Total read abundance for negative controls remaining. Steps occurred in listed left→ right order.

Sample Type	Sample ID	Raw	Cutadapt Filter	DADA2 Filter	Denoised Forward	Denoised Reverse	Merged	Chimera Removal	Min/Max Length
Field Negative Controls	**BRO_AB_02**	1,057	592	479	465	452	255	252	251
	BRO_BB_02	1,171	748	353	352	348	183	182	180
	BRO_CB_02	348	170	146	146	145	129	128	125
	BRO_AB_08	75,134	71,320	891	877	884	264	264	14
	BRO_BB_08	794	726	374	371	371	353	353	4
	BRO_CB_08	719	618	53	53	53	37	37	9
	BRO_AB_12	1,546	450	267	266	266	212	212	209
	BRO_BB_12	1,653	857	330	330	330	267	266	264
	BRO_CB_12	3,141	2,307	364	363	362	305	304	199
	WIN_CB_02	54	38	28	28	28	21	21	13
	WIN_AB_02	44	29	23	21	21	12	12	12
	WIN_BB_02	1,621	1,438	1,384	1,383	1,381	1,349	1,349	16
	WIN_AB_12	426	403	204	203	204	201	201	199
	WIN_BB_12	9,869	9,044	913	911	911	884	883	882
	WIN_CB_12	405	382	336	335	336	329	329	327
	MON_AB_02	799	456	304	304	304	248	245	237
	MON_BB_02	582	384	304	303	302	52	52	51
	MON_CB_02	1,092	604	517	512	514	263	262	240
	MON_AB_08	153	98	31	30	31	26	26	26
	MON_BB_08	311	192	22	22	22	19	19	10
	MON_CB_08	1,434	1,082	74	73	70	59	58	4
	MON_AB_01	490	451	406	406	406	401	401	401
	MON_BB_01	291	273	183	183	183	181	180	175
	MON_CB_01	1,317	1,223	1,026	1,026	1,025	1,013	855	853
	LIB_AB_03	378	138	105	105	105	91	91	19
	LIB_BB_03	84	76	13	13	13	9	9	6
	LIB_CB_03	911	781	66	66	66	57	57	12
	LIB_AB_08	2,166	1,890	124	123	123	92	92	8
	LIB_BB_08	1,219	1,139	726	724	726	697	697	5
	LIB_CB_08	390	315	233	233	232	72	72	12
	LIB_AB_12	12,279	11,112	1,093	1,067	1,086	715	715	50
	LIB_BB_12	451	270	112	111	110	92	92	91
	LIB_CB_12	1,619	1,306	372	369	371	277	277	54
	FLY_AB_03	671	623	590	590	590	578	578	578
	FLY_BB_03	150	72	57	57	57	47	47	9
	FLY_CB_03	71	54	38	38	38	37	37	30
	FLY_AB_08	89	53	35	34	35	32	32	1
	FLY_BB_08	452	259	54	51	51	21	20	18
	FLY_CB_08	906	823	82	81	80	79	79	11
	FLY_AB_12	6,118	5,281	4,866	4,865	4,863	4,803	4,800	413
	FLY_BB_12	21,809	20,077	19,438	19,436	19,438	19,198	19,198	19,168
	FLY_CB_12	742	315	119	118	118	85	85	78
	RYE_AB_02	3,692	3,058	2,556	2,549	2,551	2,180	2,171	250
	RYE_BB_02	917	527	456	455	456	413	413	338
	RYE_CB_02	149	92	61	56	55	48	48	47
	RYE_AB_09	186,243	175,824	213	176	193	30	30	13
	RYE_BB_09	3,231	3,042	2,552	2,540	2,551	2,149	2,149	5
	RYE_CB_09	830	691	15	15	15	13	13	9
	RYE_AB_12	2	1	1	1	1	0	0	0
	RYE_BB_12	4,616	4,029	260	257	255	216	215	126
	RYE_CB_12	3	2	0	NA	NA	NA	NA	NA

	TUL_AB_02	814	576	511	503	494	168	168	110
	TUL_BB_02	905	596	515	514	511	29	29	29
	TUL_CB_02	1,731	1,129	330	327	329	290	290	174
	TUL_AB_09	3,334	3,171	368	366	367	325	269	9
	TUL_BB_09	1,516	1,400	22	22	22	19	19	13
	TUL_CB_09	414	357	26	25	26	17	17	13
	TUL_AB_12	360	161	129	129	128	112	111	107
	TUL_BB_12	6	2	2	2	2	2	2	2
	TUL_CB_12	1,050	527	250	250	248	218	218	193
	WEB_AB_02	425	149	113	113	113	91	91	64
	WEB_BB_02	98	78	67	67	67	26	26	26
	WEB_CB_02	238	119	43	42	42	18	18	16
	DEC_AB_02	51	34	27	27	27	24	24	16
	DEC_BB_02	195	122	71	69	66	45	45	45
	DEC_CB_02	184	135	31	29	28	24	24	23
Extraction Negative Controls	**EXT_1**	1,460	1,127	354	354	354	351	350	345
	EXT_2	532	381	100	100	100	93	91	88
	EXT_3	1,614	653	282	281	280	167	163	163
	EXT_4	274	155	43	43	43	43	43	43
	EXT_5	682	505	489	489	489	478	477	425
	EXT_6	197	90	9	9	9	9	9	3
	EXT_7	200	57	10	10	10	10	10	10
	EXT_8	737	481	328	328	328	314	314	31
	EXT_9	602	485	33	33	33	32	32	25
	EXT_10	26	18	4	4	4	4	4	3
	EXT_11	3,937	3,583	2,685	2,682	2,682	2,629	2,626	35
	EXT_12	1,395	502	388	388	385	292	292	252
	EXT_13	1,917	746	336	336	332	247	247	246
	EXT_14	775	498	208	207	206	176	176	163
	EXT_15	331	114	19	15	14	14	14	5
	EXT_16	1,067	381	204	203	204	144	142	139
1st PCR Negative Controls	**1PCR_1**	137,294	130,502	1,048	1,028	1,043	908	908	172
	1PCR_2	6,039	5,653	40	40	40	36	36	34
	1PCR_3	41,546	39,401	124	117	120	98	98	3
	1PCR_4	35,485	33,733	248	236	246	197	197	4
	1PCR_5	28	22	18	18	18	16	15	15
	1PCR_6	1	1	1	1	1	1	0	0
	1PCR_7	1	0	0	NA	NA	NA	NA	NA
	1PCR_8	3,788	3,376	755	746	755	557	556	279
	1PCR_9	1,398	1,323	1,226	1,224	1,225	1,208	1,208	338
	1PCR_10	432	163	130	130	128	103	103	93
	1PCR_11	841	388	301	301	301	262	262	106
	1PCR_12	517	474	1	1	1	1	1	1
	1PCR_13	556	305	119	119	119	96	96	95
	1PCR_14	520	225	63	63	63	45	45	42
2nd PCR Negative Controls	**2PCR_1**	72	1	1	0	0	0	0	0
	2PCR_2	67	26	4	4	4	3	3	3
	2PCR_3	17	16	16	16	16	16	16	16
	2PCR_4	11	10	10	10	10	10	10	10
	2PCR_5	205	192	187	187	187	184	184	184
	2PCR_6	835	793	784	784	784	777	776	776
	2PCR_7	3	0	0	NA	NA	NA	NA	NA
	2PCR_8	9,657	8,752	8,572	8,555	8,554	8,427	8,425	8,190
	TOTAL	**621,019**	**569,423**	**64,894**	**64,640**	**64,686**	**58,450**	**58,188**	**39,229**
	Remaining	**100.00 %**	**91.69 %**	**10.45 %**	**10.41 %**	**10.42 %**	**9.41 %**	**9.37 %**	**6.32 %**

Table S12. Sequence filtering read pipeline for 12S assay negative controls. Total read abundance for negative controls remaining. Steps occurred in listed left→ right order.

Sample Type	Sample ID	Raw	Cutadapt Filter	DADA2 Filter	Denoised Forward	Denoised Reverse	Merged	Chimera Removal	Min/Max Length
Field Negative Controls	**BRO_AB_02**	80	58	52	51	51	44	44	44
	BRO_BB_02	104	75	67	67	66	53	50	50
	BRO_CB_02	31	21	17	17	17	17	17	17
	BRO_AB_08	87	48	40	40	40	37	36	36
	BRO_BB_08	69	37	29	29	29	28	27	27
	BRO_CB_08	19	11	8	8	8	8	8	8
	BRO_AB_12	21	14	12	12	12	12	11	11
	BRO_BB_12	25	21	20	20	20	20	20	20
	BRO_CB_12	42	36	28	28	28	28	27	27
	WIN_AB_02	36	35	34	34	32	30	29	29
	WIN_BB_02	62	38	36	36	36	35	35	35
	WIN_CB_02	18	14	10	10	10	8	8	8
	WIN_AB_12	17	8	4	4	4	4	4	4
	WIN_BB_12	26	14	11	11	11	10	10	10
	WIN_CB_12	27	10	7	7	7	6	5	5
	MON_AB_02	8,309	7,759	205	195	202	193	192	192
	MON_BB_02	9	8	7	7	7	1	1	1
	MON_CB_02	2	2	1	1	1	0	0	0
	MON_AB_08	39	19	19	19	19	17	16	16
	MON_BB_08	88	72	68	68	68	68	66	66
	MON_CB_08	117	81	71	70	69	65	60	60
	MON_AB_01	17	14	12	12	12	12	12	12
	MON_BB_01	15	8	8	8	8	8	8	8
	MON_CB_01	35	25	20	20	20	20	20	20
	LIB_AB_03	27	24	23	23	23	21	20	20
	LIB_BB_03	316	297	20	19	19	19	19	19
	LIB_CB_03	5	4	1	1	1	1	1	1
	LIB_AB_08	13	10	10	10	10	9	9	9
	LIB_BB_08	11	6	6	6	6	6	6	6
	LIB_CB_08	117	106	7	7	7	7	7	7
	LIB_AB_12	24	21	21	21	21	21	20	20
	LIB_BB_12	57	48	46	46	46	46	46	46
	LIB_CB_12	24	22	22	22	21	21	21	21
	FLY_AB_03	72	61	26	26	26	26	26	26
	FLY_BB_03	37	32	31	31	31	30	30	30
	FLY_CB_03	13,849	13,267	55	43	47	41	39	39
	FLY_AB_08	19	10	6	6	6	5	5	5
	FLY_BB_08	21	12	8	8	8	7	6	6
	FLY_CB_08	22	12	12	12	12	12	12	12
	FLY_AB_12	21	15	14	14	14	14	14	14
	FLY_BB_12	21	16	14	14	14	14	14	14
	FLY_CB_12	59	45	39	39	39	38	38	38
	RYE_AB_02	65	49	45	45	45	42	38	38
	RYE_CB_02	47	39	35	35	35	33	33	33
	RYE_AB_09	35	19	17	17	17	17	17	17
	RYE_BB_09	31	19	17	17	17	17	16	16
	RYE_CB_09	77	57	53	53	53	52	49	49
	RYE_AB_12	31	21	20	20	20	19	19	19
	RYE_BB_12	34	22	20	20	20	19	18	18
	RYE_CB_12	2	2	2	2	2	2	2	2

	TUL_AB_02	42	35	34	34	34	29	28	28
	TUL_BB_02	18	10	7	7	7	7	7	7
	TUL_CB_02	973	915	7	7	6	6	4	4
	TUL_AB_09	30	24	20	20	20	20	20	20
	TUL_BB_09	67	46	43	43	43	42	41	41
	TUL_CB_09	29	25	24	24	24	24	24	24
	TUL_AB_12	44	32	31	31	31	31	31	31
	TUL_BB_12	29	21	19	19	19	18	16	16
	TUL_CB_12	18	12	10	10	10	10	10	10
	WEB_AB_02	31	21	20	20	20	20	20	20
	WEB_BB_02	6,434	6,146	58	56	57	56	55	55
	WEB_CB_02	73	49	44	44	44	39	39	39
	DEC_AB_02	589	558	549	548	549	546	546	546
	DEC_BB_02	93	75	71	71	70	68	68	68
	DEC_CB_02	51	45	42	41	42	40	37	37
Extraction Negative Controls	**EXT_1**	7	4	2	2	2	2	2	2
	EXT_2	28	19	18	18	18	17	17	17
	EXT_3	9,357	8,957	73	65	66	62	61	61
	EXT_4	8	7	7	7	7	7	6	6
	EXT_6	14	12	9	9	9	9	8	8
	EXT_7	26	20	18	18	18	18	18	18
	EXT_8	17	12	9	9	9	9	8	8
	EXT_9	9	4	4	4	4	4	4	4
	EXT_10	2	1	1	1	1	1	1	1
	EXT_11	5,171	4,874	6	2	4	2	2	2
	EXT_12	2	2	1	1	1	1	1	1
	EXT_13	5,119	4,925	8	6	7	6	6	6
	EXT_14	89	65	60	60	60	60	58	58
	EXT_15	17	10	7	7	7	7	7	7
	EXT_16	98	73	59	59	58	57	52	52
1st PCR Negative Controls	**1PCR_15**	63	41	36	36	36	36	35	35
	1PCR_16	55	40	37	37	37	36	36	36
	1PCR_17	2	2	2	2	2	2	2	2
	1PCR_18	1,194	1,143	18	17	18	17	16	16
	1PCR_19	7,451	6,939	8	1	3	1	1	1
	1PCR_20	45	35	34	34	34	34	34	34
	1PCR_21	16	4	4	4	4	3	3	3
	1PCR_22	6	6	5	5	5	5	5	5
	1PCR_23	3	2	2	2	2	2	2	2
	1PCR_24	9	4	1	1	1	1	1	1
	1PCR_25	27	18	17	17	17	17	17	17
	1PCR_26	43	35	32	32	32	31	31	31
	1PCR_27	22	14	14	14	14	13	13	13
	1PCR_28	16	10	9	9	9	9	9	9
	1PCR_29	41	31	28	28	28	27	26	26
	1PCR_30	15	9	8	8	8	8	8	8
	1PCR_31	54	47	47	47	47	45	43	43
	1PCR_32	14	10	9	9	9	9	9	9
	1PCR_33	1	0	0	NA	NA	NA	NA	NA
	1PCR_34	5	5	0	NA	NA	NA	NA	NA
2nd PCR Negative Controls	**2PCR_9**	42	27	25	25	25	24	24	24
	2PCR_10	30	19	15	15	15	15	15	15
	2PCR_11	22	19	18	18	18	18	16	16
	2PCR_12	16	10	5	5	5	5	4	4
	2PCR_13	6	2	1	1	1	1	1	1
	2PCR_14	2	1	1	1	1	1	1	1

	TOTAL	**62,017**	**58,136**	**2,993**	**2,942**	**2,955**	**2,841**	**2,780**	**2,780**
	Remaining	**100.00 %**	**93.74 %**	**4.83 %**	**4.74 %**	**4.76 %**	**4.58 %**	**4.48 %**	**4.48 %**

Table S13. Species removed following quality sequence filtering. Listed is the number of reads detected for each species before removal. Sites: Browns Island (BRO); Montezuma Wetlands (MON); Winter Island (WIN); Liberty Island (LIB); Yolo Flyway Farms (FLY); Ryer Island (RYE); Tule Red (TUL); Webb Tract Island and Berms (WEB). ± = Restoration wetland; † = Reference site.

Assay	Species	MM/YY	BRO†	MON±	WIN±	RYE†	TUL±	LIB†	FLY±	WEB†
16S	Tule Perch (*Hysterocarpus traskii*)	02/23	4							
		08/23	12	1					2	
		09/23				2				
		12/23			2	1				
	White Sturgeon (*Acipenser transmontanus*)	02/23					3			
		12/23					2			
		01/24		5						
	Wakasagi (*Hypomesus nipponensis*)	02/23		1						
		08/23				2				
		12/23			1	1				
	Cattle (*Bos taurus*)	02/23	30	36	11	4	14			9
		03/23						1	1	
		08/23	2							
		12/23							6	
12S	Longfin Smelt (*Spirinchus thaleichthys*)	02/23		1	1					
		12/23	10		9					
		01/24		15						
	North American River Otter (*Lontra canadensis*)	03/23						9		
	American Bullfrog (*Lithobates catesbeianus*)	03/23							22	

Table S14. 16S fish-specific and 12S MiFish-U species detections for individual 1-L eDNA water samples for Ryer Island and Tule Red paired sites. Detections performed after quality sequence filtering. Blue ("1") = 16S only. Pink ("2") = 12S only. Green ("B") = both assays. Total species detection for each sample shown at bottom. ± = Restoration wetland; † = Reference site.

