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MERCURY EFFECTS ON PREDATOR AVOIDANCE BEHAVIOR OF A FORAGE FISH, GOLDEN SHINER (*NOTEMIGONUS CRYSOLEUCAS*)

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Abstract—Mercury contamination of fish is widespread in North America and has resulted in the establishment of fish consumption advisories to protect human health. However, the effects of mercury exposure to fish have seldom been investigated. We examined the effects of dietary mercury exposure at environmental levels in a common forage species, golden shiner (*Notemigonus crysoleucas*). Fish were fed either an unaltered diet (12 ng/g wet wt methylmercury [MeHg] as Hg), a low-Hg diet (455 ng/g Hg), or a high-Hg diet (959 ng/g Hg). After 90 d mean fish whole-body total Hg concentrations were 41, 230, and 518 ng/g wet wt, respectively, which were within the range of concentrations found in this species in northern U.S. lakes. There were no mortalities or differences in growth rate among groups. Groups of fish from each treatment were exposed to a model avian predator and their behavioral response videotaped for analysis. Brain acetylcholinesterase (AChE) activity was determined in fish after behavioral testing. Fish fed the high-Hg diet had significantly greater shoal vertical dispersal following predator exposure, took longer to return to pre-exposure activity level, and had greater shoal area after return to pre-exposure activity than did the other treatments, all of which would increase vulnerability of the fish to predation. There were no differences in brain AChE among treatments. We conclude that mercury exposure at levels currently occurring in northern United States lakes alters fish predator-avoidance behavior in a manner that may increase vulnerability to predation. This finding has significant implications for food chain transfer of Hg and Hg exposure of fish predators.

Keywords—Mercury Fish Behavior Diet Predator avoidance

INTRODUCTION

Mercury (Hg) contamination of freshwater fish is a widespread problem in the United States. Fish consumption advisories because of Hg contamination have been issued by 44 states and exceed the number of advisories for all other contaminants combined [1]. Although human and wildlife health effects of Hg exposure have been the subjects of a number of investigations [2,3], the effects of Hg to fish, especially sublethal effects from chronic exposure, have been seldom studied [4].

For freshwater fish, the acute toxicity of waterborne inorganic Hg ranges from 30 μg/L for guppies (*Poecilia reticulata*) to 1,000 μg/L for tilapia (*Tilapia mossambica*) [4]. However, Hg concentrations in surface waters are generally in the range of 1 to 20 ng/L [5]. Therefore, exposure of fish to acutely toxic water concentrations of Hg is unlikely. Hg accumulated by fish is largely monomethyl Hg (MeHg) [6], and at least 85% of Hg uptake by fish occurs through the diet [7]; direct uptake from water is <15%.

In all vertebrates Hg is a neurotoxin [8] at concentrations far below those causing acute toxicity. Methylmercury crosses the blood-brain barrier, preferentially accumulating in the brain [9], and, unlike in other organs, Hg is not depurated from the brain [10,11]. The biochemical effects of Hg exposure are well known, and behavioral effects in mammals have been studied extensively [12], but the toxicological importance of these effects to fish is difficult to determine. Behavioral changes may affect the ability of fish to forage, mate, compete, or avoid predation. These responses, in turn, would affect the survival of fish populations. Limited studies into the effects of Hg on fish behavior have demonstrated that Hg affects fish swimming performance [13], foraging activity [14], learning [15], activity patterns [16], and predator avoidance [17]. However, the concentration, chemical form, and route of exposure to Hg used in these studies were not environmentally realistic.

Food-borne exposures to Hg have been used to assess the pharmacodynamics of Hg poisoning in fishes. Evidence from these studies for behavioral changes is only qualitative. One-year-old walleye (*Stizostedion vitreum*) that were fed shredded pike (*Esox lucius*) with an MeHg concentration of 7.9 μg Hg/g for 240 d had whole-body Hg concentrations of 15 to 45 μg Hg/g (wet wt) and exhibited diminished coordination and escape behavior [18]. Rainbow trout (*Salmo gairdneri*) fed a laboratory diet spiked with MeHg at concentrations of 25 to 95 μg Hg/g for 105 d had whole-body Hg concentrations of 30 to 35 μg Hg/g (wet wt) and were reported to be lethargic [19]. In neither case was the behavioral change quantified, and in both cases the concentration of Hg in the fish was higher than in fish found alive in the wild [4].

There is a need to determine whether food-borne exposure to MeHg impairs fish behavior in a way that would diminish the capability for survival. The goal of this study was to determine whether dietary exposure to environmentally realistic concentrations of MeHg impaired the ability of a freshwater forage fish to avoid predation.

