The elimination of mercury by fish in the wild

By

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A Thesis Submitted In Partial Fulfillment
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Abstract

The rate of mercury elimination by fish i) is important to recovery of ecosystems from mercury contamination and ii) remains an uncertainty in mercury models due to a lack of studies under natural conditions. I addressed this problem by monitoring fish that had naturally accumulated an enriched stable isotope of mercury (spike mercury) through a whole-ecosystem experiment at Lake 658. Yellow perch (*Perca flavescens*) and northern pike (*Esox lucius*) were transported from Lake 658 to a "clean" lake, and spike mercury losses were monitored. Both species exhibited prolonged transfer of mercury into the muscle and slower losses of mercury (half-lives of 489 and 796 days respectively) when compared to past laboratory studies. A separate study on yellow perch found similar elimination rates in a lake and a reservoir. The findings presented in this thesis can improve mercury models as well as further define mercury kinetics in wild fish.

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1.1. The problem of mercury in fish

Exposure to methylmercury (an organic form of mercury) can have negative effects on human health. Symptoms of poisoning by methylmercury include numbness of the limbs, loss of hearing, and visual problems (reviewed in Clarkson 1993; Galli and Restani 1993). The accumulation of high levels of methylmercury can cause severe neurological damage, paralysis, coma, and death (reviewed in Clarkson 1993; Galli and Restani 1993). These symptoms were observed in both Minamata, Japan, and Grassy Narrows, Ontario, where the local people consumed fish highly contaminated by industrial discharges of mercury (Wheatley and Paradis 1995; Sakamoto et al. 2001).

Large industrial discharges of mercury directly into water bodies are rare, and as a result, most lakes in Canada and around the world do not have fish with methylmercury concentrations as high as those found in Minamata Bay or Grassy Narrows. Nevertheless mercury concentrations in fish from many pristine Canadian lakes can be high enough to potentially cause health problems (O.M.O.E. 2005). Fish consumption advisories (e.g. U.S.E.P.A. 2004; O.M.O.E. 2005) have been developed to protect people from consuming potentially dangerous amounts of methylmercury, and have raised awareness of this problem. For example, 32% of Ontario inland lakes monitored have consumption restrictions, 93% of which are due to mercury (O.M.O.E. 2005).

Mercury contamination of fish results from both geologic and anthropogenic sources of mercury to lakes (reviewed in Fitzgerald and Lamborg 2003). Rock types that are rich with mercury tend to occur in regions where there is collision between plates of the earth's crust (reviewed in Fitzgerald and Lamborg 2003). In addition, mercury that is

released into waterways or the atmosphere from anthropogenic sources can eventually end up in lakes (Mason et al. 1994). Currently, two-thirds of the mercury in the atmosphere is thought to be of anthropogenic origin (Mason et al. 1994).

In recognition of the global mercury problem, efforts are being made to decrease mercury inputs into the environment in Canada and around the world (Trip and Allan 2000; Environment Canada 2004). A fundamental yet unknown characteristic is how long it will take for fish mercury levels to decline in response to reduced mercury inputs into the environment. A key factor in this decline is the rate that fish lose mercury (Lockhart et al. 1972; Laarman et al. 1976). The elimination of mercury by fish under natural conditions has not been thoroughly studied and remains an area of uncertainty in predictive models used to simulate mercury concentrations in fish (Trudel and Rasmussen 1997). The studies presented in this thesis are on the elimination of mercury by two freshwater fish species under field conditions using naturally accumulated "labeled" mercury. The approach taken for this study is unique and the results are valuable to the understanding of mercury elimination by fish.

This introductory chapter will briefly describe the pathways of mercury into and within lakes, exposure of fish to mercury, and factors that affect mercury concentrations in fish. The way that fish distribute mercury among their tissues before it is eliminated is described in detail. Finally, the approach and objectives of this thesis are presented.

1.2. Mercury sources to lakes

The mercury cycle involves mercury in three oxidation states (Hg^0, Hg^{1+} and Hg^{2+}) (Wiener et al. 2003). In the atmosphere, most mercury is in the form of Hg^0 which can

be oxidized to Hg^{2+} and then deposited on land and aquatic systems in this form (reviewed in Wiener et al. 2003). Mercury in water and in sediment is primarily in the form of Hg^{2+} and is often bound to ions or molecules (Wiener et al. 2003). An organic form of mercury, methylmercury, is the dominant form found in fish (Bloom 1992) and is also highly toxic to humans (Clarkson 1997). Throughout this thesis, methyl, inorganic (which is Hg^{2+}) and total mercury are referred to. Total mercury is the sum of all mercury compounds including inorganic mercury and methylmercury.

Mercury enters lakes through atmospheric deposition, terrestrial runoff, and river inputs. Atmospheric deposition of mercury can occur through rain or particles falling on the surface of a lake (Mierle 1990; Glass et al. 1991; Mason and Sullivan 1997). Inorganic mercury is the dominant form (approximately 99%) entering lakes from the atmosphere (Watras et al. 1994). Inorganic mercury from either the atmosphere or geologic sources is also present on plants and soil, and can be washed into lakes (Evans 1986; Mierle 1990). In addition, rivers or inflows from other aquatic systems can be a source of inorganic mercury and methylmercury to lakes (Mierle 1990; Mason and Sullivan 1997; Babiarz et al. 1998). The relative importance of the different routes of entry into a particular lake depends on both the surface area of the lake and its watershed, combined with the concentration of mercury in the different sources to that lake (Evans 1986). When inorganic mercury enters the water column of a lake, it is thought to undergo one or more of the following processes: i) binding to particles and sinking to the sediments, ii) methylation leading to absorption by biota, or iii) reduction to Hg^0 and loss across the water surface into the atmosphere (Watras et al. 1994).

The major pools of mercury within a lake are the water, sediment, and biota. The mercury burden is an estimate of the mass of mercury present in a compartment and is the product of the mercury concentration and compartment mass (Watras et al. 1994). As an example, the burdens of total and methylmercury in sediment, water, and biota are shown for Little Rock Lake, Wisconsin (Table 1.1) (Watras et al. 1994).

There is both inorganic and methylmercury present in lake water (Watras et al. 1994). Watras et al. (1994) found that there was approximately 10 times more total mercury than methylmercury in the water column of Little Rock Lake (Table 1.1). The concentration of mercury in water is dilute compared to concentrations in sediment and biota (Watras et al. 1994; Wiener et al. 2003). Lakes without a large source of contamination have mercury concentrations in water ranging from 0.3 - 8 ng/L total mercury and 0.04 - 0.8 ng/L methylmercury (reviewed in Wiener et al. 2003). When a large source of industrial contamination is present, concentrations in water can range from 10 - 40 ng/L total mercury and from 1 - 2 ng/L methylmercury (reviewed in Wiener et al. 2003).

Most of the mercury burden in a lake is present in the sediment and is primarily in the form of inorganic mercury (Watras et al. 1994) (Table 1.1). Inorganic mercury that has entered a lake may be deposited on the surface sediments (Lockhart et al. 1995). Concentrations of total mercury in natural lake sediments vary widely from 0.7 - 700 ng/g dry weight (d.w.) depending on depth, substrate, organic matter and sedimentation rates (Evans 1986; Rada et al. 1993; Lockhart et al. 1995; Bowles et al. 2002). Sediment can act as a sink for mercury that has entered the lake (Rada et al. 1993; Lockhart et al. 1995) as well as a source of mercury to the water and biota (Francesconi et al. 1997).

The surface sediments are the main location where inorganic mercury is converted to the more toxic form, methylmercury (Gilmour et al. 1992). Sulfur-reducing bacteria are thought to perform this conversion by biologically methylating inorganic mercury to form methylmercury (Gilmour et al. 1992). Methylation can be enhanced by decaying organic matter, anoxia, low pH, a presence of sulfate, and warm temperatures (Furutani and Rudd 1980; Regnell 1990; Gilmour et al. 1992; Miskimmin et al. 1992; Kelly et al. 1997; Heyes et al. 2000).

A third major pool of mercury in lakes is in biota, where methylmercury can reach its highest concentrations within a lake (Watras et al. 1994). Aquatic organisms absorb both methylmercury and inorganic mercury through water and diet (Huckabee et al. 1975; Boudou and Ribeyre 1983; Post et al. 1996; Hall et al. 1997; Tsui and Wang 2004). Methylmercury is more readily absorbed and retained by invertebrates (reviewed in Huckabee et al. 1975; Wiener et al. 2003; Tsui and Wang 2004) and fish (Pentreath 1976a; Boudou and Ribeyre 1983; Trudel and Rasmussen 1997) than inorganic mercury. The organisms in a lake contain most of the methylmercury in the entire system (Watras et al. 1994). For example, the biota in Little Rock Lake contained approximately 75% of all the methylmercury and less than 18% of the total mercury present in the lake (Watras et al. 1994) (Table 1.1).

In summary, mercury enters a lake from the atmosphere or watershed primarily in the inorganic form (Watras et al. 1994; Wiener et al. 2003). Inorganic mercury in the water column binds to particles and sinks to the surface sediments, where it becomes methylated by sulfur-reducing bacteria (Gilmour et al. 1992). Lake biota readily

accumulate methylmercury (Boudou and Ribeyre 1983), and most of the methylmercury present in a lake is located in biota (Watras et al. 1994).

1.3. Methylmercury levels in fish

There are many factors that determine methylmercury levels in fish, including their age, size, growth, and metabolic rate (Norstrom et al. 1976; Braune 1987; Doyon et al. 1998; Stafford and Haines 2001). The number of trophic levels and methylmercury concentrations in water and prey can determine concentrations of methylmercury that fish are exposed to (MacCrimmon et al. 1983; Cabana et al. 1994; Kelly et al. 1997; Bowles et al. 2001). Factors that determine methylmercury concentrations in fish are described below.

1.3.1. Age, size, growth, and metabolic rate

Fish take in methylmercury by eating prey and passing water over their gills due to a presence of methylmercury in prey and water. Food is the primary route of methylmercury exposure to fish, accounting for approximately 85% of all methylmercury uptake (Hall et al. 1997). Mercury models support this conclusion; due to low concentrations of methylmercury in water (<1 ng/L), even with constant respiration, uptake over gills is not likely to account for more than 10% of methylmercury absorption by fish (Harris and Snodgrass 1993).

Fish absorb mercury quickly and lose it slowly, causing concentrations to increase over time, which is a process known as bioaccumulation (Huckabee et al. 1979). The percentage of mercury that is absorbed into the bloodstream (referred to as assimilation

efficiency) is higher for methylmercury than for inorganic mercury (Riisgard and Hansen 1990). For example, Riisgard and Hansen (1990) estimated that flounder (*Platichthys flesus*) assimilated 34% of methylmercury ingested, but only 1% of inorganic mercury ingested. Similarly, Pentreath (1976a) found that plaice (*Pleuronectes platessa*) assimilated 80 - 100% of methylmercury ingested but only 3 - 8% of inorganic mercury ingested. In addition, past studies have found that inorganic mercury is lost approximately 1.8 times more quickly than methylmercury (Trudel and Rasmussen 1997). Because methylmercury is absorbed more completely and retained longer than inorganic mercury, methylmercury bioaccumulates to a greater extent than inorganic mercury (Riisgard and Hansen 1990). As a result, almost all mercury in fish is in the form of methylmercury (Bloom 1992). Many studies on mercury in fish only measure total mercury concentrations and assume that most of it is in the form of methylmercury. Throughout the remainder of this thesis, reference to mercury in fish usually implies total mercury unless specifically indicated otherwise.

Bioaccumulation usually causes increasing mercury levels in fish with age (Huckabee et al. 1979). Fish are exposed to mercury over their lifetime and therefore mercury burdens are positively related to age (Mathers and Johansen 1985; Braune 1987; Doyon et al. 1998). In addition to age, mercury often has a positive relationship to size of the fish (e.g. Cizdziel et al. 2002). As fish increase in size, they consume larger prey (MacCrimmon et al. 1983; Mathers and Johansen 1985). Larger prey tend to be older and therefore have accumulated more mercury over their lifetime than smaller, younger prey (Huckabee et al. 1979). Both fish size and age have a joint positive relationship with mercury concentration, with age being a better predictor than size (Braune 1987).

Fish growth rate has been shown to influence mercury concentrations (MacRury et al. 2002). Mercury concentrations can decrease as a result of fast growth, which is termed growth dilution because the increase in tissue mass dilutes the existing mercury concentration (Doyon et al. 1998). Fish gain both mercury and energy from their diet, and the energy is used for metabolism, wastes, and growth (Norstrom et al. 1976; Hanson et al. 1997). Increases in energy allocated to growth result in lower mercury concentrations due to growth dilution (e.g. Braune 1987). For example, Braune (1987) found that within an age class, growth dilution caused a negative relationship between size and concentration.

The opposite of growth dilution occurs where loss in fish weight results in increased mercury concentration (Cizdziel et al. 2002). When fish use muscle tissue for energy during starvation, muscle mass will decrease without loss of the mercury present in the muscle (Cizdziel et al. 2002). A decrease in muscle mass without a similar decrease in mercury burden causes mercury concentrations to increase, and has been dubbed "starvation concentration" (Cizdziel et al. 2002).

Mercury intake is positively related to fish metabolic rate (Hanson et al. 1997). An increase in metabolic demands requires an increase in feeding and gill respiration (Norstrom et al. 1976; Hanson et al. 1997). Because fish obtain mercury through food and water, a high rate of food consumption and respiration results in greater exposure to mercury (Norstrom et al. 1976). As described earlier, fish allocate energy to metabolism, wastes, and growth (Hanson et al. 1997). Another way in which metabolic rates can impact mercury concentrations is when a high metabolic demand leaves less energy for growth, leading to less growth dilution (as suggested by Braune 1987). Therefore, fish

with a high metabolic rate could have elevated mercury concentrations for two possible reasons: greater intake of food and water, which are routes of mercury exposure (Norstrom et al. 1976), and a decrease in available energy for growth resulting in less growth dilution for a given intake of mercury (Braune 1987).

1.3.2. Food web

Mercury undergoes biomagnification in the food web (Jernelov and Lann 1971; Kidd et al. 1995; Mason and Sullivan 1997; Bowles et al. 2001), which means that mercury concentrations increase with each trophic level. The greatest increase in mercury levels within a food web is from the water to phytoplankton, where concentrations typically increase by 10^4 times (Table 1.2). Mercury concentrations are generally highest in piscivorous fish and can be up to approximately 10^6 times greater than in water, even in pristine sites (Jernelov and Lann 1971; Bowles et al. 2001) (Table 1.2). As a result of biomagnification, the concentration of mercury in muscle of piscivorous fish can surpass 500 ng/g, which is the maximum concentration allowable for sale of commercial fish in Canada (Health Canada 2002). For example, Bowles et al. (2001) found that despite low mercury concentrations in water (1.42 ng/L total mercury and 0.067 ng/L methylmercury), concentrations of methylmercury in piscivorous fish ranged from 332 - 458 ng/g. One of the reasons why the methylmercury concentrations were so high in this case was that mercury biomagnified over four trophic levels (Bowles et al. 2001). Similarly, Cabana et al. (1994) found that mercury concentrations in lake trout (*Salvelinus namaycush*) were higher when they fed at higher trophic levels (feeding on forage fish or predatory invertebrates), than when they fed directly on zooplankton.

Because methylmercury bioaccumulates and biomagnifies to a much greater extent than inorganic mercury, the percentage of total mercury in organisms present as methylmercury increases with trophic level (Table 1.2). For example, total mercury in phytoplankton is typically <15% methylmercury while mercury in fish is >90% methylmercury (Table 1.2).

Methylmercury levels in fish are known to increase in response to high methylmercury levels in the environment (e.g. Kelly et al. 1997). Elevated methylmercury levels in the food web can result from loading of inorganic mercury into a waterway, such as in the pollution of the Wabigoon-English River system in northwestern Ontario (Lockhart et al. 1972; Armstrong and Scott 1979), or from increased rates of mercury methylation (Kelly et al. 1997). Following the release of approximately 10 metric tonnes of inorganic mercury to the Wabigoon-English River (reviewed in Parks and Hamilton 1987), methylmercury concentrations in northern pike (*Esox lucius*) muscle increased to a maximum concentration of 8000 ng/g (Lockhart et al. 1972), which is 16 times greater than the limit for commercial sale. In one case where mercury methylation was enhanced through flooding, mercury concentrations in the muscle of northern pike increased from 250 - 350 ng/g to 670 - 950 ng/g (Bodaly et al. 1984). These two examples show that elevated concentrations of mercury in the environment can lead to elevated levels in fish.

Age, size, metabolic rate, biomagnification and concentration of mercury in the environment all impact the exposure of fish to mercury. The amount of mercury in a fish ultimately depends on the balance between the amount taken in and the amount eliminated (Norstrom et al. 1976). Accurate estimates of elimination are required to

understand bioaccumulation under different levels of mercury exposure (Braune 1987; Harris and Snodgrass 1993; Rodgers 1994). For remediation efforts, elimination rates are key to determining how long it will take for mercury levels in fish to decline after exposure has been reduced. Mercury elimination by fish greatly depends on where mercury is located within the fish, and how mercury is moved around inside the fish. The kinetics of mercury within fish are described below.

1.4. Kinetics of mercury within fish

When fish absorb methylmercury through either diet or water (direct) exposure, methylmercury enters the bloodstream and is transferred to tissues. Mercury is assimilated across the gills during direct exposure and across the intestine during dietary exposure (Boudou and Ribeyre 1983). Mercury that is not assimilated through the intestine is eliminated in feces. Once in the blood, methylmercury binds to red blood cells (Schultz and Newman 1997) and is transported to the organs of the fish (Boudou and Ribeyre 1985; Harrison et al. 1990; Oliveira Ribeiro et al. 1999). The distribution of mercury within fish does not change with the route of exposure, except that concentrations are high in gills following direct exposure and in the intestine following dietary exposure (Boudou and Ribeyre 1983). After the initial distribution of mercury among fish tissues, there is subsequent redistribution into muscle. This redistribution involves movement of mercury out of the initial location into blood (Oliveira Ribeiro et al. 1999). The blood then carries mercury to muscle, which is the site of long-term storage (Giblin and Massaro 1973; McKim et al. 1976; Boudou and Ribeyre 1983).

The location of mercury within a fish depends in part on how quickly the compound is redistributed among tissues. Because blood transfers mercury among tissues, the rate that mercury moves into or out of blood from particular tissues is important in the process of redistribution. In sheepshead minnows (*Cyprinodon variegatus*) methylmercury is transferred from gills to blood at the same rate as transfer in the opposite direction, from blood to gills (Leaner and Mason 2004). In contrast, the rate that methylmercury is transferred into muscle is approximately 30 times faster than the rate of transfer out of muscle (Leaner and Mason 2004). Sulfur-containing proteins that are prevalent in muscle tightly bind methylmercury (Galli and Restani 1993; Cizdziel et al. 2002), which is why movement of methylmercury out of muscle occurs slowly. As a result, methylmercury is stored in the muscle of fish (Giblin and Massaro 1973; McKim et al. 1976; Boudou and Ribeyre 1983).

To determine where methylmercury moves within fish following exposure, Oliveira Ribeiro et al. (1999) exposed arctic charr (*Salvelinus alpinus*) to a single dietary dose of radioactive methylmercury (^{203}MeHg) and followed movement through tissues of the fish. They determined that kinetics of methylmercury within fish could be described by a three-compartment model (Oliveira Ribeiro et al. 1999) (Figure 1.1). Methylmercury first entered the gut (first compartment), then was assimilated into blood and viscera (second compartment) and finally was transferred to the final compartment called "rest of body", which was 90% muscle (Oliveira Ribeiro et al. 1999) (Figure 1.1). This study found that it took 48 days for most of the methylmercury to be relocated into the "rest of body" compartment (Oliveira Ribeiro et al. 1999). In a similar study of methylmercury kinetics, Giblin and Massaro (1973) monitored ^{203}MeHg movement in

rainbow trout (*Oncorhynchus mykiss*) following exposure to a single dose of ^{203}MeHg. They found that the concentrations in viscera and blood peaked at 7 days and then declined, while concentrations in muscle increased for the first 34 days and then leveled off for the remaining 66 days (Giblin and Massaro 1973). Both studies showed a redistribution of methylmercury from viscera and blood to muscle tissue (Giblin and Massaro 1973; Oliveira Ribeiro et al. 1999).

Methylmercury storage in muscle may have developed to protect the nervous system of fish from exposure to toxic methylmercury (Wiener and Spry 1996). In a review of the literature, Wiener and Spry (1996) suggested that fish have more physical defenses against inorganic mercury than methylmercury. It is thought that fish protect against exposure to inorganic mercury by quick elimination and minimal absorption across the gastrointestinal tract (Pentreath 1976a; Boudou and Ribeyre 1983). In contrast, methylmercury is efficiently absorbed into the bloodstream and is excreted slowly (Pentreath 1976a). Redistribution of methylmercury to muscle may reduce toxicity to fish by shunting the compound away from critical areas such as the nervous system or reproductive organs (Wiener and Spry 1996).

1.5. Elimination of mercury by fish

Elimination of mercury may be facilitated through demethylation (which involves cleaving the methyl compound from inorganic mercury) in viscera (Burrows and Krenkel 1973). There is evidence that demethylation occurs in the liver and kidney followed by elimination of the resulting inorganic mercury through feces or urine (Burrows and Krenkel 1973; McKim et al. 1976; Riisgard and Hansen 1990). A similar process occurs

in the intestine of mammals where methylmercury is transformed to inorganic mercury before it is excreted (Norseth and Clarkson 1971). The dominant pathway of mercury elimination from fish is thought to be through wastes (Giblin and Massaro 1973); however, it has been suggested that some mercury may also be eliminated over the gills (Giblin and Massaro 1973), or through eggs during spawning (McKim et al. 1976).

Elimination of methylmercury in laboratory experiments is biphasic, with a fast and slow phase (Jarvenpaa et al. 1970; Burrows and Krenkel 1973; Giblin and Massaro 1973; Ruohtula and Miettinen 1975). The slow phase of methylmercury elimination includes the loss from a component of the fish where methylmercury is tightly bound (such as to sulfur proteins as suggested by Oliveira Ribeiro et al. 1999). In contrast, the fast phase of methylmercury loss results from mobilization and loss of methylmercury that is easily removed from a biological compartment. The fast phase occurs in approximately 20 days, while the slow phase can have a half-life (time for the burden to be reduced by half) that ranges from 204 - 1040 days depending on the study (Trudel and Rasmussen 1997). Ultimately, the fast phase is a combination of loss from feces and urine, the external slime coat, blood and viscera, while the slow component includes loss from muscle (Burrows and Krenkel 1973; Giblin and Massaro 1973; DeFreitas et al. 1975; Schultz and Newman 1997).

In nature, fish are likely to eliminate most of their mercury by the slow phase (Trudel and Rasmussen 1997). Fish chronically exposed to mercury are able to reach steady state where the relative concentration among different biological compartments does not change over time (Oliveira Ribeiro et al. 1999). During steady state most of the

mercury in the fish is bound in muscle and almost all elimination occurs slowly (Rowan and Rasmussen 1995; Trudel and Rasmussen 1997; Oliveira Ribeiro et al. 1999).

Most previous attempts to quantify mercury elimination by fish have been done in a laboratory setting. Past laboratory studies may have overestimated elimination rates if fish did not reach a steady state following contaminant exposure (de Boer et al. 1994; Rowan and Rasmussen 1995). Laboratory studies commonly involved a period of mercury exposure followed by a decontamination period. Unless the exposure to mercury during the contamination period is chronic, the fish involved in these studies would not have reached a steady state of mercury redistribution among their tissues prior to the decontamination period (suggested by de Boer et al. 1994; Rowan and Rasmussen 1995). If the exposure time was short, more of the mercury present in the fish would be located in the viscera and blood (Oliveira Ribeiro et al. 1999). As a result, more of the mercury would be lost through the fast phase and the rates of mercury elimination from the whole fish would be overestimated (as suggested by de Boer et al. 1994). In contrast, Trudel and Rasmussen (1997) found that experiments with chronic exposure resulted in elimination rates that were 2 times faster than studies with short-term exposure. The chronic exposure studies were based on limited data for large fish, and the effects of size on this relationship are not clear (M. Trudel, personal communication).

Due to the biphasic nature of mercury elimination by fish, the timing and length of experiments can affect estimates of elimination rates (Trudel and Rasmussen 1997). Studies should be at least 90 days in length to distinguish between the fast and slow phases of elimination, which is necessary for accurately estimating elimination rate (Trudel and Rasmussen 1997). Only one-third of past methylmercury elimination studies

were greater than 90 days in length, which suggests that most previous studies may have overestimated elimination rates (Trudel and Rasmussen 1997).

1.5.1. Effects of body size, temperature, and metabolism on mercury elimination

It is generally understood that methylmercury elimination rates are negatively correlated with weight of the fish, both within and among species (DeFreitas et al. 1975). Sharpe et al. (1977) found decreasing rates of elimination with increasing body size of goldfish (*Carassius auratus*). A comparison of methylmercury elimination rates of two fish species that commonly occur together illustrates this point further. The half-lives of methylmercury elimination are generally 2 - 10 times longer in northern pike than in their prey, yellow perch (*Perca flavescens*) (Table 1.3). This pattern is consistent with other contaminants (Hendriks 2001) including elimination of zinc from mosquitofish (*Gambusia affinis)* (Newman and Mitz 1988) and elimination of ^{137}Cs from a variety of fish species (Rowan and Rasmussen 1995). De Boer et al. (1994) suggested that the rate of contaminant elimination is negatively related to size because small fish have more surface area over which contaminants can be eliminated for a given volume of tissue. In addition, smaller fish would eliminate mercury faster than larger fish if the elimination process was related to metabolic rate; this possibility is discussed later.

Mercury elimination rates increase with temperature in studies that are longer than 90 days (Trudel and Rasmussen 1997). In a 120-day study, Ruohtula and Miettinen (1975) found that the half-life of $^{203}MeHg$ was, on average, 516 days for rainbow trout in cold water (0.5 - 4°C) compared to 348 days when fish were kept at higher temperatures (16 - 19°C). In contrast, Sharpe et al. (1977) found that goldfish of similar body weight

did not eliminate ^{203}MeHg at different rates when held at 5°, 10°, or 20° C for 60 days. Trudel and Rasmussen (1997) found that studies that were longer than 90 days produced elimination rates that had a positive relationship with temperature (r^2=0.60) while studies that were shorter than 90 days produced rates of loss that were not related to temperature (r^2=0.04). This finding suggests that the short length of the study (60 days) by Sharpe et al. (1977) prevented detection of temperature effects.

The rate at which fish eliminate mercury may increase with metabolic rate, though this has not been confirmed. Because both elimination rates and metabolic rates decrease with greater body size and increase with temperature (Trudel and Rasmussen 1997), elimination rates could be related to metabolic rate (Fagerstrom and Asell 1973). Rodgers and Beamish (1982) found that rainbow trout, presumed to have high metabolic costs from digesting large meals, eliminated mercury faster than the fish fed small meals. There is still uncertainty whether or not metabolism affects elimination rate because there has not been a direct study on the relationship between metabolism and elimination rate of mercury by fish.

1.5.2. Critical differences between past laboratory and field studies

Our current knowledge about mercury elimination from fish tissues is primarily based on laboratory studies. Conditions of laboratory studies differ from the natural environment, and therefore, their results will not always be relevant to natural populations. There were several laboratory experiments in the 1970s that exposed fish to ^{203}MeHg and then measured elimination rates (Jarvenpaa et al. 1970; Burrows and Krenkel 1973; Giblin and Massaro 1973; Ruohtula and Miettinen 1975; Pentreath 1976a; Pentreath 1976b). The

administered mercury concentrations were often higher (commonly 100 times) than would be found in nature and were commonly given as a single dose. Oral exposure often involved force-feeding fish with contaminated food via a stomach tube (Ruohtula and Miettinen 1975), while studies that focused on loss from muscle involved exposure through intramuscular injection (Jarvenpaa et al. 1970; Ruohtula and Miettinen 1975). In laboratory experiments that used a single dose of the contaminant, greater elimination occurred from the fast phase than when fish were chronically exposed in nature (suggested by de Boer et al. 1994; Rowan and Rasmussen 1995). Laboratory studies are useful for understanding mercury kinetics in fish and the effects of variables such as temperature or food consumption. Due to unrealistic methods of exposing fish to mercury, laboratory studies may not reflect the internal dynamics and elimination of mercury by fish in nature.

In the laboratory, fish consume different diets and behave in different ways than in nature (Mann 1978). Due to the potential link between metabolic rates and elimination, it is important to study elimination by fish in as natural a situation as possible. Similarly, it is difficult to mimic the availability and composition of a natural diet in a laboratory study (Mann 1978). Because prey items have unique methylmercury content and assimilation efficiencies (Beamish et al. 1974), field studies are more representative of methylmercury contamination of fish in nature.

There have been two long-term studies of the decline in mercury in fish tissues under natural conditions. Lockhart et al. (1972) transported northern pike from a lake with extremely high mercury levels to a lake with low mercury levels. After monitoring the mercury burdens in these fish for a year, Lockhart et al. (1972) estimated that the

half-life of mercury in pike was approximately 2 years. Laarman et al. (1976) transported yellow perch and rock bass (*Ambloplites rupestris*) containing 1000 ng/g mercury from a contaminated system to ponds with low levels of mercury. Over a 26-month period, the mercury burdens in fish did not drop below initial values (Laarman et al. 1976). The majority of laboratory studies report mercury elimination rates that are much faster than these two field studies. Jarvenpaa et al. (1970) is the exception, which was a 100-day study using outdoor temperatures and large fish.

Studies on other contaminants have indicated that field studies result in slower estimates of elimination rates than laboratory studies. Hamilton et al. (2002) found that estimated loss of selenium by razorback suckers (*Xyrauchen texanus*) was slower in the field (by approximately 1.5 - 3 times) than when compared to laboratory studies for fish of similar size. A field study on yellow perch found that rates of loss of cadmium from fish tissues were approximately 2 times slower than results of comparable laboratory studies (Kraemer et al. 2005). A comparison of studies with chronic and acute exposures to radiocesium indicated that acute exposure led to faster elimination (Rowan and Rasmussen 1995). This finding suggests that field studies, which typically involve chronic exposure, would have slower elimination of radiocesium than laboratory studies (Rowan and Rasmussen 1995). An 8-year study where PCB-contaminated eels (*Anguilla anguilla*) were moved to a lake with lower PCB concentrations found much slower elimination rates than reported by laboratory studies (de Boer et al. 1994). They suggested that laboratory studies had overestimated elimination rates because they were generally short-term with acute exposure to the contaminant and focused on small-bodied fish species (de Boer et al. 1994).

Mathematical mercury models can be used to predict and understand mercury concentrations in fish under different environmental conditions by taking into account fish bioenergetics and mercury exposure (Norstrom et al. 1976; Hanson et al. 1997). Rates of mercury elimination by fish remain a weakness in present models because our knowledge of elimination is primarily based on laboratory studies that are not applicable to nature. Norstrom et al. (1976) relied on elimination rates determined for goldfish in the laboratory to develop a model for 1 - 300 g yellow perch. Although this model was designed for one species at a particular size, it has been widely applied to many different species and field situations, occasionally resulting in inaccurate simulations (e.g. Rodgers 1994). To improve models of mercury elimination, Trudel and Rasmussen (1997) collected 25 of the most realistic estimates of methylmercury elimination rates from the literature to develop a new model. Although this was an improvement, 84% of the estimates used to develop the model by Trudel and Rasmussen (1997) were from laboratory studies. Given the wide use of mercury models as predictive tools, there is a need to increase the accuracy and realism of the elimination component of models using field studies.

1.6. Experimental approach and objectives

The rate at which fish lose mercury is a key part of models that predict mercury bioaccumulation by fish. Unfortunately, mercury elimination rates are poorly understood especially under natural conditions. Past studies on mercury elimination have largely been short-term and conducted in the laboratory with brief durations of exposure using artificial methods and unrealistic mercury concentrations (Trudel and Rasmussen 1997).

As summarized earlier, these methods may lead to overestimates of elimination rates (as suggested by de Boer et al. 1994). Two field studies that monitored mercury elimination under natural conditions involved transfer of fish from mercury-contaminated lakes to cleaner systems (Lockhart et al. 1972; Laarman et al. 1976). The clean lakes used in these studies had lower mercury levels than the contaminated lakes; therefore, mercury elimination was being determined while mercury uptake by fish continued (Lockhart et al. 1972; Laarman et al. 1976). Also, Lockhart et al. (1976) used fish that were contaminated to unusually high levels and it is unknown if their elimination rates apply to fish with lower mercury burdens. An estimate of methylmercury elimination in nature is needed using fish with realistic burdens that are not continually being exposed to methylmercury. The present research is able to i) estimate elimination of isotopically "labeled" mercury by fish, therefore avoiding the problem of continued uptake of mercury, and ii) measure mercury elimination under comparatively natural conditions following chronic exposure and accumulation of "labeled" mercury to realistic concentrations while in the wild.

Mercury is present in low levels in all lakes, even those without large sources of mercury. The background levels of mercury, which will be referred to as ambient mercury, originate from both weathering of geologic sources and atmospheric deposition on lakes and their watersheds (Mierle 1990). In the two field studies on mercury elimination by fish, the understanding of mercury pathways within a lake and its watershed have been obscured by the presence of ambient mercury. Questions concerning how mercury moves into lakes and biota remain unanswered because mercury that had recently entered the system could not be distinguished from previously deposited

mercury (Hintelmann et al. 2002). As a result, the relative contribution of newly deposited and previously deposited mercury to contamination in fish is not known.

A current study aims to answer some of the questions concerning mercury pathways in the environment that could not have been answered in the past. Before this study, there has not been a clear-cut way to monitor newly deposited mercury separately from mercury that has been present for longer in lakes and watersheds. This study is an on-going whole-ecosystem experiment at the Experimental Lakes Area known as METAALICUS (Mercury Experiment To Assess Atmospheric Loading In Canada and the United States). The purpose of the METAALICUS study involves the addition of different enriched stable isotopes to the lake (Hg^{202} called "lake spike"), wetland (Hg^{198} as "wetland spike") and upland (Hg^{200} as "upland spike") of Lake 658 to determine the importance of these locations to mercury fluxes into the lake and food web. Mercury has seven stable isotopes with slightly different atomic weights (Hg^{196}, Hg^{198}, Hg^{199}, Hg^{200}, Hg^{201}, Hg^{202}, and Hg^{204}). These isotopes behave similarly in the environment but can be measured separately using an inductively coupled plasma mass spectrometer (ICP/MS) for analysis (reviewed in Fitzgerald and Lamborg 2003; Hintelmann and Ogrinc 2003). An enriched isotope solution can be created where one isotope is dominant (>95% of the Hg isotope abundance) and can be tracked separately through comparisons to the isotope ratio of ambient mercury (Hintelmann and Ogrinc 2003). The application of these isotopes to the environment for METAALICUS mimicked atmospheric deposition that would occur during a light rain event (Hintelmann et al. 2002). Isotope applications have been repeated since 2000, exposing the lake and its watershed to mercury inputs at approximately 3 times the deposition rate of ambient mercury (Sandilands et al. 2005).

