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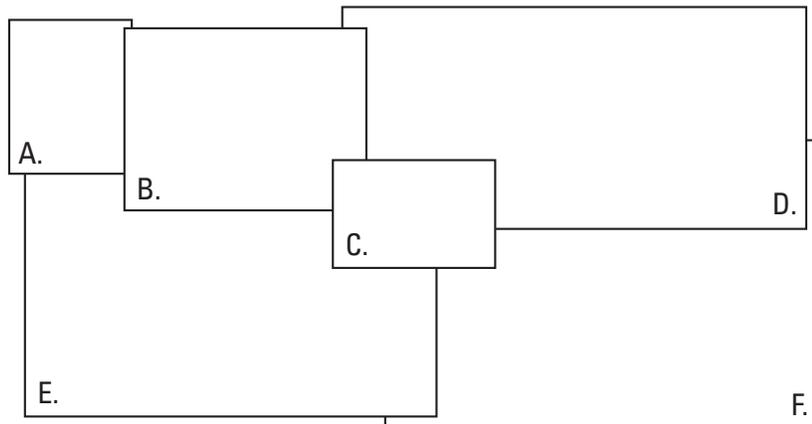
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USGS/NOAA Workshop on Mycobacteriosis in Striped Bass, May 7-10, 2006, Annapolis, Maryland



USGS Scientific Investigations Report 2006-5214
NOAA Technical Memorandum NOS NCCOS 41



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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Edited By Christopher A. Ottinger and John M. Jacobs

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The ecology of mycobacteria infecting striped bass (*Morone saxatilis*) in Chesapeake Bay: A research plan.

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The ecology of mycobacteria in estuarine and marine waters remains poorly understood. The current epizootic in Chesapeake Bay striped bass and newly described pathogens *Mycobacterium shottsii* and *M. pseudoshottsii* raise ecological questions that if answered can improve our understanding of the pathogenesis of mycobacteriosis in this fish species. Questions remain whether these mycobacteria are obligate or opportunistic pathogens. Do these species exist as free-living saprophytes in estuarine ecosystems? And if so, is their recent success as pathogens in estuarine systems facilitated by anthropogenic alterations in water quality such as nutrient enrichment or organic pollutants?

The literature describes the environmental occurrence of nontuberculous or environmental mycobacteria using the term “ubiquitous.” Numerous research papers on *Mycobacterium marinum*, for example, contain statements such as

“.....virtually any water source and water-related activity is a potential risk, including tending aquarium, fishing, skin diving, and a number of other water-related activities” (Dobos et al., 1999).

Presence does not necessarily imply function, and from an aquatic microbial ecology viewpoint such statements provide little insight toward resolution of the immediate question raised, e.g., do these organisms participate in processes of energy and material flow or behave essentially as passive and persistent allochthonous particles? A number of recent papers address mycobacterial persistence and dormancy by examining physiological responses to starvation in model species such as *M. smegmatis*. Examples from the literature will be provided in this presentation where appropriate with respect to the following questions:

1. Is there an estuarine reservoir of *M. shottsii/pseudoshottsii* in Chesapeake Bay?

Ongoing or new research needs to address the question of whether or not the dominant microorganisms now infecting striped bass are autochthonous components of Bay waters or whether they are allochthonous and transient contaminants originating from an external source and happen to infect striped bass. In addition, we need to deter-

mine if their distribution is similar to that of *M. marinum*. Does virtually any source of water serve as a reservoir for the bacterium? And are there animal reservoirs other than striped bass?

2. Are the dominant mycobacteria now infecting striped bass obligate pathogens and not found elsewhere in the environment, or are they opportunistic free-living organisms in Chesapeake Bay habitats?

If recoverable from Bay environments, isolate virulence could be assessed using a fish model or *in vitro* assays to examine the ability of mycobacteria to invade and persist in primary cultures of host cells or cell lines, as well as cytopathic effect. A recent report suggests that strain variation in *M. marinum* isolates is important to human pathogenicity. Knowing the environmental distribution and prevalence of *M. pseudoshottsii* strains possessing the IS2404 plasmid associated with mycolactone production could be relevant to the epidemiology of the ongoing epizootic.

3. What are some of the basic autecology questions to be addressed for these organisms?

Little is known regarding the autecology of *Mycobacterium* spp. Some fundamental questions need to be addressed, including the following. How do *M. shottsii/pseudoshottsii* respond to physicochemical characteristics of estuarine environments? What are the responses of these species to abiotic factors such as temperature, salinity, and light? What are the effects of nutrients, both inorganic and organic, on growth or survival? Can saprophytic growth occur in filtered estuarine water supplemented with C, N, and P or trace elements such as Fe? Can these species utilize hydrocarbons as substrates as can saprophytic mycobacteria found in soils? Is the growth/persistence of these mycobacteria facilitated by nutrient enrichment? Is the mildly alkaline pH of Bay water detrimental to these organisms? To what extent can persistence under nutrient-limiting conditions be observed with these species? As a null hypothesis, can we assume protracted persistence *in vitro* in distilled water? What about persistence under environmentally relevant conditions? What are the combined effects of temperature, light, and predation on mycobacterial densities? What are the rates of inactivation/removal that would provide information on temporal boundaries of seasonal infectivity and bacterial survival? Finally, do starved cells demonstrate stress responses similar to those observed in other species of bacteria challenged with adverse conditions? Is there evidence of an adaptive response? Can dormant cells be detected using conventional culture methods? Can dormant cells become viable/infective when conditions improve?

