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Methylmercury Content of Eggs in Yellow Perch Related to Maternal Exposure in Four Wisconsin Lakes

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We examined the influence of maternal mercury and selected lacustrine variables on the mercury content of eggs from yellow perch (*Perca flavescens*). Total mercury, methylmercury, and inorganic mercury were determined in eggs and carcasses (less eggs) from three seepage lakes with a pH range of 6.1−7.0 and a fourth lake in which pH was experimentally increased from 5.5 to 6.8 by addition of alkaline groundwater. The concentration of total mercury in eggs was strongly correlated with that in the maternal carcass. Concentrations and burdens of mercury in eggs and carcasses were inversely correlated with lake water pH, acid-neutralizing capacity, calcium, and dissolved organic carbon. In eggs containing more than 30 ng/g dry weight (4.5 ng/g wet weight) of total mercury, methylmercury averaged 91% of total mercury and ranged from 85% to 96%. Mean burdens of total mercury in individual eggs varied greatly among lakes (range, 2.3−63 pg), and the egg mass averaged 1.9% of the whole-body burden. We conclude that exposure of the developing yellow perch embryo to methylmercury is strongly affected by maternal bioaccumulation, which can vary substantially among and within lakes; however, the toxicological significance of the observed exposure of embryos to methylmercury is unclear.

Introduction

Little is known about the factors controlling the mercury content of fish eggs, the variable determining the exposure of prefeeding life stages of fish to mercury. Concentrations of mercury in the ovaries and developing eggs are less than those in most other tissues and organs of female fishes exposed to mercury in the laboratory (*1*, *2*) and in natural waters (*3*, *4*). Yet, the early life stages of fishes are considerably more sensitive than the adult to both methylmercury and inorganic mercury (*1*, *4*−*8*). A recent review (*9*) showed that the developing fish embryo can be adversely affected by a very small quantity of maternally transmitted methylmercury or inorganic mercury.

Mercury derived from the adult female seems to be the primary exposure pathway for fish embryos in natural waters, even though the amount of mercury transferred from the adult female to the eggs during oogenesis is small (*9*). Maternally derived methylmercury or inorganic mercury can adversely affect the survival and development of fish embryos in the laboratory (*1*, *8*). The zona radiata or chorion, the outermost membrane on the fertilized egg, seems to retard the uptake of inorganic mercury and methylmercury from the surrounding water into the developing fish embryo (*4*, *10*, *11*). Moreover, the waterborne concentrations of inorganic mercury and methylmercury known to cause lethal or sublethal effects in young fish in laboratory tests are typically several orders of magnitude greater than those in all but the most severely contaminated surface waters (*9*).

We quantified concentrations and masses of mercury in eggs from yellow perch (*Perca flavescens*) from four seepage lakes in northern Wisconsin and examined the utility of maternal mercury and selected lacustrine variables as predictors of the mercury content of eggs. Northern Wisconsin contains hundreds of seepage lakes (*12*), most of which are inhabited by yellow perch (*13*, *14*). High concentrations of mercury have prompted fish-consumption advisories for game fishes, such as walleye (*Stizostedion vitreum*), from many low-alkalinity lakes in the area. These lakes generally lack known on-site sources of anthropogenic mercury, and most of the mercury in these semiremote lakes is derived from atmospheric deposition (*15*−*19*).

