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Mycobacteriosis of Striped Bass (*Morone Saxatilis*) in Virginia Tributaries of the Chesapeake Bay

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MYCOBACTERIOSIS OF STRIPED BASS (*MORONE SAXATILIS*) IN VIRGINIA TRIBUTARIES OF THE CHESAPEAKE BAY

by

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ABSTRACT

MYCOBACTERIOSIS OF STRIPED BASS (*MORONE SAXATILIS*) IN VIRGINIA TRIBUTARIES OF THE CHESAPEAKE BAY

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Mycobacteriosis was first documented in Chesapeake Bay striped bass (*Morone saxatilis*) in 1997 after fish exhibited emaciation and skin lesions. Since it was first identified, studies of mycobacteriosis in the mainstem of the Chesapeake Bay (2003-2007) and the Rappahannock River (2005-2012) have shown high disease prevalence and disease-associated mortality. Until this study, no current prevalence data existed from the Rappahannock River, and no published prevalence data existed for the James River, leaving a gap in our understanding of this disease in major Chesapeake Bay tributaries. We began gathering mycobacteriosis prevalence data from an existing survey collecting striped bass from the James and Rappahannock Rivers conducted by Virginia Institute of Marine Science.

During the two years of spring collections, 2,803 striped bass were caught in 2020 and 2,169 in 2021 with 40.8% and 39.9% showing dermal disease, respectively. James River had a higher prevalence with 44.6% of fish caught through electrofishing and 45.0% of gillnet caught fish exhibiting disease compared to 39.5% of fish caught through electrofishing and 35.4% of gillnet caught fish exhibiting disease in the Rappahannock River. Logistic regression was used to examine the effect of various risk factors including age, river, and year class on disease prevalence and severity. Age had a significant positive association with disease while year class had a significant negative association with disease. Fish caught in the Rappahannock River had a significant negative association with disease in some models when compared to being caught in the James River. Disease prevalence increased with increasing age and prevalence was higher than expected in some later year classes after an initial decrease.

Differences in prevalence seen between rivers suggest that mycobacteriosis may work on a localized spatial scale. Higher than expected prevalence seen in later year classes of fish suggests that disease may also be affected on a regional spatial scale in some years. This research began filling the void of current prevalence data in Virginia tributaries of Chesapeake Bay and will help to explain the spatial scale on which disease may be influenced.

This thesis is dedicated to my mother, Ann McGilly, my grandparents Sylvia and Robert Hirschfeld, and my closest friends who have become family; Dean Mauro, Nicholas Winship, Michele Winship, Chris Laferty, Steven Alfano, Robert Aluck, and Samantha Glover. These people have supported me through all the hardships in my life and provided continual love and support during my Masters program.

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NOMENCLATURE

ASMFC	Atlantic States Marine Fisheries Commission
C	Celsius
ChesMMAP	Chesapeake Bay Multispecies Monitoring and Assessment Program
DNA	Deoxyribonucleic Acid
Hz	Hertz
km	Kilometer
mi	Mile
mL	Milliliter
mm	Millimeter
mm^2	Millimeter squared
MRG	Multispecies Research Group
NPV	Negative predictive values
PI	Principal Investigator
PPV	Positive predictive values
PSU	Practical Salinity Unit
VIMS	Virginia Institute of Marine Science
μm	Micron

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INTRODUCTION

THE STRIPED BASS

The striped bass, *Morone saxatilis* (Walbaum, 1792) is an anadromous finfish species with a historic range along the Atlantic coast from the Saint Lawrence River in Canada to the Saint Johns River in Florida (Murdy et al. 1997) and along the Gulf Coast from the Suwannee River in Florida to Corpus Christi Bay in Texas (Murdy et al. 1997). In the late 1800s, striped bass were successfully introduced to California's Pacific coast (Boyle 1980), and over the past century striped bass have been introduced to lakes and reservoirs throughout North America. Most striped bass that have access to coastal environments spend large portions of their lives in saltwater but migrate into freshwater to spawn (Kells and Carpenter 2011). Striped bass are a relatively long-lived species, being able to live upwards of 30 years (Merriman 1941) with individuals able to grow up to 1.8 meters (Smith and Wells 1977) and weigh over 57 kilograms (Tresselt 1950).

With their native range encompassing most of the Atlantic Coast, striped bass use several major estuaries along the coast for spawning. Major spawning areas that contribute fish to the migratory stock include the tributaries of the Chesapeake Bay with the James, Rappahannock, Potomac, Susquehanna Rivers, the eastern shore of Virginia, and areas in Maryland all contributing to this stock (Northeast Fisheries Science Center 2019). Outside of the Chesapeake Bay, spawning takes place in the Delaware and Hudson rivers. Small localized spawning populations in the Albemarle Sound and Roanoke Rivers located in North Carolina also contribute to the migratory stock (Boreman and Lewis 1987). Although the Atlantic stock is comprised of fish originating from many different areas, contributions are not equal. The Chesapeake Bay adds a large portion of fish to the Atlantic coastal stock (Berggren and Lieberman 1978; Van Winkle et al. 1988) with the second-largest contributor being the Hudson River. These two spawning areas alone contribute 95% of the migratory fish found in the Atlantic (Wirgin et al. 1993). The Delaware River is the third major spawning area adding to the coastal striped bass population. The Delaware River population has genetic similarities to Chesapeake Bay striped bass. In previous years, the

Delaware River experienced declining striped bass populations due to high levels of pollution affecting spawning sites and nurseries within the river (Chittenden 1971). Based on genetic patterns, striped bass from the Bay appear to be the only population that substantially contributes genetic material to other areas while this contribution of genetic material is not seen from fish originating from other spawning sites (Gauthier 2013). Genetic mixing of the United States and Canadian populations has also been seen with fish originating from US spawning sites moving north and beginning to spawn in Canadian sites (LeBlanc 2020). This migration of Bay fish to new spawning areas may help explain the genetic similarities seen between the Chesapeake Bay and Delaware River fish. Fish may have moved to the Delaware River through the Chesapeake and Delaware Canal and used this area as a new spawning site once pollution was back to low levels.

Striped bass originating from South Carolina, Georgia, and Florida stocks are mostly non-migratory with only a small portion of fish using coastal waters to reach nearby rivers or to undergo long-distance migrations to northern waters (Greene et al. 2009). For fish that do undergo an oceanic migration, in the spring they move northward and spend summer months off the coast of mid-Atlantic and New England states (Boreman and Lewis 1987; Kohlenstein 1981). Once the weather begins to cool in the fall striped bass begin their southern migration with most fish overwintering along the coast between New Jersey and North Carolina (McLaren et al. 1981; Waldman et al. 1990).

Migration behaviors appear to be age and sex-dependent (Kohlenstein 1981; Mansueti 1961; Secor and Piccoli 2007) with schools of migrating striped bass being dominated by one sex (Secor and Piccoli 1996). Merriman (1941) hypothesized that migratory schools of fish are dominated by females due to sex-specific differences in the age at which males and females reach sexual maturity. After hatching, fish under the age of 1 usually stay within their natal river systems (Mansueti 1961); the following year the same age class will begin to travel further from their natal river into connected estuaries with some entering the Atlantic Ocean (Nichols and Miller 1967). Along with differences in migration habits, the age when males and females become sexually mature differs with males becoming

mature at an earlier age than females (Kohlenstein 1981). Kohlenstiein (1981) examined tagged striped bass from the Potomac River and discussed how sexually mature males would remain within the Bay and begin spawning with larger females. Once spawning is done these larger breeding females will leave for their oceanic migration north while the smaller males remain within the Bay. Starting at the age of 2 immature females will begin leaving the Bay and migrating north in April and May (Kohlenstein 1981). Merriman (1941) hypothesized that once sexually mature, these migratory females will join the spawning group and reenter the Bay in April and May. Otolith microanalysis has more recently been used to better understand striped bass migration patterns, showing between 50 and 75 percent of female fish had resided in the ocean and large fractions of male fish do leave the Bay and enter the ocean (Secor and Piccoli 2007). Otolith microchemistry analyses also suggest that large numbers of both female and male fish live their entire lives within the Chesapeake Bay (Secor and Piccoli 2007) so it appears that not all females undergo an oceanic migration.

BAY CHARACTERISTICS

The Chesapeake Bay is the largest estuary in the United States with over 3,600 plant and animal species inhabiting the main bay and tidal margins. The main stem is approximately 320 km (200 mi) running through Maryland and Virginia where it opens to the Atlantic Ocean. The Bay's water chemistry is not constant throughout due to its varying depths, freshwater and tidal influences, and vast size. Twenty major tributaries feed into the bay draining 175,000 km^2 (67,000 mi^2) with the largest tributary being the Susquehanna River. Salinity ranges throughout the bay from saltwater (32 PSU) at the mouth of the bay in Virginia to freshwater (<0.5 PSU) at the bay's northern extremes in Maryland. Fluctuations in salinity brought on by levels of precipitation can cause monthly or yearly changes in the Bay. Localized changes to salinity, water temperature, and turbidity can take place over shorter periods with fluctuations happening during tidal cycles. The Bay also experiences the most extreme annual temperature ranges of any coastal ecosystem in the world. Nearshore water temperatures can dip to -4°C at the height of winter

and reach 30°C in the late summer, so in extreme cases, the temperature can change 34°C in 6 months (Murdy et al. 1997; National Park Service 2018).

FISHING EFFORT AND MORTALITY FOR STRIPED BASS

Striped bass are commercially and recreationally important to coastal communities along the Atlantic Coast and within the Chesapeake Bay. Striped bass are targeted by many recreational fishermen as a sport fish and many commercial fishermen who depend on this species for income. Recreational landings are often larger when compared to commercial landings with 4 times the number of fish kept through recreational fishing from 2004 to 2018. Between 1990 and 2018 the United States recreational fishery landed between 8.2 and 65 million pounds per year while the commercial fishery landed between 0.71 and 7.3 million pounds per year (Orner and Shepherd 2019). According to the Atlantic States Marine Fisheries Commission (ASMFC) in 2017 recreational landings for striped bass in the Chesapeake Bay were 1,203,060 fish and commercial landings of the same year were 456,284 fish (Northeast Fisheries Science Center 2019). Although the commercial fishery is confined within the Bay's boundaries it has accounted for 80% of the total striped bass caught along the Atlantic coast since 1990. Maryland and Virginia account for more than half of the total striped bass landings bringing in more than 200 million dollars annually to the Bay area (Kirkley et al. 2000). Besides having high economic importance striped bass have important roles in the ecosystems they inhabit. Within the Chesapeake Bay's ecosystem, the striped bass fills an important niche in food webs since it is one of the four dominant piscivores (Latour et al. 2008).

Striped bass populations have experienced overfishing and varying management methods have been put in place to help increase diminished stocks. Decreases in striped bass populations are often linked to increased commercial and recreational landings and poor recruitment years. An increase of commercial landings and mostly low yearly recruitment of juveniles were recorded from 1954 to 1970 with commercial landings reaching their peak in 1974 followed by a steep drop with almost no landings

taking place from 1986 to 1990 (Richards and Rago 1999). In response to declining populations ASMFC, the organization tasked with managing Atlantic striped bass stocks, solicited help from the United States Congress and began implementing stricter fishing regulations in the 1980s (Weaver et al. 1986). Between the 1980s and mid-1990s, ASMFC developed several management plans and amendments to help the depleted striped bass after the stock collapsed in the 1970s (Orner and Shepherd 2019; Richards and Rago 1999; USDOI (United States Department of the Interior) and USDOC (United States Department of Commerce) 1994; Weaver et al. 1986). By the end of the 1990s, the striped bass stock was back to a healthy level. Recently the ASMFC has determined that the striped bass population is again being overfished and experiencing overfishing (Northeast Fisheries Science Center 2019). Consequently, the daily limit for recreational anglers starting in 2019 was lowered from 2 to 1 fish per person per day for both Virginia and Maryland. Northern states followed suit with all states choosing to lower the daily bag limit to 1 fish per person and the introduction of a slot size limit to protect large breeding females began in 2020.

MYCOBACTERIA

Mycobacterium species are pleomorphic, acid-fast, aerobic, non-motile, rod-shaped bacilli belonging to the family *Mycobacteriaceae*, order *Actinomycetales*, suborder *Corynebacterineae*. Mycobacteria are 0.2 to 0.6 μm in diameter and between 1 and 10 μm long with a unique cell wall that includes long-chain 3-hydroxy mycolic acids (Draper 1971; Gangadharam and Jenkins 1997). Currently, the genus *Mycobacterium* is made up of 199 identified species (Parte 2018). Fish mycobacteriosis was formally called piscine tuberculosis and was first documented in carp (*Cyprinus carpio*) living in water that may have been contaminated with *Mycobacterium tuberculosis* (Bataillon et al. 1897). Mycobacteria are present throughout most aquatic systems (Falkinham III 1996) with several mycobacteria causing infection leading to a chronic progressive disease (Jacobs et al. 2009). Since these initial reports in the early and mid-20th century mycobacteria have been identified in many fish worldwide (Gauthier and Rhodes 2009) affecting fish in tropical, temperate, and cold-water environments (Dalsgaard et al. 1992;

Dos Santos et al. 2002; Rhodes 2004). Of all the reported *Mycobacterium* species *Mycobacterium. marinum, Mycobacterium fortuitum,* and *Mycobacterium chelonae* are most frequently isolated from fish worldwide (Belas et al. 1995)

Although mycobacteriosis can impact wild and captive fish populations, low mortality is usually seen in wild populations while high mortality has been documented in captive populations (Bruno 1998; Hedrick et al. 1987; Nigrelli and Vogel 1963). Like many infections, mycobacteriosis can easily spread if it has access to an enclosed high-density population allowing easy transmission from one host to the next. Aquaculture facilities with high fish densities can often see outbreaks of mycobacteriosis. *Mycobacterium marinum* outbreaks in juvenile striped bass have been attributed to high densities of fish in the aquaculture system. The increased opportunity of transmission was present due to bacteria being within the water column, in feces, and from fish feeding on smaller and dead individuals within the tanks (Hedrick et al. 1987). Salmon hatcheries have also been impacted by mycobacteriosis outbreaks. Young salmon became infected after they were fed raw infected carcasses of fish that had died from the hatchery. This introduction of mycobacteria through infected feed led to prevalence as high as 55% in some groups of fish in the hatchery (Ross 1970).

