

The Role of Mycobacteriosis in Elevated Natural Mortality of Chesapeake Bay Striped Bass: Developing Better Models for Stock Assessment and Management

A Final Report

To:

**Chesapeake Bay Office
National Oceanographic and Atmospheric Administration**

By:

**Wolfgang Vogelbein, John Hoenig, David Gauthier, Matthew Smith,
Phil Sadler, Howard Kator, & Martha Rhodes**

**Virginia Institute of Marine Science
The College of William and Mary
Route 1208
Gloucester Point, Virginia 23062
And
Old Dominion University
Norfolk, Virginia**

Award Number: NA06NMF4570295

Award Period: 1 Sept., 2006 to 31 Dec., 2011

20 August, 2012

TABLE OF CONTENTS

Executive Summary	3
Introduction	4
Study Objectives	6
Materials and Methods	6
Results	15
Objective 1.....	15
Objective 2.....	17
Objective 3.....	18
Objective 4.....	20
Discussion	21
References Cited	24
Scientific Products	27
Peer-reviewed Publications.....	27
Invited Presentations.....	28
Contributed Presentations.....	29
Tables	31
Figures	39

EXECUTIVE SUMMARY

Mycobacteriosis is a chronic systemic disease of fishes caused by bacteria belonging to the genus *Mycobacterium*. The disease currently affects striped bass throughout Chesapeake Bay and prevalence is higher than 90% in certain age groups. Two recently described species, *M. shottsii* and *M. pseudoshottsii*, are the most common isolates obtained from diseased fish and are considered the primary etiologic agents. Recent indications that natural mortality (M) has become elevated in Chesapeake Bay striped bass and that mycobacteriosis may be the underlying cause, has caused concern among fishermen, fisheries managers and scientists. However, fundamental questions, such as transmission mode, duration of disease states, impacts of disease on fish movements, feeding and reproduction remain unanswered. A central unanswered question is whether the disease causes mortality. Therefore, the aim of this study was to develop new approaches for estimating the contribution of mycobacteriosis to natural mortality in the striped bass (e.g., disease-associated mortality component of M). Based on extensive histopathological analyses of skin and spleen, we identified a small focal lesion we have called the pigmented focus, as the incipient mycobacterial skin lesion and showed that it develops into ulcers over time. Our bacteriological and molecular analyses indicated a strong relationship between *Mycobacterium shottsii* and dermal pathology. An extensive histological survey indicated that disease prevalences (both dermal and splenic) have been extremely high in Rappahannock River striped bass (60 to >95%) but stable over the course of this study (2005-2010). Evaluation of dermal and splenic disease severities showed that ~29% of the fish exhibit moderate to severe disease, whereas the majority of affected fish exhibited only mild disease. Based on our better understanding of dermal pathology and mycobacterial disease dynamics, we developed a gross “Skin Lesion Severity Index” and used it as a non-lethal estimator of overall mycobacterial disease status in bass. We applied this Index in a tag-recapture program in the Rappahannock River, VA to estimate relative survival rates of striped bass with and without mycobacterial skin disease, estimate population level effects, and determine rates and characteristics of dermal disease progression using mathematical modeling. Since fall 2005, 21,261 striped bass have been tagged and released. Additionally, Maryland DNR tagged 4,658 striped bass in Maryland waters during 2007-2010. Relative survival rates of fish released in disease severity stage 0 (no dermal disease) and stage 1 (mild disease) did not differ significantly. However, survival rates of fish released in disease severity stages 2 (moderate disease) and 3 (severe disease) were significantly different from stage 0 fish and estimated to be 0.79 and 0.56, respectively. Disease severity-specific associated increases in natural mortality rate were estimated at 0.02, 0.24 and 0.58 for disease severity stages 1, 2, and 3, respectively. With an assumed natural mortality rate of 0.15 for disease severity stage 0 fish, the adjusted natural mortality rates for the disease severity stages were 0.15, 0.17, 0.39 and 0.73 for disease severity stage 0, 1, 2 and 3, respectively. Using tag recaptures, we estimated duration of disease state 1 to be on average, 418 days (*s.e.* 19 days) and state 2 to last 609 days (*s.e.* 57 days). Of all 665 recovered fish that were released in a disease positive severity stage, only 54 (8.1%) showed reversion of dermal disease to a lower severity stage through regeneration of skin and scales. Using simulation analysis, we estimated a 16% overall reduction in striped bass abundance due to mycobacteriosis. We suggest that this reduction may not be large enough to cause alarm for the Chesapeake Bay commercial striped bass fishery. However, the estimated reductions in older fish (~50% ages 7 and 8) may have the potential to impact the charter and recreational fisheries that target these larger fish. Finally, the effects of the disease on the migratory component of the stock are presently unknown.

INTRODUCTION AND OBJECTIVES

During the late 1990s concern emerged among recreational and commercial fishermen about declining condition in Chesapeake Bay striped bass (*Morone saxatilis*). Emaciation and ulcerative skin lesions were commonly reported and first associated with a chronic bacterial disease called mycobacteriosis in the late 1990's (Vogelbein et al., 1999). The disease is now epizootic throughout the Bay with up to 98% of striped bass in some age classes, tributaries and certain time periods affected. Several hypotheses have emerged to explain the causes of this infectious disease. These include stress associated with reductions in the food forage base (starvation) due primarily to declines in menhaden stocks and the loss of summer thermal refuges as a result of eutrophication and hypoxia/anoxia in the deeper cooler portions of the Bay. The latter has been hypothesized to force striped bass into sub-optimal warmer waters during the summer, with the resulting stress making them more susceptible to the disease.

Mycobacteriosis in fishes is a chronic systemic disease caused by various species of bacteria comprising the genus *Mycobacterium* that often results in loss of body condition and ultimately in death (Colorni 1992). Mycobacteria are slow-growing, aerobic bacteria common in terrestrial and aquatic habitats. Most are saprophytes, but certain species infect both endo- and poikilothermic animals. Mycobacterial disease occurs in a wide range of species of fishes worldwide and is an important problem in aquaculture. Clinical signs are nonspecific and may include scale loss, skin ulceration, emaciation, exophthalmia, pigmentation changes and spinal defects (Nigrelli & Vogel 1963; Bruno et al. 1998). Granulomatous inflammation, a host cellular response comprised largely of phagocytic cells of the immune system called macrophages, is a characteristic of the disease. In an attempt to sequester, kill and degrade mycobacteria, macrophages encapsulate bacteria, forming nodular structures called granulomas. Skin ulceration is uncommon in most fishes and usually represents the endstage of the disease process, as captive fish with skin lesions generally do not recover and die quickly.

Mycobacteriosis was first reported from Chesapeake Bay striped bass in 1997 (Vogelbein et al. 1999). Since then, the disease has been observed to affect striped bass throughout the Bay and prevalence may be higher than 90% in certain age groups (Cardinal 2001; Vogelbein et al. 1999; Gauthier et al., 2008). Several species of *Mycobacterium* have been isolated from Chesapeake Bay striped bass, with the recently described species *M. shottsii* and *M. pseudoshottsii* being the predominant disease-associated organisms (Rhodes et al. 2002, 2003, 2004; Gauthier and Rhodes, 2009). However, a broad suite of, as yet, undescribed strains and species have also been isolated from diseased striped bass (Gauthier et al. 2011).

The impact of the disease on the population ecology of striped bass is poorly understood. Fundamental questions, such as mode of transmission, duration of disease stages, effects of disease on fish movements, feeding and reproduction, and mortality rates associated with disease, remain unanswered. Nonetheless, there are indications the disease may be having a significant impact on Chesapeake striped bass populations. Jiang et al. (2007) analyzed striped bass tagging data from Maryland and found evidence for a

significant increase in natural mortality rate at about the time when mycobacteriosis was first being detected in Chesapeake Bay striped bass. A similar analysis of Rappahannock River, Virginia, striped bass tagging data from this project also reveals an increase in natural mortality rate in recent years: natural mortality rate for fish age 2 and above was estimated to increase from $M = 0.231$ during the period 1990 – 1996 to $M = 0.407$ during the period 1997-2004. In addition, recent application of force-of-infection models to examine the epizootiology of mycobacteriosis in Chesapeake Bay striped bass provided first evidence of disease associated mortality (Gauthier et al. 2008a).

Based on experience gained from aquaculture, mycobacteriosis in fishes is generally fatal, but this has not been definitively established for wild fishes, including striped bass. Three possible distinct disease outcomes in the case of striped bass are: 1) death, 2) recovery or reversion to a non-disease state, or 3) movement of infected fish to another location. Because of the uncertainty about the fate of the infected fish, the true impacts of the disease on striped bass populations remain largely unknown. If mycobacteriosis in striped bass is ultimately fatal, the potential for significant impacts on the productivity and the quality of the Atlantic coastal migratory stock is high. At a recent symposium entitled “*Management Issues of the Restored Stock of Striped Bass in the Chesapeake Bay: Diseases, Nutrition, Forage Base and Survival*”, Kahn (2004) reported that both Maryland and Virginia striped bass tag-recaptures have declined in recent years. This suggests that survival has declined significantly, from 60-70% in the early-mid 1990’s to 40-50% during the late 1990’s and early 2000’s. Kahn (2004) and Crecco (2003) both concluded that the 20% decline in striped bass survival was not caused by fishing mortality, but rather, by an increase in natural mortality. These analyses, however, are predicated on the assumption that tag reporting rate has not changed over time. No data are currently available to evaluate this assumption. Hypotheses presented at the Symposium to explain the decline in striped bass survival included the possible role of mycobacteriosis (May et al., 2004; Vogelbein et al., 2004). However, Jacobs et al. (2004) found that decline in striped bass nutritional status during the fall was independent of disease. Uphoff (2004) reported that abundance of forage-sized menhaden, a primary food source of striped bass, declined to near historic lows during the mid-1990’s. Similar studies indicated that as the striped bass population has increased during the 1990’s, predatory demand increased coincident with a decline in menhaden populations (Hartman, 2004; Garrison et al., 2004).

Striped bass are presently managed by attempting to control fishing mortality. Fishing mortality is determined in three ways, and each method uses a value for natural mortality rate based on the assumption that natural mortality does not change over time. This is done because of the difficulty in estimating natural mortality rate. If natural mortality has increased over time, and if these increases have not been quantified, then estimates of fishing mortality will be wrong. Thus, there is the real potential of mismanaging the fishery. This is not just of theoretical concern – for the last several years the Atlantic States Marine Fisheries Commission’s Striped Bass Technical Committee and Subcommittees have struggled with the problem that the total mortality rate appears to have gone up despite the fact that the fishing regulations have been stable. But information on whether diseases may be elevating the natural mortality rate is scarce and largely circumstantial (indirect) or anecdotal. To date, no one has quantified the effects of the disease on striped bass survival rate. Indeed, to our

knowledge, quantitative estimates of infectious disease impacts on population dynamics have not been incorporated in the management plan of any marine finfish species.

The overall aim of this study therefore, was to determine the contribution of mycobacteriosis to natural mortality in the striped bass (e.g., the disease associated mortality portion of M). We have addressed this important question by designing and conducting a new striped bass tagging program in which we examined relative tag return rates of striped bass exhibiting external clinical signs of mycobacteriosis vs those that show no external signs of the disease. We hypothesized that the gross skin lesions caused by mycobacteriosis are pathognomonic (i.e., distinctively characteristic of the disease) and that they can be used as a non-lethal diagnostic of overall disease status in Rappahannock River striped bass. We therefore proposed the following study objectives:

1. To develop and validate (Year 1) gross skin lesion appearance as a non-lethal diagnostic test for the visceral disease. We will evaluate the temporal spectrum of gross skin lesions as predictors of the visceral disease, and determine the positive and negative predictive values of various forms of skin lesions for the presence of visceral disease.
2. Determine the species of mycobacteria associated with visceral and dermal disease in striped bass by traditional bacteriological methods and by newly developed molecular approaches, and integrate these measures with gross pathology and histopathology in order to examine the relationship between visceral and dermal disease.
3. To apply the non-lethal diagnostic test (gross skin pathology) to the VIMS Striped Bass tagging program in order to estimate relative survival rates of fish with and without skin disease, as well as fish with and without visceral disease as predicted by the skin lesion diagnostic. This information will be used in the future to develop predictive models that incorporate disease data in more accurate determinations of natural mortality (M).
4. To determine rate and characteristics of visceral and dermal disease progression as well as mortality rates of fish displaying dermal lesions using fish held in net pens over rising (Spring) and falling (Fall) temperature regimes.

MATERIALS & METHODS

Fish Capture and Tagging Protocols

During spring and fall of each year, striped bass were purchased from commercial fishermen operating five pound nets in the upper Rappahannock River (river miles 45-56, north of Tappahannock, VA) and five nets in the lower River (river miles 0-3). The pound net is a fixed fish trap that is presumed to be non-size selective in its catch of striped bass, and has been historically used by commercial fishermen in the Rappahannock River.

Captured striped bass were removed from the pound nets and placed by the fishermen into a floating holding pocket anchored adjacent to the pound net. Fish were dip-netted from the holding pocket, brought aboard individually, and processed for tagging and release. Fork length (FL) and total length (TL) measurements were recorded and a small sample of scales from between the dorsal fins and above the lateral line on the left side was removed to estimate age. Striped bass not previously marked and larger than 458 mm TL were tagged

with sequentially numbered internal anchor tags (Floy Tag and Manufacturing, Inc.), with the tag button inserted through a small incision made with a curved blade scalpel into the abdominal cavity. Each fish was then given a thorough external disease assessment by a pathologist (Vogelbein, Gauthier), skin disease severity was scored and recorded for both sides of the fish (see below for assessment scale), after which the fish was photographed on both sides (8 high-quality digital photographs/fish). The incision was then treated with Betadine followed by release of the fish at site of capture.

The tags used in this study were identical to the tags issued by the U. S. Fish and Wildlife Service except that they were lime green in color and had REWARD and a VIMS phone number imprinted into them. We coordinated with the Virginia Marine Resources Commission (VMRC), the Potomac River Fisheries Commission (PRFC) and the Maryland DNR to put in place fishing exemptions so that fishers could have fish with our special green tag in their possession at any time of year without penalties. We then advertised this program extensively through various media outlets in an effort to boost our tag return rates. We offered significant rewards of \$5 for recapture information and \$20 for returning the entire specimen, on ice, to VIMS personnel.

During the first year of the project Maryland DNR investigators (Mark Matsche) responsible for the striped bass tagging program in Maryland expressed strong interest in establishing an identical disease/tagging program in Maryland waters and implementing our methods and approaches already in place. During spring 2007 we initiated efforts to standardize striped bass tagging programs across Maryland and Virginia waters. We took MD DNR personnel (Mark Matsche, Larry Pieper) in the field and trained them in our approach to tagging, skin lesion nomenclature, disease severity assessment and photographic documentation. Maryland subsequently adopted our approaches and conducted an essentially identical disease study as ours. Arrangements were made where any fish tagged in Maryland waters but captured by us were returned to them and vice versa. These efforts have strengthened both programs and have provided added value to this study. It has allowed us to increase the number of tag/releases over time and has resulted in a significant increase in the number of tag returns as well as provided even greater spatial and temporal coverage to the project.

Pathology of Dermal and Splenic Mycobacteriosis in Striped Bass

To provide a solid foundation for development of a non-lethal disease diagnostic using the external clinical signs of mycobacteriosis as a surrogate of overall disease status, we undertook a thorough histopathologic investigation of striped bass skin and splenic mycobacterial disease. To better characterize the spectrum of incipient (earliest) to the most advanced changes in the skin attributable to mycobacterial infection and to better understand the relationship between dermal and visceral expression of disease, we histologically evaluated 185 specific skin lesions obtained from 163 striped bass.

Disease Dynamics

Concurrent with spring and fall tagging efforts each year, we collected random samples of striped bass for histological analyses in an effort to better understand mycobacterial disease dynamics within the Rappahannock River over broad spatial and temporal scales. We initially proposed to sample 500 fish for histology per season. However, our level of funding for year 1 was significantly lowered (24%, \$34,000). We therefore requested a reduction in the scope work (Aug. 24, 2006). We proposed to reduce some of our costs by collecting and histologically processing 400 instead of 1000 fish per year (e.g., Fall: 100 upriver, 100 downriver; Spring: 100 upriver, 100 downriver). Given our preliminary results from the prior year (05-06: funded by Virginia Marine Resources Commission and Sea Grant) and a prospective statistical analysis to identify required sample sizes, we determined that we would be able to accomplish all of our objectives using a reduced number of animals without adversely impacting the quality of results.

Histology

Fish were randomly sampled from the floating holding pocket, brought on board individually, measured (TL, SL), evaluated for external disease severity (see below), marked with a uniquely numbered mouth tag, and placed on ice. A random sample of approximately 100 fish was transported to VIMS and necropsied over the subsequent two days. Fish were measured (total and eviscerated weight), and skin samples with skin lesions and the spleen were removed and placed in Z-fix fixative. Sub-samples of selected skin lesions and spleens were collected aseptically in a BSL-2 cabinet and processed for routine bacteriological and molecular identification (see below). Tissues placed in Z-fix were processed by routine methods for paraffin histology (Prophet et al., 1992). Briefly, following fixation for a minimum of 48 hours, tissues were rinsed overnight in running tap water, decalcified (skin), and stored in 70% ethanol. To quantify disease severity, spleens were subsequently sliced transversely into six equal portions with a single-edged razor blade and placed in a histological cassette so that six transverse sections of each spleen could be analyzed simultaneously using a single histologic slide (for larger fish 2-3 slides were necessary). To avoid generating sections immediately adjacent to one another in sequential tissue pieces, each of the six tissue blocks was trimmed to remove about 0.5mm of tissue from each of the surfaces to be sectioned prior to placement into the tissue cassettes. This approach offered significantly better sampling coverage than the single section that is typically examined for this type of analysis. This approach also minimized false-negatives for visceral lesion presence and improved estimates of predictive values. Once cassetted, tissues were dehydrated in a graded ethanol series, cleared in xylene and infiltrated in melted paraffin in a Shandon Hypercenter tissue processor. They were then embedded on a Tissue Tek tissue embedding center, sectioned at 5 μ m on a rotary microtome and stained with Harris hematoxylin and eosin. Selected sections displaying granulomas were stained for the presence of acid-fast bacteria (mycobacteria) by the Ziehl-Nielsen method.

Disease Severity Estimations

Skin Lesions: A “Skin Lesion Severity Index” was devised, based on a histopathological evaluation (See Histopathology Results) of 160 characteristic skin lesions commonly

observed on Chesapeake Bay striped bass. Each tagged striped bass was examined carefully and a semi-quantitative external disease assessment was made followed by photography (Canon EOS 30D camera) at the time of tag-release. Overview and close-up photos were taken of both lateral surfaces to document the initial assessment and to provide a basis for comparison when project personnel obtained recaptured striped bass. We identified 3 discrete skin lesion categories that we consider to be pathognomonic (characteristic) of mycobacteriosis in striped bass:

Pigmented focus (PF): $\sim 1\text{mm}^2$ pale to dark brown focus

Ulceration (U): Loss of multiple adjacent scales with erosion/excavation of underlying tissue. Hemorrhage present or absent. Pigmentation present or absent.

- scale damage or extensive loss of scales
- range of severity: single small ulcers to multi-focal, coalescing ulcers occupying large portions of the body.

Putative Healing (H): Hyper-pigmented, (may not be apparent in ventral lesions). Scales present, but incomplete, malformed or abnormally organized.

To obtain semi-quantitative estimations of skin disease severity, we ranked severity from 0 to 3 (PF) or 0-4 (U and H) according to the number of pigmented foci or the number and/or size of ulcers or healing lesions. A Skin Lesion Severity Index was designated as:

Clean (DS 0): No external PFs or ulceration noted.

Light (DS 1): Less than 10 PFs and/or one small ($< 2\text{ cm}^2$) ulcer on one or both sides.

Moderate (DS 2): 10-50 PFs and/or multiple small ulcers on one or both sides.

Heavy (DS 3): > 50 PFs and/or one or more large ulcers ($> 2\text{ cm}^2$) on one or both sides.

Other (DS 4): One or more healing ulcers or areas of scale damage evident.

This semi-quantitative skin pathology diagnostic has allowed distinction between diseased and healthy fish and to estimate relative disease severity in the context of the tagging program. This is critical as a non-lethal diagnostic test (e.g., blood test) is not available for this disease in fishes. Using this diagnostic, the impacts of the disease were evaluated through differential tag return rates. Survival rates of fish with pathognomonic skin pathology were compared to survival rates of fish without mycobacterial skin pathology. This has allowed us to provide better estimates of components of natural mortality (M) and provide inputs for future multi-species modeling efforts (See Products below).

Splenic Disease: Splenic disease severity (# of granulomas/ mm^2 of splenic tissue in histologic section) was estimated using splenic tissue sections and Metamorph Image Analysis software interfaced with an Olympus AX-70 photomicroscope. Briefly, following fixation in Z-fix, spleens were divided transversely into 6 equal portions and randomized. Prior to placement into 1-3 tissue

cassettes (depending on spleen size), each piece was trimmed at least 0.5 mm on the downward facing (sectioned) face in order to avoid serial sections from adjacent pieces of spleen. Following embedment in paraffin by routine methods (see above), tissues were step-sectioned to yield two sections per slide with a 50 μm step between the two sections. Granulomas were then counted in the 6 tissue sections obtained from each spleen. Mycobacterial granuloma was defined as any nodular lesion containing epithelioid cells and not containing an obvious non-mycobacterial eliciting agent (e.g., metacercaria, microsporidians, etc). Granulomas with confluent necrotic cores were counted as single lesions. If granulomas were found in a section, lesions were counted in all 6 tissue sections and a total count obtained. Low-power digital photomicrographs were then obtained of all 6 tissue sections and total sectional area was measured using image analysis software (Metamorph). A disease severity score was obtained by dividing total number of granulomas (from the 6 tissue sections) by the corresponding area of the 6 tissue sections to obtain a Severity Index ($\text{SI} = \text{granulomas}/\text{mm}^2$ spleen tissue) between 0.001 and 20. Lightly affected fish fell within SI values from 0.001-0.1, moderate disease severities ranged from 0.1-2 and severe splenic disease ranged from 2-20 SI values.

