

Using Fish Biomarkers to Monitor Improvements in Environmental Quality

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Abstract.—The percentage of splenic tissue occupied by macrophage aggregates and hepatosomatic index (HSI) were evaluated in rock bass *Ambloplites rupestris* from Burlington Harbor, Vermont. In 1992, fish collected from the inner Burlington Harbor area had a significantly greater percentage of splenic tissue occupied by macrophage aggregates and greater HSI than did fish from reference sites. These biomarkers often are correlated with exposure to various contaminants (e.g., polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and some heavy metals, which were found in Burlington Harbor sediments during surveys in 1990 and 1991). Contaminants are believed to have entered Burlington Harbor through the city's main sewage treatment plant, which discharged effluent into the harbor for many years. In 1994, the city completed a significant upgrade of this treatment plant, which included an extension of the effluent pipe beyond the inner harbor area. In 1999, rock bass were again collected from Burlington Harbor as an index of whether there was any improvement in environmental quality. Our data showed a significantly lower percentage of splenic tissue occupied by macrophage aggregates and significantly lower HSI among nine age-4 rock bass in 1999 than among six age-4 rock bass in 1992. The significant changes in these biomarkers suggest decreased exposure to contaminants. Our study reinforces the value of macrophage aggregates and HSI as biomarkers of environmental contamination, and the correlation with remedial action shows their potential utility in documenting improvements in environmental conditions.

Biomarkers are measures of cellular, biochemical, molecular, or physiological change in an organism that indicate exposure to or the effects of environmental contaminants. Despite the fact that a large number of biomarkers have been developed and used to monitor environmental health since

the mid 1980s, there is still no consensus on the best suite of markers or on their sensitivity and reliability (Lam and Gray 2003). Macrophage aggregates, or melanomacrophage centers, are structures in the spleen, kidney, and sometimes liver of fishes that have been used as nonspecific cellular biomarkers of physiological stress due to exposure to environmental contaminants. Since the mid 1980s there has been a growing body of evidence supporting the utility of macrophage aggregates as biomarkers. Numerous studies have shown increased macrophage aggregate metrics (number, size or percentage of tissue occupied) in fishes from contaminated areas when compared with conspecifics from less contaminated reference sites (Wolke et al. 1985; Blazer et al. 1987; Macchi et al. 1992; Blazer et al. 1994; Couillard and Hodson 1996; Lindesjoo et al. 1996; Facey et al. 1999; Fournie et al. 2001). Macrophage aggregate metrics are used in a number of monitoring programs, including the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP) and the U.S. Geological Survey's Biomonitoring of Environmental Status and Trends (BEST) Program (Summers et al. 1997; Schmitt and Dethloff 2000).

Organ-level biomarkers, particularly the hepatosomatic index (HSI), also have been used as biomarkers of contaminant exposure (Goede and Barton 1990). Because the liver is so important in detoxification, exposure to contaminants can lead to an increase in liver size from hypertrophy (an increase in size), hyperplasia (an increase in number) of hepatocytes (Goede and Barton 1990; Hinton and Lauren 1990), or both. Studies evaluating the relative liver size of fishes from contaminated and reference sites often utilize the HSI, which expresses liver size as a percentage of total body weight (Facey et al. 1999).

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We are not aware of any studies that have used either of these biomarkers to monitor environmental improvement at a particular site by comparing biomarker variables before and after actions were taken to help improve environmental quality. Macrophage aggregates increase in number and area as fish age, at least in a number of fish species (Brown and George 1985; Blazer et al. 1987; Mikaelian et al. 1998). Hence, it is important to use fish of similar age when comparing contaminated and reference sites. In this study we compared the percentage of splenic tissue occupied by macrophage aggregates and hepatosomatic index of age-4 rock bass *Ambloplites rupestris* captured in inner Burlington Harbor, Vermont, in 1992 and 1999. In several studies from 1992 to 1994, macrophage aggregate area and liver size were compared from contaminated and reference sites on the Vermont side of Lake Champlain and in Sunset Lake, Vermont. These studies examined age-3 and age-4 rock bass from Burlington Harbor, which was known to have sediments contaminated with polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and some heavy metals; the rock bass had larger percentages of splenic tissue occupied by macrophage aggregates and higher HSIs than fish from reference sites (Facey et al. 1999). In 1994, the city of Burlington completed a significant upgrade of its sewage treatment facility that had been releasing effluent into Burlington Harbor. This upgrade improved the level of treatment and extended the effluent pipe far beyond the inner harbor area. In 1999 we collected rock bass from Burlington Harbor to determine whether there were any changes in the biomarkers measured in 1992.