There are no FDA-approved treatments for mycobacteriosis outbreaks in aquaculture facilities raising fish for human consumption and no unapproved products have shown promising signs for nonfood fish (Francis-Floyd 2011). Once a disease outbreak occurs, treatment of fish may be tried with minimal success (Chang et al. 2017) and fish are often euthanized to contain the bacteria. Disinfection of tanks and equipment can be done once fish and water have been removed but can be a difficult task due to bacterial resistance to certain disinfectants (Mainous and Smith 2005). In cases of ornamental fish being kept by hobbyists, infected fish can be kept alive and separated from other fish if so desired but care should be taken when doing this to stop possible zoonotic transmission of disease (Francis-Floyd 2011).

Mycobacteriosis has been documented in both the Atlantic and Pacific striped bass stocks as well as in captive populations. Disease outbreaks have been reported in US west coast populations since the early 1980s with prevalence rates reaching 68% in wild stocks and 80% in aquaculture stocks (Hedrick et al. 1987; Sakanari et al. 1983) with *M. marinum* being the main species isolated (Lansdell et al. 1993). Along the Atlantic Coast mycobacteriosis was first documented in Chesapeake Bay striped bass in 1997 after fish exhibited emaciation and skin lesions (Vogelbein et al. 1999). Jacobs (2007) examined 16 archived tissue samples of striped bass caught between 1980 to 1985 with two fish showing signs of severe mycobacteriosis. One of these positive fish was captured from the Choptank River in the northern part of the Chesapeake Bay and showed granulomatous inflammation in the liver, spleen, posterior kidney, and heart. Due to these findings, it appears that mycobacteriosis may have gone unnoticed in the Bay for over a decade before fish showed signs of physical deterioration (Jacobs 2007). Since first identified, infected striped bass have been reported from North Carolina, Chesapeake Bay, Delaware Bay, and Long Island Sound (Ottinger 2007; Stine et al. 2010; Vogelbein et al. 1999).

A diverse group of mycobacteria have been isolated from Atlantic coast striped bass mainly consisting of slow-growing mycobacteria (Rhodes 2003; Rhodes 2004; Rhodes 2005) with several species isolated from striped bass collected from the Albemarle Sound, Delaware Bay, and Long Island Sound (Ottinger 2007; Stine 2009). Two novel species of mycobacteria, *Mycobacterium shottsii* (Rhodes 2003) and *Mycobacterium pseudoshottsii* (Rhodes 2005) are major etiologic agents of disease in Chesapeake Bay striped bass. Both mycobacteria are slow-growing species related to *M. marinum* and *M. ulcerans*. Although *M. shottsii* and *M. pseudoshottsii* are regularly isolated, other slow-growing species like *M. triplex and M. interjectum* are numerically relevant in samples. *Mycobacterium shottsii* was the most frequently isolated species (57%) and was the dominant species isolated from skin lesions on infected striped bass ((Rhodes 2004). Koch's postulates have been fulfilled for *M. pseudoshottsii* and *M. shottsii* (Gauthier et al. 2021) this along with other evidence supports the idea that these pathogens contribute to mycobacteriosis in striped bass (Gauthier and Rhodes 2009).

Although *M. marinum, M. fortuitum, and M. chelonae* are commonly isolated from fish species worldwide, these three species are not often isolated from Atlantic Coast striped bass. *M. chelonae* and *M. fortuitum* have yet to be isolated from Chesapeake Bay striped bass and *M. marinum* has been isolated from only a small fraction (3%) of fish (Rhodes 2004). A single fish caught in the Delaware Bay has had *M. chelonae* isolated from it but there still have been no reports of *M. fortuitum* isolated from Delaware Bay striped bass (Ottinger 2007).

Mycobacterium pseudoshottsii is a slow-growing species (> 1 month on solid agar) at 23℃ with little growth at 30°C and no cell growth occurring at 37°C (Rhodes 2005). *Mycobacterium shottsii* is also a slow-growing (>1 month on solid agar) *mycobacterium* with rough, non-pigmented colonies and little to no growth above 30°C (Rhodes et al. 2004; Rhodes et al. 2003). Various aspects of *M. shottsii*, mainly its non-pigmented phenotype, absence from environmental reservoirs, and dysgonic nature on artificial medium, are suggestive that this bacterium is undergoing adaptation to obligate pathogenicity in a vertebrate host (Gauthier et al. in review), like that seen in other related mycobacteria such as *M. tuberculosis, M. ulcerans,* and *M. leprae* (Stinear 2007).

MYCOBACTERIA TRANSMISSION

Transmission of mycobacteria in fish is still poorly understood but several transmission routes have been presented including ingestion of bacteria through diet, environmental exposure (Gauthier et al. 2003; Nenoff and Uhlemann 2006; Ross 1963; Ross and Brancato 1959; Wood and Orddal 1958), or through vertical transmission in oviparous and viviparous fish (Ashburner 1977; Chinabut 1999; Conroy 1966). Once infected, fish may begin releasing bacteria into the water through waste or skin lesions (Noga 2010). Lab experiments using zebrafish *(Danio rerio)* infected with *M. chelonae* through ingestion or injection showed the same bacterium present in the tank's surface biofilms and detritus within 2 weeks of infection (Chang et al. 2019). Transmission of *M. marinum* has been seen in un-injected striped bass kept in a tank receiving water from tanks holding infected fish indicating that bacteria were shed from infected fish (Gauthier et al. 2003). Further studies are necessary to support the findings of this experiment.

There are several possible routes for striped bass to become infected by mycobacteria. Certain bacteria like *M. pseudoshottsii* which heavily infects striped bass in the Chesapeake Bay are present throughout the Bay's water column and bottom sediment as well as in Atlantic menhaden (*Brevoortia tyrannus*) and Bay anchovies (*Anchoa mitchilli*), two major food sources for striped bass. With *M. pseudoshottsii* being present in the environment and major prey species waterborne and ingestion transmission are possible (Gauthier 2010). Possible routes of transmission for *M. shottsii* are less obvious due to it only being isolated from striped bass; a natural reservoir outside this host species has yet to be identified.

EFFECTS OF MYCOBACTERIOSIS

Once a fish becomes infected with mycobacteria, varying physical and behavioral changes may take place. A variety of fish tissue can become impacted including eyes, gills, musculature, organs, and fins. External lesions are nonspecific to mycobacteriosis and may include skin ulcers, pigmentary changes, or scale loss (Figure 1). Other reported symptoms include deformation of the spine, emaciation, and changes in behavior (Bruno 1998; Nigrelli and Vogel 1963; Ross 1970; Snieszko 1978). Internal muscle and organs including the spleen, kidney, and liver can become impacted once infected with such organs becoming enlarged and developing grey and white nodules throughout the tissue (Chinabut 1999) in severe cases of infection, these nodules can be seen with the naked eye on the exterior of the organ. Once infected, bacterial levels can vary in fish and acute disease is sometimes seen when high bacterial loads are present (Whipps et al. 2007; Wolf and Smith 1999), but mycobacteriosis is often a chronic disease.

FIGURE 1. Striped bass with a large ulcer and scale loss (black arrow) under the pectoral fin caused by mycobacteriosis. Photo courtesy of Dr. David Gauthier

Nodules present in infected organs are referred to as granulomas (Figure 2) and are caused by an immune response triggered by varying foreign bodies within a host including bacteria and parasites. Immune granulomas are a cell-mediated immune response caused by an infection from an insoluble particle; presence of mycobacteria can cause this type of immune response. T cells produce cytokines like IL-12 and interferon-γ which help form and maintain immune granulomas (Cooper 1993; Ehlers et al. 2000; Timur et al. 1977).

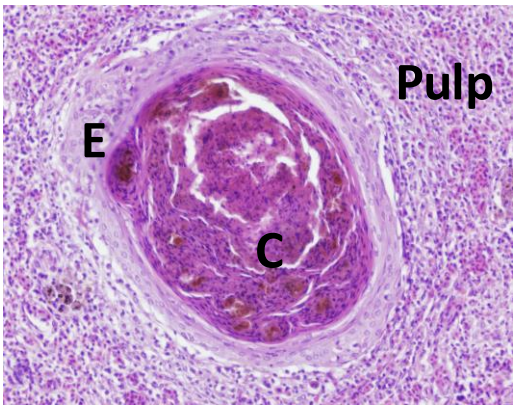

FIGURE 2. Photomicrograph (100× magnification) of a granuloma caused by mycobacteriosis within a striped bass spleen. The core (C) of granuloma is a mixture of necrotic tissue and bacteria. The epithelioid cells (E) encircle and wall off the core of the granuloma stopping the bacteria from spreading within the spleen. The granuloma is found within the spleen tissue (Pulp) made up of red and white pulp, blood vessels, arteries, and nerves.

The immune response starts with the aggregation of macrophages in an inflammatory focus around a concentration of bacteria located in organ tissue. These macrophages then begin shifting to epithelioid cell morphology. The structure of the macrophages are similar in structure to epithelial cells with open-faced nuclei, prominent nucleoli, and faintly granular cytoplasm (Cotran et al. 1999). With the formation of the granulomas, the mycobacteria become walled off from the rest of the organ eliminating its ability to spread. This process of macrophages congregating then forming epithelioid cells around bacteria is seen in most fish immune granuloma formation (Gauthier et al. 2003).

A defining feature of immune granulomas in piscine species is necrosis in the core of the granuloma; this is different than granulomas within mammalian species which may have calcified cores (Gauthier and Rhodes 2009). Because of this necrotic core, most bacteria are likely killed and broken down within granulomas. But even with this extreme environment intact bacteria and rapid outgrowth have been observed, so it seems a subpopulation of bacteria can withstand and survive within granulomas. How these few mycobacteria can survive is currently unknown (Gauthier et al. 2003). Infected fish can

have single or multiple granulomas present in organ tissue, and in severe cases, the tissue can be filled with granulomas and fluid-filled cystic. The presence or absence of granulomas is a useful diagnostic method testing for mycobacteriosis when lethal sampling of fish is being done. Paraffin histology is commonly used to examine striped bass spleen tissue for the presence of granulomas. Granulomas can be easily identified when samples of tissue are placed under a microscope and are characterized by their central area of necrosis surrounded by macrophages, epithelioid cells, and fibrous connective tissue (Talaat et al. 1997). Examining spleen tissue for granulomas to determine levels has been done to estimate disease prevalence in areas of the Chesapeake Bay (Gauthier 2008; Latour 2012). Performing histology on organ tissue is currently the only diagnostic method for identifying internal mycobacteriosis but it does require lethal sampling. Examining a fish's exterior for the presence of pigmented foci, scale loss, and ulcers is a non-lethal diagnostic method (Hoenig 2017), but it cannot be used to check for the presence of internal disease. Testing for dermal disease has limitations because striped bass can have internal disease present but show no signs of dermal disease. Although histology can be useful for determining the presence of disease it is limited to identifying disease and mycobacteria causing the infection cannot be determined using this method. To identify bacteria, DNA can be extracted, and molecular techniques used (Gauthier and Rhodes 2009), or mycobacteria can be cultured on artificial or solid media and identified based on growth rates and biochemical characteristics (Rhodes 2004).