Through natural pathways, the mercury spikes have been incorporated into the food web. In 2003, lake spike made up more than 30% of all mercury in prey fish and 10% in predatory fish (P. Blanchfield, unpublished data).

The research presented in this thesis takes a novel approach to measuring mercury elimination by fish. Fish from Lake 658 were collected and transferred to an isotopically clean lake (Lake 240) thereby ceasing all uptake of lake spike mercury (referred to as spike mercury throughout this thesis). Elimination of spike mercury by the transferred fish was followed in Lake 240. Several key strengths of this study separate it from past research on mercury elimination. First, fish were exposed to spike mercury in a natural way; the exposure was chronic, continuous, occurred via natural processes, and at realistic exposure concentrations. Second, the use of stable isotopes of mercury allowed me to distinguish between the spike mercury that is eliminated and the ambient mercury that is continually taken up by fish in the natural environment. Third, the fish experienced relatively natural conditions during the elimination study. This is important because factors in laboratory studies that may affect mercury elimination (e.g. metabolic rate, temperature) usually do not reflect nature. Fourth, elimination of mercury was monitored over a comparatively long period (between 90 and 650 days), which is important for accurate estimation of elimination rates.

The primary objective of this research was to determine the rate of mercury elimination in nature by a prey fish species (Chapter 2) and a predatory fish species (Chapter 4) and to compare the rates to those predicted by current models. Specifically, I collected yellow perch and northern pike from Lake 658 and transferred them to Lake 240. I then followed the changes in body burden of spike mercury accumulated while in

Lake 658 to estimate elimination rates. In addition, the kinetics of spike mercury in several yellow perch tissues and the implications for elimination rate are presented in Chapter 2.

An additional objective was to determine if mercury elimination by a prey fish species is different in a flooded reservoir than in a lake. This experiment is presented in Chapter 3. For this study, yellow perch from Lake 658 were moved either to Lake 240 or Lake 979 (an experimental reservoir with higher concentrations of mercury). The changes in body burdens of enriched stable isotopes of mercury were compared between these environments. Predictions of mercury models were compared to the resulting elimination rates for both lakes.

Table 1.1. Concentrations (+/- 1 standard deviation) and estimated burdens of total mercury (THg) and methylmercury (MeHg) of water, sediment, and biota of Little Rock Lake, Wisconsin as reported by Watras et al. 1994.

	Concentration (ng/L)		Estimated burden (g)	
	THg	MeHg	THg	MeHg
Water column	0.9 ± 0.3	0.1 ± 0.1	0.3	0.04
Sediment (top mm)	-	-	1.1	<0.01
Algae	25 ± 8	5 ± 3	0.1	0.02
Zooplankton	50	45	0.01	0.01
Fish	156 ± 4	150 ± 4	0.2	0.19

Table 1.2. Typical concentrations of methylmercury (MeHg) in the food web.

	MeHg (ng/g wet wt)	Total Hg present as MeHg (%)	Source
Piscivorous fish			
	650	>95	Watras and Bloom 1992, as cited in Wiener et al. 2003
	200-1000		Jernelov and Lann 1971
	2000-3200		MacCrimmon et al. 1983
	56-1058		Cizdziel et al. 2002
	392	79-94**	Bowles et al. 2001
	300-1220		Kidd et. al. 1995
	30-2680		Cabana et al. 1994
	<2700		Mathers and Johansen 1985
	39-428	99-105	Bloom 1992
	940-970	91-100	Laarman et al. 1976
Prey fish			
	100	>90	Watras and Bloom 1992, as cited in Wiener et al. 2003
	100-400		Jernelov and Lann 1971
	260		MacCrimmon et al. 1983
	43-531		Cizdziel et al. 2002
	26	54-56*	Bowles et al. 2001
	30-220		Kidd et al. 1995
	150-360	71-89	Bodaly and Fudge 1999
	36-340	96-99	Bloom 1992
Invertebrates			
	20	29	Watras and Bloom 1992, as cited in Wiener et al. 2003
	10-80		Jernelov and Lann 1971
Algae			
	4	13	Watras and Bloom 1992, as cited in Wiener et al. 2003
	<0.3	<1	Bowles et al. 2001
Water			
	0.00005	5	Watras and Bloom 1992, as cited in Wiener et al. 2003
	0.03		MacCrimmon et al. 1983
	0.000067	5	Bowles et al. 2001

*whole specimen, with gut contents intact.

**some whole body samples included (no gut contents).

Table 1.3. Half-lives of methylmercury in yellow perch (*Perca flavescens*) and northern pike (*Esox lucius*) in relation to body mass and water temperature.*

Species	Weight (g)	Water temperature (°C)	Half-life (d)	Reference
yellow perch	9	15	69	DeFreitas et al. 1975
	47	11	50	
	15	9	69	DeFreitas et al. 1974
	13	17	87	
northern pike	3920	4-10	728	Lockhart et al. 1972
	300	10	640-780	Jarvenpaa et al. 1970
	75	9	139	DeFreitas et al. 1975
	150	13	173	DeFrietas et al. 1974
	85	13	385	

* Adapted from Trudel and Rasmussen (1997)

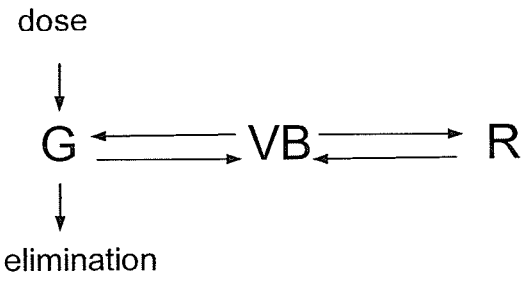

Fig. 1.1. Three-compartment model of methylmercury kinetics in arctic charr (*Salvelinus alpinus*) as presented by Oliveira Ribeiro et al. 1999. G = gut, VB = viscera and blood, and R = rest of body.

Chapter 2: Elimination of mercury by yellow perch (*Perca flavescens*) in the wild.

Introduction

Mercury contamination of fish is a potential health risk to humans and wildlife. Mercury released from anthropogenic or natural sources can be transported long distances and contaminate fish in remote lakes (Mason et al. 1994). The methylation of inorganic mercury in lake sediments forms methylmercury (Gilmour et al. 1992), which is the dominant form of mercury in fish muscle (Bloom 1992). Methylmercury is a neurotoxin, and elevated exposure to this compound through consumption of fish can cause health problems in humans (Clarkson 1997) and wildlife (Wren 1986). There are consumption advisories that warn of elevated contaminant levels in fish (e.g. U.S.E.P.A. 2004; O.M.O.E. 2005) and in Ontario inland lakes, 93% of these advisories are due to mercury (O.M.O.E. 2005).

In pristine lakes, predatory fish species that are prized by fishers can have high methylmercury levels. Elevated methylmercury levels in fish result from biomagnification and slow elimination of this compound. Biomagnification is a known cause of elevated methylmercury levels (reviewed in Wiener et al. 2003), where top predatory fish are exposed to the highest methylmercury concentrations through their diet of prey fish (e.g. Cabana et al. 1994). As an example of biomagnification, Bowles et al. (2001) found that mercury concentrations of prey fish were $3X10^5$ fold higher than in water, and 10 fold lower than in predatory fish. Slow elimination is another factor that causes elevated methylmercury levels in fish, especially in large fish (Trudel and Rasmussen 1997). The long residence time of methylmercury in fish coupled with

relatively fast uptake rates, causes increasing methylmercury concentrations in fish over time (Huckabee et al. 1979).

Though rates of mercury elimination by fish are important to our understanding of mercury bioaccumulation, our current knowledge of this topic may have limited applicability to natural populations. I have found 24 studies on methylmercury elimination by fish, 22 of which were done in the laboratory. Most of the laboratory studies involved fish that had been acutely exposed to unnaturally high concentrations of methylmercury through force-feeding or injection. In contrast, fish in natural lakes generally experience chronic exposure to lower concentrations of mercury through wild prey items. The duration of exposure to mercury is known to affect the kinetics and distribution of mercury within the fish, which in turn affects elimination rates (as suggested by de Boer et al. 1994; Rowan and Rasmussen 1995). In most past studies, elimination rates were measured in fish that were held in aquaria, where their activity levels, temperatures experienced, and food consumption patterns (all factors that may affect elimination rates because they are related to metabolic rate of the fish) were unlikely to reflect those in nature (Mann 1978). Most (90%) past studies on mercury elimination have utilized unnatural conditions that would likely result in an overestimate of mercury elimination rates (de Boer et al. 1994; Rowan and Rasmussen 1995). In addition, the results of two field studies on mercury elimination by fish (Lockhart et al. 1972; Laarman et al. 1976) may not provide accurate estimates of elimination rates because the fish were not fully removed from all sources of mercury.

Bioaccumulation of mercury by fish is often represented by mathematical models, the accuracy of which greatly depend on values used for mercury uptake and elimination

rates (Harris and Snodgrass 1993; Rodgers 1994). These models are usually based on fish bioenergetics and are able to predict changes in mercury levels under different conditions, including factors such as mercury exposure, temperature, species, and size of fish. The elimination component of these models is generally based on rates reported from laboratory studies. Rates of mercury elimination differ among models depending on which studies were incorporated in their elimination equations and on assumptions made about how mercury is lost.

Elimination rates are part of the answer to the important question: If we stop releasing mercury into the environment, how long will it take for mercury levels in fish to decline? There are industrial projects such as flooding of hydroelectric reservoirs that cause increases in the mercury concentrations in fish (Verdon et al. 1991; Bodaly et al. 1997; Bodaly and Fudge 1999). Often, industries use mercury models to estimate the duration that high mercury levels will persist in fish. The persistence of methylmercury in fish is also of interest to governments in developing regulations on mercury inputs to the environment.

In this chapter, I determine the elimination rates of mercury by yellow perch (*Perca flavescens*) in nature over 440 days. My research takes advantage of a whole-ecosystem experiment where an enriched stable isotope of mercury (referred to as spike mercury) was added to a lake for 3 years and was accumulated by all compartments of the aquatic food web. Fish collected from the experimental lake were moved to an isotopically clean lake, ending their exposure to the spike mercury. The perch were maintained in a large, flow-through enclosure in the clean lake, allowing for exposure to natural prey items and temperatures, while containing the fish so that they could be

collected for sampling. This study is different from past laboratory experiments because it involves chronic exposure to mercury through a natural food web, and the conditions experienced after the fish had been removed from spike mercury were relatively natural. This study is also longer (440 days) than most past laboratory studies. This study differs from the field studies of Lockhart et al. (1972) and Laarman et al. (1976) because the elimination of an enriched stable isotope of mercury was followed, which could be distinguished from ambient mercury continually being taken up by the study fish. Exposure to the spike mercury was completely stopped in the present study, allowing elimination to be monitored while maintaining otherwise natural conditions. I hypothesize that mercury elimination rates by yellow perch will be different in this study than past estimates of elimination rates. I test this hypothesis using mercury models, and my null hypothesis is that elimination rates are not different from those predicted by the models. By comparing the results of the present study to model predictions, I compare my study to past estimates of mercury elimination, and how they have been included in common models.

Methods

Study site

This research took place at Lake 658 and Lake 240 at the Experimental Lakes Area (ELA) in northwestern Ontario (Figure 2.1). The ELA is located on the Precambrian shield, where most lakes are naturally oligotrophic. Table 2.1 summarizes some of the geographical and biological characteristics of Lake 240 and Lake 658. Lake 658 is smaller than Lake 240 and has higher levels of ambient mercury in fish (Table 2.1). Lake

size is negatively related to mercury concentrations in fish because small lakes tend to have larger surface area to volume ratios causing greater mercury inputs for a given volume, and warmer temperatures which increase methylation rates (Bodaly et al. 1993).

Study species

Yellow perch is a common fish species that is widespread and found in both clean and metal-contaminated lakes throughout North America (Laarman et al. 1976; Rodgers and Qadri 1982; Craig 1987). Yellow perch are prey for many larger fish species such as northern pike (*Esox lucius*) and walleye (*Stizostedion vitreum*) (Mathers and Johansen 1985). There are both commercial and sport fisheries for yellow perch across North America (Craig 1987). Yellow perch reach maturity at 2 - 4 years of age and spawn in the spring (Craig 1987). Juvenile and adult yellow perch feed on zooplankton and benthic invertebrates, and can begin eating small fish at approximately 150 mm in length (Scott and Crossman 1973). The diet of perch is determined by gape size, prey availability, and individual choice of prey (reviewed in Craig 1987). Yellow perch can live for 6 - 21 years, with slower growing fish generally living longer than faster growing fish (Craig 1987).

Laboratory and field research has been done on the bioaccumulation of mercury by yellow perch (DeFreitas et al. 1975; Rodgers and Qadri 1982; Post et al. 1996; Essington and Houser 2003). The elimination of methylmercury by yellow perch has been studied in the laboratory (DeFreitas et al. 1974; DeFreitas et al. 1975), and the field (Laarman et al. 1976); however, little is currently known about how this species loses mercury under natural conditions.

Transfer and collection of fish

On June 17, 2004, 200 yellow perch were collected from Lake 658 using hoop nets (Fipec Inc., Grande-Riviere, Quebec), and a trap net. The first addition of spike mercury to the lake water for the 2004 summer season occurred on June 15, 2004. This addition is assumed to have had little impact on spike mercury in perch at the time of collection because it takes approximately 4 weeks for newly added spike methylmercury to appear in zooplankton. Only fish within a narrow size range (58 - 80 mm fork length) were selected for the study to reduce the potential variability in mercury concentrations related to the size or age of perch. The perch were transported to the ELA field station (fish laboratory) in four coolers (47.3 L or 94.6 L volumes) containing aerated water from Lake 658. Fish were lightly anaesthetized by being placed in a solution of MS222 (tricaine methane sulfonate) at 0.05 g/L for approximately 2 min or until the operculum movement slowed. Wet weight (to the nearest 0.1 g on an A&D Co. Ltd. scale) and fork length (mm) were measured and recorded for each perch. Next, each fish was marked with subcutaneous injection of pink elastomer (Biomark, Inc., Boise, Idaho) on the ventral side of the post-anal peduncle to indicate that these perch were from Lake 658. Each individual fish was tagged with a decimal sequential coded wire tag of 1.1 mm length and 0.25 mm diameter (Northwest Marine Technology Inc., Washington). Subdermal implantation of these small tags in the left cheek of each fish was done using a Single Shot Tag Injector (Northwest Marine Technology Inc., Washington). Tagging perch allowed for the determination of individual growth over the course of the experiment. Perch were then held in a 91 cm X 102 cm X 183 cm pen (0.64 cm mesh size) placed in 1 m of water in Lake 240 for 2 days. Initial mortalities were removed at

this time. On June 19, I transferred 140 of the perch to an enclosure that borders the north shore of Lake 240. The enclosure was 12 m X 20 m in size, and the deepest point was 2 m in depth. This enclosure allowed access to prey items in the water column and sediment, as it consisted of walls made of wood and wire mesh (0.64 cm) surrounding a littoral area. Algae and pollen were scrubbed off the mesh every 2 - 4 weeks to maintain the flow of water through the enclosure. The enclosure was topped with nylon net (3 cm mesh size) for protection from avian predators, and the shore side was bordered with a fence (90 cm height) to deter terrestrial predators. The water temperature within the enclosure was recorded every 30 min at 30 cm depth intervals using Stowaway Tidbit temperature loggers (Onset Computer Corp., Bourne, Massachusetts). I snorkelled in the enclosure regularly to remove mortalities and monitor perch numbers.

On June 19, 2004, 30 yellow perch (57 - 77 mm fork length) were collected from a sandy area near the north shore of Lake 240 using a beach seine net (16.8 m length, 2.4 m depth). These fish were anaesthetized and tagged as described above. Lake 240 perch were marked with yellow elastomer, rather than pink, to distinguish them from perch from Lake 658. Later that day, perch from Lake 240 were added to the enclosure with perch from Lake 658. If spike mercury was excreted from enclosed perch or released from carcasses of dead perch from Lake 658, fish in the enclosure could potentially accumulate recycled spike mercury. Thus, Lake 240 perch (that did not contain any spike mercury to begin with) were monitored for accumulation of spike mercury while in the enclosure and serve as a control. Eight enclosed perch originally from Lake 240 were collected for mercury analysis 90 days after beginning the study.

Yellow perch were collected from the enclosure at 0, 15, 30, 60, 90, 135, 180, and 240 days after transfer from Lake 658 to Lake 240. Fish were captured from the enclosure using a seine net (either a 4 m X 1.5 m pole seine or a 16.8 m X 2.4 m beach seine). Eight to ten fish were collected on days 0 through 135. In the winter, minnow traps or gill nets (8 - 10 mm mesh) were set under the ice to capture perch, yielding three perch on day 180. By day 240, the ice had damaged the enclosure, and only one perch was captured. On day 300 (April 12, 2005), there were several holes in the enclosure due to ice damage and no perch were caught. Attempts to capture the escapees by seining near the enclosure were successful on day 365 (n = 3). On day 415 and again on day 440, single escapees were captured in trap nets in Lake 240. All collected perch were immersed in 0.5 g/L MS222 in water for euthanasia. Fork length (mm) and wet weight (g) were recorded, and the fish were frozen in individual whirl-paks (Nasco, Fort Atkinson, Wisconsin). The decimal coded wire tags were removed before further processing by slicing open the left cheek of the fish. Of 54 yellow perch collected, 2 lost their tags, and both were part of the day 60 sample.

Processing of fish tissues

All fish collected had total mercury measured in their muscle tissue (Table 2.2). Approximately five individual fish from each sampling day had both total and methylmercury concentrations measured in their muscle, liver, and the remainder of the fish (Table 2.2). Samples were obtained from frozen fish and care was taken during all processing to minimize thawing of samples. Using mercury clean techniques and stainless steel or Teflon tools (similar to the methods described in Bloom 1992),

approximately 0.2 g of the dorsal muscle was removed from each frozen fish. Parasites, skin and bones were removed from muscle samples. Each muscle sample was weighed in an acid-washed 14 mm scintillation vial. Tools and surfaces were rigorously cleaned using 95% ethanol and kim-wipes (Kimberly Clark Professional) between all samples. The liver was dissected from each frozen fish, sliced into small pieces, and weighed in an acid-washed scintillation vial. Muscle and liver samples were freeze-dried in a Lyph-lock 12-L freeze dry system (Model 77545, Labconco, Kansas City, MO) until a constant weight was achieved. The gastrointestinal tract was removed from the frozen fish, sliced open, and any stomach or intestinal contents were removed and weighed. The weight of stomach contents was subtracted from the weight of perch to improve the accuracy of measured fish weight. Also, mercury levels in stomach contents may have been different than mercury levels in the fish. The whole body (referred to from now on as WB), which was the whole fish minus muscle samples, liver, and stomach contents, was freeze-dried as above. Once a constant weight was achieved, the WB sample was ground in an electric coffee grinder (KitchenWorks, Model CG81000) for approximately 30 sec, or until the tissue became a fine powder. Approximately 0.1 - 0.15 g of each freeze-dried and homogenized WB sample was weighed into an acid-cleaned scintillation vial.

Samples were shipped to Dr. H. Hintelmann (Trent University, Peterborough, Ontario) for determination of both total and methylmercury concentrations. An inductively coupled plasma mass spectrometer (ICP-MS) was used to measure the concentrations of ambient and spike mercury (Hintelmann and Ogrinc 2003).

Calculations

All short forms and symbols used in formulas below are summarized in Table 2.3.

Estimation of starting concentrations of spike mercury in yellow perch muscle

An unknown in this study is the initial concentration of mercury in individual yellow perch from Lake 658. Using perch caught at the beginning of the experiment (day 0), I tested whether fish size was an adequate predictor of mercury concentration. A strong relationship between fish size and mercury concentration would allow for back-calculation of initial mercury concentrations of fish caught at later sampling dates during the study. I used linear regression to determine the relationship between fish size (as weight) and concentrations of spike mercury in yellow perch muscle.

Fish growth

The change in weight of yellow perch was calculated below:

1) change in weight = $W_{fishdayX} - W_{fishday0}$

Where $W_{fishdayX}$ is the weight of yellow perch when sacrificed on sampling day 'X' and $W_{fishday0}$ is the weight of the same fish at the beginning of the experiment.

Mercury concentration conversions

Analysis of replicate samples found that conversion of mercury concentration in dry tissue to concentration in wet tissue added a small amount of error to the measure of concentration. The coefficient of variation was on average 1.8 times higher in wet weight concentrations. Dry weight concentrations were used when possible, except for

calculations of body burden through method 1 (defined later) and observed and expected concentrations, which required wet weight concentrations. Wet weight concentrations ($[Hg]_{ww}$) were calculated according to the following formula:

2) $[Hg]_{ww} = [Hg]_{dw} * sample_{dw} / sample_{ww}$

Where $[Hg]_{dw}$ is the dry weight concentration (ng/g), $sample_{dw}$ is the dry weight of the sample (g) and $sample_{ww}$ is the wet weight of the sample (g).

In general, dry weight concentrations can be converted to wet weight concentrations by multiplying by 0.20 for muscle tissue, 0.28 for the rest of the fish, and 0.24 for liver.

Accounting for changes in fish growth

I examined the changes in mercury levels separate from effects of growth by determining how observed changes in weight would impact mercury concentrations under an assumption of no mercury loss. The calculation of observed and expected concentrations is described later. Another method used to account for the effects of growth on mercury concentration was calculation of body burden, which is an estimate of the total mass of mercury present in a fish. A commonly used method of estimating body burden (referred to as method 1) involves multiplying the mercury concentration in a wet muscle sample by the weight of the fish (e.g. Lockhart et al. 1972). I also used another method, which involved calculating the burden in each tissue that was sampled and summing these values to estimate the mercury burden in the whole fish (referred to as method 2) (e.g. McKim et al. 1976; Boudou and Ribeyre 1983). The calculations of burden by method 1 and method 2 are described below.

Expected mercury concentrations based on observed fish growth

Calculation of "expected" concentrations isolated the effect of changes in weight from any losses or gains of mercury. This measure is different from body burden, which multiplies concentration and fish weight and therefore does not show which of these variables is responsible for the changes in burden. The "expected" mercury concentration, which is the concentration that would occur because of observed weight changes assuming no mercury loss, indicates the extent that starvation concentration or growth dilution potentially influence mercury concentration. Therefore, the difference between the expected and observed concentrations of mercury is related to the amount of mercury lost or gained. First, the mean concentration (ng/g w.w.) of spike and ambient mercury in muscle of yellow perch collected at the beginning of the experiment (avg $[Hg]_{day0}$) was calculated. This value was assumed to be the original concentration of all yellow perch moved from Lake 658 to Lake 240. Next, the original burden of mercury was estimated:

3) $B_{day0} = (\text{avg } [Hg]_{day0}) * W_{fishstart}$

The expected mercury concentration was calculated using the estimated burden at the beginning of the experiment (equation 3) divided by the individual fish weights observed at the time of sampling. The resulting value is the concentration of mercury expected in these fish after taking into account their observed growth, if the actual amount of mercury contained in the fish did not change from day 0. The expected concentration for day 'X', where 'X' is the sampling day, is abbreviated as $exp[Hg]_{dayX}$.

4) $exp[Hg]_{dayX} = B_{day0} / W_{fishdayX}$

Where $W_{fishdayX}$ is the weight of individual fish when they were collected on sampling day 'X'. The weight of stomach contents was not accounted for in the calculation of expected concentration because the stomach content weight was not known when fish were first weighed (day 0). The weight of the stomach contents accounted for approximately 2% of the wet weight of fish on average.

Calculation of body burden by method 1

Body burdens were calculated as follows:

5) $\text{Burden} = (W_{fish} - W_{sc}) * [Hg]_{muscle}$

Where W_{fish} is the wet weight of fish at the time of sacrifice and W_{sc} is the weight of the stomach contents.

Calculation of body burden by method 2

First, the burden of mercury in yellow perch muscle, liver, and rest of fish were calculated. To do this, estimates of the mass of each of these tissues were required. The weight of the entire muscle was not measured in yellow perch analyzed for mercury because complete extraction of all muscle was a time-consuming process that would result in thawing of frozen muscle tissue. Instead, 14 yellow perch not sent for mercury analysis were dissected to determine the relationship between fish weight and muscle mass. These yellow perch ranged in size (from 0.6 g - 13.9 g) and were carefully dissected to separate all muscle from the rest of the fish. Wet and dry weights of muscle and the rest of the fish (minus stomach contents) were measured (to the nearest 0.0001 g, scale: Mettler model AE163). There was a strong linear relationship between fish weight

and dry muscle weight (Figure 2.2a, r^2=0.99), which was used to estimate the mass of muscle (W_{muscle}) in yellow perch analyzed for mercury. Muscle burden (B_{muscle}) was calculated as follows:

6) $B_{muscle} = [Hg]_{muscle} * W_{muscle}$

Livers analyzed for mercury were weighed upon sample dissection (using the same scale as described above for muscle samples). The liver burden (B_{liver}) was calculated with the equation below:

7) $B_{liver} = [Hg]_{liver} * W_{liver}$

Where $[Hg]_{liver}$ is the concentration (ng/g d.w.) of mercury in the liver and W_{liver} is the dry weight of liver tissue. The WB samples analyzed for mercury contained some muscle tissue as well as skin, bones, fins, head and the visceral tissues other than the liver. Subtraction of the muscle burden in WB samples resulted in a portion referred to as RF, which represents the remaining fish after all muscle, liver, and any stomach contents were removed. To calculate RF burden (B_{rf}), first the dry muscle mass in the WB sample ($W_{wbmuscle}$) was calculated by subtracting the mass of muscle collected for mercury analysis ($W_{musclesample}$) from the estimated muscle mass in the whole fish ($W_{allmuscle}$):

8) $W_{allmuscle} = -0.0195 + 0.0813 * (W_{fish} - W_{sc})$ (Figure 2.2a).

9) $W_{wbmuscle} = W_{allmuscle} - W_{musclesample}$

The burden of the muscle present in the WB sample ($B_{wbmuscle}$) was calculated:

10) $B_{wbmuscle} = W_{wbmuscle} * [Hg]_{muscle}$

The burden of the entire WB sample (B_{wb}) was determined:

11) $B_{wb} = W_{wb} * [Hg]_{wb}$

Where $[Hg]_{wb}$ is the mercury concentration measured in the WB sample. Finally, to calculate burden in RF (B_{rf}) the burden of muscle present in the WB sample was subtracted from the burden of the entire WB sample:

12) $B_{rf} = B_{wb} - B_{wbmuscle}$

The mercury concentration in RF was estimated by dividing the RF burden by the weight determined from the regression in Figure 2.2b. This regression includes the weight of liver in RF weight, so it was necessary to subtract the weight of liver as measured for each fish. The burden in the whole fish (body burden method 2) was determined by summing the tissue burdens of muscle, liver, and RF as shown below.

13) whole fish burden = $B_{muscle} + B_{liver} + B_{rf}$

Rate of elimination by yellow perch

The elimination of mercury by fish is thought to follow first order kinetics, which means that mercury burden declines in a pattern of exponential decay as mercury is lost (Trudel and Rasmussen 1997). The natural log of the mercury burden was plotted against time (in days) and the slope of this line, 'k', is the rate of elimination. The time it takes for half of the mercury burden to be lost is called the half-life, which can be calculated using the equation below.

14) half-life (days) = $\frac{\ln (2)}{k}$

Three-parameter exponential decay models provided the best fit when the data showed an exponential decline followed by leveling off at an asymptote. In the case where three-parameter exponential models were fit to the data, 'k' is indicated in the equation below.

15) $Y = y_0 + ae^{-kt}$

Where 'Y' is burden, 'y_0' is the asymptote, 't' is days, and 'a' and 'k' are constants. The value of 'k' can then be used to determine half-life using equation 14.

Model simulations

I used three different mercury models to compare predicted rates of mercury elimination to observed losses of spike mercury. The three models chosen were different in that they represent elimination rates from different studies, they have different accessibility, and they use different assumptions of how elimination occurs. The first model used was created by Harris and Bodaly (1998), the second was the Wisconsin model version 3.0 (Hanson et al. 1997), and the third was developed by Trudel and Rasmussen (1997).

The model developed by Harris and Bodaly (referred to as the HB model) is a fish bioenergetics model linked to mercury uptake and elimination equations that are species-specific. Specifically, mercury is eliminated along with nitrogenous wastes in the model, which is ultimately linked to inputs of fish weight and water temperature. The fraction of mercury eliminated in waste is entered by the user, and the suggested value has been calibrated to fit yellow perch muscle concentrations measured in nature (Harris and Bodaly 1998). For spike mercury, this model was used to simulate zero mercury entering fish via food or water.

The Wisconsin model is similar to the HB model in that it includes fish bioenergetics equations linked to mercury uptake and elimination equations. This model also predicts elimination in relation to fish weight and water temperature, and has user-entered equation constants. The constants used in this model were developed by Norstrom et al. (1976) based primarily on goldfish (*Carassius auratus*) studies in the

laboratory, with some adjustments made later by Rodgers (1994) so that the model would better match field data. These model runs were also conducted assuming no uptake of spike mercury.

The model developed by Trudel and Rasmussen (1997) (referred to as the TR model) was designed exclusively for modeling mercury elimination by fish, and there is no bioenergetic component of this model. This model uses fish weight and water temperature to estimate methylmercury elimination rates. The elimination rates determined by this model are based on four past laboratory studies (for a total of 21 rate estimates) and one field study (for a total of four rate estimates). Elimination rates can be modeled differently in the TR model for fish that have been chronically exposed to mercury and fish that have been acutely exposed to mercury. Both chronic and acute applications of the model were compared to the observed elimination rates of spike mercury by yellow perch.

Measured growth and water temperatures experienced by yellow perch during this study were entered into each of the three models to predict the loss of spike mercury. For each model, I calibrated a growth curve to reflect observed perch growth, which was necessary so that the mercury concentration could be properly modeled for the observed fish weights. The details of the inputs and parameters used are presented in Table A.1 for the HB model, Table A.2 for the Wisconsin model, and Table A.3 for the TR model (Appendix 1).

Statistical Analyses

Linear regressions and non-linear curves were fitted and analyzed using Systat within SigmaPlot version 9.0 or SAS version 9.0. Sampling days with fewer than three fish were excluded from the following analyses. Assumptions of normality and homogeneity of variance were tested and the data were log transformed when necessary. SAS indicated extreme outliers that were then removed. Paired *t*-tests were used to determine if there were differences between starting and ending fish weights, observed and expected concentrations, and total and methylmercury concentrations within each sampling day. A one-way analysis of variance (ANOVA) was used to determine differences in mercury concentrations, or burdens among sampling days. When there were significant differences, Tukey's post-hoc test was used. A Kruskal-Wallis ANOVA on ranks was used to compare the ambient mercury burdens in the liver because the residuals did not exhibit homogeneous variance.

Results

Estimation of starting concentrations of spike mercury in yellow perch muscle

Fish collected at the beginning of the experiment had sufficient total mercury for analysis in their tissues, with spike mercury concentrations making up approximately 30 - 50% of all of the mercury (Table 2.4). The concentrations of ambient mercury were similar in the liver and the WB sample, and were 55% of the concentrations in muscle. Concentrations of spike mercury were also similar for the liver and WB sample, and were at the level of 70% of the concentration measured in muscle.

I observed a significant positive linear relationship between spike and ambient total mercury concentration in muscle of perch collected at the beginning of the experiment (Figure 2.3; $F=80.8$, $P<0.0001$, $df=1,13$).

There was no relationship between fish weight and concentration of spike total mercury in fish collected at the beginning of the experiment (Figure 2.4; $r^2= 0.0001$, $F=0.0$, $p=0.98$, $df=1,13$). Although fish were accumulating spike mercury in a natural way, the extent of accumulation was not related to the size of fish. Within the size range (2.0 g - 6.8 g), spike mercury concentrations in the muscle ranged from 364 - 564 ng/g d.w. with no relationship to fish weight. As a result, initial mercury concentration in the muscle could not be assumed for a given initial weight of the fish. The initial mercury concentrations in the muscle were assumed to be equivalent to the mean concentrations measured for all day 0 fish.

Total and methylmercury

Ambient mercury

Both total and methylmercury were measured in tissues of muscle, liver, and whole body. In general, all the ambient mercury in muscle and in the WB samples was methylmercury (Table 2.2). The mean percentage of total mercury that was methylmercury in muscle was roughly the same for all sampling days, except for day 30, which was significantly lower than days 135 ($p=0.032$) and 180 ($p=0.0008$). In the WB samples, there was no difference in the percent methylmercury among sampling days ($F=1.0$, $p=0.44$, $df= 7,28$). In contrast to muscle and WB samples, not all of the ambient mercury present in the liver was in the form of methylmercury. The percentage of methylmercury in liver had a much

broader range (56 - 114%) than other tissues. The percentage of total mercury that was methylmercury in liver was different among sampling days, with values significantly lower than starting values at days 30 (p=0.023) and 90 (p=0.002).

Spike mercury

For spike mercury, the percentage of methylmercury making up total mercury in the muscle was roughly 10% higher than the values reported for ambient mercury (Table 2.2) with an overall mean of 109%. The values varied somewhat among sampling days (from 102 - 116%) with a significantly higher percentage of methylmercury on day 90 than on days 30 (p=0.032) or 365 (p=0.012). As was found for ambient mercury, almost 100% of the mercury in the WB samples was methylmercury and there was no significant difference in this percentage among sampling days (F=0.4, p=0.91, df=7,28). Not all of the spike mercury in the liver was present in the form of methylmercury with mean values ranging from 56% - 100% for sampling days 0 - 365. The mean percent methylmercury in the liver varied among sampling days, with day 0 significantly greater than days 30 (p=0.0003), 90 (p=0.0004), 180 (p=0.018), and 365 (p=0.020). The percentage of methylmercury in perch liver measured on day 15 was significantly greater than those on days 30 (p=0.035) and 90 (p=0.039).

Total mercury is composed of all forms of mercury, including methylmercury, and therefore it is not possible to have more methylmercury than total mercury. Analyses can frequently result in higher measurements of methylmercury than total mercury due to analytical variability (Bloom 1992). The analysis used is generally accurate +/- 10% of the mean (H. Hintelmann, personal communication), which is in the range of these

results. Total mercury concentrations and burdens are presented for the remainder of this chapter. In general, all of the total mercury is in the form of methylmercury for muscle and whole body while approximately 50 - 115% of mercury in the liver was measured as methylmercury.