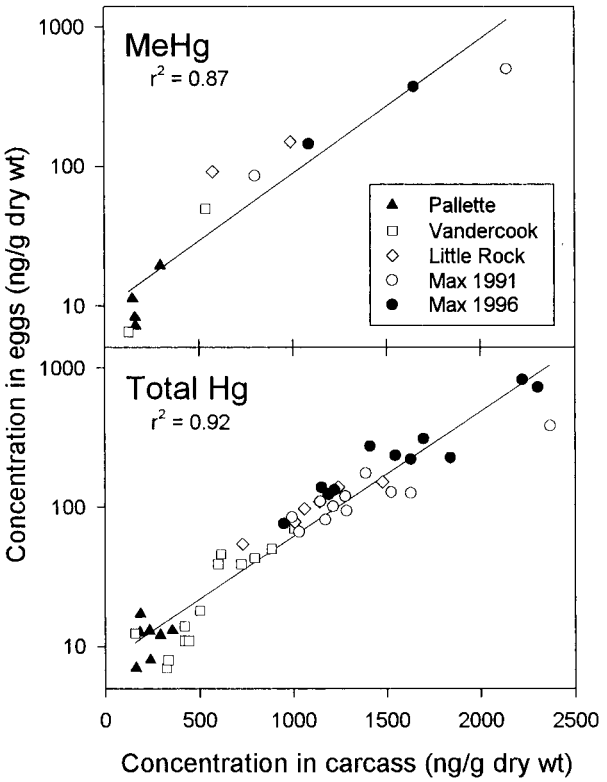

FIGURE 1. Relation between mercury concentrations in eggs and carcasses of gravid yellow perch from four seepage lakes in northern Wisconsin, shown for both methylmercury (MeHg) and total mercury.

Experimental Section

Sampling. Gravid yellow perch were sampled from four seepage lakes in northern Wisconsin (Table 1) with trap nets (0.95 cm square mesh) fished overnight in littoral habitat a few days after ice melt (usually during April) when the yellow

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TABLE 1. Physicochemical Characteristics of the Study Lakes[a]

lake (year sampled)	surface area (ha)	depth (m) max	depth (m) mean	pH	ANC (μeq/L)	calcium (μeq/L)	DOC (mg/L)
Pallette (1989)	70.0	18.2	9.6	7.03	127	111	5.3
Vandercook (1989)	43.6	7.2	4.7	6.37	42	67	4.1
Little Rock (1991)	8.1	6.5	3.1	6.12	25	47	3.0
Max (1991)	9.2	5.8	2.9	5.52	4.2	63	2.2
Max (1996)	9.2	5.8	2.9	6.77	105	103	

[a] Chemical characteristics for Max Lake in 1991 were after 1 year of groundwater addition and in 1996 were after 6 years of groundwater addition.

perch were spawning. We sampled fish in Pallette and Vandercook Lakes in 1989, Little Rock Lake in 1991, and Max Lake in 1991 and 1996; these four lakes were included in an intensive investigation of the biogeochemical cycling of mercury in temperate lakes (*18*). Each fish was measured (total length ±1 mm), weighed (±0.1 g), and stored at ≤−30 °C until dissection. Scales for age estimation were taken from each fish near the area of insertion of the left pectoral fin. The age of each fish was estimated by examination of three or more scales on a microfiche reader, as described by Jearld (*20*).

Dissection of Fish. After the frozen fish were thawed overnight in a refrigerator, we dissected the egg sac (i.e., the eggs and enclosing ovary) from each fish through a ventral incision in the peritoneal cavity. The egg sac was split with a shallow incision, and all of the eggs were removed from the ovary. Subsamples of eggs were removed for mercury determination and counting. After removal of eggs, the ovary was returned to the peritoneal cavity of all fish except those obtained from Max Lake in 1996; the ovaries of these 11 fish were analyzed separately for mercury. All dissected fish, eggs, and ovaries to be analyzed for mercury were promptly refrozen and stored at ≤−30 °C until lyophilization. Subsamples of eggs were counted under a dissecting microscope on the day of removal.

We took precautions to minimize contamination of the samples with mercury during dissection. Stainless steel implements used for dissection were rigorously cleaned with laboratory detergent and rinsed with well water. Fish were dissected inside a food-grade plastic bag. Between samples, dissection equipment was rigorously rinsed or discarded, dissection surfaces were changed, and gloves were rinsed or changed.

