FOOD AS THE DOMINANT PATHWAY OF METHYLMERCURY UPTAKE BY FISH*

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Abstract. A field experiment was conducted to determine the degree to which fish accumulated methylmercury (MeHg) via their food or via passive uptake from water through the gills. Finescale dace (*Phoxinus neogaeus*) were held in 2000 L enclosed pens floating in an undisturbed, oligotrophic lake in northwestern Ontario. Fish were exposed to water containing either low (0.10–0.40 ng L^{-1}), intermediate (0.45–1.30 ng L^{-1}), or high (0.80–2.1 ng L^{-1}) concentrations of MeHg. Zooplankton with either low (0.16–0.18 μg g^{-1} d.w.) or high (0.28–0.76 μg g^{-1} d.w.) concentrations of MeHg were added daily to each pen. Fish fed zooplankton with high concentrations of MeHg had significantly higher concentrations of mercury in muscle after 32 days than fish fed zooplankton with low concentrations of MeHg (ANCOVA, $P<0.0001$). Fish feeding on zooplankton with low concentrations of MeHg had the same amount of Hg in their tissues as fish at the start of the experiment. Uptake from water was at most 15%. This is the first experiment to confirm that food is the dominant pathway of MeHg bioaccumulation in fish at natural levels of MeHg.

Key words: contaminant pathways, Experimental Lakes Area, methylmercury, Reservoir Project, reservoirs

1. Introduction

Threats to human health resulting from the consumption of fish containing high levels of methylmercury (MeHg) justify detailed studies of MeHg in natural aquatic ecosystems. Biomagnification of MeHg in aquatic food chains resulting in elevated concentrations of MeHg in fish tissue has been well documented (Bodaly *et al.*, 1993; Cabana *et al.*, 1994; Kidd *et al.*, 1995; Spry and Wiener, 1991). Fish with elevated MeHg concentrations in their tissues have been found in lakes with point sources of mercury (Hg) (Johnels *et al.*, 1979), in remote lakes with only natural, watershed-derived and atmospheric inputs of Hg (Bodaly *et al.*, 1993), in acid lakes (Winfrey and Rudd, 1990) and in hydroelectric reservoirs (Bodaly *et al.*, 1984).

Over 90% of the total Hg (THg) in fish tissue is MeHg (Spry and Wiener, 1991). MeHg can be obtained by fish from food and from water as it passes over the gills during respiration. As well, MeHg can be produced within the fish's gastrointestinal tract (Rudd *et al.*, 1980) and on the external slime layer (Jensen and Jernelöv, 1969), but the amount of MeHg contributed to tissue concentrations by these processes has not been quantified and is assumed to be insignificant. Although studies have shown

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that the accumulation of MeHg from food and water may both be important, most researchers have assumed, without conclusive research, that food is the dominant pathway of MeHg uptake in fish (Jernelöv and Lann, 1971; Phillips and Buhler, 1978; Rodgers and Beamish, 1981). Hg models predict that uptake via the gills is relatively small compared to that from food, based on aspects of fish physiology and environmental factors (Harris and Snodgrass, 1993; Rodgers, 1994).

This experimental field study was initiated to determine the relative importance of food and water to MeHg uptake in fish at natural concentrations of MeHg. This is the first study to examine accumulation of MeHg at natural field levels, and presents strong evidence that food is the dominant pathway of MeHg uptake by fish.

2. Methods

2.1. Study design and clean techniques

The experimental design was a 2×2 factorial using food (zooplankton) and water with high and low concentrations of MeHg. Fish were held in 2000 L enclosed pens floating in Lake 240 (L240) at the Experimental Lakes Area in northwestern Ontario in the summer of 1993. The pens were constructed by fitting 2000 L impermeable nylon bags onto PVC frames equipped with floats and covered by window screening. Lake 240 was chosen because of its low MeHg water concentrations (average [MeHg] = 0.09 ng L^{-1} from May to October 1993; J. W. M. Rudd, unpublished data) to avoid contamination. Duplicate pens were randomly assigned to one of four water/food combinations (Figure 1).

