ASSESSMENT OF FISH PASSAGE USE IN FACILITATING MOVEMENT OF ANADROMOUS FISH SPECIES IN POTOMAC RIVER TRIBUTARIES

by

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A Thesis
Submitted to the
Graduate Faculty
of
George Mason University
in Partial Fulfillment of
The Requirements for the Degree
of
Master of Science
Environmental Science and Policy

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by

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Bachelor of Science
George Mason University, 2018

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Fall Semester 2020
George Mason University
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DEDICATION

This is dedicated to my parents for their unwavering support, and the friends, extended family, and mentors who have helped keep me happy and healthy along the way.

ACKNOWLEDGEMENTS

I would like to thank the many friends, relatives, and supporters who have made this happen. My parents for constantly encouraging me to keep pushing and reassuring me I have made them proud. My aunts, Karen and Ann, for being my second moms throughout my time at George Mason. Fish Lab family for being a constant source of inspiration – especially my personal hype women of science, Sara and Casey, who were always available to help reign in my frequent bouts of impending doom and send me cute pictures of their dogs. My friends and previous students who conquered thickets, ticks, snakes, deep mud, and murky water to help collect my samples and survey streams – Maddie, Lizzie, Michael, Sara, Matt, Valerie, and Adam, I would probably be lost in a stream somewhere (without snacks) without you all. Kristal, thank you for your endless friendship and commitment to helping me take breaks, either sitting with me at the swimming hole or floating on the river. Thank you Cindy Smith for providing me a safe and comfortable space equipped with cute kitties, to work during my final stretch. Finally, none of this would have been possible without the funding from Virginia Sea Grant and Friends of Accotink Creek, as well as support from my committee, Drs. de Mutsert, van der Ham, and Plough, who were always available to help answer questions and offer guidance as I pushed to complete this project. I would like to thank Kim and Joris a second time for their endless support during my undergraduate studies, without which I would have never made it to a graduate degree - you all have inspired me to put in 110% in my work and exemplify what it means to be a mentor.

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Centimeters…………………………………………………………………………...…cm
Department of Wildlife Resources…………………………..……………………….DWR
Environmental DNA…………………………………………………...………..eDNA
Feet………………………………………………………………………..…...……..ft
Meters……………………………………………………….………………………m
Second…………………………………………………..……………………….sec
National Industrial Recovery Act …….…………………………………….……..NIRA
National Forest……………………………………..……………………………NF
United States………………………………………...…………………………..US

ABSTRACT

ASSESSMENT OF FISH PASSAGE USE IN FACILITATING THE MOVEMENT OF ANADROMOUS FISH SPECIES IN POTOMAC RIVER TRIBUTARIES

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George Mason University, 2020

Thesis Director: Dr. Kim de Mutsert

Diadromous fish are particularly vulnerable to anthropogenic alterations in watersheds, such as road construction and the establishment of flow control areas like dams and weirs. In northern Virginia, two anadromous species of concern, Blueback Herring (*Alosa aestivalis*) and Alewife (*Alosa pseudoharengus*), collectively managed as river herring, rely on well-connected waterways to complete annual spawning runs from the Atlantic Ocean into inland streams. Water passage infrastructure, hereafter fish passages, are installed at road-stream intersections in order to maintain the structural integrity of roads as flow conditions fluctuate, while also supporting continued up- and downstream passage by fishes and other aquatic organisms. Successful fish passages are those that permit upstream movement by the anadromous species as they travel inland to spawn. However, little information is available surrounding which passage characteristics are most important in permitting river herring movement. This study aimed to confirm areas theorized to host river herring spawning runs in Potomac River tributaries throughout northern Virginia, while also identifying passage characteristics that promote successful upstream passage by river herring. Environmental DNA (eDNA) samples were collected at 18 road crossings, one dam, and three weirs between 2018 and 2019 to determine species presence above and below each passage. This study documented the presence of river herring in upstream reaches of 9 Potomac River tributaries previously lacking confirmation of recent use by the species. Additionally, this study found evidence to support that many currently used fish passage designs including bridges, culverts, and weirs perform equivalently in allowing the upstream movement of river herring, with the exception of large round culverts. Furthermore, environmental variables did not appear to influence river herring presence across locations evaluated, however, did influence the observed frequency of upstream passage suggesting that river herring may persist in a variety of conditions but require more specific conditions in order to move through fish passages. Understanding the variables that correspond with successful fish passage use by anadromous fish species is key to guide future management strategies and plans for the recovery of river herring populations.

Keywords: river herring, fish passages, road-stream intersections, eDNA, Potomac River

CHAPTER ONE: CURRENT STATUS AND METHODS FOR EVALUATING IMPACTS OF FISH PASSAGES ON MAINTAINING WATERSHED CONNECTIVITY FOR FRESHWATER FISHES

1.1 Introduction

In the late 19th and early 20th century, the United States (US) experienced a period of rapid urbanization brought on by increased industrial capabilities (e.g., the assembly line). This surge in industrialization provided many jobs within a small, centralized area and as a result generated a demand for new infrastructure capable of supporting the influx of residents such as housing, market-centers, and hydroelectric power facilities. As time progressed, urban development began to expand beyond the city centers and into the surrounding areas, known as the suburbs (Rees, 2016). Although this new growth was not as centralized as that experienced in prior years, it did generate a more expansive demand for additional roadways to connect the suburban community to the jobs in the city. This urban expansion came to a momentary rapid halt with the economic crash of 1929 (i.e., the Great Depression), until President Franklin D. Roosevelt began signing his “New Deal” policies into law in 1933 that would provide economic relief and incentives back to the US (Hopkins, 2011).

In 1933, the National Industrial Recovery Act (NIRA) was signed into law which eventually provided $3.3 billion to the Public Works Administration to improve nationwide infrastructure such as roads, bridges, and dams (Hopkins, 2011). In 1935, Congress passed the Emergency Relief Appropriation Act of 1935 which also established work-relief programs backed by $4 million of federal money to fund public works projects similar to those funded by the NIRA (74th Congress of the United States, 1935).

The economic depression of the late 1920s and early 1930s alongside subsequent legislation passed in an attempt to bolster the US economy, presented the opportunity for the federal government to purchase millions of acres of land from private owners at extremely low cost (Conrad, 1997). As a result, between 1931 and 1939, ten new National Forests (NF) were established in the North Central and Eastern Regions of the US (Conrad, 1997). A decade and a half after Depression Era boom of NF land acquisition, the Wilderness Act of 1975 further established six new wilderness areas in the eastern region of the US, followed by three more areas designated by Congress in 1978 (Conrad, 1997). Alongside this expansion of NF property, came a new push for NF areas to be used recreationally as camping, hiking, and fishing areas rather than solely for timber manufacturing as they had been used previously (Conrad, 1997). This increase in visitation led to the need for increased accessibility to the parks, as well as within the parks. The federal government began funding the installation of roads in NF areas and National Parks to support the onset of recreation and increase accessibility to these national resources (Steen, 2004). However, between 1945 and 1969 (i.e., post-World War II), NF lands still served as valuable source of lumber so additional roadways were installed to facilitate the export of lumber throughout the Forests (Glasser, 2004).

The expansion of roadways into National Parks and across the nation as a whole required new roads to cross over waterways varying from small creeks to large rivers, which created a need for new water passage infrastructure such as culverts and bridges to allow the flow of water to continue (Gibson et al., 2005). This roadway infrastructure was, and still is, critical in permitting the continued use of waterways by aquatic organisms in search of food, mates, and shelter (Hoffman et al., 2012). In particular, the health of migratory fish populations is strongly connected to the efficiency of these water passages (Limburg & Waldman, 2009). These fishes rely on well-connected waterways to support annual spawning migrations either within a single stream (i.e., potamodromous fishes), from the ocean to the upstream, inland spawning grounds (i.e., anadromous fishes), or from the inland streams to the ocean spawning grounds (i.e., catadromous fishes) (Metcalfe et al., 2002).

1.1.1 Maintaining watershed connectivity

Efforts to minimize the impact of stream blockages, such as the National Oceanic and Atmospheric Administration (NOAA) Fisheries Hydropower Program, are in place in order to enforce regulations meant to aid in fish passage at hydroelectric dams (The Magnuson-Stevens Fishery Conservation and Management Act, 1976). This program ensures that critical fish habitat is properly managed as required by three key federal mandates: 1) section 305 of the Magnuson-Stevens Fishery Conservation and Management Act protecting the habitat of federally-listed fishes (The Magnuson-Stevens Fishery Conservation and Management Act, 1976), 2) the Federal Power Act requiring infrastructure at dams allow for effective fish passage up and downstream of the blockage (Federal Power Act, 1920), and 3) the Fish and Wildlife Coordination Act that ensures the potential impacts of federally-funded development on the health of wildlife be assessed and accounted for prior to implementation (Fish and Wildlife Coordination Act, 1934). While these efforts are in place to encourage watershed connectivity concurrently with urban development, federal and state organizations are still concerned with the less-obvious obstructions, such as those at road-stream crossings (Hoffman et al., 2012). Within Virginia, the Virginia Department of Wildlife Resources (DWR, previously Virginia Game and Inland Fisheries) has expressed explicit interest in the success of fish passage infrastructure on migratory fish populations (*personal communication, DWR Fish Passage Coordinator Alan Weaver*).

The distribution of aquatic organisms in a watershed is determined by two predominant factors, the surrounding landscape features that influences waterscape connectivity and the specific life history strategy of species (Alexander et al., 2015). The development of urban areas inherently fragments the surrounding landscape, often through road installations, which can cause problems for organisms that rely on connected waterways to move in search of food, shelter, and mates. For example, only 17% of tributaries to the Atlantic Ocean and Gulf of Mexico along the eastern coast of the United States are considered fully connected to their terminus (McManamay et al., 2018). While terrestrial organisms have the option to traverse urban landscapes, as well as streams in some instances, aquatic organisms are typically bound by the path available via water (Samia et al., 2015).

Along the eastern US, two migratory species, Alewife (*Alosa pseudoharengus*) and Blueback Herring (*Alosa aestivalis*), collectively termed river herring, gained status as species of concern by the National Marine Fisheries Service (NMFS) in 2006. This designation was a result of population declines throughout the river herring range linked to fragmented and degraded inland spawning habitat (National Marine Fisheries Service, 2009). Later, in 2013, NMFS completed a review of the status of river herring populations in the US and determined that the listing of these species as Endangered or Threatened under the Endangered Species Act was not warranted (National Marine Fisheries Service, 2013). However, the International Union for Conservation of Nature listed Blueback Herring as 'Vulnerable and declining' on their Red List of Threatened Species citing natural systems modification through dam installation and other water management practices as a primary threat to the species (IUCN, 2011). Additionally, Alewife and Blueback Herring are considered Tier IV imperiled species, in multiple management regions throughout Virginia, by *Virginia's 2015 Wildlife Action Plan*. Maintaining connected waterways is critical for river herring, as they rely on stream connectivity to facilitate annual spawning runs from the Atlantic Ocean into freshwater tributaries each spring (Raney & Massman, 1953). In response to declines in Virginia, the Virginia DWR has expressed interest in investigating the effectiveness of passages at road-stream crossings, primarily bridges and culverts, to allow the movement of these migratory species (*personal communication, Virginia DWR Fish Passage Coordinator Alan Weave*r).

1.1.2 Fish passages and metrics for evaluating efficiency

A fish passage structure is defined as, "any structure built to facilitate the upstream passage of fish through a riverine environment" (p. 458) (Bunt et al., 2012). This definition encompasses two major groups of fish passage approaches, passive and active. Passive designs permit the movement of fishes by creating structures that act as extensions of natural riverine environments, whereas active designs incorporate additional infrastructure to aid in passage. Typically, in areas where roads or railways cross over streams, passive concrete culverts or bridges are implemented to allow the normal flow of water under the structures. In locations where water flow is blocked, such as at dams, active designs must be incorporated to aid in fish movement.

Within the passive infrastructure designs, two general classifications of structures exist for traversing substantially altered streams and are differentiated by the materials incorporated to facilitate movement. Technical and nature-like fishways both aim to promote fish migration by reducing downstream flow velocity, the former of which incorporates artificial structures (e.g., baffles and steps) and the latter employs naturally occurring elements (e.g., boulders and cobble) (Turek et al., 2016). A third type of fish passage structure differs from the first two fishways as it actively transports fish from one side of a barrier to the other (e.g., fish lifts and fish locks) (Bunt et al., 2012). Fish lifts and locks are most common in areas with large stream blockages, such as dams, but technical fishways such as fish ladders may also be incorporated at these blockages.

Following passage implementation, monitoring studies focused on fish activity are needed in order to determine if the passage is effectively promoting the movement of migratory fish (Perkin et al., 2013). Common metrics of fish passage success include attraction efficiency and passage efficiency (Bunt et al., 1999). Attraction efficiency is a measure of how well the outflow of a passage attracts fish to the entrance. Passage efficiency is a measure of how often fish that enter a passage can get to and exit the passage on the other side. In a study comparing these success metrics of 35 distinct fishways, those classified as technical fishways outperformed nature-like fishways in attraction efficiency, but not passage efficiency (Bunt et al., 2012). Interestingly, the factors that appeared to correlate to high attraction efficiency were more strongly correlated to the biology of the fishes (e.g., migration type and water-temperature tolerance). These results support the findings of McKay et al. (2013) that suggest an array of variables influence the efficiency of any passage beyond the physical design.

Confounding factors in evaluating passage efficiency include, but are not limited to, which species and age class (e.g., larval, juvenile, or adult) of fish the fishway managers are interested in promoting the movement of, what weather conditions the passage is subjected to (e.g., frequent precipitation events or drought), and even what the ambient water temperature is throughout the migration period of interest (Bunt et al., 2012; McKay et al., 2013). Forty et al. (2016) found that the successful use of fish passages by Brown Trout to migrate upstream varied based on passage design and fish length (i.e., life stage). This study illustrated the need for further assessment of the general factors that influence passage success, as well as the factors that contribute to performance variation between similarly designed structures.

Since passage efficiency is highly correlated with the biology of each individual fish species, managers aiming to promote the migration of multiple species may be less likely to implement effective passages (Perkin & Gido, 2012). Efforts to develop fish passage prioritization models have emerged in order to address the growing concern surrounding the impacts of increased watershed fragmentation on overall watershed connectivity (McKay et al., 2013). Prioritization models may be used to decide which blockages or inefficient passages would have the greatest impact on promoting migratory fishes. Having a system for prioritizing restoration locations is critical, as many regulatory agencies have insufficient funding to improve all passages across the US.

1.1.3 Status of fish passage success

As previously suggested, designing effective fish passages is a difficult task, as many migratory species tend to gravitate towards higher flow conditions that provide adequate water depth for swimming (e.g., pool-and-weir and vertical-slot fishways), but these conditions present higher velocity flows that may decrease the efficiency of fish passage upstream (Bunt et al., 2012). Additionally, the high correlation of biological variables with fish passage efficiency suggests that passage design is less important than the behavior of the specific fish species of interest further limiting the effectiveness of passages for a diverse range of fishes (Bunt et al., 2012; McKay et al., 2013). The variety of body types, range expanses, and migration behaviors contribute to the challenge of designing a universally successful fishway (Lucas et al., 2001; Williams et al., 2012). While attraction efficiency and passage efficiency are used to define the success of passages nationwide, factors such as post-capture stress following the insertion of tracking devices used to monitor fish movement in and around passages can influence the magnitude of observed attraction and passage efficiencies, and therefore influence the reliability of the estimates (Arlinghaus et al., 2007; Cooke & Hinch, 2013). As a result, factors such as capture-induced stress should be considered when interpreting attraction and passage efficiencies on a study-by-study basis.

Hydraulic models assessing the impacts of stream barriers on watershed connectivity across streams of varying diameters (i.e., distance from headwater to watershed outlet) and topologies (i.e., number of branching streams) demonstrated that longer, infrequently branching watersheds are more susceptible to stream isolation by a few number of barriers than those with larger diameters or frequently branching stream networks (McKay et al., 2013). These results suggest that longer streams with fewer tributaries should be prioritized for restoration above shorter, highly branching streams. In addition to the characteristics of the overall stream network identified as influential in magnitude of fragmentation by McKay et al. (2013), a study by Forty et al. (2016) found that spatially close fish passage structures yielded lower passage rates at the second, or upstream, passage. However, this study was unable to determine if the lower passability was a direct result of passage design or lack of energy left in the fish from previous movement through lower passages.

In general, Bunt et al. (2012) suggest that future fishway designs should employ a hybridized technical-nature-like fishway. This approach was encouraged as the technical design employed at the entrance would promote elevated stream flow to attract migratory fish, while the nature-like design towards the exit would lessen the flow velocity as the fish moved upstream making complete passage through the fishway easier. However, beyond passage design, studies ultimately found that the slope of the fish passage was the most important factor, as lower sloped fishways yield lower flow conditions that are more easily traversed by fishes (Bunt et al., 2012; Meixler et al., 2009).

Although Forty et al. (2016) were unable to identify which of the two factors, passage design or energy deficiency in the fish, contributed most to unsuccessful passage through consecutive barriers, their study suggested that serially repeated passages should be avoided by managers in the future. Instead, future fish pass implementation should consider requiring a minimum distance from other fish passes. Additionally, these authors found that even low-head barriers to fish passage (i.e., less than 3-meters high) favor adult fish over juvenile fish as a result of the larger body size (i.e., fork length) required to generate enough swimming strength to move through the barrier. From this study, we can conclude that larger than 3-meter height passages should be avoided in areas concerned with the movement of adult fishes such as a barrier blocking the downstream migration of catadromous fishes (i.e., fishes who spawn below the barrier, then return upstream as adults). Where possible, shorter-length fish passages such as those below 10-meters in length should be incorporated, as they required less sustained swimming energy, and as a result are able to support the migration of smaller-length fish (i.e., younger fish) upstream.

