2001

Mycobacteriosis in Striped Bass, Morone saxatilis, from Virginia Waters of Chesapeake Bay

Jennifer L. Cardinal
College of William and Mary - Virginia Institute of Marine Science

Mycobacteriosis in Striped Bass, *Morone saxatilis,* from Virginia Waters of Chesapeake Bay

---

A Thesis
Presented to
The Faculty of the School of Marine Science
The College of William & Mary

In Partial Fulfillment
Of the Requirements for the Degree of
Master of Science

---

by
Jennifer L. Cardinal
2001

APPROVAL SHEET

This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Science

Jennifer L. Cardinal

Approved June 2001

Wolfgang K. Vogelbein, Ph.D.
committee chairman/advisor

John M. Hoenig, Ph.D.

John E. Olney, Ph.D.

Jeffrey D. Shields, Ph.D.

Michael A. Unger, Ph.D.

Table of Contents

Acknowledgements

I would like to express my thanks to my major advisor, Wolfgang Vogelbein, and my committee members, Michael Unger, John Olney, John Hoenig, and Jeffrey Shields for helping me through my Master's project by providing scientific expertise and academic advice when needed.

I also want to acknowledge David Zwerner for all the long hours, hard work, and knowledge he put forth on this project. I will always be in his debt. Pat Blake helped with a great amount of histology, which is very much appreciated. Drew Allen, Patrice Mason, Vince Lovko, and Chris Hagar helped with many hours of field work, such as fishing pound nets and working up the striped bass in the lab.

Spring collections were provided by John Olney's crew from their striped bass monitoring studies. Phil Sadler provided ages of these striped bass. I couldn't have gotten a spring sample without their help. David Bradley, William Dean, Russell Gaskins, and Donny Green supplied me with fish from their pound nets making the rest of my project possible. Jim Owens is to thank for making contacts with these people and for picking up fish on days I could not.

Rita Crockett and Lisa Calvo deserve thanks for allowing me to work on my thesis when needed and for putting up with me while working full time and trying to finish my Master's.

Lastly, I would like to thank the many great friends I have made at VIMS that have helped me get through graduate school with fewer scars than I would have received without them. They include Sara Mirabilio, Vicki Pilon, Tripp Macdonald, Christine Conrad, Vince Lovko, Erin Burge, Drew Allen, Beth Watkins, Chris Hagar, John Foster, and Landon Ward.

Funding

Funding for this project was provided by the Recreational Fishing Advisory Board of the Virginia Marine Resources Commission through grant RF-98-2.

List of Tables

Table **Page**

List of Figures

Figure **Page**

Abstract

After a period of stock decline, striped bass, *Morone saxatilis*, made a strong recovery in Chesapeake Bay in the mid 1990's. Recently however, there have been numerous reports of skinny, "starving" bass, many of which exhibited severe ulcerative dermatitis caused by mycobacterial infections. Mycobacteriosis in fish is a chronic, systemic disease that results in loss of body condition and ultimately leads to death. In striped bass from Chesapeake Bay, many underlying factors have been postulated to modulate this disease including low dissolved oxygen, temperature constraints, overcrowding, and immunosuppression. However, starvation due to decline of forage food has gained favor in Maryland as a likely cause predisposing fish to this disease. The objective of this study was to clarify the spatial and temporal distribution of mycobacteriosis in striped bass in Virginia waters and to examine the relationship between this bacterial disease and the generally poor body condition of striped bass. Splenic and dermal lesion samples from 1899 fish from the Potomac, Rappahannock, and York Rivers were obtained from spring, 1998, through fall, 1999. Mycobacterial infections in the spleen and skin were verified histologically. Prevalence of mycobacteriosis varied temporally and between sexes, with highest prevalences generally observed in males during fall. Prevalences of splenic infections were much higher than dermal infections. Splenic infections ranged from 31.5% in the Rappahannock River in summer, 1999, to 62.7% in the York River in fall, 1999. The prevalence of dermal infections ranged from 7.5% in the Rappahannock River in summer, 1998, to 28.8% in the York River in fall, 1999. Little if any significant variance in prevalence occurred spatially among rivers. Although this study did not determine which environmental factors played a role in mycobacteriosis in striped bass, an association between condition index and mycobacteriosis suggested that adverse environmental factors along with mycobacteriosis may lead to significantly lower body condition in infected fish. The highest prevalences of infection during the fall may be a result of prolonged environmental stress beginning in early July, which also causes body condition to drop from a high in spring, to a low in summer and fall. Heavily infected fish have lower condition than uninfected fish probably due to the wasting nature of the disease.

Mycobacteriosis in Striped Bass, *Morone saxatilis,* from Virginia Waters of Chesapeake Bay

Introduction

Striped bass, *Morone saxatilis*, are anadromous fish that fill an important niche in the food web of estuarine systems. They are one of three dominant piscivores in the Chesapeake Bay (Hartman and Brandt 1995) that feed on menhaden, anchovies, silversides, and many other fishes. Distinct physical characteristics of striped bass include seven to eight dark, narrow, lateral stripes running along a compressed, elongate body with separate dorsal fins (Murdy *et al.* 1997). Broken or irregular stripes may indicate hybridization with the white bass, *Morone chrysops* (Manooch 1991).

Natural populations exist along the Atlantic coast from the St. Lawrence River, Canada, to the St. Johns River, Florida. Introduced populations exist in the southeastern United States, California, Soviet Union, and South Africa (Manooch 1991). There are four main stocks of striped bass in the U.S.: the Atlantic coastal migratory stock, the North Carolina stock, the south Atlantic stock, and the West Coast stock (Austin 1980). Striped Bass in Chesapeake Bay produce the majority of the largest stock, the Atlantic coastal migratory stock (Austin 1980). Depending on class size and sex, the young remain in the bay until they are two to six years old (Austin 1980; Kohlenstein1981). Females begin migrating to sea at age two or three while males migrate at an older age. Since males are heavily fished before they reach their migratory age, females generally make up most of the migratory stock (Kohlenstein 1981). Migratory adults from the Atlantic coastal stock are found from Virginia to Nova Scotia from spring until fall when they begin migrating southward to warmer water. Many overwinter in the deeper portions of Chesapeake Bay (Kohlenstein 1981). Spawning begins around April when the water temperature reaches 8°C (Austin 1980). Striped bass populations in the Bay have been abundant except for the 1970s and 1980s. However, implementation of federal and state laws in combination with good spawning success during the 1990s have replenished their numbers to a high level (Field 1997).

The striped bass is one of the most sought after fishes in Chesapeake Bay. During the 1920s they ranked fifth in landings and value from Virginia waters, and fourth and second in landings and value respectively, from Maryland waters. Pound nets were the most common form of capture, but gill nets, haul seines, purse seines, fyke nets, and other methods also were used (Hildebrand 1928). Although striped bass are now abundant, their numbers were low during the 1970s and 1980s. This resulted in the enactment of regulations by the Atlantic States Marine Fisheries Commission (ASMFC) and state governments to restrict commercial and recreational striped bass landings (ASMFC 1981). In the late 1980s, fish exhibited higher spawning success and a limited fishery was re-opened in 1990 (Field 1997). In 1995, the ASMFC declared Chesapeake Bay stocks to have reached benchmark levels, and states adopted an amendment to the fishery management plan that increased target fishing mortality.

Recently, a disease called mycobacteriosis has become a potential threat to the population of striped bass from Chesapeake Bay. *Mycobacterium* spp., the causative agents of mycobacteriosis, are Gram positive, acid-fast, nonmotile, non-spore forming, rod-shaped bacteria (Fryer and Rohovec 1993). They are straight or curved rods, 0.2-0.6 x 1.0-10.0 μm, with branched or filamentous growth. *Mycobacterium* spp. stain inconsistently with the Gram stain, but they can be characterized to genus by the Ziehl-

Neelsen staining method, which exploits the bacteria's acid-fast property. Acid-fast bacteria have a waxy substance in their cell walls, which allows the carbo-fuchsin stain to resist decolorization by acidified alcohol in the Ziehl-Neelsen staining method. Only one other genus of acid-fast bacteria, *Nocardia* spp., stains with Ziehl-Neelsen, and it exhibits a different microscopic morphology (Frerichs 1993).

The genus *Mycobacterium* was established in 1896 by Lehman and Neumann (Dalsgaard *et al.* 1992) and presently contains over 50 species mostly occurring in marshes, wet soil, and water (Jenkins *et al.* 1992). Many bacteria in this genus are termed "atypical" mycobacteria because they are ubiquitous, opportunistic pathogens living in the environment, unlike the well-known human pathogens *M. tuberculosis* and *M. leprae*, which are considered "typical". Atypical mycobacteria can survive a wide range of pH and temperature conditions including chlorinated water (George *et al.* 1980; Brevrey *et al.* 1982). However, Brooks *et al.* (1984) found mycobacteria to thrive best in acidic conditions. Kirschner *et al.* (1992) found the MAIS group (*M. avium, M. intracellulare, and M. scrofulaceum*) to be more abundant in waters, soils, and aerosols of the acidic brown water swamps of the southeastern US coastal plains suggesting that these habitats could be a major source of these bacteria. Members of the MAIS group are considered potential human pathogens and have been isolated from various animals including fish (Landsell *et al.* 1993). Mycobacteria can live in water without an animal host and can multiply even in nutrient-free water (Bolan *et al.* 1985). This may explain why mycobacteria have been isolated from tap water, bottled water, and hospital water systems (Bullin *et al.* 1970; Papapetropoulou *et al.* 1997; Du Moulin *et al.* 1988). Water seems to serve as a natural habitat and a means of transmission (Falkinham *et al.* 1980), but mycobacteria have been isolated from soil and other environments (Brooks *et al.* 1984).

Atypical mycobacteria most commonly affect poikilothermic animals because their body temperatures fall into the bacteria's optimal temperature range for growth, which is lower than that for typical mycobacteria. Clark and Shepard (1963) produced systemic mycobacteriosis in 50 different species of poikilotherms including reptiles, amphibians, and fishes by intraperitoneal inoculations of *Mycobacterium marinum*. Only non-systemic disease (e.g. localized in the extremities), however, was produced in mammals (mice, opossums, and bats) injected with the bacterium. Temperature was thus found to be an important factor affecting the distribution of mycobacteriosis among species and within individual organisms. Because of the temperature barrier and the opportunistic behavior of atypical mycobacteria, humans are generally susceptible to infections only in their extremities unless immunocompromised in some respect (Jenkins 1991). Humans should use caution when exposed to mycobacteria because serious skin lesions may result on extremities. Swimming pools, ocean beaches, rivers, lakes, natural bathing pools, old wells, fish tanks, and poikilothermic water animals are all sources of mycobacterial infections to humans (Huminer *et al.* 1986).

Species of Mycobacteria were first described in fish by Bataillon *et al.* (1897). Aronson (1926) described the first well-established species, *M. marinum,* from fish in the Philadelphia Aquarium. Mycobacteria are now known to affect over 150 species of marine and freshwater fishes (Nigrelli and Vogel, 1963). Many of the early reports of mycobacteriosis are attributed to *M. marinum,* the most common isolate from marine and freshwater fishes (Frerichs 1993). Ross and Brancato (1959) identified *M. fortuitum* from neon tetra, *Hyphessobrycon innesi*. Earp *et al.* (1953) described what is presently known as *M. chelonae* from Chinook salmon, but it did not receive its present name until later (Wayne and Kubica 1986). Recently, Landsell *et al.* (1993) isolated *M. simiae, M. scrofulaceum, M. marinum, M. chelonae, and M. fortuitum* from marine fishes caught in the wild including striped bass and freshwater ornamental species. Tortoli *et al.* (1996) found *M. poriferae* in snakehead, *Channa striatus*, and Bozzetta *et al.* (1995) isolated *M. gordonae* from tropical fish. *M. abscessus* (formerly *M. chelonae* subsp. *abscessus*) was recently documented from Japanese medaka (Teska *et al.* 1997).

Conventional methods of isolation, culture, and identification of mycobacteria are labor intensive and time consuming. Isolation may be done on Dorset-egg, Lowenstein-Jensen, Petragnani, or Middlebrook 7H10 media (Frerichs 1993). Culture of mycobacteria usually is done between 20^{O} to 30^{O}C for 2-30 days (Frerichs 1993). Identification of mycobacteria must account for such features as growth rate, pigmentation, colony morphology, microscopic form, and specific biochemical tests (Witebsky *et al.* 1996). Slow growth of mycobacterial colonies makes recovery difficult when samples are contaminated by an abundance of other rapidly growing microbes (Iivanainen 1995), but methods of decontaminating tissues have been developed (Frerichs 1993). More rapid identification methods using molecular probes also are being developed. Adams *et al.* (1996) have developed a somewhat reliable ELISA, a rapid screening test, for *M. marinum, M. fortuitum,* and *M. chelonei* using monoclonal antibodies produced to mycobacteria from Siamese fightingfish, *Betta splendens*. Gomez *et al.* (1996) successfully used immunohistochemical techniques to specifically label mycobacterial antigens in fish tissue. Talaat *et al.* (1997) developed a PCR method for rapid identification of fish infected with *M. marinum*, *M. fortuitum*, and *M. chelonae*.

Since atypical mycobacteria are opportunistic pathogens, mycobacteriosis is not common in fishes unless unfavorable, stressful conditions exist in the environment (Fryer and Rohovec 1993). Fish contract mycobacteriosis when they are exposed to mycobacteria in the environment at the same time they are stressed or have some injury allowing a portal of entry (Jenkins *et al.* 1992). Stress may suppress the defense mechanisms of a fish allowing the opportunistic bacteria to multiply and become pathogenic to fish (Anderson 1990). For example, public aquaria have difficulty with this disease due to sustained captivity stress.

There is no known effective treatment in fish, and control of this disease depends on good sanitation practices and maintenance of good water quality. Since infected fish can shed mycobacteria bacilli into the water (Clark and Shepard 1963) and these bacteria can live a heterotrophic existence for many years (Belas *et al.* 1995), the potential exists for adverse economic impacts on aquaria, culture operations, and possibly even commercial fisheries. Outbreaks of mycobacteriosis in farmed chevron snakehead, *Channa striata*, in Thailand have caused mortalities of 20% (Chinabut *et al.* 1990). These investigators recommended that affected fish be destroyed and that facilities be disinfected and cleaned thoroughly. In addition, parasites, which can be problematic in aquaculture operations, may infect a fish and provide a portal of entry for mycobacteria.

The host immune response to mycobacteriosis can alter immune responses to other foreign bodies such as parasites. Pyecroft (1994) described the effects of mycobacteriosis on parasite load in the Crimson-spotted rainbow fish, *Melanotaenia* *duboulayi*. The cellular inflammatory response to mycobacterial infections resulted in a lowered immune response that has compromised and may have led to higher levels of parasitism. Hatai and Lawhavinit (1993) also found an example of mycobacteriosis possibly leading to other infection. They attribute mycobacterium infections in pejerrey to be a likely initiating factor for saprolegniasis, a fungal disease.

Pollution is an example of an environmental stressor that may act in a wild fish population to contribute to mycobacteriosis. Dalsgaard *et al.* (1992) found a possible correlation between mycobacteriosis prevalences in cod and pollution. Cod were caught in Danish coastal water pond nets over an eight-year period and examined for internal granulomatous nodules. The waters were contaminated with sugar, cellulose waste, and municipal sewage waste. Over the eight-year period, the prevalence of nodules in cod dropped from 20% to 1-3%. The authors stated no explanation for this decrease, except an improvement of sewage treatment at sampling sites over the sampling period.

In fish, mycobacteriosis is a chronic disease that can exhibit internal and external clinical signs (Fryer and Rohovec 1993). External lesions may be manifestations of a heavy internal infection (Pieper *et al.* 1999), but such may not be the case. Majeed and Gopinath (1983) found no macroscopic lesions in the viscera of carp with heavy skin ulceration caused by mycobacteria. Livers, spleens, and kidneys stained with Ziehl-Neelsen showed single macrophages containing intracytoplasmic acid-fast bacilli, i.e. mycobacteria. Skin lesions on carp were unusual in that they lacked well-formed granulomas, but inflammatory cells, mainly macrophages, were present therein. A thickened epidermis was observed, and the inflammatory response occasionally extended deep into the muscle. Giavenni *et al.* (1980) attribute differences in pathogenicity to the different species of mycobacteria similar to what has been reported in the literature. Such infections can be asymptomatic for long periods (Colorni *et al.* 1993). External clinical signs vary among fish species and include listlessness, loss of appetite, swollen abdomen, granulomatous lesions (Lansdell *et al.* 1993), loss of body condition, lethargy (Abernethy *et al.* 1978), stunted growth (Colorni *et al.* 1993), emaciation, skin lesions with ulcerations, exophthalmia (Bragg *et al.* 1990), loss of scales, severe keratitis, cataract (Chinabut *et al.* 1990), fin necrosis, dyspnea, dark skin coloration (Giavenni *et al.* 1980), melanotic foci in the skin resulting in pigmented lesions, unpigmented lesions (Noga *et al.* 1990), and body deformations (Gomez *et al.* 1993). Gross internal pathology varies, too, but is characterized mainly by nodular granulomatous lesions or necrosis of body organs (Fryer and Rohovec 1993; Dulin 1976; Landsell *et al.* 1993). Nodules without mycobacteria have been reported in fish with these granulomatous lesions (Hastings *et al.* 1982; Gomez *et al.* 1993).