Mercury Determinations. For total mercury, we analyzed 48 samples of eggs, 37 carcasses with ovaries (less eggs), 11 carcasses less both ovaries and eggs, and 11 ovaries (less eggs). Frozen samples were lyophilized to a constant dry weight in Ziploc bags (carcasses) or in acid-washed polyethylene bottles (eggs and ovaries) for 72−168 h at ≤−50 °C. Lyophilized carcasses were homogenized with a stainless steel Waring blender at 20 000 rpm, and lyophilized samples of eggs were homogenized by stirring with an acid-washed polyethylene spoon. We digested 250 mg subsamples of lyophilized carcasses, 500 mg subsamples of lyophilized eggs, and entire ovaries from individual fish with 15 mL of a 4:1 (vol:vol) solution of 18 M H_2SO_4 and 16 M HNO_3 in 75 mL ignition tubes heated at 220 °C for 14 h in aluminum blocks. After cooling, each digestate was transferred to an acid-washed polyethylene bottle, and 10 mL of 12% (wt:vol) hydroxylamine hydrochloride was added before the digestate was diluted to 100 mL with 1% (vol:vol) HCl. Each digestate was analyzed by flow injection cold-vapor atomic absorption spectroscopy with a Perkin-Elmer FIMS 100.

Methylmercury and inorganic mercury in subsamples of carcasses and eggs from 12 fish were determined by Frontier Geosciences (Seattle, WA). These 12 fish were selected from all lakes to encompass the range of mercury concentrations among lakes and years of sampling, based on determined concentrations of total mercury in eggs and carcasses. For determinations of methylmercury, subsamples were prepared by KOH/methanol digestion, aqueous-phase ethylation, and isothermal GC separation and quantified with cold-vapor atomic fluorescence spectroscopy (*21*, *22*). Inorganic mercury in these same digestates was determined with cold-vapor atomic fluorescence spectroscopy after $SnCl_2$ reduction and dual gold amalgamation (*23*).

Quality Assurance. The precision of our egg counts was estimated by enumerating eggs in three or six subsamples from nine fish. Method precision (relative standard deviation) for our estimates of numbers of eggs per gram averaged 3.2% and ranged from 1.4% to 5.7%.

All glassware was acid-washed and rinsed with reagent-grade water. All acids and reagents used in digestions and analyses were suitable for use in mercury determinations (J. T. Baker, Instra-Analyzed). Reagent-grade water had a nominal resistance of ≥15 MΩ/cm. For determinations of total mercury by atomic absorption spectroscopy, standards were prepared from 1000 mg/L certified standards (Fisher Scientific). For determinations by atomic fluorescence, standards for inorganic mercury were prepared by dilution of U.S. National Institute of Standards and Technology (NIST) certified NBS-3133 mercury standard solution, and standards for methylmercury were prepared from pure powder and calibrated against NBS-3133.

For determinations of total mercury, the precision and bias of measurements for each analytical batch of samples were quantified by analyses of the following: (1) standard reference materials from NIST (albacore tuna and bovine liver) and the National Research Council of Canada (NRCC dogfish muscle-1, dogfish muscle-2, dogfish liver-2, and lobster hepatopancreas), (2) spiked (before digestion) subsamples of homogenized fish and eggs, (3) triplicate subsamples of homogenized fish and eggs, and (4) procedural blanks and standards taken through the digestion procedures. Our mean measured concentrations of total mercury in the six standard reference materials were within the certified ranges, which varied from 2−6 ng/g dry weight to 4380−4900 ng/g. The mean recovery of total mercury was 99% (95% CI, 98−101%) for 15 spiked subsamples of carcass and 101% (CI, 97−105%) for 13 spiked subsamples of eggs. Method precision (relative standard deviation) for determinations of total mercury, estimated from analyses of triplicate subsamples, averaged 3.3% (range, 1.9−4.5%) for fish carcasses and 6.6% (range, 2.1−20.5%) for eggs. Our estimated method detection limit (*24*) was 4 ng/g dry weight for total mercury in a 250 mg sample of homogenized fish tissue.