METHODS AND MATERIALS

Test organisms

Golden shiners (*Notemigonus crysoleucas*, 50–70 mm total length) were collected by seining from a man-made pond near

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Brewer, Maine, USA, and transported in a live tank to the laboratory. Golden shiners were chosen as the test species because they are ubiquitous in fresh waters of eastern North America [20]. Golden shiners consume zooplankton, especially Cladocera, and are in turn consumed by many predatory fishes and fish-eating birds [20]. They are a shoaling fish [20] and have known predator avoidance responses similar to many other cyprinid species [21,22]. They are also easily kept in the laboratory and have been used previously for toxicological studies [23].

In the laboratory the fish were placed in a 568 L holding tank (Frigid Units, Toledo, OH, USA) filled with untreated well water (mean temperature = 9°C, pH = 8.5, acid neutralizing capacity = 2,350 μEq/L, specific conductance = 480 μS). They were treated for external parasites with a 2 mg/L solution of potassium permanganate ($KMnO_4$) and fed an artificial diet (Rise; Agway, Syracuse, NY, USA). After two months of acclimation to laboratory conditions, the fish were randomly divided into six groups of 20 fish each. The groups were transferred to six black plastic tanks (378.5 L tanks filled to 227 L; loading = 0.70–0.91 g fish/L), assigned to a treatment, and fed the experimental diet described below. The tanks were supplied with untreated well water, which was recirculated through a biofilter and heated to maintain a constant 23°C temperature. One quarter of the water volume in the tanks was replaced every 10 d. The tanks were enclosed in a black plastic structure to minimize external disturbance and maintained on a cycle of 11 h light, 0.5 h dawn and dusk, and 12 h darkness.

Experimental diet

A diet was prepared following the recipe of Lochmann and Phillips [24], which was modified by the addition of MeHg. A 0.01 mg/ml (3.98×10^{-5} M) stock solution of MeHg was made by dissolving methylmercuric chloride (solid, 95+%; Alfa Aesar, Ward Hill, MA, USA) in reagent-grade ethanol (Baxter Scientific, McGaw Park, IL, USA). The appropriate amount of the stock solution was mixed with deionized water, which was then brought up to 40% weight:volume of the dry material, then extruded through a Hobart meat grinder (Madison, WI, USA) fitted with a 3 mm die. The diets were dried, then double bagged in precleaned plastic bags and stored at −18°C until they were ground. The diets were ground in a class 10 hood using a coffee grinder, passed through a 1.0 mm sieve, and retained on a 0.5 mm sieve. Three diets were produced: control (no added MeHg), low Hg (target final MeHg concentration 500 ng/g as Hg wet wt), and high Hg (target concentration 1,000 ng/g). Our preparation yielded a diet that sank slowly to the bottom of the tank. The fish ate the food while it was sinking and off the bottom of the tanks.

Behavior study

For the behavior study, the six tanks were divided into two blocks of three tanks each, and one tank in each block was randomly assigned one of the three diets: control, low Hg, or high Hg. The fish in each tank were fed 2% of their initial total body weight per day in three equivalent feedings. We recorded whether or not all the food was consumed, but no attempt was made to quantify the amount of food consumed. The person feeding the fish was naive to the treatment assignments.

After being fed a control or Hg-contaminated diet for 90 d, the predator avoidance behavior of the fish was tested. As with the feeding of the fish, the videotaping of the fish and analysis of the videotapes was done without prior knowledge of treatment. We used a 114 L (91.4 cm length × 30.5 cm width × 40.6 cm height) glass aquarium to observe and record fish behavior. A mirror mounted at a 45° angle to the top of the aquarium allowed recording of top and side views simultaneously. The aquarium was housed separate from the feeding tanks in a black plastic enclosure. The same well water, light regime, and temperature used for the feeding tanks were also used for the observation aquarium. The water was aerated prior to the introduction of fish, but the bubbles were turned off once the fish were put in the aquarium.

We used a model of a belted kingfisher (*Ceryle alcyon*), made from balsa wood and Styrofoam (wingspan = 44.5 cm, length = 29.1 cm), as the predator. Kingfishers are a common predator to golden shiners [25] and consume shiners up to 140 mm long but averaging 90 mm. Monofilament fishing line was attached to the snout of the model, which was moved across the tank 6 cm above the water at a speed of 1 m/s by a counterweight.

The 20 fish in each exposure tank were randomly divided into three groups of six fish each. A group size of six was selected because that is the number commonly used to study the behavior of shoaling fish [21,26]. The two remaining fish in each tank were analyzed for Hg content. Fish were placed into the glass aquarium and allowed to acclimate, undisturbed and unfed, for 24 h. At that time, the bird model was released and was flown over the tank. The reaction of the fish was videotaped at 60 frames/s (CCD-F55 8 mm video camera, Sony, Park Ridge, NJ, USA) with the time imprinted on the frames. Fish behavior was recorded for 10 min, the model predator was released and flown over the tank, and recording continued for an additional 10 min. The video camera was located outside the black plastic enclosure and the lens fitted through a hole in the black plastic so that the camera could be operated without disturbing the fish. The tests were run daily between 1400 and 1600 h to ensure that there were no differences in diurnal activity level to confound results. After each test the fish were removed; the water temperature, dissolved oxygen, and ammonia levels were measured; the water was replaced with fresh water; and another group of six fish was placed in the tank. Each tank of fish took 3 d to test, and the tests were run consecutively.