Precautions were taken to prevent contamination of natural low levels of MeHg used in the experiment. A small amount of MeHg leached from the nylon pen material after soaking in lake water, so the 2000 L bags were acid washed and rinsed in low MeHg L240 water prior to assembling the pens. Pumps and hoses used in water transfer were acid washed and tested as sources of contamination of MeHg. "Clean-hands dirty-hands" sampling protocols, as outlined in St. Louis *et al.* (1994), were followed for sampling water. Zooplankton were collected using tow nets (400 μm mesh) and bottles rinsed in low MeHg water prior to each use. All equipment was stored in plastic bags in a designated clean shed. Samples of both zooplankton and water were taken regularly for MeHg analysis to confirm that contamination did not take place over the course of the experiment.

2.2. Water

Water from natural sources, consisting of either high or low MeHg concentrations, was used to fill the pens. Pens holding low MeHg water were filled directly from L240 using battery operated pumps and a 400 μm filter. High MeHg water (average [MeHg] = 0.5 ng L^{-1}, June and July 1993) was taken from nearby Lake 470 (L470),

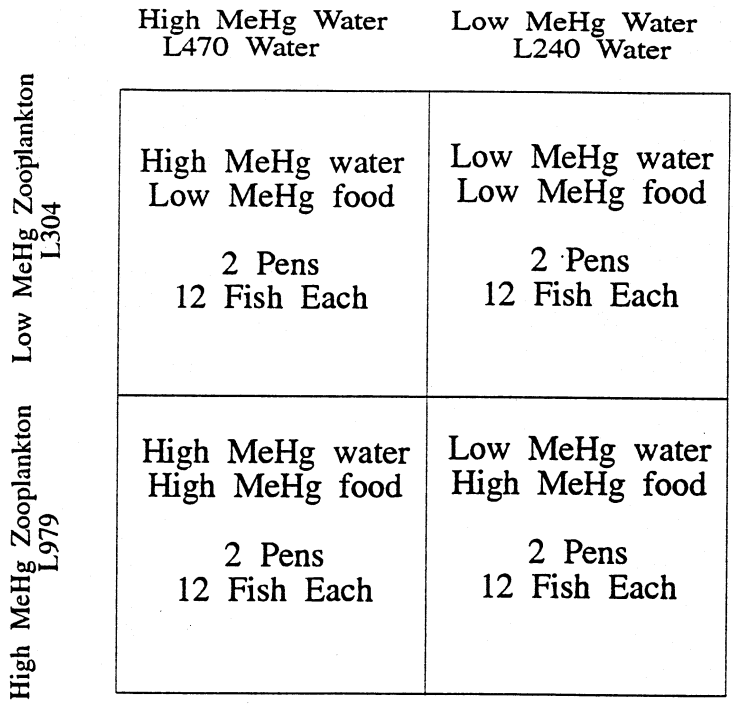

Figure 1. Design of the uptake experiment (MeHg = methylmercury).

a lake surrounded by wetlands, transferred to the pens using an acid washed PVC holding tank and added to the pens as above. Twenty-percent of the water in each of the pens was changed three times a week. Water samples were not filtered prior to analysis; however, large particles were removed. Whole water was used for MeHg analysis because MeHg associated with particulates and/or dissolved organic carbon (DOC) exists in equilibrium with, and is therefore readily exchangeable with, water (Watras *et al.*, 1994). Thus, DOC can be considered to be a reservoir of MeHg that is in flux with the surrounding physical environment and the biota (e.g. Driscoll *et al.*, 1994). In addition, analysis of whole water ensured adherence to a natural situation. Samples were analyzed by Flett Research Ltd., Winnipeg, MB, using Bloom's (1989) procedure as modified in Horvat *et al.* (1993; detection limits = 0.01–0.02 ng L^{-1} at a blank level of 0.05–0.1 ng L^{-1}). Flett Research Ltd. successfully participated in the recent interlab comparison for MeHg analysis (Bloom *et al.*, 1995).

