An Evaluation of Non-invasive Sampling Methods in Determining River Herring Abundance

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at George Mason University

by

Jessie Melton
Bachelor of Science
University of North Carolina Wilmington, 2015

Director: Kim de Mutsert, Assistant Professor
Department of Environmental Science and Policy

Summer Semester 2019
George Mason University
Fairfax, VA

DEDICATION

This is dedicated to my parents, Leslie and Stan, and my little brother, Zachary, who have always supported me and encouraged me to follow my dreams. I am so thankful for you.

ACKNOWLEDGEMENTS

I would like to first thank my advisor Dr. Kim de Mutsert, for her guidance and support throughout my graduate studies. She always encouraged me to think outside of the box, and push myself farther in my studies. She provided opportunities that have allowed me to grow into a much more critical thinker, and encouraged me to put myself out there – allowing me to grow both inside and outside of academia. Thank you for answering my many questions, teaching me how to better communicate, both inside and outside of the classroom, and for encouraging me to believe in myself.

I would also like to thank my committee members, Dr. Chris Jones and Dr. Matthew Ogburn for their time throughout this process, and the always thoughtful input. Dr. Jones and Dr. Ogburn always gave thorough feedback, and asked thought inducing questions - which forced me out of my comfort zone, making me think outside of the box.

My thanks also goes to Dr. Joris van de Ham ,who offered not only great wisdom, but also many laughs during long field days. He has taught me how to enjoy myself during the coldest and rainiest of field days. I would also like to thank my lab mates, Beverly Bachman, C.J. Schlick, Casey Pehrson, Sammie Alexander, Chris Bodner, Treda Grayson, Peter Jacobs, Rachel Kelmartin, and many others for always being encouraging both in the field and in the lab. Their support, feedback, wisdom, and humor truly kept me going through this process.

I would like to thank Amanda Sills for being my first introduction to this lab. Had it not been for her taking me under her wing as a volunteer, I do not know that I would have been able to take on this wonderful opportunity that has been my graduate career.

Finally, I would like to thank my friends and family who have sat with me as I worked through my hardest assignments, listened to me through my most frustrated times, but most importantly, always celebrated my tiniest of victories. Their support means the world to me.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF EQUATIONS

LIST OF ABBREVIATIONS

Atlantic States Marine Fisheries Commission ……ASMFC
Environmental DNA..... eDNA
National Oceanic and Atmospheric Administration……..NOAA
Potomac Environmental Research and Education Center ..... PEREC
Technical Expert Working Group……..TEWG

ABSTRACT

AN EVALUATION OF NON-INVASIVE SAMPLING METHODS IN DETERMINING RIVER HERRING ABUNDANCE

Jessie Melton

George Mason University, 2019

Thesis Director: Dr. Kim de Mutsert

Alewife (*Alosa pseudoharengus)* and Blueback Herring (*Alosa aestivalis)*, collectively known as river herring, are two anadromous species of fish that spend a majority of their life in marine waters, but migrate to freshwaters in order to spawn. The Potomac Environmental Research and Education Center (PEREC) has been conducting river herring run count studies for the last few decades in tributaries to the Potomac River, by using block nets to find adult river herring abundance, and plankton nets to examine ichthyoplankton abundance during the spawning season. The objective of this study is to compare the adult river herring abundance between block net abundance, video surveillance, and environmental DNA (eDNA) copy number, with the ultimate goal to determine if non-invasive collection methods can be effectively used to determine river herring run count. eDNA analysis allows for detection of shed DNA in a water sample from species present in the water. The study also examines the impact of abiotic factors such as salinity, pH, temperature, and flow rate on river herring eDNA, as it is expected

that these factors will have an impact on DNA shedding rates and residence time, and will allow for predictive modeling. Ichthyoplankton abundance is compared with eDNA findings as well, as ichthyoplankton may shed DNA that contributes to total eDNA found in water samples. Video counts and eDNA are both non-invasive methods, which will provide a way to determine river herring abundance without stress to the fish or damage to the environment. Both video and eDNA as methods require less staff, and are not necessarily weather dependent, in comparison to block nets. While the study showed that video surveillance is not effective in this format for finding abundance of the target species, it was beneficial in showing the impacts that the block nets have. The use of eDNA does seem beneficial in a number of cases in not only showing correlation of presence, but also species-specific presence. The aim here is to explore the relationship between eDNA copy number and river herring abundance to determine whether abundance can be deduced from copy number alone, or in conjunction with abiotic factors in the system. This study showed that there is a significant correlation between copy number and river herring abundance, and that in this case abiotic factors do not significantly impact the abundance. However, further studies should be done to further explore abiotic factors in relation to eDNA longevity within the system, as well as what determines the DNA shedding rates of both adult and larval river herring.

INTRODUCTION

River herring ecology

Alewife (*Alosa pseudoharengus)* and Blueback Herring (*Alosa aestivalis),* collectively known as river herring, are two anadromous species of fish that spend a majority of their life in marine waters, but migrate to freshwaters in order to spawn (Turner & Limburg, 2016). River herring are typically found in marine waters off the Atlantic Coast of North America from Florida to Newfoundland, and will migrate to local freshwater tributaries in order to spawn beginning in the early spring (Turner & Limburg, 2016). Depending on latitude, river herring will typically begin to travel upstream to spawn between February and May, or when temperatures have reached the optimal temperature around 10°C (Fay et al., 1983). Alewife begin their migration to freshwater and initiate spawning two to four weeks prior to Blueback Herring (Turner & Limburg, 2016). During each spawning season, an individual female can lay between 60,000 and 300,000 eggs (Haas-Castro, 2006). Once the adult river herring have spawned, they will return downstream towards marine waters, while the juveniles will remain in freshwater until a decrease in water temperature in early fall, when they will migrate to more saline waters (Atlantic States Marine Fisheries Commission, N.d.).

River herring are essential to their ecosystems as they contribute to their dynamic food webs throughout each stage of their life cycle. As adults, river herring are key prey

to large predators such as sharks and marine mammals, juvenile herring are essential to the diet of Largemouth Bass, and river herring of all stages are essential predators of zooplankton (Atlantic States Marine Fisheries Commission, 2009). Since river herring exist not only in freshwater, but also in marine water, they are essential to food webs in both ecosystems. River herring provide marine-derived nutrients to freshwater and provide food to birds and fish throughout the systems they encounter (Atlantic States Marine Fisheries Commission, 2009). Without these anadromous species, many predatory species would suffer due to the loss of prey items (Mattocks et al., 2017). Aside from this ecological importance, river herring also provide social and economic importance through commercial and recreational fishing.

River herring management

Since river herring migration patterns are repetitive and predictable, they are great target species for fisheries, as fishermen know when and where to fish for these species based on the season. Due to the predictable migration patterns, river herring were an important food source to huan populations residing along their migration routes during the Colonial period, particularly when many other food sources were scarce during the spring months (Bowden, 2013). During the mid 1900s, when fishing fleets became larger, the river herring fishery moved from being solely inshore during the spawning periods to distant-water fleets fishing for river herring during their marine stages. This increase of fishing location and time essentially decimated the adult river herring population, leaving only 5% of the average landings from 1880 to 1970 (Wienke, 2009). The combination of overfishing, spawning habitat loss and the degradation of water quality led to drastic

population declines in their natural range, with river herring now only being found as far south as South Carolina (Plough et al., 2018). Over the past few hundred years, the building of dams from has limited the accessibility for river herring to reach their spawning grounds, which has contributed to the loss of spawning habitat and productive spawning (Greater Atlantic Regional Fisheries Office, 2019).

In 2006, the National Oceanic and Atmospheric Administration (NOAA) listed river herring as a species of concern after a number of studies showed drastic population declines in many of their natural habitats (NOAA, 2006). The Atlantic States Marine Fisheries Commission (ASMFC) followed this in 2009 with an amendment to the Fishery Management Plan for Shad and River Herring, which required the development of an approved sustainable fishery management approach for all commercial and recreational fisheries (ASMFC, 2009). In accordance with this, many states either introduced, or kept existing fishing moratoria on river herring (ASMFC, 2009). The intent of the moratoria was to aide in species population recovery after a declining population pattern had been noticed over a number of years in watersheds in Connecticut, Rhode Island and Massachusetts, which already had moratoria in place at the time of the amendment (ASMFC, 2009). Since then, it has been especially important to monitor river herring to determine if moratorium efforts have been effective and if so, to what extent, or if more efforts need to be made to aide in river herring population recovery.

Since river herring have a natural habitat that spans across the Atlantic coast, conservation efforts should be carried out in each watershed utilizable by river herring, encompassing fresh and marine waters. In order to work towards collaborative

conservation efforts, the Technical Expert Working Group for river herring (TEWG) was created in 2013 to create a coordinated effort along the coast to work on river herring conservation and address gaps in the current data collections (NOAA). With the findings of the TEWG, the goal is to develop and install a conservation plan to help restore river herring in their native Atlantic coastal ecosystems – this includes addressing various conservation efforts in effect, gaps in data collection, methodology of data collection, and the monitoring and evaluation process in river herring studies. The conservation plan implemented will work to increase awareness of river herring conservation efforts to the public and to stimulate collaborative and cooperative efforts across agencies.

Fisheries-independent population estimates

Since the institution of the moratoria, it has been difficult to determine population sizes, as landings data provided by fishermen was the basis for population estiamtes (Allegood, 2012). In order to remedy this, a variety of new studies have been introduced to determine fish count for the species in each habitat. For example, scientists in North Carolina have used high-frequency sound waves in order to determine river herring count (Allegood, 2012). Through this process, echoes bounce off of fish and are returned, which are then used to measure for density and average fish size to determine fish count. While density and individual length can be determined, it cannot be assumed that all fish in the area are river herring (Allegood, 2012). This problem was later addressed by also setting gill nets and physically counting river herring, which allowed for the prediction of the percentage of river herring from the sonar findings; however, this study showed that river herring made up a very small portion of the catch (Wienke, 2009). Current studies

are also using environmental DNA (eDNA), to determine abundance of river herring. The application of eDNA is based on the understanding that all organisms leave some trace of their DNA behind in the form of urine, skin cells or feces, just to name a few (Plough et al., 2018). With this method, species presence can be determined with just a water sample, taking less of a toll on the environment itself and the fishes that live within it.

Background on Local Research

Since 1984, the Potomac Environmental Research and Education Center (PEREC) has been conducting studies on water quality and biological assessments in Gunston Cove, with one aspect of the studies focusing on fish populations and spawning activities within these tributaries of the Potomac River. Annual reports of these studies examine biotic and abiotic resources in the tidal Potomac, such as physio-chemical parameters, phytoplankton, zooplankton, benthic macroinvertebrates, submersed aquatic vegetation and adult and juvenile fish (Jones et al., 2017a). The portion of this study that focuses on river herring research began in Pohick Creek and Accotink Creek in 1988, with Cameron Run being added as a study site in 2013 (Jones, et al., 2017b). The study typically runs for ten weeks from March to May, with the intent to capture the entire spawning season. During each sampling trip, a block net is set with deer fencing stretching to the banks of each creek, so that the creek is blocked, and all fish swimming upstream are funneled into the block net. The block net is set for a 24-hour period, after which the net is taken down so that fish can be identified, measured, and kept if needed for further examination. This method of collection is labor intensive, can be impossible to accomplish if water levels are higher than the top of the net (about one meter), and can result in high stress to the

fish or mortality if they are stuck in the net too long. The block net has not been the only method used throughout this study, other methods such as spotters and electrofishing have also been used to determine abundance. In 1992, egg and larvae collections in the potential spawning creeks were added to the sampling procedures in Accotink Creek and Pohick Creek, with Cameron Run being added in 2013. At each site, two conical plankton nets are set for 20 minutes, after which the samples are preserved with ethanol so that ichthyoplankton can be identified and counted later in the lab.