For determinations of methylmercury and inorganic mercury, recovery averaged 92% (95% CI, 83−102%) for 10 spiked subsamples of fish carcasses and eggs, and method precision (relative percent difference, estimated from analyses of duplicate subsamples) averaged 7.5% and ranged from 2.5% to 15.3%. The mean recovery of mercury in NRCC dogfish muscle-2 was within the certified range for both total mercury (4547 ng/g dry weight) and methylmercury (4205 ng/g dry weight). A single analysis of methylmercury in NRCC dogfish liver-2 and NRCC lobster hepatopancreas was within 11% and 21%, respectively, of the certified concentration range. Estimated method detection limits (*24*) were 3.8 ng/g dry weight for methylmercury in eggs and carcasses, 14 ng/g for inorganic mercury in carcasses, and 0.6 ng/g for inorganic mercury in eggs.

Statistical Analyses. Data were analyzed with a microcomputer and SPSS for Windows software (version 7.5.1). Concentrations of mercury in eggs and carcasses of fish from Max Lake in 1991 and 1996 were checked for normality and homogeneity of variances before statistical tests to compare

TABLE 2. Summary Statistics and Characteristics (±1 SE) of Gravid Yellow Perch Analyzed for Total Mercury[a]

lake	lake pH	fish analyzed				eggs/g (wet weight)	total Hg (ng/g dry wt)		Hg burden (pg/egg)
		n	age range (year)	total length (mm)	fresh weight (g)		egg	carcass	
Pallette	7.03	7	2−4	170 ± 8 (134−193)	53.8 ± 7.4 (20.5−79.8)	819 ± 64	12 ± 1 (7−17)	236 ± 26 (162−356)	2.3 ± 0.4
Vandercook	6.37	13	3−6	150 ± 3 (129−165)	33.5 ± 1.4 (23.8−40.5)	584 ± 21	28 ± 6 (7−70)	555 ± 68 (156−1006)	5.9 ± 1.2
Little Rock	6.12	6	3	153 ± 2 (147−159)	28.4 ± 1.1 (25.0−32.0)	920 ± 26	105 ± 15 (54−152)	1109 ± 102 (730−1476)	17 ± 2
Max (1991)	5.52	11	3−6	200 ± 3 (185−219)	97.3 ± 4.3 (74.2−128.8)	598 ± 17	133 ± 26 (66−383)	1366 ± 115 (996−2367)	39 ± 8
Max (1996)	6.77	11	3−8	224 ± 3 (204−241)	134.1 ± 5.7 (105.3−166.0)	692 ± 30	298 ± 74 (76−819)	1560 ± 131 (951−2304)	63 ± 12

[a] Ranges are given in parentheses.

means between years. Contrasts of mercury concentrations in eggs, which were not normally distributed and had unequal variances, were made with the Mann−Whitney test. Contrasts of concentrations in carcasses, which were normally distributed and had equal variances, were made with the *t*-test. A Type I error (α) of 0.05 was used to judge the significance of all statistical tests.

Results and Discussion

Inclusion or exclusion of the ovary from the carcass sample did not measurably influence the concentration of mercury in the carcass samples. In fish taken from Max Lake in 1996, for example, the mean measured concentration of total mercury was 1574 ng/g dry weight in carcasses without ovaries, whereas we calculated that the mean concentration in these carcasses if ovaries had been included (without eggs) would have been 1560 ng/g, a difference of less than 1%. Consequently, we grouped data for carcasses with and without ovaries in all subsequent statistical analyses.