Not all fish show the same immune response once becoming infected with mycobacteria and fish of the same species can have different responses to different mycobacteria. Striped bass that were experimentally injected with *M. marinum* showed a spread of bacteria throughout the fish's tissues damaging organs and leading to the death of the affected fish. In the same study, *M. marinum* was injected into tilapia showing a less severe response compared to striped bass. Injected tilapia showed fewer and smaller granulomas and these granulomas held lower concentrations of acid-fast bacteria than the granulomas found in striped bass (Wolf and Smith 1999). Throughout the Chesapeake Bay *M. pseudoshottsii* infects striped bass as well as Atlantic menhaden but the host response is not the same

between these two species. Atlantic menhaden exhibited high densities of acid-fast organisms in both spleen and liver tissues but no host response like the formation of granulomas was seen in these fish (Gauthier 2010). The same mycobacteria species causes a host response in striped bass with some fish having large quantities of granulomas present within organs (Vogelbein et al. 2001)

PREVIOUS WORK EXAMING MYCOBACTERIOSIS IN CHESAPEAKE BAY STRIPED BASS

Mycobacteriosis prevalence in Chesapeake Bay striped bass is abnormally high for a wild fish population. Given this high prevalence of mycobacteriosis in Chesapeake Bay striped bass, and its known lethal nature, it is likely that this disease plays a role in natural mortality. Although high disease prevalence is documented in Chesapeake Bay fish, disease prevalence in other coastal systems (e.g., Hudson River, Delaware River, Roanoke River) are largely unknown, and high prevalence in these systems has not been documented. Due to the high prevalence and mortality within the Chesapeake Bay, there have been several studies estimating mycobacteriosis prevalence and observing the relative survival of diseased fish. Bay-wide trawl surveys collected fish to estimate disease prevalence and estimate relative survival for diseased versus undiseased fish in the main stem of the Chesapeake Bay (Gauthier 2008). Tag recapture surveys in the Rappahannock River have been used to look at disease prevalence and relative survival for varying disease severities compared to undiseased fish (Hoenig 2017). Groner et al. (2018) used the same tag recapture data as Hoenig et al. (2017) to investigate the impact of increasing annual summer sea surface temperatures on disease progression and relative survival of different disease severities. Diseased fish were grouped into 3 categories: mildly, moderately, and severely diseased. The likelihood of healthy fish becoming diseased was approximately 89% for fish aged 3 and older. Once diseased, fish likely stay at the same disease severity or progress in severity with few fish showing signs of regression in severity back to a healthy state. Mildly diseased fish had around 1.5% probability, moderately diseased fish had a 0.053% probability, and severely diseased fish had a 0% probability of returning to a healthy state. The impacts of increasing sea surface temperatures on varying disease states were also examined using a population projection model of 10,000 striped bass over 6 years. The chance of survival at 26°C was

estimated at 75% for mildly and moderately diseased fish and decreased to 25% at 29°C. Increasing sea surface temperature greatly lowered the chance of survival for severely diseased fish. At 26°C the chance of survival was estimated at 36 % and was reduced to 9 % at 29°C (Groner 2018). It appears that increasing sea surface temperature impacts diseased and undiseased fish with increasing summer temperatures getting close to the thermal tolerance level for striped bass. Based on projections done by Groner et al. (2019) once a fish becomes diseased progression will continue slowly finally reaching a severe state. At this point, yearly survival decreases substantially to 36% or less depending on average summer temperatures. With temperatures in the Bay steadily increasing during the summer months this could have detrimental impacts on striped bass stocks when large numbers reach a severe disease state. There has been evidence of increasing natural mortality in striped bass (Jiang 2007) and mortality caused by increasing sea surface temperature would add to the already depleting striped bass stock.

Environmental factors influencing mycobacteriosis prevalence in striped bass are largely unknown. The thermal-oxygen squeeze hypothesis (Coutant 1985) has been presented as one potential explanation. Under thermal-oxygen squeeze, striped bass in deep-water refugia are forced into thermally unfavorable upper water layers during hypoxia. Concurrent exposure to hypoxia and increased water temperature decreases the metabolic scope of striped bass with mycobacteriosis (Lapointe et al. 2014), potentially worsening disease and/or decreasing survival. Due to differences in hydrology, the seasonal hypoxic volume may vary from river to river, particularly in habitats utilized by striped bass. Progression and severity of mycobacteriosis have also been directly linked to sea-surface temperatures in the Rappahannock River, suggesting that more regional factors such as overall thermal loading and rainfall (i.e. river flow) may influence disease prevalence (Groner 2018).

Previous work looking at disease prevalence and relative survival has been done using striped bass collected by the ChesMMAP survey between 2003 and 2007 from the main stem of the Chesapeake Bay. Results demonstrated greater than 70% visceral (internal) disease prevalence in some ages with increasing disease prevalence until age five for both sexes. After age 5, disease prevalence in males

continued to increase, reaching 90% in older fish. In contrast, female fish showed a decrease in prevalence after age six. Epidemiological modeling of these data supported the presence of disease-associated mortality, with annual relative survival of diseased fish estimated as 0.69 vs. undiseased fish (Gauthier 2008; Latour 2012). In a separate study, fish in the Rappahannock River were examined for dermal disease (skin lesions) as part of a tag-recapture study between 2005 and 2012. During this study, 22,629 fish were tagged and 1,880 were recaptured over 7 years (Hoenig 2017). Dermal mycobacteriosis prevalence in Rappahannock River striped bass exceeded 50% and concordant with Gauthier et al. (2008), the dermal disease was associated with decreased survival (i.e. relative return rate), and relative survival decreased with increasing disease severity (Hoenig et al. 2017). Whereas the relative survival of fish with minor skin disease did not significantly differ from that of externally undiseased fish, fish with moderate and severe skin lesions demonstrated annual relative survival of 0.84 and 0.54, respectively. These studies demonstrate that both visceral and dermal mycobacteriosis are highly prevalent in the mainstem and tributaries of Chesapeake Bay and that the disease is associated with elevated mortality of striped bass, particularly when it progresses to the point of severe external lesions (Hoenig 2017). It has been hypothesized that during the summer months increasing sea surface temperatures and hypoxic events caused by algae blooms may force striped bass into stressful conditions. This forcing of fish out of cooler bottom waters has been referred to as a "squeeze", and this move into unfavorable water conditions lowers the chance of survival (Coutant 1985; Price 1985). It is currently not known if this combination of hypoxic events and warm waters has an impact on mycobacteriosis.

OVERVIEW OF MASTERS RESEARCH

I undertook this research project with the intent to accurately estimate the mycobacteriosis prevalence in striped bass within the James and Rappahannock River systems. Sampling was done using a combination of gillnet and electrofishing methods in collaboration with the Multispecies Research Group at Virginia Institute of Marine Science. Dermal disease data were collected for fish caught using gillnets and electrofishing. A subset of spleens was also collected from fish caught using gillnets during the 2020

season to examine for internal disease. The James and Rappahannock River systems flow through Virginia and are 2 of the 20 major tributaries feeding into the Chesapeake Bay. These two rivers are important spawning sites for striped bass contributing to the Chesapeake Bay stock and adding a large number of fish to the coastal stock (Berggren and Lieberman 1978; Van Winkle et al. 1988). Very limited data regarding mycobacteriosis prevalence has been collected for spawning tributaries of the Chesapeake Bay. No disease prevalence data have been collected from the James River and data collection for the Rappahannock has not been undertaken since 2012 (Hoenig 2017).

During my project I focused on three main research aims:

1) Collect contemporary data on visceral and dermal mycobacteriosis for the James and Rappahannock Rivers.
2) Examine age-specific differences in disease prevalence among river systems. Studies support increasing oceanic migration of striped bass with increasing age (Dorazio et al. 1994; Kohlenstein 1981; Secor and Piccoli 2007), therefore younger fish are likely to be influenced primarily by the environment in their natal river, with influences of coastal migration increasingly dominating in older fish. If factors affecting mycobacteriosis prevalence operate on local (river-by-river) spatial scales, tributary-specific pattems of disease may be detected, and further, it would be expected that these patterns would diminish with increasing fish age due to migration.
3) Examine mycobacteriosis prevalence among year classes across two major Virginia tributaries of the Chesapeake Bay. If environmental factors affecting mycobacteriosis prevalence operate on more regional spatial scales, minimal differences between tributaries would be expected, whereas prevalence may vary significantly among fish of different age classes. Age classes of striped bass would be exposed to similar cumulative environmental stressors (e.g., thermal regime, hypoxia, food availability) during their lives, and examining patterns of prevalence among age classes may allow detection of large-scale environmental variables, such as elevated summer temperatures or hypoxic extent, that may be linked to mycobacteriosis prevalence.

METHODOLOGY

COLLECTION METHODS AND SITES

Field research for this project was performed in cooperation with Virginia Institute of Marine Science research teams (R. Latour, VIMS PI) performing their "Evaluation of Striped Bass Stocks in Virginia: Tagging and Monitoring Studies" survey approval code: IACUC-2020-02-11-14100-jxgart. All striped bass were collected by the Virginia Institute of Marine Science for their yearly survey and additional data from the same fish was collected for this project; these fish were not being collected directly for this research. Spring sampling occurred for at least 10 weeks starting in mid-February and ending in mid-May in 2020 and 2021. This annual survey collects striped bass during their spring migration into Virginia tributaries to spawn. Striped bass were collected from the James and Rappahannock Rivers via electrofishing and gillnets. Fieldwork consisted of setting and retrieving gillnets and using electrofishing gear to collect striped bass. Two gillnets were set in each river once per week for at least 10 weeks during striped bass spawning season and were retrieved approximately 24 hours after being set. Each net was 300 feet long consisting of ten separate 30-foot long by 10-foot-deep panels with alternating mesh sizes ranging from 3 inches to 10 inches in diameter (Figure 3) and nets were fished in the same areas throughout the entire field season for both field seasons.

FIGURE 3. Diagram of the gillnets fished in each river. On both ends, there is a highflyer buoy attached to an anchor and the net is between these two anchors. The net is held upright by buoys on the top and a weighted lead line on the bottom. Each net is made of ten 30-foot panels of various mesh sizes. The numbers within each panel represent the mesh size measured in inches.

Nets on the Rappahannock river were set ~1 mile upriver of Carter's Wharf in Warsaw and the James River nets were set just offshore of Westover Plantation in Charles City County (Figure 4). All gillnet-caught striped bass were brought back to a Virginia Institute of Marine Science lab for data collection and processed the following day. Information regarding the dermal disease, total and fork length, weight, sex, and gonad stage was recorded for each gillnet caught fish. Otoliths were also extracted to determine the age.

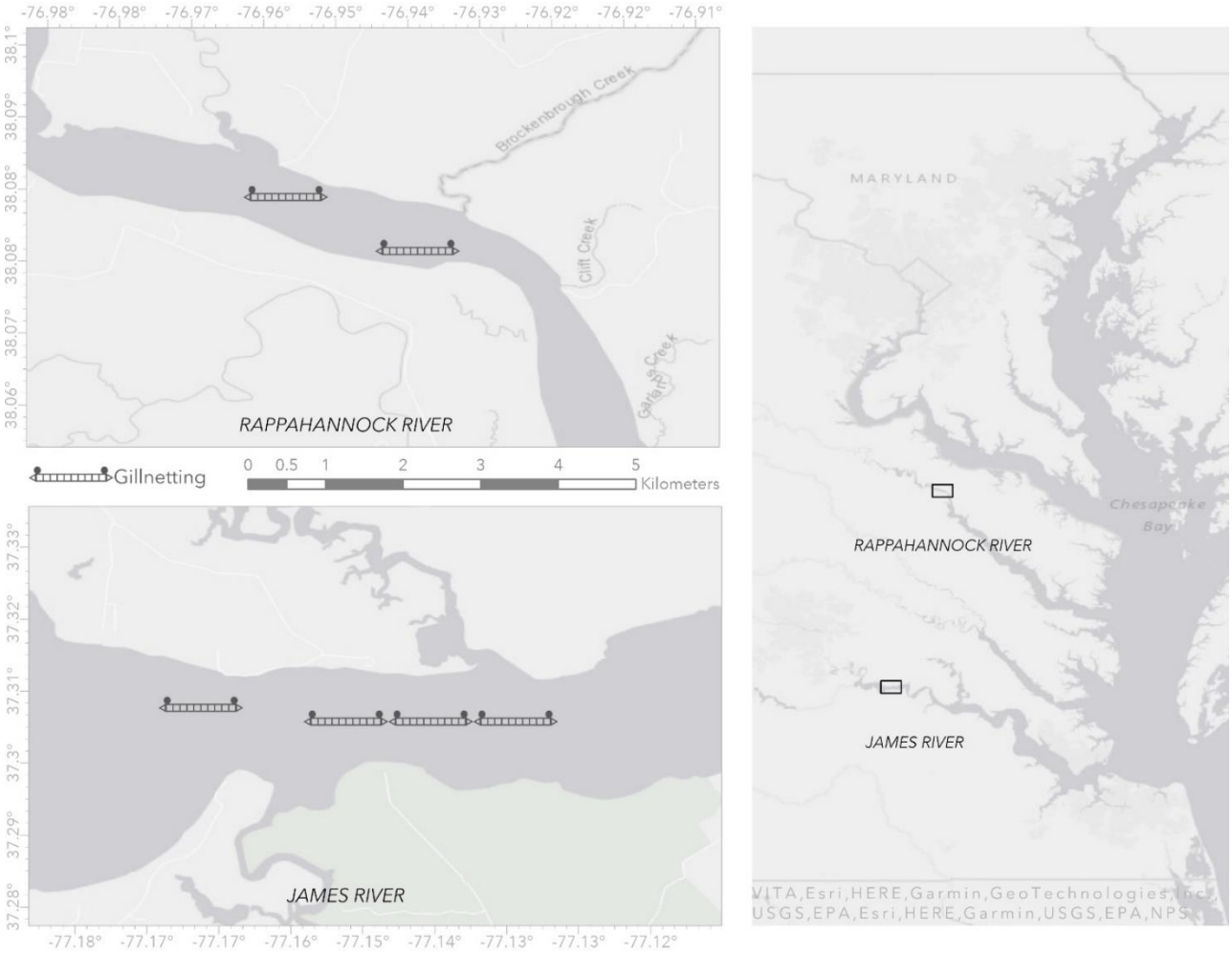

FIGURE 4. Maps showing the location of the James and Rappahannock Rivers and where gillnets were fished on both rivers. Photo courtesy of the MRG at VIMS.

A Smith Root 9.0 GPP (Smith-Root, Vancouver, WA) electrofishing system was used when tagging and releasing fish. Electrofishing typically took place along mudflats of the rivers and system settings were determined depending on the conductivity and temperature of the water. The system was set to 60 Hz and voltage was determined by the conductivity of the water each day of sampling. If conductivity was low, a higher voltage was applied and if conductivity was high lower voltage was applied. Electrofishing is done in parts of the rivers with very low salinities ranging from 0.3 to 0.7 PSU in 2020 and 0.0 to 0.1 PSU in 2021. Electrofishing in the James River took place between

Jamestown Island and Tar Bay while electrofishing within the Rappahannock river took place between the town of Tappahannock and Green Bay (Figure 5).