The adult river herring typically spawn in these tributaries of the Potomac River from March to May, while the juveniles use the tributaries as nurseries to develop (Jones et al., 2017a). Unfortunately, the predictable spawning season is part of what led to the population decline, as many fishermen became familiar with the schedule and knew when to fish for herring, leading to overfishing of the species. The decline in the population of river herring is not entirely due to overfishing though – it should be understood that habitat degradation, increased runoff and pollution are also factors that need to be addressed for full recovery. It was due to these low and sometimes non-existent population levels that a moratorium was introduced to the Virginia tidal waters in 2012, with hopes of seeing river herring return to these tributaries by combating fishing activities (De Mutsert, 2017). The PEREC study determined in 2015 that river herring have become more abundant in the Potomac River and surrounding tributaries (De Mutsert, 2017). However it is not clear whether this population increase is solely due to the implementation of the moratorium as the study has not gone on long enough to indicate whether the moratorium is the main influence in increased abundance.

PEREC sampling Area

The three research areas of the PEREC river herring survey are tributaries of the Potomac River:,Accotink Creek, Pohick Creek, and Cameron Run (Figure 1). Both Accotink Creek and Pohick Creek are located on Fort Belvoir in Fairfax County, Virginia. In order to access these two sites with a vehicle and all necessary field equipment, there is a gate that must be bypassed, which requires a key that needs to be checked out from one of the offices on Fort Belvoir – so it is not often trafficked by vehicles that do not operate on the base.

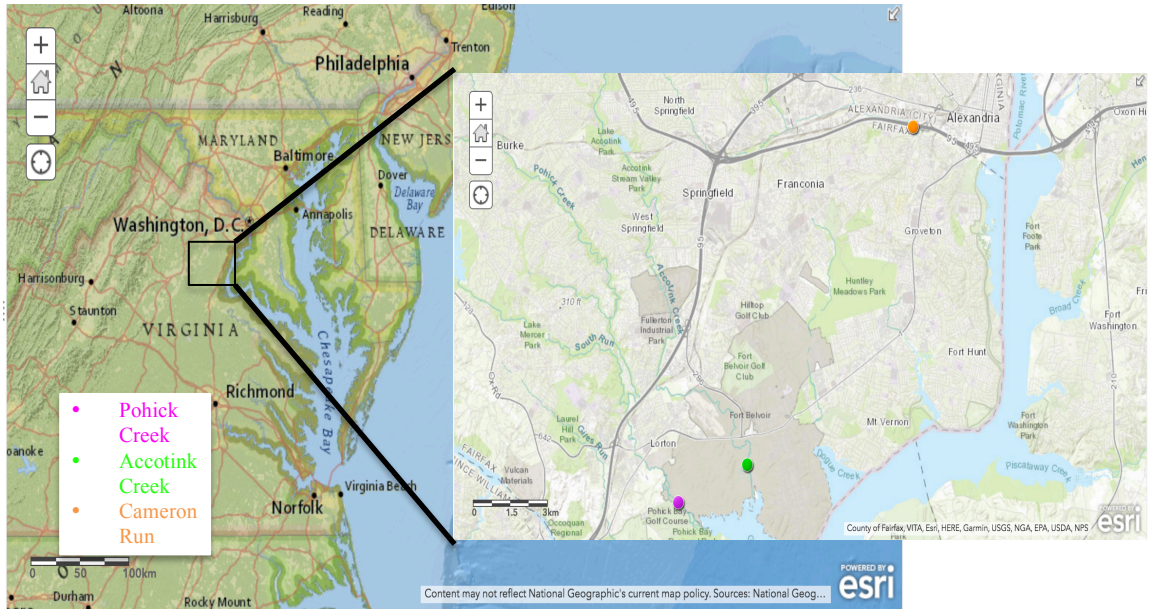


Figure 1: Locations of Accotink Creek, Pohick Creek and Cameron Run in the Potomac River watershed

The portion of Accotink Creek (Figure 2) that is used for this study is located adjacent to a walking trail. The area is heavily forested aside from the clearing for the wing trail, and

does not seem to be heavily trafficked due to the difficulty of accessing the location. The water in Accotink Creek is typically moderately clear, no more than waist deep, and has moderate discharge, except after significant rain events. There is a substantial downcutting of the channel leaving exposed, eroding banks.

Pohick Creek (Figure 3) is also located on Fort Belvoir, a short drive away from Accotink Creek. The research location in Pohick Creek is located off of a heavily

Figure 2: Fyke net set up at Acottink Creek

Figure 3: Fyke net set up at Pohick Creek

forested area, that likely does not see much traffic aside from the sampling season due to its restricted access. Pohick Creek is also typically no more than waist deep, fairly clear, but normally has higher discharge than Accotink Creek, due to sewage effluent from just upstream. The discharge is heightened after significant rain events on both creeks, which can also make it impossible to reach Pohick Creek if roads to the site or the forest itself is flooded.

On the other hand, Cameron Run is located near a highly trafficked road with many commercial businesses, and is right under a highly trafficked overpass (Figure 4). The research area itself is surrounded by forest on one side of the overpass, by rocks and sand under the overpass, and by commercial businesses on the other side of the overpass. The water is typically no deeper than waist deep with moderate discharge and is generally more turbid water than at Accotink Creek or Pohick Creek. All three watersheds are dominated by suburban land use.

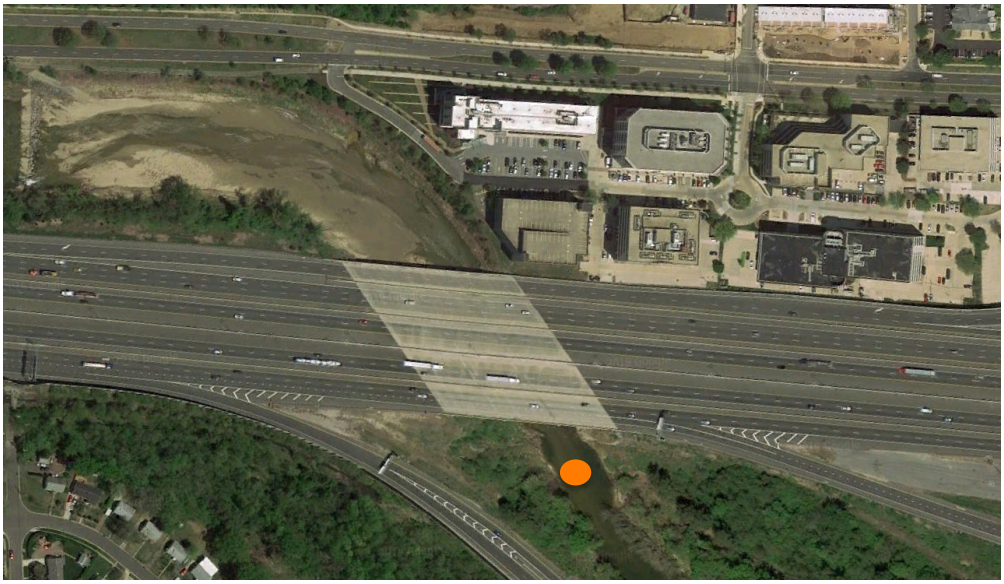


Figure 4: Location of Cameron Run Sampling

Background on Alternative Sampling Methods

In order for a collaborative conservation effort to be productive it is important that the various research agencies be able to share and understand the data from one another, which means understanding the choice of sampling methods used in each watershed, the

limitations and benefits of sampling methods used, the analysis methods, and the approach to ongoing conservation efforts. To begin progress on this, the ASMFC hosted a workshop in 2015 addressing method standardization for river herring studies and conservation efforts, which ended with the production of the *Report on the River Herring Data Collection Standardization Workshop.* This workshop produced an extensive list of sampling methods (seines, gillnets, trawls, etc.), where they are used, and their benefits and limitations in the field. When attempting to work towards conservation for one species across a large sampling area, like the Atlantic coastline where river herring are being studied, it would be beneficial if there were one standard method for collecting data in the field. From this workshop, it was determined that visual run counts are best for narrow passageways, but can be labor intensive, hydroacoustic run counts are best used in turbid waters, but provides less detailed data, and electronic run counts are best when the only species present is the target species, but can cause false positive counts with impurities in the water (ASMFC, 2016). Traditional fishery–independent surveys were also examined, which determined that seine nets can encounter adults and juveniles, but are limited to easily accessible sites, and gillnets can be easily standardized with mesh size, but should be set during peak time for target species presence (ASMFC, 2016).

Looking at each of the methods introduced, two stuck out that were non-invasive, video surveillance, and eDNA collection. According to the findings at the workshop, video surveillance is most effective when used at fish passageways where all targeted species would pass within the visual field, and where water visibility is adequate (ASMFC, 2016). Where video surveillance differ from most others, this method is

beneficial in that it is the best option when targeting multiple species, in this case, Alewife and Blueback Herring. One limitation of this method is that a power source is required, which may be demanding in labor, to either have someone present to switch out batteries, or cost, by spending money on external or additional power sources.

A variety of video sampling techniques are becoming much more prevalent in studies, but admittedly, without fully understanding the advantages and disadvantages they present (Watson, et al. 2010). One study used underwater visual census in rotation with rotating video to study fish communities in a reef and found that while these two methods differed in community observations, that they could be used complementarily to survey large areas in shorter periods of time (Delphine et al., 2014). Another similar study, conducted by Pelletier et al. (2012) removed the visual census, in the form of divers, from the study, and solely relied on video for the underwater survey. In this study it was found that video has a strong potential as a non-obtrusive technique for observing not just the target species, but also the habitat. This study also showed that the use of video increased the number of observations that could be carried out each day in comparison to the visual census. While these two studies study reef species in the ocean as opposed to anadromous species in creeks, they provide good insight on benefits that cameras provide, such as the ability for increased observations, and the ability to examine not just the target species, but also the habitat in depth, which is essential for understanding the impact the changing environment has on the population. Many other studies have used baited underwater videos, however, this presents a bias by generating presence on the camera footage that may not have been there without the presence of bait.

So while there are many studies that are utilizing video as a method for studying communities, it seems that there is still work to be done to perfect using this method.