Yellow perch growth

There was little growth by yellow perch during the first 365 days of the study (Figure 2.5). Perch were estimated to be age 1, and began the experiment at a mean weight of 3.6 g (range: 1.8 - 6.8 g) and a fork length of 69 mm (range: 58 - 80 mm). The decimal coded wire tags (DCWTs) allowed for the measurement of weight changes of individual perch between the time that they were released into the enclosure and the time they were sacrificed for sampling. All weight changes within the first 240 days of this study were within 0.5 g. A paired *t*-test comparison of the starting and final weights of perch collected on each sampling day (when $n \geq 3$) indicated that only on days 30 ($t=4.01$, $p=0.0031$, $df=9$), and 135 ($t=3.10$, $p=0.017$, $df=7$) were fish significantly heavier than at the beginning of the study. No difference in weight was observed on any other sampling days within the first year of the study (day 15: $t=0.85$, $p=0.42$ $df=7$; day 60: $t=1.48$, $p=0.20$, $df=5$; day 90: $t=0.32$, $p=0.76$, $df=8$; day 180: $t=0.51$, $p=0.66$, $df=2$). Yellow perch that escaped the enclosure were free in Lake 240 and showed no significant change in weight 1 year after the start of the study (day 365; $t=1.96$, $p=0.19$, $df=2$). Single yellow perch captured on days 415 and 440 increased in weight by 5.1 g and 8.2 g respectively (Figure 2.5b).

The effect of growth on mercury concentration

Though there was little growth in the first year of the study, concentrations of ambient and spike mercury in muscle tissue were affected by the small changes in weight observed. Expected concentrations of mercury are those that would result from observed changes in weight and no losses or gains of mercury. Observed and expected concentrations of mercury followed a similar pattern over time, suggesting that changes in weight influenced mercury concentrations (Figure 2.6). A paired *t*-test comparison indicated that observed ambient mercury concentrations in muscle were significantly higher than would be expected on all sampling days (with $n \geq 3$, day 15: $t=3.46$, $p=0.0072$, $df=9$; day 30: $t=3.28$, $p=0.0096$, $df=9$; day 60: $t=3.46$, $p=0.018$, $df=5$; day 90: $t=2.74$, $p=0.025$, $df=8$; day 135: $t=5.19$, $p=0.0013$, $df=7$) except for days 180 ($t=1.86$, $p=0.20$, $df=2$) and 365 (Figure 2.6a; $t=0.66$, $p=0.56$, $df=2$). Observed spike mercury concentrations in muscle were significantly higher than those expected only on days 15 ($t=3.07$, $p=0.013$, $df=9$) and 30 ($t=2.35$, $p=0.043$, $df=9$), after which time the observed and expected concentrations were similar (Figure 2.6b, day 60: $t=0.03$, $p=0.98$, $df=9$; day 90: $t=0.79$, $p=0.45$, $df=8$; day 135: $t=2.01$, $p=0.084$, $df=7$; day 180: $t=0.01$, $p=0.99$, $df=2$; day 365: $t=0.77$, $p=0.52$, $df=2$). The fact that the observed and expected concentrations follow a similar pattern over time, emphasizes that fish growth accounts for some of the changes in muscle mercury concentration. To truly understand changes in mercury content within a fish it is important to examine both burdens and concentrations.

Mercury concentrations and burdens

Ambient mercury

Ambient mercury concentrations in liver were not significantly different among sampling days ($F=1.3$, $p=0.29$, $df=6,23$), but were slightly (not significantly) higher in summer periods than in winter periods (Figure 2.7). In summer, concentrations were near 600 ng/g d.w. while they were closer to 400 ng/g d.w. in fall and winter (days 135 and 180). Mean ambient burdens in liver changed little over the first year of the study (Figure 2.8; Kruskal Wallis test: $\chi^2=7.77$, $p=0.35$, $df=7$). The mercury burden in this organ ranged from 4 - 6 ng over the first 365 days, followed by burdens up to 4 times higher in the two fish collected on days 415 and 440.

The concentration of ambient mercury in the RF portion of the fish was significantly different among sampling days ($F=6.4$, $p<0.0001$, $df=7,28$), but did not exhibit a regular pattern over the course of the study (Figure 2.7). The ambient burden in the RF portion of the fish differed over time ($F=5.6$, $p=0.0004$, $df=7,28$), with burdens on day 135 significantly lower than all other days (Figure 2.8). During the first 365 days, mean RF mercury burdens fluctuated, occasionally reaching nearly double the initial values. The RF burdens in the two fish sampled on days 415 and 440 were approximately 3 times higher than burdens measured at the beginning of the experiment.

Concentrations of ambient mercury in muscle of yellow perch did not change significantly over 365 days (Figure 2.7; $F=1.6$, $p=0.15$, $df=7,58$). Burdens of ambient mercury in muscle exhibited a significant increasing trend over the first 180 days of the study (Figure 2.8, linear regression: $F=12.3$, $p=0.0009$, $df=1,61$, $r^2=0.17$). By day 365, mean ambient mercury burdens in muscle were 34% lower than on day 180; however,

these values were not significantly different (Tukey's test: p=0.12). These data suggest that yellow perch accumulated ambient mercury in their muscle, with a slight but non-significant loss observed between days 180 and 365. The two perch captured on days 415 and 440 had mercury burdens in their muscle that were 1.5 and 1.7 times higher than means determined at the beginning of the study.

Spike mercury

Lake 240 perch did not accumulate any appreciable spike mercury (mean spike mercury concentration in muscle = 0.6 ng/g d.w., range=0 – 3 ng/g d.w., n=10, days 90 and 180); hence the yellow perch taken from Lake 658 were completely cut off from spike mercury. In comparison, mean spike mercury concentrations in muscle tissue of yellow perch collected from Lake 658 was 453 ng/g d.w. at the start of the study (Table 2.4). Thus, any recycling of spike mercury in the enclosure through excretion or fish mortalities did not influence the observed results.

There was an analytical error in the total spike mercury measurements in all liver samples collected on day 135 and one liver sample from day 60. These values were inaccurate (H. Hintelmann, personal communication) and were not used in any further calculations and are not presented. There were clear patterns of loss of spike mercury from the liver. Concentrations of spike mercury in liver tissue declined significantly over the first 180 days of the study ($F=18.6$, $p=0.0002$, $df=1,25$, $r^2=0.43$), and then leveled off for the remainder of the experiment (Figure 2.7). Spike mercury concentrations in liver were reduced by approximately two-thirds by day 180, and remained below 150 ng/g after this time. Similarly, the burden of spike mercury in the liver decreased steadily for

the first 90 days and then appeared to level off for the remainder of the study (Figure 2.8). Burdens of spike mercury in the liver were small, and the overall decrease of 2 ng was a reduction by 60% of the original burdens. The liver burdens of spike mercury were fit to a 3-parameter exponential decay model (Figure 2.8, r^2=0.35). The equation resulted in an estimated half-life of 24 days for the fast phase of elimination from the liver. After the fast phase of elimination was completed, the liver burdens leveled off at approximately 1.29 ng in the model.

Fish lost spike mercury from their RF portion, which included all tissues except for the muscle and liver. The concentration of spike mercury in the RF sample declined significantly (by more than 60%) in a linear manner over the first 180 days of the study ($F=38.1$, $p<0.0001$, $df=1,31$, $r^2=0.55$) and then remained at approximately 50 ng/g for the rest of the study (Figure 2.7). The spike burden in the RF sample decreased over the first 90 days and then leveled off for the remainder of the experiment (Figure 2.8). There was an initial increase in RF burden by approximately one-quarter by day 15 of the study, followed by a steady decline until day 90. Spike mercury burdens in RF tissue leveled off at approximately half the initial burden. These data were fit to a 3-parameter exponential decay model (Figure 2.8, r^2=0.43), and the resulting half-life of the fast phase of elimination for the RF portion of the fish was 44 days.

Concentrations of spike mercury in muscle tissue of yellow perch differed significantly over the course of 1 year (Figure 2.7; $F=2.5$, $p=0.024$, $df=7,58$). Most notably, the concentrations on day 365 were significantly lower than those on days 0 ($p=0.0043$), 15 ($p=0.0007$), 30 ($p=0.017$), and 135 ($p=0.015$). The two fish collected on days 415 and 440 had spike mercury concentrations in their muscle that were 25% and

30% of the mean found on day 365 respectively. Burdens of spike mercury in the muscle of yellow perch were not significantly different among sampling days ($F=1.3$, $p=0.29$, $df=7,58$). There was a subtle pattern of change over time; muscle burdens of spike mercury declined over the first 90 days, followed by an increase up to day 240, and another decrease measured at the final sampling days (Figure 2.8).

Percentage of all mercury in different tissues

When tissue burdens were converted to percentages of all mercury in fish, most of the mercury is present in muscle (60 - 93 % for ambient, and 57 - 86% for spike), while RF accounts for the next highest portion of spike mercury burden (6 - 39% ambient, and 13 - 41% for spike, Table 2.5). The percentage of mercury present in the liver consistently accounted for 1% of all mercury in fish and is not shown in Table 2.5. The percentage of ambient mercury present in muscle followed a significant increasing trend from day 0 until day 135 ($F=12.5$, $p=0.0014$, $df=1,28$, $r^2=0.31$). After day 135, the percentage in muscle decreased while the percentage in RF increased for the remainder of the study. Over the first 180 days, spike mercury in muscle tissue increased significantly by approximately 15% ($F=123.3$, $p<0.0001$, $df=1,30$, $r^2= 0.80$) followed by a decrease over the remainder of the study (Table 2.5). The changes in spike mercury in the muscle as a percentage of all of the spike mercury in the fish corresponded with opposing trends of spike mercury in the RF.

Mercury body burden in yellow perch

Ambient mercury

There was a steady increase in ambient mercury body burden (calculated by method 1) in yellow perch over the first 180 days of the experiment (Figure 2.9, F=15.8, p=0.0002, df=1,61, r^2=0.21). The mean body burden on day 365 was slightly (but not significantly) lower than on day 180 (p=0.15). Similarly, the ambient burdens in the whole fish (calculated by method 2) increased significantly over the first 180 days of the study (Figure 2.9, F=14.9, p=0.0003, df=1,61). In both method 1 and method 2 of calculating body burden, the two fish captured on days 415 and 440 had much greater burdens than those measured on earlier sampling days.

Spike mercury

The body burden of spike mercury (calculated by method 1) was not significantly different among sampling days (F=1.4, p=0.22, df=7,58). There was a pattern, however, where the body burden of spike mercury increased after 90 days, peaking at day 180, and then declined for the remainder of the study (Figure 2.9). An exponential decay curve is thought to typically represent elimination of methylmercury by fish (Trudel and Rasmussen 1997), but this type of curve does not fit the body burdens calculated by method 1. Because body burden calculated by method 1 is a product of muscle mercury concentrations, the patterns of change over time probably reflect redistribution of mercury into muscle tissue rather than loss from the whole fish. In comparison, the mean burdens of spike mercury in the whole fish calculated by method 2 showed a more consistent decline than method 1 (Figure 2.9). A 2-parameter exponential decay line was

fit to the data (Figure 2.9). The resulting half-life of spike mercury in the whole fish was 489 days.

Application of the models to method 1 and method 2 of measuring body burden

When compared to traditional measurements of body burden (method 1), all three models that were tested overestimated the rate of mercury loss by yellow perch (Figure 2.10a). None of the models captured the observed pattern of mercury redistribution into the muscle. The period of increase in spike body burden from days 135 - 240 showed the largest discrepancy between model predictions and observed values. The HB model, which incorporates the slowest rate of mercury elimination, did match the observed body burdens up to day 90 and at the end of the study. The TR model predicted elimination rates that resulted in lower burdens than those observed, especially when the model was used to predict elimination following chronic exposure to mercury. The Wisconsin model predicted that 80% of the mercury would be lost in the first 100 days, which resulted in the greatest underestimation of body burden (Figure 2.10a).

When compared to mercury body burdens based on the sum of individual tissue contributions (method 2), the model predictions were closer to fitting observed results than when compared to method 1. The Wisconsin and TR model (with chronic exposure) simulations underestimated body burdens of spike mercury with method 2, while the HB model and the TR model (with acute exposure) fit the observed data more closely (Figure 2.10b).

Discussion

I examined the elimination of an enriched stable isotope of mercury by yellow perch in a natural setting. A strength of this study compared to past research includes the use of naturally accumulated enriched stable isotopes (spike mercury), that can be distinguished from ambient mercury in the environment. Perch accumulated spike mercury through a natural diet to levels typical of chronically exposed wild fish (see Table 1.2) and eliminated spike mercury under comparatively natural conditions. Another strength of this research is the length of the study (440 days), because the accuracy of estimated elimination rates tends to increase with study length (Trudel and Rasmussen 1997). I found that elimination of mercury by yellow perch in the wild occurred 5 times more slowly than reported by laboratory studies (Table 1.3). I also found that calculating body burden based on the mercury concentrations in muscle (method 1) does not accurately reflect the loss of mercury from the whole fish.

In Lake 658, yellow perch accumulated spike mercury in a natural way, as supported by the finding that spike and ambient mercury concentrations are positively correlated. Also, the relative distribution of spike and ambient mercury was similar in different fish tissues (Table 2.4), indicating that both forms of mercury have been similarly redistributed throughout the fish. Variability in mercury levels among individual perch likely reflects diets with different levels of mercury (regardless of the perch size). For example, age-1 perch in Lake 658 have been found to eat primarily cladocerans, copepods, and chironomid larvae, with the number of these items in the stomach differing among individual fish (P. Blanchfield, unpublished data). Prey species vary in their methylmercury content among days and seasons (M. Paterson, unpublished

data); a unique combination of prey items could result in a unique amount of mercury accumulated by an individual perch. More importantly, the strong relationship between ambient and spike mercury concentrations shows that the accumulation of spike mercury occurred in a similar way as ambient mercury in the yellow perch. The natural accumulation of labeled mercury is a major difference between past studies and the present research; most elimination studies involved artificial contamination of fish either through intramuscular injection (e.g. Jarvenpaa et al. 1970; Ruohtula and Miettinen 1975), force-feeding (e.g. Jarvenpaa et al. 1970; Miettinen et al. 1970; Miettinen et al. 1970b; Massaro and Giblin 1972; Giblin and Massaro 1973; Ruohtula and Miettinen 1975) or through water (e.g. Burrows and Krenkel 1973; DeFreitas et al. 1974; McKim et al. 1976).

There was no relationship between the concentration of spike mercury and the weight of fish collected at the beginning of the experiment, and as a result, I was unable to account for individual fish that potentially had initially high or low concentrations of mercury. Fish with particularly high or low concentrations of mercury may have been collected by chance, which could have added some variability to patterns of changing mean mercury levels among sampling days. Nevertheless, the mean values showed clear patterns of spike mercury kinetics and elimination from the whole fish over the course of the study.

Total and methylmercury

Approximately all of the mercury in fish muscle and WB samples consisted of methylmercury, which agrees with most of the literature (refer to Table 1.2). The finding

that there was generally less methylmercury than total mercury in the liver is also similar to other studies. For example, laboratory studies of fish exposed to methylmercury resulted in methylmercury accounting for 65 - 85% (Riisgard and Hansen 1990), 8 - 56% (Burrows and Krenkel 1973), or 20 - 95% (Boudou and Ribeyre 1983) of total mercury in the liver. There is thought to be less methylmercury than total mercury in the liver because of demethylation of methylmercury to form inorganic mercury during the process of elimination (Burrows and Krenkel 1973; Riisgard and Hansen 1990).

Yellow perch growth

The limited yellow perch growth in the enclosure could be due to a combined effect of low water temperatures, high fish densities, and a limited habitat within the enclosure. The summer of 2004 was unusually cold, and may have limited perch growth. Yellow perch growth is generally at a maximum between 20 and 25°C (reviewed in Hanson et al. 1997). This optimum temperature for perch growth only occurred from mid-July to mid-August in 2004 (mean temperatures were 18.5°C for June-July, 20.8°C for July-August, and 16.7°C for August-September, Table A.1). In comparison, mean temperatures in the enclosure in the summer of 2005 were almost 3°C warmer than in 2004 (20.7°C for June-July, 23.6°C for July-August, and 19.2°C for August-September). Growth may have been reduced by high fish densities within the enclosure, as free-ranging perch (~age 1) from Lake 240 exhibited greater growth (1.7 g average weight gain from June to September) than fish inside the enclosure (0.2 g average reduction in weight during this time). Experimental perch were added to the enclosure at a density of 0.71 fish/m^2, which was thought to allow ample resources for these fish based on past enclosure studies in Lake

240 (e.g. Orihel 2005). Not all fish (consisting of white suckers (*Catostomus commersoni*), Johnny darters (*Etheostoma nigrum*) and older yellow perch) were successfully removed from the enclosure before perch were added from Lake 658. In addition, many tiny young-of-the-year (YOY) yellow perch and Johnny darters invaded the enclosure through the mesh walls. Throughout the study, fish that did not "belong" in the enclosure were removed, including more than 100 YOY perch, 20 darters, 20 older perch, and 3 white suckers, with most of the removal occurring on day 90. Observed growth increased little after this removal (Figure 2.5b), possibly due to seasonal effects. Day 90 occurred in the fall when perch growth slows compared to the summer (Craig 1987). The habitat inside the enclosure may have had limited availability of benthic invertebrates with a continuous supply of zooplankton flowing through the mesh walls. The enclosed habitat had little diversity of substrate and aquatic vegetation, which may have limited the habitat and diversity of prey (Wetzel 2001). The limited growth observed within the enclosure may have been similar to the reduced growth in a lake where there is competition for prey items as a result of high densities of planktivorous fish (Parrish and Margraf 1993).

The effect of growth on mercury concentration

Using decimal coded wire tags allowed me to account for the effects of weight changes in my results. The observed and expected concentrations of mercury (both spike and ambient) followed similar patterns revealing the effect of minor changes in fish weight on concentration. Many other studies have found that gains or losses of weight impact mercury concentration (Laarman et al. 1976; Doyon et al. 1998; Cizdziel et al. 2002).

Increases in concentration occur when fish lose weight because tissue mass is lost at a faster rate than mercury, which is known as starvation concentration (Cizdziel et al. 2002). Growth dilution is the opposite of starvation concentration and results when tissue mass is created more quickly than mercury is accumulated (e.g. Doyon et al. 1998). In the present study, yellow perch exhibited changes in weight that resulted in both starvation concentration and growth dilution, which in turn influenced the observed patterns of mercury concentration.

Mercury concentrations and burdens

Ambient mercury

The only past field studies on mercury elimination by fish moved fish from mercury-contaminated lakes to systems with lower mercury (Lockhart et al. 1972; Laarman et al. 1976). Therefore, their estimates of mercury elimination were confounded by the continual uptake of ambient mercury even though levels were much lower in the "clean" systems. Ambient mercury concentrations in Lake 658 are greater than in Lake 240, resulting in approximately double the mercury concentrations in yellow perch muscle (Table 2.1). A comparison of changes in ambient mercury in fish transferred to Lake 240 may provide some insight, particularly into the results of Laarman et al. (1976). Both Laarman et al. (1970) and the present study monitored yellow perch with mercury levels within the range that occurs in the wild, transferred to systems with reduced levels of ambient mercury.

Neither the ambient mercury concentrations nor burdens in liver were significantly different over the first year of the study. The liver is exposed to mercury

that has entered the body usually within <10 days (Giblin and Massaro 1973; Oliveira Ribeiro et al. 1999) and, therefore, the mercury concentration in the liver is indicative of recent exposure to this compound (Jernelov and Lann 1971; Oliveira Ribeiro et al. 1999). The relatively constant concentrations and burdens over the first year suggest that there was constant exposure to ambient mercury (which may have been slightly lower in the winter) and little storage occurring in this tissue. The two fish collected on days 415 and 440 had similar concentrations of ambient mercury in their liver as fish collected on day 365, but much higher burdens due to growth.

The RF concentration of ambient mercury did not show a consistent pattern of change, and there was little overall change in the ambient burdens. The kinetics of mercury in the RF sample are more complex than liver or muscle because there are several visceral organs and the skin and bone of the fish included, which have different affinities for mercury (Giblin and Massaro 1973; Oliveira Ribeiro et al. 1999; Leaner and Mason 2004). The burden in RF showed a slight decreasing trend from day 15 to day 135, due to greater loss than gain from this tissue, consistent with exposure to lower ambient mercury in Lake 240. The burdens in the RF were much higher on days 415 and 440 than on day 365 (due to growth), while the concentrations were similar on these days.

Both concentration and burden of ambient mercury in the muscle exhibited an increasing trend in the first 180 days, followed by a decrease. The increase in ambient mercury in muscle suggests that perch transferred accumulated ambient mercury to their muscle tissue. Laarman et al. (1976) observed a 42% increase in mercury burdens in the muscle of yellow perch over 6 months after fish were transferred from a contaminated

lake to a clean lake. This was followed by a decline in burdens for the remainder of the study (Laarman et al. 1976), which was very similar to the results shown for ambient mercury in the present study. Lockhart et al. (1972) found a 29% overall loss of mercury from the muscle of northern pike over the course of 1 year, although the initial concentrations of mercury in these pike were very high. There may also be changing availabilities of ambient mercury over the course of a year, with seasonal changes in temperature, methylation, and prey availability (Furutani and Rudd 1980; Post et al. 1996). Rodgers and Qadri (1982) found that there was less accumulation of mercury by yellow perch over the winter than the summer, emphasizing how seasonal changes in ambient mercury availability can lead to seasonal changes in mercury burdens of fish. In the present study, the decrease in ambient mercury in the muscle observed after 1 year was likely a loss resulting from reduced exposure to ambient mercury. These perch were exposed to less ambient mercury in Lake 240 than in Lake 658, as well as a potential reduction in ambient mercury availability in the winter.

In summary, yellow perch held in Lake 240 experienced constant uptake of low levels of ambient mercury, compared to Lake 658. Levels of ambient mercury in liver indicated constant exposure to ambient mercury that may have been slightly lower in winter than summer. The ambient mercury levels in muscle increased as mercury was transferred from other parts of the body, which was most obvious up to day 180. This was followed by a slight loss of ambient mercury from muscle, which reflects reduced transfer into this tissue due to lowered mercury exposure. Patterns in ambient mercury observed in muscle were similar to those found by Laarman et al. (1976).

Lockhart et al. (1972) and Laarman et al. (1976) monitored elimination of ambient mercury in the field, which they were able to do by transferring fish from highly contaminated lakes to cleaner systems. Concentrations of ambient mercury in the contaminated systems of Lockhart et al. (1972) and Laarman et al. (1976) were 5 - 50 times higher than the clean lakes used for decontamination of the fish. In comparison, ambient mercury concentrations in Lake 658 yellow perch were nearly 2 times higher than in fish from Lake 240. In the present study, low amounts of ambient mercury that had been accumulated while in Lake 240 made ambient mercury inappropriate for measuring elimination. The results of Laarman et al. (1976) and Lockhart et al. (1972) may also have been affected by slight accumulation of ambient mercury in the "clean" lakes. The perch used in the present study had spike mercury concentrations within the range of those commonly seen for ambient mercury in wild fish (refer to Table 1.2). While Lockhart et al. (1972) and Laarman et al. (1976) had to use highly contaminated fish to see results given the continued exposure to ambient mercury, spike mercury allows elimination to be monitored in fish that have experienced environmentally relevant levels of contamination.

Spike mercury

The present study had the advantage of using enriched stable isotopes of mercury (spike mercury) to monitor elimination of mercury by fish. By transferring perch from Lake 658 to Lake 240, I prevented any further exposure to spike mercury. In this way, I then monitored the elimination of spike mercury from fish tissues without further uptake of this compound.

Changes in spike mercury levels in the liver of yellow perch confirmed that mercury was lost more quickly from visceral organs than from muscle. Fish lost two-thirds of spike mercury present in liver with most of the loss occurring in the first 90 days. After 90 days, mercury burdens leveled off at approximately 1 ng, which could be due to low fluxes of spike mercury entering the liver after the period of fast elimination (Oliveira Ribeiro et al. 1999). The patterns seen in the present study are similar to those reported in the literature, though the timing often varies between experiments. For example, Leaner and Mason (2004) found that methylmercury content declined within 5 days in the liver of sheapshead minnows (*Cyprinodon variegates*) before leveling off for the remainder of the 35-day experiment (which was held at 23°C). In contrast, Giblin and Massaro (1973) found a slower loss from rainbow trout (*Oncorhynchus mykiss*) liver (at temperatures from 5 - 9°C) where the mercury content declined by 60% over 85 days after the peak concentration was reached in this tissue. The amount of time that it takes for redistribution of mercury among fish tissues may partly depend on metabolic rate (as suggested by Leaner and Mason 2004; Nichols and Playle 2004). The redistribution studies mentioned above (Giblin and Massaro 1973; Leaner and Mason 2004) were conducted at different temperatures that may have partly caused the contrasting times required for relocation of mercury among tissues. Giblin and Massaro (1973) and Leaner and Mason (2004) also used different species and body sizes that could play a role in the resulting range of redistribution rates.

Similar to liver, the RF burden of spike mercury declined and leveled off at 90 days after the beginning of the study. The RF sample contained visceral organs (which have a fast rate of elimination) and skin, bones, and fins (that tend to store a marginal

amount of mercury with little elimination) (Giblin and Massaro 1973). Therefore, the resulting decline in RF burden was a combination of the different kinetics of these two portions of the fish. The results of the present study suggest that it took roughly 90 days for spike mercury to be removed from the visceral organs by the fast phase of elimination. The low burden of mercury that remained in the RF sample after 90 days, could have been stored in the skin, fins and bones, or was mercury re-circulating to the visceral components of the body after being released from tight binding locations, such as muscle (Oliveira Ribeiro et al. 1999). Similar redistribution and loss of methylmercury from the visceral organs has been found by other studies. For example, Giblin and Massaro (1973) found that the content of mercury in tissues that were represented in RF, such as heart, spleen, and blood, lost most of their mercury content by 85 days after the concentration peaked. In contrast, Leaner and Mason (2004) found that mercury burdens in intestine, and gill, which were present in RF in the current study, declined and then leveled off 5 days after exposure. These faster losses may have been due to the higher temperatures used in Leaner and Mason (2004). The present study involves fluctuations in temperature that would be experienced by yellow perch in nature, and therefore the 90 day period where most loss occurs from the RF is likely applicable to other populations of yellow perch in the wild experiencing similar seasonal temperatures and similar mercury burdens.

The changes in spike mercury concentrations in the muscle showed a slight decline, which occurred mainly between days 180 and 365, and was partially due to a small amount of fish growth. Burdens of spike mercury did not change significantly over time, though there was a slight decline to day 90, an increase to day 180 (by 60% of the

values on day 90) followed by a decrease to the end of the experiment. The non-significant elimination of mercury from the muscle tissue was similar to the findings of Laarman et al. (1976) for larger yellow perch that were monitored over 2 years. Laarman et al. (1976) also found similar redistribution of mercury into the muscle, where the burden in muscle increased by approximately 42% over the first 6 months (roughly 180 days) followed by a slight decline for the remainder of the study. Boudou and Ribeyre (1983) also saw no noticeable decline in burden of methylmercury in the muscle of rainbow trout over 250 days after mercury exposure ended. Nevertheless, Jarvenpaa et al. (1970) and Ruohtula and Miettinen (1975) found that there was a small amount of loss of methylmercury that had been injected directly into the muscle, suggesting that elimination can occur from muscle tissue.

If spike mercury was transferred directly from RF to muscle, I would expect to see an increase in muscle burden occurring simultaneously with a decrease in RF burden. My data did not show this pattern; rather the muscle burden of spike mercury did not increase until 45 days after the RF burden leveled off. A possible route of mercury redistribution is from the RF to the intestinal contents (Riisgard and Hansen 1990), which were not analyzed for mercury. Studies have shown that methylmercury can be reabsorbed from the intestinal contents into the bloodstream and can then be relocated to muscle tissue (Norseth and Clarkson 1971; Rudd et al. 1980; Boudou and Ribeyre 1983; Leaner and Mason 2004). It is possible that the delay in movement of spike mercury into muscle occurred because most of the mercury lost from RF was first excreted into the intestine before being reabsorbed into the bloodstream and moved to muscle (supported by Rudd et al. 1980; Riisgard and Hansen 1990; Leaner and Mason 2004).

In summary, yellow perch moved from Lake 658 to Lake 240 were no longer exposed to spike mercury. There was a fast loss of spike mercury from liver and the RF sample, with most of the burden being lost from these tissues in the first 90 days. The muscle burdens of spike mercury indicated that there was redistribution into the muscle over 180 days, with a small (but non-significant) decrease by day 365 which may have been due to loss from this tissue. These patterns have been made clear by the use of enriched stable isotopes in this study, and would not have been made obvious by measurements of ambient mercury alone.

Percentage of all mercury in different tissues

Yellow perch collected from Lake 658 and moved to Lake 240 were adjusting to reduced exposure to ambient and spike mercury. As a result, mercury moved into muscle from the rest of the fish until this flux slowed due to reduced intake of mercury. The peak amount of mercury stored in muscle relative to the rest of the fish occurred at 135 days for ambient mercury (at 93%), and 180 days for spike mercury (at 86%). After the point of maximum storage in muscle, the contribution of muscle to mercury content in the whole fish declined, suggesting that there was a flux of mercury out of the muscle. This shift in the flux of mercury out of muscle has not been recorded in shorter studies, while a 26-month field study on mercury in yellow perch muscle found a similar pattern (Laarman et al. 1976).

Laboratory studies have also shown that mercury is stored in muscle, although these short-term studies usually ended before full redistribution into the muscle could occur. Also, the estimated time for mercury to be relocated into muscle differs among

studies. For example, plaice (*Pleuronectes platessa*) exposed to methylmercury translocated 80% of all accumulated mercury into muscle during a 36-day study (Pentreath 1976a). Brown bullheads (*Ictalurus nebulosus*) exposed to a single dose of methylmercury translocated 71% to muscle by the end of a 61-day study (DeFreitas et al. 1974). Even a 250-day study on the elimination of methylmercury by rainbow trout reported that the maximum percentage of mercury located in the muscle (86%) occurred at the end of the study (Boudou and Ribeyre 1983). In these reports, the percentage of methylmercury in muscle had not begun to decline within the duration of the experiments. In contrast, after 135 days for ambient, and 180 days for spike in the present study, the percentage of the mercury in fish located in the muscle declined.

Body burden

Body burden is an estimate of the total mass of mercury in a fish, and accounts for changes in both fish weight and mercury concentration. Therefore, body burden accounts for growth dilution or starvation concentration. One method of determining body burden is the product of fish mass and concentration of mercury in the muscle (e.g. Lockhart et al. 1972), which has been referred to as method 1 in this thesis. Another method of determining body burden is to sum burdens in the component parts of the fish. For example, this method of calculating body burden (referred to as method 2) was the sum of the burden in the muscle, RF, and liver in the present study.

In the present study, body burdens calculated by method 1 were from 36 - 221% higher for spike, and 54 - 125% higher for ambient than body burdens calculated by method 2. Approximately 44% of the wet mass of a yellow perch is composed of muscle

yet most (approximately 60 - 90%) of the mercury in the fish is stored there. Therefore, the assumption that concentrations in muscle apply to the whole fish (as in method 1) leads to overestimates of total burden. Body burdens calculated by method 1 reflect the kinetics of mercury in muscle including storage and slow loss. Spike mercury body burdens calculated using method 2 declined more quickly than by using method 1 because method 2 included tissues with a faster rate of elimination than muscle.

The only two past field studies on mercury elimination have followed loss from muscle, which is analogous to method 1 in this study. Laarman et al. (1976) found that the measured burdens based on concentrations in muscle increased in yellow perch after they had been removed from a contaminated site. In contrast, Lockhart et al. (1972) found that mercury burdens calculated by method 1 were only 0.5 - 6% different than the content of mercury in the whole fish determined from six homogenized whole fish samples. They also found that the ratio of mercury concentrations among tissues changed little while being monitored for mercury loss (Lockhart et al. 1972). This finding by Lockhart et al. (1972) is different from other studies of mercury elimination, where changes in mercury content in different organs is an important part of the elimination process (e.g. Massaro and Giblin 1972; Giblin and Massaro 1973). This unusual finding may have resulted from the use of extremely contaminated fish in Lockhart et al. (1972).

In the present study, spike mercury body burdens in yellow perch (calculated by using method 2) declined following a pattern of exponential decay, in agreement with past findings (Jarvenpaa et al. 1970; Miettinen et al. 1970b; Huckabee et al. 1975; Ruohtula and Miettinen 1975; Pentreath 1976a; Sharpe et al. 1977; Rodgers and Beamish 1982). The exponential curve of loss from the whole fish was shallow and nearly linear

in the current study, which was also found by Sharpe et al. (1977). This pattern may have occurred in other studies of elimination of mercury from the whole fish as well, but curves fitted to data were not presented.

In this study, the half-life of spike mercury in the whole fish was estimated as 489 days, which is more than 5.6 times slower than past laboratory studies on yellow perch (refer to Table 1.3). This finding emphasizes how past studies conducted in the laboratory have overestimated mercury elimination rates. Laarman et al. (1976) also studied mercury elimination by yellow perch in the field and found no observable elimination based on levels measured in muscle. Laboratory studies have also been shown to overestimate elimination rates of other contaminants as well including PCBs (de Boer et al. 1994), cadmium (Kraemer et al. 2005), and selenium (Hamilton et al. 2002).

Application of the models to method 1 and method 2 of measuring body burden

Models that predict the elimination of mercury from fish take into account a host of factors that can affect rates of loss. In the present study, I used three mercury models to predict losses of spike mercury by yellow perch. None of the models fit observed spike mercury body burdens when calculated by method 1. The HB model and TR model (acute exposure) fit observed burdens reasonably well when calculated using method 2, while the Wisconsin model and the TR model (chronic exposure) did not.

All three models used in this study were primarily developed based on measurements of body burdens in fish using method 2, and therefore it is expected that the models will fit data produced by method 2 better than they fit method 1. The early

mercury models (e.g. Norstrom et al. 1976) were developed to simulate mercury loss from the whole fish, which would be comparable to method 2 of determining mercury body burdens. Many early elimination studies looked solely at changes in mercury in the whole fish, by measuring the change in ^{203}Hg over time (Jarvenpaa et al. 1970; e.g. Ruohtula and Miettinen 1975; Pentreath 1976a; Pentreath 1976b; Pentreath 1976c; Sharpe et al. 1977). The Wisconsin model is based on these early models and therefore should be strictly used for predictions of elimination from whole fish (method 2 of calculating body burden).