We measured the following parameters from the videotapes: time to initiate response, time to settle, and shoal cohesion. The fish in all trials were sedentary and unmoving prior to the release of the bird model; therefore, both the time to react and the time to settle were clearly delineated. The time to react was defined as the time it took the fish, after the release of the bird model, to begin moving. The time to settle was defined as the time it took the fish to return to the level of activity that they had prior to the flight of the model, i.e., sedentary. The time to reaction and time to settle were both measured manually, using two observers, recording the times for each experimental trial 10 times per observer, and then averaging the times.

Shoal cohesion (packing or tightness of the group) was calculated by capturing the images onto a computer (TARGA+ video card, Truevision, Santa Clara, CA, USA) and on-screen digitization of fish positions (ImagePro Plus, Media Cybernetics, Silver Springs, MD, USA). Shoal cohesion was measured by taking the area of the smallest polygon that could be drawn around all the fish in the horizontal axis (looking down on the shoal from above), determining nearest neighbor dis-

Table 1. Concentrations of whole-body Hg and brain methyl Hg in fish by treatment[a]

Tissue	Treatment	*n*	Mean	SE
Whole body (total Hg)	Control	28	41	14.6
	Low Hg	36	230	59.2
	High Hg	36	536	151.6
Brain (methyl Hg)	Control	26	47	21.5
	Low Hg	23	477	148
	High Hg	25	1,118	196.2

[a] Units are ng Hg/g wet weight. In each case, mean concentrations were significantly different (ANOVA, $p = 0.0001$) among treatments. SE = standard error of the mean.

tances, and measuring shoal depth. Shoal depth was determined by measuring the distance from each fish in the shoal to the bottom of the tank and then calculating the average height of the shoal. All measures of shoal cohesion were performed at the same times before, during, and after the model predator had flown over the tank. For the low-Hg treatment tank 2, third trial, the bird model hit the right side of the tank as it was pulled across the tank. The prereaction area of the shoal and the time to initiate response were used from this trial; however, this trial was not used in any other calculations. The control treatment trials were all acceptable, as were the high-Hg treatment trials. Therefore, the behavioral data are based on replicate numbers of control, $n = 6$; low Hg, $n = 5$; and high Hg, $n = 6$, with the exception of the prereaction area of the shoal and the time to initiate response for which low-Hg $n = 6$.

After removal from the behavior tank, the fish were euthanized with an overdose of MS-222 (tricaine methane sulfonate) and total length and weight were measured. The brains of the fish were removed, weighed, and prepared for AChE activity, MeHg analysis, or both. Brains being analyzed for both AChE and MeHg were split longitudinally. The fish bodies were homogenized in a food blender and frozen at −18°C until they could be analyzed for total Hg.

Chemical analyses

One hundred eleven whole fish were analyzed for total Hg. Frozen whole fish were cut into chunks with a large stainless steel knife, rough ground in a small food processor, then finely ground in a Vertiss homogenizer (Vertiss Research Equipment, Gardiner, NY, USA). One gram of the homogenate was digested in 10 ml 70% nitric acid (TracePur Plus, instrument grade, EM Science, Gibbstown, NJ, USA) and 2 ml 30% hydrogen peroxide (Fisher Scientific, Pittsburgh, PA, USA) in a microwave digestion system (MARS-X, CEM, Matthews, NC, USA) and then diluted to 50 ml with deionized water. Total Hg concentrations were determined by atomic fluorescence spectrometry (Merlin CVAFS, PS Analytical, Sevenoaks, Kent, UK). Aliquots of the three diets were also analyzed for Hg by this method. Analytical accuracy was determined by analyzing a certified reference material (TORT-2 Lobster Hepatopancreas, National Research Council of Canada, Ottawa, ON, Canada) with each sample batch and by determining the Hg recovery from spiked homogenates. Digestion and analytical precision were determined by running duplicate digestions and analyses of one fish sample per sample run. Laboratory contamination was monitored with analysis of reagent blanks with each sample run.

Seventy-five fish brains were analyzed for MeHg concentration by the method of Liang et al. [27]. The brains were digested in a 25% potassium hydroxide (KOH) in 10% methanol solution in a 50:1 ratio (50 mg wet wt brain tissue/ml KOH/methanol) and then extracted into methylene chloride. Monomethyl mercury was back-extracted into water, ethylated, separated by gas chromatography, and analyzed by cold vapor atomic fluorescence spectrometry (Model 1, Brooks Rand, Seattle, WA, USA).

