Caging Atlantic Menhaden: Collection, Husbandry, and *in situ* Bioassays with a Sensitive Estuarine Species

John M. Jacobs,[1] *University of Maryland, Center for Environmental Science, Horn Point Laboratory, Cambridge, MD 21613*

William F. Van Heukelem, *University of Maryland, Center for Environmental Science, Horn Point Laboratory, Cambridge, MD 21613*

Charles Gieseker, *U.S. Food and Drug Administration, Center for Veterinary Medicine, Office of Research, Laurel, MD 20708*

Reginal M. Harrell, *Maryland Cooperative Extension, Wye Research and Education Center, Queenstown, MD 21658*

Abstract: Cages as tools for *in situ* bioassays have a proven track record in monitoring the effects of effluent and sediment toxicity. Application to biomonitoring, however, has received comparably little attention. With the increasing threat of harmful algal blooms to both humans and aquatic organisms, their use as sentinels for early warning and for the examination of organism response *in situ* is evident. During efforts to monitor and describe the response of juvenile menhaden *(Brevoortia tyrannus)* to the reportedly toxic dinoflagellates of the genus *Pfiesteria,* we devised a simple, inexpensive cage for the conduct of *in situ* bioassays. This design, in combination with fish husbandry and transport techniques specific for menhaden, allows for rapid, inexpensive deployment of cages in at least a 4-h radius of the holding facility with an acceptable level of mortality and minimal caging effects. Here we describe their application in Middle River, Maryland, where a high prevalence of menhaden with ulcerative lesions was detected in the presence of *Pfiesteria*-like organisms in August 1999. In all cages, no mortalities occurred that were attributable to anything other than transport stress, and physiological and neurological variables investigated proved to be in the normal range for the species. This approach demonstrates the feasibility of using inexpensive cages for biomonitoring with a sensitive piscine species.

Key words: Atlantic menhaden, cage design, biomonitoring, physiology

Proc. Annu. Conf. Southeast. Assoc. Fish and Wildl. Agencies 58:207–218

In terms of economic value and ecological role, the Atlantic menhaden *(Brevoortia tyrannus)* is one of the most important species in the mid-Atlantic region. In Chesapeake Bay, commercial landings have averaged around 200,000 metric tons since 1996. In 2001, this represented 80% of the total landings in the region and

1. Current Address: NOAA/NOS/NCCOS, Center for Coastal Environmental Health and Biomolecular Research, Cooperative Oxford Laboratory, Oxford, MD 21654.

15% of the total value of the commercial harvest (National Marine Fisheries Service, Fisheries Statistics and Economics Division, Silver Spring, Maryland, pers. commun.). Ecologically, they are an important prey species for many higher trophic order fish such as striped bass *(Morone saxatilis),* bluefish *(Pomatomus saltatrix),* and weakfish *(Cynoscion regalis)* (Hartman and Brandt 1995). In addition, adults are obligate filter feeders capable of processing large volumes of water and indiscriminately removing particles greater than 13 μm in size (Lippson 1991, Macy et al. 1999).

In the mid-Atlantic region, juvenile Atlantic menhaden have received considerable attention in recent years due to their overrepresentation in fish kills associated with harmful algal blooms (Burkholder et al. 1992, Bourdelais et al. 2002) and elevated incidence of ulcerative lesions (Blazer et al. 1999). Harmful algal blooms are ubiquitous in U.S. coastal waters, and appear to be increasing in both number of events and species diversity (Hallegraeff 1993, Bushaw-Newton and Sellner 1999). Many of the species involved can have serious impacts not only on aquatic animals, but also on human health, system ecology, and regional economics (Zingone and Enevoldsen 2000, Swinker et al. 2002). In 1997, a major fish kill in the Pocomoke River, Maryland, occurred in the presence of the reportedly toxic dinoflagellate *Pfiesteria piscicida* (Magnien 2001). The subsequent public concern that followed highlighted several key research and monitoring needs including the ability to detect harmful algal bloom activity before major kills occur.