Site	Ryer Island†																					Tule Red±																	
Date	FEB 2023									SEP 2023									DEC 2023									FEB 2023									SEP 2023					
Site Replicate	A			B			C			A			B			C			A			B			C			A			B			C			A			B			C		
Biological Replicate	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
* California Roach																																													
* Chinook Salmon										1	B																										B	B	B	B	B	B	B	B	B	B	B	B	2	B		B
*Sculpin (Cottidae *spp*)	2						1			1	1	2	1	2	B		B	2		B		B		1	2		2		1	1		2						1		B	B		B	B	B	B	B	B		2		1
* Delta Smelt				1																																											
* Hardhead																																																
* Hitch													1																																			
* Jack Silverside										B			1	B	B		1												1																			
* Longfin Smelt	1	1																												1																	
* Rainbow Trout							2																					1		B				1	1	1	B			1							
* Sacramento Blackfish																																															
* Sacramento Pikeminnow							1			B	1	1	B		2		1	B		2							2	2									B	1	1	1	B	2	B		1	B	2				
* Sacramento Splittail	B						1			B	B	B	B	B	B		B	B		B		2	B	1	B		2			1	1		1	1		B	B	B	B	B	B	B	B	B	B	B	B	2	1		1
* Sacramento Sucker																																							1		1		1	1					
* Starry Flounder																																								1				1					
* Threespine Stickleback	B	B	B	1								B	1	B	2		B	B		B			2			2		1		1		1		1		B	B	2	B	1	B	B	1	B	B	B	B	B	B	2	B	1	1
* Tule Perch										2	2		2	2	2		2	2		2								2								2				2				2	2					
* White Sturgeon				2							2	2							2			2	2	2					2																2				
American Shad												B							2																	2				1	2		1	1		1	B		B		1
Bigscale Logperch																																							2									
Black Bullhead																																															
Black Crappie																																															
Bluegill																																															
Channel Catfish																																															
Common Carp										B		B	B				B	B					2							1	1					B	B	B	B	1	1	1	1	B	B	B	B	1		1	
Fathead Minnow																												1																			
Golden Shiner																																															

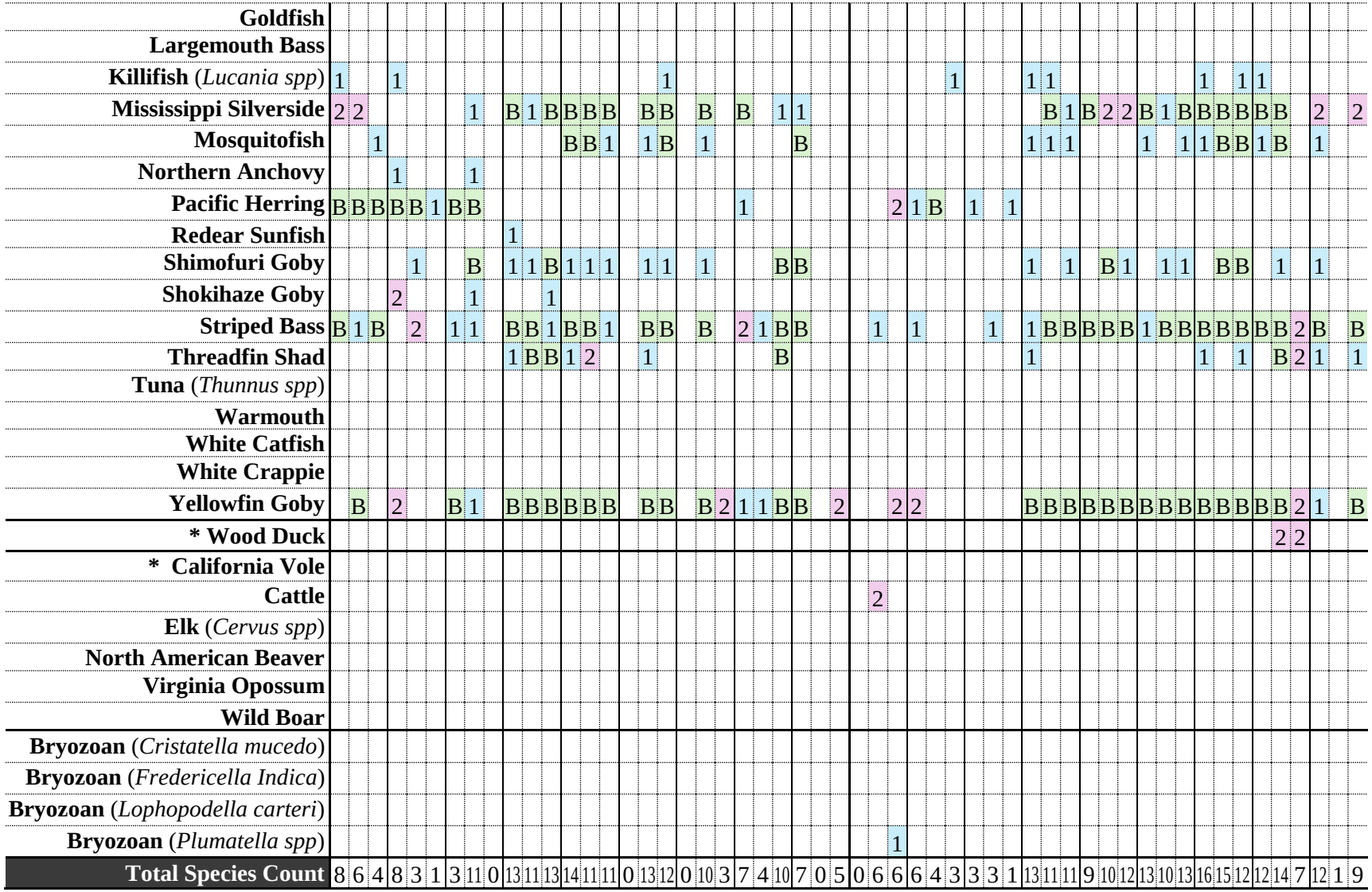

Goldfish
Largemouth Bass
Killifish (Lucania spp)
Mississippi Silverside
Mosquitofish
Northern Anchovy
Pacific Herring
Redear Sunfish
Shimofuri Goby
Shokihaze Goby
Striped Bass
Threadfin Shad
Tuna (Thunnus spp)
Warmouth
White Catfish
White Crappie
Yellowfin Goby
* Wood Duck
* California Vole
Cattle
Elk (Cervus spp)
North American Beaver
Virginia Opossum
Wild Boar
Bryozoan (Cristatella mucedo)
Bryozoan (Fredericella Indica)
Bryozoan (Lophopodella carteri)
Bryozoan (Plumatella spp)
Total Species Count
8 6 4 8 3 1 3 11 0 13 11 13 14 11 11 0 13 12 0 10 3 7 4 10 7 0 5 0 6 6 6 4 3 3 3 1 13 11 11 9 10 12 13 10 13 16 15 12 12 14 7 12 1 9

Table S15. 16S fish-specific and 12S MiFish-U species detections for individual 1-L eDNA water samples for Liberty Island and Yolo Flyway Farms paired sites. Detections performed after quality sequence filtering. Blue ("1") = 16S only. Pink ("2") = 12S only. Green ("B") = both assays. Total species detection for each sample at bottom. ± = Restoration wetland; † = Reference.

Site	Liberty Island†																					Yolo Flyway Farms±																	
Date	MAR 2023									AUG 2023									DEC 2023									MAR 2023									AUG 2023					
Site Replicate	A			B			C			A			B			C			A			B			C			A			B			C			A			B			C		
Biological Replicate	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
* California Roach				2		2					2	2	2		2						2	2		2													2	2	2	2	2		2	2					2					
* Chinook Salmon			2						1										B	B	B				1	1		2	1						1	1									B	B	B		B	B	B	B
*Sculpin (Cottidae *spp*)	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B		B	B	B	B	B	B	B	B	B	B	B	2	B	B	1	B	B	2	B	B	B	B	B	1	B		2		B	B	1		B	2	1	1
* Delta Smelt																																																		
* Hardhead	1	1																																				2										2		
* Hitch	1	B	B	B	B	B	B	B	1	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	1	1		2	1	1		B	B	1	B	B	B	B	B	B	B	B	B	B	B	B		B	B	B	B
* Jack Silverside																																																		
* Longfin Smelt		1																			1	1																														
* Rainbow Trout	B	2	1																	1																																
* Sacramento Blackfish										2																								1				2				1	1								
* Sacramento Pikeminnow	B	B					1	B	1	1	B		B	2	B	1	1	1	B	1			B		1	1	B		B	1		1	B	2	B	B	B	B	B	B	B	B	1	1		1			2	2	B
* Sacramento Splittail																B		1	B	2	2	2	1		B	B	B	B	B	B	B	B	B	B	B	B		2						B	2	B		B	B	B	2
* Sacramento Sucker	B		B				1	B	B		B					B		1	B	B	B	1		B	1		1	1	2	B		1	B	B	1	B	B	B	B	B	1	B		1	B	B	B		B	B	B	B
* Starry Flounder																1																																			
* Threespine Stickleback							1																															2												
* Tule Perch	2	2		2	2	2				2	2	2	2	2		2		2	2	2	2	2		2								2						2	2	2	2					2					
* White Sturgeon	2		2														2						2	2	2	2		2			2																			
American Shad													B	B	1	B	1	B		1				2	B													B	2	1		1	1		1			1	1			2	
Bigscale Logperch																2	1					2																B	B	B	B	B	2	2			1	1		2	1		2
Black Bullhead																																																		
Black Crappie																						2						1										B	B	B	B	B	B	B	B	B				2	2		2	
Bluegill	B	1		1		1		1								B			B	1	B				2		2	1		2		2	2				B	B	B	B	B	B	B	B	B	2	B		B	2		1	
Channel Catfish																																						2		2								1				
Common Carp	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	2	B	B	B	B	B	B	B			1	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B		B	B	B	B

| | | |

Table S16. 16S fish-specific and 12S MiFish-U species detections for individual 1-L eDNA water samples for Browns Island and Montezuma Wetlands paired sites. Detections performed after quality sequence filtering. Blue ("1") = 16S only. Pink ("2") = 12S only. Green ("B") = both assays. Total species detection for each sample at bottom. ± = Restoration wetland; † = Reference.

Site	Browns Island†																					Montezuma Wetlands±																	
Date	FEB 2023									AUG 2023									DEC 2023									FEB 2023									AUG 2023					
Site Replicate	A			B			C			A			B			C			A			B			C			A			B			C			A			B			C		
Biological Replicate	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
*** California Roach**				2											2																											
*** Chinook Salmon**			1	1		2		1												1								1	1	1	B		1	2	B			1					1		
***Sculpin** (Cottidae *spp*)	B	B	B	B	B	B	B	B	B	1	B		B	B	B	B	B	B	1		2				B	B	2	B	B	B	B	B	B	2	2	B	2	B	2	2	1	B		2		B		B	B
*** Delta Smelt**																																													
*** Hardhead**																																									1				
*** Hitch**	1	2	1	2	1		B	B	B	1	1					1			2		B				B			B		B	1	1	B	1					1		2				1		1
*** Jack Silverside**																																													
*** Longfin Smelt**	1																		1		1				1	1					1		1										1		1
*** Rainbow Trout**								2								2														2		1												
*** Sacramento Blackfish**																																													
*** Sacramento Pikeminnow**	B	1			1	1	1	B	B	B	B	B	B	B	B	B	B	B			B				1		2	1		2	1	2	1		B	B	B	2	B	B	B	B	B	2		1		2		B
*** Sacramento Splittail**		2		B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	1	B		2		B	B	B	B	B	B	B	B	B	B	B	B	2	B	B	B	B	2	2	B		B	2	B	B
*** Sacramento Sucker**			1	2	1					1		2		1							1					2	1		1	B		1	1	2	B	B	B	2	B	B	B	B	B	2		B		1		B
*** Starry Flounder**																																				1	2										1
*** Threespine Stickleback**		1	B	1	1		B		1	1	1	B	B	2	1	B	B	B			2					B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	2	2	B		B	1	B	B
*** Tule Perch**	2	2	2				2			2	2	2	2	2	2	2	2	2		2	2		2						2								2	2	2	2	2	2	2	2					
*** White Sturgeon**			2								2								2		2		2		2	2	2		2		2											2		2					
American Shad										B			1	1											1									B	B	2				2	B	2						
Bigscale Logperch															2																																
Black Bullhead																																														
Black Crappie															B	1	1	B							1				1			1																
Bluegill	1	2						1		1						B	2	1		1					1								1															
Channel Catfish			1																																												
Common Carp	2						B	B	1	1	B	B	1	B	B	B	B	B		B					B	1	B	B	B	B	1		B	B	B	B	2	B	B	B	B	B	2		B		B		1	B
Fathead Minnow																																														1

| | | |

Table S17. 16S fish-specific and 12S MiFish-U species detections for individual 1-L eDNA water samples for Browns Island and Winter Island paired sites. Detections performed after quality sequence filtering. Blue ("1") = 16S only. Pink ("2") = 12S only. Green ("B") = both assays. Total species detection for each sample at bottom. ± = Restoration wetland; † = Reference.

Site	Browns Island†																																								
Date	FEB 2023									AUG 2023									DEC 2023									FEB 2023									DEC 2023					
Site Replicate	A			B			C			A			B			C			A			B			C			A			B			C			A			B			C		
Biological Replicate	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
* California Roach				2											2																									2		
* Chinook Salmon			1	1		2		1													1					1				2	1	1		1				B	B		2		
*Sculpin (Cottidae *spp*)	B	B	B	B	B	B	B	B	B	1	B		B	B	B	B	B	B	1		2					B	B	B	B	B	B	B	B	B	B	B	2	1	B	B			2
* Delta Smelt																																										
* Hardhead																																										
* Hitch	1	2	1	2	1		B	B	B	1	1					1			2		B					B			1		2		1		1	1			1			1	
* Jack Silverside																																										
* Longfin Smelt	1																		1		1					1				1	1	1		1			1		1	1				
* Rainbow Trout								2								2																	2		B								
* Sacramento Blackfish																																										
* Sacramento Pikeminnow	B	1			1	1	1	B	B	B	B	B	B	B	B	B	B	B			B					1	1	B		B	B	B	B	B	2	B	B	B	B	B	B		2	
* Sacramento Splittail		2			B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	1	B		2			B		2	1	2	2	1	1		2	B	B	B	B	B	1	2	2	B
* Sacramento Sucker			1	2	1					1		2		1							1						1	1				1	2			B	1	B	B	B	B		2	1
* Starry Flounder																																										1
* Threespine Stickleback		1	B	1	1		B		1	1	1	B	B	2	1	B	B	B			2							2	B	B	B	1	B	B	1	B	1	B		B	B	1		2	B
* Tule Perch	2	2	2				2			2	2	2	2	2	2	2	2	2		2	2		2					2			2					2	2	2	2	2	2	2		2
* White Sturgeon			2								2								2		2		2			2				2						2	2	2	2		2		2	
American Shad										B			1	1												1			1				1				1			1	1				1
Bigscale Logperch															2																												2
Black Bullhead																																										
Black Crappie															B	1	1	B								1																		
Bluegill	1	2						1		1						B	2	1			1										1			2						1	2			
Channel Catfish			1																										1													1
Common Carp	2						B	B	1	1	B	B	1	B	B	B	B	B			B						1	B	1	1	1	1		2					1		1			
Fathead Minnow																																	1									

| | | |

Table S18. 16S fish-specific and 12S MiFish-U species detections for individual 1-L eDNA water samples for Webb Tracts Island and Berms and Decker Island paired sites. Detections performed after quality sequence filtering. Blue ("1") = 16S only. Pink ("2") = 12S only. Green ("B") = both assays. Total species detection for each sample at bottom. ± = Restoration wetland; † = Reference.

Site	**Webb Tracts Island and**									**Decker Island±**								
Date	FEB 2023									FEB 2023								
Site Replicate	A			B			C			A			B			C		
Biological Replicate	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
*** California Roach**																		
*** Chinook Salmon**											B	B	B	B	B	B	B	
***Sculpin** (Cottidae *spp*)	B	B	B	B	B	B	B	B			B	B	B	B	B	B	B	B
*** Delta Smelt**																		
*** Hardhead**																1		
*** Hitch**		1					1							B				
*** Jack Silverside**																		
*** Longfin Smelt**																		
*** Rainbow Trout**		2	B	2							2	2	1	1	2			
*** Sacramento Blackfish**																		
*** Sacramento Pikeminnow**	B	B	B	B		B		1			1	B	B	B	B	B	B	B
*** Sacramento Splittail**		1												2				
*** Sacramento Sucker**	B		2	1							B	2	1	1	1			B
*** Starry Flounder**																		
*** Threespine Stickleback**												1	2	B	B	B	B	
*** Tule Perch**				2			2				2				2			
*** White Sturgeon**																		
American Shad															1			
Bigscale Logperch			1															
Black Bullhead																2		
Black Crappie			1															
Bluegill	B	B	B	B	B	B	B	1						1		1	2	B
Channel Catfish																		
Common Carp	1	1	1	1	1	2	2				1	1				1		1
Fathead Minnow																		
Golden Shiner	B	B	B	B	B	B	1	1				1		2	1			B
Goldfish			1											1		B	1	
Largemouth Bass	B	B	B	B	1	B	B	B			2		2				2	B
Killifish (*Lucania spp*)	1	1	1	1	1	1					1	1	1	B	1	1	1	1
Mississippi Silverside											2		B	B	B	B	B	
Mosquitofish	1	B	1	B		B					B	B	B	B	B	B	B	1
Northern Anchovy																		
Pacific Herring																		
Redear Sunfish	B	B	B	B	B	2	1	B			2				1	1	1	B
Shimofuri Goby	1	1	1		1		1				1	B	B	1	B	B	B	1
Shokihaze Goby							1						2		1			
Striped Bass	1	1	1		1	1						1						1

Threadfin Shad	2	1		1							1	2				1	1	2
Tuna (*Thunnus spp*)													2					
Warmouth					1	2												
White Catfish		1					1											
White Crappie																		
Yellowfin Goby																		
*** Wood Duck**							2											
*** California Vole**																		
Cattle					2													
Elk (*Cervus spp*)																		
North American Beaver														2	2		2	
Virginia Opossum												1						
Wild Boar																		
Bryozoan (*Cristatella mucedo*)																		
Bryozoan (*Fredericella Indica*)	1	1	1	1	1	1	1	1										1
Bryozoan (*Lophopodella carteri*)																		
Bryozoan (*Plumatella spp*)																		
Total Species Count	1	4	1	7	1	4	1	2	1	2	1	3	7	0	0	1	4	1	4	1	3	1	6	1	6	1	5	1	4	1	6

Table S19. 16S and 12S species diversity and richness for 1-L eDNA water samples.
Diversity calculated using Shannon Diversity Index. Observed = Species richness. "NA" denotes samples removed after quality read filtration or had 0 reads following removal of non-fish DNA.

