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Mycobacterial infections in striped bass (Morone saxatilis) from Delaware Bay

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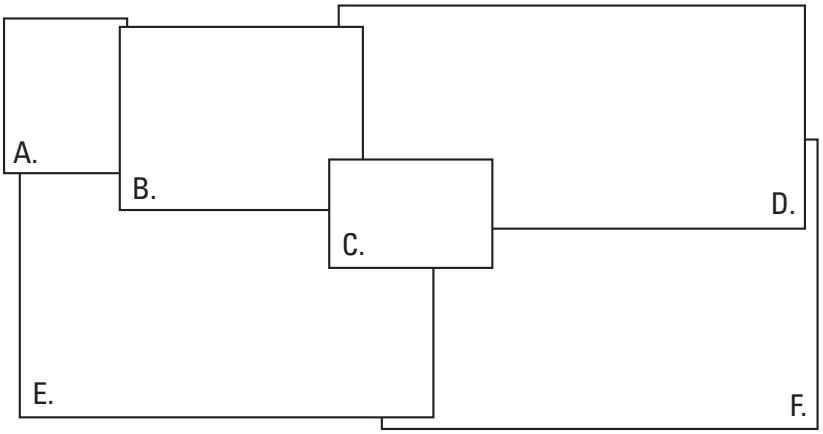

A. U.S. Fish and Wildlife Service biologist weighing Chesapeake Bay striped bass exhibiting skin lesions. Photo by Christine L. Densmore, U.S. Geological Survey

B. Stained tissue section taken from the spleen of a Chesapeake Bay striped bass exhibiting granulomatous lesions associated with a mycobacterial infection. Photo by David Gauthier, Virginia Institute of Marine Science

C. Chesapeake Bay striped bass exhibiting emaciation and skin lesions consistent with mycobacteriosis. Photo by Mark Matsche, Maryland Department of Natural Resources

D. Collection of blood sample from striped bass harvested from the Nanticoke River, Maryland, in November 2002. Photo by Mark Matsche, Maryland Department of Natural Resources

E. U.S. Geological Survey field crew processing striped bass as part of a mycobacteriosis survey conducted in the tidal portions of Chesapeake Bay rivers during 2002 and 2003. Photo by Mark Matsche, Maryland Department of Natural Resources

F. Live well transport and landing of striped bass by U.S. Fish and Wildlife personnel. Photo by Mark Matsche, Maryland Department of Natural Resources

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along with the sequence similarity present in closely related bacteria, precluded the design of absolutely specific primers. In total, six primers were constructed and tested.

Males made up 68% of the sample and 32% were females. Low-level infestations of intestinal parasites were present in nearly 80% of the fish. All of the striped bass examined had visible fat deposits in the viscera with 95% having some traces of visceral fat or moderate accumulation of fat. Granulomas were present in 83% of the fish with the vast majority being of verminous origin. Acid-fast bacteria were detected within at least one organ in 18% of the striped bass with the highest percentage (8.3%) visualized in the spleen, followed by mesenteric tissues. Low numbers of granulomas containing acid-fast bacteria were detected in the heart, liver, and head kidney. Acid-fast bacteria were detected in multiple organs in only 5% of the fish examined. The observed granulomas containing acid-fast bacteria are consistent with mycobacteriosis but no *Mycobacterium* sp. were detected by PCR amplification. All sequences recovered were from members of the bacterial order Actinomycetales, family Corynebacterineae, to which the genus *Mycobacterium* belongs. The two most common sequences were from *Corynebacterium sundsvallense* and *C. thomssenii*, two closely related species first described in 1999. *C. sundsvallense* has been isolated only from human clinical samples, while *C. thomssenii* has also been isolated from an air sample. The discrepancy between the visual observation of granulomas containing acid-fast bacteria and PCR results must be addressed in future efforts. It is clear that other factors including contamination, low levels of *Mycobacterium* in the fish samples, a higher prevalence of Corynebacteria than *Mycobacterium* in the tissues, or differences in the DNA extraction efficiency for the species involved may have influenced our results. The observed granulomas containing acid-fast bacteria are consistent with mycobacteriosis. It should also be noted that *Corynebacterium* are not acid fast and have different morphology than *Mycobacterium*. We conclude that striped bass in the Roanoke River have been exposed to acid-fast bacteria, but presumptive mycobacteriosis in the population has not been confirmed by PCR or culture.

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Mycobacterial infections in striped bass (*Morone saxatilis*) from Delaware Bay.

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Much of what is known of mycobacteriosis in wild striped bass from the mid-Atlantic region of the United States is based on our observations from Chesapeake Bay and its tributaries, where high infection prevalence and lesioned fish are frequently observed. Comparatively, the occurrence and severity of mycobacteriosis in striped bass from watersheds adjacent to Chesapeake Bay are relatively unknown. This study represents the first report on mycobacterial infection in striped bass harvested from two sites in Delaware Bay.