The Atlantic menhaden's broad distribution in eastern U.S. coastal and estuarine waters, ability to thrive in a wide salinity range (Engel et al. 1987), association with gradients of phytoplankton biomass (Friedland et al. 1996), and sensitivity to harmful algal blooms make the species an ideal candidate for use in biomonitoring with captive organisms. However, one of the limitations of this approach is the influence the cage itself may have on the variables of interest, or the so-called "cage effect." This may be evident by predator attraction or general fish aggregation, increased sedimentation around the cage, biofouling, etc. (Connell 1997). These alterations to the local environment, in addition to confinement, have the potential for changing the internal homeostasis of the caged organism, compromising results. Thus, confinement devices must be designed and evaluated for individual species to ensure their effectiveness.

Here we describe the application of capture, handling, and husbandry techniques and a unique, inexpensive cage specifically designed for biomonitoring with Atlantic menhaden. These efforts were conducted in cooperation with extensive Maryland State monitoring activities in the Chesapeake Bay of water quality and fish health in response to the potential threats associated with *Pfiesteria* sp. (Magnien 2001).

Methods

Menhaden Collection and Husbandry

In April 1999, the University of Maryland Eastern Shore/ U.S. Geological Survey, Maryland Cooperative Fish and Wildlife Research Unit initiated the collection of larval and post-larval menhaden from lower Eastern Shore rivers. A modified

ichthyoplankton tow with a collecting box similar to that described by Lewis et al. (1970) was mounted to the bow of the research vessel. After 2- to 5-min tows, menhaden were sorted from other species and placed in 50-gallon buckets for transport (Hedrick et al. 2005). Fish were transported to the Horn Point Laboratory Aquaculture Center (HPL), Cambridge, Maryland, as post-larvae and placed in 1600-L flow-through tanks at ambient temperature and salinity. Mortality to this stage often exceeded 50%. Fish were initially fed a 50/50 mixture of *Artemia* and *Isochrysis galbana* (*T. Iso* strain) at densities of 100 L^{-1} and approximately 2000 cells L^{-1}, respectively. After 1 wk of consistent feeding, fish were switched to *Artemia* at approximately 300 L^{-1} with a commercially available starter crumble mixed in *ad libitum* as suggested by Hettler (1981). By 3 wk, fish were weaned entirely onto an artificial diet fed *ad libitum.* An additional collection of 80- to 110-mm juveniles was initiated in July in the Choptank River, Maryland. Schooling menhaden were captured via cast net and transported immediately to HPL. Mortality from this process correlated strongly with the length of time from capture to the holding facility and density of individuals in the transport vessel. The lowest mortalities were achieved by selection of large schools in shallow water for capture, use of a 5-foot diameter circular transport tank on board, and transfer of fish directly from the boat to the holding facility.

Cage Design

Cages were assembled from circular molded polyethylene "fish baskets" by constructing lids out of 1.27-cm plywood and attaching concrete half-blocks to the bottom as anchors (Fig. 1). The cages have a top diameter of 48.26 cm, narrowing down to 36.83 cm at the base, a height of 36.83 cm, and 1.27 cm x 0.79 cm openings cut 1.9 cm on center horizontally. Plywood tops were cut to fit with 1.27 cm holes drilled randomly for increased water circulation. Tops were secured with plastic cable ties and floats and line attached to plastic handles for deployment. Two cages were initially placed in 500-gallon circular tanks for behavioral observations. After a brief period of acclimation (30 min), up to 25 menhaden swam in characteristic circular patterns. An additional deployment at HPL's boat basin confirmed the usefulness of the design with no mortalities or abrasions noted after 48 h.

Transport and Application of Cages

On 21 August 1999 large numbers of menhaden in Middle River, Maryland, with ulcerative lesions were reported to the Maryland Department of Natural Resources (MDNR), triggering immediate sampling efforts for harmful algal activity. Lesion prevalence initially exceeded 80%, and declined to 50% over a 2-wk period, while *Pfiesteria* sp. presumptive counts ranged from 250 cells mL^{-1} to less than 50 cells mL^{-1} with consistent detection with genetic probes (MDNR, unpublished data). The lesions discovered were typical of those reported by Noga and Dykstra (1986) and by Blazer et al. (1999) characterized by extensive myonecrosis and granulomatous mytosis.

