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Is Diversity Dammed? Analysis Of Fish Diversity In Dammed And Undammed Estuary Streams Within The Virginia Peninsula Using Environmental Dna (Edna)

Melinda Ann Spence
College of William and Mary - Arts & Sciences, Yeckley5287@hotmail.com

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Melinda Ann Spence

Williamsburg, Virginia

Bachelor's of Science, Penn State, 2021

Bachelor's of Arts, Penn State, 2010

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Melinda Ann Spence

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Committee Chair
James Skelton, Assistant Professor, Biology
College of William & Mary

Harmony Dalgleish, Associate Professor, Biology
College of William & Mary

John Swaddle, Professor, Biology
College of William & Mary

ABSTRACT

Anthropogenic activities are the leading cause of biodiversity decline worldwide. For example, the construction of dams has negatively impacted riverine biodiversity globally. It has been well documented that large dams (generally larger than 45 m high) disrupt dispersal and alter local habitat often leading to less diverse aquatic systems. Yet, it is not known whether smaller dams have similar impacts, nor is it known how numerous small dams influence regional patterns of fish biodiversity at a landscape level. Coastal waterways of Virginia are impounded by hundreds of small dams. These dams are especially likely to impact fish communities in this estuary system because migration and dispersal are critical parts of the ecosystem and impoundments dramatically change the local environmental conditions from tidal lotic systems to freshwater lentic systems. Here we used environmental DNA (eDNA) to assess the alpha (diversity of species within a particular locality), beta (differences in species composition among localities), and gamma diversity (the overall diversity of species across a large geographical area) of fish species at 35 sites within the Virginia Peninsula in the lower Chesapeake Bay. We then compare patterns of diversity among sites with an undammed connection to the estuary to sites situated above small to medium sized dams (less than 25 m high). We hypothesized that dams impact local and regional fish diversity by degrading local habitats and reducing dispersal by creating barriers. Specifically, we predicted that we would 1) observe higher local fish species richness in undammed sites than in dam sites, 2) that dammed sites would have a significantly different species composition and different beta diversity than undammed sites, and 3) dammed sites would have higher frequencies of non-native species. To test our predictions, we collected 522 eDNA samples from 16 undammed waterways and 19 impoundments. We documented a total of 61 species of fish.

Congruent with our predictions, undammed sites had significantly higher species richness (alpha diversity) by approximately 10 fish species per site compared to dammed sites. Multivariate analysis showed that dammed sites had significantly different fish species composition compared to undammed sites. Furthermore, undammed sites had higher beta diversity; i.e. undammed sites had higher variability in species composition from site to site, whereas dammed sites had a more homogenous species composition. Gamma diversity of these sites across the landscape showed that overall diversity was highest within undammed areas across the peninsula, with a total of 41 species detected in dammed sites compared to 58 species detected in undammed sites. This indicates that dams do not function as additional habitats for new or additional fish species as only 3 species were exclusively detected in dammed sites, while undammed sites showed much higher species richness and diversity. These results suggest that the numerous small dams present in coastal waterways disrupt dispersal and alter habitats in estuary networks which leads to a loss of biodiversity at local sites and across the landscape.

Key Words: eDNA, biodiversity, local (alpha) diversity, regional (beta) diversity, fish communities, dams, dispersal

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1. INTRODUCTION

Anthropogenic activities are the leading cause to biodiversity decline worldwide (Ceballos et al. 2015). Biodiversity loss can lead to a breakdown in ecosystem functioning, services, and reduce resiliency of a system after disturbance (Oliver et al. 2015). This is of particular concern in the Chesapeake Bay estuaries where anthropogenic activities have caused significant impacts (Chapin III et al. 2000). Biodiversity of the Chesapeake Bay has been impacted by several anthropogenic activities including land use changes such as dams, pollution, excess nutrients, sedimentation, overfishing, and introduction of non-native species (Mcallister et al. 2001, Jansson 2006, Phillips and McGee 2016, "Living Planet Report 2020 | Publications | WWF" 2021, Dal Pont et al. 2021).

The Chesapeake Bay is the largest estuary in the United States which provides critical habitat for thousands of plant and animal species, including close to 350 species of fish, and it supports over 17 million people. This estuary is an important area for migration and reproduction of fish, birds, and other wildlife species. Some of the migratory species in this system include American Shad (*Alosa sapidissima*), Atlantic Sturgeon (*Acipenser oxyrynchus* endangered species), American Eel (*Anguilla rostrate*), and Striped Bass (*Morone saxatilis*). These species are a critical part of the ecology of the Chesapeake Bay as well as the local economies supported by recreational and commercial fishing (Estrada 2007, Callihan et al. 2015, Zydlewski et al. 2021, Mensinger et al. 2021, Annapolis and Us 2021). Additionally, the Bay provides numerous benefits related to climate change mitigation, such as carbon sequestration and flood control (Duarte et al. 2013). The biodiversity of the Chesapeake Bay is essential to maintaining its ecological and economic value, as well as the sustainability of the surrounding communities. While there has been considerable research to examine factors contributing to the degradation of

the Bay's health (Phillips and McGee 2016) the landscape-scale impacts of numerous small dams on the fish metacommunity within the Bay's estuary network remains largely unknown.

In this study we specifically focus on how dams have altered coastal fish communities within the Virginia Peninsula in the Chesapeake Bay due to changes in the abiotic conditions of coastal waterways and disruption of dispersal brought about by dams. Although a considerable amount of research has been done on the effects of large dams on fish in large rivers, less research has been done in small estuary stream networks with smaller dams. And even less work has looked at the changes to patterns of regional diversity across an entire landscape (Mcallister et al. 2001, Freedman et al. 2014, Dal Pont et al. 2021). It is particularly important that we understand the effects of small dams in the Virginia Peninsula region because this region encompasses a vast estuary system that has been impeded by over one hundred documented small dams built for mill production, recreation, and water supply, as well as many undocumented small dams ("National Inventory of Dams" 2022).

Dams affect local diversity by changing local conditions including water temperature, dissolved oxygen levels, and nutrient availability (Anderson 2006, Jansson 2006, Borges et al. 2020). Water temperatures inside dams can increase due to decreased water flow and canopy cover in impounded reaches, which have the potential to increase stream temperatures downstream which can have significant impacts on the survival of fish (Lessard and Hayes 2003, Zaidel et al. 2021). The availability of dissolved oxygen is also critical for fish, and changes in dissolved oxygen levels can occur because of dams (Ali et al. 2022, Abbott et al. 2022). Nutrient availability can also be affected by dams, as they can alter the natural flow of sediments and nutrients downstream sometimes causing eutrophication leading to fish kills (Maavara et al. 2020, Wang et al. 2022).

Dams can also facilitate the establishment of invasive species by creating new or disturbed habitats and altering the natural flow of rivers, allowing invasive species to establish and outcompete native species (Johnson et al. 2008, Turgeon et al. 2018). Furthermore, physical barriers created by dams can limit the movement of fish and restrict their ability to access different parts of the metacommunity within the estuary network (Porto et al. 1999, Pelicice et al. 2014, Carpenter-Bundhoo et al. 2020). Therefore, migratory species and species that are sensitive to changes in environmental conditions can be negatively impacted by dams.

The research summarized above clearly shows that large dams often decrease biodiversity on a local level. However, there is little understanding of the effects that numerous small dams have on patterns of dispersal and regional habitat availability, and the resulting changes in patterns of diversity at a landscape level. This knowledge is necessary to provide better management practices to protection agencies and development companies to preserve regional biodiversity, not just local biodiversity. A major challenge to studying patterns of regional diversity is that it requires taxonomically comprehensive data for a large area, with sufficient temporal and/or spatial resolution to detect spatial or temporal patterns in community composition. Environmental DNA offers a cost- and labor-efficient means for obtaining the requisite data to assess the effects of dams on patterns of regional fish diversity.