The use of eDNA is still a relatively new method for monitoring species, but seems to be an option that is efficient and cost effective on a large scale. Unlike setting block nets, eDNA requires very little field labor, as it only requires a water sample in order to determine if river herring are present. The idea behind eDNA as a collection method is that aquatic species leave DNA remnants in the water column in the form of cells, gametes and feces, and that this DNA can be detected in a water sample (Plough et al., 2018). River herring DNA can be detected and quantified using the specific PCR assay, which targets and amplifies the DNA specific to river herring (Plough et al., 2018). Dr. Louis Plough from the University of Maryland Center for Environmental Science has developed an assay that distinguishes Alewife from Blueback Herring (Plough et al., 2018).

To understand eDNA, it is also important to collect several abiotic parameters, such as pH, water temperature, discharge and specific conductivity. Surface pH readings are important to this study, not just in examining eDNA degradation, but these measurements also allow insight into fish development. Water that shows pH levels above 8.3 threatens the development of eggs and larvae, while water that is even slightly acidified to below 6.5, can be lethal for certain species (Chase et al., 2010).

Most studies of eDNA in lentic systems result in reliable species detection; however, it is important to consider the impacts of environmental variability when examining lotic systems. One study states that DNA can degrade within minutes in lotic

systems due to the variability within the system (Seymour et al., 2018). A study by Jerde et al., (2016) found that in a flowing system eDNA is simply transported out of the system within minutes. Similarly, Wilcox et al., (2016) found that more than half of the target species eDNA was no longer detectable within 100 m of the source due to the variability within the flowing system. In flowing systems though, it is important to consider that eDNA is transported horizontally and vertically, so while some eDNA is transported out of the system horizontally, some is also transported into the sediment vertically, reducing the concentration of free eDNA (Stoeckle et al., 2017). Many studies, though, are showing that river herring migration increases during increased water flow, which may lead to varying physical count and copy number, as increased flow might result in a lower, or inaccurate, copy number (ASMFC, 2016).

It should be noted that these abiotic factors, discharge, pH, water temperature and conductivity, may not just have an impact on the degradation rates of eDNA, but also on the shedding rates of DNA. In addition to that, it should be considered that adult river herring and juveniles may also exhibit varying shedding rates in general, and in relation to the abiotic factors considered. Studies have shown that shedding rates increase with higher temperatures, perhaps correlated to when spawning occurs, and with larger body size, which may correlate with the larger spawning size of the adult river herring (Jo et al., 2019). There are four main factors associated with shedding: biomass of organisms, developmental stage, behavior and stressors – all of which can point to increased adult shedding during spawning (Jo et al., 2019). There is heightened biomass as all adults are migrating to spawn, are all of a certain reproductive age, exhibiting the same behavior of

spawning, and are experiencing the same stressors of swimming upstream, perhaps getting caught in nets.

Studies have suggested that not only do temperature and pH positively impact the degradation rates, UVB also increases the degradation rates, and that each respective impact relies on the levels of the other factors (Machler et al., 2018). Future studies could take this into consideration and measure for UVB in addition to pH and temperature in order to further determine how these factors impact the degradation rates of eDNA.

Plan and Purpose of Study

The purpose of this study is to examine alternative sampling methods, namely eDNA and video recording, in relation to the standard method of block nets, to determine if either can be used as effective methods for determining abundance in place of block nets. This will be done by comparing the adult river herring abundance between block net results, video surveillance, and eDNA collection. Abiotic factors will also be examined during this study, as it is thought that pH, water temperature, discharge and specific conductivity can impact eDNA presence and abundance. In order to determine how effective these two new sampling methods are, they will both be introduced while conducting the routine sampling survey that is done with the block nets. The routine sampling survey consists of setting block nets for a 24-hour period, to collect all fish that are swimming upstream during that time. After this period, all fish caught in the net are identified, measured and released, though it should be known that Alewife and Blueback Herring can be quite difficult to identify in the field.

Hopefully this study can form the basis of a move towards implementing one or both of these methods as standardized collection methods for all river herring count studies. For gear to be beneficial to all of the agencies in this study, the method must be something that is viable for each agency to use, meaning cost effective, not too labor intensive, and a tool that can be easily taught, specifically for the agencies that use volunteers and civilian scientists for field work. Some volunteers may turn away when it comes to helping in the field when the work is too labor intensive, or takes intense training.

The introduction of these new sampling methods could be beneficial in that they both have less negative impacts on the environment as they are non-invasive, could attract more volunteers through the ease of use, and if used across the board could allow for standardization of data collection for large scale studies. These two methods will also add a new approach that the current methods do not represent, in that the current methods are a reflection of abundance based on "catchability" – which is a reflection on the effectiveness of the fishing method and therefore cannot truly reflect the total abundance. By using the block nets, the total abundance is based on whether the fish are "catchable" or not, so with this method, the fish can avoid being caught by finding holes in the nets, or by turning around at the entrance of the net, impacting the overall total catch. With the video, fish only have to be seen on film, so they must swim by the camera, or within the view of the camera, and with eDNA, their eDNA simply has to be present in the water, so these rely less on actually being caught. Hopefully the introduction of these two methods,

which vary in gear efficiency and detectability, will result in a more robust and reliable collection of abundance (Thomsen et al., 2016).

CHAPTER 2. EVALUATION OF VIDEO COUNTS AND ENVIRONMENTAL DNA AS COLLECTION METHODS FOR DETERMINING RIVER HERRING ABUNDANCE

Introduction

A number of studies examining river herring populations have incorporated the use of videos as both a stand-alone method, and as a count verification method (ASMFC, 2016). Video surveillance should typically be incorporated at fish passageways or where there are bottlenecks, and where visibility is adequate (ASMFC, 2016). Video surveillance is also beneficial in areas where there are many species present aside from the target species, as the video can be analyzed to differentiate between species. It is often suggested that if volunteers are used to determine physical abundances that counts should be validated, and the addition of video recordings can be beneficial in this aspect. Lastly, the use of video can be extremely important for educational and outreach purposes.

Though there are many benefits to using video surveillance as a sampling method, the limits of this method must also be examined. When it is necessary to take video for longer sampling periods, such as 24 hours, which is the length the block net is set for in the routine sampling, a single battery charge will not suffice, nor will a single memory card hold all the footage. The purchase and upkeep of additional cameras (if needed for multiple sites or angles), batteries and memory cards must be considered with this sampling method. This means considering how long each battery lasts, having backup memory cards and charging packs for the batteries, and a place to charge new batteries in the field if necessary. Another limitation is that the camera must be waterproof or be

accommodated by waterproof housing, which can be more expensive depending on camera. Another limitation is that the quality of footage depends on day-to-day water conditions; if the water is murky, the footage may not be of good quality. In addition, the quality of footage may make it difficult to even determine species present based on distance from camera or water quality. There are also limitations when it comes to how the video data will be examined, whether it means video will be examined and abundance determined by a researcher or if a software will be applied with video surveillance, which could include an extra fee for software. There are also technical glitches to consider, for example, the camera can malfunction resulting in missing data for a period of time. In some cases where there are issues with the video, counts have still been used, but were reported as minimum or partial counts (ASMFC, 2016). Video surveillance also doesn't allow researchers to handle and collect individuals if necessary to the study.

The use of eDNA as a sampling method has been recently introduced as a tool to determine presence of species for which assays have been intentionally developed to detect, most commonly for invasive species and rare or endangered species, as these are ones that may not often be found easily in the environment. eDNA can also be used with barcoding, which consists of a library of DNA barcodes for a variety of species, to be able to determine species through their DNA barcodes, though Alewife and Blueback Herring are so similar that a specific assay must be used to tell the difference. This tool is based on the idea that all organisms that live in water bodies leave some trace of their DNA behind, whether it is in the form of urine, skin cells or feces, just to name a few (Plough et al., 2018). This tool requires very little effort by researchers in the field, and is

more efficient at detecting species than other sampling methods that require physical or visual efforts. eDNA collection is also much more cost-effective than other typical sampling methods, depending on the number of samples that are collected and need to be processed (Plough et al., 2018). This method is even less invasive than video collection, since the field process simply depends on water collection, which also makes it less labor intensive.

Plough et al. (2018) introduced a specific assay that allows for the detection of both Alewife and Blueback Herring. With this assay, it was seen that creeks with slower flow resulted in more positive hits of eDNA and that the highest significance was found between eDNA and ichthyoplankton (Plough et al., 2018). Overall their study showed high target specificity between eDNA and visual surveys, linking ichthyoplankton river herring to eDNA, and that eDNA worked well in a variety of environments. One drawback of eDNA use is that the assay developed requires additional qPCR validation in order to differentiate between Blueback Herring and Alewife, which means additional labor and costs (Plough et al., 2018). Additionally, this study showed that eDNA corresponded very well to many traditional abundance metrics (Plough et al., 2018).

While eDNA has been found to be a more sensitive sampling method than traditional methods, there are limitations in that one must know where to sample to take advantage of the methods sensitivity to detect presence (Smart et al., 2016). Though eDNA has been used to locate invasive species, such as the Burmese Python and the Smooth Newt, and is being implemented in conservation studies, there are still limitations in understanding how eDNA is released by fish and how abiotic factors impact its

longevity within the water column (Hunter at al., 2015 & Smart et al., 2015). While studies have shown that detection of presence has been established, this has typically been done in closed systems, so the understanding of eDNA in open environments is limited. On top of that, the specification of abundance from eDNA is still not fully understood, and will also need some more work in an open system compared to a closed system. PERECs current method for abundance estimation relies on the setting of block nets for a 24 hour period, which can be labor intensive, costly, and can have negative impacts on local fishes. It is thought that if an alternative sampling method that is non-invasive can be introduced, that this would not only reduce intensity of labor, but would also reduce negative impacts on local fishes.

This study serves to find sampling methods that are less invasive than the current methods, with the hopes to one day eliminate these sampling methods by perfecting the practice of new, non-invasive methods. This is particularly important with species of concern because it is important to protect these species while they are being monitored, and invasive methods can cause stress or harm to the target species. This will be done by implementing the use of eDNA collection and video surveillance in conjunction with the current sampling methods that are used. In this study, video surveillance and eDNA copy number will be examined in comparison to the routine sampling method findings from the block net, with the hope being that one or both of these methods will result in a successful prediction model to determine adult abundance through the non-invasive method in conjunction with the abiotic factors that are collected in the routine sample collection.

Objectives

The objective of video collection as a sampling method is to compare the adult abundance from the block net, which was set for 24 hours, with the adult abundance from the video sampling, and determine the relationship between these methods. The adult abundance is used as a sample for what the total run count is over the spawning season. This study will also aim to determine advantages and disadvantages to video as a sampling method, at least in relation to the methods that are in place, and in relation to the environment within which they are used. The overall aim is to determine whether or not a relationship can be determined between block net and video abundances, and if so, how cameras can best be used to determine river herring abundance.

The second objective of this study is to explore eDNA as a method to determine adult river herring abundance and abundance of river herring eggs and larvae. For this objective adult river herring abundance as determined by block net collections will be compared with video results, and eDNA collection. I also aim to examine the effects of factors such as conductivity, pH, temperature, flow rate, and ichthyoplankton abundance on river herring count found by eDNA, as it is expected that these factors will have an impact on eDNA presence and will allow for predictive modeling to determine abundance.