Menhaden held in the HPL hatchery were transported on 8 September 1999 to Middle River, Maryland, a densely populated tidal tributary of the Chesapeake Bay approximately 15 miles northeast of Baltimore, Maryland. All fish were transported

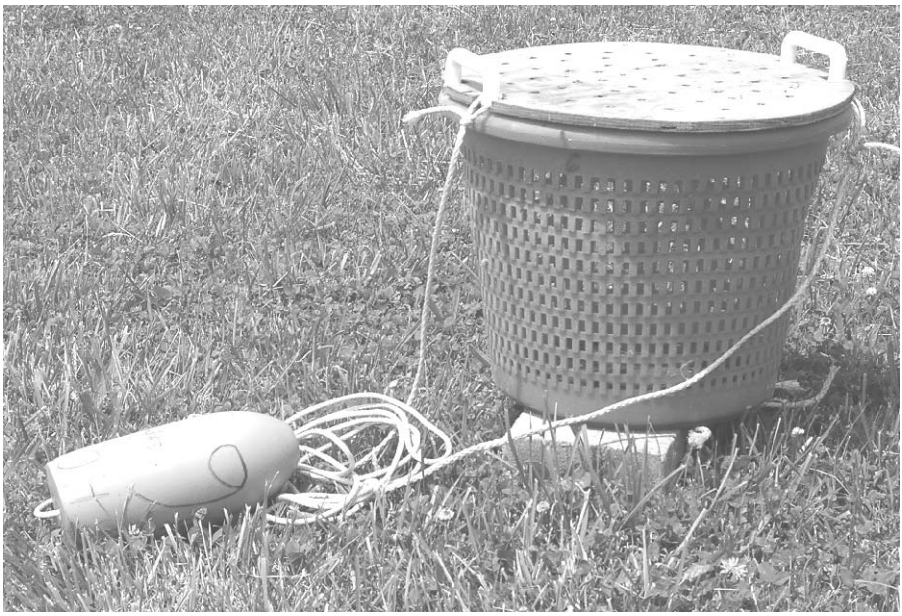

Figure 1. Plastic 36.83 cm (H) x 48.26 cm (W) "fish baskets" modified for caging of juvenile Atlantic menhaden.

in live shipping boxes at a density of 15 per box (90–110 mm fish). Two days prior to transport, salinity in the holding tanks was gradually lowered to 10 ppt and feeding ceased. Plastic fish transport bags were one-third filled with 10 ppt tank water while inside insulated fish transport boxes. The remainder of each bag was then filled with pure oxygen and tightly sealed. A single ice pack was placed in each box to maintain temperature during the 4-h vehicle trip to Middle River. Upon arrival at the launch location, all fish boxes were removed from the vehicle and transferred to the boat for transport to each site.

On site, each cage was tied to the gunnel with the top off and half of the basket in the water (Fig. 2). Fish bags were then removed from shipping boxes and placed into cages to acclimate to temperature differences. During temperature acclimation, gradual addition of ambient water to the bags over a 20-min period was conducted to allow for water quality and salinity differences. At the end of the acclimation period, bags were removed and plywood tops secured with plastic cable ties. Cages were then gradually lowered to the bottom to ensure they rested in the upright position.

Sites were selected based on elevated density of *Pfiesteria*-like-organisms in the week prior to deployment (> 100 cells mL^{-1}). Hopkins and Norman creeks in Middle River, Maryland, were chosen as well as a control site on the Choptank River, Maryland. Tides in Chesapeake Bay are mixed (semidiurnal with differing heights) with Hopkins and Norman creek sites having tidal ranges of approximately 0.4 m and the Choptank site, 0.5 m. Three cages were deployed in each creek to a depth of 1.2 m

Figure 2. Deployment of cages and acclimation of fish in transport bags.

with a single cage pulled at 48, 96, and 144 h from each location. An additional cage was added to each site on 12 September 1999 and allowed to remain for 312 h. Two cages were deployed on 11 September 1999 (1.2 m depth) in the Choptank River adjacent to the HPL pier to serve as controls and a single cage removed at 96 and 288 h.

