



## Investigations on Fish Health in Selected Tributaries of the Chesapeake Bay and Linkages to Water Quality, Land Use, and Other Environmental Factors

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**Statement of Problem:** During the summer and fall of 1996 and 1997, an unusually high prevalence of skin lesions in fishes from the Pocomoke River, Maryland and other tributaries of Chesapeake Bay stimulated significant public concern and scientific interest. These skin lesions ranged from small petechial hemorrhages to abrasions to deep ulcers penetrating underlying muscle and visceral organs. A variety of fish species were involved as indicated by results of surveys conducted by several state and federal agencies during this time period. In addition, two fish kills involving primarily juvenile Atlantic menhaden (*Brevoortia tyrannus*) occurred in the Pocomoke River during August 1997. The fish kills as well as the variety of fish lesions were attributed to the presence of the toxic dinoflagellate, *Pfiesteria piscicida* or *Pfiesteria*-like (*Pfiesteria*- complex organisms) dinoflagellates. Because menhaden were the most frequent target of acute fish kills and episodes of fish lesions in the Chesapeake Bay, the penetrating ulcers so common in this species are now viewed by many as "*Pfiesteria*-related" and thought to be caused by exposure to *Pfiesteria* toxin.

In 1997 we examined histologically over 100 menhaden collected from both fish kills and areas where "significant numbers" of lesioned menhaden were observed. Consistently, these lesions are composed of a chronic inflammatory response around very invasive, penetrating fungal hyphae. The lesions we observed in the menhaden are identical to those described for epizootic ulcerative syndrome (EUS) in the IndoPacific, mycotic granulomatosis (MG) in Japan and red

spot disease (RSD) in Australia. These ulcerative syndromes are all now known as EUS and *Aphanomyces invadans* has been confirmed as the cause by other researchers.

In spring 1998, we began the first year of a three-year study to evaluate general fish health in selected tributaries. This research utilizes white perch as the test species because we can obtain the appropriate size specimens in all of the tributaries during the three time periods sampling occurs. This study includes a comprehensive fish health assessment, immune function evaluation and correlation of findings with land use. During the sampling trips in August 1998 we were also able to capture lesioned menhaden from the Wicomico and Pocomoke Rivers. There were no fish kills in 1998, the fish were not showing clinical signs of toxin exposure, nor were significant numbers of *Pfiesteria*-like dinoflagellates

### **Objectives:**

- 1) Identify and characterize the fungal pathogen isolated from menhaden.
- 2) Determine infectivity of fungal isolate in a number of fish species.
- 3) Evaluate environmental factors that may affect susceptibility of fish to fungal infections in the laboratory.
- 4) Determine the factors that may influence development of lesions in menhaden in the Chesapeake Bay tributaries.
- 5) Evaluate white perch health in selected tributaries of the Chesapeake Bay as an indicator of environmental health.

**Approach:** For the *Aphanomyces* studies: We will utilize the isolates made in 1998 from the Wicomico and Pocomoke Rivers, *A. invadans* (provided by Dr. James Lilley), and the ATCC *Aphanomyces* sp. (84-1240) previously associated with ulcerative mycosis of menhaden in North Carolina, for the comparisons described below.

Morphological description, culture characteristics, temperature growth curves, infectivity and pathogenesis in selected fish species, and genetic comparisons are the data that will be collected under objectives 1 and 2.

Factors that affect fungal growth, infectivity and fish susceptibility, progression and temporal observations of lesions in the wild, and site characteristics of areas with high incidences of lesions are the data that will be collected under objectives 2 and 3.

For the white perch studies: Comprehensive fish health assessments that include documenting gross lesions, immune function, histopathological changes and reproductive health will be conducted in June, August and October 1998-2003.

We will continue the health assessments of white perch in the Pocomoke and Patuxent rivers. In the Pocomoke we will have 6 years of samples collected in June, August and October of each year. This is allowing us to evaluate the influences of various water conditions (high flow/low salinity versus low flow/high salinity), water temperatures and contaminant levels on presence of

gross lesions, changes in immune function and reproductive health of the perch. In addition, in the Patuxent the fish collected this year should have been eggs or fry at the time of a major oil spill. This allows us to evaluate potential long-term, chronic effects of an oil spill.

We will continue assessing the effects of various environmental conditions on the growth and sporulation of *Aphanomyces invadans*, complete manuscripts on infectivity studies and develop methods to measure zoospore concentrations in natural water samples.

