



THESIS APPROVAL SHEET

Title of Thesis: Investigating the Effects of the Reel Rewards Program on the
Ecosystems & Communities of the Middle Branch

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Master of Science 2025

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Molatore Carmen, I, Mendelson, T, Lohan, K, Malabanan., V. “Investigating the Effects of the Reel Rewards Program on the Ecosystems and Communities of the Middle Branch.” American Fisheries Society Conference. Poster Presentation.

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ABSTRACT

Title of Document: INVESTIGATING THE EFFECTS OF THE REEL REWARDS PROGRAM ON THE ECOSYSTEMS & COMMUNITIES OF THE MIDDLE BRANCH

Isabella Carmen Molatore, M.S, 2025

Directed By: Professor and Associate Chair of Biological Science, Dr. Tamra Mendelson, Department of Biological Science

My research examines the Reel Rewards Program, an invasive fish removal initiative in Baltimore's Middle Branch. Designed to reduce invasive Blue Catfish (*Ictalurus furcatus*), Flathead Catfish (*Pylodictis Olivares*), and Northern Snakehead (*Channa argus*) and reward recreational anglers, the program also gauges community impact. South Baltimore faces multiple types of environmental injustice and degradation, and this program is part of the Middle Branch Resiliency Initiative, a broader equitable urban wetland restoration project. Methods included environmental DNA (eDNA) sampling and angler interviews to detect invasive species, assess fish community composition, and document community perceptions of environmental change. eDNA samples were collected in spring and summer using metabarcoding of the Cytochrome c oxidase subunit I (COI) gene to estimate seasonal fish diversity and relative abundance. Interviews with paid summer anglers helped identify recreational fish species and community perception of environmental change in the Middle Branch. While no invasive species were detected using eDNA, anglers removed 256 Northern Snakeheads and 37 Blue Catfish, with almost \$9,000 paid to anglers for their catch. The research establishes a baseline for future assessments of the program's effectiveness. eDNA metabarcoding proved useful for measuring species richness and relative abundance, with

moderate fish species detection. Anglers reported that improved water quality overall, though sewage, litter, and industrial runoff remain concerns of the community.

INVESTIGATING THE EFFECTS OF THE REEL
REWARDS PROGRAM ON THE ECOSYSTEMS &
COMMUNITIES OF THE MIDDLE BRANCH

By

Isabella Carmen Molatore

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore County, in partial fulfillment of
the requirements for the degree of
Master of Sciences
2025

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Dedication

To my mother who taught me to protect nature not to fear it. And to all the outsiders, others, and weridos our unique perspective is invaluable to society and brings delight to the dull.

Acknowledgements

I want to thank my advisor and true mentor Dr. Tamra Mendelson; you gave me the freedom to be genuine and the support and wisdom to grow. My Committee members, ICARE cohort, and EJJI team members, helped me in so many countless ways. Dr. Katrian Lohan graciously took me into her lab and made my dream of working with environmental DNA come true. I am a more versatile scientist and not intimidated by molecular science anymore. I also want to thank Dr. Eric Schott for the wealth of Baltimore estuary history and local ecology. You helped me to recalibrate to the Atlantic ecosystems from my Pacific background. My ICARE peers created a safe space and a sort of chosen family. Moving 2,000 miles from home was/is hard and you all made it more survivable. A special thanks to my coresearcher, Veronica Malabanan Lucchese you showed me how to think like a social scientist and how to analyze the data. Also, thanks to my community organizer, Donzell Brown, for making connections and space for people who care.

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Chapter One: The Effect of Coastal Restoration on Communities Through the Lens of Recreational Fishing

Introduction

Human interactions with the capture and harvest of aquatic resources have well-documented cultural, economic, and spiritual value (Booi et al., 2022; Arlinghaus 2005; Romanazzi et al. 2023). In some regions, recreational harvest accounts for more total catch than commercial harvest, showing the heavy impact of recreational fishing on ecosystems (Booi et al., 2022). A person's first or only interaction with wildlife and the environment may be from fishing, providing a unique wildlife education and conservation opportunity. Baltimore's riverine estuary is in a time of significant change, with the collapse of the iconic Francis Scott Key Bridge and ambitious shoreline construction and restoration projects. Invasive fish are another source of change anticipated to impact ecosystem stability (Densmore 2020) and the success of wetland restoration (Crooks 2005). I, therefore, conducted a socioecological analysis to 1) assess the fishing community's interactions with invasive Northern Snakehead (*Channa argus*), Blue Catfish (*Ictalurus furcatus*), and Flathead Catfish (*Pylodictis olivaris*) and 2) the effects of an invasive fish bounty program (Reel Rewards program) on the community.

The Reel Rewards Program is an invasive fish removal and monitoring program that compensates local recreational anglers for the ecological services they

provide by removing invasive fish. The program is funded by the South Baltimore Gateway Partnership (SBGP) and is a key component of an urban wetland restoration.

The Reel Rewards program aims to decrease the population of Northern Snakehead (*C. argus*), Blue Catfish (*I. furcatus*), and Flathead Catfish (*P. olivaris*) by increasing the rate of removal by recreational fishing efforts. Blue Catfish (*I. furcatus*) and Northern Snakehead (*C. argus*) are consistently mentioned as moderate to high management concerns as aquatic invasive species in multiple states (Densmore 2020). Fish of these species can grow to a large size quickly, outcompeting native fish and negatively impacting Maryland's economically important seafood industry, such as the Blue Crab fishery (Desmore 2020; Moore 2023), with Blue Catfish and Flathead Catfish diet analysis revealing Blue Crab as a prey item (Aguilar et al., 2016; McCabe, 2019). Reel Rewards aids in detecting invasive fish species, has created a method for cost-effective species monitoring, and has developed a mode of educational outreach and community engagement. More research and data are required to improve management methods for aquatic invasive species, and the Reel Rewards Program can be a blueprint for monitoring and removing invasive species informed by and for the community.

Restoration and Place

The Reel Rewards program serves as a case study for restorative fishing, where fishing is transformed from an extractive process into a source of ecological restoration through fishing of invasive Northern Snakehead (*C. argus*), Blue Catfish

(*I. furcatus*), and Flathead Catfish (*P. olivaris*) in the Patapsco estuary. Restorative fishing is a term used to address the restorative service provided by removing species from an ecosystem through fishing efforts. Fishing is referred to as restorative due to removing a species that is creating ecological harm or disruption to an ecosystem. Examples of restorative fishing can be observed in Florida fishing of invasive Lionfish (*Pterois volitans*) or in California fishing of native, but locally overabundant Purple Sea Urchin (*Strongylocentrotus purpuratus*) barrens (Pasko and Goldberg 2013). The Reel Rewards program facilitates restorative fishing of invasive fish in the Middle Branch of the Patapsco River, which forms the shoreline of South Baltimore.

The Middle Branch is an example of an urban estuary that should perform many vital ecological services, including sustaining ecosystems and providing economic, social, and spiritual benefits to surrounding communities (Mahoney and Bishop 2017; EPA Report; Booi et al., 2022; Romanazzi et al. 2023). In the Chesapeake Bay, salt marshes and seagrass meadows serve as nurseries that support the production and recruitment of valuable fish (e.g., Striped Bass) and invertebrate species (e.g., Blue Crab) and enhance local recreational and commercial fisheries (Mahoney and Bishop 2017). Shoreline restoration in the Middle Branch will create wetland habitats to perform the ecological services of supporting fish communities, sequestering nitrogen and phosphorus, and aiding in shoreline stability and water quality (EPA Report; Mahoney and Bishop 2017; Woodland et. al., 2021; Rome et al. 2023).

Shoreline restoration will also create opportunities for revitalizing the surrounding neighborhoods. South Baltimore is impacted by historical and systematic environmental injustice, suffering from redlining and racial covenants, resulting in

multiple types of environmental degradation (Zec, 2024) such as polluted air and water (Mahoney and Bishop 2017; Woodland et. al., 2021). Only recently has research and real-time monitoring of the air and water quality in South Baltimore's Middle Branch been conducted, by organizations such as Blue Water Baltimore.

To address these issues, the South Baltimore Gateway Partnership received \$67 million in private (i.e., Horseshoe Casino), federal, state, and local government funding for the Middle Branch Resiliency Initiative (MBRI). Included in this funding, NOAA has provided \$5 million in Transformational Habitat Restoration and Coastal Resilience Grants to design and restore wetlands in South Baltimore's Middle Branch Harbor, as part of The Reimagine Middle Branch Project (RMBP). The MBRI is a multifaceted environmental justice wetland restoration project that will create living shorelines and green infrastructure, expand environmental education, and address safety concerns with dredge material.

The MBRI is a subset of the Reimagine Middle Branch Plan (RMBP), a multifaceted restoration and revitalization plan. Over \$175 million in Federal, State, Local, Casino, and Philanthropic funds were raised for RMBP. This project also aims to mitigate flooding of critical infrastructures such as the MedStar Harbor Hospital and Baltimore Gas and Electric Company's Spring Gardens. The project also restores wetlands in the Middle Branch and includes various monitoring programs for air and water quality and the abundance of river herring and invasive fish species.

In addition to restoring the wetland ecosystems, new community spaces will be constructed to improve the community's connection to the shoreline. Project goals of the RMBI include creating a wetland educational center and updating South

Baltimore parks. South Baltimore parks will be updated with the creation of new community gathering spaces, connecting eleven miles of walking trails around the Middle Branch, and trash removal programs. The project will address environmental justice, equity, diversity, and inclusion issues and create a thriving ecosystem for wildlife and people in Baltimore Harbor. This goal is accomplished by the involvement of community members, non-government organizations (NGOs), local government, and academia in the planning and implementation of projects. Issues of JEDI (Justice, Equity, Diversity, and Inclusion) will also be addressed by interdisciplinary research in the Middle Branch, including the research described here, performed in conjunction with the Reel Rewards program.

Historic and Contemporary Fishing and Anglers

The Patapsco River's fish community includes anadromous and catadromous species that migrate between the ocean, river, and tributaries, as well as resident fish that spend most of their lives in the river and estuary. Notable migratory species include Alewife and Blueback Herring, American Shad, Hickory Shad, White Perch, Yellow Perch, Striped Bass, and American Eel (Stence et al., 2013). Declining populations of ecologically and commercially crucial migratory Striped Bass (Mahoney and Bishop 2017), Shad sp. (Stence et al., 2014), and Herring sp. (Stence et al., 2014) are correlated with overfishing (Mahoney and Bishop 2017), loss of nursery habitats (Mahoney and Bishop 2017), and invasive species (Densmore 2020), indicating the need for wetland restoration projects (Densmore 2020). American Shad

was once the most important commercial fishery, but declining populations closed the fishery in the 1980s (Stence et al., 2014).

The Piscataway chiefdoms were the first subsistence fishers and stewards of the Potomac, Anacostia, and Patapsco Rivers. The Piscataway chiefdom historically consisted of multiple subgroups, including the Patuxent, Nacotchtank (Anacostans), Pamunkeys, Nanjemoyo, Potapacos (in southern Maryland), as well as the Doegs and Patawomekes of Virginia (Fiske et al., 2020). Indigenous fishers harvested oysters, clams, mussels, sturgeon, shad, and herring using weirs, traps, fish spears, and clam rakes (Fiske et al., 2020). African American fishers also have long relied on subsistence fishing in the Chesapeake Bay. The Potomac, Anacostia, and Patapsco Rivers served as both physical and symbolic boundaries between the North and South during colonial times. Amidst colonialism, a small population of free Black fishers harvested bass, catfish, herring, and shad from the Potomac and Anacostia Rivers (Fiske et al., 2020). Fishing offered an alternative to chattel slavery, and it became a source of wealth and dignity for the Black community (Fiske et al., 2020).

Today, subsistence fishing continues to support families and communities, helping to alleviate food insecurity (Fiske et al., 2020; Booi et al., 2022). Perch, Spot, and native and invasive catfish are recreationally important in the Chesapeake Bay and commonly targeted by subsistence anglers in the Middle Branch.

Multigenerational fishing traditions remain strong, with Black fishers being the most likely to have learned fishing from a family member (Fiske et al., 2020). However, subsistence fishing practices face challenges, including modern consumption advisories, or lack thereof, and declines in native fish distribution and populations,

creating environmental justice issues that conflict with historical fishing practices (Fiske et al., 2020).

Economic Value of Maryland Fisheries and Impact of Invasive Fish Species

The introduction of invasive species is often linked to declines in native fish populations and disruptions in food webs and ecosystems (Densmore 2020). Invasive fish species have negatively impacted many of Maryland's valuable commercial fishers through predation (Odenkirk and Owens 2007; Schloesser et al., 2011; McCabe, 2019;) and competition (Lapointe et al., 2012; Love and Newhard 2012) and may destabilize food webs (Mahoney and Bishop 2017; Sakai et al., 2001). In 2023 the Governor of Maryland declared invasive fish species a federal fisheries disaster to commercial fisheries and requested federal funding under the MagnusonStevens Fishery Conservation and Management Act of 2007 (Moore 2023). Governor Moore called for financial incentives for anglers to remove invasive fish species for the management of invasive fish (Moore 2023).

The management of invasive fish is imperative, as many of Maryland's fisheries are economically impacted by invasive fish species. The Maryland commercial blue crab fishery, valued at \$55 million (Moore 2023), has been impacted by invasive fish preying upon the valuable crustacean. The Striped Bass fishery, valued at \$5 million (Moore 2023), is also impacted by competing for prey items with all three invasive fish, upper tributary spawning grounds, and small bass, often directly consumed by Blue Catfish (*I. furcatus*). American Eel (Moore 2023), Channel Catfish (Moore 2023), and White Perch (Moore 2023) fisheries, valued at

around \$1 million annually, are also impacted as invasive fish consume native fish as prey items.

Many common prey items of Northern Snakehead (*C. argus*) are valuable fisheries in the Chesapeake Bay, such that anglers are competing with invasive fish for many of the same prey species. Northern Snakehead (*C. argus*) introductions were correlated with declines in native fish populations used as prey by the invasive species (Odenkirk & Owens, 2007). A two-year gut study on Northern Snakehead (*C. argus*) found a variety of valuable native fish species: Banded Killifish (*Fundulus diaphanus*), White Perch (*Morone americana*), Yellow Perch (*Perca flavescens*), Bluegill Sunfish (*Lepomis macrochirus*), Pumpkinseed Sunfish (*Lepomis gibbosus*), American Eel (*Anguilla rostrata*), and Largemouth Bass (*Micropterus salmoides*) were common prey items (Odenkirk & Owens, 2007). Flathead Catfish (*P. olivaris*) have a generalist diet of forage fish, crustaceans, and catfish, which impacts valuable baitfishes (shad sp. and centrarchids) but also results in declines of native Bullhead Catfish populations through predation and competition (Cimino et al., 2005). Flathead Catfish (*P. olivaris*) are well known to prey on larger crustaceans, mostly crayfish, but more information is required to understand the species' ecological interactions with Blue Crabs.

Despite disruptive or damaging ecosystem interactions, invasive fish are desirable sport species, growing relatively quickly and considered ideal for human consumption (Crowley et al., 2017; Fiske et al., 2020). Blue Catfish (*I. furcatus*) was initially established as a non-native sport species in adjacent tributaries of the Chesapeake Bay (Graham 1999). Blue Catfish (*I. furcatus*) landings are valued at \$276,000 annually (Moore 2023), while Northern Snakehead (*C. argus*) landings are

valued at \$13,000 (Moore 2023); expectations are that the value of these fisheries may increase as markets grow. However, the economic value of invasive fisheries is outweighed by its adverse impacts on more valuable native fisheries (e.g., Blue Crab). In the James River, the economic value of the recreational invasive Blue Catfish (*I. furcatus*) peaked at \$2.5 million in 2002 (Invasive Catfish Management Strategy 2020). However, it later fell as trophy fish were removed and replaced by moderate sized fish (Invasive Catfish Management Strategy 2020). Catfish remain a popular recreational fishery in the Chesapeake Bay, especially among subsistence fishers (Fiske et al., 2020). Subsistence anglers ranked Blue Catfish (*I. furcatus*), Striped Bass, Yellow Perch, and Channel Catfish as the most common catch for consumption. (Fiske et al., 2020). Northern Snakehead (*C. argus*) is a popular target species among the growing bow fishing community. However, the composition of subsistence versus sport fishers within the bow fishing community for Northern Snakehead in the Chesapeake Bay remains unclear.

The research described here seeks to understand the effects of the Reel Rewards program and restorative fishing on the ecosystem and fishing communities of the Middle Branch. Centering human interactions with wildlife management offers individuals the opportunity to engage in urban estuary restoration, broadening the network of estuary stewards. Recreational and subsistence anglers have a unique connection to wildlife, which was investigated to improve knowledge of community use of green spaces, environmental justice, and the identity of species of cultural value. The RMBI recognizes the cultural importance of living shorelines and their role in functional ecosystems and healthy communities as a type of environmental

justice. Restoration projects are an opportunity to restore the historically underserved communities of South Baltimore and their ecosystems. I therefore performed surveys of recreational anglers participating in the Reel Rewards program to document their perceptions of this program and its impact on their communities. My questions also allowed me to assess the presence of invasive fish species in the Middle Branch from the anglers' perspective. In my second chapter, I discuss how I analyzed environmental DNA samples for the detection of invasive fish and to assess fish diversity in the Middle Branch.

Method

Angler Interviews- Qualifying Angler Perceptions

The Reel Rewards management team created two sets of questions, one to be answered by anglers when they registered for the program (“registration questions”), and one to be answered by anglers while they were fishing during the summer (“angler survey questions”). Registration questions were used to enroll eligible anglers in the program, confirm anglers' eligibility to fish recreationally, and verify anglers' ability to identify invasive fish from native fish (Table 1). Angler surveys were conducted in person, and questions asked were similar to registration questions but also included questions about environmental perception and concerns in the Middle Branch (Table 2). Questions were open-ended, qualitative and interdisciplinary formulated to encompass subject categories of fish biology, environmental justice, fisheries management, and social science. Experts in biology, social science, and local community organizers were represented in the management

of the Reel Rewards program. Both registration and angler interview surveys started with simple questions, such as common catch, and ended with more complex questions on ecology, environmental justice, and community perceptions. A proportion of anglers who enrolled in the Reel Rewards program participated in summer angler interviews.

Registration Questions

Anglers registered for the Reel Rewards program by completing registration questions online (Table 1). Registration questions allowed the program to gather demographic information, confirmed anglers' ability to identify invasive fish species, and confirmed that anglers possessed a proper fishing license. In addition, anglers were also asked questions about fishing frequency and common species caught in the Middle Branch. It was important for registration to confirm that anglers were adequately permitted and would fish legally when participating in the program. Registration questions also acted as a gauge for public interest in the program, served to increase public awareness of invasive fish, and offered links to additional information on restoration projects.