Sample Collection and Analysis

All fish were examined for external abnormalities and 6 fish per cage were bled for hematocrit and protein determination, and preserved for histology. Fish were bled via dorsal aortic puncture with a 21-ga, 3-cc syringe. Blood samples were immediately transferred to replicate micro-capillary tubes and spun at 13,000 g for 5 min. Percent red cell volume was determined with a standard hematocrit reader. Immediately following, tubes were broken at the cell plasma^{-1} interface and protein determined via veterinary refractometer. An additional 2–6 fish per cage were frozen whole on dry ice for subsequent acetylcholinesterase (AChE) determination. Six fish per cage were preserved whole in 10% neutral buffered formalin after partial necropsy to ensure adequate fixation of the viscera. Tissue sections were processed using routine paraffin embedding, microtome sectioning (5 μm), and slide preparation with standard hematoxylin and eosin staining. Water chemistry and samples for the pres-

Table 1. Middle and Choptank river water quality for selected parameters collected during cage sampling. PCR reflects probe detection results while Pres. Count are presumptive cell counts from water samples. PCR and cell count data provided by the Maryland Department of Natural Resources.

Date	Location	Time (hr)	Depth (M)	Temp (C)	pH	D.O. (mg/L)	Salinity (ppt)	PCR (+/–)	Pres. Count (cells/ml)
10 Sep 1999	Hopkins Creek	48	0.5	26.3	7.3	5.2	7.3	+	<50
10 Sep 1999	Norman Creek	48	0.5	26.6	7.5	6.2	7.8	+	<50
12 Sep 1999	Hopkins Creek	96	0.5	27.1	8.5	11.0	7.5	+	<50
12 Sep 1999	Norman Creek	96	0.5	25.7	7.1	8.0	7.9	–	<50
13 Sep 1999	Choptank HPL	96	0.5	23.4	7.0	5.7	15.4	–	<50
14 Sep 1999	Hopkins Creek	144	0.5	26.4	8.4	10.8	7.5	+	<50
14 Sep 1999	Norman Creek	144	0.5	26.5	7.8	9.2	7.9	+	<50
21 Sep 1999	Choptank HPL	288	0.5	21.4	6.6	10.2	7.3	–	<50
27 Sep 1999	Hopkins Creek	312	0.5	22.0	7.0	11.2	5.9	+	<50
27 Sep 1999	Norman Creek	312	0.5	21.4	7.4	6.0	6.2	+	<50

ence and enumeration of *Pfiesteria* sp. were taken from each system during each sampling day in accordance to the State of Maryland's protocols (Magnien 2001), and processed separately by the Maryland Department of Natural Resources and contractors.

Acetylcholinesterase (AChE) activity was determined from whole brain homogenates following the methods of Ellman et al. (1961) with modification for use with a micro-plate reader at a wavelength of 405 nm. Optimal substrate concentration was determined to be $10^{-2.5}$ with a 120-fold tissue dilution. Standard curve development with electric eel AChE proved linear throughout the range of hydrolysis (R^2 of 0.986). This method and optimization for menhaden is described in detail by Jacobs et al. (2000). Multivariate analysis of variance and least square means comparison procedures (Tukey's adjustment) were utilized to examine site, time, and site x time (cage) for all physiological variables (SAS 1990).

Results

Pfiesteria spp. activity was minimal during this time period, with presumptive counts never exceeding 50 cells mL^{-1} and PCR results variable as to detection (Table 1). Water quality during the cage study showed no atypical trends and is also presented in Table 1.

Mortality in cages was highly variable and not significantly different by site ($P = 0.299$, $F = 1.14$, $df = 2$) (Fig. 3). Almost all of the mortality occurred within the first 48 h as noted by the progressive decomposition in later samples. By 144 h, mortalities were assumed by the absence of fish since little evidence remained. Hematocrit and total protein values were not significantly different among individual cages (multivariate, location x time, $P > 0.05$), averaging 41.67 ± 0.963% and 4.09 ± 0.166 mg dL^{-1} respectively, although protein values were slightly depressed at 48 h (Fig. 4). Rates of AChE hydrolysis did not differ among locations, times, or individual cages ($P = 0.245$) (Fig. 4).