1.1.4 Global challenges to fish passage success

In many instances, historic fishways are already in place and complete removal for the implementation of a new, hybrid design is not economically feasible (Lejon et al., 2009). Agencies in charge of fishway management have limited funds, and as such need cheaper improvement options. Some examples provided by Bunt et al. (2012) of fishway modifications included the addition of supplemental attraction flows at the entrance of pre-existing fishways, or the diversion of high flow conditions away from suboptimal passages that are outcompeting nearby optimal passages with lower flow. Additional low-cost modifications include alteration of the entrance shape and location, but more research is needed to determine if these are effective options (Bunt et al., 2012). One method of prioritization suggested by McKay et al. (2013) was to assign values to species of interest ranking based on conservation priorities and concerns (e.g., higher values for vulnerable species and lower for invasive species), and then incorporate these values as multipliers in a watershed connectivity model. This approach is unique in that it accounts for the potential benefits as well as detrimental effects of restoring watershed connectivity via passage restoration or implementation.

While the ultimate goal is to restore stream connectivity, Jackson and Pringle (2010) warned against the potential dangers associated with only partially restoring flow at areas once blocked by human infrastructure. In instances where stream flow is not restored enough to support the movement of target organisms, downstream areas may still receive pollutants accumulated in upstream sediments and water that were previously contained. In some instances, the challenge of maintaining, or reestablishing, watershed connectivity extends beyond the societal pressure to preserve human infrastructure. Attempting to expand watershed connectivity in landscapes that have already been highly modified and fragmented by human activity presents the opportunity for newly arrived nonnative species to expand their range into previously protected stream reaches (Jackson & Pringle, 2010; Lejon et al., 2009).

1.1.5 Local challenges to fish passage success

Approximately a quarter to one third of the fish diversity within the Potomac River is accounted for by non-native species (Starnes et al., 2011). In 2004, the first known population (i.e., 20 individuals ranging across six year-classes) of the non-native fish species, Northern Snakehead (*Channa argus*), was documented in the mainstem and associated tributaries of the Potomac River (Odenkirk & Owens, 2005). Odenkirk and Owens (2005) predicted that this founder population originated from Dogue Creek, a tributary to the Potomac River, as half of the initially discovered population were recovered from this stream. In a study using radio tags to track the movement of Northern Snakehead, nearly one-third of the surviving tagged population exhibited a rapid range expansion between the end of April and beginning of June, with 92% of the group moving upstream from their winter home range (Lapointe et al., 2013). The majority of individuals that moved upstream, then settled and established new home ranges.

Although this study found that the tagged fish moved upstream, the predicted maximum salinity tolerance of this species, 10 parts per thousand (Lapointe et al., 2013), suggests that during spring peaks in freshwater outflow the Northern Snakehead may be capable of dispersing further downstream into the Chesapeake Bay where they can then access entrance points to middle and lower tributaries to the Bay. Following the initial discovery of a breeding population in a Potomac River tributary, concern arose over the potential of this species to spread downstream into the Chesapeake Bay and decimate regional fish populations. However, due to the habitat preference of Northern Snakehead for shallow, soft sediment with high macrophyte cover (Lapointe et al., 2010), it is unlikely that this species will attempt to colonize the Bay.

Although not considered a major threat to mainstem freshwater tidal tributaries to the Chesapeake Bay, the shallow habitat preference exhibited by Northern Snakehead make many freshwater tributaries prime areas for colonization. Coinciding with the dispersal period observed by Lapointe et al. (2013), Gascho Landis et al. (2011) also determined that Northern Snakehead experience a distinct pre-spawning period from April to June, wherein the feeding rates of mature individuals rapidly increases in preparation for a subsequent period of slowed feeding during the spawning season from July to mid-September. This preference for movement into tributaries during a peak feeding season presents the opportunity for this piscivorous species to decimate inland populations of freshwater fishes via predation. While Lapointe et al. (2013) found that the average minimum dispersal distance for the Northern Snakeheads in their study was only 18 km, they predicted that these individuals may have continued seeking new home ranges had they not been blocked by anthropogenic barriers, such as dams, and natural barriers, such as Great Falls. Overall, the high dispersal potential of this piscivorous species contributes to the risks associated with connecting upstream areas to previously disconnected downstream areas via fish passages.

Another regional threat to fish passage success is the potential for use in range expansion by another invasive species, the Blue Catfish (*Ictalurus furcatus*), that switches to a primarily piscivorous diet once individuals achieve a total length between 500- and 900-mm (Schmitt et al., 2019). Blue Catfish were introduced into the Chesapeake Bay in 1974 by the Virginia Department of Game and Inland Fisheries (now Virginia DWR) and the U.S. Fish and Wildlife Service to establish a recreational sport fishery. However, due to high salinity tolerance and an opportunistic generalist diet this species has now established populations throughout the upper reaches of the Bay (Fabrizio et al., 2018; Higgins, 2006; Schloesser et al., 2011; Schmitt et al., 2019). A species once introduced intentionally in batches of approximately 300,000 fry per river system has now exploded in numbers, exceeding millions of adult individuals in the streams they were initially introduced in, not including the newly colonized freshwater stream networks (Fabrizio et al., 2018; Higgins, 2006). The ability of Blue Catfish to traverse various salinity gradients has allowed this species to travel from freshwater tidal streams out into oligohaline (0.5-5.0 ppt) and mesohaline (5-18 ppt) estuaries, and back into new freshwater systems. Currently, there are commercial and recreational Blue Catfish fisheries, but the rate of harvest needed to eradicate this species is not known (Fabrizio et al., 2018). Salinity may limit the distribution of Blue Catfish throughout the

Chesapeake Bay, although some have been documented in salinities as high as 21.5 ppt (Fabrizio et al., 2018). Unfortunately, these gradients are in constant fluctuation due to regional weather conditions (e.g., rainfall) making containment of the species challenging (Schloesser et al., 2011). The only known barrier to Blue Catfish spread are physical impoundments, such as dams and weirs (Higgins, 2006), which also block the movement of anadromous species of conservation interest, such as river herring (Limburg & Waldman, 2009).

This unique conservation challenge raises the question: which is more important, migratory fish passage upstream or the protection of once isolated native species from invaders? In addition to the potential threat of nonnative species, streams in densely urbanized areas may experience elevated chemical pollutants originating from the surrounding watershed following precipitation events (Limburg & Waldman, 2009). In the case of the Coho Salmon, five previously blocked tributaries were reopened in Seattle, Washington to facilitate spawning runs upstream. However, these efforts were generally considered unsuccessful as the majority of the population experienced mortality before they were able to spawn due to toxic concentrations of chemicals in the urban streams, likely originating from stormwater runoff (Scholz et al., 2011). These scenarios highlighted the need for site-specific evaluations of potential fish passage locations that assess biodiversity and water quality above and below a future or restored passage.

Overall, the currently utilized fish passage designs are not entirely ineffective, but in many instances struggle to efficiently promote the migration of targeted fish species

(Bunt et al., 2012). When possible, passive fishways are the most ideal option to promote migration as this approach minimizes alterations to the landscape features such as slope and flow. However, in instances where infrastructure completely blocks or alters stream flow for anthropogenic activities, such as irrigation and power generation, active fishways may be unavoidable. Although the type of passage is important, fishway utilization is highly dependent on the species of fish in combination with the passage design. Therefore, species-specific studies should be conducted within the region of interest in order to determine the optimal fishway design for the targeted species (Limburg & Waldman, 2009; Perkin et al., 2013). In the case of river herring along the eastern US, due to their annual use of natal spawning streams, river-specific studies are needed to provide insight on the health of spawning populations throughout their range (ASMFC, 2012).

1.1.6 Non-invasive approach to assessing fish passage use

Passive integrated transponder (PIT) tagging is a frequently used method for monitoring movement by target fish species, as logging stations can be established above and below locations of interest (Cathcart et al., 2018). Specifically, PIT tagging is commonly employed to evaluate the attraction of fish to fish passages, as well as movement through target passages (Forty et al., 2016). Although a relatively effective method, PIT tagging requires target species be captured and tags inserted into individual fish, so may not be ideal for organisms of known or potential population concern as handling-induced stress may occur (Cooke & Hinch, 2013). Additionally, PIT tagging relies on the ability of researchers to first capture target fish species for tagging, which may be difficult for particularly elusive fish species or species with low population abundances. This method also only provides insight into passage use by a select number of species, as it is limited by the specifically tagged individuals.

Alternatively, environmental DNA (eDNA) is a passive sampling procedure, wherein researchers collect soil, water, or other environmental samples to extract and amplify the DNA left by organisms residing in the associated environment (Thomsen & Willerslev, 2015). This DNA can be deposited via metabolic waste processes (e.g., defecation or urination) or natural shedding of external cells (e.g., scales, mucus, and fur; Ficetola et al., 2008; Kelly et al., 2014; Thomsen & Willerslev, 2015). Once DNA is deposited, the DNA-containing environmental sample (e.g., water or soil) is collected and undergoes a series of filtering processes to consolidate the DNA. Following DNA consolidation, the DNA is extracted and then amplified via one or many types of polymerase chain reactions (PCR). Researchers can then determine which species came in contact with the environmental sample based on the DNA that is amplified. This approach is ideal for organisms of conservation interest, as it allows for species detection without handling the target organisms, as well as elusive organisms as the detection of DNA does not require direct observation (Ruppert et al., 2019; Thomsen & Willerslev, 2015).

In some instances, species-specific primer sets or assays have been developed and allow researchers to specifically target and amplify the DNA of one or a few closely related organisms. Particularly useful along the eastern coast of the US was the creation of a river herring specific assay developed by Plough et al. (2018) that allowed the rapid detection and differentiation of fishes in the *Alosa* genus using quantitative PCR (qPCR) amplification. This assay differentiates river herring (*Alosa aestivalis* and *Alosa pseudoharengus*), from two closely related species, American Shad (*Alosa sapidissima*) and Hickory Shad (*Alosa mediocris*), that are also found in natal streams used for river herring spawning runs. Having species-specific or group-specific assays help expedite the detection of species of management concern, and ultimately allows for more expansive population assessments. Although this river herring assay does not differentiate between Alewife and Blueback Herring, these organisms utilize many of the same stream reaches so this method is sufficient for many rapid assessment surveys. In instances where differentiation between the two species is required, an additional step using Sanger sequencing can achieve this objective.

When species-specific primers are not available or when researchers are interested in multiple species from a single sample, "universal" primers are used to amplify regions of specific genes found across taxa of interest (Wood et al., 2013). Assessing multiple species within an eDNA sample requires a highly variable yet short (e.g., less than 400 base pairs; Engelbrektson et al., 2010; Huber et al., 2009) DNA region with well-documented reference sequences for each taxa of interest (Leray et al., 2013). Although there are many community assessment studies that utilize ribosomal markers on DNA to identify individual taxa in eDNA samples (Cowart et al., 2020; Horton et al., 2017), these markers are not widely accessible for public use (Leray et al., 2013). The mitochondrial

Cytochrome c oxidase subunit 1 gene (CO1), however, is the largest publicly available sequence region made available through online platforms, such as the GenBank® sequence database (https://www.ncbi.nlm.nih.gov/genbank/). This database is produced and maintained by the National Center for Biotechnology Information as a part of the International Nucleotide Sequence Database Collaboration. This gene has great diversity in a relatively small sequence region, which is an ideal quality for determining species richness in a sample (Leray et al., 2013). Serrao et al. (2014) had great success sequencing the mitochondrial 5' CO1 barcoding region for 25 of the 36 known species of Snakehead (family Channidae), an invasive species found locally in Potomac River tributaries, which helped develop a more precise tool for identifying species that otherwise often have insufficient morphological keys available for species identification.

DNA degradation is a common challenge associated with eDNA surveys and can minimize the range of sequencing approaches appropriate for species-level identification. The DNA extracted from environmental samples are frequently fragmented, which limits surveyors to choosing genetic markers on relatively short base pair chains (Taberlet et al., 2012). Although flowing water may dilute the amount of DNA present in an eDNA sample collected from a stream, Deiner and Altermatt (2014) demonstrated that DNA could still be detected kilometers away from the host organism. A common uncertainty associated with the interpretation of eDNA as a valid representation of biodiversity, in streams specifically, is the potential of DNA originating upstream to flow to the location of interest downstream.

River herring present a unique situation along the eastern US, in which the limitations of eDNA sampling are minimized by the fishes' anadromous life history strategy that require they travel upstream from the ocean into freshwater tributaries to spawn. Since river herring would be migrating from the Atlantic Ocean into inland streams, a positive DNA detection upstream of the crossing is sufficient to suggest that river herring were able to access, at a minimum, the location where the eDNA sample was collected, and therefore were able to move through the target fish passage. A positive eDNA detection of a species at any one location in a stream suggests that the species was either present in the immediate vicinity of the eDNA collection location, present further upstream and the DNA was transported downstream by streamflow, or present at and above the collection location. Nakagawa et al., (2018) found that fish community composition reflected in eDNA was most similar to human-observed fish communities when compared to upstream observations made within 6 km of eDNA collection location. Data surrounding distribution of river herring in Potomac River tributaries is currently limited to three streams, so a widespread assessment of distributions is needed. Additionally, beyond the Potomac River watershed, the overall effectiveness of fish passages at road-stream intersections are still relatively unknowns, so more studies are needed to provide information to inform future fish passage designs and restoration projects. The status of river herring populations is classified as decreasing or unknown in many major rivers along the eastern U.S. (ASMFC, 2012), so eDNA collection is an ideal sampling method to assess these populations as it can passively detect species presence across a large number of streams in a relatively short time period, even if there are low numbers of target organisms in each location (Ficetola et al., 2008). This thesis aims to add to the limited knowledge of fish passage success and spawning range of river herring in the Potomac River watershed.

1.2 Study Overview

1.2.1 Chapter Two: Assessment of Fish Passage Use by Two Migratory Fish Species, Alewife (Alosa pseudoharengus) and Blueback Herring (Alosa aestivalis), in Potomac River tributaries

The purpose of this study is to determine if river herring can utilize fish passages to migrate upstream to reach inland spawning grounds. This study applies eDNA collection methods to evaluate stream and fish passage use by Alewife (*Alosa pseudoharengus*) and Blueback Herring (*Alosa aestivalis*), grouped as river herring, in 11 Potomac River tributaries. A river herring-specific assay developed by Plough et al. (2018) is used in combination with quantitative polymerase chain reaction (qPCR) to identify: (1) the current range extent of the species and (2) fish passage use at road-stream intersections within each tributary. Rivers theorized to host river herring were selected using the Chesapeake Fish Passage Prioritization (CFPP) project tool developed by the Nature Conservancy (Martin, 2019). The assessment of fish passage use includes identifying which features at each location correspond to species presence and successful upstream movement by the fishes. Features assessed include: (1) CFPP tool "modeled" barrier ranks including insignificant, minor, and moderate; (2) structural design type including bridges, small round culverts (diameter <2 m), large round culverts (diameter

>2 m), large square culverts (diameter >2m), and weirs; (3) stream segment type including riffle, run, and pool; and (4) physio-chemical variables related to habitat quality including percent coverage of seven bottom substrate types (bedrock, boulder, cobble, gravel, sand, silt-clay, and organic muck), percent coverage of seven stream cover attributes (leaf litter, large woody debris, other woody debris, algae, floating aquatic vegetation, submerged aquatic vegetation, and no cover), as well as four water quality parameters (temperature (°C), dissolved oxygen (%), salinity, and pH). An outline of the management applications of these findings will be presented, focusing on lessons learned during passage assessments and how these results can be incorporated into the existing CFPP project tool developed by the Nature Conservancy.

1.2.1.1 Study rationale

Fish passages are implemented to aid in the movement of native species, including spawning runs of anadromous fishes, but managers currently lack information to support that these passages allow fish movement. River herring rely on stream connectivity to support annual spawning runs meaning that they rely on streams to be unobstructed by human development to persist. However, little is known regarding the efficacy of fish passages at road crossings, so determining which passage types facilitate river herring movement is critical to ensure future spawning runs are a success.

Understanding which passage types facilitate river herring migrations is important so that management actions can focus on restructuring the passages that are detrimental for river herring and turning them into passages that promote river herring movement.

Beyond passage type, physical conditions in the surrounding environment are anticipated to influence river herring presence at each passage. For example, it is known that Alewife and Blueback Herring both rely on a narrow range of environmental conditions to trigger upstream spawning migrations including temperatures of at least 10°C (Pardue, 1983) and 14°C (Loesch & Lund, 1977), respectively, so it is expected that areas above these thresholds should detect the presence of each species and areas below will not. Ultimately, the passages that pose the greatest risk for preventing river herring spawning success will become the highest priority locations for passage restoration. Although the most recent assessment of Alewife classified local spawning populations in the Potomac River as stable, the status of the co-managed species, Blueback Herring, is still classified as unknown which warrants the inclusion of both species in this study (ASMFC, 2017).

Kelly et al. (2014) affirm that eDNA is a cost-effective method for quickly assessing the presence, as well as range, of policy-relevant species, such river herring. River herring are an ideal candidate for detection via eDNA, as this method minimizes potential stress on individual members of the population and thereby does not disrupt regular spawning activities. This study may act as the first tier in future two-tiered monitoring studies, wherein passage locations are rapidly assessed using eDNA collection methods to determine if the site hosts the species of interest. Sites that do not detect the species of interest will then be removed from consideration for restoration, while sites yielding positive DNA detections of targeted species will then become candidates for further assessment using traditional monitoring protocol, such as sampling via nets. Ultimately, the results of this project can guide future monitoring studies by reducing the time necessary to identify locations worth monitoring via more involved traditional sampling methods.

The population status for river herring is unclear for the majority of the eastern coast of the US, so this study would contribute to filing a large knowledge gap concerning the areas utilized by the species (ASMFC, 2017). The Virginia DWR wants to know if the fish passages they have implemented are working, and a confirmation of usage by river herring would directly support their mission to promote the persistence of wildlife for future generations. The analysis of environmental parameters and species distributions will determine which areas are optimal for river herring. These results will contribute to the development of informed management strategies for end-users such as DWR that will target areas that have the greatest potential to benefit the most from increased monitoring. If there are man-made blockages preventing species from utilizing areas of the watershed, then there may be increased competition between organisms, which could potentially harm the recovery of river herring.

