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Effects of Environmental Mercury on Gonadal Function in Lake Champlain Northern Pike (*Esox lucius*)

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Levels of mercury in the environment have increased steadily over the past two centuries, primarily because of human activity (Nater and Grigal 1992). Common point sources of this heavy metal include industrial waste discharge from chloralkali and paper pulp plants. More diffuse emissions, which become widely distributed by global wind currents, result from the combustion of fossil fuels and incineration of municipal wastes. Stricter laws in the United States have decreased the amount of pollution from point sources. In contrast, mercury from diffuse atmospheric origins has been increasing, causing a rise in rainwater concentrations and aquatic environments frequently distant from the source of pollution. Once in aquatic systems, mercury is readily converted to the more toxic methylated form and is the only heavy metal that indisputably biomagnifies through the food web (WHO 1990). Acid rain compounds the environmental impact of anthropogenic mercury because aquatic organisms concentrate more mercury when living in waters with lower alkalinity (Spry and Wiener 1991). The persistance of this heavy metal in teleosts is illustrated by the finding that mercury, unlike cadmium, arsenic, and lead, did not decrease in North American freshwater fish between 1976 and 1984 (Schmitt and Brumbaugh 1990).

Recently, interest has focused on the potential of pollutants to disrupt reproductive endocrinology in vertebrates (Colborn *et al.* 1993). Increasing levels of mercury in the environment, though not high enough to produce the dramatic effects observed during the 1950's in Minimata Bay, Japan (Kitamura 1968), may still pose a threat to wild fish populations. Unfortunately, little is known about the ability of mercury to alter the endocrine function of fish in natural systems. Most laboratory investigations into the effect of mercury on fish populations employ concentrations and methods of exposure that are not environmentally relevant. Studies designed to elucidate the influence of mercury on teleost reproduction, an important determinant of fish populations, have exposed fish primarily to water-borne mercury concentrations in the µg/l range (McKim *et al.* 1976; Weis and Weis 1977; Snarski and Olson 1982; Kirubagaran and Joy 1988, 1992; Dey and Bhattacharya 1989; Bano and Hasan 1990; Dave and Xiu 1991; Devlin and Mottet 1992; Wester and Canton 1992). In contrast, mercury levels in natural waters of North America rarely exceed the ng/l range (Wiener and Spry 1995). Furthermore, in laboratory

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studies that add high concentrations of mercury to the water, uptake occurs primarily through the gills, whereas the primary route of mercury exposure for fish in natural waters is via the diet (Mathers and Johansen 1985; Spry and Wiener 1991). We investigated the influence of natural exposure to mercury by examining reproductive function in northern pike (*Esox lucius*) caught from Lake Champlain. Indices of gonadal function included the gonadosomatic index (GSI) and gonadal sex steroid levels. Northern pike were selected as the target of this study because, as upper-level predators, they tend to accumulate mercury to a greater extent than other fish.

MATERIALS AND METHODS

Fourteen northern pike from Lake Champlain were collected from anglers during a one-week period in January of 1994. All fish were obtained from the Missisquoi Bay at the northern end of the lake, in order to reduce the effects of varying environments within the lake. Fish were taken through the ice by hook and line and kept in live wells until they could be processed. Body length and weight were recorded, and a muscle biopsy removed from just behind the head on both sides of the midline. The gonads were removed, weighed and frozen by placing them on the ice. Frozen gonads were transported to the laboratory, where they were allowed to thaw, homogenized with distilled water (equal volume for ovaries, 2 x volume for testes), and stored at -20° C.

The muscle biopsy from each fish was analyzed for mercury content (wet weight) using cold vapor atomic absorption spectrometry (AAS) by Hazleton Environmental Services, Inc. (Madison, WI) (Hatch and Ott 1968). The detection limit for mercury in biological samples analyzed by this laboratory is 0.01 µg/g, well below the mercury concentration of most fish caught in the wild.

The gonadosomatic index (GSI) was determined by the ratio of gonadal weight to total body weight, expressed as 1-g gonad/100-g body weight.