It is important to note that mycobacteria may be present even if there is an absence of acid-fast bacteria in histologic sections (Van Duijn 1981). The inability to consistently detect acid-fast bacteria in granulomas may be due to some mycobacteria being poorly acid fast unless pretreated with oxidants (Harada 1977). Complete bacteriological investigation may be needed, and even then mycobacteria may be difficult to isolate.

All major organs may be affected by mycobacteriosis (Hedrick *et al.* 1987). The most commonly affected organs in teleosts include the kidney, spleen, liver, heart, and peritoneal serosa (Agius 1985). However, the spleen is often the most consistently affected internal organ (Colorni *et al.* 1993). Microscopic pathology is characterized by the presence of granulomatous inflammation (Lansdell *et al.* 1993). Interestingly, salmonid fishes appear to lack this typical inflammatory response (Parisot and Wood 1960). Lesions may or may not be encapsulated by fibrous connective tissue as seen in some tropical fish species (Abernethy *et al.* 1978; Nigrelli and Vogel 1963). Although reports of multi-nucleated giant cells are rare in mycobacteriosis in fish, they have been reported in white cloud mountain minnows (Gomez 1998) and plaice (Timur 1977). Granulomas caused by mycobacteria in fishes are composed of tightly apposed macrophages, often with an epithelioid appearance, containing mycobacteria (Hatai and Lawhavinit 1993). The younger granulomas usually consist of macrophages with an epithelioid appearance, while older granulomas consist of centers of necrotic cellular debris surrounded by compact concentric rings of flattened cells without an epithelioid appearance (Sakanari *et al.* 1983). Mycobacteria are considered intracellular parasites since they survive and live inside macrophages (Armstrong and Hart 1971).

Different studies have shown that transmission and epizootiology vary among fish species. Conroy (1966) has documented vertical (ovarian) and horizontal (shedding of bacilli into water) transmission of this disease. Fish in aquaculture facilities have acquired infections from contaminated trash fish used for food suggesting entry through the gastro-intestinal tract (Wood and Ordal 1958; Ross 1970). Bragg *et al.* (1990) suggest the severity and occurrence of this disease is probably related to age, nutritional status, oxygen levels, and stocking density. Abernethy *et al.* (1978) and Hastings *et al.* (1982) found evidence that disease intensity and severity increased with age. MacKenzie (1988) indicated that mycobacteriosis prevalence in Northeast Atlantic mackerel increased with fish age, did not differ between sexes, and somewhat varied with season. The mean condition factor also was greater for uninfected than infected fish. He attributed regional variations in prevalences to optimal temperature ranges for mycobacterial growth or varying susceptibility of genetically different stocks.

Mycobacteriosis in striped bass has been reported in public aquaria from the East Coast (Aronson 1926; Winsor 1946; Nigrelli and Vogel 1963). Hedrick *et al.* (1987) reported mycobacteriosis in striped bass reared in West Coast hatcheries. No reports have been found of mycobacteriosis in striped bass in East Coast hatcheries. The first report of this disease in wild striped bass came in 1983 from 192 striped bass from four locations in northern California and Coos Bay, Oregon (Sakanari *et al.* 1983). The prevalences of internal lesions ranged from 25%-68% at these five sites. No external lesions were noted, and internal lesions were histologically similar to those described in salmon (Parisot and Wood 1960) but differed from those in tropical fishes described by Amlacher (1970) and Beckwith and Malsberger (1980). Early granulomas in these striped bass exhibited necrotic centers with acid-fast bacteria surrounded by an epithelioid cell layer and a collagenous connective tissue capsule while older granulomas had no epithelioid cell layer. Sakanari *et al.* (1983) attributed the annual striped bass die-off reported by Kohlhorst (1975) during the warm summer months to possible mycobacteriosis since the warmer water temperatures could support mycobacterial growth at the time (Clark and Shepard 1963).

Since 1997, numerous cases of striped bass from Chesapeake Bay exhibiting mild to severe ulcerative dermatitis have been submitted to the Aquatic Animal Disease Diagnostic Lab (AADDL) at VIMS and to the Maryland Department of Natural Resources (MD DNR). Fish examined at VIMS consistently have had *Mycobacterium* spp. associated with the skin lesions. The impact of this bacterial disease on striped bass from Chesapeake Bay, however, is not yet understood. Many environmental stressors have been postulated which may influence disease development. These include overcrowding, dissolved oxygen, temperature constraints, and immunosuppression. The decline of forage food has gained favor as a likely cause predisposing striped bass to this disease. At a workshop during July 1998 entitled "Trophic Changes in Chesapeake Bay Open Water Habitat: a Workshop to Evaluate and Interpret Recent Trends", Drs. E. May and S. Jordan from the MD DNR attributed ulcerative dermatitis in striped bass to immunosuppression associated with starvation. Support for this hypothesis was based on the increasing abundance of striped bass in the bay, with and without clinical signs of this disease, lacking abdominal fat and having low somatic condition indices. Mycobacteriosis, however, is a chronic, wasting disease, which may cause the fish to exhibit emaciation, loss of abdominal fat, and low condition indexes without involving starvation. Clearly, the environmental factors that potentially influence or modulate disease development in striped bass are not well understood. Because of the immense popularity of this species with recreational anglers and its great importance to the commercial fishery in Virginia and elsewhere, it is critical that this disease and its potential impacts on striped bass stocks be more fully understood.

Project Goals and Objectives

The goals of this study were to understand the epizootiology of mycobacteriosis in striped bass from Chesapeake Bay by 1) determining the temporal and spatial distribution of mycobacteriosis in striped bass from Virginia waters of the Chesapeake Bay, 2) investigating the association between splenic mycobacteriosis and fish age, 3) examining the association between splenic and dermal mycobacteriosis, and 4) determining the relationship between this bacterial disease and the generally poor body condition of striped bass from the Chesapeake Bay using the condition factor of the fish and histopathological disease diagnosis. These goals were accomplished by investigating the following objectives:

1) seasonal prevalence of mycobacteriosis in the Rappahannock River
2) gender differences in disease prevalence
3) spatial variation in prevalence of mycobacteriosis
4) effect of age on disease prevalence (using length as estimate of age)
5) association between dermal and splenic mycobacteriosis
6) association between mycobacteriosis and fish condition

Materials and Methods

Striped bass were collected from pound nets in the Rappahannock, Potomac, and York Rivers during spring, summer, and fall. Age, sex, and measurements for condition factor were taken from each fish. All gross dermal and spleen lesions were histopathologically verified for the presence of mycobacteriosis.

Sampling protocol:

Striped bass were collected from commercial pound nets in the Rappahannock, Potomac, and York Rivers. Sample location, therefore, was dependent on net locations selected by the waterman. The Rappahannock River samples were obtained from nets at the mouth and up river, past Tappahannock, VA. The Potomac River samples were taken from nets on the VA and MD sides near the mouth of the river. The York River samples were obtained from nets at the mouth and one half mile upriver (Figure 1). Striped bass were placed on ice in coolers and transported to VIMS for necropsy. If the net had less than 200 striped bass, all fish were saved for necropsy. If more than 200 striped bass were present, the fish were selected haphazardly by standing in one spot on the boat and taking the nearest fish until the quota of 200 fish was met. During spring, striped bass from the Rappahannock River were collected in a similar manner from pound nets by fisheries staff and brought to VIMS. Prevalence data are potentially biased due to haphazard sampling when the pound nets contained over 200 fish. The variability in the geographic locations of pound nets also may have introduced some unavoidable spatial bias.

Necropsy Protocol

All striped bass were examined and assigned unique ID numbers within 3 days from date of capture. Total length (TL), fork length (FL), standard length (SL), total weight (WT1), gutted weight (WT2), and sex then were recorded. The fish were cut open from the anus to the gill region. Macroscopic lesions on the skin and spleen (mycobacterial granulomas) were noted: the skin was scored as positive or negative, splenic (spleen) lesions were scored from 0-3 (0 = no lesions, 1 = 1-2 lesions, 2 = 3-4 lesions, 3 = 5+ lesions). The spleen was chosen because it often is reported as the most consistently affected visceral organ (Colorni *et al.* 1993). Spleen and dermal samples were fixed in Bouin's fluid for identification of mycobacterial infections histologically. Gonad samples were fixed in Bouin's fluid to determine sex in immature fish. Liver weight was taken to calculate a hepatosomatic index for potential analyses.

Histology Protocol:

Representative samples of skin and spleen were trimmed with a single edged razor blade and placed in tissue cassettes, fixed in Bouin's fluid for 48 hours, and rinsed in running tap water overnight. Skin samples were decalcified (de-ionized water, calcium citrate, and formic acid) overnight before the water rinse. All tissues were placed in 50% ethanol/lithium carbonate for 4-6 hours to remove soluble picrates, followed by dehydration in 50% ethanol for 1 hour, 50% ethanol for 1 hour, 70% ethanol for 1 hour, and held in 70% ethanol until embeddment. All tissues were placed in 95% ethanol for 15-30 minutes and placed in a tissue processor (Shandon Hypercenter) for further dehydration and paraffin infiltration. The tissues were embedded in TissuePrep paraffin and sectioned at 5 μm on an Olympus or American Optical rotary microtome. Tissue ribbons were floated in a warm water bath, mounted on slides, and warmed in an oven at 41^{O} C overnight. The slides were rehydrated, stained with Harris hematoxylin and eosin (H&E) (Luna 1968), dehydrated, and mounted for microscopic examination. To eliminate bias, spleen and skin sections were read blind.

Data Analysis:

Comparisons of disease prevalences for seasons (spring, summer, and fall), sexes, and rivers (Rappahannock, Potomac, and York) were made using r x c contingency tables analyzed by Mantel-Haenszel chi-square using the SAS system (Stokes 1995) and G_H tests (Sokal and Rohlf 1981).

R x c contingency tables, chi-square analyses, and G_H tests also were used to investigate the association between mycobacteriosis and age and the association between splenic and dermal mycobacteriosis.

Condition factors were calculated by the formula (gutted weight/length^3)*10^6. Fulton's condition factor was chosen because of its ease and simplicity in obtaining a number indicative of the nutritional state and nutritional history of a fish (Pederson and Jobling 1989). However, this formula assumes isometric growth: growth with unchanged body proportions and specific gravity over time (Bolger and Connolly 1989). Lambert and Dutil (1997) showed that this assumption is met if the condition factors do not correlate with fish lengths. Condition factors for fish infected with *Mycobacterium* spp. versus uninfected fish were examined using multiple factor regressions (Zar 1996).

Results

Mycobacterium Identification

Dermal lesions appeared as coalescing or multifocal, and some had a gritty texture (Figure 2). Splenic lesions consisted of white nodules dispersed throughout the spleen (Figure 3). Figure 4 illustrates a histologic section of spleen stained with H&E exhibiting a granuloma and the corresponding section shown at a higher magnification and stained with the Ziehl-Neelsen method for acid-fast bacteria. Note the reddish colored acid-fast mycobacterial rods. Figure 5 is an example of mycobacterial granulomas in a histologic section of skin stained with H&E. The disease diagnostic center at the Virginia Institute of Marine Science found 91% of striped bass with splenic granulomas and 94% with dermal lesions to be infected with mycobacteria using the Ziehl-Neelsen stain; therefore, the presence of granulomas was considered diagnostic for mycobacteriosis in this study. Granulomas associated with other recognizable parasites were omitted from the analysis (Figure 6).

The infection intensity of mycobacteriosis in the spleen was determined for each fish by estimating the abundance of granulomas in the spleen with 0 = no infection (0 granulomas in 40X field), 1 = lightly infected (1-2 granulomas in 40X field), 2 = moderately infected (3-4 granulomas in 40X field), and 3 = heavily infected (>5 granulomas in 40X field). Figure 7 shows four spleen sections displaying the intensity range. The 0.5 splenic intensity category was created for samples with 0 intensity in the

40X random field but had a positive overall reading. The entire section of spleen was examined microscopically. The prevalence of dermal lesions was histologically determined by examining the entire skin section for the occurrence of granulomatous inflammation.

Collection

A total of 1899 striped bass was collected from summer, 1998, through fall, 1999, in the Rappahannock, Potomac, and York Rivers. A total of 1133 fish from the Rappahannock River, 546 fish from the Potomac River, and 220 fish from the York River were collected (Table 1). The number of females collected during this study was much lower than that of males for all time points and rivers (Table 2). Thus, most analyses were done on data from male fish unless otherwise stated.

Seasonal Prevalence of Mycobacteriosis in the Rappahannock River

The prevalences of dermal and splenic mycobacteriosis showed no significant differences between 1998 and 1999 summer samples (chi-square, df = 1, $p_{splenic} = 0.274$, $p_{dermal} = 0.956$). Similarly, there were no differences in mycobacteriosis prevalence in the 1998 and 1999 fall samples (chi-square, df = 1, $p_{splenic} = 0.809$, $p_{dermal} = 0.771$). Consequently, like seasons were pooled for subsequent analyses.

The prevalence of disease showed significant seasonal differences for fish from the Rappahannock River (chi-square, df = 1, $p_{splenic} = 0.001$, $p_{dermal} = 0.001$). Only fish from the Rappahannock River were used in this analysis because it was the only river with a spring sample. The highest prevalences of both the splenic and dermal infections occurred during fall (Figure 8). Prevalence of splenic disease in summer, however, was found to vary significantly from spring and fall ($G_H = 10$), but prevalence of splenic disease did not differ between spring and fall ($G_H = 4$, $X^2_{(0.05)(2)} = 5.991$). The prevalence of dermal mycobacteriosis was significantly different among the three seasons ($G_H = 6{,}10$, $X^2_{(0.05)(2)} = 5.991$) (Figure 8). The seasonality of infection also was apparent when sexes were analyzed separately (chi-square, df = 1, $p_{splenic,\ female} = 0.002$, $p_{splenic,\ male} = 0.001$, $p_{dermal,\ male} = 0.002$) (Table 2). Females with dermal infections were omitted from the analysis due to inadequate sample size.

Gender Differences in Disease Prevalence

Prevalence of splenic mycobacteriosis was significantly higher in males than females (chi-square, df = 1, $p < 0.025$) except for the spring sample of fish from the Rappahannock (chi-square, df = 1, $p = 0.636$) (Figure 9). The York River samples were inadequate in sample size, with splenic infection prevalence in females during the fall (80%) higher than that in males (61%) probably being spurious (Table 2).

Prevalence of dermal mycobacteriosis was significantly higher in males than females (chi-square, df = 1, $p < 0.008$) for samples with adequate size (Figure 10). Spring samples from the Rappahannock, Potomac, and all of the York Rivers could not be used in the analysis due to the low number of females obtained.

Spatial Variation in Prevalence of Mycobacteriosis

By season, location (Rappahannock River, Potomac River, and York River) had no significant effect on prevalence of splenic mycobacteriosis in male striped bass (chi-square, df = 1, $p > 0.293$) (Figure 11). Female fish and all fish from the spring samples were omitted from these analyses due to low sample sizes. Similarly, the prevalence of dermal mycobacteriosis was not significantly different between locations in summer (chi-square, df = 1, $p = 0.951$); however, a subtle but significant effect was observed in the fall samples (chi-square, df = 1, p = 0.046), with male bass from the York River exhibiting a higher prevalence of dermal lesions than fish from the Rappahannock and Potomac Rivers (Figure 11).

Effect of Age on Disease Prevalence

Using fish scales, Phil Sadler of the Anadromous Fishes Research Program (VIMS) aged 335 fish from the spring, 1999, Rappahannock River sample. Total length was significantly correlated with age (p = 0.0001, $r^2 = 0.9609$, df = 333) (Figure 12). The ages of the remaining 1564 fish in this sample were then estimated using this linear regression (Y = -2.65 + 0.0137X) (Figure 12). Fish 0 to 350 mm in total length were assigned an age range of 0 to 2 years (N = 10); fish 351 to 600 mm were assigned an age of 3-5 years (N = 291); fish >601 mm were assigned an age of >6 years (N = 34).

