Methylmercury bioaccumulation in zooplankton: an assessment of exposure routes and accumulation in newly flooded reservoirs

by

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

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Abstract

This thesis examines methylmercury (MeHg) bioaccumulation in zooplankton. The specific objectives are: 1) assess the influence of flooding upland forested regions on MeHg accumulation in zooplankton, and 2) to test experimentally the relative importance of food versus water as routes of MeHg uptake in zooplankton.

Bulk zooplankton samples were collected for MeHg analysis in three flooded upland forested regions for three flooding seasons at the Experimental Lakes Area (ELA) in northwestern Ontario. The three reservoirs differed in soil and vegetative carbon storage, and were classified as the High Carbon, Medium Carbon and Low Carbon reservoirs. We hypothesized that concentrations of MeHg in zooplankton would be proportional to rates of mercury methylation that, in turn, would be proportional to carbon availability. Zooplankton exhibited large increases in MeHg concentrations immediately after flooding in all three years, and concentrations were inversely related to carbon storage in the first two years of flooding. MeHg concentrations in zooplankton steadily declined in the Low and Medium C reservoirs in the second and third year of flooding. In the High C reservoir, however, mean MeHg concentrations remained high, indicating that the duration of elevated MeHg accumulation may be related to C storage in newly flooded reservoirs. MeHg concentrations in zooplankton closely followed MeHg concentrations in water; therefore, zooplankton appear to be good indicators of MeHg concentrations in aquatic systems.

A laboratory experiment was conducted to determine the relative importance of food versus water as routes of MeHg exposure in the cladoceran, *Daphnia pulicaria* (Forbes). *D. pulicaria* were exposed to MeHg in food and water in three treatments. In the first treatment, *Daphnia* were exposed to high concentrations of MeHg in both food and water. In the second treatment, *Daphnia* were exposed to high concentrations of MeHg in food only. The third treatment acted as a control, with low concentrations of MeHg in both food and water. After 48 hours, concentrations of MeHg in *Daphnia* from the "food + water" treatment were 9 - 11 times greater than those in the "food only" and control treatments. No significant differences in uptake of MeHg by *Daphnia* were observed between the "food only" and control treatments, an indication that water was the dominant route of MeHg exposure in *D. pulicaria*.

Zooplankton make up an important part of the aquatic food web, providing an important source of food for planktivorous fish. As a consequence of flooding, increases of MeHg in zooplankton provide an important link for MeHg movement up the food chain into higher organisms, including humans. This thesis clarified some of the questions surrounding MeHg dynamics in zooplankton; however, it is evident that more studies on MeHg dynamics at the base of the food chain are needed.

General Introduction

Background information

In the past few decades, there has been growing concern over the impact of humans on the environmental cycling of mercury (Hg) in aquatic systems. Anthropogenic inputs of Hg have increased well beyond natural inputs and it is estimated that 30 to 60% of atmospheric Hg comes from man-made sources (Nriagu 1989; Benoit *et al.* 1994). Existing in many forms, Hg is a prevalent and important contaminant in all types of aquatic systems. From a biological standpoint, the most important and toxic form of Hg is methylmercury (MeHg). MeHg can biomagnify up the aquatic food web, reaching concentrations in piscivorous fish that often exceed human consumption guidelines (Driscoll *et al.* 1994, 1995; Wren and Stephenson 1991). MeHg exposure from contaminated fish can lead to serious health problems in humans (Rosenberg *et al.* 1995, 1997) and in other animals such as birds (Pollard and Hultman 1997; Wolfe *et al.* 1998). An extensive literature is available for Hg dynamics in fish and it is well known that diet is the most important route of MeHg exposure in fish (Hall *et al.* 1997). Information is limited with respect to factors affecting the uptake and accumulation of MeHg by lower trophic level organisms, specifically zooplankton, which serve as important food items for fish (Paterson *et al.* 1997, 1998; Monson and Brezonik 1999; Pickhardt *et al.* 2002).

Biogeochemistry of Hg

There are many sources of Hg to the aquatic environment. It naturally occurs in soils and rocks that may erode, so Hg is carried into lakes via surface run-off. Large amounts of Hg are also found in the atmosphere (Lindqvist 1994). Seventy to eighty percent of the mercury in the atmosphere comes from human activities such as coal combustion, chlorine alkali plants and waste incineration (Fitzgerald 1995; Lindqvist 1994; Nriagu and Wong 1997). Elemental Hg (Hg^0) constitutes the majority of the total gaseous Hg in the atmosphere. Photochemical reactions in the atmosphere result in the oxidization of Hg^0 into Hg(II) (Iverfeldt and Lindqvist 1986), which is deposited onto lakes or terrestrial environments in dust or precipitation (Hultberg *et al.* 1994; Schroeder 1994).

Once in a lake, abiotic and microbial processes transform Hg(II) into MeHg. In anoxic regions of lake sediments, Hg(II) is currently believed to be microbially methylated into MeHg by sulfate reducing bacteria (Gilmour and Henry 1991). Demethylation of MeHg may occur within lakes in the presence of demethylating bacteria, which may result in the formation of Hg^0 before being volatized to the atmosphere (Fitzgerald and Mason 1997).

Ullrich *et al.* (2001) provide a good review of the many factors affecting Hg methylation in aquatic systems. Some of these factors include temperature (Callister and Winfrey 1986; Wright and Hamilton 1982), amount of sulfate present (Gilmour *et al.* 1992), pH (Winfrey and Rudd 1990), organic carbon (C), and the amount of Hg available for methylation (Callister and Winfrey 1986; Choi and Bartha 1994; Pak and Bartha 1998). All of these factors influence the amount of MeHg being transformed and the

amount of MeHg entering the aquatic system, which ultimately affects the amount of MeHg available for movement up the food chain.

The problem of MeHg in hydroelectric reservoirs

MeHg accumulation is an especially serious problem in hydroelectric reservoirs (Bodaly *et al.* 1997). The dynamics of MeHg production and availability are assumed to be similar in reservoirs and natural lakes, except that MeHg production often occurs at faster rates in reservoirs. These faster rates occur because bacterial activity increases after flooding, presumably because the amount of carbon available for bacteria increases from flooding of vegetation and soils (Morrison and Therein 1994). With an increased amount of decomposing vegetation, anoxic regions develop quickly and allow the methylating bacterial community to thrive, thus increasing MeHg concentrations in reservoirs (Kelly *et al.* 1997; Matilainen and Verta 1995). Fish living in reservoirs often have MeHg concentrations exceeding human-consumption guidelines, therefore creating problems for people using reservoirs for domestics consumption and commercial harvest (Bodaly *et al.* 1997; Rosenberg *et al.* 1997).

Factors affecting MeHg uptake in zooplankton

Zooplankton along with other aquatic invertebrates, are important food items for fish. Because fish get the majority of MeHg from their diet (Hall *et al.* 1997), zooplankton are important in studying MeHg movement up the food web. There is a great degree of variability in MeHg concentrations in zooplankton from manipulated lakes and non-manipulated lakes (Table 1). To understand this variability fully, it is important to

consider the many factors affecting MeHg availability and bioaccumulation in zooplankton. Here, availability is defined as the amount of MeHg available for uptake into zooplankton, whereas bioaccumulation refers to the concentrations of MeHg in zooplankton relative to those in water or in food.

The most important factor affecting MeHg concentrations in zooplankton is the concentration of MeHg in the surrounding environment. If concentrations of MeHg in water increase in a lake or reservoir, concentrations of MeHg will also increase in zooplankton (Paterson *et al.* 1998; Tremblay *et al.* 1998b).

Variations in dissolved organic carbon (DOC) concentrations also affect MeHg bioaccumulation in zooplankton. DOC is the aqueous phase of organic carbon with a particle size of usually <0.45 μm, although the size of the particle is usually user-defined (Kolka *et al.* 1999). DOC can affect availability in different ways. When combined with DOC, MeHg availability for uptake into organisms is usually reduced and bioaccumulation in organisms decreases. DOC may also complex Hg(II) making it less available for methylation (Driscoll *et al.* 1995). Evidence for the complexing effects of DOC was seen in several Wisconsin Lakes where a decrease in zooplankton bioaccumulation of MeHg was observed with increasing DOC concentrations (Back and Watras 1995). Another theory suggests that once MeHg is bound to DOC, it is unable to pass across gill membranes due to the large size and polar nature of DOC (Choi *et al.* 1998).

In contrast, several studies have shown increases in MeHg concentrations in zooplankton with an increase in DOC concentrations (Westcott and Kalff 1996; Plourde *et al.* 1997, Watras *et al.* 1998; Garcia and Carignan 1999). These increases may occur

because DOC also acts as a carrier for terrestrially derived Hg(II) and MeHg into lakes. Mercury that is bound to DOC may enter the aquatic system in the form of surface runoff (Mierle and Ingram 1991; St. Louis *et al.* 1994; Lee and Iverfeldt 1991). Depending on the type of lake and its characteristics, this Hg-organic matter complex may stimulate the in-lake production of MeHg or enhance the retention of Hg(II) and MeHg by lakes (Watras *et al.* 1995; Miskimmin 1991; Wren and Stephenson 1991). Dramatic increases in DOC are observed immediately after flooding (Paterson *et al.* 1998; Chapter 1-this thesis) making DOC a potentially important factor in newly formed reservoirs. Increases in MeHg in zooplankton with greater DOC may also occur because humic matter may serve as a food source for zooplankton (Westcott and Kalff 1996; Tremblay *et al.* 1995; Plourde *et al.* 1997).

High MeHg concentrations in zooplankton often occur with low pH concentrations in lakes and reservoirs (Westcott and Kalff 1996; Watras and Bloom 1992; Watras *et al.* 1998; Garcia and Carignan 1999; Meili 1991). At lower pH, Hg methylation apparently increases (Xun *et al.* 1987; Miskimmin *et al.* 1992) and bioaccumulation may also increase as a result of greater uptake of MeHg (Westcott and Kalff 1996).

Changes in phytoplankton trophic structure and increases in phytoplankton biomass in newly formed reservoirs have been well documented (Paterson *et al.* 1997; Campbell *et al.* 1998; Ostrofsky and Duthie 1980; Ostrofsky 1978). Increases in primary production mainly occur because of a release of nutrients from newly flooded areas (Paterson *et al.* 1998; Grimard and Jones 1982). Increases in phytoplankton biomass and productivity can also result in increases in zooplankton biomass. Increased MeHg

concentrations and increased zooplankton biomass can provide a great deal of MeHg for movement up the food chain and into fish in newly formed reservoirs (Paterson *et al.* 1998; Chapter 1-this thesis).

On the other hand, a recent study showed that a large increase in algal biomass actually reduced the amount of MeHg stored per cell of algae due to "bloom dilution" (Pickhardt *et al.* 2002). *Daphnia spp.* exposed to algal blooms in Pickhardt *et al.* (2002) showed lower MeHg concentrations than in treatments where *Daphnia* were exposed to lower algal biomass. Consequently, blooms of algae may result in less Hg movement into higher trophic levels because less MeHg is transferred to zooplankton. Pickhardt *et al.* (2002) also noted lower concentrations of MeHg in *Daphnia* from treatments where densities of *Daphnia* were allowed to increase, indicating that the dilution of MeHg may also occur at the organism level, resulting in less MeHg available for movement through the food web.

Zooplankton taxa often accumulate differing amounts of MeHg; for example, copepods had lower MeHg concentrations than cladocerans in several studies (Watras *et al.* 1998; Rask *et al.* 1994). Larval *Chaoborus* spp. collected in different lakes and reservoirs also exhibited lower MeHg concentrations than their zooplankton prey (e.g. *Daphnia*) (Paterson *et al.* 1998; Back and Watras 1995). These findings may be due to higher growth rates of *Chaoborus* and less MeHg accumulated per gram of tissue due to growth biodilution (Visman 1995). Growth biodilution occurs when an organism adds new tissue at a faster rate than MeHg uptake, thereby diluting the concentration of MeHg per animal. Growth biodilution in newly formed reservoirs, where high nutrient levels may influence growth rates of particular invertebrate and zooplankton species, could be

an important factor influencing the amount of MeHg available for biomagnification through the food web and into fish.

Thesis Objectives

This thesis is focused on MeHg concentrations in zooplankton through field and laboratory studies. The first chapter focuses on the problem of MeHg accumulation in zooplankton from newly impounded areas and used data from three experimental reservoirs at the Experimental Lakes Area in northwestern Ontario, Canada. The second chapter investigates the relative significance of water or food as the major route of MeHg uptake in zooplankton and used of the cladoceran, *Daphnia pulicaria* (Forbes), in a controlled experiment in the laboratory.

Table 1. Reported ranges for methylmercury concentrations in zooplankton collected from natural and manipulated lakes e.g. reservoirs. Values in parentheses indicate mean values when reported in the source paper.

Lake Type	Type of Sample	Site Description	MeHg concentrations ($ng\ g^{-1}$ dw)	Reference
Natural Lakes	**Mixed Zooplankton**			
		Swedish forest lakes	34 - 400	Meili 1991
		Duncan and Detcheverry lakes	50 – 200	Plourde *et al.* 1997
		15 Wisconsin lakes	6 – 161 (53)	Watras *et al.* 1998
		12 Wisconsin lakes	1 - 479	Back and Watras 1995
		5 N. Quebec lakes	18 – 82	Tremblay *et al.* 1998*a*
		38 Canadian lakes 20 reference lakes	35 – 377 (112)	Garcia and Carignan 1999
		73 Canadian lakes (Ontario and Quebec)	25.5 – 377 (108)	Tremblay *et al.* 1995
		80 N. Minnesota lakes	10 - 210	Sorensen *et al.* 1990
	Cladocerans	24 S. Ontario lakes	19 - 448	Westcott and Kalff 1996
		15 Wisconsin lakes	(73)	Watras *et al.* 1998
		Finnish forest lake	20 - 250	Rask *et al.* 1994

Table 1. continued...

Lake Type	Type of Sample	Site Description	MeHg concentrations (ng g^{-1} dw)	Reference
	Copepods	15 Wisconsin Lakes	(45)	Watras *et al.* 1998
		Finnish forest lake	20 - 250	Rask *et al.* 1994
		Little Rock Lake - reference basin	12 – 28	Watras and Bloom 1992
		Reference lake	11 - 54	Paterson *et al. 1998*
Manipulated Lakes				
Acidified Lake	**Mixed Zooplankton**	Little Rock Lake – acidified basin	52 – 56	Watras and Bloom 1992
Reservoirs		2 Quebec reservoirs	100 – 800	Plourde *et al.* 1997
		Peatland reservoir	79 – 615	Paterson *et al.* 1998
		La Grande reservoir littoral zones pelagic zones	 282 – 430 25 – 452	Tremblay *et al.* 1998*a*
Logged catchments		9 Canadian lakes	135	Garcia and Carignan 1999
Burned catchments		9 Canadian lakes	97	Garcia and Carignan 1999

Chapter 1 – Methylmercury bioaccumulation in zooplankton from newly flooded upland reservoirs; The Flooded Uplands Dynamics Experiment (FLUDEX)

Abstract

As part of the Flooded Uplands Dynamics Experiment (FLUDEX), concentrations of methylmercury (MeHg) in zooplankton were measured in 1999-2001 from three experimental reservoirs that flooded boreal forest uplands. The three reservoirs differed in soil and vegetative carbon storage, and were classified as the High Carbon, Medium Carbon and Low Carbon reservoirs. We hypothesized that concentrations of MeHg in zooplankton would be proportional to rates of mercury methylation that, in turn, would be proportional to carbon availability. MeHg concentrations in zooplankton increased in all reservoirs relative to reference zooplankton immediately after flooding in all three years. On average, zooplankton in the Low C reservoir had the highest concentrations in 1999 and 2000 followed by the Medium C site and the High C reservoir, indicating that in the first two years of flooding, MeHg accumulation in zooplankton was inversely related to carbon storage. After the third year of flooding, however, a relationship between carbon storage and MeHg concentrations in zooplankton became direct. Over the 3-year period of study, concentrations of MeHg in zooplankton declined in the Low C and Medium C reservoirs at a faster rate than in the High C reservoir. The rate of decline among years was greatest in the Low C reservoir, suggesting that the duration of elevated concentrations of MeHg in new reservoirs may be correlated with C storage in soils and vegetation. MeHg concentrations in zooplankton were strongly correlated with those in unfiltered water ($r = 0.82$) and bioaccumulation factors did not change after flooding despite large changes in zooplankton biomass and species composition. Concentrations of MeHg were always low in zooplankton from the lake that was the source of water for the reservoirs, indicating that flooding had a direct effect on MeHg concentrations in zooplankton.

Introduction

Humans have dramatically influenced the amount of methylmercury (MeHg) entering the food chain by the creation of hydroelectric reservoirs (Bodaly *et al.* 1997). Increases in MeHg concentrations occur typically in fish (Bodaly *et al.* 1984), zooplankton (Plourde *et al.* 1997, Paterson *et al.*, 1998) and other invertebrates (Hall *et al.* 1998; Tremblay *et al*, 1998b, 1996a, 1996b). These increases are the result of

the decomposition of flooded organic matter that stimulates the activity of bacteria that convert inorganic Hg to MeHg. MeHg is the most toxic form of Hg and it predominates in fish and other higher biota. Over 90% of the total-Hg in fish is MeHg (Bloom 1992). In northern Canadian reservoirs, total Hg concentrations in fish often exceed the Canadian limit of 0.5 $\mu g\ g^{-1}$ for consumption. High concentrations of MeHg in fish from reservoirs have resulted in the closure of affected commercial and subsistence fisheries. Social and health impacts are especially prevalent in aboriginal communities that heavily use fish from northern Canadian reservoirs for harvest and consumption (Rosenberg *et al.* 1995, 1997; Bodaly *et al.* 1997). Elevated concentrations of MeHg typically persist in new reservoirs for 20-30 years (Bodaly *et al.* 1997).

Fish obtain MeHg almost solely from their diet (Hall *et al.* 1998), which usually consists of zooplankton, benthic organisms and other fish. Because zooplankton are important dietary components for many fish, it is important to understand how changes in zooplankton community structure and water chemistry affect MeHg accumulation in zooplankton in newly flooded reservoirs. In addition to increased MeHg production following flooding, bacterial colonization and decomposition of submerged vegetation results in large releases of nutrients and other decomposition by-products (Morrison and Therein, 1991; Paterson *et al.* 1997). This increased nutrient availability has been shown to stimulate primary production (Ostrofsky and Duthie 1980; Grimard and Jones 1982) and change zooplankton community structure in newly formed reservoirs (Paterson *et al.* 1997; Campbell *et al.* 1998; Ostrofsky 1978).