Bacterial Identification: Culture & Molecular Analysis

Culture Methods: Bacteriology was performed on spleen samples aseptically collected from striped bass captured in the Rappahannock River (upriver sites) during fall, 2006. Bacteriological methods followed the procedures outlined in Rhodes et al. (2004) without decontamination steps. Colonies growing on Middlebrook Agar (MDA) were identified as *M. pseudoshottsii* or *M. shottsii* when possible based on biochemical and phenotypic criteria, and additional acid-fast isolates were archived for future study (see, Gauthier et al. 2011).

DNA Extraction: Striped bass spleens were dissected aseptically and fixed in 95% ethanol. Grossly visible skin lesions were excised with a wide margin from striped bass and fixed in 95% ethanol. Negative skin samples were sampled in a similar manner from areas in which no skin lesions were visible. Prior to DNA extraction, spleen samples and skin lesions were dissected to 5-10 mg pieces, including affected dermal layers for the latter. Extraction was performed as described (Gauthier et al. 2008b) with a DNeasy Tissue Kit (Qiagen, Valencia, CA), using a modification of the manufacturers protocol. Briefly, samples were digested with Proteinase K overnight at 55°C, followed by three cycles of freeze/thaw in liquid nitrogen and a 70°C water bath. Samples were then held at 95°C for 5 min prior to final lysis in buffer AL and processed according to kit directions.

Nested PCR/RFLP Analysis: Extracts from spleen and skin samples were assayed for the presence of mycobacterial DNA as described (Gauthier et al. 2008b). Briefly, a primary amplification was performed with primers m23.12f/11r followed by genus-level secondary amplification with primers m23.27f/27r and sub-genus level secondary amplification with primers m23.8f/8r1. The latter primer set is specific to *M. pseudoshottsii*, *M. shottsii*, and *M. marinum*. Amplification products of m23.8f/8r1 reactions were digested with restriction enzymes *HinF* I and *Hpa* I as described. In addition to nested PCR, DNA extracted from skin samples was also tested for the presence of IS2404 (present in *M. pseudoshottsii*) with primer set m2404.7f/7r.

Fish Aging

Scales for fish age estimation were obtained from the left lateral surface between the spinous and soft dorsal fins above the lateral line, using the method established by Merriman (1941), except that impressions made in acetate sheets replaced the glass slide and acetone. In order to be consistent with ageing techniques of other agencies, all striped bass were considered to be one year older on 1 January of each year.

Statistical Analyses/Modeling

Estimating Relative Survival: The ratios of the survival rates of animals released with signs of disease (disease severity stages 1, 2, & 3) to those released with no dermal signs of disease (disease severity stage 0) were estimated using logistic regression. Separate models were run for disease severity stages 1, 2, and 3 in order to obtain severity based estimates of disease-associated mortality. Each model run used the same group of disease severity stage 0 fish as the baseline from which estimates of disease-associated reductions in survival were obtained. Since all cohorts were released in the fall at approximately the same time, we collapsed the tagging data over years and tagging program (Maryland and Virginia) to have a parsimonious model. Due to the close association of the Virginia tagging program with commercial fishing gear, recaptures occurring within seven days of release were omitted from the dataset. This was done to allow time for dispersal and mixing of tagged animals as well as to reduce the effects of any differential tagging-induced mortality. All of the Maryland recapture data were included in the analyses. The logistic model has the following form:

$$\ln(\text{PR}(\omega_i = 1)/\text{PR}(\omega_i = 0)) = \alpha + \beta * \text{YAL}_i + \varepsilon_i,$$

where ω is an indicator of disease state at the time of release (positive = 1 or negative = 0), YAL is the years at liberty of fish, α is the estimated intercept, β is the estimated slope of the logistic regression and ε is the error term. The β parameter estimates the force of infection (difference in the total instantaneous mortality rates of the two cohorts). Exponentiation of β estimates relative survival (ratio of survival rates) of the disease positive group to the disease negative group. Ninety-five percent confidence intervals were calculated using a profile likelihood method for each relative survival estimate. Logistic models are widely applied standard methods for the fitting and interpretation of these models exist (see Hoenig et al. 1990, Hueter et al. 2006). For the above analysis to be valid, it is necessary to assume that the ratio of tag reporting rates for the two groups remains constant over time but not that the reporting rates for the two groups are equal or that the rates are unchanging. All analyses were done using functions available in the base packages of program R.

Estimating Population Level Natural Mortality Rate: We assumed that the instantaneous rate of fishing mortality is equal for all disease categories. Therefore the relative survival estimates represent changes in survival due to disease-associated changes in natural mortality rate (M) based on the following relationship:

$$\Delta M_i = -\log_e(\text{RS}_i); \quad i = 1, 2, \& 3$$

where ΔM_i is the change in natural mortality rate (ΔM) associated with disease severity stage i and RS_i is the relative survival estimate of disease severity stage i with $\Delta M_0 = 0$. To calculate absolute disease category-specific natural mortality rates we added the appropriate ΔM_i to the assumed natural mortality rate for disease negative fish of 0.15. A rate of 0.15 has historically been used as the striped bass natural mortality rate, however, its accuracy is unknown especially for lower age classes where it is likely an underestimate (see Jiang et al. 2007). Using the disease category-specific M values and the cross sectional disease prevalence data we estimate the age-independent population level natural mortality rate as:

$$M_{pop} = \sum_{i=0}^3 P_i M_i$$

where P_i is the prevalence of disease severity stage i and M_i is the natural mortality rate for disease severity stage i , and the subscript 0 refers to animals without signs of disease. The cross sectional disease prevalence estimates (P_i) were obtained by pooling data across years and tagging programs.

Modeling Impact of Disease on Study Population: The percentage reduction in population size N due to disease is $100(1 - N_{with}/N_{without})$ where the subscript refers to whether disease is present or absent. We assumed constant survival rates over time and age. The population size of animals \geq age 3 in the absence of disease was computed by projecting a cohort of animals forward from age 3 to age 8 with a constant survival rate of 0.42. This is the target mortality rate adopted by the management agency (ASMFC 2003). Projection of abundance over time and age in the presence of disease was done recursively with the relationship $\mathbf{S}\mathbf{A}N_t = N_{t+1}$ where \mathbf{S} is a diagonal matrix with the disease severity stage-specific semi-annual survival rates (combining the target mortality with additional mortality due to disease), \mathbf{A} is a probability transition matrix specifying annual progression probabilities (a_{11} is the probability an animal without signs of the disease will remain without signs of the disease and a_{21} is the incidence of the disease), and N_t is a vector of numbers in the different disease severity stages at time t . The matrix \mathbf{S} is $\text{diag}([S_0, S_1, S_2, S_3]^{1/2})$ where the S_i values are $\exp(-0.42 - \Delta M_i)$ and the ΔM_i values are derived as described above. The probability transition matrix \mathbf{A} gives the probability a_{ij} of a fish going to disease severity stage $i-1$ from disease severity stage $j-1$ for $i, j = 1, 2, 3, 4$ such that $\sum_i a_{ij} = 1.0$. It is constructed as:

$$\mathbf{A} = \begin{bmatrix} a_{11} & 0 & 0 & 0 \\ a_{21} & x_1 & 0 & 0 \\ 0 & x_2 & x_3 & 0 \\ 0 & 1 - x_1 - x_2 & 1 - x_3 & 1 \end{bmatrix}$$

The zeros above the main diagonal arise from the assumption that disease progresses unidirectionally.

Estimating components of the transition matrix A: Incidence (a_{21}) and the probability a disease negative fish remains disease negative (a_{11}) were estimated using cross sectional

disease prevalence data for age three and four fish calculated as the median observed prevalence over 5 years of sampling. We assumed that all three year old fish that become infected remain in disease severity stage 1 for at least a year because the duration of stage 1 is assumed to be longer than one year (see Objective 4 results). We also assumed that the survival rate of fish in disease severity stage 1 was equal to that of fish in disease severity stage 0 (see Objective 3 results). Then the percentage reduction in the prevalence of disease severity stage 0 fish from age three to age four provides an estimate of disease incidence. Thus, the probability of staying in disease severity stage 0 is $1 - a_{21} = a_{11}$. The remaining coefficients in **A** were estimated using observed disease severity stage composition at ages 3 and 4. We estimated the coefficients by least squares by minimizing

$$\min_{x_1, x_2, x_3} \sum_{i=0}^3 \left(\hat{P}_{4i} - P_{4i} \right)^2,$$

where P_4 is the observed vector of the average proportion of 4 year olds in the disease severity stages and \hat{P}_4 is the projected proportions of the 4 year olds in the disease states from the average observed prevalence at age 3, i.e.,

$$\hat{P}_4 = \frac{SASN_3}{(SASN_3)^T \bar{\mathbf{1}}},$$

where $\bar{\mathbf{1}}$ is a vector of ones.

Disease progression: The duration of time a fish spends in the various disease severity stages (i.e., the time it takes to progress from one condition to the next) was estimated from the tagging data. We assumed that transitions from one disease severity stage to another are asynchronous across the population. This means that at the time of tagging, a fish can be anywhere in the time interval it takes to progress from one stage to the next. The methodology is analogous to that used to estimate inter-molt periods in crustaceans and insects (Willoughby and Hurley 1987, Restrepo and Hoenig 1988, Hoenig and Restrepo 1989, Millar and Hoenig 1997). Data for this analysis were obtained from fish returned to VIMS or MDDNR for secondary assessment and necropsy. Comparison of the release disease severity stage (and accompanying photos) with the severity stage assessed at recapture provided a measure of disease progression (or remission) for each recaptured striped bass. The relevant data obtained were disease severity stage at tagging, time at liberty, and disease severity stage at recapture. Thus, the data reduce to time at liberty and an indicator of whether the animal has progressed to the next disease condition class.

The simplest model to handle this situation was developed by Munro (1974, 1983). The recaptures are tabulated in three month time period bins. Then, under the assumptions that:

- 1) the duration of a disease severity stage is a constant, g

- 2) at the time of tagging, the time elapsed since the animal entered the disease severity stage is a uniform random variable over the interval 0 to g
- 3) the probability of recapture does not vary by disease severity stage

the proportion of animals, p_t , having made the transition to a higher disease severity stage at time t is a linear function of the time at liberty, t , up until g units of time have passed, and is 1.0 for $t > g$. That is,

$$p_t = \begin{cases} \frac{t}{g}, & 0 \leq t < g \\ 1.0, & t > g \end{cases}$$

Thus, a plot of the proportion of recaptures in a time interval that show a transition to a higher disease severity stage should describe a linear relationship with time up until the proportion reaches 100%; the slope of the regression line estimates $1/g$. The stage duration, g , is estimated by

$$g = 1/\text{slope}.$$

The categories for disease stage duration analysis are as defined above. Only animals tagged and released in the light and moderate disease severity stages were analyzed using this method since animals released in the heavy severity stage could not progress (heavy is the terminal classification level) and stage duration estimates for animals released in severity stage zero would be confounded with incidence of the disease latent period limiting their interpretability. Some truncation of the data was required due to the fact that animals at liberty for long periods of time are likely to experience differential survival based on their disease stage which violates the third assumption of the model. All recaptures occurring within 15 months of release were included in the analysis. Reversion of disease severity was examined for all returned fish that were released in a disease positive stage. Any animal with a secondary severity stage score that was lower than its release score was classified as showing signs of disease severity reversion.

RESULTS

Objective 1: Develop & validate gross skin pathology as a non-lethal diagnostic for disease

Pathology of Dermal and Splenic Mycobacteriosis

The histologic data obtained from microscopic evaluation of skin lesions on 163 Rappahannock River striped bass are summarized in **Table 1** and **Figures 1 and 2**. Approximately 160 specific mycobacterial skin lesions of three types were evaluated histologically. These included 72 pigmented foci (PF), 80 ulcers (U) and 8 healing areas (H). They were scored in dermis and hypodermis/muscle for the presence of diffuse inflammation, granuloma formation, scale damage, presence of acid-fast bacteria

(mycobacteria) in inflammatory tissues, and occurrence of visceral (splenic) disease. PFs and Us exhibited higher prevalence of inflammation/granuloma formation, scale damage, acid-fast bacteria than Hs within dermis (**Table 1**). Hypodermis was less affected by the disease. **Figure 1** summarizes absolute and relative (lesion percentages) values for presence of inflammation, granuloma formation and occurrence of acid-fast bacteria in PFs, Us, and Hs. The majority of PFs and Us exhibited dermal inflammation (>95%) and granulomas (>85%), whereas Hs exhibited less (inflammation 65%; granulomas <40%) (**Figure 1**). Acid-fast bacteria were observed in only about 50-70% of the lesions. **Figure 2** illustrates the relationship between skin lesion types and occurrence of the visceral (splenic) disease. Presence of ulcers was a strong predictor (Positive predictive value: 0.987) of visceral disease, whereas PFs and Hs were less so (PPV: ~0.85)

Figure 3 illustrates the gross pathology of a suggested temporal progression for dermal mycobacteriosis in striped bass. The earliest gross expression of dermal disease (**Fig. 3a**) is a 0.5-1 mm pigmented focal lesion (brown pigmentation) we have termed the “pigmented focus” (PF). These incipient mycobacterial lesions (see below) vary in shape, size and abundance (**Fig. 3b, c**) but larger PFs often exhibit a clear peripheral halo suggestive of scale involvement (**Fig. 3b**) and as they become even larger, they begin to resemble a small focal ulceration (**Fig 3c**). As these foci enlarge they exhibit loss of overlying scales, exposure of the dermis and initially a relatively smooth pale surface (**Fig. 3d**). As these early ulcers enlarge they exhibit foci of brown to black pigmentation, reddening and further scale loss (**Fig. 3e**). As ulceration progresses the lesions can enlarge greatly, covering a significant portion of the surface of the fish. They exhibit a gritty surficial texture with multi-focal pigmentation, extensive scale loss and hemorrhage (**Fig 3f**) but they remain surficial, almost never penetrating into, or involving, the underlying somatic musculature. Developing mycobacterial ulcers are often associated with PFs.

Figure 4 illustrates the gross pathology of advanced ulcerous and healing dermal mycobacteriosis in striped bass. A somewhat rare manifestation of dermal disease is presentation with large numbers of pigmented foci (**Fig. 4a**) over much of the body surface without development of the larger ulcers. However, in the majority of cases expression of ulcerous lesions typifies advanced disease. **Figure (4b)** illustrates advanced mycobacterial ulceration. Even the largest ulcers are superficial and do not penetrate underlying somatic muscle. Surface texture is gritty, hemorrhagic and often hyper-pigmented, especially towards the dorsum of the fish. Extensive scale loss is usually observed in active ulceration and many fish exhibit poor body condition. Lesions considered to be undergoing healing are rare in striped bass. Most we have observed exhibit disorganized re-scaling and re-epithelialization with restoration of mucus coat (**Fig. 4c**). Some represent re-scaling with hyperpigmentation (**Fig. 4d**). In both types the scale pattern and orientation is atypical.

Figure 5 shows the gross appearance of splenic mycobacteriosis in the striped bass. The spleen (Sp) is a thin leaf-shaped, dark reddish brown, friable visceral organ that, when harboring mycobacterial infection presents grossly with pale gray nodular foci within the parenchyma (**Fig. 5a**). These foci (arrows) represent granulomas and are an attempt by the host to sequester and kill the mycobacteria. In severe splenic mycobacteriosis (**Fig. 5b**) much of the normal splenic parenchyma can be replaced with granulomatous inflammatory tissue

(arrow) and the spleen may become extremely enlarged. The spleen illustrated here weighed over 3 lbs.

Histologically, the healthy spleen consists of a red and white pulp, often exhibiting numerous macrophage aggregates (**Fig. 6a**). Splenic veins and arteries are prominent, as are splenic nerves. Affected striped bass exhibit varying levels of granuloma formation in response to infection. In severe mycobacterial disease much of the normal parenchyma is replaced by the granulomatous inflammatory tissue (**Fig.6b: arrows**). Granulomas represent a cellular host response to chronic infection in which the macrophage is the predominant cell type. A typical granuloma is illustrated in **Fig. 4c**. It consists of a multi-layered band of tightly apposed epithelioid macrophages (E) (**Fig. 6d**) and most have a necrotic central core (N). In many granulomas a thin outer layer of fibrotic elements containing leukocytes is observed. Ziehl-Nielsen staining (**Fig. 6e, f**) highlights the presence of acid-fast mycobacteria within the necrotic core and inner cellular layers of many granulomas, however, a large percentage is negative for acid-fast bacteria at the histologic level.

Figure 7 illustrates the histology of healthy skin in the striped bass. Skin consists of 4 layers, including the outer mucus cuticle, the stratified squamous epidermis that secretes the mucus coat, the dermis, a connective tissue layer divided into stratum spongiosum containing the scales and stratum compactum, comprised of dense fibrous connective tissue and a hypodermis that overlies somatic muscle.

The histopathology of incipient dermal mycobacteriosis in striped bass is detailed in **Figure 8**. Based on careful histological evaluation of numerous pigmented foci (PFs from 72 fish, see below), we have determined this to be earliest lesion of dermal mycobacteriosis. Histologically the vast majority of PFs are characterized histologically by the presence of a single dermal granuloma (**Fig. 8a**) with a highly characteristic thinning or actual perforation of the over- and under-lying scales (**Fig. 8a, b**). Some PFs exhibit evidence of scale remodeling with both osteoblasts and osteoclasts observed in association with developing scales (**Fig. 8b, inset**). Many granulomas associated with PFs are positive by Ziehl-Nielsen for acid-fast bacteria (**Fig. 8c**), however some are not. In most PFs the overlying epidermis has been entirely lost, however in some, re-epithelialization (E) is apparent (**Fig. 8b**). SEM analysis indicates surface erosion of PF-associated scales (**Fig. 8d, e**), as well as perforation of the scales in some cases (**Fig. 8e, f**).

The histopathology of mycobacterial ulceration and healing in striped bass is detailed in **Figure 9**. Ulcers were characterized by examining 80 individual samples histologically. They generally exhibited widespread loss of epidermis and scales and granulomatous dermatitis with multiple granulomas and severe diffuse inflammation throughout affected dermis and hypodermis (**Fig. 9a**). Granulomas occurred in various stages of development and mature epithelioid lesions were common (**Fig. 9b**). Granulomas were often but not always positive for acid-fast bacteria (**Fig. 9d**). In some cases inflammation extended into the somatic muscle resulting in zones of severe myodegeneration/myolysis (**Fig. 9c**). Healing lesions were rare and only 8 were evaluated histologically. These were generally characterized by prominent re-epithelialization, development of new of scales and remodeling of underlying connective tissues, reductions of inflammatory infiltrates, but

continuing evidence of granulomatous inflammation, with some granulomas being positive for acid-fast bacteria (**Fig. 9e, f**).

Disease Prevalence and Severity in Rappahannock River Striped Bass

From 2005 to 2010, 1491 randomly-sampled striped bass were evaluated for dermal (gross pathology) and splenic (histopathology) mycobacteriosis. Disease prevalence and severity data are summarized in **Tables 2, 3 and 4** and **Figures 10, 11 and 12**. Both dermal and splenic disease prevalence appeared stable over the study period (2005-2010), however, splenic disease exhibited higher prevalence (~80-90%) than dermal disease (~60-80%) (**Figure 10**). No significant differences in disease prevalence were observed based on year, season (spring, fall), location (up, down river). **Figures 11 and 12** summarize relative severity of dermal and splenic disease in the 1491 Rappahannock River striped bass sampled randomly during 2005-2010. Of the fish that harbored mycobacterial disease (both dermal and splenic), the majority exhibited only mild disease, with moderate to severe disease seen only in about 20% of the fish, irrespective of year, season, or location.

Objective 2: Mycobacterial species associated with disease

Table 5 presents a summary of nPCR/RFLP results for skin ($n=35$). Skin lesion-positive samples included 9 pigmented foci (PF), 7 mixed pigmented foci/ulcers (PF/U), and 6 ulcers (U). Nineteen of 22 skin lesions were positive by nested PCR, while all skin lesion-negative samples were PCR negative. Three pigmented foci (PF) samples were negative by PCR. All PCR-positive samples were positive by both m23.27f/27r and m23.8f/8r1 primer sets. All restriction digests of m23.8f/8r1 amplification products were consistent with the presence of *M. shottsii*. No skin lesions were positive for IS2404, indicating the absence of *M. pseudoshottsii*. **Table 6** presents molecular assay results for spleen samples ($n=129$) as compared to visceral disease severity category. Seventy-nine percent of samples were positive for mycobacteria amplifiable by the m23.8f/8r1 primer set. Of these, 19% were positive for *M. pseudoshottsii* alone, 31% were positive for *M. shottsii* alone, and 24% were positive for both *M. pseudoshottsii* and *M. shottsii*. Six fish were positive by the m23.8f/8r1 primer set, but restriction fragment digest patterns were inconclusive for species identification. No clear patterns were evident for association of *M. pseudoshottsii* and/or *M. shottsii* with disease severity, and Stage 3 (severely diseased) fish were found with *M. pseudoshottsii* alone, *M. shottsii* alone, and coinfections. Of PCR-negative fish ($n=27$), 5 were negative for visceral disease, and the remainder had evidence of mild disease (Stage 1). Finding of mild disease without PCR positive results is not entirely unexpected due to the heterogeneous distribution of mycobacterial granulomas in the spleen and the relatively greater amount of spleen examined with histological techniques. No Stage 2 or 3 fish returned negative PCR results. Comparison of PCR results with skin lesion status (**Table 7**) indicated an association of visceral presence of *M. shottsii* and severity of skin lesions. Severe (Stage 3) skin lesions were only seen in fish with visceral *M. shottsii* infections, while fish with only *M. pseudoshottsii* did not progress past Stage 1 dermal disease. Three fish that were negative by PCR did show Stage 2 dermal disease. Chi-square of PCR-positive fish recategorized to *M. shottsii*-positive or -negative revealed a highly significant association between visceral *M. shottsii* and presence of severe skin disease (χ^2 , 3df, $p<0.001$). The

relationship between splenic density of *M. shottsii* and skin disease was examined using fish for which *M. shottsii* was cultured with traditional bacteriology (**Figure 13**). Mean log density of splenic *M. shottsii* increased significantly with increasing skin disease category (ANOVA, 3df, $p < 0.001$).