The HB model is also based on elimination of mercury from the whole fish; however, parameter values were calibrated using concentrations of mercury in the muscle of fish (Harris and Bodaly 1998). Predicted concentrations were altered from representing whole body mercury to mercury in muscle by multiplying by 1.5 (Harris and Bodaly 1998). Concentrations of mercury in muscle and viscera are known to change at different rates from each other (Oliveira Ribeiro et al. 1999; Leaner and Mason 2004). As a result, concentrations of mercury in the muscle will not consistently be 1.5 times that in the rest of the fish as mercury levels change with time. The HB model was calibrated to muscle concentrations, and transfer in and out of the muscle is slow, which may partly cause the slow elimination rates predicted by this model.

The TR model is based primarily on studies of loss from the whole body (Jarvenpaa et al. 1970; Burrows and Krenkel 1973; Ruohtula and Miettinen 1975; Pentreath 1976c) while also including one study of loss from muscle (Lockhart et al. 1972). Therefore, some of the error of the TR model may be due to using elimination rates from both muscle and whole body of the fish.

In the description of the TR model, fish that were chronically exposed to methylmercury displayed a faster rate of elimination than fish that were not chronically exposed (Trudel and Rasmussen 1997). This finding is in contrast to other studies that suggest contaminants are eliminated faster when fish are acutely exposed (de Boer et al. 1994; Rowan and Rasmussen 1995). Most of the chronic exposure data used by Trudel and Rasmussen (1997) came from the study by Lockhart et al. (1972), which also had the largest fish (>1000 g) of all studies considered. It was possible that the weight of fish had an impact on the finding that chronically exposed fish eliminated mercury faster than acutely exposed fish (M. Trudel, personal communication). Using the TR model so that chronically exposed fish lose mercury faster than acutely exposed fish results in an overestimation of elimination rates in the present study. This finding suggests that the chronic application of this model does not apply to small fish.

Suggestions for model use

The models used in this study generally overestimated the elimination of spike mercury when calculated by method 1. Method 1 of calculating body burden only considers the slow exchange in and out of muscle, which respond differently to changes in mercury exposure than the rest of the fish. If models are to be used based on body burden method 1, they should be made to reflect the kinetics of mercury storage and loss from muscle.

When using method 1 and 2 to calculate the body burden of mercury in a fish, it is important to consider how these measures may respond differently to changes in mercury exposure. By only measuring mercury in muscle tissue, method 1 tends to overestimate burdens of mercury in the whole fish by 36 - 221% in the present study. This

overestimation occurs because mercury is redistributed into muscle, while it declines in other organs that have not been included in the calculation. Method 2 is a more accurate measurement of all of the mercury in the fish because the whole fish is analyzed rather than just the muscle. Method 2 should be considered for studies that investigate food web transfer of mercury, because predators (other than humans) consume the whole fish, rather than just muscle. In these cases, understanding mercury losses from the whole prey fish is important to understanding changes in this source of mercury to predators. On the other hand, humans are most concerned with mercury in the muscle of fish, because this is the part of the fish that people eat. In this case, method 1 is suitable, as long as it is understood that the actual amount of mercury in the whole fish may differ and mercury models may not be appropriate to predict changing mercury body burdens calculated in this way. For future studies only concerned with declines in mercury in the muscle, method 1 is a suitable estimate of body burden that is less labour intensive than method 2.

Conclusions

The results of this study provide important new insights into mercury cycling in fish.

1) This study used enriched stable isotopes (spike mercury) to monitor mercury elimination by fish in the wild. It was possible to monitor elimination of spike mercury separately from the continual accumulation of ambient mercury.
2) Net reductions of mercury in muscle were not immediate. There was a lag of approximately 135 days before mercury redistributed from other tissues appeared in muscle. A peak of mercury storage in muscle was reached after approximately

180 days, followed by a period of loss. No past studies have monitored the elimination of labeled mercury in nature, and few studies have lasted long enough to detect losses from muscle.

3) Yellow perch that had naturally accumulated spike mercury in the wild, resulting in a burden of 213 ng, exhibited half-lives of elimination of 24 days (fast phase) from liver, 44 days (fast phase) from the non-muscle or liver tissues, and 489 days from the whole fish. There was an apparent fast and slow phase of elimination from liver and RF tissues. These rates of elimination of mercury from the whole fish are more than 5 times slower than past laboratory estimates for this species.

4) Two methods of estimating body burden of mercury in fish were compared. Method 1 involved multiplying concentrations of mercury in muscle by the weight of the fish, and method 2 involved summing burdens in the component parts of the fish. Method 1 resulted in overestimates of body burdens, and was not a realistic measure of changing mercury levels in fish.

5) The models were generally not applicable to method 1 of calculating body burden and this would also be true for other models based on mercury elimination from the whole fish. The HB model was the best for modeling mercury loss from the whole fish (as calculated by method 2). The TR model was also adequate, but only when it was used to represent acutely exposed fish, despite the fact that the exposure was chronic. The Wisconsin model (which is also the most widely used model) greatly overestimated elimination rates. Therefore, the current parameters of the Wisconsin model do not represent elimination of mercury by yellow perch in nature.

Table 2.1. The bathymetric, geographic, and biological characteristics of Lake 240 and Lake 658 of the Experimental Lakes Area. The mean weights, fork lengths (FL), and concentrations of spike and ambient mercury (THg) in yellow perch (*Perca flavescens*) muscle from June 2004 are also shown.

Lake	Location	Mean depth (m)	Max. depth (m)	Surface area (m^2)	Volume (m^3)	Lake type	Common fish species*	Weight (g)	FL (mm)	[spike THg] (ng/g d.w.)	[ambient THg] (ng/g d.w.)
240	49°39'15"N 93°43'35"W	6.0	13.1	441,800	608,000	oligotrophic, dimictic	yellow perch northern pike	3.6 ± 0.2	70 ± 2	0	645 ± 74
658	49°39'14"N 93°43'18"W	6.8**	13.2	84,655**	577,595**	oligotrophic, dimictic	white sucker yellow perch northern pike white sucker lake whitefish blacknose shiner	3.6 ± 0.3	67 ± 2	453 ± 16	1077 ± 43

*northern pike (*Esox lucius*), yellow perch (*Perca flavescens*), white sucker (*Catastomus commersoni*), lake whitefish (*Coregonus clupeaformis*), blacknose shiner (*Notropis heterolepis*).

**The average of values recorded for low and high water periods.

Table 2.2. Mean (+/- 1 standard error of the mean) percentages of total mercury that is methylmercury for ambient and spike mercury in muscle (M), liver (L), and whole body (WB) samples of yellow perch (*Perca flavescens*) over 440 days after being transferred from Lake 658 to Lake 240. The number of samples analyzed for total (THg) and methylmercury (MeHg) are also shown.

	Ambient			Spike			THg (n)*			MeHg (n)		
Day	M	L	WB	M	L	WB	M	L	WB	M	L	WB
0	97± 1	114± 12	103±4	109± 2	100± 13	104± 3	15	4$^{\phi}$	5	4	4*	4
15	98± 1	89± 7	104±3	109± 1	86± 8	104± 2	10	5	5	5	5	5
30	92± 2	66± 5	101±5	105± 2	56± 4	105± 5	10	5	5	5	5	5
60	99± 2	86± 5	108±4	108± 2	86± 11	103± 2	8	5	5	5	5	5
90	98± 2	56± 5	95±4	115± 3	56± 6	107± 5	9	5	5	5	5	5
135	100± 1	98± 11	104±2	113± 1		106± 2	8	5	5	5	5	5
180	104± 2	69± 5	102±3	109± 2	65± 10	109± 5	3	3	3	3	3	3
240	101			105			1			1		
365	98± 4	68± 4	103±1	102± 4	67± 5	108± 2	3	3	3	3	3	3
415	103	75	107	110	51	89	1	1	1	1	1	1
440	95	76	99	116	41	100	1	1	1	1	1	1

*Only fish that were analyzed for both THg and MeHg were used for the above THg:MeHg comparisons (n≤5)

$^{\phi}$ n= 4 after 1 extreme outlier removed.

Table 2.3. List of abbreviations used in calculations.

Symbol	Definition
WB	whole body of fish minus liver, stomach contents and samples of muscle
RF	whole body of fish minus liver, stomach contents, and all muscle
$sample_{dw}$	sample dry weight
$sample_{ww}$	sample wet weight
W_{fish}	weight of fish at time of sacrifice
$W_{fishstart}$	weight of fish at beginning of experiment
$W_{fishdayX}$	weight of fish at time of sacrifice, where X is sampling day
W_{sc}	weight of stomach contents
$W_{allmuscle}$	weight of muscle in the whole fish, estimated from regression in Figure 2.2a
$W_{musclesample}$	weight of muscle sampled for mercury analysis
W_{liver}	weight of liver
W_{rf}	weight of RF in the whole fish, estimated from regression in Figure 2.2b
W_{wb}	weight of WB
$W_{wbmuscle}$	weight of muscle in WB
$B_{allmuscle}$	burden of mercury in muscle
$B_{wbmuscle}$	burden of mercury in WB due to muscle
B_{rf}	burden of mercury in RF
B_{liver}	burden of mercury in liver
B_{wb}	burden of mercury in WB
$[Hg]_{dw}$	dry weight concentration of mercury
$[Hg]_{ww}$	wet weight concentration of mercury
$[Hg]_{muscle}$	dry weight concentration of mercury in muscle
$[Hg]_{liver}$	dry weight concentration of mercury in liver
$[Hg]_{wb}$	dry weight concentration of mercury in WB
$[MeHg]_{urine}$	concentration of methylmercury in urine as estimated by the HB model
$[MeHg]_{tissue}$	concentration of methylmercury in fish tissue as estimated by the HB model
$avg[Hg]_{day0}$	mean wet weight concentration of mercury in muscle of fish collected at day 0
B_{day0}	estimated starting body burden of yellow perch collected at later sampling days
$exp[Hg]_{dayX}$	expected wet weight concentration of mercury in muscle of fish collected at day X if there was no change in burden from day 0
$obs[Hg]_{dayX}$	observed wet weight concentration of mercury in muscle of fish collected at day X

Table 2.4. Mean concentrations (+/- 1 standard error of the mean) of total mercury (THg) and percentages of mercury made up of spike in tissues of yellow perch (*Perca flavescens*) collected from Lake 658 on day 0.

Tissue	n	Ambient (ng/g d.w.)	Spike (ng/g d.w.)	% spike
liver	5	584 ± 120	342 ± 59	37.9 ± 1.9
RF	5	174 ± 50	137 ± 24	50.3 ± 8.0
muscle	15	1077 ± 43	453 ± 16	29.7 ± 0.3

Table 2.5. Mean (+/- 1 standard error of the mean) percentage of all fish mercury (THg) in the whole fish present in muscle and RF for ambient and spike mercury measured in yellow perch (*Perca flavescens*) over 440 days after being transferred from Lake 658 to Lake 240 (n=5).

	Ambient		Spike	
Day	Muscle	RF	Muscle	RF
0	81.9 ± 4.7	17.0 ± 4.7	67.0 ± 2.0*	31.7 ± 2.1*
15	69.9 ± 0.8	29.2 ± 0.9	61.9 ± 0.9	37.0 ± 0.9
30	70.5 ± 1.9	28.2 ± 1.9	67.1 ± 2.1	31.5 ± 2.2
60	77.9 ± 1.5	21.2 ± 1.5	72.5 ± 1.2	26.7 ± 1.1
90	75.8 ± 2.0	23.3 ± 2.1	75.2 ± 1.1	23.9 ± 1.2
135	93.2 ± 2.4	5.8 ± 2.4	85.0 ± 1.9	14.6 ± 1.9
180	80.3 ± 2.3	18.8 ± 2.3	85.9 ± 1.8	13.3 ± 1.7
365	69.0 ± 4.4	29.6 ± 4.4	73.0 ± 1.2	25.9 ± 1.0
415	64.5	34.4	57.8	41.2
440	59.7	38.8	58.7	40.1

*n=4 after 1 extreme outlier removed

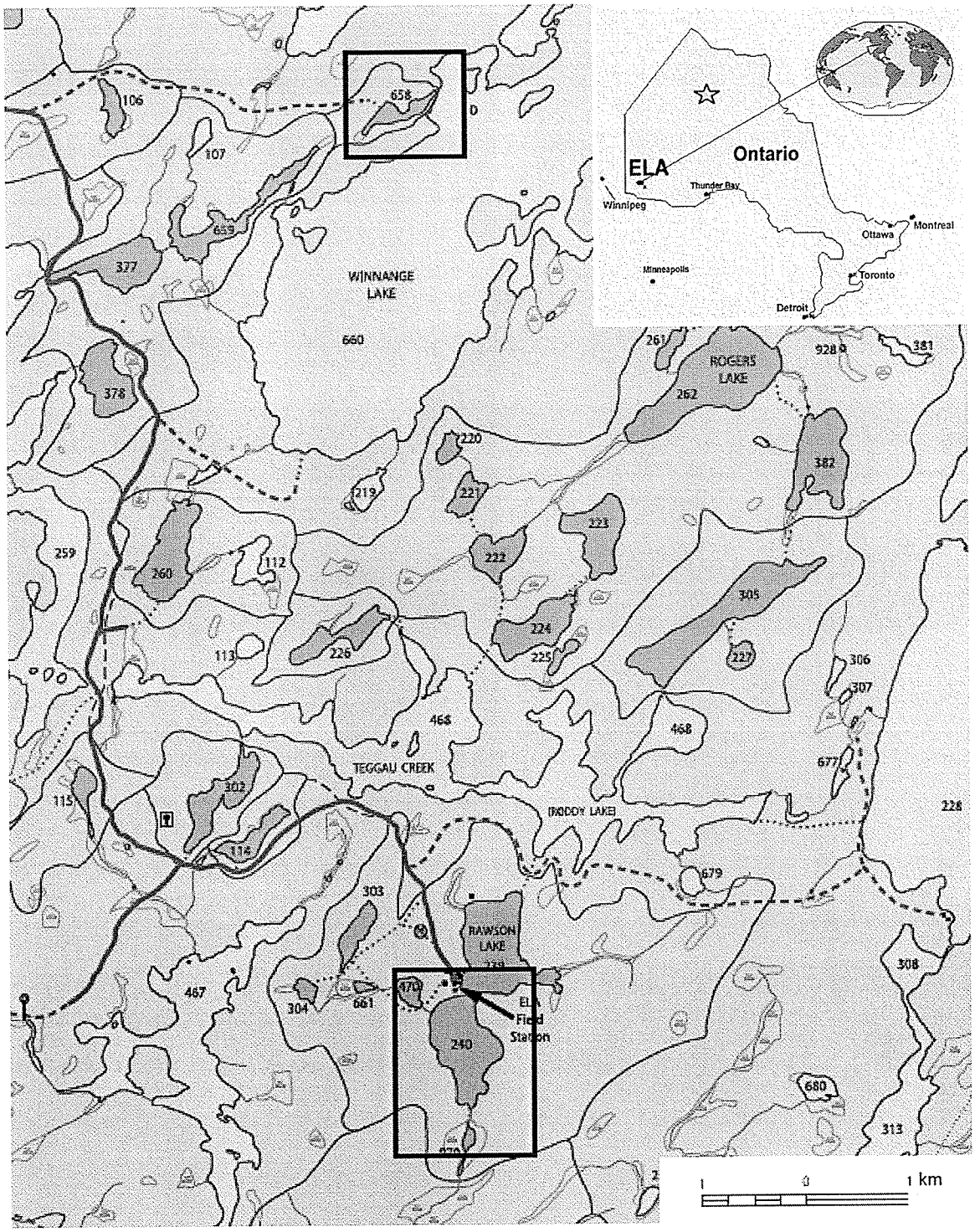

Fig. 2.1. Partial map of the Experimental Lakes Area (ELA) in northwestern Ontario. Lake 658 (top) and Lake 240 (bottom) are indicated by the black squares. The location of the ELA in Ontario is shown in the upper right corner.

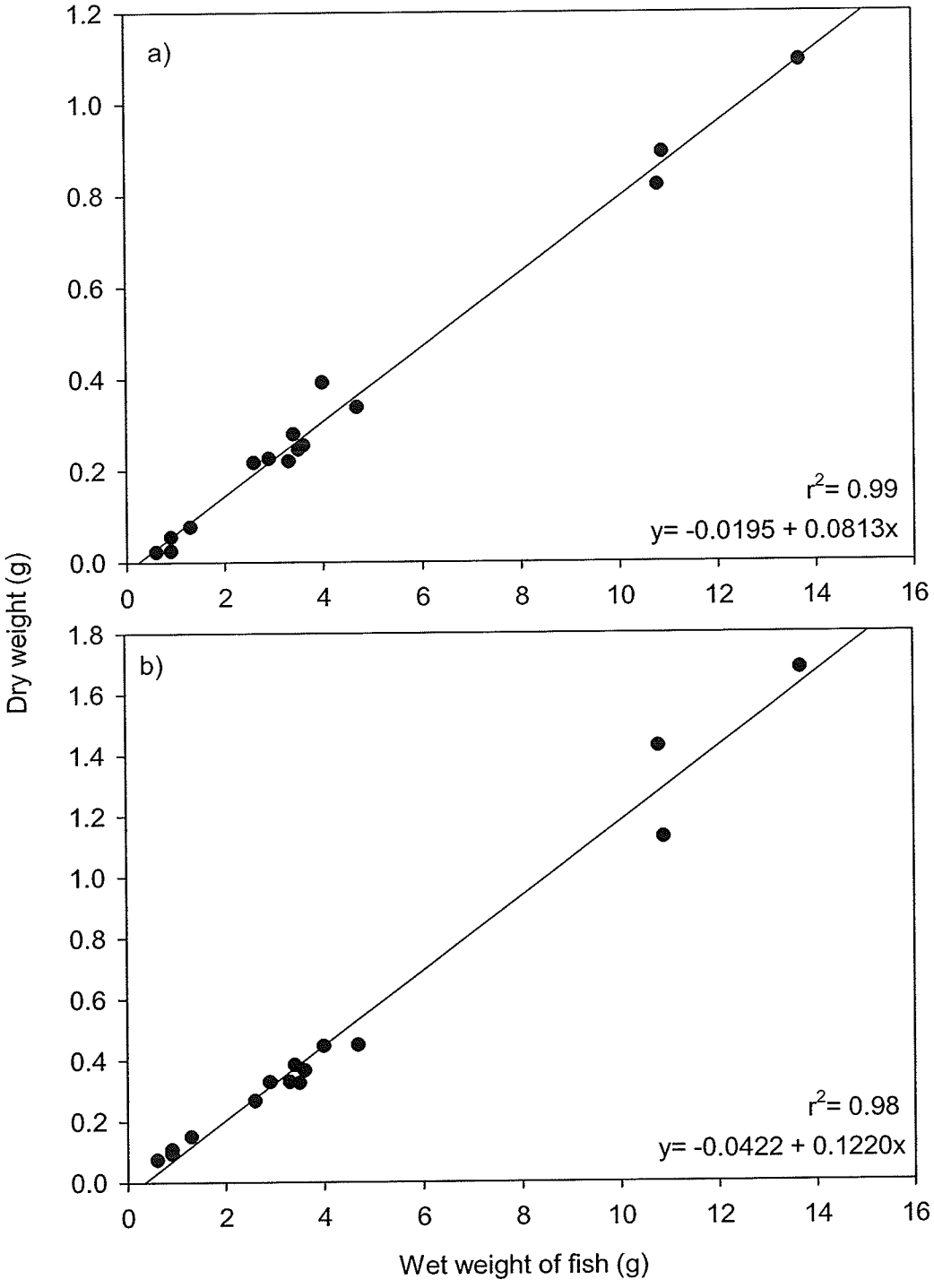

Fig. 2.2. Dry masses of a) muscle and b) the rest of the fish compared to the whole weight of 14 yellow perch (*Perca flavescens*) with stomach contents removed collected from Lake 658 and Lake 240.

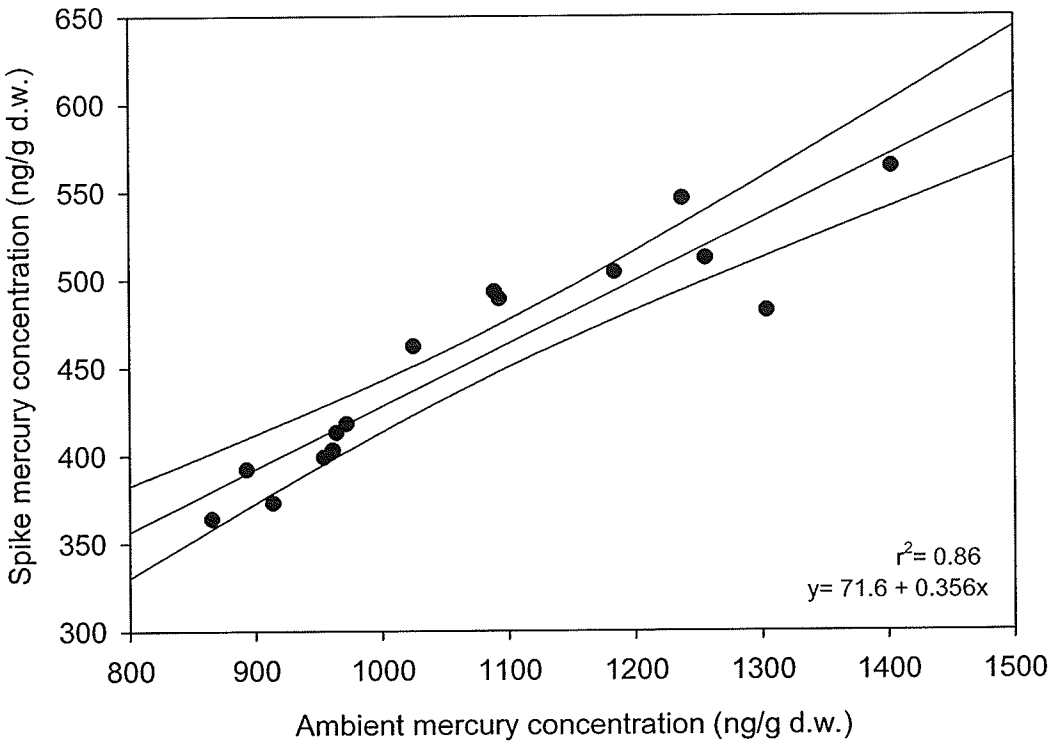

Fig. 2.3. Concentrations of spike mercury (THg) and ambient mercury (THg) in muscle of 15 yellow perch (*Perca flavescens*) collected from Lake 658 at the beginning of the study (day 0). Outer bands represent 95% confidence intervals.

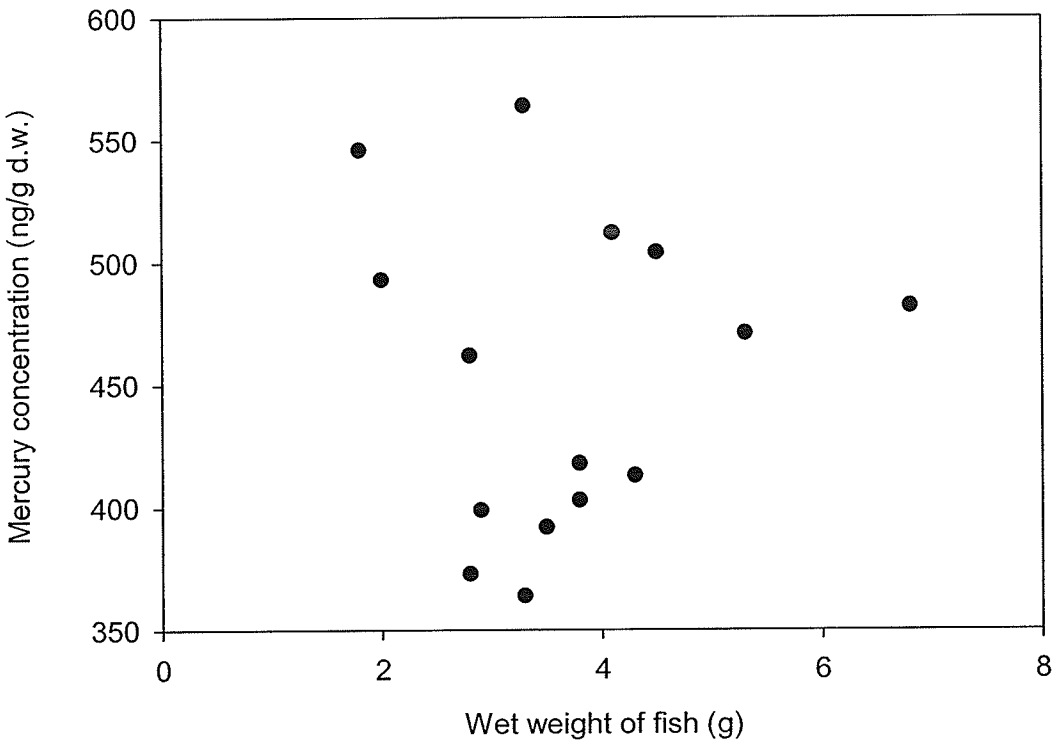

Fig. 2.4. Concentration of spike mercury (THg) in muscle compared to the weight of 15 yellow perch (*Perca flavescens*) with stomach contents removed collected from Lake 658 at the beginning of the study (day 0).

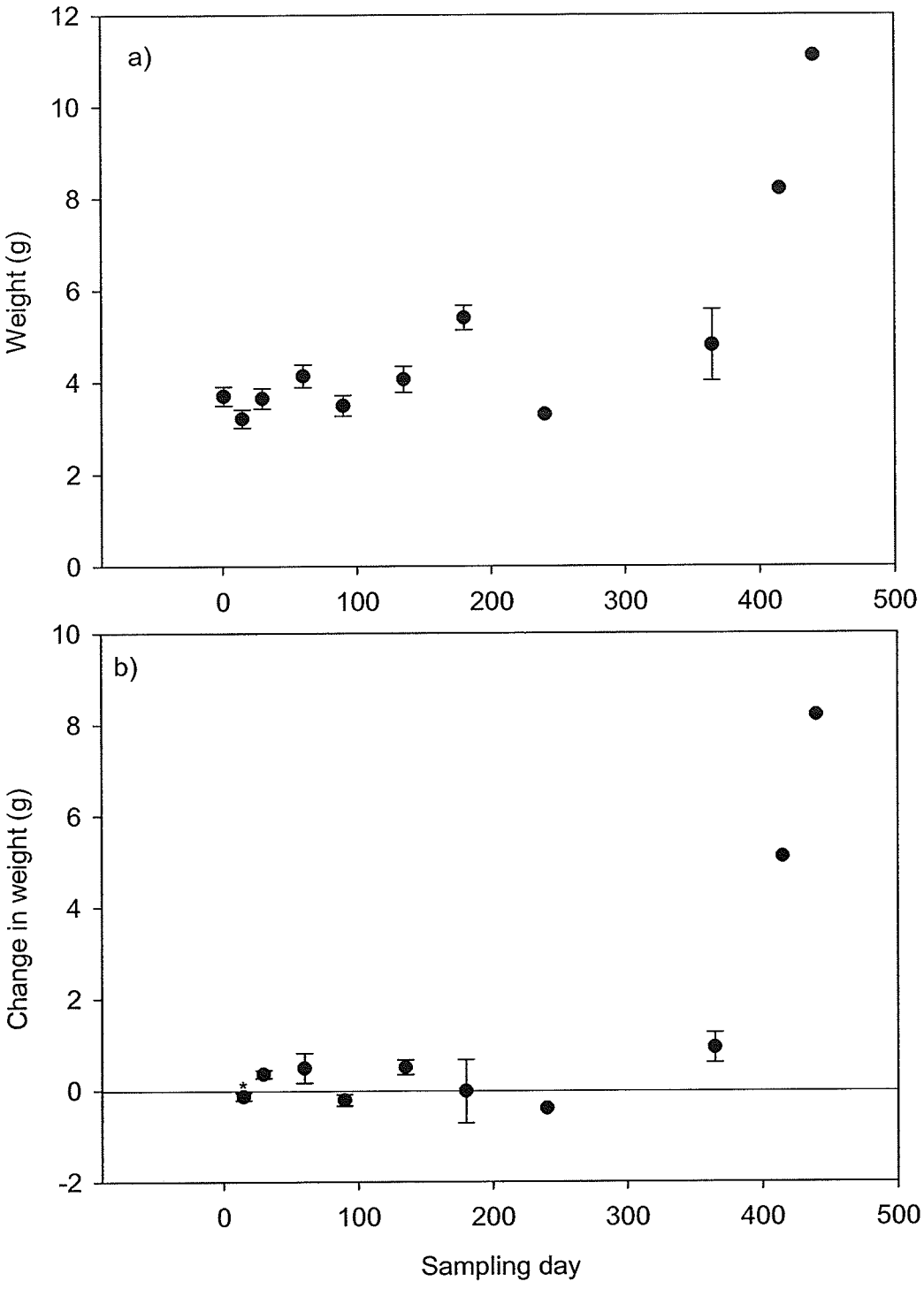

Fig. 2.5. Mean (+/- 1 standard error of the mean) a) weight and b) change from the initial weight of individual yellow perch (*Perca flavescens*) over 440 days after being transferred from Lake 658 to Lake 240. * 2 extreme outliers removed n = 8.

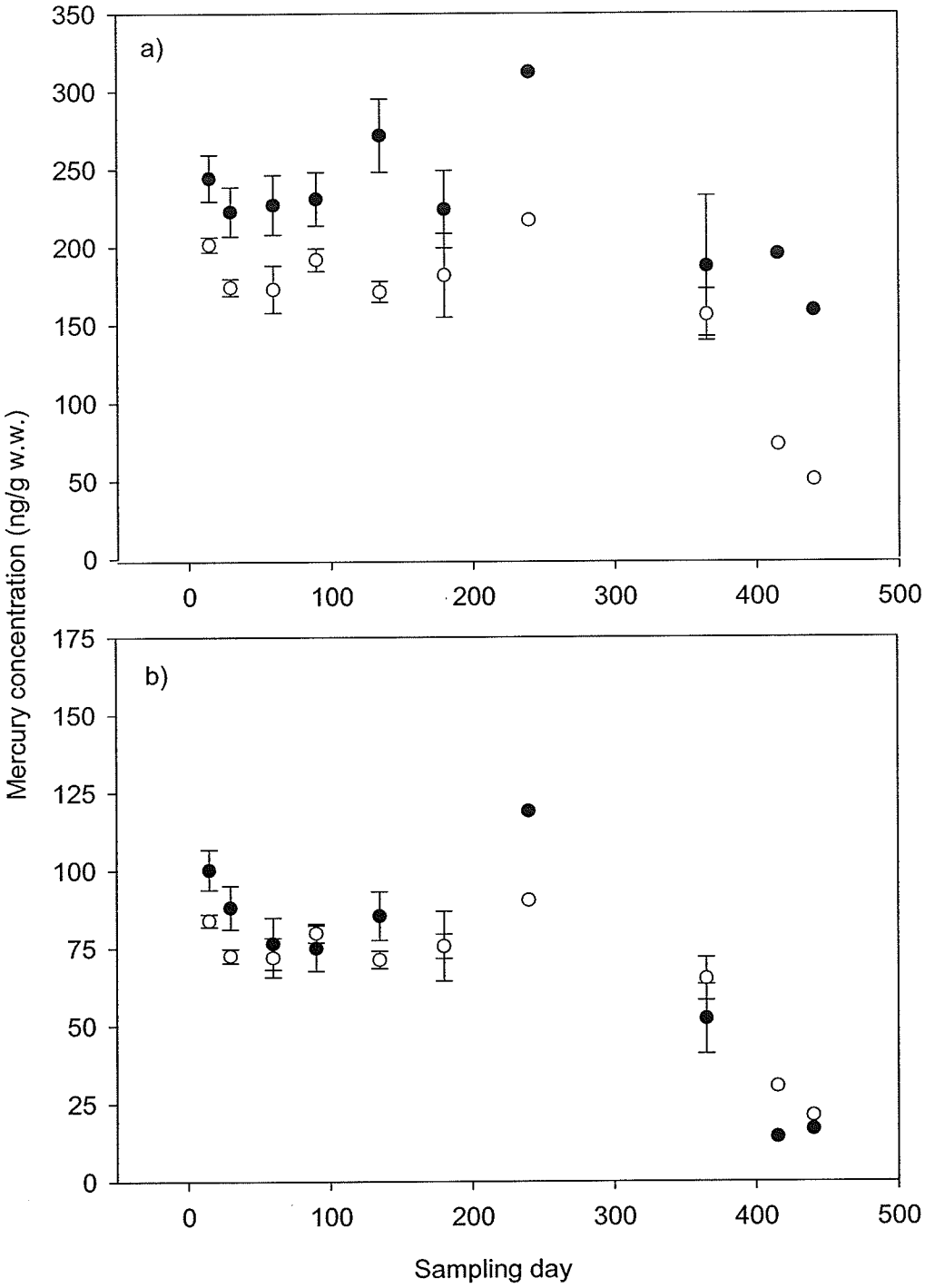

Fig. 2.6. Mean observed (closed circles) and expected (open circles) concentrations (+/- 1 standard error of the mean) of a) ambient and b) spike mercury in muscle tissue of yellow perch (*Perca flavescens*) over 440 days after being transferred from Lake 658 to Lake 240.