An increase in water MeHg concentrations with the addition of high MeHg zooplankton resulted in the fish being exposed to water containing either low (0.10–0.40 ng L^{-1}), intermediate (0.45–1.30 ng L^{-1}), or high (0.80–2.10 ng L^{-1}) concentrations of MeHg (Figures 2, 3). The unexpected elevated MeHg concentrations in the water resulted from either leaching of MeHg during decomposition

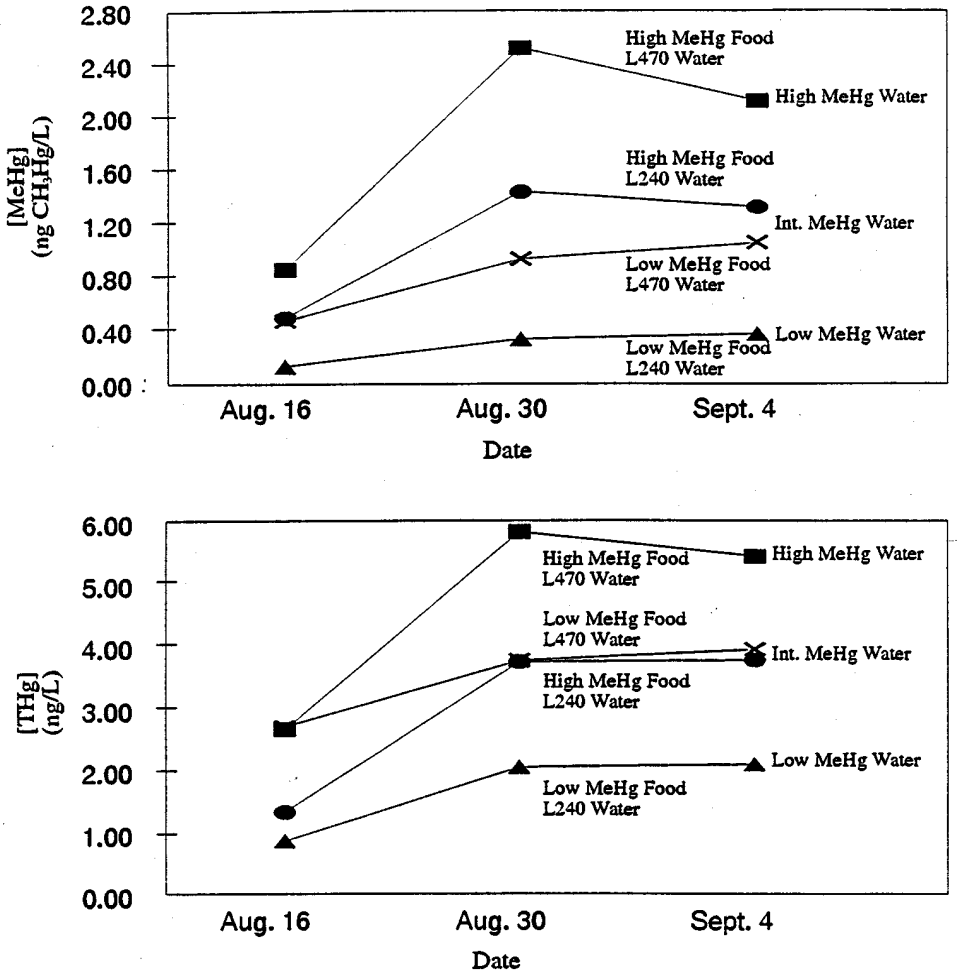

Figure 2. Concentrations of methylmercury (MeHg) and total mercury (THg) in the water held in the experimental pens. A sample was taken near the beginning, middle and end of the 32-day experiment (L = Lake; Int. = Intermediate).

of dead zooplankton or equilibration of levels of MeHg in living zooplankton with the water.