After enrollment, anglers fished in the Middle Branch using the iAngler app to log their catches and confirm their location by GPS. Fish were turned into Reel Rewards staff and volunteers at various locations in the Middle Branch, and anglers were paid \$30 in gift cards for each invasive fish turned in (fish heads or whole fish body). The Reel Rewards project manager and assistant manager oversaw payment for invasive fish dropped off at collection sites. The restoration project is ambitious

and will require multiple years to accomplish. However, the project is unique in its approach to not only restoring the wetlands but also investing in the people who call South Baltimore home.

Angler Survey Questions

Anglers were asked a series of questions from across subject categories, with the primary questions being in fish biology, environmental justice, fisheries management, and social science categories (Table 2). Biological questions identified common species caught, contrasted with common species targeted by anglers. Anglers were also asked what is done with their catch, and this question was intended to estimate the proportion of anglers eating their catch in contrast to fishing recreationally. Anglers were also asked questions on demographics, such as if they live in South Baltimore and if they perceive that people who fish at this location are from South Baltimore. Demographic questions were asked to estimate community participation in the Reel Rewards program and addressed how equitably financial incentives were distributed amongst residents and non-residents of Baltimore. Demographic information could also identify which neighborhoods use the waterfront and green spaces. Respondents were asked a series of environmental questions, such as if they had noticed changes in the health of the Middle Branch Harbor, where they received information on environmental issues and regulations, and what organizations were impacting the harbor's health. These questions address the community's perception of the health of the harbor and restoration successes and shortcomings. Questions also identified sources of environmental justice issues. They improved the

understanding of information networks in the fishing community, an important element in disseminating information and increasing public awareness of the threats of invasive fish.

Angler interviews were conducted between May 15, 2024, and September 28, 2024, with 44 respondents participating. All participants were compensated with \$10 virtual gift cards emailed to participants after the interviews were completed. Gift cards were administered using the Tremendous app (app.tremendous.com). Angler interviews were completed in person at various locations in the Middle Branch, such as Middle Branch Park, Broening Boat Ramp, West Covington Park, or Middle Branch Trailhead 9 (Fig. 13; Table 3). All completed surveys were scanned and transcribed into Microsoft Excel. Survey data were cleaned and formatted using RStudio (R Core Team 2020) and Microsoft Excel for accuracy and repeatability. We are interested in observing patterns in the data collected from anglers, such as how anglers' perception of the fish community matches with species detected by eDNA (see Chapter 2). The data collected will establish a basic qualitative description of the fishing community members' use of and perception of their natural resources in the Middle Branch before the onset of an ambitious shoreline restoration project designed to increase the sustainable use of these resources.

Angler Survey Analysis

The Reel Rewards program applied for IRB approval in accordance with testing on human subjects and was found exempt by the UMBC IRB Board on March 15, 2024. The following steps were completed using RStudio (R Core Team 2020)

and Microsoft Excel. Data collected by angler interviews were processed in RStudio and sorted by binning survey answers into qualitative categories. Quantifiable answers, such as fish catch and target fish species are represented in histograms generated in RStudio.

In the first step, raw survey response data were imported from an Excel spreadsheet into Rstudio and created into a dataframe. The following libraries: ‘readxl’, ‘dplyr’, ‘stringr’, and ‘tidyverse’ were used to import Excel spreadsheets and were used in later steps. In the second step, Dataframe column names were reformatted by replacing spaces with underscores and adding “Q” prefix to any integer column names (e.g., “Q1” for question 1). Only relevant columns for analysis were retained, with coding work split between two researchers, each with questions pertinent to their research in fisheries management and social science of policy. All responses were converted to lowercase. Extra spaces and special characters, except commas and semicolons, were removed from standardized text.

For the third step, redundant responses such as “yes,” “no,” “not sure,” and “anything” were removed. Comma-separated values were split into a new column; for example, “Q1_1” and “Q1_2....” are responses to question 1 that were split into a new column. Data were further normalized by replacing the semicolon with a comma and removing commas for consistent formatting. Similar words were grouped together (e.g., “crabs” to “crab”) to standardize terms across responses by converting from plural to singular. During the fourth step, Dataframe columns were collapsed back into one column for each question as a cleaned dataframe. All rows with no values are removed from the final dataframe. Finally, ‘ggplot’ was used to create histograms, pie charts, and tables for survey questions.

Results

Registration Survey

Responses were collected from March 10, 2024, to September 28, 2024, with 347 individuals registering for the Reel Rewards program and 44 participating in angler interviews. A high proportion of registered individuals were Maryland residents (67%), with a moderate proportion of Baltimore residents (19%) treated separately from Maryland residents for this study. The remaining responses (13%) were from neighboring states of Virginia (2%), Pennsylvania (7%), and the District of Columbia (0.5%). Two people were residents of the District of Columbia (0.5%), Michigan (0.5%), and one person each from Delaware (0.2%), New Hampshire (0.2%), Ohio (0.2%), Georgia (0.2%), with a small minority (0.8%) of participants did not provide their state of residence (Fig. 2).

Fishing Frequency and Typical Catch

The most common (30%) response to the question, “About how often do you fish in the Middle Branch?”, was that this was the respondents’ first time fishing in the Middle Branch. Other common reactions were fishing weekly (~24%) and monthly (~22%) in the Middle Branch (Fig. 3). Fishing daily was the least common response, accounting for only 3% percent of respondents' answers. Residents of Baltimore were found to be more likely to have fished in the Middle Branch than non-residents. One exception to this trend was the response, “This is my first time”, which was selected by a higher proportion of non-residents than residents of Baltimore. Weekly and monthly residents.

In response to the question, “What do you usually catch here?”, answers ranged greatly among respondents. Some responses provided one species, and some respondents gave lists of commonly caught species and provided insight into the species richness of commonly caught species (Fig. 4).

Information Source

Social media (32%), word of mouth (27%), and internet search (17%) accounted for the majority of responses for how respondents heard of the Reel Rewards program (Fig. 5). Over half of the responses identified internet-based information sources (e.g., social media, internet search, EJJI website) as how they learned about the Reel Rewards program. When comparing residents' and nonresidents' responses, non-residents were about 10% more likely to have learned about the Reel Rewards program from an internet-based information source. Conversely, residents were more likely to have learned about the program through TV/News or email.

Perception of Environmental Improvements

In response to the question, “What have you heard about environmental improvements for the Middle Branch shoreline?,” most respondents (72%) were unaware or had little knowledge of any improvements or restoration efforts in the Middle Branch. Of the other responses, “Reel Rewards,” “invasive fish,” and “habitat restoration” were the next most common responses. Respondents were asked if they were interested in learning more about environmental improvements for the Middle

Branch Shoreline. Almost two-thirds (61%) responded “Yes” and said they would like to learn more (Fig. 6).

Angler Survey

Fish Taxa Caught and Targeted

In response to the question, “What do you usually catch here?,” catfish of unspecified species (14%) were the most mentioned catch. Also frequently mentioned as common catch were White Perch (9%), Spot (9%), unspecified perch species (7%), and Striped Bass (7%). Some anglers interviewed were unfamiliar with any species commonly caught in the Middle Branch, which is represented by the “Any fish” category (5%). Blue Crab (4%) was mentioned rarely, which was surprising considering the valuable commercial fishery in the region (Fig. 7).

In response to the question, “What are you trying to catch?,” catfish of unspecified species were also the most commonly mentioned target species, at 15%. This response was followed by Northern Snakehead (15%), unspecified perch species (7%), Blue Catfish (7%) and Blue Crab (7%). White perch and Striped Bass were mentioned more often by anglers as a common catch but appeared to be targeted less (Fig. 8). Anglers targeted Northern Snakehead at higher frequency than the fish was reportedly caught. With multiple common recreation species, White Perch, Spot and Striped Bass caught at much higher frequencies than the species were targeted (Fig. 9).

Reasons for Fishing: Location and Fish Use

In response to the question, “Why do you like fishing here?,” the most common reasons that people fished in the Middle Branch were to relax/spend time in nature (36%). Other reasons included spending time with family/people while using the park (23%), the location being close to home/work (19%), good fishing (8%), and generational fishing (8%). One respondent had been fishing the middle branch for 40 years. Anglers also mentioned that the area supported a “large fish population” with “lots of species,” and there was “less competition for larger crabs.”

In response to the question, “What do you do with the fish when you catch them?,” almost half of the anglers interviewed (44%) said they would release their catch. About a third (38%) of anglers said they intended to eat their catch, with some anglers gifting (13%) fish to friends and family to be consumed. Some respondents said they would also be selling (3%) the fish with the intent for human consumption. Some respondents specified that they would eat certain fish or invertebrate species only or specify certain-sized fish of specific species for consumption. Other respondents specified which species caught were to be used as bait and which were the primary target species. Unfortunately, our analysis was unable to include this information or specifics such as species or size for this response.

Invasive Fish Catch

In response to the question, “Have you caught Blue Catfish, Flathead Catfish, or Northern Snakehead here? If yes, which ones?,” half of all anglers interviewed had never caught an invasive fish in the Middle Branch. Blue Catfish were the most

mentioned (27%) species of invasive fish caught, followed by Flathead Catfish (15%) and Northern Snakehead (5%) (Fig. 10).

Residents vs. Nonresidents

We also asked “Do you live in Baltimore? If yes, Which neighborhood? If no, what city do you live in?” Analysis of this question was perplexing; many respondents said they lived in Baltimore but then identified a city outside Baltimore city lines. Baltimore has many neighborhoods, some of which are a couple of blocks away and only known by locals, which creates an issue for verification. The majority (65%) of respondents lived outside of Baltimore City. This question was asked to measure the proportion of Reel Rewards anglers who were Baltimore City residents to nonresidents as an estimate of community participation. Baltimore is a city and a county, a distinction that may have confused some respondents.

Perception of Residents vs. Nonresidents Fishing the Middle Branch

In response to the question, “Do you feel like people who fish here, live in the neighborhood?,” the majority (77%) of respondents felt that people who fished the Middle Branch lived in the neighborhood, with some respondents perceiving anglers as nonresidents (11%) and mostly nonresidents (11%) (Fig. 11). The community perception, therefore, is that most anglers who fish in the Middle Branch are residents of Baltimore. However, participation data collected by question 6 suggests the opposite, with most (65%) Reel Rewards participants living outside Baltimore City. This discrepancy could be explained by non-residents being more likely to participate

in angler interviews and residents being more likely to be fishing the Middle Branch but not agreeing to be surveyed. It is likely that community perception is correct and that most anglers are from the neighborhood or are Baltimore City residents.

Community Use of Green Space in the Middle Branch

We also asked, “What else do you come here [to the Middle Branch] to do?” The three most common responses for community use of green spaces in the Middle Branch were “recreation/ exercise” (38%), spending time with “family/kids” (27%), and “relaxing/ fresh air/ open spaces” (25%). Respondents mentioned park use for picnicking, playgrounds, other community uses, and crabbing (Fig. 12).

Community Perceptions of the Reel Rewards Program

In response to the question, “What are your opinions on the Reel Rewards program? (Note, if they did not understand the question, we explained Reel Rewards by saying “paying people for the invasive fish that they catch”)., the majority (84%) of respondents had positive attitudes towards the Reel Rewards Program. Positive responses can be summarized as saying that the program was good for the environment and anglers. Participants also mentioned they enjoyed contributing to science and receiving payment for removing invasive fish. Only one respondent (~2%) viewed the program negatively without elaborating why. Six respondents (13%) had neutral responses, two of whom responded that they had never heard of the program.

Community Perceptions of Changes in the Harbors' Health

In response to the question, “Have you noticed any changes in the health of the Harbor or fishing here? If yes, What do you think is causing these changes?,” most (64%) respondents noticed changes to the health of the harbor, with some (25%) noticing no change and a few (11%) unsure of changes to the harbor's health. Of the changes noticed by participants, most (57%) noticed positive changes, with some (39%) noticing negative changes. The top three positive changes noticed were improvements in wildlife (56%), water quality (25%), and pollution/trash (18%). The top three negative changes noticed were declines in wildlife (54%), pollution/trash (36%), and water quality (9%). Respondents were also asked to identify the causes of change to the harbor but answered inconsistently. Many respondents were quick to identify environmental changes, but few were able to provide the mechanism of change.

Sources of Local Environmental Information:

To determine how anglers are receiving information about environmental issues, we asked, “Who or where do you get information from about local environmental or water issues, and what kind of information do you get from them?” The three most common responses were apps/internet/social media (31%), business/industry (13%), and people (11%) (Fig. 13). The most common types of information anglers sought were regarding fishing spots/type of fish/bait type (53%), water conditions/water quality/tides (25%), and fishing regulations/park closures (12.5%). Government agencies, nonprofits, and academia were mentioned in low numbers. These responses illustrate the angling communities' reliance on the internet

and word of mouth for environmental information and dissemination of news and information.

To characterize anglers' perception of local organizations that may have an impact on the environmental health of the Middle Branch, we asked, "What organizations do you know of that affect the health of the Harbor [good, bad, or other]?" Answers were variable, with one-third (34%) of respondents unaware of any organizations impacting the harbor's health. Half (48%) of respondents identified organizations that caused negative change. One-quarter (18%) of respondents identified organizations that positively affect the harbor's health.

Unspecified industry was the most commonly mentioned organization negatively affecting the harbor's health (~28%), followed by Baltimore Gas and Electric combined with the BRESKO waste incinerator (~14%). Amazon (~3%), Under Armor (~3%), and Topgolf Baltimore (~3%) were each mentioned once during interviews as negatively impacting the harbor's health. Of the organizations causing positive impacts, the Reel Rewards Program (~7%) was mentioned twice. The following organizations were mentioned once, each positively impacting the harbor's health: the Mr. Trash Wheel project (~3%), the Chesapeake Bay Foundation (~3%), the Maryland Department of Natural Resources (~3%), and the Maryland University systems (~3%).

Although not required, most respondents specified the type of effects and changes to the harbor's health caused by organizations. Functioning as a secondary response to question 10, we asked "Have you noticed any changes in the health of the Harbor or fishing here?" The most commonly mentioned negative impact on the harbor was water quality, which was mentioned 13 times; trash/ pollution, which was

mentioned 9 times; and air quality, which was mentioned 3 times. During interviews, habitat loss caused by waterfront construction and negative impacts on ecosystems caused by invasive fish were mentioned once each. Positive organizational changes were shoreline restoration, trash removal, and invasive fish removal programs. Shoreline restoration projects that utilize oysters to restore water quality were mentioned twice in interviews as having positive impacts. Organizations that removed and mitigated trash in waterways were mentioned as improving water quality and the safety of the fish for human consumption.

Summary of Invasive Fish Caught, Payment, and Community Engagement

The Reel Rewards Program received 256 Northern Snakeheads, 37 Blue Catfish, and 6 fish identified as Flathead Catfish, but which were later identified as native Bullhead Catfish. These fish were turned in by recreational anglers participating in the program. Fish were collected at drop-off collection sites located in the Middle Branch from May through August 2024. Just under \$9,000 was paid to anglers for their invasive fish catch, and \$400 was paid in digital gift cards to anglers who participated in the angler surveys. The Reel Rewards Program engaged with 466 individuals at fish drop-off, community engagement events, and angler surveys. The age of participants ranged from children to senior citizens, with noteworthy involvement from the veteran community.

Discussion

I conducted angler interviews as part of the Reel Rewards invasive fish removal program. Angler interviews were conducted to assess species of importance, establish if anglers often catch invasive fish in the Middle Branch and what is done with fish catch. Interviews also established a baseline of community engagement with the shoreline prior to a massive restoration project. Interviews allowed us to compare community perception of local fish population with eDNA results (Chapter 2). Interviews also document environmental success of improving water quality and highlights ongoing environmental justice issues with air quality and trash. Angler interviews allowed the Reel Rewards program to build trust and credibility with community members as we become known. The interview format allowed for candid conversations to occur, highlighting adjacent concerns of the community, such as gentrification, and allowed interviewees to ask follow-up questions or reword questions if anglers didn't understand or address the questions. These conversations allowed feedback for improving the Reel Rewards program and identifying unseen barriers to participation. One such barrier was the phone requirement and correct use of an app (iAngler), which proved troubling for some older or less technologically advanced users. In-person interviews also increased awareness of the program to anglers unaware of the program disseminating information and addressing community questions about the program, wetland restoration, and invasive fish. However, angler interviews were conducted in person, decreasing the number of participants to individuals fishing in the Middle Branch on a given day. Registration questions were completed online, allowing for higher participation by a broader range

of members of the angling community, with accommodations for disability or language barriers.

Biological Takeaways

Anglers noticed improvements in wildlife and biology over the past decade, but fewer fish and more small fish were commonly perceived as negative environmental changes. Anglers attributed declines in the size of recreationally important croaker, perch, and trout to increased fishing effort as more people fished and harvested from new fishing sites. Declines in recreational fish species were attributed to invasive fish, pollution, and shoreline construction, reducing habitats. Mode of fishing could be a factor in angler dissatisfaction with the fish size and species composition. Most interviews were conducted with shore-based anglers, with a few boat-based anglers that we were able to intercept and interview at the Broening boat ramp. The few (n=2) boat-based anglers I interviewed had caught multiple large (12"-14") White Perch and Yellow Perch. Future research should consider the effect of fishing mode on environmental perception and should be incorporated into the second year of the Reel Rewards program.

Most anglers interviewed perceived invasive fish as a risk to native fish and crab species. Anglers were enthusiastic about removing invasive fish from the Middle Branch. Reasons for removing invasive fish ranged from aiding in restoration efforts, engaging in subsistence fishing and Reel Rewards financial incentives. Northern Snakehead was commonly targeted by anglers however few anglers interviewed caught Northern Snakehead in the Middle Branch. In contrast, Blue Catfish were the

most mentioned invasive fish caught in the Middle Branch but were rarely mentioned by name; anglers mentioned catfish but rarely specified the species. Flathead Catfish was also not mentioned by common name during interviews. However, catfish were the most targeted fish taxon, representing high community value of fishing for catfish species. High enthusiasm for invasive fish removal from the South Baltimore fishing community will aid in sustained restoration efforts in the Middle Branch.

Flathead Catfish have high variance in coloration and were difficult for anglers to distinguish from native Bullhead Catfish species. The Flathead Catfish collected by the Reel Rewards anglers were only heads. They were later discovered after further inspection not to be Flathead Catfish and are likely a native catfish species but could not be identified with only heads collected. The policy of accepting heads creates a verification issue between “similar” looking Bullhead Catfish species. Flathead Catfish catch will not be accepted in the second year of the Reel Rewards program due to anglers' difficulty distinguishing between native and invasive fish. In addition, Flathead Catfish do not tolerate the average salinity (6-11 ppt) in the Middle Branch and are rarely detected. Flathead Catfish were not mentioned as a common catch in the Middle Branch during angler interviews.

Community Environmental Perceptions and Environmental Equity Takeaways

Water quality was a common community concern, with wastewater contaminated with chemical sewage and oil often mentioned along with trash at the shoreline. Source points were attributed to Baltimore Gas and Electric, the BRESO Waste incinerator, the Patapsco and Backwater wastewater treatment plants, and the harbor docking station. Evidence can partially support the community's claim with

the Blue Baltimore and Patapsco Waterkeeper documenting that both wastewater treatments were not compliant with allowable *E.coli* fecal bacteria counts. However, many anglers perceive water quality as improving, noting the presence of marine mammals (e.g., dolphins and otters), a high number of fish offspring, and large waterfowl populations as indicators of good water quality and an improving ecosystem. Oyster restoration programs and city and community trash removal efforts were attributed to water quality improvements. Water quality continues to be monitored in Baltimore Harbor by the Eyes on the Bay and Blue Water Baltimore. Despite this, our results indicate that water quality is still a pressing issue for the community of the Middle Branch.