Site	Sample Event	Sample ID	16S Observed	16S Shannon	12S Observed	12S Shannon
Tule Red±	**FEB 2023**	**TUL_A1_02**	NA	NA	NA	NA
		TUL_A2_02	4	1.277442	1	0
		TUL_A3_02	2	0.358942	3	0.419928
		TUL_B1_02	4	0.904873	2	0.321851
		TUL_B2_02	3	0.901417	2	0.248936
		TUL_B3_02	3	0.697861	NA	NA
		TUL_C1_02	3	0.893683	NA	NA
		TUL_C2_02	3	0.794982	NA	NA
		TUL_C3_02	1	0	NA	NA
	SEP 2023	**TUL_A1_09**	12	1.687034	7	1.307549
		TUL_A2_09	10	1.268229	8	1.880984
		TUL_A3_09	10	1.685835	7	1.362682
		TUL_B1_09	9	1.217357	7	1.579646
		TUL_B2_09	9	1.651319	7	1.20486
		TUL_B3_09	8	1.417726	10	1.653632
		TUL_C1_09	13	1.533349	7	1.356692
		TUL_C2_09	9	1.539249	5	1.231491
		TUL_C3_09	13	1.453424	8	1.615345
	DEC 2023	**TUL_A1_12**	15	1.996236	10	1.86487
		TUL_A2_12	12	2.012081	14	1.861624
		TUL_A3_12	12	1.909283	10	1.845103
		TUL_B1_12	12	1.987351	7	1.271163
		TUL_B2_12	12	1.619285	11	1.987728
		TUL_B3_12	NA	NA	6	1.368926

		TUL_C1_12	10	1.755086	6	1.519383
		TUL_C2_12	1	0	NA	NA
		TUL_C3_12	8	1.641236	4	0.738224
Ryer Island[†]	**FEB 2023**	**RYE_A1_02**	6	1.531866	6	1.400632
		RYE_A2_02	5	1.483853	4	1.152689
		RYE_A3_02	4	0.923766	3	0.519916
		RYE_B1_02	5	0.359059	4	1.304988
		RYE_B2_02	2	0.313679	2	0.00388
		RYE_B3_02	1	0	NA	NA
		RYE_C1_02	3	0.773539	2	0.138048
		RYE_C2_02	10	1.895905	3	0.192371
		RYE_C3_02	NA	NA	NA	NA
	SEP 2023	**RYE_A1_09**	12	1.605072	8	1.136816
		RYE_A2_09	9	1.590305	7	1.678345
		RYE_A3_09	11	1.499391	10	1.100545
		RYE_B1_09	13	1.298064	8	1.335274
		RYE_B2_09	8	0.769037	10	1.379131
		RYE_B3_09	8	1.120521	8	1.669251
		RYE_C1_09	NA	NA	NA	NA
		RYE_C2_09	12	1.244719	8	1.72479
		RYE_C3_09	10	1.41848	10	1.84349
	DEC 2023	**RYE_A1_12**	NA	NA	NA	NA
		RYE_A2_12	8	1.859587	8	1.360208
		RYE_A3_12	NA	NA	3	0.70063
		RYE_B1_12	4	1.154453	5	1.076243
		RYE_B2_12	3	0.954525	2	0.577342
		RYE_B3_12	7	1.553503	7	1.439263
		RYE_C1_12	6	1.525161	6	0.752246
		RYE_C2_12	NA	NA	NA	NA
		RYE_C3_12	NA	NA	5	1.381866
Winter Island[±]	**FEB 2023**	**WIN_A1_02**	11	1.852154	5	0.951798
		WIN_A2_02	9	1.733279	6	1.745177
		WIN_A3_02	11	2.034358	5	1.054022
		WIN_B1_02	13	1.783978	13	1.975452
		WIN_B2_02	15	2.01929	9	1.724258
		WIN_B3_02	17	2.064595	10	1.615457
		WIN_C1_02	12	2.144701	8	1.282292
		WIN_C2_02	13	2.15709	10	1.607298
		WIN_C3_02	6	1.55717	7	0.9761
	DEC 2023	**WIN_A1_12**	12	1.833909	8	1.720011
		WIN_A2_12	14	1.811804	11	1.755195
		WIN_A3_12	9	1.642319	9	1.782262
		WIN_B1_12	17	2.203559	10	1.834563
		WIN_B2_12	13	2.073839	14	2.291556
		WIN_B3_12	8	1.937981	8	1.687842
		WIN_C1_12	NA	NA	8	1.675185
		WIN_C2_12	NA	NA	10	2.11605
		WIN_C3_12	9	1.664527	5	1.248223
Montezuma Wetlands[±]	**FEB 2023**	**MON_A1_02**	11	1.373158	9	1.207685
		MON_A2_02	8	1.48095	7	1.621313
		MON_A3_02	10	1.858493	7	1.227045
		MON_B1_02	9	1.717197	8	1.550827
		MON_B2_02	11	1.769242	9	1.657268
		MON_B3_02	9	1.607923	8	1.548867
		MON_C1_02	12	1.64058	8	1.3179

		MON_C2_02	14	1.863911	7	1.318376
		MON_C3_02	15	1.672816	6	1.201542
	AUG 2023	**MON_A1_08**	14	1.616691	13	1.963451
		MON_A2_08	14	1.585593	13	1.951804
		MON_A3_08	17	1.785652	14	2.023478
		MON_B1_08	NA	NA	13	2.032302
		MON_B2_08	15	1.751635	13	1.807739
		MON_B3_08	11	1.761588	11	2.007752
		MON_C1_08	13	1.703675	14	2.057545
		MON_C2_08	12	1.452892	13	1.780849
		MON_C3_08	15	1.647666	14	2.094881
	JAN 2024	**MON_A1_01**	NA	NA	NA	NA
		MON_A2_01	NA	NA	10	1.47139
		MON_A3_01	NA	NA	3	0.815074
		MON_B1_01	13	1.34407	12	1.503706
		MON_B2_01	NA	NA	NA	NA
		MON_B3_01	14	1.715929	10	1.563166
		MON_C1_01	2	0.691847	1	0
		MON_C2_01	10	1.547173	5	1.106282
		MON_C3_01	17	1.919977	9	1.218722
Browns Island[†]	**FEB 2023**	**BRO_A1_02**	9	1.511947	6	0.822272
		BRO_A2_02	8	1.428635	11	1.578721
		BRO_A3_02	9	1.772202	10	1.322694
		BRO_B1_02	8	1.009091	7	0.795149
		BRO_B2_02	14	1.690422	7	1.044715
		BRO_B3_02	9	1.32196	5	0.708571
		BRO_C1_02	12	1.744953	10	1.346823
		BRO_C2_02	15	1.79819	12	1.175442
		BRO_C3_02	16	1.907526	10	1.297632
	AUG 2023	**BRO_A1_08**	18	1.969784	7	1.540604
		BRO_A2_08	13	1.656752	11	1.801367
		BRO_A3_08	10	1.390318	11	1.632337
		BRO_B1_08	16	1.523709	13	1.79135
		BRO_B2_08	16	1.743788	13	1.947733
		BRO_B3_08	15	1.380603	14	1.878939
		BRO_C1_08	18	1.946022	14	2.27639
		BRO_C2_08	16	1.805113	16	2.177209
		BRO_C3_08	17	1.945112	15	2.223887
	DEC 2023	**BRO_A1_12**	7	1.513589	4	0.999375
		BRO_A2_12	4	1.191133	1	0
		BRO_A3_12	13	1.263827	11	1.921249
		BRO_B1_12	1	0	NA	NA
		BRO_B2_12	NA	NA	5	1.108408
		BRO_B3_12	NA	NA	NA	NA
		BRO_C1_12	NA	NA	NA	NA
		BRO_C2_12	NA	NA	NA	NA
		BRO_C3_12	13	1.019073	6	1.549249
Yolo Flyway Farms[±]	**MAR 2023**	**FLY_A1_03**	10	1.2987	14	1.835996
		FLY_A2_03	9	0.858851	10	1.372512
		FLY_A3_03	14	1.338243	11	1.707768
		FLY_B1_03	10	0.989069	8	1.304331
		FLY_B2_03	6	0.796246	5	1.037776
		FLY_B3_03	12	0.976341	9	1.534915
		FLY_C1_03	14	1.048139	12	1.740025
		FLY_C2_03	11	0.941846	9	1.41073

		FLY_C3_03	13	1.128383	12	1.390496
	AUG 2023	**FLY_A1_08**	20	2.222344	20	2.532414
		FLY_A2_08	20	1.77292	21	2.435097
		FLY_A3_08	20	2.096398	20	2.32099
		FLY_B1_08	17	1.814073	17	2.348812
		FLY_B2_08	19	1.857765	16	2.28872
		FLY_B3_08	18	1.819118	13	2.066336
		FLY_C1_08	17	1.778613	17	1.891338
		FLY_C2_08	19	1.707081	14	1.779961
		FLY_C3_08	17	1.836173	12	1.879576
	DEC 2023	**FLY_A1_12**	19	1.632764	13	1.974665
		FLY_A2_12	15	1.451231	14	2.010205
		FLY_A3_12	18	1.563397	13	1.965462
		FLY_B1_12	NA	NA	NA	NA
		FLY_B2_12	15	1.304073	14	1.836812
		FLY_B3_12	14	1.308159	15	1.962278
		FLY_C1_12	14	2.024066	13	1.791258
		FLY_C2_12	16	1.728187	17	1.920949
		FLY_C3_12	NA	NA	NA	NA
Liberty Island[†]	**MAR 2023**	**LIB_A1_03**	13	1.180984	10	0.682436
		LIB_A2_03	12	0.881982	6	0.368485
		LIB_A3_03	8	0.747264	9	0.868076
		LIB_B1_03	9	1.448692	9	1.09319
		LIB_B2_03	8	1.312903	7	1.291719
		LIB_B3_03	10	1.38116	9	1.4046
		LIB_C1_03	11	1.601232	5	1.205521
		LIB_C2_03	10	1.218174	8	1.001312
		LIB_C3_03	9	0.983668	5	0.834912
	AUG 2023	**LIB_A1_08**	13	0.864683	12	1.507111
		LIB_A2_08	14	0.928265	14	1.522723
		LIB_A3_08	11	0.854645	12	1.528761
		LIB_B1_08	13	1.034154	12	1.599874
		LIB_B2_08	13	1.123003	10	1.872902
		LIB_B3_08	13	1.089916	12	1.794905
		LIB_C1_08	19	2.367457	15	2.187806
		LIB_C2_08	7	1.27978	5	0.593802
		LIB_C3_08	15	2.189139	9	1.708373
	DEC 2023	**LIB_A1_12**	18	2.026981	17	2.279971
		LIB_A2_12	17	2.198419	12	1.870632
		LIB_A3_12	16	2.147194	17	2.194369
		LIB_B1_12	14	1.651249	13	1.916842
		LIB_B2_12	13	1.701113	8	1.860251
		LIB_B3_12	13	1.93704	12	1.893246
		LIB_C1_12	11	1.563743	8	1.656403
		LIB_C2_12	10	1.55661	9	1.622841
		LIB_C3_12	12	1.761585	8	1.596088
Webb Tracts Island and Berms[†]	**FEB 2023**	**WEB_A1_02**	12	1.414418	8	1.47999
		WEB_A2_02	15	1.898325	8	1.322368
		WEB_A3_02	15	1.65649	8	1.009844
		WEB_B1_02	11	1.797898	9	1.582455
		WEB_B2_02	10	2.015204	4	0.854996
		WEB_B3_02	8	1.572828	9	1.759016
		WEB_C1_02	9	1.708032	5	1.099874
		WEB_C2_02	6	1.56717	3	0.608698
		WEB_C3_02	NA	NA	NA	NA

Decker Island[±]	**FEB 2023**	**DEC_A1_02**	NA	NA	NA	NA
		DEC_A2_02	9	1.124257	9	0.767214
		DEC_A3_02	10	1.454089	8	0.557005
		DEC_B1_02	9	1.065031	10	0.705773
		DEC_B2_02	13	1.361322	10	0.76527
		DEC_B3_02	13	1.276718	9	0.835625
		DEC_C1_02	14	1.495611	9	0.526753
		DEC_C2_02	11	1.333375	9	0.855405
		DEC_C3_02	13	1.40028	9	0.979665

Table S20. 16S and 12S native species diversity and richness for 1-L eDNA water samples. Diversity calculated using Shannon Diversity Index. Observed = Species richness. "NA" denotes samples removed after quality read filtration or had 0 reads following removal of non-fish DNA.

Site	Sample Event	Sample ID	16S Observed	16S Shannon	12S Observed	12S Shannon
Tule Red[±]	**FEB 2023**	**TUL_A1_02**	NA	NA	NA	NA
		TUL_A2_02	2	0.614953	1	0
		TUL_A3_02	2	0.358942	1	0
		TUL_B1_02	2	0.432969	1	0
		TUL_B2_02	2	0.492836	1	0
		TUL_B3_02	1	0	NA	NA
		TUL_C1_02	1	0	NA	NA
		TUL_C2_02	2	0.671608	NA	NA
		TUL_C3_02	0	0	NA	NA
	SEP 2023	**TUL_A1_09**	5	0.911618	5	0.944238
		TUL_A2_09	4	0.404606	3	0.934517
		TUL_A3_09	4	0.878186	4	0.665343
		TUL_B1_09	5	0.261549	3	0.796187
		TUL_B2_09	5	0.820827	3	0.646543
		TUL_B3_09	4	0.424765	6	1.230894
		TUL_C1_09	7	0.950314	5	0.988465
		TUL_C2_09	4	0.67098	2	0.461539
		TUL_C3_09	7	0.918432	4	1.297143
	DEC 2023	**TUL_A1_12**	7	1.523104	6	1.386643
		TUL_A2_12	5	1.396346	7	1.45796
		TUL_A3_12	4	1.331668	4	1.286388
		TUL_B1_12	5	1.148382	4	0.939159
		TUL_B2_12	5	0.720691	5	1.470422
		TUL_B3_12	NA	NA	3	0.989603
		TUL_C1_12	3	1.077956	3	1.047078
		TUL_C2_12	1	0	NA	NA
		TUL_C3_12	4	1.126581	1	0
Ryer Island[†]	**FEB 2023**	**RYE_A1_02**	3	0.863233	3	0.768042
		RYE_A2_02	2	0.650416	1	0
		RYE_A3_02	1	0	1	0
		RYE_B1_02	2	0.054295	1	0
		RYE_B2_02	0	0	0	0
		RYE_B3_02	0	0	NA	NA
		RYE_C1_02	0	0	0	0
		RYE_C2_02	3	0.890968	1	0
		RYE_C3_02	NA	NA	NA	NA
	SEP 2023	**RYE_A1_09**	5	0.734318	4	0.857934