The prevalence of splenic mycobacteriosis increased with fish age (chi-square, df = 1, N = 1893, p = 0.001) (Figure 13). Disease prevalence in fish ages 3 - 5 was not significantly different from that in fish ages >6 ($G_H = 2$), but a significant difference was observed in fish ages 0 - 2 and fish ages 3 - 5 ($G_H = 10$, $X^2_{(0.05)(2)} = 5.991$) (Figure 13). Similar results were observed when sexes were analyzed separately (chi-square, df = 1, $N_{female} = 319$, $p_{female} = 0.002$, $N_{male} = 1574$, $p_{male} = 0.001$) (Figure 13).

Association Between Dermal and Splenic Mycobacteriosis

The prevalence of dermal lesions significantly increased with the intensity of splenic infections (chi-square, df = 1, N = 1896, p = 0.001) (Figure 14). No significant difference in the prevalence of dermal mycobacteriosis was found between fish with splenic mycobacterial severity scores of 0 and 0.5 ($G_H = 4$), 0.5 and 1 ($G_H = 2$), 1 and 2($G_H = 8$), and 2 and 3 ($G_H = 0$), but differences were found between 0, 0.5, and 1 ($G_H =$

20) and 1, 2, and 3 ($G_H = 42$) creating a continuum of increasing dermal mycobacteriosis with splenic mycobacterial intensity (G_H, $X^2_{(0.05)(4)} = 9.488$) (Figure 14).

Although the prevalence of dermal lesions closely tracked increasing severity of splenic mycobacteriosis (as estimated by granuloma counts), a small proportion of the total sample had histologically confirmed dermal mycobacteriosis (3.5%, 65 specimens) but no splenic disease (Table 3).

Association Between Mycobacteriosis and Fish Condition

The natural log of Fulton's condition factor had a significant negative relationship with the natural log of the total length for uninfected ($r^2 = 0.004$, $p = 0.048$, df = 984), infected ($r^2 = 0.017$, $p < 0.001$, df = 903), and uninfected and infected striped bass ($r^2 = 0.003$, $p = 0.017$, df = 1889); but the coefficients of determination were low suggesting that factors other than length affected condition (Figure 15). This significant relationship violates the assumption of isometric growth because the condition factors correlate with fish lengths, but the relationship was not great. Slopes from the regression equations for uninfected and infected fish were found not to be significantly different, suggesting similar trends in growth with increasing lengths in both groups. Thus, Fulton's condition factor meets the criteria as a suitable indicator of fish condition (t-test, $t = 0.222$, $p > 0.001$, df = 1887). Appendix 1 and Figure 19 show the deviation of larger fish from the regression lines in figure 15.

The condition index of striped bass collected during spring was significantly higher than that of fish collected during fall ($p < 0.001$), but condition index of striped bass from summer samples was not ($p = 0.514$). Striped bass from the Rappahannock River had a significantly higher condition index ($p = 0.001$) than fish from the Potomac River, but fish from the York River were not significantly different from the Potomac River samples ($p = 0.826$). Female striped bass had significantly higher condition indices ($p = 0.003$) than males. Splenic ($p = 0.121$) and dermal ($p = 0.308$) mycobacteriosis were found to have a much lesser and non-significant effect on condition index (Figures 16 (splenic) and 17(dermal)).

Substituting disease intensity status for the disease variable gives similar results, except that intensity of infection has a significant effect on condition index. Intensities of splenic mycobacteriosis were grouped into two categories (low = 0,0.5,1 and high = 2,3) for ease of analysis. Compared to fall, condition index of striped bass from spring was significantly higher ($p < 0.001$), but condition index of striped bass from summer was not ($p = 0.776$). Striped bass from the Rappahannock River had a significantly higher condition index ($p < 0.001$) than those from Potomac River; York River fish were not significantly different from Potomac River fish ($p = 0.933$). Female striped bass had a significantly higher condition index ($p < 0.001$) than males. Condition index of striped bass with higher intensities of splenic mycobacteriosis was significantly lower than condition index from fish with low intensities ($p = 0.004$) (Figure 18).

Discussion

This study indicates that mycobacteriosis in the striped bass, *Morone saxatilis*, is widespread in Virginia waters of Chesapeake Bay. High prevalences of the disease were observed in fish from 3 tributaries investigated during 1998-99. Prevalence of mycobacteriosis varied temporally and between sexes, with highest prevalences generally observed in males during fall. Little if any significant variance in prevalence occurred spatially among the three rivers. Prevalences of splenic infections were much higher than the dermal infections, ranging from 31.5% in the Rappahannock River in summer, 1999, to 62.7% in the York River in fall, 1999. Dermal infection prevalence ranged from 7.5% in the Rappahannock River in summer, 1998, to 28.8% in the York River in fall, 1999. Severity of mycobacteriosis increased with age from a low of 25% for fish that were 2 years old and less to a high of around 50% for fish 3 years old and up. There was a lack of association between condition indices of striped bass and mycobacteriosis, but temporal and spatial effects on condition indices were observed.

There has been much speculation about the factors that influence mycobacterial infections in striped bass from Chesapeake Bay. Several hypotheses were put forward at a workshop during July 1998 entitled "Trophic Changes in Chesapeake Bay Open Water Habitat: a Workshop to Evaluate and Interpret Recent Trends". These included oxygen/temperature constraints, overcrowding, starvation, immunosuppression, and possibly a combination of these environmental factors. The decline of forage food gained favor as a likely cause predisposing striped bass to this disease. Overton *et al.* (2000)

suggested that a change in striped bass prey abundance in Chesapeake Bay may result in changing prey consumption by striped bass affecting immune function and ultimately susceptibility to bacterial diseases such as mycobacteriosis.

Prevalences of dermal and splenic mycobacteriosis in this study were found to vary seasonally in the Rappahannock River with highest disease prevalences occurring in the fall. Dermal mycobacteriosis increased significantly from spring through fall, but splenic mycobacteriosis in striped bass during the summer was significantly lower than in the spring and fall. Condition indices of striped bass sampled during fall and summer were significantly lower than in spring. These seasonal variations in disease prevalence and fish condition support the views of Coutant (1990a) who found that adult striped bass preferred temperatures from 19°C to 23°C and strongly avoided temperatures above 25°C. Coutant (1990a) postulated that as temperatures increase during the summer months, there is increasingly less suitable habitat for striped bass in Chesapeake Bay. Temperature throughout the entire Bay is above 25°C from early July through mid-September, with anoxia widespread in the deeper, cooler portions. Thus, these waters are suboptimal habitat for striped bass during the summer months. Using the EPA Chesapeake Bay Program's computerized data set, Coutant (1990b) found a statistically significant decline in suitable habitat for sub-adult and adult striped bass in relation to temperature increases and dissolved oxygen decreases since the 1960s. As a result of these temperature and oxygen constraints on striped bass throughout much of the Chesapeake Bay, these fish are thought to congregate in thermal refuges in the Bay (Coutant 1990b). Increased disease and reduced body condition due to crowding in summer thermal refuges have been observed, and the annual carrying capacity for striped bass in the Bay is most likely compromised by the summer thermal and dissolved oxygen structure (Coutant 1990b). Environmental stress may suppress the immune defense mechanisms of fish allowing opportunistic microbial agents such as mycobacteria to multiply and produce disease (Anderson 1990). The stressful summer conditions experienced by striped bass in Chesapeake Bay may lower condition indices and allow possible latent mycobacterial infections to become pathogenic. Lowered body condition results when adult striped bass exposed to unsuitable high temperatures undergo physiological distress (Coutant 1990a). Their metabolic rates increase with increases in ambient water temperature creating a higher physiological demand for dissolved oxygen and food. If food is depleted in the thermal refuges to which these striped bass may be restricted during the summer, body condition may be expected to decline. Pederson and Jobling (1989) suggest that fish with low condition factor have probably fed poorly. Hartman (1994) found that striped bass food demand exceeded prey supply in Chesapeake Bay during spring and summer, and only returns close to demand during late summer and fall when clupeid consumption is high. Overton *et al.* (2000) suggest that menhaden, which constituted almost half of the total biomass of striped bass diet in their study, are declining in the Bay. They also found that menhaden constituted the greatest biomass in the diet of striped bass during November through December. Overton *et al.* (2000) suggest that the decline in menhaden may be a result of increased density of striped bass in the Bay. Also, Coutant has noted a decrease in condition over short periods where temperatures are high because energy is diverted from somatic growth and reproduction to the elevated metabolic demands associated with stressful summertime conditions. Condition indices of striped bass during the summer also may be lower because of spawning stresses from spring. This declining body condition from heat stress, starvation, spawning, and a shift of energy reserves could lead to a higher susceptibility to disease. Coutant (1990b) suggests that the temperature and dissolved oxygen constraints on critical habitat refuges during the summer may vary from year to year with cyclic climatic and other environmental factors. Hence, these stressors may not be present every year.

By the fall, fish subjected to summer stressors such as elevated temperatures, dissolved oxygen declines, and possible depletion of food resources in thermal refuges experience reduced condition indices and higher disease prevalences. Such fish would have experienced three to four months of stressful conditions possibly resulting in lowered body condition and allowing mycobacteriosis to progress. However, during the fall, decreasing temperatures, increasing oxygen concentrations, and increasing food resources may result in some recovery and an increase in the condition index. In fact, it is possible that the most heavily infected fish might drop out of the population during the very stressful summer months. The decline in the prevalence of the splenic disease observed in the summer during this study may be an indication of such mortality. Alternatively, prevalence of the splenic and dermal disease may increase during fall as a result of commercial and recreational fishers actively selecting the highest quality fish and releasing the most emaciated and infected fish. The striped bass fishery in Chesapeake Bay is most active during fall, and it is likely that most commercial and recreational fishers release unmarketable fish with visible lesions or emaciation because of their impalatability. The prevalence of splenic mycobacteriosis in spring was not significantly different from that in fall, suggesting that latent infections may overwinter in fish. Conversely, dermal lesion prevalences were lowest in the spring suggesting some resolution of dermal clinical signs over the winter months or loss of the most heavily affected individuals. Similarly, Haeseker *et al.* (1996) found that striped bass in Albemarle Sound, North Carolina, have a higher prevalence of red sores of undetermined etiology and lower relative weight during the summer when temperatures were above $25^{\circ}C$.

In general, females had significantly lower prevalences of dermal and splenic mycobacteriosis and significantly higher condition indices than males. At age 2, females begin migrating out of the Bay to cooler waters, but we currently have no data on the infection in these migrants. In contrast, males have higher splenic and dermal prevalence and lower condition because they probably do not move out of the Bay until they are older (5-6 years) (Kohlenstein 1981). Male striped bass from Chesapeake Bay contribute little to the coastal migratory stock because age classes 2 - 6 are subjected to heavy fishing pressure in the Bay. They, therefore, are subjected repeatedly to stressful summer conditions possibly accounting for the observed higher disease prevalence and lower body condition. Also, possible environmental contamination may allow for higher susceptibility to disease by reducing striped bass percent body fat (Korn *et al.* 1976) and bone health (Mehrle *et al.* 1982). Males may be at a higher risk to contaminants since they generally do not leave the Bay, and females may rid their bodies of accumulated toxicants through spawning.

Location had no effect on prevalence of dermal and splenic mycobacteriosis in striped bass; however, dermal mycobacteriosis was significantly elevated in the fall York River sample. Location did have a significant effect on condition indices of striped bass.

Condition indices of striped bass from the Potomac River were not significantly different from those of fish in the York River samples, but they were significantly lower than those of fish from the Rappahannock River. This may be the result of varying environmental factors such as prey availability or suitable habitat in the three tributaries investigated.

Striped bass in Chesapeake Bay may constitute genetically distinct stocks that may exhibit differential susceptibility to mycobacteriosis and factors influencing condition index, but no data was found showing differential susceptibility of striped bass stocks to mycobacteriosis or varying condition indices. In mackerel, *Scomber scombrus*, MacKenzie *et al.* (1988) attributed regional variations in prevalence of mycobacteriosis in the Northeast Atlantic to optimal temperature ranges for mycobacterial growth or varying susceptibility of genetically different stocks. Massmann and Pacheco (1961) found evidence of striped bass subpopulations in the York, Rappahannock, and James Rivers. Using otolith microprobe analysis, Secor (1999) found 3 different contingents of striped bass in the Hudson River - resident, lower estuary, and coastal migratory - and revealed the complex nature of striped bass populations. The complex of substocks in the Chesapeake Bay has created confusion in identifying the origins of the Atlantic coastal migratory stock (Wirgin *et al.* 1997). There is poor understanding of the contigents of striped bass in the Chesapeake Bay resulting in poor understanding of the complex life history dynamics of striped bass in the Chesapeake Bay. This complex population structure may be partially at fault for differences in disease prevalence and condition indices in striped bass from the Chesapeake Bay.

Age was found to be a significant factor influencing prevalence of splenic mycobacteriosis. Abernathy *et al.* (1978), Hastings *et al.* (1982), MacKenzie *et al.*

(1988), and Haeseker *et al.* (1996) found that intensity and severity of mycobacteriosis increased with age. Coutant (1990a) found that juvenile striped bass preferred temperatures from 24°C to 28°C, whereas adult fish preferred temperatures from 19°C to 23°C. Since adult striped bass are less tolerant to high temperatures, the older fish may be the first to succumb to disease such as mycobacteriosis when exposed to high temperatures. Another explanation may be that the older fish simply have had the infection for a longer period of time. We do not understand how and at what age striped bass become infected with mycobacteria.

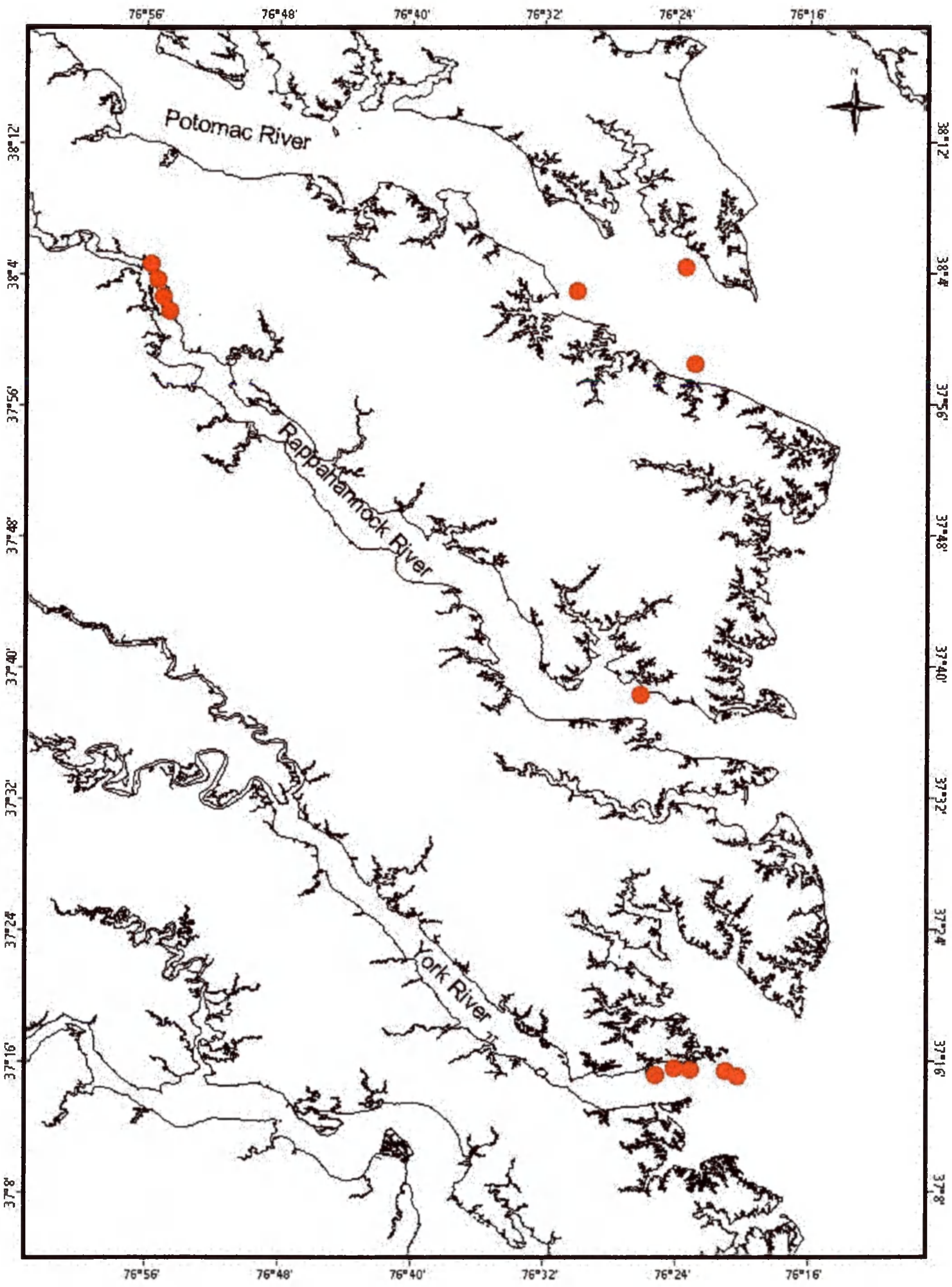
The condition of fish with heavy splenic infection was significantly lower than that of uninfected and lightly infected fish. Mycobacterial infections may be asymptomatic for long time periods (Colorni *et al.* 1993). This suggests that a fish must be heavily infected internally before the disease begins to negatively affect body condition. MacKenzie (1988) found that the mean condition factor was greater in uninfected mackerel than for infected fish from the Northeast Atlantic. This, again, suggests that striped bass in the Chesapeake Bay may carry latent *Mycobacterium* spp. that become pathogenic when fish condition is decreased by environmental stressors such as heat, low dissolved oxygen, starvation, and spawning stress.