Further research is needed to update fish consumption advisories to include invasive fish for the Middle Branch. The lack of consumption guidelines for invasive fish in the Patapsco Middle Branch is an information gap that the Maryland Department of the Environment must address for public health. The Reel Rewards team tried to partner with scientists to test fish samples for heavy metals but was unsuccessful in its first year. Many invasive catfish were received as heads only, suggesting their bodies were used as bait or for human consumption. However, many bowfishers donated whole Northern Snakehead bodies, suggesting sport fishing, not subsistence fishing. Interestingly, White Perch and Striped Bass are less targeted species (3% each) despite being a common catch (11 %, 9%). This illustrates a difference between how commonly caught (abundant) a fish may be, compared to how much fishing effort there is.

Invasive Northern Snakehead and Blue Catfish were found to be present in the Middle Branch, however Flathead Catfish were not detected by Reel Rewards

anglers. Community perception was positive of the Reel Rewards program and oyster programs, which were both found to improve the environment. High enthusiasm to participate in the Reel Rewards program was noted among anglers; however, effort should be made to increase the proportion of Baltimore residents involved in the program. Catfish species were found to be of high community value for both recreational and subsistence anglers of the Middle Branch. Community concerns of litter, and air pollution continue with individuals interviewed noting that water quality has improved over the years despite continued pollution sources of sewage and runoff.

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Table 1. Reel Rewards Registration Questions and Research Categories Addressed.

Question	Categories Addressed
1. Have you registered as an angler on iAngler? If no, please register on iAngler before filling out this form	Program Management • Required for enrollment • Confirm legal recreational fishing
2. Name (First Last)	Program Management • Required for enrollment
3. Email address	Program Management • Required for enrollment
4. Chesapeake Bay and Coastal License number (MD DNR)	Program Management & Regulatory • Required for enrollment • Confirm legal recreational fishing
5. Chesapeake Bay and Coastal License validation dates (ex. Month/Day/2023 - Month/Day/2024)	Program Management & Regulatory • Required for enrollment • Confirm legal recreational fishing
6. What Baltimore neighborhood do you live in? If you live outside of Baltimore, what city do you live in?	Program Management & Social Science • Established anglers lived in the region • Identified the demographics of residents to non-residents
7. About how often do you fish in the Middle Branch?	Fisheries Management • Experience and familiarity with fishing the Middle Branch
8. What do you usually catch here?	Fisheries Management & Biological • Identify a range of species present • Identify the most common species

9. What have you heard about environmental improvements for the Middle Branch shoreline?	Social Science Gauges community awareness of restoration
10. Would you like to learn more about environmental improvements for the Middle Branch shoreline? If yes, we will use your contact information to follow up.	Community Outreach - Provide resources - Increases community awareness - Pathway to community engagement
11. How did you hear about the “Reel Rewards” invasive species removal program?	Social Science & Program Management - Understand information pathways in the fishing community - Improved communication with fishing communities
12. I confirm that I can identify the following fish species targeted by this program (select all):	Fisheries & Program Management Confirm anglers remove invasive fish species
13. I have read and agree to the above program policies.	Program Management & Regulatory Confirm anglers are responsible for legal fishing and program participation

Table 2. Summer Angler Survey Questions and Research Categories Addressed

Question	Categories Addressed
1. What do you usually catch here? [as many as you know]	Fisheries Management & Biological • Identify a range of species present • Identify the most common species
2. What are you trying to catch? [as many as you know]	Fisheries Management & Biological • Identify the most common target species
3. Why do you like fishing here?	Social Science, Environmental Justice & Program Management • Identify positive attributes of fishing sites • Improvised understanding of factors anglers consider for site selection • Identify potential access issues or disturbances to community spaces caused by restoration construction
4. What do you do with the fish when you catch them?	Environmental Justice & Fisheries Management • Identify fish and invertebrates commonly consumed and valued • Highlight consumption of urban fish implications on public health
5. Have you caught Blue Catfish, Flathead Catfish, or Northern Snakehead here? □ [If yes] - Which ones?	Fisheries Management & Biological • Estimates which invasive species are most likely to be caught • Establishes that anglers are catching invasive species in the Middle Branch

6. Do you live in Baltimore? □ [If yes] - Which neighborhood? □ [If no] - What city do you live in?	Program Management, Social Science & Environmental Justice • Established anglers lived in the region • Identified the demographics • Documents program participation by community members or non-residents
7. Do you feel like people who fish here, live in the neighborhood?	Program Management, Social Science & Environmental Justice • Records the perceptions of the fishing community's composition from the perspectives of residency in Baltimore • Investigate the difference in community perception of residency fishing participation
8. What else do you come here [to the Middle Branch] to do?	Social Science & Environmental Justice • Understand community use and value of green space and waterways • Identify potential access issues or disturbances to community spaces caused by restoration construction
9. What else do you come here [to the Middle Branch] to do?	Social Science & Environmental Justice • Understand community use and value of green space and waterways • Identify potential access issues or disturbances to community spaces caused by restoration construction

10. What are your opinions on the Reel Rewards program? [If they don't understand, say "paying people for the invasive fish that they catch"]	Program Management & Environmental Justice • Gauge community perception and sentiments • Address community issues or barriers to improving the program
11. Have you noticed any changes in the health of the Harbor or fishing here? □ [If yes] - What do you think is causing these changes?	Environmental Justice • Document environmental changes observed by community members • Identify mechanics of environmental change in the community
12. Who or where do you get information from about local environmental or water issues, and what kind of information do you get from them?	Social Science & Environmental Justice • Understand information pathways in the fishing community • Identify the most effective mode of communication • Improved understanding of what type of information anglers value and seek
13. What organizations do you know of that affect the health of the Harbor [good, bad, or other]?	Environmental Justice • Document and identify which organizations are perceived as impacting environmental health

Table 3. Summer Angler Survey Sites

Site	GPS coordinates
Broening Boat Ramp	39.255487, -76.615861
Middle Branch Park	39.256061, -76.621522
Middle Branch Trailhead 9	39.248023, -76.611607
West Covington Park	39.261502, -76.615968

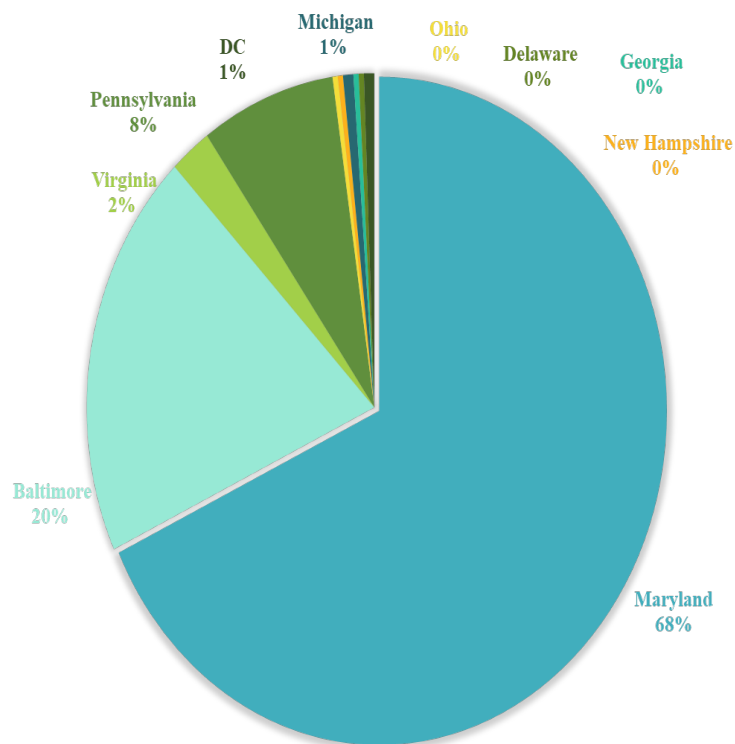


Figure 1. Registration Question: State of residency. Data represents 347 participants.

Resident and Nonresident Fishing Frequencies at Middle Branch Park

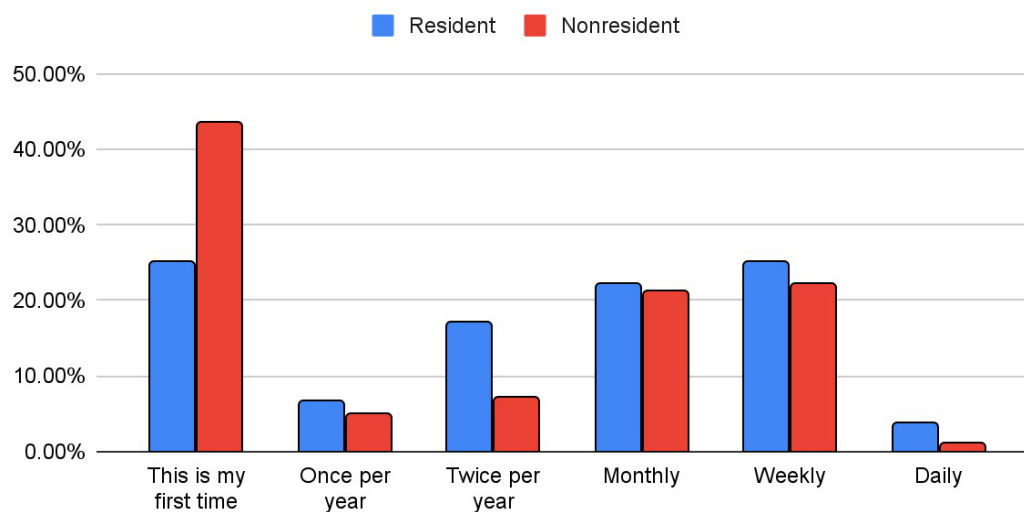


Figure 2. Registration Question: Fishing frequency grouped by Baltimore residents and NonBaltimore residents. Data represents 347 participants. Image adapted from EJJI Reel Rewards Project Summary 2024.

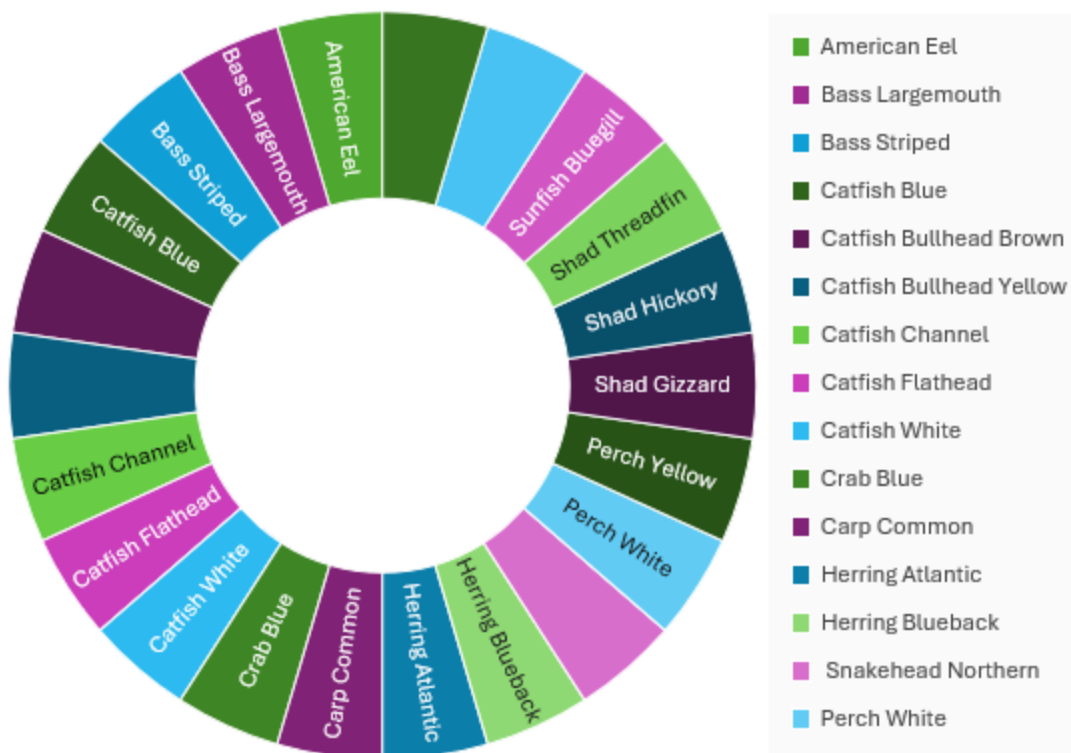


Figure 3. Registration Question: Most common catch in the Middle Branch. Data represents 347 participants.

Responses to "How did you hear about the Reel Rewards invasive species program?"

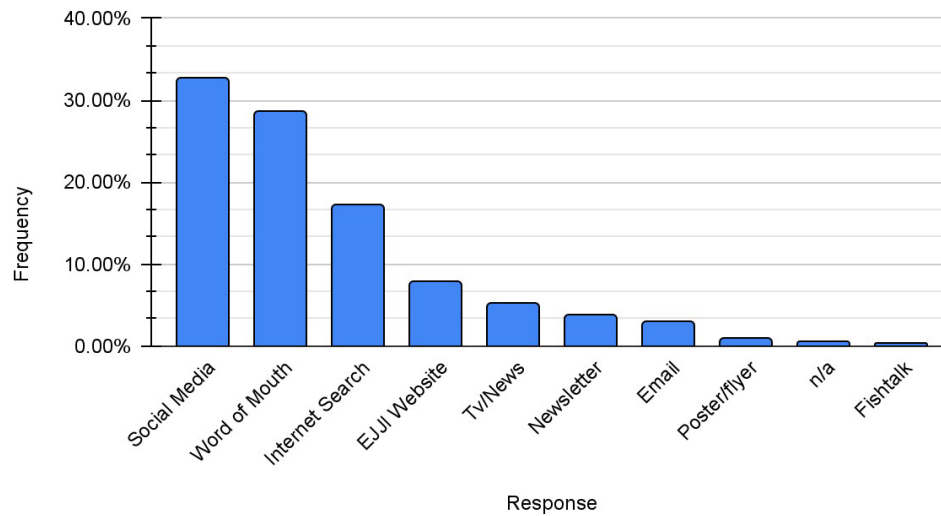


Figure 4. Registration Question: Information source for the Reel Rewards program. Data represents 347 participants. Image adapted from EJJI Reel Rewards Project Summary 2024.

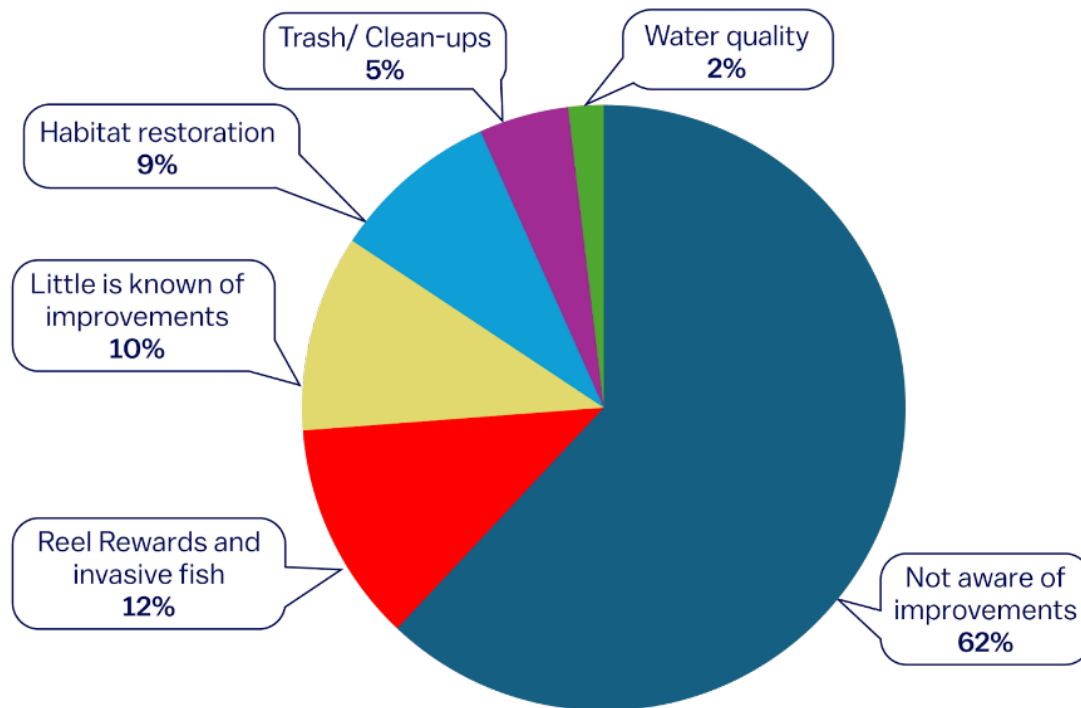


Figure 5. Registration Question: Angler awareness of improvements in the Middle Branch. Data represents 347 participants. Image adapted from EJJI Reel Rewards Project Summary 2024.

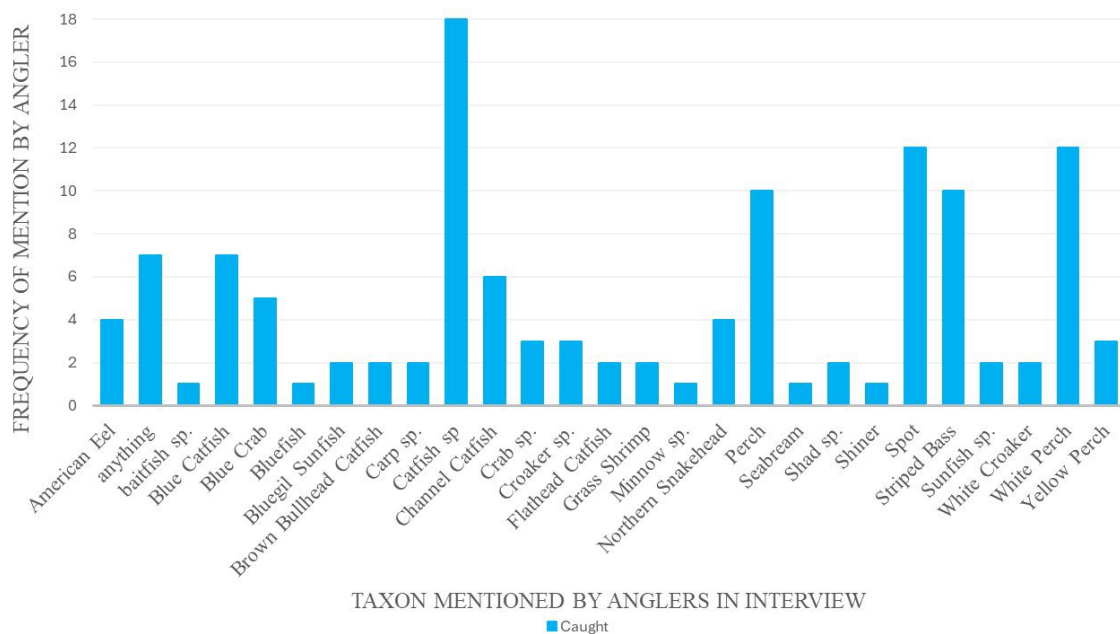


Figure 6. Angler Interview Question: Most commonly caught species in the Middle Branch. Data represents 44 participants average of 1.5 responses per participant.