Homogenized gonads were extracted three times with ethyl ether (anaesthetic grade, Fisher) using 2 x volume for ovaries and 5 x volume for testes. The combined extracts were dried under nitrogen at 37° C and stored at -20° C. The procedure for removal of fat from ovarian extracts was based on that described by Hollander and Hollander (1958). Dried extract (from 4 g tissue) was reconstituted with 10 ml petroleum ether (Certified Grade, Fisher) and extracted three times with an equal volume of 70% ethanol to remove steroids. These combined extracts were back-extracted with 10 ml petroleum ether to remove additional lipids. The remaining aqueous fraction was dried overnight under nitrogen at 40° C. All extractions were carried out in 50-ml polypropylene tubes. Aqueous and organic phases were separated by centrifugation at 1500 x G (4° C).

Extraction efficiencies for the above procedure were determined by adding tritiated hormone to tubes containing homogenized gonadal tissue. Eighty percent of testosterone (n = 6) and 64% of 17β-estradiol (estradiol) (n = 5) was recovered from ovarian tissue, and 78% of testosterone (n = 6) and 72% of estradiol (n = 6) was recovered from testes. Following the measurement of hormone by radioimmunoassay, mean variability with duplicate extractions was calculated to be 5.2% for testosterone and 7.4% for estradiol using the following formula: 100*(duplicate #1 - duplicate #2)/mean.

Dried extracts were reconstituted with assay buffer (the respective zero calibrators). Estradiol and testosterone were determined using coated tube radioimmunoassay kits purchased from Diagnostic Products Corporation, Los Angeles, CA (Sumi *et al.* 1989). All radioimmunoassay values were corrected for extraction efficiencies. The estradiol antiserum is highly specific, has low cross-reactivity to other naturally occurring steroids, and has a lower limit of sensitivity to estradiol of 8 pg/ml(0.008 ng/ml). Similarly, the testosterone antiserum is highly specific with the notable exception of 11-ketotestosterone (cross-reactivity of 16%). Since 11-ketotestosterone is thought to be a major product of northern pike testis (Kime and Hews 1978), measurements from the testosterone assay are referred to as androgen immunoreactivity. This assay has a lower limit of sensitivity to testosterone of 40 pg/ml (0.04 ng/ml). To validate the estradiol and testosterone assays for northern pike, reasonable dilution linearity was demonstrated on extracts from an ovary and a testis (correlation coefficient, r = 0.99 for both assays).

The interassay coefficient of variation (C.V.) for the estradiol assay was 9.2% based on 162 separate measurements of a control sample with a mean value of 113 pg estradiol/ml, and 6.3% based on 161 separate measurements of a control sample with a mean value of 439 pg estradiol/ml. Similarly, the interassay C.V. for the testosterone assay was 8.2% and 8.3%, both based on 39 separate measurements of control samples with mean values of 1.17 and 6.75 ng testosterone/ml, respectively. The intraassay C.V. for estradiol ranged from 4.0% to 7.0% and was based on assays performed using control samples containing between 50 and 1082 pg estradiol/ml. Similarly, the testosterone intraassay C.V. ranged from 4% to 6.5% between concentrations of 1 and 16 ng testosterone/ml.

Simple statistics were calculated (mean, standard error) for mercury content, GSI, and steroid levels by sex. Differences between males and females were compared by Student's t test. Within each sex, the relationships between mercury content and fish length, gonadosomatic index, and steroid levels were examined using simple regression and calculation of a correlation coefficient (r). $P < 0.05$ was taken to be significant.

RESULTS AND DISCUSSION

The results of this study are summarized in Table 1. There was no significant difference between the mercury content of males and females. As in other teleost species, GSI was significantly higher in females than in male, and ovaries contained significantly higher concentrations of estradiol than testes. The difference between males and females in androgen immunoreactivity was not significant. Standard histological analysis of gonads revealed that vitellogenic oocytes and spermatozoa were the predominant cell types in ovaries and testicular lobules, respectively.

Within each sex, there was a four-fold variation in mercury content and at least a two-fold variation in GSI and steroid levels. Although this amount of variation in seven fish was sufficient to detect a significant correlation between body length and mercury content (Fig 1), no significant correlations were found between mercury content and GST or gonadal sex steroid concentration.