The prevalence of dermal lesions in striped bass from Chesapeake Bay was positively associated with the intensity of splenic mycobacteriosis suggesting that the internal infection ultimately may lead to development of dermal lesions. The dermal lesions thus may be an expression of the terminal stages of this disease. Dermal infections, however, represent a minor fraction of mycobacteriosis found in striped bass from Chesapeake Bay. In contrast, Majeed and Gopinath (1983) found no macroscopic lesions in the viscera of carp, *Cyprinus carpio*, with heavy dermal ulceration caused by mycobacteria. In this study, 3.28% or 62 of the 1899 striped bass exhibited histologically verified dermal mycobacteriosis in the absence splenic infection. These findings suggest that internal and dermal mycobacteriosis are related but may not always be caused by the same infection. Rhodes (2001) isolated multiple *Mycobacterium* spp. from striped bass collected from Chesapeake Bay during the time of this study. The *Mycobacterium* spp. isolated from dermal lesions resembled *M. peregrinum*, *M. gordonae*, *M. terrae* complex, *M. marinum*, *M. flavescens/szulgai*, *M. interjectum*, *M. scrofulaceum*, and other unindentified species. Species isolated from the spleen resembled *M. scrofulaceum*, *M. simiae*, a new species designated as M 175, and other unidentified species. Although dermal and splenic mycobacteriosis in striped bass from Chesapeake Bay may not have the same etiologic agents, this provides support for stressful conditions in the Chesapeake Bay because mycobacteria are ubiquitous, opportunistic pathogens that usually do not cause disease unless fish are subjected to unfavorable, stressful conditions (Fryer and Rohovec 1993).

Although this study did not determine which environmental factors play a role in mycobacteriosis in striped bass, there is evidence that the temperature and dissolved oxygen constraints in the Chesapeake Bay described by Coutant (1990b) may contribute to the observed prevalence of mycobacteriosis. The highest prevalences of infection occurring during the fall may be the result of prolonged environmental stress beginning in early July, which may also cause body condition to decline from a high during spring, to a low in summer and fall. Heavily infected fish have lower condition indices than uninfected fish possibly because of the chronic, wasting nature of this disease. Dermal mycobacteriosis was found to be positively associated with severity of splenic mycobacteriosis; however, the significant number of fish with skin lesions and no internal lesions suggests that skin pathology is not always a manifestation of the later stages of the disease. Males exhibited higher disease prevalence than females possibly because they are residents of the Chesapeake Bay for several years longer than the females. Although location had no affect on disease prevalence, it did have an effect on fish condition indices possibly as a result of varying environmental conditions in the three tributaries studied. Age was found to be a significant factor in prevalence of splenic mycobacteriosis, which can be explained by older fish requiring lower temperatures and therefore experiencing more stress than younger fish.

Figure 1. Sample locations in the Potomac River, Rappahannock River, and York River.

Figure 1



76°56'
76°48'
76°40'
76°32'
76°24'
76°16'
38°12'
38°4'
37°56'
37°48'
37°40'
37°32'
37°24'
37°16'
37°8'
Potomac River
Rappahannock River
York River
N

Figure 2. Dermal lesions caused by mycobacteriosis in striped bass, *Morone saxatilis*. *A) coalescing lesion, B) multifocal lesion, C) a higher magnification of Figure 2A. Note the shallow nature, hemorrhage, hyperpigmentation, and gritty texture.*

Figure 2

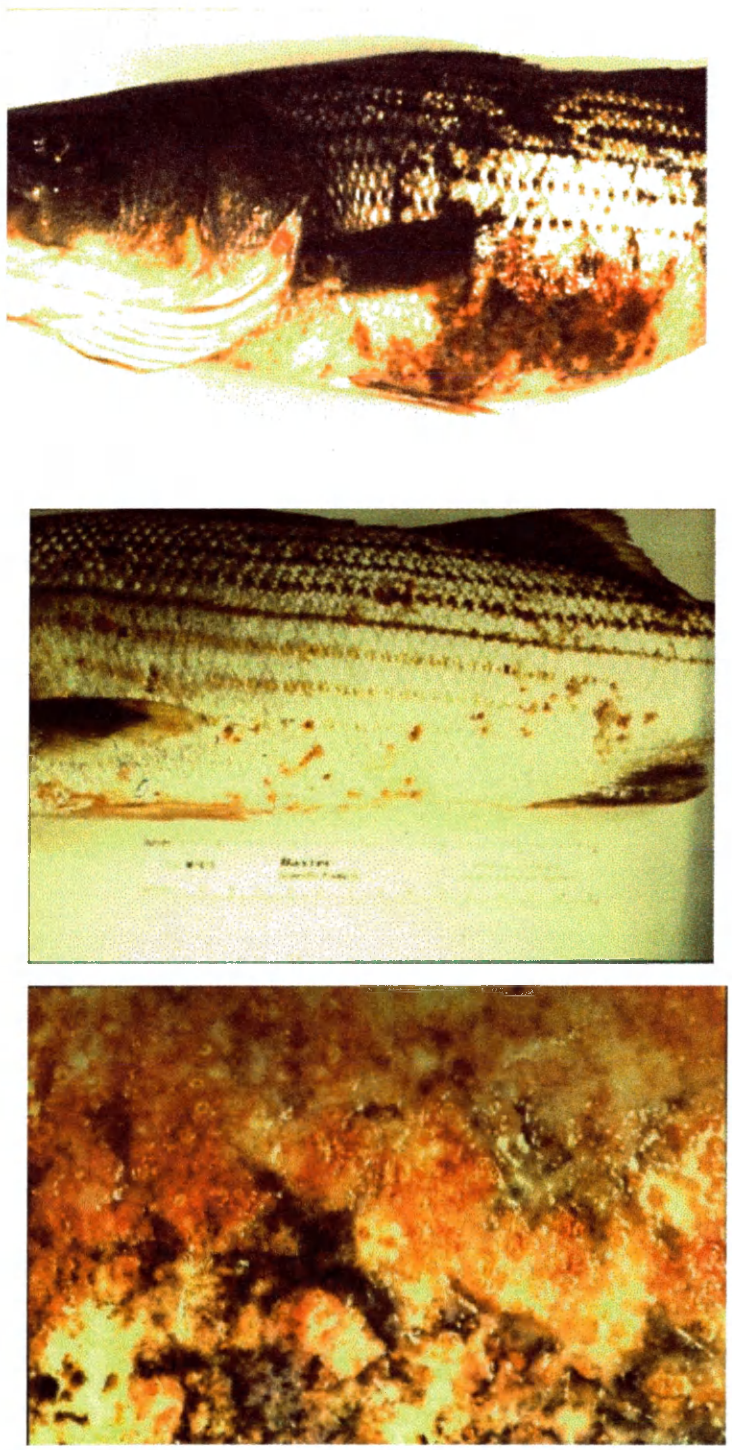


Figure 3. Pale focal nodular splenic lesions caused by mycobacteriosis in striped bass, *Morone saxatilis.*

Figure 3

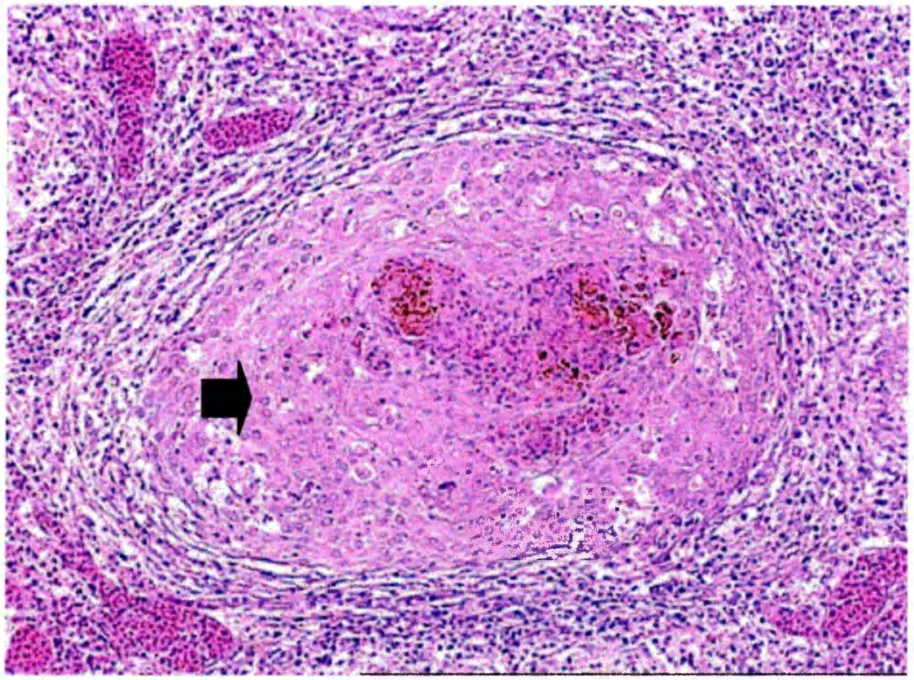
Figure 4. Splenic granulomas associated with mycobacterial infections.

A) granuloma with epithelioid cells (arrow) with necrotic core, H&E, 100X

B) same granuloma with mycobacterial rods (arrow), Z-N, 1000X

Figure 4

A)



B)

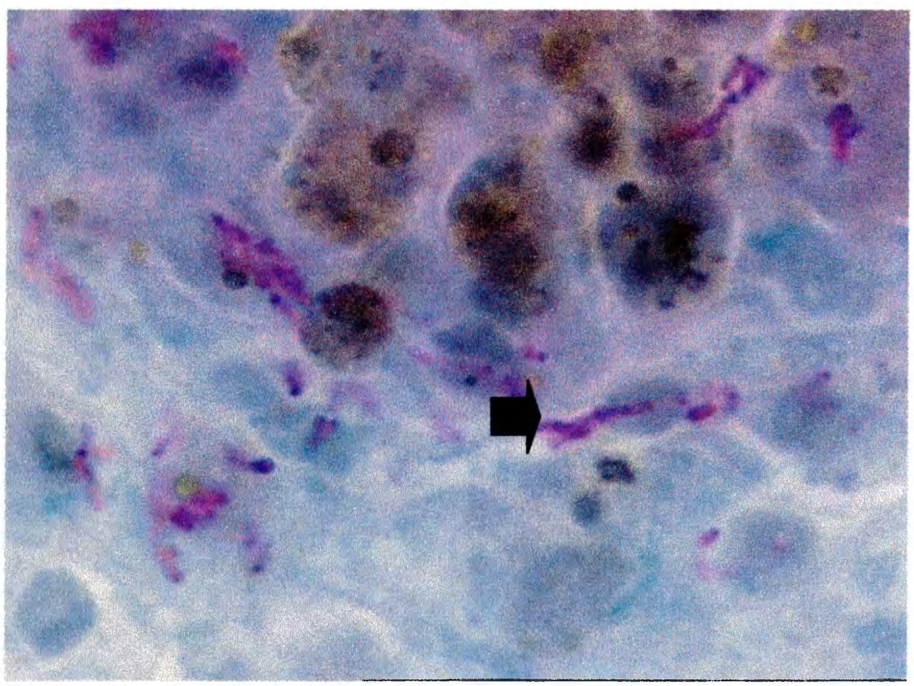


Figure 5. Dermal granulomas associated with mycobacterial infections, H&E, 100X.

Figure 5

Figure 6. Splenic granuloma associated with a parasite, H&E, 200X.

Figure 6

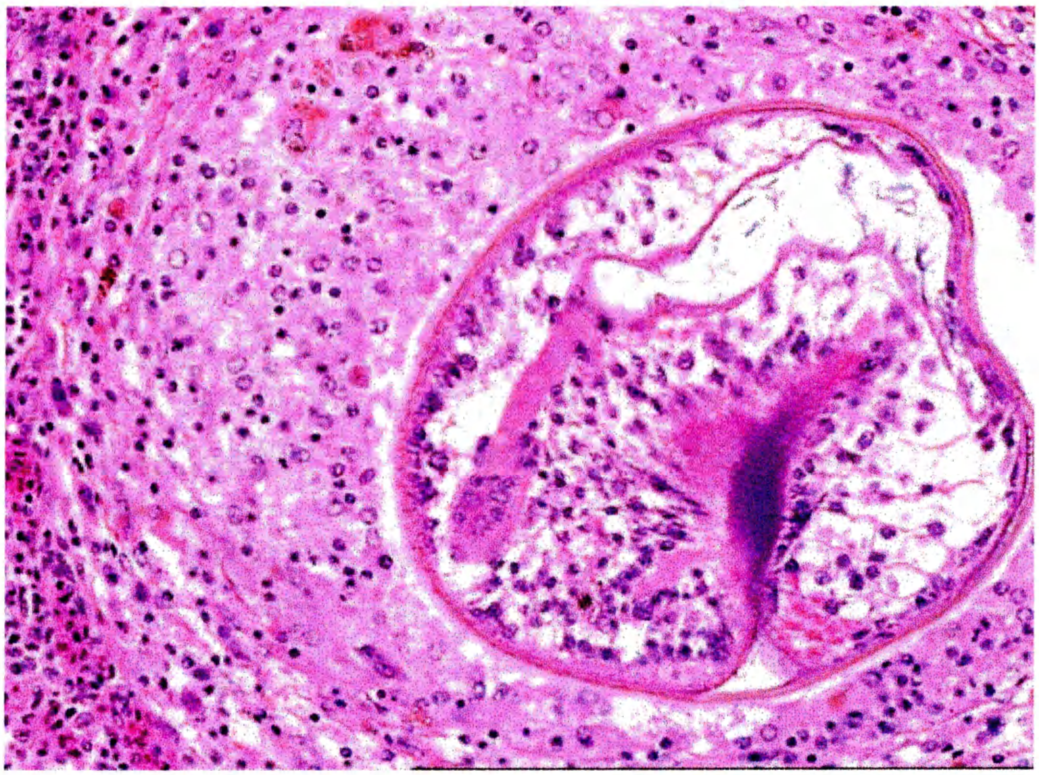


Figure 7. Splenic granulomas associated with mycobacterial infections, H&E, 40X.