Traditional survey methods such as electrofishing and gill netting used in local biodiversity studies often struggle to achieve high sample sizes and comprehensive species lists, making it difficult to conduct large-scale regional biodiversity studies. However, the use of environmental DNA (eDNA) has emerged as an alternative to traditional sampling methods, offering a more efficient and effective way to collect data from sites not only at a local scale but also at a regional scale while achieving high species coverage for each site (Deiner et al. 2017).

eDNA refers to the DNA that organisms release into their surrounding environment through shed skin cells, blood, excrement, or other biological materials. eDNA is a rapidly evolving field that has revolutionized the way in which we detect and monitor species in their natural habitats (Deiner et al. 2017). The use of eDNA has several benefits over traditional survey methods, including better detection of elusive species that are difficult to observe directly, reduced cost and time of surveys, and it does not disrupt the sampled habitats or organisms. eDNA detection has equal or greater accuracy compared to traditional survey methods (Deiner et al. 2017) and consequently eDNA methods have become a valuable tool for conservation biologists, wildlife managers, and other researchers interested in monitoring and protecting biodiversity. The use of eDNA analysis in this study provided broadscale species detection and community characterization across a large estuarian ecosystem in single 2-month long field season.

We addressed three main questions in this study. Our first question was: Do dams reduce local (alpha) fish diversity? We hypothesized that dams reduce local fish diversity by limiting fish immigration and degrading the local habitat. Therefore, we predicted that we would observe higher local fish species richness in undammed sites than in dam sites.

Our second question was: Do dams change the species composition and regional variability (beta diversity) of fish communities? We hypothesis that dams influence local species composition in two ways; first by limiting dispersal from the regional network and therefore excluding migratory species and secondly by altering the local habitat from lotic to lentic and therefore selecting for a different set of the regional species pool. Therefore, we predicted that dammed sites would have a significantly different species composition than undammed sites. We had two competing alternative hypotheses regarding the effects of dams on variability among local communities (beta diversity). On one hand, dams were hypothesized to create barriers that

limit dispersal causing disconnection from the estuary network and leading to highly variable species composition in dammed communities due to ecological drift. From this hypothesis we predicted that that there would be higher variation in species composition (higher beta diversity) among dammed sites than undammed sites. Alternatively, we hypothesized that dams cause biotic homogenization by reducing variability in important habitat variables (i.e., species composition among dammed sites are similar due to similar habitat characteristics of dam ponds). From this hypothesis we predicted that there would be less variation in species composition (lower beta diversity) among dammed sites than in undammed sites.

Our third question was: Do dams facilitate invasive species? We predicted dammed sites would have higher proportions of non-native to native species because dams create new niches of habitat that did not previously occur in coastal Virginia in which non-natives have a higher fitness for than the native species.

2. MATERIALS AND METHODS

2.1. Study Area

I collected eDNA samples between May 9th and July 11th, 2022, at 35 sites within the Virginia Peninsula. The Virginia Peninsula is situated between the James and York Rivers (Figure 1). The James River drains a catchment comprising 27,020 km^2. The watershed includes about 4% open water and an area with a population of 2.5 million people ("Virginia Watersheds Interactive Map | Northern Virginia Regional Commission - Website" 2022)(USGS 2010). The James River forms near Iron Gate on the border between Alleghany and Botetourt counties, from the confluence of the Cowpasture and Jackson rivers in the Appalachian Mountains. It flows into the Chesapeake Bay at Hampton Roads. Larger tributaries draining to the tidal portion include

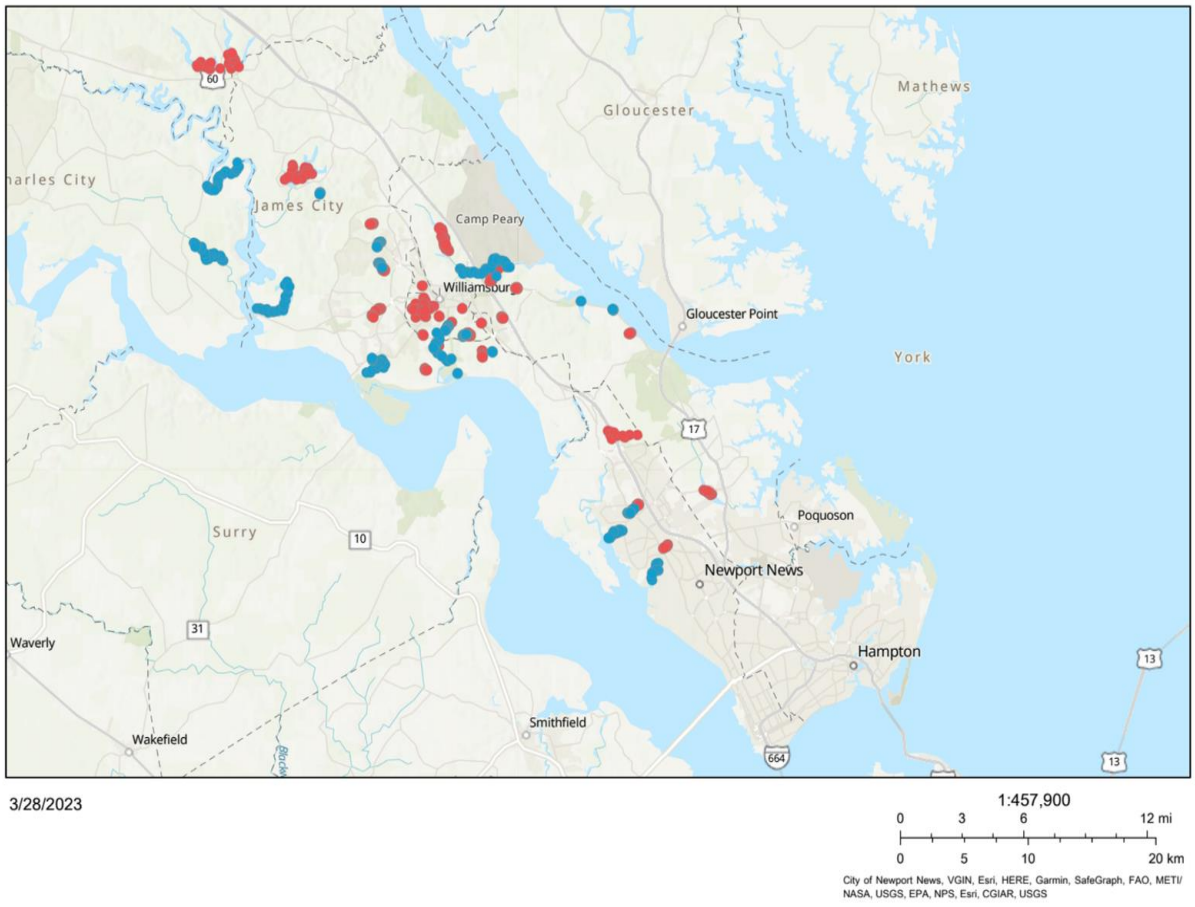

Figure 1: Map of the Virginia Peninsula showing sampling site locations. Each dot represents a sampling event. Samples from undammed waterways are indicated with blue dots; samples from dammed waterways indicated with red dots.

the Appomattox River, Chickahominy River, Warwick River, Pagan River, and the Nansemond River. Due to its potential for generating mechanical power for rotating machinery such as grist mills, hydroelectric power, and as a water route for trade, many dams have been built across the James River since the time of European settlement of the region (USGS 2010). While most of these dams have been removed or failed, several dams still exist along the upper course of the river. From the head of the river downstream to Richmond are found the following dams as identified by the current US Army Corps of Engineers National Inventory of Dams: The Cushaw Hydroelectric Project near Glasgow and Big Island, The Bedford Hydropower Project near Big Island, The Big Island Dam near Big Island, The Coleman Falls Dam in Coleman Falls, The Holcomb Rock Dam near Lynchburg, The Reusen's Dam near Lynchburg, The Scotts Mill Dam in Lynchburg, and The Bosher Dam in Richmond ("National Inventory of Dams | FEMA.gov" 2022) (USGS 2010).

The York River is a navigable estuary, approximately 55 km long, in eastern Virginia in the United States. It ranges in width from 1.6 km at its head to 4.0 km near its mouth on the west side of Chesapeake Bay (USGS 2010). Its watershed drains an area of the coastal plain of Virginia north and east of Richmond. The York River is formed at West Point, approximately 56 km east of Richmond, by the confluence of the Mattaponi River and the Pamunkey River. It drains into the Chesapeake Bay towards the southeast, entering the bay approximately 8 km east of Yorktown, VA.