		RYE_A2_09	4	0.773822	4	1.189664
		RYE_A3_09	3	0.359403	4	0.763965
		RYE_B1_09	6	0.334413	3	0.60354
		RYE_B2_09	3	0.066922	5	0.781159
		RYE_B3_09	3	0.227957	6	1.217485
		RYE_C1_09	NA	NA	NA	NA
		RYE_C2_09	5	0.337102	4	1.066045
		RYE_C3_09	3	0.621716	5	1.313354
	DEC 2023	**RYE_A1_12**	NA	NA	NA	NA
		RYE_A2_12	3	0.85675	4	0.857642
		RYE_A3_12	NA	NA	2	0.190915
		RYE_B1_12	1	0	3	0.676582
		RYE_B2_12	1	0	2	0.577342
		RYE_B3_12	2	0.59812	2	0.004703
		RYE_C1_12	1	0	2	0.086621
		RYE_C2_12	NA	NA	NA	NA
		RYE_C3_12	NA	NA	4	1.11771
Winter Island[±]	**FEB 2023**	**WIN_A1_02**	3	0.805493	2	0.376019
		WIN_A2_02	3	0.841997	3	1.038114
		WIN_A3_02	4	1.115435	3	0.497369
		WIN_B1_02	4	0.966702	6	1.49482
		WIN_B2_02	5	1.245801	4	1.209129
		WIN_B3_02	6	1.187104	4	1.087746
		WIN_C1_02	5	1.386888	5	0.770339
		WIN_C2_02	5	1.179497	3	0.842368
		WIN_C3_02	3	0.668806	5	0.932482
	DEC 2023	**WIN_A1_12**	7	1.455288	6	1.513417
		WIN_A2_12	6	1.156465	6	1.332355
		WIN_A3_12	4	0.962467	6	1.495724
		WIN_B1_12	7	1.548659	7	1.558766
		WIN_B2_12	6	1.63072	7	1.710409
		WIN_B3_12	6	1.650849	5	1.462134
		WIN_C1_12	NA	NA	3	0.897128
		WIN_C2_12	NA	NA	6	1.595197
		WIN_C3_12	5	1.290175	4	1.179066
Montezuma Wetlands[±]	**FEB 2023**	**MON_A1_02**	4	0.711731	5	1.058837
		MON_A2_02	3	0.793539	5	1.371881
		MON_A3_02	5	1.376641	4	0.838782
		MON_B1_02	4	1.095623	5	1.20679
		MON_B2_02	5	1.49887	6	1.427456
		MON_B3_02	5	1.18594	4	1.165627
		MON_C1_02	7	1.078671	5	1.102721
		MON_C2_02	8	1.428703	4	1.158614
		MON_C3_02	6	1.017507	4	0.986639
	AUG 2023	**MON_A1_08**	5	0.771185	7	1.417267
		MON_A2_08	4	0.784703	6	1.231436
		MON_A3_08	7	0.912876	6	1.436577
		MON_B1_08	NA	NA	8	1.580499
		MON_B2_08	6	0.877203	7	1.089204
		MON_B3_08	4	0.90428	6	1.403155
		MON_C1_08	5	0.728707	6	1.340176
		MON_C2_08	5	0.616256	5	0.94942
		MON_C3_08	6	0.703503	7	1.294066
	JAN 2024	**MON_A1_01**	NA	NA	NA	NA
		MON_A2_01	NA	NA	6	1.294314
		MON_A3_01	NA	NA	2	0.311551

		MON_B1_01	5	0.842922	5	1.169868
		MON_B2_01	NA	NA	NA	NA
		MON_B3_01	7	1.290852	4	1.102789
		MON_C1_01	1	0	1	0
		MON_C2_01	4	0.975831	3	1.029196
		MON_C3_01	8	1.379462	5	1.194577
Browns Island[†]	**FEB 2023**	**BRO_A1_02**	4	0.834327	3	0.257769
		BRO_A2_02	3	0.63184	4	0.928993
		BRO_A3_02	5	1.204385	4	1.180689
		BRO_B1_02	3	0.277651	4	0.69867
		BRO_B2_02	6	1.016116	2	0.279956
		BRO_B3_02	3	0.552362	3	0.638596
		BRO_C1_02	5	1.086835	5	1.037333
		BRO_C2_02	5	0.931863	5	0.820487
		BRO_C3_02	5	1.16363	4	0.920928
	AUG 2023	**BRO_A1_08**	6	0.922859	3	0.817918
		BRO_A2_08	5	0.834653	5	1.13152
		BRO_A3_08	3	0.255845	5	0.941182
		BRO_B1_08	4	0.457019	5	1.116882
		BRO_B2_08	4	0.663702	5	1.392274
		BRO_B3_08	4	0.552991	5	1.163184
		BRO_C1_08	5	1.06485	5	1.392451
		BRO_C2_08	4	0.931414	6	1.288054
		BRO_C3_08	4	0.813891	5	1.41071
	DEC 2023	**BRO_A1_12**	3	0.785573	3	0.854777
		BRO_A2_12	1	0	1	0
		BRO_A3_12	6	0.767004	7	1.40503
		BRO_B1_12	0	0	NA	NA
		BRO_B2_12	NA	NA	3	0.72907
		BRO_B3_12	NA	NA	NA	NA
		BRO_C1_12	NA	NA	NA	NA
		BRO_C2_12	NA	NA	NA	NA
		BRO_C3_12	6	0.784154	4	1.032645
Yolo Flyway Farms[±]	**MAR 2023**	**FLY_A1_03**	4	0.214068	5	1.441198
		FLY_A2_03	3	0.442739	3	0.691425
		FLY_A3_03	4	0.65611	4	0.638052
		FLY_B1_03	5	0.500264	4	0.626945
		FLY_B2_03	2	0.092815	1	0
		FLY_B3_03	5	0.904173	4	0.867132
		FLY_C1_03	5	1.011188	6	1.520632
		FLY_C2_03	5	0.401005	4	0.771912
		FLY_C3_03	6	0.670141	4	0.783238
	AUG 2023	**FLY_A1_08**	4	1.088738	6	1.331591
		FLY_A2_08	4	0.886169	8	1.370322
		FLY_A3_08	4	0.933661	8	1.412376
		FLY_B1_08	4	0.668005	6	1.40708
		FLY_B2_08	4	0.960186	4	0.982981
		FLY_B3_08	4	0.834095	3	0.92631
		FLY_C1_08	4	0.685349	4	0.479
		FLY_C2_08	3	0.493251	4	0.714669
		FLY_C3_08	3	0.964914	1	0
	DEC 2023	**FLY_A1_12**	6	1.274871	6	1.703378
		FLY_A2_12	4	1.103257	6	1.513283
		FLY_A3_12	6	1.179805	4	1.105415
		FLY_B1_12	NA	NA	NA	NA
		FLY_B2_12	5	1.271925	5	1.4056

		FLY_B3_12	4	1.236657	6	1.031281
		FLY_C1_12	5	1.397737	6	1.415831
		FLY_C2_12	5	1.386285	5	1.37091
		FLY_C3_12	NA	NA	NA	NA
Liberty Island[†]	**MAR 2023**	**LIB_A1_03**	6	0.591608	6	0.269394
		LIB_A2_03	5	0.460262	5	0.285153
		LIB_A3_03	4	0.508183	5	0.568104
		LIB_B1_03	2	0.502399	4	0.54824
		LIB_B2_03	2	0.641041	3	0.365053
		LIB_B3_03	2	0.588868	4	0.717877
		LIB_C1_03	4	0.611624	2	0.059264
		LIB_C2_03	4	0.772944	3	0.037478
		LIB_C3_03	5	0.707331	2	0.011187
	AUG 2023	**LIB_A1_08**	4	0.616563	5	0.968812
		LIB_A2_08	3	0.630571	5	0.832873
		LIB_A3_08	3	0.646188	5	0.901154
		LIB_B1_08	3	0.418538	5	0.809687
		LIB_B2_08	2	0.247163	4	1.01615
		LIB_B3_08	3	0.379531	4	0.890545
		LIB_C1_08	6	1.111876	5	1.135106
		LIB_C2_08	2	0.687332	1	0
		LIB_C3_08	5	0.961721	4	0.799731
	DEC 2023	**LIB_A1_12**	6	1.061854	7	1.261143
		LIB_A2_12	6	1.170896	6	1.153714
		LIB_A3_12	5	0.901594	7	1.35001
		LIB_B1_12	4	0.526199	5	0.734193
		LIB_B2_12	3	0.214055	2	0.388206
		LIB_B3_12	4	0.719957	6	1.488867
		LIB_C1_12	5	1.133219	4	1.303571
		LIB_C2_12	5	1.048277	3	0.975588
		LIB_C3_12	4	1.260082	3	0.760372
Webb Tracts Island and Berms[†]	**FEB 2023**	**WEB_A1_02**	3	0.577236	3	0.154245
		WEB_A2_02	4	0.638736	3	0.077244
		WEB_A3_02	3	0.495973	4	0.600382
		WEB_B1_02	3	0.942293	4	0.641811
		WEB_B2_02	1	0	1	0
		WEB_B3_02	2	0.683119	2	0.583044
		WEB_C1_02	2	0.465539	2	0.443281
		WEB_C2_02	2	0.553549	1	0
		WEB_C3_02	NA	NA	NA	NA
Decker Island[±]	**FEB 2023**	**DEC_A1_02**	NA	NA	NA	NA
		DEC_A2_02	4	0.570625	5	0.459301
		DEC_A3_02	4	0.596569	5	0.267511
		DEC_B1_02	5	0.518844	4	0.402748
		DEC_B2_02	7	0.860445	6	0.511844
		DEC_B3_02	5	0.65001	6	0.56655
		DEC_C1_02	5	0.80782	4	0.29953
		DEC_C2_02	4	0.659728	4	0.629675
		DEC_C3_02	7	0.887894	5	0.619887

8 | Supplementary Materials (Figures)

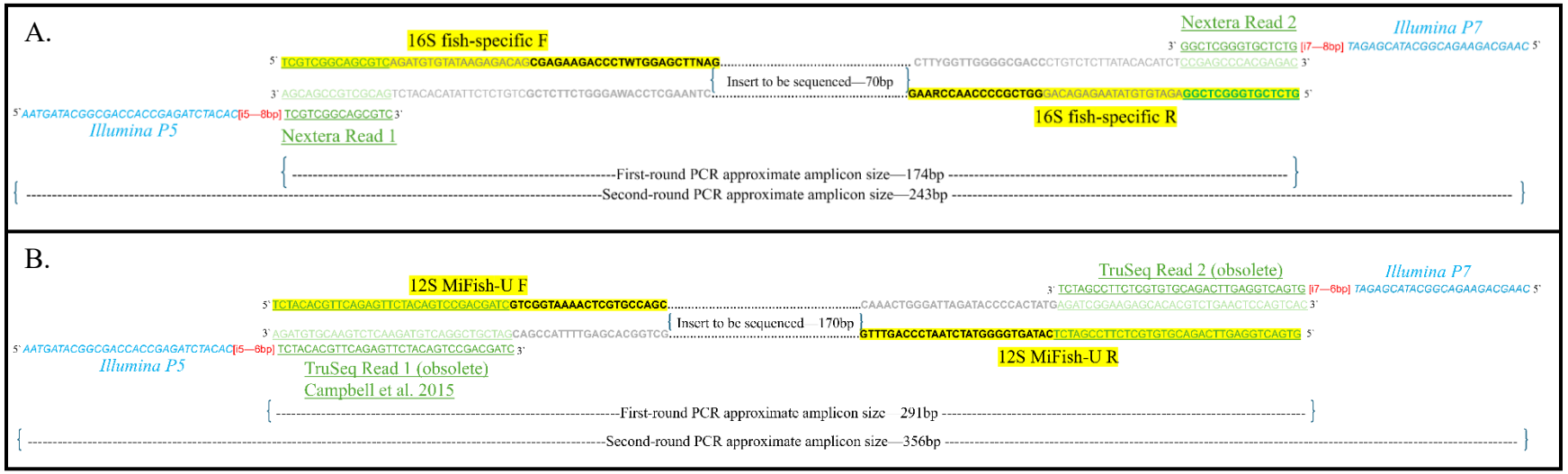


Figure S1. Amplicon and primer visualization of the paired-end library preparation. (A) 16S fish-specific primers (yellow; Shaw et al. 2016) sequence approximately 70 bp of the 16S rRNA gene. (B) 12S MiFish-U primers (yellow; Miya et al. 2015) sequence approximately 170 bp of the 12S rRNA gene. The **bold** regions bind to the target locus; green binds to Read 1/2 regions of the sequencing primers for the second round of PCR. The blue regions (P5/P7) of the sequencing primers bind to the flow cell of the Illumina MiSeq system. Oligonucleotide sequences © 2007-2015 Illumina, Inc. All rights reserved.

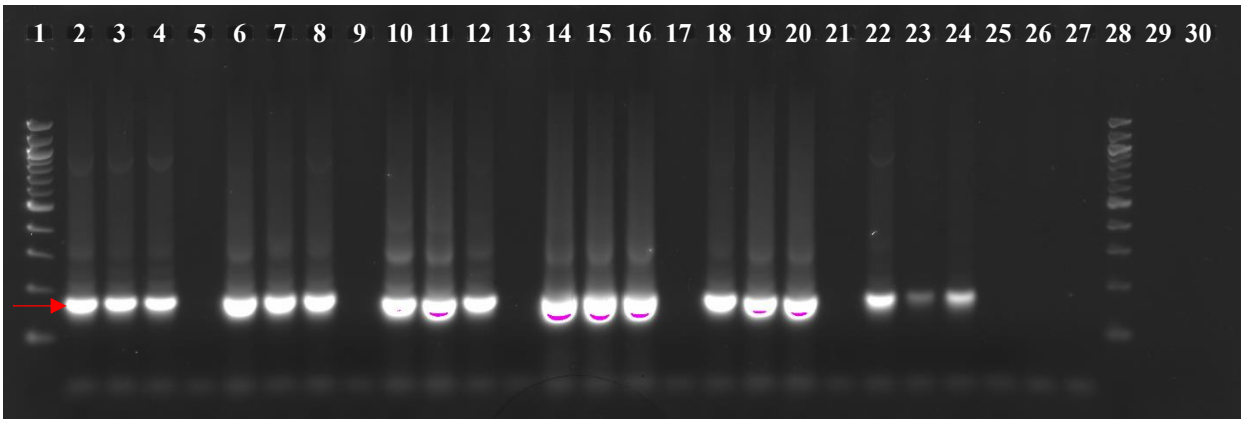


Figure S2. First-round PCR with 16S fish-specific primers. 2 % agarose gel (0.5 cm thick) run at 147V for 48m. Red arrow = target band.
Lane 1 & 28: Quick-Load® Purple 100bp DNA Ladder (New England Biolabs, USA; Product #: N0551S). DNA ladders were diluted 2:3 with DNase/RNase-free water.
Lanes 2-4, 6-8, and 10-12: eDNA samples from Yolo Flyway Farms (collected on 08/16/2023).
Lanes 14-16, 18-20, and 22-24: eDNA samples from Liberty Island (collected on 8/17/2023).
Lanes 5, 9, 13, 17, 21, and 25: Field negative controls.
Lanes 26: Extraction negative control.
Lanes 27: First-round PCR-negative control.
Lanes 29-30: Empty.

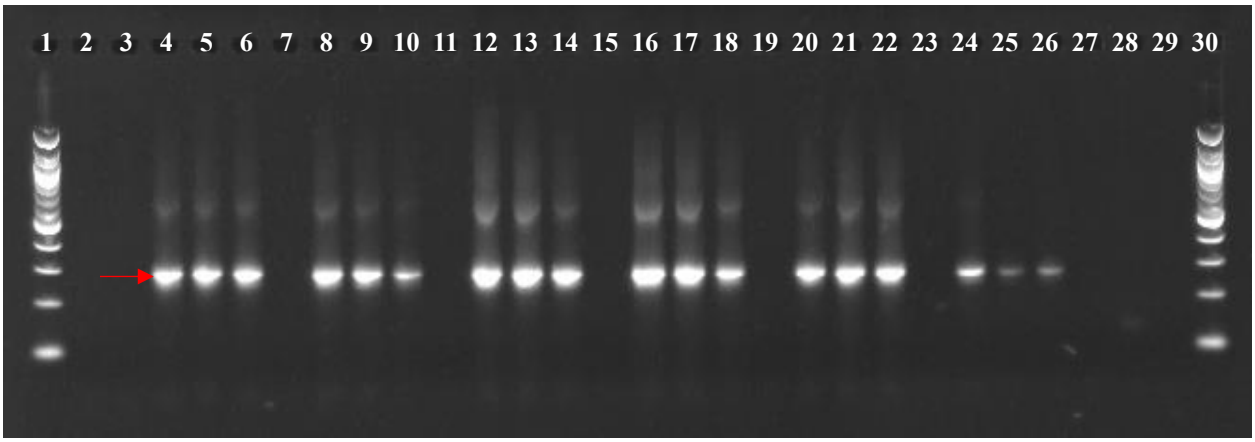


Figure S3. First-round PCR with 12S MiFish-U primers. 2 % agarose gel (0.5 cm thick) run at 147-V for 1hr 7m. Red arrow = target band.
Lane 1 & 30: Quick-Load® Purple 100bp DNA Ladder (New England Biolabs, USA; Product #: N0551S). DNA ladders were diluted 2:1 with DNase/RNase-free water.
Lanes 4-6, 8-10, and 12-14: eDNA samples from Yolo Flyway Farms (collected on 08/16/2023).
Lanes 16-18, 20-22, and 24-26: eDNA samples from Liberty Island (collected on 8/17/2023).
Lanes 7, 11, 15, 19, 23, and 27: Field negative controls.
Lanes 28: Extraction negative control.
Lanes 2-3, and 29: First-round PCR-negative controls.