The results of this study suggest that the mercury content of northern pike in Lake Champlain has not yet reached a level that dramatically impairs gonadal function. Our findings, at levels of mercury typical in the natural environment, are in contrast

Table 1. Mercury content (µg/g-muscle), total length (mm), gonadosomatic index (GSI) and gonadal hormone levels (ng/g-gonad) in northern pike, *Esox lucius,* taken from Lake Champlain. Asterisk indicates significant difference ($P < 0.001$) between means for males and females.

Fish	Mercury	Length	GSI*	Estradiol*	Androgen
Male	0.117	435	0.9	0.15	24.7
Male	0.161	535	1.0	0.14	10.6
Male	0.334	570	1.9	0.55	12.3
Male	0.368	630	1.2	0.14	13.5
Male	0.399	521	1.1	0.10	11.0
Male	0.436	580	1.6	0.26	10.5
Male	0.612	704	1.2	0.38	11.8
Female	0.139	538	7.6	2.59	7.2
Female	0.175	502	11.8	2.34	8.6
Female	0.195	575	16.5	3.56	9.6
Female	0.273	665	13.3	6.61	9.6
Female	0.315	650	13.2	3.61	6.8
Female	0.404	745	10.4	2.92	16.3
Female	0.623	745	14.2	5.85	8.5
Mean±SE					
Males	0.347±0.06	568±32	1.3± 0.1	0.2± 0.1	13.5±1.9
Females	0.303±0.06	631±36	12.4±1.1	3.9± 0.6	9.5 ± 1.2

to those of others who, at artificially high concentrations of mercury, have demonstrated a decrease in GSI (Channa punctatus, 16.7 µg Hg/l) (Dey and Bhattacharya 1989) and impaired testicular 3β-hydroxy-Δ^5-steroid dehydrogenase activity (*Clarias batrachus,* 32-37 µg Hg/l) (Kirubagaran and Joy 1988). High concentrations of mercury have also been shown to affect other aspects of teleost reproduction. Developmental abnormalities have been observed in a variety of teleost species, including *Oncorhynchus kisutch* (53-120 µg Hg/l) (Devlin and Mottet 1992), *Salvelinus fontinalis* (0.93-2.9 µg Hg/l) (McKim *et al.* 1976), and *Fundulus heteroclitus* (20-40 µg Hg/l) (Weis and Weis 1977). Kirubaragaran *et al.* (1992) reported retardation of gamete maturation in *C. batrachus* (32-37 µg Hg/l), and Bano and Hasan (1990) and Wester and Canton (1992) described inhibition of oogenesis in *Heteropneustes fossilis* (148 µg Hg/l) and spermatogenesis in *Poecilia reticulata* (4.5-8.0 µg Hg/l), respectively. Mercury also inhibited spawning in *Pimephales promelas* (0.26-3.7 µg Hg/l) (Snarski and Olson 1982), and altered the hatching rate of *Danio* (*Brachydanio*) *rerio* embryos (16-512 µg Hg/l) (Dave and Xiu 1991).

These laboratory studies reveal the potential of mercury to impair teleost reproduction, but they were not designed to assess the effect of this heavy metal in