Acetylcholinesterase activity was determined for 54 fish brains using a modification of the Ellman method [28] run on a Gilford Stasar spectrophotometer (Gilford Systems, Oberlin, OH, USA). Tissue was homogenized at a ratio of 20 mg tissue per milliliter 0.05M Tris buffer (Trizma, Sigma Chemical, St. Louis, MO, USA), and absorbance at 412 nm was recorded every 30 s for 3 min. Acetylcholinesterase activity was calculated by the following relationship: [Δ absorbance/time elapsed (min)] · 2.87×10^{-5} = moles of substrate hydrolyzed per minute per gram tissue.

Statistical analyses

Growth, Hg and MeHg concentrations, AChE activity, and behavioral data were tested for normality and homoscedasticity with Levine's test. Treatment differences were determined by randomized complete block ANOVA using the tank as the replicate for those data meeting the assumptions. Mean separation was determined by least squares difference. If the assumptions of the ANOVA were not met, then the data were transformed or analyzed nonparametrically with the Kruskal-Wallis one-way ANOVA by rank. Because of the inherent variability in individual behavior, we defined means that were different with a probability of 0.05 or less as significant, and with a probability 0.1 to 0.05 as marginally significant.

RESULTS

General fish health and growth

The fish appeared healthy and ate well for the duration of the feeding period, except that fish in one tank receiving the low-Hg diet consistently did not consume all of the food (in 42% of the feedings there was food left in the tank vs 9–24% for the other five tanks). No fish died during the 90 d of feeding, and no fish died during behavior testing.

The fish grew throughout the experiment, increasing an average of 38% of their initial body weight. There was no significant difference in growth between the control and the high-Hg fish (38.5% and 42.7%, respectively). However, the low-Hg fish grew less than both the control and high-Hg fish (32.8%, Kruskal-Wallis one-way ANOVA = 0.1005), which is a result of the fish in one low-Hg tank that consistently ate less food (see above).

Mercury analyses

Standard reference material samples run with each batch of fish samples were within the certified range, and the highest digestion blank was 0.2% of the lowest sample result. Recovery of matrix spikes averaged 104%, and relative percent difference of duplicate samples was <10%.

The control diet (no added MeHg) had an average Hg concentration of 12 ng/g (standard error [SE] = 3, $n = 9$); the low-Hg diet averaged 455 ng Hg/g (SE = 29, $n = 9$); and the high-Hg diet was 959 ng/g (SE = 94, $n = 12$). In all cases, all of the diet Hg was in the form of MeHg. Whole-body Hg concentration in fish fed the low-Hg diet was five times higher than the fish fed the control diet (Table 1) and was doubled

Table 2. Fish brain acetylcholinesterase activity by treatment[a]

Treatment	*n*	Mean	SE	Range
Control	15	34.75	0.83	26.53–38.98
Low Hg	17	35.12	1.21	25.90–43.14
High Hg	22	34.39	0.70	26.90–39.09

[a] Units are micromoles substrate hydrolyzed per minute per gram (μmol substrate hydrolyzed · min^{-1} · g^{-1}) brain tissue. Differences among treatments were not significantly different (ANOVA, $p > 0.05$). SE = standard error of the mean.

again in fish fed the high-Hg diet. Assuming that all of the food presented to the fish was consumed, the low-Hg fish assimilated 34.4% of the Hg in the diet and the high-Hg fish assimilated 38.2%. Because not all food was consumed, especially in one low-Hg tank, these values are underestimates of true assimilation.

The brain Hg concentration in the control fish was slightly higher (1.4 times) than the whole-body Hg concentration (Table 1). However, the brain Hg concentrations in the low-Hg fish and the high-Hg fish were both 2.24 times the whole-body Hg concentration. There was a significant difference (ANOVA, $p = 0.0001$) between the control and the two Hg treatments. The percent of Hg body burden found in the brain was 1.00% for control fish, 1.77% for low-Hg fish, and 1.47% for high-Hg fish. There was a significant difference between the control and the two Hg treatments (ANOVA, $p = 0.0036$).

Acetylcholinesterase activity

Acetylcholinesterase activity in brain tissue averaged approximately 35 μmol substrate hydrolyzed · min^{-1} · g^{-1} brain tissue in all three treatments (Table 2). There was no significant difference (ANOVA, $p = 0.8425$) in AChE activity between the treatments.

Behavioral results

The fish were calm before the presentation of the bird. They were close together, not moving, and usually in the front center of the tank. The fish reacted immediately to the release of the bird. The bird was released 0.76 m to the left of the tank, and the fish reacted before the bird was over the tank. Initially the fish swam away from the bird model, that is, in the same direction that the bird model was being flown. When the bird approached the position of the fish they turned and swam back under the bird model. The fish then swam separately, with no pattern, until they regrouped and settled to their pre-exposure activity level.