Weekly water chemistry samples were taken from each pen and analyzed for DOC (OI Corporation model 700 Carbon Analyzer with calibration to glucose standard), pH (*in situ* measurements with an Orion Ross Sureflow pH electrode) and calcium (Ca^{+2}) concentrations (Stainton *et al.*, 1977). Average error between replicate pens was 2.3% (range: 0–11%). Temperature and oxygen (YSI oxygen probe) in the pens were monitored regularly and were similar to the levels in L240. Mid-day temperatures ranged from 20.6–22.3 °C. Water held in experimental pens remained at or near oxygen saturation.

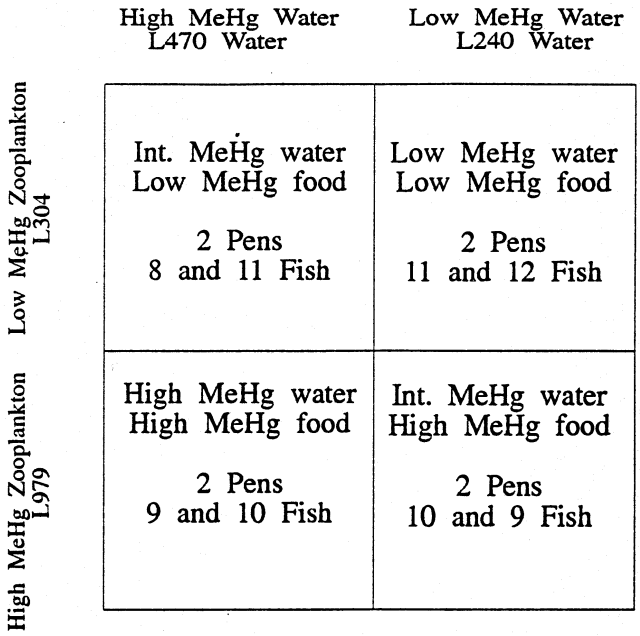

Figure 3. Revised experimental design after zooplankton addition and consequent increase in water methylmercury (MeHg) concentrations (L = Lake; Int. = Intermediate).

2.3. FOOD

Zooplankton was analyzed in the Experimental Lakes Area Reservoir Project (ELARP) Mercury Laboratory at the Freshwater Institute by atomic absorption spectrophotometry (AAS) after organic partitioning into hexane and methylene chloride (Malley *et al.*, in press). This method measures all organic forms of Hg with a method detection limit of 10 ng Hg g^{-1}. We assumed that the organic Hg concentrations measured were all MeHg, although small amounts of dimethylmercury (DMHg) may have been present (Horvat *et al.*, 1993). Zooplankton with either high (0.28–0.76 μg g^{-1} d.w.) or low (0.16–0.18 μg g^{-1} d.w.) concentrations of MeHg were collected and added to each pen daily. Zooplankton with low concentrations of MeHg were collected from Lake 304 (L304), a small fishless lake. Lake 979 (L979), an experimentally flooded wetland pond, was the source of the high MeHg zooplankton. Zooplankton community structure differed in the two lakes so, to ensure fish were receiving similar amounts of sustenance, dry/wet weight relationships were determined weekly and used to calculate the quantity of live zooplankton added to each pen on a dry weight basis. On a given day, all pens received the same dry weight of zooplankton. Amount per day varied from 0.025–0.125 g d.w. per fish. Neither the growth of the fish (Table I) nor mortality (Figures 1, 3) were related to the source of zooplankton fed to fish.