Because Hg methylation is a bacterial process linked to carbon (C) decomposition, it is reasonable to expect that the amount of stored carbon in flooded areas will have a positive relationship with MeHg production in newly formed reservoirs (St. Louis *et al.* 1994; 1996). Some support for this assumption has been provided by recent studies at the Experimental Lakes Area (ELA) in northwestern Ontario. First, St Louis *et al.* (1994; 1996) found greater MeHg concentrations in outflows from catchments with wetlands (with high C storage) vs. uplands (with lower C storage). Second, the Experimental Lakes Area Reservoir Project (ELARP) followed changes in the production of greenhouse gases (GHGs) and MeHg following impoundment of a small lake (Lake 979) surrounded by a wetland (Kelly *et al.* 1997). Immediately following impoundment, there were large increases in both MeHg production and carbon decomposition. The latter process produced large increases in GHG fluxes (Kelly *et al.* 1997). The strong correlation between GHG and MeHg production suggests that the two processes were intimately linked.

If C decomposition and MeHg production are tightly linked, a flooded peatland might represent a "worst-case scenario" for MeHg production and accumulation because of the high amounts of stored carbon available for bacterial methylation (St. Louis *et al.* 1994; 1996). Therefore, careful selection of impoundment areas with lower amounts of stored carbon may result in less severe increases of MeHg. To test this idea, a whole ecosystem experiment, named the **FL**ooded **U**plands **D**ynamics **EX**periment (FLUDEX), was initiated at ELA to examine MeHg cycling and accumulation in newly created upland forest catchments. In this study, 3 small experimental reservoirs were created that flooded catchments

that differed in moisture regimes and amounts of above- and below-ground stored carbon.

The present study examined changes in MeHg accumulation in zooplankton collected from the newly formed upland reservoirs as a part of the FLUDEX project. To assess the influence of flooding on MeHg accumulation in zooplankton, the reservoirs were sampled for 3 years following initial flooding. The following questions were addressed: 1) Are MeHg concentrations in zooplankton related to differences in above- and below-ground carbon storage in the newly flooded reservoirs? 2) Are MeHg concentrations in zooplankton related to changes in MeHg concentrations in water? 3) Are changes in zooplankton MeHg bioaccumulation affected by changes in community structure or water chemistry?

Methods

Site descriptions

Three upland forested catchments at the ELA in northwestern Ontario, Canada (49°38'N, 93°43'W) were chosen as study sites. The three sites had similar areas and volumes, but differed in moisture regimes, vegetation communities, and above- and below-ground carbon storage (Table 1). The Moist Forest, High Carbon Site (High C) had the greatest amount of total carbon. It was made up of essentially two communities, one dominated by a *Pinus banksiana* (Lamb) (jackpine) forest with an understory community of *Sphagnum* spp. and *Ledum groenlandicum* (Oeder) (Labrador tea). The other community was largely *P. banksiana* with an understory of *Polytrichum* spp. The Dry Forest, Medium Carbon (Medium C) site had intermediate

amounts of total stored carbon. It was dominated by a relatively homogeneous stand of *P. banksiana*. The Very Dry forest, Low carbon (Low C) site had thin soil and patches of exposed bedrock and the lowest amounts of carbon in vegetation and soil. Two vegetation communities dominated the Low C site, a treed community of mostly *P. banksiana* and a lichen community.

The reservoir walls were constructed of wood and earthen dikes lined with plastic to reduce leakage. Irrigation pipes were placed around the top edge of the reservoir walls to supply water to the reservoirs (Fig. 1). The three reservoirs were initially flooded in early July 1999 and again in early June of 2000 and 2001 by continuously pumping water through the irrigation pipes from nearby oligotrophic Roddy Lake. The average depths of all three flooded reservoirs were 1.2 m with maximum depths greater than two meters. Water renewal times were approximately 6-8 days for all three reservoirs. Drawdown of the reservoirs occurred in late September to preserve the reservoir walls and to simulate winter water demands due to seasonal energy use in real hydroelectric reservoirs. Because the reservoirs were completely drawn down in winter, they most realistically simulate the near-shore drawdown regions of larger reservoirs.

Invertebrate communities including zooplankton were allowed to establish themselves in the reservoirs following flooding in all three years. In every year of flooding, finescale dace (*Phoxinus neogaeus* (Cope)) were added to the reservoirs in densities between 3500 and 4100 fish/hectare.

Sample Collection

Water chemistry and hydrology

Water flows into the reservoirs were continuously monitored with inline flow meters and outflows were measured with calibrated V-notch weirs (K. Beaty and M. Lyng 1999 – Freshwater Institute, Winnipeg, MB unpublished data). Water temperature profiles were measured at 0.2 m intervals using a temperature probe every few days throughout the flooding season. Water temperatures were also measured at 0.2 m intervals with Onset Tidbit data loggers at 5-minute intervals for the entire flooding season. Dissolved oxygen profiles were collected weekly at 9-12 sites within each reservoir with a YSI-Model 58 oxygen probe and meter. Samples for water chemistry analysis were taken at 1-2 week intervals at the outflow weir and at 3 floating rafts placed in each reservoir. Samples were routinely analyzed by the ELA chemistry laboratory for dissolved and particulate nutrients, pH, and chlorophyll *a* (Chl *a*) according to Stainton *et al.* (1977). Only chemistry data for samples collected at the outflows are used here because they were the most complete data set. Chemistry data collected at the rafts within each reservoir (including depths 0-1.0m) were always highly correlated with values in the outflow ($r = 0.552 – 0.934$; $p < 0.05$ for all chemistry variables used).

Water samples for MeHg analysis were collected from inflow pipes and outflow weirs once every week for the first month of flooding and then bi-weekly for the rest of the flooding season. Samples were collected using the "clean hands, dirty hands" technique to minimize contamination (St. Louis *et al.* 1994).

Zooplankton

Zooplankton collection methods closely followed those used in the ELARP study (Paterson *et al.* 1997; 1998). Separate samples were collected for the determination of MeHg concentrations and zooplankton biomass. Samples for zooplankton MeHg analysis were collected weekly in the first month of flooding and bi-weekly in the months following. Samples were taken at several sites within each reservoir using horizontal sweeps of a 150-μm tow net mounted on a wooden pole until enough biomass was collected for MeHg analysis. Comparison of samples collected at different sites indicated that MeHg concentrations did not vary substantially within reservoirs. Variability in MeHg concentrations among samples within reservoirs was similar to variability among samples collected at a single site (M. J. Paterson, Freshwater Institute, Winnipeg, MB – unpublished data).

Care was taken to avoid submersed vegetation and substrate in the reservoirs. Samples were immediately placed in Whirl-pak® bags and frozen until MeHg analysis. A small subsample was taken and preserved in a 3% formalin-sugar mixture (Prepas 1978) to assess the species composition of the Hg sample. Zooplankton for MeHg analysis were also collected from Roddy Lake as a reference and as an estimate of MeHg inputs to the reservoir in zooplankton.

Samples for the determination of zooplankton biomass ($\mu g\ L^{-1}$) and community structure were obtained with a 7-cm diameter wire-enforced tube sampler (Pennak 1962; Paterson *et al.* 1997). Samples were collected at 9-10 stations per reservoir (Figs. 2a-c). Zooplankton were sieved through a 53-μm net, narcotized with 95% methanol (Gannon & Gannon 1975) and preserved in a 3% formalin-sugar

mixture. Samples for zooplankton biomass and species composition were also collected at the inflow of the Low C site by pouring 50 L through a 53-μm net, to analyze the species composition entering the reservoirs from Roddy Lake. Zooplankton were counted and identified to species by an experienced zooplankton taxonomist. Lengths of zooplankton in MeHg samples were measured using an automated sizing system (ZEBRA2; Allen *et al.* 1994) and were converted to biomass using length-weight regressions in Downing and Rigler (1984) and Malley *et al.* (1989). Rotifer biomass was estimated by assigning average weights for each taxon as determined by Malley et al (1989).

Methylmercury Determinations

Zooplankton samples were freeze-dried and then weighed into conical Teflon tubes. A minimum dry mass of 2 mg of zooplankton was needed for MeHg analysis. MeHg concentrations were analyzed using gas chromatography and cold vapour atomic fluorescence spectrophotometry (GC-CVAFS) after aqueous phase ethylation at Flett Research Ltd. (Winnipeg, Manitoba) (Watras and Bloom 1992). With the exception of 3 dates in Aug-Sept. 2000, visual scans of the samples confirmed that the total biomass was composed primarily (>90%) of zooplankton. MeHg in zooplankton was determined from duplicate analyses on 43 out of 100 sampling days over the three years of flooding. Two samples were determined from four replicates; one was from a sampling site in the Low C site in 1999; the other was from Roddy Lake in 2000. Except for one date, standard errors were always less than 15% for replicated samples. The mean coefficient of variation for all replicated samples was

18%, which is similar to CVs from other studies (Paterson *et al.* 1998; Westcott and Kalff 1996). Concentrations of MeHg in zooplankton are expressed as ng g^{-1} dry weight (dw).

Statistical Procedures

To examine relationships between MeHg in zooplankton and environmental factors, we used residuals from a model II regression (Ricker 1973) relating MeHg concentrations in zooplankton and MeHg concentrations in unfiltered water. We used a model II regression because the independent and dependent variables were measured with comparable error. The level of significance for statistical tests was $p<0.05$ and the Bonferroni method was used to correct for multiple comparisons. All variables were logarithmically transformed as necessary based on results of residual analyses. All statistical analyses were performed using SYSTAT 8.0.

The relationship between MeHg in zooplankton and water can also be expressed using the bioaccumulation factor (BAF). The BAF is a relative measure of the transfer of MeHg from the physical-chemical environment (unfiltered water) to zooplankton and is defined as:

$$BAF = \frac{\text{MeHg in zooplankton (ng } g^{-1} \text{ dw)}}{\text{MeHg in unfiltered water (ng } mL^{-1})}$$

Results

Physical-chemical parameters

Temperature differences were minimal among the reservoirs within the three-year study period. Temperature differences from bottom to surface were always within 2°C. In all reservoirs, oxygen concentrations at 0-1m and >1m depths were lower in the first year of flooding compared to those in 2000 and 2001 (Table 2). The Low C site consistently had the highest average dissolved oxygen concentrations in all three years. In 1999 and 2000, the Medium C site had the lowest deep-water oxygen levels, with most concentrations less than 2.0 p.p.m. At one time in the Medium C site, 0-1 m depth O_2 concentrations reached a low of 0.5 p.p.m. At all times in the Low and High C reservoirs, the 0-1 m depth zone had O_2 concentrations in excess of 2 p.p.m, which is sufficient for zooplankton survival (Pennak 1953).

In 1999, all reservoirs had greater nutrient concentrations than oligotrophic inflow water (Figs. 3 A - C). Dissolved organic carbon (DOC) and total phosphorus (TP) data suggested that decomposition and leaching of material from flooded vegetation and soil were greatest in the Medium C site for the first two years of flooding, followed by the High C site and the Low C reservoir (Figs. 3 A and C). In the last year of flooding, the High C site had the highest DOC and TP concentrations followed by the Medium and Low C sites, although differences were very small. Decomposition and leaching of materials was less severe in all reservoirs in the third year of flooding than in the previous two years. Total phosphorus concentrations in 2001 were nearly equal to those seen in 2000, but were much lower than concentrations seen in 1999. Chl*a* and suspended carbon data indicated that flooding

stimulated primary production and increased the presence of water column particulates (either living or dead) (Figs. 3 B and D).

In all years, MeHg concentrations in unfiltered water rapidly increased after the initiation of flooding (Fig. 4.; B. D. Hall, University of Alberta – unpublished data). For example, MeHg concentrations in water increased from the inflow value of 0.05 ng L^{-1} to 1.0 ng L^{-1}, 1.1 ng L^{-1}, and 0.70 ng L^{-1} in the High, Medium and Low C reservoirs, respectively. In September, concentrations typically decreased with declines in water temperatures and methylation rates.

The Medium C site consistently had the highest average unfiltered MeHg concentrations over the three years of flooding, whereas the High C site had intermediate values. The Low C site had the lowest average MeHg concentrations for the three years. Concentrations of MeHg in unfiltered water were highest in the first year of flooding in all reservoirs and decreased by 2001. Average concentrations were similar in the High C site among all three years of flooding (0.56, 0.56 and 0.50 ng L^{-1} in 1999, 2000 and 2001 respectively). In contrast, values in the Medium C and Low C site decreased from the first year of flooding to the third year of flooding.

Zooplankton biomass and species composition

In 1999, zooplankton biomass was over 100x greater in the Medium and Low C sites than in the inflow (Fig. 5). This increase was due to high densities of *Daphnia* spp. (primarily *D. rosea*) (Sars) (Fig. 6a). Zooplankton biomass in the High C site was also elevated compared to the inflow, but lower than in the other two reservoirs. Only at the end of the season did *Daphnia* increase in the High C site.

In 2000, zooplankton biomass was lower in all three reservoirs than in 1999 (Fig.5). Seasonal differences among the three reservoirs were minimal, with biomass increasing in all reservoirs at the beginning of flooding and decreasing in July to values similar to those seen in inflows. In June-July, *Daphnia* spp. dominated in the Medium C site, but then declined (Fig. 6A). Subsequently, rotifers dominated in this reservoir (Fig. 6D). Similar decreases of cladoceran biomass were observed in the Low C reservoir. *Bosmina* spp. dominated the High C site throughout 2000 (Fig. 6B).

In 2001, average zooplankton biomass in the inflow and all three reservoirs was higher than in 2000; however, biomasses never reached those seen in 1999 (Fig. 5). *Daphnia* spp. were, again uncommon in the High C site in 2001 and other Cladocera such as *Bosmina* spp. dominated. In the Medium and Low C sites, the patterns of species composition resembled those in 2000 (Fig. 6B-C). *Daphnia* appeared early in the year and then declined in proportion to other Cladocera and Cyclopoid copepods.

MeHg concentrations in zooplankton

Impoundment invariably resulted in large increases in MeHg concentrations in zooplankton (Fig. 7). In all three years of flooding, concentrations of MeHg in zooplankton from all the reservoirs were much higher than those in Roddy Lake (Fig. 8). Mean MeHg concentrations from zooplankton from the flooded wetland (L979) during the first 3 years of impoundment were also within the range of MeHg concentrations seen in zooplankton from the three upland reservoirs (Fig. 8). Mean concentrations in the reservoirs were also much higher than concentrations observed

in zooplankton collected from natural lakes (Watras *et al.* 1998; Tremblay *et al.* 1998b; Sorensen *et al.* 1990; Westcott and Kalff 1996), and from other lakes at ELA (M. J. Paterson, unpublished data).

In 1999, average concentrations of MeHg in zooplankton were inversely correlated with patterns of C storage among reservoirs. Zooplankton in the Low C site had the highest average MeHg concentrations (469 ng g^{-1} dw), followed by the Medium C (374 ng g^{-1} dw) and High C reservoir (282 ng g^{-1} dw) (Fig. 8). Zooplankton MeHg was not related to carbon storage in 2000 although differences between the three reservoirs were minimal (Low C: 371 ng g^{-1} dw, Medium C: 359 ng g^{-1} dw; High C: 307 ng g^{-1} dw). Visual scans of the samples collected between August-September 2000 (Aug. 15th, Sept. 12th) in the High and Medium C reservoirs and Aug. 28th and Sept. 12th in the Low C site indicated that they contained a large amount of non-zooplanktonic material. Thus, these dates may not reflect true concentrations in zooplankton at that time. Removal of these points from the data set did not substantially change any of the results.

In 2001, there was a positive relationship between C storage and MeHg concentrations in zooplankton. Concentrations of MeHg in zooplankton from the Low C site decreased substantially from previous years, with average concentrations as low as 170 ng g^{-1} dw (Fig. 8). The maximum MeHg concentration in zooplankton from this reservoir was 213 ng g^{-1} dw, down from maximum values of 634 ng g^{-1} dw in 2000 and 924 ng g^{-1} dw in 1999 (Fig. 7). Average concentrations in zooplankton from the Medium C site also decreased from 359 ng g^{-1} dw in 2000 to 314 ng g^{-1} dw in 2001. In contrast, average zooplankton concentrations in the High C site from 2001

were similar to those seen in 2000 and both years had higher average MeHg concentrations than in the first year of flooding, indicating that concentrations in zooplankton may be decreasing at a slower rate than in the other two reservoirs.

Mass of MeHg in Zooplankton

The amount of MeHg stored in zooplankton L^{-1} of water is a function of both zooplankton biomass and MeHg concentrations. Changes in zooplankton storage provide an indication of how much MeHg is available for movement through the food web and of MeHg partitioning in the water column of newly formed reservoirs. In 1999, zooplankton from the Medium and Low C reservoirs stored similar amounts of MeHg and levels were 2 – 2.5 orders of magnitude greater than those in the inflow (Fig. 9). The amount of MeHg stored in zooplankton from the High C site was less than in the other two reservoirs because zooplankton densities were comparatively low in 1999. In the Medium C and Low C reservoirs, the amount of MeHg stored in zooplankton was also much lower in 2000 and 2001 than in 1999.

In terms of the percentage of unfiltered water concentrations that MeHg in zooplankton constituted, there were differences from year to year depending on zooplankton biomass in each reservoir. In 1999, MeHg storage in zooplankton from the Low and Medium C reservoirs was often equal to or greater than (between 0.95 – 1.91x) that in water. In the High C site, values were between 2-6%, which is typical of most lakes and reservoirs (M. J. Paterson - unpublished data). In 2000 and 2001, zooplankton constituted less than 10% of water MeHg concentrations in all reservoirs.

Bioaccumulation of MeHg in zooplankton

MeHg concentrations in zooplankton were significantly correlated with MeHg concentrations in unfiltered water when considering all reservoirs over the three years of flooding ($r = 0.82$; $p<0.0001$) (Fig. 10). Pearson correlation factors for MeHg in water and MeHg in zooplankton were also similarly significant for individual reservoirs across the three years of flooding (High C: $r = +0.67$; Medium C: $r = +0.71$; Low C: $r = +0.73$; $p<0.05$).