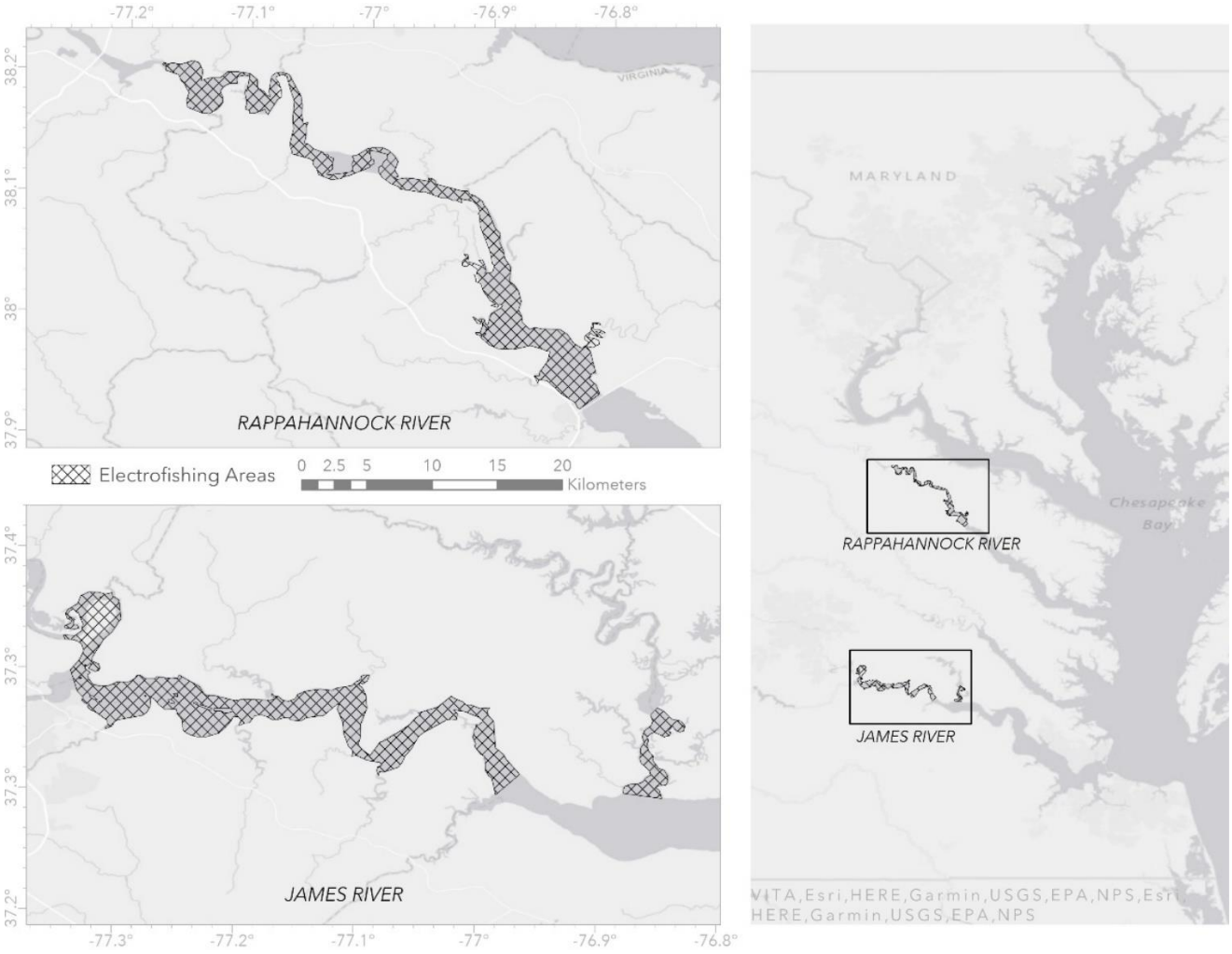

FIGURE 5. Maps showing the location of the James and Rappahannock Rivers and where electrofishing took place on both rivers. Photo courtesy of the MRG at VIMS.

Striped bass caught through electrofishing had total and fork length taken and were checked for dermal disease (Hoenig 2017) while sex was determined to be either male or unknown based on if the fish was running ripe or not. Scale samples were also taken from all fish caught to determine the age. A small incision is also made on the left side of the fish where a nylon anchor tag is placed inside the body cavity. Once data collection and tagging were completed fish were immediately released back into the river. Data were only collected on fish greater than 457 mm, anything under this minimum was immediately released back into the river without a tag. With collections occurring during the spawning season when a new growth ring is added to a fish's otoliths and scales, a year was added to the number of growth rings to account for the new growth ring that would have been placed shortly after collection. The only case when a year was not added was when the formation of a new growth ring was seen on the end of the hard part, if a new ring was seen only the number of rings were counted to determine age. Year class was determined for all fish by subtracting the established age from the calendar year the fish was collected.

Electrofishing and gillnets were used near each other in both rivers, so there was a chance that a fish caught and tagged through electrofishing could be recaptured the same year in a gillnet. If a fish were to be recaptured in the gillnet the same year it was tagged and released the fish would be released back into the river if still alive. If the fish was not alive it would be brought back to the lab for data collection but this fish would be taken out of the tagging data set so it would not be counted twice. Between 2017 and 2021, only 1 of ~6500 fish tagged through electrofishing has been recaptured in a gillnet (Jameson Gregg, personal communication).

ESTIMATING DISEASE

Disease prevalence was measured by performing dermal and visceral examinations of collected fish. Dermal examinations were conducted as described by Hoenig et al. (2017), and fish were placed into an undiseased category or one of four severity categories based on the number of pigmented foci (Table 1) and the number and size of skin ulcers present (Table 2) on either side of the fish. Fish were placed into a severity category based on the highest severity level seen on either side of the fish.

TABLE 1. Definitions of pigmented foci severity levels based on the number of foci present on each side of the fish (Hoenig 2017).

Severity Level	Number of Foci
1	1 to 10
2	11 to 50
3	51 or more

TABLE 2. Definitions of skin lesion severities based on the number and size of skin lesions present on each side of the fish (Hoenig 2017).

Severity Level	Focal or Multifocal	Size of Lesion
1	Focal	Lesion < 2 cm
2	Multifocal	All Lesions < 2 cm
3	Focal	Lesion > 2 cm
4	Multifocal	Some Lesions > 2cm

Paraffin histology was performed on 312 spleens from gillnet-caught fish harvested during the 2020 field season. Fish spleens collected from necropsied fish were preserved in 35-45 mL of formalin (Z-fix, Anatech, Battle Creek, MI)**.** Preserved spleens were divided transversely into six approximately equal sections; removal of 1-2 mm portions were done in between each section to avoid examination of contiguous cross-sections (Gauthier 2008) and spleen sections were processed for routine paraffin histology (Prophet 1992). A Thermo Shandon Excelsior ES tissue processor was used to dehydrate, clear, and paraffin infiltrate spleens. Tissues were subsequently embedded in paraffin wax using a Thermo Scientific Histostar Embedding Center. When possible, all 6 sections of the spleen were mounted in one paraffin block and placed on a single microscope slide but in cases where the tissues were too large, sections were randomly placed in multiple cassettes and processed onto multiple slides. Paraffin blocks were sectioned at 5 µm thick using a Tanner Scientific Titan 500 microtome and stained with hematoxylin and eosin (HE) using a Thermo Scientific Varistain Gemini ES. Sections of embedded specimens were mounted on a microscope slide and a 50 µm step section from the same block was also placed on that slide. Tissue samples were observed through a compound light microscope to check for the presence of granulomas to determine if an individual was disease positive or negative (Gauthier 2008). Once the disease state was determined, the total area of the spleen tissue examined on a slide was measured (mm^2) using an Epson scanner (Model v700, Epson America Inc, Los Alamitos, CA). The severity of visceral disease was quantified as granulomas/mm^2 for all sections of spleen for a fish thus binning that fish into one of the five disease severity categories (Latour 2012).

STATISTICAL MODELS

The open-source program R 4.0.1 (R core team 2020) was used for all analyses, modeling, and graphing of data. Packages used in this study included tidyverse, ggplot2, DescTools, and MuMin. Generalized logistic regression was the statistical model used to examine the effects that age, year class, and river had on disease prevalence and severity. The two years of data collection for each fishing method were combined for the statistical analysis. The age variable could include fish originating from different

year classes but for the year class variable fish could only be placed into the year class it was hatched in. Age-based models included river and age as independent variables [Model (M) 1]. Year class-based models included river and year class as independent variables [Model (M) 2]. Interaction terms were also added to all full models to examine how the independent variables affected disease when combined.

glm(Prevalence ~ (River + Age)^2, family = binomial (M) 1

glm(Prevalence ~ (River + Year Class)^2, family = binomial (M) 2

Ordinal logistic regression was first attempted for running statistical models for disease severity due to severity being a multilevel categorical outcome. To properly run ordinal logistic regression 4 assumptions must be met regarding the independent variables. The 4 assumptions were "the dependent variable is measured on an ordinal level, one or more of the independent variables are either continuous, categorical or ordinal, no multi-collinearity, and proportional odds" (Lee 2019). The last two assumptions could not be met, so analyses using this model were not performed. Diseased fish were separated into their severity categories and compared to undiseased fish.

The function dredge (R package MuMin) was used on each full model to run all possible combinations of independent variables to determine the best-fitting models. Akaike information criterion (AIC) scores were used to rank best-fitting models with any model having a delta score of 2 or less considered well-supported. Model averaging (R package MuMin) was then done by combining the best fit model with all well-supported models. This combined model proportionally incorporated all models depending on their weight.

Subsets of fish from the 2020 gillnet collections were used to compare different aging methods and internal and external disease prevalence. Spleens were collected to compare visceral and dermal disease prevalence. Fish aged using both otoliths and scales were used to compare aging methods. Positive predictive value (PPV), negative predictive values (NPV), sensitivity, and specificity were determined to understand how accurate using the presence of external disease is for determining internal

disease. Comparing aging methods was done using a linear model (R package tidyverse [Model (M) 3]) and ages were plotted along with residual values (R package ggpmisc).

(lm (Otolith Age ~ Scale Age, na.action = na.omit)) (M) 3

RESULTS

During the 2 years of field sampling, a total of 4,252 striped bass were collected using gillnets and electrofishing. Ages 0 to 26 were represented in the sample originating from year classes ranging from 1995 to 2021 with the majority being between the ages of 2 and 8 and originating from the 2015 to the 2018 year classes. Of the total sample, 3,843 fish were male, 154 were female, and sex could not be identified for 255 (Table 3). During the 2020 field season, 2,083 striped bass were collected while in the 2021 field season 2,169 striped bass were collected; overall skin mycobacterial disease prevalence rates of 40.8% and 39.9% were seen respectively (Figure 6). During both field seasons, a total of 2,035 striped bass were collected by electrofishing: 911 from the James River and 1,124 from the Rappahannock River. Using gillnets 2,217 striped bass, 857 from the James River, and 1,360 from the Rappahannock River (Figure 7). The James River had a higher disease prevalence overall compared to the Rappahannock River with 44.6% of the fish caught using electrofishing and 45.0% of gillnet caught fish being diseased. In the Rappahannock River, 39.5% of fish caught using electrofishing and 35.4% of gillnet caught fish were diseased (Figure 7).

TABLE 3. Sample sizes from both field seasons are separated into the catch method, the river caught, total catch size, and the total number of each sex caught. Fish were identified as male, female, or sex unidentified.

Collection Method	**Gillnet**		**Electrofishing**	
	James River	Rappahannock River	James River	Rappahannock River
Males	733	1,330	771	1,010
Females	123	29	0	1
Unidentified sex	1	1	140	113
Total Catch	857	1,360	911	1,124

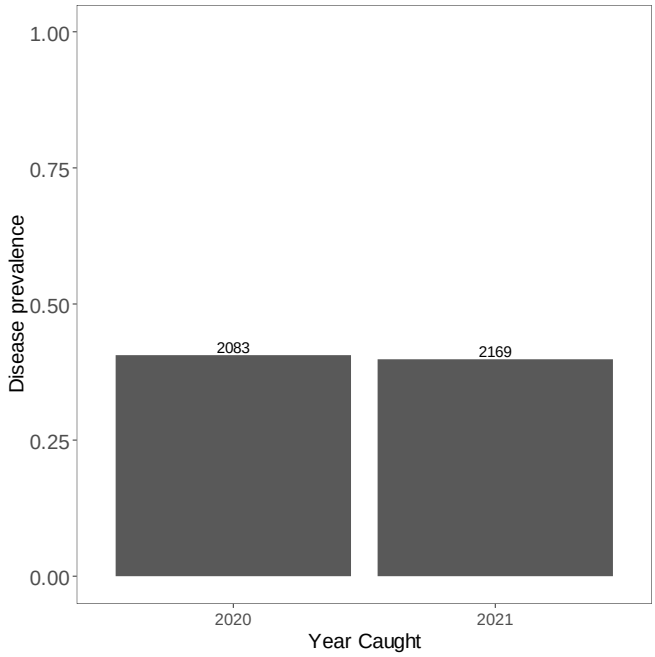

FIGURE 6. Skin disease prevalence for both field collection seasons (38.8% prevalence for 2020 and 39.7% prevalence for 2021). Sample sizes are shown above each bar.