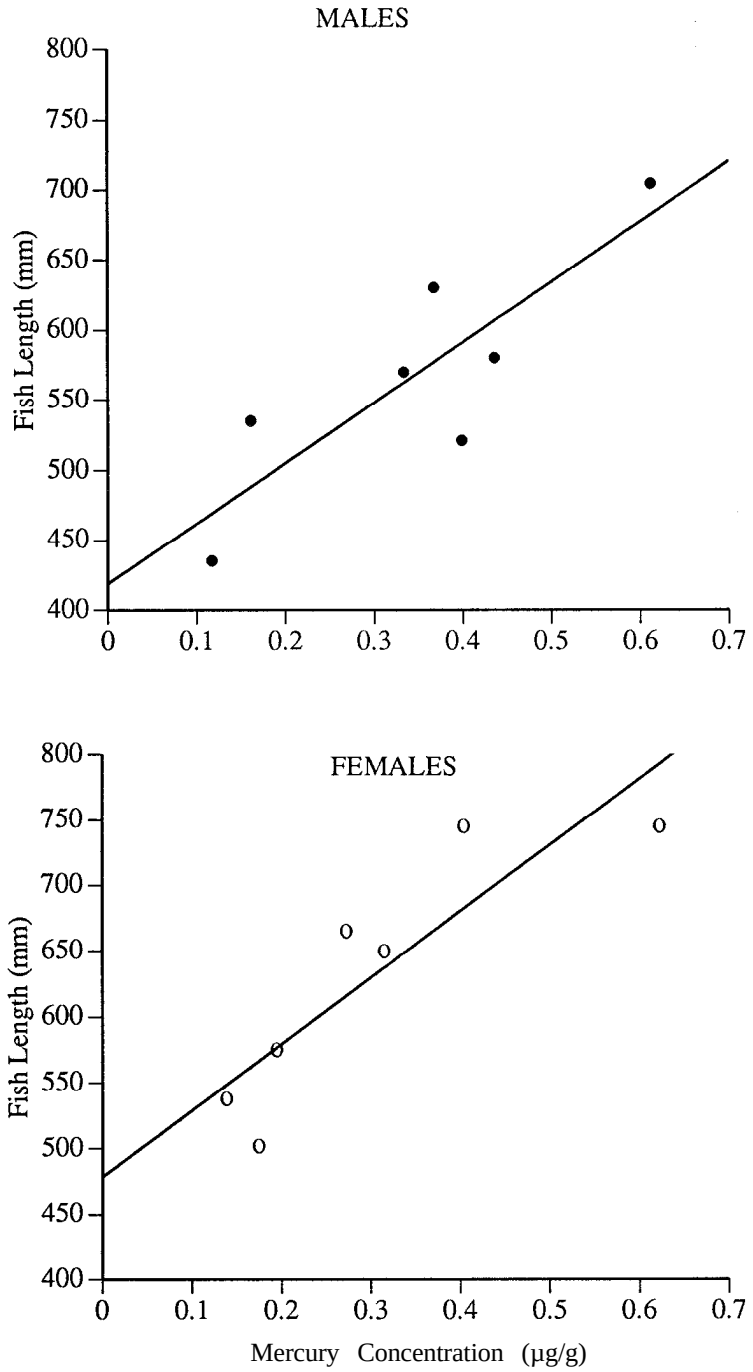

Figure 1: Mercury concentration and total length in male and female northern pike (*Esox lucius*) taken from Lake Champlain. Regression analysis revealed a significant correlation between fish length and mercury content for both males ($r = 0.85$; $P < 0.02$) and females ($r = 0.88$; $P < 0.01$).

a natural setting. Fish were exposed to levels of mercury that are rarely, if ever, encountered in natural waters of North America. In light of mercury's increasing presence in the aquatic environment, there is a need for toxicological investigations that employ realistic exposure scenarios. Evaluating wild-caught fish that contain varying concentrations of mercury permits the examination of environmentally relevant mercury exposure on teleost reproductive potential. The fourteen northern pike taken from the Missisquoi Bay in this study were found to have a mean muscle content of 0.325 µg Hg/g, slightly higher than a nationwide body burden average of 0.1 µg Hg/g determined in 1984 (Schmitt and Brumbaugh 1990), but much lower than the concentrations achieved in many laboratory studies [reviewed in Wiener and Spry 1995]. The failure to observe a significant correlation between mercury content, GSI and gonadal sex steroids in these northern pike raises the possibility that in natural settings, mercury exposure might not exert as dramatic an effect on teleost reproduction as was suggested by earlier laboratory work. It is also possible however, that the mercury levels present in predatory fish of Lake Champlain have a more subtle influence on reproductive physiology, one that might be discernable if a larger sample population of fish was examined. With this latter possibility in mind, we are continuing studies of this kind on walleye (*Stizostedion vitreum*), another upper-level predator that typically contains slightly higher concentrations of mercury.

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