In this study, I collected samples from nine sub-watersheds within the greater James and York watersheds of the Virginia Peninsula. Sixteen collection sites were considered undammed waterways because they had undammed connections to the Chesapeake Bay. Of the sixteen undammed sites eleven were tidal creeks and five were non-tidal freshwater creeks. Nineteen

sites were located within impoundments created by dams and varied in size from moderate reservoirs, such as Diascund Reservoir (4.6545 km²) to small ponds (< 1 km^2). Sampling sites were chosen to provide approximately equal representation of dammed and undammed sites within sub-watershed, as well as researcher accessibility and safety. In instances in which a waterway had multiple dams, we sampled just above the dam furthest downstream, and not above other dams. Therefore, any observed effects of dams were the result of a single dam, not the cumulative effect of multiple dams along the watercourse. Samples were collected from along the shore and from small vessels. Sampling was done from a canoe inside restricted access sites on York Town Naval Base. A small "Jon boat" equipped with electronic motor was used to sample larger reservoirs and most of the tidal creeks, and an outboard powerboat was used to sample larger tidal creeks. At small freshwater creeks and small ponds where boat access was not possible, sampling was done from the shoreline. Tidal sites were only sampled during low tide or receding tide to limit the effects of eDNA being transported into the site on a rising tide because it is not yet well known how high or incoming tides may influence the spatial signal of eDNA (Barnes et al. 2014, Matsuoka et al. 2019).

2.2. Collection of Water Samples, eDNA Collection

Collection of eDNA was done using a Smith-Root eDNA backpack sampler (Thomas et al. 2018). The backpack sampler used a peristaltic pump to filter the water through a membrane filter in a single-use cartridge in the field. The sampler also recorded the GPS location of each sample and allowed precise control and standardization of the volume of water filtered, filtering flow rate, and vacuum pressure. All the filter cartridges used were sterile, single-use, and replaced between each sample. The first 240 samples were taken using commercially available sterile cartridges (Smith-Root, SKU: 10995). Due to supply issues the remaining 282 cartridges

were reused filter housing cartridges with a new sterile collection filter. A sterilization protocol was developed using UV light and bleach for the filter housing cartridges and a sterile collections filter was loaded (protocol available by request). Negative and positive controls were run with these cartridges prior to use to ensure prior DNA material on the housing was destroyed and that residue of bleach was not destroying new eDNA being collected. Molecular grade water was filtered, DNA extracted, PCR amplified and then run on a gel to ensure sterility. All negative controls were clean. Then positive controls on collected samples were put through the same protocol for these cartridges and they all had successful DNA extracted and PCR amplification. Single-use gloves were used to avoid contamination and changed between sites. The backpack sampler and hosing were flushed with at least 5 L of de-ionized water at the end of each field day.

The target volume was 2 L per sample. The eDNA was collected on 5-micron polyethersulfone (PES) filters. Due to filters clogging at sites with high total suspended solids (TSS) some samples had lower than target volume (see additional materials for individual sample information). The minimum accepted volume was 1.5 L and the filter was changed as needed to collect this volume. Filters from samples that required multiple filters were pooled together in the same tube for each sampling event. Filters were removed from the collection cartridge using sterile forceps for each sample to prevent cross-contamination and stored in 2 ml of DNA sterile cetyltrimethyl ammonium bromide (CTAB) cell lysis solution in a 5 ml centrifuge tube. Filters were placed immediately in the buffer in the field after samples were collected to prevent degradation of the DNA. During the middle of the season supply issues caused a shortage of 5 ml centrifuge tubes, consequently 77 samples were stored in 1 ml of CTAB in 1.5 ml centrifuge tubes until the 5 ml tubes were restocked. Upon returning to the lab

the filters in CTAB were then stored in -80 °C freezer until DNA extraction (approximately 2 months later). Each sample was processed individually prior to metabarcode amplicon sequencing.

Between 10 and 20 field replicate samples were collected within each site. The samples within undammed sites were collected working downstream to upstream to avoid sampling disturbed sediments. In undammed small creeks, samples were spaced 50 -100 m apart and collected from areas where the water could be accessed safely. At each site a negative field control was taken to control for field contamination. Negative field controls consisted of a dry replacement 5-micron filter that was placed in a field collection tube containing CTAB and subsequently processed in an identical manner and in parallel with other field samples. Undammed sites required more field replicate samples than dammed areas to capture the target of 85-90% species saturation; approximately 10 for dammed sites and 20 for undammed. These sample size estimates were chosen after producing an extrapolation using the Chao estimator (Hsieh et al. 2016) from preliminary samples taken the prior summer, August 2021. Using the same methods described above, we sampled Queens Creek (37°18'04.5"N 76°39'25.7"W) and Queens Lake (37°17'45.5"N 76°39'30.5"W) where 8 samples were taken above the dam at Queens Lake and 10 were taken from an adjacent undammed branch of the creek.

Ten field replicate samples were collected within each dammed site. In two dammed sites twenty replicate samples were collected to determine if 85-90% saturation of species was collected within the standard 10 samples for dammed sites. These sites were two of the largest impoundments in the study, Waller Mill and Little Creek Reservoir. Each sample from lentic sites were collected near the shore because prior studies have shown that the eDNA concentration is highest in these areas because the majority of fish in lakes occupy the littoral

zone (Hänfling et al. 2016, Eichmiller et al. 2016). A few samples were also collected away from the shoreline in lakes in attempt to sample open-water species. Samples were spaced out to cover the entire perimeter of each dam when it was possible to access the entire area.

I collected point measurements of water quality at each site concurrent to eDNA sampling using at YSI proDSS multiparameter digital water quality meter with probes for temperature and conductivity, pH, turbidity, and dissolved oxygen (Xylem Inc., Washington DC, USA). Water quality measurements were taken at the same depth as eDNA samples at approximately 10 cm beneath the water surface. To calculate the catchment area and watershed size for each study site, sample coordinates from the backpack sampler were uploaded into GIS software along with the National Hydrography Dataset (NHD) layer. The NHD layer was used to identify the nearest stream network to each site and to delineate the watershed for that stream network, which allowed for the calculation of catchment area and watershed size.

2.3. DNA Extraction

DNA extractions from CTAB and PES filter membranes were performed under a UV-sterilized positive pressure sterile hood with bleached and UV-treated instruments to eliminate possible contamination and gloves were changed frequently. For each extraction batch, a field negative control and an extraction negative control were processed in parallel with eDNA samples to assess potential contamination. eDNA was extracted from the samples using a CTAB/Glass Milk procedure (Lindner and Banik 2009) as modified in Jusino et al. (2014) (Jusino et al. 2014). Filters in CTAB were thawed to room temperature prior to DNA extraction. Samples were then vigorously agitated by vortexing for 30 seconds to release DNA and cellular material from the filter into the CTAB. Then samples were placed in 65 °C water bath for 15 min, vortexed again for 30 s, placed in 65 °C water bath for 30 min, and then vortexed again for

30 s. Samples were then centrifuged at 20 °C at 10,000 rfc for one minute to spin down sediment. Then, in the PCR hood, 100 µl of supernatant was transferred to a new centrifuge tube and the remainder of the sample was stored back in a -80 °C freezer.

To prevent contamination, all equipment and bench spaces were cleaned before use with 10% bleach solution followed by UV light for 20 min. All pipetting was conducted using sterile barrier filter tips. To begin extraction samples were again spun in centrifuge at 20 °C at 10,000 rfc for one minute. Then, 150 µl of -20 °C molecular grade isopropanol alcohol was added to each sample, samples were gently inverted several times to mix, and then they were placed in the -80 °C freezer for seven minutes. Samples were then centrifuged at 0 °C for 20 min to separate DNA and form a pellet on the bottom of the tube. Then the supernatant was removed without disturbing the DNA pellet, 175 µl 70% molecular grade ethanol was added to each sample to wash pellet, and then centrifuged at 20 °C at 10,000 rfc for five minutes. The supernatant was removed again without disturbing the pellet and the tubes were then left open in the PCR hood to air dry until all traces of isopropanol alcohol had evaporated (approximately 15 min). Then, 45µl of molecular grade sterile water was added along with 135 µl of NaI solution, and 3µl of glass milk. Tubes were then flicked to dislodge pellet from the bottom of the tube then placed upside down in vortex attachment for 5 minutes at 1.5 setting. Following the vortex samples were centrifuge at 20 °C 10,000 rfc for 8 s. Supernatant was then removed and discarded without disturbing the pellet. Then 175µl of New Wash was added, vortexed for 5 minutes upside down and centrifuged again at 20 °C 10,000 rfc for 8 s. New Wash buffer (MP Biomedicals, Santa Ana, CA USA) was removed and discarded then pellet in the tubes was air dried for 30 min to ensure New Wash was completely evaporated.