Mean concentrations and burdens of total mercury in eggs and fish carcasses varied considerably within and among lakes (Table 2). In eggs, the mean concentration of total mercury ranged from 12 ng/g dry weight in Pallette Lake to 298 ng/g in Max Lake in 1996. Calculated mean burdens of total mercury in individual eggs ranged from 2.3 pg in Pallette Lake to 63 pg in Max Lake in 1996. The mean concentration of total mercury in fish carcasses ranged from 236 ng/g dry weight in fish from Pallette Lake to 1560 ng/g in fish sampled from Max Lake in 1996. Within individual lakes, the mean concentration of total mercury in eggs ranged from 5% to 20% of that in the carcass.

Concentrations of mercury in eggs were positively correlated with those in the carcass of the maternal fish (Figure 1). With data from all lakes combined, the relation between the concentration of total mercury in eggs (C_e) and the maternal carcass (C_m) was described by the regression equation

$$\log C_e = 0.884 + (9.03 \times 10^{-4})C_m \quad (1)$$

which had a coefficient of determination (r^2) of 0.92. There was a positive correlation between concentrations of total mercury in eggs and fish carcasses in three of the four lakes (r^2 ranged from 0.80 to 0.93), whereas in fish from Pallette Lake the concentrations in eggs and carcasses were not correlated ($r^2 = 0.05$). For the 12 fish analyzed for methylmercury, the relation between the concentration in eggs (C_e) and the carcass (C_m) was also significant ($r^2 = 0.87$), yielding the regression equation

$$\log C_e = 0.977 + (9.69 \times 10^{-4})C_m \quad (2)$$

Concentrations of total mercury in carcasses and eggs of fish sampled in 1989 and 1991 were inversely related to the pH, acid-neutralizing capacity, calcium, and dissolved organic carbon concentration of the four lakes (Tables 1 and 2). For these samples, mean dry-weight concentrations were lowest (12 ng/g in eggs, 236 ng/g in carcasses) in fish from Pallette Lake (pH 7.0) and highest (133 ng/g in eggs, 1366 ng/g in carcasses) in fish from Max Lake (pH 5.5). The samples taken from Max Lake in 1996 (pH 6.8 after experimental alkalization by groundwater addition) did not follow this pattern, averaging 298 ng/g in eggs and 1560 ng/g in carcasses. Mean concentrations of total mercury in fish carcasses collected in 1991 and 1996 from Max Lake did not differ (*t*-test, $p = 0.28$), whereas concentrations and burdens in eggs differed considerably between 1991 and 1996 (Mann−Whitney test, $p = 0.01$). We suspect that the differences observed in mean concentrations of mercury in eggs between 1991 and 1996 reflected interyear variation in dietary methylmercury uptake during oogenesis. In general, mercury levels did not decline in any length or age group of yellow perch in response to the alkalization of Max Lake (J. G. Wiener, U.S. Geological Survey, La Crosse, WI, unpublished data).

Most of the mercury in eggs and carcasses of yellow perch was methylmercury, the highly toxic form of the metal. In eggs ($n = 12$ samples), methylmercury averaged 80% of total mercury and ranged from 53% to 96%. However, the fraction of total mercury in the methyl form increased concomitantly with increasing concentration of total mercury in the eggs (Figure 2). In the seven samples of eggs with total mercury exceeding 30 ng/g dry weight (equivalent to 4.5 ng/g wet weight, given a mean water content of 85%), methylmercury averaged 91% and ranged from 85% to 96% of total mercury. We suspect that the small amount of inorganic mercury found in our egg samples resulted from handling contamination, which adds inorganic mercury, not methylmercury, to the samples (*25*). In the eight carcasses with quantifiable concentrations of inorganic mercury, methylmercury averaged 95% and ranged from 84% to 97% of total mercury. In both eggs and carcasses, concentrations of methylmercury and total mercury were strongly correlated with a regression slope of 0.96, indicating that most of the mercury was methylmercury (Figure 3). Our results for eggs and carcasses agree with recent reports showing that methylmercury accounts for nearly all of the mercury in the skeletal muscle of fish (*25*, *26*).

