The Influence of Temperature on Uptake of Methylmercury-203 by Bluntnose Minnows, *Pimephales notatus* (Rafinesque)

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INTRODUCTION

It is recognized that physical and chemical characteristics of aquatic ecosystems may limit, enhance, or otherwise modify uptake of mercury directly from water by fishes. Direct sorption from water is reported to be an important route for mercury uptake by fishes (JERNELOV and LANN, 1971). Information relating the effect of water temperature to incorporation of methylmercury by fishes is lacking. Consideration of temperature in aquatic environments includes thermal inputs from electric generating installations as well as diel, temporal, and spatial temperature fluctuations experienced routinely by fishes. Determining the influence of temperature on the uptake of mercury by fish will facilitate interpretation of field data depicting mercury levels.

In order to learn something about the relationship between subtle temperature changes and methylmercury uptake from water by fish, I exposed bluntnose minnows maintained at 18, 21, and 24 C to equal concentrations of methylmercury-203 ($CH_3{}^{203}HgCl$; specific activity approximately 7 mCi/g; 47-day half life; Amersham-Searle, Arlington Heights, Illinois).

METHODS

The bluntnose minnows used in this experiment were seined from the Olentangy River near Columbus, Ohio. These animals were returned to the laboratory and kept in a tank with about 400 liters of aerated tap water maintained at 17 C (this temperature corresponds to the stream temperature at the time the fish were collected). Lighting inside the lab was on a 12-hr photoperiod. Individuals were segregated by visual estimation into small, large, and intermediate-length groups, and the number of fish comprising each group were recorded. Several fish representing each length were weighed and then discarded. These procedures negated excessive handling and possible injury to the fish prior to experimentation but still permitted adequate estimation of average weights. Such data were required for apportionment of fish biomass to water volume in the tests. The minnows

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were fed granulated trout chow for 2 months during acclimation to laboratory conditions before tests were begun.

The experiment consisted of three tests conducted at 18, 21, and 24 C, respectively. In each test, 30 fish and 35 liters of water were removed from the holding tank and transferred to a polyethylene-lined aquarium immersed in a waterbath maintained at the desired test temperature (± 0.2 C). The bluntnose minnows were given a final feeding several hours prior to transfer to the experimental apparatus. Fish from each length group were randomly selected and removed from the holding tank in such a manner that nearly equal numbers of similar-sized individuals were used for each test. The fish were allowed a 48-hr period to become acclimated to the conditions of the test. The methylmercury concentration used in each of the three tests was 10 μg $CH_3{}^{203}HgCl$/liter. The aquaria were shielded so that routine movements within the laboratory would not excite the fish.

Samples of the water supply were obtained and analyzed for Si, Fe, Ca, Mg, Mn, K, Na, SO_4, Cl, PO_4, hardness, total alkalinity, pH, and dissolved oxygen. Analyses were completed according to procedures in Standard Methods (A.P.H.A., 1971); cation concentrations were determined by atomic absorption spectrophotometry. Knowledge of ambient water chemistry will allow comparisons to be made in the future between known conditions of this experiment and those found in aquatic environments. Table 1 depicts the chemical characteristics of the water used in this

TABLE 1.

CHEMICAL ANALYSIS OF AQUARIUM WATER
BEFORE METHYLMERCURY ADDITION

Chemicals Analyzed	mg/ℓ
Silica	3.17
Iron	0.00
Calcium	23.0
Magnesium	7.2
Manganese	0.0
Potassium	3.3
Sodium	24.5
Total alkalinity ($CaCO_3$)	35.0
Sulfate	87.3
Chloride	34.6
Total hardness ($CaCO_3$)	87.1
Total phosphate	0.59

experiment. The pH ranged from 6.9 to 7.1 and did not drift from neutral during the experiment. Also, D.O. (mg/ℓ) ranged from 5.4 at 18 C to 4.5 at 24 C. The aquaria were not aerated artificially.

Exposure to the isotopic methylmercury lasted 48 hours. All fish survived the exposure to the mercury compound and exhibited no behavioral abnormalities. One individual from the group of fishes tested at 18 C was "lost" in a fold of the polyethylene

TABLE 2.

WEIGHT GROUP CLASSIFICATION OF TEST FISH LISTING SAMPLE SIZE, MEAN WEIGHT, AND MEAN METHYLMERCURY CONCENTRATION WITHIN AND BETWEEN TEST TEMPERATURES

	18 C			21 C			24 C		
Wt Class	N	Wt, grams	µg Hg/g	N	Wt, grams	µg Hg/g	N	Wt, grams	µg Hg/g
>0-0.5	1	0.50	4.82						
0.5-1.0	7	0.79 (0.04)[a]	4.70 (0.25)	12	0.78 (0.03)	6.18 (0.29)	15	0.81 (0.03)	5.76 (0.10)
1.0-1.5	16	1.19 (0.04)	4.61 (0.18)	11	1.21 (0.04)	5.87 (0.25)	11	1.21 (0.04)	5.85 (0.23)
1.5-2.0	5	1.74 (0.06)	4.16 (0.21)	5	1.67 (0.08)	6.04 (0.09)	4	1.65 (0.04)	4.98 (0.14)
2.0-2.5				2	2.31	5.12			