Objective 3: Application of non-lethal skin lesion diagnostic to tagging program: Relative survival rates

Summary Results for Tagging Program

A total of 21,261 striped bass have been assessed, tagged and released in the Rappahannock River since fall 2005 (**Table 8**). The variability in the numbers tagged reflects differences in spatial and temporal availability. Fall releases (resident Chesapeake Bay striped bass) from the Rappahannock River mouth were 30.2% clean, 42.6% light infections, 16.8% moderate infections, 9.8% heavy infections and 0.6% other (healing or no assessment). Fall releases from the upper Rappahannock River were 31.1% clean, 38.9% light infections, 17.5% moderate infections, 11.5% heavy infections and 1.0% other. Spring releases (mixture of resident and coastal migrants) from the Rappahannock River mouth were 39.3% clean, 38.2% light infections, 11.5% moderate infections, 9.7% heavy infections and 1.2% other.

In addition, Maryland DNR tagged 4,658 striped bass throughout the Maryland portions of Chesapeake Bay during summers and early falls 2007-2010 (**Table 9**). These releases were 43.9% clean, 25.7% light infections, 10.2% moderate infections, 14.2% heavy infections and 6% other. The Maryland releases are a mixture of resident and migrant striped bass in June, transitioning to resident striped bass in July – October.

The striped bass tagged in the Rappahannock River ranged from three – seven years of age in the fall and three – ten years of age in the spring, although most of the fish were age five or younger (Maryland DNR did not determine the age of their releases). There is clear evidence of progression in both the prevalence and the severity of mycobacterial infection with age in the resident population with the proportion of externally uninfected striped bass decreasing and the proportion of moderate and heavily infected striped bass increasing with age in both the lower and upper Rappahannock River (**Figures 14, 15**). However, there is a much weaker trend with age in the spring releases (**Figure 16**) which may be indicative of differences in the prevalence and progression of mycobacterial infection between the resident and coastal migrant populations of Chesapeake Bay striped bass.

Estimating Relative Survival: The numbers of fish used in the relative survival analysis were 601, 667, 271 and 222 for disease severity stages 0, 1, 2, and 3 respectively. The survival rate of fish released in disease severity stage 1 was estimated to be slightly lower than that of fish released in disease severity stage 0; however this result was not statistically significantly at $\alpha = 0.05$ (**Table 10, Figure 17**). Relative survival rates of fish released in disease severity stages two and three were estimated to be 0.79 and 0.56 respectively. Both these results were statistically significantly at a level $\alpha = 0.05$. (**Table 10, Figures 18, 19**).

Estimating Population Level Natural Mortality Rate: Using the relative survival estimates from the previous section, the disease severity-specific associated increases in natural mortality rate were estimated at 0.02, 0.24 and 0.58 for disease severity stages 1, 2, and 3 respectively. With an assumed natural mortality rate of 0.15 for disease severity stage 0 fish, the adjusted natural mortality rates for the disease severity stages are 0.15, 0.17, 0.39 and 0.73 for disease severity stage 0, 1, 2 and 3 respectively. Five years of prevalence data from two tagging programs were combined to create an overall prevalence estimate. Year to year variability in disease prevalence was low (**Figs. 10-12**), providing evidence that disease prevalence is relatively stable throughout the time series. The combined cross sectional disease prevalence was estimated at 0.35, 0.39, 0.15 and 0.11 for disease severity stages 0, 1, 2, and 3 respectively. Disease severity stage components of the population natural mortality rate were estimated to be 0.05, 0.07, 0.06 and 0.08 for disease severity stages 0, 1, 2 and 3 respectively. Summing these components gives an estimated population-level natural mortality rate in the presence of mycobacteriosis as 0.26.

Estimating components of the disease transition matrix: The median observed proportion of animals in disease severity stage 0 at age 3 was 0.42. The median observed proportion of animals in stage 0 at age 4 was 0.30. This corresponds to a 28% reduction in severity stage 0 fish between the ages of 3 and 4. Therefore, the incidence rate is estimated to be 0.28 and corresponds to cell a_{21} in the transition matrix **A**. Since we assume fish cannot transition from disease severity stage 0 to severity stages 2 or 3, we estimate the probability that a severity stage 0 fish remains in stage 0 as $1 - 0.28$ which equals 0.72 (cell a_{11} in the **A** matrix).

The average observed disease stage prevalence for age three was 0.37, 0.40, 0.15 and 0.08 for severity stages 0, 1, 2 and 3 respectively. The average observed disease stage prevalence for four year olds was 0.31, 0.41, 0.17 and 0.11 for severity stages 0, 1, 2 and 3 respectively. Only ages three and four were used to estimate the transition probabilities because the sample sizes of animals older than age four were not sufficient to enable us to incorporate them into the analysis. Using least squares, we estimated the unknown disease transition probabilities. The probability that a severity stage 1 fish remained in that stage was estimated to be 0.71. The probability that a severity stage 1 fish progresses to stage 2 or 3 was estimated to be 0.19 and 0.10 respectively. The probability that a severity stage 2 fish remained in that stage was estimated to be 0.78. The probability that a severity stage 2 fish progressed to stage 3 was estimated to be 0.22. The transition matrix took on the following form:

$$\begin{bmatrix} 0.72 & 0 & 0 & 0 \\ 0.28 & 0.71 & 0 & 0 \\ 0 & 0.19 & 0.78 & 0 \\ 0 & 0.10 & 0.22 & 1 \end{bmatrix},$$

where the values on the main diagonal are the probability that an animal in a given severity stage remains in that severity stage and the values below the main diagonal are the progression transition probabilities.

Modeling the Impact of Disease on Study Population: The annual survival rates associated with the different disease severity stages were estimated to be 0.66, 0.62, 0.51 and 0.39 for stages 0, 1, 2 and 3 respectively. Projections of simulated cohort abundances at age are shown in **Table 11**. Overall it is estimated that mycobacteriosis results in a 16% reduction in the striped bass population size when the population is restricted to animals ranging from 3 to 8 year olds. Individual age deviations vary widely with older age groups experiencing nearly a 50% reduction in abundance due to the cumulative effects of disease on survival.

Objective 4: Application of non-lethal skin lesion diagnostic to tagging program: Dermal disease progression (net pen study)

Net pen disease progression study: Our net pen holding study during fall 2006 was unsuccessful. We conducted a preliminary pen holding study during fall 2005 at the up-river study site (mile 43). However, we were unable to maintain striped bass in the net pens for any length of time, losing them rapidly to secondary microbial infections. We hypothesized that the warm temperatures and low salinity at the upper river site were too stressful to the fish and that moving the net pen into higher salinity waters for subsequent studies would allow us to maintain these fish in the pen. We therefore moved the net pen to a protected cove at the mouth of the Rappahannock River for a second net pen study during fall 2006. Again, we were unable to maintain the striped bass in our net pen and rapidly lost most of them to secondary infections. During year one of this study we therefore requested that we be allowed to terminate this aspect of the grant and to be permitted to focus on the tag/release of as many fish as possible.

Stage duration estimation results: A total of 1,017 tagged striped bass have been returned to either VIMS or MDDNR for secondary assessment and necropsy. 372 of these fish were released in disease severity stage 1 and 148 were released in disease severity stage 2. After right-truncation methods were applied to the data, 247 of the fish released in stage 1 and 97 of the fish released in stage 2 remained for use in the analysis. Using these recaptures of tagged fish, we estimated the duration of state 1 to be 418 days (*s.e.* 19 days); state 2 was estimated to last 609 days (*s.e.* 57 days) (**Figures 20, 21**). Of all 665 recovered fish that were released in a disease positive severity stage, 54 (8.1%) showed reversion of dermal disease to a lower severity stage through regeneration of skin and scales.

DISCUSSION

Objective 1

Detailed gross and histopathological characterization of dermal and splenic mycobacteriosis has allowed us to develop an effective non-lethal, semi-quantitative “Skin Lesion Severity Index” for Chesapeake Bay striped bass. Our extensive histopathological analyses have definitively identified the pigmented focus (PF) as the earliest, grossly visible lesion associated with mycobacterial disease in the striped bass. The PF is characterized by focal scale erosion/perforation in association with an underlying dermal granulomatous inflammatory focus. Further, our analyses suggest that PFs enlarge over time and progress towards development into ulcers. Granulomatous inflammation is a characteristic diagnostic

feature in both these lesions, confirmed histologically in 87.5% of PFs and 86.4% of Us. Acid-fast bacteria are sufficiently abundant in the majority of these lesions (PFs: 58%, ulcers: 69.2%) to be apparent in Ziehl-Nielsen stained histologic sections. Although a number of striped bass pathogens can cause skin pathology in striped bass (e.g., lymphocystis virus, other bacterial agents, protozoan and metazoan parasites etc.), the typical gross appearance of both pigmented foci and ulcers is pathognomonic (highly characteristic) for striped bass mycobacterial disease in our extensive experience. This has allowed us to easily distinguish between affected and unaffected fish and to estimate skin disease severity in the field and apply our Skin Lesion Severity Index in the context of a multi-year tag-recapture program that has focused on determining if Chesapeake Bay striped bass are experiencing disease-associated mortality. This is an extremely important point because a non-lethal diagnostic for mycobacteriosis, a simple blood test for example, is not available for fishes. We have therefore used gross dermal pathology as a surrogate of overall disease status in this study, realizing that its use for estimating if a fish has mycobacteriosis is conservative. This is because a significant number of fish that are negative for the dermal disease actually exhibit splenic infections. Nevertheless, this was the best approach available to us for addressing the question of disease-associated mortality.

Development of a gross pathological approach to quantifying dermal disease severity and application of histomorphometric methods for quantifying splenic disease severity have allowed a more critical examination of mycobacterial disease dynamics in Rappahannock River striped bass. Dermal and splenic disease prevalences for resident striped bass were extremely high (~70 and ~90% respectively) but have remained stable over the 6 years for which we have data. However, despite these very high disease prevalences, only a small proportion of all fish exhibited moderate to severe disease (~20%). The majority of affected fish exhibited only mild disease (both dermal and splenic). Disease severity varied somewhat based on year, season and location, however clear trends are not readily apparent in the data. More rigorous statistical analyses of disease severity trends are underway.

Objective 2

Considerable effort has gone into identifying the etiologic agents of striped bass mycobacteriosis. Early studies by our group have employed traditional bacteriological culture approaches for species identification. We have determined that two recently described species, *Mycobacterium shottsii* and *M. pseudoshottsii*, represent the most common isolates obtained from diseased striped bass (Rhodes et al., 2004; Gauthier et al., 2008b). However, a suite of more than 10 unidentified species has also been isolated from diseased fish (Rhodes et al., 2004; Gauthier et al., 2011). Development and application of new molecular tools (Gauthier et al., 2008b; 2010; 2011) have allowed us to better evaluate potential relationships between bacterial species and dermal and splenic disease expression. Our results indicate a strong relationship between the skin disease and *M. shottsii*. The large majority of skin lesions of various categories are *M. shottsii*-positive by PCR, and in this data set, fish with severe (Stage 3) skin lesions that were tested via PCR always had detectable *M. shottsii* DNA in the spleen. Further, density of splenic *M. shottsii*, as determined by bacteriology, increased significantly with increasing skin disease category. It should be noted that we have not yet been able to reproduce wild-type skin lesions in laboratory striped bass, therefore the

relationship between *M. shottsii* and skin lesions remains an association, and causation has not yet been established. It does appear, however, that of the two mycobacteria thought to be major factors in the striped bass disease, *M. shottsii* is more worthy of close examination as the agent of dermal signs.

Objective 3

The results of our tag-recapture analyses establish some important points. First, this project has provided a direct measurement of disease-associated mortality by disease severity state. Moderately and severely diseased fish exhibited annual survival rates that are significantly lower than those of outwardly disease-negative fish. Fish with mild dermal disease appear to have no significant reduction in survival in comparison to fish without signs of the disease. It should be noted that the fish tagged without external clinical signs of disease are a mixture of viscerally uninfected and infected fish. Thus, as mentioned above, a comparison of the two groups underestimates the disease-associated mortality because some fish in the “clean” group may already be experiencing some disease-related mortality. We believe this bias, however, to be small due to the fact that survival rate of mildly affected fish is nearly equal to that of the group exhibiting no external signs of disease.

Objective 4

We attempted a net pen holding study during the first year of the project to examine temporal fate of the disease. The goal of this study was to determine if the disease is mainly progressive, ultimately leading to death or if fish have the ability to overcome the infection over time. This study was unsuccessful. We conducted a preliminary pen holding study during fall 2005 at the up-river study site (mile 43) but were unable to maintain striped bass in the net pens for any length of time, losing them rapidly to secondary microbial infections. We hypothesized that the combination of warm temperatures, low salinity and handling at the upper river site was too stressful and that moving the net pen into higher salinity waters for subsequent studies would allow us to hold the fish for longer periods. We therefore moved the net pen to a protected cove at the mouth of the Rappahannock River for a second net pen study during fall 2006. Again, we were unable to maintain the striped bass in our net pen and rapidly lost most of them to opportunist microbial infections. We requested and received permission to terminate this aspect of the grant and to focus on the tag/release of as many fish as possible and address aspects of disease progression/regression by analysis of tag recaptures. We have determined that the majority of striped bass will progress in disease severity on a nearly annual basis and that very few resident (fall) striped bass remain outwardly uninfected by age five. We estimate that fish exhibiting mild disease take on average, 418 days to progress to the moderate disease state, whereas fish exhibiting moderate signs of dermal disease take on average, 609 days to progress to the severe disease state. Thus mycobacteriosis in wild Chesapeake Bay striped bass is a slowly progressing disease. The Munro method used to estimate disease stage duration is generally robust (Restrepo and Hoenig 1988) but it is inefficient because a) it requires recaptures to be binned into time intervals rather than using exact times of recapture, and b) it does not use the information from animals at liberty for a long period of time. Hoenig and Restrepo (1989) developed a likelihood approach to estimating disease stage duration but their model was based on the

assumption that there is no individual variability in stage duration. This assumption can cause a serious positive bias in estimates of stage duration. Millar and Hoenig (1997) generalized the approach of Hoenig and Restrepo (1989) to allow for individual variability in stage duration. Despite the fact that we truncated our data, it remains possible that differential mortality and individual stage duration variability positively biased our results, especially in the case of progression for the moderate severity stage to severe disease.

We have estimated transitional probabilities that conform with our findings on stage duration. This is evident in that for all disease severity stages except 3 the annual probability of remaining in the current severity stage is around 70% indicating that the disease is in fact chronic and that stage durations likely last for more than a year. This provides both a check on our understanding of disease progression dynamics as well as a functional means to simulate disease impacts on the population. The simulation we conducted is one of the first attempts to estimate the impacts of mycobacteriosis on striped bass and effectively synthesizes all the results of this study into a unified model of striped bass disease dynamics. The estimated 16% reduction in abundance may not be large enough to cause alarm for the Chesapeake Bay commercial striped bass fishery. However, the estimated reductions in older fish (~50% ages 7 and 8) have the potential to dramatically affect the charter and recreational fisheries that target these larger fish. In addition, reductions in older fish are potentially damaging to the spawning stock abundance which in turn could lower future Chesapeake Bay recruitment events and affect all striped bass fisheries.

We were recently invited to update the Atlantic States Marine Fisheries Commission (ASMFC) Striped Bass Technical Committee on our most recent work regarding the impacts of mycobacteriosis on the striped bass. This Special Session entitled “Mycobacteriosis and Natural Mortality in Atlantic Striped Bass” was part of the Atlantic Striped Bass Benchtop Stock Assessment Data Workshop held in Philadelphia, PA from July 31 – Aug 3, 2012 by ASMFC. Their goal was to use the most recent science to better estimate natural mortality (M) for management of the striped bass fisheries. An important and overarching goal of our studies and indeed, the central focus of the work we have done for this NCBO project, has been to determine if this disease is having population level impacts and if better fisheries management strategies that account for potential disease impacts on the striped bass can be devised. Four of us presented findings to the ASMFC (Vogelbein, Gauthier, Hoenig/Smith and Latour). It is likely that ASMFC will use the data summarized in this report to more effectively manage the fishery.

It is clear that we have met, if not exceeded, each of our stated four objectives. We have exerted considerable efforts to present our findings through scientific and public venues, published several significant scientific papers partially supported by this contract, and interacted effectively with fisheries management entities to provide scientifically sound data and management advice. We presently have two manuscripts in preparation for publication, one detailing results of the tag-recapture studies and modeling efforts and the other detailing disease pathology and dynamics.

REFERENCES CITED

- Atlantic States Marine Fisheries Commission. Amendment #6 to the Interstate Fishery Management Plan for Atlantic Striped Bass. Atlantic States Marine Fisheries Commission, Washington, DC. Fisheries Management Report No. 41 (2003). (<http://www.asmfc.org/speciesDocuments/stripedBass/fmps/sbAmendment6.pdf>)
- Brownie, C., D.R. Anderson, K.P. Burnham, and D.R. Robson. 1985. Statistical inference from band recovery data: a handbook, 2nd ed., U.S. Fish and Wildl. Serv. Resour. Publ. No. 156.
- Bruno DW, J Griffiths, CG Mitchell, BP Wood, ZJ Fletcher, FA Drobniewski, and TS Hastings. 1998. Pathology attributed to *Mycobacteria chelonae* infection among farmed and laboratory - infected Atlantic salmon (*Salmo salar*). Dis Aquat Org. 33:101-109
- Cardinal JL. 2001. Mycobacteriosis in striped bass, *Morone saxatilis*, from Virginia waters of Chesapeake Bay. Master's Thesis. School of Marine Science, Virginia Institute of Marine Science. Pp.83.
- Colomi A. 1992. A systemic mycobacteriosis in the European sea bass *Dicentrarchus labrax* cultured in Eilat (Red Sea). Bamidgeh – Isr J Aquacult 44:75-81
- Crecco, V. 2003. Methods of estimating fishing (F) and natural (M) mortality rates from total mortality (Z) and exploitation (u) rates for striped bass. Final Report. Connecticut Marine Fisheries Division. 40 pp.
- Garrison, LP, JS Link and G White. 2004. A multispecies modeling approach to evaluate interactions between Atlantic menhaden and its predators. Abstract: 60th Annual Northeast Fish and Wildlife Conference. 25-28 April, 2004. Ocean City, Maryland.
- Gauthier, DT, RJ Latour, DM Heisey, CF Bonzak, J Gartland, EJ Burge and WK Vogelbein. 2008a. Mycobacteriosis-associated mortality in wild striped bass (*Morone saxatilis*) from Chesapeake Bay, USA. Ecol. Appl. 18: 1718-1727.
- Gauthier DT, Vogelbein WK, Rhodes MW, Reece K. 2008b. Nested PCR assay for detection of *Mycobacterium shottsii* and *Mycobacterium pseudoshottsii* in striped bass (*Morone saxatilis*). J Aquat Anim Health 20:192-201
- Gauthier DT and MW Rhodes. 2009. Mycobacteriosis in fishes: A review. The Veterinary Journal 180:33-47.
- Gauthier DT, KS Reece, J Xiao, MW Rhodes, HI Kator, RJ Latour, Cf Bonzek, JM Hoening and WK Vogelbein. 2010. Quantitative PCR assay for *Mycobacterium pseudoshottsii* and *Mycobacterium shottsii* and application to environmental samples and fishes from Chesapeake Bay. Appl. Environ. Microbiol. 76(18):6171-6179.

- Gauthier DT, Helenthal AM, Rhodes MW, Vogelbein WK, Kator HI. 2011. Characterization of photochromogenic *Mycobacterium* spp. from Chesapeake Bay striped bass (*Morone saxatilis*). *Dis Aquat Organ* 95:113-124
- Hartman, KJ. 2004. Increases in coastal striped bass predatory demand and implications of declines in Atlantic menhaden populations. Abstract: 60th Annual Northeast Fish and Wildlife Conference. 25-28 April, 2004. Ocean City, Maryland.
- Jacobs JM, CS Stine, AM Baya, ML Kent. 2009. A review of mycobacteriosis in marine fish. *J Fish Dis* 32:119-130.
- Hoenig, J.M. and V.R. Restrepo. 1989. Estimating the Intermolt Periods in Asynchronously Molting Crustacean Populations. *Biometrics* 45:71-82.
- Hoenig JM, P Pepin, and WD Lawing. 1990. Estimating relative survival rate for two groups of larval fishes from field data: Do older larvae survive better than young? *Fish. Bull.* 88:485-491.
- Hueter, R.E., C.A. Manire, J. Tyminski, J.M. Hoenig and D.A. Hepworth. 2006. Assessing mortality of released or discarded fish using a logistic model of relative survival derived from tagging data. *Trans. Am. Fish. Soc.* 135:500-508.
- Jacobs JM, HL Rogers, WF Van Heukelem, B Coakley, C Giesecker and M Matsche. 2004. Nutritional health of Chesapeake Bay striped bass *Morone saxatilis* in relation to disease. Abstract: 60th Annual Northeast Fish and Wildlife Conference. 25-28 April, 2004. Ocean City, Maryland.
- Jiang, H, K.H. Pollock, C. Brownie, R.J. Latour, J.M. Hoenig, B.K. Wells, and J.E. Hightower. 2007. Tag Return Models Allowing for Harvest and Catch and Release: Evidence of Environmental and Management Impacts on Striped Bass Fishing and Natural Mortality Rates. *North American Journal of Fisheries Management.* 27:387-396.
- Kahn, DM. 2004. Tag-recapture data from Chesapeake Bay resident striped bass indicate that survival has declined. Abstract: 60th Annual Northeast Fish and Wildlife Conference. 25-28 April, 2004. Ocean City, Maryland.
- May, EB, V Pernell Lewis, AM Overton, J Jacobs and L Alade. 2004. Potential impacts of mycobacteriosis in striped bass on Chesapeake and Atlantic coastal stocks. Abstract: 60th Annual Northeast Fish and Wildlife Conference. 25-28 April, 2004. Ocean City, Maryland.
- Merriman, D. 1941. Studies on the striped bass (*Roccus saxatilis*) of the Atlantic Coast. *Fish. Bull. U.S. Fish Wildl. Serv.* 50(35):1-77.