River herring were once an economically valuable species along the eastern United States, but now are protected under moratoriums in many parts of their range with the exception seven states that have met the qualifications to have operational river herring fisheries by implementing River Herring Sustainable Fishery Management Plans or gaining approval for Alternative Management Plans (ASMFC, 2009). The results of this study will contribute to the development of effective management strategies that could increase the population sizes of river herring enough for the moratorium to be lifted and for river herring to become a source of recreation or income for communities surrounding the coasts of their range.

CHAPTER TWO: ASSESSMENT OF FISH PASSAGE USE BY TWO MIGRATORY FISH SPECIES, ALEWIFE (*ALOSA PSEUDOHARENGUS*) AND BLUEBACK HERRING (*ALOSA AESTIVALIS*), IN POTOMAC RIVER TRIBUTARIES

2.1 Introduction

Diadromous fish species rely heavily on stream connectivity to facilitate their spawning migrations, either to the sea (i.e., catadromous) or into freshwater tributaries (i.e., anadromous). These life-history patterns make diadromous fish particularly vulnerable to anthropogenic activities such as the development of dams that completely block streams, as well as roads that often partially block or redirect streams (Hoffman et al., 2012; Limburg & Waldman, 2009). In northern Virginia, two anadromous fish, Blueback Herring (*Alosa aestivalis*) and Alewife (*Alosa pseudoharengus*), return from the Atlantic Ocean to Potomac River tributaries to spawn from March to May. These fishes remain in the tributaries throughout the juvenile stage of their life until September, and then they return to the Atlantic Ocean to continue their development as adults (De Mutsert, 2013). Physically, these fish are nearly identical and, as a result, are typically managed together and collectively referred to as river herring. Due to dramatic declines in population sizes throughout much of their range and limited knowledge on the remaining population sizes and distributions, in 2006, the National Marine Fisheries Service deemed river herring a national "species of concern" (National Marine Fisheries Service, 2009). Additionally, Alewife and Blueback Herring are individually classified as

Tier IV imperiled species throughout numerous management regions, including Northern Virginia, by the Virginia Wildlife Action Plan (*Virginia's 2015 Wildlife Action Plan*, 2015).

In 2014, *the Chesapeake Bay Watershed Agreement* set a ten-year goal to increase stream connectivity by 1,000 stream miles in order to reestablish fish migratory routes to previous ranges. Although the additional 1,000 stream miles are a new goal, the 1987 *Chesapeake Bay Agreement* first established the goal to increase stream connectivity to promote migratory species, beginning in 1989. Either installing fish passages at dams or removing dams should achieve this increase in connectivity; however, limited data is available to confirm if the adjusted passages have been successful (Hoffman et al., 2012). In 2013, the Nature Conservancy developed the Chesapeake Fish Passage Prioritization (CFPP) tool for classifying dams based on predicted severity of blockage to aquatic species movement in an effort to prioritize dam restoration projects to increase watershed connectivity (Martin, 2019). While dams were quickly identified as barriers to fish movement, road-stream crossings were slower to gain recognition as a means of fragmenting a stream network and were not initially included in the CFPP tool until revisions were incorporated beginning in 2017.

In theory, fish passages, such as bridges and culverts, implemented at road-stream crossings should be successful in allowing fish movement above and below the structure as they are designed specifically to allow water flow. In practice, however, the landscape surrounding a fish passage may influence passage characteristics (e.g., size, shape, and severity of altered streamflow), resulting in varying levels of blockages to fish movement

(Limburg & Waldman, 2009). The updated CFPP tool incorporated estimated (i.e., "modeled") and ground-truthed (i.e., "surveyed") locations of road-stream crossings, with six possible barrier ranks: 1) No Barrier, 2) Insignificant Barrier, 3) Minor Barrier, 4) Moderate Barrier, 5) Significant Barrier, and 6) Severe Barrier. This barrier layer can then be viewed against distribution layers of Alewife and Blueback Herring representative of three distribution types: 1) "current", 2) "potential current", and 3) "historic". The CFPP tool defines a current range as one with current data to support the presence of a target species in that stream, while a potential current range is one that lacks current data to support the presence of a target species but was once documented in a historic context. A historic range is an area that has historic accounts of a target species in the stream but is now cut off from downstream aquatic organisms (e.g., upstream of an impassable dam).

Assessments of fish passage usage following installation are needed to evaluate the success of currently employed passages in supporting upstream migrations of regionally important fish species, specifically river herring. The CFPP tool provides a platform for identifying passages of varying levels of estimated stream blockage, but many locations still lack the ground-truthing necessary to classify the barrier level definitively (i.e., currently classified as "modeled", instead of "surveyed" barriers). Additionally, information regarding the passage characteristics (e.g., CFPP defined barrier rank, shape, and size) contributing to successful migratory fish movement is sparse. Using environmental DNA (eDNA) collection methods, fish passage success was evaluated, and the current distribution of river herring throughout the northern Virginia portion of the Potomac River watershed was reevaluated.

eDNA is a relatively new tool utilized by ecologists and ecosystem managers to determine the presence or absence of species within an area (Pilliod et al., 2013). Water samples are collected and tested utilizing primers that target specific DNA sequences in traces of biologic material left by each species of interest, such as saliva, skin, and scales (Thomsen & Willerslev, 2015). This novel method is less invasive than traditional assessment techniques and therefore is ideal for monitoring species in ecosystems sensitive to disturbance. Additionally, the utilization of eDNA monitoring in aquatic systems reduces the amount of time and energy spent in the field collecting samples, which reduces cost over time. Collecting water samples rather than fish samples saves time and money, without interfering with river herring spawning runs.

2.1.1 Objectives

The purpose of this study was to survey fish passages identified as "modeled" barriers by the CFPP tool with the goal of classifying which passage ranks, types, and surrounding water quality parameters yield successful fish passage in northern Virginia portions of Potomac River tributaries. Three primary objectives were addressed regarding passage use by fish in order to inform future passage designs and restoration efforts: 1) determine if anadromous river herring pass through various levels of CFPP "modeled" barriers following the tiered ranking outlined by the tool (i.e., greater fish passage through lower-ranking barriers and lower fish passage through higher ranking barriers); 2) determine if there is a difference between fish passage success through various structural designs (e.g., bridges, culverts, weirs, and a dam) and if so which designs generally promote higher levels of passage; and 3) determine if certain physio-chemical stream characteristics (i.e., stream segment type, substrate type, cover attributes, and water quality) were associated with the presence and/or passage success of each species. Additionally, the methods employed to assess passage use will aid in validating the current river herring range.

I anticipated positive detections of river herring DNA in all samples representing the areas sampled below each fish passage, as these areas are an extension of reported current ranges of each species with an absence of known stream blockages up to these points. By addressing the objectives outlined above, this study will provide extensive information for use by fish passage managers to determine which passage characteristics (i.e., rank, structural design, and surrounding physio-chemical parameters) yield the greatest successful upstream passage by river herring. This information can then be used to decide which types of passages should be implemented in the future and which types are good candidates for restoration to promote the movement of Alewife and Blueback Herring. This study addressed five core hypotheses surrounding overall river herring presence, as well as fish passage use in regard to CFPP "modeled" barrier ranks, structural designs of fish passages, and physio-chemical parameters at each fish passage.

Hypothesis 1 (Barrier Ranks):

$\boldsymbol{H_{01}}$**:** There is no difference in the frequency of detecting successful upstream passage by river herring between passages of different CFPP "modeled" barrier ranks, including insignificant, minor, and moderate.

***H_{A1}*:** There is a difference in the frequency of detecting successful upstream by river herring between passages of different CFPP "modeled" barrier ranks, including insignificant, minor, and moderate.

Hypothesis 2 (Structural Designs):

***H_{02}*:** There is no difference in the frequency of detecting successful upstream passage by river herring between passages of different structural designs, including bridges, small round culverts (diameter <2 m), large round culverts (diameter >2 m), large square culverts (diameter >2 m), weirs, and a dam.

***H_{A2}*:** There is a difference in the frequency of detecting successful upstream passage by river herring between passages of different structural designs, including bridges, small round culverts (diameter <2 m), large round culverts (diameter >2 m), large square culverts (diameter >2 m), weirs, and a dam.

Hypothesis 3 (Stream Type Below Passages)

H_{03}: There is no difference in detections of river herring DNA below passages in relation to stream segment type below the passage, including riffles, runs, and pools.

H_{03}: There is a difference in detections of river herring DNA below passages in relation to stream segment type below the passage, including riffles, runs, and pools.

Hypothesis 4 (Physio-chemical Variables Influencing Species Presence):

H_{04}: There is no difference in overall detections of river herring DNA around passages in relation to water quality, including temperature (°C), salinity (ppt), dissolved oxygen (%), and pH, and site parameters, including bottom substrate type and stream cover attributes.

H_{A4}: There is a difference in overall detections of river herring DNA around passages in relation to water quality, including temperature (°C), salinity (ppt), dissolved oxygen (%), and pH, and site parameters, including bottom substrate type and stream cover attributes.

Hypothesis 5 (Physio-chemical Variables Influencing Successful Passage Use):

H_{05}: There is no difference in the frequency of detecting successful upstream passage by river herring in relation to water quality, including temperature (°C), salinity (ppt), dissolved oxygen (%), and pH, and site parameters, including bottom substrate type and stream cover attributes.

H_{A5}: There is a difference in the frequency of detecting successful upstream passage by river herring in relation to water quality, including temperature (°C), salinity (ppt), dissolved oxygen (%), and pH, and site parameters, including bottom substrate type and stream cover attributes.

2.2 Materials and Methods

2.2.1 Study site

The study systems for this assessment are tributaries to the northern VA portion of the Potomac River, subregion hydrologic unit code 0207, which includes 11 streams: Accotink Creek, Bull Neck Run, Cameron Run, Dogue Creek (mainstem and north fork), Donaldson Run, Marumsco Creek, Neabsco Creek, Powell's Creek, Quantico Creek (mainstem, Mary Bird Branch, and south fork), an unnamed stream in Prince William Forest Park, and an unnamed stream near Huntley Meadows Park. In total, 22 fish passage location were assessed and comprised of road-stream intersections (n=18), a dam (n=1) and multiple weirs (n=3) (Figure 1; Appendix I). Slightly over half of the passages assessed (n=12) occurred in waters adjacent to or within either regional, state, or national park properties across Fairfax and Prince William Counties including Lake Accotink Regional Park, Huntley Meadows Park, Potomac Overlook Regional Park, Veterans Memorial Park, Leesylvania State Park, and Prince William Forest Park (National Park). Residential areas accounted for nearly one-fourth of the passages assessed (n=5) and were characterized as being surrounded primarily by houses, condos, and apartments. The remaining passages occurred in industrial areas (n=4) and were surrounded by shopping complexes.

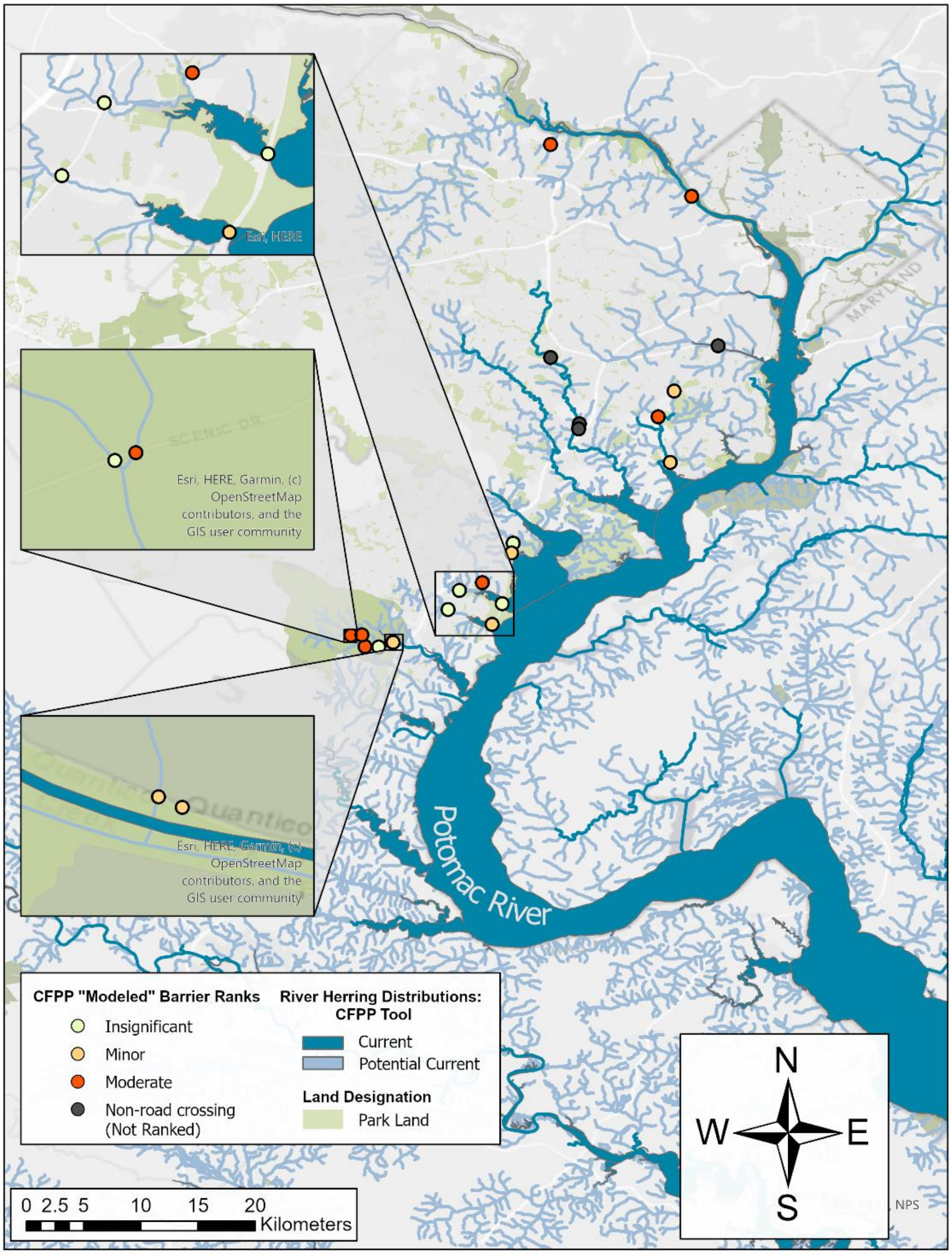


Figure 1. Fish passage locations sampled during spring 2018 and 2019 with colors indicating CFPP "modeled" barrier ranks: insignificant (green), minor (orange), moderate (red), and barriers not defined by the tool as a road crossing barrier, which were the dam and weirs (grey).

Target road crossings were selected manually using the CFPP tool (https://maps.freshwaternetwork.org/chesapeake/) based on three criteria: 1) located in Virginia, 2) located in the “potential current” range of river herring (i.e., the stream is in the historic river herring range and not obstructed by any major downstream blockages, but there is no collection data to support the continued use of the area by river herring), and 3) categorized as one of the lowest three road crossing barrier rankings assigned by the tool, after “no barrier”, “insignificant” (n=6), “minor” (n=5), and “moderate” (n=7) (Figures 2 and 3). Road crossings occurring in the “current” river herring range were ignored, as it was assumed that these crossings do not result in failed passage because there is data to support the current use of those areas by migrating river herring. Next, the first encountered road crossings occurring in the “potential current” range (i.e., those closer to the mainstem of the Potomac River) were considered for potential sampling locations.

Figure 2. Examples of three CFPP “modeled” barrier ranks surveyed: (left) a bridge ranked as an insignificant barrier on Marumsco Creek, (middle) two large round culverts ranked as a minor barrier on Marumsco Creek, and (right) a large square culvert ranked as a moderate barrier branching from Dogue Creek (full list of barriers surveyed with pictures in Appendix I).

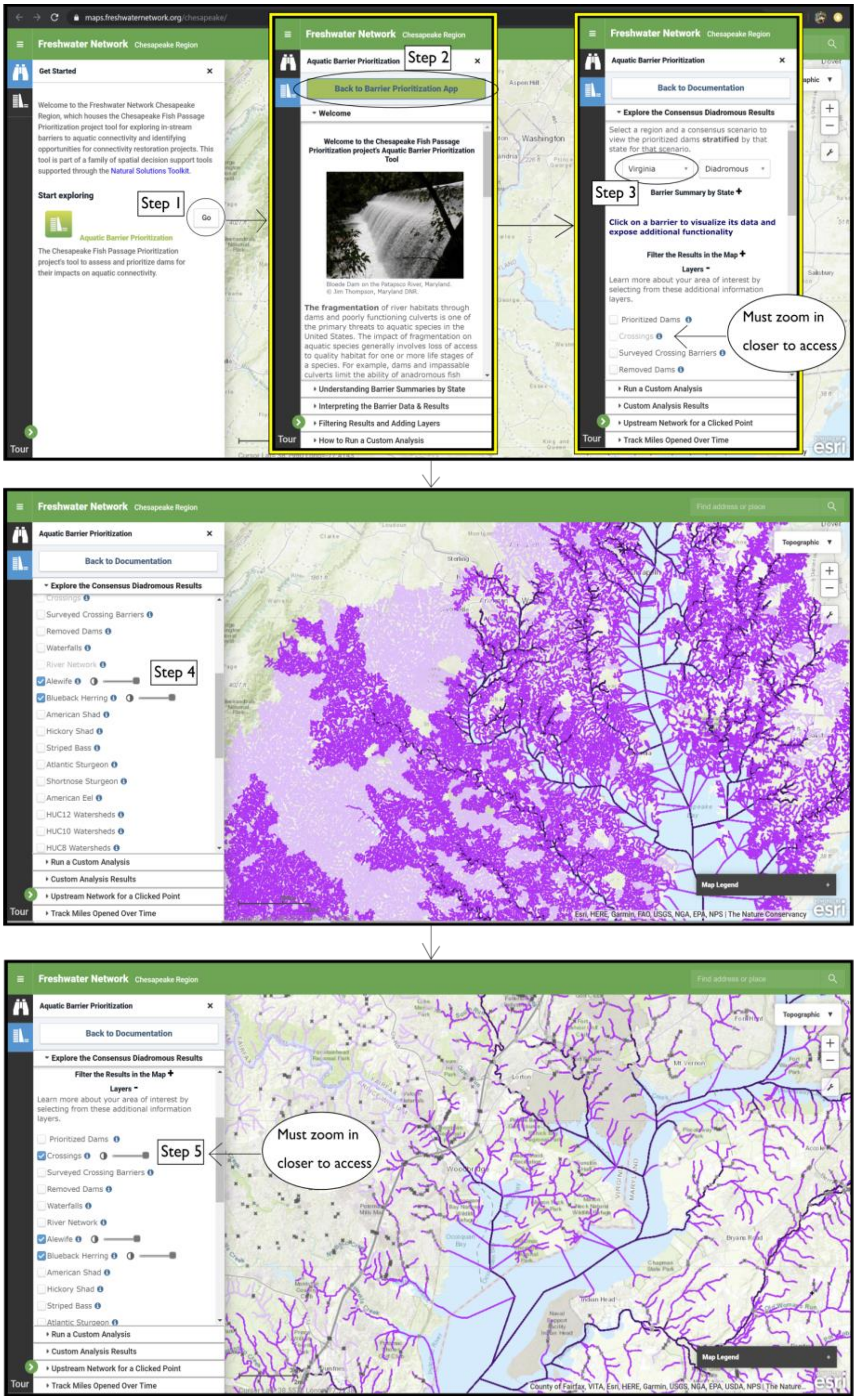


Figure 3. Flow diagram of barrier selection process using the CFPP tool (https://maps.freshwaternetwork.org/chesapeake/).