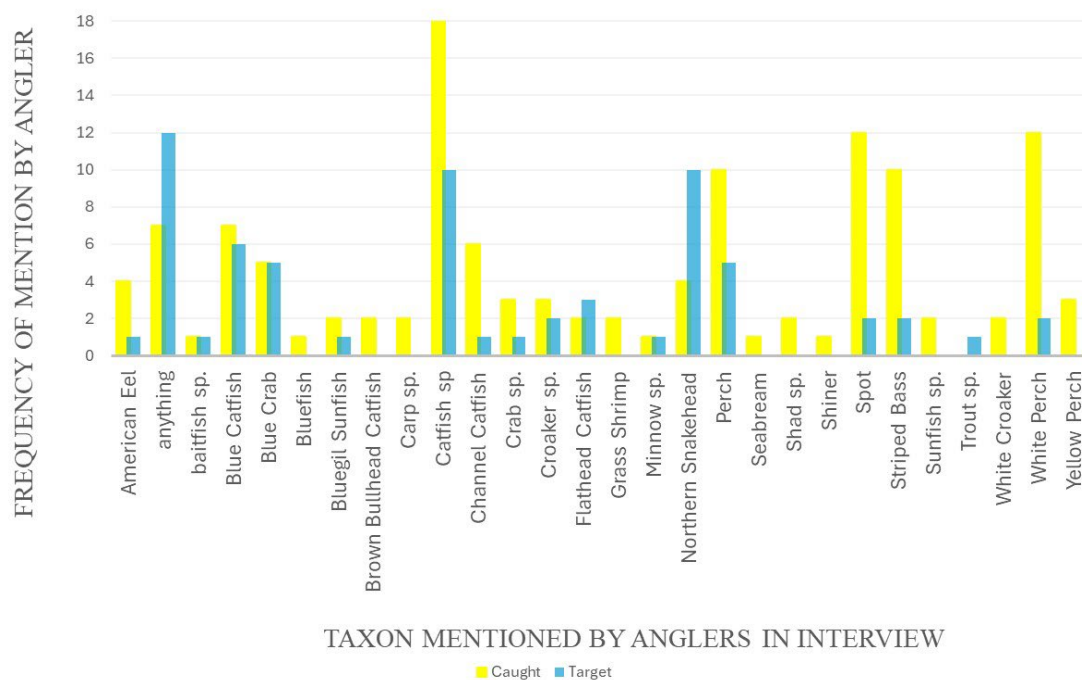


Figure 7. Combined Angler Interview Questions: Commonly caught species (Fig. 6) and commonly targeted (Fig. 8) species in the Middle Branch. Data represents 44 participants.

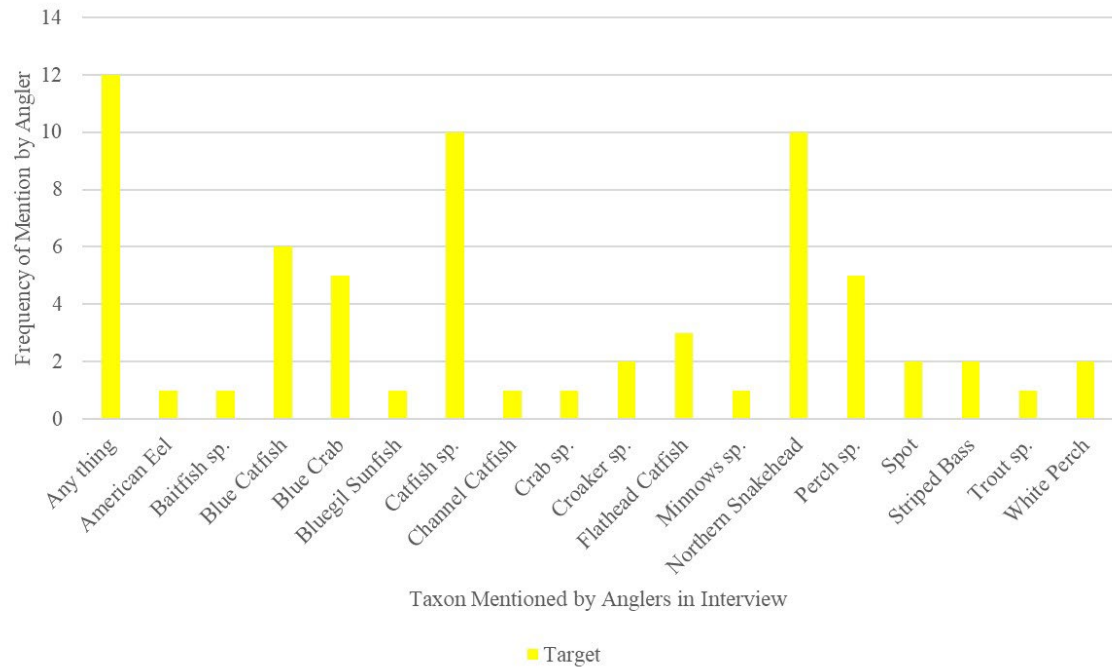


Figure 8. Angler Interview Question: Commonly targeted species in the Middle Branch. Data represents 44 participants and an average of 2.8 responses per participant.

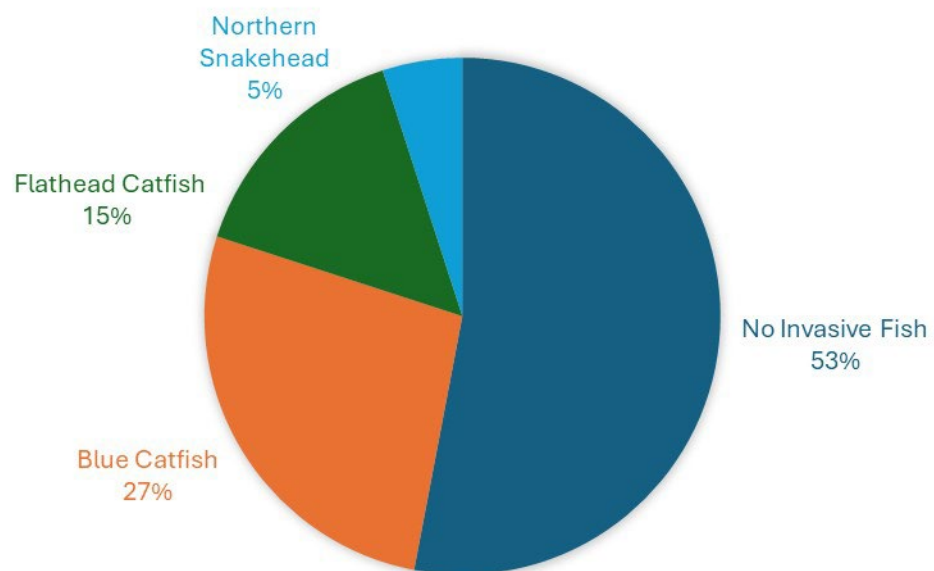


Figure 9. Angler Interview Question: Invasive fish catch in the Middle Branch. Data represents 44 participants.

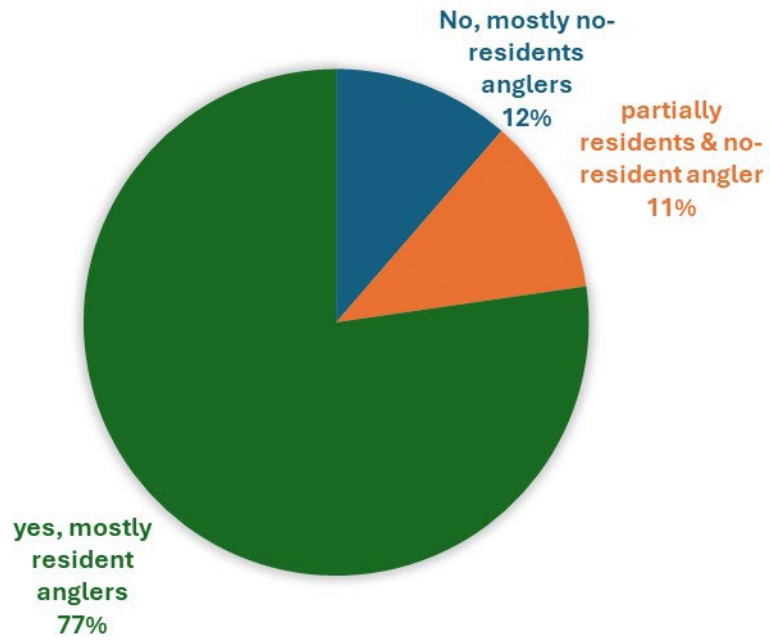


Figure 10. Angler Interview Question: Anglers' perception of Baltimore residents to non-Baltimore resident anglers who fish the Middle Branch. Data represents 44 participants.

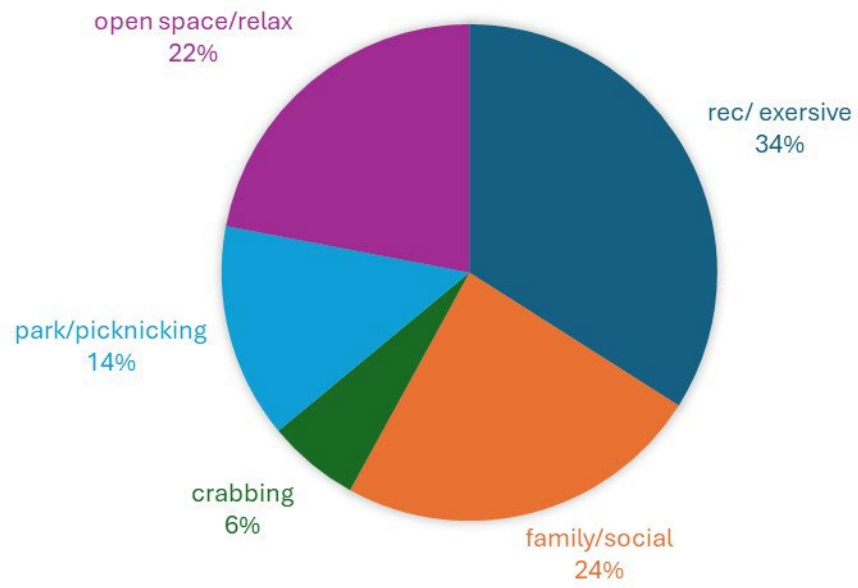


Figure 11. Angler Interview Question: Other activities for which anglers use the Middle Branch. Data represents 44 participants.

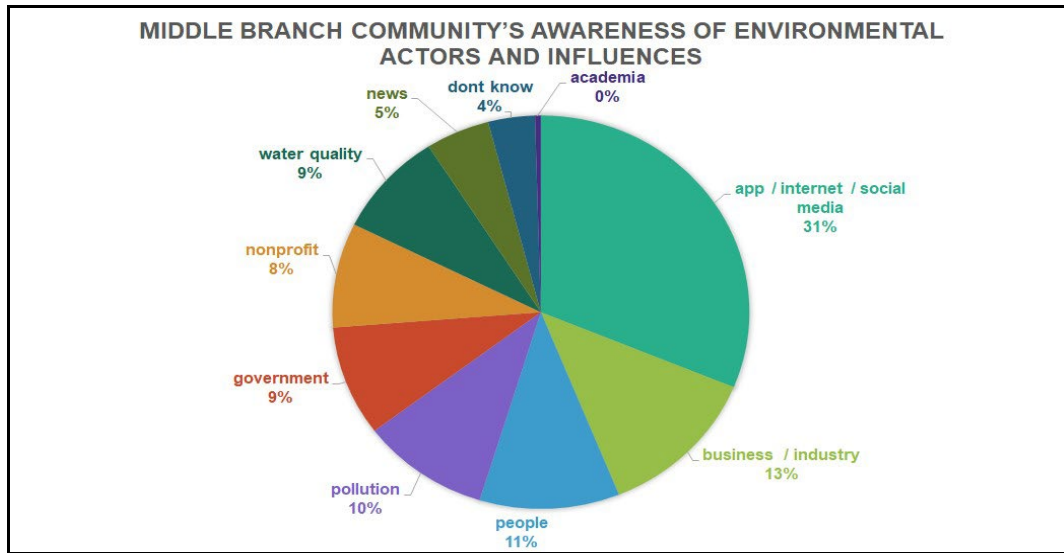


Figure 12. Angler Interview Question: Anglers' information sources, type of environmental information, and what organizations affect the health of the harbor. Data represents 44 participants. Image adapted from EJJI Reel Rewards Project Summary 2024.

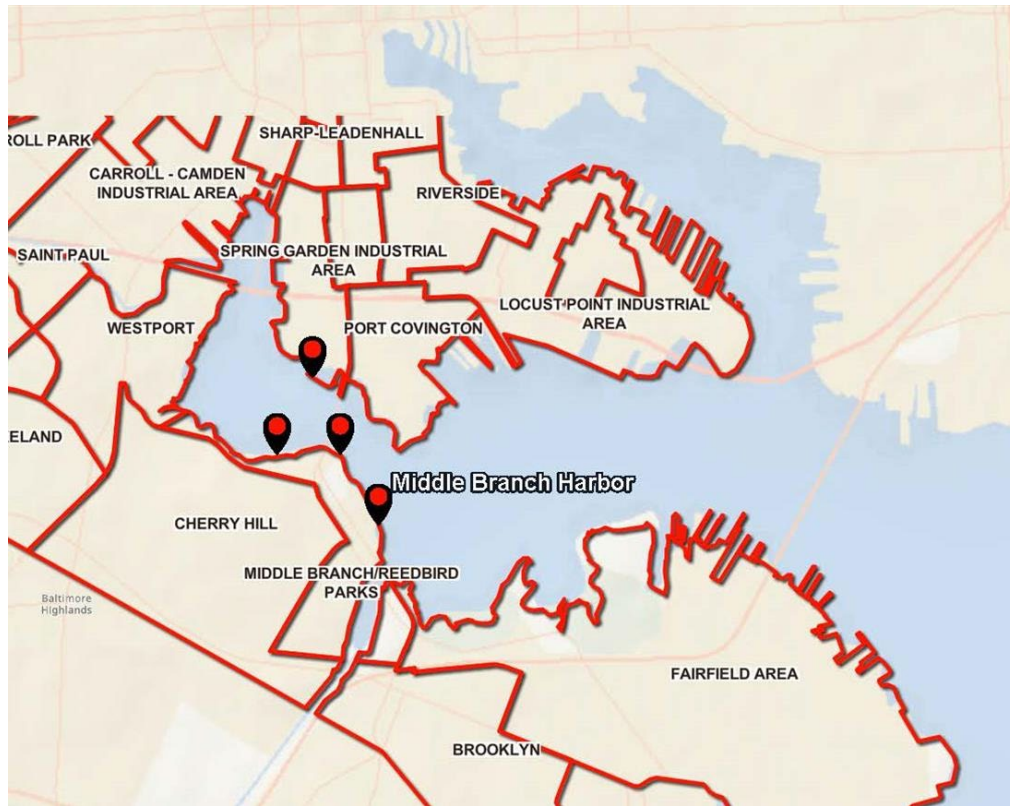


Figure 13. Map of the Baltimore Harbor with the Middle Branch on the left. Black and red pins indicate Angler interview sites; West Covington Park, Middle Branch Park, Broening Boat Ramp, Middle Branch Trailhead 9. Image adapted from EJJI Reel Rewards Project Summary 2024.

Chapter Two: Species Composition of Fishes of the Middle Branch as Assessed with Environmental DNA (eDNA)

Introduction

My research used environmental DNA (eDNA) to achieve three goals: (1) to assess the presence or absence of invasive Blue Catfish (*Ictalurus furcatus*), Flathead Catfish (*Pylodictis olivares*), and Northern Snakehead (*Channa argus*) in the Middle Branch (Patapsco River) estuary, (2) to estimate teleost fish species richness, (3) to create a baseline of the teleost fish community relative sequence abundance for the mitochondrial gene cytochrome oxidase I (COI). There is a lack of research on the ecosystems of the Middle Branch, with very little documentation of fish species composition or the prevalence of invasive Blue Catfish, Flathead Catfish, and Northern Snakehead. As part of a larger shoreline restoration project spearheaded by the South Baltimore Gateway Partnership, my research will provide information to the community, aid in the management and detection of invasive fishes, and support further studies of the ecosystems of South Baltimore.

Environmental DNA Fish Monitoring

DNA is present in the environment in many forms and can be found in tissues, cells, waste products, and decomposing organisms. Most fish species' DNA is 0.2-10 um in size (Barnes et al., 2021). "Metabarcoding" is a gene sequencing method that

allows for the identification of multiple species from a single environmental sample, as common genes shared across taxa are amplified using primers in polymerase chain reactions (PCR). The results of the metabarcoding analysis allow for a baseline of fish species richness (as ASV richness), which serves as a reference point for continued species monitoring in the Middle Branch. The results also will create a presenceabsence baseline for invasive Blue Catfish (*I. furcatus*), Flathead Catfish (*P. olivares*), and Northern Snakehead (*C. argus*), which are targeted by the Reel Rewards invasive fish removal program.

Environmental DNA analyses have become an effective and relatively lowcost method for invasive species monitoring. eDNA sampling has no habitat degradation and does not require live fish handling, which is stressful and harms to fish. Collecting water samples for eDNA analysis also presents an opportunity to involve the community in participatory science of their local environments. Appropriately done, participatory science provides an educational experience and community outreach to the people of South Baltimore, while also including those individuals in the collection of data that could be used to understand their waterways. As the best way to control invasive species is to prevent their introduction into ecosystems (Densmore 2020), educating the public could lessen the occurrence of non-native species being introduced into ecosystems (Densmore 2020). Connecting people with wildlife and ecosystems is essential to fostering an interest in preserving and restoring these spaces.

The Reel Rewards Program is an invasive fish removal and monitoring program that compensates local recreational anglers for the ecological services they

provide by removing invasive fish. The program is funded by NOAA and is part of a larger urban wetland restoration project. The program aims to decrease the populations of Blue Catfish, Flathead Catfish, and Northern Snakehead by increasing the rate of removal by recreational fishing efforts. Blue Catfish and Northern Snakehead are consistently mentioned as aquatic invasive species with moderate to high management concerns in multiple states (Densmore 2020). These fishes can grow to a large size quickly, outcompeting native fish and negatively impacting Maryland's economically important seafood industry, such as the Blue Crab (*Callinectes sapidus*) fishery. Reel Rewards aids in detecting invasive fish species, creates a method for inexpensive species monitoring, and has developed a mode of educational outreach and community engagement. More research and data are required to improve management methods for aquatic invasive species, and the Reel Rewards Program can be a blueprint for monitoring and removing invasive species informed by and for the community.