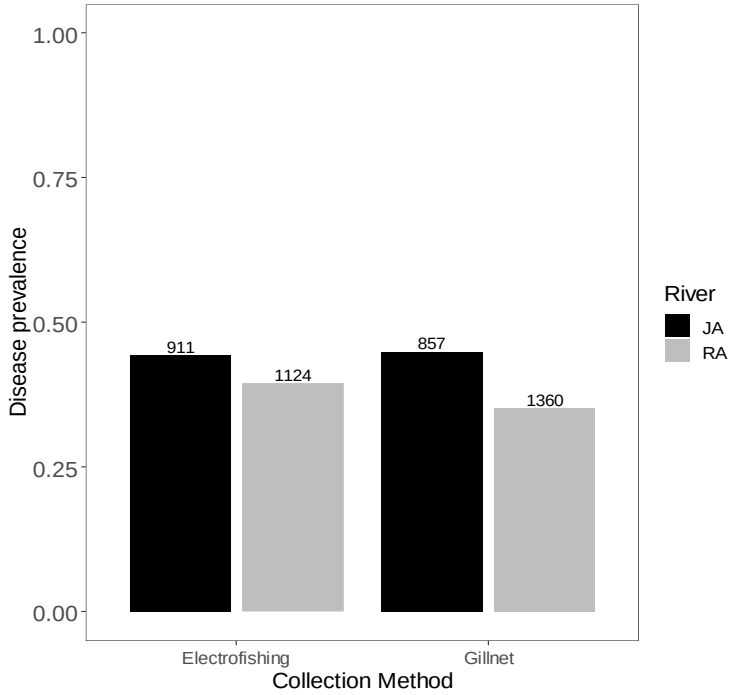

FIGURE 7. Skin disease prevalence for fish caught using gillnets (45.0% prevalence for JA and 35.3% prevalence for RA) and electrofishing (44.5% prevalence for James and 39.5% prevaelnce for Rappahannock) from the James (JA) and Rappahannock (RA) rivers. Sample sizes are shown above each bar.

With low catches for females and fish under age 2 and over age 8, only males between 2 and 8 years old from the original 4,252 fish were included in the final analysis. After restricting the data sets, 1,755 fish remained in the electrofishing data set and 2,037 in the gillnet data set. Both data sets had to be further restricted when using Model 2 due to low sample sizes for some year classes. Only fish spawned between 2015 and 2018 were included in the analysis resulting in 1,646 fish for the electrofishing data set and 1,847 fish for the gillnet data set. Severities 3 and 4 were grouped for all analyses also due to low sample sizes for these two disease categories.

Striped bass caught using gillnets and electrofishing were analyzed separately due to different methods of aging. Fish caught in gillnets were aged using otoliths, while fish caught by electrofishing were aged using scales because these animals were tagged and released after determination of dermal disease status (Atlantic States Marine Fisheries Commission 2003). A subset of 652 fish from the 1,082 caught using gillnets from the 2020 field season were aged using both otoliths and scales. Residual plots were used to determine if age determined using both methods agreed. Ages agreed when plots were 0 on the y axis, if aging methods disagreed the point on the graph was placed above or below 0. Most age compressions did agree and most of the disagreements for age differed by 1 year. Analysis of residuals from a regression (Model 3) of scale and otolith ages indicated using otoliths had a bias towards under-aging of fish from age 2 to 5; at age 5 under and over-aging of fish occurred, and for 6-8-year-old fish a bias towards over-aging occurred (Figure 8). When aging fish using scales, under and over-aging of fish from 3 to 6 years occurred (Figure 9).

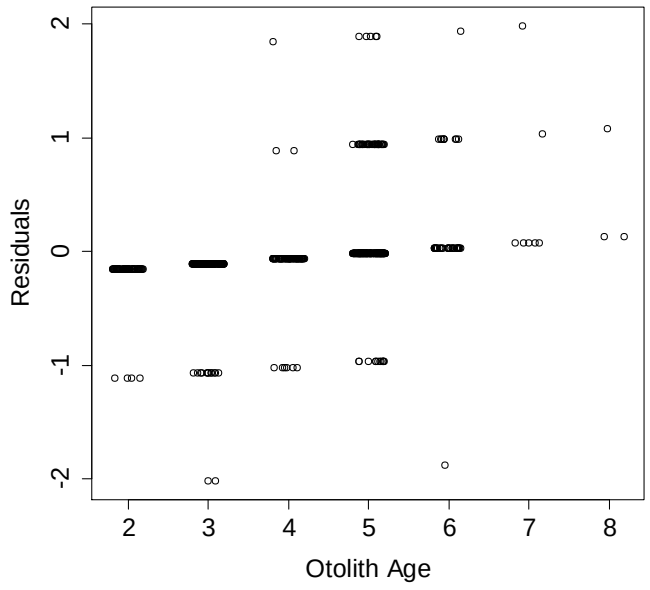

FIGURE 8. Otolith age residual values for the 652 striped bass aged using both otoliths and scales from the 2020 gillnet collection season.

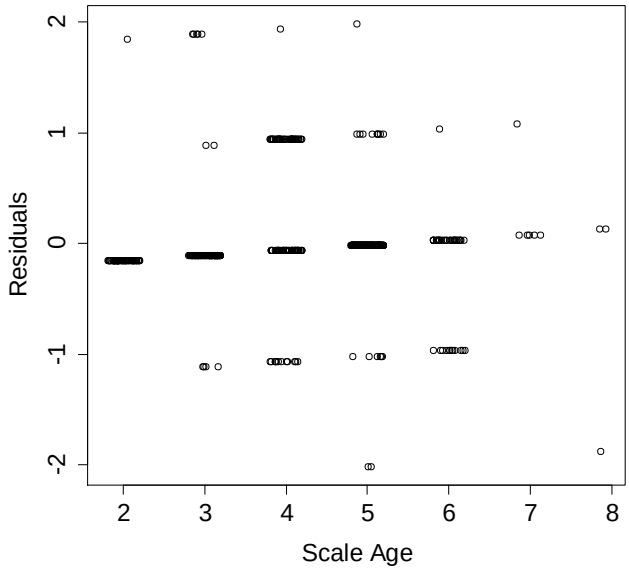

FIGURE 9. Scale age residual values for the 652 striped bass aged using both otoliths and scales from the 2020 gillnet collection season.

Dermal disease prevalence increased from age 2 to 8 for fish caught using gillnets (Figure 10). Prevalence also increased from age 3 to 8 after an initial decrease in prevalence from age 2 to 3 for fish caught using electrofishing (Figure 11) in both rivers. Most diseased fish caught using gillnets (Figure 12) and by electrofishing (Figure 13) exhibited mild disease signs and were categorized into disease severity category 1 and fish with higher disease severities were mainly seen from age 3 to 8. Severity 4 was seen only in 5 and 6-year-old fish in the James River and age 3 to 6 and age 8 fish in the Rappahannock River.

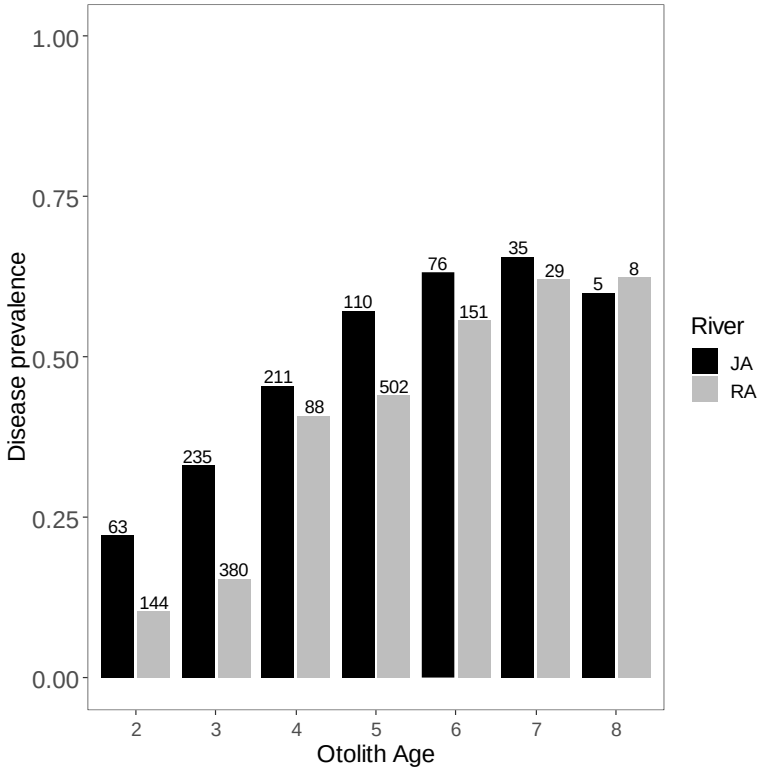

FIGURE 10. Age vs. skin disease prevalence for fish caught using gillnets during the 2020 and 2021 field seasons from the James (JA) and Rappahannock (RA) rivers. Sample sizes are shown above each bar.

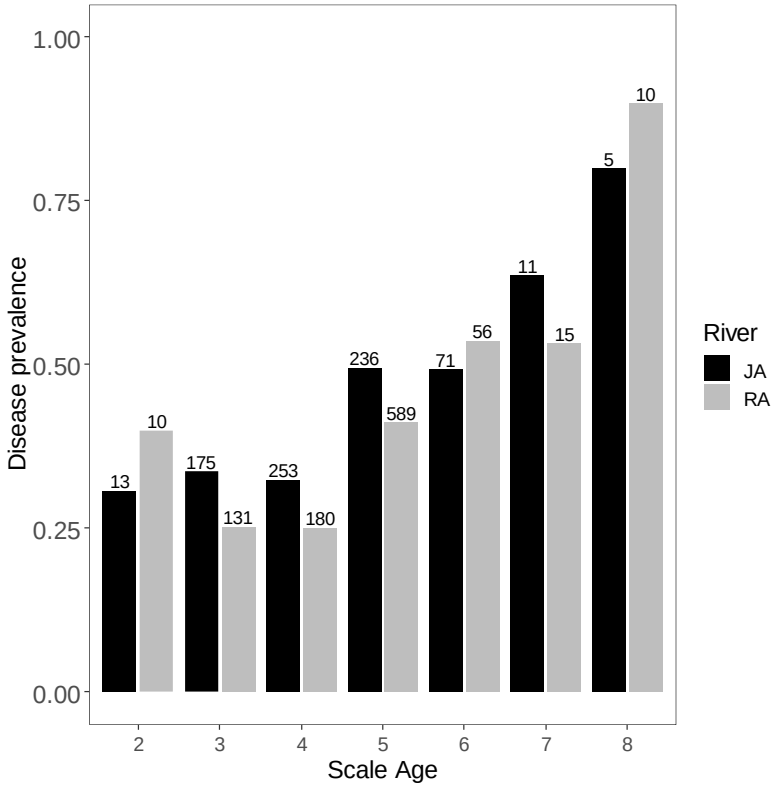

FIGURE 11. Age vs. skin disease prevalence for fish caught using electrofishing during the 2020 and 2021 field seasons from the James (JA) and Rappahannock (RA) rivers. Sample sizes are shown above each bar.

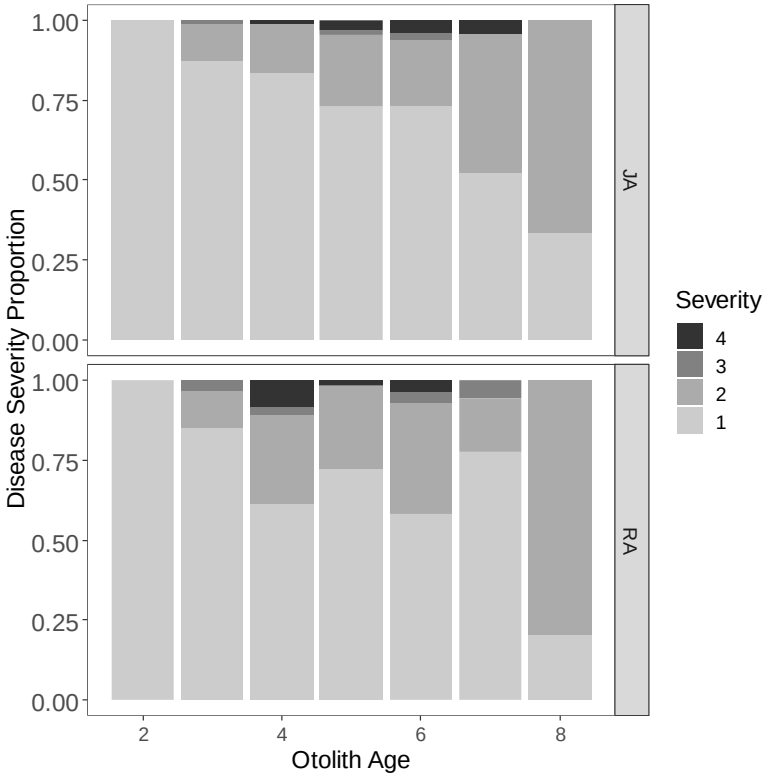

FIGURE 12. Age vs. skin disease severity for disease-positive fish aged 2-8 caught using gillnets during the 2020 and 2021 field season from the James (JA) Rappahannock (RA) rivers. Increasing severity is shown by increased shading.