- Millar, R.B. and J.M. Hoenig. 1997. A Generalized Model for Estimating Intermolt Periods of Asynchronously Molting Insects and Crustacea from Field or Laboratory Data. *J. Agric., Biol. & Environ. Statistics* 2(4):1-14.
- Munro, J.L. 1974. The biology, ecology and bionomics of spiny lobsters (Palinuridae), spider crabs (Majidae) and other crustacean resources. Part VI in J. L. Munro, ed. The biology, ecology, exploitation and management of Caribbean reef fishes: Scientific Report of the ODA/UWI Fisheries Ecology Research Project 1969-1973: University of the West Indies, Jamaica. Research Reports from the Zoology Department Number 3, University of the West Indies, Kingston. 57 pp.
- Nigrelli RF and H Vogel. 1963. Spontaneous tuberculosis in fish and other coldblooded vertebrates with special reference to *Mycobacterium fortuitum* from fish and human lesions. *Zoologica* 48:131-144.
- Prophet EB, B Mills, Arrington, LH Sobin. 1992. Laboratory Methods in Histotechnology. Washington DC. American Registry of Pathology, Armed Forces Institute of Pathology.
- Restrepo, V.R. and J.M. Hoenig. 1988. Munro's method for estimating intermolt periods of tropical decapods is robust. *Bull. Mar. Sci.* 42:488-492.
- Rhodes MW, Kator H, Kaattari I, Gauthier D, Vogelbein WK, Ottinger CA. (2004). Isolation and characterization of mycobacteria from striped bass *Morone saxatilis* from the Chesapeake Bay. *Dis Aquat Organ* 61:41-51
- Rhodes MW, H Kator, S Kotob, P van Berkum, I Kaattari, WK Vogelbein, F Quinn, MM Floyd, WR Butler and CA Ottinger. 2003. *Mycobacterium shottsii* sp. nov., a slow growing species isolated from Chesapeake Bay striped bass (*Morone saxatilis*). *Int. J. Syst. Evol. Micro.* 53:1-5.
- Rhodes MW, H Kator, S Kotob, P van Berkum, I Kaattari, WK Vogelbein, F Quinn, MM Floyd, WR Butler, CA Ottinger. 2002. *Mycobacterium shottsii* sp. nov., a slow growing species isolated from Chesapeake Bay striped bass, (*Morone saxatilis*). *Int. J. System. Environ. Microbiol.* 53:421-424.
- Rosner, B. 1990. Fundamentals of Biostatistics, 3rd edition. PWS-Kent Publishing Company, Boston.
- Uphoff, JH Jr. 2004. Striped bass and Atlantic menhaden: Is there a predator-prey imbalance in Chesapeake Bay? Abstract: 60th Annual Northeast Fish and Wildlife Conference. 25-28 April, 2004. Ocean City, Maryland.
- Vogelbein WK, DE Zwerner, H Kator, MW Rhodes and J Cardinal. 1999. Mycobacteriosis of striped bass from Chesapeake Bay. pages 53-58. In J.E. Olney (ed.), *Research on Recreational Fishes and Fisheries*, VIMS Spec. Sci. Rept. 139, 82 pp.

Vogelbein WK, DT Gauthier, MW Rhodes, H Kator, R Latour, C Bonzek and C Ottinger. 2004. Mycobacteriosis in striped bass (*Morone saxatilis*) from Chesapeake Bay. Abstract: 60th Annual Northeast Fish and Wildlife Conference. 25-28 April, 2004. Ocean City, Maryland.

Willoughby, L.G. and M.A. Hurley. 1987. "Echo" moulting used to estimate moulting periodicity of mayflies (Ephemeroptera) and stoneflies (Plecoptera) in nature. *Aquatic Insects* 9:221-227.

SCIENTIFIC PRODUCTS

Peer-Reviewed Scientific Publications:

Gauthier DT, RJ Latour, H Gaff, WK Vogelbein. Mycobacteriosis in Chesapeake Bay striped bass (*Morone saxatilis*). In: Northeast Atlantic Coast Striped Bass Fisheries Management. P. Perra and M. Armstrong, eds. (In Press).

Latour RJ, DT Gauthier, J Gartland, CF Bonzek, KA McNamee, WK Vogelbein. 2012. Impacts of mycobacteriosis on the growth dynamics and condition of striped bass (*Morone saxatilis*) in Chesapeake Bay. *Can. Journ. Fish. Aquat. Sci.* 69:247-258.

Gauthier DT, AM Helenthal, MW Rhodes, WK Vogelbein, HI Kator. 2010. Characterization of Photochromogenic *Mycobacterium* spp. from Chesapeake Bay Striped Bass (*Morone saxatilis*). *Dis. Aquat. Orgs.* 95:113-124.

Gauthier DT, KS Reece, J Xiao, MW Rhodes, HI Kator, RJ Latour, Cf Bonzek, JM Hoenig and WK Vogelbein. 2010. Quantitative PCR assay for *Mycobacterium pseudoshottsii* and *Mycobacterium shottsii* and application to environmental samples and fishes from Chesapeake Bay. *Appl. Environ. Microbiol.* 76(18):6171-6179.

Gauthier, DT, WK Vogelbein, MW Rhodes and KS Reece. 2008. Nested PCR assay for detection of *Mycobacterium shottsii* and *Mycobacterium pseudoshottsii* in striped bass (*Morone saxatilis*). *J. Aquat. Anim. Health* 20:192–201,

Gauthier, D.T. and M.W. Rhodes. 2008a. Mycobacteriosis in Fishes: A Review. *The Veterinary Journal.* 180:33-47.

Gauthier DT, RJ Latour, DM Heisey, CF Bonzek, J Gartland, EJ Burge, WK Vogelbein. 2008b. Mycobacteriosis is associated with mortality in wild striped bass (*Morone saxatilis*) from Chesapeake Bay, USA. *Ecol. Appl.* 18(7):1718–1727.

Invited Presentations:

Vogelbein WK, DT Gauthier, R Latour, JM Hoenig, M Smith, P Sadler, and H Gaff. Mycobacteriosis in Chesapeake Bay striped bass: population impacts. Atlantic Striped Bass

Benchmark Stock Assessment Data Workshop. Atlantic States Marine Fisheries Commission. Philadelphia, July 2012.

Gauthier DT, H Gaff, R Latour, C Bonzek, W Vogelbein. Mycobacteriosis in Chesapeake Bay striped bass (*Morone saxatilis*): Etiology, Ecology, and Epidemiology. Atlantic Striped Bass Benchmark Stock Assessment Data Workshop. Atlantic States Marine Fisheries Commission. Philadelphia, July 2012.

Hoenig, J.M., J.D. Shields, M.W. Smith, W.K. Vogelbein, D.T. Gauthier, P. W. Sadler, M. Matsche, A. Haines and H.J. Small. Impact of mycobacteriosis on striped bass in the Rappahannock River and the Maryland portion of the Chesapeake Bay. Atlantic Striped Bass Benchmark Stock Assessment Data Workshop. Atlantic States Marine Fisheries Commission. Philadelphia, July 2012.

Vogelbein WK, DT Gauthier, RJ Latour, HD Gaff, J Hoenig and MW Smith. Mycobacteriosis in Chesapeake Bay striped bass. Disease Ecology session of the 141st Annual Meeting of the American Fisheries Society. 4-8 Sept., 2011. Seattle Washington.

Gauthier, D.T., R Latour, C Bonzek, WK Vogelbein. Epidemiology of mycobacteriosis in Chesapeake Bay striped bass (*Morone saxatilis*). Atlantic States Marine Fisheries Commission Striped Bass Management Board, Baltimore, MD. March 31, 2010.

Vogelbein, WK, M Smith, J Hoenig, D Gauthier, R Latour, M Matsche and P Sadler. The role of mycobacteriosis in elevated natural mortality of Chesapeake Bay striped bass: Developing better models for stock assessment and management. Annual NOAA Chesapeake Bay Office Fisheries Symposium. 21-23 April, 2009. Laurel MD.

Vogelbein WK, D Gauthier, R Latour, M Smith, J Hoenig, K Reece, M Matsche and P Sadler. Mycobacteriosis in Chesapeake Bay striped bass: Etiology, ecology and Impacts. Third Bilateral Fish Health Conference between the United States and Russia. 12-17 July, 2009. Shepardstown, WV.

Vogelbein, WK, D Gauthier, J Hoenig, C Ottinger, M Rhodes, H kator, K Reece, R Latour, and C Bonsek. Integrated research program of striped bass mycobacteriosis at VIMS. NOAA Chesapeake Bay Office 9th Annual Fisheries Science Symposium. Williamsburg, VA. 26, April, 2006.

Vogelbein, W, J Hoenig, and D Gauthier. Epizootic mycobacteriosis in Chesapeake Bay striped bass: What is the fate of infected fish? USGS/NOAA Workshop on Mycobacteriosis in Striped Bass. 7-10 May, 2006. Annapolis, MD.

Gauthier DT, R Latour and WK Vogelbein. Epizootiology of mycobacteriosis in Chesapeake Bay striped bass (*Morone saxatilis*): large-scale field survey. USGS/NOAA Workshop on Mycobacteriosis in Striped Bass. 7-10 May, 2006. Annapolis, MD.

Contributed Presentations

Vogelbein WK, DT Gauthier, RJ Latour, HD Gaff, J Hoenig and MW Smith. Mycobacteriosis in Chesapeake Bay striped bass. Disease Ecology session of the 141st Annual Meeting of the American Fisheries Society. 4-8 Sept., 2011. Seattle Washington.

Gauthier, DT, C Bonzek, H Gaff, K Reece, J Hoenig, H Kator, R Latour M Rhodes, WK Vogelbein, J Xiao. Ecology of pathogenic mycobacteria in Chesapeake Bay. Eastern Fish Health Workshop, 28-31 March, 2011. Charleston, South Carolina.

Vogelbein WK, DT Gauthier, HI Kator, M Rhodes, R Latour, J Hoenig. Mycobacteriosis in Chesapeake Bay striped bass (*Morone saxatilis*): Etiology, pathology ecology and population impacts. 2010 Joint Meeting of the American College of Veterinary Pathologists and American Society for Veterinary Clinical Pathology. 30 Oct. – 3 Nov., 2010. Baltimore MD.

Gauthier, D.T., Xiao, J., Reece, K., Vogelbein, W.K. 2011. Ecology of pathogenic mycobacteria in Chesapeake Bay, USA. American Fisheries Society, Tidewater Chapter, March 12-14, 2011, Gloucester Point, VA

Latour R, DT Gauthier, H Gaff, C Bonzek, WK Vogelbein. Impacts of mycobacteriosis on the growth of striped bass (*Morone saxatilis*) in Chesapeake Bay. American Fisheries Society, Tidewater Chapter, March 12-14, 2011, Gloucester Point, VA

Gauthier DT, RJ Latour, WK Vogelbein. 2010. Application of force-of-infection models to epidemiology of mycobacteriosis in Chesapeake Bay striped bass (*Morone saxatilis*). Northeast Fisheries and Wildlife Conference, April 26-27, 2010, Newton, Massachusetts

Gauthier DT, J Xiao, K Reece, WK Vogelbein. Ecology of pathogenic mycobacteria in Chesapeake Bay. Virginia Water Research Conference, Oct. 15-16, 2010, Richmond, VA

Vogelbein WK, D. Gauthier, M. Smith, J. Hoenig, R. Latour and K. Reece. Mycobacteriosis in Chesapeake Bay Striped Bass: An Update. 34th Annual Eastern Fish Health Workshop. 26 April to 1 May, 2009. Lake Placid, NY.

Smith M.W., J.M. Hoenig, W.K. Vogelbein, D.T. Gauthier, A. Haines, and P.W. Sadler. Utility of mark and recapture in the study of mycobacteriosis: application to Striped Bass Rappahannock River, VA. 139th AFS Annual meeting, Nashville, Tennessee. 2009.

W.K. Vogelbein, M.W. Smith, J.M. Hoenig, D.T. Gauthier, R. Latour, M. Matsche and P.W. Sadler. The Role of Mycobacteriosis in Elevated Natural Mortality of Chesapeake Bay Striped Bass: Developing better models for stock assessment and management. 2009 Fisheries Science Symposium, Laurel Maryland.

Vogelbein WK, K Reece, MW Rhodes, R Latour and J Hoenig. Mycobacteriosis in the

Striped Bass: Recent Findings. Annual Meeting of the International Committee for the Exploration of the Seas (ICES). 22-26 Sept, 2008. Halifax, Nova Scotia, Canada.

Vogelbein WK, D Gauthier and J Hoenig. Mycobacteriosis in Chesapeake Bay striped bass: Monitoring disease status and determining stock impacts. American Fisheries Society Annual National Fish Health Section Meeting. 9-12 July, 2008. Charlottetown, Prince Edward Island, Canada.

Vogelbein W.K., D.T. Gauthier, J.M. Hoenig. Epizootic mycobacteriosis in Chesapeake Bay striped bass: Use of skin pathology in a tagging program to investigate disease impact. Program and Abstracts. 48th Western Fish Disease Workshop and AFS Fish Health Section Annual Meeting. June 4-6, 2007. Grand Teton National Park, Wyoming.

Gauthier D.T, R. Latour and W.K. Vogelbein. Application of force of infection models to epidemiology of mycobacteriosis in Chesapeake bay striped bass (*Morone saxatilis*). Program and Abstracts. 48th Western Fish Disease Workshop and AFS Fish Health Section Annual Meeting. June 4-6, 2007. Grand Teton National Park, Wyoming.

Vogelbein WK, JM Hoenig, and DT Gauthier. Epizootic mycobacteriosis in Chesapeake Bay striped bass: What is the fate of infected fish? Program and Abstracts, Fifth International Symposium on Aquatic Animal Health. Sept. 2-6, 2006. San Francisco, CA

Gauthier, DT, R Latour and WK Vogelbein. Epizootiology of mycobacteriosis in Chesapeake bay striped bass *Morone saxatilis*: large-scale field survey. Program and Abstracts, Fifth International Symposium on Aquatic Animal Health. Sept. 2-6, 2006. San Francisco, CA

Hoenig JM, WK Vogelbein and DT Gauthier. Impact of mycobacteriosis on striped bass, as inferred from tagging data. 37th Annual Meeting of the American Fisheries Society. Lake Placid, New York. Sept. 10-14, 2006.

Latour J, DT Gauthier, CF Bonzek and WK Vogelbein. Epizootiology of mycobacteriosis in Chesapeake Bay striped bass (*Morone saxatilis*). 136th Annual Meeting of the American Fisheries Society. Lake Placid, New York. Sept. 10-14, 2006.

TABLES

Numbers

Percentages

Dermis	H	PF	U	Grand Total	Dermis	H	PF	U	Grand Total
0 (normal)	3	3	2	8	0 (normal)	38%	4%	2%	5%
1 (inflammation)	2	6	9	17	1 (inflammation)	25%	8%	11%	11%
2 (granuloma)	3	63	70	136	2 (granuloma)	38%	88%	86%	84%
Grand Total	8	72	81	161	Grand Total	100%	100%	100%	100%
Hypodermis/Muscle	H	PF	U	Grand Total	Hypodermis/Muscle	H	PF	U	Grand Total
0 (normal)	5	46	21	72	0 (normal)	63%	64%	27%	45%
1 (inflammation)	0	7	19	26	1 (inflammation)	0%	10%	24%	16%
2 (granuloma)	3	19	39	61	2 (granuloma)	38%	26%	49%	38%
Grand Total	8	72	79	159	Grand Total	100%	100%	100%	100%
Scales	H	PF	U	Grand Total	Scales	H	PF	U	Grand Total
1 (present, intact)	3	5	3	11	1 (present, intact)	38%	7%	4%	7%
2 (eroded, focal)	1	61	29	91	2 (eroded, focal)	13%	85%	37%	58%
3 (eroded, extensive)	2	3	31	36	3 (eroded, extensive)	25%	4%	40%	23%
4 (absent)	2	3	15	20	4 (absent)	25%	4%	19%	13%
Grand Total	8	72	78	158	Grand Total	100%	100%	100%	100%
Acid-Fast Bacteria	H	PF	U	Grand Total	Acid-Fast Bacteria	H	PF	U	Grand Total
Negative	4	29	24	57	Negative	50%	42%	31%	37%
Positive	4	40	54	98	Positive	50%	58%	69%	63%
Grand Total	8	69	78	155	Grand Total	100%	100%	100%	100%
Visceral Disease	H	PF	U	Grand Total	Visceral Disease	H	PF	U	Grand Total
Negative	1	10	1	12	Negative	13%	14%	1%	8%
Positive	7	62	79	148	Positive	88%	86%	99%	93%
Grand Total	8	72	80	160	Grand Total	100%	100%	100%	100%

Table 1. Data summary from 163 striped bass (*Morone saxatilis*) evaluated histologically for dermal and splenic mycobacteriosis. Analyses focused on three skin lesion types (pigmented focus [PF], ulcer [U] and healing [H]), and evaluated disease signs (inflammation, granuloma) in dermis, hypodermis/muscle and scales and relationships between skin lesion types and presence of acid-fast bacteria and occurrence of visceral (splenic) mycobacteriosis.

			SKIN DISEASE				SPLENIC DISEASE			
			n	PREV	95% CI lo	95% CI hi	n	PREV	95% CI lo	95% CI hi
2010	spring	lower	36	0.5556	0.16232	0.16232	36	0.83333	0.121741	0.121741
		upper	88	0.7273	0.093052	0.093052	88	0.863636	0.0717	0.0717
	fall	lower								
		upper								
2009	spring	lower	100	0.53	0.097823	0.097823	100	0.83	0.073624	0.073624
		upper	100	0.8	0.0784	0.0784	100	0.9	0.0588	0.0588
	fall	lower	100	0.8	0.08784	0.08784	100	0.91	0.056092	0.056092
		upper	82	0.732	0.0959	0.0959	82	0.939024	0.051792	0.051792
2008	spring	lower	108	0.6944	0.08688	0.08688	108	0.851852	0.06699	0.06699
		upper	102	0.6176	0.09431	0.09431	102	0.84313	0.070577	0.070577
	fall	lower								
		upper								
2007	spring	lower	100	0.56	0.09729	0.09729	100	0.81	0.076891	0.076891
		upper	151	0.5656	0.075789	0.075789	151	0.70861	0.072478	0.072478
	fall	lower	100	0.62	0.09514	0.09514	100	0.85	0.069985	0.069985
		upper	100	0.66	0.09285	0.09285	100	0.98	0.02744	0.02744
2006	spring	lower								
		upper								
	fall	lower	100	0.75	0.08487	0.08487	100	0.87	0.06592	0.06592
		upper	57	0.544	0.1293	0.1293	56	0.96428	0.04861	0.04861
2005	spring	lower								
		upper								
	fall	lower	89	0.652	0.0551	0.0551	89	0.831	0.0752	0.0752
		upper	78	0.705	0.0602	0.0602	78	0.769	0.0602	0.0602

Table 2. Data summary of dermal and splenic disease prevalence, sample size and 95% confidence intervals for striped bass randomly sampled in the upper and lower Rappahannock River, Virginia from 2005-2010.

RAW NUMBERS			SKIN DISEASE						SPLENIC DISEASE					
			n	PREV	S0	S1	S2	S3	n	PREV	V0	V1	V2	V3
2010	spring	lower	36	0.56	16	11	4	5	36	0.8333	6	21	1	8
		upper	88	0.727	24	49	13	2	88	0.863636	12	66	4	6
	fall	lower												
		upper												
2009	spring	lower	100	0.53	49	36	9	6	100	0.83	17	52	3	28
		upper	100	0.8	20	64	11	5	100	0.9	10	68	4	18
	fall	lower	100	0.8	20	59	17	4	100	0.91	9	83	3	5
		upper	82	0.73	22	40	12	8	82	0.93902	5	65	3	9
2008	spring	lower	108	0.694	33	53	14	8	108	0.85185	15	61	3	28
		upper	102	0.617	41	42	14	5	102	0.84313	16	64	7	15
	fall	lower												
		upper												
2007	spring	lower	100	0.56	44	35	12	9	100	0.81	19	66	13	2
		upper	151	0.6556	53	76	15	7	151	0.708609	44	87	13	7
	fall	lower	100	0.61	39	40	8	13	100	0.85	15	53	13	19
		upper	100	0.65	35	46	17	2	100	0.98	2	75	11	12
2006	spring	lower												
		upper												
	fall	lower	100	0.73	27	47	16	10	100	0.87	13	47	25	15
		upper	57	0.491	29	18	8	2	54	0.944	3	37	10	4
2005	spring	lower												
		upper												
	fall	lower	89	0.652	31	20	13	25						
		upper	78	0.705	23	37	13	5						

Table 3. Data summary of dermal and splenic mycobacterial disease prevalence and sample size broken out by absolute animal numbers per disease severity state for striped bass randomly sampled in the upper and lower Rappahannock River, Virginia from 2005-2010.