Table I

Mercury (Hg) concentrations and weights of experimental fish (±SEM). Roman numerals in column three are explained in the text. (MeHg = methylmercury, Int. = intermediate)

Treatment	Hg Conc. (μg g^{-1} wet wt.)		Percent difference from time zero	Average initial weight (g)	Average final weight (g)
	per pen	mean			
Time Zero	0.117±0.009				5.08±0.112
Low MeHg food,	0.112±0.011	(I) 0.123	4.6	4.77	4.08±0.187
Low MeHg water[1]	0.133±0.013		12.0	4.42	4.05±0.234
Low MeHg food,	0.135±0.015	(II) 0.136	13.3	5.08	4.34±0.244
Int. MeHg water[2]	0.136±0.006		14.0	4.96	3.83±0.192
High MeHg food,	0.212±0.007	(III) 0.221	44.8	4.60	4.60±0.111
Int. MeHg water[3]	0.229±0.015		48.9	4.98	4.53±0.637
High MeHg food,	0.236±0.019	(IV) 0.240	50.4	4.73	4.06±0.122
High MeHg water[4]	0.243±0.023		51.9	4.76	4.05±0.246

Note: Results from ANOVAs testing differences between pens: [1] p = 0.544, [2] p = 0.932, [3] p = 0.288, [4] p = 0.827.

2.4. FISH

Finescale dace (*Phoxinus neogaeus*; Cyprinidae) were obtained from a commercial bait fisherman and transported to L240 in oxygen-saturated water in plastic bags. After acclimatization to pen temperature for 1/2 h, 12 fish were added to each pen. THg was determined on one fillet from each fish randomly selected from the initial stock at the start of the experiment and on all penned fish surviving at the end of the 32-day experiment. Cold vapour AAS was used (method detection limit of 10–25 ng Hg g^{-1}; Hendzel and Jamieson, 1976; Armstrong and Uthe, 1971). Reference material (National Research Council of Canada (NRCC) dogfish muscle, DORM-1) was analyzed coincidentally with experimental fish samples. Average concentration of DORM-1 material was 768.5 ±10% ng g^{-1} d.w., which was within the certified range of 724–862 ng g^{-1} d.w. To determine significance of differences of THg concentrations in fish from different treatments, an ANCOVA was performed on log-transformed fish THg concentrations. High and low MeHg zooplankton were the main effects and MeHg concentrations in water was the covariate. The two different food sources were used as a main effect in a one-way ANOVA to test the significance between any differences in fish weight.