4. What is the distribution of *M. shottsii/pseudoshottsii* in Chesapeake Bay aquatic habitats?

What are possible sources? Can *M. shottsii/pseudoshottsii* be isolated from soils or stormwater runoff? If restricted to water, is their distribution dependent or independent of salinity or rainfall? Can they be isolated from sewage or other types of wastewaters? What is the extent of genomic strain variation among isolates and what genetic mechanisms are involved? Are there subtypes across physical-chemical gradients that vary in virulence?

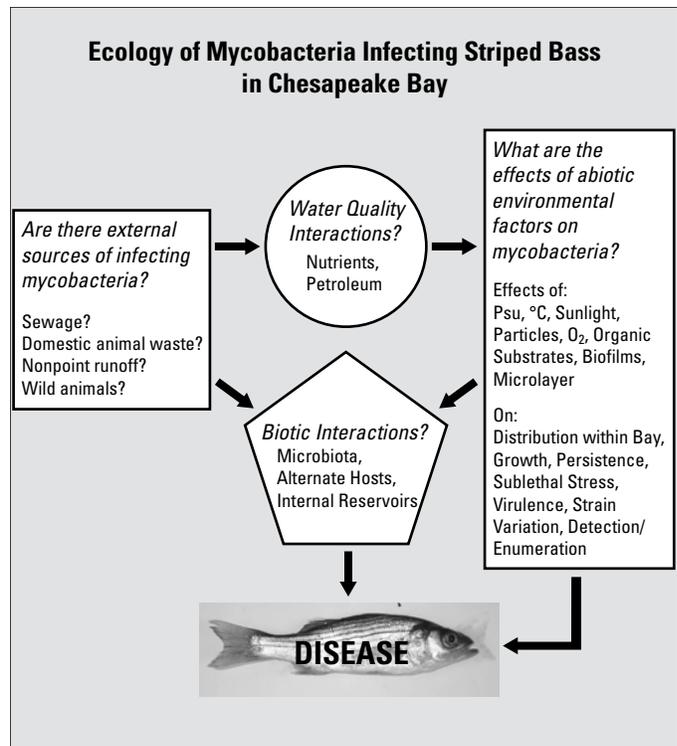
Other specific niche habitats include microlayer, water column, and biofilms. Microlayers concentrate nonpolar molecules, which may be substrates for mycobacteria. Can these organisms be detected in surface water as well as below seasonal thermoclines? Can they be found in sediment? Biofilms offer unique microenvironments for pathogens and provide protection from predators, enhance potential to achieve high densities, select for virulent strains, and increase the infective dosage. Do swampy freshwater areas contiguous with Bay watersheds provide unique habitats of comparatively low pH supporting mycobacterial growth?

5. What is the relationship of *M. shottsii/pseudoshottsii* to other indigenous aquatic microbiota?

Using *in situ* exposure methods described in the literature, it is possible to evaluate the effect of microbial predation on these species. However, given the recent literature describing how mycobacteria use amoebae as an intracellular refuge for replication, can similar interactions occur with the seasonally diverse estuarine microheterotrophic plankton community? If so, is there potential for mycobacterial transfer up the food chain as a plausible mechanism to infect tertiary consumers such as striped bass?

Although the research questions articulated above are important and relevant, and to some extent testable using conventional cultural methods, it is obvious that efforts to address them will be retarded without improved detection/enumeration techniques.

The classic problem with enumeration of environmental mycobacteria in a mixed microbial community is that existing methods for isolation of mycobacteria employ harsh decontaminants that can significantly bias recovery and render quantitative results inaccurate. Moreover, this limitation becomes more acute should sample concentration be required. There is thus a critical need for improved methods. These include development and validation in environmental waters of species-specific polymerase chain reaction (PCR) primer sets (and TaqMan probes) for real-time PCR quantitative detection. While access to such methods would be extraordinarily beneficial, we cannot ignore that the problem of detecting nonviable cells with PCR will require supplementary approaches to confirm cell viability. Use of live/dead penetrating fluorescent



stains, PCRs targeting specific genes or gene products, and perhaps developing a workable fluorescent *in situ* hybridization (FISH) method combining direct counting with a metabolic assay would facilitate the study of mycobacterial ecology in aquatic systems.

Understanding the ecology of the mycobacteria infecting striped bass is a crucial step toward identifying environmental factors and processes that influence the epidemiology of the current epizootic.

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