Overall, the objective is to determine whether the number of DNA copies correlates to the count of river herring. Since abiotic factors as well as ichthyoplankton abundance are expected to have an impact on the number of DNA copies (or copy

number) in the water, a predictive model that include these factors will be developed to estimate abundance from eDNA signal strength.

Under these objectives, I aim to answer the following research questions:

1. Do video recordings yield river herring abundances comparable to that of block net river herring abundances?
2. Do eDNA samples yield river herring abundances comparable to that of block net river herring abundances?
3. What impacts do ichthyoplankton abundance, discharge, pH, specific conductivity, and water temperature have on eDNA abundance in a flowing system?

Rationale

The use of video surveillance as a collection method is of interest for a variety of reasons, one main reason being that it is non-invasive in the field. Since river herring in the study sites are currently under a moratorium due to their low population levels, it seems that less invasive collection options are best. Another reason why video surveillance is of interest is because the footage can be revisited if need be. With video surveillance as a method, not only can video be reassessed to validate data, but video can also be reanalyzed at later dates if data needs to be checked again. It is hypothesized that by using video surveillance as a sampling method, there would be a beneficial impact on both individual agencies, as well as on programs that require a standardized sampling method for large-scale studies. It is hypothesized that even if video surveillance in this study cannot be effective solely on its own as a sampling method, it can be beneficial in

validating data and adding supplemental findings. Having video surveillance as a standard method for river herring spawning population size estimates can be beneficial even if it is only used as a supplemental method if all agencies take advantage of the method.

The use of eDNA as a sampling method is beneficial in many ways when it comes to a standardized method for the river herring monitoring project. This sampling method, even more so than video surveillance, can be very easily performed with little training for collection, is much more cost effective, requires much less labor in the field, and can be replicated very easily in many different watersheds. Though studies have showed that eDNA corresponds very well to many traditional abundance methods, studies have not yet been done to compare eDNA to the setting of block nets for 24 hours. The use of eDNA has already been used to link physical abundance with eDNA copies in mesocosm experiments, with hopes to translate this to field studies (Nevers et al., 2018), but it is important to understand the environmental parameters that impact eDNA abundance in terms of physical abundance. Perhaps the biggest challenge of this study is that this study takes place in flowing waters, which impacts the longevity of eDNA in the system as it flows with the waters. There are a number of studies that have discussed the impact of flowing waters on eDNA longevity within a system, so hopefully with the correct modeling, it can be understood how flow impacts eDNA within the system. The implementation of eDNA as a sampling method is also less invasive than block nets, as they require just a sample of water instead of blocking off a creek for 24 hours with a net, which can result in mortality of captured fish by sudden temperature change or predation

by other fishes (Portt et al., 2006). Even if fish exits the net alive, it is possible that they experience high stress due to the capture. While video surveillance has the possibility for error both through technical issues, such as a camera malfunction, and researcher error, through incorrect camera handling, it seems that in the case of eDNA, most errors would stem from researcher error, which is more easily avoided than technical errors. Studies have also shown that eDNA is extremely effective in studies that deal with anadromous species, and has been helpful in many habitat restoration projects because of this (Evans and Lamberti, 2018).

Materials and Methods

Study Sites

The three sites that are used for the routine sampling are Cameron Run, Pohick Creek and Accotink Creek, but due to the time required for set up, collection of video material and review of that material, it was deemed necessary to use cameras at just one site to determine whether or not they were effective sampling tools. The site chosen for camera set up was Accotink Creek (Figure 5), which worked out best logistically, and would be easiest to deploy cameras at due

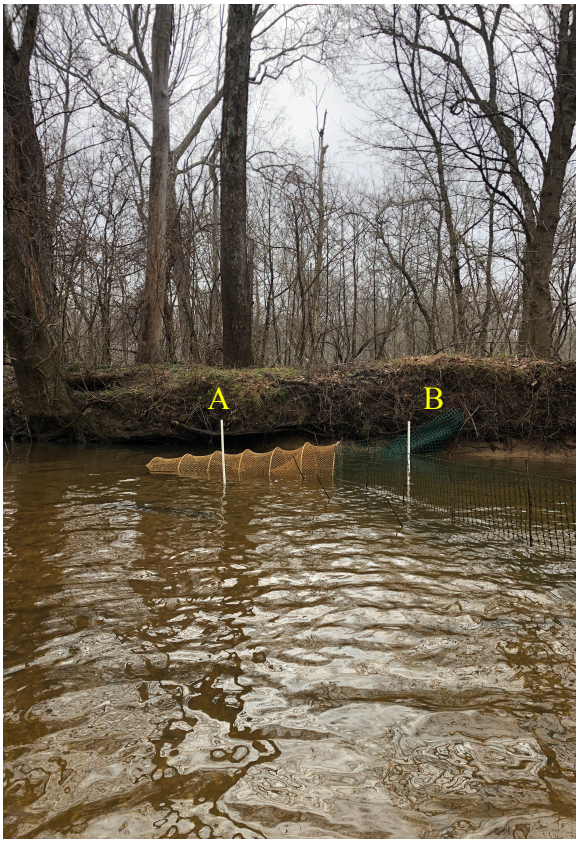


Figure 5: Camera positioning during second season. **A** shows position of camera placed to see into back of fyke net. **B** shows position of camera placed

to sediment type. The portion of Accotink Creek studied is located on Fort Belvoir, right off of a walking trail. The site itself has moderate vegetation, with trees, shrubs and grasses along the banks, but to each side of the creek are forested areas. Since the site is on Fort Belvoir, it seems much of the activity around the site is restricted to those who live or work on base. The creek itself typically has a fairly shallow depth, no deeper than waist high, unless there have been recent events with heavy rain.

While video collection was conducted only at one site, eDNA was conducted at all three sites where block nets are normally set. eDNA samples were collected at Accotink Creek, Pohick Creek, and Cameron Run, in addition to setting the block net at each of these sites. At each of the three sites, the water samples were collected just upstream of where the block nets were to be set.

Field methods

The sampling season typically runs from early March to late May. In 2017 sampling began on March 23, and ended on May 18, and in 2018 sampling began on March 15, and ended on May 24 – typically with sampling occurring on the Thursday and Friday of each week during this time period; all sampling dates and collection methods are show in Table 1, and all data in Appendix A.

For the video portion of this study, two GoPro Hero 5 session cameras were used. These cameras were chosen because they are waterproof, and therefore did not need additional waterproof housing. The GoPro Hero 5 Session also had one of the longer battery lives, approximately two hours. Cameras were deployed at the same time that the net was set in Accotink Creek, with the hopes that anything that was captured in the net

Date	Location	Net Set	Ichthyoplankton Set	eDNA Sampled	YSI	Discharge Found	Camera Set
3/23/17	Accotink Creek	Y	Y	Y	Y	Y	Y
	Pohick Creek	Y	Y	Y	Y	Y	N
	Cameron Run	Y	Y	Y	Y	Y	N
3/30/17	Accotink Creek	Y	Y	Y	Y	Y	Y
	Pohick Creek	Y	Y	Y	Y	Y	N
	Cameron Run	Y	Y	Y	Y	Y	N
4/6/17	Accotink Creek	N	Y	Y	Y	Y	N
	Pohick Creek	N	Y	Y	Y	Y	N
	Cameron Run	Y	Y	Y	Y	Y	N
4/13/17	Accotink Creek	N	Y	Y	Y	Y	Y
	Pohick Creek	Y	Y	Y	Y	Y	N
	Cameron Run	Y	Y	Y	Y	Y	N
4/20/17	Accotink Creek	Y	Y	Y	Y	Y	Y
	Pohick Creek	Y	Y	Y	Y	Y	N
	Cameron Run	Y	Y	Y	Y	Y	N
4/27/2017	Accotink Creek	Y	Y	Y	Y	Y	Y
	Pohick Creek	Y	Y	Y	Y	Y	N
	Cameron Run	Y	Y	Y	Y	Y	N
5/4/2017	Accotink Creek	Y	Y	Y	Y	Y	Y
	Pohick Creek	Y	Y	Y	Y	Y	N
	Cameron Run	Y	Y	Y	Y	Y	N
5/11/17	Accotink Creek	N	Y	Y	Y	N	N
	Pohick Creek	N	N	N	N	N	N
	Cameron Run	N	Y	Y	Y	N	N
5/18/17	Accotink Creek	Y	Y	Y	Y	Y	Y
	Pohick Creek	Y	Y	Y	Y	Y	N
	Cameron Run	Y	Y	Y	Y	Y	N
5/25/17	Accotink Creek	N	Y	Y	Y	N	N
	Pohick Creek	N	Y	Y	Y	N	N
	Cameron Run	N	Y	Y	Y	N	N
3/15/18	Accotink Creek	Y	Y	Y	Y	Y	Y
	Pohick Creek	Y	Y	Y	Y	Y	N
	Cameron Run	Y	Y	Y	Y	Y	N
3/29/18	Accotink Creek	Y	Y	Y	Y	Y	Y
	Pohick Creek	Y	Y	Y	Y	Y	N
	Cameron Run	Y	Y	Y	Y	Y	N
4/5/18	Accotink Creek	Y	Y	Y	Y	Y	Y
	Pohick Creek	Y	Y	Y	Y	Y	N
	Cameron Run	Y	Y	Y	Y	Y	N
4/12/18	Accotink Creek	Y	Y	Y	Y	Y	Y
	Pohick Creek	Y	Y	Y	Y	Y	N
	Cameron Run	Y	Y	Y	Y	Y	N
4/19/18	Accotink Creek	Y	Y	Y	Y	Y	Y
	Pohick Creek	Y	Y	Y	Y	Y	N
	Cameron Run	Y	Y	Y	Y	Y	N
4/26/18	Accotink Creek	Y	Y	Y	Y	Y	Y
	Pohick Creek	Y	Y	Y	Y	Y	N
	Cameron Run	N	N	N	N	N	N
5/3/18	Accotink Creek	Y	Y	Y	Y	Y	Y
	Pohick Creek	Y	Y	Y	Y	Y	N
	Cameron Run	Y	Y	Y	Y	Y	N
5/10/18	Accotink Creek	Y	Y	Y	Y	Y	Y
	Pohick Creek	Y	Y	Y	Y	Y	N
	Cameron Run	Y	Y	Y	Y	Y	N
5/17/18	Accotink Creek	N	N	Y	N	N	N
	Pohick Creek	N	N	Y	N	N	N
	Cameron Run	N	N	Y	N	N	N
5/24/18	Accotink Creek	Y	Y	Y	Y	Y	Y
	Pohick Creek	Y	Y	Y	Y	Y	N
	Cameron Run	Y	Y	Y	Y	Y	N

Table 1: Dates of Sampling events and collection methods that occurred. Y = Yes, N= No.

during the time the cameras were recording, would also be seen on camera. During the first sampling season, one camera was placed underwater, facing across the creek, to capture any fish swimming up or down stream. This camera was attached to a PVC pipe using specific GoPro attachments. The PVC piping was then placed over a piece of rebar that had been secured into the sediment, positioning the camera so that a cross section of the creek could be viewed. The second camera was placed roughly 10 feet above the water with the help of PVC and a tree that the PVC could rest on. This camera was also attached to the PVC pipe with GoPro attachments, with the camera facing down toward the creek. This camera set up also required rebar to be secured into the sediment to ensure the PVC pipe would not get washed away. This camera was used to get an aerial view of what species were swimming up or down stream. It was determined after the first sampling season, that the aerial camera was not the most beneficial as it was often difficult to identify fish species from such a height. For the second season, the first camera remained underwater, but was moved to be near the mouth of the net, to see which species were swimming near the net and entering the net. The second camera was moved to be underwater as well, and was placed facing the back end of the net to see if any fish were captured in the net, and were then eaten or managed to get out. Both of these set ups required the cameras be attached to PVC, which would then be set atop the secured rebar into the sediment in their required locations. The camera set up for the second season can be seen in Figure 5, with both cameras being attached underwater where the poles are shown.