2.2.2 Identification of species presence and passage usage

eDNA sample acquisition was used to determine the presence of river herring species in this study. Water samples were collected at spaced intervals representative of the relative beginning, middle, and end of the known river herring spawning season in northern Virginia. Overlapping this study period, Melton (2019) evaluated spawning populations of river herring in three Potomac River tributaries using traditional 24-hour hoop net sampling protocols concurrently with non-invasive eDNA sampling. This study determined that the two methods were comparable for use assessing species presence, and that eDNA copy numbers had a positive correlation with target species abundances.

2.2.2.1 eDNA collection, storage, and filtration

During the spring of 2018 (April and May) and spring of 2019 (May), a total of 22 fish passage locations were assessed (n_{2018}=10, n_{2019}=10, and $n_{BothYears}$=2) (Figure 1). Approximately 1-L of water was collected from the center of the water column halfway across the diameter of the stream channel. These collection points were approximately 0.5 km downstream of the targeted fish passage when accessible or directly below the targeted passage if 0.5 km was not accessible (e.g., unsafe terrain or in a restricted area), and then directly upstream of the fish passage at each site, excluding one moderate barrier over an unnamed stream that was assessed in 2018 and did not have enough flowing water to completely fill the bottle (i.e., approximately 3-cm deep). Samples at each location were collected below the target passage first and then above to reduce the potential for DNA contamination from one position to the next. Additionally, the field technician collecting the eDNA sample remained downstream of the collection bottle at all times in order to avoid potential DNA contamination from DNA transferred from waders used across locations. In all, each sampling location resulted in a total of six samples collected (i.e., 3 collection days with 1 above and 1 below sample each), except at the few locations where only above samples could be collected due to lack of access to below passage locations resulting in three total samples.

In 2018, a total of 69 eDNA samples were collected in the field. In 2019, a total of 64 primary eDNA samples were collected in the field, while four negative controls (i.e., disinfected Nalgene bottles filled with deionized water) were carried into the field and opened then closed at random sample sites. These controls were then placed in the cooler alongside the rest of the eDNA samples for the remainder of the time in the field, and then were later stored in the same freezer in the lab until they could be thawed and filtered to begin the DNA analysis. Additionally, at two CFPP "minor" ranked fish passages sampled in both 2018 and 2019, one on Quantico Creek and Dogue Creek, the below sample locations were moved to approximately 0.5 km below the passage, as opposed to directly below (i.e., where sampled in 2018). At one of these locations, the minor passage on Quantico Creek, an additional eDNA sample was collected during the first collection day of 2019 directly below the passage to compare to the new below location.

Prior to field collection of eDNA, each 1-L Nalgene bottle was disinfected using 15-minute 10% bleach bath and rinsed twice with deionized (DI) water. In the field, each sample bottle was rinsed three times using the water at the location and the rinsed solution was discarded downstream of the final eDNA collection location. After rinsing the 1-L Nalgene bottle three times, the final eDNA collection was taken (i.e., the fourth filling of the bottle) and the sample bottle was stored in a cooler of ice before getting transported to the -20°C freezer at the Potomac Science Center in Woodbridge, VA. Concurrent eDNA and box sampling occurred at Cameron Run, Pohick Creek, and Accotink creek and acted as controls for the eDNA sampling methodology.

If time permitted, eDNA samples were filtered within the same day of collection. If there was not enough time to filter an eDNA sample on the day of collection, then the sample was stored in a -20°C freezer until a later date. A study by Hinlo et al. (2017) found that DNA copy number varied significantly across a 28-day period for all storage methods (i.e., room temperature at approximately 20°C and refrigerated at 4°C), except freezing at -20°C wherein the copy number had no significant variation. Although Hinlo et al. (2017) also found that refrigeration (4°C) was the best preservation method for short-term periods of 3-5 days, the constraints of this study could not guarantee that samples would be processed within 5 days, so freezing (-20°C) was deemed the most suitable method of preservation for this study prior to filtration. Once an eDNA water sample was filtered, the filter paper was then folded and inserted into a Falcon TM 15-mL conical centrifuge tube with the sample ID number written in permanent marker on the outer label. The Falcon tube was then placed in a -80°C freezer until the next trip to the Horn Point Lab in Cambridge, MD for DNA extraction and amplification, wherein the tubes were transported in a cooler filled with ice.

Frozen eDNA samples (i.e., 1-L Nalgene bottles) were thawed under running water at a temperature that varied between 30-35°C with intermittent periods of mixing (i.e., inverting the Nalgene bottle). Following the thawing period, samples were transferred to a vacuum filtration apparatus containing a 47-mm diameter cellulose nitrate (CN) filter paper with 1-micron pore size. In the study conducted by Hinlo et al. (2017) comparing the DNA yield and filtration efficiency of various filter paper types used to filter environmental DNA samples, CN filters were found to yield comparable copy numbers to the other top performing filter type (i.e., mixed cellulose ester) and had the second fastest filtration efficiency behind the glass fiber filter. If the amount of water in a sample was less than 1-Liter, then the approximate volume was noted for later comparison with DNA copy numbers returned from positive river herring qPCR products.

Between each eDNA sample, all filter apparatuses and forceps used in the eDNA capture process were disinfected in a 10% bleach solution bath for 15-minutes, followed by 2 DI rinse cycles separated by 5-minute drying periods. New 10% bleach solutions were made daily during the filtration process in order to ensure the bleach concentration did not degrade below 10%.

2.2.2.2 eDNA amplification

All DNA extractions and PCR amplifications were conducted in the Plough Laboratory at the University of Maryland's Center for Environmental Science Horn Point Laboratory between spring 2018 and fall 2019. Extraction methods changed between sampling years in order to conserve time but were deemed comparable after tests conducted by the Plough lab (personal communication, Dr. Louis Plough, UMCES). The DNA from spring 2018 eDNA samples was extracted using the Omega bio-tek E.Z.N.A.® Water DNA Mini Kit Protocol, while the DNA from spring 2019 eDNA samples was extracted following the cetyl trimethylammonium bromide (CTAB) protocol adapted from Renshaw et al. (2015). Following extraction, all samples were stored in a PCR-product-free -20°C freezer until they could be amplified at a later date.

Prior to DNA amplification, samples containing DNA concentrations of greater than 13ng/µL were diluted to produce a product with a concentration of 10ng/µL or less of DNA, in order to minimize inhibition during DNA amplification.

All eDNA samples were amplified using quantitative PCR (qPCR) in triplicate alongside an inhibition control sample to determine if river herring were present at each location. Additionally, two replicates of serially diluted river herring DNA standards ranging from 300,000 to 30 copies of DNA were run with each cluster of qPCR to represent a standard curve for comparison against eDNA amplifications, as well as two water blanks as controls for lab contamination. The river herring standard represents a synthesized oligo (Integrated DNA Technologies GeneBlocks) of a 164 base pair (bp) region of the Cytochrome c oxidase subunit 1 (CO1) gene region of river herring, in which Alewife and Blueback Herring vary by 8-10 bp. A river herring specific molecular beacon assay (i.e., probe) developed by Plough et al. (2018) was used during qPCR to amplify the same 164 bp region of the CO1 gene. The control inhibition samples were comprised of 1µL of eDNA sample and 1µL of the river herring standard. Since these samples were comprised of known quantities of the target DNA (i.e., synthesized river herring DNA) they were expected to achieve a number of quantification cycles (i.e., Cq-value) during qPCR equivalent to the Cq-values produced by the standard samples amplified without eDNA sample added. These samples acted as controls to indicate if the qPCR amplification of the eDNA sample was successful, as the Cq-value achieved would shift away from the Cq-value of the standard without an eDNA sample added, if an inhibitory compound was present in the eDNA sample. If the inhibition control sample did not amplify, then that indicated that an inhibition inducing contaminant was present and the associated eDNA samples in the row needed to be reamplified using a lower concentration of DNA. DNA samples that experience inhibition during qPCR amplification were diluted to 1:10 of original DNA sample, and then reamplified. In instances where inhibition persisted (n=2), a second dilution was performed resulting in a 1:100 dilution of the original DNA sample, and then reamplified.

2.2.2.3 Data Processing

Following qPCR amplification, the number of Cq-value outputs for each triplicate sample and inhibition well were manually assessed to determine which eDNA samples amplified using the river herring assay. Within each eDNA sample, a minimum of two of the three amplifications needed to exceed 15 quantification cycles and at least two of the amplifications needed to not differ by more than 8 cycles relative to one another when amplified, to be automatically considered a positive detection of river herring DNA. Samples that differed by more than 8 cycles were evaluated on a case-by-case basis. Amplification curves were visually assessed for samples exhibiting Cq-values that deviated from 30 to verify if these values warranted a positive detection classification.

2.2.3 Assessment of passage properties

In addition to collecting water samples for DNA extraction at each site, water quality measures, depth profiles and flow profiles were assessed following a modified (i.e., reduced) version of the assessment protocol utilized by McIninch and Garman (1999) to assess the suitability of each stream for hosting river herring spawning runs in the Rappahannock River basin. Based on the extensive analysis conducted by McIninch and Garman (1999), many variables that were initially assessed were ultimately removed from their final analysis. This reduction was a result of preliminary findings which suggested that the excluded variables exhibited either minimal variance, high co-linearity with other variables, or did not contribute to the explanation of variance in species presence. The present study avoided assessing habitat variables excluded from the analysis by McIninch and Garman (1999), with the exception of five variables - four that were excluded on the basis of a lack of variance (i.e., bedrock substrate, boulder substrate, floating aquatic vegetation cover, and leaf pack cover) in the study areas and one that was excluded on the basis of correlation with other variables (i.e., visual assessment of gravel substrate).

A YSI multiparameter sonde was used to measure the following water quality parameters: temperature (°C), dissolved oxygen (%), pH, and salinity (ppt). The sonde used to measure water quality was not equipped to measure total suspended solids, so this water quality parameter was not assessed. These water quality measurements were taken above and below each passage each time an eDNA sample was collected, except for a few samples due to lack of adequate water depth or when measurements were forgotten to be taken.

Physical characteristics including quantitative flow (m/sec) and depth (cm) profiles were converted into average discharge (m^3/sec), as well as qualitative assessments of bottom substrate type, cover attributes (i.e., types of materials available for use as shelter by aquatic organisms), and stream segment type (i.e., riffle, run, or pool), were recorded above and below passage locations in order to assess the suitability of each stream for hosting river herring spawning runs. Flow profiles were generated across the width of each stream using a portable flowmeter by measuring flow, or current velocity (m/sec), every meter until reaching the opposite shoreline. For each measurement, the flowmeter was placed midway in the water column. Concurrently, depth profiles were generated across the width of each stream with a meter stick by measuring depth (cm) every 1-meter until reaching the opposite shoreline. Depth and flow measurements were averaged, and then used to calculate average discharge (m^3/sec) above and below each fish passage – except at locations that were only assessed above the target fish passage due to accessibility issues.

Substrate type was classified by assessing the percent coverage of the following bottom substrates: bedrock, boulder, cobble, gravel, sand, silt-clay, and organic muck (Table 1). Cover attributes were classified by assessing the percent cover of the following attributes: leaf, large woody debris, woody debris (other), algae, submerged aquatic vegetation (SAV), floating aquatic vegetation, and none (Table 2). Both substrate and cover types were visually assessed at three randomly selected locations across the width of the target stream reach within a 0.5-m^2 PCV pipe square marked every 5-cm, or every 1/10 of the total length, on one side and at 25-cm, or the halfway point, on the remaining three edges (Figure 4). Assessment locations across the width of the stream were randomly selected using the Random UX random number generator application for Android operating system. These locations were selected by entering the total distance (m) across the width of the stream, rounded down to the nearest whole number, and applying the "No repeat" parameter to ensure no location on the stream was selected more than once. After three locations were selected, bottom substrate and cover were assessed within the middle of each 1-m sample location. For example, if the 3-m distance was selected then the edge of the 0.5-m^2 square would be placed at 3.25-meters. In instances where the width of the stream was less than 3-meters wide, substrate and cover were assessed for every whole meter available. Substrate and cover were estimated by two observers at two-thirds of locations surveyed, and then later averaged to represent the 0.5-m^2 area assessed. Only one observer estimated substrate and cover types at the remaining one-third of locations assessed. Finally, the water type of each sampling location was classified as either a riffle, run, or pool as defined by the Virginia Department of Environmental Quality (Table 3).

Table 1. Definitions of each substrate type assessed above and below each fish passage.

Substrate Type	Definition
Bedrock	Continuous sheet of rock, no granulation. Assessed visually.
Boulder	Particles sized approximately greater than 250 mm diameter. Assessed visually.
Cobble	Particles sized between approximately 250 mm and 60 mm diameter. Assessed visually.
Gravel	Particles sized between approximately 60 mm and 2 mm diameter. Assessed visually.
Sand	Particles sized between approximately 2 mm and 0.06 mm diameter. Assessed visually.
Silt-clay	Particles sized approximately less than 0.06 mm diameter. Assessed visually.
Organic muck	Decomposing, mud-like substrate. Assessed visually.

Table 2. Definitions of each cover attribute assessed above and below each fish passage.

Cover Attribute	Definition
Leaf	A collection of leaves (i.e., a leaf pack) anywhere in the water column. Assessed visually.
Large woody debris	Woody debris that exceeds 20 cm in diameter. Assessed visually.
Woody debris (other)	Woody debris that is less than 20 cm in diameter. Assessed visually.
Algae	Chlorophyll-containing non-vascular plant-like organisms.
Submerged aquatic vegetation (SAV)	Vegetation growing under water, completely submerged. Assessed visually.
Floating aquatic vegetation	Vegetation growing on the surface of the water (i.e., leaves and flowers), roots may be partially or completely submerged. Assessed visually.
None	Bare or without any material.

Figure 4. PVC 0.5-m^2 square used to mark area for substrate and cover assessments.

Table 3. Definitions of each cover type assessed above and below each fish passage.

Water Type	Definition
Riffle	Shallow, turbulent areas along narrower portions of a stream where the water has a tendency to churn and flow rapidly. In smaller streams, riffles are defined as areas of a distinct change in gradient where flowing water can be observed (DEQ).
Run	Deep with fast-moving water and little or no turbulence.
Pool	Areas of slow-moving water, where the stream widens and deepens, little to no turbulence.

2.2.4 Statistical Analysis

Due to the nature of this sampling method, these results can only confirm the presence of target species but cannot confirm the absence of the species. For this analyses, I assumed that a positive DNA detection above a passage indicated that the target species was able to pass upstream (i.e., completed successful passage), even if the below eDNA sample did not return positive eDNA detection on the same sampling day. This assumption is based on the fact that DNA is not guaranteed to be equally distributed throughout a stream, so it is possible to have "patches" of DNA (Strickland & Roberts, 2019). Additionally, river herring in Potomac River tributaries reside downstream in the Atlantic Ocean for the majority of their lives, aside from the time spent migrating upstream in the spring to spawn, so DNA should only be detected upstream if individuals were able to move through the passage.

In total, between 2018 and 2019, 133 eDNA samples were collected and amplified, along with four negative controls. The four negative controls were not included for statistical analyses to address each hypothesis. Additionally, of the 133 eDNA samples collected in the field, one sample was lost post-filtration and three samples were unable to amplify due to inhibition. These four samples were not included in subsequent statistical analyses as they did not yield river herring presence or absence information. Therefore, 129 eDNA samples were considered for final analysis.

A Kruskal-Wallis test comparing the frequency of successful upstream passage by river herring between passages of difference CFPP "modeled" barrier ranks was done to determine if there was a difference in successful passage at each fish passage based on the calculated ranks (Hypothesis 1). Frequency of successful upstream passage was defined as the fraction of the time that river herring were present above a specific fish passage out of the total number of times they were present in general either above or below on a sampling day. For example, if a fish passage was sampled above and below three times throughout the season, resulting in six total samples, and two sampling days returned positive river herring detections above the passage and one sampling day did not have a positive detection above or below, then the frequency of passage would be 1. If river herring were not detected at all on the third sampling day, this does not indicate that the passage was impassible that day but rather that river herring were not at the location at all that day. The value applied to the third day towards calculating passage frequency is null instead of a zero value, resulting in a passage frequency of 1 rather than 0.67. Fish passages that did not have a single positive river herring detection either above or below were removed from this analysis, as applying a zero value would indicate that the river herring were present but not successfully moving upstream through the passage and I found no evidence to confirm that they were present. This calculation for frequency of successful upstream passage was applied to address Hypotheses 2 and 4 as well. For Hypothesis 1, only samples ranked by the CFPP tool were considered, so the dam and all weirs were excluded for this analysis. A second Kruskal-Wallis test was run to determine if there was a difference in the frequency of successful upstream passage based on structural design, including bridges, small round culverts (<2m wide), large round culverts (>2m wide), large square culverts (>2m wide), and weirs (Hypothesis 2). When a Kruskal-Wallis test indicated a significant difference, it was followed by a post hoc Steel-Dwass test to determine which ranks/structures were significantly different from which. A Pearson's chi-squared test was performed to determine if there was a difference in river herring presence below fish passages based on the stream segment type below the passage, including riffles, runs, and pools (Hypothesis 3). The above-mentioned analyses were performed using JMP Software from SAS.