PERCENTAGES			SKIN DISEASE						SPLENIC DISEASE					
			n	PREV	V0	V1	V2	V3	n	PREV	S0	S1	S2	S3
2010	Spring	lower	36	0.56	0.444	0.306	0.111	0.139	36	0.8333	0.167	0.583	0.028	0.222
		upper	88	0.727	0.273	0.557	0.148	0.023	88	0.863636	0.136	0.75	0.045	0.068
	Fall	lower												
		upper												
2009	Spring	lower	100	0.53	0.49	0.36	0.09	0.06	100	0.83	0.17	0.52	0.03	0.28
		upper	100	0.8	0.2	0.64	0.11	0.05	100	0.9	0.1	0.68	0.04	0.18
	Fall	lower	100	0.8	0.2	0.59	0.17	0.04	100	0.91	0.09	0.83	0.03	0.05
		upper	82	0.73	0.268	0.488	0.146	0.098	82	0.93902	0.061	0.793	0.037	0.11
2008	spring	lower	108	0.694	0.306	0.491	0.13	0.074	108	0.85185	0.139	0.565	0.028	0.259
		upper	102	0.617	0.402	0.412	0.137	0.049	102	0.84313	0.157	0.627	0.069	0.147
	fall	lower												
		upper												
2007	spring	lower	100	0.56	0.44	0.35	0.12	0.09	100	0.81	0.19	0.66	0.13	0.02
		upper	151	0.6556	0.351	0.503	0.099	0.046	151	0.708609	0.291	0.576	0.086	0.046
	fall	lower	100	0.61	0.39	0.4	0.08	0.13	100	0.85	0.15	0.53	0.13	0.19
		upper	100	0.65	0.35	0.46	0.17	0.02	100	0.98	0.02	0.75	0.11	0.12
2006	spring	lower												
		upper												
	fall	lower	100	0.73	0.27	0.47	0.16	0.1	100	0.87	0.13	0.47	0.25	0.15
		upper	57	0.491	0.509	0.316	0.14	0.035	54	0.944	0.056	0.685	0.185	0.074
2005	spring	lower												
		upper												
	fall	lower	89	0.652	0.348	0.225	0.146	0.281						
		upper	78	0.705	0.295	0.474	0.167	0.064						

Table 4. Data summary of dermal and splenic mycobacterial disease prevalence and sample size broken out by relative percentage of animals per disease severity state for striped bass randomly sampled in the upper and lower Rappahannock River, Virginia from 2005-2010.

PCR	Skin lesion status		Total
	(+)	(-)	
(+)	19	0	19
(-)	3	13	16
Total	22	13	35

Table 5. Results of nPCR/RFLP on DNA extracted from gross skin lesions. PCR (+) indicates positive reaction on both m23.27f/27r (genus-level) and m23.8f/8r1 (*M. pseudoshottsii*, *M. shottsii*, and *M. marinum*-specific) secondary amplifications. Three skin lesions that were negative by PCR were pigmented foci (PF). All PCR-positive samples were identified as *M. shottsii* via restriction digest (*HinF* I/*Hpa* I) of amplification products. Agreement between presence of gross lesions and molecular test results was highly significant (χ^2 , 1df, $p < 0.001$).

PCR species ID	Visceral Lesion Severity				Total
	0	1	2	3	
Negative	5	22	0	0	27
<i>Mps</i>	2	15	5	3	25
<i>Ms</i>	3	20	12	5	40
<i>Mps/Ms</i>	1	15	11	4	31
Unidentified	1	0	1	4	6
Total	12	72	29	16	129

Table 6. Visceral lesion severity and PCR-based *Mycobacterium* spp. identification in spleen. Visceral lesion severity is presented in stages from 0 (no disease) to 3 (severe disease) as explained in text. *Mps* = positive for *M. pseudoshottsii*. *Ms* = positive for *M. shottsii*. *Mps/Ms* = positive for *M. pseudoshottsii* and *M. shottsii*. Unidentified=positive by m23.8f/8r1 primer sets, but RFLP did not yield a clear species identification.

PCR species ID	Skin Lesion Severity				Total
	0	1	2	3	
Negative	15	10	3	0	28
<i>Mps</i>	15	10	0	0	25
<i>Mps/Ms</i>	8	15	3	5	31
<i>Ms</i>	8	8	19	5	40
Unidentified	2	0	1	3	6
Total	48	43	26	13	130

Table 7. Skin lesion severity and PCR-based *Mycobacterium* spp. identification in spleen. Skin lesion severity is presented in stages from 0 (no disease) to 3 (severe disease) as explained in text. Abbreviations are as for Table 2.

A)	release totals							recapture totals						
	DATE	n	Disease assessment					n	disease assessment					
			0	1	2	3	4		5	0	1	2	3	4
fall 2005	1566	398	653	315	188	10	2	153	27	66	32	26	1	1
spring 2006	502	166	232	61	39	3	1	126	35	62	20	8	1	0
fall 2006	3314	1006	1366	544	377	19	2	416	131	139	70	73	3	0
spring 2007	656	307	203	72	62	10	2	124	42	48	20	10	4	0
fall 2007	987	371	376	140	95	5	0	112	37	42	21	11	1	0
spring 2008	169	69	65	14	19	2	0	60	26	17	4	13	0	0
fall 2008	2594	801	1133	387	260	13	0	432	138	169	69	55	2	0
spring 2009	347	136	127	46	36	2	0	83	37	20	18	8	0	0
fall 2009	3001	772	1384	591	254	0	0	181	57	76	31	18	0	0
spring 2010	232	95	83	24	30	0	0	38	18	12	6	2	0	0
fall 2010	2517	777	1090	421	195	34	0	332	101	149	46	33	6	0
spring 2011	270	103	98	35	28	6	0	76	30	23	16	9	2	0
fall 2011	2498	844	1023	370	247	14	0	343	109	139	47	46	3	0
spring 2012	237	73	114	26	21	3	0	28	6	10	5	6	1	0
falls	16477	4969	7025	2768	1616	95	4	1969	600	780	316	262	16	1
springs	2413	949	922	278	235	26	3	535	194	192	89	56	8	0
total	18890	5918	7947	3046	1851	121	7	2504	794	972	405	318	24	1

B)	release totals							recapture totals						
	DATE	n	Disease assessment					n	disease assessment					
			0	1	2	3	4		5	0	1	2	3	4
fall 2005	250	66	109	48	24	3	0	43	10	21	7	5	0	0
spring 2006	68	26	31	9	0	2	0	7	4	1	2	0	0	0
fall 2006	399	197	111	60	21	10	0	36	14	17	1	3	1	0
fall 2007	597	229	233	71	61	3	0	59	12	31	10	6	0	0
fall 2008	278	78	109	45	42	4	0	61	19	21	11	7	3	0
fall 2009	277	42	124	69	41	0	1	56	7	25	13	11	0	0
fall 2010	502	105	210	111	75	1	0	96	24	33	27	15	0	0
falls	2303	717	896	404	264	21	1	351	86	148	69	47	4	0
spring	68	26	31	9	0	2	0	7	4	1	2	0	0	0
total	2371	743	927	413	264	23	1	358	90	149	71	47	4	0

Table 8. Annual and seasonal release and recapture totals, by myco severity index, of striped bass from A) the lower Rappahannock and B) upper Rappahannock River, fall 2005 – spring 2012.

DATE	n	release totals						recapture totals						
		Disease assessment						disease assessment						
		0	1	2	3	4	5	n	0	1	2	3	4	5
2007	1082	562	261	85	123	46	5	35	14	9	5	6	1	0
2008	1497	557	406	251	166	108	9	71	29	18	8	10	6	0
2009	865	375	235	90	124	34	7	147	76	34	16	16	5	0
2010	1214	549	295	47	247	72	4	125	61	33	4	18	8	1
total	4658	2043	1197	473	660	260	25	378	180	94	33	50	20	1

Table 9. Annual release and recapture totals, by skin disease severity index, from Maryland portions of the Chesapeake Bay, 2007-2010.

Model	Disease state	Relative survival	95% lower bound	95% upper bound	p-value	Sample size
DC 1 vs DC 0	Light	0.98	0.83	1.15	0.789	667
DC 2 vs DC 0	Moderate	0.79	0.63	0.99	0.047	271
DC 3 vs DC 0	Heavy	0.56	0.41	0.74	< 0.0001	222

Table 10. Estimated relative survival with confidence bounds for dermal mycobacteriosis of striped bass and estimated prevalence by disease state. DC = disease condition. Sample size for DC 0 is 601 recaptured fish. Sample sizes for the other disease states are given in the last column.

	Age					
	3	4	5	6	7	8
Disease present	1,000,000	586,000	336,000	188,000	104,000	56,000
Disease absent	1,000,000	660,000	436,000	287,000	190,000	125,000
Ratio	1.00	0.89	0.77	0.65	0.55	0.45

Table 11. Simulated population projections under scenarios where mycobacteriosis is present and absent from the population. In the absence of disease annual survival rate is fixed at 0.66 for all years/ages. In the presence of disease annual survival rate depends age and is a function of the disease severity stage relative survival rates and age specific prevalence estimates. Simulation projects a 16 % decline in the study group (ages 3 - 8) population due to disease.

FIGURES

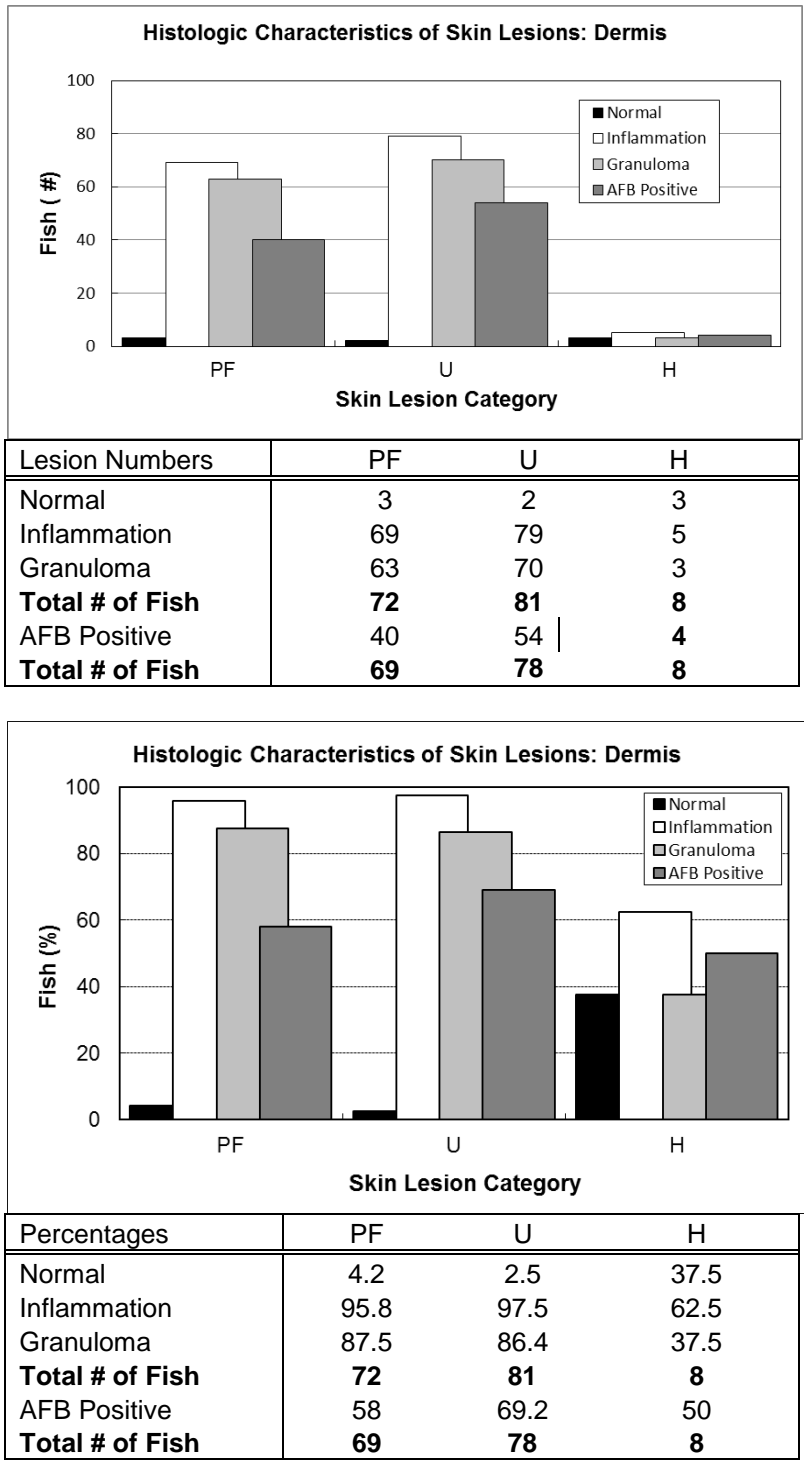
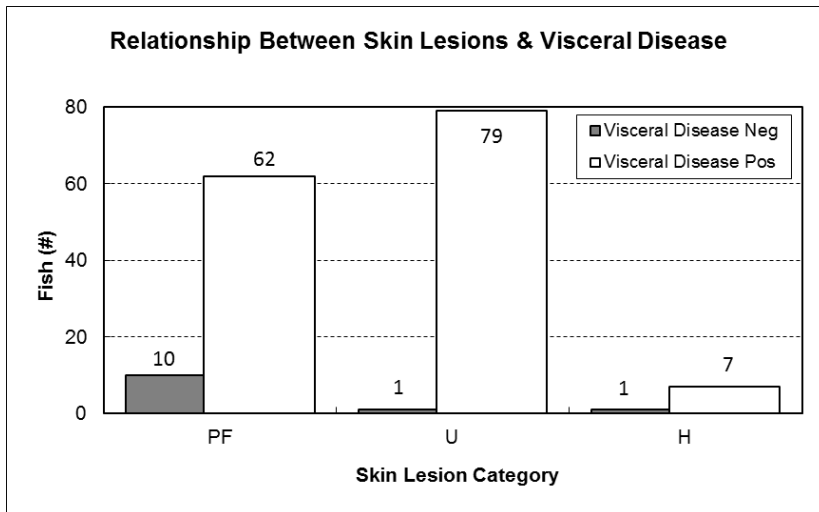
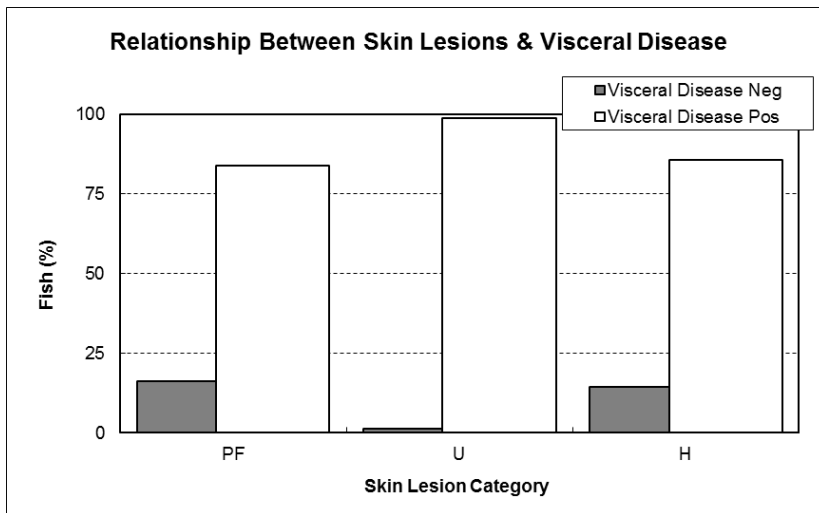


Figure 1. Histologic features (e.g., no pathology, inflammation, granuloma formation, presence of acid fast bacteria) for three grossly identified skin lesion categories. Top: absolute numbers, Bottom: relative values.



# of Fish	PF	U	H
Visceral Disease Neg	10	1	1
Visceral Disease Pos	62	79	7
Total Lesions Examined	72	80	8



% of Fish	PF	U	H
Visceral Disease Neg	16.1	1.3	14.3
Visceral Disease Pos	83.9	98.7	85.7
Total Lesions Examined	72	80	8

Figure 2. Relationship between specific histologically identified skin lesions and splenic mycobacteriosis. Top: absolute values (actual skin lesion numbers), Bottom: relative values (percentages).

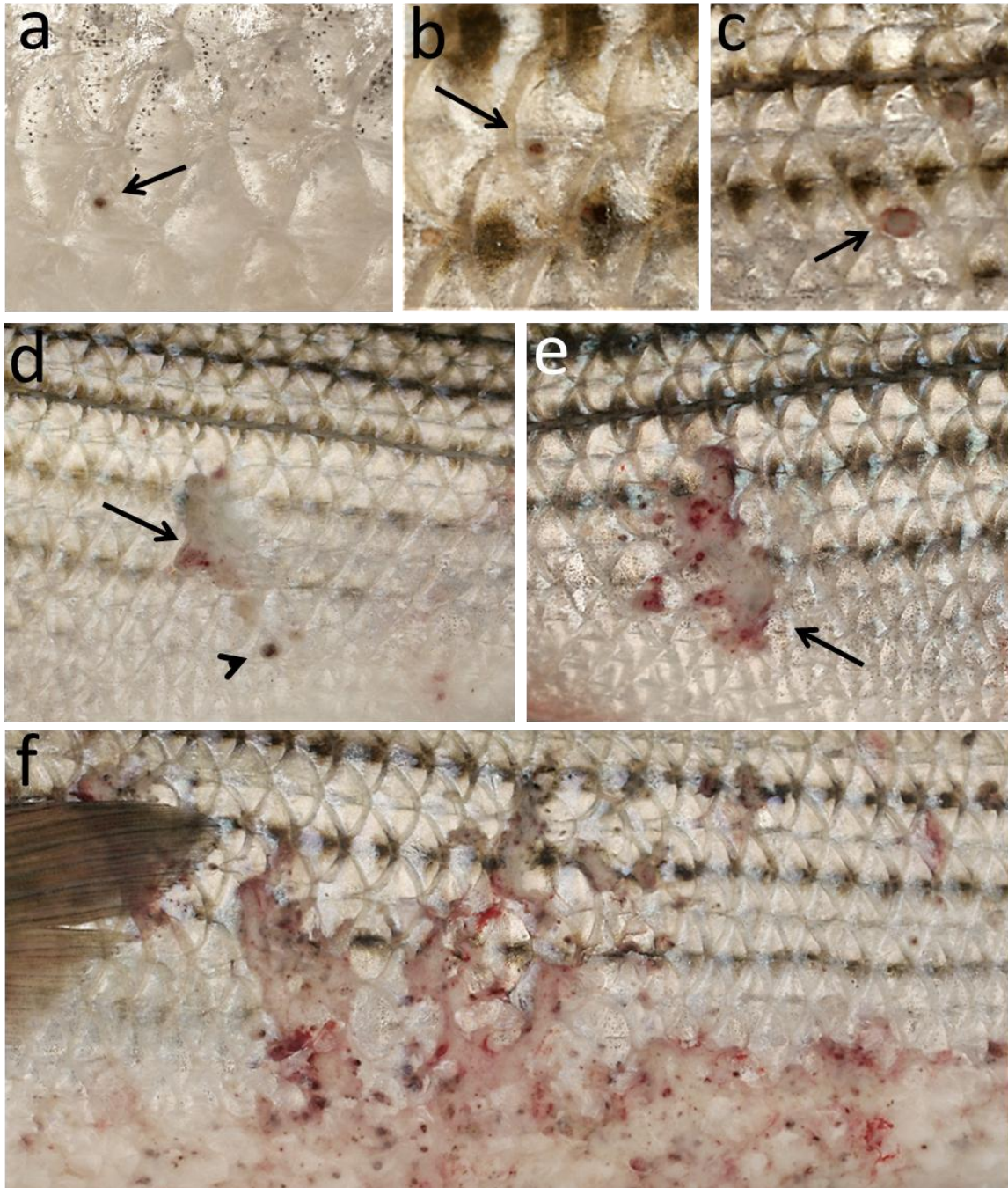


Figure 3. Proposed skin lesion progression of dermal mycobacteriosis in striped bass (*Morone saxatilis*). **a)** small pigmented focus (PF: arrow), the earliest recognizable mycobacterial lesion, **b)** PF showing light halo around pigmented center (arrow), **c)** enlarging pigmented focus (arrow) exhibiting erosion/perforation of overlying scales, **d)** incipient ulcer (arrow) exhibiting scale loss and adjacent pigmented focus (arrowhead), **e)** enlarging ulcer (arrow) with pigmented focal areas and adjacent pigmented foci, **f)** advanced shallow ulcer with focal areas of pigmentation and surrounding pigmented foci.