Concentrations of total mercury in eggs of yellow perch were similar to the mean values reported for eggs in five species of fishes from lakes Ontario and Erie, which varied from 27 ng/g dry weight in white bass (*Morone chrysops*) to 73 ng/g in rainbow trout (*Oncorhynchus mykiss*) (*3*). Eight yellow perch from the central basin of Lake Erie (*3*) had mean concentrations (total mercury) of 33 ng/g dry weight in eggs and 335 ng/g in carcasses without eggs. Niimi (*3*) reported

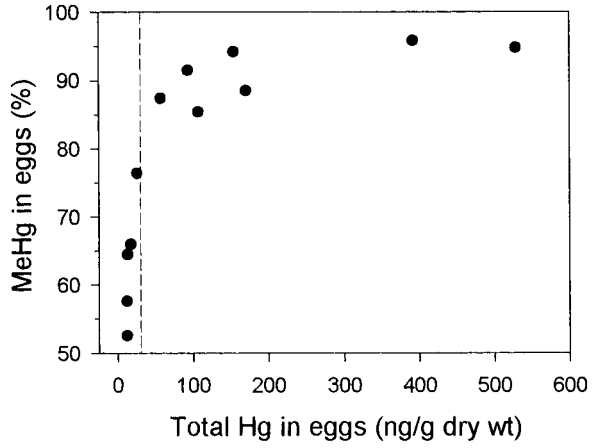

FIGURE 2. Relation between percent methylmercury (fraction of total mercury present as methylmercury) and concentrations of total mercury (calculated as methylmercury + inorganic mercury) in eggs from gravid yellow perch from four seepage lakes in northern Wisconsin. The dashed vertical line denotes a total mercury concentration of 30 ng/g dry weight in eggs.

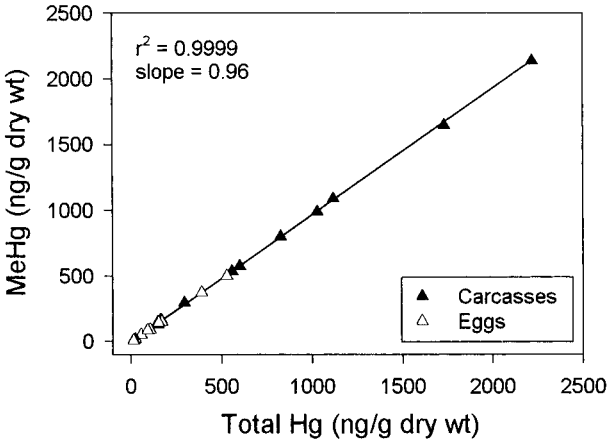

FIGURE 3. Relation between concentrations of methylmercury (MeHg) and total mercury present in eggs and carcasses of gravid yellow perch from four seepage lakes in northern Wisconsin.

wet-weight concentrations, which we converted to the preceding dry-weight values assuming that water content in his samples averaged 85% in the eggs and 80% in the carcass.

Concentrations of total mercury in the ovaries of 11 fish taken from Max Lake in 1996 averaged 862 ng/g dry weight, ranging from 285 to 2153 ng/g. Concentrations in the ovary were strongly correlated with those in the eggs ($r = 0.99$) and the carcass ($r = 0.93$). Concentrations of total mercury in individual ovaries averaged 50% of those in the carcass and ranged from 30% to 97%. When expressed as a fraction of the total burden of mercury in the whole fish, the percentage of total mercury in the ovaries varied little among fish, averaging 1.0% and ranging from 0.4% to 1.7% of the whole-body burden.

Concentrations of total mercury in the ovaries averaged 3-fold greater than those in the eggs. The fraction of total mercury in the ovaries averaged 23% and ranged from 10% to 46% of the total burden in the egg sac (i.e., the eggs plus ovary). Therefore, mercury concentrations in the eggs would be overestimated if portions of the ovary are included and analyzed with the egg sample.