The final step of the extraction was to collect the clean DNA from the pellet. To elute the DNA, 50 µl of molecular grade sterile water was added and tip mixed to disperse pellet, vortexed to ensure homogeneity, and then centrifuged at RT 10,000 rfc for 35 seconds. Supernatant containing clean DNA was then removed and transferred to a new 1.5 ml centrifuge tube. After extraction the DNA from each sample was quantified for concentration using a Qubit 4 fluorometer with a Qubit dsDNA HS Assay kit (Thermo Fisher Scientific) and concentrations were recorded (data for individual Qubit reads available by request). Of the 522 samples processed, 10 samples yielded concentrations that were below detection and were re-extracted. All field negative controls yielded concentrations that were below detection on the Qubit Fluorometer.

2.4. Mock Community

To verify that our methodology would successfully detect the phylogenetically diverse fish species in our sampling area, and to investigate possible amplification or sequencing bias of metabarcoding due to the difference in affinity between the primers and the binding site of the different species sequences of the primer regions, we built a mock community using genomic DNA from 28 bony fish species. We choose species for the mock community that were known to be common in the sampling area or of particular economic or ecological importance, with a focus on species did not yet have representation in the NCBI Genbank database for our chosen marker (a region of the mitochondrial 12s gene). The mock community also served as a positive control during sequencing and informed calibration of our bioinformatics pipeline (Palmer et al. 2018).

Table 1: List of species used to construct the mock community sample that was used as a positive control during eDNA PCR, sequencing, and data analysis. Species were chosen to represent a taxonomically diverse sample of common species in the Chesapeake Bay and surrounding area as well as species of conservation concern.

Family	Common Name	Scientific Name
Acipenseridae	Atlantic Sturgeon	*Acipenser oxyrinchus*
Achiridae	Hogchoker	*Trinectes maculatus*
Anguillidae	American Eel	*Anguilla rostrata*
Batrachoididae	Oyster Toadfish	*Opsanus tau*
Centrarchidae	Bluespotted Sunfish	*Enneacanthus gloriosus*
Centrarchidae	Pumpkinseed Sunfish	*Lepomis gibbosus*
Centrarchidae	Bluegill Sunfish	*Lepomis macrochirus*
Centrarchidae	Redear Sunfish	*Lepomis microlophus*
Centrarchidae	Largemouth Bass	*Micropterus salmoides*
Clupeidae	Atlantic Menhaden	*Brevoortia tyrannus*
Clupeidae	Atlantic Silverside	*Menidia menidia*
Clupeidae	Bay Anchovy	*Anchoa mitchilli*
Clupeidae	Gizzard Shad	*Dorosoma cepedianum*
Fundulidae	Striped Killifish	*Fundulus majalis*
Fundulidae	Mummichog	*Fundulus heteroclitus*
Gobiidae	Naked Goby	*Gobiosoma bosc*
Ictaluridae	Brown Bullhead	*Ameiurus nebulosus*
Ictaluridae	Blue Catfish	*Ictalurus furcatus*
Moronidae	Striped Bass	*Morone saxatilis*
Moronidae	White Perch	*Morone americana*
Mugilidae	Striped Mullet	*Mugil cephalus*
Sciaenidae	Atlantic Croaker	*Micropogonias undulatus*
Sciaenidae	Red Drum or Redfish	*Sciaenops ocellatus*
Sciaenidae	Silver Perch	*Bairdiella chrysoura*
Sciaenidae	Spot	*Leiostomus xanthurus*
Sciaenidae	Weakfish	*Cynoscion regalis*
Sparidae	Pinfish	*Lagodon rhomboides*
Syngnathidae	Chain Pipefish	*Syngnathus_louisianae*

Muscle or fin tissue was extracted from each specimen using forceps and a scalpel and were sterilized using ethanol and a flame between specimens. DNA was extracted using Qiagen

DNeasy Blood & Tissue Kit. The concentration of DNA extracted from each specimen was measured using a Qubit 4 fluorometer with a Qubit dsDNA HS Assay kit (Thermo Fisher Scientific). Samples were then diluted to the lowest specimen concentration of 141 ng/µl, so all samples were of equal concentration of genomic DNA prior to PCR amplification. Then to test the MiFish primer binding at varying concentrations, the equilibrated samples were pooled together, and a dilution series was made by dilution with molecular grade water at ratios of 1:1, 1:10,000, 1:100,000, and 1:1,000,000 combined DNA template to water. The 1:1 dilution served primarily as a test for translation of DNA concentration to read numbers. The 1:10,000 dilution represented an approximation of the concentration at which eDNA is likely to be present in the environment and the 1:100,000 and 1:1,000,000 dilutions were an estimation of eDNA in the environment that would be in very low concentration or in low quality due to environmental degradation (Schultz and Lance 2015, Mauvisseau et al. 2022). The highest concentration tested was 1:1 at 141 ng/µl of genomic DNA and the lowest concentration tested was diluted to 1:1,000,000, or 0.141 pg/µl total genomic DNA. PCR amplification, indexing and sequencing were as described below for field samples. Electrophoresis showed that all four dilutions of the mock community had amplification and the negative control was negative. PCR product from the mock community was then added to a plate and treated identically to the other samples for the remainder of the processing including indexing and PCR cleanup.

2.5. PCR amplification of 12S mtDNA

Metabarcoded amplicon sequencing was performed using two-stage PCR. The initial PCR amplifications were carried out using the MiFish primers (Miya et al. 2015) with Illumina NextEra v2 adapters (Illumina Inc., San Diego, CA, USA), and the modifications to the binding regions described in Skelton et al. (2022) (Skelton et al. 2022). These primers amplify a ~170 bp

fragment of the mitochondrial 12S rRNA gene. Initial PCR was conducted in 15 µl reactions containing 0.3 µl of each forward and reverse primer, 0.12 µl bovine serum albumin (BSA), 0.1 µl GoTaq DNA polymerase (Promega), 3.0 µl of GoTaq Green buffer (Promega), 0.3 µl of dNTPs, 7.88 µl of molecular grade water, and 3.0 µl of DNA template. Amplification proceeded under the following conditions: initial denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 s, 61.5 °C for 30 s, 72 °C for 1 min., and finishing at 72°C for 10 min with an indefinite hold at 12°C . At least one PCR negative control was included on each PCR run along with two or three field negative controls. PCR amplified products were visualized on 1% agarose gels to ensure amplification of samples and to assess DNA contamination of negative controls. No contamination was detected in the negative controls. After initial amplification, the samples were dual indexed by PCR (8 cycles) using Illumina Nextera v2 indices and adapters following the manufacturer's protocol (Illumina Inc., San Diego, CA, USA). Each indexed sample was then purified using ZYMO DNA Clean & Concentrator MagBead Kit following the manufacturer's protocol. All samples were pooled by equal volumes in preparation for 2 by 150 paired-end sequencing on two Illumina MiSeq runs at the University of California Riverside Genomics Core Facility, Riverside, CA, USA. Prior to sequencing, pooled libraries were bead cleaned, quantified by qPCR, and quality checked by Agilent 2100 Bioanalyzer. All negative controls (field, extraction, and PCR negatives) were sequenced with the field samples.

2.6. Bioinformatics

Raw sequences were processed to create amplicon sequence variants (ASVs) using the DADA2 pipeline (Callahan et al. 2016) implemented in R version 4.1.2. Forward and reverse reads were separately filtered and primer sequences removed using the filterAndTrim() function with a maximum expected error rate of 2 errors per sequence. Filtered reads were pooled during sequence inference with a minimum hamming distance of 3 required for new sequence variants. After merging the forward and

reverse reads, chimeras were removed using the removeBimeraDenovo() function with the "consensus" method and all sequences outside the expected range of 163 to 185 bases in length were removed. A total of 453 ASVs remained after filtering. Each ASV was then manually searched against the NCBI nucleotide database using the BLASTn algorithm using default parameters. BLASTn results were used to identify ASVs to the lowest possible taxonomic rank following the criteria that a match had at least 80% query coverage, 97% sequence similarity, and represented a fish species known to occur within the Chesapeake Bay region. All ASVs that were identified to non-fish species, and those that did not have matches meeting the above criteria, were excluded from further analysis. To minimize the likelihood of false detections in our study, we used a very conservative subtraction filter to control for tag-switching and or background contamination: For each ASV, the maximum number of reads observed for that ASV in all field and lab negative controls was subtracted from each sample with resulting negative numbers or numbers smaller than 10 converted to zeros. We then aggregated the number of reads for each ASV according to species; i.e., summing the number of reads for all ASVs within a sample that were identified to the same fish species. Read numbers for each fish species that were greater than zero were converted to one to create a species occurrence (presence/absence) matrix. Lastly, within each site, the number of detections for each species was divided by the total number of field replicate samples for that site to give the detection frequency of each species at each sampling site.

