NOTES

EFFECTS OF LONG-TERM MERCURY EXPOSURE ON HEMATOLOGY OF STRIPED BASS, *MORONE SAXATILIS*

The striped bass, *Morone saxatilis*, is found along the shore of the heavily populated Atlantic coast of North America; hence, it is subjected to considerable domestic and industrial pollution. Even higher pollutant concentrations may be encountered when the fish migrate into rivers to spawn. Because of the availability of the species, its value to both commercial and sport fisheries, and its normal habitat in many areas where pollution is a problem, the striped bass may be a particularly appropriate indicator species for pollution studies. In spite of these factors, information about sublethal effects of metal on striped bass is limited; even the response of the species to mercury, perhaps the most widely studied heavy metal, has received little attention in the literature.

That fish accumulate mercury from water has been demonstrated both in the laboratory and in the wild. Pentreath (1976), in a study of accumulation, distribution, and retention of mercury, found a gradual uptake and slow loss of ^{203}mercury in the plaice, *Pleuronectes platessa*, during laboratory exposures up to 90 d. Olson et al. (1973) showed that the rainbow trout, *Salmo gairdneri*, accumulates mercury through the gills. Our laboratory demonstrated uptake of mercury into the winter flounder, *Pseudopleuronectes americanus*, during a 60-d laboratory exposure (Calabrese et al. 1975). Mercury analyses on fish taken from their natural environment corroborate the laboratory findings: An increasing mercury concentration correlated with increasing weight has been demonstrated in the pike, *Esox lucius*; the bluefish, *Pomatomus saltatrix*; the blue hake, *Antimora rostrata*; and the striped bass (Johnels et al. 1967; Alexander et al. 1973; Cross et al. 1973).

The sensitivity of striped bass to pollution in general has been reported by a number of investigators. Raney (1952) noted that, although the striped bass had formerly used as spawning areas most of the large rivers along the Atlantic coast of the United States, the species had virtually disappeared from many of these areas, notably the Delaware, Connecticut, and Roanoke Rivers; he attributed its disappearance to gross pollution. Chittenden (1971) also attributed the lack of striped bass in the Delaware River to gross pollution and suggested that a major limiting factor was the river's very low oxygen content. An earlier study in our laboratory demonstrated sublethal responses of the striped bass to mercury. Juvenile striped bass were exposed to 5 and 10 parts per billion (ppb) mercury for periods ranging from 30 to 120 d. Measurements of gill-tissue oxygen consumption showed changes whose magnitude and direction varied with length of exposure (Dawson et al. 1977).

The present study was undertaken to determine the nature and extent of physiological disturbance to striped bass caused by mercury exposure using a variety of hematological tests. Variables related to the oxygen-carrying capacity of the blood, such as hemoglobin and hematocrit, were considered important because of earlier indications that mercury exposure affects respiration (Dawson et al. 1977), because of the suggestion that low oxygen levels eliminate striped bass from certain polluted environments (Chittenden 1971), and because of evidence that mercury affects these measurements in other fish (Calabrese et al. 1975; Dawson 1979). In addition, because my earlier work indicated that mercury disrupts osmo- and ion-regulation in winter flounder (Dawson 1979), I included these aspects of plasma chemistry in the present study.

Methods

Exposure

Striped bass were obtained from the Edenton National Fish Hatchery, U.S. Fish and Wildlife Service, Edenton, N.C., where they had been reared in freshwater. Upon arrival at the Northeast Fisheries Center Milford Laboratory, they were placed directly into flowing Milford Harbor seawater and allowed to acclimate for 2 wk prior to exposure. Throughout the acclimation and the exposure period the fish were fed Purina Trout Chow[1] ad libitum, daily. The fish

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

were exposed in 80 l glass aquaria filled to 60 l with sand-filtered seawater by a proportional-dilution apparatus (Mount and Brungs 1967). The dilutor controlled the intermittent delivery of mercury-treated and control water at a flow rate of 1 l to each tank every 3 min throughout the test period. This provided a flow of 480 l/tank per d and an estimated 90% replacement time of 7 h (Sprague 1969). Mercury, as mercuric chloride, was added at concentrations of 5 and 10 ppb. The concentrations are nominal concentrations of the metal ion in solution, not including the background level, which was below 0.3 ppb. The fish were exposed for 60 d and then removed for testing. Each tank contained 5 fish, for a total of 15 fish at each mercury concentration and 15 controls. The fish averaged 17.1 cm long (range 13.1-19.0) and 59.3 g (range 22.7-79.3). The exposure ran from late November 1976 to January 1977. The temperature ranged from a high of 8°C at the beginning of the exposure period to a low of 0°C at the termination of the exposure. Salinity during the exposure period averaged 27.0‰ and ranged from 26.0 to 29.6‰ with the exception of 1 d when it fell to 20‰.