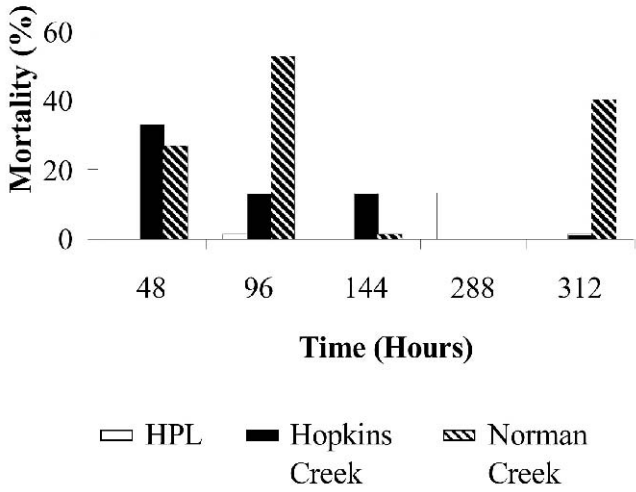

Figure 3. Total mortality in cages at Horn Point (control), Norman, and Hopkins creeks. No trend in mortality is apparent over time, but total mortality was higher at Norman Creek overall. This observation is not statistically significant due to within site variability (P = 0.299, f = 1.14, 2 df).

Microscopic examination of tissue sections revealed few departures from normal, with the noted exception of fish held for 312 h at both Norman and Hopkins creeks. All fish sampled had elevated prevalence of macrophage aggregates in spleen and head kidney. In addition, myxozoan plasmodia were apparent in the oropharynx, gill filaments, and lining of the branchial cavity of all fish sampled at 312 h. The plasmodia are most likely associated with the myxozoan parasite *Kudoa* sp.

Cage performance in the Middle and Choptank rivers proved exceptional. The cages did not move from their site, suggesting the concrete block anchoring system was sufficient. Also, very little fouling or sedimentation was evident on or in any cage. All lids remained secure until retrieval and little evidence of physical damage consistent with cage interaction was present on fish.

Discussion

The use of caged fish to monitor host response to anthropogenic inputs and environmental changes is apparent in the literature. Escher et al. (1999) examined the effect of treated wastewater from a sewage plant on health of brown trout *(Salmo trutta fario).* By caging trout in areas of sewage influence, river water only, and a control, the authors were able to document increased mortality, incidence of ulcerative conditions, and other histological and physiological differences associated with the impacted site. A similar study was conducted by Birtwell et al. (1983). Frodge et al. (1995) successfully caged largemouth bass *(Micropterus salmoides)* and steel-

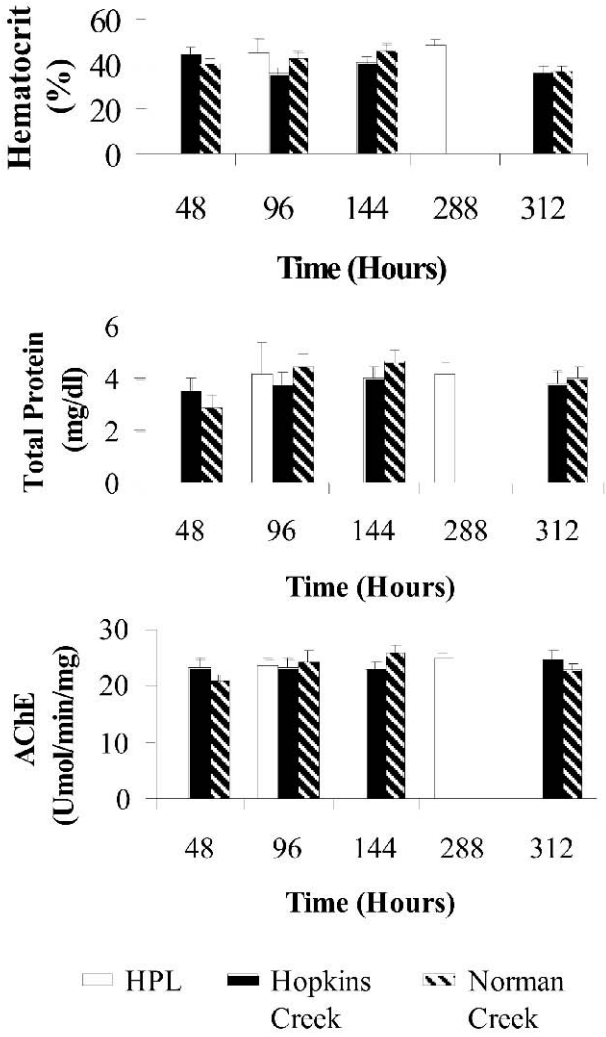

Figure 4. Least square mean hematocrit, total protein, and brain acetylcholinesterase levels in caged Atlantic menhaden. Time represents hours caged in river system. No significant differences were noted by location, time, or location x time for any variable ($P > 0.05$).

head trout *(Oncorhynchus mykiss)* in two densely vegetated Washington lakes with routine hypoxia. By placing replicate cages at different depths, the investigators were able to demonstrate a local effect of aquatic macrophytes on fish survival. Caged fish have also been used to examine the impact of chlorinated discharge from cooling towers (Dickson et al. 1974), and are widely used in predation studies in fisheries ecology (Steele 1996, Connell 1997).