Methods

Rock bass were collected in June 1992 and July 1999 by boat electrofishing along the rock breakwater that protects Burlington Harbor. Fish were transported live back to the laboratory, and within 24 h were euthanized with a lethal dose of tricaine methanesulfonate (Finquel, Argent Chemical Laboratories, Redmond, Washington). Within 1 h after death all fish were measured and weighed, scales were removed to determine age, livers were removed whole and weighed to determine the hepatosomatic index ($HSI = \text{weight of liver} \times 100 / \text{total fish weight}$), and spleens were removed and fixed in 10% buffered formalin. We examined both male and female fish, and did not try to compare the sexes because of small sample size.

Fish age was estimated by two independent eval-

uators who examined and counted scale annuli (Helfman et al. 1997). Pieces of spleen were routinely processed for histopathology, embedded in paraffin, sectioned at 10 μm , and stained with hematoxylin and eosin (Luna 1992). Macrophage aggregate variables (number and area) were measured using computer-assisted image analysis as described by Fournie et al. (2001). Briefly, all aggregates were measured in 10 randomly distributed fields. The system was calibrated, and all measurements were taken at $25\times$ magnification. Total screen area was determined, and 10 fields represented approximately 2 mm^2 of tissue. From this the total number of aggregates per square millimeter, mean size of aggregates and the percentage of tissue occupied by aggregates were calculated. A size discriminator was used to eliminate aggregates smaller than 50 μm^2 (the approximate size of three aggregated macrophages). We used Student's *t*-tests to compare characteristics of fish caught in 1992 versus those caught in 1999. The comparisons of the mean area of spleen tissue occupied by macrophage aggregates and the mean HSI required the use of a *t*-test for groups with significantly different variance, as determined by an *F*-test.

Results

The results from 1999 and those from 1992 were significantly different ($P < 0.05$) for both biomarkers (i.e., mean area of spleen tissue occupied by macrophage aggregates and the mean HSI; Table 1). The fish collected in 1992 had a significantly larger percentage of their splenic tissue occupied by macrophage aggregates than did the fish collected in 1999. The six age-4 fish from 1992 had an average of nearly 10% of their splenic tissue occupied by macrophage aggregates, one of which was over 20% and none were below 2.5%. In contrast, all nine of the age-4 fish caught in 1999 had 3.1% or less of their splenic tissue occupied by macrophage aggregates. This great difference in the ranges of the values between the 1992 and 1999 groups accounts for the large difference in standard deviations.

Our results also showed the fish from 1999 had significantly smaller livers and lower HSI values than the fish from 1992. In addition, the age-4 rock bass caught in 1999 were significantly smaller and weighed less than did the fish caught in 1992 (Table 1).

Discussion

Numerous studies have documented that macrophage aggregates are valuable indicators of en-

TABLE 1.—Percent of splenic tissue occupied by macrophage aggregates (MAs), hepatosomatic index, weight, liver weight, and standard length presented as means in age-4 rock bass captured in Burlington Harbor, Vermont, in 1992 and 1999. *P*-values from *t*-tests show that all fish metrics were significantly lower in 1999 than in 1992 ($P < 0.05$); comparisons of percent spleen tissue occupied by macrophage aggregates and hepatosomatic indices required *t*-tests based on unequal variance, as determined by an *F*-test.