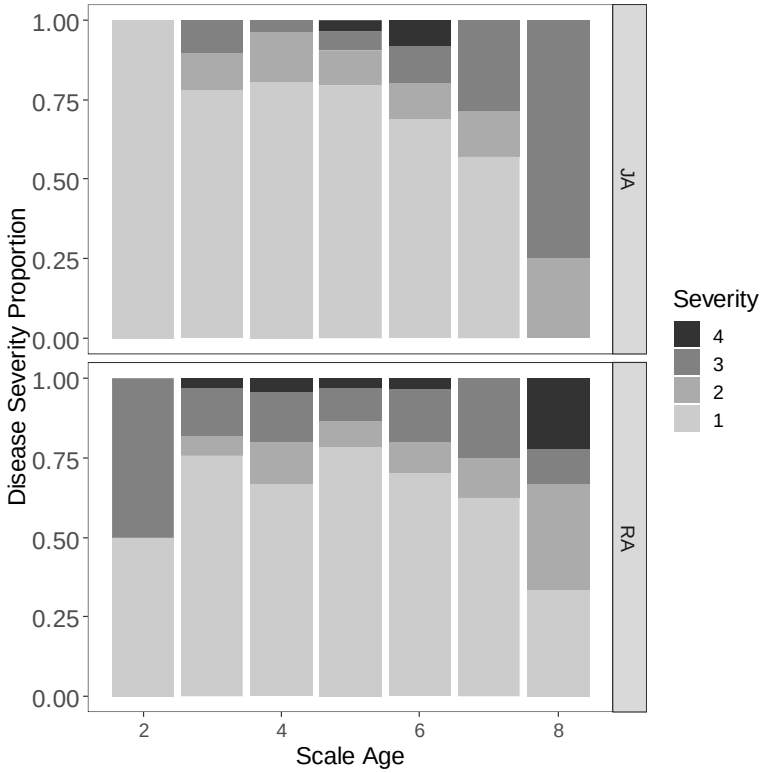

FIGURE 13. Age vs. skin disease severity for disease-positive fish aged 2-8 caught using electrofishing during the 2020 and 2021 field season from the James (JA) Rappahannock (RA) rivers. Increasing severity is shown by increased shading.

Dermal disease prevalence decreased in gillnet-caught fish with increasing year class (Figure 14) for both rivers. Prevalence of skin lesions in fish caught during electrofishing generally decreased with increasing year class, however, this trend was broken by increased prevalence from the 2016–2017-year classes in Rappahannock River fish and from 2017 to the 2018 year classes in James River fish (Figure 15). Fish caught using gillnets (Figure 16) and electrofishing (Figure 17) were mainly categorized into disease severity 1 but fish in higher disease severities were seen in all year classes at very low prevalence.

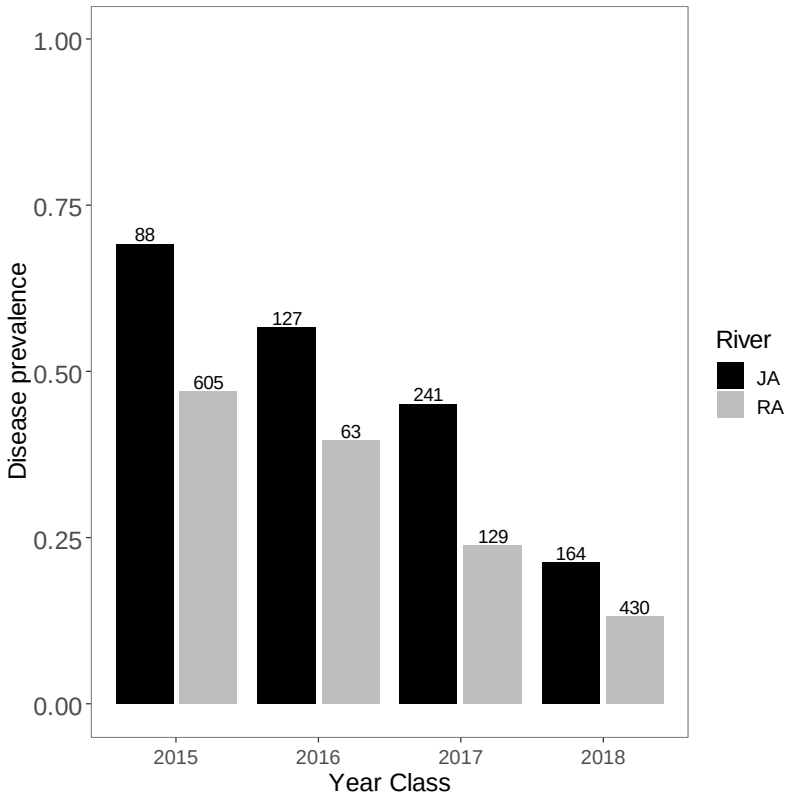

FIGURE 14. Year Class vs. skin disease prevalence for fish caught using gillnets during the 2020 and 2021 field seasons from the James (JA) and Rappahannock (RA) rivers. Sample sizes are shown above each bar.

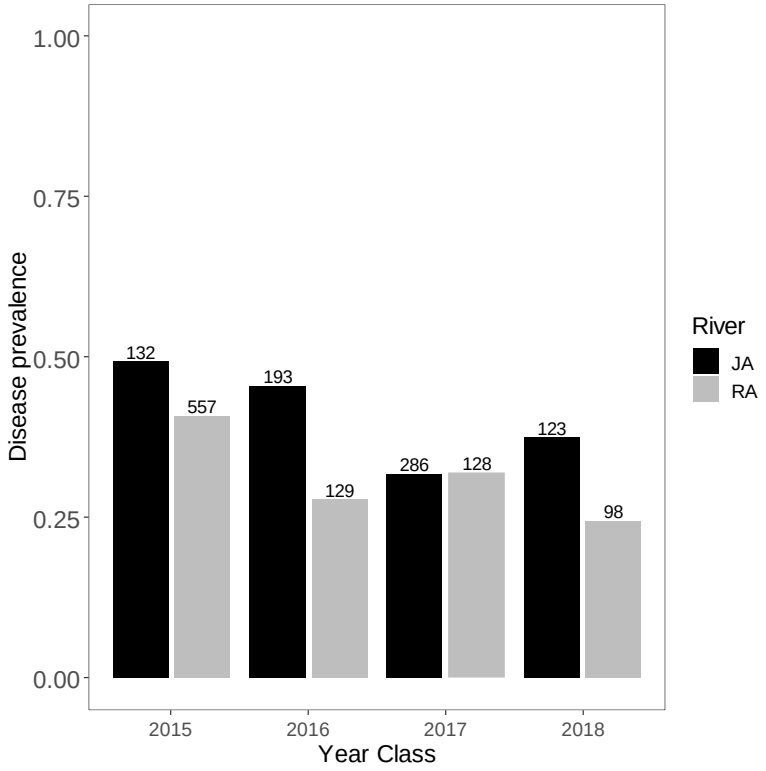

FIGURE 15. Year Class vs. skin disease prevalence for fish caught during electrofishing from the 2020 and 2021 field seasons from the James (JA) and Rappahannock (RA) rivers. Sample sizes are shown above each bar.

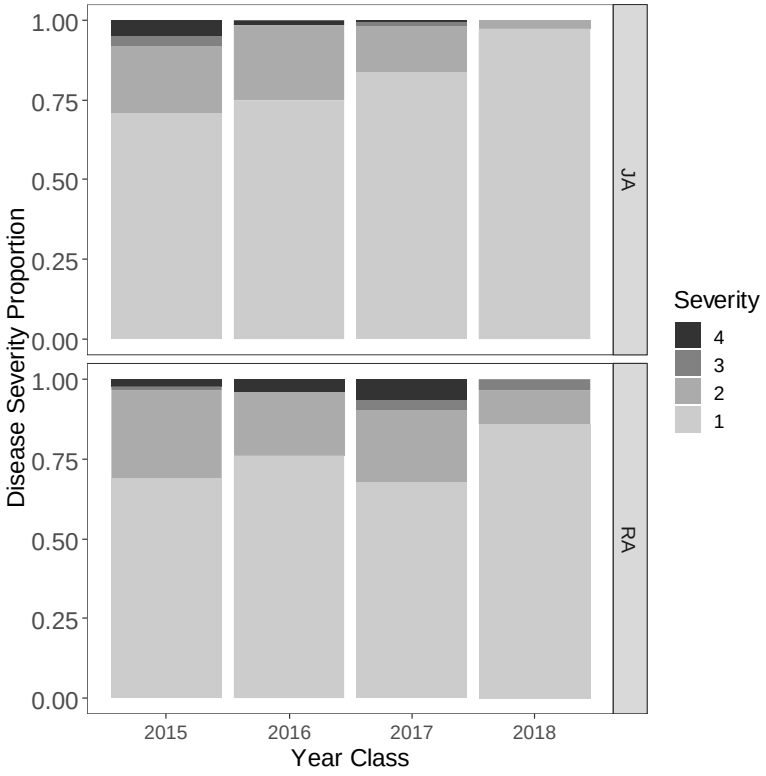

FIGURE 16. Year Class vs. skin disease severities for disease-positive fish from the 2015-2018 year classes caught using gillnets during the 2020 and 2021 field seasons from the James (JA) and Rappahannock (RA) rivers. Increasing severity is shown by increased shading.

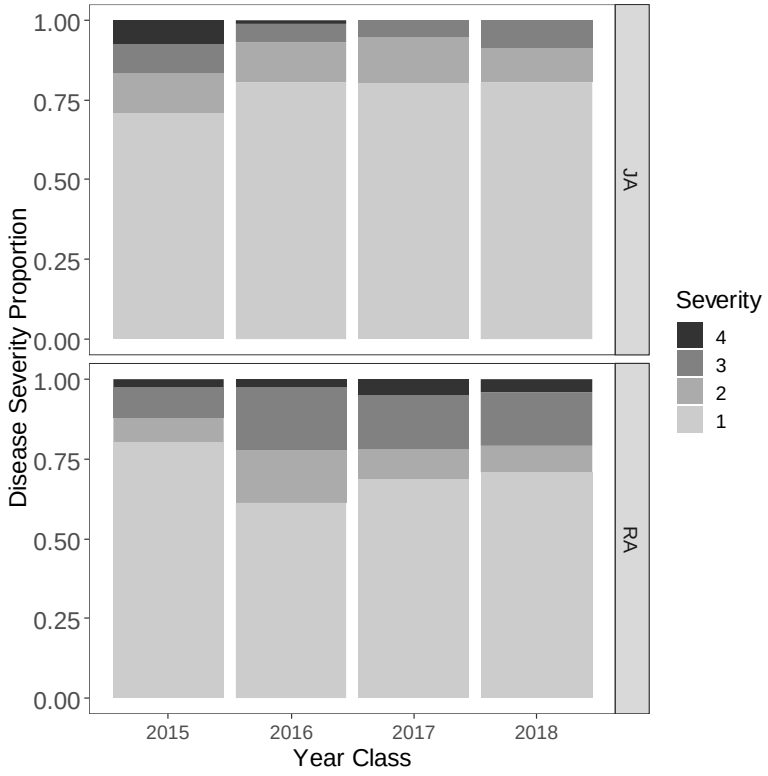

FIGURE 17. Year Class vs. skin disease severities for disease-positive fish from the 2015-2018 year classes caught using electrofishing during the 2020 and 2021 field seasons from the James (JA) and Rappahannock (RA) rivers. Increasing severity is shown by increased shading.

Logistic regression was used on both data sets to examine the effects of the independent variables on disease. The same models were used to test the effects on disease prevalence and all severity categories. Outcome variables were: disease prevalence, disease severity 1, disease severity 2, and disease severity 3 and 4 combined. For each odds ratio table the reference variable "River RA" is comparing fish caught in the Rappahannock River to fish caught in the James River. Age had a significant positive association with disease while being caught in the Rappahannock Rive had a significant negative association with disease for the prevalence and severity 1 models. Only age had a significant positive association with disease for the severity 2 and the combined severity 3 and 4 models (Table 4). Year class and being caught in the Rappahannock River had a significant negative association with disease for the prevalence, severity 1, and severity 2 models. Year class and being caught in the Rappahannock River had a significant negative association with disease but the interaction between these variables had a positive association with disease for the combined severity 3 and 4 model (Table 5).

TABLE 4. Odds ratio table for the gillnet data set representing all variables and interactions from the best fit and all well-supported models from [Model (M) 1]. Variables, Odds Ratios (OR), and p-values for models comparing disease prevalence to undiseased fish and different disease categories to undiseased fish are separated by thin black lines. Odds ratios include 95% confidence intervals (CI).

Variables	OR (95% CI)	p-value
Prevalence		
Intercept	1.033 (0.923 -1.155)	0.576
Otolith Age	1.107 (1.078 - 1.137)	<2e-16***
River RA	0.832 (0.720 - 0.963)	0.013*
Otolith Age: River RA	1.026 (0.995 - 1.058)	0.107
Severity 0:1		
Intercept	1.073 (0.970 - 1.187)	0.174
Otolith Age	1.083 (1.057 - 1.120)	< 2e-16***
River RA	0.847 (0.750 - 0.955)	0.007**
Otolith Age: River RA	1.020 (0.987 - 1.053)	0.238
Severity 0:2		
Intercept	0.835 (0.785 - 0.889)	<2e-16***
Otolith Age	1.080 (1.064 - 1.096)	<2e-16***
River RA	0.989 (0.918 - 1.066)	0.782
Otolith Age: River RA	0.990 (0.966 - 1.015)	0.449
Severity 0:3 and 4		
Intercept	0.966 (0.943 - 0.989)	0.005**
Otolith Age	1.014 (1.009 - 1.020)	1e-06***
River RA	0.996 (0.980 - 1.012)	0.640

*p < 0.05.** p < 0.01. *** p < 0.001

TABLE 5. Odds ratio table for the gillnet dataset representing all variables and interactions from the best fit and all well-supported models from [Model (M) 2]. Variables, Odds Ratios (OR), and p-values for models comparing disease prevalence to undiseased fish and different disease categories to undiseased fish are separated by thin black lines. Odds ratios include 95% confidence intervals (CI).