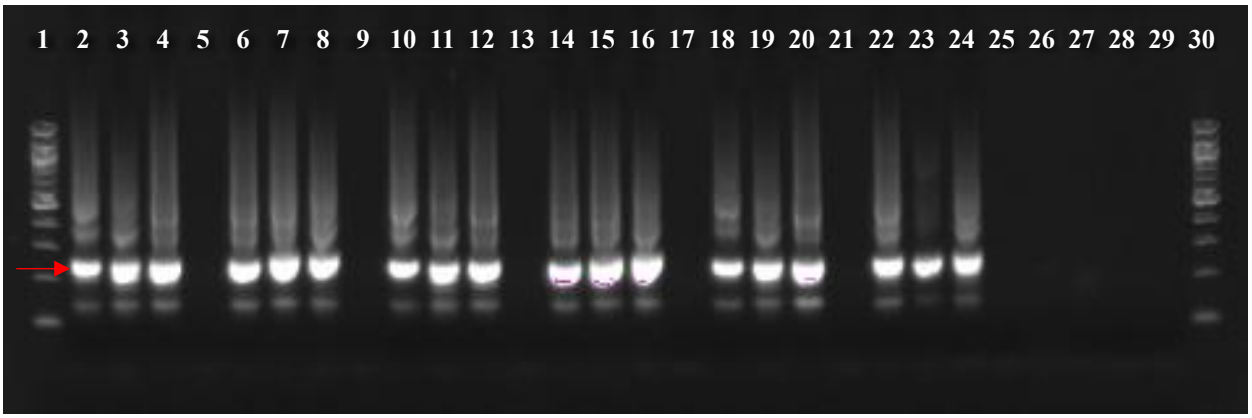


Figure S4. Second-round PCR for indexing 16S fish-specific amplicons. 2 % agarose gel (0.5 cm thick) run at 147V for 43m. Red arrow = target band.
Lane 1 & 30: Quick-Load® Purple 100bp DNA Ladder (New England Biolabs, USA; Product #: N0551S). DNA ladders were diluted 2:1 with DNase/RNase-free water.
Lanes 2-4, 6-8, and 10-12: eDNA samples from Yolo Flyway Farms (collected on 08/16/2023).
Lanes 14-16, 18-20, and 22-24: eDNA samples from Liberty Island (collected on 8/17/2023).
Lanes 5, 9, 13, 17, 21, and 25: Field negative controls.
Lanes 26: Extraction negative control.
Lanes 27: First-round PCR-negative control.
Lanes 28-29: Second-round PCR-negative controls.

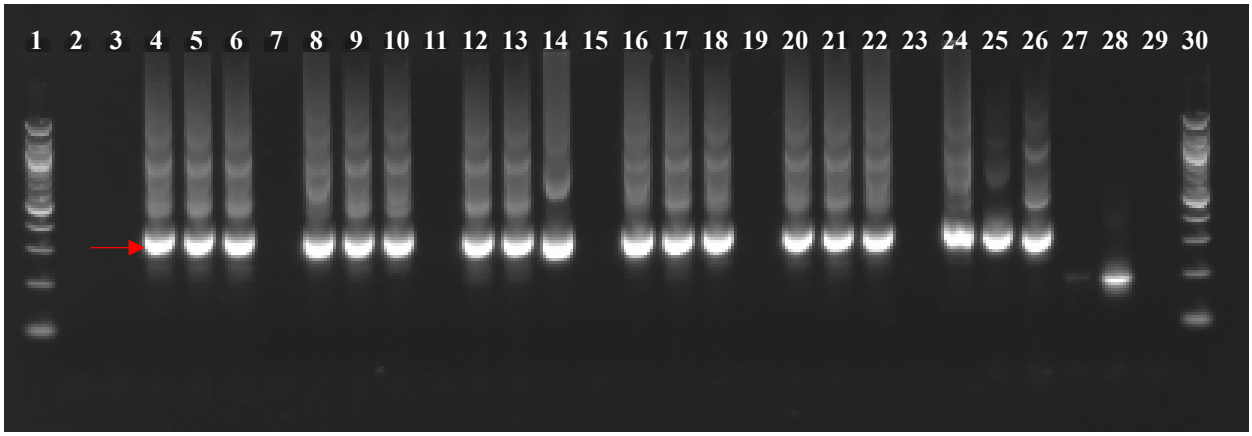


Figure S5. Second-round PCR for indexing 12S MiFish-U amplicons. 2 % agarose gel (0.5 cm thick) run at 147V for 1hr 5m. Red arrow = target band.
Lane 1 & 30: Quick-Load® Purple 100bp DNA Ladder (New England Biolabs, USA; Product #: N0551S). DNA ladders were diluted 2:1 with DNase/RNase-free water.
Lanes 4-6, 8-10, and 12-14: eDNA samples from Yolo Flyway Farms (collected on 08/16/2023).
Lanes 16-18, 20-22, and 24-26: eDNA samples from Liberty Island (collected on 8/17/2023).
Lanes 7, 11, 15, 19, 23, and 27: Field negative controls.
Lanes 28: Extraction negative control.
Lanes 2-3, and 29: First-round PCR-negative controls.

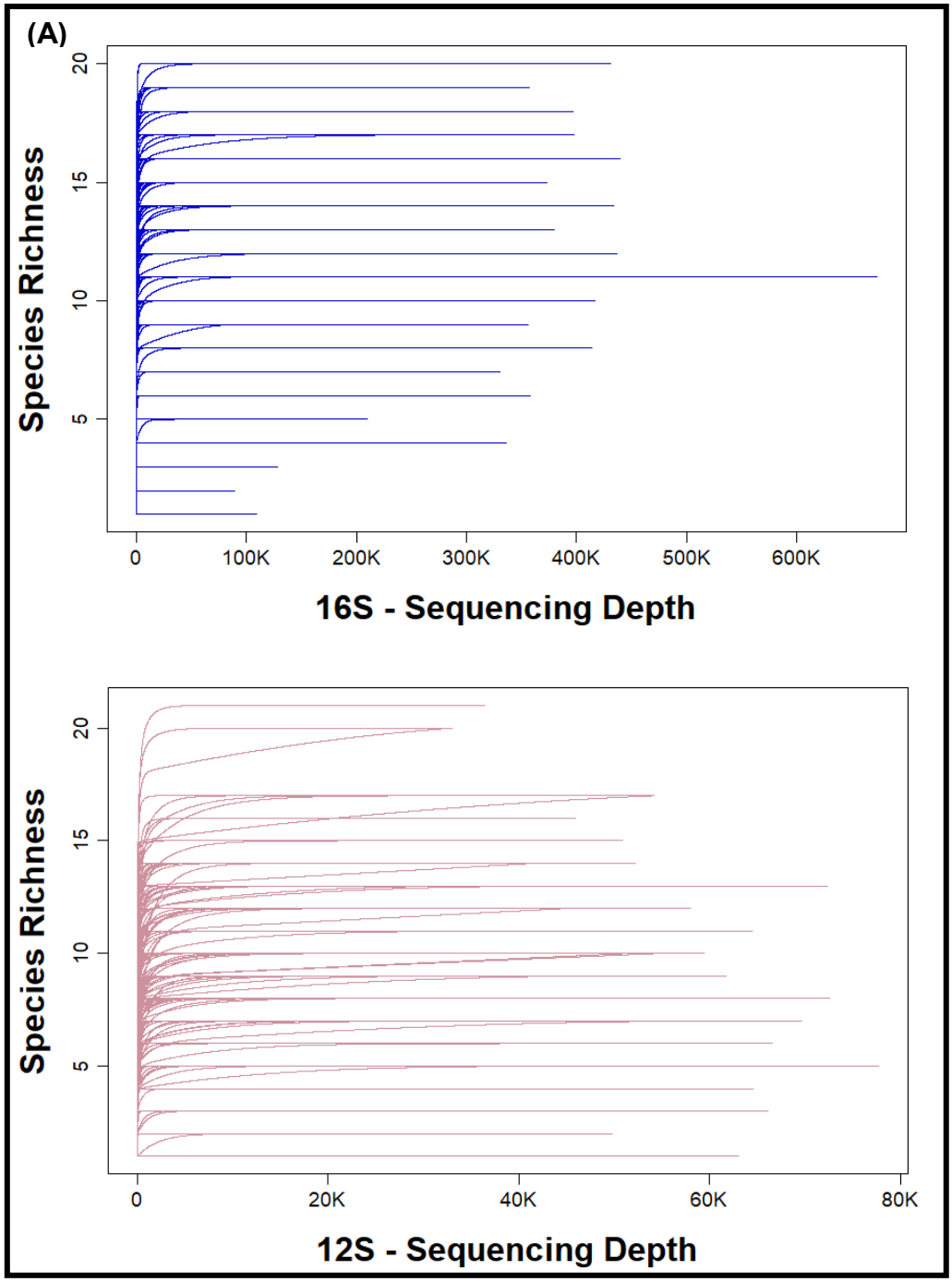


Figure S6. Fish species rarefaction curves. Accumulated fish species richness against sequencing depth is shown. Curves represent individual 1-L eDNA water samples. (A) 16S fish-specific barcode (across 175 samples). (B) 12S MiFish-U barcode (across 178 samples).

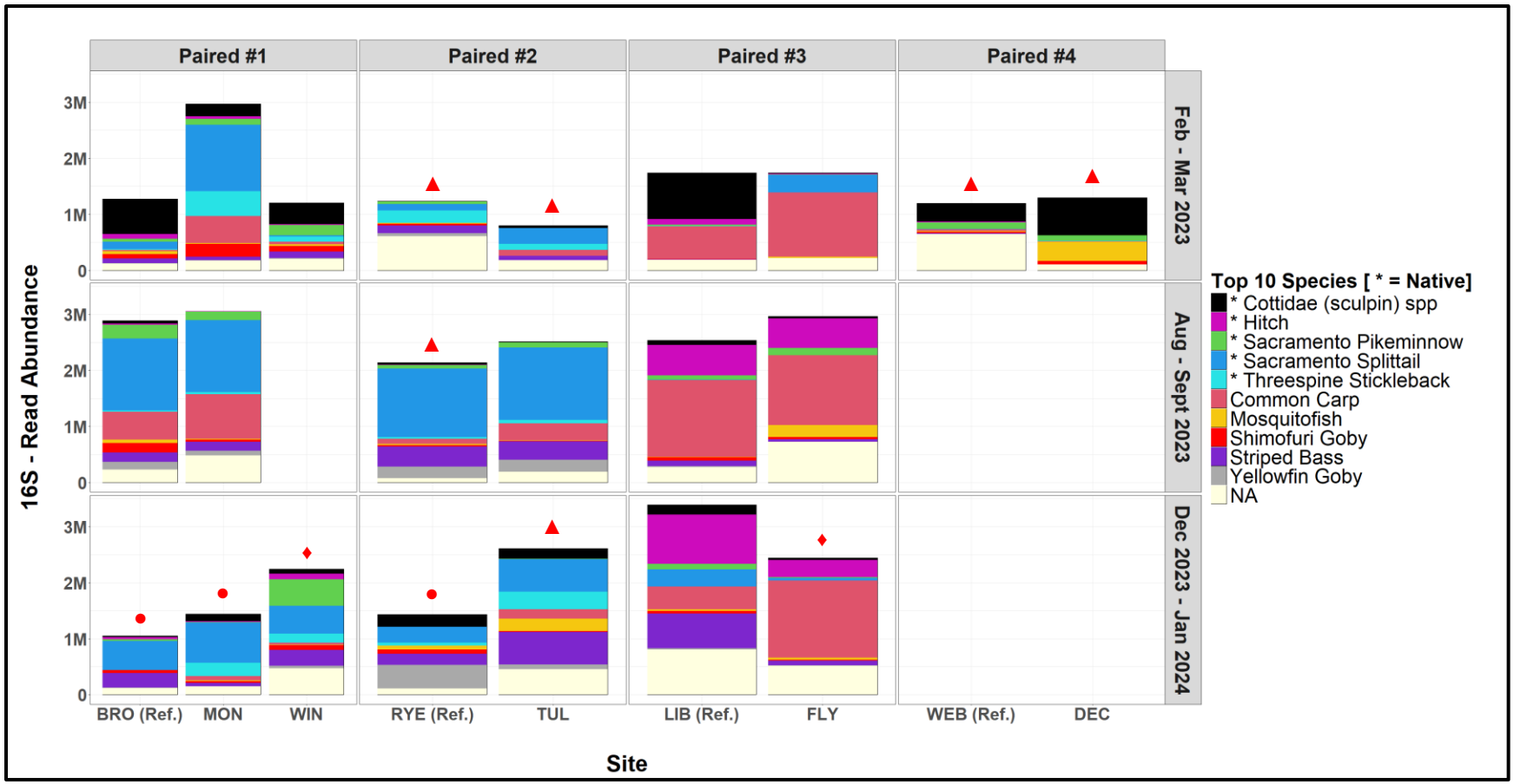


Figure S7. 16S read abundances for the top 10 species with the highest read counts. Read abundances from 3 sub-sites and 3 biological replicates from each sub-site are pooled together for each site. There are 9 samples max per site, but some sites had samples removed during sequence filtering process. ▲ = Sites with 8 samples. ♦ = Sites with 7 samples. ● = Sites with 5 samples. **NA** = Remaining species. **Ref.** = Reference site. Paired sites include Browns Island (BRO)/Montezuma Wetlands (MON)/Winter Island (WIN); Liberty Island (LIB)/Yolo Flyway Farms (FLY); Ryer Island (RYE)/Tule Red (TUL); Webb Tract Island and Berms (WEB)/Decker Island (DEC).

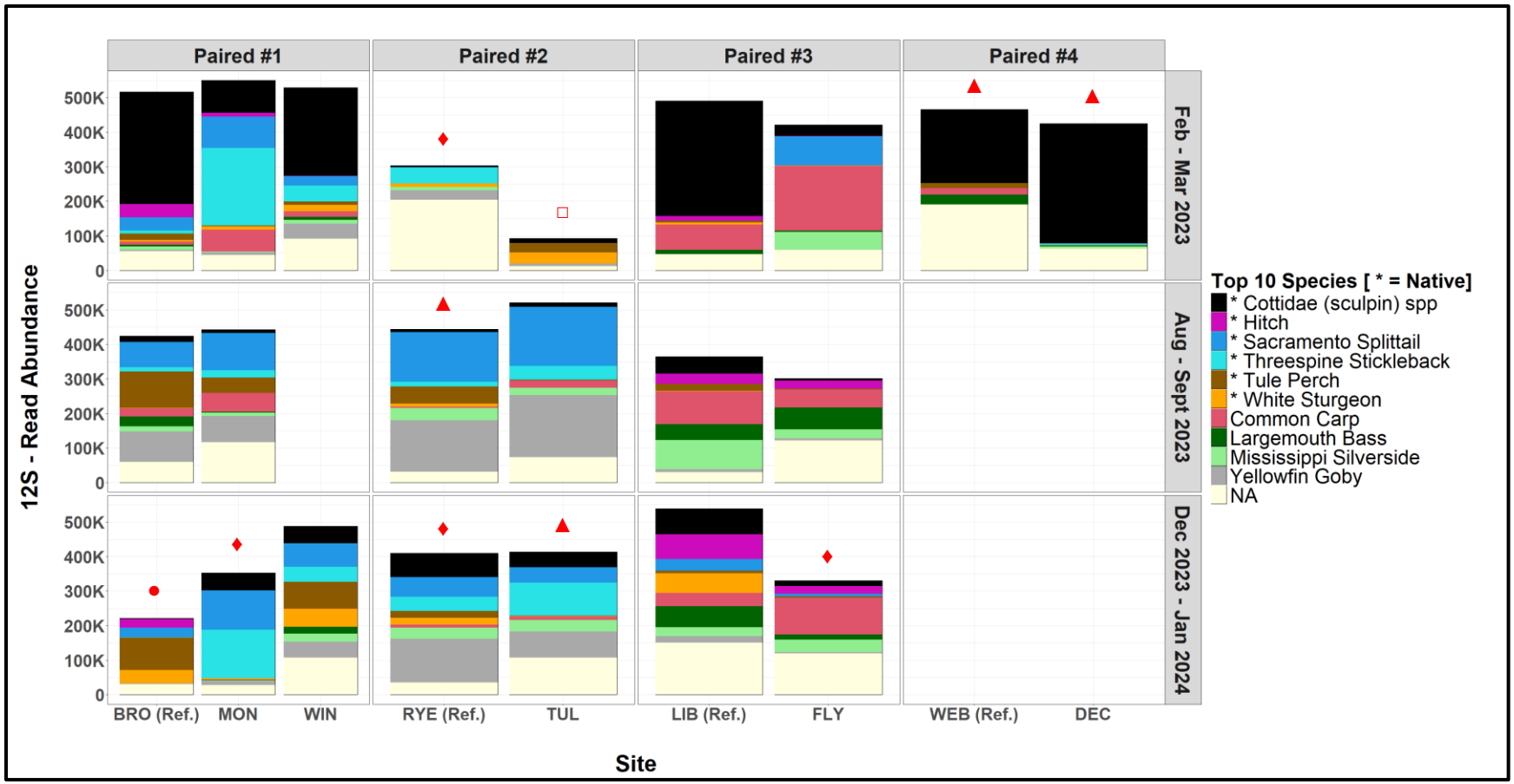


Figure S8. 12S read abundances for the top 10 species with the highest read counts. Read abundances from 3 sub-sites and 3 biological replicates from each sub-site are pooled together for each site. There are 9 samples max per site, but some sites had samples removed during sequence filtering process. ▲ = Sites with 8 samples. ♦ = Sites with 7 samples. ● = Sites with 5 samples. □ = Sites with 4 samples. **NA** = Remaining species. **Ref.** = Reference site. Paired sites include Browns Island (BRO)/Montezuma Wetlands (MON)/Winter Island (WIN); Liberty Island (LIB)/Yolo Flyway Farms (FLY); Ryer Island (RYE)/Tule Red (TUL); Webb Tract Island and Berms (WEB)/Decker Island (DEC).