Our findings indicate that the methylmercury content of eggs reflects the maternal exposure history, with the concentration in the eggs increasing concomitantly with that in the maternal fish. The number of fish analyzed from each lake was small, yet the concentration of mercury in eggs was significantly correlated with that in the maternal carcass in all lakes except Pallette Lake, which had fish (eggs and carcasses) with the lowest mean concentrations of mercury. Exposure of adult yellow perch to methylmercury in the study lakes presumably resulted almost entirely from dietary uptake, given the low concentrations of waterborne methylmercury in these lakes (*18*) and recent findings showing that fish obtain methylmercury almost entirely from the diet (*9*, *27*, *28*). Reported mean concentrations of waterborne mercury in oxic waters of the study lakes ranged from 0.7 to 2.1 ng Hg/L for total mercury and from 0.05 to 0.33 ng Hg/L for methylmercury (*18*).

We attribute the observed variation in the mercury content of eggs among lakes to the influence of lake chemistry. The observed pattern in concentrations of mercury in eggs and carcasses in the present study mirrors that reported for whole yellow perch and axial muscle tissue of walleye from the study area; that is, mercury concentrations were inversely correlated with lake pH and associated chemical variables (*15*, *17*, *29*).

Methylmercury, unlike the more lipophilic organic contaminants (*30*), does not seem to concentrate in the eggs of fish. Burdens of total mercury in eggs of all 48 yellow perch averaged 1.9% of the whole-body burden. Similarly, Niimi (*3*) found that the eggs contained from 0.3% to 2.3% of the mean whole-body burden of total mercury in five species of fish, whereas the quantities of 12 organic contaminants in the eggs averaged from 5.5% to 25.5% of the whole-body burden. Given the relatively small fraction present in the eggs, we infer that yellow perch eliminate little methylmercury during spawning.

The toxicological significance of the concentrations reported here for eggs of yellow perch is unknown. Methylmercury damages the central nervous system, and in those vertebrate organisms that have been intensively studied, the developing embryo is the most sensitive life stage (*31*, *32*). In birds, for example, the effects of methylmercury on embryos and chicks are much more severe than those on adults, and low-level dietary exposures that cause no measurable effect in adults can significantly impair egg fertility, hatching success, and overall reproductive success (*31*). Comparatively little is known about the effects of maternally transmitted methylmercury on the survival and growth of embryolarval fishes. Most information on the effects of methylmercury on early life stages of fish is from studies involving exposures of fertilized eggs to unrealistically high concentrations of waterborne methylmercury (*9*).

Yet, the margin of safety between present and toxic exposure levels may be small for some fish populations. Laboratory studies of rainbow trout exposed to mercuric chloride, for example, showed that increased mortality of fertilized eggs was associated with total-mercury concentrations in eggs as low as 70 to 100 ng/g wet weight (*8*). In comparison, overt toxicity in adult rainbow trout was associated with reported tissue concentrations of about 10 000 to 30 000 ng/g (*9*). In a laboratory study of brook trout (*Salvelinus fontinalis*), embryos containing 2200 ng Hg/g wet weight after both maternal and waterborne exposure to methylmercuric chloride were deformed and did not survive more than 3 weeks after hatching (*1*). The maximum concentration of methylmercury in eggs of yellow perch in our study was within the range of concentrations associated with increased mortality in embryos of rainbow trout (*8*) but only about $^1/_{20}$ of that associated with 100% mortality of embryos of brook trout (*1*). It is unclear whether the apparent differences in mercury burdens associated with toxicity reported in these two laboratory studies (*1*, *8*) resulted from differing sensitivity to mercury between rainbow trout and brook trout, from the different forms of mercury used in the

tests, from other methodological differences between the studies, or a combination of these factors. Critical evaluation of the toxicological significance of maternally derived methylmercury to early life stages of fishes will be needed to understand the consequences of environmental mercury contamination on fish populations.

Acknowledgments

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