A permutational multivariate analysis of variance (PERMANOVA) was ran in Plymouth Routines in Multivariate Ecological Research (PRIMER-e) version 7 followed by a principal component analysis (PCA) to address whether certain combinations of physio-chemical variables were indicative of overall detection of river herring DNA around a passage (Hypotheses 4). All samples lacking measurements for water quality parameters were removed from the test (n=6). River herring presence was treated as a factor, while each water quality parameter was a variable. A second PERMANOVA was ran followed by a PCA to address whether the same physio-chemical variables that were indicative of species presence were also indicators of the frequency of successful upstream passage by river herring at each fish passage (Hypothesis 5). The variable 'stream discharge' was entirely removed from the analysis associated with Hypotheses 4 and 5 due to missing values in some of the streams which the PERMANOVA test and subsequent PCA did not allow for.

2.3 Results

In total, 132 of the 133 eDNA samples collected in the field between spring 2018 and 2019 underwent qPCR using the river herring-specific assay developed by Plough et al. (2018). Three eDNA samples did not amplify during qPCR and subsequent inhibition removal protocols, so were excluded from further analysis resulting in 129 eDNA outputs informing river herring presence. Field collected eDNA samples returned 82 negative and 37 positive river herring detections, while 3 negative control samples were negative for river herring DNA and 1 was positive.

Of the 22 fish passage locations assessed, including 18 road-stream intersections, 3 weirs, and 1 dam, I found evidence to suggest that river herring were present at 15 of the locations (Figure 5). These 15 locations were comprised of fish passages at road-stream intersections, 1 weir, and 1 dam. Positive river herring DNA detections occurred in 9 of the 11 surveyed streams: Accotink Creek, Bullneck Run, Cameron Run, Dogue Creek (mainstem and north fork), Donaldson Run, Marumsco Creek, Neabsco Creek, Powell's Creek, and Quantico Creek (mainstem, south fork, and Mary Bird Branch). Four streams detected river herring at multiple locations including 3 fish passages on Neabsco Creek, 3 fish passages across the mainstem, south fork and Mary Bird Branch of Quantico Creek, 2 fish passages along the north fork of Dogue Creek and 2 fish passages on Powell's Creek. River herring were also detected at the weir on Cameron Run and the dam on Accotink Creek. All locations experienced at least one successful upstream passage event as indicated by a positive eDNA detection above the targeted passage, weir, or dam.

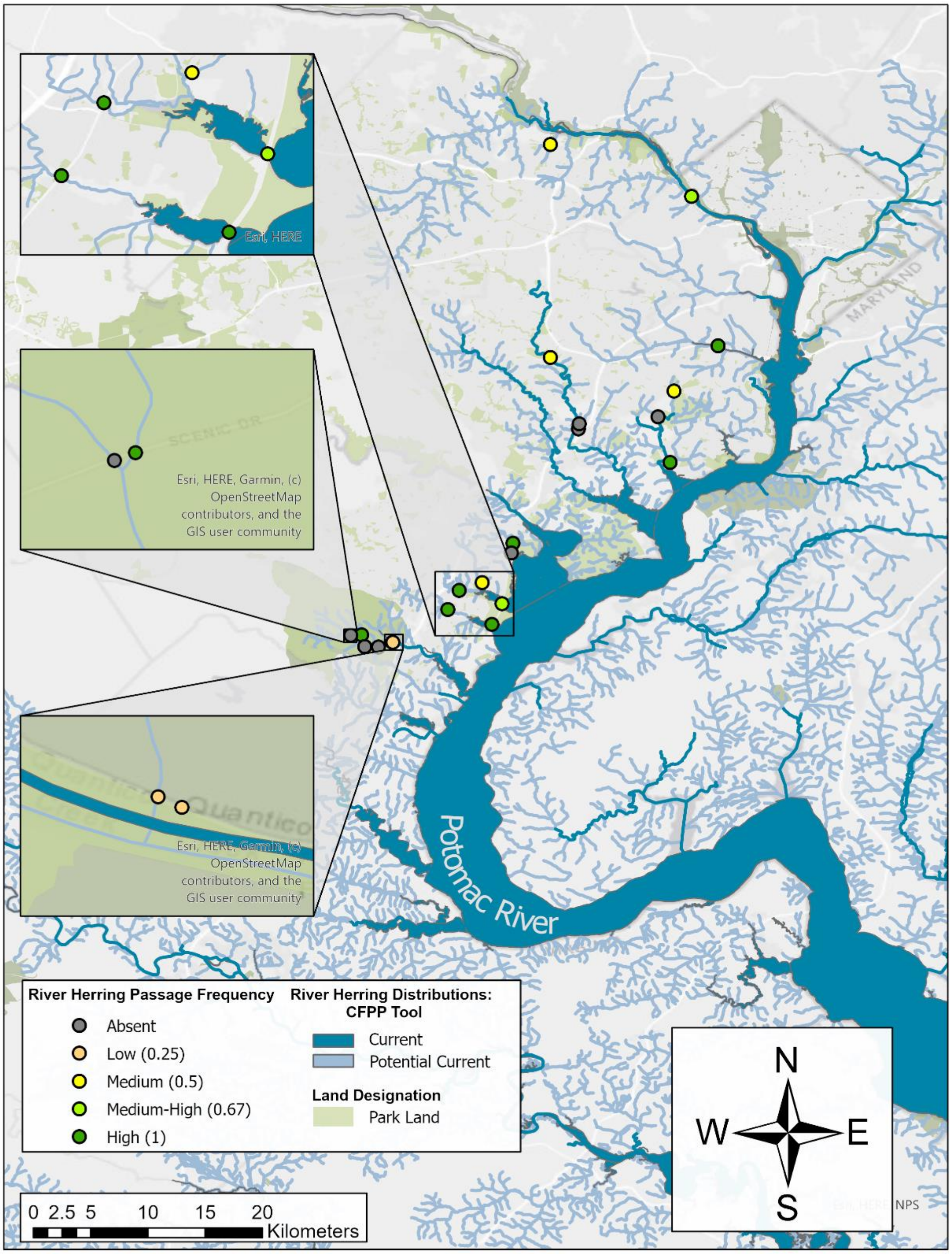


Figure 5. Frequency of river herring passage at each fish passage location assessed in relation to current and potential current river herring ranges determined by the CFPP tool, as well as known areas designated as either regional, state, or national park land.

2.3.1 River Herring Presence and Passage

I reject the null hypothesis (Hypothesis H_{01}) of no difference between the frequency of successful upstream passage between the three barrier ranks, insignificant (n=13), minor (n=36), and moderate (n=27) (*P*=.03; Figure 6). The post hoc Steel-Dwass test determined that there was a significant difference in passage frequency between Insignificant and Moderate barrier ranks (*P*=.04), but that all other barrier ranks performed equivalently to one another (Table 4).

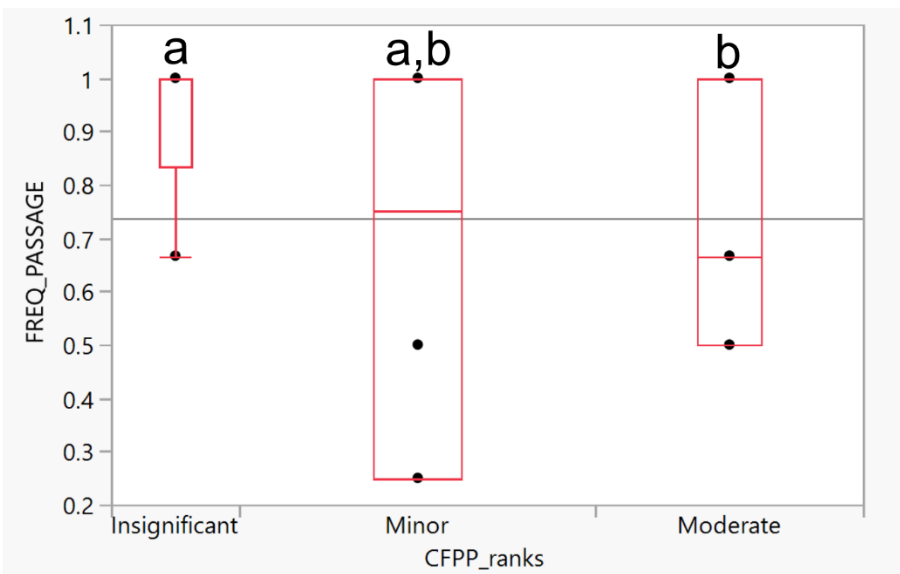


Figure 6. Comparison of successful passage frequencies (FREQ_PASSAGE) observed across CFPP "modeled" barrier ranks (CFPP_ranks), Insignificant, Minor, and Moderate, illustrating significant differences in representation of four passage frequencies (0.25, 0.5, 0.67, and 1) across groups (P=.03) with significant differences occurring between Insignificant and Moderate passage frequencies (*P*=0.04). The letters (a,b) indicate the significant differences.

Table 4. Outputs from the Steel-Dwass post hoc comparisons, between CFPP "modeled" barrier ranks (Level, -Level) based on frequency of successful upstream passage, including score mean difference, standard error difference, Z value, *P*-value, Hodges-Lehman value, lower and upper confidence intervals (CL), with significant *P*-values indicated in red.

Level	- Level	Score Mean Difference	Standard Error Difference	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL
Moderate	Minor	4.63426	4.332454	1.06966	0.5330	0	0	0.25
Moderate	Insignificant	-8.49003	3.53809	-2.39961	**0.0433**	-0.166667	-0.5	0
Minor	Insignificant	-9.37073	4.127565	-2.27028	0.0600	-0.083333	-0.5	0

Additionally, I reject the null hypothesis (Hypothesis H_{02}) of no difference between the frequency of successful upstream passage between the six structural designs, bridge, small round culvert (diameter < 2 m), large round culvert (diameter > 2 m), large square culvert (diameter > 2 m), weir, and dam (*P*<.0001; Figure 7). Further comparisons using a post hoc Steel-Dwass test, with the alpha level lowered from 0.05 to 0.01 to account for the increased chance of false positive detections of significant differences due to the high amount of multiple comparisons, suggested that large round culverts performed worse than all other design types (maximum p-value, *P*=0.0007). Alternatively, all remaining design types performed equivalently against other designs, except when comparing bridges and dams (*P*=.002) (Table 5). Bridges performed significantly better than dams (*P*=.002) and large round culverts (*P*<.0001), but equally as well as the remaining three structure types, large square culverts (diameter >2 m), small round culverts (diameter <2 m), and weirs (Table 5).

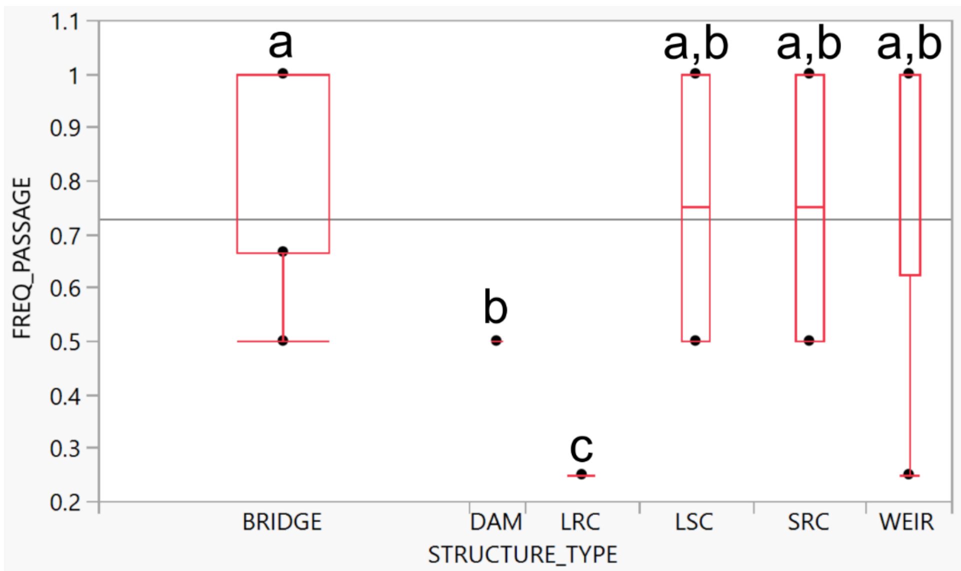


Figure 7. Comparison of successful passage frequencies (FREQ_PASSAGE) between five structural designs, bridge, dam, large round culvert (LRC; diameter <2 m), large square culvert (LSC; diameter >2 m), small round culvert (SRC; diameter < 2 m), and weir illustrating significant differences in passage frequency across groups (*P*<.0001), and between groups with significant differences indicated by differences in letter assignment a, b, and c.

Table 5. Outputs from post hoc Steel-Dwass comparison, between five structural design levels – large square culvert (LSC; diameter >2 m), large round culvert (LRC; diameter >2 m), small round culvert (SRC; diameter <2 m), weir, dam, and bridge, based on frequency of successful upstream passage, including score mean difference, standard error difference, Z value, *P*-value, Hodges-Lehman value, lower and upper confidence intervals (CL), with significant *P*-values indicated in red.

Level	- Level	Score Mean Difference	Standard Error Difference	Z	p-Value	Hodges-Lehmann	Lower CL	Upper CL
LSC	LRC	11.9167	2.653955	4.49015	**0.0001**	0.5	0.25	0.75
LSC	DAM	4.375	2.182821	2.00429	0.3397	0.25	0	0.5
LSC	BRIDGE	-6.8109	4.148795	-1.64166	0.5707	0	-0.5	0
LRC	DAM	-8.875	2.182821	-4.06584	**0.0007**	-0.25	-0.25	-0.25
LRC	BRIDGE	-25.4455	4.486132	-5.67204	**<.0001**	-0.75	-0.75	-0.416667
SRC	LRC	11.9167	2.653955	4.49015	**0.0001**	0.5	0.25	0.75
SRC	DAM	4.375	2.182821	2.00429	0.3397	0.25	0	0.5
SRC	LSC	0	2.502173	0	1	0	-0.5	0.5
SRC	BRIDGE	-6.8109	4.148795	-1.64166	0.5707	0	-0.5	0
WEIR	LSC	1.6528	2.354074	0.70209	0.9817	0	-0.25	0.5
WEIR	LRC	8.0694	2.236534	3.60801	**0.0042**	0.75	0	0.75
WEIR	SRC	1.6528	2.354074	0.70209	0.9817	0	-0.25	0.5
WEIR	BRIDGE	0.3419	4.144683	0.08249	1	0	-0.25	0.333333
WEIR	DAM	4.0278	2.154729	1.86927	0.4212	0.5	-0.25	0.5
DAM	BRIDGE	-18.9423	5.031153	-3.765	**0.0023**	-0.5	-0.5	0

The Pearson's chi-squared test found no significant difference between the ratio of species presence and absence detected below passages when comparing stream segment types observed below passages (Hypothesis H_{03}; *P*=.13), although deeper, faster moving water yielded a higher proportion of present detections than absent (Figure 8).

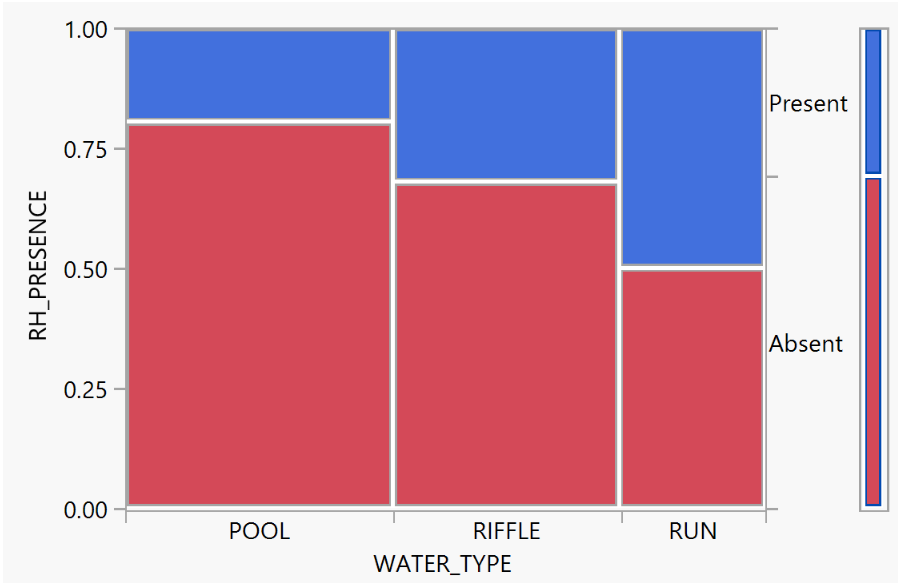


Figure 8. Mosaic plot illustrating the ratio of river herring (RH) detections below fish passages based on stream segment type, either pool, riffle, or run.