The success of caging experiments relies heavily on the selection of appropriate species, knowledge of the species requirements, minimizing additional stressors, and understanding and avoiding caging effects. In this application, the Atlantic menhaden was identified as the primary species of interest due to the high prevalence of external lesions and its association with systems where *Pfiesteria* sp. was present. Over the course of two years, techniques were honed for the capture, transport, and husbandry of the species. Behavioral observations of caged menhaden in shallow tanks and pilot deployments clearly demonstrated minimal departures from normal condition once caged. Finally, experimental deployment in an effected system showed that any toxic activity that may have been present was no longer active.

It is apparent that cage studies with juvenile menhaden are feasible, even at locations great distances from the fish source. However, the Middle River study and high post-capture mortality associated with collection efforts suggest that improvements in transport techniques are still warranted. Post-capture mortality most often occurred after two days of acclimation, with no clinical evidence presented, suggesting physiological mechanisms. It is apparent that transport under iso-osmotic conditions (about 10 ppt), cool water temperatures (18–20 C), and adequate oxygen reduce post-capture mortality; however, providing strong directional water flow both in the transport vessel and during post-transport acclimation has not proven beneficial. In our experience, the variables having the greatest impact on post-capture survival are extended transport time and excessive handling.

Mortality at the caging sites was higher in Norman Creek than at Hopkins, although not statistically significant due to the high variability among the cages within each site (Fig. 3). There was little difference in water quality between the sites (Table 1) or other physical factors which may aid in explanation. The stage of decomposition of dead fish remaining in cages suggested that most mortality occurred within the first 48 h, leading us to conclude that transport stress was the predominant cause. However, transport mortality alone was not assessed; and without complete security and real-time monitoring of the site, other factors disturbing individual cages cannot be excluded.

Samples taken from caged fish at all sites for histopathology proved to be normal for the species, with the exception of those with the longest environmental exposure and sampled after 312 h. In some cases, spores of the myxosporean parasite *Kudoa* sp. were detected. However, this is very common for menhaden and the spore stage does not illicit a host response (Reimschuessel et al. 2003). Fish sampled at 312 h, however, all contained invasive myxozoan plasmodium that may represent an early stage of *Kudoa*. This plasmodium has been associated with ulcerative lesions in juvenile menhaden (Reimschuessel et al. 2003); however, it is likely that the lesions do not lead to mortality under normal conditions (Jacobs et al. 2001). Blood chemistry parameters were also in the normal range for the species, and were not significantly different among sites or cages suggesting little extraneous stress in the caged fish. Finally, acetylcholinesterase (AChE) hydrolysis was unchanged. AChE is the enzyme responsible for the removal of acetylcholine, the primary neurotransmitter in fish. Its inhibition can lead to uncontrolled stimulation of the post-synaptic membrane, which

can cause unregulated muscle contraction, erratic swimming behavior, and eventually death. Considerable data concerning environmental toxicants (primarily pesticides) that are AChE inhibitors exist, most notably those containing high levels of organophosphates (Olson and Christensen 1980, Mineau 1991). Toxins inhibiting AChE have also been found in the bacteria *Aeromonas hydrophila* with 90% of the strains examined testing positive for secretion of the causative protein (Rodriguez et al. 1993). In addition, elevated levels of AChE have been associated with a general stress response (Pavlov et al. 1994). Behavioral changes from erratic swimming to lethargic movements noted in fish exposed to *Pfiesteria* toxins and at fish kills where *Pfiesteria*-like organisms are present are consistent with AChE inhibition; however, full evaluation of the usefulness of this assay awaits the availability of a *Pfiesteria* toxin.