Fish Diversity of the Middle Branch

The Patapsco River's fish community comprises anadromous and catadromous fishes that migrate between the ocean, river, and tributaries as well as resident fish that spend the majority of their lives in the river and estuary of the Patapsco. Noteworthy migratory fish include the river herring complex (Alewife (*Alosa pseudoharengus*) and Blueback (*Alosa aestivalis*)), shad (American (*Alosa sapidissima*), Gizzard (*Dorosoma cepedianum*), and Hickory (*Alosa mediocris*)), perch (White (*Morone americana*) and Yellow (*Morone mississippiensis*)), Striped

Bass (*Morone saxatilis*), and American Eel (*Anguilla rostrata*) (Stence et al., 2014; Harbold et al., 2013). Declining populations of the Patapsco River's ecologically and commercially important migratory baitfish led to restoration projects to enhance the fish population. American Shad (*A. sapidissima*) historically was the most important commercial fishery, but declining populations closed the fishery in the 1980s (Stence et al., 2014).

Resident fish of the Patapsco River include Sunfish (Bluegill (*Lepomis macrochirus*), Green Sunfish (*Lepomis cyanellus*), Pumpkinseed (*Lepomis gibbosus*), Redbreast Sunfish (*Lepomis auritus*)), native catfishes (Brown Bullhead (*Ameiurus nebulosus*), Yellow Bullhead (*Ameiurus natalis*), naturalized Channel Catfish (*Ictalurus punctatus*), Margined Madtom (*Noturus insignis*), White Catfish (*Ameiurus catus*)), shiners (Common Shiner (*Luxilus cornutus*), Satinfish Shiner (*Cyprinella analostana*), Spottfin Shiner (*Cyprinella spiloptera*, Spottail Shiner (*Cyprinella spiloptera*), Swallowtail Shiner (*Notropis procne*)), bass (Largemouth Bass (*Micropterus salmoides*), Smallmouth Bass (*Micropterus dolomieu*)), trout (Rainbow Trout (*Oncorhynchus mykiss*), Brown Trout (*Salmo trutta*)), suckers & carp (White Sucker (*Catostomus commersonii*), Northern Hogsucker (*Hypentelium nigricans*), Common Carp (*Cyprinus carpio*), Quillback (*Carpoides cyprinus*)), minnows (River Chub (*Nocomis micropogon*), Bluntnose Minnow (*Pimephales notatus*), Fallfish (*Semotilus corporalis*), Central Stoneroller (*Campostoma anomalum*)), darters (Shield Darter (*Percina peltata*), Tessellated Darter (*Etheostoma olmstedii*)), and other fish (Banded Killifish (*Fundulus diaphanus*), Black Crappie (*Pomoxis nigromaculatus*), Inland Silverside (*Menidia beryllina*), Chain Pickerel (*Esox niger*), Rock Bass (*Ambloplites rupestris*)), (Stence et al., 2014; Harbold et al., 2013). The invasive Blue

Catfish, Flathead Catfish, and Northern Snakehead appear to have established themselves among these residents. A fish is defined as invasive if it is not native to its introduced ecosystem and the species has the capability to disperse, reproduce in the wild and cause negative impacts to native fish populations, habitats or ecosystems function (Britton et al., 2011).

Invasive Fish of the Chesapeake Bay

Blue Catfish (Ictalurus furcatus)

Blue Catfish were intentionally introduced to Virginia from the 1970s to 1980s to establish a recreational fishery on the James, Rappahannock, Mattaponi, and Potomac Rivers (Higgins et al., 2006). Many conflicting interests exist in managing Blue Catfish in the Chesapeake Bay, as multiple fisheries --commercial, trophy, and recreational -- rely on the continued presence of the fish in the region. Despite Blue Catfish's economic value to the region, the invasive species may be economically impacting other valuable fisheries in the Chesapeake Bay, raising questions about just how profitable the fishery is when factoring in the hidden costs (Desmore 2020; Crowley, et al., 2017; Graham 1999; Moore 2023).

Blue Catfish are indigenous to the Mississippi River drainage basin and the Gulf of Mexico to Guatemala (Graham, 1999). The species is found in freshwater reservoirs, backwaters, and main channels of rivers with strong currents and turbid water in the Chesapeake Bay (Graham, 1999). Blue Catfish are also found in Atlantic slope estuaries in the region, with large individuals tolerating salinities as high as 17 ppt (Nepal and Fabrizio, 2019; Schloesser et al., 2011; Graham 1999). The species'

high salinity tolerance suggests a potential for populations to expand their range into brackish water (Nepal & Fabrizio, 2019). Blue Catfish can reach sexual maturity in 47 years (Nepal and Fabrizio, 2021) but may mature later in life (6-10 years). The species exhibits “population-specific differences in reproduction” (Nepal and Fabrizio, 2021), adapting and tailoring life history to exploit different environmental conditions (Nepal et al., 2020) Males build nests and guard eggs; brood size is small with large eggs (Nepal and Fabrizio, 2021).

Blue Catfish are generalist omnivores consuming a range of macroinvertebrate and fish prey items, with medium size fish (300- 600 mm) shifting to a piscivorous diet (Schloesser et al., 2011). Amphipods were the primary prey item for small Blue Catfish (< 300 mm) in the Virginia riverine system (Schloesser et al., 2011). Medium-size Blue Catfish prey items vary by tributary, with Atlantic menhaden (*Brevoortia tyrannus*) dominating the diet of Rappahannock River Blue Catfish (Schloesser et al., 2011). Crabs and mollusks were dominant prey items in other Virginia rivers and accounted for more than 55% of the diets of medium-sized Blue Catfish (Schloesser et al., 2011). Blue Catfish will likely affect native aquatic communities through direct and indirect interactions and habitat alterations (Sakai et al., 2001; Schloesser et al., 2011; Schmitt et al., 2018).

Flathead Catfish (Pylodictis olivaris)

Flathead Catfish were initially stocked in the Potomac and James Rivers in 1965, with a second stocking in 1977 in the James River (Cimino et al., 2005). The species has been introduced in 11 other states (Jackson 1999), and it is disputed if the species was introduced or native to the Great Lakes (Illes et al., 2020). Flathead

Catfish are native to the Mississippi and Rio Grande drainages and Northern Mexico (Jackson 1999; Bringolf et al., 2005).

Flathead Catfish were described as “fluvial generalists” by Jackson (1999), as they show a preference for main channels but can be found in a variety of freshwater habitats such as lakes and reservoirs (Jackson 1999, Brown et al., 2005). Flathead Catfish are associated with undercut banks, woody debris (Jackson 1999, Illes 2020), and turbidity (Illes 2020). The salinity tolerance for the species ranges from 11ppt to 15 ppt (Bringolf et al., 2005), with larger individuals having a higher salinity tolerance than smaller individuals (Nepal and Fabrizio, 2019). The species has great migration potential, covering 100 km a year, with three distinct seasonal migrations triggered by temperature and other factors (Montague and Shoup, 2021). However, Flathead Catfish prefer to move small distances to feed nocturnally in shallow water (Jackson 1999) and exhibit high site fidelity over the years, even among highly migratory individuals (Montague and Shoup, 2021).

Flathead Catfish are long-lived, with individuals living past 19 years and estimates of wild individuals' lifespans reaching their maximum at 28 years (Brown et al., 2005). Sexual maturity is reached at 3-4 years for males and 4-5 years for females (Brown et al., 2005), with total lengths for the fish ranging from 375-539 mm (Illes et al., 2020). Low-velocity sites with cavities are selected for nest building (Jackson 1999; Montague and Shoup, 2021), and spawning occurs from June- July when water temperatures reach 19-24C (Jackson 1999; Illes et al., 2020). Females can produce 100,000 eggs (Jackson 1999), with 31- 579 eggs per clutch (Illes et al., 2020) commonly observed.

Flathead Catfish are opportunists (Pine et al., 2005; Schmitt 2019; Montague and Shoup, 2021), generalist predators (Montague and Shoup, 2021) that feed nocturnally (Brown et al., 2005) and select prey mostly in proportion to their abundance (Pine 2005). Flathead Catfish and Blue Catfish have similar diets, often competing with juvenile and adult Striped Bass (Cimino et al., 2005) for American Shad, Gizzard Shad, and Bay Anchovy (Pine et al., 2005; Schmitt 2019; Montague and Shoup 2021). Small (< 100mm) Flathead Catfish diet is primarily composed of insects; once larger than 100mm, fish transition into a piscivore diet with occasional large crustaceans (mostly crayfish) in their diets (Jackson 1999; Brown et al., 2005). Native Bullhead Catfish and Redbreast Sunfish populations have declined after the introduction of Flathead Catfish (Brown et al., 2005). Competition for prey items with Striped Bass and the decline in native fish populations have caused management concerns about the invasive fish's impact on native fish and commercially important fisheries.

Northern Snakehead (Channa argus)

Since its first discovery in the Maryland riverine system in 2004, the Northern Snakehead has caused concern due to its high risk of becoming invasive (Love and Newhard, 2012) due to many aspects of the species' biology and life history. Northern Snakeheads are native to the eastern rivers of China, Korea, and Russia. In the Chesapeake Bay, Northern Snakehead are found in the upper portions of the bay in slow-moving fresh water with submerged vegetation and soft substrates (Lapointe et al., 2012; Love and Newhard, 2012). Northern Snakeheads are predatory piscivores with no local predators as adults. The species' primary prey item in the Chesapeake

Bay is Banded Killifish (*F. diaphanus*), but it commonly consumes a range of commercial and ecologically important fish and invertebrates, such as White Perch (*M. americana*), (Courtenay & Williams, 2004; Odenkirk & Owens, 2007; Odenkirk & Owens, 2006).

Parental care is provided by both male and females, who care through guarding for 4-9 weeks post-hatch (Lapointe et al., 2012). Nests contain small clutches with large eggs that rapidly hatch in warm waters within 28 hours at temperatures of 31C and 45 hours at 25C (Qin, 2023). High parental care results in an enhanced survival rate of offspring past earlier life stages but may lead to reduced growth of parents (Qin, 2023; Courtenay and Williams, 2004). Northern Snakehead has a high thermal tolerance, is adapted to low dissolved oxygen, and can survive and locomote outside water for prolonged periods if maintained moist. Despite a reportedly low salinity tolerance (>0.6 -1 ppt), (Courtenay & Williams, 2004), Northern Snakehead have been found at salinities of 12 ppt by U.S. Fish and Wildlife, suggesting a wider salinity tolerance. These factors allow for establishing a breeding population and range expansion despite poor water quality and terrestrial boundaries.

The data collected here will be the first time eDNA is used to assess fish and invertebrate diversity in the Middle Branch, establishing a qualitative baseline description of the fish and invertebrate community in the Middle Branch. Monitoring the fish community through eDNA collections will continue for multiple years as the restoration projects continue. The development of the Reel Rewards Program was informed by and created to serve the angling community of South Baltimore by compensating local anglers for the ecological services they provide. Researching the

effects of restoration efforts on not just the fish population but the community of South Baltimore is a vital step in ensuring that the improvements are made with community involvement and consent.

Methods

Water Collections for eDNA

An initial test collection occurred on March 1, 2024, at Trailhead 9 (Table 4; Fig. 14) where two, three, and four liters of water were collected to assess the optimal volume for eDNA collection in the Middle Branch portion of the Patapsco subestuary. It was determined that 2L was ideal for the estuarine environment due to the high presence of non-target eDNA (algae, phytoplankton, and zooplankton) collected at higher volumes and increased filtration time per sample. Water samples were then collected on April 15, 2024 (spring) and July 24, 2024 (summer) for analysis of the presence of fish eDNA and to provide snapshots of spring migration and summer peaks. Two-liter samples were collected with the Smith-Root citizen eDNA sampler (Smith-Root, Inc.) and filtered with Smith-Root glass filters with a pore size of 1.5µm. Samples were collected from five fishing piers in the Middle Branch and one boat launch upstream (Table 4; Fig. 14). Sites were divided into restoration sites (Cherry Hill Park Fishing Pier, Reed Bird Island Fishing Pier, and Middle Branch Trailhead 9 Fishing Pier) and sites adjacent to restoration sites (Middle Branch Park Fishing Pier, Masonville Cove Fishing Pier, Southwest Area Park Boat Ramp). Four samples were collected at each site, with a negative control of store-bought drinking water taken at the start of sampling. All samples were stored on dry ice in a cooler for

transport to the University of Maryland, Baltimore County for short-term storage (80C) and then to the Smithsonian Environmental Research Center (SERC) for extraction and amplification. SERC scientists specializing in the fish and invertebrate community were consulted to select ideal sampling dates to capture seasonal changes in migration and peak density.

Restoration Survey Sites

Cherry Hill Park Pier (hereafter “Cherry Hill”), Reed Bird Island Pier (hereafter “Reed Bird”), and Middle Branch Trailhead 9 Piers (hereafter “Trailhead 9”) were selected as survey sites because they are fishing piers in the Middle Branch and due to proximity to current and planned wetland restoration sites. Reed Bird is on the edge of ongoing wetland restoration. Cherry Hill and Trailhead 9 are neighboring piers to the Reed Bird Island site.

Comparison Sites

Masonville Cove Fishing Pier (hereafter “Masonville Cove”), Middle Branch Park Fishing Pier (hereafter “Middle Branch Park”), and the Southwest Area Park Boat Ramp (hereafter “Southwest”) were selected as comparison sites due to their proximity to survey sites and bordering wetlands. All comparison sites also had fishing piers, allowing consistent water collection methods across all sites.

Masonville Cove could not be sampled in the spring due to seasonal closures for bald eagle nesting.

Metabarcoding

DNA extraction and amplification were conducted at SERC's Coastal Disease Ecology Laboratory under the supervision of Dr. Katrina Lohan and Ruth DiMaria. Smith-Root 1.5um glass filters were cut into quarters and placed in Qiagen PowerWater Bead Pro tubes for genomic DNA extraction using a Qiagen DNeasy PowerWater extraction Kit. Extractions followed the Qiagen DNeasy PowerWater protocol with SERC modifications to maximize DNA eluted from PowerWater Spin Columns. DNA amplification targeted the mitochondrial cytochrome oxidase I sequence (COI) gene, an approximately 310-base-pair (bp) long fragment, using the primers mlCOLintF (Leray et al. 2013) and jgHCO2198R (Geller et al. 2013). Nextera indices were added to the 5' end and degenerate base pairs (0, 1, 2, and 3 bp) were added in equal concentration across all samples. All polymerase chain reactions (PCRs) were performed in triplicate, PCR amplicons were then pooled for each sample corresponding to the brightness of amplified gel bands. After amplicons were pooled, dual indexing with Nextera adapters were performed to attach a unique tag combination to each amplicon allowing for amplicons to be traced back to collection sites. Samples were then bead-cleaned to remove small fragments and quantified. Samples were then pooled into final libraries and were sequenced using MiSeq v3 600 Reagent kit (Illumina) kit on an Illumina MiSeq platform by Laboratories of Analytical Biology of the Smithsonian Natural Museum of Natural History.

Bioinformatics

Primer sequences were removed using cutadapt (Martin 2011). Sequences were then quality trimmed and merged. Chimeras, singletons, and small sequences were removed using DADA2 package (Callahan et al. 2016) in R (R Core Team

2020). Taxonomic identification was assigned to amplicon sequence variants (ASV), which include intraspecies diversity, using COI library from the Chesapeake Bay Barcode Initiative (CBBI; Ogburn et al., BioProjects PRJNA396533 and PRJNA498040) and the National Center for Biotechnology Information (NCBI) for COI sequences. Sequences from negative controls were identified and enumerated using decontam (Callahan et al. 2024), and negative controls were removed. Sequences were then pruned to only Animalia sequences and further pruned to remove mammalian sequences. Animalia sequences were then binned by taxonomic class and analyzed for ASV richness by class and relative sequence abundance by site and season. Additionally, sequences were further pruned to select only Teleost sequences, which were then analyzed for ASV richness and relative sequence abundance by site and season. Box plots of ASV richness using the Chao1 index (Chao 1984) and taxonomic bar plots were calculated in the phyloseq package in R. Chao1 index estimates community richness by relative abundance of individuals in the community, using OTU or ASV amplicons as individuals (CD Genomics 2021). Thus, ASV richness estimates may overestimate species richness, as some species may have more intraspecific diversity in COI. Chao1 Index:

$$S_{chao1} = S_{obs} + \frac{n_1(n_1-1)}{2(n_2+1)}$$

S_{obs} are the number of species observed;
 n_1 is the number of species occurring only once;
 n_2 is the number of species that occur twice.

(CD Genomics
2021)

Results

Alpha Diversity Estimates of ASV Richness

Filtering eDNA Sequences by Taxonomic Rank

A total of 8,028,318 raw reads were generated from 47 samples (including 2 negative controls). This initial total was reduced to 8,026,795 sequences after sequences were filtered and merged and chimeras and singletons were removed, which accounts for 99.9% of initial raw sequences. Sequences were then further filtered for removal of all unicellular sequences and all mammals. After the removal of non-Animalia sequences, 247,862 sequences remained, which comprised 3.08% of the raw sequences. Many of the sequences removed during the Animalia filtering stage were Myzozoa and Fungi. After the removal of mammal sequences, 246,909 sequences remained with 659 ASVs. Animalia sequences with mammals omitted were grouped by taxonomic class and analyzed as Animalia alpha diversity estimates by site and season. Animalia sequences were further filtered for only Teleost species, resulting in 6,349 sequences with 54 ASVs, which represented 0.08% of initial raw reads.

ASV Richness for Animalia Grouped as Class

ASV richness for each site is summarized for Animalia sequences grouped by class (Table 5; Fig. 15; Appendix i) The Southwest site displayed the greatest alpha diversity for all Animalia taxa, with 140 ASVs in a single sample and the greatest mean of 69 ASVs per site with sequences grouped by class. Cherry Hill had the second-greatest alpha diversity of Animalia, with 161 ASVs observed in a single

sample and the second-greatest mean of 52 ASVs. However, samples at this site were variable as the site exhibited the largest range (11-161 ASV). Third, Trailhead 9 ASVs maximum was 89 ASVs with a mean of 45 ASVs, followed by Reed Bird with a maximum of 79 ASVs, a mean of 25 ASVs, and the lowest minimum value of 3 ASVs. Of the comparison sites, Middle Branch Park had a maximum of 67 ASVs in which season and a mean of 30 ASVs. Masonville Cove had the lowest maximum, 21 ASVs in which season, and the lowest mean, with 16 ASVs sequences. Masonville Cove exhibited the narrowest range observed across sites, of 10- 21 ASVs. This narrow range could be explained by the fact that this site was sampled only once, compared to other sites sampled twice.