A) zero infection score (0)
B) light infection score (1)
C) moderate infection score (2)
D) heavy infection score (3)

Figure 7

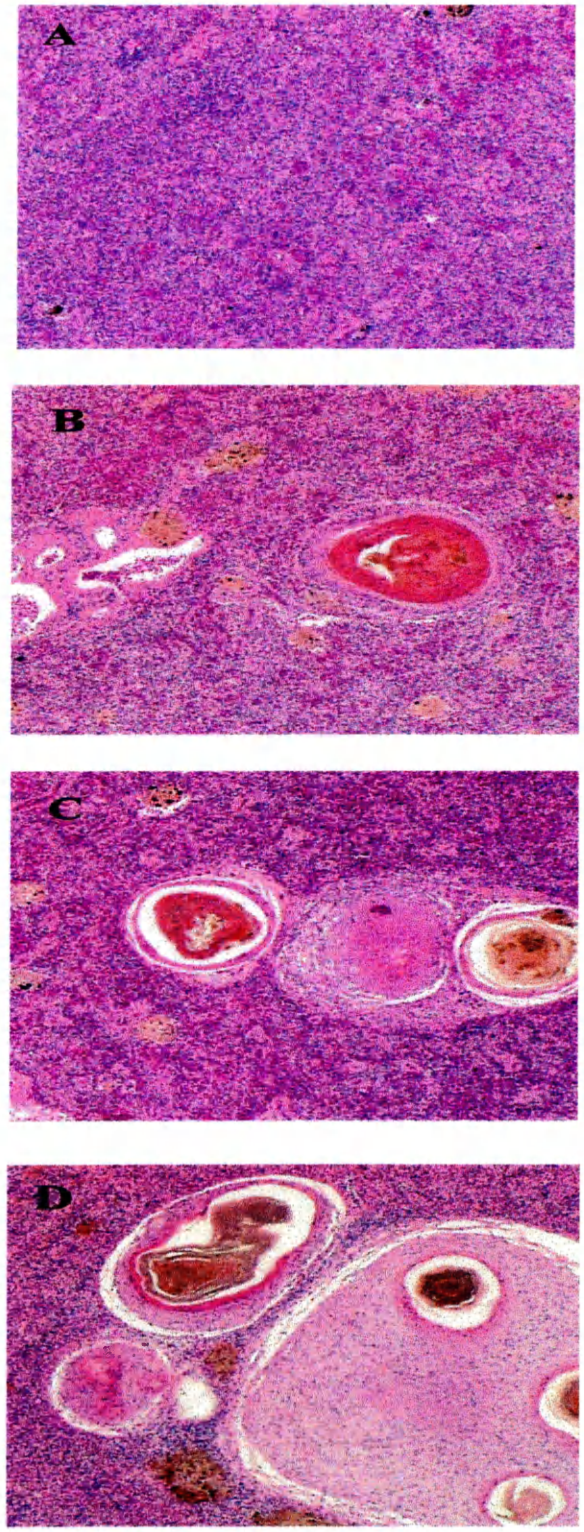


Table 1. Prevalence of splenic and dermal mycobacteriosis in striped bass, *Morone saxatilis*, from the Rappahannock, Potomac, and York Rivers during summer 1998 through fall 1999.

	Rappahannock River				Potomac River				York River			
	N_{Total}	$N_{Splenic}$ (%)	N_{Dermal} (%)	**Collection Dates**	N_{Total}	$N_{Splenic}$ (%)	N_{Dermal} (%)	**Collection Dates**	N_{Total}	$N_{Splenic}$ (%)	N_{Dermal} (%)	**Collection Dates**
Sum '98	239	89 (37.2)	18 (7.5)	9/2/98 – 9/16/98	234	103 (44.0)	40 (17.1)	7/14/98 – 8/24/98	0	---------	---------	------------
Fall '98	297	159 (53.5)	44 (14.8)	10/19/98 – 10/20/98	203	109 (53.7)	32 (15.8)	10/28/98	0	---------	---------	------------
Sp '99	335	159 (47.5)	11 (3.3)	3/30/99 – 4/26/99	0	--------	---------	------------	0	---------	---------	------------
Sum '99	130	41 (31.5)	10 (7.7)	7/21/99 – 7/26/99	0	--------	---------	------------	102	53 (52.0)	22 (21.6)	7/21/99 – 9/14/99
Fall '99	132	69 (52.3)	21 (15.9)	9/29/99 – 10/6/99	109	48 (44.0)	17 (15.6)	11/8/99	118	74 (62.7)	34 (28.8)	10/4/99 – 11/18/99
Total	1133	517 (45.6)	104 (9.2)	------------	546	260 (47.6)	89 (16.3)	------------	220	127 (57.7)	56 (25.5)	------------

This page has no text or graphics.

Figure 8. Seasonal variation in splenic and dermal mycobacteriosis of striped bass, *Morone saxatilis*, from the Rappahannock River (N = 1133).
* *results of* G_H *test indicate significant differences for splenic* ($G_H = 10$, $X^2_{(0.05)(2)} = 5.991$) *and dermal* ($G_H = 6,10; X^2_{(0.05)(2)} = 5.991$) *infection prevalence among the three seasons; splenic infection prevalence was not significantly different between spring and fall* ($G_H = 4$).

Figure 8

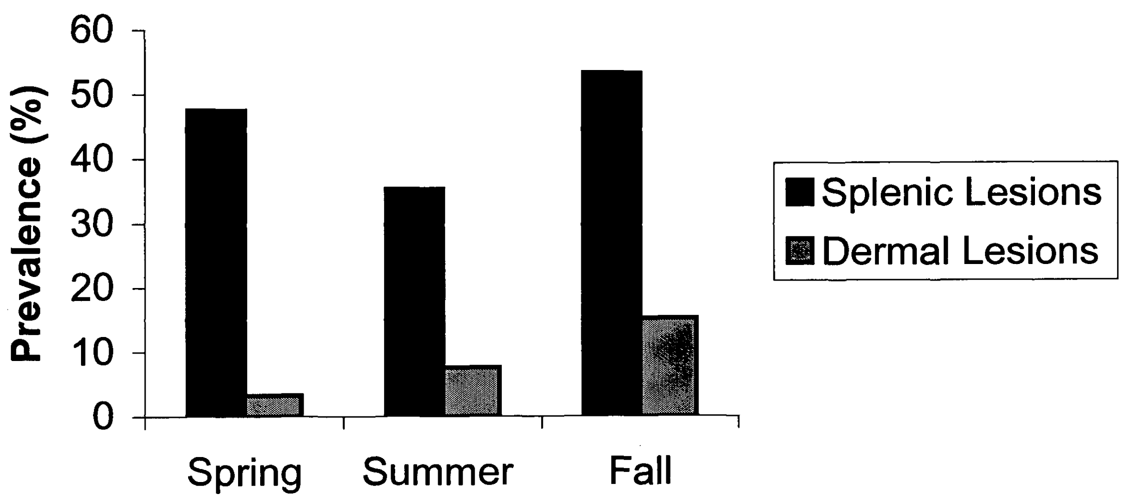

60
50
40
30
20
10
0
Prevalence (%)
Spring
Summer
Fall
Splenic Lesions
Dermal Lesions

Table 2. Temporal trends in lesion prevalence of female and male striped bass, *Morone saxatilis*, from three Virginia rivers.
T = total; S = splenic; D = dermal
$^{+,\,++}$ = *significant differences in splenic ($^{+}$) and dermal ($^{++}$) prevalence between females and males by season and river*

	Rappahannock River					
	Female			Male		
	N_T*	N_S (%)	N_D (%)	N_T	N_S (%)	N_D (%)
Spring	30	13 (43.3)	1 (3.3)	305	146 (47.9)	10 (3.3)
Summer$^{+,\,++}$	114	22 (19.3)	0 (0.0)	255	108 (42.4)	28 (11.0)
Fall$^{+,\,++}$	76	30 (39.5)	2 (2.6)	352	198 (56.3)	63 (17.9)

	Potomac River					
	Female			Male		
	N_T*	N_S (%)	N_D (%)	N_T	N_S (%)	N_D (%)
Spring	-----	------	------	-----	------	------
Summer$^{+,\,++}$	33	6 (18.2)	0 (0.0)	199	96 (48.2)	39 (19.6)
Fall$^{+,\,++}$	53	20 (37.7)	2 (3.8)	256	140 (54.7)	47 (18.4)

	York River					
	Female			Male		
	N_T*	N_S (%)	N_D (%)	N_T	N_S (%)	N_D (%)
Spring	----	------	------	----	------	------
Summer^{+}	9	4 (44.4)	0 (0.0)	92	48 (52.2)	22 (23.9)
Fall^{+}	5	4 (80.0)	1 (20.0)	112	69 (61.6)	33 (29.5)

Figure 9. Seasonal prevalences of splenic mycobacteriosis in female and male striped bass, *Morone saxatilis*, from three Virginia rivers.
**Potomac and York Rivers had no spring samples.*

Figure 9

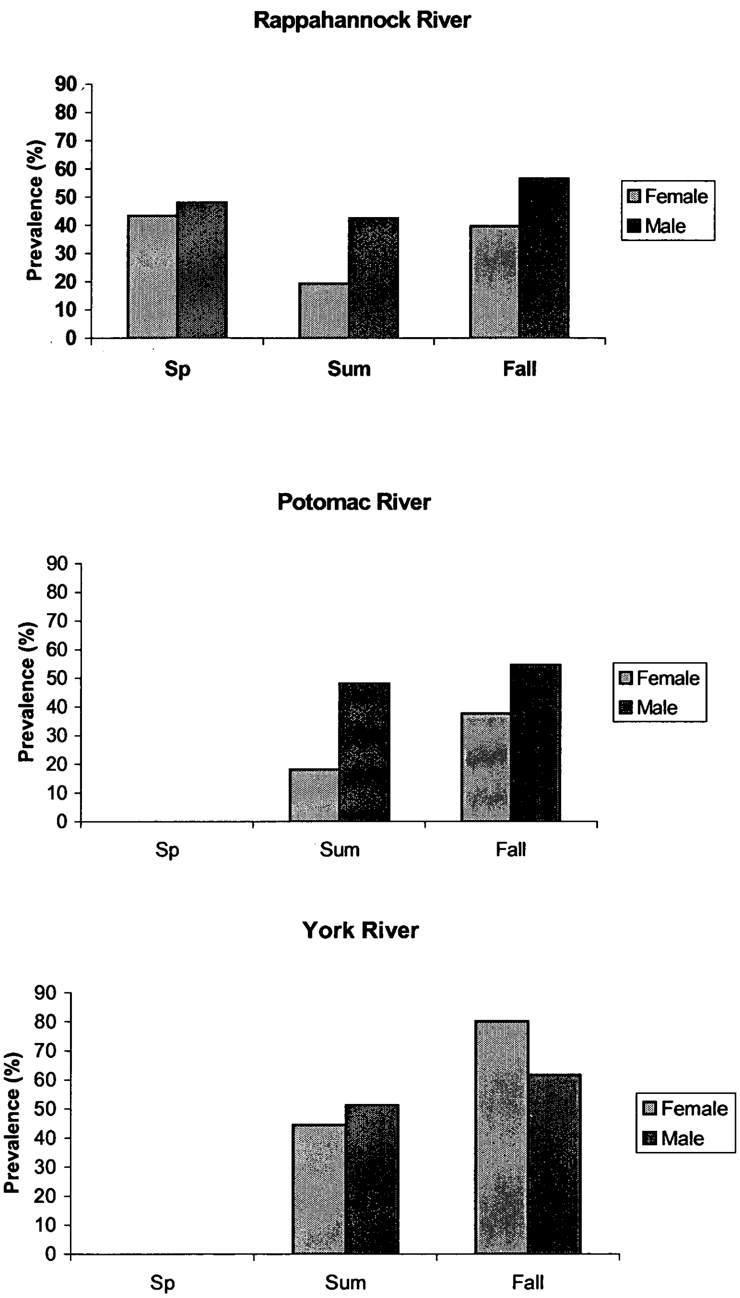
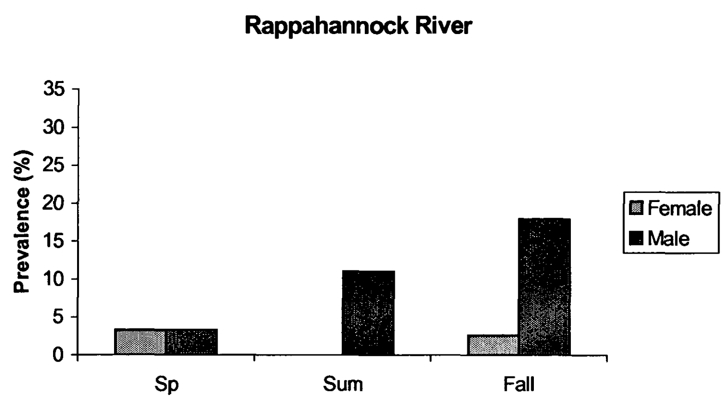
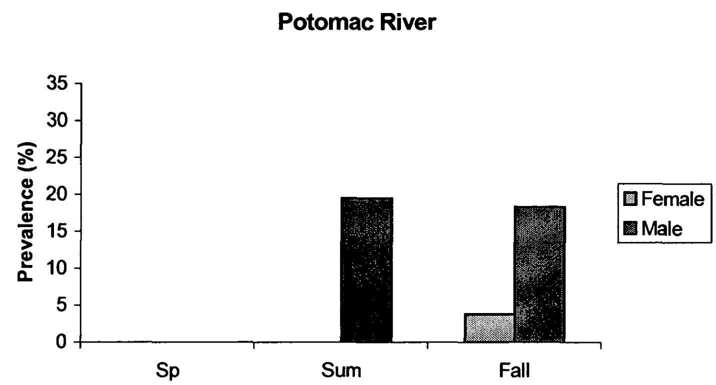

Rappahannock River
Prevalence (%)
90
80
70
60
50
40
30
20
10
0
Sp
Sum
Fall
Female
Male
Potomac River
Prevalence (%)
90
80
70
60
50
40
30
20
10
0
Sp
Sum
Fall
Female
Male
York River
Prevalence (%)
90
80
70
60
50
40
30
20
10
0
Sp
Sum
Fall
Female
Male

Figure 10. Seasonal prevalences of dermal mycobacteriosis in female and male striped bass, *Morone saxatilis*, from three Virginia rivers.
**Potomac and York Rivers had no spring samples.*

Figure 10


Rappahannock River
Prevalence (%)
35
30
25
20
15
10
5
0
Sp
Sum
Fall
Female
Male


Potomac River
Prevalence (%)
35
30
25
20
15
10
5
0
Sp
Sum
Fall
Female
Male

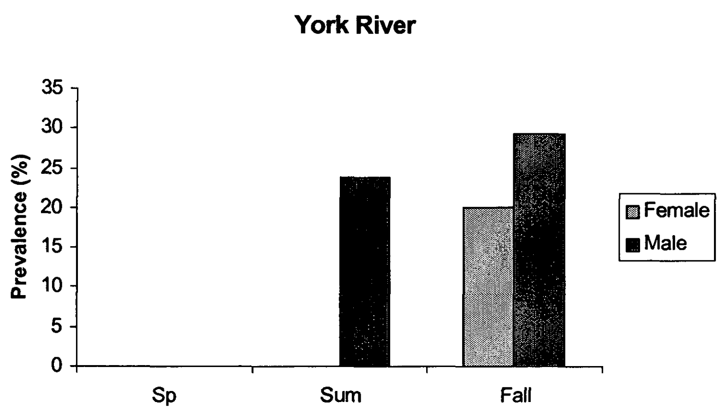
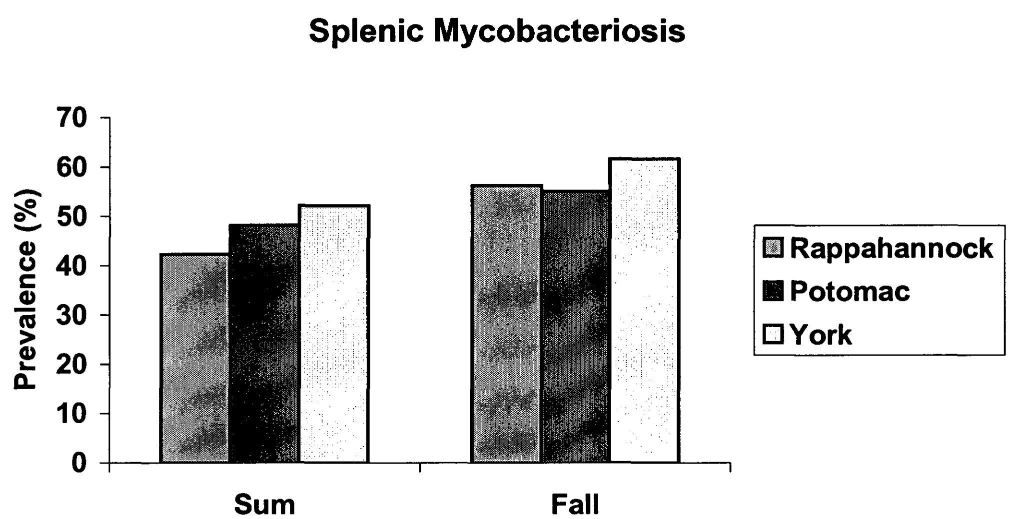

York River
Prevalence (%)
35
30
25
20
15
10
5
0
Sp
Sum
Fall
Female
Male

Figure 11. Effect of location (Rappahannock, Potomac, and York Rivers) controlling for season on splenic (chi-square, $p_{summer} = 0.866$; $p_{fall} = 0.293$) and dermal (chi-square, $p_{summer} = 0.951$; $p_{fall} = 0.046$) mycobacteriosis in male striped bass, *Morone saxatilis*.