This work demonstrates the feasibility of conducting *in-situ* bioassays with a sensitive estuarine species. With the increasing threat of harmful algal blooms, we envision the use of Atlantic menhaden as "sentinels" providing early detection of organisms indicative of ecosystems capable of affecting human health on a river or system specific basis. This approach could also be applied to studies of bacterial loading, source tracking, and pollution monitoring, as long as appropriate control sites are chosen.

Acknowledgments

We are grateful to Jim Hedrick, Lara Hedrick, Brett Coakley, Amy Jacobs, Bart Rogers, and Megan O'Connor in assistance with field collection of menhaden and cage deployment. Gratitude is also extended to Carol McCollough, Dr. Dave Goshorn, and Dr. Stephen Jordan (MDNR) for histology services, and provision of water quality data, and general project support. Finally, we appreciate the critical reviews of Gretchen Messick and Jane Keller (NOAA/NOS/Oxford Lab) and Dr. Ray Morgan (UMD Appalachian Lab). This work was funded by the State of Maryland.

Literature Cited

Birtwell, I. K., G. L. Greer, M. D. Nassichuk, and I. H. Rogers. 1983. Studies on the impact of municipal sewage discharged onto an intertidal area within the Fraser River estuary, British Columbia. Canadian Technical Report of Fisheries and Aquatic Science 1170.

Blazer, V. S., W. K. Vogelbein, C. L. Densmore, E. B. May, J. H. Lilley, and D. E. Zwerner. 1999. *Aphanomyces* as a cause of ulcerative skin lesions of Atlantic menhaden from Chesapeake Bay tributaries. Journal of Aquatic Animal Health 14:1–10.

Bourdelais, A. J., C. R. Tomas, J. Naar, J. Kubanek, and D. G. Baden. 2002. New fish-killing alga in coastal Delaware produces neurotoxins. Environmental Health Perspectives 110(5): 465–470.

Burkholder, J. M, E. J. Noga, C. H. Hobbs, and H. B. Glasgow. 1992. New 'phantom' dinoflagellate is the causative agent of major estuarine fish kills. Nature 358(30):407–410.

Bushaw-Newton, K. L. and K. G. Sellner. 1999. Harmful algal blooms. NOAA's State of the Coast Report. National Oceanic and Atmospheric Administration, Silver Spring, Maryland. http://state-of-coast.noaa.gov/bulletins/html/hab_14/hab.html

Connell, S. D. 1997. Exclusion of predatory fish on a coral reef: the anticipation, pre-emption, and evaluation of some caging artifacts. Journal of Experimental Marine Biology and Ecology 213:181–198.

Dickson, K. L., A. C. Hendricks, J. S. Crossman, and J. Cairns, Jr. 1974. Effects of intermittently chlorinated cooling tower blowdown on fish and invertebrates. Environmental Science and Technology 8(9):845–849.

Ellman, G. L., K. D. Courtney, V. Andres, and R. Featherstone. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 7:88–95.

Engel, D. W., W. F. Hettler, L. Coston-Clements, and D. E. Hoss. 1987. The effect of abrupt salinity changes on the osmoregulatory abilities of the Atlantic menhaden *Brevoortia tyrannus*. Comparative Biochemistry and Physiology 86A(4):723–727.

Escher, M., T. Wahli, S. Buettner, W. Meier, and P. Burkhardt-Holm. 1999. The effect of sewage plant effluent on brown trout *(Salmo trutta fario):* a cage experiment. Aquatic Sciences 61(2):93–110.

Friedland, K. D., D. W. Ahrenholz, and J. F. Guthrie. 1996. Formation and seasonal evolution of Atlantic menhaden juvenile nurseries in coastal estuaries. Estuaries 19(1):105–114.

Frodge, J. D., D. A. Marino, G. B. Pauley, and G. L. Thomas. 1995. Mortality of largemouth bass *(Micropterus salmoides)* and steelhead trout *(Oncorhynchus mykiss)* in densely vegetated littoral areas tested using in situ bioassay. Lake and Reservoir Management 11(4):343–358.

Hallegraeff, G. M. 1993. A review of harmful algal blooms and their apparent global increase. Phycologia 32:79–99.

Hartman, K. J. and S. B. Brandt. 1995. Predatory demand and impact of striped bass, bluefish, and weakfish in the Chesapeake Bay: applications of bioenergetics models. Canadian Journal of Fisheries and Aquatic Science 52(8):1667–1687.