Relative Sequence Abundance of Animalia Grouped as Class

Here I summarize the relative sequence abundance of Animalia grouped as taxonomic class across site and season (Table 5; Fig. 16; Appendix i). At Cherry Hill in the spring sampling period, the most relatively abundant Animalia species (excluding terrestrial mammals) when grouped by taxonomic class were *Hexanauplia* (67%), *Eurotatoria* (15%), *Clitellata* (6.16%), *Polychaeta* (3%), with *Teleostei* (1%) as the seventh most abundant classes. At this site in the summer sampling period, the most relatively abundant Animalia species were from class *Eurotatoria* (35%), *Branchiopoda* (14%), *Hexanauplia* (10%), and *Bivalvia* (9%), with *Teleostei* (2%) as the eighth most abundant class.

At Masonville Cove in the summer sampling period, the most relatively abundant Animalia species were from class *Branchiopoda* (48%), *Bivalvia* (22%),

Hexanauplia (15%), and *Gymnolaemata* (5%) with *Teleostei* (~0.4%) as the eighth most abundant class.

At Middle Branch Park in the spring sampling period, the most relatively abundant Animalia species were from class *Hexanauplia* (45%), *Teleostei* (17%), *Polychaeta* (17%), *Thecostraca* (15%), and *Clitellata* (2%). At this site in the summer sampling period, the most relatively abundant Animalia species were from class *Chromadorea* (40%), *Eurotatoria* (30%), *Hexanauplia* (7%), and *Branchiopoda* (4%), with *Teleostei* (~4%) as the sixth most abundant class.

At Reed Bird in the spring sampling period, the most relatively abundant Animalia species were from class *Thecostraca* (38%), *Hexanauplia* (37%), *Teleostei* (8%), *Polychaeta* (6%), and *Eurotatoria* (4%). At this site in the summer sampling period, the most relatively abundant Animalia species were from class *Polychaeta* (38%), *Bivalvia* (22%), *Branchiopoda* (11%), and *Gymnolaemata* (11%), with *Teleostei* (10%) as the fifth most abundant class.

At Southwest in the spring sampling period, the most relatively abundant Animalia species were from class *Insecta* (38%), *Eurotatoria* (29%), *Hexanauplia* (18%), and *Clitellata* (6%), with *Teleostei* (1.5%) as the sixth most abundant class. Southwest summer, the most relatively abundant Animalia species were from class *Hexanauplia* (69%), *Branchiopoda* (11%), *Eurotatoria* (8%), and *Gymnolaemata* (4%), with *Teleostei* (~0.6%) as the eighth most abundant class.

At Trailhead 9 in the spring sampling period, the most relatively abundant Animalia species were from class *Thecostraca* (53%), *Hexanauplia* (21%), *Eurotatoria* (10%), *Polychaeta* (6%) with *Teleostei* (~1.5%) as the seventh most

abundant class. At Trailhead 9 in the summer, the most relatively abundant Animalia species were from class *Bivalvia* (28%), *Branchiopoda* (22%), *Gymnolaemata* (21%), *Hexanauplia* (8%) with *Teleostei* (~2.5%) as the eighth most abundant class, and *Chondrichthyes* (~0.01%).

Class *Eurotatoria* (Rotifers) and *Hexanauplia* (Copepods) were detected in all collections, often in high relative abundance (based on number of sequences), and were often observed as the most or second most abundant class in collections. Class *Thecostraca* (Barnacles) was also present in all collections but its relative sequence abundance was much more variable, accounting for (0.02 - 55%) relative sequence abundance. *Teleostei* (Ray-finned fish) were detected at all site samples during spring and summer collections, along with one *Chondrichthyes* (cartilaginous fish) detection at the Trailhead 9 summer collection. *Malacostraca* (crabs, lobsters, isopods) was present at all collections except for the Middle Branch Park spring sampling period, but in extremely low relative sequence abundance (0.08% - 1.3%). *Bivalvia* (oysters, mussels) was present in all collections but ranged from high (28%) to very low (0.3%).

ASV Richness and Diversity of Teleost Species

When estimating alpha diversity by sites, the Southwest site displayed the greatest with a maximum of 16 fish species and a mean of almost 7 fish species across the two sampling periods (Table 6; Fig. 17). Cherry Hill displayed the second highest ASV richness with a maximum of 15 fish species detected and a mean of 4 fish species across the sampling periods. Middle Branch Park has the third highest ASV richness, with 14 fish species detected and a mean of 5 fish species.

Trailhead 9 had a maximum of 11 fish species and a mean of almost 6 fish species. Reed Bird had a maximum ASV richness of 6 fish species and a mean of 4 fish species. Lastly, Masonville Cove had a maximum ASV richness of 3 and a mean of almost 2 fish species. As observed for non-mammalian Animalia taxa, Masonville Cove has the lowest mean and narrowest range, likely because it was only sampled once. However, all sites except Reed Bird had the same lowest minimum ASV richness value of one fish species detected in at least one sample. Considering that the Reed Bird site had the highest minimum value for fish ASV richness, it could suggest a unique attribute that can sustain the highest minimum ASV richness.

Relative Sequence Abundance of Teleost Species

The Cherry Hill spring sample contained 491 ASV Animalia sequences and detected 10 Teleost species (Table 7; Table 8; Table 9; Fig. 18). The most relatively abundant *Teleost* species in this sample were *Engraulis mordax* (45.8%), *Notropis hudsonius* (25.3%), *Dorosoma cepedianum* (8.1%), *Ameiurus nebulosus* (5.9%), *Cyprinus carpio* (5.1%), *Menidia menidia* (3.3%), *Lepomis macrochirus* (2.2%), *Perca flavescens* (1.6%), *Luxilus cornutus* (1.4%), and *Carassius auratus* (1.2%). At this site in the summer sample, 166 ASV sequences were amplified, including 4 Teleost species. The most relatively abundant Teleost species were *Engraulis mordax* (42.2%), *Menidia menidia* (32.5%), *Dorosoma cepedianum* (24.1%), *Leiostomus xanthurus* (1.2%).

The Masonville Cove summer sample contained 57 ASV 5 Teleost species, with the most relatively abundant Teleost species being *Brevoortia tyrannus* (52.6%),

Notropis hudsonius (21.1%), *Morone saxatilis* (15.8%), *Leiostomus xanthurus* (5.3%) and *Menidia menidia* (5.3%).

The Middle Branch Park spring sample contained 159 sequences and detected 1 Teleost species, *Engraulis mordax* (relative sequence abundance 100%). The Middle Branch Park Summer sample yielded 1,137 amplified sequences, of which 10 were Teleost species. Relative sequence abundance of those Teleost species were *Dorosoma cepedianum* (30.6%), *Ameiurus nebulosus* (12.9%), *Menidia menidia* (12.4%), *Engraulis mordax* (12.1%), *Carassius auratus* (9.9%), *Lepomis macrochirus* (7%), *Leiostomus xanthurus* (4.6%), *Brevoortia tyrannus* (3.6%), *Notropis hudsonius* (3.6%), *Micropogonias undulatus* (3%).

The Reed Bird spring sample contained 637 sequences and detected 4 Teleost species, with the most relatively abundant Teleost species being *Menidia menidia* (68.6%), *Engraulis mordax* (30.6%), *Ameiurus nebulosus* (0.5%) and *Brevoortia tyrannus* (0.3%). The Reed Bird summer sample exhibited the most Animalia sequences of all sites and seasons, with 1,805 sequences amplified and 7 Teleost species. The most relatively abundant Teleost species at Reed Bird in the summer were *Menidia menidia* (70.7%), *Fundulus heteroclitus* (18.5%), *Engraulis mordax* (5%), *Leiostomus xanthurus* (2.5%), *Fundulus majalis* (2.2%), *Dorosoma cepedianum* (0.9%), and *Brevoortia tyrannus* (0.2%).

The Southwest spring sample contained 831 sequences and detected 10 Teleost species. The most relatively abundant Teleost species at this site and season were *Notropis hudsonius* (33.8%), *Engraulis mordax* (16.7%), *Ameiurus nebulosus* (15.4%), *Dorosoma cepedianum* (14.2%), *Hypentelium nigricans* (4.8%), *Carassius auratus* (4.2%), *Catostomus commersonii* (4.1%), *Menidia menidia* (3.4%), *Fundulus*

heteroclitus (1.7%), and *Gambusia holbrooki* (1.7%). The Southwest summer sample contained 134 samples and detected 1 Teleost species, *Engraulis mordax* (relative sequence abundance 100%).

The Trailhead 9 spring sample contained 512 sequences and detected 10 Teleost species. Here, the most relatively abundant Teleost species were *Engraulis mordax* (63.9%), *Notropis hudsonius* (14.5%), *Dorosoma cepedianum* (5.5%), *Fundulus heteroclitus* (4.5%), *Menidia menidia* (3.7%), *Ameiurus nebulosus* (3.1%), *Alosa sapidissima* (1.4%), *Catostomus commersonii* (1.4%), *Carassius auratus* (1.2%), and *Lepomis* sp. (1%). The Trailhead 9 summer sample contained 300 sequences and detected 10 Teleost species. Here, the most relatively abundant Teleost species were *Engraulis mordax* (53.7%), *Brevoortia tyrannus* (10.7%), *Menidia menidia* (10.7%), *Fundulus heteroclitus* (7%), *Micropogonias undulatus* (4.7%), *Dorosoma cepedianum* (4%), *Leiostomus xanthurus* (3.7%), *Cyprinodon variegatus* (2.3%), *Lepomis macrochirus* (2%), and *Notropis hudsonius* (1.3%).

The most common fish species across the site-season combinations were *Menidia menidia* (Atlantic Silverside) and *Engraulis mordax* (Pacific Anchovy), which were found at nearly all sites during both spring and summer collections (Table 7; Table 8; Table 9; Fig. 18). *Engraulis mordax* was present at all sites and seasons except Masonville Cove. *M. menidia* was at all sites and seasons except for Middle Branch Park in the spring and Southwest in the summer and was the most abundant species at Reed Bird in both spring and summer. Several other species, like *Dorosoma cepedianum* (Gizzard Shad), *Leiostomus xanthurus* (Spot), and *Notropis hudsonius* (Spottail Shiner), were commonly detected across multiple locations.

Discussion

I investigated how effective eDNA was in achieving three goals: (1) assessing the presence or absence of invasive Blue Catfish (*Ictalurus furcatus*), Flathead Catfish (*Pylodictis Olivares*), and Northern Snakehead (*Channa argus*) in the Middle Branch estuary, (2) estimating ASV richness of Teleost fish, and (3) creating a baseline of the Teleost fish community relative sequence abundance for COI. Metabarcoding of COI did not detect any invasive fish species at the study sites. However, eDNA metabarcoding of the COI gene was found to be highly effective at detecting multiple fish species and Animalia taxa across collection sites and seasons. The presence of the COI gene across taxa provides species-level resolution across vertebrate and invertebrate taxa. These data have provided the most detailed assessment of fish composition in the Middle Branch and summary of the aquatic animalia community in this location to date.

Summary of Metabarcoding

Environmental DNA collections revealed that the sequence abundance of Animalia species was higher during the spring when compared to summer collections at most sites. Spring samples from Cherry Hill, Southwest, and Trailhead 9 all had substantially higher relative sequence abundance of Animalia sequences compared to their summer collections. However, Reed Bird and Middle Branch Park both exhibited low Animalia sequence abundance in the spring and higher sequence abundance during the summer. Interestingly the same trend was observed with fish sequence abundance at Reed Bird and Middle Branch Park, with fish sequence abundance accounting for most of the summer sequence abundance. Relatively low

fish sequence abundance was observed at all other sites when comparing summer relative sequence abundance to spring. I hypothesize that Southwest, Cherry Hill, and Trailhead 9 exhibit environmental attributes in the spring making the site more habitable for invertebrate communities which in turn supports the fish community at these sites. In contrast, Middle Branch Park and Reed Bird possess environmental attributes in the summer making them habitable for certain fish species.

Southwest was a unique site when compared to the other sites sampled. Southwest was the farthest from the estuary, situated up the Patapsco River and on the margin of a wetland with many bird and insect species observed during sampling. The high relative sequence abundance of Animalia sequences at this site seems unsurprising in an ecosystem supporting a noteworthy insect and avian population. Cherry Hill was the closest site to Southwest and I hypothesize that there is some level of connectivity between these two sites. Cherry Hill also was not located in the estuary but in a shallow wide region of the Patapsco River just upriver from the estuary. I expected that Cherry Hill would have low ASV richness due to a lack of habitat complexity and shallow depths. My hypothesis may be correct as low fish sequence abundance was detected for the Cherry Hill summer collection, however summer algae blooms may have resulted in low species detection of Animalia at Cherry Hill. Trailhead 9 was a deeper water site, positioned on the shore where the Patapsco River meets the estuary. Trailhead 9 was expected to have connectivity to the neighboring sites of Cherry Hill situated upriver and Reed Bird which was directly across from Trailhead 9. I expected that Trailhead 9 site sequence abundance would be a “Goldilocks” site, with intermediate sequence abundance between the upriver Southwest site and Middle Branch Park’s urbanized estuary site. I also

expected to detect spring fish migrations upriver from Trailhead 9 to Cherry Hill and possibly at Southwest, which was observed in some species.

The habitat at Reed Bird appeared to be most similar to Cherry Hill, as Reed Bird was at the end of the same section of wide shallow river channel as Cherry Hill. Reed Bird was on a section of hardened shoreline under a road overpass and although supporting shorebirds seemed an unlikely fish habitat. Many factors could explain the high summer sequence abundance of fish sequences at the site. It may be that the shallow slow-moving habitat is used as summer spawning grounds as the site had low ASV richness (n=4) but extremely high relative sequence abundance. Alternatively, the shallower water resulted in improved eDNA collected as the surface waters and benthic sources of DNA are both sampled. Another explanation for the high summer sequence abundance was the presence of the aforementioned shorebirds. Water samples were collected downriver from shorebirds, and it is possible that their fecal matter contained high amounts of fish DNA. Reed Bird is the first site for shoreline wetland construction, and eDNA results suggest that the site has a high sequence abundance of Atlantic Killifish (*Menidia menidia*) and Mummichog (*Fundulus heteroclitus*).

Middle Branch Park was the most popular fishing spot of all sites sampled and displayed the highest Shannon-weaver diversity and evenness indices. Middle Branch Park is the most urbanized site and is situated the farthest from the Patapsco River in the heart of the Middle Branch. Middle Branch Park is a moderate depth with muddy sediments and a living shoreline, and the site supports a moderate population of aquatic bird species. Summer eDNA collections indicate that Middle Branch Park supports a diverse Animalian and Teleost community. The productive invertebrate

community at Middle Branch Park, observed in Animalia collections, could be explained by moderate water depth allowing connectivity between the benthic and pelagic food webs (Woodland et al., 2021). Also, Middle Branch Park had a complexity of habitats with a rocky shoreline, human-made structures (piers, docks, and marina), and muddy benthos providing a variety of niches and structures for sessile invertebrates to settle upon.

Migratory Fishes

Spring sample was timed to capture spring migrations of fish species moving up the Patapsco River when abundance was expected to be high. Multiple migratory fishes were detected only during the spring indicating that the April collection partially captured fish migrations. Migratory American Shad (*Alosa sapidissima*), American Eel (*Anguilla rostrata*), and White Perch (*Morone americana*) were detected only during the spring, with Yellow Perch (*Perca flavescens*) found at more sites during the spring. Striped Bass (*Morone saxatilis*), another migratory fish, was only detected in the summer, at Masonville Cove, but this site had no spring sample with which to compare abundances across seasons. Migrating river herring (Blueback Herring (*Alosa aestivalis*) and Alewife (*Alosa pseudoharengus*)) were not detected at any sites sampled. Atlantic Menhaden (*Brevoortia tyrannus*) postspawning summer migration was observed in eDNA collections with the fish detected at more sites during the summer than in the spring.

Estuary Resident Fishes

Many resident fishes of the Patapsco River and estuary were detected in eDNA samples. A high summer relative sequence abundance of Gizzard Shad

(*Dorosoma cepedianum*) could indicate spawning occurring at Middle Branch Park or that the species has high intraspecies diversity in sequences of the COI gene. High summer relative sequence abundance estuary resident Atlantic Killifish (*Menidia menidia*), Gizzard Shad (*Dorosoma cepedianum*), and Mummichog (*Fundulus heteroclitus*) at Reed Bird in the summer sample could indicate summer reproduction or high intraspecies sequence diversity across COI. Surprisingly only one species of catfish was detected, the Brown Bullhead Catfish (*Ameiurus nebulosus*). This is surprising since anglers have reported catching multiple catfish species in the Middle Branch as indicated in angler interviews, and I expected to detect the diversity of catfish species in the Middle Branch.

Invasive Fish Species Detection

Despite no detections of invasive fish in the Middle Branch by eDNA metabarcoding, Reel Rewards anglers catch documents of a high relative sequence abundance of invasive fish species present (Chapter 1). Reel Rewards anglers removed Northern Snakehead (n = 256) and Blue Catfish (n = 37) documenting that these fish are present in the Middle Branch during water collections. Flathead Catfish were absent from any Reel Rewards collections and are a rarely detected species in the estuary as the average salinity is not tolerable to the species. The lack of properly identified Flathead Catfish species by Reel Rewards anglers coupled with the low salinity tolerance of this species, indicate that the species was likely not present during water collection, and thus was not detected.

Multiple factors could explain why invasive Northern Snakehead and Blue Catfish were not detected in the Middle Branch estuary during spring and summer

sampling. I hypothesize that the COI gene's broad taxonomic application likely decreased the proportion of amplified sequences from targeted Teleost species. A high relative sequence abundance of non-targeted species sequences amplifying (Fungi, Bacteria, and Archaea) was also observed in other studies (Barnes et al., 2020). Here, Teleost sequences comprised only 0.08% of total sequences, with Animalia sequences faring slightly better at 3.08%. Summer algae blooms are another factor to consider, as algae blooms were present at most summer sites sampled, clogging filters and contributing to a high proportion of non-Animalia sequences. Filter size was carefully considered to account for sampling eutrophic water, to ensure that filter pore size was fine enough to capture DNA (0.45um-1.5um) but coarse enough to accommodate for high summer algae. Water samples were collected slightly (2'-5' inches) lower in the water column at allowable sites to attempt to collect from below the algae bloom.

Another factor that could explain the low detection of invasive fish species and lower-than-expected catfish numbers is where the water is collected. Detection of different fish and invertebrate assemblages by eDNA may depend on where in the water column samples are collected. Most fishes are detected in eDNA samples from surface water (unpubl. data), but a few benthic species were only detected in collections from the lower water column. eDNA behaves like a particle and also a dissolved solute in aquatic environments suggesting uneven mixing and dispersal (Shogren et al., 2017). Environmental DNA sources (e.g., tissues, feces) can travel down a river or settle in lakes. Water samples were collected from surface waters from approximately 4 in- 12in adjusting for summer algae blooms, for sampling logistics and repeatability. The three invasive fish species as well as the Patapsco

catfish are mostly associated with benthic environments with vegetation. It may be that fish were detected in low relative sequence abundance and lower ASV richness than expected because fish cells accounted for a smaller proportion in contrast to the diversity of the invertebrate communities inhabiting the surface at the shoreline and piers.