Variables	OR (95% CI)	p-value
Prevalence		
Intercept	2.675 (1.894 -3.824)	3.9e-08***
Year Class	0.505 (0.422 - 0.601)	3.5e-14***
River RA	0.340 (0.230 - 0.497)	3.7e-08***
Year Class: River RA	1.111 (0.908 - 1.364)	0.309
Severity 0:1		
Intercept	1.730 (1.287 - 2.327)	2.9e-04***
Year Class	0.580 (0.506 - 0.664)	< 2e-16***
River RA	0.364 (0.266 - 0.498)	< 2e-16***
Year Class: River RA	1.067 (0.831 - 1.321)	0.554
Severity 0:2		
Intercept	0.608 (0.378 - 0.975)	0.039*
Year Class	0.398 (0.305 - 0.520)	<2e-16***
River RA	0.421 (0.253 - 0.700)	8.5e-04**
Year Class: River RA	1.174 (0.785 - 1.757)	0.435
Severity 0:3 and 4		
Intercept	0.168 (0.059 - 0.393)	1.7e-04***
Year Class	0.224 (0.083 - 0.484)	6e-04***
River RA	0.199 (0.068 - 0.644)	0.004**
Year Class: River RA	2.832 (1.168 - 8.169)	0.031*

*p < 0.05.** p < 0.01. *** p < 0.001

Age had a significant positive association with disease for all four models. No other variable had a significant association with disease for any model (Table 6). Year class and being caught in the Rappahannock River had a significant negative association with disease for the disease prevalence and severity 1 models. Being caught in the Rappahannock River had a significant negative association with disease for the severity 2 model. Year class had a significant negative association with disease, and the interaction between year class and being caught in the Rappahannock River had a significant positive association with disease for the severity 3 and 4 model (Table 7).

TABLE 6. Odds ratio table for the electrofishing data set representing all variables and interactions from the best fit and all well-supported models from [Model (M) 1]. Variables, Odds Ratios (OR), and p-values for models comparing disease prevalence to undiseased fish and different disease categories to undiseased fish are separated by thin black lines. Odds ratios include 95% confidence intervals (CI).

Variables	OR (95% CI)	p-value
Prevalence		
Intercept	1.053 (0.928 - 1.194)	0.425
Scale Age	1.085 (1.055 - 1.112)	<2e-16***
River RA	0.914 (0.782 - 1.068)	0.258
Scale Age: River RA	1.021 (0.976 - 1.068)	0.366
Severity 0:1		
Intercept	1.068 (0.927 - 1.231)	0.363
Scale Age	1.067 (1.033 - 1.102)	8.4e-05***
River RA	0.889 (0.730 - 1.082)	0.241
Scale Age: River RA	1.031 (0.982 - 1.081)	0.217
Severity 0:2		
Intercept	0.953 (0.883 - 1.030)	0.224
Scale Age	1.031 (1.013 - 1.049)	7.7e-04***
River RA	0.952 (0.873 - 1.037)	0.259
Scale Age: River RA	1.010 (0.981 - 1.040)	0.511
Severity 0:3 and 4		
Intercept	0.889 (0.830 - 0.953)	9.4e-04***
Scale Age	1.045 (1.029 - 1.061)	<2e-16***
River RA	1.010 (0.979 - 1.042)	0.514

*p < 0.05.** p < 0.01. *** p < 0.001

TABLE 7. Odds ratio table for the electrofishing data set representing all variables from the best fit and all well-supported models comparing from [Model (M) 2]. Variables, Odds Ratios (OR), and p-values for models comparing disease prevalence to undiseased fish and different disease categories to undiseased fish are separated by thin black lines. Odds ratios include 95% confidence intervals (CI).

Variables	OR (95% CI)	p-value
Prevalence		
Intercept	1.619 (1.533 - 1.710)	<2e-16***
Year Class	0.945 (0.920 – 0.971)	4.7e-05***
River RA	0.923 (0.871 – 0.979)	0.007**
Year Class: River RA	1.004 (0.958 - 1.052)	0.871
Severity 0:1		
Intercept	0.744 (0.566 – 0.977)	0.033*
Year Class	0.783 (0.677 – 0.907)	1.1e-03**
River RA	0.698 (0.514 – 0.948)	0.021*
Year Class: River RA	0.896 (0.713 - 1.127)	0.348
Severity 0:2		
Intercept	0.102 (0.058 - 0180)	<2e-16***
Year Class	0.807 (0.603 - 1.082)	0.152
River RA	0.528 (0.287 – 0.972)	0.040*
Year Class: River RA	1.126 (0.678 - 1.871)	0.647
Severity 0:3 and 4		
Intercept	0.132 (0.068 - 0.236)	1e-10***
Year Class	0.548 (0.356 – 0.828)	5e-03**
River RA	0.657 (0.329 - 1.381)	0.248
Year Class: River RA	1.795 (1.094 – 2.968)	0.021*

*p < 0.05.** p < 0.01. *** p < 0.001

From the 2020 field season, spleens were collected from 310 fish caught using gillnets. Positive and negative predictive values for using skin disease prevalence to estimate internal disease prevalence, as well as sensitivity and specificity, were calculated after comparing internal and external disease prevalence. The positive and negative predictive values were 0.776 and 0.389, respectively (Table 8).

TABLE 8. Predictive values were estimated by comparing skin disease testing using the presence of pigmented foci and skin ulcerations to internal disease testing determined by the presence of granulomas in the fish's spleen tissue. Values in each box represent the number of fish in each testing category, skin testing positive and internal testing positive (125), skin testing positive and internal testing negative (36), skin testing negative and internal testing positive (91), and skin testing negative and internal testing negative (58).

Internal Disease

Skin Disease		Positive	Negative	Row Total
	Tests Positive	125	36	161
	Test Negative	91	58	149
	Column Total	216	94	

125/161= 0.776 58/149=0.389 125/216=0.579 58/94 =0.617

Positive Predictive Value (PPV)	Negative Predictive Value (NPV)	Sensitivity	Specificity
0.776	0.389	0.579	0.617

DISCUSSION

Based on these two years of research it seems mycobacteriosis works yearly on a localized spatial scale but some years disease also works on a regional spatial scale adding to the localized effects. During the 2020 and 2021 spawning seasons, mycobacteriosis prevalence data were collected from fish caught from 2 of Virginia's tributaries to the Chesapeake Bay. These two tributaries examined in this research contribute to the Chesapeake Bay stock, the largest contributor of striped bass to the Atlantic Coastal stock. The main research objectives for this project were to collect current disease prevalence and severity data for striped bass in two important Chesapeake Bay tributaries, the Rappahannock and James Rivers. This study was initiated to better understand the spatial and temporal scale of this infectious disease. Before this study, the most recent prevalence data for the Rappahannock River was collected in 2012 (Hoenig 2017) and no published disease data existed for the James River.

Disease prevalence mainly increased in both rivers as age increased (Figures 10, 11) and most fish between the age of 2 and 8 were found to exhibit mild dermal disease (disease severity category 1) but fish with higher severities were seen in all ages (Figures 12, 13). Increasing prevalence with increasing age was also seen in male striped bass caught in the main stem of the Chesapeake Bay from 2003 to 2005 with an internal disease prevalence of over 70% seen after the age of 6 (Gauthier 2008). Age-based logistic regression analyses indicated that age had a significant positive association with disease prevalence and all severity categories. However, fewer fish exhibited severe dermal disease (e.g., grouped into severity 3 and severity 4 categories), whereas most of the fish examined fell into disease severity categories 1 and 2. Despite these findings, there is convincing evidence that fish in these lower severity categories have a low probability of recovering from this infectious disease (Groner 2018). Mycobacteriosis is a chronic and progressive infection and it is widely held that infected fishes do not recover from it (Decostere et al. 2004; Frerichs 1993; Van Diujn Jr 1981). With little evidence of mycobacterial disease regression, the significantly lower numbers of fish collected with severe infections (e.g., severity 3 and 4 categories) may be due to disease-associated mortality which has been documented

in the main stem of the Bay and the Rappahannock River (Gauthier 2008; Hoenig 2017). Fish caught from the main stem of the Bay had a 69% chance of annual survival compared to undiseased fish (Gauthier 2008) while moderately and severely diseased fish caught in the Rappahannock River had annual relative survival of 84% and 54 % respectively (Hoenig 2017). With these previous studies seeing decreased relative survival in diseased fish caught in different areas of the Bay, the low catches of highly diseased fish are likely due to disease-associated mortality

Disease prevalence for the Rappahannock River from this study was lower than previously documented (Hoenig 2017). Comparing disease prevalence rates for the James River could not be done due to no previously published prevalence data. Hoenig et al (2017) reported skin disease prevalence often exceeding 50% from striped bass tagged and released from 2005 to 2012 in the Rappahannock River while the prevalence for this study was between 38% and 39.5% for the Rappahannock River. The lower disease prevalence seen in the Rappahannock during this work may be caused by differences between collection methods and/or changes within the river. Hoenig et al (2017) collected fish using pound nets in areas closer to the mouth of the Rappahannock River where it drains into the Chesapeake Bay. Differences in overall prevalence may be due to these differences in collection methods and areas fished within the river. Recaptures of severely diseased fish when using pound nets were seen at a much higher rate compared to recaptures of undiseased and mildly diseased fish when fishing pound nets (Hoenig 2017). Due to the higher recapture rate seen in higher severity fish, pound nets may have a bias towards collecting fish in higher disease severities. Along with differences in collection methods and areas fished with the Rappahannock River, localized changes may have also taken place. During the 8 years between studies, the hydrology and dominant prey species harboring mycobacteria may have changed within the Rappahannock River resulting in a decrease in disease prevalence.

Disease prevalence levels significantly differed between rivers, with the James River having a higher prevalence for most ages and year classes while the Rappahannock River had a higher number of severity 4 in some ages and year classes. With higher overall disease prevalence in the James River, this

may suggest that disease may work on a localized spatial scale, in this case, may be influenced by currently unidentified river-specific factors. The transmission of mycobacteriosis is still poorly understood but likely transmission routes include ingestion of bacteria through diet and exposure to bacteria present in environmental matrices such as water and sediment(Gauthier et al. 2003; Nenoff and Uhlemann 2006; Ross 1963; Ross and Brancato 1959; Wood and Orddal 1958). Within the Chesapeake Bay, *M. pseudoshottsii* has been isolated from water and sediment samples, and in the tissues of Atlantic menhaden (*Brevoortia tyrannus*) and Bay anchovy (*Anchoa mitchilli*), two main food sources for Bay striped bass. Currently, *M. shottsii* has only been isolated from striped bass and has not been isolated from the Bay's environment. With *M. pseudoshottsii* present in major prey species as well as throughout the Bay, this may represent a major reservoir of pathogenic mycobacteria to striped bass (Gauthier 2010). Varying prevalence levels between rivers could be caused by different factors specific to river systems. The abundance of *M. pseudoshottsii*, in prey species, and throughout the water column may differ from river to river due to environmental conditions and species present. A river system may have a higher abundance of prey species that harbor mycobacteria while another river may have fewer of these species, resulting in different exposure rates through ingestion. Differences in hydrology between rivers may also result in various levels of mycobacteria present in the environment leading to variable prevalence. If there are differences between rivers leading to increased disease prevalence in specific rivers, these differences would primarily affect younger fish. Young striped bass tend to stay within and around their natal rivers for up to 2 years and these fish mainly experience environmental factors within and around their natal rivers possibly resulting in higher disease prevalence rates in certain rivers.