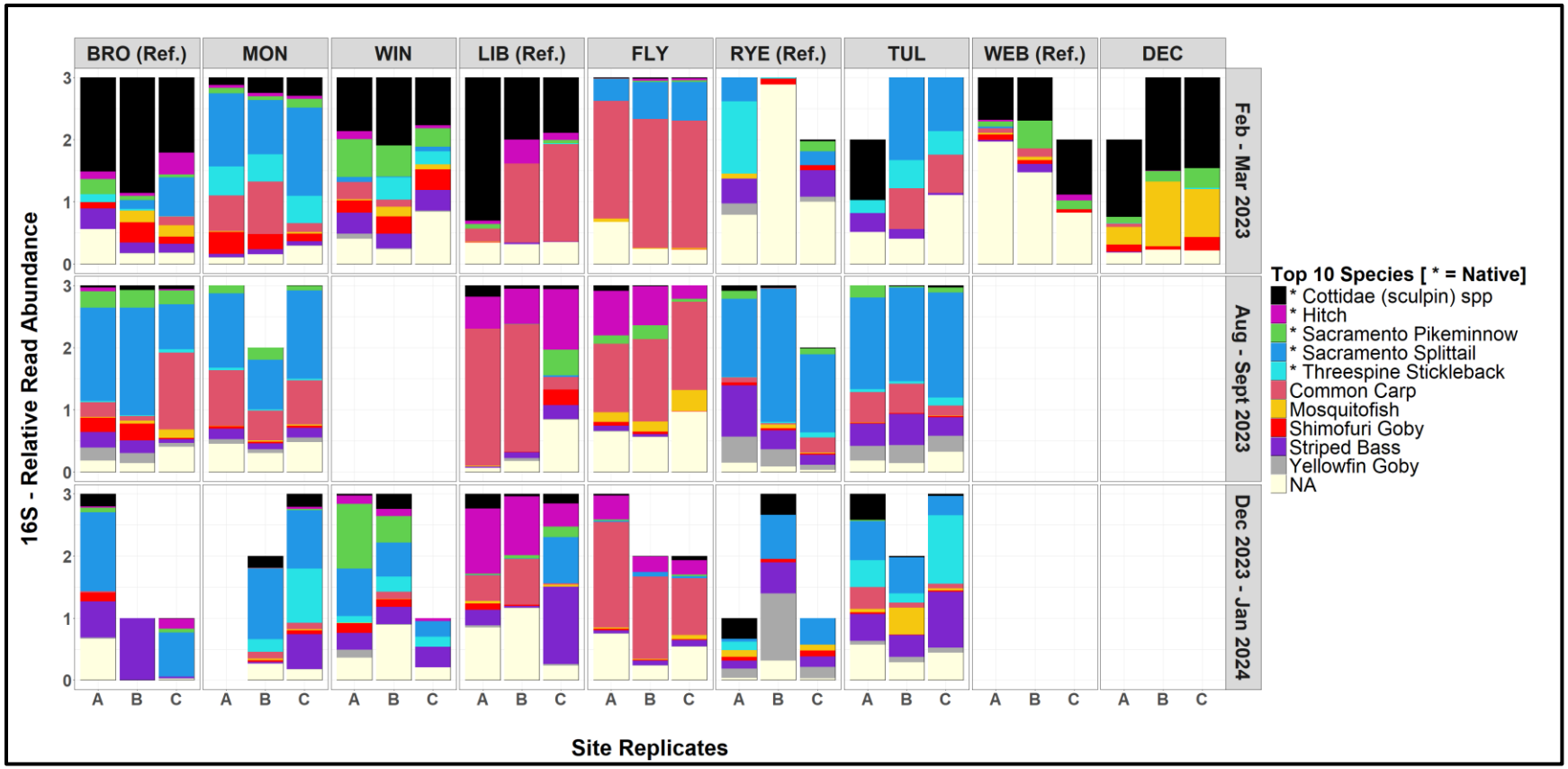


Figure S9. 16S sub-site relative read abundances for the top 10 species with the highest read counts. Relative read abundances from 3 sub-sites (A/B/C) within each site are displayed. Each sub-site consists of 3 biological replicates 'added' together. In addition to compounded relative read abundance, the y-axis also corresponds to the number of samples included in each site. There are 3 samples max per sub-site, but some sites had samples removed during sequence filtering process. **NA** = Remaining species. **Ref.** = Reference site. Paired sites include Browns Island (BRO)/Montezuma Wetlands (MON)/Winter Island (WIN); Liberty Island (LIB)/Yolo Flyway Farms (FLY); Ryer Island (RYE)/Tule Red (TUL); Webb Tract Island and Berms (WEB)/Decker Island (DEC).

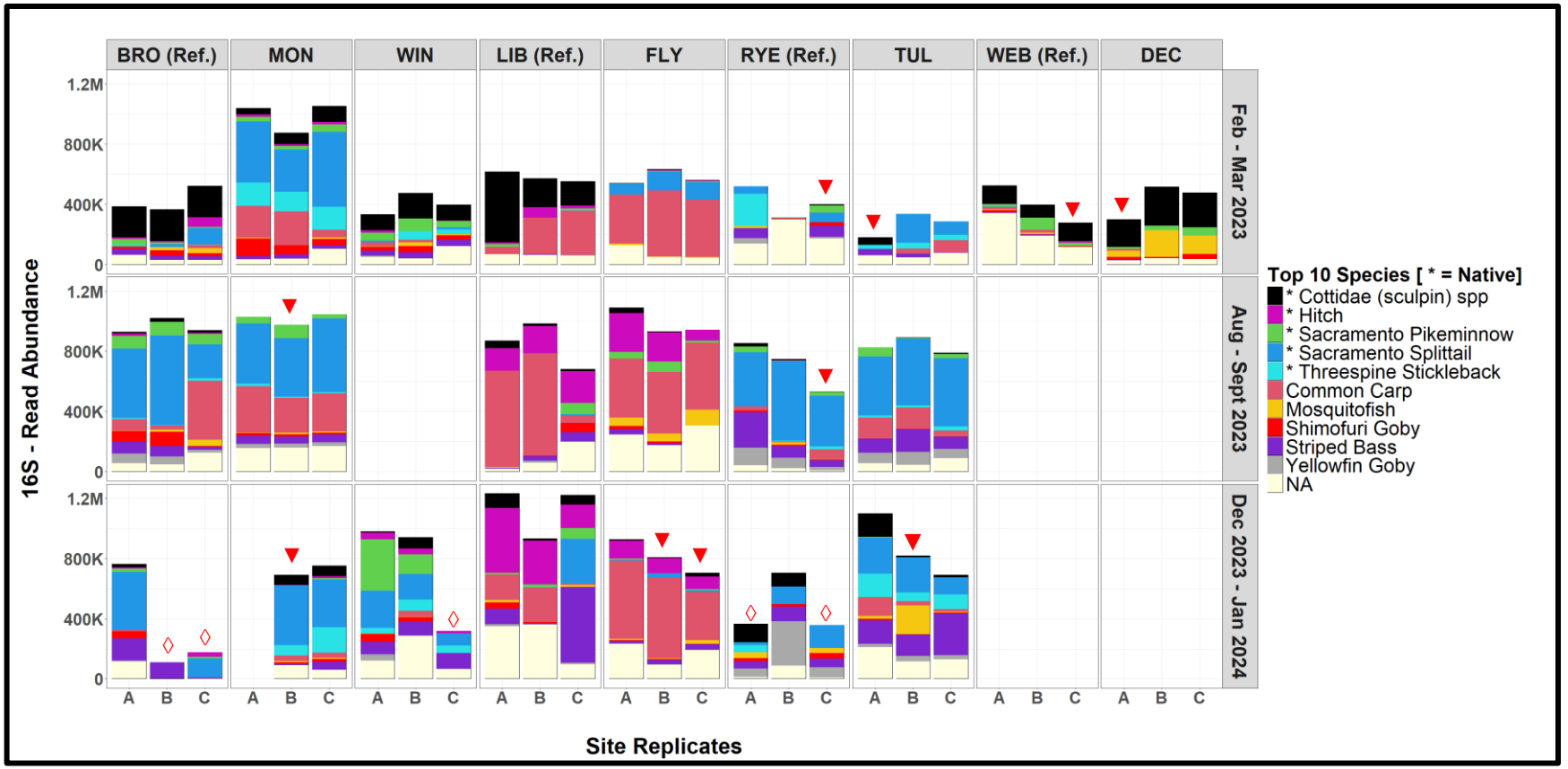


Figure S10. 16S sub-site read abundances for the top 10 species with the highest read counts. Read abundances from 3 sub-sites (A/B/C) within each site are displayed. Each sub-site consists of 3 biological replicates pooled together. There are 3 samples max per sub-site, but some sites had samples removed during sequence filtering process. ▼ = Sub-sites with 2 samples. ◊ = Sub-sites with 1 sample. MON-A in Dec-Jan had no samples remaining. **NA** = Remaining species. **Ref.** = Reference site. Paired sites include Browns Island (BRO)/Montezuma Wetlands (MON)/Winter Island (WIN); Liberty Island (LIB)/Yolo Flyway Farms (FLY); Ryer Island (RYE)/Tule Red (TUL); Webb Tract Island and Berms (WEB)/Decker Island (DEC).

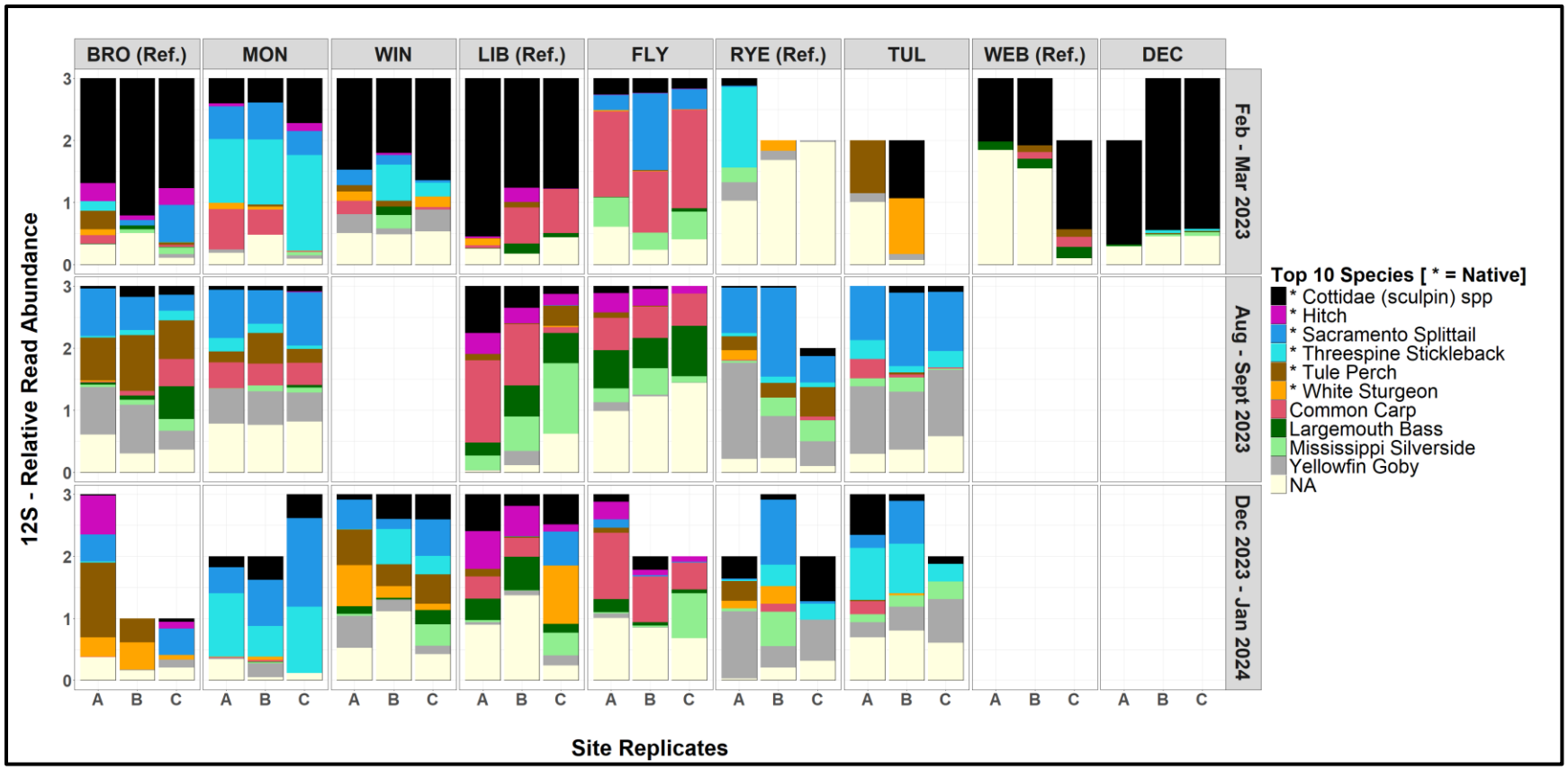


Figure S11. 12S sub-site relative read abundances for the top 10 species with the highest read counts. Relative read abundances from 3 sub-sites (A/B/C) within each site are displayed. Each sub-site consists of 3 biological replicates 'added' together. In addition to compounded relative read abundance, the y-axis also corresponds to the number of samples included in each site. There are 3 samples max per sub-site, but some sites had samples removed during sequence filtering process. **NA** = Remaining species. **Ref.** = Reference site. Paired sites include Browns Island (BRO)/Montezuma Wetlands (MON)/Winter Island (WIN); Liberty Island (LIB)/Yolo Flyway Farms (FLY); Ryer Island (RYE)/Tule Red (TUL); Webb Tract Island and Berms (WEB)/Decker Island (DEC).

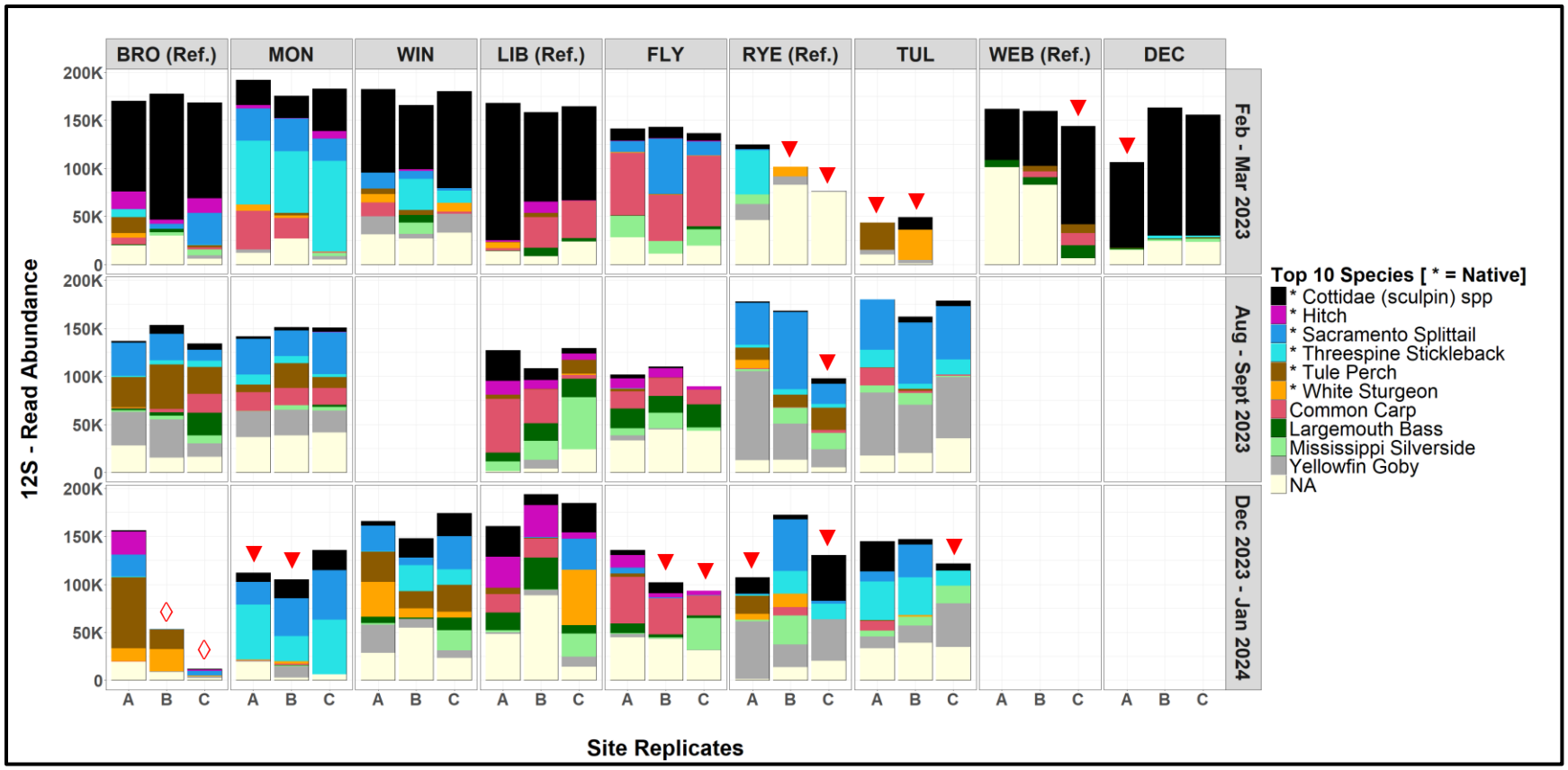


Figure S12. 12S sub-site read abundances for the top 10 species with the highest read counts. Read abundances from 3 sub-sites (A/B/C) within each site are displayed. Each sub-site consists of 3 biological replicates pooled together. There are 3 samples max per sub-site, but some sites had samples removed during sequence filtering process. ▼ = Sub-sites with 2 samples. ◊ = Sub-sites with 1 sample. TUL-C in Feb-Mar had no samples remaining. **NA** = Remaining species. **Ref.** = Reference site. Paired sites include Browns Island (BRO)/Montezuma Wetlands (MON)/Winter Island (WIN); Liberty Island (LIB)/Yolo Flyway Farms (FLY); Ryer Island (RYE)/Tule Red (TUL); Webb Tract Island and Berms (WEB)/Decker Island (DEC).