Log mean BAFs changed little over the three years of flooding (Fig. 11). Log values ranged from 5.53 to 6.29 in 1999, with average log BAFs highest in the inflow and lowest in the Medium C reservoir. Patterns were similar in 2000, where values for all three reservoirs and the inflow ranged from 5.29 to 6.55. In 2001, the inflow had the lowest average BAF, whereas the three reservoirs had similar log BAFs ranging from 5.40 to 6.22.

The potential effects of various chemical and biotic variables on bioaccumulation were investigated by examining correlations with residuals from the Model II regression between MeHg in water and zooplankton (Fig. 10). No significant relationships were found between residuals from the zooplankton – water regression and zooplankton biomass, % copepods, % cladocerans and % *Daphnia*. Similarly, residuals were not significantly correlated with water chemistry variables (Suspended C, DOC, TP and Chl *a*).

Discussion

Effects of flooding on MeHg concentrations in zooplankton

MeHg concentrations in zooplankton greatly increased as a result of flooding upland areas of the boreal forest. In the first 2 years of impoundment, there was no support for the hypothesis that MeHg concentrations in zooplankton were positively related to differences in C storage among reservoirs. On average, zooplankton in the Low C reservoir had the highest concentrations in 1999 and 2000 followed by the Medium C site and the High C reservoir indicating a negative relationship between C storage and MeHg concentrations in zooplankton. Similar increases of MeHg concentrations in zooplankton in response to flooding have been seen in other studies (Bodaly *et al.* 1997; Plourde *et al.* 1997; Tremblay *et al.* 1998b; Paterson *et al.* 1998). In particular, concentrations of MeHg in zooplankton from the three upland reservoirs were similar to changes observed in Lake 979, where average zooplankton MeHg concentrations of 231, 331 and 256 ng g^{-1} dw were seen in the first, second and third years of flooding (Fig. 8) (Paterson *et al.* 1998).

Although a positive relationship did not exist between zooplankton MeHg and carbon storage in the first two years of flooding, there was a positive relationship in 2001. Over the 3-year period of study, average concentrations of MeHg in zooplankton declined in the Low C and Medium C reservoirs at a faster rate than in the High C reservoir. The rate of decline among years was greatest in the Low C reservoir (Fig. 8), which may suggest that the duration of elevated concentrations of MeHg in new reservoirs is correlated with C storage in soils and vegetation. Further data collections in 2002-2003 from the FLUDEX reservoirs will help to confirm or

disprove this suggestion. For now, the hypothesis is strengthened by the addition of zooplankton data from L979, a reservoir with very high C storage (Fig. 8). Concentrations of MeHg in zooplankton increased after the first year of flooding and have remained high over the last nine years of impoundment indicating that the high amounts of C storage in this reservoir is still stimulating MeHg production (Paterson *et al.* 1998; M. J. Paterson – unpublished data).

Mass of MeHg in Zooplankton

In 1999, large increases in zooplankton biomass and MeHg concentrations resulted in large increases in MeHg stored in zooplankton in the Medium and Low C reservoirs. This result suggests that MeHg availability for zooplankton predators, such as fish and invertebrates, increased substantially after impoundment. In 1999, storage of MeHg in zooplankton equalled or exceeded concentrations in water, indicating that it may be occasionally important to consider this pool in mass balance budgets.

Bioaccumulation of MeHg in zooplankton

MeHg concentrations in unfiltered water increased in response to flooding, but patterns of increase did not conform to the hypothesis that MeHg concentrations follow patterns of C storage (Fig. 4). Conditions in the flooded uplands, specifically, low dissolved oxygen concentrations and high amounts of flooded vegetation were conducive to high rates of decomposition and MeHg production in all of the reservoirs.

Significant relationships were found between MeHg in unfiltered water and MeHg in zooplankton for all three reservoirs. In L979, a strong relationship was also observed between concentrations of MeHg in unfiltered water and MeHg in zooplankton after the first three years of flooding ($r^2 = .86$, $p < 0.0001$; Paterson *et al.* 1998). Although significant relationships were found between these two variables, these results are not necessarily an indication that zooplankton receive the majority of their MeHg exposure through water alone. Unfiltered water also contains suspended materials used as a food source by zooplankton. Plourde *et al.* (1997) suggested that MeHg-contaminated suspended particulate matter (SPM) that has been re-suspended by wave action in shallow areas of reservoirs may be a potential source of food for zooplankton. Plourde *et al.* (1997) and Paterson *et al.* (1998) found that MeHg concentrations in both SPM and zooplankton were highly correlated, although it was not demonstrated that SPM was in fact a food source for zooplankton.

One objective of my study was to identify variables contributing to MeHg accumulation by zooplankton following flooding. Bioaccumulation refers to the process whereby organisms obtain MeHg from their environment from all applicable exposure routes. Bioaccumulation of MeHg in zooplankton is often correlated with several chemistry variables including pH, TP and DOC and temperature (Watras *et al.* 1998; Westcott and Kalff, 1996; Watras and Bloom 1992; Sorensen *et al.* 1990). Equally important, variations in MeHg uptake may also occur among differing zooplankton species (Back and Watras 1995; Rask *et al.* 1994; Watras *et al.* 1998). In Watras *et al.* (1998), the strength of correlations increased between MeHg in zooplankton and pH, DOC concentrations and dissolved MeHg with increasing

trophic position of several zooplankton taxa in several Wisconsin Lakes. In my study, no significant relationships were found between residuals from water – zooplankton regressions and any of the measured chemical variables in the reservoirs. However, the nature of the data is such that colinearity among variables may have obscured the results of the correlations tested. In addition, ranges within all variables were limited due to the similarity of the three reservoirs. Nevertheless, within this field study, water chemistry variables did seem particularly important in directly regulating the uptake of MeHg in zooplankton. Indirectly however, these variables may affect the supply or bioavailability of MeHg to primary producers, which serve as a food source to zooplankton.

My study did not directly focus on species-specific differences within the zooplankton community. My analysis simply showed a lack of statistically significant relationships between percent composition and total biomass with residuals from zooplankton – water regressions. Lack of relationships occurred despite large changes in zooplankton species composition and zooplankton biomass in the reservoirs after flooding. Similarly, there were no relationships were found between MeHg concentrations in zooplankton and changes in species composition and biomass in L979 (Paterson *et al.* 1998). Plourde *et al.* (1997) also found that MeHg concentrations in cladocerans (*Daphnia* spp. and *Bosmina* spp.) and copepods (*Senecella* spp.) were also not significantly different from one another. However, species-specific differences in other studies have been documented in lakes with smaller ranges of MeHg concentrations than in the reservoirs (Rask *et al.* 1994; Watras *et al.* 1998). For example, differences in MeHg accumulation by copepods

and cladocerans were found in a small lake in Finland in (Rask *et al.* 1994) and in copepods and cladocerans in several Wisconsin Lakes (Watras *et al.* 1998).

Conclusions

My study was unique with regard to scale and our ability to examine MeHg production and accumulation in all aspects of the aquatic environment immediately after flooding in three different upland regions. Zooplankton are considered an integral part of the lower aquatic food chain and serve as important food items for fish and other invertebrates. It is therefore important to understand factors that affect MeHg uptake by zooplankton as they provide a link for MeHg movement in contaminated systems. In the three FLUDEX reservoirs, zooplankton were quick to accumulate MeHg in all reservoirs soon after flooding; however, concentrations in the first two years were not positively correlated with patterns of C storage. Concentrations declined in the Low and Medium C reservoirs in 2000 and 2001. In the High C reservoir, mean MeHg concentrations remained high which may indicate a positive relationship between MeHg accumulation and C storage in newly flooded reservoirs, although additional years of flooding data will help to strengthen this hypothesis. MeHg concentrations in zooplankton closely followed MeHg concentrations in water; therefore, zooplankton appear to be good indicators of MeHg concentrations in aquatic systems.

Table 1. Carbon stores (kg C ha^{-1}) in the FLUDEX reservoirs prior to flooding.

	High C Site	Medium C Site	Low C site
Areas of Reservoirs (ha)	0.76	0.64	0.68
Dominant vegetation (per cent coverage)	*Pinus/Ledum/Sphagnum* (53%) *Pinus/Polytrichum* (47%)	*Pinus/Betula* (100%)	*Pinus/Vaccinium* (73%) *Polytrichum/Cladina* (22%) *Organic pillows (5%)*
Carbon in trees	26 240	27 600	19 560
Carbon in shrubs, herbs, and mosses[1]	1 400	130	200
Carbon in litter and fungal/humic layer[2]	15 400	5 700	8 700
Carbon mineral layer[2]	2 900	1 500	2 400
Total soil carbon (including litter)[2]	18 300	7 200	11 100
Total carbon in above ground vegetation[1,2]	27 640	27 730	19 760
Total carbon (kg C ha^{-1})	**45 890**	**34 930**	**30 860**

[1]D. Huebert, unpublished data
[2]from N. Boudreau and J. Venkiteswaran, University of Waterloo - unpublished data.
[3]determined from litterfall measurements.

Table 2. Means and ranges for dissolved oxygen concentrations (ppm) in the FLUDEX reservoirs at depths 0-1m, and >1m for 1999-2001 (unpublished data A. Majewski and R.A. Bodaly).

	1999		2000		2001	
Depths	**0-1 m**	**> 1m**	**0-1 m**	**> 1m**	**0-1 m**	**> 1m**
High Carbon						
Mean	3.5	1.8	4.5	2.9	5.4	2.8
Range	2.1 – 5.8	0.0 – 4.4	2.8 – 6.7	0.4 – 5.5	3.3 – 8.1	0.6 – 5.4
Medium Carbon						
Mean	2.7	1.2	5.6	2.6	5.9	3.1
Range	0.5 – 5.3	0.3 – 3.7	3.4 – 7.6	0.3 – 6.0	4.8 – 7.5	0.4 – 5.9
Low Carbon						
Mean	4.5	2.6	5.7	4.8	6.2	5.3
Range	2.7 – 6.85	0.3 – 6.5	3.8 – 7.8	3.3 – 7.4	4.7 – 7.7	3.9 – 6.7

Figure 1. View of the dikes surrounding the Low Carbon reservoir.

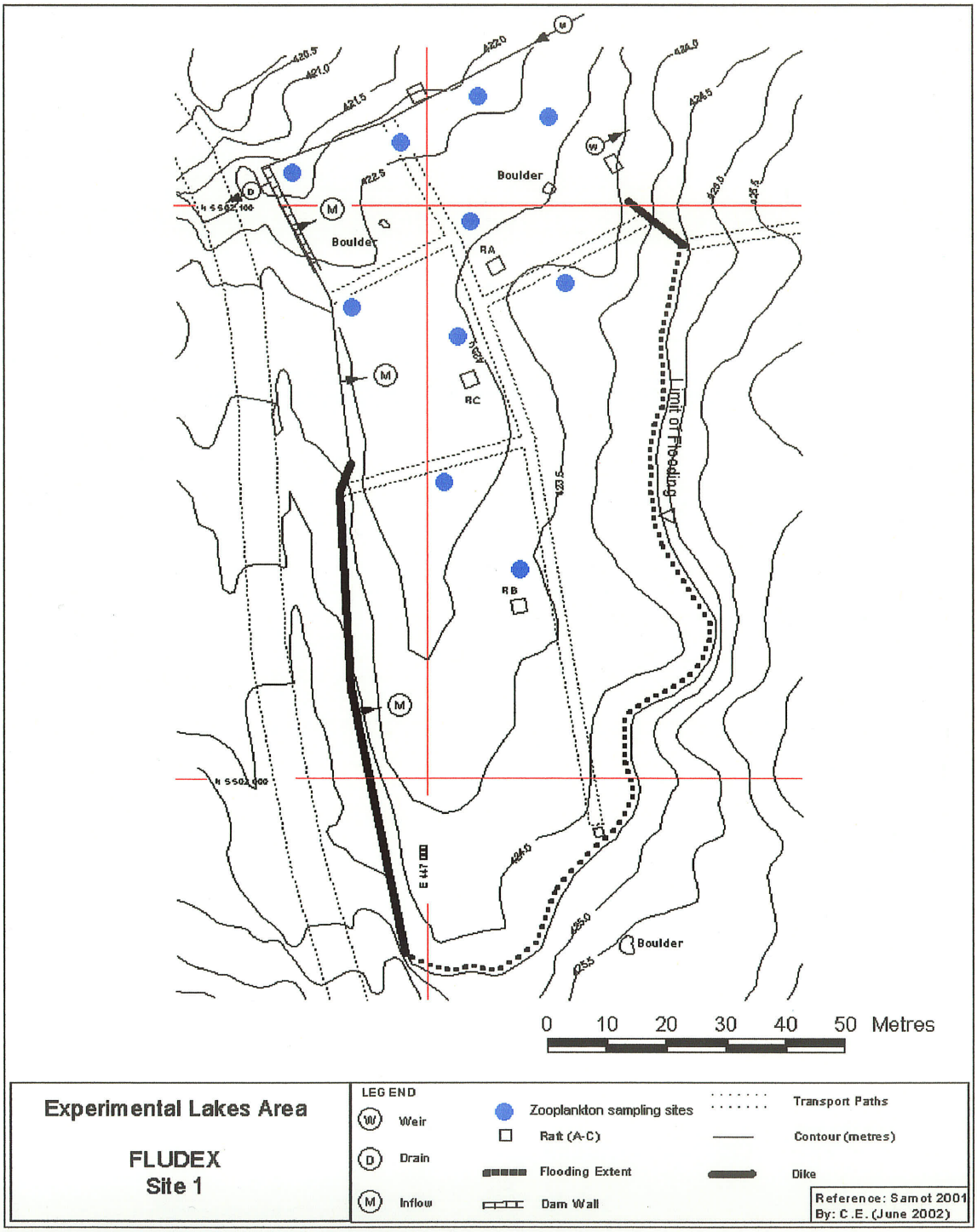

Figure 2a. Zooplankton sampling sites in the High C reservoir.

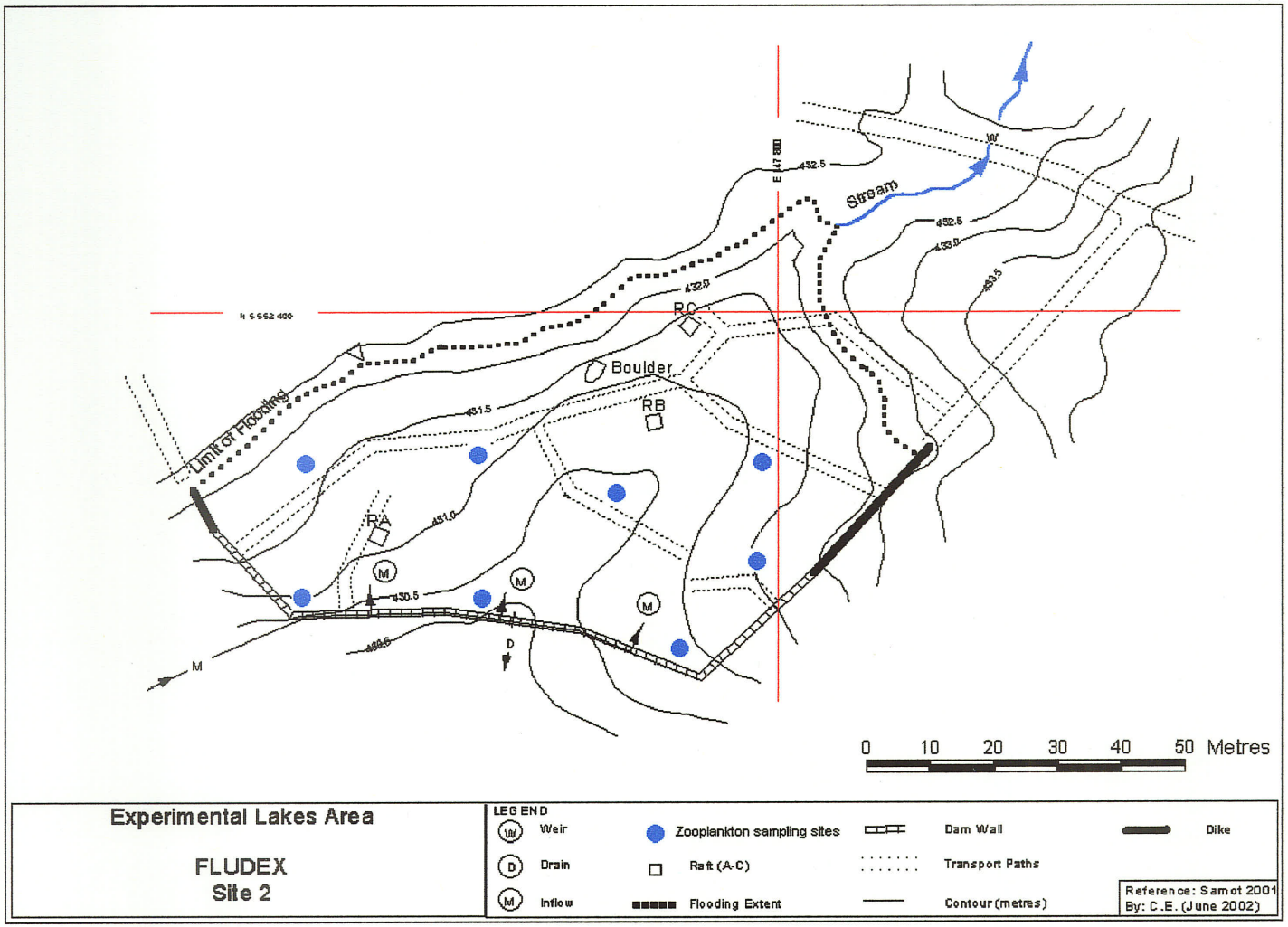

Figure 2b. Zooplankton sampling sites in the Medium C reservoir.