I failed to reject the null hypothesis (Hypothesis H_{04}) of no difference between areas where river herring were detected and not detected in relation to environmental variables including water quality, bottom substrate type, and habitat cover (*P*=.07, *Pseudo-F*=1.60). I still performed a PCA to evaluate site differences based on environmental variables. Although 8 principal components (PCs) exhibited eigenvalues above 1, a clear distinction occurred between the second and third PC wherein eigenvalues dropped below 2 (Table 6). The first two axes together accounted for 24.8% of the variation, whereas the first five axes accounted for 53.6% (Table 6). Higher values on PC1 indicate an increase in percent coverage of floating aquatic vegetation (FAV.%) and algae (ALGAE.%) habitat coverage, silt-clay (SILT_CLAY.%) and organic muck

(ORGANIC_MUCK.%) bottom substrates, as well as salinity (SAL.PPT), whereas higher values on PC2 indicate an increase in percent leaf coverage (LEAF.%), boulder substrate (BOULDER.%), and pH, and a decrease in cobble substrate (COBBLE %) and temperature (TEMP.C; Table 7). There seems to be no clear habitat preference in these samples due to the lack of distinct clusters between streams with river herring absent or present (Figure 9).

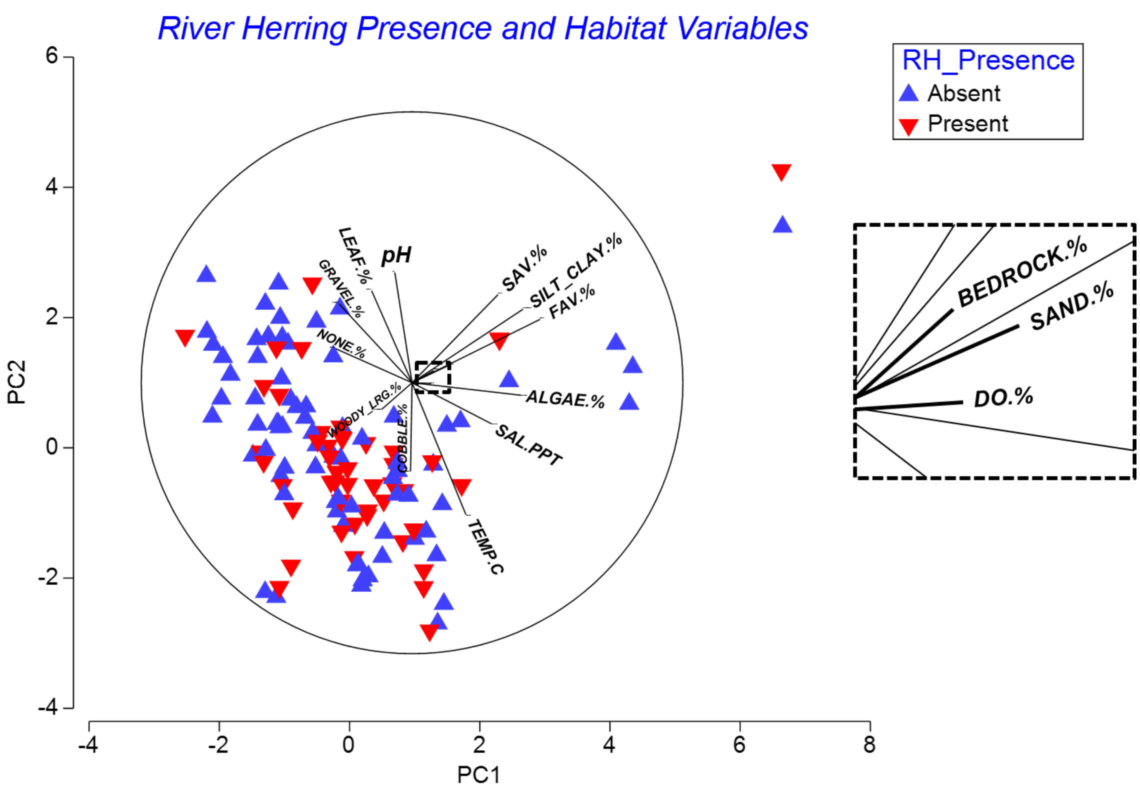


Figure 9. PCA examining overall river herring (RH) presence in streams in relation to three general habitat characteristics, water quality, substrate types and stream cover, representing 18 variables: temperature (TEMP.C), percent dissolved oxygen (DO.%), pH, salinity (SAL.PPT), bedrock (BEDROCK.%), boulder (BOULDER.%), cobble (COBBLE.%), gravel (GRAVEL.%), sand (SAND.%), silt-clay (SILT_CLAY.%), organic muck (ORGANIC_MUCK.%), leaf litter (LEAF.%), large woody debris (WOODY_LRG.%),

other woody debris (WOODY_OTHER.%), algae (ALGAE.%), submerged aquatic vegetation (SAV.%), floating aquatic vegetation (FAV.%), and no cover (NONE.%).

Table 6. Principle component analysis (PCA) output for 10 principle components (PC) including associated eigenvalues, percent (%) variation, and cumulative percent (Cum.%) variation assessing relationship between river herring presence and habitat variables.

PC	Eigenvalues	%Variation	Cum.%Variation
1	2.43	13.5	13.5
2	2.03	11.3	24.8
3	1.92	10.7	35.5
4	1.76	9.8	45.2
5	1.51	8.4	53.6
6	1.28	7.1	60.7
7	1.16	6.5	67.2
8	1.06	5.9	73.1
9	0.903	5	78.1
10	0.896	5	83.1

Table 7. Eigenvector outputs, or coefficients in the linear combination of variables making up principle components (PCs), from principle component analysis (PCA) assessing the relationship between river herring presence and habitat variables across 10 PCs, including: temperature (TEMP.C), percent dissolved oxygen (DO.%), pH, salinity (SAL.PPT), bedrock (BEDROCK.%), boulder (BOULDER.%), cobble (COBBLE.%), gravel (GRAVEL.%), sand (SAND.%), silt-clay (SILT_CLAY.%), organic muck (ORGANIC_MUCK.%), leaf litter (LEAF.%), large woody debris (WOODY_LRG.%), other woody debris

(WOODY_OTHER.%), algae (ALGAE.%), submerged aquatic vegetation (SAV.%), floating aquatic vegetation (FAV.%), and no cover (NONE.%).

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
TEMP.C	0.25	-0.341	0.001	0.196	0.256	-0.015	0.201	-0.096	-0.195	-0.488
DO.%	-0.014	-0.037	0.292	-0.049	-0.368	-0.33	-0.141	-0.294	-0.238	-0.516
SAL.PPT	0.261	-0.058	0.184	0.223	-0.271	-0.304	-0.369	0.13	-0.046	0.252
PH	-0.131	0.365	0.169	-0.096	0.108	-0.349	-0.429	-0.038	0.033	-0.098
BEDROCK.%	0.048	0.177	0.027	0.28	-0.339	0.013	0.105	0.655	-0.057	-0.122
BOULDER.%	-0.14	0.441	-0.012	0.05	0.067	-0.25	0.443	-0.258	-0.076	-0.039
COBBLE.%	-0.026	-0.36	0.155	-0.079	-0.075	-0.458	0.352	0.17	0.3	0.129
GRAVEL.%	-0.286	-0.069	0.137	-0.45	0.02	0.278	-0.081	0.209	0.086	-0.337
SAND.%	0.177	-0.069	-0.172	-0.195	-0.502	0.255	-0.122	-0.437	0.068	0.192
SILT_CLAY.%	0.384	0.19	0.047	-0.229	-0.065	0.173	0.007	0.252	-0.187	-0.101
ORGANIC_MUCK.%	0.292	-0.055	-0.478	-0.197	0.119	-0.224	-0.103	0.082	-0.171	-0.168
LEAF.%	-0.137	0.428	-0.3	0.029	-0.308	0.042	0.207	0.03	-0.064	-0.143
WOODY_LRG.%	0.079	-0.07	-0.589	-0.011	-0.102	-0.301	-0.008	0.004	0.227	-0.083
WOODY_OTHER.%	-0.163	-0.152	0.011	-0.234	-0.077	-0.129	0.215	0.036	-0.75	0.371
ALGAE.%	0.319	-0.025	0.26	-0.034	-0.313	0.12	0.371	-0.115	0.19	-0.091
SAV.%	0.237	0.287	0.186	-0.285	0.186	-0.184	0.169	-0.048	0.222	0.15
FAV.%	0.458	0.139	0.073	-0.335	0.177	-0.016	-0.021	0.08	-0.069	0.052
NONE.%	-0.253	-0.167	-0.064	-0.479	-0.208	-0.157	0.019	0.179	0.137	-0.058

I reject the null hypothesis (Hypothesis H_{05}) of no significant difference between the environmental variables of sites with different river herring passage frequency (P=.0001, *Pseudo-F*=4.451). To determine exactly which sites with different passage frequencies were significantly different from one another, based on environmental variables, a post-hoc pair-wise PERMANOVA was conducted. Accepted alpha values were reduced from 0.05 to 0.01 to account for the increased chance of detecting artificially generated differences due to multiple comparisons across frequency of passage categories. This comparison indicated that there were significant differences between all passage frequency groups (P<.01), except when comparing the two highest passage frequencies (Table 8). The PCA examining how sites with different passage frequencies by river herring differed based on their environmental variables found that 8 PCs exhibit eigenvalues above 1, but a clear distinction occurred between the third and fourth PC wherein eigenvalues dropped below 2 (Table 9). The first three axes accounted for 38.1% of the variation, whereas the first five axes accounted for 56.2% of the variation (Table 9). Each passage frequency category formed clear clusters, with samples from the highest frequency category overlapping with all other categories (Figure 10). Cluster separation occurred as PC1 and PC2 values increased, and were most heavily influenced by percent coverage of floating aquatic vegetation (FAV.%) followed by submerged aquatic vegetation (SAV.%) and silt-clay (SILT_CLAY.%) bottom substrate (Table 10). Locations with the lowest frequency of passage corresponded to areas with the lowest FAV, SAV, and silt-clay coverage, whereas mid-range passage frequencies corresponded to locations with the highest coverage of these three variables (Figure 10). The locations demonstrating the two highest frequencies of passage corresponded to mid-range levels of FAV, SAV, and silt-clay coverage (Figure 10).

Table 8. Outputs for post-hoc pair-wise PERMANOVA comparing habitat variables between frequency of successful passage by river herring (Groups), including pseudo-*t* (t), *P*-values by permutation (P(perm)), and number of unique permutations performed (perms), and with significant *P*-values indicated in red.

Groups	t	P(perm)	perms
0.25, 0.5	2.7135	**0.0001**	9951
0.25, 0.67	2.591	**0.0003**	7790
0.25, 1	2.4106	**0.0001**	9938
0.5, 0.67	1.8981	**0.0058**	9814
0.5, 1	2.0094	**0.0001**	9926
0.67, 1	1.6394	0.0132	9929

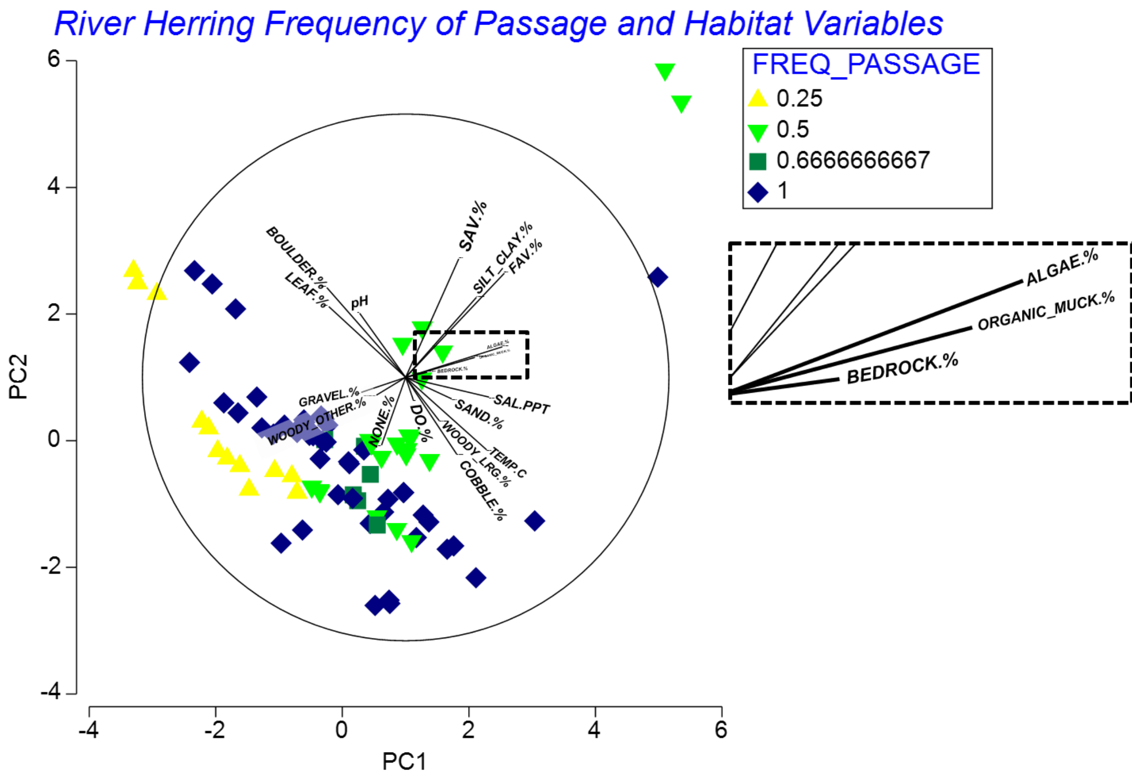


Figure 10. PCA examining frequencies of successful passage by river herring in relation to three general habitat characteristics, water quality, substrate types and stream cover, representing 18 variables: temperature (TEMP.C), percent dissolved oxygen (DO.%), pH, salinity (SAL.PPT), bedrock (BEDROCK.%), boulder (BOULDER.%), cobble (COBBLE.%), gravel (GRAVEL.%), sand (SAND.%), silt-clay (SILT_CLAY.%), organic muck (ORGANIC_MUCK.%), leaf litter (LEAF.%), large woody debris (WOODY_LRG.%), other woody debris (WOODY_OTHER.%), algae (ALGAE.%), submerged aquatic vegetation (SAV.%), floating aquatic vegetation (FAV.%), and no cover (NONE.%).

Table 9. Principle component analysis (PCA) output for 10 principle components (PCs) including associated eigenvalues, percent (%) variation, and cumulative percent (Cum.%) variation assessing relationship between river herring frequency of successful upstream passage and habitat variables.

PC	Eigenvalues	%Variation	Cum.%Variation
1	2.61	14.5	14.5
2	2.19	12.2	26.6
3	2.06	11.5	38.1
4	1.64	9.1	47.2
5	1.6	8.9	56.2
6	1.33	7.4	63.6
7	1.2	6.7	70.2
8	1.05	5.8	76.1
9	0.858	4.8	80.8
10	0.816	4.5	85.4

Table 10. Eigenvector outputs, or coefficients in the linear combination of variables making up principle components (PCs), from principle component analysis (PCA) assessing the relationship between river herring frequency of successful upstream passage and habitat variables across 10 PCs, including: temperature (TEMP.C), percent dissolved oxygen (DO.%), pH, salinity (SAL.PPT), bedrock (BEDROCK.%), boulder (BOULDER.%), cobble (COBBLE.%), gravel (GRAVEL.%), sand (SAND.%), silt-clay (SILT_CLAY.%), organic muck (ORGANIC_MUCK.%), leaf litter (LEAF.%), large woody debris (WOODY_LRG.%), other woody debris (WOODY_OTHER.%), algae (ALGAE.%), submerged aquatic vegetation (SAV.%), floating aquatic vegetation (FAV.%), and no cover (NONE.%).

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
TEMP.C	0.308	-0.275	0.038	-0.066	0.126	-0.136	0.133	-0.297	0.257	0.568
DO%	0.029	-0.086	0.479	0.136	-0.144	0.19	0.256	0.021	-0.165	0.431
SAL.PPT	0.317	-0.075	0.328	0.13	0.25	-0.101	0.061	0.338	-0.235	-0.012
PH	-0.175	0.243	0.347	-0.227	0.095	0.044	0.018	0.368	-0.331	0.108
BEDROCK.%	0.082	0.019	0.069	0.502	-0.099	-0.509	0.151	0.087	-0.007	-0.159
BOULDER.%	-0.299	0.341	-0.061	-0.069	0.14	0.05	0.493	-0.198	0.066	0.159
COBBLE.%	0.194	-0.291	0.085	-0.316	0	-0.103	0.487	-0.199	-0.099	-0.4
GRAVEL.%	-0.169	-0.057	0.039	-0.168	-0.58	-0.162	-0.321	-0.126	-0.152	0.137
SAND.%	0.175	-0.082	-0.18	0.37	-0.245	0.521	0.007	0.18	-0.119	0.071
SILT_CLAY.%	0.269	0.304	-0.014	0.281	-0.196	-0.303	0.064	0.021	0.083	-0.049
ORGANIC_MUCK.%	0.263	0.076	-0.429	-0.187	0.05	-0.178	-0.015	0.284	-0.017	0.412
LEAF.%	-0.292	0.269	-0.246	0.31	-0.028	-0.011	0.301	-0.125	-0.161	0.155
WOODY_LRG.%	0.127	-0.164	-0.485	-0.066	0.135	0.082	0.164	0.153	-0.446	-0.066
WOODY_OTHER.%	-0.111	-0.068	-0.022	-0.117	-0.229	0.12	0.304	0.568	0.618	-0.079
ALGAE.%	0.368	0.116	0.056	0.086	-0.221	0.426	0.094	-0.274	0.044	-0.152
SAV.%	0.202	0.45	0.061	-0.282	-0.143	0.007	0.088	-0.045	-0.121	-0.095
FAV.%	0.375	0.396	-0.015	-0.23	-0.136	-0.036	-0.052	0.029	0.066	0.039
NONE.%	-0.092	-0.257	-0.067	-0.139	-0.516	-0.189	0.271	0.075	-0.237	0.069

2.4 Discussion

This study found that the CFPP modeled barrier ranks were moderately indicative of fish passage success, as there were significant differences between the lowest and highest ranks, but not between either the low or high and mid-range. Since there was not a complete distinction between the performance of passages based on CFPP ranks, this suggests that other factors not yet accounted for in the CFPP tool may be influencing passage use. Still, this tool should continue to be used as a resource for selecting passages for preliminary evaluation and not the direct selection of passages in need of restoration. Additionally, all culvert designs, aside from large round culverts, performed equally as well at permitting the upstream passage of river herring as bridges, indicating that all designs are viable options for use in future fish passage installations. There was no discernable difference between locations with and without river herring present based on habitat characteristics; however, between locations with river herring present, variations in habitat characteristics were strongly associated with observed differences in passage frequency. These differences did not follow a hierarchical progression, rather, as FAV, SAV, and silt-clay coverage increased passage frequencies rapidly increased then returned back to a moderate level of passage. This finding suggests that river herring can persist in a variety of habitat conditions but require more specific conditions to support movement through artificial passages. Areas with greater frequencies of upstream passage had high to moderate levels of FAV, SAV, and silt-clay coverage, which corresponds to the preferred spawning habitat characteristics documented in the literature (Mather et al., 2012).