Fish from all treatments were closely grouped before the bird model was released, with a small nearest-neighbor distance that was not different among treatments (Fig. 1). The pattern of change in nearest-neighbor distance in response to the model predator was complex. Initially, the fish in all treatments repeatedly dispersed and then regrouped. After 2 s the control fish regrouped, and their nearest-neighbor distance further decreased by 5 s, and was unchanged at 10 s. The Hg-exposed fish remained separated, and both the low-Hg and high-Hg fish were spread farther apart than were the control fish 10 s after the bird model had been flown across the tank (Kruskal-Wallis one-way ANOVA, $p = 0.0754$, marginally significant). The time required to respond to the model ranged from 0.27 s for the high-Hg group to 0.38 s for the low-Hg group and did not differ significantly among groups.

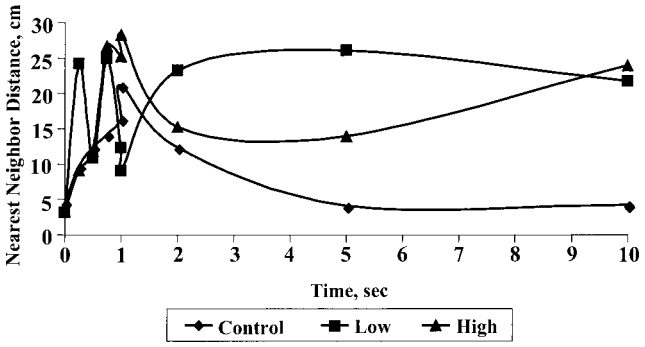

Fig. 1. Change in mean nearest neighbor distance in golden shiner shoals from initiation of exposure to the model predator to 10 s after exposure. After 10 s nearest neighbor distance in mercury-exposed fish was marginally significantly greater than in control fish (Kruskall-Wallis one-way test, $p = 0.0754$).

There was no significant difference (ANOVA, $p = 0.5203$) in shoal area among treatment groups at the moment prior to release of the model predator. The maximum shoal area reached after exposure to the predator was about three times that of the prereaction area, but did not differ among treatments (Kruskal-Wallis one-way ANOVA, $p = 0.4705$). However, the control fish formed more compact shoals after exposure and the Hg-exposed fish formed less compact shoals (Table 3). The area at the time of settling was 75% of the prereaction area for the control fish, 111% for the low-Hg fish, and 248% of the prereaction area for the high-Hg fish. There was a significant difference among treatments (Kruskal-Wallis one-way ANOVA, $p = 0.0463$) in shoal area after settling. As for nearest-neighbor distance and shoal area, shoal height did not differ among treatments prior to exposure to the predator. After exposure to the predator, the control and low-Hg fish had similar mean maximum shoal heights, but the high-Hg fish had a mean about twice this level (Table 3). There was no significant difference between the control and low-Hg fish; however, there was a significant difference (ANOVA, $p = 0.0417$) between those two treatments and the high-Hg fish. Similarly, the control and low-Hg fish ceased activity in about the same time, but the high-Hg fish took nearly three times longer to return to pre-exposure activity (Table 3). This difference was marginally significant (Kruskal-Wallis one-way ANOVA, $p = 0.0702$).

Table 3. Shoal height, shoal area after settling, and time to settling for fish from the three treatments[a]

Metric	Treatment	Mean	SE
Shoal height (cm)	Control	5.9	0.6
	Low Hg	7.7	2.1
	High Hg	57.0	7.2
Shoal area after settling (cm^2)	Control	120	25
	Low Hg	185	27
	High Hg	322	193
Time to settling (s)	Control	7.4	2.2
	Low Hg	8.7	4.8
	High Hg	58.5	7.3

[a] For shoal height and area, fish from the high-Hg treatment were significantly different from control and low-Hg treatment fish (ANOVA, $p < 0.05$), and for time to settling the difference was marginally significant (ANOVA, $p = 0.07$). SE = standard error of the mean.

DISCUSSION

Predator avoidance in shoaling fish, such as golden shiners, is achieved through a behaviorally mediated suite of actions. If that suite is altered, probability of predation may increase. Behavior of prey fish is more important than physical appearance in influencing predator choice and, therefore, prey survival [29]. In this study, we found that exposure to environmentally realistic concentrations of mercury, over a period of 90 d, disrupted several predator avoidance behavior patterns of golden shiners following exposure to a model predator.