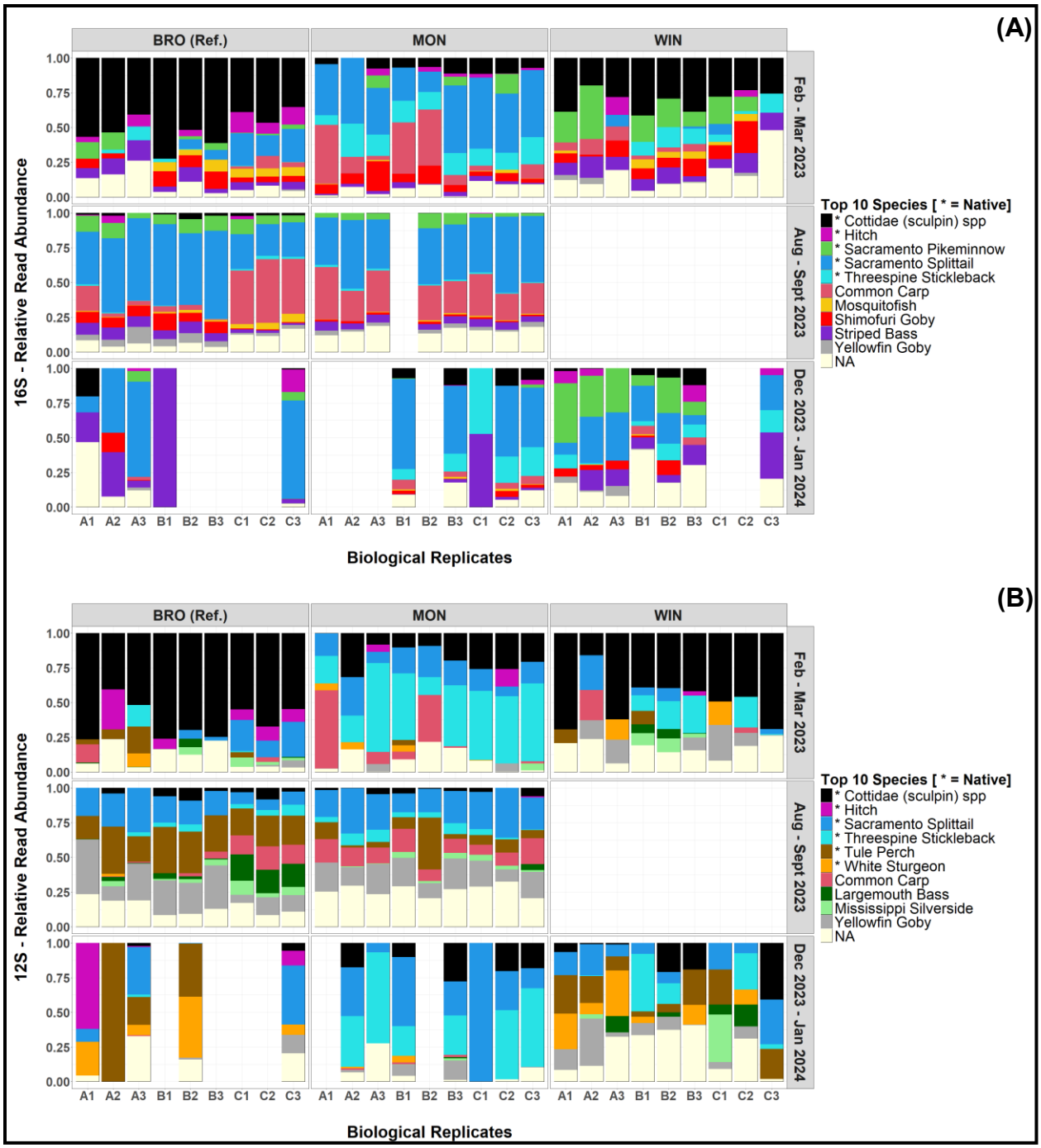


Figure S13. Browns, Montezuma, and Winter biological replicate relative read abundances for the top 10 species with the highest read counts. Displayed in grids are 3 sub-sites (A/B/C), with 3 biological replicates (1/2/3) for each site. Each bar represents a single 1-L water sample. (A) 16S fish-specific assay. (B) 12S MiFish-U assay. **NA** = Remaining species. **Ref.** = Reference site. Sites include Browns Island (BRO)/Montezuma Wetlands (MON)/Winter Island (WIN).

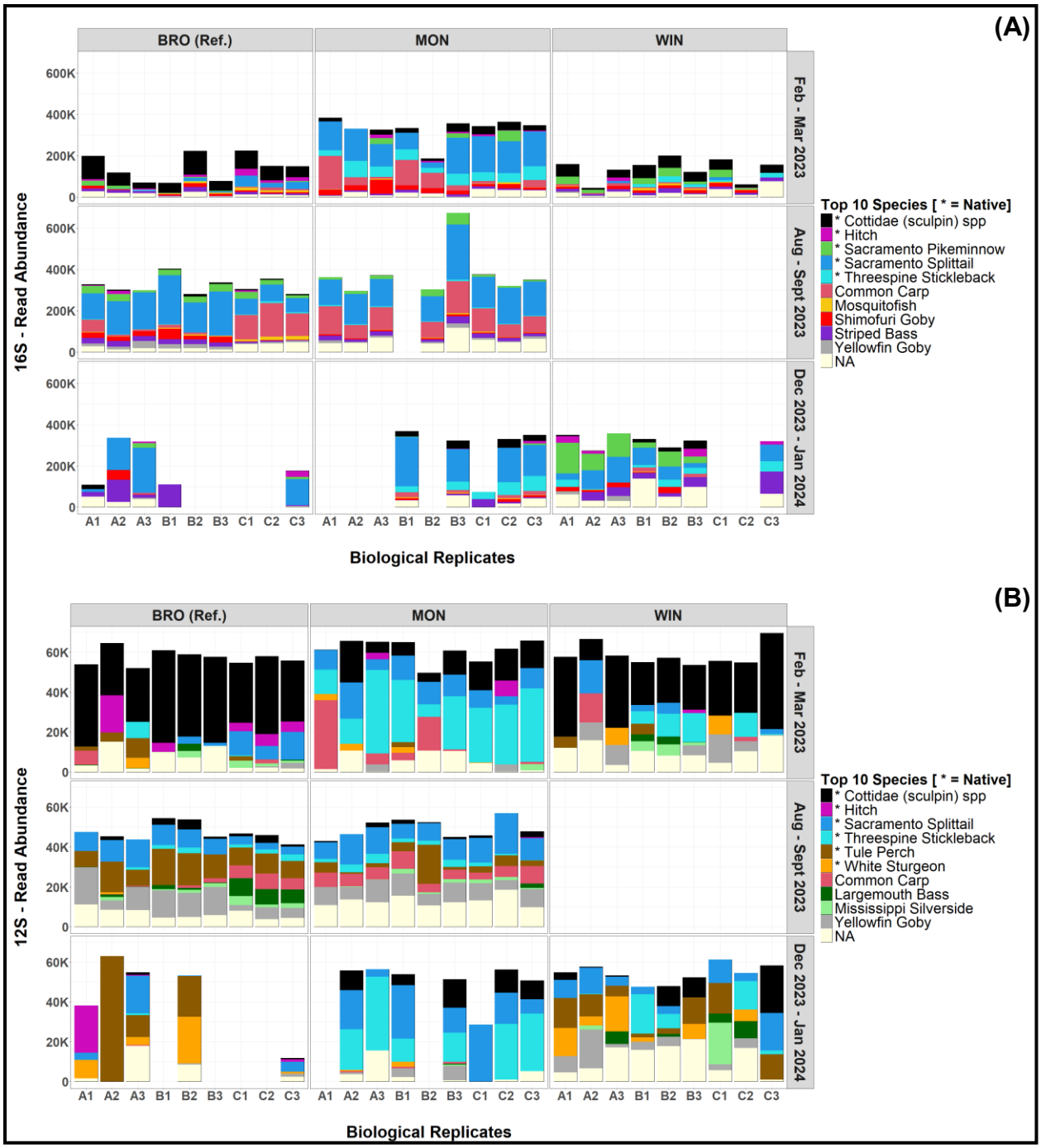


Figure S14. Browns, Montezuma, and Winter biological replicate read abundances for the top 10 species with the highest read counts. Displayed in grids are 3 sub-sites (A/B/C), with 3 biological replicates (1/2/3) for each site. Each bar represents a single 1-L water sample. (A) 16S fish-specific assay. (B) 12S MiFish-U assay. **NA** = Remaining species. **Ref.** = Reference site. Sites include Browns Island (BRO)/Montezuma Wetlands (MON)/Winter Island (WIN).

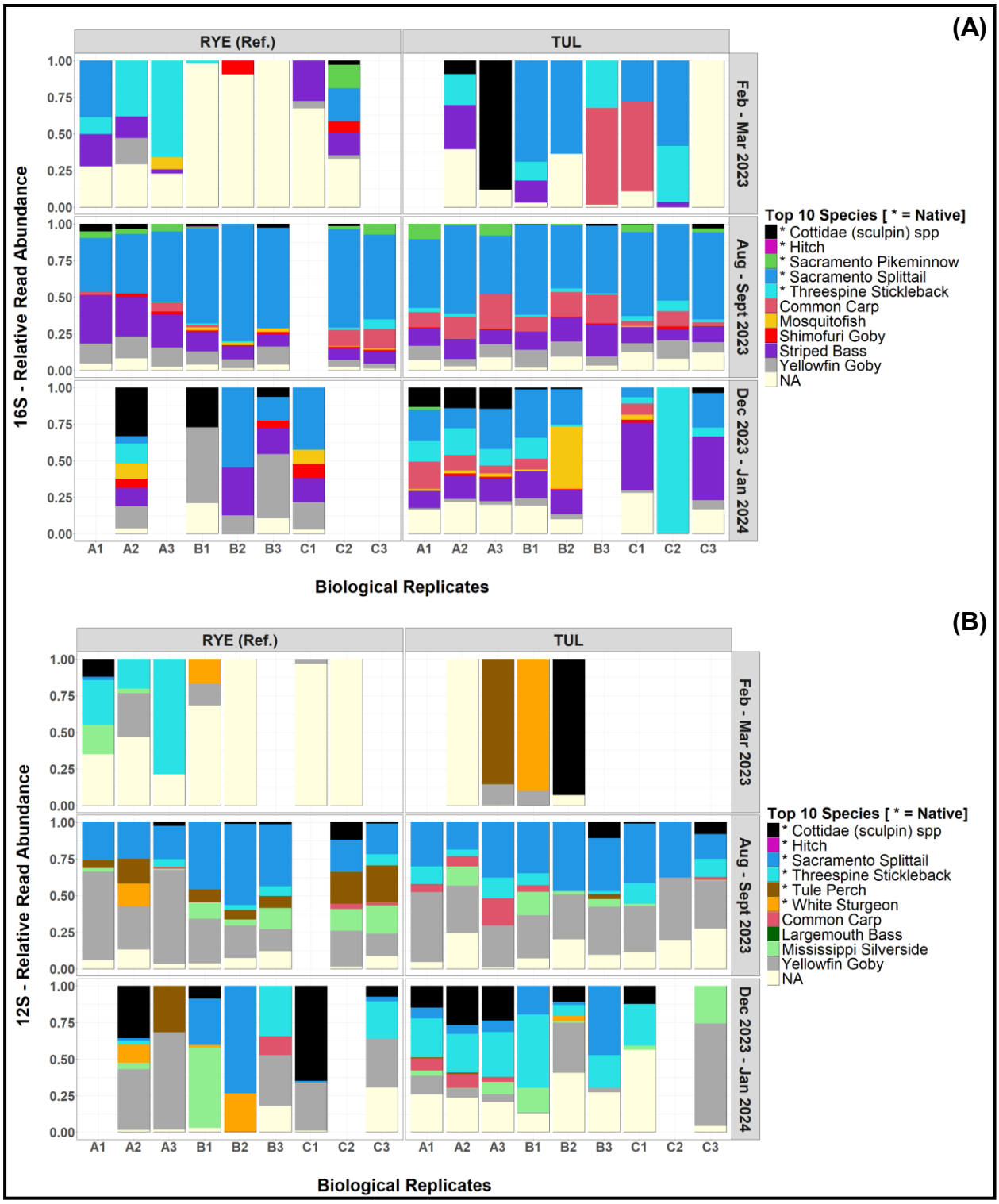


Figure S15. Tule and Ryer biological replicate relative read abundances for the top 10 species with the highest read counts. Displayed in grids are 3 sub-sites (A/B/C), with 3 biological replicates (1/2/3) for each site. Each bar represents a single 1-L water sample. (A) 16S fish-specific assay. (B) 12S MiFish-U assay. **NA** = Remaining species. **Ref.** = Reference site. Sites include Ryer Island (RYE)/Tule Red (TUL).

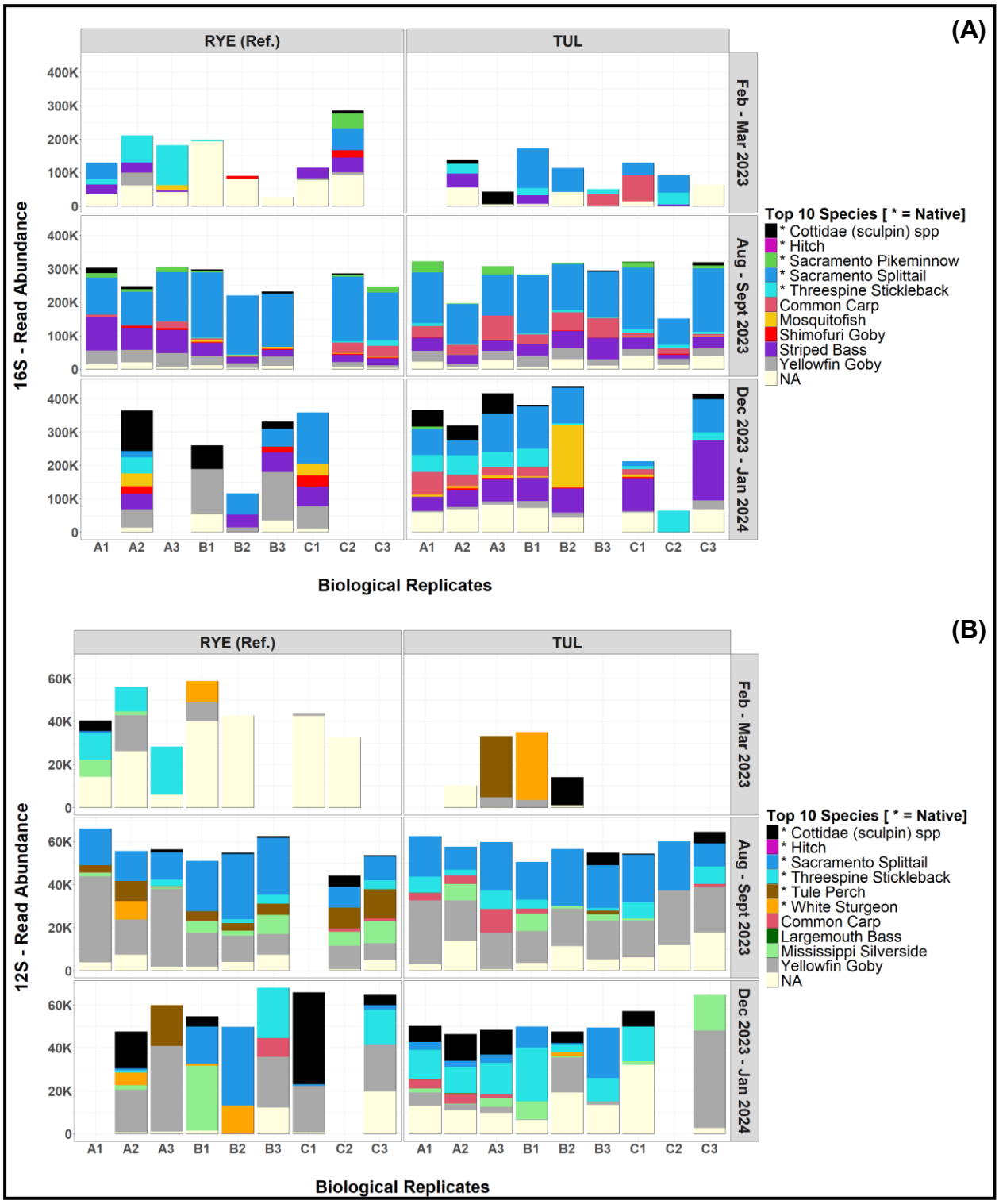


Figure S16. Tule and Ryer biological replicate read abundances for the top 10 species with the highest read counts. Displayed in grids are 3 sub-sites (A/B/C), with 3 biological replicates (1/2/3) for each site. Each bar represents a single 1-L water sample. (A) 16S fish-specific assay. (B) 12S MiFish-U assay. **NA** = Remaining species. **Ref.** = Reference site. Sites include Ryer Island (RYE)/Tule Red (TUL).