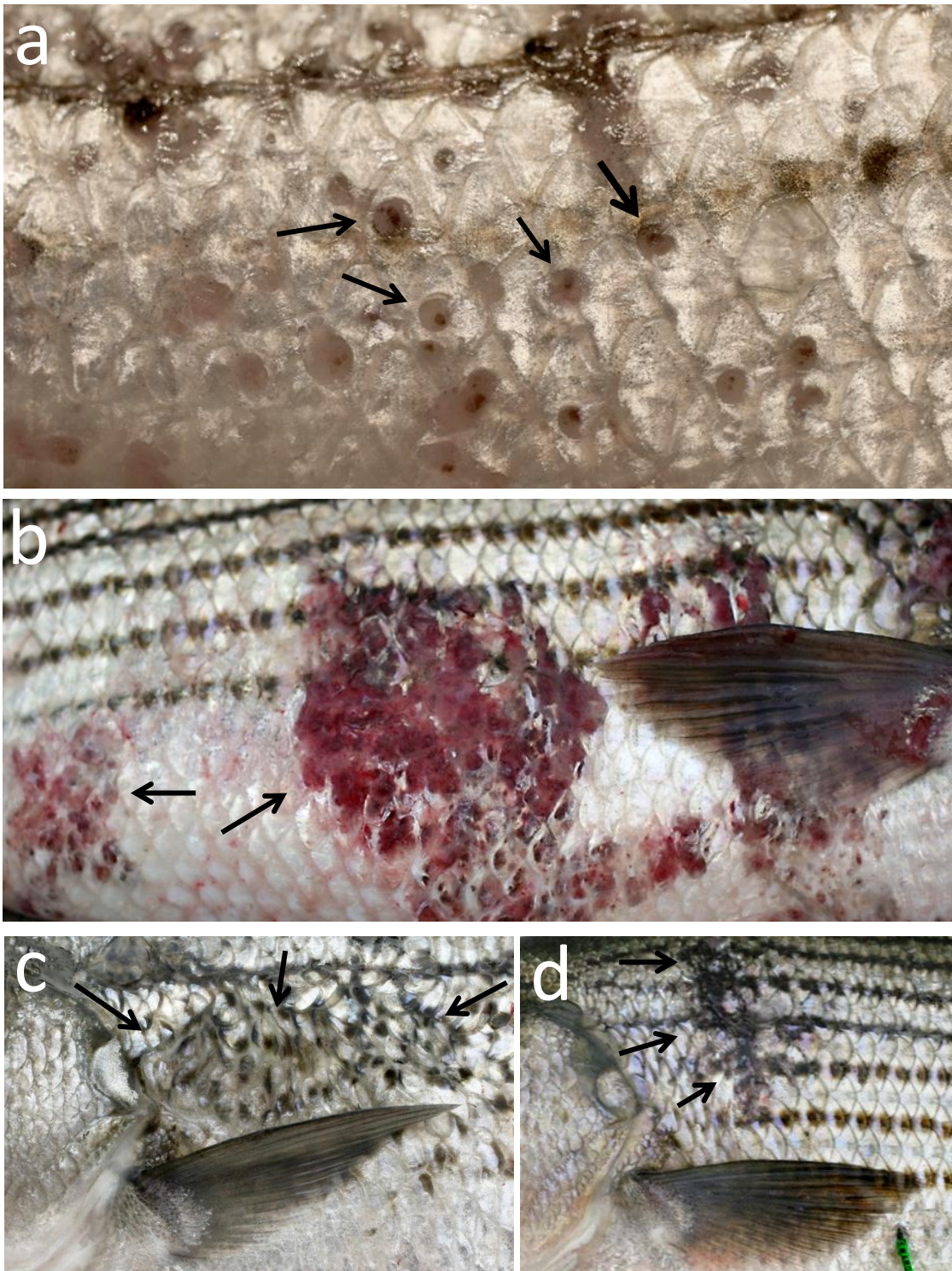


Figure 4. Advanced dermal mycobacteriosis in the striped bass (*Morone saxatilis*). **a)** severe development of pigmented foci (PF) (arrows), **b)** advanced dermal ulceration associated with mycobacterial infection (arrows), **c)** disorganization of regenerating scales in a healing skin lesion (arrows), **d)** hyperpigmentation often associated with healing skin lesions (arrows).

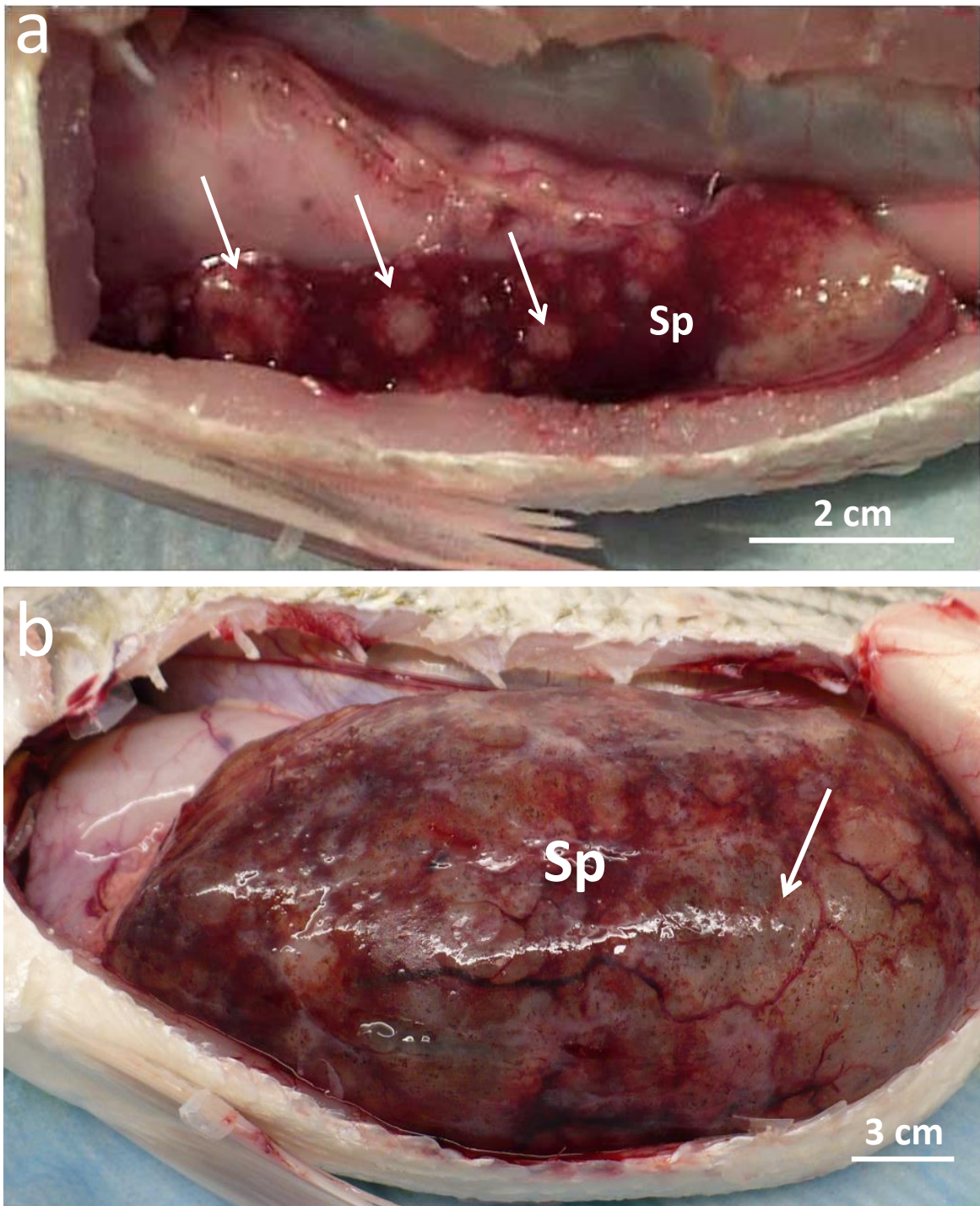


Figure 5. Splenic mycobacteriosis in the striped bass (*Morone saxatilis*). **a)** Spleen (Sp) is a thin leaf-shaped dark red organ. Granulomatous inflammation presents as pale gray nodules within splenic parenchyma (arrows), **b)** severely affected fish exhibiting advanced granulomatous disease (gray nodular masses: arrow) and severe splenomegaly.

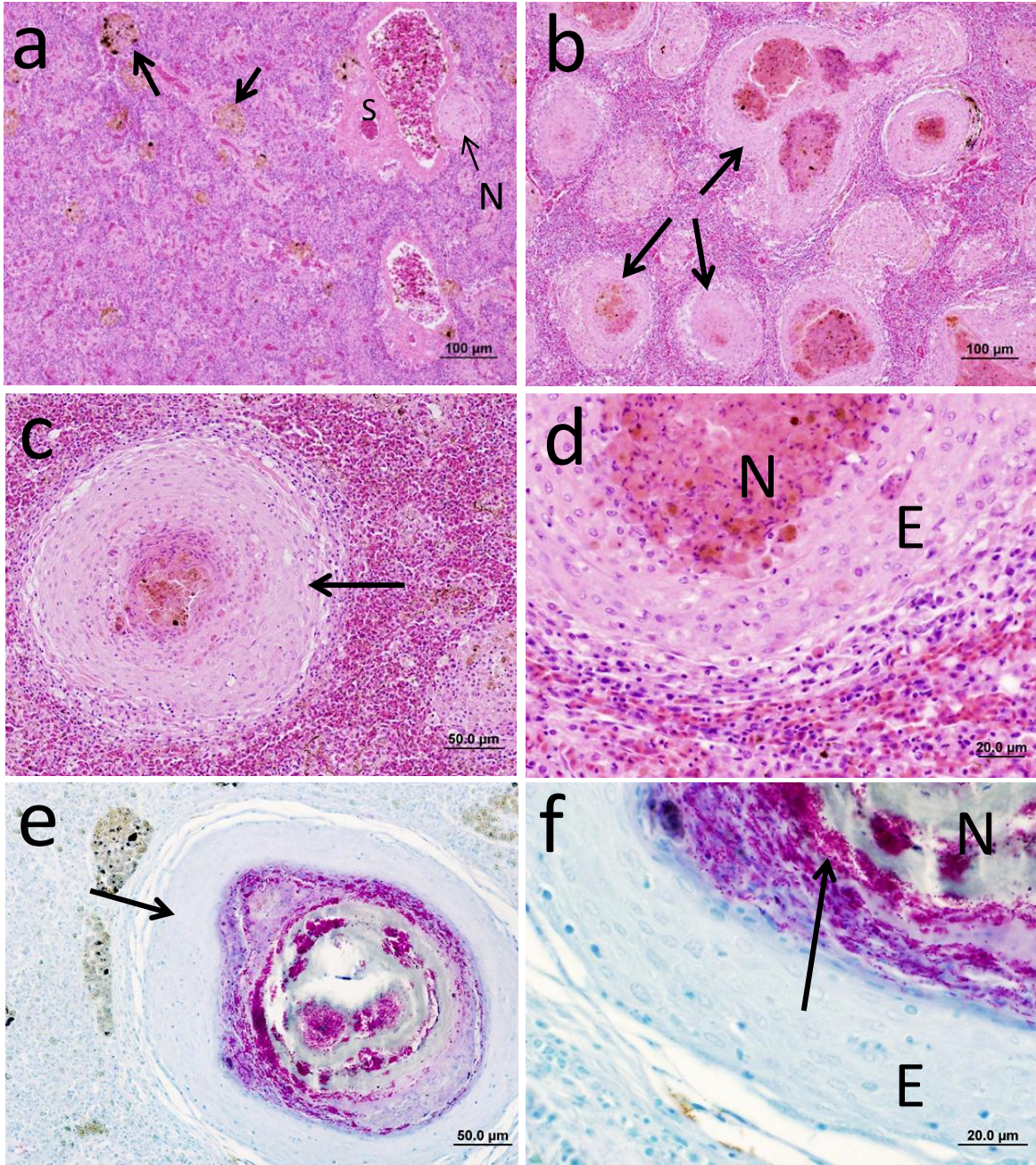


Figure 6. Histopathology of splenic mycobacteriosis in the striped bass (*Morone saxatilis*). **a)** Healthy uninfected splenic parenchyma. Macrophage aggregates (arrows), splenic blood vessels (S), splenic nerve (N), **b)** severely affected spleen exhibiting advanced granulomatous inflammation; granulomas (arrows), **c)** higher magnification of single splenic granuloma showing typical architecture (arrow), **d)** higher magnification of granuloma showing outer layer of inflammatory leukocytes, epithelioid cell layer (E), and necrotic center (N), **e)** granuloma (arrow) stained by Ziehl Neelsen method for acid-fast bacteria (stained red) showing bacterial localization to central necrotic core and inner cellular layers, **f)** higher magnification showing abundant acid-fast bacteria within splenic granuloma (arrow), N: necrotic center of granuloma, E: epithelioid cell layer.

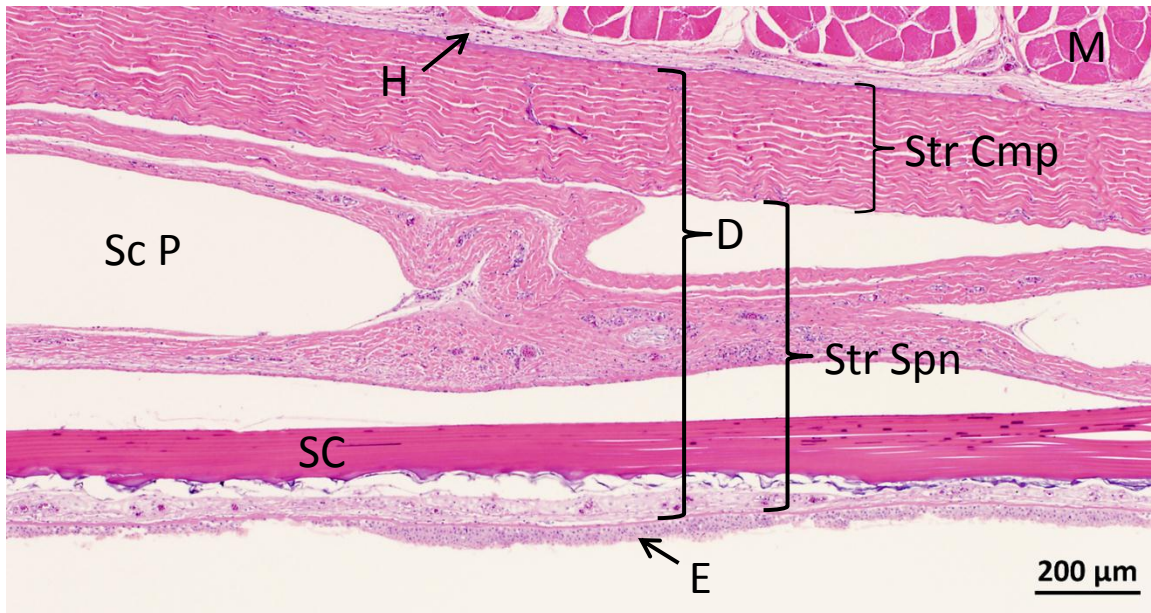


Figure 7. Histology of healthy skin in the striped bass (*Morone saxatilis*). Skin consists of four layers, an outermost “cuticle” comprised of a thin layer of mucus secreted by specialized goblet cells, a stratified outermost squamous epithelial layer called the epidermis (E), the underlying dermis (D) and a hypodermis (H) storing variable amounts of lipid. The dermis is subdivided into an outer loosely organized connective tissue layer called the stratum spongiosum (Str Spn) and an inner stratum compactum (Str Cmp) composed of more dense connective tissue. The stratum spongiosum forms the scale pockets (Sc P) in which are found the scales (SC). Underlying the hypodermis are the somatic muscles (M).

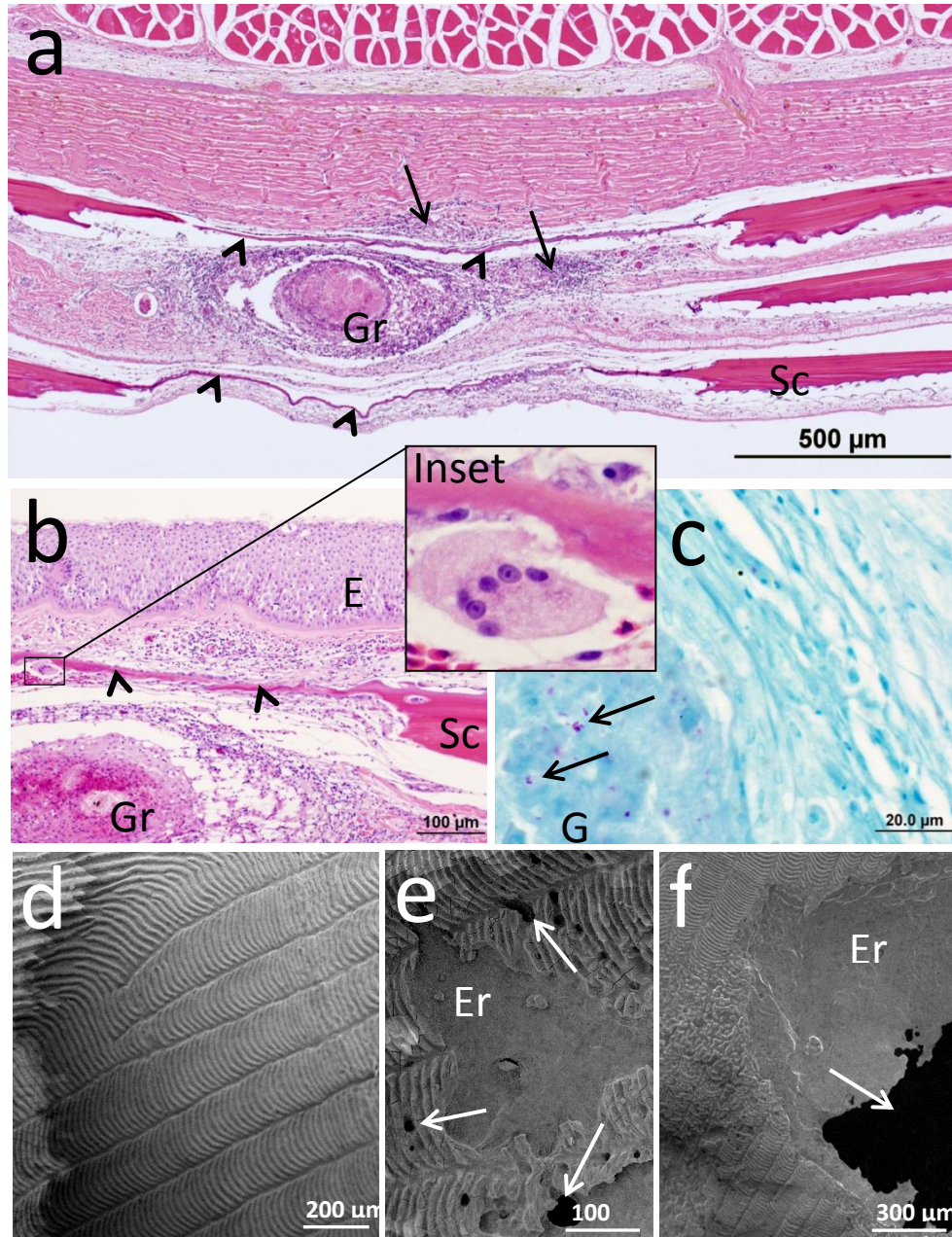


Figure 8. Histopathology and scanning electron microscopy (SEM) of the pigmented focus (PF) in the striped bass (*Morone saxatilis*). **a)** low magnification overview of a PF showing salient features including localization of a single mycobacterial granuloma (Gr) within the dermal stratum spongiosum, surrounding diffuse cellular inflammatory infiltrates (arrows), significant thinning/erosion (arrowheads) of under- and overlying scales (Sc), and complete erosion of outer epidermis, **b)** higher magnification illustrating granuloma (Gr), thinning (arrowheads) of overlying scale (Sc), re-epithelialization (E) and a multi-nucleated osteoclast suggesting scale remodeling (inset), **c)** Ziehl Neelsen stain illustrating acid-fast bacteria (arrows) in PF granuloma, **d)** SEM of normal scale, **e)** SEM of scale overlying a PF exhibiting surficial erosion (Er) and scale micro-perforations (arrows), **f)** SEM illustrating advanced surficial scale erosion and large perforation (arrow) in scale over-lying a PF.

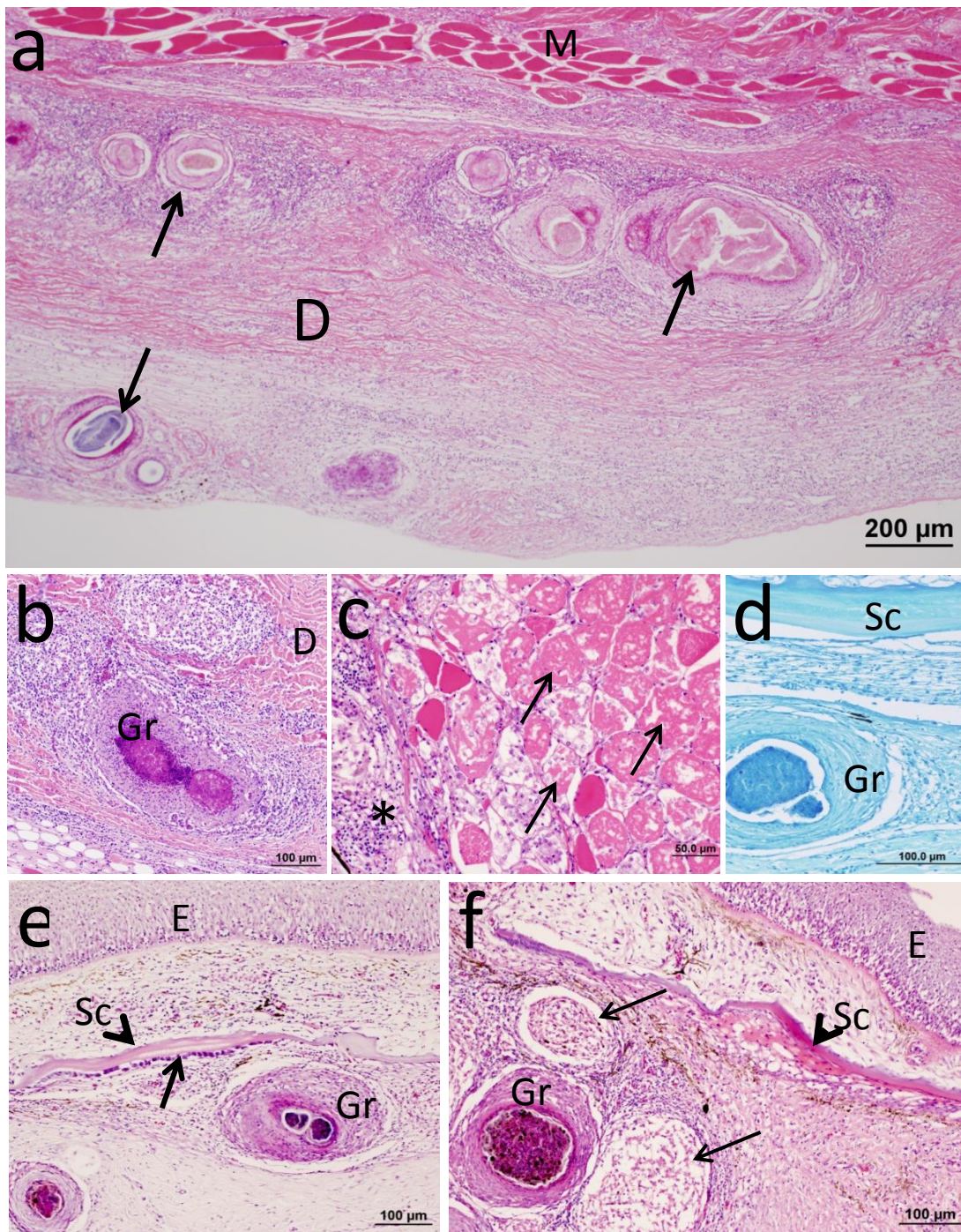


Figure 9. Histopathology of mycobacterial ulceration and healing in striped bass (*Morone saxatilis*). **a)** low magnification overview of mycobacterial ulcer exhibiting granulomatous (arrows) and diffuse inflammation in dermis (D) extending into somatic muscle (M) and with complete loss of scales and outer epidermis, **b)** closeup granulomatous (Gr) inflammation in the dermis (D), **c)** extensive myolysis (arrows) in underlying somatic muscle associated with inflammation (*), **d)** Ziehl Neelsen stain in many instances shows granulomas (Gr) negative for acid-fast bacteria, **e)** healing ulcer exhibiting strong re-epithelialization (E), remodeling and formation of new scales (Sc) lined by osteoblasts (arrow) but with evidence of continuing granulomatous inflammation (Gr), **f)** healing ulcer exhibiting similar changes as in **e)** but with degenerating granulomas (arrows).