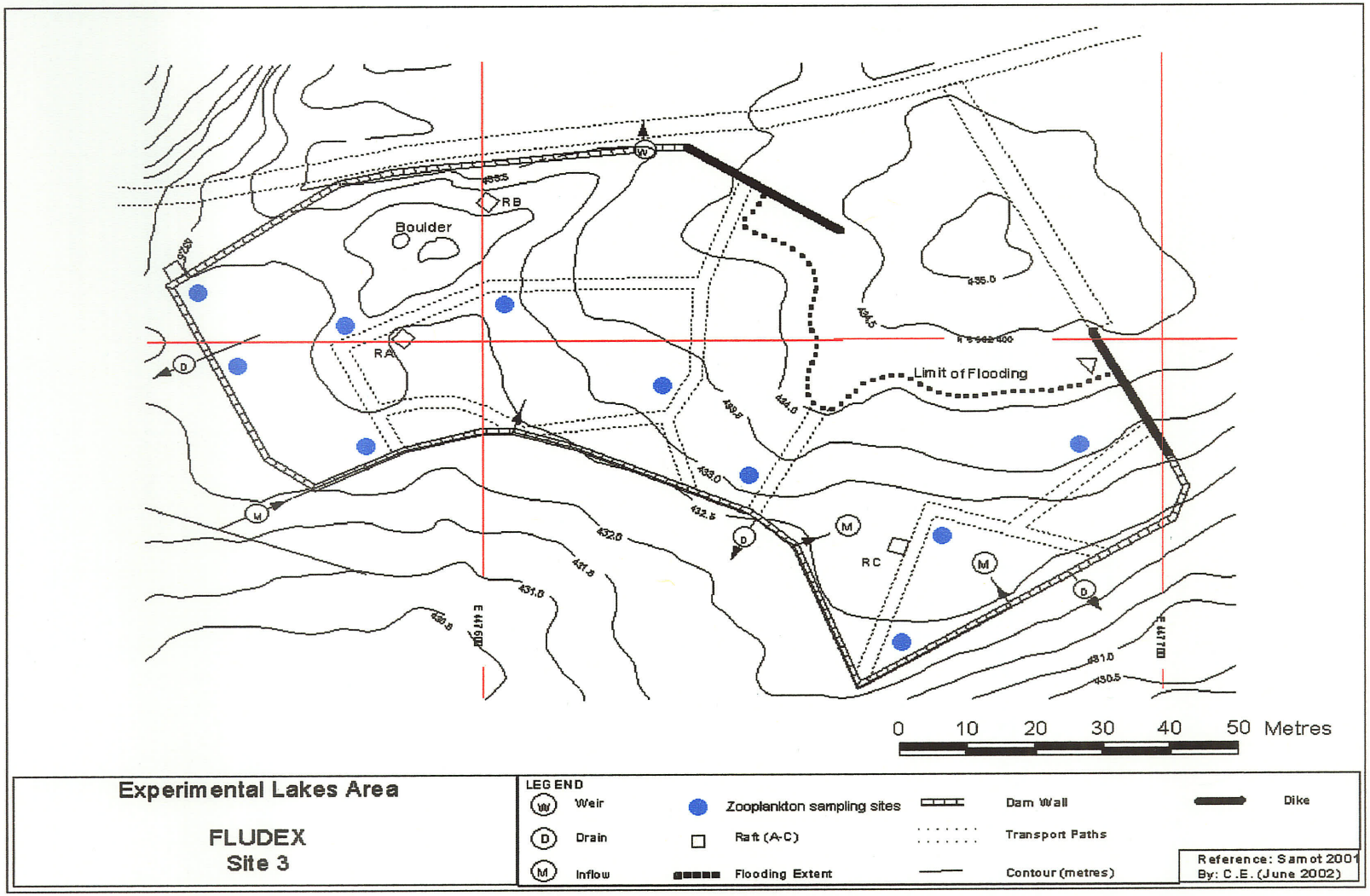

Figure 2c. Zooplankton sampling sites in the Low C reservoir.

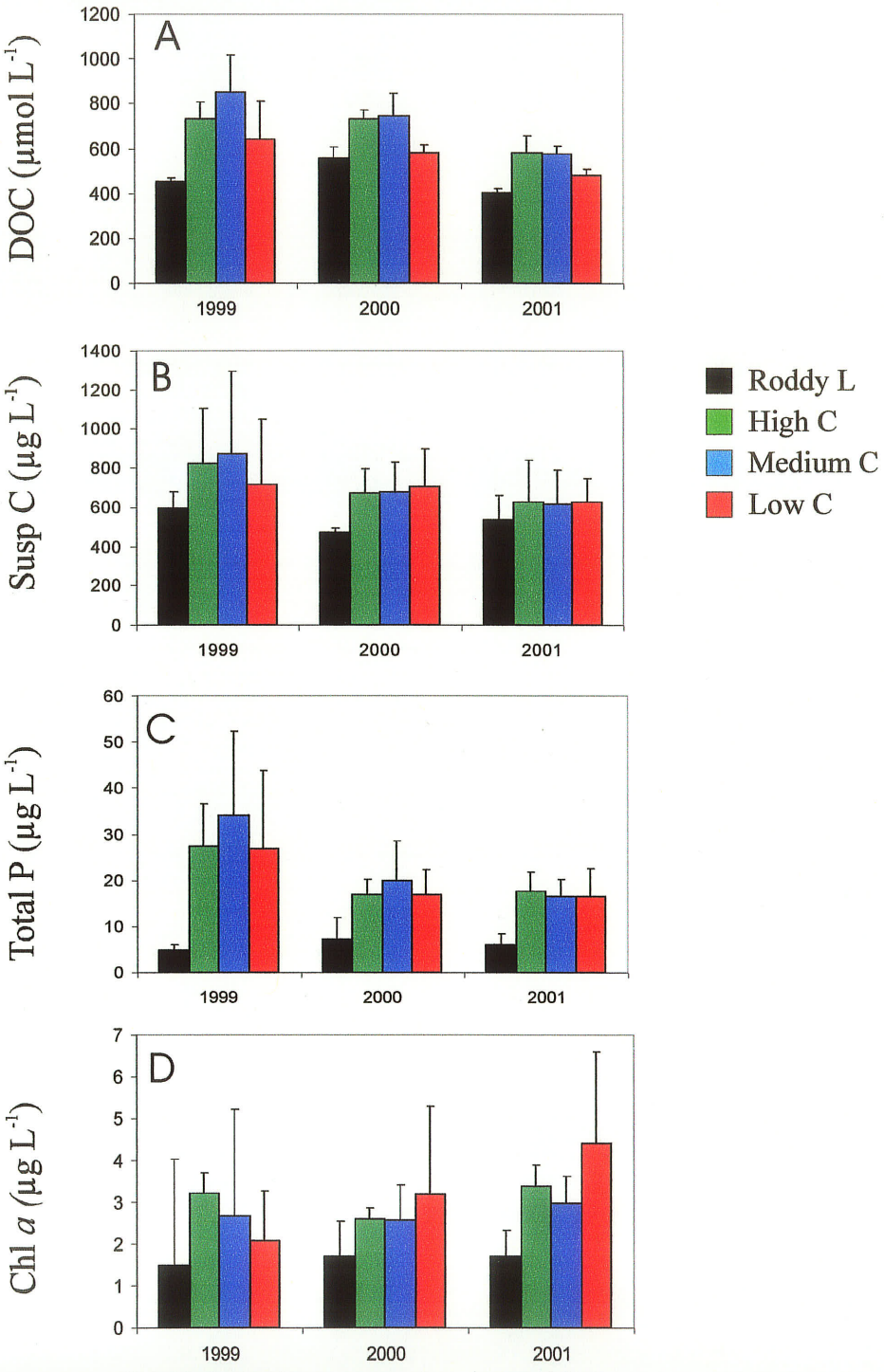

Figure 3. Mean (A) dissolved organic carbon (B) suspended C (C) total phosphorus and (D) chlorophyll *a* concentrations for 1999-2001 from Roddy L and the High C, Medium C and Low C reservoirs. Vertical bars indicate standard deviation of the mean for each reservoir (n = 9 in 1999 & 2000; n = 10 in 2001).

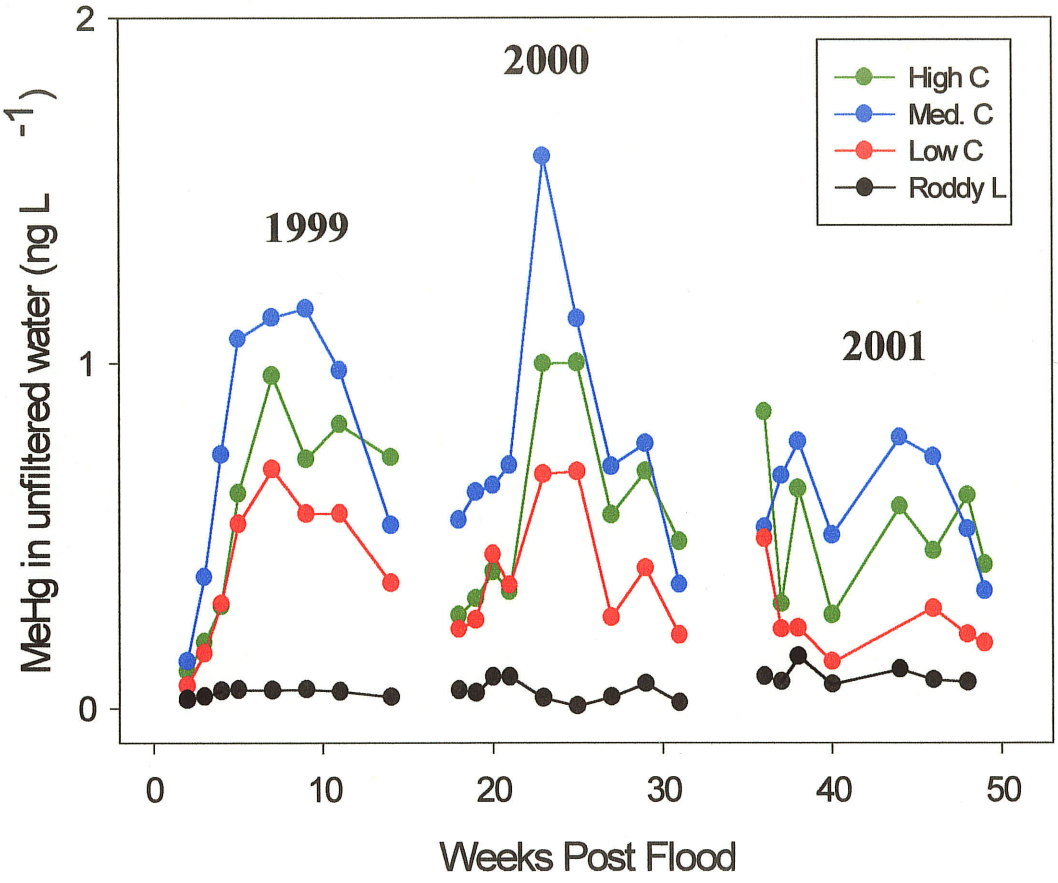

Figure 4. Changes in concentrations of MeHg in unfiltered water in the inflow (Roddy L) and the outflows of the High, Medium, and Low C reservoirs for the three years of flooding (B. D. Hall, University of Alberta – unpublished data). Note that the x-axis is total weeks of inundation after initiation of flooding in 1999. The breaks in weeks post-flood from year to year include a 2 week period of draining and filling the reservoirs at the end and beginning of each flooding season.

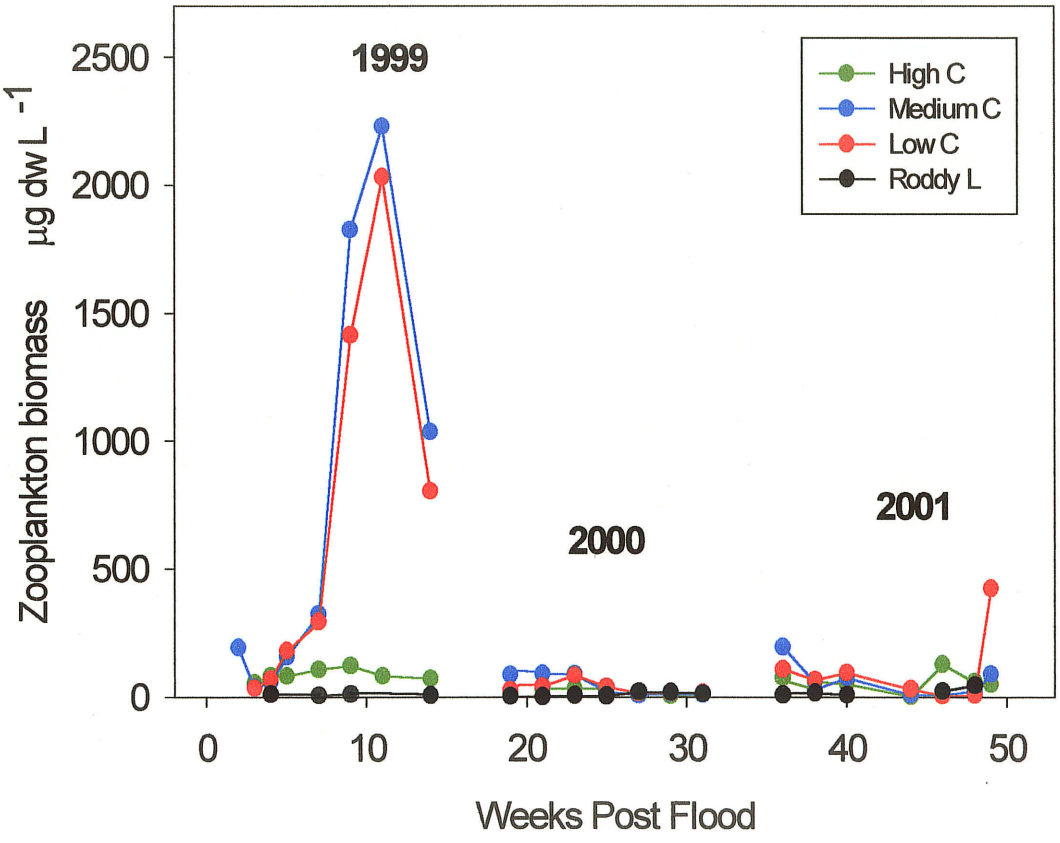

Figure 5. Changes in total zooplankton biomass (µg dw L^{-1}) for the three FLUDEX reservoirs for 1999-2001.

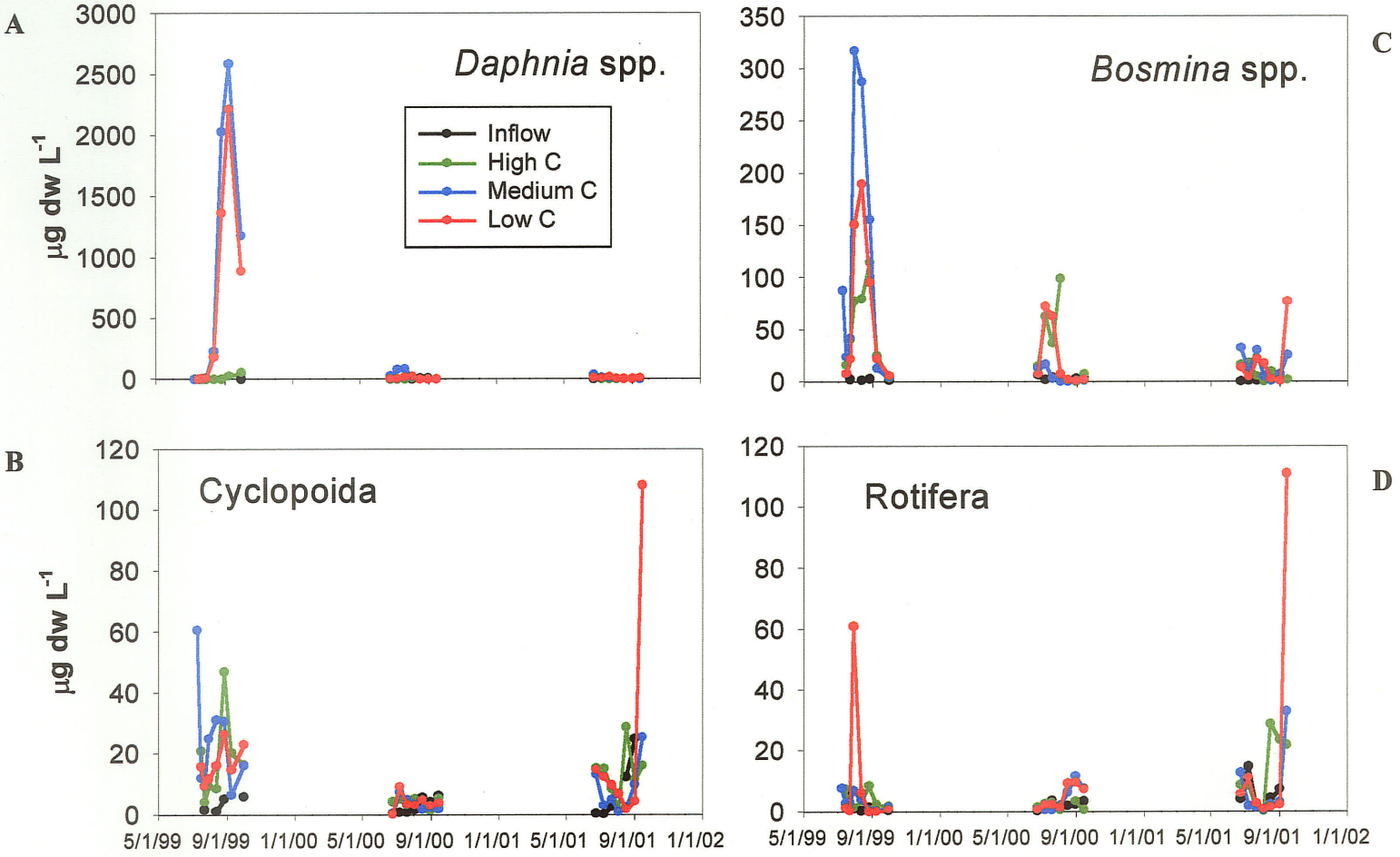

Figure 6. Changes in (A) *Daphnia* spp. (B) *Bosmina* spp. (C) Cyclopoida and (D) Rotifera biomass in mg dw L^{-1} for the three FLUDEX reservoirs from 1999 – 2001. Note differences in y-axis scales.