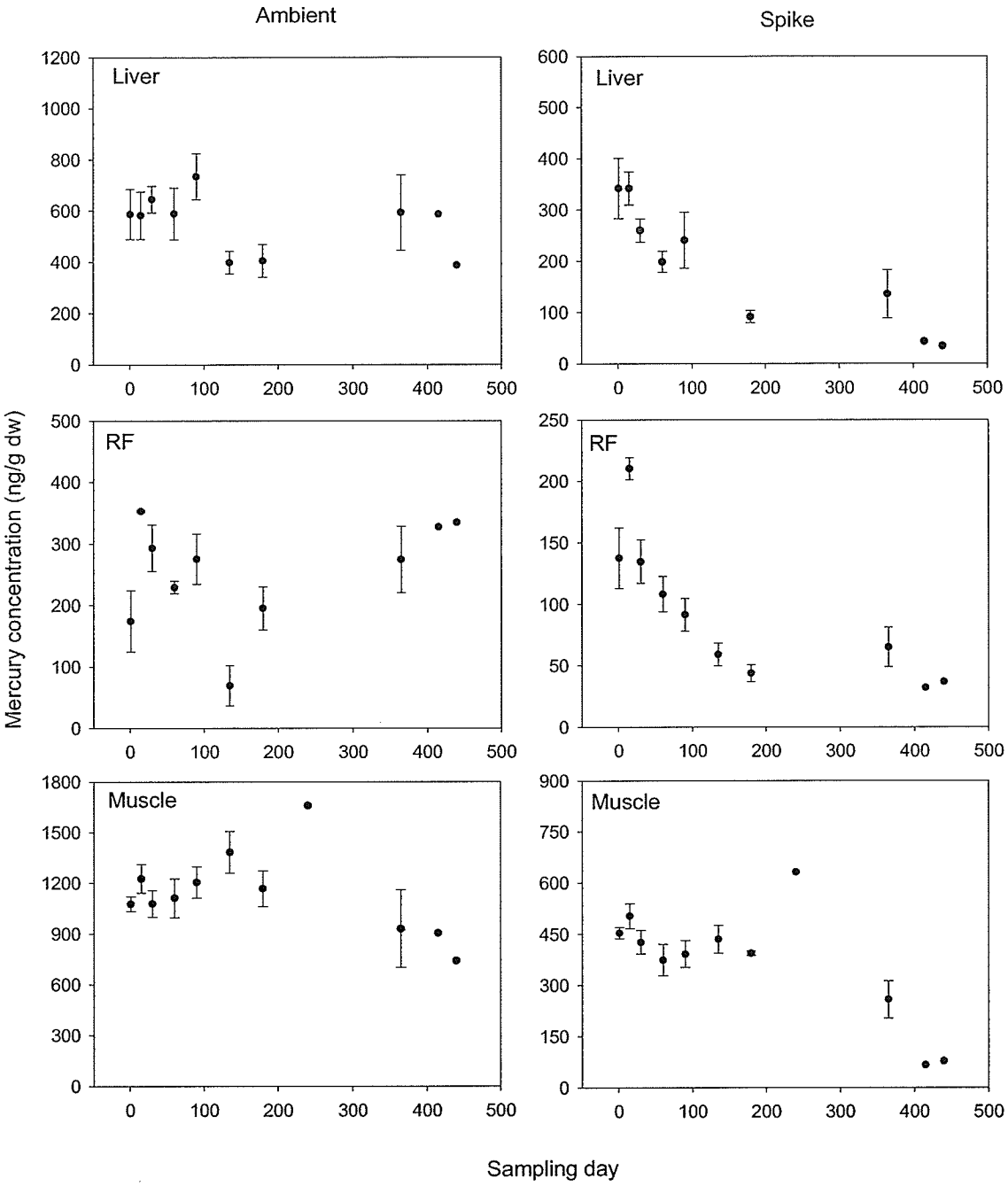

Fig. 2.7. Mean concentrations (+/- 1 standard error of the mean) of ambient and spike mercury (THg) in the liver, RF, and muscle of yellow perch (*Perca flavescens*) over 440 days after transfer from Lake 658 to Lake 240. Note the different y-axis scales.

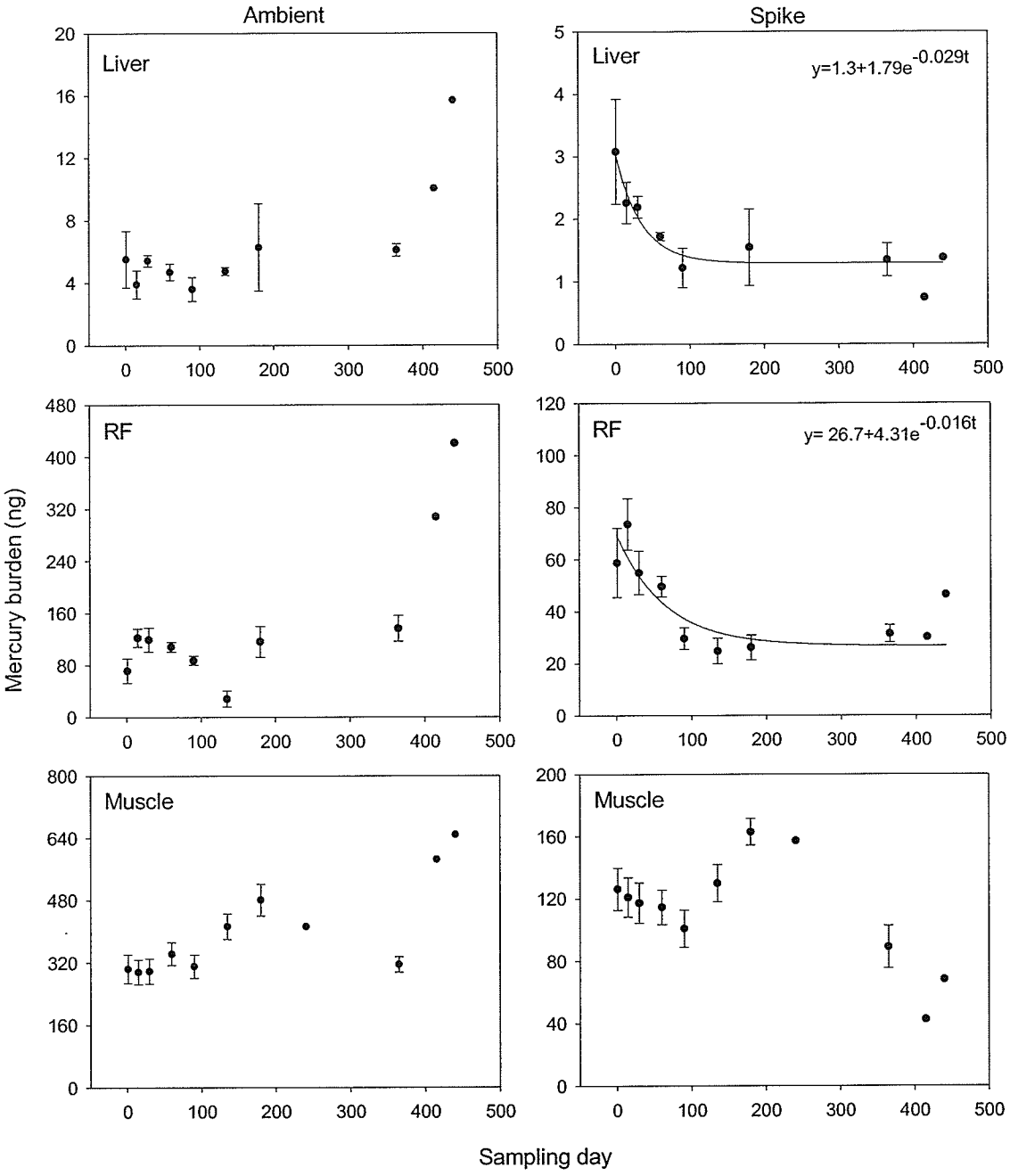

Fig. 2.8. Mean burdens (+/- 1 standard error of the mean) of ambient and spike mercury in liver, RF, and muscle of yellow perch (*Perca flavescens*) over 440 days after being transferred from Lake 658 to Lake 240. The 3-parameter exponential decay equations are shown for spike mercury in the liver and RF. Note the different y-axis scales.

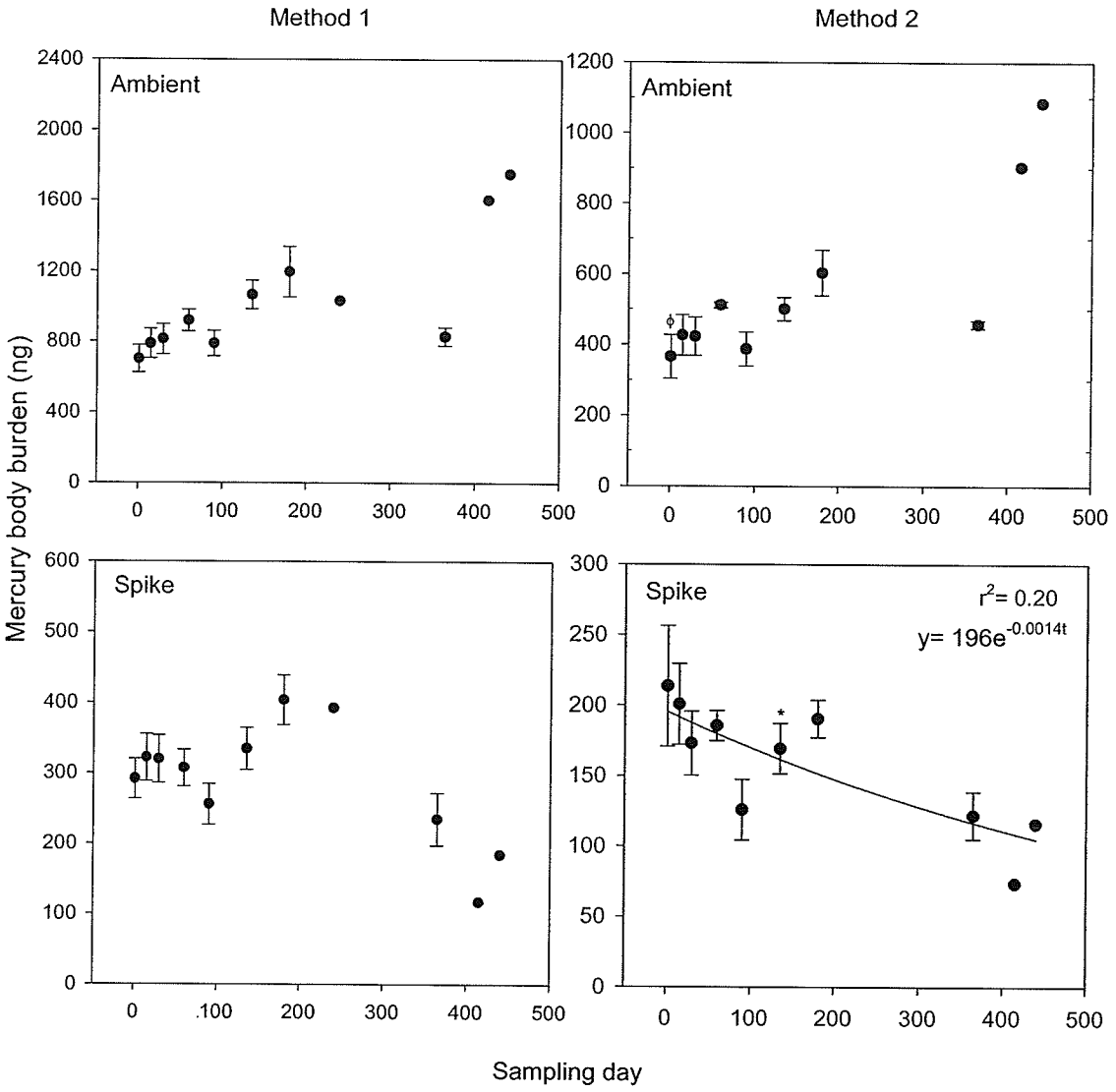

Fig. 2.9. Mean (+/- 1 standard error of the mean) ambient and spike mercury body burdens calculated by method 1 and method 2 (see text) for yellow perch (*Perca flavescens*) over 440 days after transfer from Lake 658 to Lake 240. *liver burden was not included in the body burden calculation due to analytical errors. $^{\phi}$ 1 extreme outlier removed (n=3). Note the different y-axis scales.

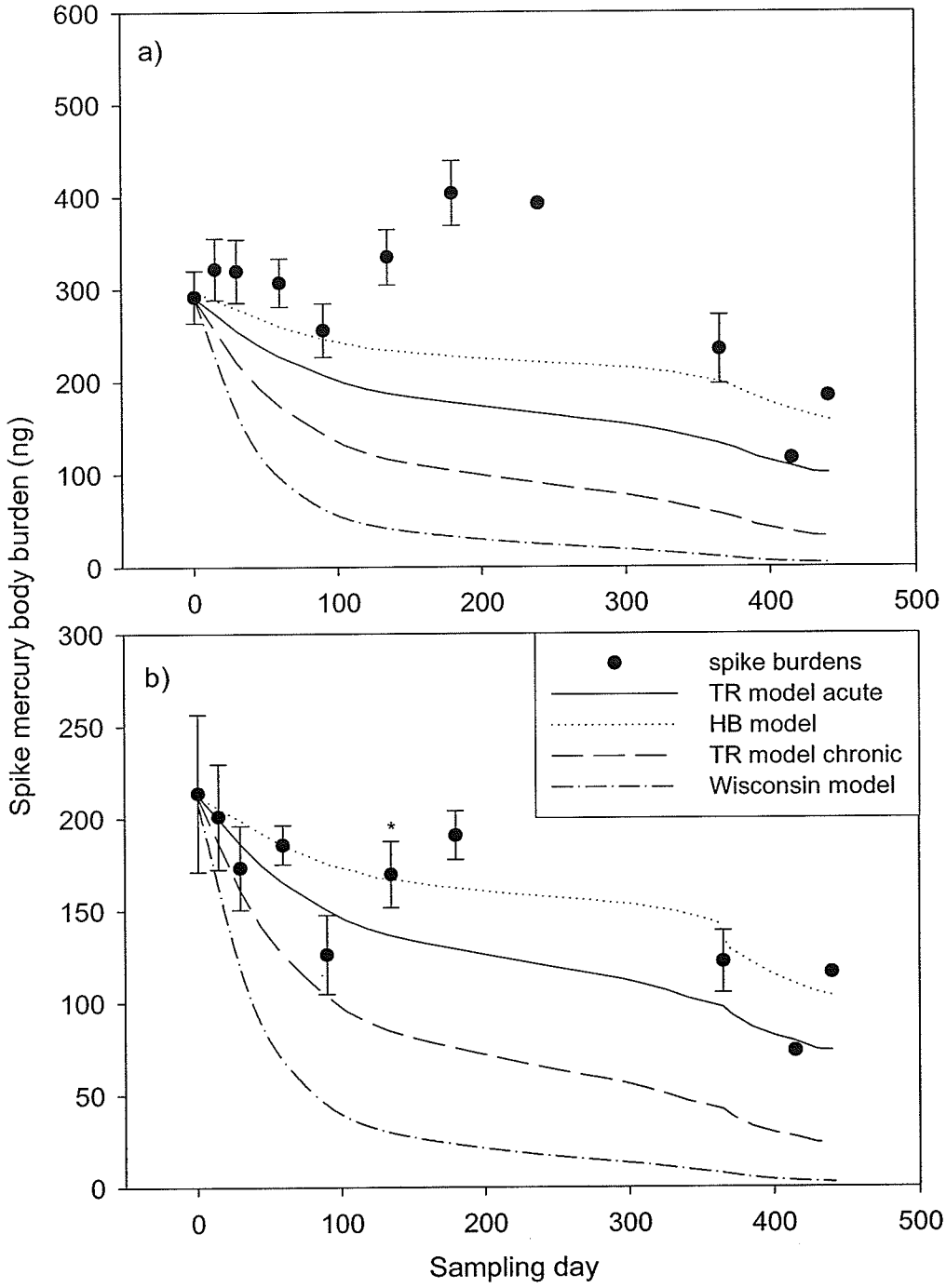

Fig. 2.10. Mean (+/- 1 standard error of the mean) spike mercury body burdens calculated by a) method 1 and b) method 2 (see text) for yellow perch (*Perca flavescens*) over 440 days after transfer from Lake 658 to Lake 240. Predicted spike mercury loss from these yellow perch by the HB model, Wisconsin model, and TR model (acute and chronic exposures) are shown. * liver burden was not included in the body burden calculation due to analytical errors.

Chapter 3: Elimination of mercury by yellow perch (*Perca flavescens*) in a reservoir and an unimpounded lake.

Introduction

Flooding of hydroelectric reservoirs commonly causes mercury levels in fish to increase and surpass guidelines for safe consumption (reviewed in Bodaly et al. 1997). Fish consumption guidelines for Ontario recommend that children and women of child-bearing age do not eat fish with mercury concentrations exceeding 520 ng/g, while people in the general population should avoid fish with greater than 1840 ng/g of mercury (O.M.O.E. 2005). Studies on Canadian and Finnish reservoirs showed that northern pike (*Esox lucius*) had muscle mercury concentrations greater than 500 ng/g (the limit for commercial sale) due to increases by 2 - 9 times pre-flooding levels (reviewed in Bodaly et al. 1997). In the La Grande reservoir complex in Quebec, mercury concentrations in walleye (*Stizostedion vitreum*), a piscivorous fish, increased from 680 - 2800 ng/g (Verdon et al. 1991). Two benthivorous species in this reservoir, longnose sucker (*Catostomus catostomus*) and lake whitefish (*Coregonus clupeaformis*), increased 4-fold to 670 ng/g and 570 ng/g, respectively (Verdon et al. 1991). Similarly, concentrations of mercury increased in northern pike and walleye by 2.5 - 3 times, reaching 670 - 950 ng/g and 570 - 750 ng/g respectively as a result of flooding of the Churchill River Diversion (Bodaly et al. 1984). Elevated mercury levels in reservoir fish pose a potential health risk to people that eat these fish. Understanding the changes of mercury levels in reservoirs over time is important to predicting the duration of these risks.

Flooding and decay of flooded organic matter causes conditions that increase the availability of mercury to the food web. As flooded matter decays, it releases bound

inorganic and methylmercury and supplies nutrients to methylating bacteria (as suggested by Heyes et al. 2000). Decomposition increases bacterial mercury methylation, which is thought to be the most important process leading to elevated methylmercury levels in reservoirs (Bodaly et al. 1997; Kelly et al. 1997; Heyes et al. 2000). Microorganisms decompose flooded matter and their elevated respiration depletes oxygen levels in the water. Resulting anoxic zones further increase bacterial mercury methylation (Regnell 1990; Bodaly et al. 1997). Methylmercury is accumulated by the food web and biomagnifies with each trophic level (reviewed in Wiener et al. 2003). As a result, the increased methylmercury production caused by flooding leads to elevated methylmercury concentrations in fish (Bodaly and Fudge 1999). Throughout this chapter, references to 'mercury' in fish imply total mercury, which is known to be primarily composed of methylmercury (refer to Table 1.2).

A concern with reservoir creation is the persistence of mercury in fish for many years after the initial impoundment. Concentrations of methylmercury in zooplankton eventually decline to pre-impoundment levels approximately 10 years following flooding (reviewed in Bodaly et al. 1997). In comparison, mercury levels in omnivorous and predatory fish remain high for much longer after flooding: 15 - 20 and 20 - 30 years, respectively (Verdon et al. 1991; Bodaly et al. 1997). These long recovery times are partly caused by slow rates of elimination of mercury by fish. In other words, the number of years that mercury levels in fish remain high (and possibly unsafe to eat) is related the rate at which mercury is lost from the muscle tissue of various species.

The rate at which fish eliminate mercury has not been studied in reservoirs. The effect of mercury exposure on elimination rates by fish has been briefly investigated and

is relevant to reservoirs because of elevated mercury levels in these systems. In the only study on this topic, Rodgers and Beamish (1982) found that increased mercury exposure resulted in greater elimination rates. Rainbow trout (*Oncorhynchus mykiss)* were fed diets with added mercury (either <0.1, 23.2, or 76.5 mg/g) for either 1, 28, or 56 days at feeding rates of 1% or 2% of the body weight per day. Then the fish were fed labeled mercury at the same concentrations that they had been exposed to earlier. Next, the fish were returned to the non-labeled mercury diets for an additional 20 days, after which time their elimination of labeled mercury was determined. This 20-day period will be referred to as the decontamination period for the rest of this chapter, because this is when elimination of labeled mercury occurred. Rodgers and Beamish (1982) found that rainbow trout continually exposed to methylmercury at a dose of 76.5 mg/kg, eliminated labeled methylmercury up to 76% faster than when clean food was given. Rodgers and Beamish (1982) concluded that a higher dose of mercury during the decontamination period stimulated and increased the elimination processes. This theory has not been tested at lower or more environmentally realistic concentrations of methylmercury. Rodgers and Beamish (1982) used exposure treatments of methylmercury approximately 16 - 60 times higher than the highest levels recorded in zooplankton over 9 years of an experimental reservoir study at the Experimental Lakes Area (referred to as ELARP: Experimental Lakes Area Reservoir Project) (St. Louis et al. 2004). It is currently not known if the findings of Rodgers and Beamish (1982) are relevant in nature. The goal of the present study is to test if altered elimination rates that result from high levels of mercury exposure (as found by Rodgers and Beamish 1982), would also result from the elevated mercury levels that are typical of reservoirs.

There are mathematical models that can be used to predict mercury concentrations in fish, taking into account physical and biological factors found in reservoirs. These models need to be accurate so that industry can predict the duration of mercury contamination by fish following impoundment. Current models do not adjust for differential elimination of mercury based on concentrations of mercury absorbed (R. Harris, personal communication). If mercury elimination rates were different in systems with high levels of mercury, the accuracy of the reservoir model predictions could be compromised. To examine the effects of mercury exposure on elimination rates, I conducted a field test of mercury elimination in a reservoir. The purpose of this study was to test if yellow perch (*Perca flavescens*) eliminate mercury differently in a low-mercury lake compared to the high-mercury conditions of a reservoir. Yellow perch that had naturally accumulated labeled mercury (see Chapter 2) were transferred to either the ELARP experimental reservoir or a low-mercury lake. This study tests whether environmentally relevant levels of mercury exposure affect elimination rates under natural conditions. From this study, I will determine whether elimination rates in reservoir models should be adjusted to account for the bioavailability of mercury.

Methods

Study site

This research took place at Lake 658, Lake 240 and Lake 979 of the Experimental Lakes Area in northwestern Ontario. The Experimental Lakes Area is located on Precambrian shield, consisting of naturally oligotrophic lakes, in an area isolated from outside anthropogenic influences. Some characteristics of these three lakes are summarized in

Table 3.1. Lake 658 is the site of a whole-ecosystem experiment called METAALICUS (Mercury Experiment To Assess Atmospheric Loading In Canada and the United States), where enriched stable isotopes of mercury have been added for the past four field seasons. The fish in the lake have accumulated the added mercury (referred to as spike mercury) (Table 3.1), which can be distinguished from the ambient mercury that is prevalent in freshwater systems. Lake 240 has comparatively low levels of ambient mercury in the food web (Table 3.1). There have not been any additions or manipulations of Lake 240 (it is "clean" with respect to isotopically enriched mercury). Ambient mercury levels in Lake 240 have been monitored for the METAALICUS study as a reference for Lake 658. Lake 979 is a small peatland that was first experimentally flooded in 1993 for the ELARP study (Experimental Lakes Area Reservoir Project). Flooding caused the surface area to increase by 300% and raised the water level by 1.3 m (St. Louis et al. 2004). The water level has been drawn down each fall and re-flooded each spring since 1993 to simulate changes in water level in hydroelectric reservoirs. Flooding caused increases in methylmercury levels in water, the lower food chain, and fish (Hall et al. 1998; Paterson et al. 1998; Bodaly and Fudge 1999; St. Louis et al. 2004) (Table 3.1).

For the present study, yellow perch containing spike mercury were relocated from Lake 658 to enclosures in either Lake 240 (which has low ambient mercury) or Lake 979 (which has high ambient mercury), and loss of spike mercury was monitored for 90 days. Enclosures in these two lakes had mesh walls and were open to the sediment, allowing access to typical prey items of age 1 yellow perch. The 240 m^2 rectangular enclosure in Lake 240 was located on sandy substrate and had a maximum depth of 2 m. The

enclosure in Lake 979 was a 19.6 m^2 cylinder sandbagged to the sediment, with a floating ring on the surface of the water. This enclosure was located on muddy sediment in the central pond of the flooded peat bog at a depth of approximately 2.4 m. Algae and pollen were scrubbed off the mesh every 2-4 weeks to maintain flow of water and floating prey through the enclosures. Enclosures were topped with nylon mesh for protection from avian predators. The water temperature within enclosures was recorded every 30 min using three Stowaway Tidbit temperature loggers (Onset Computer Corp., Bourne, Massachusetts) placed so that they spanned the depth profile in the enclosures (60 cm intervals of depth in Lake 240, and 120 cm intervals in Lake 979).

Measurements of ambient mercury in the food web

To estimate exposure of perch to ambient mercury in Lakes 240 and 979, ambient methylmercury concentrations were measured in samples of zooplankton collected from these lakes. A sample of zooplankton was collected on July 4, July 26, and September 26, 2005 from Lake 979 using a zooplankton net with 150 μm mesh. Zooplankton were collected from center buoy of Lake 240 using a 0.5 m zooplankton net (150 μm mesh) every 2 - 5 weeks from May 14, 2005 to October 9, 2005 as part of the METAALICUS sampling. Collected samples were emptied into whirl-pak bags (Nasco, Fort Atkinson, Wisconsin) and frozen. Later, the zooplankton were freeze-dried and a sample was weighed into Teflon vials before analysis of methylmercury concentrations. Zooplankton from both Lake 240 and Lake 979 were analyzed by cold-vapour atomic fluorescence spectrophotometry (CVAFS) by Flett Research Ltd in Winnipeg, MB.

Transfer and collection of fish

On June 9, 2005, 280 age 1+ yellow perch were collected from Lake 658 using a pole seine net (4 m length, 1.5 m deep), and hoop nets (Fipec Inc., Grande-Riviere, Quebec). Due to cool temperatures in the summer of 2004, age 1 perch were somewhat smaller than those caught in the previous year (see Chapter 2), fitting within a size range of 35 - 69 mm fork length. Mean weights and mercury concentrations of the yellow perch collected at the beginning of the experiment are shown in Table 3.1. Fish were anaesthetized by being placed in a solution of MS222 (tricaine methane sulfonate) at 0.05 g/L for approximately 2 min or until operculum movement slowed. Wet weight (to the nearest 0.1 g on an A&D Co. Ltd. scale) and fork length (mm) were measured and recorded for each perch. Next, each fish was marked with subcutaneous injection of orange elastomer (Biomark, Inc., Boise, Idaho) on the ventral side of the post-anal peduncle to provide an external visual mark that these fish were collected from Lake 658. A decimal sequential coded wire tag (Northwest Marine Technology Inc., Washington) of 1.1 mm length and 0.25 mm diameter was implanted subdermally in the left cheek of each perch using a Single Shot Tag Injector (Northwest Marine Technology Inc., Washington). Fish were held in a holding pen (91 cm X 102 cm X 183 cm dimensions with 0.64 cm mesh) in Lake 240 for 2 days after tagging and initial mortalities were removed. I then transferred 114 perch to an enclosure in Lake 240, and 90 perch to an enclosure in Lake 979.

On June 10, 2005, 50 yellow perch (42 - 77 mm fork length) were collected from Lake 240 using a beach seine net (45.7 m X 1.8 m). These perch were marked with green elastomer dye and each was injected with a decimal coded wire tag. Thirty of these fish

were included in the enclosure in Lake 240, and 20 in the enclosure in Lake 979. This was a control to measure if there was recycling and accumulation of spike mercury that may have been released from Lake 658 fish (see Methods of Chapter 2).

Ten perch were collected at the time of transfer as a day 0 sample. Four to ten yellow perch were collected from the enclosures at 15, 30, 60, and 90 days after transfer from Lake 658 (Table 3.2). Capture of fish in the enclosure in Lake 240 was done using a seine net (either a 4 m X 1.5 m pole seine or a 16.8 m X 2.4 m beach seine), while perch were captured in the Lake 979 enclosure using gillnets with 8 - 10 mm mesh. On day 90, all remaining fish were removed from the Lake 979 enclosure using a purse seine net (16.8 m length, 2.4 m depth). The control perch were collected for analysis at 90 days after the beginning of the experiment in Lake 979. In Lake 240, fish remaining after day 90 were left over winter in an attempt to extend the experiment. Due to ice damage, all perch had escaped the enclosure before spring. As a result, control perch were not collected from the Lake 240 enclosure for the present study. In 2004, recycling of spike mercury did not occur in this enclosure (refer to Chapter 2), and therefore it is likely that there was no recycling during the present study. Collected perch were immersed in 0.5 g/L MS222 in water for euthanasia. Fork length (mm) and wet weight (g) were recorded, and the fish were frozen in individual whirl-pak bags. Before the fish were processed further, decimal coded wire tags were removed from the left cheek and read. There were 2 out of 64 fish collected that had lost their tags: 1 on day 30 in Lake 979, and 1 on day 90 in Lake 240.

Processing of fish tissue

Yellow perch were processed as described in Chapter 2, except that the livers were not analyzed separately, but were included in the RF (whole fish minus all muscle) sample. All samples were analyzed for total mercury using an ICP-MS at Trent University in the laboratory of Dr. H. Hintelmann.

Calculations

Mercury concentrations (ng/g d.w.), expected concentrations based only on changes in weight, and tissue and whole fish burdens were calculated using methods described in Chapter 2.

I calculated a daily growth rate by subtracting the starting weight of a fish from its weight at the time of sampling, and dividing this by the number of days the fish had been in the enclosure.

Model simulations

I compared observed elimination rates with rates predicted by three models: a model developed by Harris and Bodaly (1998) (the HB model), the Wisconsin model version 3.0 (Hanson et al. 1997), and a model developed by Trudel and Rasmussen (1997) (the TR model). These models are described in detail in Chapter 2 and Appendix 1. The inputs used for these models are shown in Table A.4 for the HB model, Table A.5 for the Wisconsin model, and Table A.6 for the TR model. The Wisconsin model was also used to determine ambient mercury concentrations in diet that could result in the mercury levels reached by yellow perch held in Lake 979 (Table A.10).

Statistical analyses

Differences in yellow perch mercury levels in Lake 240 and Lake 979 were tested using a 2-factor analysis of variance (ANOVA), with factors sampling day and lake (α=0.05). Differences between observed and expected concentrations were compared using a paired *t*-test on each sampling day. Extreme outliers indicated by SAS were removed. When necessary, variables were log transformed so that residuals were normally distributed (tested by the Shapiro-Wilk test) with homogeneous variance (by Bartlett's test). When significant differences occurred, Scheffe's post-hoc test was used. All of the above analyses were done using SAS version 9.0. Half-life was calculated using linear regressions of the natural log of burden and time, which were produced in SigmaPlot version 9.0.

Results

Fish growth

Yellow perch held in the enclosure in Lake 240 grew steadily, quadrupling in size over 90 days (Figure 3.1a). In the Lake 979 enclosure, growth rate increased substantially in the last 30 days, when the weights increased by more than 3-fold. Over 90 days, the perch held in Lake 979 increased their weight by roughly twelve times (Figure 3.1a). Yellow perch had greater growth rates in Lake 979 than in Lake 240, especially in the last 30 days of the study (Figure 3.1b, $F=73$, $p<0.0001$, $df=1$).

Ambient mercury in zooplankton

Because zooplankton samples were not collected on the same dates for both Lake 240 and Lake 979, it was difficult to compare the two lakes statistically. Concentrations of methylmercury in zooplankton in Lake 979 appeared to be less than 1.6 times higher than on similar sampling days in Lake 240. Seasonal changes in concentrations were similar in both lakes, with low levels in the spring and fall, and a peak in mid-summer, approximately 50 - 65 days after the beginning of the experiment (Figure 3.2).

Ambient mercury in yellow perch

I examined the extent that observed changes in ambient mercury in perch muscle tissue were affected by growth by comparing observed concentrations to values expected due solely to weight changes (Figure 3.3). The difference between these estimates indicates the amount of mercury lost or gained independent of changes in weight. Assuming no change in mercury uptake or loss, concentrations should decrease steadily over 90 days in both lakes due to weight changes alone (see the expected concentrations in Figure 3.3). Instead, observed concentrations of ambient mercury were greater than the expected values, indicating that accumulation occurred (Figure 3.3). After day 15 in Lake 240, concentrations of ambient mercury were significantly higher than expected on all sampling days (day 15: $t=1.93$, $p=0.095$, $df=7$; day 30: $t=3.49$, $p=0.0069$, $df=9$; day 60: $t=8.58$, $p<0.0001$, $df=8$; day 90: $t=8.72$, $p=0.0010$, $df=4$). For all sampling days in Lake 979, perch muscle contained concentrations of ambient mercury significantly higher than expected by changes in fish weight alone (day 15: $t=11.28$, $p<0.0001$, $df=9$; day 30: $t=7.83$, $p<0.0001$, $df=8$; day 60: $t=13.10$, $p=0.0002$, $df=4$; day 90: $t=76.26$, $p<0.0001$,

df=3). There was a greater difference between observed and expected concentrations in Lake 979 than in Lake 240 (Figure 3.3, $F=14.6$, $p<0.0001$, df=3,24), indicating that there was greater ambient mercury accumulation in Lake 979.

Burdens of ambient mercury in yellow perch muscle were significantly greater in Lake 979 than in Lake 240 (Figure 3.4, $F=71.0$, $p<0.0001$, df=1). By the end of the experiment, perch in Lake 979 had ambient mercury burdens in muscle that were 4.6 times higher than the perch held in Lake 240. There was also an increase in ambient mercury burden among sampling days for both lakes ($F=48.5$, $p<0.0001$, df=4). Over 90 days, ambient mercury burdens in muscle of yellow perch increased by 2.5 times over original levels in Lake 240, and 12 times original levels in Lake 979.

Similar to patterns in muscle burdens, the burdens of ambient mercury in RF were greater in Lake 979 than Lake 240 (Figure 3.4, $F=75.8$, $p<0.0001$, df=1). Within each lake, there were significant differences in RF burden among sampling days (Figure 3.4, $F=49.0$, $p<0.0001$, df=4). Fish kept in Lake 240 experienced increases of 2.5 times original ambient mercury burdens in the RF portion of the fish, with the highest values occurring at day 60. Fish in Lake 979 experienced an increase to 15 times their starting burdens in non-muscle tissue (Figure 3.4). Patterns were similar to those seen in muscle burdens, except that overall burdens in RF were approximately two-thirds lower than those found in the muscle tissue.

When burdens of ambient mercury in muscle and the RF were summed to represent burdens in the whole fish, the burden increased with sampling day in both lakes ($F=55.5$, $p<0.0001$, df=4). Burdens were greater in Lake 979 than in Lake 240 (Figure 3.4, $F=83.7$, $p<0.0001$, df=1). Yellow perch experienced substantial increases in burdens

of ambient mercury compared to levels measured at day 0, with a 2.5-fold increase occurring in Lake 240 and a 13-fold increase occurring in Lake 979 (Figure 3.4).

In summary, it is clear that the yellow perch accumulated ambient mercury over the course of the study. The effect of fish growth on mercury concentration has been taken into account in the above calculations of ambient mercury burdens and observed and expected concentrations. The extent of accumulation was consistently greater in Lake 979 than Lake 240, allowing for the comparison of spike mercury elimination rates under conditions of high and low ambient mercury accumulation.

Spike mercury in yellow perch

Control fish from Lake 240 that were added to the Lake 979 enclosure (mean weight on day 90 = 5.3 g) did not show any measurable spike mercury in their muscle or RF (all muscle and RF samples: 0 ng/g, n=4). Therefore, perch held in Lake 979 were not accumulating any spike mercury from their environment. There was also no accumulation of spike mercury by control fish while held in Lake 240 in 2004 (refer to Chapter 2). Given these findings, I assumed that perch held in the Lake 240 enclosure for the present study also did not accumulate additional spike mercury.