Eighty striped bass *(Morone saxatilis*) were obtained from Delaware Bay using commercial gill-nets located adjacent to Woodland Beach (39.333°N, 75.475°W; n = 70) and Bowers Beach (39.058°N, 75.397°W; n = 10) in December of 2003. Bowers Beach and Woodland Beach samples were obtained over an 8-day period on one and three sample dates, respectively. Nets were fished for approximately 24 h, and live striped bass were removed and transported to shore in a water tank fed by pumped surface water. Fish were euthanized with a lethal dose of an anesthetic agent, packed in ice, and transported to the USGS National Fish Health Laboratory (Leetown, WV) for further processing. At necropsy for each specimen, fish were examined for gross external and internal lesions as well as the presence of parasites; total lengths and eviscerated weights were determined to calculate condition factor (K); and tissue samples were collected. Portions of spleens were aseptically harvested for bacterial culture, and tissue specimens were collected in Z-fix preservative for histological examination. Tissue specimens for histological

and bacteriological analyses were processed routinely using methods previously described and utilized for similar studies with Chesapeake Bay striped bass populations. Spleen, liver, kidney, and gonad were evaluated histologically for lesions, including presence and severity of granulomatous inflammation; presence of acid-fast bacteria associated with splenic, hepatic, and renal granulomas; and presence of parasites. Homogenates of aseptically harvested spleen tissue were serially diluted and plated onto Middlebrook and Brain Heart Infusion agars for bacterial isolation and enumeration. Recovered acid-fast and non-acid-fast bacteria were further characterized biochemically. Statistical analyses of results were performed using analysis of variance (size, condition factor), Pearson chi-square and two-tailed Fisher exact test (bacterial and parasite infection intensities, granuloma occurrence). All statistical analyses were performed using SYSTAT 11 (SYSTAT Software, Inc., Point Richmond, CA). Statistical significance was designated as $p < 0.05$.

No differences were noted based on size or condition of the fish. The size distribution of the striped bass was relatively homogeneous with mean total lengths of about 600 mm in all samples. Mean condition factors exceeded 0.95 in all samples and were not significantly different among the samples. Pooled condition factors for mycobacterial infected (n = 14) and noninfected (n = 66) striped bass were also not significantly different. With the exception of rectal nodules associated with acanthocephalan infections, no gross abnormalities were observed either externally or internally among these striped bass. The percentage of fish with coelomic nematode infections ranged from 26.6 – 69.2% and did not differ significantly among sample collections. The percentage of striped bass with rectal acanthocephalan infections was significantly different among samples, such that collections made at both sites on 12/09/03 and 12/10/03 (61.5 - 70.0%) were significantly higher than the Woodland Beach sample collected on 12/17/03 (21.4%) but were not significantly different from each other. No significant differences in granuloma prevalence were observed among samples. Prevalence of acid-fast bacilli observed in association with splenic granulomas was low overall (6.25%, n = 80), and acid-fast bacilli were not observed in association with any liver or kidney granulomas. The occurrence of encysted helminthes in spleens was a common observation, but parasites were very seldom noted in association with granulomas in the liver, kidney, or gonad. Significant differences in mycobacterial infection prevalence (as determined by the combination of bacterial culture and histopathology) were observed among samples, with those obtained at Woodland Beach on 12/10/03 (53.8%, n = 13) and 12/17/03 (7.1%, n = 42) exhibiting the most striking differences. Mycobacterial infection intensities ranged from about 10^2 to 10^7 CFU g^{-1} spleen. Ten acid-fast isolates were obtained from the Delaware Bay striped bass in all, and only one presumptive *Mycobacterium* sp. was isolated from each positive sample. Seven of the ten acid-fast isolates were characterized by biochemical phenotyping. Only one of the seven Mycobacterium isolates, identified as *M. chelonae*, exactly fit existing species descriptions. Non-acid-fast bacterial infection prevalence was also significantly different among the samples. Infection intensities ranged from 10^3 to 10^5 CFU g^{-1} spleen. *Lactococcus lactis* ssp. *lactis*, and *Pseudomonas fluorescens* were the most frequently identified non-acid-fast species.

Mycobacterial infection prevalences observed in the Delaware Bay samples were substantially lower than that reported for striped bass in Chesapeake Bay. In general, the infection intensities in these Delaware Bay striped bass were also lower than those previously reported for mycobacteria culture-positive striped bass of similar size from Chesapeake Bay. Although overall condition of these striped bass among the four Delaware Bay samples was rather homogeneous, differences in mycobacterial infection prevalence, non-acid-fast bacterial infection prevalence, and acanthocephalan infection prevalence were apparent. Differences in microbial exposures, diet, contact with environmental stressors, or genetic background might explain the intersample variation in bacterial and parasitic infections observed among these fish.