### **Selected Reports and Other Products:**

Posters, Delivered: Page, S., J. Young, V. Blazer, C. Ottinger, C. Densmore, L. Iwanowicz and E. Frankenberry, 2002, The role of GIS in the analysis of fish health and environmental data. (poster). Chesapeake and Potomac Regional SETAC meeting, Reston, VA, April 18.

Presentations, Delivered: Blazer, V., C. Densmore, C. Ottinger, L. Iwanowicz, E. Frankenberry and L. Pieper, 2002, White perch health indicators in the Patuxent river. Chesapeake and Potomac Regional SETAC meeting, Reston, VA, April 18.

Presentations, Delivered: Blazer, V.S., 2002, EUS in the United States – a Case Definition. 5th Symposium on Diseases in Asian Aquaculture, Nov. 24-27, Surfers Paradise, Queensland, Australia.

Presentations, Delivered: Blazer, V.S., W.B. Schill, C.L. Densmore, L. Pieper, Y. Kiryu and S. Page, 2002, Overview of *Aphanomyces invadans* in menhaden along the East Coast of the United States. 4th International Symposium on Aquatic Animal Health, Sept. 1-5, New Orleans, Louisiana.

Presentations, Delivered: Cartwright, D.D., V.S. Blazer and R.R. Reimschuessel, 2002, Myxosporean infections in Atlantic menhaden in tributaries of the Chesapeake Bay. Myxozoan Parasites of Fish Conference, July 31-August 2, Nanaimo, British Columbia.

Presentations, Delivered: Ottinger, C., V. Blazer, C. Densmore, D. Cartwright, L. Iwanowicz, S. Page, L. Pieper, Modulation of white perch immune function: Investigations on fish health in selected tributaries of the Chesapeake Bay. Fourth International Symposium on Aquatic Animal Health. New Orleans, LA. September 1 – 5, 2002. INVITED.

Report, Delivered: Blazer, V.S., J.H. Lilley, W.B. Schill, Y. Kiryu, C.L. Densmore, V. Panyawachira & S. Chinabut, 2001, AF349614 *Aphanomyces invadans* isolate WIC internal transcribed spacer 1, complete sequence.

Report, Delivered: Blazer, V.S., J.H. Lilley, W.B. Schill, Y. Kiryu, C.L. Densmore, V. Panyawachira and S. Chinabut. 2002. *Aphanomyces invadans* in Atlantic menhaden along the east coast of the United States. J. Aquatic Animal Health 14:1-10.

Report, Delivered: Blazer, V.S., W.K. Vogelbein, C. Densmore, H. Kator, D. Zwerner and J. Lilley, 2000, Etiology and pathogenesis of skin ulcers in menhaden, *Brevoortia tyrannus*: Does *Pfiesteria piscicida* play a role? Marine Environmental Research 50:487-488.

Report, Delivered: Gauthier, D.T., D.D. Cartwright, C.L. Densmore, V.S. Blazer and C.A. Ottinger, 2003, Measurement of in vitro mitogenesis in fish: ELISA-based detection of the thymidine analogue 5'-bromo-2'-deoxyuridine: Fish and Shellfish Immunol. 14:279-288.

Report, Delivered: Kiryu Y., J.D. Shields, W. K. Vogelbein, H. Kator, and V. S. Blazer, 2003, Infectivity and pathogenicity of the oomycete, *Aphanomyces invadans*, in Atlantic menhaden, *Brevoortia tyrannus*. Diseases of Aquatic Organisms 54:135-146.

Report, Delivered: Kiryu, Y., J. Shields, W.K. Vogelbein, D.E. Zwerner, H. Kator and V.S. Blazer, 2002, Induction of skin ulcers in Atlantic menhaden by injection and water-borne exposure to the zoospores of *Aphanomyces invadans*: J. Aquatic Animal Health 14:11-24.

Report, Delivered: Reimschuessel, R., C.M. Gieseke, C. Driscoll, A. Baya, A.S. Kane, V.S. Blazer, J.J. Evans, M.L. Kent, J.D.W. Moran and S.L. Poynton, 2003, Myxosporean plasmodial infection associated with ulcerative lesions in young-of-the-year Atlantic menhaden in a tributary of the Chesapeake Bay, and possible links to *Kudoa clupeiidae*: Dis. Aquat. Org. 53:143-166.

**Relevance and Benefits:** This task addresses the Fisheries and Aquatic Resources Program's Goal to foster aquatic animal health.