3. STATISTICAL ANALYSIS

3.1. Aim 1 – Do dams reduce local (alpha) fish diversity?

Our sampling effort varied among sampling sites according to the estimates of sample size needed to achieve 80-90% species detection in a preliminary study. To account for variation in sampling effort in out comparison of alpha diversity between dammed and undammed sites, we first verified that our sampling effort was sufficient using species accumulation created in R using the specaccum() function in the Vegan Package (Oksanen et al. 2019). Not all sites showed saturating species accumulation curves. Therefore we then used the Chao estimator to estimate

the species richness of each site if it were sampled comprehensively based on the observed occurrence data using the iNEXT package for R (Chao 1984, Hsieh et al. 2016). Our analysis of alpha diversity was done using the observed species richness from each site, and again using the estimated species richness of each site from the Chao estimator.

A generalized linear model with normal Gaussian error distribution for both observed and estimated species was chosen because the residuals of the response variables (observed and estimated species) had an approximately normal distributions allowing the use of the simpler gaussian model. Collinearity among available predictor variables were checked and collinear variables in water quality data were removed, because they were different metrics calculated from the same point measurement such as salinity and conductivity. Conductivity was removed from the model in favor of salinity because the measurement of salinity was the target measurement and it was derived from conductivity. Predictor variables in this analysis were dam (categorical, "yes" or "no"), salinity (psu), dissolved oxygen (DO in mg/L), turbidity (FNU), pH, temperature residuals, catchment area (km^2), and watershed. Temperature residuals were the residuals of a linear model of water temperature on the day of sampling regressed against Julian day to remove the effect of sampling date on temperature.

We mixed models to account for random effects of watershed on species richness. Three potential variance structures for both Gaussian models (observed & estimated) were compared – the generalized linear models with no random effects, a GLMM with random intercept for watershed, and a GLMM with a random intercept for watershed and a random slope for dam within each watershed. By treating watershed as a random effect, our model could account for variation among watersheds that would cause non-independence among observations, without adding excessive coefficients to the model. Following the suggestions of Zuur et al (Zuur et al.

2009), we first selected variance structure using the most inclusive model (all fixed effects included) before proceeding to stepwise backwards model selection.

The variance structures were compared by AIC and the model with the lowest AIC score was chosen. Based on AIC, the variance structure of the fixed effects only models were chosen for both the observed and estimated species. Therefore, accounting for random effects of watershed had no detectable improvement on our model and was therefore removed in favor of the more parsimonious fixed-effects only models.

Next, stepwise removal of predictor variables was done to reduce the model to only significant terms. Following the suggestions of Zuur et al. 2009 (Zuur et al. 2009), we started with the most inclusive model and then used the drop1() function in base R to implement a chi-square test to compare likelihood-ratios of the competing models (the inclusive model compared to a model with the corresponding variables removed) (R Core Team 2021). The only predictor variable of statistical significance that remained after model selection was dam for both the observed and estimated species models.

Two model validation procedures were used to check model assumptions and evaluate model adequacy. First, over dispersion of residuals was checked using the testdispersion() for correct error distribution. The residuals were evenly dispersed in both models. Then plotted the residuals against the significant predictor variable dam and the other predictors variables that were dropped from the model to assess potential non-independence or missing co-variates that would lead to strong patterns in the residuals. No patterns were detected in the residuals for both models.

3.2. Aim 2 – Do dams change the species composition and regional variability of fish communities (beta diversity)?

We used ordination to visualize the fish community composition of our sites and to compare dammed and undammed sites. We made two ordinations; one using the Bray-Curtis distance matrix based on a matrix of sampling sites by species detection frequencies and a second using a Raup-Crick distance matrix based on a matrix of sampling sites by detection/non-detection of fish species. Ordination based on Bray-Curtis distance of detection frequencies emphasizes differences in the relative detection frequencies of common species among sites, whereas ordination based on detection/non-detection data emphasizes differences in the occurrences of rarely detected taxa. Therefore, the two ordinations provide two different perspectives of possible community-level differences between sites. Distance matrices and ordinations were made using the vegan package in R (Oksanen J et. al. 2022). Detection frequencies for each species at each site were calculated as the proportion of replicates per site that a given species was detected. Detection frequencies were used as an approximation of species abundances because they are less likely to be biased by PCR amplification than read numbers. Each ordination was fit using the metaMDS() function with default settings. The standard deviation around the centroids for dammed and undammed groups were plotted using ordiellipse() function in the vegan package. For both distance matrices, we used a PERMANOVA (Anderson 2001) to assess if there was a statistically significant difference in species composition between the dam and undammed sites using the function adonis2() in the vegan package.

What are the effects of dams on variability among local communities? We had two competing predictions for this question. We predicted that that there would be higher variation in species composition (higher beta diversity) among dammed sites than undammed sites because

dams create a barrier limiting dispersal causing disconnection from the estuary network leading to different species in dammed communities. Alternatively, we predicted that dammed sites would have less variable community composition than undammed sites because they homogenize aquatic habitats and select for a subset of local taxa, i.e. that dammed sites would have lower beta diversity. We used a permutations-based test of the homogeneity of multivariate dispersion to test for a statistical difference in beta diversity in dammed compared to undammed sites (Anderson et al. 2006a). This test was conducted using the betadisper() function of the vegan package (Anderson et al. 2006b). As described above for ordination and PERMANOVA, we conducted this test on a Bray-Curtis distance matrix to emphasize effects on the relative abundances of common species, as well as the Raup-Crick occurrence-based matrix to emphasize differences in species composition, especially rare species.

3.3. Aim 3 – Do dams facilitate invasive species?

To compare the number of native versus non-native fish species in dammed versus undammed habitats, we conducted a chi-square test using the statistical software in base R. First, we created a 2×2 contingency table with rows representing the two types of habitats (dammed and undammed) and columns representing the two categories of fish species (native and non-native). We used the "chisq.test()" function in R to perform the chi-square test, the chi-square test was used to determine if there was a significant association between habitat type and the number of native versus non-native fish species in these habitats.

We considered fish species as native or non-native based on the information provided by the United States Geological Survey's Nonindigenous Aquatic Species (NAS) database (USGS 2023). The NAS database is an up-to-date comprehensive source of information on the distribution and status of introduced and native aquatic species in the United States. We used the

database to verify the native range of each species and to determine if their occurrence in our study area was outside of their native range. This information was then used to categorize each fish species as either native or non-native in our study.

To assess the effects of dams on individual fish species, we used the manyGLM function of the mvabund package (Wang et al. 2012) to fit individual binomial GLMs (family = binomial("cloglog")) for the effect of dams and environmental covariates on the probability of detecting each species at each site, as well as a global model for the overall multivariate effect of dams on species detections. To reduce the effect of false detection, a species had to be detected in at least 2 samples from a site to be considered as detected. Non-significant environmental covariates were sequentially removed using the drop1 function and AIC criterion.

4. RESULTS

4.1. Mock Community

We recovered 26 of the 28 species (93%) in the mock community. All species recovered were part of the mock community, indicating that there was no contamination and the primers were effective in amplifying the DNA for the majority of the phylogenetic diversity present in the mock community (Table 2). Two species from the mock community were not recovered at any concentration, Oyster Toadfish (*Opsanus tau)* and Silver Perch (*Bairdiella chrysoura*). While Silver Perch was not detected in the mock community it was detected in the field samples. PCR bias could have caused the lack of reads for Silver Perch and Oyster Toadfish in the mock community. PCR bias (the preferential amplification of one DNA sequence over another) can occur due to various factors such as primers having a higher affinity for some sequences than others, template quantity, PCR conditions, tag switching during indexing/library preparation, and

DNA quality (Miya et al. 2015, O'Donnell et al. 2016, Jusino et al. 2019, Zizka et al. 2019, Skelton et al. 2019).