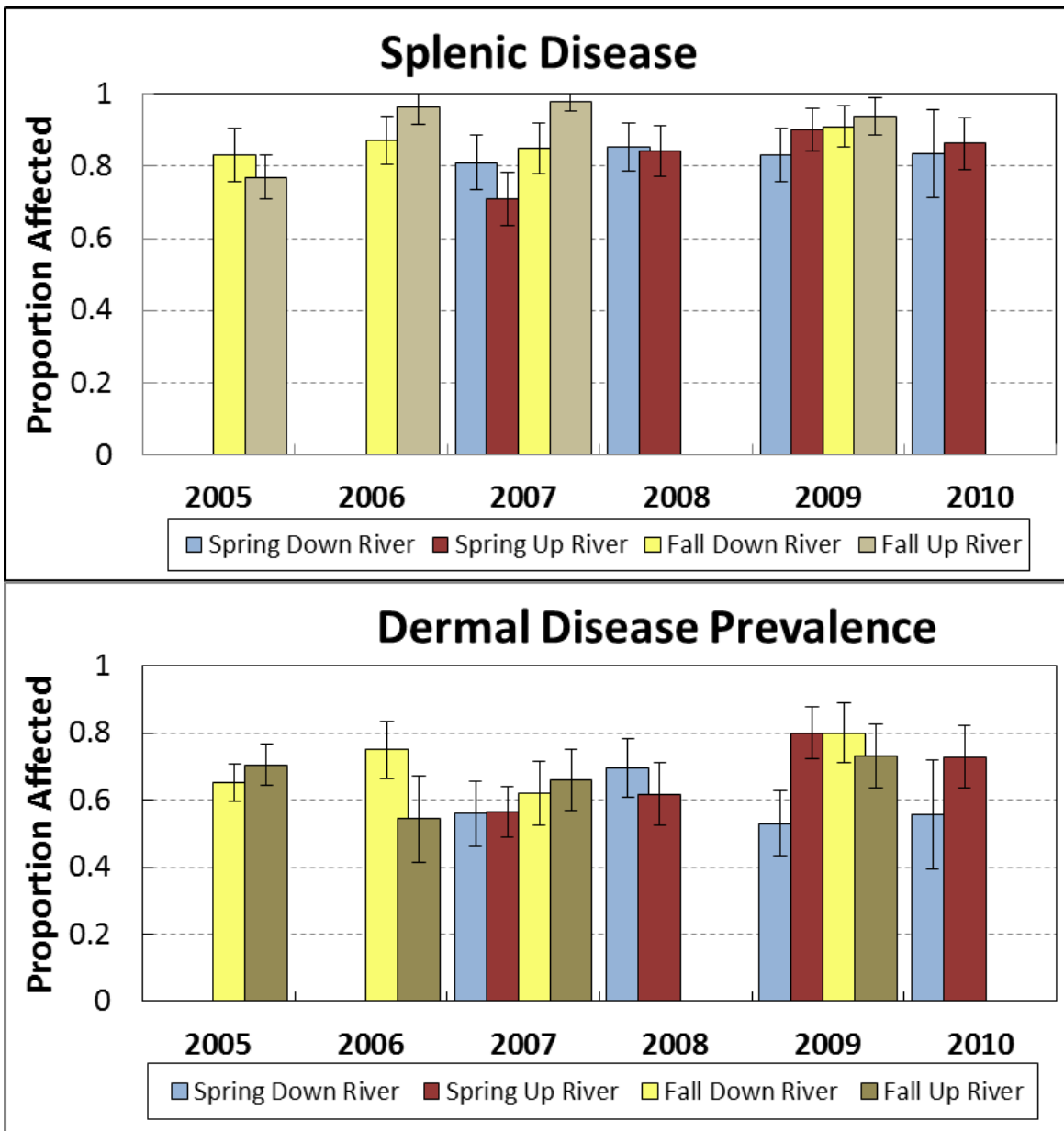


Figure 10. Splenic (histology) and dermal (gross pathology) mycobacterial disease prevalence for 2005-2010 during spring and fall in striped bass randomly sampled in the upper and lower Rappahannock River, VA. (error bars represent 95% confidence intervals).

SPRING

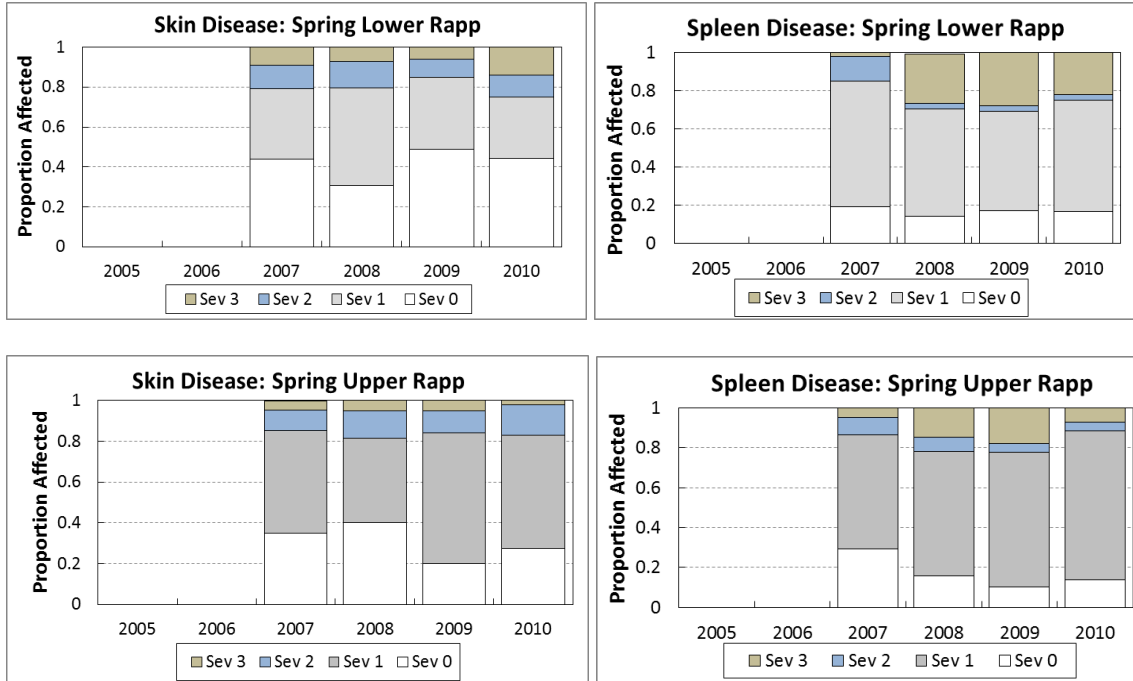


Figure 11. Relative dermal and splenic disease severity in random samples of striped bass collected during spring 2007-2010 in the upper and lower Rappahannock River.

FALL

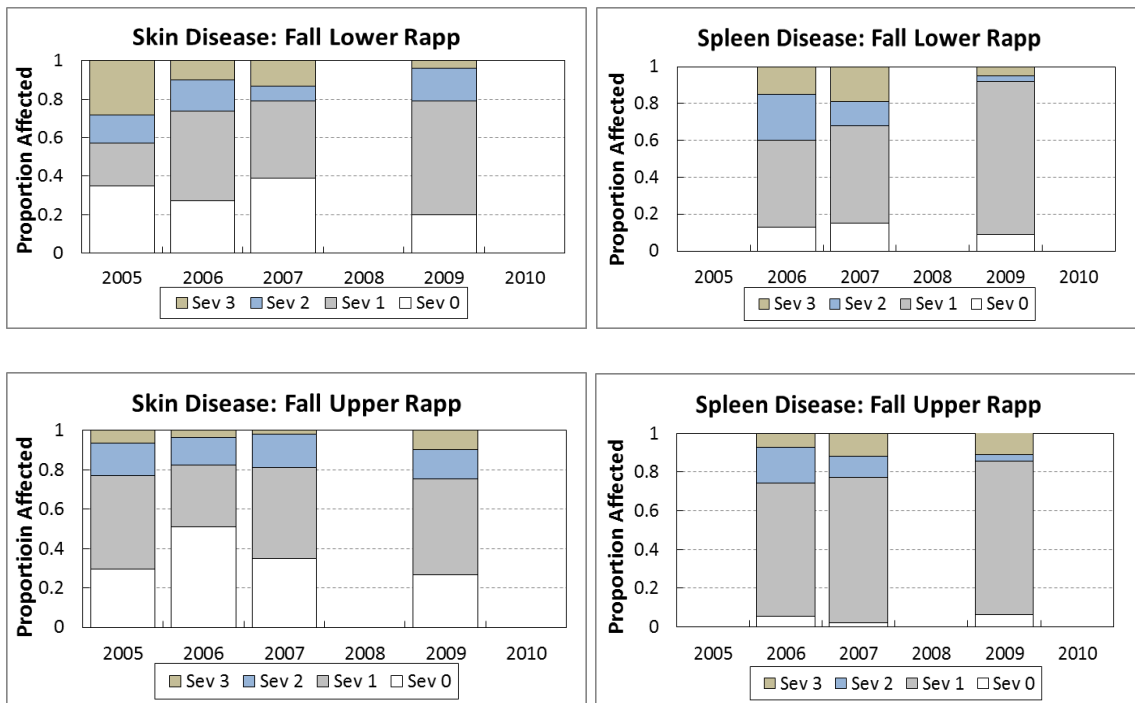


Figure 12. Relative dermal and splenic disease severity in random samples of striped bass collected during fall 2007-2009 in the upper and lower Rappahannock River.

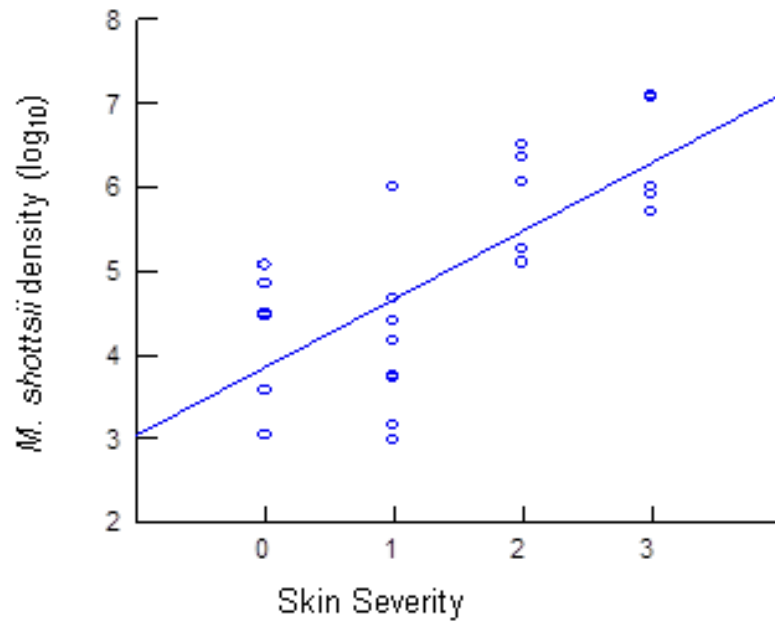


Figure 13: Scatterplot comparing log of *M. shottsii* splenic density with skin severity category. Data points represent fish for which bacteriological culture was positive for *M. shottsii*. Line is linear fit.

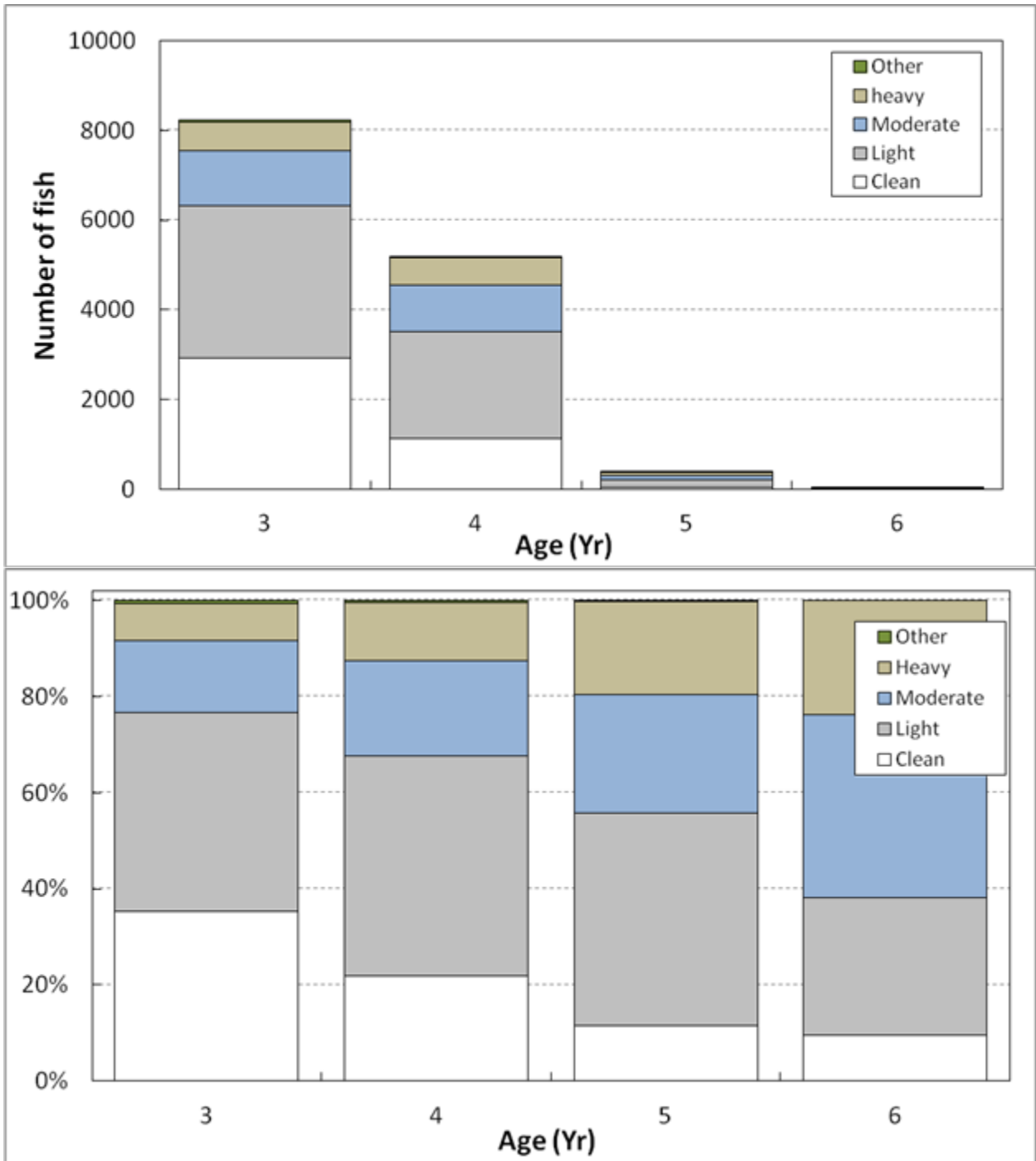


Figure 14. Absolute (top) and relative (bottom) age composition, by myco severity index, of striped bass released in the lower Rappahannock River, fall 2005-2010.

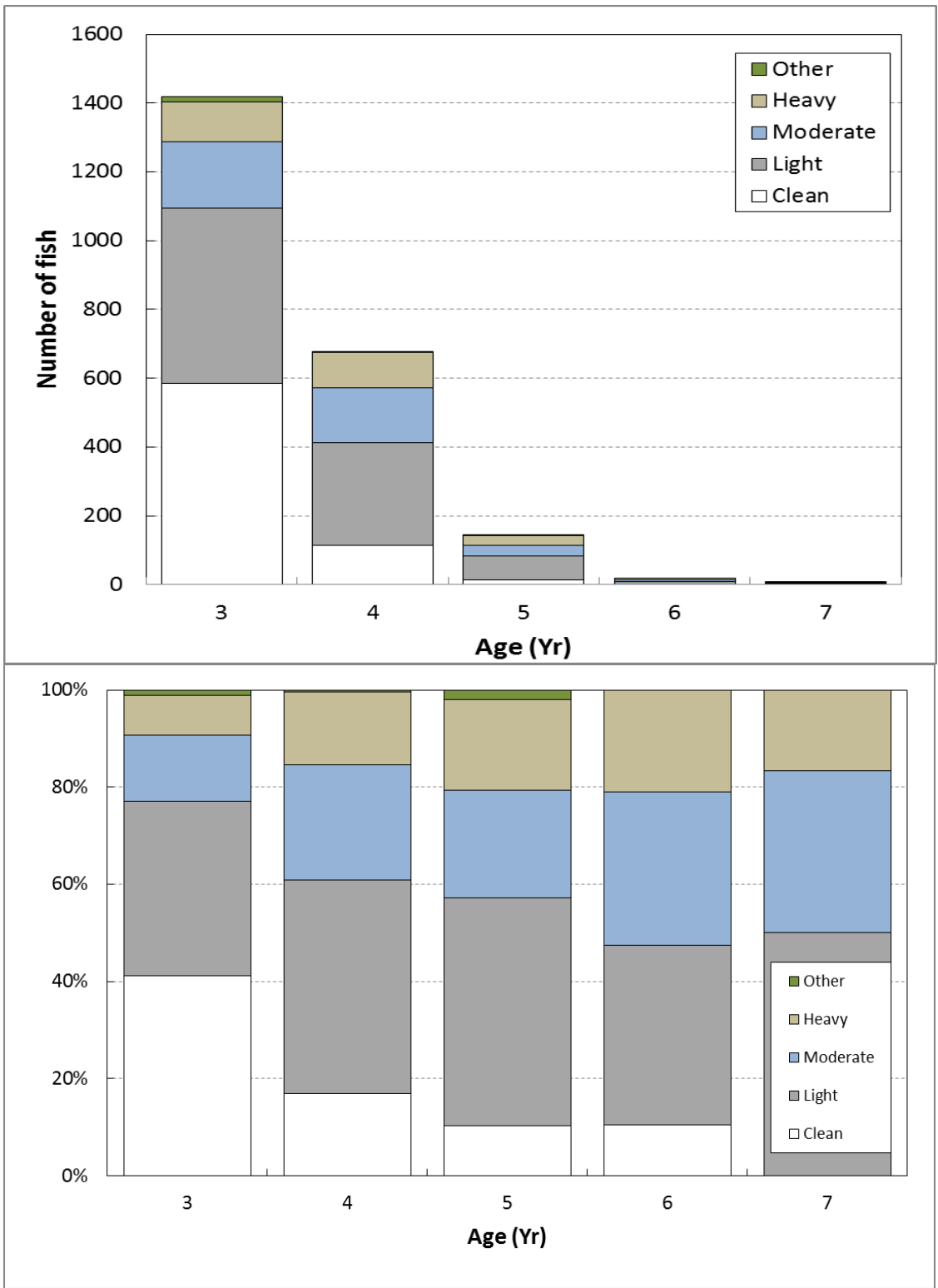


Figure 15. Absolute (top) and relative (bottom) age composition, by myco severity index, of striped bass released in the upper Rappahannock River, fall 2005-2010.