Disease prevalence for fish caught using gillnets (Figure 14) and electrofishing (Figure 15) were highest for both rivers in earlier year classes. Disease prevalence decreased as year class increased for gillnet caught fish, but prevalence did not continually decrease for fish caught using electrofishing. Fish caught using electrofishing had higher than expected disease prevalence in both rivers after the 2016-year class. Additionally, all four severity categories were seen in all year classes of fish caught from the

Rappahannock River using electrofishing. All four severities were only seen in the 2015 and 2016 year classes of fish caught in the James River, severity 4 was not seen in the 2017 and 2018 year classes. The absence of severity 4 in these two-year classes is likely caused by these younger fish that have become diseased but have yet to progress to higher severities. Year class had a significant negative association with disease for prevalence and all severity categories for the year class-based logistic regression analyses conducted in this study. The higher-than-expected prevalence and break in decreasing prevalence seen in later year classes of fish caught using electrofishing suggests that disease may work on a larger spatial scale. Unusual environmental anomalies that don't take place yearly in the Bay like high sea surface temperature, abnormal river flow, and large hypoxic areas may be causing this increased prevalence in later year classes. Hypoxic events happening during the warm summer months may "squeeze" young pre-migratory fish out of cooler deeper water in their natal rivers exposing them to unfavorable water temperatures (Coutant 1985; Price 1985). Striped bass with mycobacteriosis that are regularly exposed to unfavorable water conditions and hypoxia have decreased metabolic scope (Lapointe et al. 2014). Striped bass staying within the Bay during the summer months may experience water temperatures over 30°C, which is near the thermal tolerance (Coutant 1985; Groner 2018). Exposures to these higher temperatures are believed to increase disease prevalence and decrease the relative survival of fish. Though increasing water temperature does negatively affect striped bass, *M. shottsii* and *M. pseudoshottsii* are also negatively affected by these warmer temperatures reaching 30°C. Captive striped bass injected with *M. shottsii* and *M. pseudoshottsii* saw a decrease in bacterial load within the splenic tissue with increasing temperatures (Gauthier et al. 2021). This does not mean exposure to elevated water temperatures may not affect disease. With striped bass reaching their thermal tolerance near 30°C, diseased fish may become stressed even though bacterial loads may be decreasing within their spleen. When water temperatures begin decreasing, the stress on the fish is lowered but bacterial loads may again start increasing within the fish. This exposure to unfavorable water temperatures followed by increasing bacterial loads when in cooler water may result in increased disease severity. If unfavorable abiotic conditions negatively affect fish already diseased, these same conditions may also play a role in initial infection of young fish. High

summer water temperature and hypoxia may be causing younger fish that stay in and around their natal rivers to become diseased at earlier ages. Due to differences in hydrology, these environmental events may or may not affect the James and Rappahannock Rivers similarly leading to increases in prevalence in the same or varying year classes.

The interaction between year class and being caught in the Rappahannock River compared to year class and being caught in the James River for the severity 3 and 4 models had a positive association with disease for the year class-based analyses. This differs from all other models looking at disease prevalence and severity 1 and 2. In other models, being caught in the Rappahannock River had a significant negative association or no significant association with disease. The positive association with disease may support the idea that disease works on larger spatial scales increasing disease prevalence in some year classes. Disease may constantly work on a localized spatial scale with disease affected by environmental factors specific to rivers, but regional environmental events may add to the localized factors causing variability in disease prevalence.

A comparison of dermal and visceral disease prevalence was undertaken to better understand the relationship between internal and external mycobacteriosis in Chesapeake Bay striped bass. Both *M. shottsii* and *M. pseudoshottsii* have been isolated from striped bass tissues (Rhodes 2005) but only *M. shottsii* has been isolated from skin ulcers (Rhodes 2003), thus internal and external disease may be caused by exposure to different mycobacteria. It is also possible that *M. shottsii* is undergoing adaptation to obligate pathogenicity in a vertebrate host resulting in it only being isolated from Bay striped bass (Gauthier et al. in review). Based on the subset of spleens examined most fish showing signs of external disease also had internal disease, but a small group of fish only showed external disease. Conversely, some fish had no dermal disease that showed no signs of external disease but had internal disease. The possibility that fish may only have internal or external disease helps support the idea that they are caused by different exposure events to mycobacteria leading to reinfections. Internal and external disease may

also be caused by different routes of exposure to mycobacteria resulting in some fish only having internal or external disease.

Aging fish for this study was done two different ways due to lethal sampling needed for extracting otoliths from fish. Although striped bass can be aged using scales or otoliths (Atlantic States Marine Fisheries Commission 2003; Secor et al. 1995) residual plots were created to compare the ages determined using both methods for the 652 striped bass caught using gillnets from the 2020 field season. The ages for these fish did not completely agree and each aging method had over and under aging occurring. The formation of the new annulus on the otolith and scales of striped bass happens around the time of spawning between April and June (Atlantic States Marine Fisheries Commission 2003). The discrepancies between aging methods may be caused by the structure of the scales taken from each fish. Scales can have damage, have regeneration, or were taken from the wrong place on the fish, resulting in unclear or missing rings resulting in incorrectly aging fish (Atlantic States Marine Fisheries Commission 2003). In the case of severely diseased striped bass, the use of scales may be difficult due to dermal disease damaging scales. Combining data sets that included highly diseased fish aged using scales and otoliths could have led to incorrect results. Due to possible differences with aging, the gillnet and electrofishing data sets were kept separate.

This Masters project had three main research goals; 1) to collect current mycobacteriosis prevalence and severity data for James and Rappahannock River striped bass, 2) to compare disease prevalence data between these two important rivers, and 3) to compare disease prevalence between year classes in these two rivers. Throughout the two-year sampling period, 4,252 striped bass were caught from both rivers and 3,792 males between the age of 2-8 were used in the final analysis. The low samples of females compared to males is regularly seen each year during this survey run by VIMS using gillnet and electrofishing within these two rivers. This may be due to larger numbers of males spending most of their lives within the Bay and reaching sexual maturity earlier compared to females (Kohlenstein 1981; Merriman 1941; Secor and Piccoli 2007). With males reaching maturity earlier and having a shorter

migration to spawning tributaries compared to females coming into tributaries from the ocean this may explain why the sample sizes for males are much higher than females. The three research goals for this project were completed and the results can be very beneficial to better understanding mycobacteriosis in Chesapeake Bay striped bass. This project updated prevalence data for the Rappahannock River and is the first to publish James River mycobacteriosis prevalence. The disease prevalence data was used to begin to better understand the spatial scale that mycobacteriosis may work on within the Bay. Prevalence was compared between rivers and year classes to see if disease may work on a small localized spatial scale or a larger regional spatial scale. After using statistical models to compare disease prevalence between these groups it seems disease may work on both small and large spatial scales.

FUTURE WORK

This Masters research started to collect current disease prevalence data for two Virginia spawning tributaries. The results of this project can help understand the effects mycobacteriosis has on Chesapeake Bay striped bass and the spatial scale disease may work on within the Bay. Until this study, no published data existed for the James River and there was an 8-year gap in prevalence data for the Rappahannock River. The two years of prevalence data were explored, and it appears that mycobacteriosis works yearly on a localized spatial scale and some years it also works on a regional spatial scale within major Virginia tributaries of the Chesapeake Bay. These regional events increase the disease prevalence caused by localized environmental factors within specific rivers. Though this work had interesting findings further work needs to be done to expand the findings from this project. Continued collection of mycobacteriosis prevalence data for these rivers needs to be done and used to further understand the spatial scale on which disease works. The hydrology between rivers should be compared to help explain the differences in disease prevalence for the James and Rappahannock Rivers. Higher than expected disease prevalence seen in specific year classes should also be explored and possibly linked to large environmental anomalies that may directly affect disease. Possible regional events that may affect mycobacteriosis are hypoxic events, higher sea surface temperature, or the presence of specific prey species known to harbor

mycobacteria. Direct connections between these regional events and mycobacteria would be a great addition to the knowledge of mycobacteria and its effects on Bay striped bass.

Little is known about mycobacteriosis prevalence and its effects on Atlantic stock striped bass outside the Chesapeake Bay with only several studies looking at small samples of fish from different areas along the coast (Ottinger 2007; Stine 2009). Expanding monitoring outside the Bay would be beneficial to understanding the coast-wide effect of mycobacteriosis on striped bass. Collecting and comparing disease prevalence data between fish originating from different spawning areas would be interesting to explore to see if prevalence varies between fish originating from different stocks. This type of study could be beneficial to further explore the spatial scale disease may work. Three major spawning areas contribute to the Atlantic stock and are spread across the Mid-Atlantic. These different spawning areas have variable hydrology and major commercial industry along the shorelines. With differences between spawning areas, disease prevalence may vary between these different populations of fish.

When calculating positive and negative predictive values for this study, the focus was on comparing disease prevalence data for internal and external disease for the 310 fish that had histology performed on them. These predictive values determined by disease prevalence can be applied to future work dealing with skin disease data. The PPV can estimate how many striped bass showing external disease may also have internal disease, while the NPV can estimate how many fish may have internal disease but show no signs of external disease. Further work comparing internal and external disease severities was explored but the sample sizes for fish in the higher disease severity categories were low compared to severity 1 and overall disease prevalence. Comparing different skin disease severities to internal disease resulted in some interesting results. Negative predictive values do not change between disease prevalence and different severities due to these fish having no external disease present. The positive predictive values did vary when comparing disease prevalence and the 3 severity categories. PPV was very similar when using disease prevalence and severity 1 data, severity 2 had the highest PPV, and severity 3 and 4 group had the lowest PPV (Table 9). The differences in PPV between disease severities

are interesting and should be further explored when larger data sets are available for fish grouped into higher disease severities. Currently, limited information is known about the relationship between internal and external disease in striped bass and this is the first published work calculating predictive values comparing skin disease prevalence and severity to internal disease.

TABLE 9. Predictive values were estimated by comparing skin disease severity categories used in the age-based and year-class based models to fish with internal disease determined by the presence of granulomas in the fish's spleen tissue.

Severity 1

Positive Predictive Value (PPV)	Negative Predictive Value (NPV)	Sensitivity	Specificity
0.770	0.389	0.516	0.667

Severity 2

Positive Predictive Value (PPV)	Negative Predictive Value (NPV)	Sensitivity	Specificity
0.828	0.389	0.209	0.921

Severity 3 and 4 grouped

Positive Predictive Value (PPV)	Negative Predictive Value (NPV)	Sensitivity	Specificity
0.697	0.389	0.704	0.382

Comparing disease prevalence for fish caught at different times during the spring surveying period would be useful to see if disease prevalence changes at different points during the spawning season. Disease prevalence should be compared between fish caught in the same rivers but caught at different weeks during the survey. Prevalence should also be compared between fish caught from different rivers during the same weeks of the survey. Comparing prevalence of fish caught in the same river but at different times could show if the disease has any effect on spawning habits. If there are differences between prevalence rates during the spawning season within the same river it would also be interesting to see if similar trends take place between rivers. Comparing prevalence between these groups would be beneficial to understand if diseased fish have differences in their spawning habits compared to undiseased fish.

During the first year of this project, disease prevalence was compared between fish caught through different collection methods. This comparison was done by combining all fish caught through

electrofishing and the 652 gillnet caught fish that were aged using otoliths and scales, scales were used for age for all fish in this model. This model was not explored after the 2021 collection season due to low numbers of gillnet caught fish aged with scales and the data sets being kept separate for the rest of the project. The collection-based model included river, age, and collection method as independent variables [Model (M) 4]. The one year of data run through this model did suggest being caught through gillnets did have a significant positive association with disease compared to being caught through electrofishing. This significant positive association was interesting to see and further development and use of this model may be beneficial to see if disease does differ between collection methods. If further use of this model showed there is a tendency of a certain collection method to collect fish in higher disease severity categories, this may suggest there are behavioral differences between different disease severities. Exploring the idea that disease does impact striped bass behavior would be important to understand the impact mycobacteriosis has on Bay striped bass.

$$\text{glm(Prevalence} \sim (\text{River} + \text{Age} + \text{Collection Method})\text{\^{}}2, \text{family} = \text{binomial} \qquad \text{(M. 4)}$$

The continued collection of mycobacteriosis prevalence data can become very useful in future stock assessment models created by ASMFC. Currently, mycobacteriosis is grouped into natural mortality which includes all diseases affecting striped bass, predation, and fish dying out of the population due to old age. With the high disease prevalence documented in this study as well as in previous studies (Gauthier 2008; Hoenig 2017), a mycobacteriosis-specific portion to stock assessment models would be beneficial to striped bass health. Given the high prevalence of mycobacteriosis in Chesapeake Bay striped bass, and its known lethal nature, it is likely that this disease plays a large role in natural mortality that is not properly estimated in current models. Proper stock estimates can only be achieved with further collections of disease prevalence data from within and outside the Bay and a mycobacteriosis-specific portion to stock assessment models is created.

This research was the third study looking at disease prevalence within the Bay with data collected from two major spawning tributaries within Virginia. This project was the first to publish prevalence data for the James River and updated the prevalence data for the Rappahannock River after 8 years without monitoring. This study was the first to collect prevalence data from the upper portions of these rivers closer to areas used for spawning. Regardless of collection method or area within the Bay high disease prevalence has been reported in all studies (Gauthier 2008; Hoenig 2017), but prevalence seems to vary between striped bass caught in different areas of the Bay using different collection methods. This study was a good start to begin collecting disease prevalence data and using this data to try to understand the spatial scale disease works on within the Bay. The study design for this project can easily be applied to other studies taking place within and outside the Chesapeake Bay increasing available prevalence data. Further studies need to be done to add to these two years of prevalence data and to explore possible environmental events that directly impact mycobacteriosis. Further comparisons between disease prevalence should be done to see if different collection methods have a bias of collecting higher or lower disease severities. Comparing disease prevalence to collection methods could show diseased fish change their habits compared to undiseased fish resulting in higher catch rates. Applying predictive values determined from this study to future work looking exclusively at skin disease prevalence should be done to estimate how many fish are diseased but show no signs of external disease. Further comparisons should also be done comparing different skin disease severities to the presence of internal disease once larger samples of highly diseased fish have been collected. Lastly, continued collections of disease prevalence data could also have direct effects on striped bass stock assessment models. With high disease prevalence documented within the Bay and this being a chronic disease, a portion of striped bass are likely taken out of the population each year due to mycobacteriosis. The current grouping of mycobacteriosis into natural mortality in models may not be an appropriate way to estimate the impact of this disease on striped bass stocks. These two years of work were a good starting point for researching mycobacteriosis and its effects on Chesapeake Bay striped bass, but further work should be undertaken to expand on the findings of this project.

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