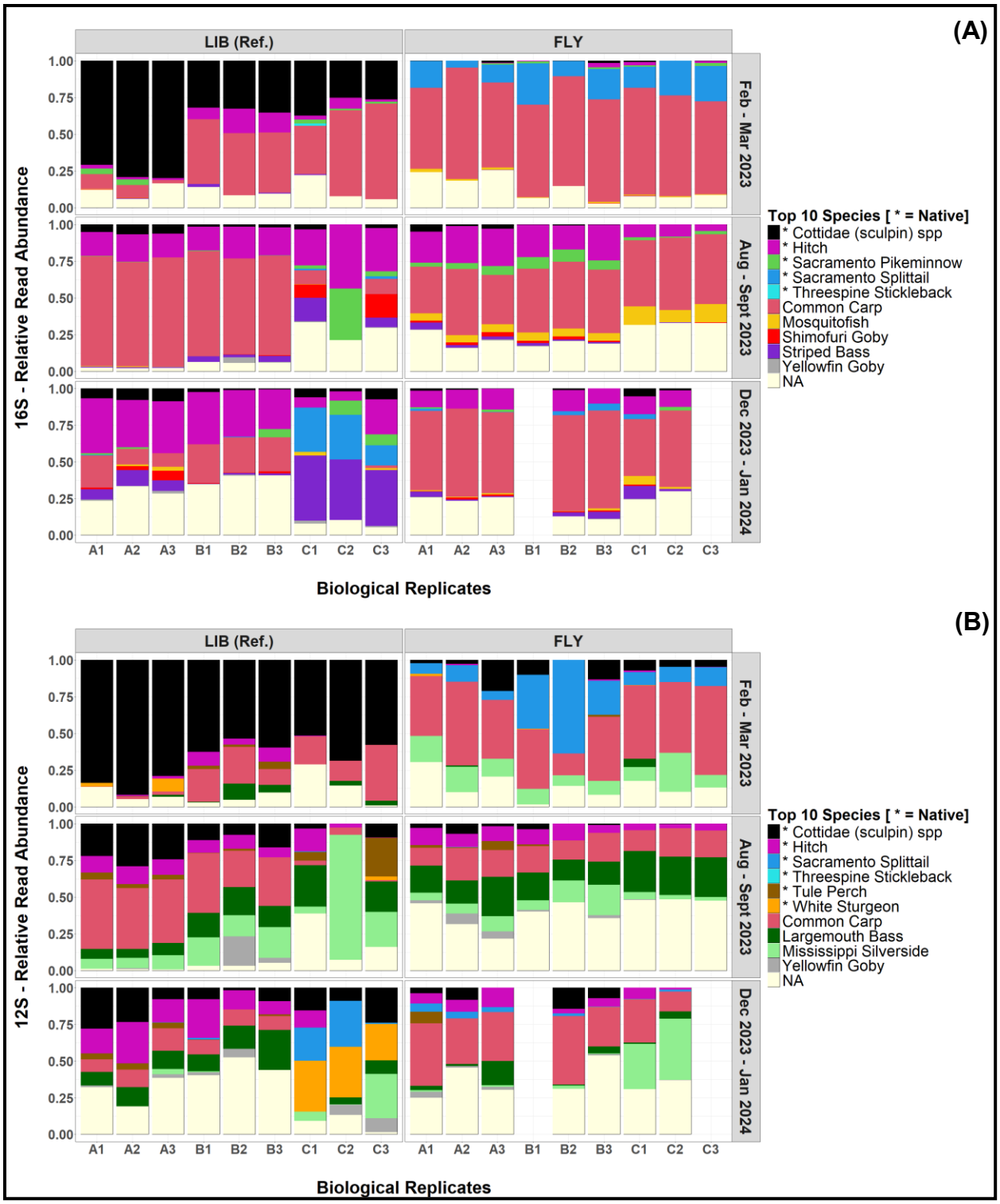


Figure S17. Liberty and Flyway biological replicate relative read abundances for the top 10 species with the highest read counts. Displayed in grids are 3 sub-sites (A/B/C), with 3 biological replicates (1/2/3) for each site. Each bar represents a single 1-L water sample. (A) 16S fish-specific assay. (B) 12S MiFish-U assay. **NA** = Remaining species. **Ref.** = Reference site. Sites include Liberty Island (LIB)/Yolo Flyway Farms (FLY).

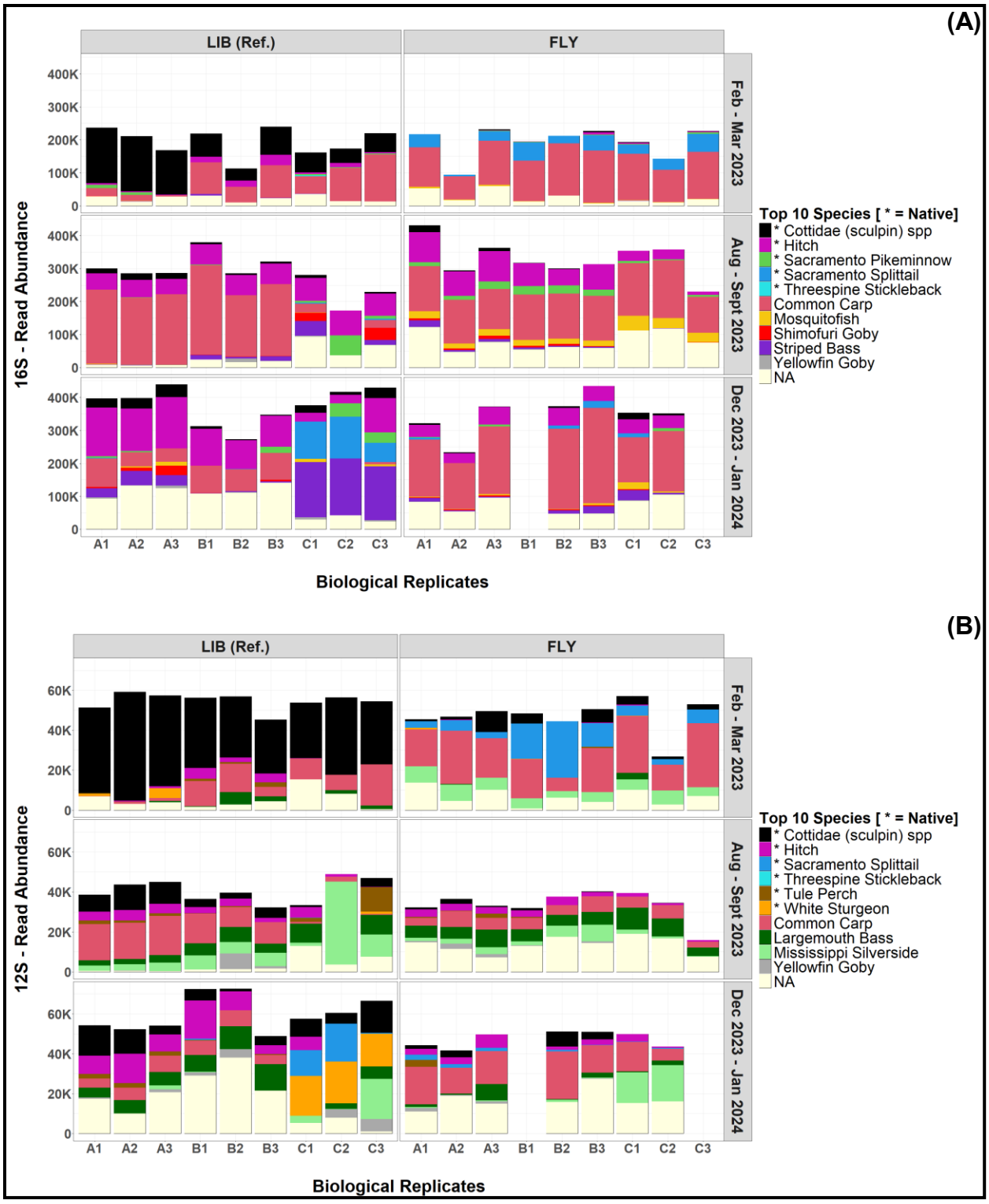


Figure S18. Liberty and Flyway biological replicate read abundances for the top 10 species with the highest read counts. Displayed in grids are 3 sub-sites (A/B/C), with 3 biological replicates (1/2/3) for each site. Each bar represents a single 1-L water sample. (A) 16S fish-specific assay. (B) 12S MiFish-U assay. **NA** = Remaining species. **Ref.** = Reference site. Sites include Liberty Island (LIB)/Yolo Flyway Farms (FLY).

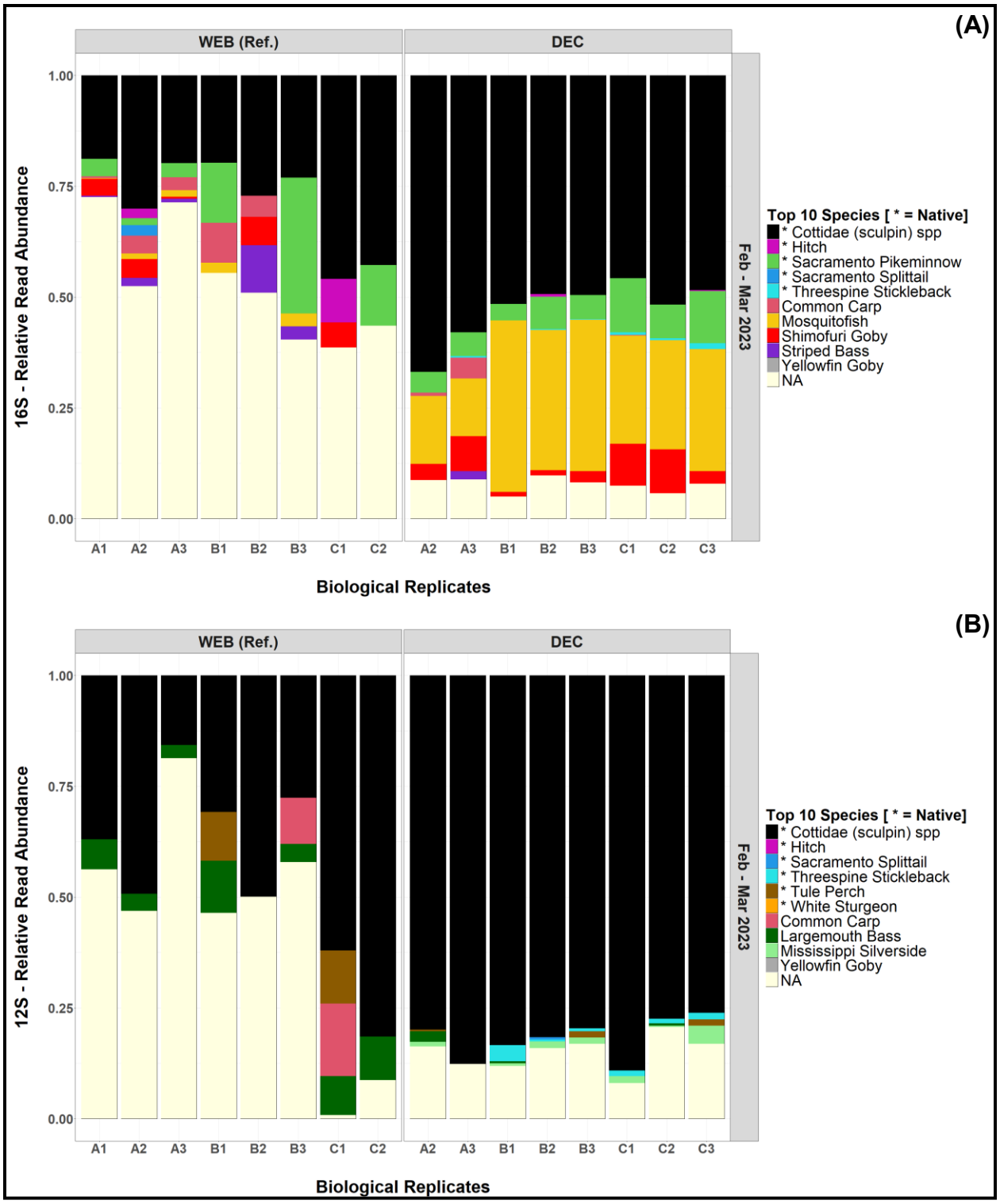


Figure S19. Decker and Webb biological replicate relative read abundances for the top 10 species with the highest read counts. Displayed in grids are 3 sub-sites (A/B/C), with 3 biological replicates (1/2/3) for each site. Each bar represents a single 1-L water sample. (A) 16S fish-specific assay. (B) 12S MiFish-U assay. **NA** = Remaining species. **Ref.** = Reference site. Sites include Decker Island (DEC)/Webb Tracts Island and Berms (WEB).

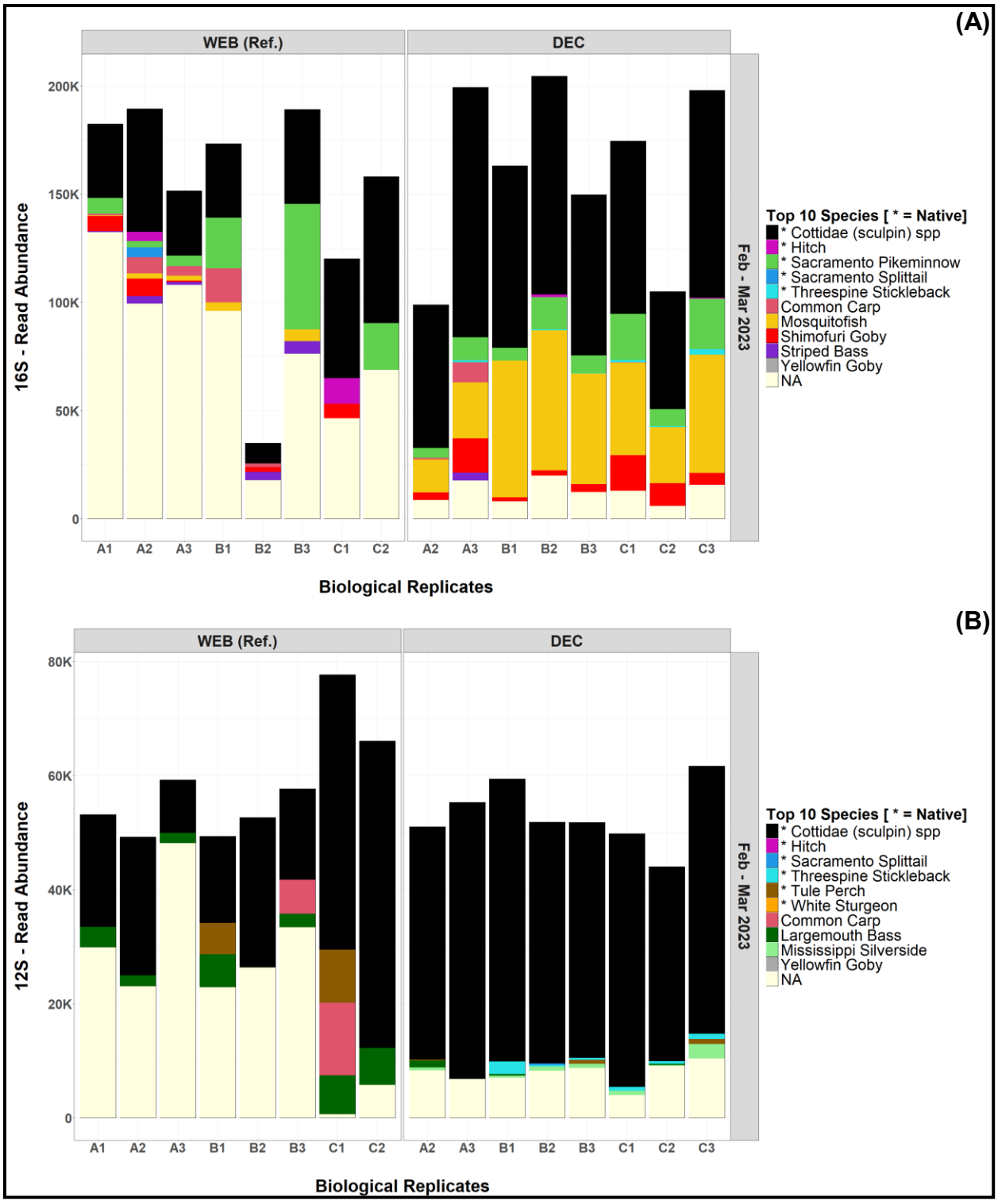


Figure S20. Decker and Webb Tract biological replicate read abundances for the top 10 species with the highest read counts. Displayed in grids are 3 sub-sites (A/B/C), with 3 biological replicates (1/2/3) for each site. Each bar represents a single 1-L water sample. (A) 16S fish-specific assay. (B) 12S MiFish-U assay. **NA** = Remaining species. **Ref.** = Reference site. Sites include Decker Island (DEC)/Webb Tracts Island and Berms (WEB).