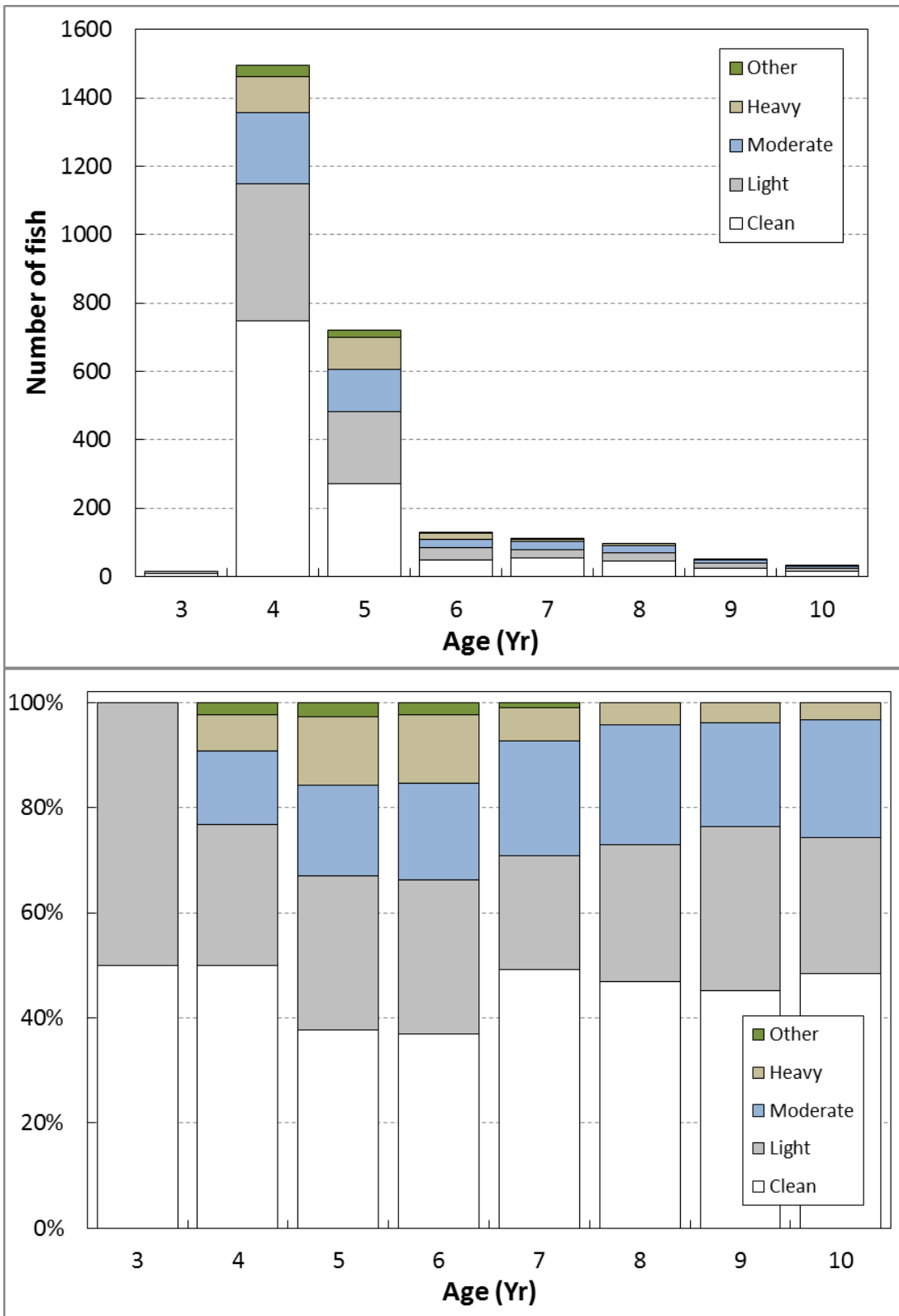


Figure 16. A) absolute and B) relative age composition, by disease severity index, of striped bass released in the lower Rappahannock River, spring 2006-2011.

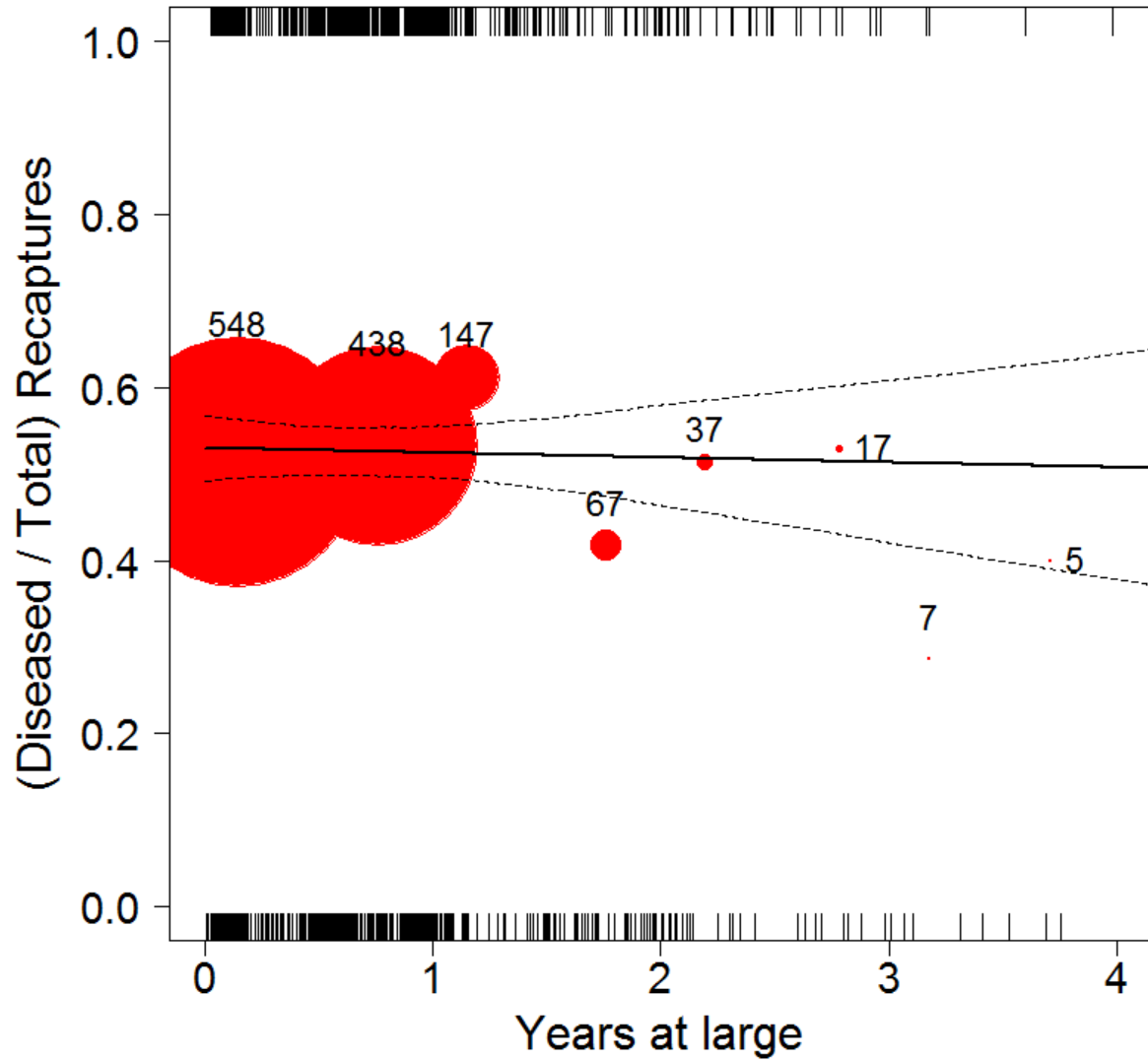


Figure 17. Logistic regression to estimate relative survival of 0.98 for striped bass with light (DC 1) dermal mycobacteriosis relative to fish with no external signs of the disease (DC 0). Rug displays show individual recaptures (disease positive on top, disease negative on bottom). Circle area is proportional to the logarithm of sample size, with numbers indicating recaptured fish. Dashed lines are 95% bootstrapped confidence intervals.

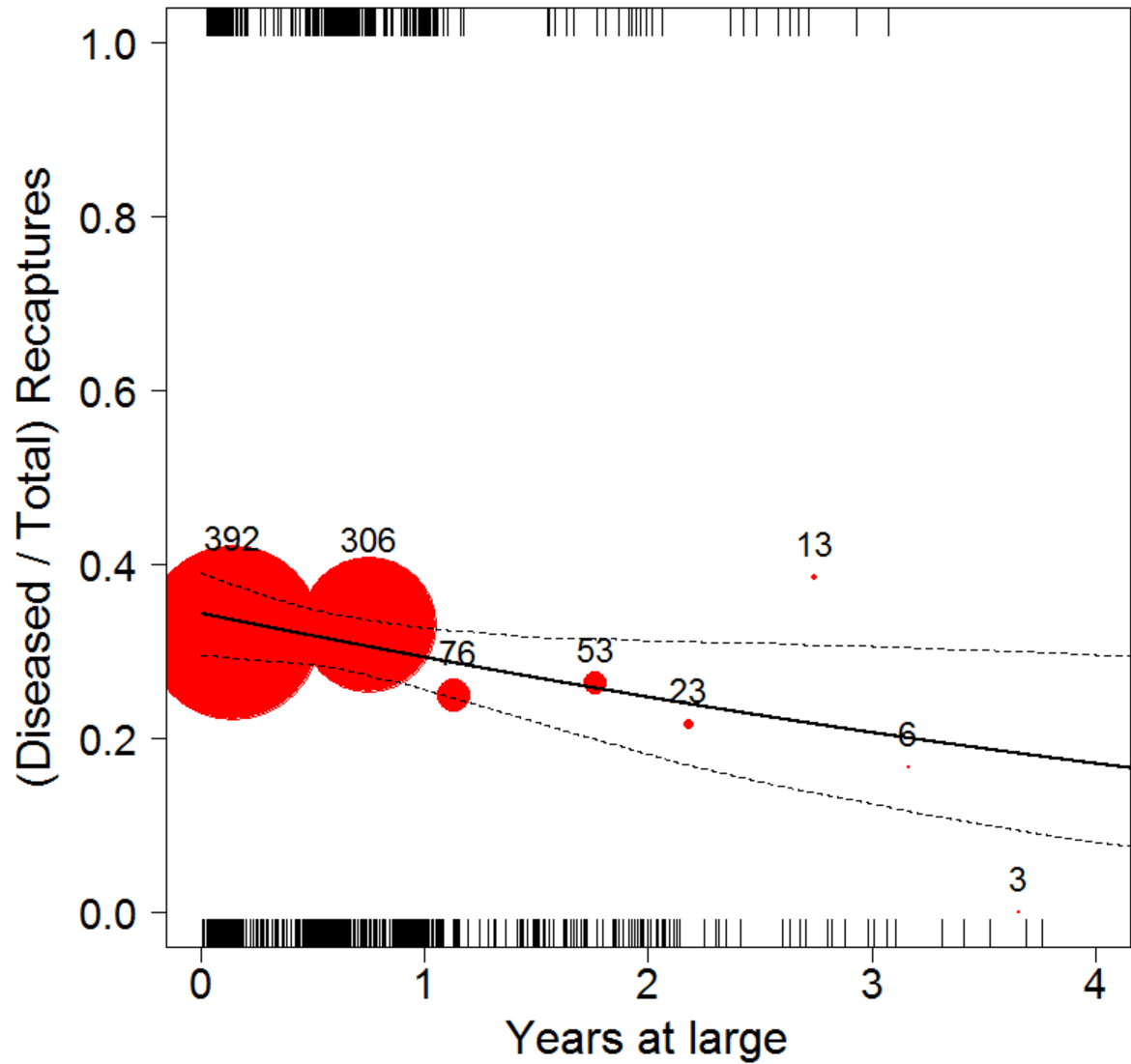


Figure 18. Logistic regression to estimate relative survival of 0.79 for striped bass with moderate (DC 2) dermal mycobacteriosis relative to fish with no external signs of the disease (DC 0). Rug displays show individual recaptures (disease positive on top, disease negative on bottom). Circle area is proportional to the logarithm of sample size, with numbers indicating recaptured fish. Dashed lines are 95% bootstrapped confidence intervals.

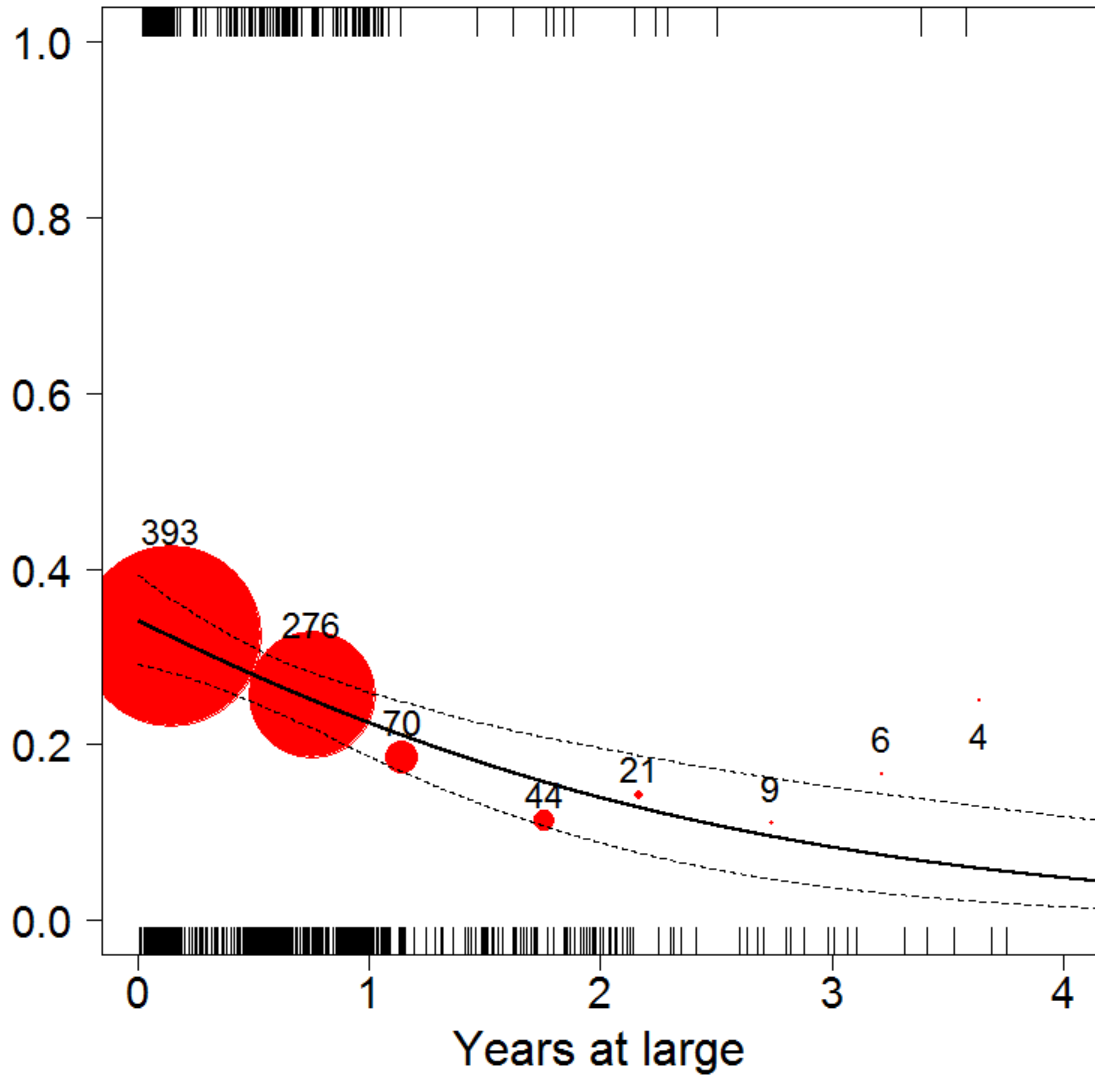


Figure 19. Logistic regression to estimate relative survival of 0.56 for striped bass with heavy (DC 3) dermal mycobacteriosis relative to fish with no external signs of the disease (DC 0). Rug displays show individual recaptures (disease positive on top, disease negative on bottom). Circle area is proportional to the logarithm of sample size, with numbers indicating recaptured fish. Dashed lines are 95% bootstrapped confidence intervals.

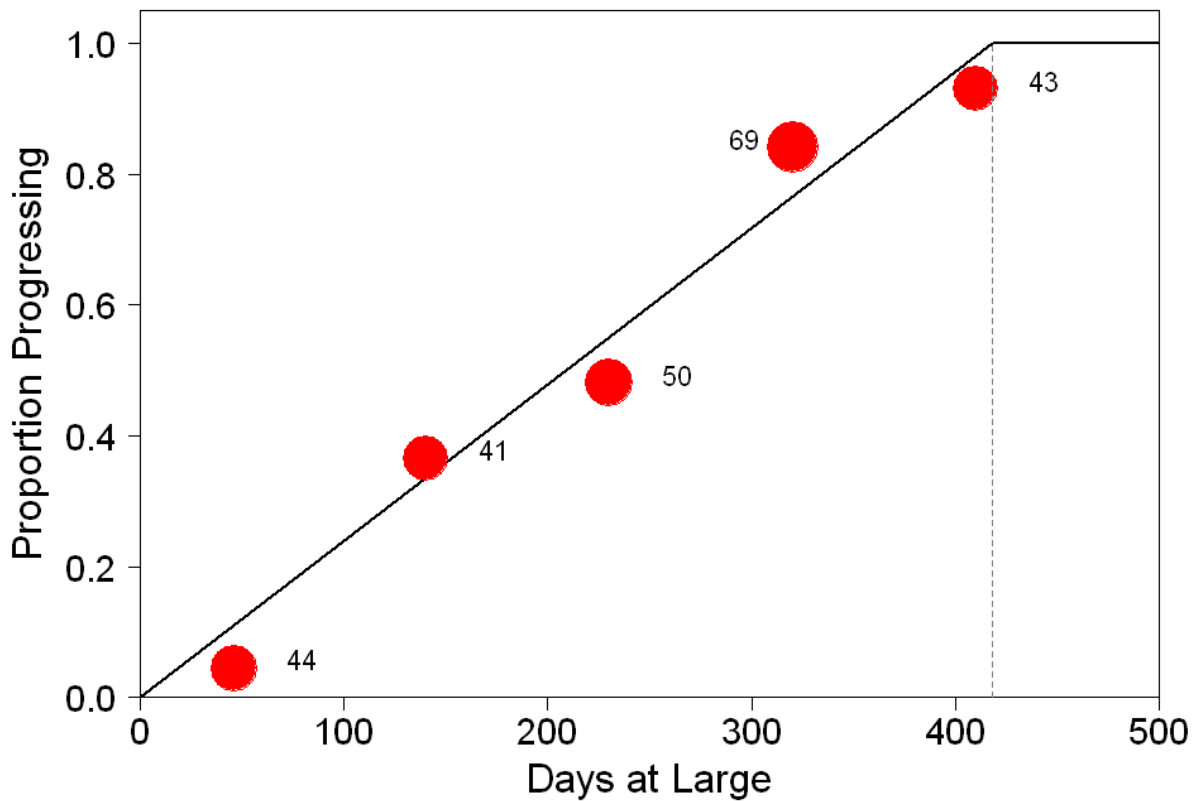


Figure 20. Progression of dermal disease from stage 1 (light infection) to stage 2 (moderate infection) in striped bass as a function of days at large. Stage duration is estimated by the reciprocal of slope and is 418 days (s.e. = 19 days). Circle area is proportional to the logarithm of sample size, with numbers indicating recaptured fish.

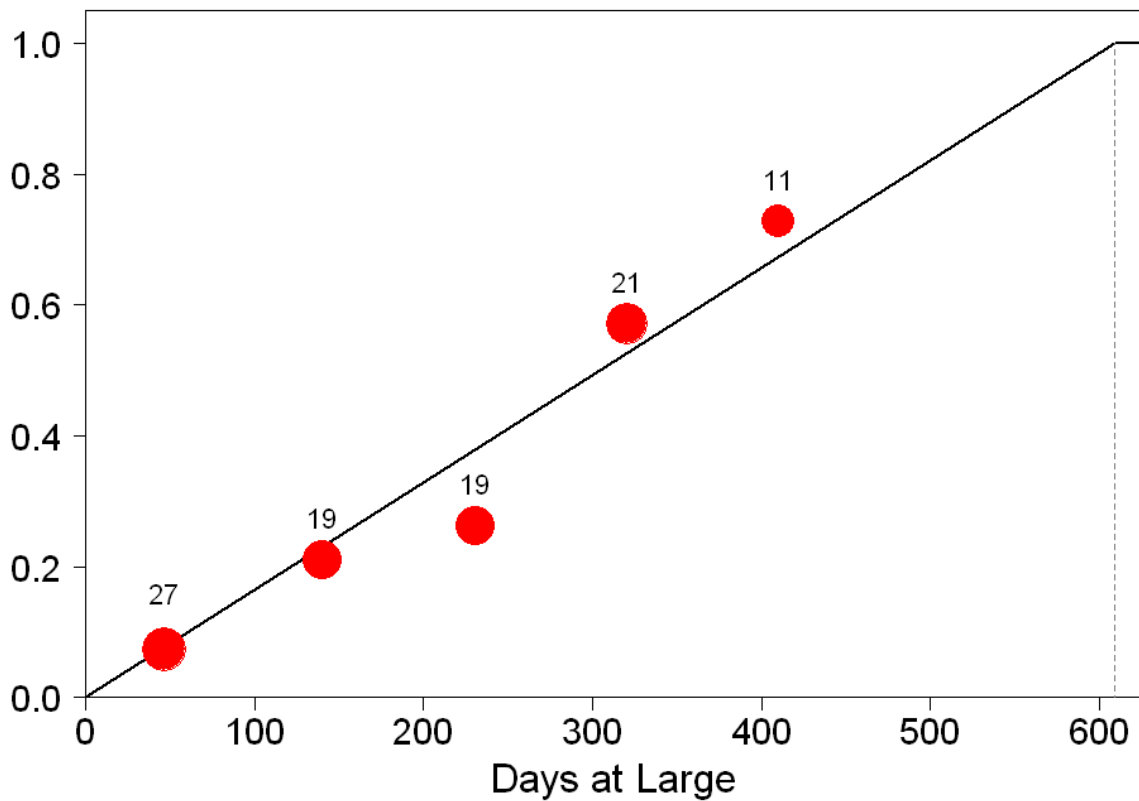


Figure 21. Progression of dermal disease from stage 2 (moderate infection) to stage 3 (heavy infection) in striped bass as a function of days at large. Stage duration is estimated by the reciprocal of slope and is 609 days (s.e. = 57 days). Circle area is proportional to the logarithm of sample size, with numbers indicating recaptured fish.