Figure 11


Splenic Mycobacteriosis
70
60
50
40
30
20
10
0
Prevalence (%)
Sum
Fall
Rappahannock
Potomac
York

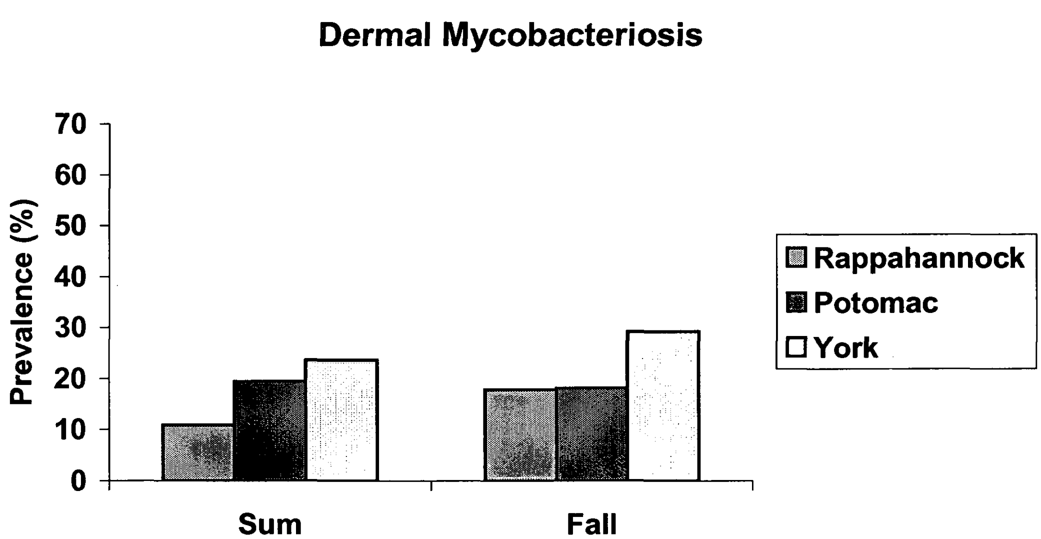

Dermal Mycobacteriosis
70
60
50
40
30
20
10
0
Prevalence (%)
Sum
Fall
Rappahannock
Potomac
York

Figure 12. Relationship between total length and age of striped bass, *Morone saxatilis*, from the Rappahannock River collected in spring, 1999.
$Y = -2.65+0.0137X$; $r^2 = 0.9601$; $N = 335$; $p = 0.001$, $df = 1$
**data provided by Phil Sadler, Anadromous Fishes Research Program, VIMS*

Figure 12

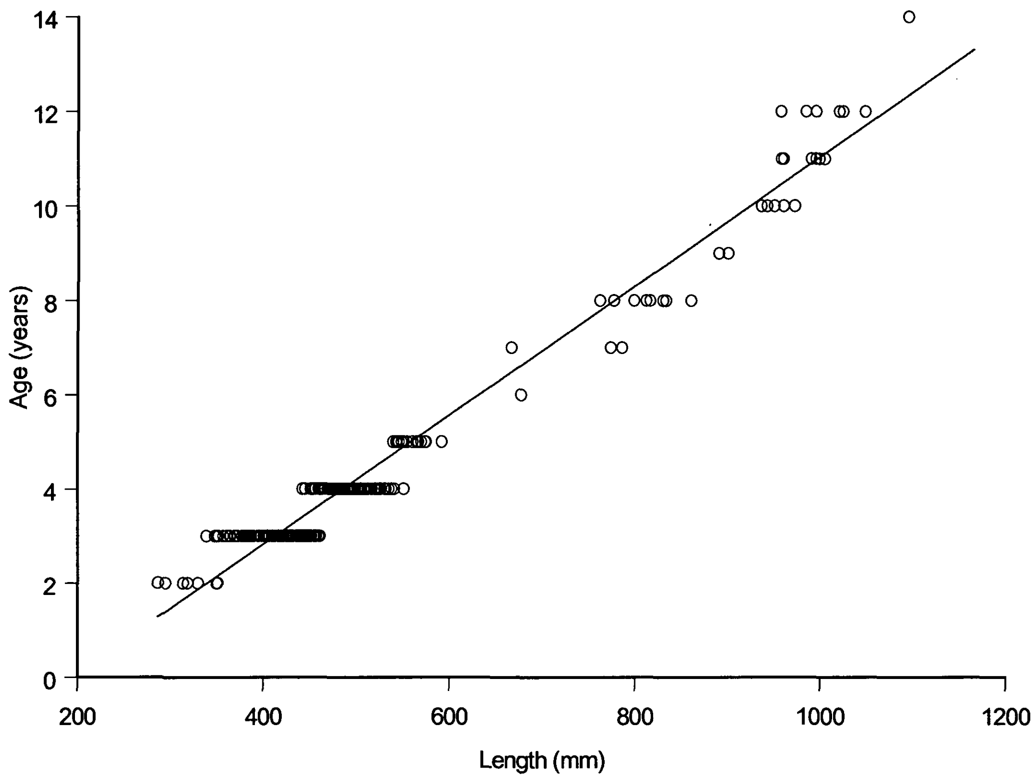


Figure 13. Prevalence of splenic mycobacteriosis in three age groups of striped bass, *Morone saxatilis*.

* *results of G_H test indicate no significant difference between age groups 3 through 5 and 6+ ($G_H = 2$), but the age group 0 through 2 was significantly different from the other two groups ($G_H = 10$, $X^2_{(0.05)(2)} = 5.991$).*

Figure 13

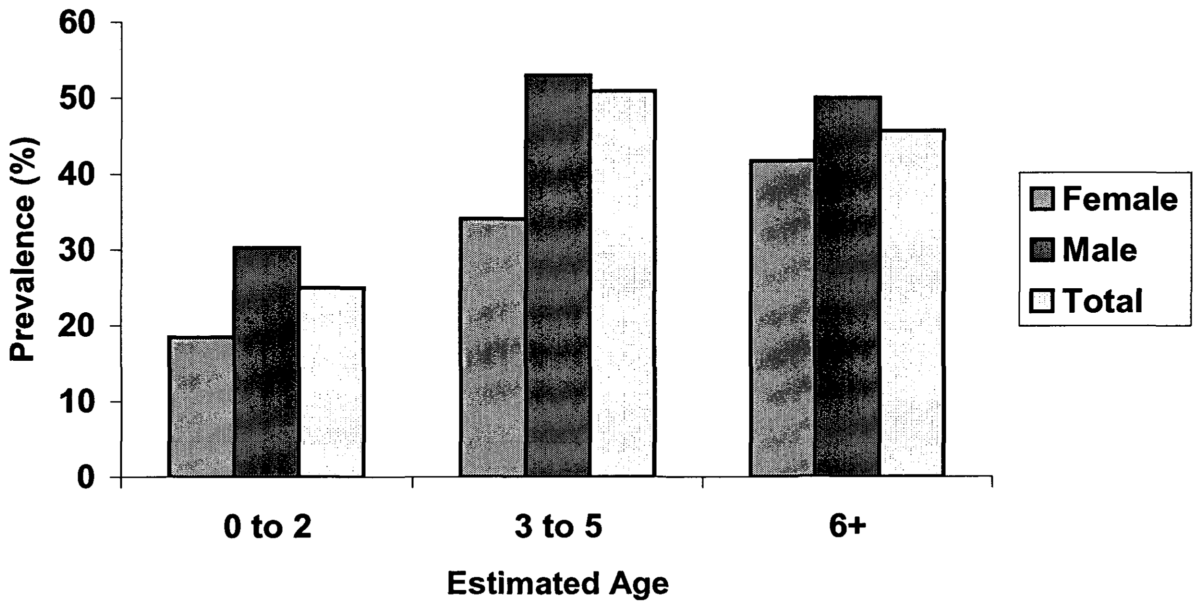

60
50
40
30
20
10
0
Prevalence (%)
0 to 2
3 to 5
6+
Estimated Age
Female
Male
Total

Figure 14. Comparison of intensity of splenic mycobacteriosis with prevalence of dermal lesions from striped bass, *Morone saxatilis*.
$N_0 = 990$; $N_{0.5} = 310$; $N_1 = 328$; $N_2 = 80$; $N_3 = 188$
* *results of* G_H *test indicate no significant differences in dermal prevalence between fish with splenic intensities 0 and 0.5* ($G_H = 4$), *0.5 and 1* ($G_H = 2$), *1 and 2* ($G_H = 8$), *and 2 and 3* ($G_H = 0$); *significant differences were observed between fish with splenic intensities 0 and 1* ($G_H = 20$) *and 1 and 3* ($G_H = 42$, $X^2_{(0.05)(4)} = 9.488$).

Figure 14

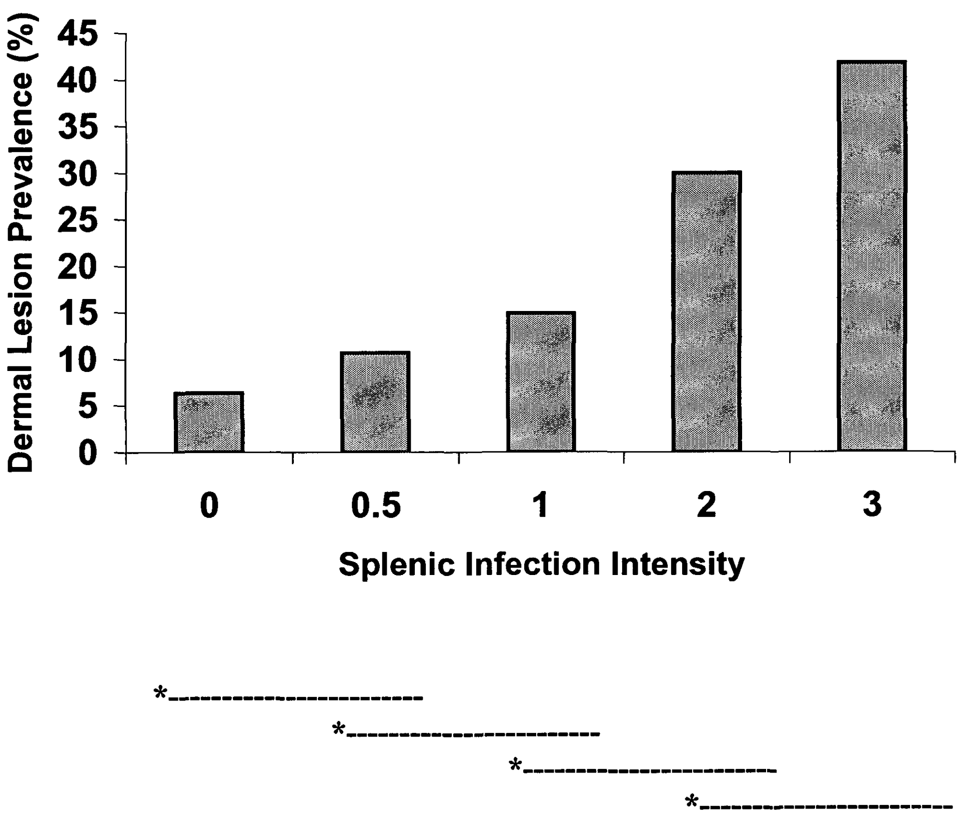

Dermal Lesion Prevalence (%)
45
40
35
30
25
20
15
10
5
0
0
0.5
1
2
3
Splenic Infection Intensity

Table 3. Prevalences of dermal versus splenic mycobacteriosis.

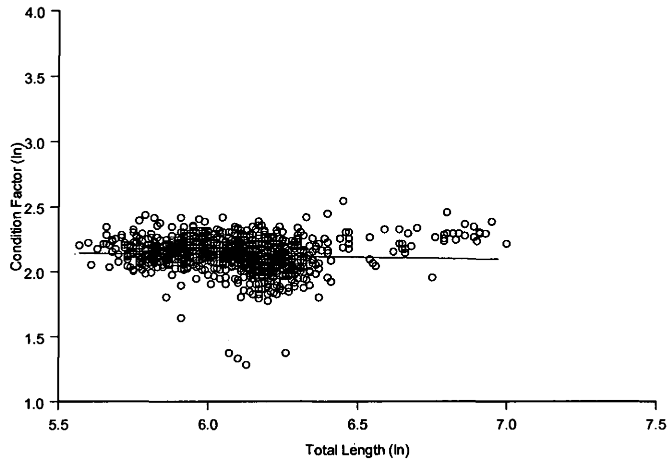
	Splenic -	**Splenic +**
Dermal -	48.92%	38.03%
Dermal +	3.28%	9.77%

Sample Size = 1893

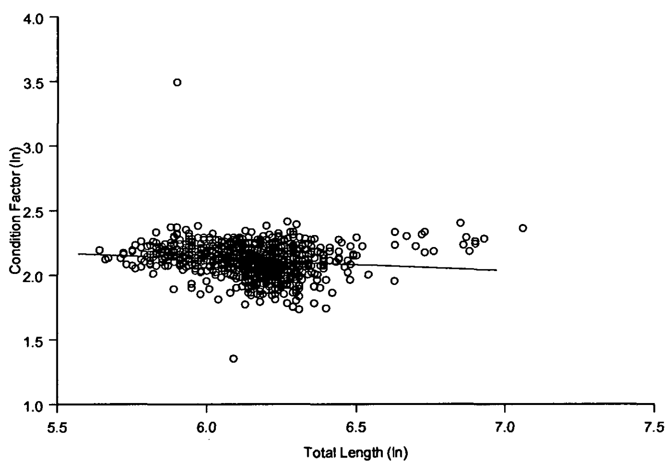
Figure 15. Linear regressions of natural log of condition factor with natural log of total length for uninfected, infected, and uninfected and infected striped bass, *Morone saxatilis*.
A) $Y = 2.34-0.0356X$, $r^2 = 0.004$, $p = 0.048$, $df = 1$
B) $Y = 2.67-0.0910X$, $r^2 = 0.017$, $p = 0.000$, $df = 1$
C) $Y = 2.33-0.0338X$, $r^2 = 0.003$, $p = 0.017$, $df = 1$

Figure 15

A) Uninfected



B) Infected



C) Uninfected + Infected

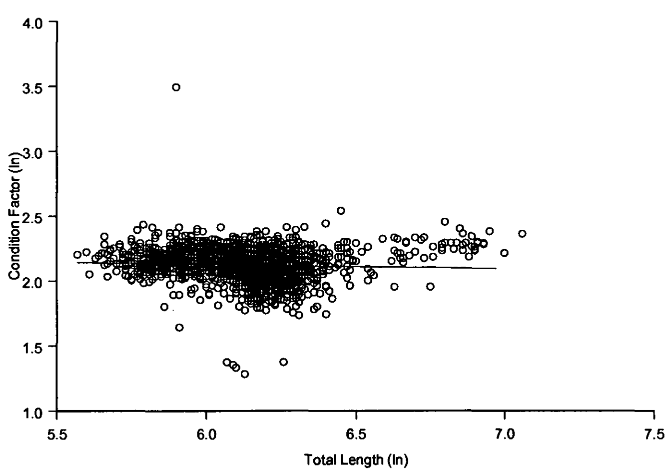
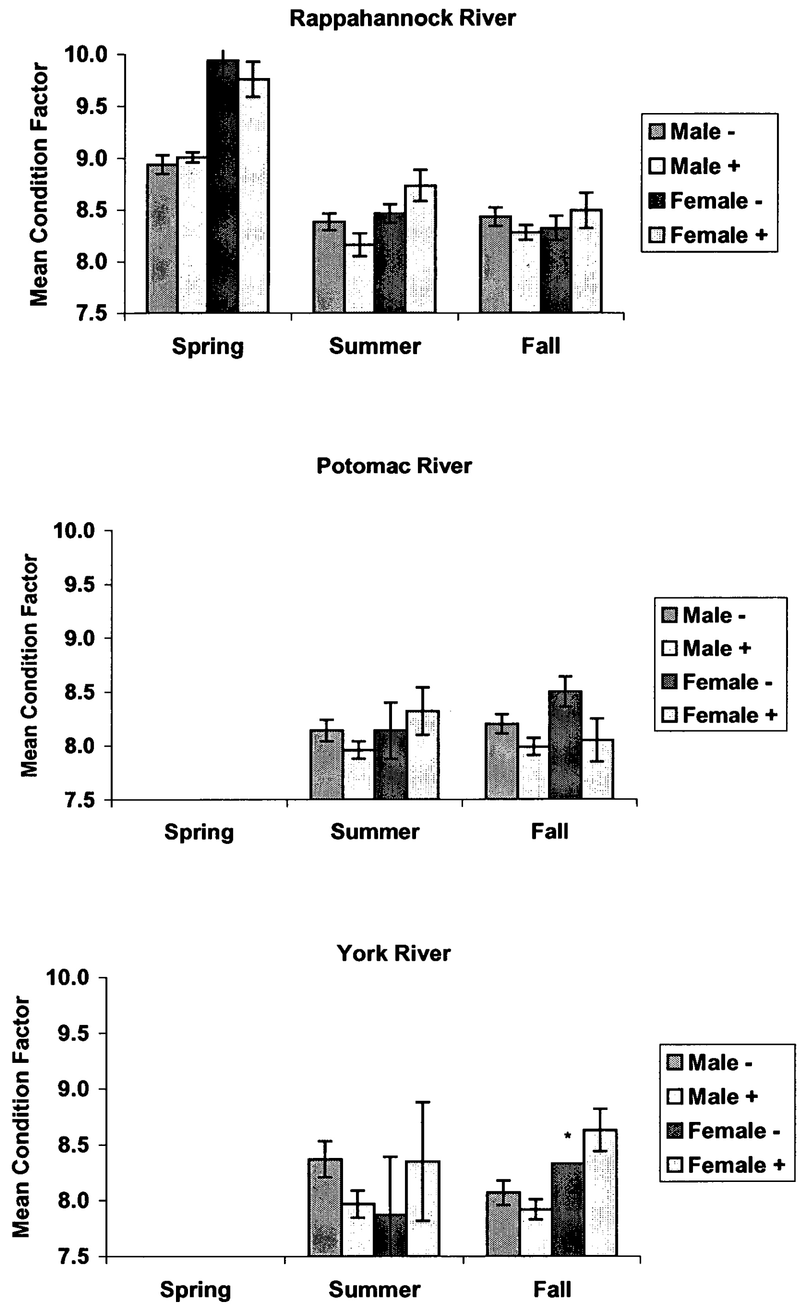


Figure 16. Variation in condition factor with season, location, gender, and status of splenic infection (- = uninfected, + = infected) of striped bass, *Morone saxatilis*.
** bars without standard errors only contained 1 sample (Table 2)*
(regression, r^2 = 0.08, ln condition factor = 2.09 + 0.0794 fish from spring - 0.00467 fish from summer + 0.0025 fish from York + 0.263 fish from Rappahannock + 0.0267 female fish - 0.0101 fish with dermal mycobacteriosis - 0.0103 fish with splenic mycobacteriosis).