Mercury concentrations in our test fish were similar to Hg concentrations reported for wild forage fish from lakes in Maine, USA. Concentrations of Hg in wild forage fish ranged from 100 ng/g wet weight in age zero- to one-year-old yellow perch (*Perca flavescens*) [30] to 280 to 590 ng/g in rainbow smelt (*Osmerus mordax*, 18–22 cm total length) [31]. Golden shiners of approximately the same size as the ones in this study had Hg concentrations ranging from 150 ng/g (Little Beaver Lake, ME, USA) to 630 ng/g wet weight (Attean Lake, ME, USA) [32]. The whole-body Hg concentrations attained by the fish fed the Hg-amended diets in the present study (230 and 518 ng/g, respectively, for the low-Hg and high-Hg diets) are therefore similar to those found in wild golden shiners. The assimilation efficiency of dietary Hg in our study (0.3–0.4) was low compared with values obtained from mass balance studies but similar to the values of 0.29 and 0.33 obtained in a study using channel catfish (*Ictalurus punctatus*) [33].

Acetylcholinesterase inhibition has been reported in fish living downstream of chlor-alkali plants [34], with a 26% inhibition in activity in fish having brain Hg concentrations of 702 ng/g wet weight, which is less than the concentration of Hg in the high-Hg treatment fish in our study. Our results showed no such effect of Hg on AChE activity. Further, no inhibition of AChE activity by organic Hg compounds was found in electric rays (*Torpedo ocellata*) [35], and, generally, organic Hg is not thought to inhibit AChE in mammals [36], which agrees with our findings. The method used for determining AChE activity in these studies was different; Shaw and Panigrahi [34] used a sucrose gradient method, whereas Eldefraw et al. [35] and our study used the Ellman method [28]. It is possible that the sucrose gradient method gives different results from the Ellman method (i.e., different sensitivity, different enzymes, or different forms of enzymes react with the substrate), or that some other factor than Hg in the chlor-alkali discharge is responsible for the effect.

In this study, exposure to MeHg elicited an overall increase in activity as indicated by vertical dispersal and time to settling. Hyperactivity has been observed in other low-concentration Hg studies. Exposure of mummichogs (*Fundulus heteroclitus*) as embryos to 10 μg/L methylmercury increased activity of resulting larvae [17]. This increased activity was inversely proportional to survival against grass shrimp (*Palaemonetes pugio*) predators. Rainbow trout fed 10 μg Hg/g every 5 d were unusually nervous, showing aimless movement or agitation [37]. Largemouth bass (*Micropterus salmoides*) were also hyperactive after exposure to 10 μg/L Hg for 24 h [16].

After the initiation of a predator avoidance response and prior to regrouping behind the predator, individual fish are most vulnerable to predation [38]. The fish do not have the benefit of confusing the predator by presenting more than one target, and the fish are also active, therefore calling attention to themselves. In this study, the high-Hg treatment fish took about three times longer to settle than did the control or the low-Hg treatment fish. Overreaction to stimuli has also been seen in grass shrimp exposed to parathion [39]. This overreaction rendered the shrimp more easily detected by the predator (gulf killifish, *Fundulus grandis*), and more easily fatigued when chased.

Compression of forage fish along the plane perpendicular to the predator (or predatory threat) is considered to increase the confusion effect. Compression increases the number of prey that are in the same plane of focus to the predator, thereby maximizing confusion and limiting the chance of the predator attacking any one of the fish. This was observed in black chin shiners (*Notropis heterodon*) when in the presence of largemouth bass [40]. The shiners will present themselves spread on the vertical plane in the presence of the bass. Similarly, in our study the control golden shiners in the presence of a kingfisher model exhibited shoal compression, such that more fish were in the same plane of focus. In contrast, the shoal height of the Hg-treated fish increased. In the case of the high-Hg treatment fish, some of the fish came to the surface of the water and two broke the surface. This behavior would have the survival disadvantage of decreasing the distance between predator and prey, and also of allowing the fish to be separated from the shoal so that the predator could focus on that fish.

Golden shiners normally regroup more densely after a predatory attack than they were before the attack [41]. In this way the fish return to the presentation of multiple targets to the predator, and can also initiate predator avoidance tactics again. In this study, both the low- and high-Hg treatment fish were less closely grouped after the threat of predation had passed, whereas the control fish were more tightly packed, as expected. Loose repacking after predator threat also occurred in goldfish (*Carassius auratus*) exposed to DDT [42]. The conclusion of that study was that the behavior of the DDT-contaminated fish would have increased their susceptibility to predation.

Several other studies have reported behavioral changes in fish related to body or brain Hg concentrations similar to those used in this study. Fjeld et al. [43] found impaired feeding efficiency and reduced competitive ability in grayling (*Thymallus thymallus*) with whole-body Hg concentration of 270 ng/g or higher. Smith and Weis [44] reported that mummichogs from a polluted stream had significantly higher brain Hg concentration than fish from a reference stream (mean Hg concentration 120 vs 30 ng/g), and that the high-Hg fish had a reduced ability to capture prey (grass shrimp) and suffered significantly greater mortality in the presence of a predator (blue crab, *Callinectes sapidus*).