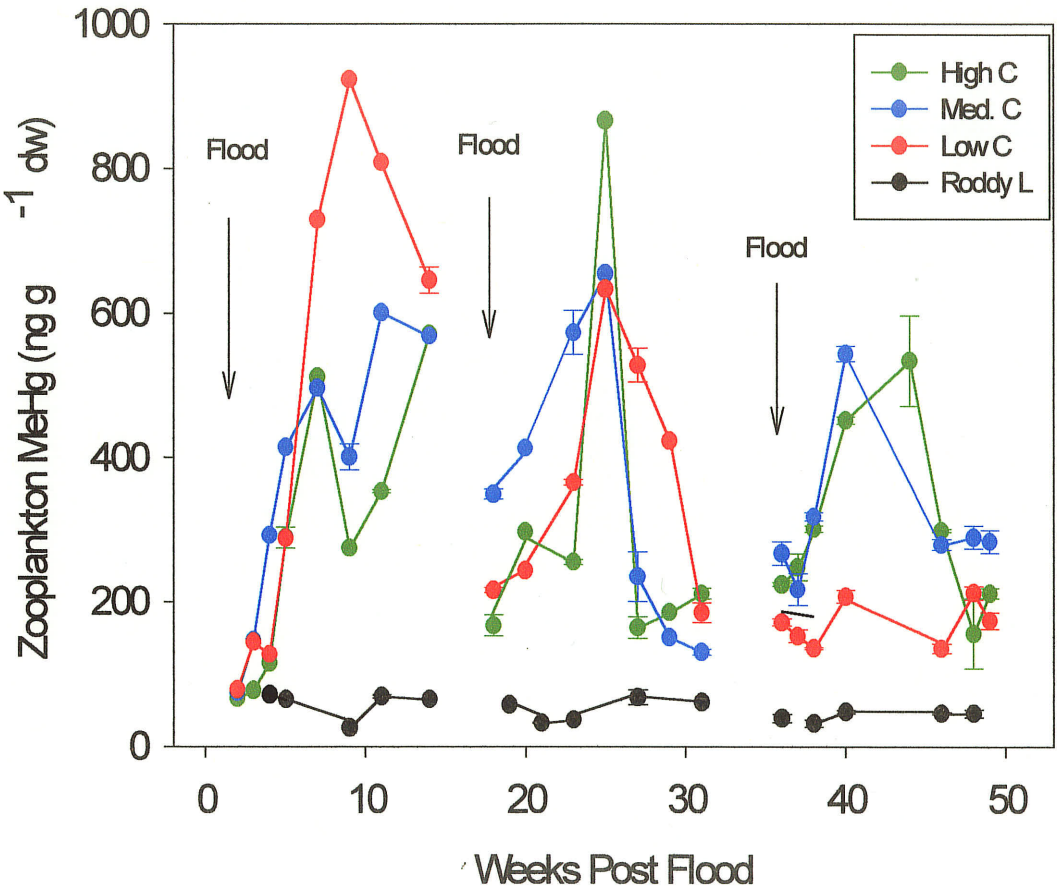

Figure 7. Changes in concentrations of MeHg in zooplankton in the High C, Medium C and Low C reservoirs. Samples for inflow analysis were collected in Roddy L. Note that x-axis is total weeks of inundation after the initiation of flooding in 1999. Vertical bars represent standard error for replicate MeHg analysis (n = 2 - 4).

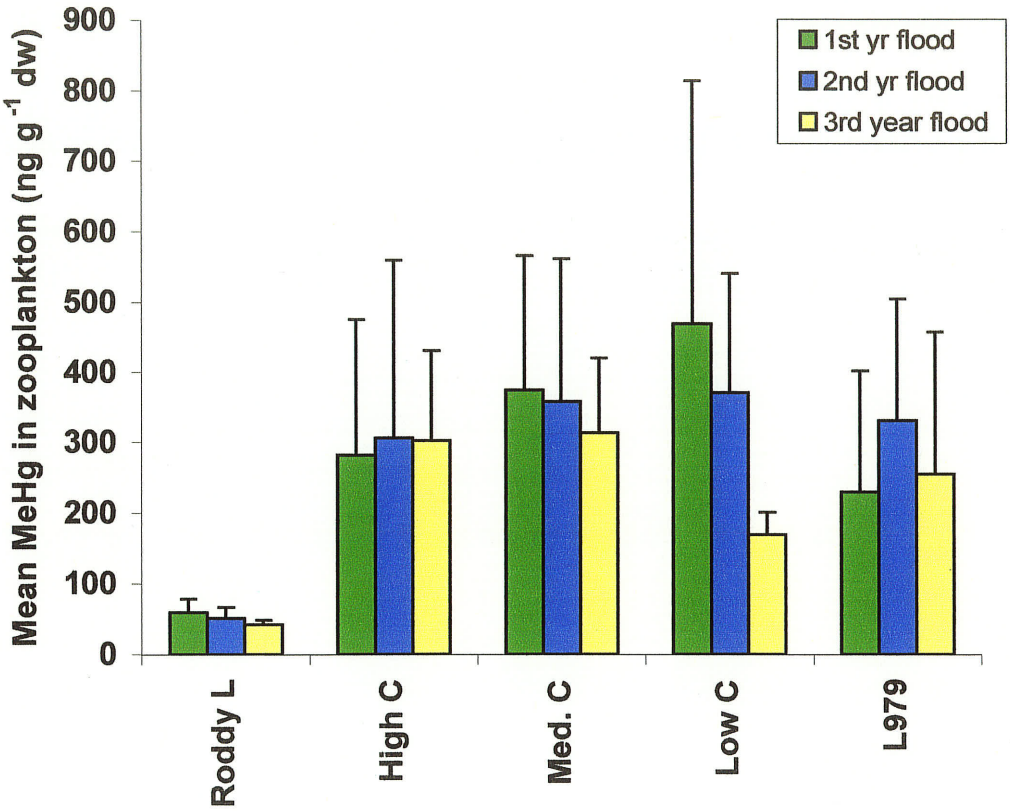

Figure 8. Average concentrations of MeHg in zooplankton from the three reservoirs, inflows (Roddy Lake) and L979 (data from Paterson *et al.* 1998) for the first, second and third years of flooding. Vertical bars represent one standard deviation from the mean (n = 5 – 8).

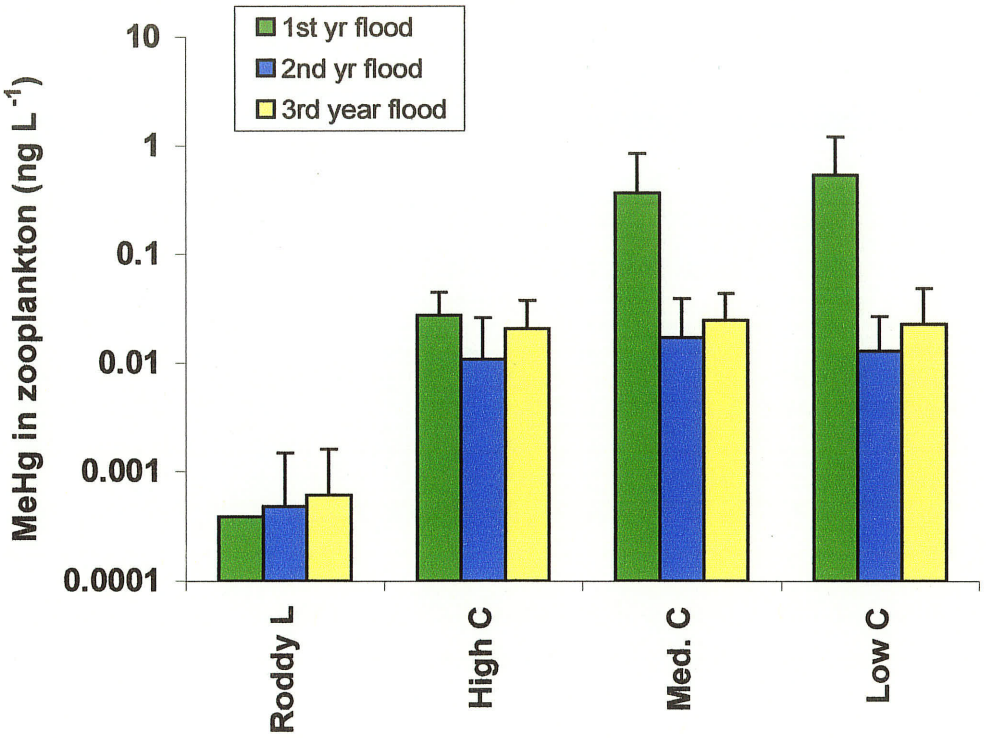

Figure 9. Average mass of MeHg in zooplankton as ng L^{-1} from Roddy L (inflow), and the FLUDEX reservoirs from 1999-2001. Note log scale. Vertical bars represent one standard deviation from the mean (n = 4 - 7).

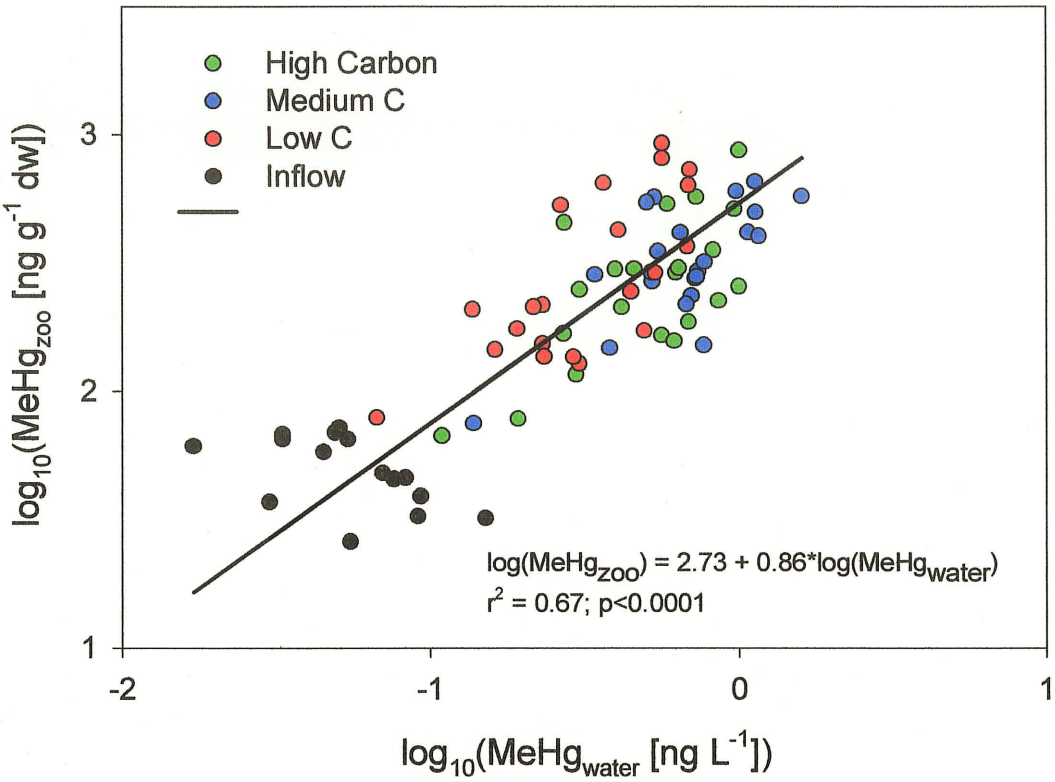

Figure 10. Model II regression relating concentrations of MeHg in zooplankton ($MeHg_{zoo}$) and MeHg in unfiltered water ($MeHg_{water}$) in the FLUDEX reservoirs and the inflow (from Roddy Lake). Data collected from 1999-2001.

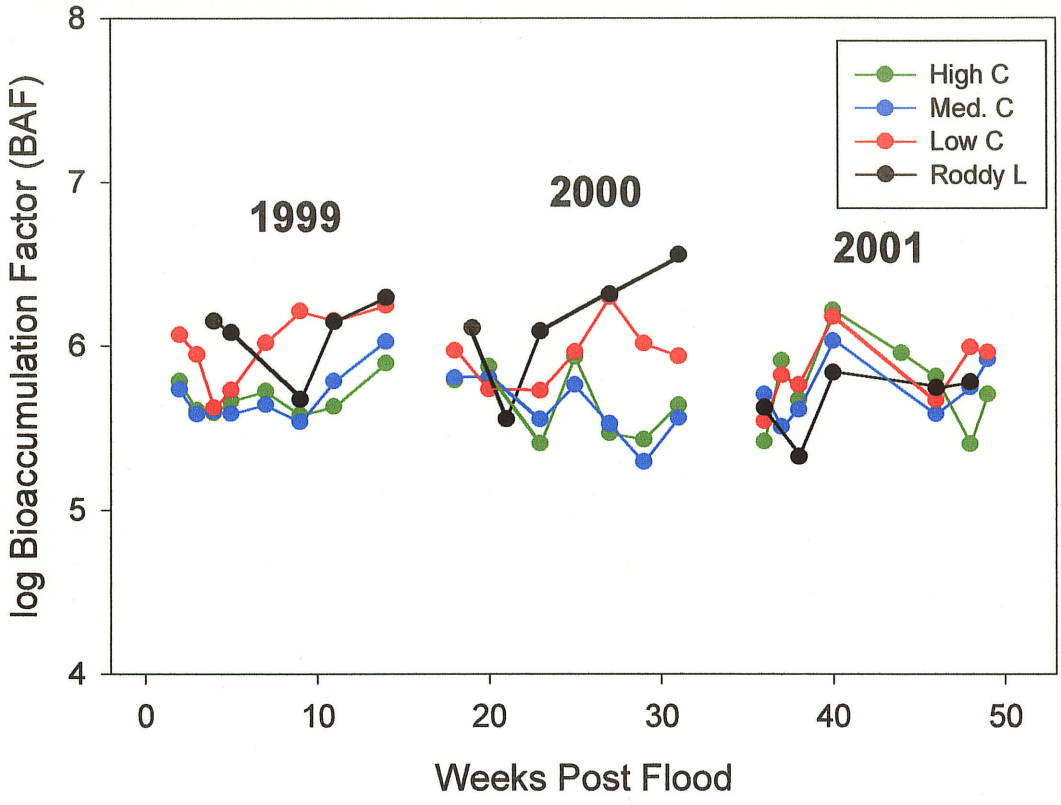

Figure 11. Changes in zooplankton bioaccumulation factors (BAFs) for MeHg during three years of flooding for all three reservoirs and Roddy L (inflow).

Chapter 2 – The relative importance of food and water for methylmercury accumulation in *Daphnia pulicaria* (Forbes)

Abstract

The relative importance of food and water for methylmercury (MeHg) accumulation in *Daphnia pulicaria* was examined using an incomplete 2 x 2 factorial design. *D. pulicaria* were exposed to MeHg in both algae and water in one treatment and were exposed to MeHg in food only in another. A control treatment exposed *Daphnia* to algae and water with low concentrations of MeHg. After 48 hours of exposure, MeHg concentrations in *Daphnia* from the food and water treatment were 6-9 times higher than initial concentrations of 198 ng g^{-1} dw. Minimal changes in MeHg concentrations were found in the food only and control treatments. This suggests that MeHg uptake from food was negligible.

Introduction

Diet is the most important route of methylmercury (MeHg) exposure in top predators such as fish, birds and mammals (Hall *et al.* 1998; Wolfe *et al.* 1998). Zooplankton are an important dietary component for planktivorous fish, and factors affecting contaminant uptake in zooplankton may also affect contaminant concentrations up the aquatic food web. Zooplankton may be exposed to contaminants such as MeHg through two main pathways: 1) through direct sorption of dissolved MeHg in water, and 2) through the ingestion of MeHg-contaminated food. It is typically assumed that an important first step in MeHg movement into zooplankton occurs at the phytoplankton level where MeHg is taken up directly from MeHg in the surrounding water column (Miles *et al.* 2001; Moye *et al.* 2002; Hill *et al.* 1996).

To date, only one study has directly tested the relative importance of MeHg uptake by zooplankton from water and food (Monson and Brezonik 1999). After a 48-h exposure to MeHg in both food and water or water only, Monson and Brezonik (1999) found no statistical differences in *Daphnia magna* (Strauss) MeHg levels from the two treatments. In another study with *D. magna*, Boudou and Ribeyre (1981) observed increased MeHg concentrations in *Daphnia* at higher temperatures because of a corresponding increase in the ingestion of MeHg-contaminated food. In their study, there was very little Hg in the water and they did not consider the exposure of MeHg through water as a possible exposure route for zooplankton. Pickhardt *et al.* (2002) found that changes in algal biomass resulted in different MeHg concentrations in algae and in *Daphnia*, with little change in dissolved concentrations. These results suggested that the trophic pathway may be the predominant route of uptake.

Several studies have suggested that MeHg accumulation in larger invertebrates occurs primarily through ingestion of food (Mason *et al.* 2000; Tremblay *et al.* 1996a, 1996b; Tremblay and Lucotte 1997). In two streams in Maryland, trophic transfer was the most important route of transfer for several aquatic Plecoptera, Trichoptera and Odonata species (Mason *et al.* 2000). MeHg uptake in *Hexagenia rigida* (McDunnough) from the water column was also minimal compared to uptake from ingested organic matter in MeHg-rich sediments in a mesocosm (Odin *et al.* 1997). MeHg accumulation may also occur through the direct transfer from the water column into large invertebrates. However, concentrations of MeHg in food sources for these organisms are often much larger than concentrations in water, so the trophic route is relatively more important. Because results for large invertebrates and

higher taxa suggest that MeHg uptake is primarily from food, it has typically been assumed that this path also predominates for zooplankton. It is possible, however, that a larger proportion of MeHg is taken up from water by zooplankton because of their smaller size and greater relative surface area.

It is important to establish the routes of MeHg exposure for zooplankton because factors affecting MeHg uptake through water or food may be very different. For example, if the major route of MeHg exposure to zooplankton is food, then food-related variables such as food quality and quantity (Meili 1991) and physiological variables affecting assimilation may be important determinants of MeHg availability to zooplankton (Wang and Fisher 1999). Alternatively, if zooplankton obtain MeHg primarily from the dissolved phase, water chemistry variables affecting the speciation and availability of MeHg for uptake such as pH (Winfrey and Rudd 1990) and dissolved organic carbon (DOC) concentrations would be much more important.

MeHg concentrations in zooplankton are frequently related to those in unfiltered water from natural lakes and reservoirs (Paterson *et al.* 1998; Watras *et al.* 1998; Back and Watras 1995; Becker and Bigham 1995). It is very difficult to separate exposure pathways for zooplankton in the field, however, because of rapid dynamics between dissolved and particulate phases (Pickhardt *et al.* 2002; Fujita and Hashizume 1975; Glooschenko 1969). For this reason, an attempt was made to separate pathways of MeHg exposure to zooplankton in a controlled laboratory experiment.

I examined the relative importance of food and water as routes of MeHg uptake by a common zooplankter, *Daphnia pulicaria* (Forbes). *Daphnia* have

frequently been used as test organisms in contaminant studies (e.g. Keddy *et al.* 1995; Toussaint *et al.* 1995) and are common in many lakes, including the lakes and reservoirs at the Experimental Lakes Area of northwestern Ontario (ELA).