For the eDNA portion of this study, the collection method consisted of collecting one-liter samples of water from each sample location on each sampling event. However, it should be noted that the eDNA samples were collected before the block nets were set – so the eDNA data should represent what is present in the water directly prior to the 24 hour period when the block nets are set. The samples are collected prior to any of the other sampling equipment entering the water to ensure there is no contamination from other equipment. The thought is that the run over the following 24 hours will be similar to run when the eDNA sample is taken – assuming no extreme variations within the system between the sample collections. Since the samples are not collected at the exact same day or time, this could potentially be a source of error if error arises. One-liter water samples were required in order to efficiently detect eDNA presence, meaning one liter of water was collected from Accotink Creek, Pohick Creek, and Cameron Run for each sampling day. However, it should be noted that there were sampling events where sites were not accessible, so samples were not always collected. Due to the ease of collecting these samples though, there were sampling events where block nets could not be set, but eDNA samples could be collected, so there were more sampling events where eDNA was collected than sampling events where block nets were set (Table 1). The one-liter bottles were sterilized prior to sample collection, to ensure that there was no cross contamination. Sterilization was achieved with either a 10% bleach bath for 15 minutes, and a through rinse with DI water, or by autoclaving. Samples bottles were kept separate from all other gear, in a sterilized cooler filled with ice. Water samples were collected right before the net was set, again, to avoid cross contamination from equipment. Gloves

were worn while collecting the water samples, and samples were collected upstream from where the net was to be set, being careful to avoid disturbing the water and sediment too much. The sample bottle was filled once, emptied, and filled again to allow for one more rinse, then capped tightly and stored in the cooler on ice. This process was done each week that nets were set, from mid-March to late May, typically even when nets were not able to be set due to water level.

In addition to block nets, two conical plankton nets were set at each site for 20 minutes (unless inclement weather calls for less time), after which the samples were removed from the conical nets and preserved in one-liter bottles with ethanol so that ichthyoplankton can be identified and counted in the lab. A YSI hydrosonde was deployed at each site to collect data points for pH, water temperature, and specific conductivity. A depth profile was conducted, measuring the width of the creek, and the depth at each meter across to determine the depth and width of the creek. At each meter across the width of the creek, a flowmeter was used to determine the flow at each meter. With these data, discharge was calculated for each sampling event, using the following equation:

Discharge = Velocity * Depth * Width

Lab Methods

Once video was collected, the video was shortened, taking out the first and last few minutes while cameras were being set up and taken down, and while the site was heavily disturbed. This allowed for a constant video time of one hour and thirty minutes for analysis. The video is then broken up into three, thirty-minute segments to allow for

multiple samples from each sampling event. The video was then physically examined, looking for and counting all river herring that swam through the video frame in each thirty-minute segment.

The one-liter water samples from eDNA collected were required to be filtered through a Cellulose Nitrate filter (47 mm, 1.0 micron), using multiple filters per sample if filters clogged. For filtration, a vacuum hand-pump was used with a magnetic filter funnel (47 mm, 350 mL) atop a one-liter vacuum propylene flask. All equipment was sterilized before filtering, and sterilized again between filtering each new location's sample. If samples could not be filtered immediately after field sampling, they were stored in a -80 °C freezer until filtration was possible. Samples would then need to be completely thawed before filtration, by setting into a refrigerator until thawing was complete. During the filtration process, sterile techniques were applied, sterilizing forceps between each filter, and gloves and lab coat were worn. Once filtration was complete, filters were stored in falcon tubes in the -80 °C freezer until they could be transported to the University of Maryland Center for Environmental Studies at Horn Point for eDNA extraction and processing.

Filters were then processed using the Omega E.Z.N.A. Water DNA Kit, which has been used to successfully isolate target DNA, to extract DNA from them, then qPCR was performed to determine presence or absence of river herring.

The ichthyoplankton were also sorted and identified in the lab. Ichthyoplankton were first sorted out from the rest of the debris in the sample, and then counted and identified to species level if possible.

Analysis methods

The video surveillance results were to be compared to the abundance found in the block net using a linear regression to determine correlation between the two. The two methods were also to be examined in reference to their respective units of effort. The camera footage from each date was broken down into three 30-minute segments to allow for multiple samples on each date, with each half hour of footage being a unit of effort. The total fish caught in the net per sampling day were then be broken down into how many on average were caught per half hour. This allows for the comparison on the half hour unit between the footage and abundance in the block net.

The first purpose of eDNA use in this study was to determine whether or not abundance as determined by block net collections can be predicted by number of DNA copies, particularly when considering pH, discharge, specific conductivity, water temperature, and ichthyoplankton count. There were a number of sampling events where not all parameters of the study were collected, for example, at days where inclement weather prevented block nets to be set, but eDNA could be collected. For the purpose of analysis, only sampling events where all variables were collected were used, totaling 47 sampling events. All analyses for this portion of the study were run using JMP 14. A simple linear regression was first used as a predictor model, with copy number as the predictive variable and adult abundance as the dependent variable to determine how well copy number alone could predict abundance. For the copy number and adult abundance, a log(x+1) transformation was used to create data normality and to account for the zeros within the data set. For this portion of the study, Blueback Herring and Alewife were

grouped together as there are more data points for Alewife and Blueback Herring combined from eDNA copy number. A Spearman correlation was then conducted between adult abundance in the block net and larval count, abiotic factors and eDNA copy number to look for significance before running a multiple regression. The Spearman correlation was used because the data was non-normal, and allowed for measuring the strength and direction of correlation between two variables. An additional Spearman's correlation was run looking at copy number versus each of the abiotic variables that were collected. This was done to determine if the abiotic factors, pH, specific conductivity, water temperature, and discharge had a significant correlation with copy number as this may show whether these have impact on shedding rates or longevity of eDNA within the system. A multiple regression was run with adult abundance as the dependent variable, and copy number, plus the abiotic factors pH, discharge, water temperature and specific conductivity as predictive variables to determine whether including these abiotic factors in the regression model improves the relationship between copy number and adult abundance. Lastly, a correlation was run between the total run count at each location for each sampling year, and the total copy number for each location at each sampling year. This was done to look at the sampling years as a whole, and to remove the error that could come from looking at the copy number from a sample that was collected 24 hours prior to the adult abundance sample was collected.

Results

After the video recordings were examined for river herring presence, it was determined that there were zero sightings of river herring on the video recordings. Due to

this, I was unable to conduct the intended comparison between river herring abundance collected with block nets and video counts.

Luckily, I did get results from the eDNA samples. Figure 6 shows data for copy number and adult abundance in each of the creeks for 2017, and 2018. Similarly, Figure 7 shows data for the abiotic components across the two sampling years, Figure 7A showing data from 2017, and Figure 7B showing data from 2018.

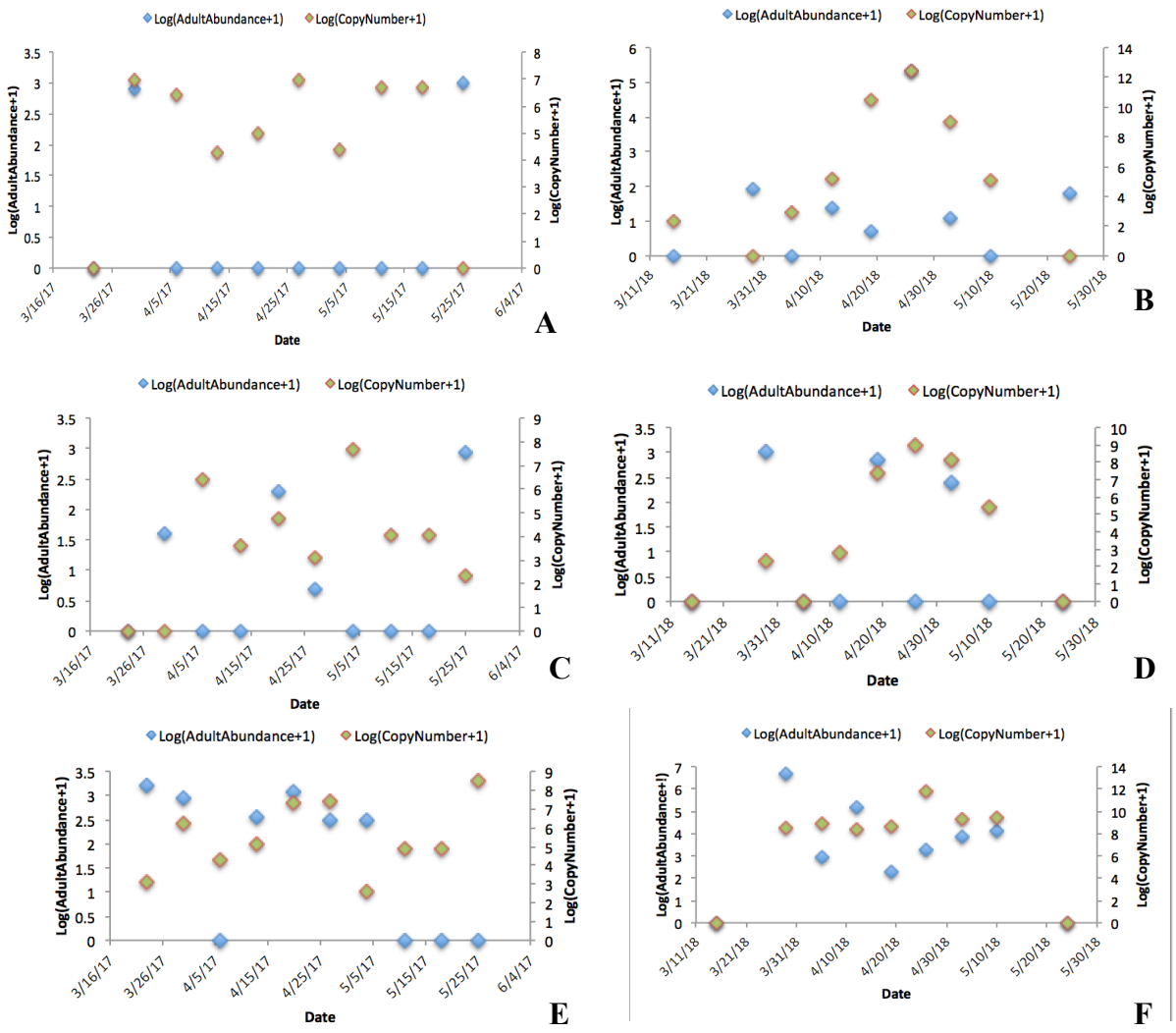
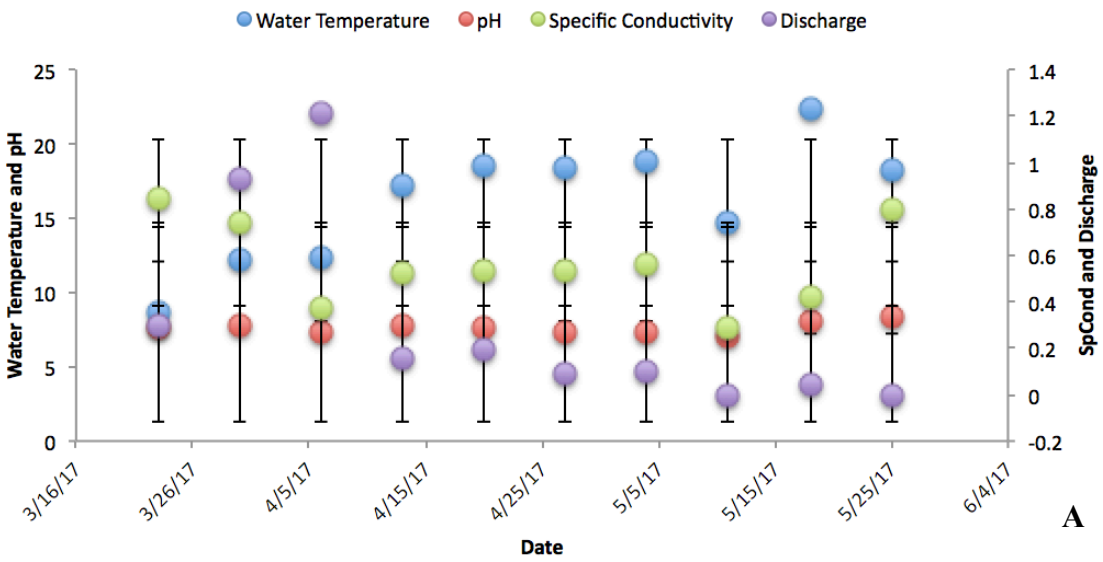