Hedrick, J. D., L. R. Hedrick, and F. J. Margraf. 2005. A sampler for capturing larval and juvenile Atlantic menhaden. North American Journal of Fisheries Management 25:245–250.

Hettler, W. F. 1981. Spawning and rearing Atlantic menhaden. Progressive Fish-Culturalist 43(2): 80–84.

Jacobs, J. M., W. F. Van Heukelem, and R. M. Harrell. 2000. Physiological effects of fish skin abnormalities in Atlantic menhaden *(Brevoortia tyrannus).* Technical Series Project Completion Report, TS-238-00, University of Maryland, Center for Environmental Science, Cambridge, Maryland.

_____, R. Reimshuessel, C. Gieseker, H. Rogers, B. Coakley, W. F. Van Heukelem, and R. Harrell. 2001. Fate of YOY Atlantic menhaden *(Brevoortia tyrannus)* with external lesions associated with a myxosporidian. Center for Disease Control and Prevention (CDC) National Conference on *Pfiesteria:* From biology to public health, Conference Abstracts. CDC, Atlanta, Georgia.

Lewis, R. M., W. F Hettler, E. P. H. Wilkens, and G. N. Johnson. 1970. A channel net for catching larval fishes. Chesapeake Science 11:196–197.

Lippson, R. L. 1991. Atlantic Menhaden. Pages (7) 1–6 *in* S. L. Funderburk, S. J. Jordan, J. A. Mihursky, and D. Riley, editors. Habitat requirements for living resources. Second edition, June 1991. Chesapeake Research Consortium, Solomons, Maryland.

Macy, W. K. III, A. G. Durbin, and E. G. Durbin. 1999. Metabolic rate in relation to temperature and swimming speed, and the cost of filter feeding in Atlantic menhaden, *Brevoortia tyrannus.* Fisheries Bulletin 97(2):282–293.

Magnien, R. E. 2001. State monitoring activities related to *Pfiesteria*-like organisms. Environmental Health Perspectives 109(5):711–714.

Mineau, P., editor. 1991. Cholinesterase-inhibiting insecticides: Their impact on wildlife and the environment. Elsevier, New York.

Noga, E. J. and M. J. Dykstra.1986. Oomycete fungi associated with ulcerative mycosis in menhaden, *Brevoortia tyrannus* (Latrobe). Journal of Fish Diseases 9:47–53.

Olson, D. L. and G. M. Christensen. 1980. Effects of water pollutants and other chemicals on fish acetylcholinesterase *(in vitro).* Environmental Research 21: 327–335.

Pavlov, D. F., G. M. Chuiko, and A.G. Shabrova. 1994. Adrenaline induced changes of acetylcholine activity in the brain of perch (*Perca fluviatilis* L.). Comparative Biochemistry and Physiology 108C: 113–115.

Reimschuessel, R., C. M. Gieseker, C. Driscoll, A. Baya, A. Kane, V. S. Blazer, J. J. Evans, M. L. Kent, J. D. W. Moran, and S. L. Poynton. 2003. Myxosporean plasmodial infection associated with young-of-the-year Atlantic menhaden in a tributary of the Chesapeake Bay, and possible links to *Kudoa clupeidae.* Diseases of Aquatic Organisms 53:143–166.

Rodriguez, L. A., A. I. G. Fernandez, and T. P. Nieto. 1993. Production of the lethal acetylcholinesterase toxin by different *Aeromonas hydrophila* strains. Journal of Fish Diseases 16:73–78

SAS. 1990. SAS procedures guide: reference, version 6 edition. SAS Institute, Inc., Cary, North Carolina.

Steele, M. A. 1996. Effects of predators on reef fishes: separating cage artifacts from effects of predation. Journal of Experimental Marine Biology and Ecology 198:249–267.

Swinker, M., P. Tester, D. Koltai Attix, and D. Schmechel. 2002. Human health effects of exposure to *Pfiesteria piscicida:* a review. Microbes and Infection 4:751–762.

Zingone, A. and H. O. Enevoldsen. 2000. The diversity of harmful algal blooms: a challenge for science and management. Ocean and Coastal Management 43(8–9):725–748.