At a 1:1 concentration, all species except Silver Perch and Oyster Toadfish were successfully identified. At a 1:10,000 concentration, the same species were detected, but the relative read numbers varied more among species. However, with decreasing mock community concentrations, the number of species detected also decreased. The read numbers for each species were generally lower in lower concentrations than at higher concentrations, though the relative proportions of reads among species changed considerably. For example, the Atlantic Sturgeon (*Acipenser oxyrinchus)* went from a few percent of the total reads at the high concentration to 100% of reads at the lowest concentration. These results indicate that using either raw read numbers or relative read numbers as measures of abundance or relative abundance may be unreliable, especially when the concentration of the sample is low, supporting previous findings (Jusino et al. 2019, Skelton et al. 2022). Furthermore, some species were more sensitive to lower concentrations than others, which suggests that eDNA methods may not detect all species present in a sample. These findings may have implications for the use of similar eDNA methods for detecting species in other types of studies, where accurate identification of all species present is critical.

Table 2: The results of the mock community dilution series that measured the sequence reads at various concentrations. Species in the mock community were chosen to represent phylogentic coverage of the bony fish clade, have economic value, or ecological value it the region. The number of reads recovered from each species in the mock community dilution series is shown below the dilution concentrations of 1:1, 1:10,000, 1:100,000, 1:1,000,000. The fish mock community was PCR amplified from the concentrations prior to sequencing, read numbers recovered are not equal. 26 of 28 species were recovered.

Species	1:1	1:10,000	1:100,000	1:1,000,000
Acipenser_oxyrinchus	1309	1483	664	6092
Ameiurus_nebulosus	757	613	0	0
Anchoa_mitchilli	2183	2613	0	0
Anguilla_rostrata	454	258	0	0
Brevoortia tyrannus	2297	987	0	0
Cynoscion_regalis	3401	3373	0	0
Dorosoma_cepedianum	1008	418	0	0
Enneacanthus_gloriosus	1870	1552	0	0
Fundulus_heteroclitus	2371	1811	0	0
Fundulus_majalis	3972	3499	2238	0
Gobiosoma_bosc	3428	2668	0	0
Ictalurus_furcatus	3391	2705	0	0
Lagodon_rhomboides	1674	963	0	0
Leiostomus_xanthurus	4350	3231	0	0
Lepomis_gibbosus	6065	5851	0	0
Lepomis_macrochirus	3451	1946	0	0
Lepomis_microlophus	1684	1464	0	0
Menidia_menidia	3221	1166	3076	0
Micropogonias_undulatus	4906	7646	3883	0
Micropterus_salmoides	55	84	0	0
Morone_americana	472	303	0	0
Morone_saxatilis	1402	1563	2609	0
Mugil_cephalus	2055	1284	0	0
Sciaenops_ocellatus	1910	1081	526	0
Syngnathus_louisianae	818	656	0	0
Trinectes_maculatus	449	544	0	0

4.2. Aim 1 – Do dams reduce local (alpha) fish diversity?

Dams showed the strongest effect of any of the variables that we considered for explaining the number of observed and estimated fish species. Dam sites had significantly fewer

species detected per site than undammed sites (Figure 2). Both models clearly support the prediction from our hypothesis that there is reduced alpha (local) diversity of fish species within dammed watercourses compared to sites in the estuary that are not dammed (Figure 2).

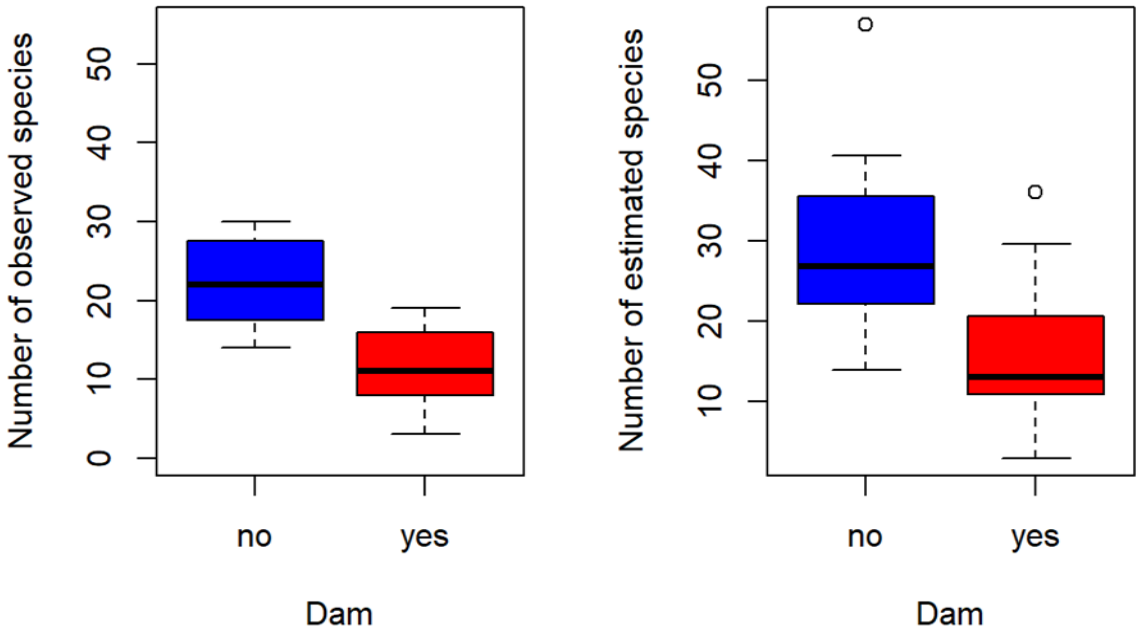

Figure 2: Significantly more species were detected at undammed sites than dammed sites. The blue boxes represent undammed sites whereas the red boxes represent dammed sites. The box and whisker plot on the left shows the number of species that were detected in all samples for dammed and undammed sites. The plot on the right shows the Chao estimator to extrapolate the number of projected species per site type of species saturation was achieved in sampling. Linear models showed significant negative effects of dams on both observed species richness ($p < 0.001$) and estimated species richness ($p < 0.001$).

During model selection for both the observed and estimated species richness, all variables were removed from the model except for the variable dam (present or not) (Table 3). Based on the estimated coefficients in the observed richness model (Model 1), we detected 10 fewer species per site in dammed sites compared to undammed sites (Table 3). Based on the estimated total richness model (Model 2), we estimated an average of 13 fewer species in dammed sites compared to undammed sites (Table 3).

Table 3: Statistical results of linear models. Model 1 shows the results of the glm for the effect of dams on observed fish richness after model selection. Model 2 shows the results (after model selection) of the glm using the Chao estimater for the estimated fish richness. The significant p-values indicated by *** indicate a statistical difference between the average number of species detected between dammed and undammed sites. In each model the estimated coeffcient indicates on average how many fewer species were detected per dam site compaired to undammed sites.

Model 1: Observed richness

Variable	Estimated Coefficient	Std. Error	t value	P-value
Dam present	-10.105	1.823	-5.542	P < 0.001***

Model 2: Estimated richness

Variable	Estimated Coefficient	Std. Error	t value	P-value
Dam present	-13.610	3.254	-4.182	P < 0.001***

4.3. Aim 2 – Do dams change the species composition and regional variability of fish communities (beta diversity)?

Community composition was significantly different between dammed and undammed sites regardless of the distance metric that was used (Figure 3). Stress for the Bray-Curtis NMDS ordination was 0.15. Stress for the Raup-Crick NMDS was 0.18. Both ordinations show clear separation of dammed and undammed sites along NMDS axis 1. The difference in species composition between dammed and undammed sites observed in both ordinations were statistically significant (Table 4). This result supports the prediction that dams cause a change in the species composition of local fish communities.