The vulnerability that hyperactivity may induce in forage fish should be tested using live predators and in the presence of prey refuges. It seems intuitive that hyperactivity would bring the prey fish to the attention of a predator. However, in the presence of a live predator, the underlying behavioral changes that may result in differential predation are often obscured. Without first knowing what underlying behavioral changes may be bringing about an increase in predation, we would know nothing new about Hg intoxication.

The route and duration of Hg exposure in this study were used to simulate environmental exposure to Hg experienced by wild forage fish. If it was an accurate exposure, then it is possible that wild fish may experience subtle survival-limiting behavioral changes that would be undetected in standard bioassay or toxicity tests. High- and low-Hg concentration wild

fish should be tested against either a simulated predator or a real predator to determine whether the behavioral changes seen in this study are seen in wild fish and whether they do reduce survival. Further, if high-Hg fish are more susceptible to predation than fish with lower Hg concentration, the Hg dose to the predator will be higher than would be predicted based on a prey population mean Hg concentration.

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REFERENCES

1. U.S. Environmental Protection Agency. 2002. Update: National listing of fish and wildlife advisories. EPA-823/F/02/007. Office of Water, Washington, DC.
2. Meyers G, Davidson P, Cox C, Shamlaye C, Cernichiari E, Clarkson T. 2000. Twenty-seven years studying the human neurotoxicity of methylmercury exposure. *Environ Res* 83:275–285.
3. Bouton S, Frederick P, Spalding M, McGill H. 1999. Effects of chronic, low concentrations of dietary methylmercury on the behavior of juvenile great egrets. *Environ Toxicol Chem* 18:1934–1939.
4. Wiener JG, Spry DJ. 1996. Toxicological significance of mercury in freshwater fish. In Heinz G, Beyer N, eds, *Interpreting Concentrations of Environmental Contaminants in Wildlife Tissues.* Lewis, Boca Raton, FL, USA, pp 297–340.
5. Morel F, Kraepiel A, Amyot M. 1998. The chemical cycle and bioaccumulation of mercury. *Annu Rev Ecol Syst* 29:543–566.
6. Watras C, Back R, Halvorsen S, Hudson R, Morrison K, Wente S. 1998. Bioaccumulation of mercury in pelagic freshwater food webs. *Sci Total Environ* 219:183–208.
7. Hall B, Bodaly R, Fudge R, Rudd J, Rosenberg D. 1997. Food as the dominant pathway of methylmercury uptake by fish. *Water Air Soil Pollut* 100:13–24.
8. Chang L. 1977. Neurotoxic effects of mercury: A review. *Environ Res* 14:329–373.
9. Kerper LE, Ballatori N, Clarkson TW. 1992. Methylmercury transport across the blood-brain barrier by an amino acid carrier. *Am J Physiol* 31:R761–R765.
10. Gilbin FJ, Massaro EJ. 1973. Pharmacodynamics of methyl mercury in the rainbow trout (*Salmo gairdneri*): Tissue uptake, distribution and elimination. *Toxicol Appl Pharmacol* 24:81–91.
11. Weisbart M. 1973. The distribution and tissue retention of mercury-203 in the goldfish (*Carassius auratus*). *Can J Zool* 51: 143–150.
12. Department of Health and Human Service. 1994. Toxicological profile for mercury (update). TP-93/10. Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA, USA.
13. Weis JS, Weis P. 1995. Swimming performance and predator avoidance by mummichog (*Fundulus heteroclitus*) larvae after embryonic exposure to methylmercury. *Can J Fish Aquat Sci* 52: 2168–2173.
14. Kamchen R, Hara TJ. 1980. Behavioral reactions of whitefish to food extract: An application to sublethal toxicity bioassay. *Can Tech Rep Fish Aquat Sci* 975:182–191.
15. Weir PA, Hine CH. 1970. Effects of various metals on behavior of conditioned goldfish. *Arch Environ Health* 20:45–51.
16. Morgan WSG. 1979. Fish locomotor behavior patterns as a monitoring tool. *J Water Pollut Control Fed* 51:580–589.
17. Zhou T, Scali R, Weis P, Weis JS. 1996. Behavioral effects in mummichog larvae (*Fundulus heteroclitus*) following embryonic exposure to methylmercury. *Mar Environ Res* 42:45–49.
18. Scherer E, Armstrong FAJ, Nowak SH. 1975. Effects of mercury-contaminated diet upon walleyes, *Stizostedion* vitreum vitreum (Mitchell). Technical Report 597. Canadian Fisheries and Marine Service Research Branch, Winnipeg, MB.