Variable	Mean (SD)		<i>P</i>
	1992	1999	
Sample size	6	9	
Percent splenic tissue occupied by MAs	9.86 (6.71)	1.85 (0.72)	0.033
Hepatosomatic index	1.41 (0.27)	1.16 (0.17)	0.044
Fish weight (g)	116.8 (14.9)	82.7 (17.7)	0.002
Liver weight (g)	1.64 (0.35)	0.96 (0.25)	0.001
Fish standard length (cm)	14.9 (0.72)	13.2 (0.89)	0.002

environmental stress in fish (Wolke et al. 1985; Blazer et al. 1994; Facey et al. 1999; Fournie et al. 2001). To the best of our knowledge, this is the first study in which macrophage aggregates were used to suggest recovery from pollution in a population of fish or evaluate the benefits of remediation. The significant decreases in splenic macrophage aggregate area and HSI suggest that rock bass captured in 1999 experienced less environmental stress than those captured in 1992. This is only one study based on a small number of fish, so it should only be considered as one piece of evidence suggesting an improvement in environmental quality of Burlington Harbor. Other studies by researchers at the University of Vermont also support the conclusion that overall environmental quality of Burlington Harbor has improved since the early 1990s; manuscripts for several of these studies were being prepared at the time that our paper was submitted (Dr. Mary C. Watzin, Director, Rubenstein Ecosystem Science Laboratory, University of Vermont, personal communication).

With the extension of the effluent pipe from the sewage treatment plant out of the Burlington Harbor in 1994 and increased clean-up efforts, it is quite likely that the harbor was cleaner in 1999 than in 1992. Treated effluent had not been emptying into the harbor for 5 years before the 1999 collection, allowing improvement in water quality and also allowing contaminated surface sediments to be covered by cleaner deposits that thereby limited the availability of pollutants to the invertebrates and small fishes consumed by rock bass. Our results suggest that the physiological stress on the rock bass population had decreased with this reduction in exposure to pollution. Because the fish examined were all age 4, it is likely that those collected in 1999 were never exposed to the levels of contamination that the fish in the 1992 study had been, and that contaminant levels had already

been reduced by the time they were hatched. Studies on the pattern of movement of rock bass indicate they are a sedentary species, generally staying within 50–100 m (Gerking 1953; Gatz and Adams 1994), and therefore should be a good indicator species for certain areas of the lake, such as Burlington Harbor.

Another factor that could have had an effect on the level of contaminant exposure of fish is the invasion of zebra mussels *Dreissena polymorpha*. Zebra mussels are an invasive species that became established in Burlington Harbor in 1994, the same year that the sewage treatment plant upgrade was completed. It is possible that their filtering of water for food also removed a number of contaminants from the water, thereby making the contaminants less available to other species in the ecosystem. Zebra mussels were not present in Burlington Harbor in 1992, but by 1999 covered nearly the entire breakwater. Because the rock bass we caught were taken from the crevasses among the boulders of the breakwater, their exposure to pollutants may have been decreased by the high number of zebra mussels filtering the water around them. The high density of zebra mussels also would filter a great deal of plankton from the water column, thereby reducing food availability and perhaps limiting growth for higher trophic levels. In addition, the sewage treatment plant upgrade would have reduced nutrient input to the harbor, further reducing productivity. If rock bass growth decreased due to reduced food availability, because of either reduced nutrient input or plankton consumption by zebra mussels, then this might explain the apparent decrease in growth rate in the rock bass (i.e., age-4 fish caught in 1999 were significantly smaller and weighed less than those caught in 1992). In addition, the reduction in the HSI might be due to decreased liver size due to less fat storage rather than improved environmental quality. Unfortu-

nately, the arrival of zebra mussels at the same time that the sewage treatment plant upgrade was completed makes it impossible to know the impact of each independent of the other.

In conclusion, this study has documented the use of macrophage aggregates as indicators of apparent reduction of environmental stress in a fish population previously exposed to contamination. Our results suggest that rock bass caught in 1999 experienced less stress from pollution than those caught in 1992, which suggests, in turn, improvement in the environmental quality of Burlington Harbor.

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