Figure 16



Rappahannock River
Mean Condition Factor
10.0
9.5
9.0
8.5
8.0
7.5
Spring
Summer
Fall
Male -
Male +
Female -
Female +
Potomac River
Mean Condition Factor
10.0
9.5
9.0
8.5
8.0
7.5
Spring
Summer
Fall
Male -
Male +
Female -
Female +
York River
Mean Condition Factor
10.0
9.5
9.0
8.5
8.0
7.5
*
Spring
Summer
Fall
Male -
Male +
Female -
Female +

Figure 17. Variation in condition factor with season, location, gender, and status of dermal infection (- = uninfected, + = infected) of striped bass, *Morone saxatilis.*
** bars without standard errors only contained 1 sample (Table 2) (regression, $r^2 = 0.08$, ln condition factor = 2.09 + 0.0794 fish from spring - 0.00467 fish from summer + 0.0025 fish from York + 0.263 fish from Rappahannock + 0.0267 female fish - 0.0101 fish with dermal mycobacteriosis - 0.0103 fish with splenic mycobacteriosis).*

Figure 17

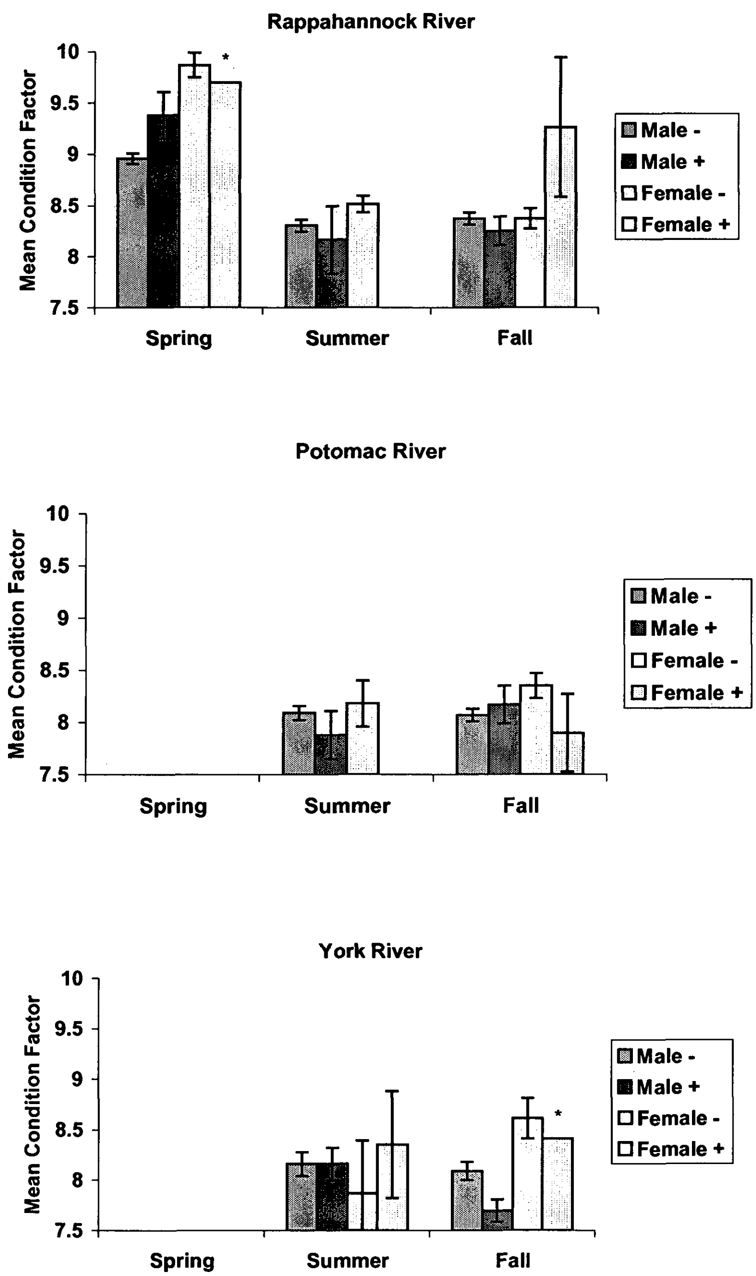
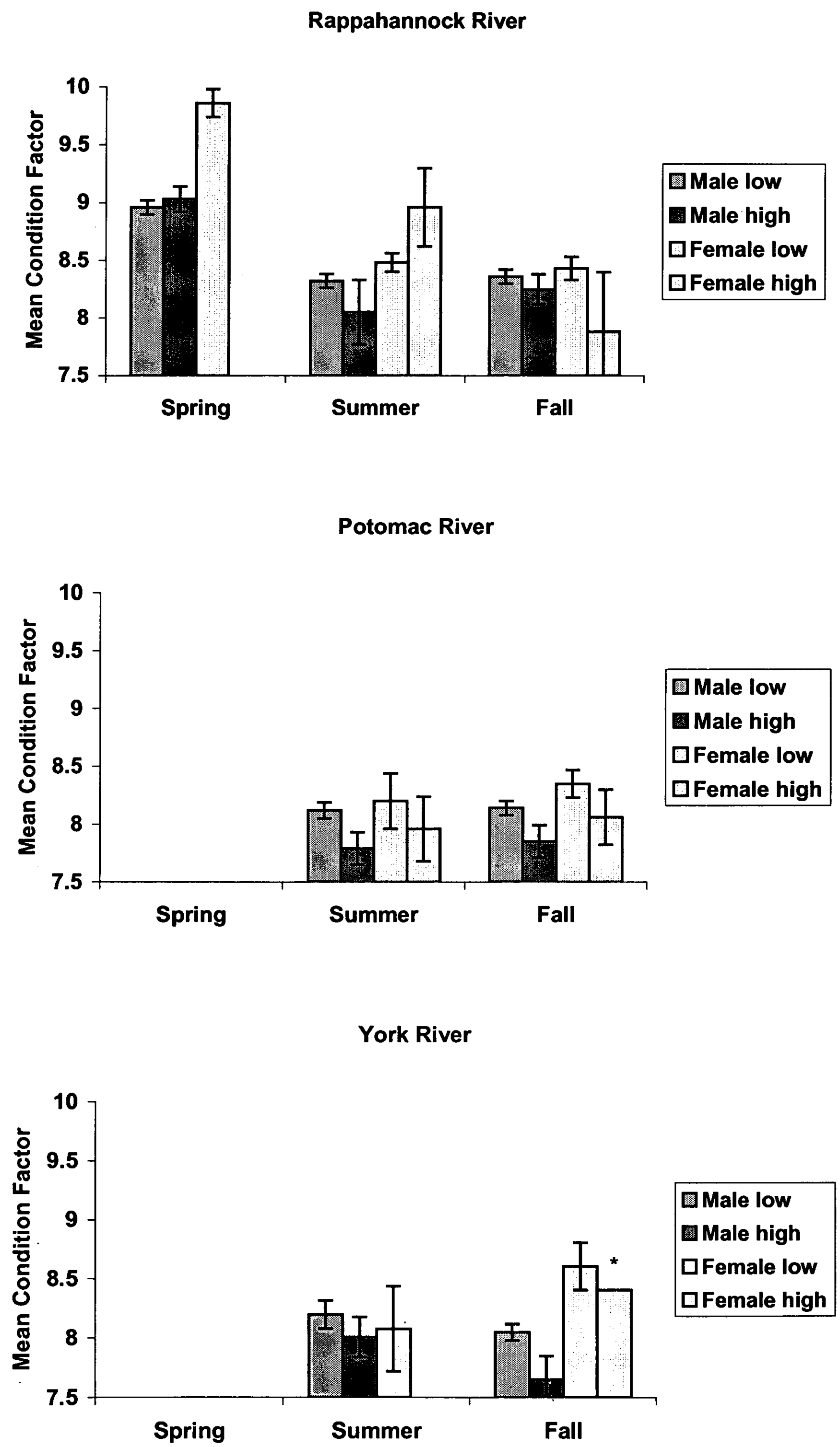

Rappahannock River
Mean Condition Factor
10
9.5
9
8.5
8
7.5
Spring
Summer
Fall
Male -
Male +
Female -
Female +
Potomac River
Mean Condition Factor
10
9.5
9
8.5
8
7.5
Spring
Summer
Fall
Male -
Male +
Female -
Female +
York River
Mean Condition Factor
10
9.5
9
8.5
8
7.5
Spring
Summer
Fall
Male -
Male +
Female -
Female +

Figure 18. Variation in condition factor with season, location, gender, and intensity of splenic mycobacteriosis in striped bass, *Morone saxatilis.*
(low = intensity of splenic mycobacteriosis of 0, 0.5, 1)
(high = intensity of splenic mycobacteriosis of 2, 3)
* *bars without standard errors only contained 1 sample (Table 2)*
(regression, r^2 = 12.1 ln condition factor = 8.10 + 0.722 fish from spring – 0.0139 fish from summer – 0.0065 fish from York + 0.227 fish from Rappahannock + 0.223 female fish - 0.185 fish with high splenic intensity).

Figure 18


Rappahannock River
Mean Condition Factor
10
9.5
9
8.5
8
7.5
Spring
Summer
Fall
Male low
Male high
Female low
Female high
Potomac River
Mean Condition Factor
10
9.5
9
8.5
8
7.5
Spring
Summer
Fall
Male low
Male high
Female low
Female high
York River
Mean Condition Factor
10
9.5
9
8.5
8
7.5
Spring
Summer
Fall
*
Male low
Male high
Female low
Female high

Appendix 1

When determining if Fulton's condition factor was suitable, the regression (Figure15) indicated that striped bass with a natural log of length larger than 6.5 tended to deviate above the regression line. Figure 19 supports this (regression, A) $n = 1832$, $r^2 = 0.05$, df = 1833 B) $n = 57$, $r^2 = 0.21$, df = 55); however, this is overlooked for this study because the fish constituting the deviation only account for 3% of the total, and the r^2 value for them is low.

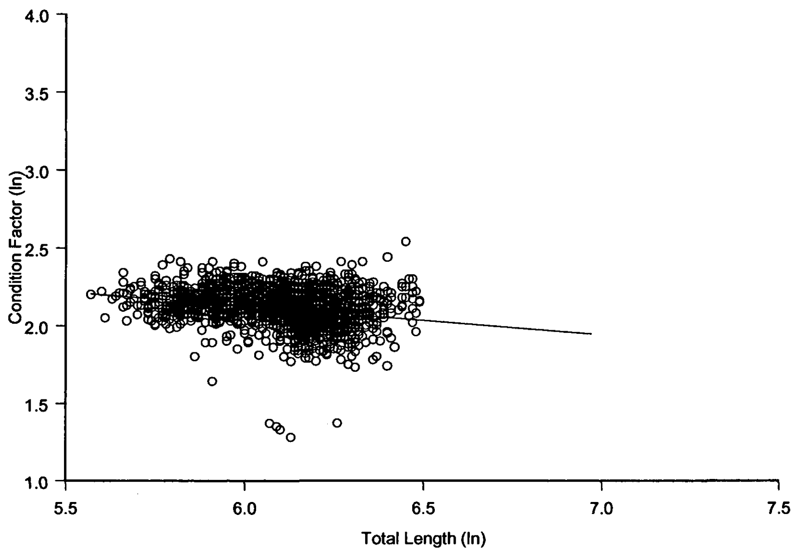
Figure 19. Linear regressions of natural log of condition factor with natural log of total length for striped bass, *Morone saxatilis,* separated into lengths. < 6.5 and lengths > 6.5

A) $Y = 3.23 - 0.184X$, $r^2 = 0.05$, $p = 0.000$, $df = 1$

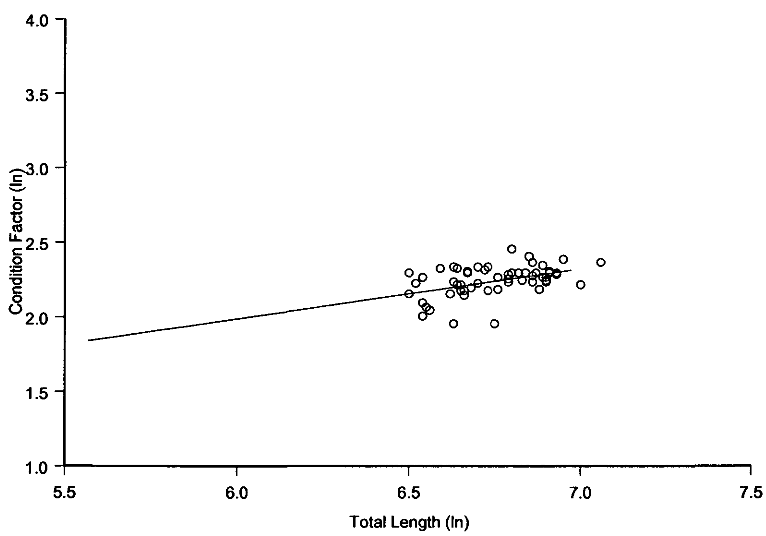
B) $Y = -0.02 + 0.334X$, $r^2 = 0.21$, $p = 0.000$, $df = 1$

Figure 19

A) Length < 6.5



A) Length > 6.5



Literature Cited

Abernethy, CS; Lund, JE (1978): Mycobacteriosis in mountain whitefish (*Prosopium williamsoni*) from the Yakima River, Washington. Journal of Wildlife Diseases 14, 333-337.

Adams, A; Thompson, KD; McEwan, H; Chen, SC; Richards, RH (1996): Development of monoclonal antibodies to *Mycobacterium* spp. isolated from chevron snakeheads and siamese fightingfish. Journal of Aquatic Animal Health 8, 208-215.

Agius, C (1985): The Melano-macrophage centers in fish: a review. In: Fish Immunology. Academic Press, London, 85-104.

Amlacher, E (1970): Textbook of fish diseases. TFH Publishing, Jersey City. 302 pages.

Anderson, DP (1990): Immunological indicators: effects of environmental stress on immune protection and disease outbreaks. American Fisheries Society Symposium 8, 38-50.

Armstrong, JA; Hart, D (1971): Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations on fusion of lysosomes with phagosomes. Journal of Experimental Medicine 134(3), 713-741.

Aronson, JD (1926): Spontaneous tuberculosis in salt water fish. Journal of Infectious Diseases 39, 315-320.

Atlantic States Marine Fisheries Commission (1981): Interstate management plan for the striped bass of the Atlantic coast from Maine to North Carolina. Fisheries Management Report of the Atlantic States Marine Fisheries Commission No. 1.