19. Rodgers DW, Beamish FWH. 1982. Dynamics of dietary methylmercury in rainbow trout, *Salmo gairdneri*. *Aquat Toxicol* 2: 271–290.
20. Scott WB, Crossman EJ. 1973. *Freshwater Fishes of Canada.* Bulletin 184. Fisheries Research Board of Canada, Ottawa, ON.
21. Litvak MK. 1993. Response of shoaling fish to the threat of aerial predation. *Environ Biol Fish* 36:183–192.
22. Magurran AE, Pitcher TJ. 1987. Provenance, shoal size and the sociobiology of predator-evasion behaviour in minnow shoals. *Proc R Soc London B* 229:439–465.
23. Hartwell SI, Jin JH, Cherry DS, Cairns J Jr. 1989. Toxicity versus avoidance response of golden shiner, *Notemigonus crysoleucas*, to five metals. *J Fish Biol* 35:447–456.
24. Lochmann RT, Phillips H. 1994. Dietary requirement of juvenile golden shiners (*Notemigonus crysoleucas*) and goldfish (*Carassius auratus*) in aquaria. *Aquaculture* 128:277–285.
25. Salyer JC II, Lagler KF. 1946. The eastern belted kingfisher, *Megaceryle alcyon alcyon* (Linnaeus), in relation to fish management. *Trans Am Fish Soc* 76:96–117.
26. Hunter JR. 1966. Procedure for analysis of schooling behavior. *J Fish Res Board Can* 23:547–562.
27. Liang L, Horvat M, Cernichiari E, Gelein B, Balogh S. 1996. Simple solvent extraction technique for elimination of matrix interferences in the determination of methylmercury in environmental and biological samples by ethylation-gas chromatography-cold vapor atomic fluorescence spectrometry. *Talanta* 43:1883–1888.
28. Ellman JL, Courtney KD, Andres IR, Featherstone RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 7:88–95.
29. Coble DW. 1973. Influence of appearance of prey and satiation of predator on food selection in northern pike (*Esox lucius*). *J Fish Res Board Can* 30:317–320.
30. Bodaly RA, Rudd JWM, Fudge RJP, Kelly CA. 1993. Mercury concentrations in fish related to size of remote Canadian Shield lakes. *Can J Fish Aquat Sci* 50:980–987.
31. Akielaszek JJ, Haines TA. 1981. Mercury in the muscle tissue of fish from three northern Maine lakes. *Bull Environ Contam Toxicol* 27:201–208.
32. Evers D, Reaman P. 1998. A comparison of mercury exposure between artificial impoundments and natural lakes measured in common loons and their prey. BioDiversity, Falmouth, ME, USA.
33. McCloskey JT, Schultz IR, Newman MC. 1998. Estimating the oral bioavailability of methylmercury to channel catfish (*Ictalurus punctatus*). *Environ Toxicol Chem* 17:1524–1529.
34. Shaw BP, Panigrahi AK. 1990. Brain AChE activity studies in some fish species collected from a mercury contaminated estuary. *Water Air Soil Pollut* 53:327–334.
35. Eldefraw ME, Mansour NA, Eldefrawi AT. 1977. Interactions of acetylcholine receptors with organic mercury compounds. *Adv Exp Med Biol* 84:449–463.
36. Eccles CU, Annau Z. 1987. *The Toxicity of Methylmercury.* Johns Hopkins University Press, Baltimore, MD, USA.
37. Hartmann AM. 1978. Mercury feeding schedules: Effects on accumulation, retention, and behavior in trout. *Trans Am Fish Soc* 107:369–375.
38. Webb PW. 1982. Avoidance responses of fathead minnows to strikes by four teleost predators. *J Comp Physiol* 147:371–378.
39. Farr JA. 1977. Impairment of antipredator behavior in *Palaemonetes pugio* by exposure to sublethal doses of parathion. *Trans Am Fish Soc* 106:287–290.
40. Abrahams MV, Colgan PW. 1985. Risk of predation, hydrodynamic efficiency and their influence on school structure. *Environ Biol Fish* 13:195–202.
41. Nursall JR. 1973. Some behavioral interactions of spottail shiners (*Notropis hudsonius*), yellow perch (*Perca flavescens*) and northern pike (*Esox lucius*). *J Fish Res Board Can* 30:1161–1178.
42. Weis P, Weis JS. 1974. DDT causes changes in activity and schooling behavior in goldfish. *Environ Res* 7:68–74.
43. Fjeld E, Haugen TO, Vøllestad LA. 1998. Permanent impairment in the feeding behavior of grayling (*Thymallus thymallus*) exposed to methylmercury during embryogenesis. *Sci Total Environ* 213:247–254.
44. Smith GM, Weis JS. 1997. Predator-prey relationships in mummichogs (*Fundulus heteroclitus* (L.)): Effects of living in a polluted environment. *J Exp Mar Biol Ecol* 209:75–87.