3. Results and Discussion

Fish fed zooplankton with high concentrations of MeHg had significantly higher

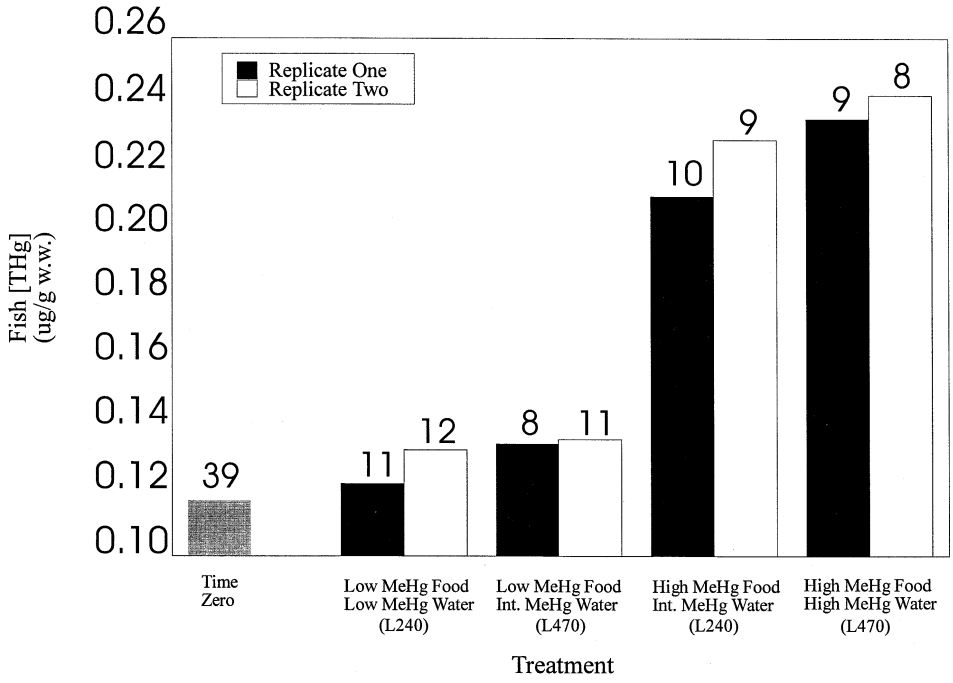

Figure 4. Total mercury (THg) in fish tissue at the beginning (Time Zero) and end of the experiment. Number of fish analyzed is shown above bars (Int. = Intermediate).

concentrations of Hg in muscle than fish fed zooplankton with low concentrations of MeHg (ANCOVA, $p<0.0001$; Figure 4). The Hg concentrations of fish that fed on zooplankton with low concentrations of MeHg were not significantly different from those in fish at the start of the experiment. The significant increase in fish Hg concentrations in those fish eating high MeHg zooplankton indicates that food was the dominant pathway of MeHg uptake by fish. One-way ANOVAs revealed that differences between average Hg concentrations of fish from duplicate pens were not significant (Table I).

The fish either maintained their weight or lost between 0.3 and 1.1 g over the course of the experiment (Table I). However, weight loss was not dependent on the type of food fed to the fish (one-way ANOVA, $p = 0.982$). The relatively small weight loss indicates significant feeding. Using parameters from the Wisconsin Bioenergetics model (Hewett and Johnson, 1992) and the average ambient temperature of the water in the pens, the theoretical amount of food required for fish to experience the moderate weight loss over the course of the experiment was determined. The model predicts that fish consumed 10.3 g of food over 30 days. This ration is equivalent to 0.34 g wet weight per day or $\sim$7% of a 5 g fish's body weight. During the experiment, a mean of 0.78 g w.w. (range: 0.25–1.25 g w.w., using a 10% wet weight/dry weight conversion factor) of zooplankton was fed to

the fish each day, which is similar to the theoretical consumption, indicating that the fish consumed most of the food added to the pens. Therefore, fish were eating enough food to assimilate Hg into body tissues.

The data also indicate a small but measurable uptake of MeHg from the water. Changes in fish MeHg concentrations attributable to uptake of MeHg from the water were equal to the difference in the mean concentrations of THg in fish tissue between fish fed the same food but held in water with different MeHg concentrations (Table I, Col. 3: II-I = 0.013 and IV-III = 0.019 μg g^{-1} THg). The resulting concentrations (0.013 and 0.019 μg g^{-1}) were relatively small compared to those attributable to uptake of MeHg from food. The latter were calculated by comparing the mean THg concentrations of fish fed high MeHg food to those fed low MeHg food and held in water with comparable MeHg concentrations (Table I, Col. 3: III-I = 0.098, III-II = 0.085, and IV-II = 0.104 μg g^{-1} THg). Thus, direct absorption from the water may have been responsible for $\sim$15% of the Hg uptake in fish muscle.

If elevated MeHg concentrations in water were a result of loss of MeHg from zooplankton into water, then MeHg concentrations in zooplankton may have decreased before consumption by fish. Thus, fish fed high MeHg zooplankton may have been exposed to less MeHg via food than was measured by analyzing zooplankton, and the estimated 85% uptake from diet would be conservative.