Future eDNA studies should collect ecological metadata on water and habitat parameters to better interpret the ecological factors that can affect detection, such as water visibility, dissolved oxygen, water depth, as well as habitat complexity, and benthic substrate. Future research on invasive fish species should also consider quantitative eDNA for single species detection to complement metabarcoding of 12s, 18s, or COI for broader taxonomic community composition. Amplification of DNA samples was initially conducted with Mifish mitochondrial 12S ribosomal DNA primer. MiFish primer only amplifies Teleost species, making it the ideal primer for research on fish community and invasive fish detection and addressing the issue of a high relative sequence abundance of non-target taxon amplification. However, MiFish primer required extensive troubleshooting and manual cutting amplification bands out of agarose gels. For these reasons, a pivot was made away from the 12s Mifish primer to COI. Estimating species diversity for animal taxa will be valuable research for the community of South Baltimore, especially community-valued bivalves. Water quality improvements in the Middle Branch have partially been attributed to increased populations of bivalves and other filter-feeding species. ASV richness estimates and relative sequence abundance of Teleost and other Animalia species will serve as a baseline for the Middle Branch aquatic community. Despite no detection of invasive Blue Catfish (*I. furcatus*), Flathead Catfish (*P. olivares*), and

Northern Snakehead (*C. argus*), the COI metabarcoding conducted here provided insight into the invertebrate community for shareholders who are interested in increasing knowledge of the ecosystem as a whole.

Appendices

Appendix i. ASV relative abundance of Animalia (excluding terrestrial mammals) grouped by taxonomic class across site and season.

Site	Season	Class	Relative Abundance
Cherry Hill Park Pier	Spring	Hydrozoa	0.0110
Cherry Hill Park Pier	Spring	Echinamoebida	0.0199
Cherry Hill Park Pier	Spring	Flabellinia	0.0199
Cherry Hill Park Pier	Spring	Demospongiae	0.0442
Cherry Hill Park Pier	Spring	Thecostraca	0.0486
Cherry Hill Park Pier	Spring	Testudines	0.0729
Cherry Hill Park Pier	Spring	Himatismenida	0.0773
Cherry Hill Park Pier	Spring	Malacostraca	0.0884
Cherry Hill Park Pier	Spring	Arachnida	0.1038
Cherry Hill Park Pier	Spring	Gymnolaemata	0.2385
Cherry Hill Park Pier	Spring	Bivalvia	0.4926
Cherry Hill Park Pier	Spring	Gastropoda	0.5036

Cherry Hill Park Pier	Spring	Teleostei	1.1176
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Cherry Hill Park Pier	Spring	Insecta	1.2480
Cherry Hill Park Pier	Spring	Branchiopoda	3.2425
Cherry Hill Park Pier	Spring	Polychaeta	3.5385
Cherry Hill Park Pier	Spring	Clitellata	6.1691
Cherry Hill Park Pier	Spring	Eurotatoria	15.5807
Cherry Hill Park Pier	Spring	Hexanauplia	67.3830
Cherry Hill Park Pier	Summer	Tentaculata	0.0344
Cherry Hill Park Pier	Summer	Arachnida	0.0516
Cherry Hill Park Pier	Summer	Hydrozoa	0.0688
Cherry Hill Park Pier	Summer	Thecostraca	0.4298
Cherry Hill Park Pier	Summer	Malacostraca	0.8597
Cherry Hill Park Pier	Summer	Insecta	1.3239
Cherry Hill Park Pier	Summer	Teleostei	2.8542
Cherry Hill Park Pier	Summer	Gastropoda	7.8576

Cherry Hill Park Pier	Summer	Polychaeta	8.4250
Cherry Hill Park Pier	Summer	Gymnolaemata	8.5282

Cherry Hill Park Pier	Summer	Bivalvia	9.2847
Cherry Hill Park Pier	Summer	Hexanauplia	10.4195
Cherry Hill Park Pier	Summer	Branchiopoda	14.3054
Cherry Hill Park Pier	Summer	Eurotatoria	35.5571
Masonville Cove Pier	Summer	Anthozoa	0.0140
Masonville Cove Pier	Summer	Gastropoda	0.0140
Masonville Cove Pier	Summer	Rhabditophora	0.1049
Masonville Cove Pier	Summer	Malacostraca	0.1468
Masonville Cove Pier	Summer	Insecta	0.2656
Masonville Cove Pier	Summer	Teleostei	0.3984
Masonville Cove Pier	Summer	Polychaeta	1.4889
Masonville Cove Pier	Summer	Eurotatoria	2.7261
Masonville Cove Pier	Summer	Thecostraca	3.8655

Masonville Cove Pier	Summer	Gymnolaemata	4.9979
Masonville Cove Pier	Summer	Hexanauplia	15.6927
Masonville Cove Pier	Summer	Bivalvia	22.1376

Masonville Cove Pier	Summer	Branchiopoda	48.1476
Middle Branch Park Pier	Spring	Bivalvia	0.4315
Middle Branch Park Pier	Spring	Gymnolaemata	0.4315
Middle Branch Park Pier	Spring	Gastropoda	0.5394
Middle Branch Park Pier	Spring	Arachnida	1.2945
Middle Branch Park Pier	Spring	Insecta	1.4024
Middle Branch Park Pier	Spring	Clitellata	2.1575
Middle Branch Park Pier	Spring	Thecostraca	14.7789
Middle Branch Park Pier	Spring	Polychaeta	16.9364
Middle Branch Park Pier	Spring	Teleostei	17.1521
Middle Branch Park Pier	Spring	Hexanauplia	44.8759
Middle Branch Park Pier	Summer	Longamoebia	0.0067

Middle Branch Park Pier	Summer	Himatismenida	0.0101
Middle Branch Park Pier	Summer	Testudines	0.0303
Middle Branch Park Pier	Summer	Ostracoda	0.0505
Middle Branch Park Pier	Summer	Phylactolaemata	0.0674

Middle Branch Park Pier	Summer	Malacostraca	0.3570
Middle Branch Park Pier	Summer	Thecostraca	0.4210
Middle Branch Park Pier	Summer	Bivalvia	0.4278
Middle Branch Park Pier	Summer	Clitellata	0.7747
Middle Branch Park Pier	Summer	Gymnolaemata	0.9128
Middle Branch Park Pier	Summer	Insecta	1.1250
Middle Branch Park Pier	Summer	Gastropoda	1.1587
Middle Branch Park Pier	Summer	Collembola	1.8660
Middle Branch Park Pier	Summer	Polychaeta	3.1089
Middle Branch Park Pier	Summer	Teleostei	3.9375
Middle Branch Park Pier	Summer	Hydrozoa	4.1093

Middle Branch Park Pier	Summer	Branchiopoda	4.4932
Middle Branch Park Pier	Summer	Hexanauplia	6.6826
Middle Branch Park Pier	Summer	Eurotatoria	30.8498
Middle Branch Park Pier	Summer	Chromadorea	39.6106
Reed Bird Island Pier	Spring	Hydrozoa	0.0399

Reed Bird Island Pier	Spring	Gastropoda	0.3321
Reed Bird Island Pier	Spring	Gymnolaemata	0.3321
Reed Bird Island Pier	Spring	Arachnida	0.6111
Reed Bird Island Pier	Spring	Bivalvia	0.6510
Reed Bird Island Pier	Spring	Insecta	0.7706
Reed Bird Island Pier	Spring	Branchiopoda	0.8636
Reed Bird Island Pier	Spring	Clitellata	1.1293
Reed Bird Island Pier	Spring	Malacostraca	1.3153
Reed Bird Island Pier	Spring	Eurotatoria	4.6765
Reed Bird Island Pier	Spring	Polychaeta	6.0316

Reed Bird Island Pier	Spring	Teleostei	8.4629
Reed Bird Island Pier	Spring	Hexanauplia	36.8540
Reed Bird Island Pier	Spring	Thecostraca	37.9301
Reed Bird Island Pier	Summer	Clitellata	0.0112
Reed Bird Island Pier	Summer	Echinamoebida	0.0112
Reed Bird Island Pier	Summer	Arachnida	0.0224

Reed Bird Island Pier	Summer	Testudines	0.0336
Reed Bird Island Pier	Summer	Tentaculata	0.0561
Reed Bird Island Pier	Summer	Rhabditophora	0.0617
Reed Bird Island Pier	Summer	Gastropoda	0.3924
Reed Bird Island Pier	Summer	Malacostraca	0.5326
Reed Bird Island Pier	Summer	Insecta	0.5382
Reed Bird Island Pier	Summer	Eurotatoria	0.5887
Reed Bird Island Pier	Summer	Thecostraca	1.4128
Reed Bird Island Pier	Summer	Hexanauplia	4.3561

Reed Bird Island Pier	Summer	Teleostei	10.1194
Reed Bird Island Pier	Summer	Gymnolaemata	11.1286
Reed Bird Island Pier	Summer	Branchiopoda	11.3528
Reed Bird Island Pier	Summer	Bivalvia	21.1975
Reed Bird Island Pier	Summer	Polychaeta	38.1847
Southwest Boat Launch Pier	Spring	Chaetonotida	0.0107
Southwest Boat Launch Pier	Spring	Eutardigrada	0.0107

Southwest Boat Launch Pier	Spring	Hyperoartia	0.0196
Southwest Boat Launch Pier	Spring	Malacostraca	0.0214
Southwest Boat Launch Pier	Spring	Thecostraca	0.0267
Southwest Boat Launch Pier	Spring	Amphibia	0.0303
Southwest Boat Launch Pier	Spring	Himatismenida	0.0445
Southwest Boat Launch Pier	Spring	Flabellinia	0.0623
Southwest Boat Launch Pier	Spring	Gymnolaemata	0.0623
Southwest Boat Launch Pier	Spring	Arachnida	0.0801

Southwest Boat Launch Pier	Spring	Demospongiae	0.1762
Southwest Boat Launch Pier	Spring	Hydrozoa	0.2261
Southwest Boat Launch Pier	Spring	Bivalvia	0.3774
Southwest Boat Launch Pier	Spring	Gastropoda	0.5643
Southwest Boat Launch Pier	Spring	Branchiopoda	0.6604
Southwest Boat Launch Pier	Spring	Teleostei	1.5719
Southwest Boat Launch Pier	Spring	Polychaeta	5.4615
Southwest Boat Launch Pier	Spring	Clitellata	5.6004

Southwest Boat Launch Pier	Spring	Hexanauplia	17.6983
Southwest Boat Launch Pier	Spring	Eurotatoria	28.9915
Southwest Boat Launch Pier	Spring	Insecta	38.3035
Southwest Boat Launch Pier	Summer	Malacostraca	0.0606
Southwest Boat Launch Pier	Summer	Rhabditophora	0.1025
Southwest Boat Launch Pier	Summer	Teleostei	0.6246
Southwest Boat Launch Pier	Summer	Polychaeta	1.2911

Southwest Boat Launch Pier	Summer	Thecostraca	2.4704
Southwest Boat Launch Pier	Summer	Bivalvia	3.2768
Southwest Boat Launch Pier	Summer	Gymnolaemata	3.6730
Southwest Boat Launch Pier	Summer	Eurotatoria	8.4367
Southwest Boat Launch Pier	Summer	Branchiopoda	10.7812
Southwest Boat Launch Pier	Summer	Hexanauplia	69.2831
Trailhead 9 Pier	Spring	Flabellinia	0.0084
Trailhead 9 Pier	Spring	Hydrozoa	0.0362
Trailhead 9 Pier	Spring	Malacostraca	0.0612

Trailhead 9 Pier	Spring	Demospongiae	0.0668
Trailhead 9 Pier	Spring	Arachnida	0.0835
Trailhead 9 Pier	Spring	Chromadorea	0.0863
Trailhead 9 Pier	Spring	Gymnolaemata	0.1113
Trailhead 9 Pier	Spring	Gastropoda	0.1392
Trailhead 9 Pier	Spring	Bivalvia	0.3563

Trailhead 9 Pier	Spring	Insecta	1.0298
Trailhead 9 Pier	Spring	Teleostei	1.4780
Trailhead 9 Pier	Spring	Clitellata	2.0124
Trailhead 9 Pier	Spring	Branchiopoda	4.4895
Trailhead 9 Pier	Spring	Polychaeta	6.0621
Trailhead 9 Pier	Spring	Eurotatoria	10.4375
Trailhead 9 Pier	Spring	Hexanauplia	20.7526
Trailhead 9 Pier	Spring	Thecostraca	52.7889
Trailhead 9 Pier	Summer	Chondrichthyes	0.0167
Trailhead 9 Pier	Summer	Tentaculata	0.0167
Trailhead 9 Pier	Summer	Insecta	0.5261
Trailhead 9 Pier	Summer	Malacostraca	0.6931
Trailhead 9 Pier	Summer	Gastropoda	1.3026
Trailhead 9 Pier	Summer	Teleostei	2.5217
Trailhead 9 Pier	Summer	Thecostraca	2.9476

Trailhead 9 Pier	Summer	Eurotatoria	5.8200
Trailhead 9 Pier	Summer	Polychaeta	7.2478
Trailhead 9 Pier	Summer	Hexanauplia	7.8574
Trailhead 9 Pier	Summer	Gymnolaemata	20.7665
Trailhead 9 Pier	Summer	Branchiopoda	22.0608
Trailhead 9 Pier	Summer	Bivalvia	28.2231

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Table 4. Water collection sample sites for eDNA in the Middle Branch.

Site	GPS coordinates
Cherry Hill Fishing Pier	39°14'44.9", 76°36'49.4"W
Masonville Cove Fishing Pier	39°14'04.9"N 76°37'21.9"W
Middle Branch Park Fishing Pier	39.256061, -76.621522
Reed Bird Island Fishing Pier	39°14'48.5"N 76°36'39.2"W
Southwest Area Park Boat Ramp	39°14'04.9"N 76°37'21.9"W
Middle Branch Trailhead 9 Fishing Piers	39.248023, -76.611607

Table 5. Chao1 diversity index for ASV of Animalia species, grouped by taxonomic class at six sample sites, with terrestrial mammalian taxa excluded.

Location	Mean Chao1 Diversity	Min sequences	Max sequences
Cherry Hill Park Pier	52	11	161
Masonville Cove Fishing Pier	16.7	10	21
Middle Branch Park Fishing Pier	30	6	67
Reed Bird Island Fishing Pier	25.3	3	79
Southwest Area Park Boat Ramp	69.5	11	140
Trailhead 9 Fishing Piers	45.6	14	89

Table 6. Chao1 diversity index for community richness for ASV of Teleost species at sample sites.

Location	Mean Chao1 ASV Diversity	Min	Max
Cherry Hill Park Pier	4.3	1	15
Masonville Cove Fishing Pier	1.7	1	3
Middle Branch Park Fishing Pier	5.4	1	14
Reed Bird Island Fishing Pier	4.0	2	6
Southwest Area Park Boat Ramp	6.8	1	16
Trailhead 9 Fishing Piers	5.8	1	11

Table 7. ASV relative sequence abundance of Teleost by site for Spring collection.

	April 2024				
Taxon	Cherry Hill Park Pier	Middle Branch Park Pier	Reed Bird Island Pier	Southwest Boat Launch Pier	Trailhead 9 Pier
<i>Alosa sapidissima</i>	0	0	0	0	7
<i>Ameiurus nebulosus</i>	29	0	3	128	16
<i>Anguilla rostrata</i>	5	0	0	0	0
<i>Brevoortia tyrannus</i>	0	0	2	10	0
<i>Carassius auratus</i>	6	0	0	35	6
<i>Carpionodes cyprinus</i>	5	0	0	12	0
<i>Catostomus commersonii</i>	0	0	0	34	7
<i>Cyprinella analostrana</i>	0	0	0	4	0
<i>Cyprinodon variegatus</i>	0	0	0	0	0
<i>Cyprinus carpio</i>	25	0	0	0	0
<i>Dorosoma cepedianum</i>	40	0	0	118	28
<i>Engraulis mordax</i>	225	159	195	139	327
<i>Fundulus heteroclitus</i>	0	0	0	14	23
<i>Fundulus majalis</i>	0	0	0	0	0
<i>Fundulus waccamensis</i>	2	0	0	2	3
<i>Gambusia holbrooki</i>	0	0	0	14	0
<i>Hypentelium nigricans</i>	0	0	0	40	0
<i>Leiostomus xanthurus</i>	0	0	0	0	0
<i>Lepomis macrochirus</i>	11	0	0	0	0

Lepomis sp	0	0	0	0	5
Lucania parva	0	0	0	0	5
Luxilus cornutus	7	0	0	0	3
Menidia menidia	16	0	437	28	19
Micropogonias undulatus	0	0	0	0	0
Morone americana	0	0	0	5	3
Morone saxatilis	0	0	0	0	0
Nocomis micropogon	3	0	0	3	0
Notropis hudsonius	124	0	0	281	74
Notropis procne	0	0	0	7	0
Perca flavescens	8	0	0	5	5
Rhinichthys cataractae	0	0	0	4	0
Channa argus	0	0	0	0	0
Ictalurus furcatus	0	0	0	0	0
Pylodictis olivaris	0	0	0	0	0

Table 8 .ASV relative sequence abundance of Teleost by site for Summer collection.

	July 2024					
Taxon	Cherry Hill Park Pier	Middle Branch Park Pier	Reed Bird Island Pier	Southwest Boat Launch Pier	Trailhead 9 Pier	Masonville Cove Pier
<i>Alosa sapidissima</i>	0	0	0	0	0	0
<i>Ameiurus nebulosus</i>	0	147	0	0	2	0
<i>Anguilla rostrata</i>	0	0	0	0	0	0
<i>Brevoortia tyrannus</i>	0	41	3	0	32	30
<i>Carassius auratus</i>	0	113	0	0	0	0
<i>Carpiodes cyprinus</i>	0	0	0	0	0	0
<i>Catostomus commersonii</i>	0	0	0	0	0	0
<i>Cyprinella analostana</i>	0	0	0	0	0	0
<i>Cyprinodon variegatus</i>	0	0	0	0	7	0
<i>Cyprinus carpio</i>	0	13	0	0	0	0
<i>Dorosoma cepedianum</i>	40	348	16	0	12	0
<i>Engraulis mordax</i>	70	138	90	134	161	0
<i>Fundulus heteroclitus</i>	0	5	334	0	21	0
<i>Fundulus majalis</i>	0	0	40	0	0	0
<i>Fundulus waccamensis</i>	0	0	0	0	0	0
<i>Gambusia holbrooki</i>	0	3	0	0	0	0
<i>Hypentelium nigricans</i>	0	0	0	0	0	0

Leiostomus xanthurus	2	53	45	0	11	3
Lepomis macrochirus	0	80	0	0	6	0
Lepomis sp	0	0	0	0	0	0
Lucania parva	0	0	0	0	0	0
Luxilus cornutus	0	0	0	0	0	0
Menidia menidia	54	141	1277	0	32	3
Micropogonias undulatus	0	35	0	0	14	0
Morone americana	0	0	0	0	0	0
Morone saxatilis	0	0	0	0	0	9
Nocomis micropogon	0	0	0	0	0	0
Notropis hudsonius	0	41	0	0	4	12
Notropis procne	0	0	0	0	0	0
Perca flavescens	0	11	0	0	0	0
Rhinichthys cataractae	0	0	0	0	0	0
Channa argus	0	0	0	0	0	0
Ictalurus furcatus	0	0	0	0	0	0
Pylodictis olivaris	0	0	0	0	0	0

Table 9. Heatmap for Teleost species total sequence abundance by site and season. ASV relative sequence abundance heatmap of Teleost species by site for Spring and Summer.