Experimental Design

Daphnia pulicaria were exposed to different treatments to investigate whether they received the majority of their MeHg through food or through water. Ideally, this test would use a 2X2 factorial design exposing *Daphnia* to all combinations of low and high concentrations of MeHg in food (algae) and the dissolved phase. This design was deemed impractical because of the extremely rapid uptake of dissolved MeHg by algae. Previous studies by Fujita and Hashizume (1975) and Glooschenko (1969) have demonstrated that phytoplankton achieve equilibrium with the dissolved phase within the first several hours of exposure (<10 h). As a result, it is not possible to present MeHg-free algae in water with high concentrations of MeHg in the dissolved phase.

As an alternative, I used a design utilized by Munger and Hare (1997) to study Cadmium uptake by *Chaoborus punctipennis* (Say). *D. pulicaria* were exposed to MeHg in food and water in three treatments (Fig. 1). In the first treatment, *Daphnia* were exposed to high concentrations of MeHg in both food and water ("food + water"). In the second treatment, *Daphnia* were exposed to high concentrations of MeHg in food only ("food only"). The third treatment acted as a control, with low concentrations of MeHg in both food and water. Each treatment was replicated three

times and samples were collected at the onset and end of the experiment and at two sampling intervals (24-h and 48-h) within 48 hours.

An important assumption of this design is that MeHg losses from algae in the "food only" treatment (high-MeHg algae in low-MeHg water) are slow enough to maintain differences between the "food only" and "control" treatments. Although there are few data for MeHg, studies with other contaminants have frequently indicated that rates of desorption from particles are slower than rates of uptake (reviewed in Autenrieth and DePinto 1991). With respect to MeHg, Glooschenko (1969) found that the rate of uptake of radioactive MeHg in phytoplankton in the first 7 hours was faster than losses during the next 24 hours.

Methods

Culturing of phytoplankton

Algae were raised in 1 L chemostats (Fig. 2; Healy and Hendzel 1975) with the freshwater growth medium COMBO (Kilham *et al.* 1998). The algal chemostats were maintained at 20°C with a 24-h light exposure and had turnover rates of 250 mL per 24-h period. Air was constantly bubbled through the chemostats to keep algal cells in suspension. *D. pulicaria* were fed a free-swimming, flagellated chlorophyte, *Ochromonas* sp. The feeding experiment was undertaken using this taxon as the food resource.

Culturing of *D. pulicaria* for the feeding experiment.

Daphnia pulicaria were collected from Lake 110 at the ELA and were maintained in algal/zooplankton chemostats similar to those used by Lampert (1975) (Fig. 2). *Daphnia* were held in two 4 L containers filled with filtered dechlorinated tap water. An algal chemostat fed *Ochromonas* sp. into the two zooplankton containers at a rate of 125 mL / 24 h. The turnover of dechlorinated water in the zooplankton containers was 4 L/ 24 h. The algal/zooplankton chemostat was kept in a controlled temperature room (20°C) with continuous light.

Contamination of algae for *D. pulicaria* feeding experiment

Two algal chemostats with *Ochromonas* sp. were established for the *Daphnia* feeding experiment; one with MeHg added (high-MeHg) for the "food + water" and "food only" treatments and one with no MeHg added (low-MeHg) for the control treatment. From the time that both chemostats were started, I completed cell counts and optical density measures daily to determine when the chemostats were at steady state. Cell counts were completed with a bright-lined double Neubauer haemocytometer. Cell counts at the beginning of the feeding experiment were 11.99 x 10^6 cells mL^{-1} for the low-MeHg chemostat and 11.97 x 10^6 cells mL^{-1} from the high-MeHg. Optical density measurements using a spectrophotometer at a wavelength of 750 nm acted as a check on the cell counts.

In a preliminary experiment with the algae *Ankistrodesmus* spp., I found substantial losses of MeHg over a 24-h time period from the culture medium used to grow the algae. As a result, we added 40 µL of MeHg (as CH_3Hg dissolved in

isopropanol) to the 8-L reservoir of COMBO growth medium for the high MeHg chemostat to achieve a target concentration of 20 ng MeHg L^{-1}. MeHg concentrations were increased in the growth media to 20 ng L^{-1} to offset expected losses to container walls or demethylation. Subsequent analyses of unfiltered media at the end of the experiment indicated that a concentration of 12 ng L^{-1} was actually achieved. After MeHg additions to the growth medium, the chemostat was left for 4 days to establish a steady state, which was enough time for total contents (algae and water) in the chemostat to turn over once. Average MeHg concentrations in algae from the high MeHg chemostat were 1545 ng g^{-1} C at the beginning of the experiment. These concentrations were similar to those found in particulates from L979 after impoundment (Paterson *et al.* 1998).

Daphnia pulicaria feeding experiment

Eighteen, 1 L separatory funnels were established for use in this feeding experiment (3 treatments X 2 replicates X 3 time intervals – 24 and 48 h) (Fig. 1). Six containers were also set up at the beginning and end of the experiment to analyze initial and final concentrations of MeHg in water and algae drawn from the chemostat. The "food only" treatment was not sampled initially under the assumption that algal and water concentrations would be the same as in the "food and water" and control treatments respectively. Unfiltered samples were also taken directly from the high-MeHg chemostat and the high-MeHg medium reservoir the end of the experiment. Filtered low-MeHg water was added to the "food only" and to the control treatments, whereas filtered water with an initial MeHg concentration of 3.0 ng L^{-1}

was added to funnels being used in the "food + water" treatment. We aimed for an initial target concentration of 10 ng L^{-1} in the high-MeHg water but after losses, obtained concentrations of only 3.0 ng L^{-1}. These losses of MeHg were probably the result of processes such as adsorption to the sides of the containers or demethylation.

Before algae were added to the experimental containers, equal volumes of algae were taken out of each of the two chemostats (low-MeHg and high-MeHg) and optical density readings were completed. Algae from the chemostats were then placed in acid-washed tubes and centrifuged at 2900 rpm for 2 minutes, resuspended in 100 mL of fresh low-MeHg media and centrifuged a second time under the same conditions. After centrifugation, the supernatant was drained and algae from the high-MeHg chemostat were re-suspended in enough fresh high-MeHg media to reach the initial optical density reading as when they were taken from the chemostats. Algae from the low-MeHg chemostat were similarly diluted, except that the low-MeHg medium was used to reach the initial optical density reading. Equal amounts of algae were added to each of the separatory funnels in each treatment.

Approximately 100 *D. pulicaria* were randomly allocated to each of the 18 experimental containers used in the experiment. All added *Daphnia* were similar in size and care was taken not to use *Daphnia* with eggs. During the experiment, the experimental containers were maintained at 20°C with 4 sets of fluorescent growth lights behind the funnels. No *Daphnia* mortality was noticed throughout the experiment.

Three-quarters of the water in all separatory funnels was replaced every 12 hours with either low-MeHg or high-MeHg dechlorinated water to maintain MeHg

concentrations throughout the experiment. Water was slowly drawn from the bottom of the separatory funnels and losses of *Daphnia* were prevented by a 48-μm Nitex sieve placed near the bottom of the separatory funnel (Fig. 3). Fresh low-MeHg and high-MeHg algae were also added every 12 hours to each of the separatory funnels to ensure a constant supply of contaminated food for zooplankton.

In a preliminary experiment, losses of MeHg from another algal species, *Ankistrodesmus* sp. were observed over a 24-h time period. Although we observed losses of MeHg from *Ankistrodesmus,*, concentrations of MeHg were still substantially elevated after 12 hours. As a result, the decision was made to change the algae and media in the feeding experiments every 12 hours. A shorter exchange interval was not practical given the large amount of effort required to complete sampling.

Visual observation of gut contents indicated that *Daphnia* were eating *Ochromonas* throughout the experiment. In addition, cultures of *Daphnia* were kept at high populations for several months prior to the start of the experiment using cultures of *Ochromonas* sp. as a food source.

At each sampling interval (24 and 48 h), zooplankton, algae and water were sampled from three separatory funnels in each treatment. Contents from each separatory funnel were poured through a 48-μm Nitex sieve to catch the *Daphnia.* *Daphnia* were then washed from the sieve with low-MeHg water and placed in small clean beakers with low-MeHg water for 15 minutes to clear their gut contents. *Daphnia* were then placed into acid-washed scintillation vials, frozen, freeze-dried, weighed, and analyzed for MeHg content. Funnel contents passing through the sieve

were drained into an acid washed 1000-mL beaker for particulate and water sampling. Particulates were filtered for suspended carbon (C) and MeHg using an acid-washed Teflon filtering apparatus. Suspended C was determined using an ignited GF/C filter and the methods of Stainton *et al.* (1977). Algae for MeHg analysis were retained on ashed QM/A quartz-fiber filters. Filters were placed in conical acid-washed Teflon tubes and frozen before MeHg analysis. Filtrate through the QM/A filters was collected in acid-washed Teflon bottles and was frozen for dissolved MeHg analysis. The different filters used to collect suspended C and MeHg were required for methodological reasons but both have the same nominal pore size.

MeHg in water, algae and zooplankton was determined by Flett Research Ltd. (Winnipeg, Canada) using acid distillation, ethylation and reduction before gas chromatograph - cold vapour atomic fluorescence detection (GC-CVAFS). The detection limit for water samples was 0.04 ng L^{-1}, 0.02 ng g^{-1} for algae and 0.02 ng g^{-1} for zooplankton. Values below the detection limit were divided by one-half the detection limit for use in statistical analyses.

Data Analysis

Treatment effects were determined using One-way Analysis of Variance (ANOVA) and an $\alpha = 0.05$. Tukey's test was used to determine which treatments were significantly different from one another. Loss-rate calculations were used to estimate exposure of MeHg in zooplankton from algae and water. Loss rates were calculated as:

$$(\ln C_{(t-1)} - \ln C_t)/(t_0-t_1) \qquad [1]$$

where C is the algal concentration at time 0 and time t (in hours). All statistical tests were done using SYSTAT version 8.0.

Results

Daphnia feeding experiment

At the start of the experiment, mean concentrations of dissolved MeHg were 0.02 ng L^{-1} in the low-MeHg water and 3.0 ng L^{-1} in the high-MeHg water. Algae grown without added MeHg had an initial mean concentration of 28.0 ng g^{-1} C (SE = 3) whereas MeHg-rich algae had an initial average concentration of 1545 ng g^{-1} C (SE = 162).

Changes in MeHg concentrations were observed in algae drawn from the chemostats at the end of the experiment. Although the average initial concentration of algae from the high-MeHg chemostat was 1545 ng MeHg g^{-1} C, final concentrations from the same chemostat were 3174 ng MeHg g^{-1} C (SE = 103). Mean carbon measures from algae in the high-MeHg chemostat were 3077 μg L^{-1} (SE = 134) at the beginning of the experiment and decreased to 1290 μg L^{-1} (SE = 21) at the end of the experiment.

Algae: After 24 hours, concentrations of MeHg in algae from the "food only" treatment were only 6% of initial concentrations (Fig. 4). The average loss rates of MeHg from *Ochromonas* in the feeding experiment was 60.2 ng g^{-1} MeHg/h or 8%

MeHg loss/h. At 24 and 48 h, MeHg concentrations in algae from the "food only" treatment were not statistically different from algae in the control treatment (Tukey's $p = 0.621$ (24 hr), ANOVA ($F(2,6) = 365.21$, $p < 0.0001$); $p = 0.622$ (48 hr), ANOVA ($F(2,6) = 79.384$, $p < 0.0001$). Concentrations of algae in the control and "food + water" treatments did not change significantly over the 48-h time period.

Dissolved phase: Although there were large losses of MeHg from algae in the "food only" treatment, dissolved MeHg remained low throughout the experiment (Fig. 5). Concentrations of dissolved MeHg in the "food only" treatment and the control were not significantly different at 24 h and 48 h (Tukey's $p = 0.659$ (24 h), ANOVA ($F(2,6) = 4298.618$, $p < 0.0001$) and $p = 0.903$ (48 h), ANOVA ($F(2,6) = 621.468$, $p < 0.0001$). Concentrations of dissolved MeHg in the control treatment ranged between 0.002 – 0.02 ng L^{-1} (SE = 0.003) throughout the experiment. Concentrations of dissolved MeHg from the "food + water" treatment decreased from 2.72 ng L^{-1} to 1.0 ng L^{-1} within 24 hours, after which no changes were detected.

***Daphnia*:** Concentrations of MeHg in *Daphnia* from the "food + water" treatment were six and nine times higher at 24 and 48 h than initial concentrations of 198 ng g^{-1} dw (Fig. 6). Comparatively, zooplankton MeHg concentrations did not increase in the "food only" or control treatments. There were no significant differences in zooplankton MeHg concentrations in the control and "food only" treatments at 24 or 48 h (Tukey's $p = 0.951$, $F(2,6) = 294.755$, $p < 0.0001$ and 0.226, $F(2, 6) = 4167.336$, $p < 0.0001$, respectively).

Mass Balance: A mass balance for each treatment was constructed by summing MeHg L^{-1} in algae, zooplankton and the dissolved phase (Fig. 7). With new additions every 12 hours, algae in the "food only" treatment lost an average of 8.7 ng L^{-1} over 24 hours, yet MeHg concentrations increased by only 0.01 ng L^{-1} in the dissolved phase. Comparatively, MeHg concentrations in algae from the "food + water" treatment only lost 1.2 ng L^{-1} over 24 hours. Similarly, concentrations of MeHg in the dissolved phase decreased by 3.4 ng L^{-1} over 24 hours in the "food + water" treatment.

Discussion

Changes in chemostat

The observed increases in MeHg concentrations in algae from the MeHg-rich chemostat can probably be explained by differences in particle dilution. In order to provide sufficient food for *Daphnia*, algae were removed from the chemostats every 12 hours. These withdrawals resulted in large decreases in cell densities over the course of the experiment. Decreases in cell densities coincided with the increases in MeHg concentrations in algae, which lends support for the results of Pickhardt *et al.* (2002), who also found that decreases in algal biomass result in increases of MeHg concentrations per cell of algae.

Loss of methylmercury in algae

At the end of the experiment, MeHg concentrations in algae were less than 10% of initial concentrations in the "food only" treatment. MeHg was also lost from

algae in the "food + water" treatment although concentrations of MeHg in algae were not significantly different from each other at each sampling period. By using mass balance equations, it is evident that the rates of MeHg loss from the two treatments were different from each other.

Much of the MeHg lost from algae in the "food + water" and "food only" treatments cannot by accounted for. It is also unclear why losses from the "food only" treatment were so much greater than those from the "food + water" treatment. Demethylation and/or photodegradation of MeHg are possible explanations (e.g. Sellers *et al.* 1996; Ullrich *et al.* 2001). Alternatively, there may have been sorption of MeHg to the walls of the separatory funnels although sorption of such large quantities is unlikely (Miles *et al.* 2001). If MeHg was lost primarily by sorption, it is also hard to reconcile why smaller losses were observed in the "food + water" treatment versus the "food only" treatment.

Large losses of MeHg from *Ankistrodesmus* (another species of algae) were also seen in a preliminary experiment. Culturing and contamination methods in this experiment were similar to those used in the *Daphnia* feeding experiment using *Ochromonas*. MeHg concentrations in *Ankistrodesmus* showed a 70% decrease from initial concentrations over the 24-h time period. The loss rate of MeHg from *Ankistrodesmus* was 3% MeHg loss/h, which was less than the loss rate for *Ochromonas* in the feeding experiment. It is unclear why the loss rates of MeHg differed in the two experiments. Possible non-exclusive explanations include: 1) the two algal taxa may have naturally different loss rates; 2) the presence of *Daphnia* in the second experiment may have enhanced loss rates; or 3) the greater difference in

the gradient between concentrations of MeHg in the algae and dissolved phase in the second experiment may have resulted in greater loss rates.

Importance of food vs. water as sources of MeHg to *D. pulicaria*

Large increases in MeHg were found in *Daphnia* from the "food + water" treatment, whereas minimal changes were found for *Daphnia* in the "food only" and control treatments. There are two possibilities: 1) accumulation of MeHg in *D. pulicaria* was greater from the water pathway as opposed to the food pathway, or 2) insufficient difference was generated between the "food only" and control treatments to truly test the hypothesis that *D. pulicaria* obtain the majority of their MeHg from food.

To distinguish the above possibilities, exposure of MeHg to *Daphnia* from food and water in our experiment was estimated using a simple model. The model depended on two assumptions: 1) MeHg concentrations in algae increased linearly after each withdrawal from the high-MeHg chemostat over time and 2) MeHg was lost from algae in the separatory funnels according to a first-order loss process described using equation [1]. First-order losses are the simplest possible description and similar dynamics have been observed for the desorption of contaminants such as PCBs from sediments and algae (e.g. Wood *et al.* 1987). Other dynamic processes have also been described; however, and follow-up studies are required. For example, loss rates may be proportional to the concentration gradient between algae and water (Brown *et al.* 1982) or display multi-phase partitioning (e.g. Jannasch *et al.* 1988).

Under the assumption of first-order losses, a plot was generated of predicted MeHg dynamics in algae from the "food + water", "food only", and control treatments (Fig. 8). Algae in the separatory funnels were replaced every 12 hours with fresh algae, which is why the predicted values on the graph increase intermittently. The predictions from the model suggest that average MeHg concentrations in algae from the control and "food only" treatments were considerably different (Table 1). Therefore, zooplankton were exposed to algae at significantly different concentrations in all treatments throughout the experiment. Despite these predicted differences in exposure, *Daphnia* did not accumulate substantial MeHg through their diet. If this description of MeHg dynamics is accurate, *Daphnia* obtained most of their MeHg from the dissolved phase.

Exposure to contaminated food and water in different treatments did not produce significant differences in MeHg concentrations in *D. magna* after a 48 h exposure in the study of Monson and Brezonik (1999). Another experiment in the Monson and Brezonik (1999) study showed a linear dose-uptake response for *D. magna* exposed to MeHg in water alone ranging in concentrations from 0 ng L^{-1} to 4 ng L^{-1}. If all other factors were held constant, as MeHg increased in water it similarly increased in daphnids (Monson and Brezonik 1999). This study used realistic concentrations of MeHg (2 ng L^{-1}), although only one combination of food and water was used to test differences between uptake from food versus water. As well, the food source was a yeast-alfalfa-trout-chow mix, which is very different from algal assemblages found in natural lakes and is dissimilar in composition to the phytoplankton species used in my feeding experiment.