All changes in spike mercury concentrations in the muscle of yellow perch held in Lake 979 and Lake 240 can be explained by growth dilution. With no loss or gain of spike mercury by the yellow perch, the concentrations should decline over time in relation to the rate of fish growth (Figure 3.3). Any differences between observed and expected concentrations suggest a gain or loss of mercury from muscle tissue. In Lake 240, I found no significant differences between observed and expected concentrations of

spike mercury in fish muscle (day 15: t=1.58, p=0.16, df=7; day 30: t=0.92, p=0.38, df=9; day 60: t=0.90, p=0.40, df=8), except for day 90 when observed concentrations were significantly lower than expected (t=3.26, p=0.031, df=4). In Lake 979, there was also no significant difference between the observed and expected concentrations of spike mercury (day 30: t=1.64, p=0.14, df=8; day 60: t=1.69, p=0.17, df=4; day 90: t=0.38, p=0.73, df=3) except on day 15 when observed concentrations were higher than expected (t= 2.80, p = 0.021, df= 9). With the exception of the two sampling days mentioned above, observed and expected concentrations in muscle were similar throughout the study, suggesting that fish growth was responsible for most of the observed changes in spike mercury concentrations.

Burdens of spike mercury in muscle tissue of yellow perch were not significantly different between lakes (F=1.8, p=0.19, df=1) or among sampling days (F=1.2, p=0.34, df= 4). There was no change in the burden occurring over 90 days in either lake (Figure 3.5). Mean burdens of spike mercury in muscle began at 27.5 ng and remained low throughout the study, ranging to a maximum of 39.3 ng in Lake 979 on day 90, and 37.3 ng in Lake 240 on day 60 (Figure 3.5). There is not a clear increasing trend indicating a redistribution of spike mercury into the muscle during this study.

There were no significant changes in the spike mercury burdens measured in the RF over the course of the experiment (F=2.2, p=0.078, df=4) and there was no difference between Lake 979 and Lake 240 (Figure 3.5, F=1.0, p=0.31, df=1). The data indicate that there was little loss or redistribution of spike mercury from non-muscle tissue over 90 days in either lake. Similar to the results for ambient mercury, burdens of spike mercury in RF were approximately one-third of burdens in muscle (Figure 3.5).

Spike mercury burdens in whole fish did not change over 90 days in either Lake 240 or Lake 979 (Figure 3.5, $F=0.9$, $p=0.54$, $df=9,74$), suggesting that elimination of mercury during this time was negligible. Whole fish burdens are the sum of muscle and RF burdens, which showed no significant changes over time (Figure 3.5). Fish held in Lake 240 and Lake 979 did not experience differing rates of elimination.

The calculation of half-life (the amount of time it would take for the burden of spike mercury in the fish to be reduced by half) requires a decreasing trend in the natural log of the spike burden over time. Half-life could not be calculated for this study because the slope of the regression line was not significantly different from zero in either lake (Lake 240 slope=0.0010, $p=0.28$; Lake 979 slope=0.0022, $p=0.62$). Nevertheless, I compared these elimination rates to those produced by Rodgers and Beamish, and those measured in Chapter 2 of this thesis (Table 3.3). The elimination rates of Rodgers and Beamish were consistently higher than those found in the field by both this chapter and Chapter 2 (Table 3.3).

Model simulations

The models tended to overestimate the elimination of mercury compared to field data from this study (Figure 3.6). The HB model predictions were closest to observed, but predicted mercury levels in yellow perch diverged from the observed burdens after day 30. The Wisconsin model provided the fastest elimination rates, resulting in a drastic underestimation of burdens of spike mercury over time (Figure 3.6). The TR model, which was applied as if fish were chronically exposed to mercury, provided intermediate elimination rates as compared to the other two models.

The models were not designed to have elimination rates change with mercury bioavailability, and as a result, the predicted elimination rates were similar for the two lakes. The models generally predicted a slightly faster elimination rate in fish enclosed in Lake 240, as a result of the smaller body size (due to the slower growth rate in Lake 240) and slightly warmer temperatures. It is worth noting that observed and expected concentrations of spike mercury suggested a potential loss in Lake 240 that did not occur in Lake 979, which agrees with the patterns in the models. Fish in Lake 240 were predicted to lose mercury at a marginally faster rate than those fish in Lake 979, resulting in an overall difference of 2 ng by the TR model, and 4 ng by the Wisconsin model after 90 days. The HB model predicted that the fish in Lake 979 would end the experiment with burdens that were 2.5 ng less than those in Lake 240, which is the opposite of what the other two models predicted, and was unexpected. Closer examination of the HB model predictions show that the difference was caused by an increase in the elimination rate for 1 day on day 60, which was the first day of a programmed period of faster growth. The difference between fish held in Lake 240 and Lake 979 as predicted by the models was equal to approximately 5% of the spike burden in the whole fish.

Discussion

I experimentally tested the hypothesis that different levels of mercury exposure influence rates of mercury elimination by fish. The major finding of this study was that loss of spike mercury was not different in a reservoir with high mercury levels (Lake 979) and an unimpounded lake with relatively low mercury levels (Lake 240). This occurred despite the different accumulation of ambient mercury by yellow perch in the two lakes.

Fish growth

Growth rates of yellow perch in Lake 979 were faster than those in Lake 240, especially during the last 30 days of the study. There were fewer perch put into the Lake 979 enclosure, but due to the smaller size of this enclosure, densities of perch were always higher than in Lake 240. Despite this fact, growth rates were higher in Lake 979, which was presumably a result of the very high densities of prey for yellow perch in this lake. Though the density of prey items in Lake 979 waters was not measured for this experiment, past measurements of zooplankton biomass in Lake 979 were 27 μg/L (pre-flood, 1992), 300 μg/L (<2 years post-flood, 1993 and 1994), and 82 μg/L (3 years post-flood, 1995) (Paterson et al. 1998). In contrast, the biomass of zooplankton in Lake 240 ranged from 9.4 - 15.6 μg/L between 2000 and 2003 (M. Paterson, unpublished data). The density of insects also increased in Lake 979 following flooding (St. Louis et al. 2004). It is typical to see increases in biomass of prey items that remain high for more than 10 years after flooding (Tremblay et al. 1998; Gerrard and St. Louis 2001). The greater abundance of prey items was a key reason for greater observed growth of yellow perch in Lake 979.

Ambient mercury

Fish in both Lake 979 and Lake 240 accumulated ambient mercury in their muscle and non-muscle tissues. Accumulation was more substantial in Lake 979, where fish were exposed to higher levels of ambient mercury. The ratio between RF and muscle burdens was consistent at approximately 1:3, suggesting that transfer from the RF to muscle occurred continuously as the fish accumulated mercury. Muscle tissue is the main

storage site for mercury and mercury burdens in muscle of yellow perch increased substantially over time in this study. This finding agrees with many other studies that report a transfer of methylmercury from the RF into the muscle (Massaro and Giblin 1972; Giblin and Massaro 1973; McKim et al. 1976; Boudou and Ribeyre 1983; Riisgard and Hansen 1990; Oliveira Ribeiro et al. 1999; Leaner and Mason 2004) as well as Chapter 2 of this thesis.

Accumulation of ambient mercury by perch was much greater in the flooded reservoir (Lake 979) than in Lake 240. This level of accumulation cannot be explained by the measured methylmercury levels in zooplankton alone, because they were only 1.6 times greater in Lake 979 than in Lake 240. It is possible that the peak in methylmercury concentrations in zooplankton in Lake 979 occurred between days 45 and 90 and was missed by the sampling. Past measurements in Lake 979 have found that mercury concentrations in zooplankton varied greatly over the season, with lows of <50 ng/g d.w. in the spring and peaks ranging from 600 - 1500 ng/g d.w. in midsummer (St. Louis et al. 2004).

Perch in Lake 979 must have had a diet with higher mercury concentrations than those measured in zooplankton to achieve the levels of ambient mercury observed. As the perch grew larger in Lake 979, they would have access to larger predatory benthic invertebrates or even young-of-the-year (YOY) fish. The switch to larger species in the diet could result in greater mercury intake, particularly if fish or predatory insects are consumed (MacCrimmon et al. 1983; Hall et al. 1998). There are fewer long-term data on benthic invertebrates in Lake 979 compared to zooplankton. In 1993 and 1994, mean methylmercury concentrations in benthic organisms ranged from approximately 50 - 125

ng/g d.w. for species classified as collectors or shredders, and from 230 - 350 ng/g d.w. for predatory insects (Hall et al. 1998). The mercury concentrations in YOY fish are likely lower than those in older fish (Post et al. 1996). As an example mercury concentration for fish in Lake 979, Bodaly and Fudge (1999) found that finescale dace (mean weight of 2.8 g) held in Lake 979 for 3.5 months reached methylmercury concentrations up to 360 ng/g w.w. (approximately 1800 ng/g d.w.).

Using the Wisconsin model (with elimination rates set to zero), I was able to match observed concentrations in Lake 979 using two different diet scenarios as general examples. The first scenario involved a gradual shift to a diet of benthic invertebrates with mercury concentrations 3.5 times higher than those in zooplankton. This scenario is possible as the Lake 979 experimental reservoir is 13 years old and therefore could be nearing a time when methylmercury levels begin to decline in the prey items of yellow perch (particularly zooplankton) (Bodaly et al. 1997). It is possible that concentrations of mercury in benthic organisms persist at high levels for longer following impoundment than concentrations in zooplankton. For example, in Quebec the concentrations of methylmercury were higher in benthic invertebrates collected from old reservoirs (14 - 16 years old) than in young reservoirs (1 - 3 years old) (Tremblay and Lucotte 1997) but the opposite was true for zooplankton (reviewed by Bodaly et al. 1997). Therefore, despite relatively low methylmercury concentrations measured in zooplankton from the 13-year-old reservoir in the present study, benthic invertebrates may have had higher concentrations of methylmercury that could account for the observed increases in ambient mercury levels in yellow perch.

The second modeling scenario involved similar mercury concentrations in zooplankton and benthos, with a shift to consumption of YOY fish (composing 10% of the diet) after day 60 (refer to Appendix 1, Table A.10). There were a small number of YOY white suckers (*Catostomus commersoni*) observed in the Lake 979 enclosure during the study, which could have been a food source for the enclosed perch. Both scenarios 1 and 2 can fit observed ambient mercury burdens in yellow perch held in Lake 979, and there are likely a number of other scenarios that could match observed patterns. The most important factor causing the observed concentrations in yellow perch was a shift to prey with higher mercury concentrations after day 60.

Spike mercury

There was no difference in elimination rates of spike mercury between fish in a flooded reservoir and an unimpounded lake despite different levels of ambient uptake. This finding is in contrast to observations by Rodgers and Beamish (1982). They found that elimination rates were consistently higher than those in the present research (Table 3.3). Several features set these two studies apart; the fish species examined, the duration that fish were exposed to labeled mercury, and the duration of the decontamination period. Rodgers and Beamish exposed rainbow trout to two meals of labeled mercury (at very high doses) before the elimination of labeled mercury was measured for a 20-day decontamination period. In contrast, the research presented in this chapter involved the exposure of age 1 yellow perch to spike mercury over their lifespan followed by a 90-day decontamination period. Rodgers and Beamish (1982) found that within treatments there was faster elimination (shown by a larger k) in fish experiencing higher doses of

mercury, especially between the control values and the lowest treatment concentrations (25 000 ng/g w.w.). The range in elimination rates reported by Rodgers and Beamish (1982) translates to half-lives of mercury ranging from 54 - 107 days. In comparison, the present study found no measurable elimination and a half-life could not be calculated. Using data from the first 90 days of the study presented in Chapter 2 resulted in a half-life of 141 days.

Rodgers and Beamish (1982) fed rainbow trout labeled mercury (<100, 25 000, or 75 000 ng/g) and then monitored the loss of this mercury as fish were continually fed the same concentrations of non-labeled mercury. Rodgers and Beamish (1982) concluded that the concentrations of mercury given during the decontamination phase could impact elimination rates. The findings of Rodgers and Beamish are confounded by the concentrations of labeled mercury given before the decontamination period. Trudel and Rasmussen (1997) found that the burden of mercury in a fish should not impact elimination rates. Nevertheless, several studies have found that elimination rates are higher when initial burdens of mercury to be eliminated are greater (Miettinen 1973; DeFreitas et al. 1975; Ruohtula and Miettinen 1975). It is possible that the faster elimination rates found by Rodgers and Beamish (1982) were due to the dose of labeled mercury given prior to the decontamination period rather than the concentration of non-labeled mercury given during the decontamination period.

Exposure concentrations used by Rodgers and Beamish (1982) ranged from <100 ng - 76 500 ng/g w.w. compared to approximately 9.1 - 230 ng/g w.w. in the present study (Table 3.3). To put the exposure concentrations into context, the greatest concentration of methylmercury in insects collected from various reservoirs of northern

Quebec was approximately 258 ng/g w.w., while the lowest concentration was 3 ng/g w.w. (assuming 90% moisture, Tremblay and Lucotte 1997). Both the estimated mercury concentrations of prey in the present study (in both Lake 240 and Lake 979) and the background concentrations in control feed used by Rodgers and Beamish are within the wide range of recorded values of prey items in reservoirs. There was no consistent positive relationship between elimination rate and exposure concentration at levels relevant to reservoirs. Though elimination rates were faster in Rodgers and Beamish (1982), I suspect that these differences are not due to mercury concentrations in the diet, but rather to fundamental differences among the experiments.

Another factor affecting elimination rates studied by Rodgers and Beamish (1982) was the level of food consumption. They found that fish with greater food consumption eliminated mercury faster. In contrast, Riisgard and Hansen (1990) found that there was no difference in elimination of methylmercury by flounders (*Platichthys flesus*) that had been starved or fed. Food consumption rates may be an important factor in reservoirs, because flooding increases the abundance of prey items for small fish (Paterson et al. 1998). Food consumption was higher in Lake 979 than in Lake 240, as shown by the greater growth of yellow perch; however, there was no difference in elimination rates between the two lakes. The meal sizes used by Rodgers and Beamish were 1 or 2% of the fish's body weight per day, and resulted in considerable differences in elimination. Riisgard and Hansen (1990) had food consumption rates of approximately 0.4% of the fish's body weight per day, which resulted in similar mercury elimination rates compared to fish that were not fed. The food consumption rates that resulted in the proper growth for the suspected diets in Lake 240 and Lake 979 ranged from averages of 4% (Chapter 2

data) to 11% (Lake 979) of the fish's body weight per day (determined using the Wisconsin model). Despite the greater range in food consumption in the present study, there were no differences in mercury elimination rates, which agrees with the findings of Riisgard and Hansen (1990).

Conclusions

Yellow perch exposed to differing levels of ambient mercury and food availability showed no difference in their rate of elimination of enriched stable isotopes of mercury. This study reflects a realistic range of high and low mercury environments and shows that mercury elimination rates were not different in these two environments. Therefore, under environmentally realistic "high" concentrations of methylmercury, such as a flooded reservoir, there was no induction of elimination as was documented at higher concentrations by Rodgers and Beamish (1982). The elimination rates of mercury by fish were consistent under very different environments with respect to mercury bioavailability, which agrees with general elimination models such as Harris and Bodaly (1998), Trudel and Rasmussen (1997) and the Wisconsin model (Hanson et al. 1997). Models used to predict the changes of mercury in fish do not need to have the elimination component adjusted to account for mercury exposure. Similar to the findings in Chapter 2, an adjustment to slower elimination rates in the models needs to be made to represent loss by yellow perch, especially for the Wisconsin model and the TR model.

Table 3.1. The bathymetric, geographic, and biological characteristics of Lake 658, Lake 240, and Lake 979 of the Experimental Lakes Area. The mean weights, and concentrations of spike and ambient mercury (THg) in muscle of yellow perch (*Perca flavescens*) or finescale dace (*Phoxinus neogaeus)* (data from Bodaly and Fudge 1999) are also shown.

Lake	Location	Max. depth (m)	Surface area (m^2)	Volume (m^3)	Common resident fish species*	Species* and collection time	Weight (g)	[spike THg] (ng/g d.w.)	[ambient THg] (ng/g d.w.)
658	49°39'14"N 93°43'18"W	13.2	85,387	607,225	NP, YP, WS, LWF, BNS	YP, June 2005 (day 0 sample)	1.1	422	1048
240	49°39'15"N 93°43'35"W	13.1	441,800	608,000	NP, YP, WS	YP, October 2004	1.2	0	**400
979	immediately downstream from Lake 240	~2.5	167,000	164,542	transient, WS	FD, October 1995 after held in Lake 979 for 4 months	3.6	0	**2150

*NP= northern pike (*Esox lucius*), YP= yellow perch (*Perca flavescens*), WS= white sucker (*Catastomus commersoni*), LWF= lake whitefish (*Coregonus clupeaformis*), BNS= blacknose shiner (*Notropis heterolepis*), FD= finescale dace (*Phoxinus neogatus*).

** concentrations converted to dry weight assuming 80% moisture.

Table 3.2. Number of fish collected on each sampling day for mercury (THg) analysis.

Location of enclosure	Sampling day	n
Lake 240	0	10
	15	10
	30	10
	60	9
	90	6
Lake 979		
	15	10
	30	10
	60	5
	90	4

Table 3.3. Elimination rates of mercury for yellow perch (*Perca flavescens*) and rainbow trout (*Oncorhynchus mykiss*) from field studies and a laboratory study (Rodgers and Beamish, 1982) under different levels of mercury exposure during elimination.

Study	Species	Mercury exposure concentration (ng/g w.w.)	Food consumption (% of body weight per day)	Duration of decontamination period	Elimination rate (k)*
Lake 240 2005	yellow perch	9.1	9	90	-0.001032
Lake 979 2005	yellow perch	9.1-360	11	90	-0.001327
Lake 240 2004	yellow perch	7.5	4	90	0.0049
Rodgers and Beamish (1982)	rainbow trout	<100	1	20	0.00647
			2	20	0.00939
		23200	1	20	0.00832
			2	20	0.0122
		76500	1	20	0.0101
			2	20	0.0127

*Elimination rates shown from Rodgers and Beamish (1982) were based on fish first exposed to methylmercury for 28 days before the elimination study began (see text).

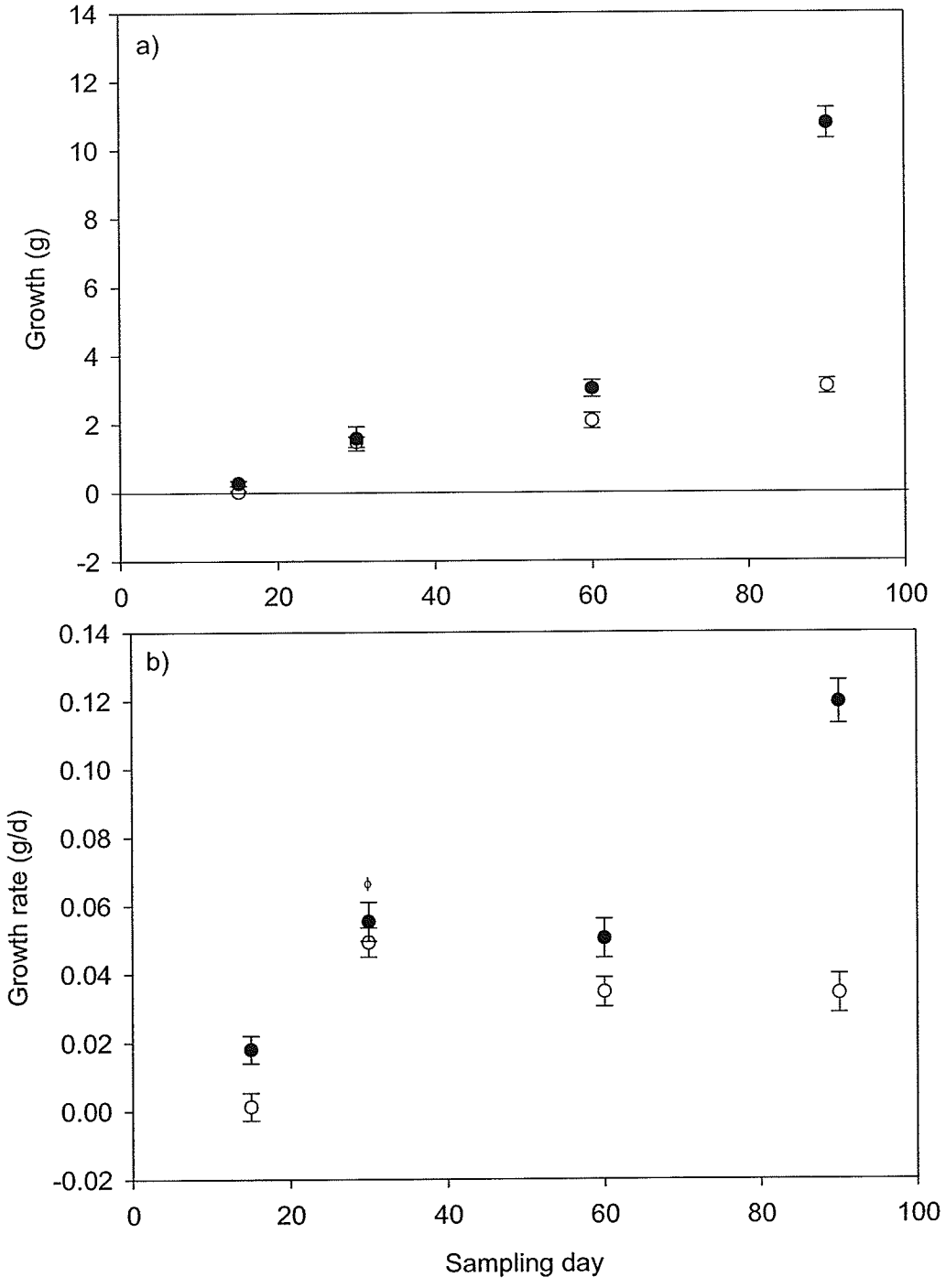

Fig. 3.1. Mean (+/- 1 standard error of the mean) a) growth and b) growth rate of yellow perch (*Perca flavescens*) over 90 days after being transferred from Lake 658 to Lake 240 (open circles) or Lake 979 (closed circles). $^{\phi}$ extreme outliers removed from both lakes.

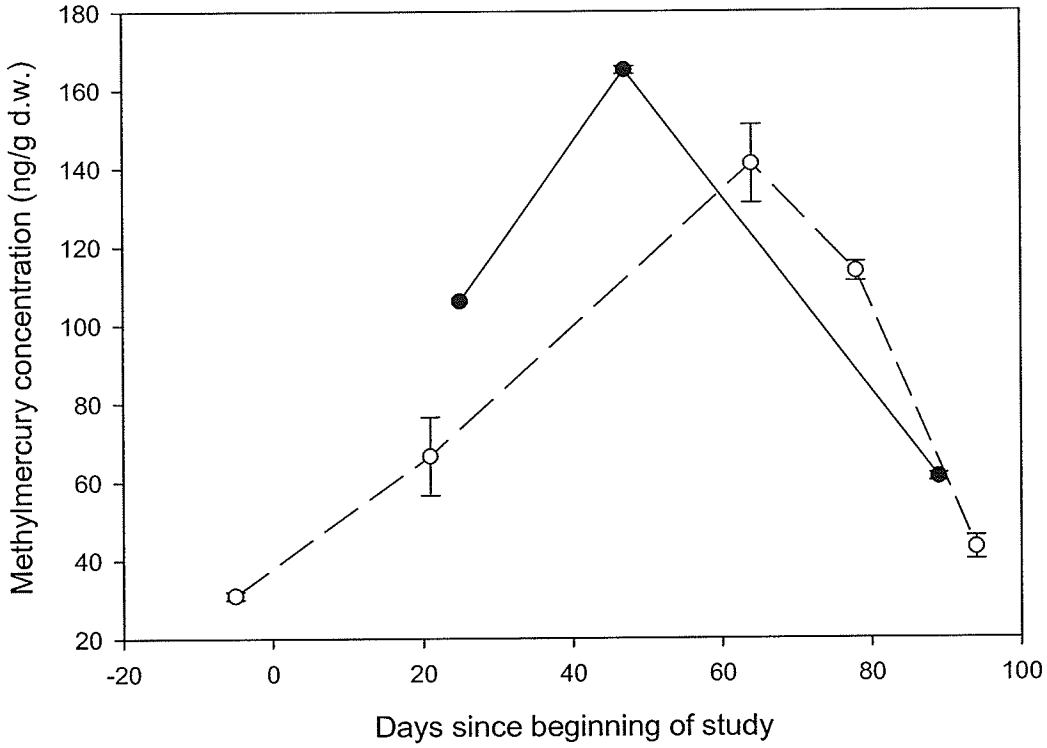

Fig. 3.2. Mean concentrations (+/- 1 standard error of the mean) of methylmercury in zooplankton collected from Lake 240 (open circles) and Lake 979 (closed circles).

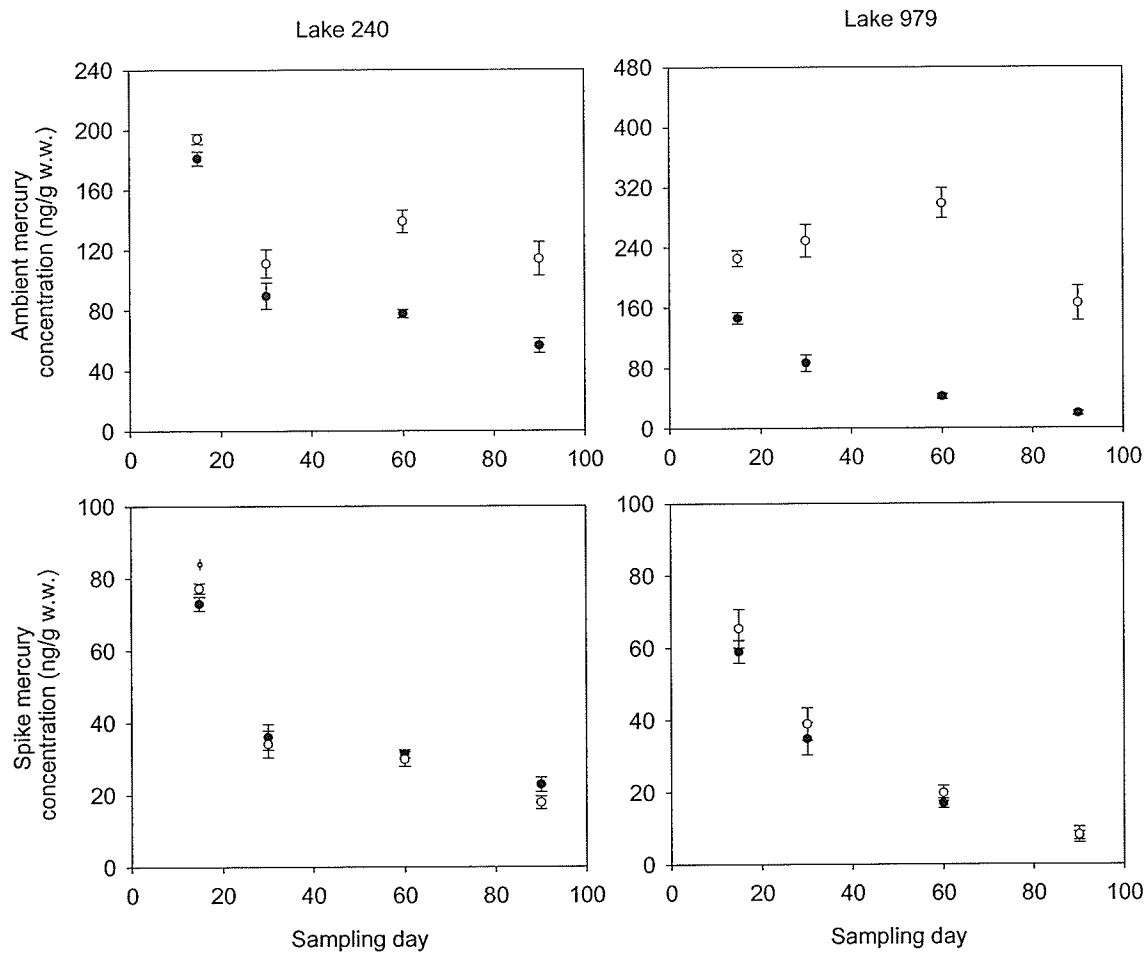

Fig. 3.3. Mean (+/- 1 standard error of the mean) concentrations of ambient and spike mercury (THg) (open circles) and the concentrations expected due to growth dilution alone (closed circles) in the muscle of yellow perch (*Perca flavescens*) over 90 days after transfer from Lake 658 to Lake 240 or Lake 979. $^{\phi}$ n=8 after two extreme outliers removed.

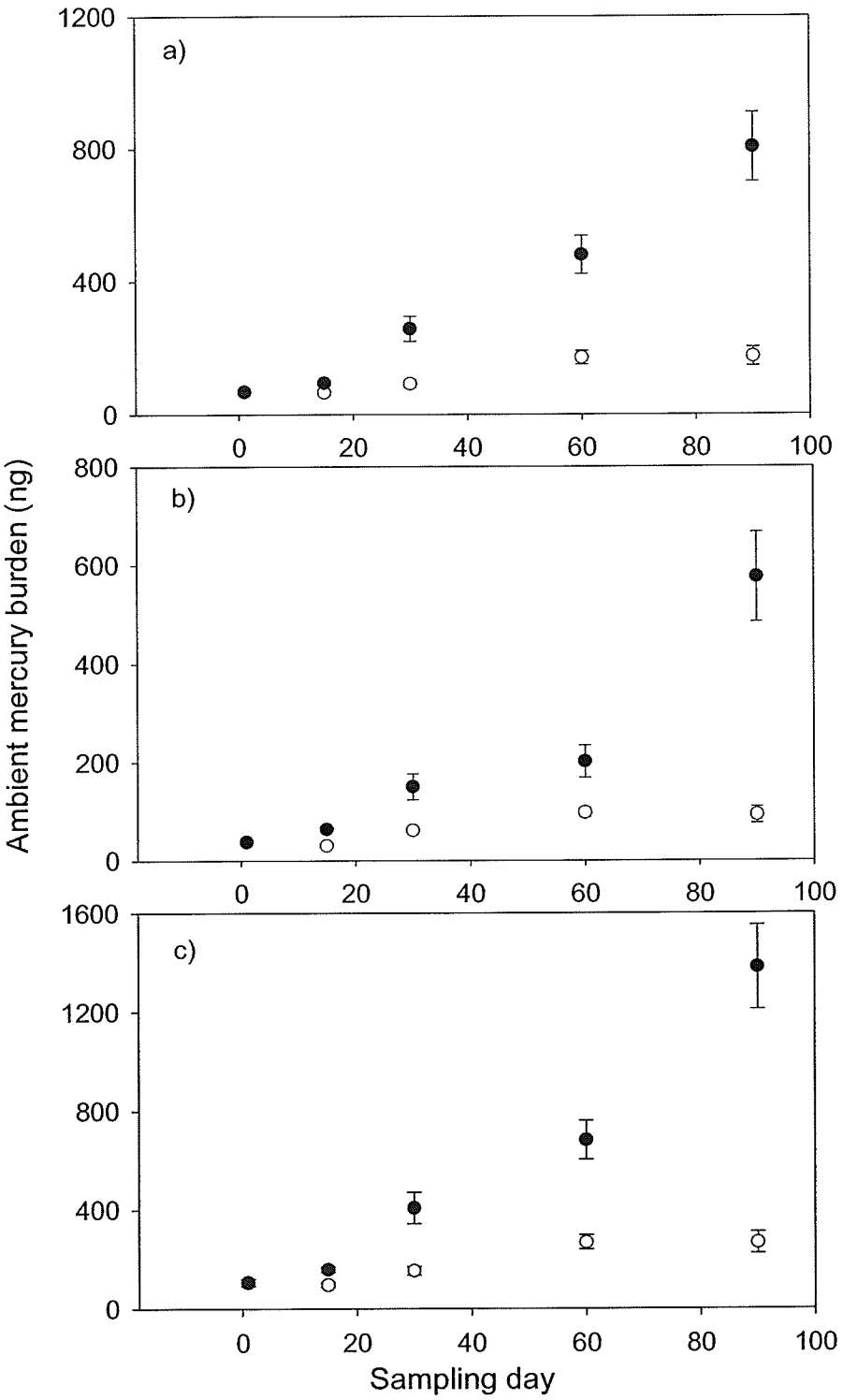

Fig. 3.4. Mean (+/- 1 standard error of the mean) burdens of ambient mercury (THg) in the a) muscle b) non-muscle tissue (RF) and c) whole fish tissue of yellow perch (*Perca flavescens*) that had been transferred from Lake 658 to Lake 240 (open circles) or Lake 979 (closed circles). Note the different y-axis scales.

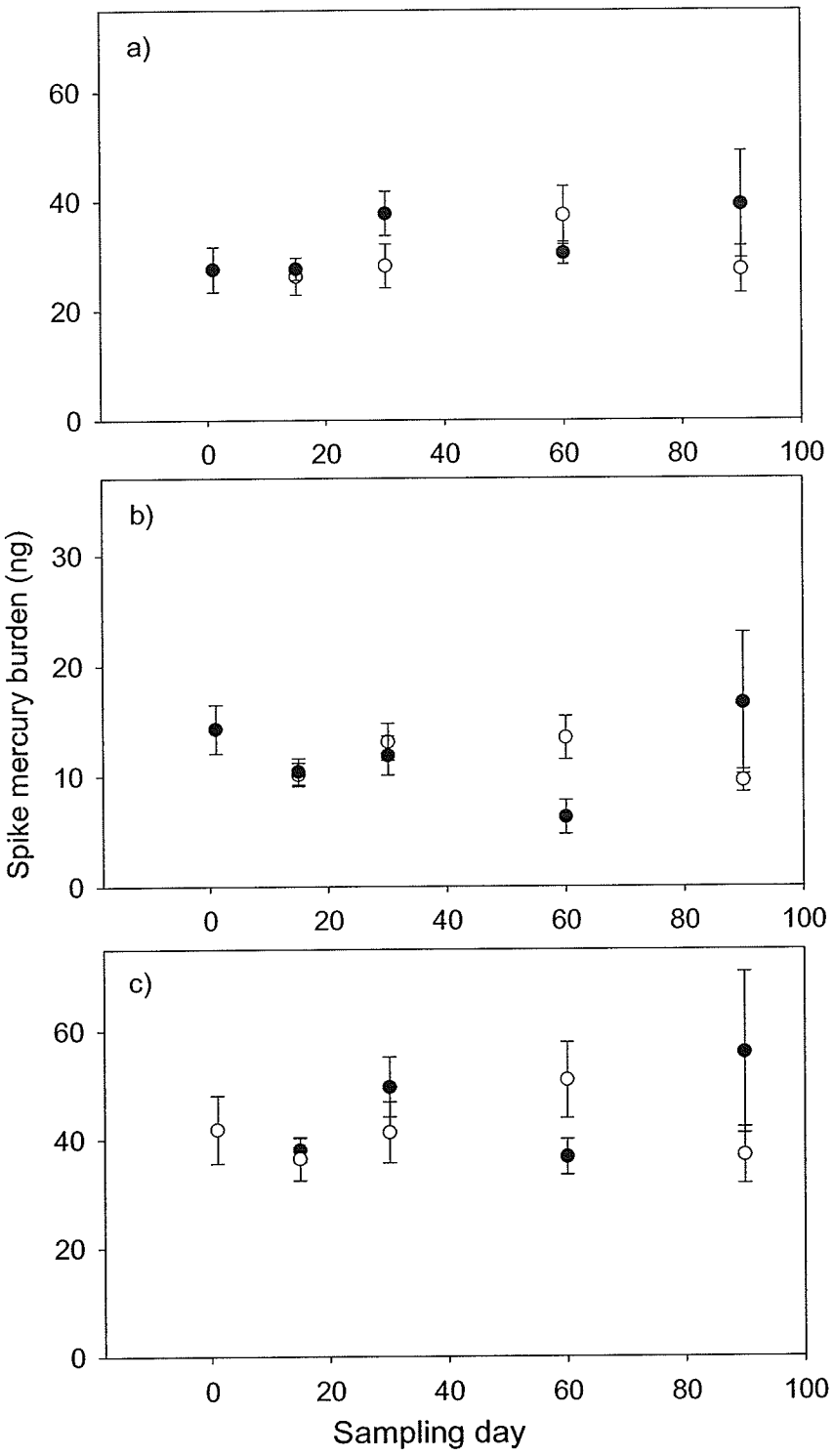

Fig. 3.5. Mean (+/- 1 standard error of the mean) burdens of spike mercury (THg) in a) muscle b) non-muscle tissue (RF) and c) whole fish tissue of yellow perch (*Perca flavescens*) that had been transferred from Lake 658 to Lake 240 (open circles) or Lake 979 (closed circles). Note the different y-axis scales.