Figure 6: 2017 Log(CopyNumber+1) and Log(AdultAbundance+1) over sampling dates for **A**. Accotink Creek 2017, **B.** Accotink Creek 2018 , **C.** Pohick Creek 2017, **D.** Pohick Creek Creek 2018, **E.** Cameron Run 2017, and **F.** Cameron Run 2018.



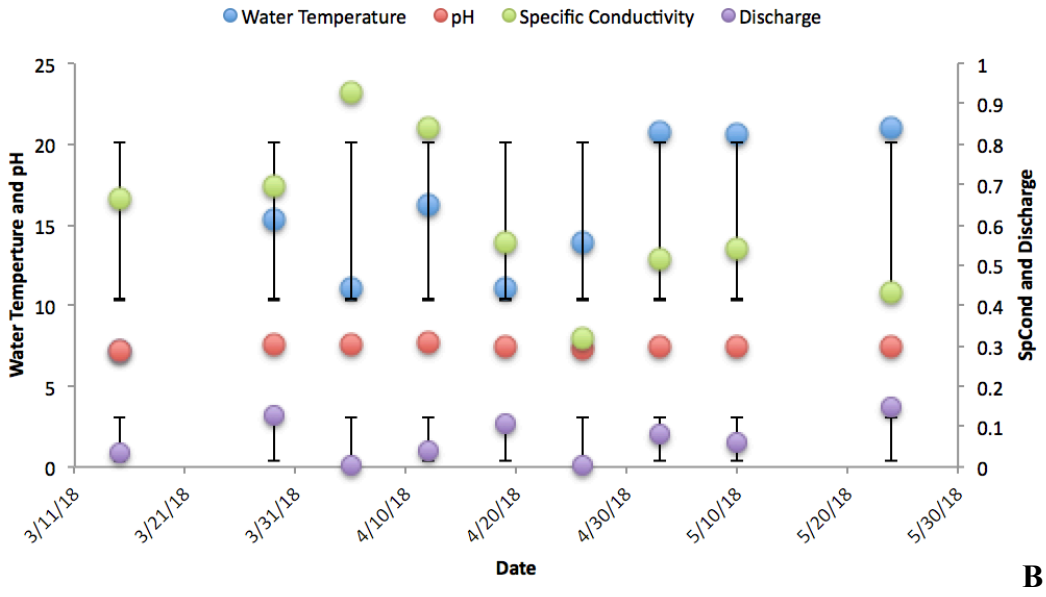


Figure 7: **A**. 2017 data for average pH, SpCond (Specific Conductivity, µS/cm), Water Temperature (°celcius) and Discharge (m^3/s) and their standard deviation error bars. **B.** 2018 data for average pH, SpCond (Specific Conductivity, µS/cm), Water Temperature (°celcius) and Discharge (m^3/s) and their standard deviation error bars.

Of the 47 sampling events where both nets were set and eDNA samples collected, 19 of these also resulted in positive sequencing for species identification (Figure 8). Of these events, 42% of the events showed solely Alewife presence through eDNA, and also showed only Alewife presence through adult abundance. Roughly 26% of the samples resulted in events where there were no river herring found in the physical abundance, but eDNA identified alewife presence. Another 10% of the events showed both species present through eDNA, majority species being Alewife, but only Alewife were present in the physical adult abundance, while just 5% showed both present with eDNA sequencing, with a majority attributing to Blueback Herring, with both being present in the adult abundance, but a majority being Blueback Herring. Similarly, 5% of samples showed both species present through eDNA sequencing, with most attributed to Alewife, but zero fish were found in the adult abundance. Lastly, and perhaps most interesting is the 10% of events that showed both Alewife and Blueback Herring present in the physical adult abundance, but the eDNA sequencing showing only presence of Alewife.

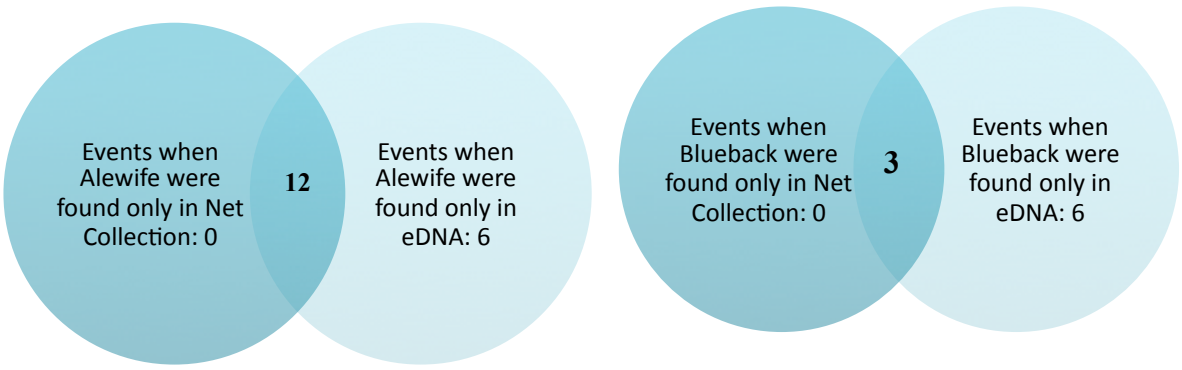


Figure 8: Species identification Venn Diagram showing when Alewife were identified by species in net collection versus eDNA and when Blueback Herring identified by species in net collection versus copy number.

The linear regression of copy number versus adult abundance (Figure 9) showed a significant relationship ($p = <0.0001$), and a coefficient of determination of 0.297, which could suggest there are more parameters that have an impact on predicting abundance.

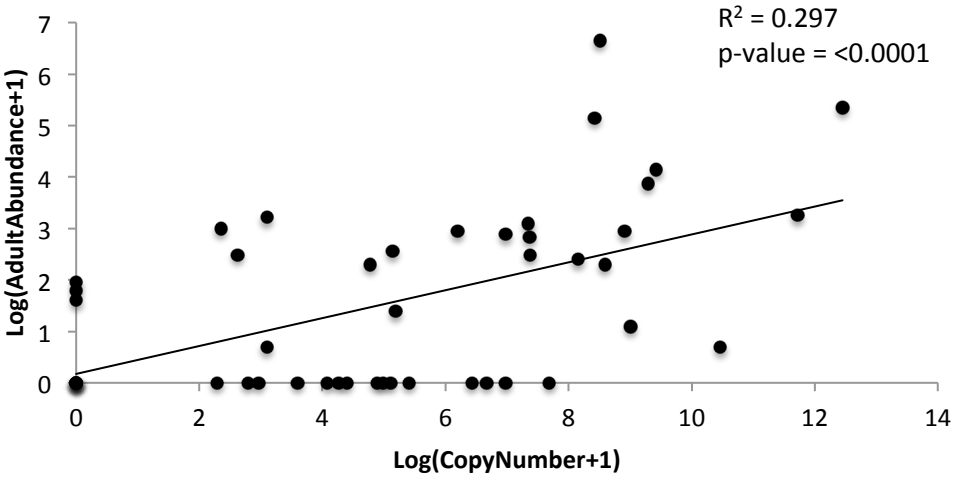


Figure 9: Linear Regression of Log (CopyNumber+1) and Log(AdultAbundance+1)

When looking at the correlation between copy number and adult abundance, a Spearman Correlation coefficient of 0.547 was found, showing a moderate relationship, with a p-value of <0.0001. When examining the correlation between larval abundance and adult abundance, both transformed using log(x+1), the Spearman Correlation coefficient was 0.0208, which was not significant ($p = 0.155$). The Spearman Correlation found no relationship between larval abundance and copy number ($p = 0.411$). All abiotic factors were also examined using the Spearman's Correlation, and none showed a relationship to copy number or adult abundance.

The multiple regression with adult abundance as the response variable, and larval abundance, pH, water temperature, specific conductivity, discharge and copy number as predictive variables, resulted in a coefficient of determination of 0.37 for the full model with $p = <0.0028$. This model showed many extremely insigificant values that had no impact on the model as a whole at first (i.e. larval abundance, discharge), so factors were removed until only signifcant factors remained. This meant removing discharge, followed by larval count, water temperature, pH, and finally specific conductivity. This left only copy number as the significant contributor to the model with a coefficient of determination of 0.297, but with significance at $p = <0.0001$, and the lowest Akaike Information Criterion. The same is seen using a stepwise regression – that copy number has the most significant impact on finding total abundance, which resulted in the following model:

Total Adult Abundance = 0.173 + 0.271 * Log(CopyNumber+1)

Lastly, when looking at total abundance and total copy number at each location, each year, over the two years, there was a strong Pearson's Correlation of 0.8857, with significance at 0.0188.