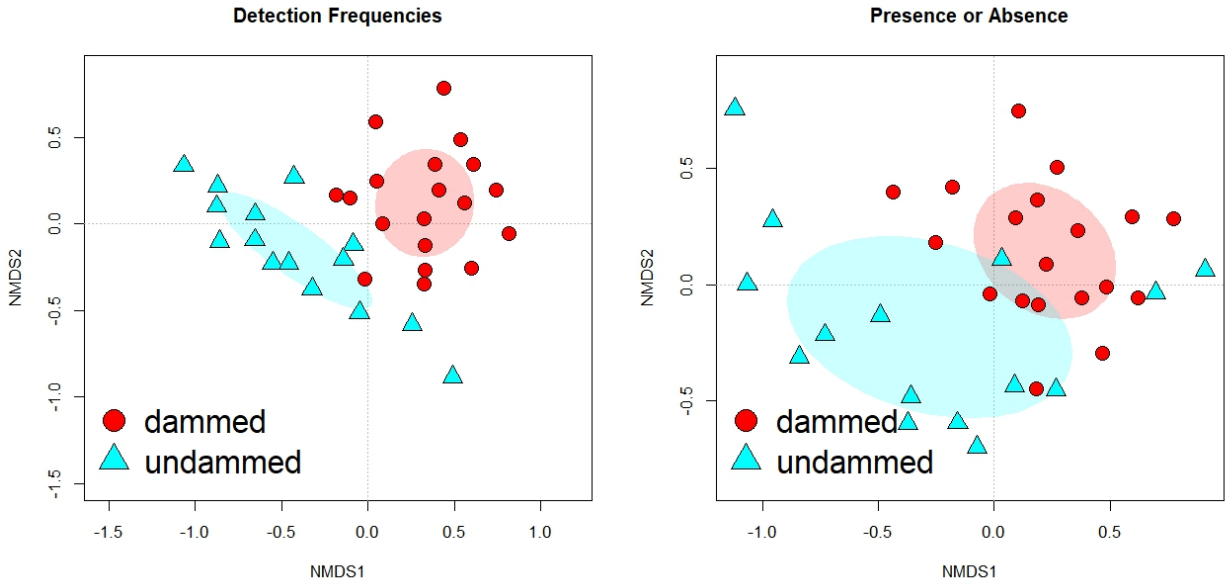

Figure 3: Dammed and undammed sites have different species composition. Non-metric multidimensional scaling ordination with sites coded by site type (dammed= red circles, undammed = blue triangles). (left) ordination of Bray-Curtis distance calculated from detection frequencies of each species and (right) ordination of Raup-Crick dissimilarity based on detection only of each species.

Congruent with our predictions, we found that undammed sites had higher beta diversity whereas dammed sites were more homogenous in species composition. However, this was only true when the analysis emphasized the differences in species occurrences which is sensitive to differences in rare species (i.e. in the analysis of presence/absence data) using the Raup-Crick metric – we observed significantly higher dispersion in the community composition (i.e. beta diversity) of undammed sites (Table 5).

In contrast, when the analysis emphasized the relative abundances of common species (i.e. Bray-Curtis of frequency data), there was no significant differences in beta diversity detected (Table 5). This result shows that dammed sites have relatively homogenous fish species composition while undammed sites are more variable in species composition. Because only the presence/absence based analysis showed significantly different beta diversity and because analyses based on presence/absence data emphasizes the effects of rare species, our results

suggest that the higher beta diversity observed in undammed sites is largely due to variation in the detections of rare species.

Table 4: Permutations based multivariate ANOVA assessing the effect of dams on observed fish community composition using the Bray-Curtis distance based on detection frequencies (Model 1) and Raup-Crick distance (Model 2) based on detection only (i.e. presence/absence). P-value < 0.001 for both models indicate community composistion between dammed and undammed sites is different.

Model 1: Bray -Curtis distance

Variable	F-Stat	R^2	P-value
Dam (undammed or dammed)	9.586	0.23051	P < 0.001 ***

Model 2: Raup-Crick distance

Variable	F-Stat	R^2	P-value
Dam (undammed or dammed)	6.9255	0.17346	P < 0.001 ***

Table 5: Analysis of beta dispersion assessing the effect of dams on variability in observed fish community composition using the Bray-Curtis distance based on detection frequencies (Model 1) and Raup-Crick distance (Model 2) based on detection only (i.e. presence/absence). P-value with ** indicates signifcantly higher variabililty within undammed communities compared to dammed communities.

Model 1: Bray-Curtis Distance

Variable	F-Stat	P-Value
Dam (undammed or dammed)	0.5364	0.4693

Model 2: Raup-Crick Distance

Variable	F-Stat	P-Value
Dam (undammed or dammed)	7.966	0.008 **

4.4. Aim 3 – Do dams facilitate invasive species?

We predicted that in dammed sites the frequency of nonnative species detected would be higher than in the undammed sites because the dams provide a novel lentic habitat that did not previously occur in coastal Virginia. Our comparison of the number of native versus non-native fish in dammed versus undammed habitats revealed that in dammed habitats, there were 32 native and 9 non-native fish, while in undammed habitats, there were 49 native and 9 non-native

fish. The chi-squared test yielded no statistically significant difference in the frequency of non-native species between the dammed and undammed sites (X^2= 0.305, d.f. = 1, p > 0.05). Therefore, we failed to reject the null hypothesis that there was no association between habitat type and the frequency of native versus non-native fish species.

Results of the multiple generalized linear models for multivariate data showed that dams had a significant impact on the detection probability of twenty-one species at an alpha of 0.10 (Figure 4). Only four of these species (Grass Carp [*Ctenopharyngodon idella*], Largemouth Bass [*Micropterus salmoides*], Yellow Perch [*Perca flava*], and Black Crappie [*Pomoxis nigromaculata*]) were more likely to be detected in dammed sites; the other 17 were less likely to be detected in dammed sites. In addition to dams, water salinity and sub-watershed also had significant effects on species detections.

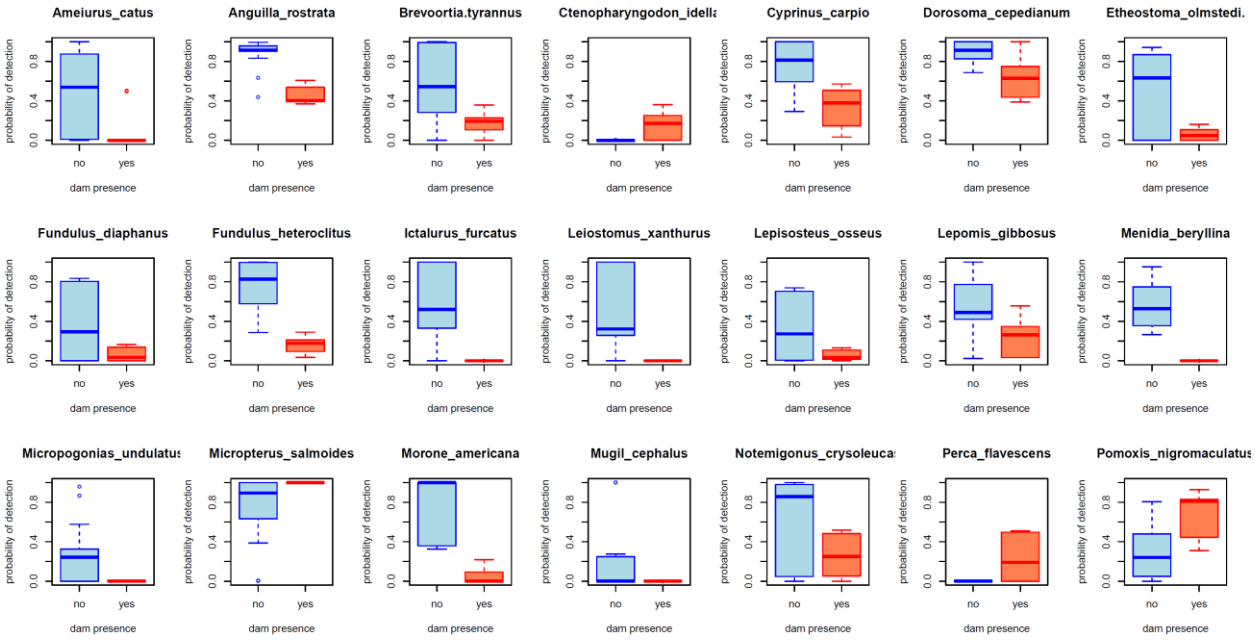

Figure 4: Effects of dams on the detection probability of 21 fish species. Box and whiskers represent the model fits for the multiple binomial GLM model with blue representing undammed sites and red representing dammed sites. Only species for which the presence of a dam had a significant effect on detection probability are shown (alpha = 0.1).