(a) Standard error given in parentheses.

liner inside the aquarium, however. Each fish was removed from the water, placed separately into a tared 5-oz polyethylene container, and weighed (wet). Individual weight ranged from 0.5 to 2.4 g, and the ratio (g/ℓ) of total fish biomass to water volume was 0.99 at 18 C, 1.0 at 21 C, and 0.92 at 24 C. The fish were digested by adding 40 mℓ of concentrated nitric acid to each container. Digestion was complete after 72 hr at room temperature followed by a 12-hr period inside an oven at 30 C. A 4-mℓ aliquot of the digest was removed from each container and placed into a 1-dram vial for radio assay.

Assays were conducted using a Packard 3002 gamma spectrometer employing a 3 x 5" NaI(Tl) well crystal. The vials containing the digest were inserted into the well of the crystal and assayed for isotopic methylmercury. The concentration of $CH_3{}^{203}Hg$ in each 4-mℓ aliquot of digest was such that a 1-minute counting period generally yielded a count rate exceeding twice the counting error (95% level). Count data obtained from the aliquots were converted to μg $CH_3{}^{203}Hg$/4-mℓ by comparison with count data from carefully prepared standards made up from a stock solution of the methylmercury-203 compound. Multiplying the calculated μg $CH_3{}^{203}Hg$/4-mℓ by a factor of 10 (which yields the total μg $CH_3{}^{203}Hg$ in the 40 mℓ of digest) and then dividing by the weight of the corresponding fish gave the μg $CH_3{}^{203}Hg$/g (wet) of fish. This procedure eliminated back calculations to correct for isotopic decay and allowed for the data to be expressed in terms of micrograms of $CH_3{}^{203}Hg$ accumulated by each fish.

RESULTS

A two-way analysis of variance was conducted on the data presented in Table 2 (observations in the > 0-0.5 and 2.0-2.5 weight classes were excluded). At the 5 percent signficance level, the analysis of variance test indicates significant differences in methylmercury burden due to temperature, but no significant difference due to weight and weight-temperature interactions. On the assumption that weight might influence methylmercury uptake, it is appropriate to demonstrate that, although individual weight varied, the distribution of differently sized fish within a single test group was similar to that of the other groups. Thus, fish were segregated into half-gram weight classes and sample size, mean weight, and mean mercury concentration within and between temperature groups compared. A comparison of number and mean weight of minnows comprising each weight class illustrates that size was distributed regularly throughout the three test groups. Mean weights were equal or nearly so for every size class of the three test groups.

Data by weight groups were combined for each test temperature, and these data are illustrated in Figure 1. Mean methylmercury concentration was 4.56 μg/g at 18 C, 5.97 μg/g at 21 C, and 5.72 μg/g at 24 C. Duncan's Multiple Comparison Test indicates that concentration means at 21 and 24 C are not significantly different ($P = 0.05$), but each differed significantly from the mean methylmercury concentration obtained at 18 C.

DISCUSSION

The results of this experiment indicate that environmental temperature altered the amount of methylmercury accumulated directly from water by bluntnose minnows. Accumulation of the mercury compound by the test fish increased initially with a rise in water temperature, as evidenced by the difference in mean body burden of methylmercury in the 18 and 21 C groups. However, accumulation did not continue to rise with increasing temperature, as indicated by the lack of significant difference between mean methylmercury concentrations at 21 and 24 C.

Axiomatically, one might suppose that an increase in water temperature should have resulted in an increased accumulation of the mercury compound elicited by higher metabolic rates in the