Discussion

This study shows that the use of cameras on their own, for short periods of time, and in non-contained areas, is not the best way to determine abundance of river herring. A previous study conducted by Bowen et al. (2006) used both cameras and human observers at a fish ladder to determine if the camera was an equivalent alternative to

using human observers at the ladder. This study concluded that the camera was a viable alternative, as the count and identification observed on the camera and the human observer count and identification were the same in a number of trials. However, this study was not looking for specific species, simply looking to see which species accessed the fish ladder. With the cameras used, other species were seen, so they do work if the purpose is not species specific, based on the study. Future experiments could explore options for extending battery length of the cameras, and setting cameras both during the day and night to see if river herring are more compelled to swim upstream based on time of day.

Though there were no river herring seen on the footage, it is important to understand the benefits that come from the footage that was captured. Through the footage it became apparent that the setting of the block net has hindered the movement of various species that live in the area. On multiple sampling dates there were turtles, fishes, small mammals, and snakes that were seen being either stopped entirely, or deterred for lengths of time from getting to the other side of the net. The use of video recordings effectively demonstrated how invasive the setting of block nets are, and enhance the understanding for needing non-invasive sampling methods in this study. This is particularly important to understand as this is a species of concern, and finding methods to study this species is imperative in the case that invasive sampling methods become illegal.

While almost half of the sampling events where eDNA sequencing for species identification show a match between the species found through eDNA sequencing and

adult abundance, there are quite a few events where the relationship is not so direct. There were five sampling events where there were no fish found in the block net, but there was presence of Alewife found through sequencing. On three of these events there was larval presence, which could be indicative of Alewife sequencing. However, there were still two events where neither larval or adult river herring were found, but it is thought that the Alewife presence suggested through eDNA sequencing could simply be from larval or adult presence that was just not captured (perhaps missed in the time between when eDNA samples were collected and nets set), or could be representative of river herring presence at another location in the stream where eDNA has flowed from. This could also be the cause for the sampling event where sequencing showed both species present, but no adult river herring were found, as there was larval presence of river herring. Similarly, there were two sampling events that showed both species present through eDNA sequencing, but solely Alewife was found in the block net, one of these events also had larval river herring present, which could indicate Blueback Herring presence, but the other sample did not show any larval presence. Again, this could simply indicate that the methods used did not capture everything that was present, or could indicate that eDNA flowed from another location in the creek. Lastly, there were two events where sequencing stated the presence of just Alewife, but both species were found present in the block net, and low larval abundance was found. Both of these events happened on the same day, April 26, 2018, in two different creeks. The specific conductivity at both of these events was noted to be notably lower than the average of all events, as did the discharge, but it does not seem that these small variances would have

any significant impact on Blueback Herring eDNA not being found through sequencing. Considering the time of year itself, this sampling date would be around the height of Blueback Herring spawning, which could reflect a decrease in shedding rates while spawning, however this would require further examination.

The findings from the Spearman Correlation of both adult abundance in the block net and larval count to copy number, adult abundance is found to have moderate correlation to copy number and showed significance, while larval abundance is found to have no relation to copy number or abundance. For the correlation, adult abundance combined both Alewife and Blueback Herring as there were more data points with them combined than separated from eDNA. This leads to the notion that eDNA is a significant predictor for adult abundance, but that there are more parameters that factor into this relationship as the strength of the relationship could be increased. Though there was no relationship found between larval abundance and copy number in this study, there are other studies that have found correlations between larval abundance and copy number. For example, one study found a moderate (Spearman's Rho = 0.52) between larval abundance and eDNA (Plough et al., 2018).

There was also interest in finding correlations between the abiotic factors and eDNA copy number. Based on the findings from the Spearmans correlation that specific conductivity and discharge have no relation with copy number, it seems this can be attributed to longevity of eDNA within the environment. With higher discharge, it can be thought that more eDNA is being washed out of the system, so less will be present, however this does not seem to be present in this case. Similarly, it has been found that

specific conductivity is a great predictor and negatively correlates with eDNA decay rates (Collins, et al., 2018). Water temperature also does not show any correlation with copy number in the Spearman Correlation to copy number or total abundance. Climate change should also be considered going forward when examining temperature as a factor in river herring presence, as the changes in mean temperature could not only alter the seasonal run time of river herring, but could also increase the rate at which eDNA is degraded. Similarly, highly acidic environments have been shown to speed up the eDNA degradation process to non-detectable levels in less than two days (Seymour et al., 2018). As mentioned, pH levels can play a significant role in fish development, but there was only one event that gave way to an extreme pH level 8.52, this sampling event yielded no adult river herring, no ichthyoplankton, and a very low copy number of 57.77 – but to confirm whether this level had any impact on development, more studies would need to me done.

In the end, none of the four abiotic factors showed a significant role in the regression model for determining abundance with copy number. It is of interest to keep these factors in the study though to further understand whether they impact abundance in relation to copy number in other setting, perhaps in lentic systems, or whether they have impact on shedding or degradation rates of eDNA.

Conclusions

While a significant correlation was found between adult abundance and copy number, the relationship itself was moderate [Spearman's rho = 0.5269], but not far off from a strong relationship. It is thought that this could be due to the sample size, and that

with a larger sample size or longer sampling period, that this relationship could become better understood. The same can be said for the coefficient of determination in the multiple regression model [$R^2 = 0.297$], that with longer and broader studies in examining the relationship between eDNA and abundance, and the impact that abiotic factors have on this relationship, that the coefficient of determination will only get stronger. The multiple regression model did not how significant improvement from the simple regression model in predicting adult abundance from copy number by including each of the four abiotic factors. Larval abundance did not have any impact on the model, but could still be important to the study in examining reproduction levels each year, particularly as other studies have shown a relationship between these two, so it would be necessary to further explore to find why there was no relationship found here. If river herring abundance increases each year, this could lead to the increased abundance of river herring eggs and ichthyoplankton, which could ultimately lead to their increased impact on the model. This leads to the conclusion that eDNA presence is more significantly impacted by adult presence, however more studies should be done on eDNA presence and longevity within lotic systems. While each of the abiotic factors showed a significant relation to copy number in this study, it seems that more studies should be focused on examining these individual factors to see exactly how they impact eDNA longevity and movement within a system as well as shedding rates of the species themselves.

This study found that while copy number was a main factor in being able to determine adult abundance in this system, that the abiotic factors did not contribute to this relationship. The model developed here could be of use to further the study in

determining adult abundance from strictly copy number, where abiotic factors are not found to contribute to the relationship. Further recommendations for the study would be to construct lab controlled mesocosms to further explore how the abiotic facors impact both the shedding and degradation rates of eDNA within the flowing system. It would also be interesting to consider using each season as a single sample, and continuing this study for many years, and examine each years total adult abundance and total copy numbers to look at total run count through the sampling season to see how these two correlate over the total of the sampling season. This seemed to be a good indicator over the two years for this study, so it is of interest to see how this relationship continues over the years. Additionally, further studies could be explored with video surveillance by setting cameras at varying times of the day to determine if river herring are most likely to run at certain times of the day.

APPENDIX

Date	Location	Alewife Abundance	Blueback Abundance	Total Abundance	Larval Abundance	Copy #	Water Temperature (° Celcius)	pH	Specific Conductivity (µS/cm s)	Discharge (m^3/s)
3/23/17	Accotink Creek	0	0	0	0	0	6.44	7.53	1.16	0.006
	Pohick Creek	24	0	24	0	21.21	12.43	7.44	0.64	0.006
	Cameron Run	0	0	0	0	0	7.27	7.75	0.74	0.89
3/30/17	Accotink Creek	17	0	17	0	1072.35	11.2	7.92	0.96	0.009
	Pohick Creek	18	0	18	7	490.28	15.23	7.41	0.65	0.007
	Cameron Run	4	0	4	0	0	10.28	7.98	0.61	2.76
4/6/17	Cameron Run	0	0	0	0	620.76	11.08	7.45	0.5	3.6
4/13/17	Accotink Creek	0	0	0	2	70.11	14.84	7.71	0.37	0.004
	Pohick Creek	7	5	12	60	169.26	18.26	7.55	0.61	0.008
	Cameron Run	0	0	0	18	35.59	18.49	7.97	0.6	0.46
4/20/17	Accotink Creek	0	0	0	4	144.73	15.7	7.5	0.41	0.005
	Pohick Creek	10	11	21	13	1543.56	19.55	7.5	0.6	0.008
	Cameron Run	9	0	9	2	117.13	20.16	7.7	0.61	0.56
4/27/17	Accotink Creek	0	0	0	3	1070.04	16.11	7.5	0.41	0.005
	Pohick Creek	6	5	11	12	1594.04	18.6	7.08	0.58	0.008
	Cameron Run	1	0	1	4	21.19	20.53	7.32	0.6	0.25
5/4/17	Accotink Creek	0	0	0	0	80.72	16.91	7.03	0.41	0.005
	Pohick Creek	8	3	11	6	12.71	20.28	7.26	0.63	0.009
	Cameron Run	0	0	0	0	2165.96	19.45	7.7	0.63	0.29
5/18/17	Accotink Creek	0	0	0	0	788.05	20.2	7.94	0.25	0.004
	Pohick Creek	0	0	0	0	131.4	21.4	7.93	0.54	0.009
	Cameron Run	0	0	0	0	57.77	25.6	8.52	0.48	0.12
3/15/18	Accotink Creek	0	0	0	0	8.91	3.58	6.77	0.65	0.002
	Pohick Creek	0	0	0	2	0	13.51	7.36	0.71	0.007
	Cameron Run	0	0	0	0	0	4.54	7.62	0.65	0.97
3/29/18	Accotink Creek	6	0	6	0	0	12.2	7.12	0.22	0.002
	Pohick Creek	772	0	772	5	4959.55	17.03	7.64	0.73	0.009
	Cameron Run	19	0	19	0	9.48	16.62	8.03	1.13	0.37
4/5/18	Accotink Creek	0	0	0	0	18.36	8.91	7.48	1.27	0.009
	Pohick Creek	18	0	18	46	7432.08	14.81	7.43	0.71	0.008
	Cameron Run	0	0	0	8	0	9.6	7.67	0.81	0
4/12/18	Accotink Creek	3	0	3	46	178.22	12.38	7.74	1.07	0.01
	Pohick Creek	172	0	172	126	4555.57	17.75	7.69	0.73	0.009
	Cameron Run	0	0	0	9	15.31	18.7	7.82	0.72	0.1
4/19/18	Accotink Creek	1	0	1	0	34861.74	8.49	7.32	0.41	0.003
	Pohick Creek	9	0	9	0	5387.77	13.61	7.26	0.65	0.006
	Cameron Run	16	0	16	0	1576.55	11.12	7.87	0.62	0.32
4/26/18	Accotink Creek	179	29	208	3	255307.11	12.63	7.2	0.24	0.002
	Pohick Creek	24	1	25	1	123437.29	15.13	7.48	0.39	0.004
5/3/18	Accotink Creek	2	0	2	26	8145.05	18.67	7.5	0.35	0.005
	Pohick Creek	17	30	47	23	10771.12	19.61	7.33	0.63	0.009
	Cameron Run	10	0	10	0	3486.99	23.84	7.59	0.57	0.22
5/10/18	Accotink Creek	0	0	0	14	163.54	17.68	7.23	0.41	0.005
	Pohick Creek	5	57	62	67	12308.22	20.16	7.3	0.64	0.009
	Cameron Run	0	0	0	14	221.58	24.17	7.75	0.58	0.17
5/24/18	Accotink Creek	0	0	0	87	0	18.92	7.48	0.28	0.004
	Pohick Creek	0	0	0	132	0	20.08	7.29	0.58	0.008
	Cameron Run	0	0	0	1277	0	23.9	7.56	0.43	0.44

Table A1: All data used for eDNA analyses.