Austin, HM (1980): Biology of adult striped bass, *Morone saxatilis*. In: Marine Recreational Fisheries. Vol. 5. (Ed: Clepper, Henry) Sport Fishing Institute, Washington, DC, 125-140.

Bataillon, E; Dubard, R; Terre, L (1897): Un nouveau type de tuberculose. Compte Rendu des Seances de la Societe de Biologie et de ses Filiales 49, 446-449.

Beckwith, DG; Malsberger, RG (1980): Kidney tumour virus -- tumour or mycobacterial tubercle? Journal of Fish Diseases 3, 339-348.

Belas, R; Faloon, P; Hannaford, A (1995): Potential applications of molecular biology to the study of fish mycobacteriosis. Annual Review of Fish Diseases 5, 133-173.

Beurey, J; Weber, M; Vignaud, JM; Dailloux, M (1981): Mycobacteriosis cutanees: enquete epidemiologique. Annales de Dermatologie et de Venereologie 108, 439-442.

Bolan, G; Reingold, AL; Carson, LA; Silcox, VA; Woodley, CL; Hayes, PS; Highwater, AW; McFarland, L; Brown III, JW; Peterson, NJ; Favero, MS; Good, RC; Broome, CV (1985): Infections with *Mycobacterium chelonei* in patients receiving dialysis and using processed hemodialyzers. Journal of Infectious Diseases 152(5), 1013-1019.

Bolger, T; Connolly, PL (1989): The Selection of Suitable Indices for the Measurement and Analysis of Fish Condition. Journal of Fish Biology 34, 171-182.

Bozetta, E; Prearo, M; Penati, V; Pungkachonboon, T; Ghittino, C (1995): Isolamento etipizzazione di micobatteri in pesci tropicali d'allevamento. Bollettino Societa Italiana di Patologia Ittica 16, 13-21.

Bragg, RR; Huchzermeyer, HFAK; Hanisch, MAM (1990): *Mycobacterium fortuitum* isolated from three species of fish in South Africa. Onderstepoort Journal of Veterinary Research 57, 101-102.

Brooks, RW; Parker, BC; Gruft, H; Falkinham, JO, III (1984): Epidemiology of infection by nontuberculous mycobacteria. American Review of Respiratory Disease 130, 630-633.

Bullin, CH; Tanner, EI (1970): Isolation of *Mycobacterium xenopei* from water taps. Journal of Hygiene 68, 97-100.

Chinabut, S; Limsuwan, C; Chanratchakool, P (1990): Mycobacteriosis in the snakehead, *Channa striatus* (Fowler). Journal of Fish Diseases 13, 531-535.

Clark, FH; Shepard, CC (1963): Effect of environmental temperatures on infection with *Mycobacterium marinum* (Balnei) of mice and a number of poikilothermic species. Journal of Bacteriology 86, 1057-1069.

Colorni, A; Ankaoua, M; Diamant, A; Knibb, W (1993): Detection of mycobacteriosis in fish using the polymerase chain reaction technique. Bulletin of the European Association of Fish Pathologists 13(6), 195-198.

Cone, SR (1989): The Need to Reconsider the Use of Condition Indices in Fishery Sciences. Transactions of the American Fisheries Society 118, 510-514.

Conroy, DA (1966): Observaciones sobre casos espontaneos de tuberculosis ictica. Microbiologia Espanola 19, 1-21.

Coutant, C (1990) [a]: Temperature-oxygen habitat for freshwater and coastal striped bass in a changing climate. Transactions of the American Fisheries Society. 119, 240-253.

Coutant, C (1990) [b]: Summer habitat suitability for striped bass in Chesapeake Bay: reflections on a population decline. Transactions of the American Fisheries Society. 119, 757-778.

Dalsgaard, I; Mellersgaard, S; Larsen, JL (1992): Mycobacteriosis in cod (*Gadus morhua* L.) in Danish coastal waters. Aquaculture 107, 211-219.

Dulin, MP (1979): A review of tuberculosis (mycobacteriosis) in fish. Veterinary Medicine 74, 731-735.

du Moulin, GC; Stottmeier, KD; Pelletier, PA; Tsang, AY; Hedley-Whyte, J (1988): Concentration of *Mycobacterium avium* by hospital hot water systems. Journal of the American Medical Association 260(11), 1599-1601.

Earp, BJ; Ellis, CH; Ordal, EJ (1953): Kidney disease in young salmon. Special Report Series Number 1 State of WA Dept. of Fisheries and University of WA.

Falkinham, JO, III; Parker, BC; Gruft, H (1980): Epidemiology of infection by nontuberculous mycobacteria. American Review of Respiratory Disease 121, 931-937.

Field, JD (1997): Atlantic striped bass management: where did we go right? Fisheries 22(7), 6-8.

Frerichs, GN (1993): Mycobacteriosis: Nocardiosis. In: Bacterial diseases of fish. (Eds: Inglis,Valerie; Roberts, Ronald J; Bromage, Niall R) John Wiley & Sons, Inc., New York, 219-223.

Fryer, JL; Rohovec, JS (1993): Bacterial diseases of fish. In: Pathobiology of marine and estuarine organisms. (Eds: Couch, John A; Fournie, John W) CRC Press, Inc., Boca Raton, FL, 53-72.

George, KL; Parker, BC; Gruft, H; Falkinham, JO, III (1980): Epidemiology of infection by nontuberculosis mycobacteria II.Growth and Survival in natural waters. American Review of Respiratory Disease 122, 89-94.

Giavenni, R; Finazzi, M; Poli, G; Grimaldi, K (1980): Tuberculosis in marine tropical fishes in an aquarium. Journal of Wildlife Diseases 16(2), 161-168.

G¢mez, S (1998): Unusual morphopathological features in a case of fish tuberculosis. Journal of Fish Diseases 21, 237-239.

G¢mez, S; Bernab, A; G¢mez, MA; Navarro, JA; Sanchez, J (1993): Fish mycobacteriosis: morphopathological and immunocytochemical aspects. Journal of Fish Diseases 16, 137-141.

G¢mez, S; Navarro, JA; G¢mwz, MA; Sanchez, J; Bernab, A (1996): Comparative study of immunohistochemical methods to diagnose mycobacteriosis in swordtail *Xiphophorus helleri*. Diseases of Aquatic Organisms 24, 117-120.

Haeseker, Steven L; Carmichael, John T; Hightower, Joseph E (1996): Summer distribution and condition of striped bass within Albemarle Sound, North Carolina. Transactions of the American Fisheries Society 125, 690-704.

Harada, K (1977): Staining mycobacteria with periodic acid-carbol-pararosanilin: principle and practice of the method. Microscopica Acta 79(3), 224-236.

Hartman, KJ (1994): Similarities in production cycles and predator-prey relationships among clupeid-feeding piscivores in Chesapeake Bay and Lake Erie. 37th Conference of the International Association for Great Lakes Research and Estuarine Research Federation: Program and Abstracts. Buffalo, NY, IAGLR. 166.

Hartman, KJ; Brandt, SB (1995): Trophic partitioning, diets, and growth of sympatric estuarine predators. Transactions of the American Fisheries Society 124, 520-537.

Hastings, TS; MacKenzie, K; Ellis, AE (1982): Presumptive mycobacteriosis in mackerel (*Scomber scombrus* L.). Bulletin of the European Association of Fish Pathologists 2, 19-21.

Hatai, K; Lawhavinit, O (1993): Mycobacterium infection in pejerrey, *Odonthestes bonariensis* Cuvier & Valenciennes. Journal of Fish Diseases 16, 397-402.

Hedrick, RP; McDowell, T; Groff, J (1987): Mycobacteriosis in cultured striped bass from California. Journal of Wildlife Diseases 23(3), 391-395.

Hildebrand, SF (1928): Fishes of the Chesapeake Bay [by] Samuel F Hildebrand and William C Schroeder. 2nd ed. Smithsonian Institution Press, Washington.

Huminer, D; Pitlik, SD; Block, C; Kaufman, L; Amit, S; Rosenfeld, JB (1986): Aquarium-borne *Mycobacterium marinum* skin infection. Archives of Dermatology 122, 698-703.

Iivanainen, E (1995): Isolation of mycobacteria from acidic forest soil samples: comparison of culture methods. Journal of Applied Bacteriology 78, 663-668.

Jenkins, PA (1991): Mycobacteria in the environment. Journal of Applied Bacteriology Symposium Supplement 70, 137S-141S.

Jenkins, PA; Duddridge, LR; Yates, MD; Grange, JM (1992): Identification of pathogenic and environmental mycobacteria. Identification Methods in Applied and Environmental Microbiology 29, 311-325.

Kirschner, RA, Jr; Parker, BC; Falkinham, JO, III (1992): Epidemiology of infection by nontuberculous mycobacteria. American Review of Respiratory Disease 145, 271-275.

Kohlenstein, LC (1981): On the proportion of the Chesapeake Bay stock of striped bass that migrates into the coastal fishery. Transactions of the American Fisheries Society 110, 168-179.

Kohlhorst, DW (1975): The striped bass (*Morone saxatilis*) die-offs in the Sacramento-San Joaquin estuary in 1973 and a comparison of its characteristics with those of the 71 and 72 die-offs. California Department of Fish and Game, Anadromous Fisheries Branch Administration Report 73-7, Sacramento, CA. 21 pages.

Korn, S; Struhsaker, JW; Benville, P, Jr (1976): Effects of benzene on growth, fat content, and caloric content of striped bass. U.S. National Marine Fisheries Service Fisheries Bulletin. 74, 694-698.

Lambert, Y; Dutil, JD (1997): Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of Atlantic cod (*Gadus morhua*)? Canadian Journal of Fisheries and Aquatic Science 54, 104-112.

Lansdell, W; Dixon, B; Smith, N; Benjamin, L (1993): Isolation of several *Mycobacterium* species from fish. Journal of Aquatic Animal Health 5, 73-76.

Luna, LG (Ed.) (1968): Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd ed. McGraw-Hill, NY. 258 pages.

MacKenzie, K (1988): Presumptive mycobacteriosis in north-east Atlantic mackerel, *Scomber scombrus* L. Journal of Fish Biology 32, 263-275.

Majeed, SK; Gopinath, C (1983): Cutaneous tuberculosis in the carp, *Cyprinus carpio* L. Journal of Fish Diseases 6, 313-316.

Manooch III, CS (1991): Fisherman's guide to the fishes of the southeastern United States. 3rd ed. North Carolina State Museum of Natural History, Raleigh. 362 pages.

Massmann, WH; Pacheco, AL (1961): Movements of striped bass tagged in Virginia waters of Chesapeake Bay. Chesapeake Science 2, 37-44.

Mehrle, PM; Haines, TA; Hamilton, S; Ludke, JL; Mayer, FL; Ribick, MA (1982): Relationship between body contaminants and bone development in east coast striped bass. Transactions of the American Fisheries Society. 111, 231-241.

Murdy, EO; Birdsong, RS; Musick, JA (1997): Fishes of Chesapeake Bay. Smithsonian Institution Press, Washington. 324 pages. pp. 216-220.

Nigrelli, RF; Vogel, H (1963): Spontaneous tuberculosis in fishes and in other cold-blooded vertebrates with special reference to *Mycobacterium fortuitum* Cruz from fish and human lesions. Zoologica 48, 131-143.

Noga, EJ; Wright, JF; Pasarell, L (1990): Some unusual features of mycobacteriosis in the cichlid fish *Oreochromis mossambicus.* Journal of Comparative Pathology 102, 335-344.

Overton, AS; May, EB; Griffin, J; Margraf, JF; (2000): A bioenergetics approach for determining the effect of increased striped bass population on its prey and health in the Chesapeake Bay. Final report to: Maryland Department of Natural Resources, Fisheries Service. Annapolis, MD.

Papapetropoulou, M; Tsintzou, A; Vantarakis, A (1997): Environmental mycobacteria in bottled table waters in Greece. Canadian Journal of Microbiology 43, 499-502.

Parisot, TJ; Wood, EM (1960): A comparative study of the causative agent of a mycobacterial disease of salmonoid fishes. American Review of Respiratory Disease 82, 212-222.

Pederson, T; Jobling, M (1989): Growth rates of large, sexually mature cod, *Gadus morhua*, in relation to condition and temperature during an annual cycle. Aquaculture 81, 161-168.

Pieper, L; Weedon, C; May, E (1999): Ulcerative dermatitis in striped bass a concern or problem for the Chesapeake Bay. American Fisheries Society 129th Annual Meeting.

Pyecroft, SB (1994): Relationship between experimental mycobacteriosis and monogenean infections on gills of the atherinoid fish *Melanotaenia duboulayi.* Bulletin of the European Association of Fish Pathologists 14(4), 124-127.

Rhodes, Martha (2001): Personal communication. Virginia Institute of Marine Science. Gloucester Point, VA.

Ross, AJ (1970): Mycobacteriosis among Pacific salmonid fishes. In: Symposium on Diseases of Fishes and Shellfishes. (Ed: Snieszko,SF) American Fisheries Society, Washington, 270-283.

Ross, AJ; Brancato, FP (1959): *Mycobacterium fortuitum* Cruz from the tropical fish *Hyphessobrycon innesi*. Journal of Bacteriology 78, 392-395.

Sakanari, JA; Reilly, CA; Moser, M (1983): Tubercular lesions in Pacific coast populations of striped bass. Transactions of the American Fisheries Society 112, 565-566.

Secor, DH (1999): Specifying divergent migrations in the concept of stock: the contigent hypothesis. Fisheries Research 43, 13-34.

Scientific and Technical Advisory Committee of the Chesapeake Bay Program (1998): Trophic changes in Chesapeake Bay open water habitat: a workshop to evaluate and interpret recent trends. Solomons, MD.

Sokal, RR; Rohlf, FJ (1981): Biometry. W.H. Freeman and Company. San Fransisco, CA. 859 pages.

Stokes, ME; Davis, CS; Koch, GG (1995): Categorical data analysis using the SAS system. SAS Institute Inc., Cary, NC. 499 pages.

Talaat, AM; Reimschuessel, R; Trucksis, M (1997): Identification of mycobacteria infecting fish to the species level using polymerase chain reaction and restriction enzyme analysis. Veterinary Microbiology, 1-9.

Teska, JD; Twerdok, LE; Beaman, J; Curry, M; Finch, RA (1997): Isolation of *Mycobacterium abscessus* from Japanese medaka. Journal of Aquatic Animal Health 9, 234-238.

Timur, G; Roberts, RJ; McQueen, A (1977): The experimental pathogenesis of focal tuberculosis in the plaice (*Pleuronectes platessa* L.). Journal of Comparative Pathology 87, 83-87.

Tortoli, E; Bartoloni, A; Bozzetta, E; Burrini, C; Lacchini, C; Mantella, A; Penati, V; Simonetti, MT; Ghittino, C (1996): Identification of the newly described *Mycobacterium poriferae* from tuberculous lesions of snakehead fish (*Channa striatus*). Comparative Immunology and Microbiology of Infectious Diseases 19(1), 25-29.

Van Duijn, C, Jr (1981): Tuberculosis in fish. Journal Small Animal Practice 22, 391-411.

Wayne, LG; Kubica, GP (1986): Family Mycobacteriaceae. In: Bergey's manual of systematic bacteriology. Vol. 2. (Eds: Sneath,PHA; Mair,NS; Sharpe,ME) Williams and Wilkins, Baltimore, 1436-1457.

Winsor, H (1946): Cold-blooded tuberculosis from the Fairmont Aquarium, Philadelphia. Proceedings of the Pennsylvania Academy of Science, 20, 43-46.

Wirgin, II; Waldman, JR; Maceda, L; Stabile, J; Vecchio, VJ (1997): Mixed-stock analysis of Atlantic coast striped bass *Morone saxatilis* using nuclear DNA and mitochondrial DNA markers. Canadian Journal of Fisheries and Aquatic Sciences 54(12), 2814-2826.

Witebsky, FG; Kruczak-Filipov, P (1996): Identification of mycobacteria by conventional methods. Clinical Mycobacteriology 16(3), 569-601.

Wood, JW; Ordal, EJ (1958): Tuberculosis in Pacific salmon and steelhead trout. Fish Commission of Oregon 25, 1-38.

Zar, JH (1996): Biostatistical Analysis. 3rd ed. Prentice-Hall, Inc, Upper Saddle River. 662 pages.

Vita

Jennifer Lynn Cardinal

Born in Tell City, Indiana, 29 September 1975. Graduated from Conway High School in Conway, South Carolina in 1993. Earned B.S. in marine science and biology from Coastal Carolina University in Conway, South Carolina in 1997. Entered M.S. program at the Virginia Institute of Marine Science in fall of 1997.