The frequency of successful upstream passage metric used in this study parallels the passage efficiency metric commonly used in assessments of fish passage use and success (Bunt et al., 2012), which is indicative of how well a fish passage is able to support fish movement. Although I was not documenting individual fish passage events, this assessment was still able to detect passage use by river herring for the duration of their spawning season. Incorporating this metric in future fish passage assessments using eDNA will allow for the differentiation between low- and high-performing passages, which will ultimately aid in the prioritization of low-performing passages as sites for passage restoration.

The approximation of attraction efficiency, through stream segment classification, did not perform well as a diagnostic metric for river herring presence below passages. High levels of attraction efficiency frequently correlate to higher flow output leading up to a fish passage (Bunt et al., 2012), however, there was not significant variation between the ratio of river herring presence and absence below passages when comparing across stream segment types, either riffle, run, or pool. Based on these findings, I infer that this metric was not precise enough to account for the variations in flow associated with passages attracting river herring. While these classifications were unable to yield as precise of measurements as those produced by calculating average discharge, they are still valuable as they allowed for the rapid, qualitative evaluation of water flow around fish passages. Furthermore, by definition, these stream segment classifications correspond to relative stream flow, and therefore estimated attraction efficiency, increasing from pool to riffle to run. Since frequency of passage, in the study, was calculated only for locations that detected the presence of river herring DNA at least once, locations with zero river herring detections were not incorporated into the subsequent analyses associated with frequency of passage. Therefore, the stream segment types found below passages not attracting river herring may have been underrepresented in analysis.

While this study found no significant trends in variables pertaining to attraction efficiency, there were significant distinctions between the frequency of successful upstream passage metric and structural design types. All fish passages at road-stream intersections performed equally across locations with the exception of large round culverts (diameter >2 m) which performed significantly worse than all other passage types. Upon visual inspection, both large round culverts assessed experienced partial blockage during the river herring spawning season from debris accumulation above the passage (Appendix I, Passages 10A and 11A). Larger culverts are generally anticipated to permit higher stream flows, which may increase the likelihood of larger debris washing downstream and subsequently blocking passage entrance. This outcome suggests that passages should be assessed, particularly during peak seasons of use by species of interest such as river herring, to ensure debris accumulation is not blocking either upstream or downstream access to passages. Future anadromous fish passage use studies should pre-treat passages identified as important by clearing debris early in the spawning season to ensure a lack of passage was due to an issue in structural design and not unrelated blockages from debris. Passages identified as experiencing frequent debris accumulation while also hosting river herring spawning runs, should be cleared annually in late April to prepare for the river herring spawning runs that year.

Although I anticipated bridges to outperform all other fish passage structures due to the openness of the passage type, as well as dedication to preserving the nature-like bottom substrate, I found that they performed comparably to nearly all passage types with the exception of the two lowest performing passages, large round culverts and the dam. However, of the passages deemed “insignificant” barriers to fish movement (i.e., the lowest ranked barrier), bridges appeared to have the least impact on natural streamflow when visually assessed (Figure 11). Smaller passages, such as small round culverts, were frequently observed clogging with various items including, trash and fallen limbs. These eDNA results suggest that many of the structural designs support fish movement, yet visual inspections found that over time, structures with artificially created bottom substrate often outlast the surrounding natural features, resulting in eroded undercuts near passage exits (Appendix I, Passages 10, 15, 18).

Figure 11. Variations in observed stream flow between bridge (left), small round culvert (middle), and weir (right).

River herring were not detected around the two lowest stream barriers on Accotink Creek, one artificially created weir and one naturally occurring weir, however, the species were detected directly above and below Accotink Dam upstream of the two lowest barriers. Based on these findings, there are four primary possibilities for how river herring DNA was detected around the dam but not lower in the stream: 1) one or many river herring were transported to the stream reach above the dam, 2) a once migratory population of river herring settled in the waters above the dam and the DNA traces have been diluted so far as to severely decrease the potential for detection at the lowermost barriers, 3) a migratory population of river herring have traversed the steep grade of the dam in search of spawning habitat above the barrier, or 4) contamination of the eDNA sample occurred at a point between collection, filtration, and amplification. The first option seems the most likely, as there are numerous fish-eating raptor and wading bird species inhabiting the areas surrounding Accotink Creek and Lake Accotink, such as Bald Eagles (*Haliaeetus leucocephalus*), Osprey (*Pandion haliaetus*), and Great Blue

Herons (*Ardea Herodias*) (*personal observation*), that could transport river herring DNA to upstream reaches via partial or whole transport of individuals following capture or defecation following consumption. Deiner et al. (2017) recognize the potential of predator fecal matter to influence species presence documented by eDNA. The second hypothesis is less likely, but an interesting idea that should be explored further. Although most land-locked river herring, primarily Alewife, are believed to be anthropogenically introduced, some regions such as southern Connecticut host populations believed to have become landlocked as a result of dam installation more than 200 years ago (Phillips et al., 1987). According to the CFPP tool, Accotink Dam is 28.0 feet high with a steep gradient and no documented fish passage facility (Appendix I, Passage 3), so the possibility of river herring traversing the face of the dam seems unlikely. However, the CFPP tool reports that 57.31 miles of upstream functional network length exist above Accotink Dam, also known as the number of miles of a stream that a fish could theoretically access upstream of a specified point based on known barriers to movement, which is an ample amount of stream miles for river herring persistence. Furthermore, Lake Accotink covers approximately 50-acres which is comparable to other lakes hosting landlocked populations of Alewife (Palkovacs et al., 2007). Therefore, a scenario where a founder population of river herring landlocked upstream prior to the development of Accotink Dam in 1918 is a possibility.

Finally, there is still the possibility, although low, that the three instances of river herring DNA detection around the dam, once upstream and twice below, were a result of error during eDNA processing. The positive river herring detection in one of the four deionized water negative control samples filled in the lab then opened while in the field and stored with subsequently collected eDNA samples supports this possibility. Contamination likely occurred during preparation for DNA amplification wherein many DNA samples were handled in close proximity to one another. Ultimately, to account for possible errors in processing, future studies should consider collecting multiple eDNA samples at each location, above and below targeted barriers.

Overall, streams with river herring present or absent did not have significantly different habitat characteristics but were different when comparing frequency of passage across fish passages. This finding suggests that habitat characteristics are more important in determining where river herring will successfully traverse a fish passage. The PCA examining the relationship between habitat variables and frequency of successful river herring passage found that a greater presence of FAV and SAV coverage, and silt-clay bottom substrate were positively associated with mid-range passage frequencies. The highest passage frequencies were associated with mid-range FAV, SAV, and silt-clay coverage. These three habitat characteristics are indicative of areas with lower streamflow, and as such potentially more easily traversed fish passages and more optimal for use as spawning areas (Pardue, 1983). These results warrant the prioritization of further passage evaluation in streams supporting these three key habitat features, as they are more indicative of streams likely to support river herring spawning runs.

2.4.1 Management Applications

Since river herring were once a valuable fisheries species and are currently protected to regain population strength, rapid assessment survey methods like the eDNA protocol executed in this study are key tool to help in the monitoring of populations to inform future recovery strategies. The findings in this study indicate that eDNA is capable of differentiating between localized (i.e., within 1km) differences in anadromous species presence around fish passages, which supports the application of this detection method for the assessment of passage use by anadromous fishes in northern Virginia. This protocol can serve as a feasible option for assessment of passage use by river herring, and other anadromous fish species, moving forward. Having evidence to support the use of this method in assessing passage use is valuable as it can be executed across more streams in the same amount of time required by traditional monitoring methods, such as 24-hour hoop nets, further building upon known species ranges and requirements for successful passage use.

The results of the present study, including updated confirmed river herring ranges (Figure 12), will be shared with natural resource managers, such as the Virginia DWR, interested in implementing their own fish passage assessment. Additionally, the information surrounding ground-truthed passage characteristics and river herring passage frequencies will be shared with the Nature Conservancy in hopes that these assessments be incorporated into future iterations of the CFPP tool. These results suggest that fish passage restoration should focus on road-stream intersections marked as more severe barriers to fish movement by the CFPP tool, as these were demonstrated to perform worse than lower ranked barriers. More importantly, though, the visual observations from this study suggest that lower ranked barriers in potential river herring ranges should be evaluated each year prior to the start of river herring spawning season to determine if stream debris such as fallen branches and trash have accumulated since the previous spring. As demonstrated in this study, many passage designs are capable of permitting anadromous fish passage but may need more frequent upkeep to ensure they do not become obstructed over time. These outcomes can be used to guide future site selection for studies interested in quantifying the effects of watershed fragmentation on aquatic organism health.

The results of this study are only able to infer information on overall river herring presence and passage success, differentiating Alewife and Blueback Herring as a group from other Alosine species. To better inform management decisions, future studies using the river herring specific assays developed by Plough et al. (2018) should also send PCR products for additional Sanger sequencing to specifically differentiate between occurrences of Alewife, Blueback Herring, or both species DNA detections. This differentiation has a growing importance as Alewife populations in Potomac River tributaries are believed to be stabilizing, while the status of Blueback Herring populations are still unknown (ASMFC, 2017).

Establishing well-defined sampling protocols for assessing fish passage use for migratory species is especially important now, as the processing costs for eDNA sampling and subsequent analysis is projected to continue to decline as time progresses supporting the use of this method in future species monitoring projects (Kelly et al., 2014). This study focused on Alewife and Blueback Herring, but the assessment protocols used could aid in future studies focused on other of interest species such as

Atlantic Sturgeon (*Acipenser oxyrinchus oxyrinchus*) or American Shad (*Alosa sapidissima*).

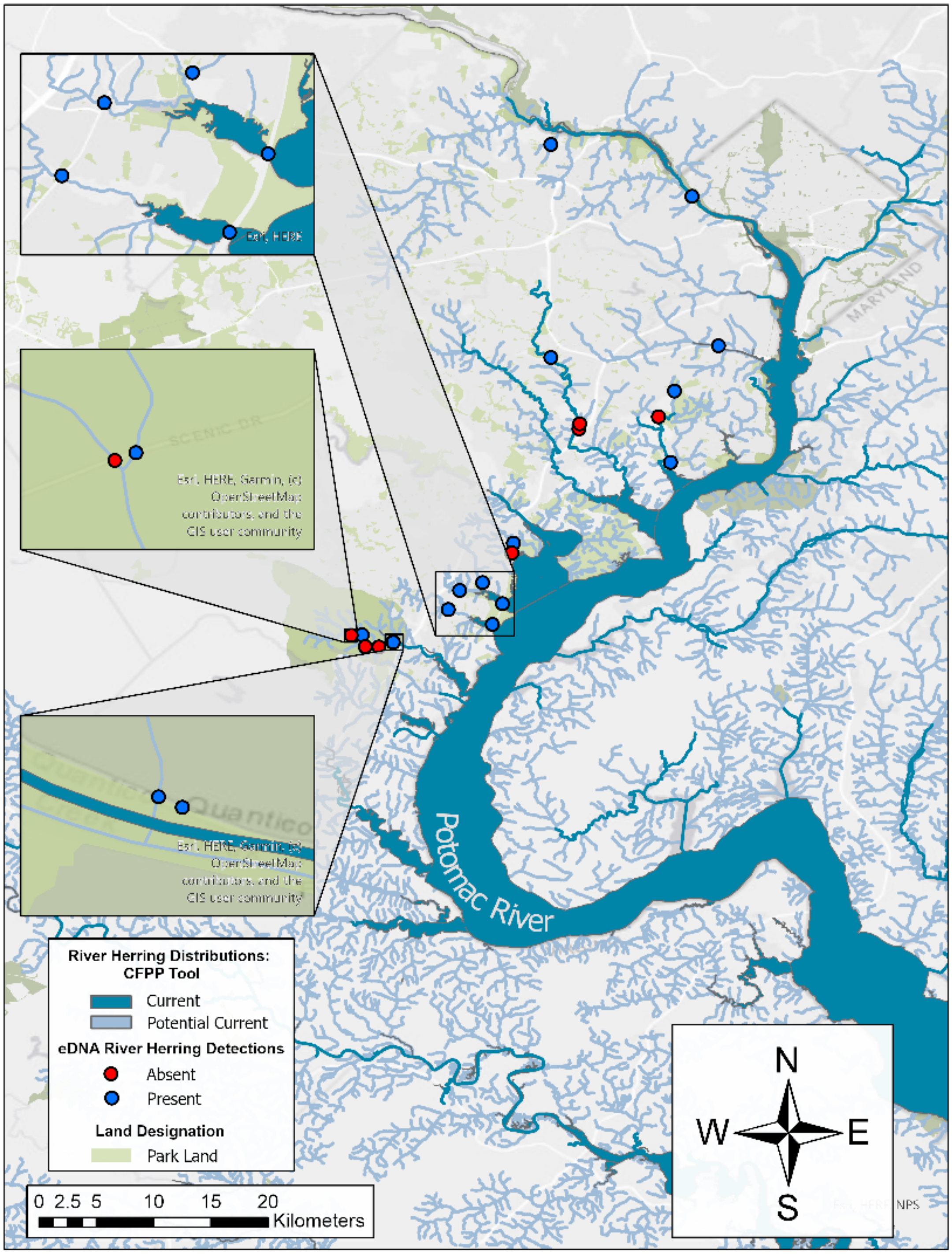


Figure 12. Updated river herring range based on eDNA detections of fish passage use throughout northern Virginia in relation to the current and potential current ranges documented in the CFPP tool.

2.5 Conclusions and Future Directions

This study employed eDNA monitoring to confirm theorized river herring distributions and evaluate fish passage use throughout northern Virginia. This was the first known fish passage assessment using eDNA sampling to document upstream movement through passages by anadromous fishes. I found that river herring spawning runs were occurring in 9 Potomac River tributaries throughout northern Virginia, and that road-stream intersections were not significant barriers to fish movement. Aside from the current long-term monitoring of river herring populations in Accotink Creek, Pohick Creek, Cameron Run, and previous monitoring in Dogue Creek, by the Potomac Environmental Research and Education Center (De Mutsert, 2013, 2017; Jones & Kraus, 2010), this study is the only other assessment of river herring distributions in the northern Virginia portion of the Potomac River watershed.

Overall, future passage assessments should focus on maintaining clear waterways above and below current passage infrastructure and then reevaluating use by target species, as all structural designs at road-stream intersections permitted some level of upstream passage by river herring when present. Since the lowest ranked barriers, or the less severe “modeled” barriers, to fish movement outperformed the highest rank tested, or the more severe “modeled” barriers, the lower ranked sites would benefit the most from pre-season maintenance, most notably debris removal. Additionally, these pre-season preparations should focus first on larger passages anticipated to have greater streamflow, as these passages have the greatest potential to permit movement of larger adult fishes. While the CFPP tool is available for use by resource managers to explore known and predicted blockages to aquatic species, as well as visualize current species distributions, new data surrounding known species ranges and additional factors influencing passage accessibility are constantly emerging and should be continuously incorporated into the tool.

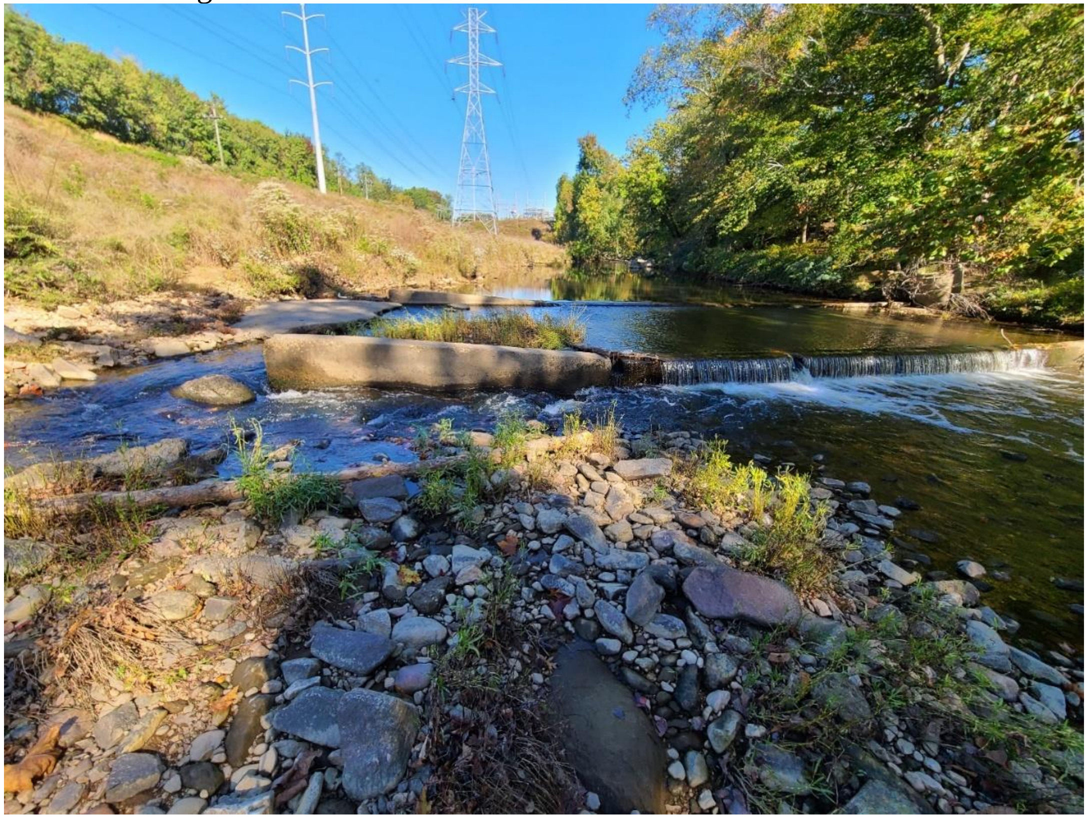
This study filled a knowledge gap identified by the Nature Conservancy's CFPP online mapping tool by evaluating fish passages identified as lower "modeled" barriers to fish anadromous fish migrations within the potential current river herring range. We have now confirmed the presence of river herring at 15 of our 22 evaluated fish passage locations, and will be sharing these updated, confirmed current range extents with the Nature Conservancy. These confirmations can be used to change fish passages classified as "modeled" in the CFPP tool to "surveyed" and provide an outline for how to conduct future fish passage assessments for anadromous species using eDNA protocols. Additionally, this study found preliminary evidence to suggest that river herring may be active around Lake Accotink, warranting future investigations into the 57 miles of stream above Lake Accotink to further inform river herring presence in this area.