Although uptake of MeHg from water was small and relatively insignificant as compared to uptake from food, chemical differences between the two sources of water may have had an effect on the uptake of MeHg from water. This could have happened either by affecting gill permeability (Rogers and Beamish, 1983) or by changing the amount of bioavailable (dissolved) MeHg (Watras *et al.*, 1994). With respect to gill permeability, fish from waters with elevated Ca^{+2} concentrations (Rodgers and Beamish, 1983) or high pH (Winfrey and Rudd, 1990) tend to have lower tissue MeHg concentrations than fish from waters with low Ca^{+2} concentrations and low pH. Ca^{+2} concentrations and pH in this study were examined to determine if differing chemical characteristics of water had any effect on uptake from water. If the chemical composition of high MeHg water was preventing MeHg uptake from water, then high MeHg water should have higher Ca^{+2} concentrations and higher pH than low MeHg water. However, high MeHg water had lower Ca^{+2} concentrations and lower pH than low MeHg water (Figure 5). Therefore, Ca^{+2} concentration and pH characteristics of water with high concentrations of MeHg are opposite to what would be expected if the chemical characteristics of the water source was confounding the results of the experiment.

A similar trend was observed for chloride (Cl^{-}) ions, which may enhance uptake of MeHg from water by forming membrane permeable MeHg complexes (MeHgCl; Boudou *et al.*, 1983). Average Cl^{-} concentrations in low MeHg water and high MeHg water in 1993 were 0.327 and 0.134 mg L^{-1} (M.P. Stainton, unpublished data), respectively, opposite to the expected effect if uptake from water via MeHgCl complexes were observed.