REFERENCES

Allegood, J. (2012). Hoping for a comeback: Researchers eye River Herring. Coastwatch. Retrieved from https://ncseagrant.ncsu.edu/coastwatch/previous-issues/2012-2/spring-2012/hoping-for-a-comeback-researchers-eye-river-herring/

Atlantic States Marine Fisheries Commission. N.d. Shad & River Herring. Retrieved from http://www.asmfc.org/species/shad-river-herring

Atlantic States Marine Fisheries Commission. (2016). Report on the River Herring data collection standardization workshop. Retrieved from http://www.asmfc.org/uploads/file/56fc3c6dRH_DataCollectionStandardizaitionWorkshopSummary_March2016.pdf

Bowen, M.D., Marques, S., Silva, L.G.M., Vono, A., Gondinho, H.P. (2006). Comparing on Site Human and Video Counts at Igarapava Fish Ladder, South Easten Brazil. *Neotropical Ichthyology.* 2016: 4(2): 291-294.

Bowden, A. (2013). Towards a comprehensive strategy to recover River Herring on the Atlantic seaboard: lessons from Pacific salmon. ICES Journal of Marine Science. 71(3), 666-671. Retrieved from https://academic-oup-com.mutex.gmu.edu/icesjms/article/71/3/666/632784

Chase, B.C., Callaghan, T., Dechant, M.B., Patel, P. (2010). River herring spawning and nursery habitat assessment: Upper Mystic Lake, 2007-2008. Technical Report, Commonwealth of Massachusetts, Division of Marine Fisheries. Retrieved from https://www.mass.gov/files/documents/2016/08/sj/tr-44.pdf

Collins, R.A., Wangensteen, O.S., O'Gorman, E.J., Mariani, S., Sims, D.W., & Genner, M.J. (2018). Persistence of environmental DNA in marine systems. *Communications Biology.* 2018:1:185.

De Mutsert, K. (2017). Anadramous Fish Survey. In *An ecological study of Gunston Cove* (pp: 139-150). Retrieved from https://www.fairfaxcounty.gov/publicworks/sites/publicworks/files/assets/documents/monitoring-program_2.pdf

Delphine, M., Laurent, W., Soazig, L., Laurent, V., & Dominique, P. (2014). Complimentarity of rotating video and underwater visual consensus for assessing species richness, frequency and density of reef fish on coral reef slopes. PLoS ONE. 2014: 9(1), 1-12. Retrieved from: http://web.a.ebscohost.com.mutex.gmu.edu/ehost/detail/detail?vid=1&sid=4334821c-8824-4983-bb89-4997cd0140fe%40sdc-v-sessmgr02&bdata=JnNpdGU9ZWhvc3QtbGl2ZQ%3d%3d#db=a9h&AN=94233820

DiMaggio, M.A., Breton, T.S., Kenter, L.W., Diessner, C.G., Burgess, A.I., & Berlinsky, D.L. (2016). The effects of elevated salinity on River Herring embryo and larval survival. *Environmental Biology of Fishes.* 2016: 99(5), 451-461. Retrieved from http://web.b.ebscohost.com.mutex.gmu.edu/ehost/pdfviewer/pdfviewer?vid=1&sid=ad613bb7-2e78-46b4-a71a-ff135f77ea64%40sessionmgr101

Evans, N.T. and Lamberti, G.A. (2018). Freshwater fisheries assessment using environmental DNA: A primer on the method, its potential, and shortcomings as a conservation tool. *Fisheries Research*. 197, 60-66. Retrieved from https://www-sciencedirect-com.mutex.gmu.edu/science/article/pii/S0165783617302618

Greater Atlantic Regional Fisheries Office. (2019). Town Brook Restoration Complete with Removal of Holmes Dam. *NOAA.* Retrieved from https://www.fisheries.noaa.gov/feature-story/town-brook-restoration-complete-removal-holmes-dam

Haas-Castro, R. River Herring. National Oceanic and Atmospheric Administration. Retrieved from https://www.nefsc.noaa.gov/sos/spsyn/af/herring/

Hunter, M, OYler-McCance, S., Dorazio, R., Fike, J., Smith, B., Hunter, C., Reed, R., & Hart, K. (2015). Environmental DNA (eDNA) sampling improces occurrence and detection estimates of invasive Burmese pythons. PLOS One. 10(4). Retrieved from https://search-proquest-com.mutex.gmu.edu/docview/1673379000?accountid=14541&rfr_id=info%3Axri%2Fsid%3Aprimo

Jerde, C. L., Olds, B.P., Shogren, A.J., Andrusz kiewicz, E.A., Mahon, A.R., Bolster, D., Tank, L. (2016). Influence of stream bottom substrate on retention and transport of vertebrate environmental DNA. *Environ. Sci. Technol.* **50**, 8770–8779.

Jo, T., Murakami, H., Yamamoto, S., Madudo, R., Minamoto, T. (2019). Effect of

temperature and fish biomass on environmental DNA shedding, degradation, and size distribution. Ecology and Evolution.

Jones, R.C., De Mutsert, K., Fowler, A. (2017a). An Ecological Study of Gunston Cove. Retrieved from https://www.fairfaxcounty.gov/publicworks/sites/publicworks/files/assets/documents/monitoring-program_2.pdf

Jones, R.C., De Mutsert, K., Jonas, R., Foster, G., Huff, T., & Fowler, A. (2017b). An Ecological Study of Hunting Creek. Retrieved from http://mars.gmu.edu/bitstream/handle/1920/10634/Hunting%20Creek%202015%20Final%5B1%5D.pdf?sequence=1&isAllowed=y

Mattocks, S., Hall, C.J., & Jordan, A. (2017). Damming, lost connectivity, and the historic role of anadramous fish in freshwater ecosystem dynamics. Bioscience. 67(8), 713-728. Retrieved from http://web.b.ebscohost.com.mutex.gmu.edu/ehost/pdfviewer/pdfviewer?vid=1&sid=d33c9a20-8ee6-4ff6-b0de-69a34046a8b3%40sessionmgr103

Machler, E., Osathanunkul, M., Altermatt, F. (2018). Shedding light on eDNA: neither natural levels of UV radiation nor the presence of a filter feeder affect eDNA-based detection of aquatic organisms. PLOS One. 13(4).

National Oceanic and Atmospheric Administration. (2006). Species of Concern: River Herring. NOAA. Retrieved from http://www.nmfs.noaa.gov/pr/pdfs/species/riverherring_detailed.pdf

Nevers, M.B., Byappanahalli, M.N., Morris, C.C., Shively, D., Przybyla-Kelly, K., Spoljaric, A.M., Dickey, J. & Roseman, E.F. (2018). Environmental DNA (eDNA): A tool for quantifying the abundant but elusive round goby (Neogobius melanostomus). PLos ONE. 13(1). Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5777661/

Pardue, G.B. (1983). Habitat suitability index models: alewife and blueback herring. U.S. Dept. Int. Fish Wildl. Serv. FWS/0Bs-82/10.58. 22pp. Retrieved from: https://www.nwrc.usgs.gov/wdb/pub/hsi/hsi-058.pdf

Pelletier, D., Leleu, K., Mallet, D., Mou-Tham, G., Herve, G., Boureau, M,. & Guilpart, N. (2012). Remote high-definition rotating video enables fast spatial survey of marine underwater macrofauna and habitats. PLoS ONE. 7(2). Retrieved from: https://journals-plos-org.mutex.gmu.edu/plosone/article?id=10.1371/journal.pone.0030536

Plough, L., Ogburn, M., Fitzgerald, C., Gerano, R., Marafino, G., & Richie, K. (2018). Environmental DNA analysis of river herring in Chesapeake Bay: a powerful tool for monitoring threatened keystone species. PLOS One. 13(5). Retrieved from http://web.b.ebscohost.com.mutex.gmu.edu/ehost/pdfviewer/pdfviewer?vid=1&sid=34024210-87df-4f5a-a240-fc8535be6c46%40pdc-v-sessmgr03

Seymour, M., Durance, I., Cosby, B.J., Ransom-Jones, E., Deiner, K., Ormerod, S.J., Colbourne, J.K., Wilgar, G., Carvalho, G.R., de Bruyn, M., Edwards, F., Emmett, B.A., Bik, H.M., Creer, S. (2018). Acidity promotes degradation of multi-species environmental DNA in lotic mesocosms. Communications Biology. 1(4).

Smart, A., Weeks. A., Van Rooyen, A., Moore, A., McCarthy, M., & Tingley, R. (2015). Environmental DNA sampling is more sensitive than a traditional survey technique for detecting an aquatic invader. Ecological Society of America. 25(7), 1944-1952. Retrieved from https://esajournals-onlinelibrary-wiley-com.mutex.gmu.edu/doi/epdf/10.1890/14-1751.1

Smart, A., Weeks. A., Van Rooyen, A., Moore, A., McCarthy, M., & Tingley, R. (2016). Assessing the cost efficiency of environmental DNA sampling. British Ecological Society. 7, 1291-1298. Retrieved from https://besjournals-onlinelibrary-wiley-com.mutex.gmu.edu/doi/epdf/10.1111/2041-210X.12598

Stoeckle, B.C., Beggel, S., Cerwenka, A.F., Motivans, E., Geist, J. (2017). A systematic approach to evaluate the influence of environmental conditions on eDNA detection success in aquatic ecosystems. PLOS One. 12(12).

Wienke, R. (2009). Species on the brink: Latest challenges for River Herring research. Coastwatch. Retrieved from https://ncseagrant.ncsu.edu/coastwatch/previous-issues/2009-2/winter-2009/species-on-the-brink-latest-challenges-for-river-herring-research/

Wilcox, T. M., McKelvey, K.S., Young, M.K., Sepulveda, A.J., Shepard, B.B., Jane, S.F., Whiteley, A.R., Lowe, W.H., Schwarts, M.K. (2016). Understanding environmental DNA detection probabilities: a case study using a stream-dwelling char *Salvelinus fontinalis*. *Biol. Conserv.* **194**, 209–216

BIOGRAPHY

Jessie Melton graduated from JEB Stuart High School, Falls Church, Virginia, in 2011. She received her Bachelor of Science from the University of North Carolina at Wilmington in 2015. She was employed as a graduate teaching assistant for two and a half years while she worked on her Master of Science in Environmental Science and Policy at George Mason University. During her last two semesters she was awarded the Becker Fellowship which allowed her to focus solely on research.