	April 2024					Jul-24					
Taxon	Cherry Hill Park Pier	Middle Br.	Reed Bird	Southwes	Trailhead 9	Cherry Hill Park Pier	Middle Br.	Reed Bird	Southwes	Trailhead	Masonville
<i>Alosa sapidissima</i>	0	0	0	0	7	0	0	0	0	0	0
<i>Ameiurus nebulosus</i>	29	0	3	128	16	0	147	0	0	2	0
<i>Anguilla rostrata</i>	5	0	0	0	0	0	0	0	0	0	0
<i>Brevoortia tyrannus</i>	0	0	2	10	0	0	41	3	0	32	30
<i>Carassius auratus</i>	6	0	0	35	6	0	113	0	0	0	0
<i>Carpodius cyprinus</i>	5	0	0	12	0	0	0	0	0	0	0
<i>Catostomus commersoni</i>	0	0	0	34	7	0	0	0	0	0	0
<i>Cyprinella analostana</i>	0	0	0	4	0	0	0	0	0	0	0
<i>Cyprinodon variegatus</i>	0	0	0	0	0	0	0	0	0	7	0
<i>Cyprinus carpio</i>	25	0	0	0	0	0	13	0	0	0	0
<i>Dorosoma cepedianum</i>	40	0	0	118	28	40	348	16	0	12	0
<i>Engraulis mordax</i>	225	159	195	139	327	70	138	90	134	161	0
<i>Fundulus heteroclitus</i>	0	0	0	14	23	0	5	334	0	21	0
<i>Fundulus majalis</i>	0	0	0	0	0	0	0	40	0	0	0
<i>Fundulus waccamensis</i>	2	0	0	2	3	0	0	0	0	0	0
<i>Gambusia holbrooki</i>	0	0	0	14	0	0	3	0	0	0	0
<i>Hypentelium nigricans</i>	0	0	0	40	0	0	0	0	0	0	0
<i>Leiostomus xanthurus</i>	0	0	0	0	0	2	53	45	0	11	3
<i>Lepomis macrochirus</i>	11	0	0	0	0	0	80	0	0	6	0
<i>Lepomis sp.</i>	0	0	0	0	5	0	0	0	0	0	0
<i>Lucania parva</i>	0	0	0	0	5	0	0	0	0	0	0
<i>Luxilus cornutus</i>	7	0	0	0	3	0	0	0	0	0	0
<i>Menidia menidia</i>	16	0	437	28	19	54	141	1277	0	32	3
<i>Micropogonias undulatus</i>	0	0	0	0	0	0	35	0	0	14	0
<i>Morone americana</i>	0	0	0	5	3	0	0	0	0	0	0
<i>Morone saxatilis</i>	0	0	0	0	0	0	0	0	0	0	9
<i>Nocomis biguttatus</i>	3	0	0	3	0	0	0	0	0	0	0
<i>Notropis hudsonius</i>	124	0	0	281	74	0	41	0	0	4	12
<i>Notropis procerus</i>	0	0	0	7	0	0	0	0	0	0	0
<i>Perca flavescens</i>	8	0	0	5	5	0	11	0	0	0	0
<i>Rhinichthys cataractae</i>	0	0	0	4	0	0	0	0	0	0	0
<i>Channa argus</i>	0	0	0	0	0	0	0	0	0	0	0
<i>Ictalurus furcatus</i>	0	0	0	0	0	0	0	0	0	0	0
<i>Pylodictis olivaris</i>	0	0	0	0	0	0	0	0	0	0	0

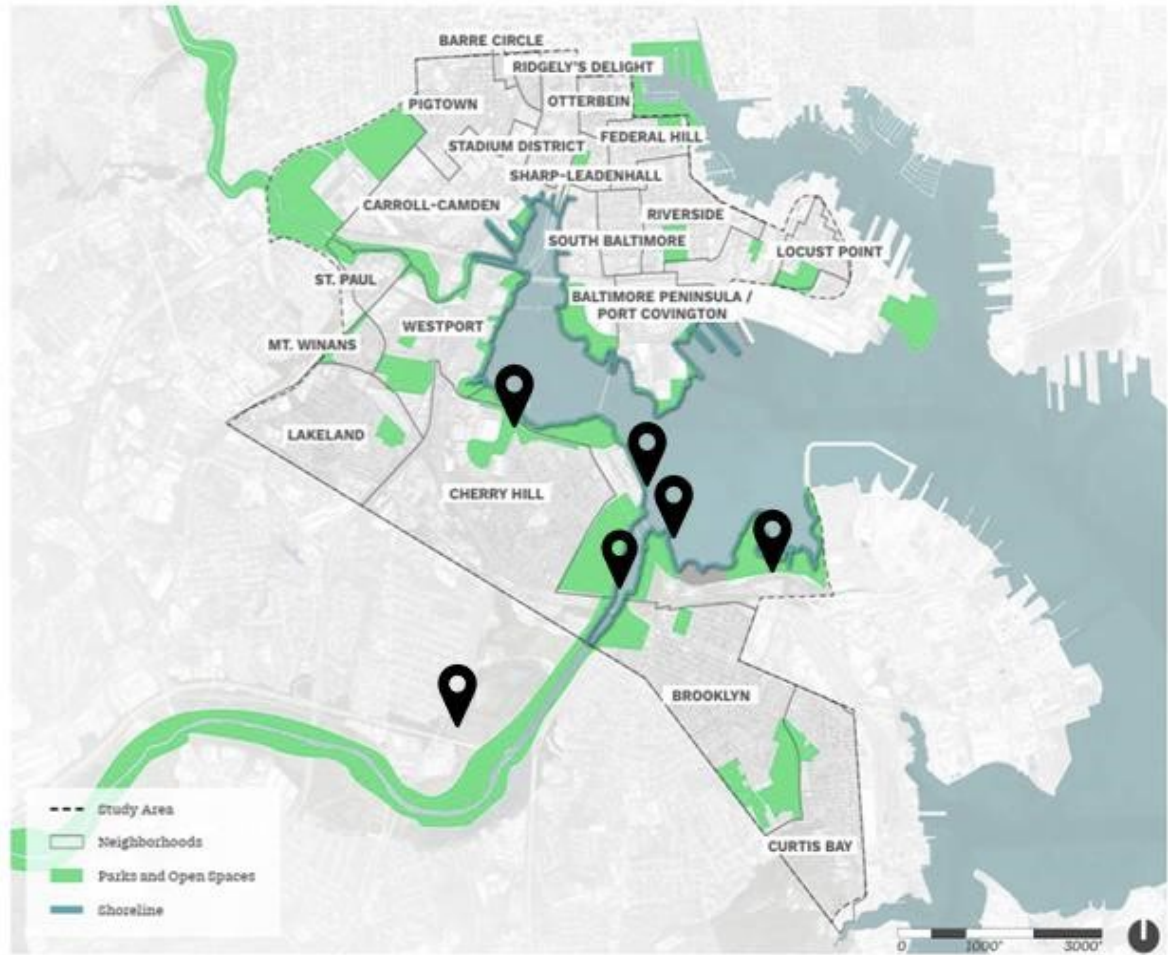


Figure 14. Map of the Baltimore Harbor with the Middle Branch on the left. Black pins indicate eDNA collection sites: Cherry Hill Fishing Pier, Masonville Cove Fishing Pier, Middle Branch Park Fishing Pier, Reed Bird Island Fishing Pier, Southwest Area Park Boat Ramp, Middle Branch Trailhead 9 Fishing Pier.

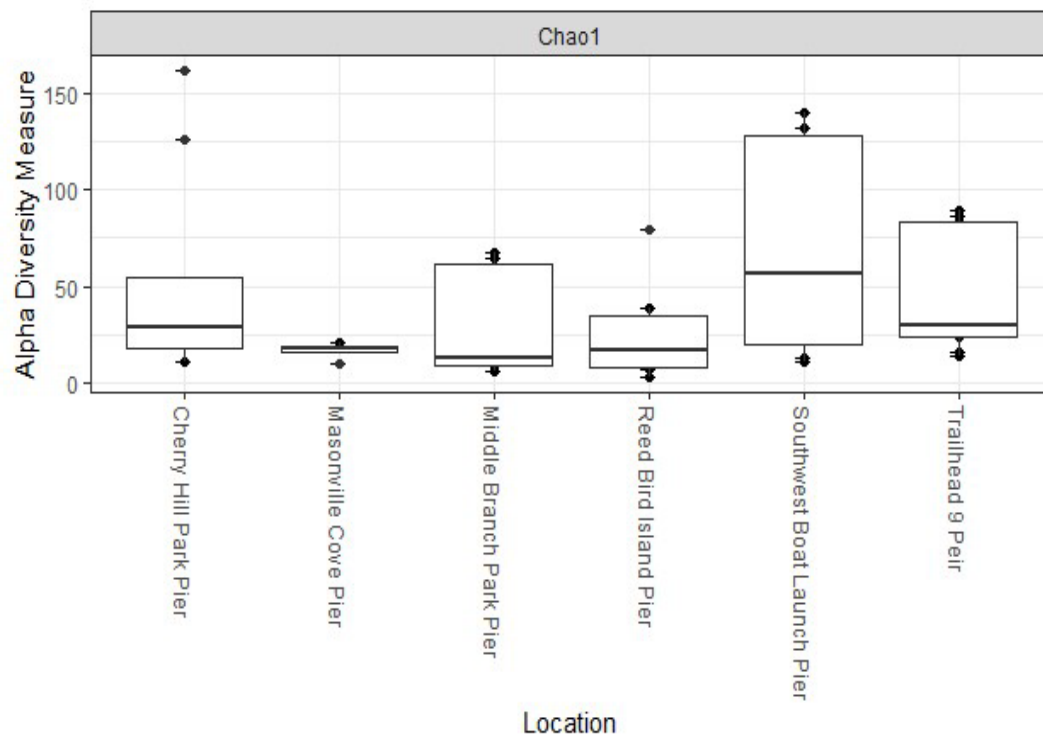


Figure 15. Chao1 diversity index for ASV of Animalia species grouped by taxonomic class at sample sites, with terrestrial mammalian taxa excluded.

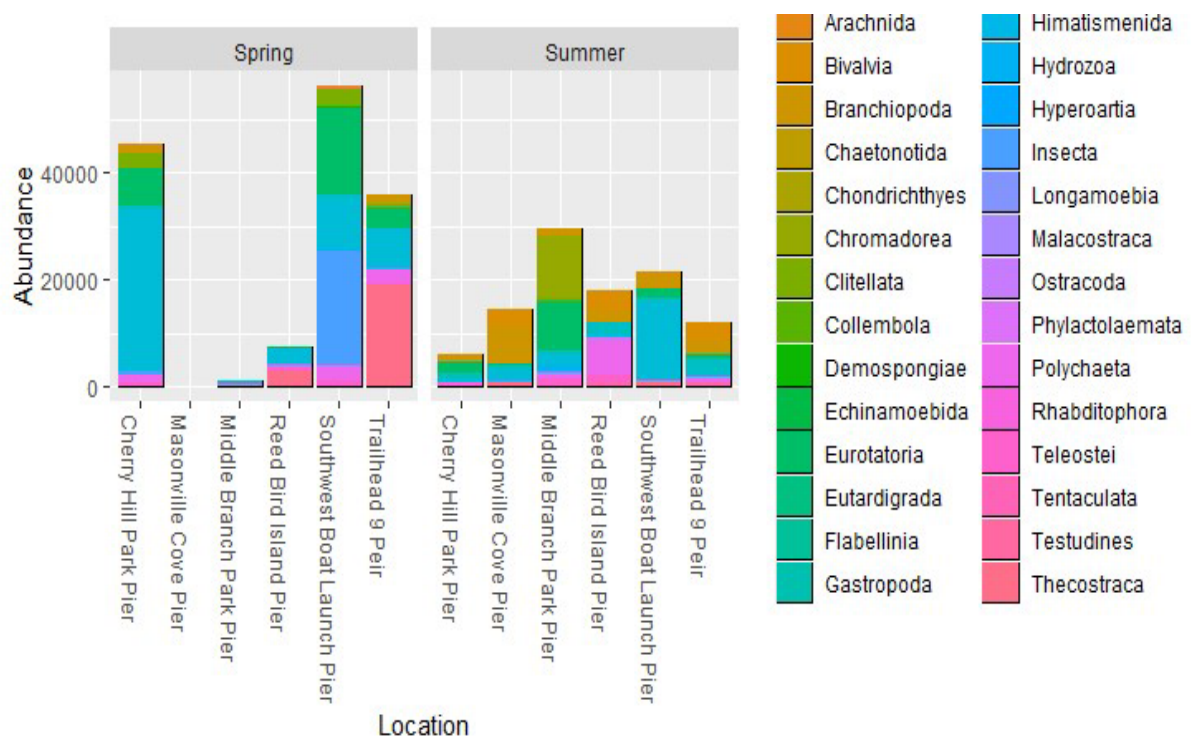


Figure 16. ASV relative sequence abundance of Animalia (excluding terrestrial mammals) grouped by taxonomic class across site and season.

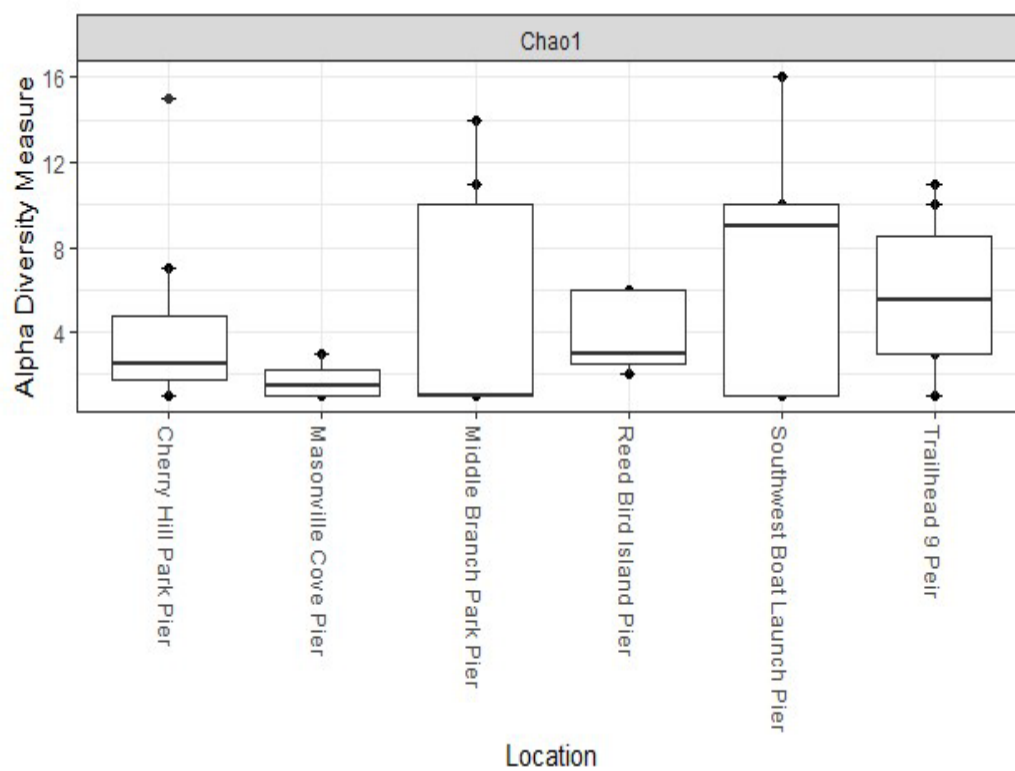


Figure 17. CHao1 diversity index for community richness for ASV of Teleost species at sample sites.

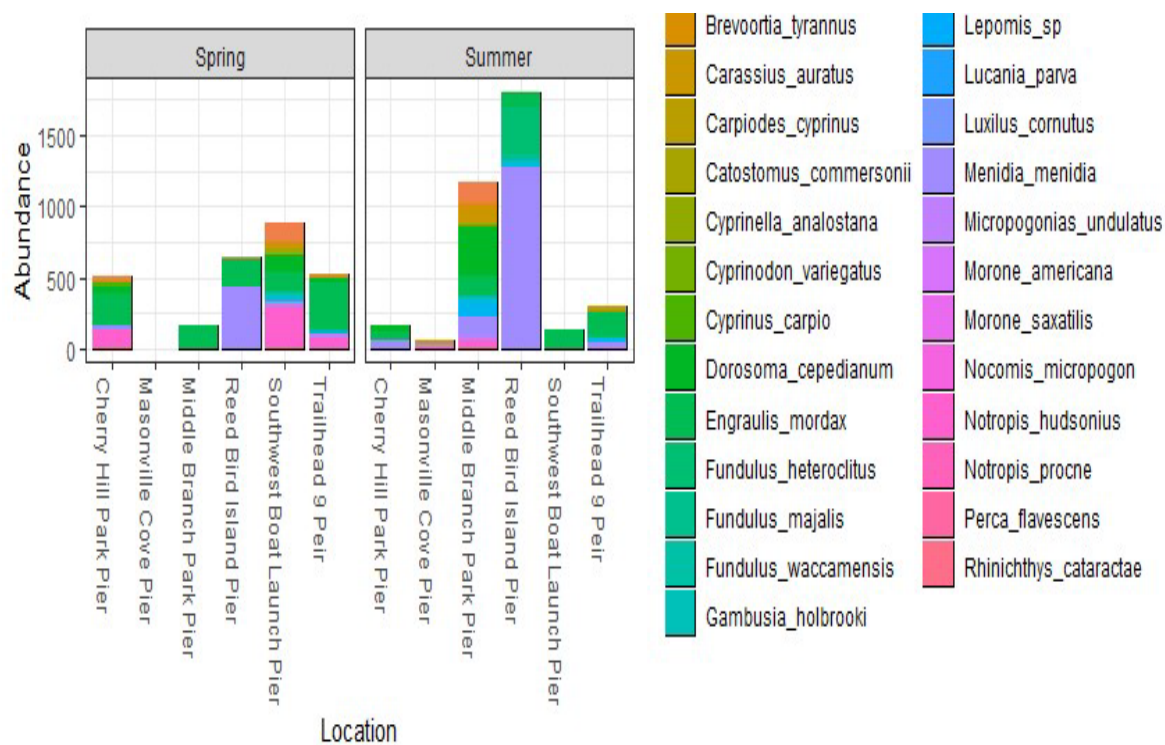


Figure 18. ASV relative sequence abundance of Teleost by site and season.