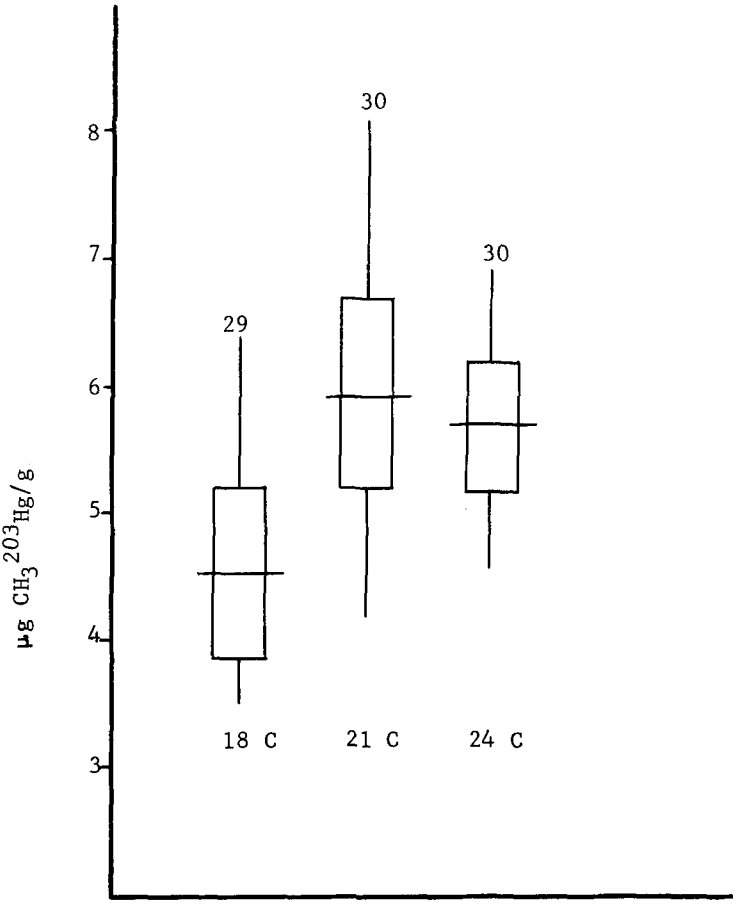

Figure 1. Uptake of methylmercury by bluntnose minnows, Pimephales notatus. Bar includes mean ± one standard deviation; vertical lines represent ranges. Numbers above figures indicate sample size.

test fish, providing that uptake and metabolic rate were linearly related. Considering the amount of methylmercury sorbed by the fish during the short exposure period (48 hr), it was judged that gill surfaces served as the major entry route for the methylmercury. OLSON, et al. (1973), reported that uptake of methylmercury-203 by rainbow trout appeared to be by way of the gills. Further, I assumed that contact of the contaminant with gill membranes was related directly to the routine metabolic rate of the minnows, i.e., the rapidity with which water is pumped over gill lamellae is related directly to metabolic rate. Given that a Δt of 10 C ideally would double the metabolic rate of the fish tested, would accumulation of the contaminant increase correspondingly? The temperature differential between the 18 and 21 C groups, 3C, is 30% of a 10 C increment, and the difference between the mean methylmercury burdens at these temperatures is 1.41 µg, a 31% increment over that observed at 18 C. A direct interrelationship among temperature, metabolic rate, and uptake may have existed between 18 and 21 C but not between 21 and 24 C.

One of the following three hypotheses may provide a tentative explanation for the lack of significant difference in accumulation between the 21 and 24 C groups: (1) the body burden of methylmercury found at 21 C could represent the upper limit of concentration of the contaminant in the fish tissue, i.e., equilibrium was achieved between uptake and excretion, (2) methylmercury had a depressant effect on metabolic rate above 21 C, and (3) routine activity, and therefore routine metabolic rate of the test fish, peaked at 21 C or slightly above. None of these possibilities is definitive at this time, but there are indications as to the plausibility of each.

First, for each temperature group the magnification factor between methylmercury concentration in the water at the start of the experiment versus that concentration measured in the fish following exposure averaged approximately 500X. Although experiments by others have demonstrated much higher magnification factors for fishes (HANNERZ, 1968; JOHNELS, et al., 1967), mercury concentrations found in fishes from nature suggest that concentrations of up to 4-6 µg Hg/g fish (which occurred in the bluntnose minnows used in this experiment) are exceptionally high (LAMBOU, 1972; LOFROTH, 1969).

Second, assuming that contact of methylmercury with gill membranes was related directly to the routine metabolic rate of these fish, than any depressant influence upon metabolic rates wrought by exposure to methylmercury as water temperatures were increased would account for the trend produced. MACLEOD and PESSAH (1973) reported that mercuric chloride depressed the active metabolic rate of rainbow trout fingerlings and higher temperatures augmented the depressant effect. Maximum depressant effect was calculated to be 10 percent and occurred at 20 C with a concentration of 100 µg $HgCl/\ell$. Whether or not methylmercury in my experiments had a similar depressant effect is indefinite.

Last, any compensatory response by the fish to an elevated water temperature would affect their metabolic rates and hence their accumulation of methylmercury. Since the fish used in this experiment were not immobilized, it seems likely that if compensation occurred, it was due largely to the behavioral aspects of

metabolic compensation. Data reported by FRY (1964) showed how spontaneous activity at different temperatures influenced the oxygen consumption of undisturbed fish; for goldfish and carp maximum oxygen consumption occurred at approximately 21 and 24 C, respectively. If random activity of the bluntnose minnows reached a peak somewhere in the range from 21 to 24 C, then this would account for the initial increase followed by a leveling off in accumulation of methylmercury.

Since under the conditions of this experiment small temperature differences altered the total body burden of methylmercury in the bluntnose minnows, it therefore is appropriate that water temperature be carefully considered when mercury concentration data obtained from fishes collected in the field are evaluated. Also, anyone contemplating *in situ* studies using fishes as biological monitors for detection of mercury pollution must recognize the interactions of metabolic rates, temperature and sorption. Additionally, if future research reveals a general trend of temperature influence on uptake of methylmercury and other pollutants by fishes, then thermal additions to aquatic systems from electric generating stations will need to be evaluated in terms of their potential influence upon biotic uptake of contaminants already present in the water system.

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