Hematology

Blood was collected from each fish by cardiac puncture using a 3 ml plastic syringe and a 22-gage needle. The sample was transferred gently into an 8 ml glass vial containing 150 units of dried ammonium heparinate as an anticoagulant. Microhematocrits (packed red cell volumes) were determined following centrifugation for 5 min at 13,500 × *g*. Hemoglobin concentrations were determined by the cyanmethemoglobin method using the Hycel reagent; absorbance was read on a Bausch and Lomb Spectronic 20 spectrophotometer at 540 nm. Erythrocytes were counted in a hemacytometer using Natt-Herricks diluting fluid (Natt and Herrick 1952) at a 1:200 dilution. Within 4 h after collection, the remaining blood sample was centrifuged at 12,000 × *g* for 4 min and the plasma frozen for later determination of osmolality, protein, sodium, potassium, and calcium. Plasma sodium, potassium, and calcium concentrations were measured with a Coleman 51 flame photometer. Plasma protein was determined by the Biuret method as modified by Layne (1957). Plasma osmolalities were determined on an Advanced 3L osmometer using a 0.2 ml sample. Samples were pooled as necessary to obtain a 0.2 ml volume. The effect of the added heparin on the osmolality was negiligible. Three indices were computed from the measured values: mean corpuscular volume in cubic micrometers/cell = Hct/RBC × 10, mean corpuscular hemoglobin in picograms/cell = Hb/RBC × 10, and mean corpuscular hemoglobin concentration in grams/100 ml packed red cells = Hb/Hct × 100. All data were analyzed using Student's *t*-test.

Results

Control fish had a mean hematocrit of 47%, a hemoglobin concentration of 8.7 g/100 ml, and a red cell count of 4.10×10^6 cells/mm^3 (Table 1). These values resemble those reported by other investigators: Courtois (1976), using striped bass of similar size acclimated to cold seawater, reported a mean hematocrit of 46 and a hemoglobin of 8.4. More recently, Westin (1978) reported a hematocrit of 47.9, a hemoglobin of 9.11, and a red cell count of 3.79 for adult striped bass during the spawning season.

The effects of mercury on the erythrocyte component of the blood were pronounced (Table 1). Hematocrit, hemoglobin, and RBC all decreased following mercury exposure. In each case the response was significantly greater at the higher mercury concentration. The reduction in hemoglobin was proportional to the reduction in red cell count: About 10% in the 5 ppb-exposed animals and about 25% in the 10 ppb-exposed animals. This is reflected in the lack of change of the mean corpuscular hemoglobin and indicates that the lowered hemoglobin concentration in exposed fish is the result of a lower number of circulating erythrocytes and not of a smaller quantity of hemoglobin in each cell. The de-

TABLE 1.—Effects of 60-d exposure to mercuric chloride on erythrocytes of striped bass (means ± SE with ranges in parentheses).

Test	Controls	5 ppb	10 ppb
Hematocrit, % packed cells	47±1.3 (38-56)	36±1.2** (30-47)	26±1.6** (17-36)
Hemoglobin, g/100 ml whole blood	8.7±0.05 (7.8-9.9)	7.8±0.13** (7.2-8.9)	6.6±0.32** (4.9-8.8)
Red blood cells, 10^6 cells/mm^3	4.10±0.106 (3.69-4.81)	3.82±0.106 (3.19-4.45)	3.03±0.180** (2.10-4.57)
Mean corpuscular volume, μm^3/cell	115.2±3.70 (96-147)	96.6±3.69* (69-118)	86.2±3.58** (65-114)
Mean corpuscular hemoglobin, pg/cell	21.4±0.52 (18.5-25.9)	20.3±0.54 (16.6-23.4)	21.9±0.73 (18.7-29.0)
Mean corpuscular Hb concentration, g/100 ml packed red cells	18.7±0.44 (17.0-21.6)	21.8±0.57** (18.9-27.3)	25.6±0.58** (22.8-28.8)
Number of samples	14	15	13

*Significantly different from controls at 0.005 level, **Significantly different from controls at 0.001 level.

creases in hematocrit were greater proportionately: 23% and 45% of the control value in the 5 and 10 ppb-exposed groups. The greater decrease in hematocrit represents not only a lower number of red cells but, in addition, a smaller mean volume in red cells of exposed animals, reflected in the significantly lower mean corpuscular volume for exposed animals.