An important finding of my study was the extremely rapid dynamics of MeHg uptake in *Daphnia* as well as MeHg loss from algae. Several researchers have used correlations of MeHg concentrations in zooplankton, phytoplankton, bacteria, suspended organic matter and the dissolved phase to examine possible routes of MeHg uptake by plankton in the field (Paterson *et al.* 1998; Plourde *et al.* 1997; Watras *et al.* 1998; Westcott and Kalff 1996). Uptake and loss of MeHg at the base of the food chain are too rapid to obtain separation of exposure routes using correlations in the field, a conclusion reached by other researchers as well (e.g. Paterson *et al.* 1998).

Results from my study suggest that *D. pulicaria* take up MeHg mainly from water and that the dynamics of MeHg accumulation in *Daphnia* are very fast (<24 hours). Of course, it is possible that in the natural environment, *Daphnia* may receive considerable amounts of MeHg from both food and water. It is also possible that uptake of MeHg occurs so quickly that relative exposure routes of MeHg in zooplankton do not pose a concern when considering MeHg uptake up the food chain into higher organisms.

Several researchers have assumed that food is the major route of MeHg exposure to zooplankton (Boudou and Ribeyere 1981; Pickhardt *et al.* 2002; Plourde *et al.* 1997); thus, much research has focused on MeHg loss and uptake in primary producers (e.g. Miles *et al.* 2001; Moye *et al.* 2002; Mason *et al.* 1996). The results of my study show that zooplankton may receive the majority of their MeHg from the dissolved phase. If so, zooplankton may be the first point of entry for MeHg into the

food chain and research should focus on factors affecting MeHg uptake by these organisms.

Conclusion

There were large differences in MeHg accumulation by *Daphnia* exposed to MeHg in both food and water compared to those that were exposed to MeHg in food alone. After 48 hours, concentrations of MeHg in *Daphnia* from the "food + water" treatment were 9 - 11 times greater than those in the "food only" and control treatments. No significant differences in uptake of MeHg by *Daphnia* were observed between the "food only" and control treatments. Replicates were similar in all treatments. Simple modeling suggested that substantial differences in MeHg exposure from food versus water may have existed in the "food only" and "food + water" treatments, despite large losses of MeHg from the algae. Despite the limitations of experimental design, these results suggest that MeHg in water is the main route of exposure in zooplankton.

Several further studies are needed to confirm this conclusion. First, a more complete assessment is needed of MeHg loss dynamics from MeHg-rich *Ochromonas* spp. resuspended in low-MeHg water. Second, estimation of *D. pulicaria* grazing rates on *Ochromonas* spp. would be useful in determining how much MeHg-contaminated food was actually ingested in the experiment. Last, determination of the fate of MeHg lost from algae in the "food only" treatment is needed. Included in this assessment should be the measurement of Total-Hg and Hg^0 to assess losses by

demethylation. It is also recommended that MeHg sorption to container walls be estimated using acid rinses (e.g. Miles *et al.* 2001).

Table 1. Predicted average concentrations of MeHg in algae for the first and second 24-hour periods using the model described in the text. Mean concentrations of water measured from samples at 0, 12 and 24 hours. (SE for water = 0.003 – 0.02)

	Measured average MeHg in water (ng L^{-1})			Predicted average MeHg in algae (ng g^{-1} C)	
Treatment	**0 hours**	**24 hours**	**48 hours**	**0-24 hours**	**24-48 hours**
Food + water	3.0	1.0	1.0	1580	1613
Food only	0.02	0.01	0.03	600	693
Control	0.02	0.01	0.01	29	28

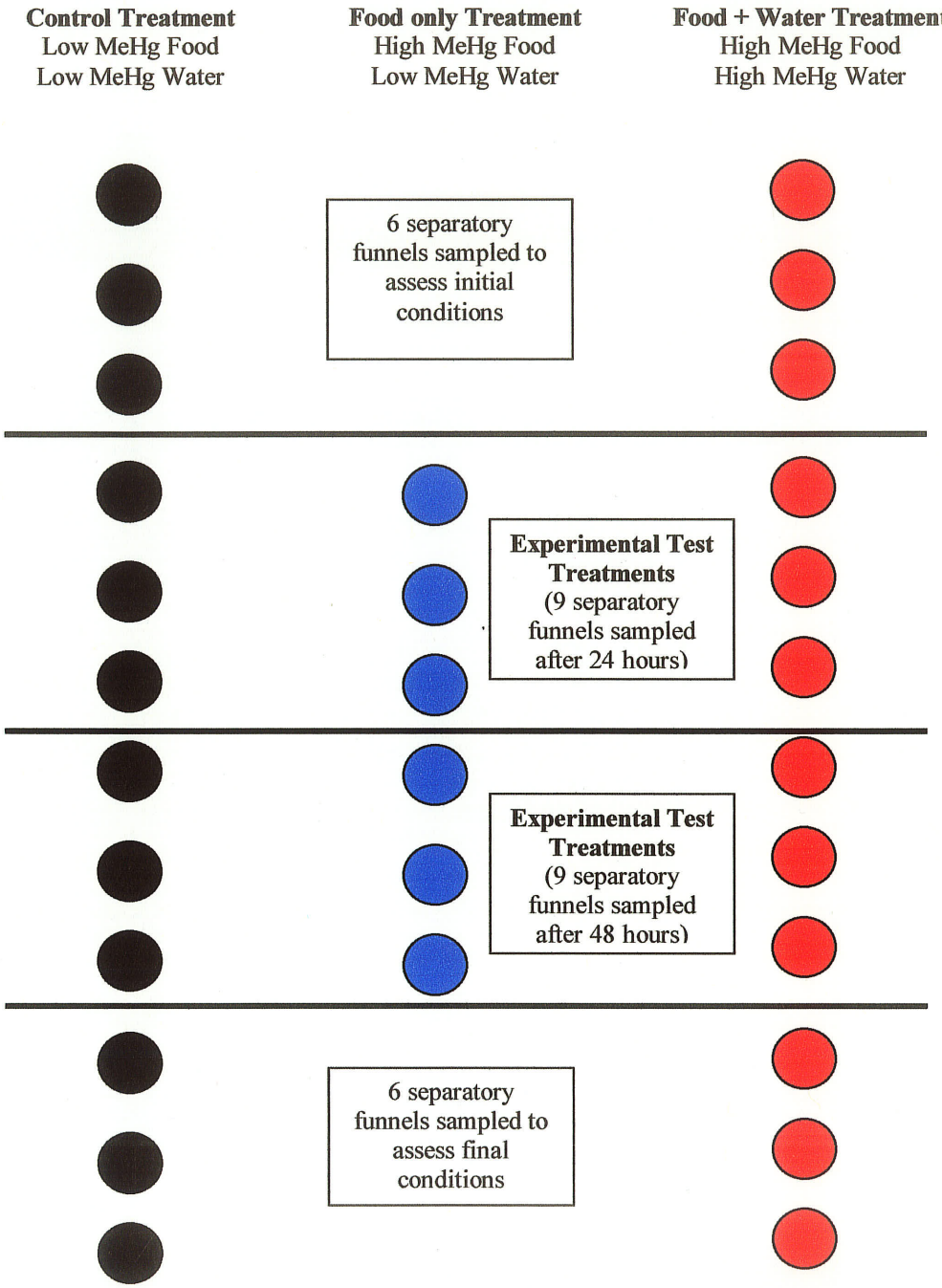

Figure 1. Treatments used in the feeding experiment. Each treatment was replicated three times for each sampling time period. Each separatory funnel contained 100 *Daphnia pulicaria*, a pre-determined amount of low or high-MeHg algae and 1 L of low or high-MeHg water.

Figure 2. The algal/zooplankton chemostat used for culturing *Daphnia pulicaria* and algae for the feeding experiment.

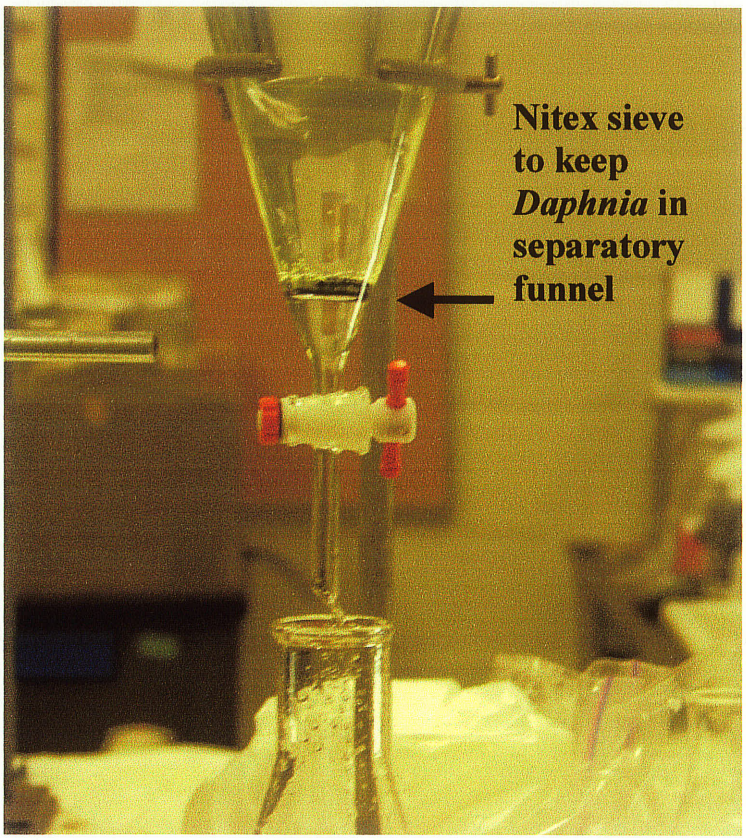

Figure 3. Separatory funnel showing drainage spout and sieve used to keep *Daphnia* inside.

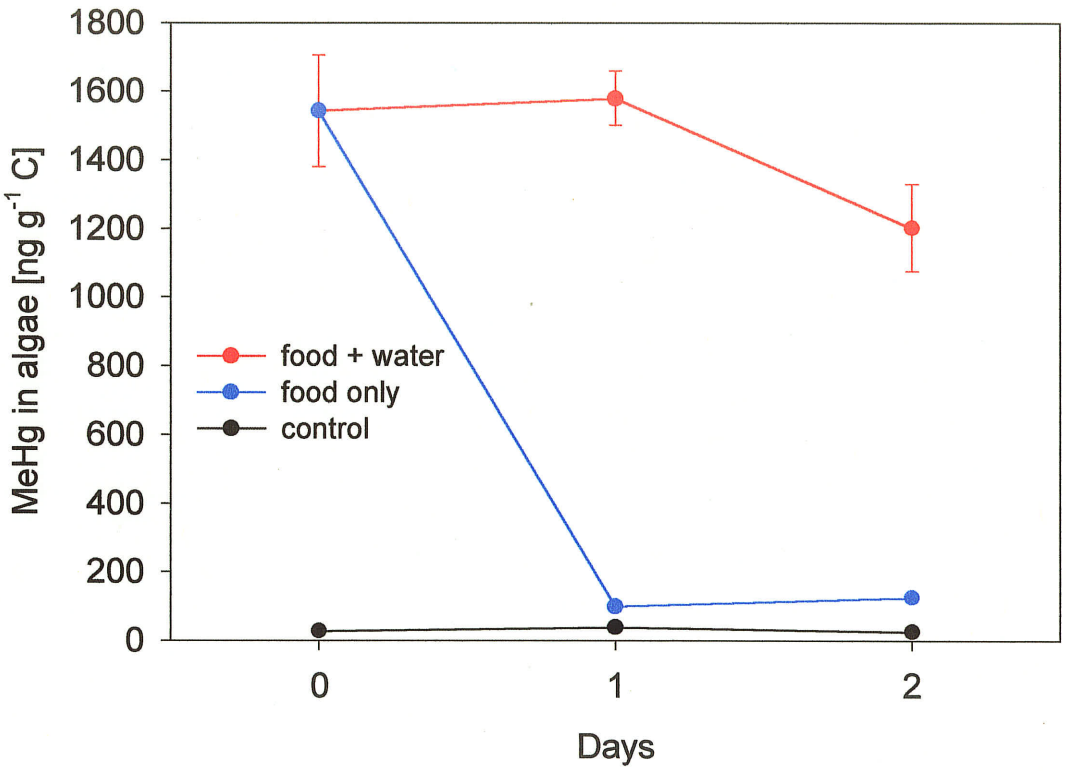

Figure 4. Changes in algae concentrations of MeHg. Samples were taken initially, at 24-h and at 48-h. Error bars represent one standard error of the mean (n = 3 for each sampling point).

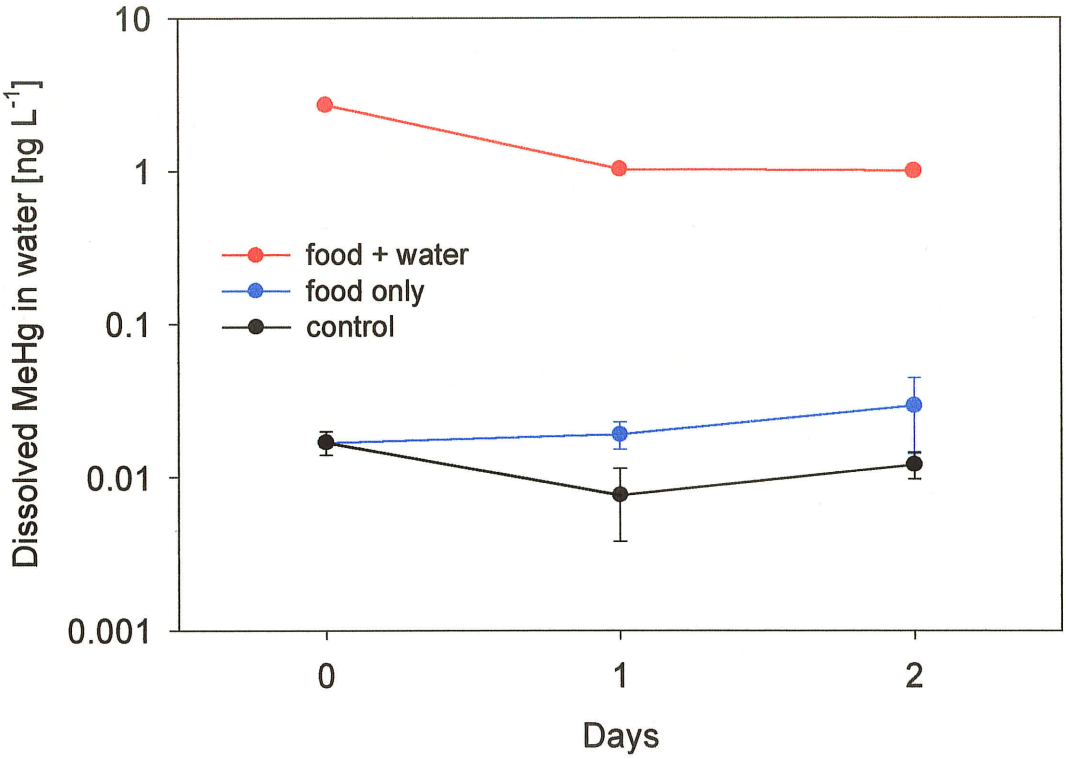

Figure 5. Changes in average dissolved MeHg concentrations. Samples were taken at 0, 24 and 48 h. Note log scale. Error bars represent one standard error of the mean (n = 3 for each point on graph).

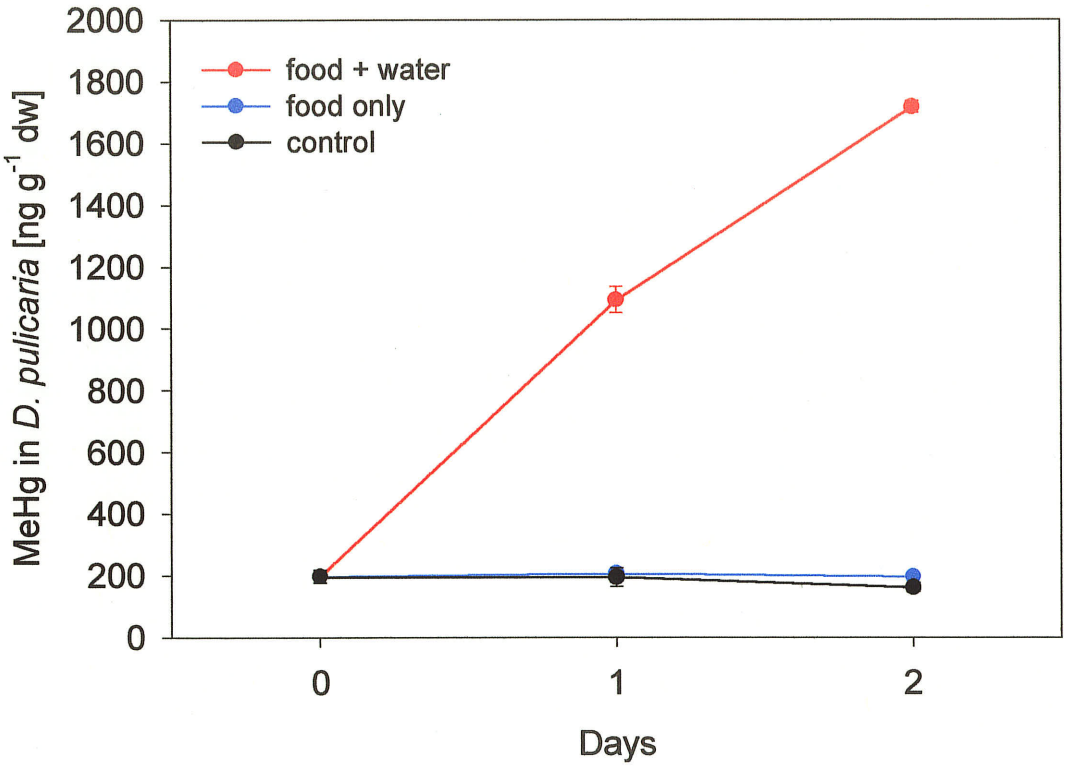

Figure 6. Average changes in zooplankton MeHg concentrations. Samples were taken at 0, 24 and 48 h. Error bars represent standard error (n = 3 for each point on graph).