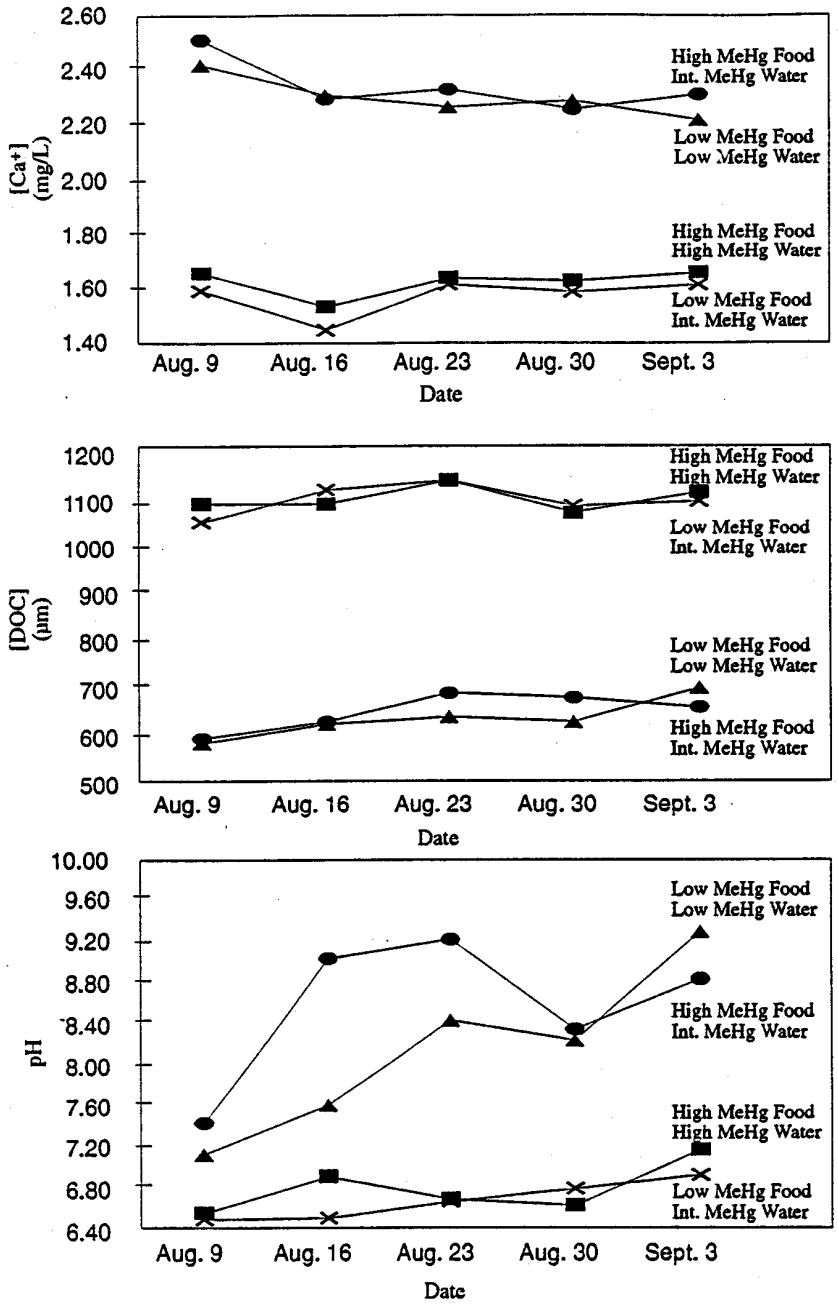

Figure 5. Average results of weekly water chemistry analysis from each pen (MeHg = methylmercury; Lake 470 = ■ and X; Lake 240 = ● and ▲; (Int. = Intermediate).

DOC may also have affected MeHg uptake from water. Humic and fulvic acids bind MeHg to varying degrees depending on their concentrations and the pH of the water (Hintlemann *et al.*, 1995). This binding decreases the free MeHg concentration and its uptake from water. The DOC concentrations in our experiment (650–1100 μM, Figure 5) were similar to the humic acid concentrations (830 μM) used in experiments of Hintlemann *et al.* (1995). Assuming that the relationship between pH and dissolved MeHg was the same in the ELA water as in Fawn Lake (Hintlemann *et al.*, 1995), we estimated that in our experiment about 20% of the MeHg was free at pH 8.5 and about 25% at pH 6.5 (Figure 5). This small difference in percent free MeHg among our treatments would not have affected our interpretation of MeHg uptake from water. Although examination of the chemical characteristics of the water is important in evaluating the effects of water chemistry on MeHg uptake from water, the results of this study indicate that water is contributing, at most, 15% of the Hg to fish. Thus, water chemistry is not an important determinant of MeHg bioaccumulation by fish.

This investigation supports conclusions obtained from past studies (Jernelöv and Lann, 1971; Rodgers and Beamish, 1981; Phillips and Buhler, 1978), which were laboratory based and done at unnaturally high MeHg concentrations. Also, these studies were done prior to the development of clean-sampling procedures and ultra-sensitive analytical techniques for measurement of low concentrations of MeHg. Another group of studies with designs similar to the experiment described here (Rodgers and Beamish, 1981; Phillips and Buhler, 1978) showed that most MeHg taken up by fish was from the diet, and that water contributed $\sim$10% of the MeHg assimilated by fish. A factorial field experiment done by Parks *et al.* (1987) using crayfish in field situations (Hg-contaminated and -uncontaminated rivers) also showed the importance of food to MeHg uptake.

The results of this experiment also agree with predictions made from bioenergetic mercury models. For example, Rodgers (1994) did three simulations using yellow perch (*Perca flavescens*) and lake trout (*Salvelinus namaycush*) and reported that diet was responsible for a large proportion of MeHg uptake. Harris and Snodgrass (1993) predicted that food pathways were responsible for 90% of MeHg uptake in walleye (*Stizostedion vitreum*) and yellow perch. Both of these models used MeHg concentrations at the ng L^{-1} level in water, which approximates concentrations in natural waters.

4. Conclusions

This experiment indicates that food is the dominant pathway of MeHg uptake by planktivorous fish at natural concentrations of MeHg. At most, 15% of MeHg was taken up from the water, given the chemical characteristics and MeHg concentrations prevailing in the experiment. These results confirm theoretical modelling

studies. They also indicate the need for increasing emphasis on food-chain factors affecting the transfer of MeHg to fish.

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