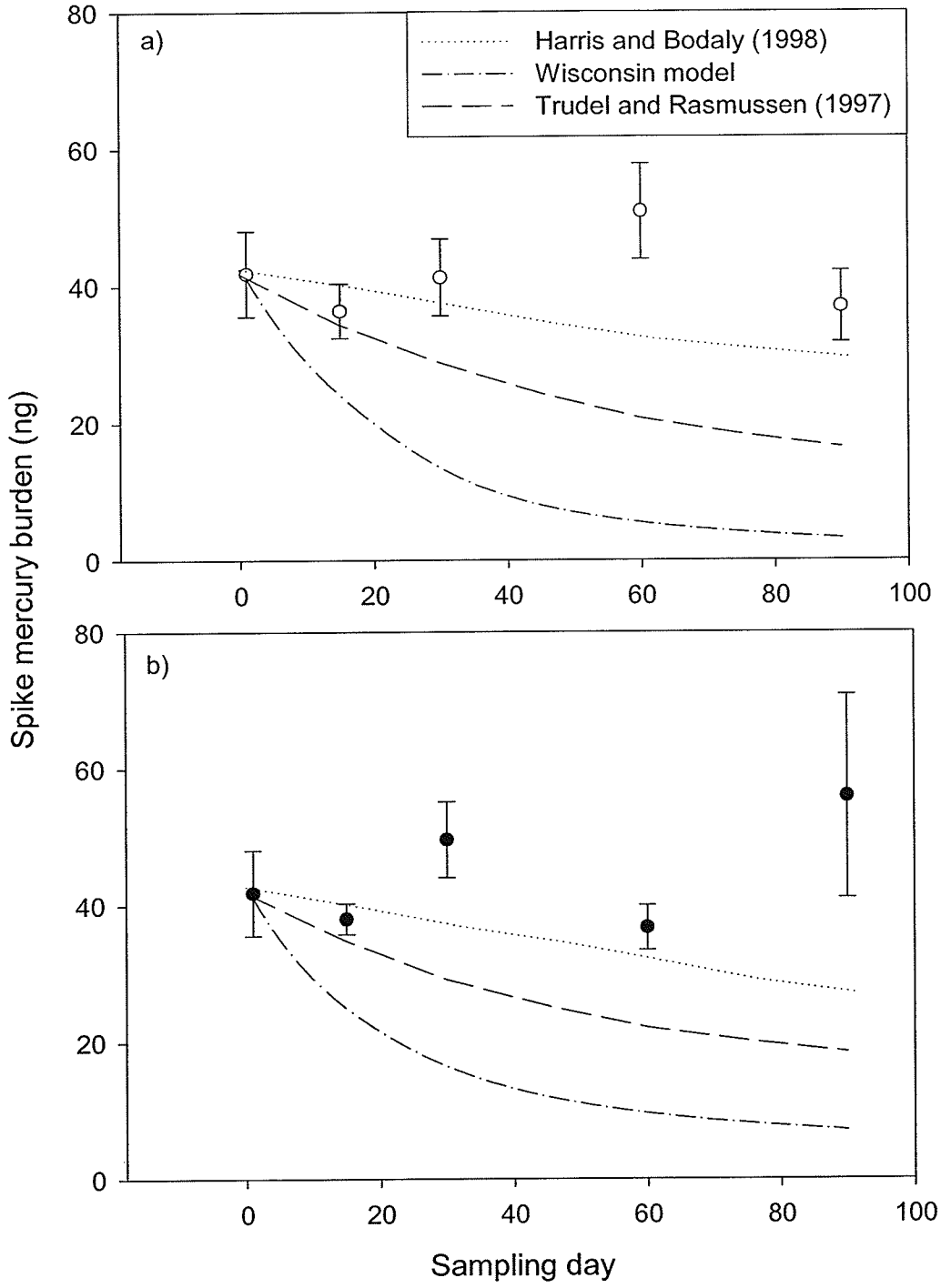

Fig. 3.6. Mean (+/- 1 standard error of the mean) burdens of spike mercury (THg) in the whole body of yellow perch (*Perca flavescens*) over 90 days after being transferred from Lake 658 to a) Lake 240 or b) Lake 979. The observed burdens are compared to predictions of three mercury elimination models: Harris and Bodaly (1998), the Wisconsin model 3.0, and Trudel and Rasmussen (1997).

Chapter 4: Elimination of mercury by northern pike (*Esox lucius*) in the wild.

Introduction

Mercury concentrations within freshwater food webs are usually highest in predatory fish (Cabana et al. 1994; Bowles et al. 2001). Concentrations of mercury in predatory fish can be up to 10 times higher than concentrations in their prey (refer to Table 1.2). Many people prize and enjoy eating large predatory fish; however, consumption of many fish with high methylmercury levels can be a health risk (O.M.O.E. 2005). It is common for mercury concentrations in top predatory fish to exceed the 500 ng/g limit for commercial sale even in remote lakes (refer to Table 1.2) (U.S.E.P.A. 2004; O.M.O.E.).

Two factors that lead to elevated mercury concentrations in large predatory fish include biomagnification and slow elimination. Mercury concentrations biomagnify with each trophic level causing fish at the top of the aquatic food web to have the highest concentrations (Bowles et al. 2001). In addition, large fish lose mercury more slowly than small fish, which contributes to greater accumulation of this compound (Huckabee et al. 1979; Trudel and Rasmussen 1997). Mercury elimination is a key factor that determines mercury levels in fish and is a particularly important determinant of concentrations in systems recovering from elevated mercury levels. Little is known about how large fish lose mercury in the wild.

Laboratory and field studies on mercury elimination by large fish have shown that loss occurs slowly. Half-lives (the amount of time for the burden to be reduced by half) of methylmercury reported for fish greater than 300 g range from 92 - 780 days depending on the study (reviewed in Trudel and Rasmussen 1997). Rates of mercury elimination appear to be positively related to temperature (Ruohtula and Miettinen 1975;

Trudel and Rasmussen 1997), and negatively related to fish weight (Sharpe et al. 1977; Trudel and Rasmussen 1997) and duration of contamination (DeFreitas et al. 1975; Ruohtula and Miettinen 1975; Rowan and Rasmussen 1995). Some studies also suggest that mercury elimination rates are related to metabolic rate (Fagerstrom and Asell 1973; Harris and Bodaly 1998). Of 22 laboratory studies on the elimination of mercury by fish, most have involved artificial conditions that did not resemble exposure to mercury as it occurs in the wild (Trudel and Rasmussen 1997). In addition, only two of these laboratory studies were conducted using fish that were larger than 300g in weight (Jarvenpaa et al. 1970; Miettinen et al. 1970). The only two field studies on mercury elimination monitored loss of ambient mercury that could not be distinguished from mercury continually accumulated by fish from their environment (Lockhart et al. 1972; Laarman et al. 1976). Therefore, little is known about how fish lose mercury in the wild, especially large fish (Trudel and Rasmussen 1997).

Mathematical models can be used to predict mercury concentrations in wild fish. These models consider a variety of environmental conditions and are useful for long-term monitoring of mercury concentrations in fish. Mercury models are often based on both fish bioenergetics and mercury kinetics equations. The general bioenergetics equation that is used for fish is summarized below:

food consumption = metabolic costs + wastes + growth

Rates of food consumption and gill respiration are then combined with assimilation efficiencies and mercury concentrations in prey and water to determine how much mercury is absorbed through these routes. The mercury component of the models generally follows the format:

mercury burden = mercury from food + mercury from water – mercury eliminated

The rate of mercury elimination is important for estimating the final mercury burden in the fish, and has a large impact on model predictions (Harris and Snodgrass 1993; Rodgers 1994). Embedded within the metabolic costs of the bioenergetics equation, is the rate of activity:

metabolic costs = active metabolism + costs of digestion + cellular respiration

Active metabolism is the energy used for all activity including foraging and spontaneous swimming (Norstrom et al. 1976; Hanson et al. 1997). The activity part of the model has an important impact on predicted metabolic costs, which in turn impact final predictions of fish mercury levels (Trudel and Rasmussen 2001; Rennie et al. 2005). The accuracy of models relies on our understanding of bioenergetics and mercury kinetics within fish (Norstrom et al. 1976).

Several studies have emphasized the importance of estimating activity rates based on field data rather than using estimates from laboratory experiments (Trudel and Rasmussen 2001; Klumb et al. 2003). The use of field activity rates in bioenergetics models can greatly increase the accuracy of predicted mercury concentrations (Trudel and Rasmussen 2001). Though it has been occasionally suggested that mercury elimination is related to metabolic rate (Fagerstrom and Asell 1973; Norstrom et al. 1975), this has not been tested aside from measuring elimination under different temperatures and body sizes (e.g. DeFreitas et al. 1974; DeFreitas et al. 1975; Ruohtula and Miettinen 1975; Sharpe et al. 1977). The relationship between metabolism and elimination could be further explored by testing whether different rates of activity impact rates of mercury loss.

The present study takes a novel approach to monitoring elimination of mercury by a large predatory species, northern pike (*Esox lucius*), in nature. Elimination of a naturally accumulated enriched stable isotope of mercury (referred to as spike mercury) was measured in the field. Field activity rates of these same pike were estimated by monitoring their movements in a lake. Objectives of the study include i) determining the rate of mercury elimination by northern pike in the wild; ii) quantifying activity rates of these pike in the wild; iii) developing a relationship between rates of activity and mercury elimination and iv) comparing predictions of mercury models to natural elimination rates.

Methods

Study site

This study was conducted at Lakes 240 and 658 at the Experimental Lakes Area in northwestern Ontario (refer to Figure 2.1). As discussed in earlier chapters, Lake 658 is the site of the METAALICUS study where enriched stable isotopes of mercury (referred to as spike mercury) were added to the lake from 2001 through 2005. At the start of the present study, spike mercury added to the lake (^{202}Hg) had entered the food chain and accounted for over 8% of total mercury in the muscle of northern pike (P. Blanchfield, unpublished data). Lake 240 is the "clean" reference system for the METAALICUS study. Ambient mercury concentrations in fish from Lake 658 are generally higher than those in fish from Lake 240 (refer to Table 2.1). Winnange Lake is a large, double-basin, oligotrophic lake (49° 45' 00'' N; 93° 42' 00'' W) that receives outflow water from Lake 658 (refer to Figure 2.1).

Study species

Northern pike is a predatory fish species found in freshwaters through most of the northern hemisphere (Scott and Crossman 1973). This species often has high mercury concentrations in muscle compared to prey fish species in the same lakes (Mathers and Johansen 1985). Optimum temperature for pike growth is 23 - 26°C under favorable feeding conditions (reviewed in Paat 1988). Young-of-the-year pike depend on aquatic insects as a food source, but switch to a fish-dominated diet when they reach 50 mm in length (Scott and Crossman 1973). As pike grow, they can consume larger prey and the species composition of their diet may change (Mathers and Johansen 1985). Northern pike are ambush predators that typically hide in vegetation to capture prey (reviewed in Paat 1988). Pike reach maturity after 2 - 6 years, depending on the environment, with males maturing approximately 1 year before females (Scott and Crossman 1973). Spawning by this species occurs in spring, in shallow water (Priegel and Krohn 1975). The life expectancy of pike varies among environments, ranging from 5 - 25 years, with males having a shorter lifespan than females (reviewed in Paat 1988).

Mercury elimination

The approach of this study is similar to that described for yellow perch (*Perca flavescens*) in Chapter 2. I monitored mercury loss by a predatory fish species (northern pike) after being transferred from a lake receiving spike mercury additions (Lake 658) to a lake with no spike mercury added (Lake 240). Since 2000, individual northern pike captured for the first time in Lake 658 were injected with PIT tags (Biomark, model TX1410L) in their left cheek, allowing for identification of the fish when recaptured. From June 22 to

August 3, 2004, 10 pike greater than 750 g in weight were captured from Lake 658 by angling and using trap nets. Four additional pike greater than 750 g in weight were collected from Lake 658 from June 3 to June 15, 2005. Four of these northern pike had entered Lake 658 from Winnange Lake during a high water period in the spring of 2004. One of these pike originally from Winnange Lake was transferred to Lake 240 in 2004 after spending approximately 1 month in Lake 658 and the remaining three were transferred in 2005. Pike were transported to Lake 240 in coolers (94.6 L) and held overnight in a wire mesh pen (3 m X 1.3 m X 1.2 m) in approximately 1 m of water in Lake 240. Pike were anaesthetized with 0.06 g/L MS222 (tricaine methane sulfonate), and a biopsy sample of muscle was collected using the dermal punch method (Baker et al. 2004). This method of measuring mercury in fish muscle is non-lethal and therefore allows for repeated sampling of the same individual fish over time. Fork lengths (mm) and weights (to the nearest 1 g using an A&D Co. Ltd. scale) of pike were measured. Four pike had continuous, acoustic temperature-sensing transmitters (weight 22 g in air, 62 mm length, 16 mm diameter, Model CTT-83-3, Sonotronics, Tuscon, AZ) surgically inserted in their abdomen in 2003 while in Lake 658. The remaining pike underwent this procedure after transfer from Lake 658, using the surgical methods described in Wagner and Stevens (2000). Pike were anaesthetized with 0.06 g/L MS222 until they did not respond to a squeeze of their caudal peduncle. The gills were bathed in 0.03 g/L MS222 solution throughout the surgery. An incision was made anterior to the pelvic girdle, the sterilized tag was inserted, and the incision was closed with three sutures (Monocryl Suture, Novartis Animal Health). The entire process of biopsy and surgery generally

lasted 8 - 15 min. Once surgery was complete, pike recovered from anaesthesia in a tub of fresh lake water before being released into Lake 240.

Ten pike were recaptured at different periods over 670 days after they had been transferred to Lake 240 (Table 4.1). The recapture periods (range of days shown in brackets) were categorized as 80 (75 - 88), 320 (296 - 345), 460 (446 - 473), and 650 (630 - 670) days after pike had been transferred to Lake 240. These categories were used for purposes of comparing mercury levels among time periods, while the exact days were used for the calculation of half-life. Pike were in spawning condition during the day 650 recapture period (April 23 - 27, 2006), and sex was determined on most of the pike recaptured at this time. The pike were located using a hydrophone (Model DH4, Sonotronics) and receiver (USR-5W) and recapture was achieved by use of seine nets, trap nets, and short-term (5 - 30 min) gill net sets (multipanel nets with mesh from 1.5 - 6.5 cm). Fish were anaesthetized as above before a muscle biopsy was collected and weight and fork length were measured. Muscle samples were inserted into 0.6 mL plastic vials (Cat. No. 502N, Rose Scientific Ltd., Edmonton, Alberta), immediately put on ice, and frozen within 30 min.

Biopsy samples were also taken from a Lake 658 northern pike mortality as a standard to evaluate the variability in mercury measurements (i.e. due to slight differences in analytical calibrations and error among different samples from the same fish). This "standard" pike was kept frozen and one biopsy sample was collected each year (2005 - 2006) and sent for analyses with other samples from free-ranging pike. These samples sent in December 2005 and May 2006 had a coefficient of variation of 2% and 27% for concentrations (ng/g w.w.) of spike and ambient mercury respectively.

Each muscle sample was weighed to the nearest 0.0001 g (scale: Mettler AE 163) before and after freeze-drying to obtain wet and dry sample weights. Total mercury analysis was done on the freeze-dried muscle samples at Trent University in the laboratory of Dr. H. Hintelmann (refer to Chapter 2).

Presentation of mercury data

Concentration and body burden

Wet weight concentrations of spike and ambient mercury are used throughout this chapter and were calculated by the following formula:

$$1)\ [Hg]_{ww} = [Hg]_{dw} * sample_{dw} / sample_{ww}$$

Where

$[Hg]_{ww}$ = the concentration of mercury in the wet sample

$[Hg]_{dw}$ = the concentration of mercury in the dry sample

$sample_{dw}$ = the dry weight of the muscle sample

$sample_{ww}$ = the wet weight of the muscle sample

Body burden was calculated by the following formula:

$$2)\ Burden = [Hg]_{ww} * W$$

Where W = weight of fish.

The expected concentrations of mercury were calculated for each fish in a similar manner as in Chapters 2 and 3, except initial concentrations of each pike were known:

$$3)\ B_{day0} = [Hg]_{day0} * W_{day0}$$

$$4)\ exp[Hg]_{dayX} = B_{day0} / W_{dayX}$$

Where

B_{day0} = initial burden of a pike

$[Hg]_{day0}$ = original concentration of mercury in the pike muscle

W_{day0} = original weight of the fish

W_{dayX} = weight of the fish at sampling day X

$exp[Hg]_{dayX}$ = expected concentration of mercury in the pike muscle on sampling day X assuming no loss or gain of mercury

Percent spike mercury retained

The amount of spike mercury retained by the pike on each sampling day was estimated using equation 5.

$$5)\ \%retained = B_{dayX}/B_{day0} * 100$$

Calculation of half-life

Calculation of half-life was based on the percentage of spike mercury retained by each fish, similar to the approach of Lockhart et al. (1972). The calculation of half-life requires a decreasing trend in the natural log of spike mercury burden over time. First, the percentage of spike mercury retained by individual pike was calculated for each day they were sampled. The percent retained is analogous to estimates of body burden using method 1 (in Chapter 2) because it is based on the mercury concentrations in muscle rather than the whole fish. While most mercury elimination studies determine a rate of loss based on several sampling periods over time, Lockhart et al. (1972) used only their last sampling day to estimate half-life. To compare the findings of Lockhart et al. (1972) to the present study as well as past studies, I used all of the data reported by Lockhart et

al. (1972) to calculate a new half-life of mercury loss. This was done in a similar matter as the half-life calculations for the present study.

Fish activity

Monitoring fish activity

The pike transferred to Lake 240 in 2004 were periodically manually tracked to estimate swimming activity. The pike and times of tracking were randomly chosen. The locations of individual pike were determined using a hydrophone (Model DH4, Sonotronics) and receiver (USR-5W) following manual tracking methods described in Blanchfield et al. (2005). The term "track" is used to refer to each period that fish were monitored. Activity tracks lasted for a duration of approximately 1 h during which time the exact location of the fish was recorded repeatedly (approximately every 5 min). The number of locations recorded per activity track ranged from 5 - 14. When the boat was located above the monitored pike, the time (to the nearest second) and location indicated by a GPS (Magellan) were recorded. Temperature signals from the transmitters were recorded during tracking, indicating the temperature experienced by the pike while being tracked. Forty-seven 1-h tracks were conducted on northern pike in each of the study years, 2004 and 2005, resulting in a total of 94 tracks on 10 pike. In 2004, the 47 activity tracks took place during the day between the hours of 7:00 and 20:00 from July to September. In 2005, 34 tracks took place between the hours of 7:00 and 20:00 with the remaining 13 tracks at night. Activity of the same individuals was monitored in both years and pike transferred from Lake 658 in 2005 were not tracked. Of 10 pike tracked in 2004, 2 died

and the transmitter failed in 1 other before the 2005 season. As a result, seven pike were tracked in 2005.

Calculation of swimming speed

Swimming speed was calculated for individual pike that had been tracked. The total two-dimensional distance (as number of body lengths) travelled in each 1-h track was divided by the total duration of the tracking period (sec). In 2004, the fork length measured at the beginning of the study was used to calculate body lengths traveled per second (bl/sec). The fork lengths of fish recaptured in spring 2005 were used to calculate their swimming speeds for summer 2005. There was a mean increase in fork length by 3.4 cm between June 2004 and June 2005. Pike that were not recaptured in 2005 were assumed to be 3.4 cm longer than they were in 2004 for the calculation of swimming speed. The resolution of the tracking procedure was 2 cm/sec, which was determined by tracking a stationary telemetry tag on the lake bottom for 1 h and calculating the speed of movement.

Mercury model applications

I compared the elimination of spike mercury by northern pike after exposure ended using the same three models described in Chapters 2 and 3. The first model was created by Harris and Bodaly (1998) (the HB model), the second was the Wisconsin model version 3.0 (Hanson et al. 1997), and the third was developed by Trudel and Rasmussen (1997) (the TR model). The TR model has different equations for chronically exposed and acutely exposed fish, and both equations were compared in this study. Further

descriptions of the models are provided in Chapter 2. Not all pike were released into Lake 240 on the same date, and therefore temperatures experienced by each pike were different between each sampling day of the study. In addition, starting weights of the pike ranged from 752 - 1233 g. To run the models for this group of pike, I used a fast elimination scenario and a slow elimination scenario for each model. The scenarios included actual temperatures experienced by the pike in Lake 240 and body weights measured upon recapture. Fast elimination scenarios involved the smallest fish at the warmest temperatures during that period, while slow elimination scenarios involved the largest fish at the coolest temperatures. These model scenarios were intended to cover the range of possible mercury elimination rates for these pike. Model inputs for the HB model (Table A.7), Wisconsin model (Table A.8) and TR model (Table A.9) are shown in Appendix 1.

Statistical analyses

Observed and expected mercury concentrations of northern pike on the various sampling days were compared using paired *t*-tests (when n>2). I also used paired *t*-tests to compare differences between original and recapture burdens of mercury in pike. Assumptions that the residuals were normally distributed with homogeneous variance were tested using Shapiro-Wilk's test and Bartlett's test respectively. A Kruskal-Wallis analysis of variance (ANOVA) on ranks was done to test for differences among swimming speeds of individual pike. Analyses were completed using SAS version 9.0. The slope of the line used to calculate half-life was determined using SigmaPlot version 9.0.

Results

Fish growth

Pike transferred from Lake 658 to Lake 240 grew considerably, exhibiting significant increases in weight over time (Table 4.2, $F=30.6$, $p<0.0001$, $df=1,16$). On average, fish that were recaptured approximately 650 days after they had been moved to Lake 240, had gained 704 g in weight, which is an increase of 1.6 - 2.4 times their original weight. There were no periods of weight loss recorded.

Mercury levels

Ambient mercury

Observed concentrations of ambient mercury in northern pike muscle were not different than expected on day 80 ($t=0.57$, $p=0.60$, $df=4$). Values observed on days 320 ($t=2.83$, $p=0.037$, $df=5$) and 650 ($t=4.37$, $p=0.012$, $df=4$) were significantly higher than those expected from no loss or gain of mercury (Figure 4.1a). Comparisons of mercury concentrations in pike among sampling periods were not made because each sampling period contained a different group of individuals, and the mean concentrations were influenced by which pike were sampled.

Most of the individual fish experienced an increase in ambient burden while in Lake 240 that generally occurred by day 320 (Figure 4.2a). A paired *t*-test comparing the original and recapture burdens for each sampling period indicated that fish captured on day 80 had similar burdens (105%) to when they were first released into Lake 240 ($t=0.81$, $p=0.46$, $df=4$). Pike recaptured on days 320 and 460, had mercury burdens that were not significantly higher than those measured at the beginning of the study (day 320:

t=2.48, p=0.056, df=5; day 460: t=10.09, p=0.063, df=1, Table 4.3) despite mean increases of 63% and 77%, respectively. The mean ambient mercury burdens of the fish captured on day 650 had significantly increased (by 57%) from the beginning of the study (t=3.40, p=0.027, df=4, Table 4.3).

Spike mercury

Observed concentrations of spike mercury in pike were similar to those expected due solely to changes in fish weight (Figure 4.1b). There was no significant difference between observed and expected concentrations for each day tested (day 80: t=1.38, p=0.24, df=4; day 320: t=1.41, p=0.22, df=5; day 650: t=2.33, p=0.080, df=4). There was a general trend in which observed concentrations of spike mercury were slightly lower than expected on days 80 and 650, and higher than expected on days 320 and 460.

The burdens of spike mercury changed differently over time for individual fish (Figure 4.2b). The dominant pattern observed in fish that were recaptured several times was an increase in burden over the first 320 - 460 days and a loss by day 650 (Figure 4.2b). A paired *t*-test showed no significant change in burdens of spike mercury between original and recapture periods, which was most obvious for the first 460 days of the study (day 80: t=1.48, p=0.21, df=4; day 320: t=1.27, p=0.26, df=5; day 460: t=1.39, p=0.40, df=1). Spike burdens measured on day 650 were nearly significantly lower than the original burdens for those fish (t=2.56, p=0.062, df=4). Though the spike burdens of mercury did not change significantly from the original levels, there is a pattern of decline within the first 80 days, followed by an increase and another decrease by day 650 (Table 4.4).

Half-life of spike mercury

Ideally, mercury half-life is calculated using losses from the whole fish, which was not possible using the non-lethal periodic sampling in this study. Retained body burdens of spike mercury did not follow a typical pattern of first order loss, likely due to redistribution of mercury into muscle. Hence, I removed the day 320 and 460 data for estimations of half-life. By doing this, I was able to incorporate the timing of mercury loss from the fish, but ignored the redistribution kinetics where the burden temporarily increases on days 320 - 460. The natural logs of the percent retained for days 0, 80, and 650 were plotted against time for the calculation of half-life (linear regression r^2=0.47). The resulting half-life of spike mercury in northern pike was 796 days.

Fish activity

The 1 h activity tracks showed that the swimming speeds ranged up to 4.04 bl/sec, although nearly 80% of these speeds fell below 0.14 bl/sec (Figure 4.3). When pike were visually observed during activity tracks, they were often stationary. On rare occasion, pike would swim quickly for the entire hour-long tracking period, resulting in the few higher swimming speeds (Figure 4.3). In these situations, pike could swim across a large portion of Lake 240 within an hour. Pike traveled throughout most of the lake, with a tendency to frequent areas with submerged macrophytes, boulders or logs (Figure 4.4).

A range of swimming speeds were recorded for individual pike due to monitoring activity when fish were stationary as well as when they were swimming. There was greater variation in swimming speeds exhibited by individual pike than among

individuals. Most of this variation was caused by infrequent fast periods of swimming. Overall, there was no difference among the median swimming speeds of individual pike (Figure 4.5, χ^2=8.95, p=0.44, df=9). As a result, I was not able to further investigate the effects of individual activity differences on spike mercury elimination.

Modeling mercury elimination

Predicted declines in average spike mercury burdens were compared among three models: the HB model, Wisconsin model, and TR models. Fast and slow elimination scenarios were applied to the models to represent a range of possible elimination rates for these fish. Elimination scenarios did not involve altering the rate of elimination in the model, but rather entering water temperatures and body sizes from the outer range of those recorded during this study. The slow elimination scenario included the largest fish experiencing cool temperatures, while the fast elimination scenario used the smallest fish experiencing warm temperatures. Predicted losses were slowest by the HB model, moderate by the Wisconsin model, and fastest by the TR model. None of the models captured the observed pattern of redistribution of mercury into muscle, but some predictions fit observed losses at the end of the study (day 650).

When compared to day 650, the HB model predicted that loss would occur more slowly than was observed (Table 4.4). The difference between the fast and slow elimination scenarios (i.e. the impact of temperature and weight on elimination) was smallest for the HB model. The Wisconsin model predicted losses of mercury that neared observed values on sampling day 650 (Table 4.4). The TR model has different equations for chronic and acute exposures to mercury. The chronic equation resulted in

predicted declines in mercury burden that were considerably faster than the observed rates of mercury elimination by northern pike. When the model was run for acute exposure, the observed elimination rates fell within the range of those predicted for day 650. Also, the difference between the fast and slow elimination scenarios was greatest for the TR model (chronic exposure), indicating that these predictions were most heavily impacted by weight of fish and water temperature.

Discussion

Concentrations of mercury in fish are impacted by the rate that they lose mercury. Little is known about how fish lose mercury, particularly in the wild and for large-bodied species. Understanding mercury kinetics in large-bodied fish species is important because people consume large fish. I measured the loss of naturally accumulated spike mercury by northern pike in the wild. A major finding of this study was that mercury was redistributed into muscle tissue for a long period after which time there was some loss. The half-life of mercury was estimated as 796 days, which is among the slowest elimination rates recorded for this species.

Mercury levels

Ambient mercury

The pike transferred from Lake 658 to Lake 240 experienced increases in ambient mercury over 650 days. Changes in ambient mercury burdens in individual pike suggest that redistribution into muscle was most substantial up to the day 320 sampling period (Figure 4.2a). After this time, ambient mercury burdens of individual pike do not

continue to increase at the same rate observed up to day 320 (Figure 4.2a). Lake 240 has lower ambient mercury concentrations than Lake 658 (refer toTable 2.1). The pattern of changes observed were likely due to redistribution of Lake 658 ambient mercury into the muscle (peaking near day 320) as well as accumulation of lower levels of ambient mercury in Lake 240. After day 320, there would likely be combined effects of low accumulation of ambient mercury in Lake 240 and losses of mercury that had been absorbed while in Lake 658. Laarman et al. (1976) observed similar changes in ambient mercury levels in yellow perch (*Perca flavescens*) and rock bass (*Ambloplites rupestris*) that had been transferred from a contaminated lake to cleaner ponds and monitored for 26 months. They found that ambient mercury levels increased in the muscle following transfer of the fish, and then did not decline below the levels seen at the beginning of the study (Laarman et al. 1976). Lockhart et al. (1972) monitored the loss of ambient mercury in northern pike after transferring them from a highly contaminated lake to a cleaner system. They found a steady loss of mercury (up to 29%) over 1 year, though the rates of redistribution and elimination in that study may have been impacted by the use of pike with extremely high mercury levels (Lockhart et al. 1972). It is also important to note that elimination rates estimated by Lockhart et al. (1972) and Laarman et al. (1976) were confounded by continual uptake of ambient mercury by fish after transfer to "clean" lakes. The present study had the advantage of monitoring elimination of spike mercury, which was not accumulated by the pike after transfer to Lake 240.

Spike mercury

Spike mercury was continually redistributed into the muscle tissue of northern pike up to 460 days after they were transferred to a clean system and exposure to spike mercury had ended. All of the pike captured during the day 650 sampling period had lost spike mercury from muscle as compared to the levels measured for the same fish on days 320 and 460. This finding indicates that net loss of mercury from muscle was delayed by redistribution of accumulated mercury into this tissue for up to approximately 460 days. Although not statistically significant, there was a trend in which concentrations were higher than expected on days 320 and 460, and lower than expected on days 80 and 650. This pattern suggests that redistribution and eventual loss of mercury from the muscle occurred.

The lack of a statistically significant decline in spike mercury was likely due to the variation in fish size, temperatures experienced, and initial mercury burdens. The conditions of the study are very realistic, and the resulting variability is in part due to the realism of the study. Because this study used pike with naturally accumulated spike mercury, each pike monitored had a different mercury content at the beginning of the study. Due to a low availability of large pike (>750 g) in Lake 658, there was a 481-g range of initial body sizes in this study. Each fish would not have experienced the exact same temperatures and growth while in Lake 240 because the movements of pike were not restricted in this lake. As a result, there was large variability in the data and significant differences between original and recapture spike mercury burdens did not occur. Observed concentrations were not significantly different than those expected if there was no loss of mercury. On its own, this finding suggests that all of the changes in

concentration were due to growth dilution rather than mercury elimination. The lack of significant differences is partly due to the variation in the dataset caused by differing rates of redistribution and elimination of mercury within this group of northern pike (Figure 4.2b). It is unclear what has caused these different redistribution times; fish that had earlier or later redistribution did not have distinct characteristics with respect to size, sex, original mercury concentration, activity rate, or temperature experienced. Different durations of exposure to spike mercury (due to the range of transfer times and including pike originally from Winnange Lake) also did not relate to observed differences in mercury redistribution times among pike. Nevertheless, the overall pattern shown for most pike was a slight decrease in spike mercury burden by day 80, followed by an increase that carried through days 320 and 460, and a final decrease below starting levels for day 650. This pattern suggests redistribution of mercury into the muscle resulting in a peak near days 320 - 460 followed by some loss from this tissue by day 650.

Disadvantages of studying free-ranging fish in the wild were that fewer samples could be collected and not every fish could be recaptured on each sampling period. Patterns of redistribution may have been clearer if more frequent sampling was possible; however, this could not be done because the pike needed sufficient healing time between biopsy collections. Capturing all pike on each sampling day would have improved the dataset, but was not possible.