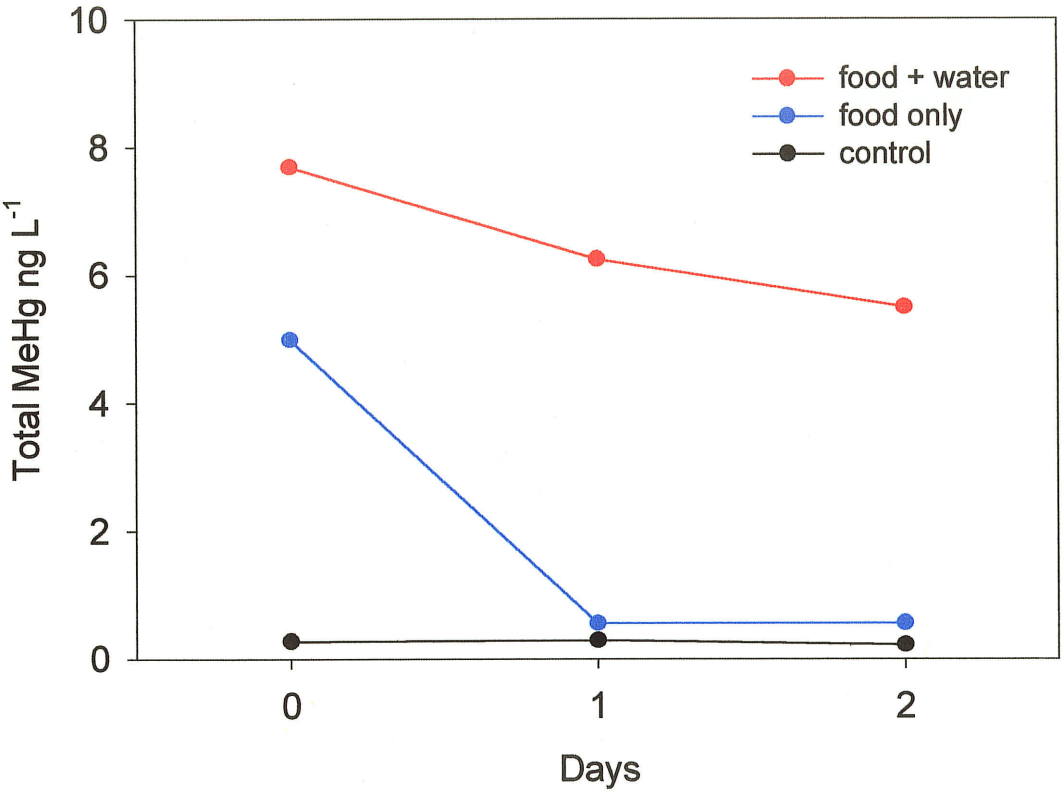

Figure 7. The sum of MeHg L^{-1} in algae, water and zooplankton from each of the three treatments.

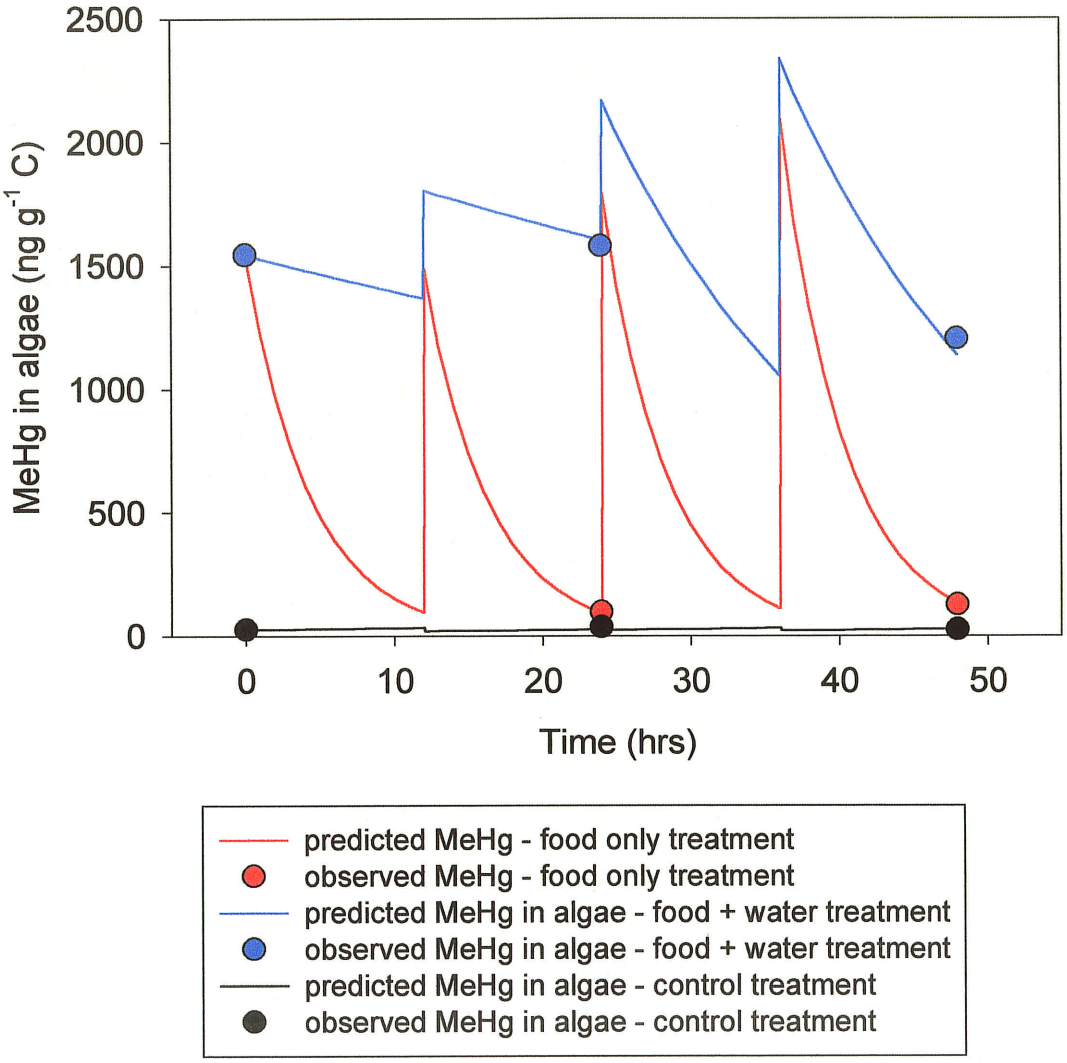

Figure 8. Changes in algal MeHg concentrations in the food + water, food only and control treatments calculated using the model described in the text.

General Discussion, Implications and Recommendations

Methylmercury (MeHg) in zooplankton is studied because of its importance in the diet of fish. Concentrations of MeHg in fish lead to MeHg contamination in humans that rely on fish for food. It is evident from numerous studies that MeHg concentrations in fish are the result of interactions at the base of the food chain between MeHg concentrations in water, primary consumers and zooplankton. Moreover, water chemistry variables such as pH, and DOC can have effects on the amount of MeHg produced as well as the amount of MeHg available for uptake by organisms. Therefore, I indicate areas where future research should focus to strengthen understanding of MeHg dynamics in the lower food web.

From the feeding experiment in Chapter 2, I determined that food may not be important for determining MeHg concentrations in *Daphnia*. This suggests that the concentration of MeHg in *Daphnia* may be directly related to the bioavailable fraction of MeHg in water. Strong correlations between MeHg in water and MeHg in zooplankton may strengthen the argument that concentrations of MeHg in zooplankton are indicative of those in water (Watras *et al.* 1998). Questions remaining to be answered include whether all MeHg in water is bioavailable or whether there are certain fractions that cannot be taken up by *Daphnia*. In addition, what affects the supply of bioavailable MeHg in water? We know that increased methylation rates in flooded areas may affect the amount of MeHg in the water column (Kelly *et al.* 1997). In addition, pH and DOC may affect the amount and

availability of MeHg for zooplankton (Watras and Bloom 1992; Back and Watras 1995; Garcia and Carignan 1999). Although no relationship was observed between MeHg concentrations in zooplankton and concentrations of DOC in the FLUDEX reservoirs, when DOC concentrations and MeHg concentrations are included from a wide range of lakes at ELA, a strong negative relationship exists between the two variables (M. J. Paterson, Freshwater Institute – unpublished data). These results indicate the limitations of concluding biological relationships from a narrow range of field data. Conceivably, relationships between MeHg and chemistry variables could be tested in a laboratory experiment with zooplankton to see whether or not uptake is affected by changes in MeHg speciation in the dissolved phase as well as by changes in pH and DOC concentrations.

The question remains whether results of the feeding experiment can be applied to natural populations of zooplankton like those in the FLUDEX reservoirs. It was only feasible to study MeHg dynamics in one species of zooplankton using only one species of algae as a food source. In the FLUDEX reservoirs, zooplankton populations, although frequently dominated by *Daphnia* spp., were made up of a variety of zooplankton species. These species may accumulate different amounts of MeHg (Watras *et al.* 1998; Rask *et al.* 1994) and may have different physiological characteristics affecting the amount of MeHg accumulated. Some zooplankton species may be more efficient at accumulating MeHg than other species, thereby affecting how much MeHg is available for MeHg movement in the food web (Chen et al. 2000). Differences in size, growth rates, feeding habits, assimilation rates and

depuration rates may all play a role in determining how much MeHg enters the food web through zooplankton.

The FLUDEX reservoirs contained a variety of phytoplankton species and it has been noted that uptake of MeHg among different species of algae can occur at different rates (Moye et al. 2002). My research do not suggest that the trophic route of MeHg uptake can be discounted for zooplankton in the FLUDEX reservoirs because there is still a lack of understanding of processes occurring in individual zooplankton species of the larger zooplankton assemblage. Future studies should therefore focus on MeHg accumulation efficiencies in different species of zooplankton as well as different species of phytoplankton. For example, the feeding experiment in Chapter 2 should be repeated for other species of zooplankton that have different physiological attributes than those of *Daphnia* to provide support for the results obtained in Chapter 2.

Pickhardt *et al.* (2002) observed decreases in MeHg concentrations in phytoplankton with increases in algal biomass. As the density of algal cells increased, MeHg per cell of algae decreased, essentially "biodiluting" MeHg within the algal community. MeHg concentrations in some species of zooplankton may also decrease over the course of their lifetime by means of growth dilution. Growth dilution of MeHg has been noted in other species in the lower food web (Visman 1995) and it is reasonable that the same processes may occur in certain species of zooplankton. The importance of biodilution and its effects on MeHg accumulation in zooplankton should be examined in field situations to fully understand how these

processes affect MeHg accumulation in the food web. Research should also focus on whether differences in zooplankton MeHg concentrations due to growth dilution affect MeHg accumulation in fish.

Changes in the length and complexity of food webs may alter MeHg concentrations in predatory fish in Canadian lakes (Cabana *et al.* 1994). Mercury levels in predatory fish from lakes with longer food chains often have higher Hg concentrations than fish from lakes with fewer trophic levels (e.g. the absence of *Mysis relicta* and/or forage fish). These results have implications for food-web dynamics in newly formed reservoirs where large changes in zooplankton species composition and biomass occur immediately after flooding (Chapter 1). It is possible that the length of the pelagic food web in newly formed reservoirs can change after flooding due to the presence or absence of particular zooplankton species that may act as a source of food for planktivorous fish. These changes in trophic-level complexity may account for some of the variability in MeHg concentrations seen in predatory fish from various reservoirs. Characteristics of the different trophic levels such as physiology and growth rates may affect MeHg concentrations and may also be important to consider for MeHg biomagnification. For example, if a particular species of zooplankton accumulates more or less MeHg than another species because of physiological differences or growth dilution, the presence or absence of that particular species in the food web may affect the amount of MeHg taken up by fish.

When discussing MeHg dynamics in the food web throughout this thesis, I often assumed that zooplankton were the main source of MeHg for planktivorous

fish. This assumption implies that the "food *chain*" is linear which oversimplifies the complexity of the "food *web*" which is found in natural systems. There are many possible routes of MeHg movement in natural systems, leading to elevated concentrations in higher organisms (Fig. 1). It may not be sufficient to consider only zooplankton as the source of MeHg in planktivorous fish when it is also possible that they receive a large portion of MeHg from larval invertebrates such as *Chaoborus* spp. or Chironomid spp. It is also apparent from Fig. 1 that various zooplankton species may accumulate different amounts MeHg from different food sources including water, algae, bacteria or particulates. It is important to consider all of these trophic routes until experiments like those in Chapter 2 can rule out specific exposure routes for individual zooplankton species.

In order to provide true replication of the results described in Chapter 1, a duplicate set (or more) of upland reservoirs would have to be created. The lack of statistical tests from the lack of replication in whole-ecosystem studies is often overlooked by the amount of useful information arising from large-scale ecological studies. The purpose of most large-scale experiments is to provide information on processes and interactions occurring on an ecosystem level; it is often hard to replicate realistic interactions in small laboratory or mesocosm studies. In the FLUDEX experiment, the goal was to simulate processes and interactions occurring in shallow areas of real hydroelectric reservoirs. It is evident from Table 1 in the General Introduction and from results in Chapter 1 that MeHg concentrations occurring in zooplankton from the FLUDEX reservoirs are similar to observed

dynamics in zooplankton from larger reservoirs. These similarities allow results from FLUDEX to be used in predicting and possibly mitigating MeHg problems in real reservoirs.

Conclusions

According to R. B. Brennan, President and Chief Executive Officer of Manitoba Hydro, the future of hydroelectric energy growth in Manitoba looks promising. In a report released in November of 2001, he wrote:

> "Over the last 12 years, revenues from electricity exports have multiplied eight-fold, reaching an all-time high this year with over $480 million in sales...Manitoba Hydro is also continuing to study three potential sites for hydroelectric generation in the north, the Wuskwatim and Notigi sites along the Burntwood River system and the Gull Rapids site on the Nelson River. The development of one or more of these sites will further enhance Manitoba Hydro's ability to take advantage of the lucrative market for electricity in the United States. ("Insights", November, 2001. Manitoba Hydro website: http://www.hydro.mb.ca/news/insights/insights_01_11.shtml).

The proposed increases in hydroelectric exports and the possible expansion of hydroelectric generation sites in the near future suggests an increased need for new hydroelectric reservoirs in northern Manitoba. The flooding of new areas brings with it environmental, social, and economic concerns, which is why studies such as those described in this thesis are important and must continue. The FLUDEX study has provided valuable information on MeHg dynamics in sediments, water, and the

upper and lower food web after three years of flooding. Further sampling programs are planned in the reservoirs in upcoming years and I anticipate more information will be obtained about the effects of flooding in boreal forest regions.

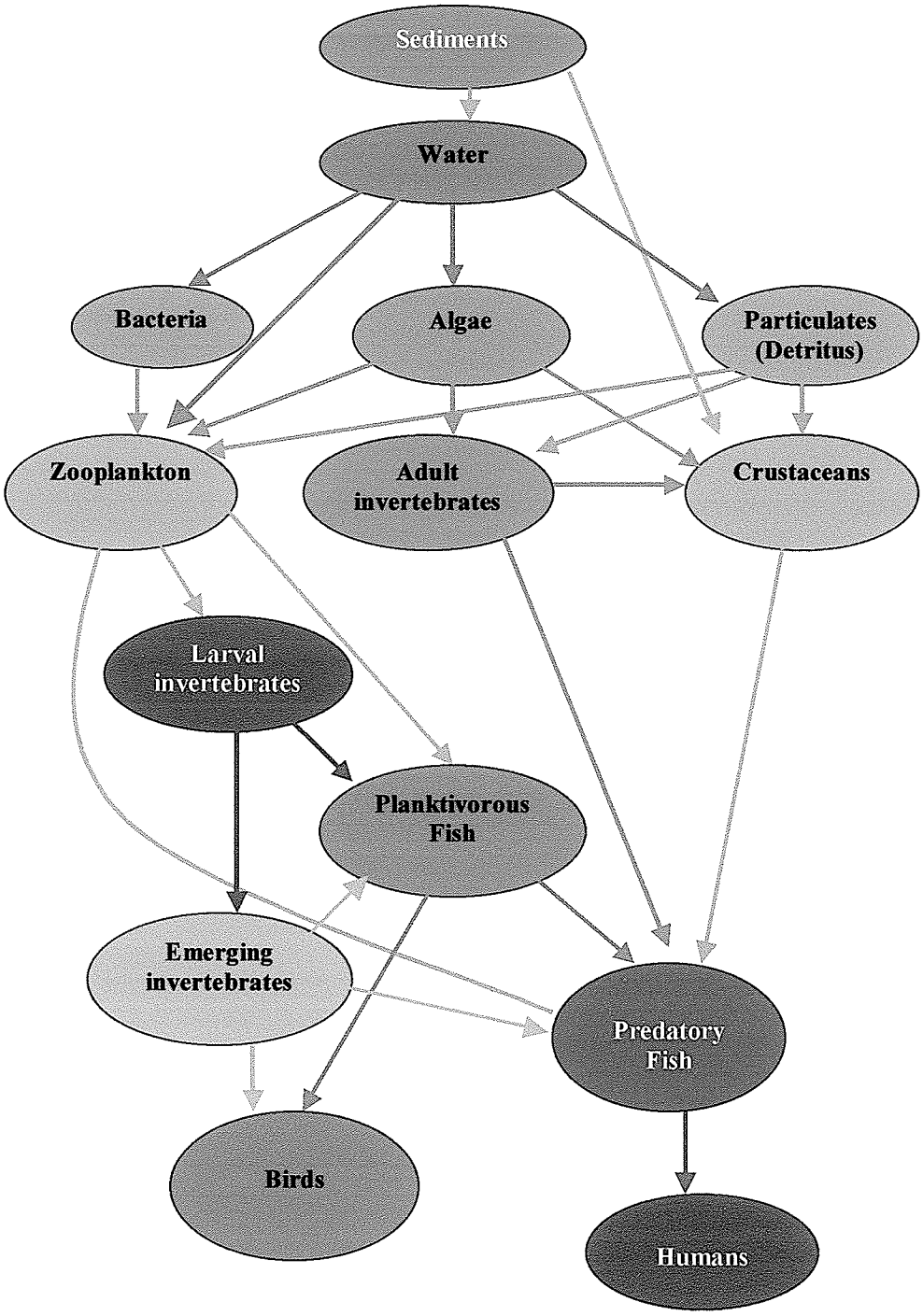

Figure 1. Potential food web in a newly formed reservoir, showing paths of MeHg transfer.

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