Overall, this study has created a path for future investigations interested in exploring the extent of anadromous fish spawning ranges, as well as evaluate barriers to their movement. This framework for fish passage assessment using eDNA will allow future studies to perform expansive investigations into anadromous fish movements throughout a watershed.

2.6 Appendix

Fish Passage 1: Accotink Creek, AC_1 to AC_2 (lower weir)

CFPP Rank: None (Not included in my analysis on CFPP "modeled" barrier ranks)
Structural Design: Weir



A) Orientation: Upstream view from below lower weir on Accotink Creek, below dam. October 2020.


B) Orientation: Upstream view from below lower weir on Accotink Creek, below dam. October 2020.

Fish Passage 2: Accotink Creek, AC_2 to AC_3 (upper weir)

CFPP Rank: None (Not included in my analysis on CFPP "modeled" barrier ranks)
Structural Design: Weir

A) Orientation: Downstream view from above upper weir (nature-like water control area) on Accotink Creek, between AC_1 and AC_2, and below dam. May 2019.

B) Orientation: Downstream view from above upper weir (nature-like water control area) on Accotink Creek, between AC_1 and AC_2, and below dam. May 2019.

Fish Passage 3: Accotink Creek, AC_3 to AC_4 (dam)

CFPP Rank: Dam (Not included in my analysis on CFPP "modeled" barrier ranks)
Structural Design: Dam

A) Orientation: Upstream view from below Lake Accotink Dam, between AC_3 (below) and AC_4 (above), in Lake Accotink Park. April 2020.

B) Orientation: Upstream view from below Lake Accotink Dam, between AC_3 (below) and AC_4 (above), in Lake Accotink Park. April 2020.

C) Orientation: Upstream view from below Lake Accotink Dam, between AC_3 (below) and AC_4 (above), in Lake Accotink Park. August 2020.

Fish Passage 4: South Fork Quantico Creek, Insignificant_PC1/2

CFPP Rank: Insignificant
Structural Design: Bridge

Orientation: Upstream view from below Insignificant_PC1/2 in Prince William Forest Park. October 2020.

Fish Passage 5: Marumsco Creek, Insignificant_PC22

CFPP Rank: Insignificant
Structural Design: Bridge

A) Orientation: Upstream view from below Insignificant_PC22 in Veterans Memorial Park. October 2020.


B) Orientation: Upstream view from above Insignificant_PC22 in Veterans Memorial Park. May 2019.

C) Orientation: Downstream view from below Insignificant_PC22 in Veterans Memorial Park. October 2020.

Fish Passage 6: Powell's Creek, Insignificant_PC23

CFPP Rank: Insignificant
Structural Design: Bridge

Orientation: Downstream view from above Insignificant_PC3 beside Route 1. October 2020.

Fish Passage 7: South Fork Quantico Creek, Insignificant_PC3

CFPP Rank: Insignificant
Structural Design: Bridge

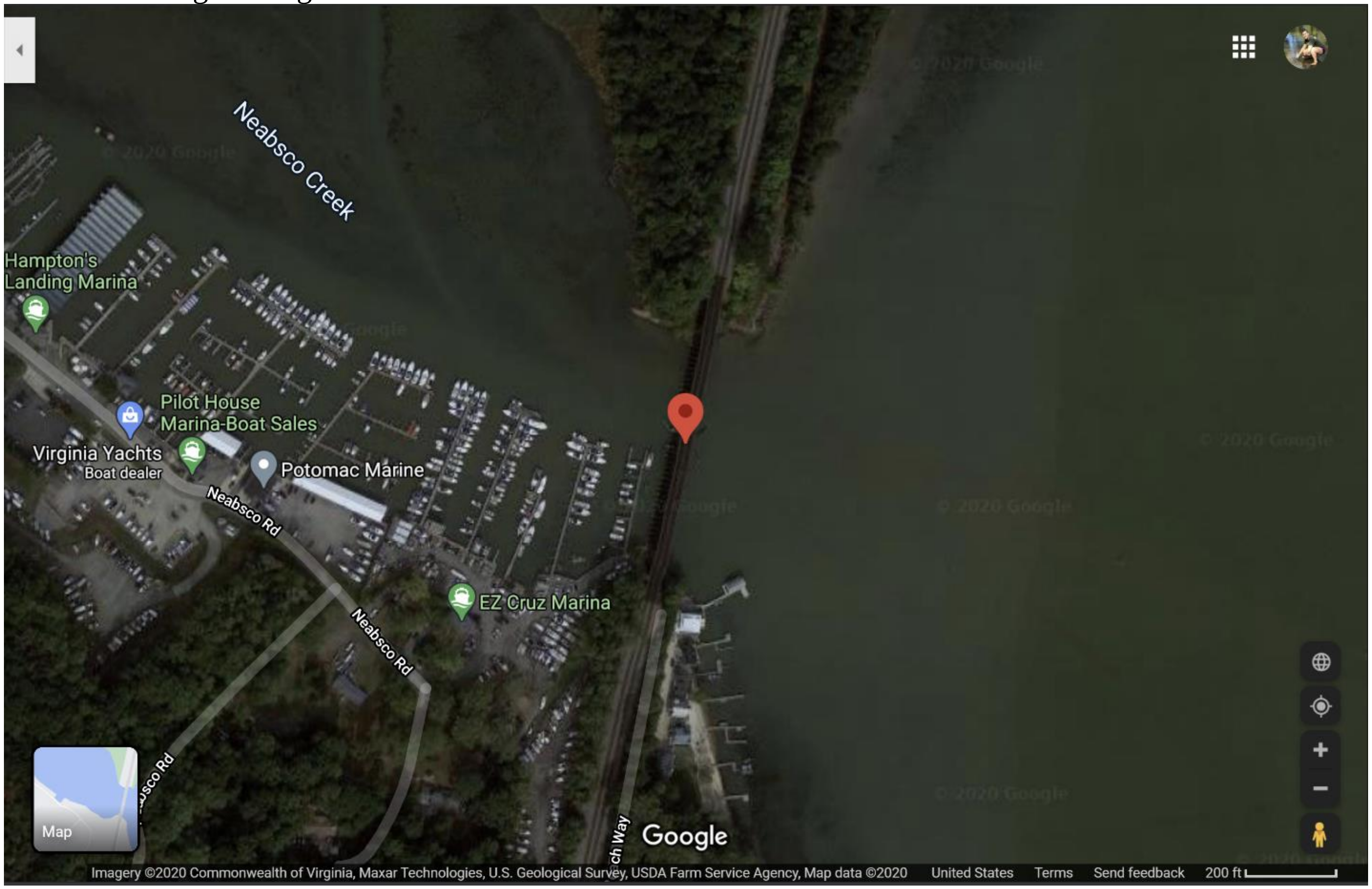
A) Orientation: Upstream view from below Insignificant_PC3 in Prince William Forest Park. April 2018.

B) Orientation: Upstream view from below Insignificant_PC3 in Prince William Forest Park. October 2020.

C) Orientation: Downstream view from below Insignificant_PC3 in Prince William Forest Park. October 2020.

Fish Passage 8: Neabsco Creek, Insignificant_PC4

CFPP Rank: Insignificant
Structural Design: Bridge



Orientation: Aerial satellite view from Google Maps of Insignificant_PC4 between Featherstone National Wildlife Refuge and Leesylvania State Park. Large bridge allowing water to easily pass from upstream (left) to downstream (right).

Fish Passage 9: Neabsco Creek, Insignificant_PC5

CFPP Rank: Insignificant
Structural Design: Bridge

Orientation: Downstream view from above Insignificant_PC5 beside Route 1. October 2020.

Fish Passage 10: Quantico Creek, Minor_PC1

CFPP Rank: Minor
Structural Design: Large round culvert (>2m)

A) Orientation: Downstream view from above Minor_PC1 in neighborhood on the outer boundary of Prince William Forest Park. Fallen trees partially obstructing passage opening. April 2018.

B) Orientation: Upstream view from below Minor_PC1 in neighborhood on the outer boundary of Prince William Forest Park. April 2018.

C) Orientation: Downstream view from below Minor_PC1 in neighborhood on the outer boundary of Prince William Forest Park, looking out towards approximate 2-meter drop from passage exit down concrete slope into approximately 1-1.5-meters deep pool before flowing through far-right exit into mainstem Quantico Creek. April 2018.

D) Orientation: Upstream view from below Minor_PC1 in neighborhood on the outer boundary of Prince William Forest Park. May 2019.

E) Orientation: Downstream view from above Minor_PC1 in neighborhood on the outer boundary of Prince William Forest Park. Fallen trees from spring 2018 and 2019 have been cleared away. October 2020.

F) Orientation: Upstream view from below Minor_PC1 in neighborhood on the outer boundary of Prince William Forest Park. October 2020.

Fish Passage 11: Marumsco Creek, Minor_PC21

CFPP Rank: Minor
Structural Design: Large round culvert (>2m)

A) Orientation: Downstream view from above Minor_PC21 in Veterans Memorial Park. Fallen branches partially block water flow in both culverts. May 2019.

B) Orientation: Upstream view from above Minor_PC21 in Veterans Memorial Park. Water movement is still aside from edge of marsh area where water flows into two large round culverts (>2m). May 2019.

C) Orientation: Upstream view from below Minor_PC21 in Veterans Memorial Park. Water movement is still aside from two culvert openings. Observed large, Northern Snakehead (*Channa argus*) active within right culvert, suggesting fish entry into passage is possible from downstream entrance (below). May 2019.

Fish Passage 12: Dogue Creek, Minor_PC3

CFPP Rank: Minor
Structural Design: Bridge

Orientation: Upstream view from below Minor_PC3 at entrance to Huntley Meadows Park. October 2020.

Fish Passage 13: Powell's Creek, Minor_PC4

CFPP Rank: Minor
Structural Design: Bridge

Orientation: Aerial satellite view from Google Maps of Minor_PC4 in Leesylvania State Park. Large bridge allowing water to easily pass from upstream (left) to downstream (right).

Fish Passage 14: North Fork Dogue Creek, Minor_PC5

CFPP Rank: Minor
Structural Design: Bridge

Orientation: Downstream view from above Minor_PC5 in residential neighborhood beside golf course. May 2019.

Fish Passage 15: South Fork Quantico Creek, Moderate_PC1

CFPP Rank: Moderate
Structural Design: Small round culvert (<2m)

A) Orientation: Upstream view from below Moderate_PC1, next to Insignificant_PC3, in Prince William Forest Park. April 2018.

B) Orientation: Upstream view from below Moderate_PC1, next to Insignificant_PC3, in Prince William Forest Park. October 2020.

Fish Passage 16: Neabsco Creek, Moderate_PC21

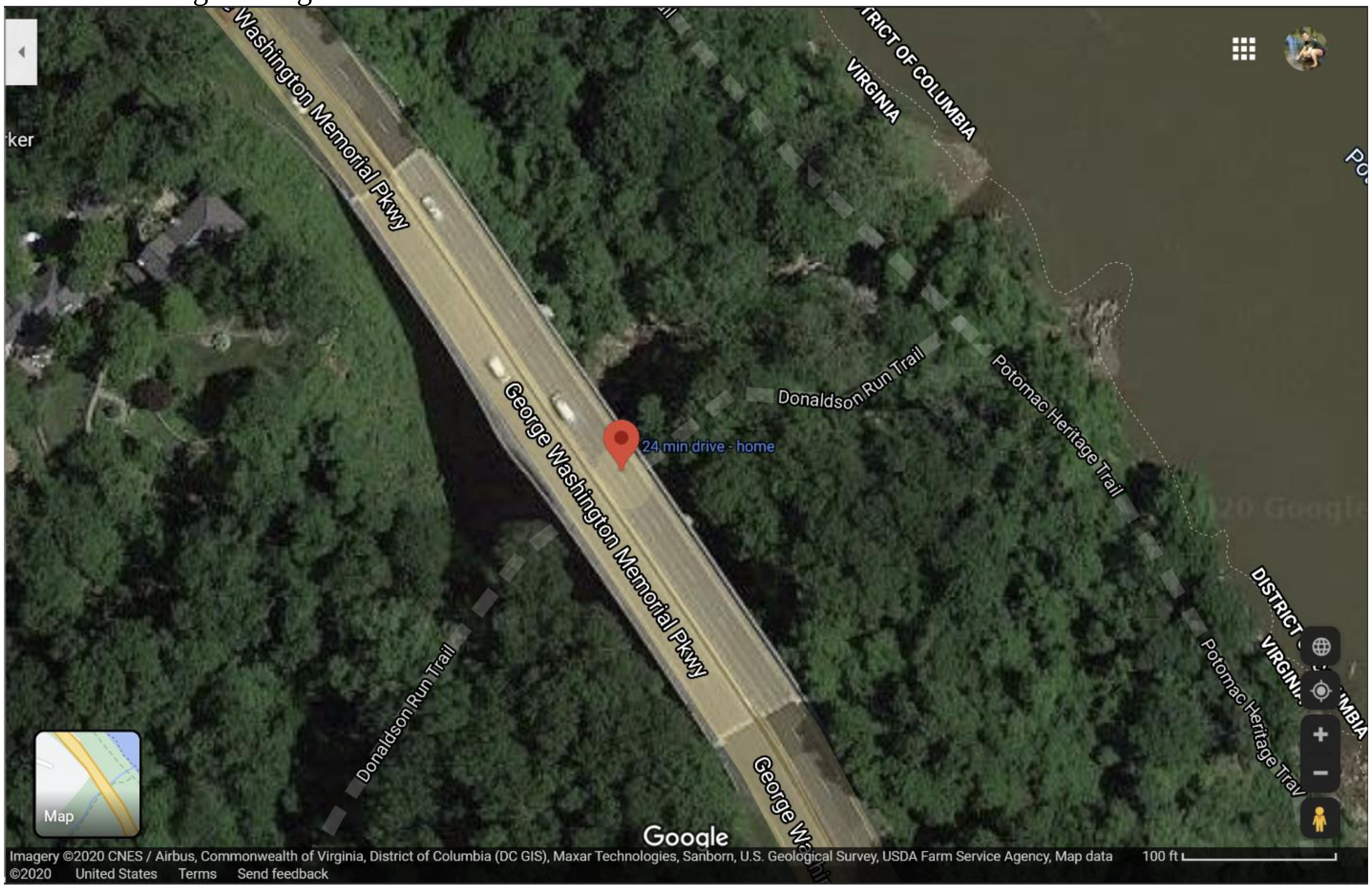
CFPP Rank: Moderate
Structural Design: Small round culvert (<2m)

A) Orientation: Downstream view from above Moderate_PC21 in residential neighborhood off Rippon Boulevard. May 2019.

A) Orientation: Downstream view from above Moderate_PC21 in residential neighborhood off Rippon Boulevard. October 2020.

Fish Passage 17: Donaldson Run, Moderate_PC22

CFPP Rank: Moderate
Structural Design: Bridge



Orientation: Aerial satellite view from Google Maps of Moderate_PC22. Large bridge with water flowing from upstream (left) to downstream (right).

Fish Passage 18: Bullneck Run, Moderate_PC23

CFPP Rank: Moderate
Structural Design: Large square culvert (>2m)

A) Orientation: Upstream view from below Moderate_PC23 under Georgetown Pike. May 2019.

B) Orientation: Upstream view from within Moderate_PC23 under Georgetown Pike. October 2020.

C) Orientation: Upstream view from below Moderate_PC23 under Georgetown Pike. October 2020.

Fish Passage 19: Mary Bird Branch (Quantico Creek), Moderate_PC3

CFPP Rank: Moderate
Structural Design: Large square culvert (>2m)

A) Orientation: Downstream view from above Moderate_PC3 in Prince William Forest Park. April 2018.

B) Orientation: Upstream view from below Moderate_PC3 in Prince William Forest Park. April 2018.

Orientation: Upstream view from below Moderate_PC3 in Prince William Forest Park. October 2020.

Fish Passage 20: Unnamed Stream (Prince William Forest Park), Moderate_PC4

CFPP Rank: Moderate
Structural Design: Bridge

Orientation: Upstream view from below Moderate_PC4 in Prince William Forest Park. October 2020.

Fish Passage 21: Unnamed Stream (Huntley Meadows Park), Moderate_PC5

CFPP Rank: Moderate
Structural Design: Large Square Culvert

Orientation: Downstream view from above Moderate_PC5 in residential area near Huntley Meadows Park. October 2020.

Fish Passage 22: Cameron Run, Weir_CR

CFPP Rank: Not Assigned (Not included in my analysis on CFPP “modeled” barrier ranks)
Structural Design: Weir

A) Orientation: View of weir on Cameron Run with water flowing from upstream (left) to downstream (right). October 2020.


B) Orientation: View of weir on Cameron Run with water flowing from upstream (left) to downstream (right). October 2020.

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