Exposure to mercury also affected the osmo- and ion-regulatory capacity of the striped bass (Table 2). There was no significant difference between 5 ppb-exposed fish and controls in any of the five plasma chemistry variables measured. In the 10 ppb-exposed animals, the plasma sodium and the osmolality increased to 238 mEq/l and 462 mOsm. Plasma calcium dropped to 3.98 mEq/l in the 10 ppb-exposed group, significantly lower than the control value of 4.52. Although the mean calcium concentration in the 5 ppb-exposed fish was even lower, because of the greater variability in that group, it was not significantly different from that of controls. There was no significant difference between controls and 10-ppb-exposed fish in plasma protein or potassium.

TABLE 2.—Effects of 60-d exposure to mercuric chloride on plasma chemistry of striped bass (means ± SE with ranges in parentheses). Plasma samples were pooled as necessary to obtain volume of material required.

Test	Controls	5 ppb	10 ppb
Na, mEq/l	195±2.6 (180-202)	198±2.1 (174-204)	238±6.2** (210-262)
K, mEq/l	6.57±0.799 (3.00-9.60)	5.19±1.088 (2.20-9.80)	5.00±0.588 (2.40-9.00)
Ca, mEq/l	4.52±0.135 (4.00-5.30)	3.90±0.303 (2.98-5.80)	3.98±0.146* (3.26-4.74)
Protein, g/100 ml	3.70±0.218 (3.14-4.58)	3.51±0.016 (3.05-4.15)	3.34±0.446 (2.38-5.18)
Osmolality, mOsm	354±10.8 (321-395)	369±6.9 (346-398)	462±11.8** (435-519)
Number of samples	8	8	10

*Significantly different from controls at 0.05 level, **Significantly different from controls at 0.001 level.

Discussion

Mercury had a major disruptive influence on the hematology of the striped bass, affecting both the red cell component of the blood and the plasma chemistry. Mercury had similar effects on winter flounder in an earlier study (Dawson 1979). In general, the changes demonstrated in winter flounder paralleled those of striped bass although the magnitude of change was smaller in the winter flounder in spite of higher mercury concentrations, namely, 10 and 20 ppb. The one exception was that the mean corpuscular volume increased in winter flounder and decreased in striped bass. The greater sensitivity to mercury in striped bass may represent a real species difference or may simply reflect the smaller size of the striped bass used.

The alterations in plasma sodium and osmolality following mercury exposure may be caused by gill-tissue damage. Meyer (1952) found decreased uptake and increased loss of sodium in the gills of mercury-exposed goldfish in freshwater. Olson et al. (1973) found ultrastructural damage in rainbow trout gills following mercury exposure. Renfro et al. (1974) demonstrated mercury uptake by the gill of the killifish, *Fundulus heteroclitus*, in freshwater and concomitant inhibition of sodium uptake. Our laboratory has demonstrated mercury uptake from seawater into the gills of winter flounder (Calabrese et al. 1975).

At least two sites of mercury accumulation have been described which could account for changes in the red cell component of the blood. Olson et al. (1973) and Pentreath (1976) reported the uptake of mercury into the blood of rainbow trout and plaice which could lead to direct cell damage. Perhaps more relevant are reports of mercury accumulation in the kidneys of teleosts; this would very likely affect renal hemopoiesis and, hence, such variables as hematocrit, hemoglobin, and RBC. Olson et al. (1973) reported a high mercury concentration in the kidney rainbow trout following a 24-h exposure. Pentreath (1976) reported that, following a 60-d exposure of the plaice to ^{203}Hg, the kidney was among the organs highest in ^{203}Hg.

Hematology is a valuable tool for assessing a variety of stresses in fish. Its main limitation lies in the lack of information about the normal range of values in fish. Wedemeyer and Yasutake (1977) have noted that, in general, hematological measurements show a greater variation in fish than in many other animals. Fish are subjected to a wide range of temperature, salinity, and nutrient availability, all of which are likely to be reflected in their hematology. Courtois (1976) has demonstrated hematological changes in striped bass exposed to varying conditions of temperature and salinity. Bridges et al. (1976) have demonstrated significant seasonal variation in winter flounder hematology. Hesser (1960), Blaxhall and Daisley (1973), and Wedemeyer and Yasutake (1977) have attempted to standardize and interpret

hematological tests as applied to fish. The gradual accumulation of the necessary background information should make fish hematology an even more useful tool in the future.

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MARGARET A. DAWSON

Northeast Fisheries Center Milford Laboratory
National Marine Fisheries Service, NOAA
Milford, CT 06460-6499