5. DISCUSSION

With biodiversity in decline worldwide, identifying the driving factors behind the decline is critical to provide better management practices and preserve biodiversity and ecosystem function. We know from prior scientific research that large dams often impede migration patterns (Mcallister et al. 2001), reduce local biodiversity (Jansson 2006), alter local food webs (Power et al. 1996), and change the hydrology of streams with confounding effects such as increased sedimentation accumulation (Doyle et.al. 2003). But there have been few studies on how small dams impact local biodiversity, and even fewer on the effect of ubiquitous small dams on landscape-scale patterns of beta and gamma diversity. Our work reveals comprehensive effects of small dams on fish diversity at scales from the local site to landscape-levels.

Our results suggest that dams reduce local diversity through multiple mechanisms. Many studies have shown dams to have a negative effect on alpha diversity (Turgeon et al. 2018). A principal mechanism for these declines is a loss or reduction of migratory species (Liermann et al. 2012). Congruently, our results show that small dams do reduce migratory species as the probability of detection of several migratory species was significantly reduced by the presence of a dam including White Perch (*Morone americanus*), Menhaden (*Brevoortia tyrannus*), American Eel (A*nguilla rostrate*), Atlantic Croaker (*Micropogonias undulates*), and Striped Mullet (*Mugil cephalus*). This result confirms existing evidence that dams create isolation from the ecological network and negatively impact migratory populations.

However, we also found evidence that dams decreased species richness due to habitat alteration. Damming coastal waterways can reduce salinity by blocking tidal flow and we observed reduced detection probabilities for several non-migratory species that depend on estuarine habitats including Mummichog (*Fundulus heteroclitus*.), Naked Goby (*Gobiosoma bosc*), Hogchoker (*Trinectes maculatus*), and Atlantic Silversides (*Menidia menidia*). Our study

also shows that dams have impacts on non-migratory freshwater species, suggesting that alteration of the physical environment also has an impact. Detection was reduced significantly in non-migratory freshwater species such as Tessellated Darters (*Etheostoma olmstedi*), White Catfish (*Ameiurus catus*), Longnose Gar (*Lepisosteus osseus*), and Banded Killifish (*Fundulus diaphanous*). These results suggest that dams negatively impact coastal fish diversity through habitat alteration, in addition to blocking migratory pathways.

Intriguingly, none of the water quality variables remained during model selection in local species richness models. However, it is important to note that this study only examined single point measures for these variables, and long-term averages may provide more predictive information. Therefore, caution should be taken in interpreting the lack evidence here as a definitive conclusion regarding the role of water quality in this system as other studies have suggested that water quality variables such as turbidity, DO, and temperature can also influence biodiversity along with dams (Berger et al. 2020, Li et al. 2022). However, these studies varied in sampling methods and habitat conditions with larger water quality datasets but suggest that dams are affecting habitat variables. While results in this study suggest that disruption of dispersal is the main force driving differences in local diversity between sites, however the loss of non-migratory species as mentioned above, and the loss of rare taxa suggest habitat alteration is also influencing changes to species diversity in this system.

The results of our study demonstrate that dams can significantly reduce beta diversity in fish communities, particularly among rare taxa. This finding is significant because biodiversity at regional and landscape levels is still understudied (Socolar et al. 2016), and the individual local effects of dams may not necessarily represent the total impact of numerous dams on the fish diversity of the region as suggested in prior studies (Fencl et al. 2015, Morden et al. 2022). The

homogenization of local communities across landscapes and the loss of rare species can reduce the resilience of regional ecosystems, leading to long-term changes in ecosystem structure and function (Magurran et al., 2015). Our study's findings provide further evidence that dams are contributing to the loss of species diversity not only at a local community level but also across a significant portion of a large estuary system that has been previously understudied.

While dams have the potential to increase gamma diversity, especially through the addition of non-native species (Johnson et al. 2008), it is crucial to consider the wider ecological impacts on estuarine biodiversity when constructing and managing dams (Doyle et.al. 2003). Dams create reservoirs that provide additional habitat for aquatic species, though the resulting habitat differs significantly from natural habitats. Dams modify freshwater flow, leading to changes in water quality, sediment transport, and the movement of aquatic organisms(Hoque et al. 2022, Abbott et al. 2022). These changes can negatively impact estuarine ecosystems and decrease overall biodiversity.

In this study, our results show that dams did not increase gamma biodiversity by providing novel habitat for new species, but rather led to a loss of species within the dammed areas. Of the twenty-one species dams had a significance of detection on only four of these species (Grass Carp [*Ctenopharyngodon idella*], Largemouth Bass [*Micropterus salmoides*], Yellow Perch [*Perca flava*], and Black Crappie [*Pomoxis nigromaculata*]) were more likely to be detected in dammed sites than undammed sites. In contrast, 17 species were less likely to be detected in dammed sites. Also, out of a total of 61 species detected in the study, only 41 were detected within dammed areas while 58 species were detected in undammed areas, thus only 3 species were detected at only dammed sites. Two of those 3 were non-native species; Grass Carp and Flathead Catfish (*Pylodictis olivaris*). In this estuary system, diversity at all scales, local to

regional, was lower in waterways that were dammed. These findings indicate that dams in coastal estuaries have a negative impact on regional fish diversity.

While other studies have shown that the presence of dams can facilitate invasions of non-native species (Johnson et al. 2008, Turgeon et al. 2018) we did not find similar evidence in this study. However, our study found that different non-native species benefited from different mechanisms in dammed sites. Grass Carp appeared to benefit from stocking practices, while Common Carp and Blue Catfish benefited from free dispersal routes to undammed sites. Furthermore, we did not find evidence of a general increase in non-native species due to damming, but rather a decrease in non-migratory species.

It is possible that the difference in results regarding non-native species could be due to differences in the habitat characteristics of the dammed sites and the species present, as well as differences in the methods used to sample and analyze the fish communities. For example, some studies may have focused on larger, older dams with a longer history of colonization by non-native species, whereas our study included a range of dam sizes and ages. Additionally, differences in the types and quantities of data collected and analyzed may have contributed to different findings. Future research could focus on exploring the specific factors that influence the establishment and spread of non-native species in dammed sites, including the role of human activities such as fish stocking, as well as the potential interactions between different non-native species.

Although there is growing interest in removing dams to restore stream quality, it remains a highly experimental practice (Shahady and Cleary 2021). To ensure that the removal of dams does not negatively impact biodiversity and ecosystem health at the regional and landscape level, it is important to conduct research and develop effective management strategies while

considering dam removal. Studies like this are valuable in providing insights into how multiple small dams in stream networks affect regional diversity and resiliency, which is essential for assessing the net impacts of dam removals. Understanding the reduction of beta diversity at a regional level can help in planning and managing dam removals to mitigate any negative impacts on biodiversity and maintain ecosystem health.

To further explore the impact of dams on biodiversity in estuarine ecosystems, it could be valuable to perform an analysis that assess the relationship between the number of dams on the landscape and the reduction in beta and gamma diversity. Such an analysis would provide insight into whether there is a threshold beyond which the presence of dams has a significant impact on biodiversity. One potential way to assess the relationship between the number of dams and biodiversity is to use the Chao estimator to estimate gamma diversity based on random subsets of the observed data while controlling for the number of dam sites that are re-sampled. By comparing the Chao estimate of gamma diversity to the number of dams sampled, it may be possible to determine if there is a correlation between the number dams and decreased biodiversity. This analysis could help inform future decision-making about the construction and management of dams in estuarine ecosystems and could potentially lead to the identification of thresholds beyond which the negative impact of dams on biodiversity becomes significant.

6. CONCLUSION

In conclusion, this study found that small dams within the Virginia Peninsula estuary in the lower Chesapeake Bay have reduced fish diversity at local and regional scales. The results indicate that dams have a significant negative impact on alpha (local) diversity of fish species, with fewer species per dammed site compared to undammed sites. This study also shows that dams change the species composition and regional variability of fish communities (beta

diversity) by making dammed sites more homogenous in species composition. The overall gamma diversity of the region was lower in dammed sites compared to undammed sites. Additionally, the study did not find a higher proportion of non-native species in dammed sites as predicted. Overall, this research suggests that small dams in the Virginia Peninsula estuary system have negative impacts on local and regional fish diversity, affecting both local and regional diversity, as well as regional variability of fish communities.

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