Overall, the present study observed a loss of 29% from the original burdens after 650 days. In a study similar to the present one, Lockhart et al. (1972) found that 29% of the mercury body burden had been lost after 1 year. The redistribution period where mercury was transferred into muscle probably delayed the detection of net elimination

from muscle in the present study. Redistribution of mercury into fish muscle from other tissues is known to occur (Massaro and Giblin 1972; Giblin and Massaro 1973; McKim et al. 1976; Boudou and Ribeyre 1983; Riisgard and Hansen 1990; Oliveira Ribeiro et al. 1999; Leaner and Mason 2004). Lockhart et al. (1972) also found that there was redistribution of mercury into the muscle, leading to 105% of the original burdens, but this occurred within 3 months following transfer to the cleaner lake. The present study found that net redistribution of mercury into muscle took longer to begin (at some time between 80 and 320 days), lasted for a longer period (>120 days), and caused greater increases (up to 316% of original burdens) than the findings of Lockhart et al. (1972). Chapter 2 of this thesis found a similar delay (90 days) before the redistribution of spike mercury into the muscle tissue occurred for yellow perch. In comparison, the field study of Laarman et al. (1976) found a redistribution of mercury into the muscle that lasted for 6 months to a year after yellow perch were transferred to a clean lake. It is unclear why the present study, Lockhart et al. (1972), and Laarman et al. (1976) all showed different periods of redistribution of mercury into the muscle. The starting concentrations of mercury were approximately 80 ng/g w.w. (spike) and 769 ng/g w.w.(ambient) for the present study, 1000 ng/g w.w. for Laarman et al. (1976), and 8000 ng/g w.w. for Lockhart et al. (1972). Therefore, it is possible that the extent of redistribution is related to the original mercury concentration in studied fish. In Lockhart et al. (1972) the amount of mercury redistributed into muscle after transfer may have been minor relative to the amount already stored in muscle. For example, an increase of 80 ng/g w.w. spike mercury in the present study would have doubled the percent retained compared to the start of the study. In comparison, an increase of 80 ng/g w.w. in Lockhart et al. (1972)

would only result in an added increase of 1% of the mercury burden retained. Therefore, the percentage of mercury redistributed into the muscle may have been negatively related to the concentration of mercury measured in fish at the beginning of the study. This possibility would explain why the present study had a greater percentage of the original mercury burdens redistributed into the muscle than Laarman et al. (1976) and Lockhart et al. (1972). The fact that redistribution into the muscle lasted for longer in the present study than the other two field studies, may have been caused by a different distribution of mercury within the compartments of the fish at the time of transfer (Rowan and Rasmussen 1995; Oliveira Ribeiro et al. 1999). The location of mercury among different fish tissues over time was not tested in the present study and, therefore, cannot be compared to other studies.

The half-life of spike mercury estimated in the present study was longer than what has previously been estimated for northern pike in both field and laboratory studies (Table 4.5). As mentioned above, net redistribution into muscle is long-lasting, which delays net elimination from this tissue. Past studies have suggested that factors such as exposure time and fish weight also play a role in measured elimination rates (suggested by de Boer et al. 1994; Trudel and Rasmussen 1997). In particular, studies that are shorter in duration tend to estimate faster elimination rates (Table 4.5) (Trudel and Rasmussen 1997). Some studies have also suggested that elimination rates are positively related to starting concentrations of mercury (DeFreitas et al. 1975; Ruohtula and Miettinen 1975); however, Trudel and Rasmussen (1997) disagreed. The present study is further evidence that elimination of mercury in nature occurs more slowly than predicted by short-term laboratory studies.

The elimination of mercury by northern pike appears to be highly dependent on elimination from muscle tissue, as this is where most mercury is stored. I suspect that my estimate of half-life might change if the experiment was extended, because half-life was impacted by a long period of redistribution of mercury into muscle tissue. By 650 days after the beginning of the study, net storage of spike mercury into muscle had ceased and there was an outward flux (Table 4.4). The decline from the peak in mercury burdens appears to have occurred relatively quickly. If this loss from the muscle continued to occur quickly and fish were sampled again later, the calculated half-life would shorten. The half-life estimated in the present study was based on method 1 of calculating body burden, which would not directly apply to mercury elimination rates from the whole fish (Chapter 2). Nevertheless my data is comparable to Lockhart et al. (1972), which is the only other field study on mercury elimination by northern pike, because mercury losses in that study were also tracked using body burden method 1.

Fish Activity

It is unclear whether mercury elimination by fish is related to their metabolic rate (DeFreitas et al. 1975; Norstrom et al. 1975; Rodgers 1994). Past studies have indicated that mercury elimination is negatively related to weight of the fish and positively related to water temperature (Trudel and Rasmussen 1997). There have been no studies on possible relationships between mercury elimination and metabolic costs due to activity. I monitored activity rates of northern pike in a natural habitat over two summer seasons. These pike were the same individuals whose mercury elimination rates were being estimated in the wild. By monitoring rates of both activity and mercury elimination, I

attempted to determine whether there was a relationship between mercury loss and metabolic costs of activity.

The observation that pike were often stationary with short bursts of faster swimming is similar to other field observations of this species (Diana 1980; Lucas et al. 1991). Diana (1980) found a mean swimming speed of 0.45 bl/sec, which was greater than the mean of 0.095 bl/sec (approximately 5.1 cm/s) found by this study; however, Diana (1980) only included moving fish in his estimate. Another field study on pike movements estimated that average swimming speed was 5.5 cm/s which was very similar to the findings of the present study (Poddubnyi et al. 1970). Lucas (1992) found that activity rates of pike differed by sex during the spawning season, where males were more active than females. This was also the case for walleye (*Stizostedion vitreum*) to the extent that mercury concentrations were increased in males due to their higher activity levels and allocating less energy to growth (Henderson et al. 2003). In the present study, two of the three pike with the fastest swimming events (as shown by the 95th percentile in Figure 4.5, ID numbers = 2, 7, 9) were males, and one was not sexed. Pike were not tracked during the spawning season and median values of swimming speed were similar among pike. This finding suggests that the overall activity rates are similar among northern pike when they are not spawning.

Modeling mercury elimination

Three mercury models were used to predict losses of spike mercury by northern pike. These models were the HB model, Wisconsin model, and TR models, which take into account factors including water temperature and fish body mass to predict losses of

mercury. None of the models accurately reflected the observed redistribution of mercury into muscle. It is important to keep in mind that these models have been designed to predict mercury elimination from the whole fish, not redistribution based on mercury kinetics in muscle. Therefore, the models are not expected to fit the redistribution period of the data, but can be compared to a longer overall trend of the slow phase of elimination best shown by sampling days 0, 80 and 650. These results further support the finding of Chapter 2 that method 1 of calculating body burden did not fit model predictions of changing mercury levels in fish.

Based on sampling days 0, 80, and 650, the HB model predicted elimination rates that were slightly slower than those observed. The HB model also predicted the slowest elimination rates for yellow perch (Chapters 2 and 3). The elimination rate inputs used for northern pike were those recommended for walleye based on field calibrations done in Harris and Bodaly (1998). Potential species differences in mercury kinetics between walleye and northern pike could have caused some error in the predictions for northern pike.

The Wisconsin model provided the best fit of any of the models to the day 0, 80, and 650 data. This is very different from predictions by this model for yellow perch, which overestimated elimination rates (Chapters 2 and 3). The elimination component of the Wisconsin model is based on equations developed by Norstrom et al. (1976). Norstrom et al. (1976) developed their model to fit the longer-term, large fish studies of Lockhart et al. (1972) (field) and Jarvenpaa et al. (1970) (laboratory), while for small fish, the model was primarily based on short-term laboratory studies (DeFreitas et al. 1974; Sharpe et al. 1977). As short-term studies tend to overestimate elimination rates in

particular (Trudel and Rasmussen 1997), the Wisconsin model predictions poorly fit field data for small-bodied fish as shown in Chapter 2. In contrast, the slow elimination rates incorporated into the Wisconsin model from Lockhart et al. (1972) and Jarvenpaa et al. (1970) resulted in predictions that closely fit the observed rates of elimination by northern pike in this study. The long-term studies of Lockhart et al. (1972) and Jarvenpaa et al. (1970) produced half-lives that were 2 - 8 times longer than those found by studies $\leq$60 days in length. Although the half-lives found by Lockhart et al. (1972) and Jarvenpaa et al. (1970) were slightly shorter than those found by the present study, the Wisconsin model resulted in accurate predictions of spike mercury levels in northern pike. This may have been due to the relationships among mercury loss, body size and temperature in the model.

The TR model predicted the fastest elimination rates for northern pike. The application of the additional increase in elimination rates due to chronic exposure in the TR model caused predictions that poorly fit the observed data. The chronic equation also resulted in overestimates of elimination rates when applied to yellow perch in Chapter 2. Most other studies suggest that elimination rates would be slower in fish that are chronically exposed (de Boer et al. 1994; Rowan and Rasmussen 1995), rather than faster. It is possible that the chronic application of the TR model is not accurate, because it does not apply to either northern pike or yellow perch in the wild, and goes against findings of other studies.

The application of the acute exposure equations of the TR model resulted in predictions that fit observed data. The Wisconsin and TR models were developed based on the same elimination studies on large fish, mainly Lockhart et al. (1972) and

Jarvenpaa et al. (1970). The acute equation of the TR model and the Wisconsin model produce similar results, with slightly faster elimination predicted by the TR model.

Conclusions

I set out to determine the elimination rates of naturally accumulated mercury by northern pike in the wild. Other studies have been done either in the laboratory or in the field using fish more highly contaminated than found in most lakes (Trudel and Rasmussen 1997). The present study is unique compared to other field studies because it involved the use of enriched stable isotopes, which could be distinguished from ambient mercury present in all lakes. This study provided insight into the movement of mercury into and out of muscle of wild fish over a long period (650 d). I found a lengthy period of redistribution of mercury into muscle that lasted longer and caused greater relative increases in calculated body burdens than other field studies. Overall elimination of spike mercury by northern pike was very slow, with an estimated half-life of approximately 796 days. This estimate up to 8 times longer than previously determined for this species in short-term studies. The half-life was impacted by the lengthy period of net mercury redistribution into the muscle before any detectable loss occurred. As the experimental pike are currently still alive in the study lake, it would be interesting to extend the study for a longer period to better determine rates of loss from muscle. Three mercury models were tested to see how well they predicted declines in spike mercury burdens in northern pike, and none of these models reflected the period of redistribution into the muscle. This discrepancy was likely due to comparing these models to spike mercury body burden calculated from the concentrations in muscle (method 1), while the

models are designed to predict elimination from the whole fish. When models were compared to spike mercury burdens recorded at the beginning and end of the experiment, the Wisconsin model produced the best fit. The HB model underestimated spike mercury loss. The TR model fit observed data more closely when acute exposure equations were used, even though the fish were chronically exposed to mercury. Pike were found to have similar activity rates among individuals based on tracking that occurred from June through September. As a result, the impacts of activity rate on mercury elimination could not be compared in this study.

Table 4.1. Characteristics of the northern pike (*Esox lucius*) collected from Lake 658 and transferred to Lake 240 for monitoring of spike mercury elimination and activity rates.

Pike ID	Starting weight (g)	Starting concentration (ng/g w.w.)		Sex	Original lake	Year of transfer	Activity tracks	Recapture Hg data
		Spike	Ambient					
1	754	4	325	female	Winnange	2004	yes	yes
2	782	42	963	-	658	2004	yes	yes
3	1201	60	704	female	658	2004	yes	yes
4	752	75	994	-	658	2004	yes	yes
5	1233	83	695	female	Winnange	2005	no	yes
6	1074	86	823	-	658	2004	yes	yes
7	783	88	790	male	658	2004	yes	yes
8	1182	91	631	-	Winnange	2005	no	yes
9	803	101	837	male	658	2004	yes	yes
10	773	179	783	-	658	2005	no	yes
11	827	50	641	-	658	2004	yes	no
12	625	66	849	-	658	2004	yes	no
13	809	66	957	-	658	2004	yes	no

Table 4.2. Weights (g) of northern pike (*Esox lucius*) recaptured at intervals after transfer from Lake 658 to Lake 240.

Pike ID	day 0	day 80	day 320	day 460	day 650
1	754	919	1182	1610	1786
2	782	-	-	-	1415
3	1201	-	1547	-	1935
4	752	979	-	-	-
5	1233	1403	1442	-	-
6	1074	-	1338	1534	-
7	783	-	1199	-	1418
8	1182	1479	-	-	-
9	803	-	1185	-	1289
10	773	1017	-	-	-

Table 4.3. Mean (+/- 1 standard error) ambient mercury burdens in northern pike (*Esox lucius*) recaptured at intervals after transfer from Lake 658 to Lake 240.

Sampling period	Number of pike	Original mercury burden (ng)	Recapture mercury burden (ng)
day 80	5	640175 ± 106595	679347 ± 118243
day 320	6	686959 ± 98858	1120562 ± 192534
day 460	2	564284 ± 319474	996366 ± 276647
day 650	5	626782 ± 102909	989624 ± 143399

Table 4.4. Mean (+/- 1 standard error) percentages of original spike mercury burdens retained in northern pike (*Esox lucius*) recaptured at intervals after transfer from Lake 658 to Lake 240. The predicted percentages of mercury retained that were produced by the HB model, Wisconsin model, and TR model (acute and chronic) are shown based on the range of possible body sizes and water temperatures experienced by these pike.

Sampling period	No.	% burden retained	HB	Wisconsin	TR (chronic)	TR (acute)
day 80	5	112 ± 23	97-99%	90-97%	78-90%	89-95%
day 320	6	217 ± 73	95-97%	81-90%	59-73%	77-86%
day 460	2	316 ±149	92-95%	71-85%	40-59%	64-78%
day 650	5	64 ± 8	90-94%	67-82%	33-51%	59-73%

Table 4.5. Half-lives of methylmercury in large fish species. Duration of exposure, mercury concentrations, body weights, tissues monitored, and duration that elimination was studied are shown for experiments in both field and laboratory settings.

Type of study	Species	Duration of exposure	Mean starting [Hg] (ng/g w.w.)	Mean starting weight (g)	Tissue monitored for elimination	Duration of elimination study (d)	Mercury Half-life (d)	Source
Field	northern pike (*Esox lucius*)	between 1 and 5 years (except for 1 fish at 30 days)	80	933	muscle	650	796	the present study
		between 2 and 7 years	8341	3920	muscle	365	**528	Lockhart et al. 1972
Laboratory	northern pike (*Esox lucius*)	one oral dose	5.8*	300	whole body	130	640-750	Jarvenpaa et al. 1970
		one dose injected	5.8*	300	whole body	130	780	Jarvenpaa et al. 1970
		<6 days	13700	300	whole body	30	94	Miettinen et al. 1970
		<6 days	24700	330	whole body	18	110	Miettinen et al. 1970
		<1 day	-	75	whole body	59	139	de Freitas et al. 1974
		<1 day	-	150	whole body	59	173	de Freitas et al. 1974
		one oral dose	-	85	whole body	60	385	de Freitas et al. 1975
	eel (*Anguilla vulgaris*)	one oral dose	18*	100	whole body	130	910-1030	Jarvenpaa et al. 1970
		one dose injected	18*	100	whole body	130	1030	Jarvenpaa et al. 1970
	flounder (*Platichthys flesus*)	one oral dose	9.7*	180	whole body	100	700-780	Jarvenpaa et al. 1970
		one dose injected	9.7*	180	whole body	100	1200	Jarvenpaa et al. 1970

* as reported in Trudel and Rasmussen (1997)
**calculated based on all of Lockhart's data

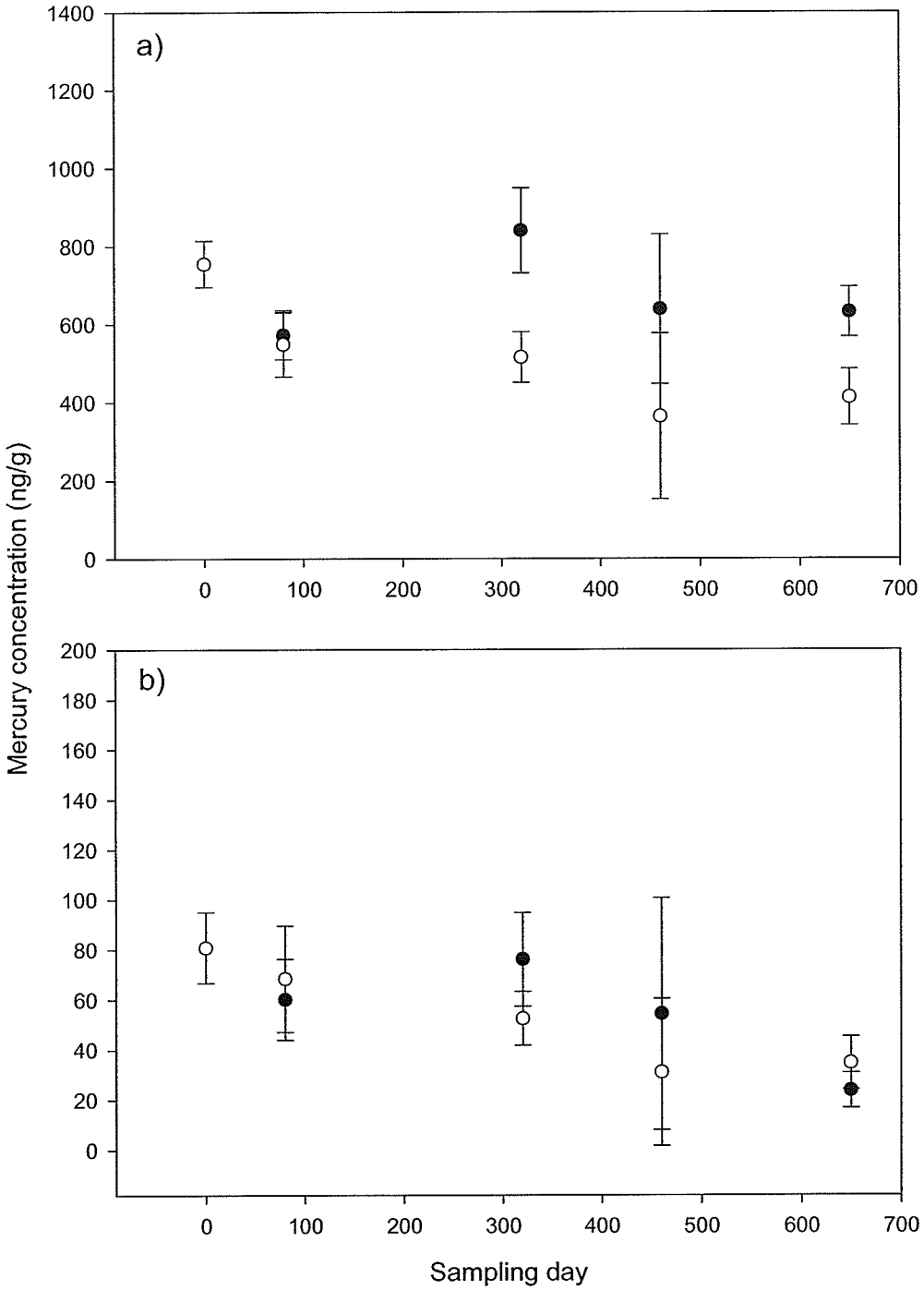

Fig. 4.1. Mean (+/- 1 standard error of the mean) observed (closed circles) and expected (open circles) concentrations of a) ambient and b) spike mercury in northern pike (*Esox lucius*) muscle over approximately 650 days following transfer from Lake 658 to Lake 240.

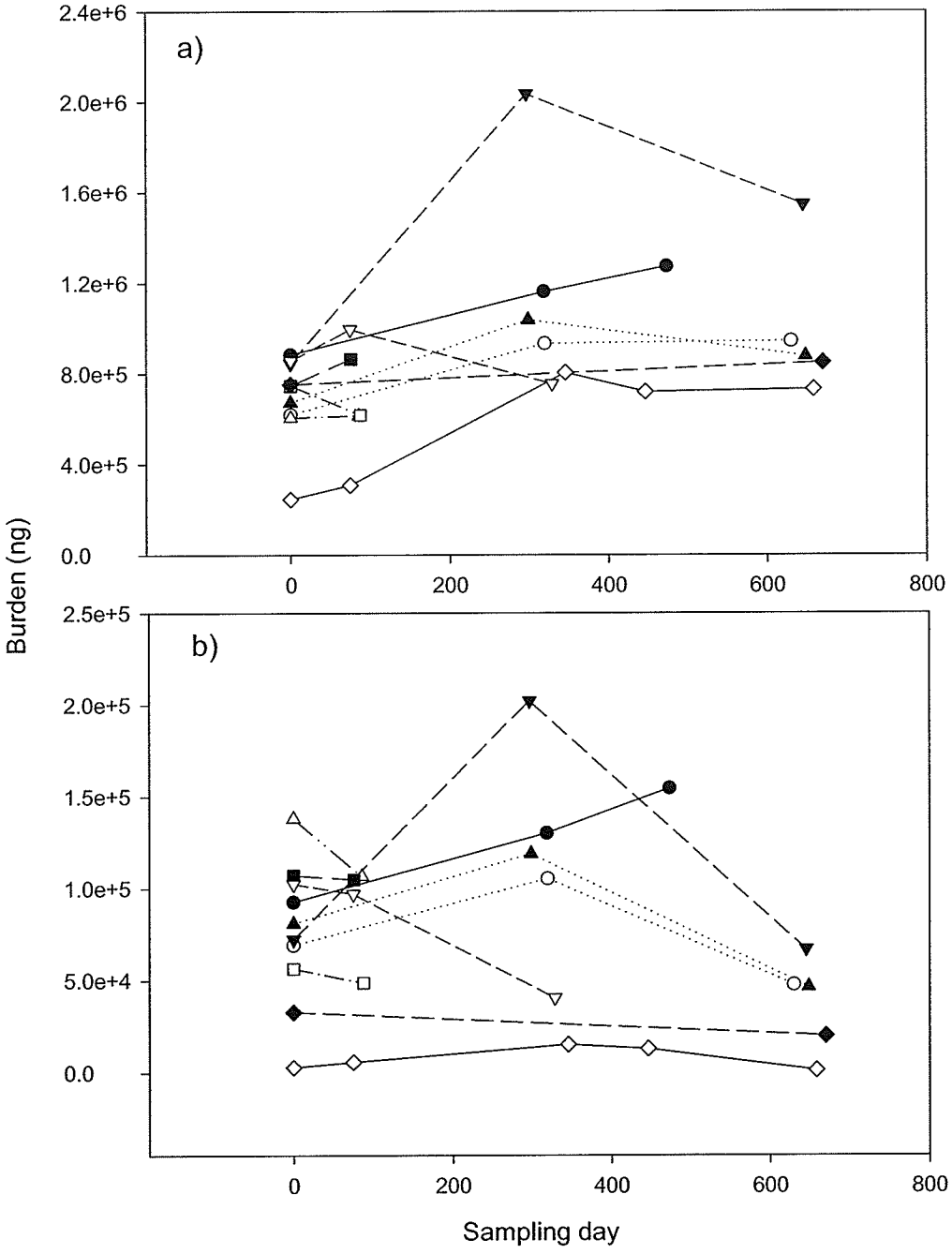

Fig. 4.2. Body burdens of a) ambient and b) spike mercury in individual northern pike (*Esox lucius*) over approximately 650 days after being transferred from Lake 658 to Lake 240. Different symbols represent individual fish.

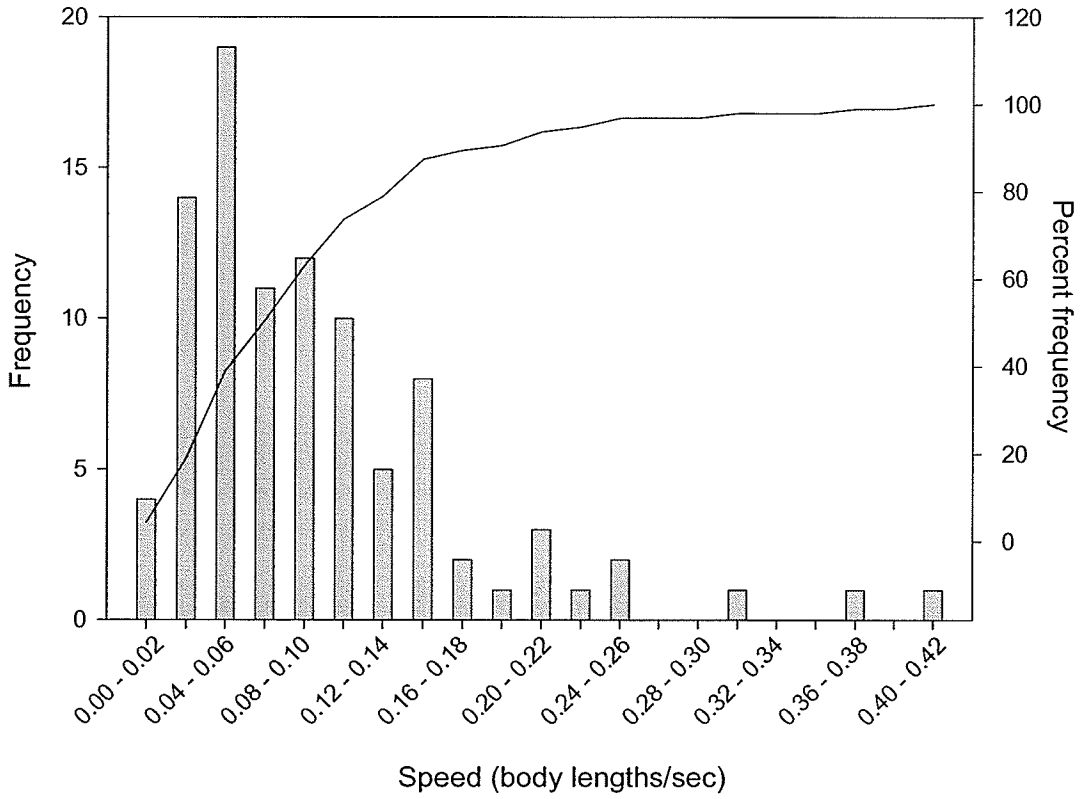

Fig. 4.3. The frequency of swimming speeds during 1 h periods recorded for northern pike (*Esox lucius*) manually tracked over two summers after being transferred from Lake 658 to Lake 240. The solid line shows the cumulative percent frequency of all speeds.

Fig. 4.4. Locations of 10 northern pike in Lake 240 (indicated by dots) when manually tracked in 2004 (left) and 2005 (right) after being transferred from Lake 658.

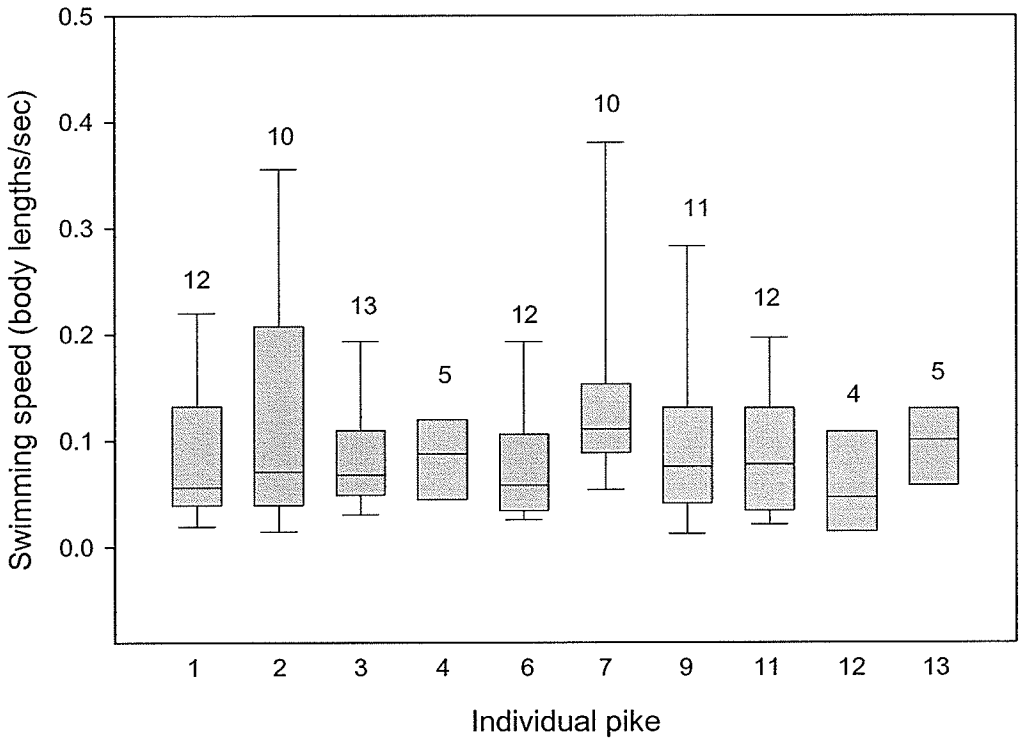

Fig. 4.5. Swimming speeds of individual northern pike (*Esox lucius*) manually tracked in 2004 and 2005 after being transferred from Lake 658 to Lake 240. Solid lines inside the box indicate medians, outer edges of the box indicate the 25th and 75th percentile, and error bars indicate the 5th and 95th percentile. The number of activity tracks is shown above the error bars. The x-axis shows the pike IDs (refer to Table 4.1).

Chapter 5: Synthesis

1) The slow elimination of mercury by fish is an important factor that leads to elevated mercury levels. In reservoirs, mercury concentrations in fish can become elevated beyond those safe for consumption and remain high for 20 - >30 years (reviewed in Bodaly et al. 1997). The elimination rates used in current mercury models are primarily based on short-term laboratory studies that would have little applicability to natural systems or reservoirs. To understand and better predict mercury levels in fish under different environmental conditions and in reservoirs, it is important to understand mercury elimination rates in nature.

2) The approach of the research presented in this thesis sets it apart from past laboratory and field research on this topic. I monitored the loss of a naturally accumulated form of isotopic mercury by yellow perch and northern pike while they experienced the conditions of a natural lake. The isotopic mercury could be distinguished from ambient mercury continually accumulated by fish in lakes. The concentrations of isotopic and ambient mercury were in the range of those that are typically found in fish in the wild. Two past field studies on elimination used non-isotopic mercury, and studied fish that were highly contaminated.

3) I found that a high-mercury environment did not impact elimination of mercury by yellow perch because elimination was the same in a reservoir as in an unimpounded lake. Therefore, it is likely that the findings of this thesis will apply

to reservoirs and other systems regardless of whether there are low or high concentrations of available mercury.

4) A major finding of this thesis is the pattern and timing of mercury redistribution into and out of the muscle tissue. Once exposure to isotopic mercury ended, it slowly moved from the other tissues into the muscle. There is a time lag between when the mercury leaves the rest of the fish and enters the muscle, which lasts approximately 90 d in yellow perch (Chapter 2) and between 90 - 320 days in northern pike (Chapter 4). Mercury is then stored in the muscle, reaching peak storage at 180 days (yellow perch) and 320 - 460 days (northern pike) after exposure has ended. After peak storage, mercury is slowly lost from the muscle tissue. Decreases in mercury were observed in yellow perch after 1 year and northern pike after 650 days. The timing of mercury elimination is different for yellow perch and northern pike, but the pattern is similar for both species. This finding suggests that this pattern may apply to wild fish of a range of sizes (3 - 1200 g) that have been naturally exposed to mercury.

5) Loss of mercury from the whole fish is more continuous than loss from the muscle, as was found for yellow perch in Chapter 2. The method of calculating body burden of mercury (using the concentration in muscle only (method 1, Chapter 2) versus concentration in the whole fish (method 2, Chapter 2)) changes the observed patterns of loss. The models are not able to predict the increases in mercury burdens calculated by method 1 that occur due to redistribution of

mercury into the muscle. Therefore, if body burdens are to be calculated based on method 1 when monitoring changing mercury levels in fish, the associated models should be changed so that they reflect the storage and loss of mercury from the muscle tissue.

6) Half-lives of mercury (the amount of time it takes for the mercury burden in a fish to be reduced by half) were calculated as 489 days for yellow perch and estimated as 796 days for northern pike. These half-lives are approximately 2 - 8 times longer than those reported by short-term laboratory studies, indicating that elimination of mercury occurs more slowly in nature than originally implied by laboratory studies. These half-lives should apply to yellow perch (1 - 11 g) and northern pike (750 - 2000 g) with concentrations of approximately 80 - 300 and 80 - 800 ng/g w.w. of mercury in their muscle, respectively. In theory, these elimination rates will also apply to perch and pike with different starting burdens (Trudel and Rasmussen 1997).

7) Three different mercury models were compared in their abilities to predict mercury loss by yellow perch and northern pike. The model developed by Harris and Bodaly (1998) had the slowest elimination rates of the three models, and accurately predicted mercury losses by yellow perch, but underestimated losses by northern pike. The Wisconsin model version 3.0 (Hanson et al. 1997) overestimated losses of mercury by yellow perch, but was suitable for northern pike. The model developed by Trudel and Rasmussen (1997) provided accurate

simulations for both species only if their equation for acute exposure was used. The half-lives found by the present study should be incorporated into models to reflect realistic rates of mercury loss by fish experiencing water temperatures typical of a temperate dimictic lake.

8) Long-term monitoring of reservoirs has found that mercury concentrations in fish increase to approximately 1000 - 3500 ng/g w.w. by 1 - 10 years following flooding and can remain high for 20 - >30 years (reviewed in Bodaly et al. 1997). The slow mercury elimination rates by fish are important to the amount of time that it takes for mercury levels to decline in reservoirs following flooding. Models used for predicting mercury concentrations in fish in reservoirs should include the elimination rates presented in this thesis for more accurate predictions of long-term trends and recovery from elevated mercury levels. Models that take into account a range of elimination rates reported by different studies, such as the one developed by Trudel and Rasmussen (1997), should also incorporate the elimination rates presented in this thesis and remove short-term (<90 d) laboratory studies to increase the realism of their models.

Appendix 1: Description of model inputs

The inputs and equations of three mercury models are included in this appendix. These models include one developed by Harris and Bodaly (1998) (the HB model), the Wisconsin model version 3.0 (Hanson et al. 1997), and a model developed by Trudel and Rasmussen (1997) (the TR model). All three models can be used to predict mercury losses by fish. The HB model and Wisconsin model include bioenergetic components, while the TR model does not.

Bioenergetics

The HB model and the Wisconsin model have bioenergetics components that are described for each chapter below. The data collected for weight and temperature were also used for the TR model.

Fish growth

For the HB and Wisconsin models to properly represent concentration and burden of mercury, first the predicted growth rates had to reflect those observed.

Chapter 2

There are two different rates of growth of the yellow perch used for this experiment because perch inside the enclosure grew at a slower rate than escaped perch. These two periods were modeled separately with different growth rates (Figure A.1). The parameters used to create the growth curves are shown in Table A.1 for the HB model and Table A.2 for the Wisconsin model. Rather than using a growth curve that does not

include weight losses, the actual weights were entered into the TR model (Table A.3). Sampling days did not occur on every 15th day, so a linear change in weight between sampling days was assumed.

Chapter 3

The model inputs were chosen to reflect the steady growth of yellow perch studied in this chapter (HB model: Table A.4; Wisconsin model: Table A.5). The weights of yellow perch captured on sampling days were entered into the TR model (Table A.6).

Chapter 4

This chapter required estimates of the growth of the largest and the smallest fish for the slow and fast elimination scenarios respectively. As the smallest or largest pike was not caught on each sampling day, the intermediate weights were estimated based on growth rates of the other pike of similar weights (HB model: Table A.7; Wisconsin model: Table A.8; TR model: Table A.9).

Water temperature

Chapter 2

The HB model requires mean monthly water temperatures (Table A.1), while the inputs to the Wisconsin model (Table A.2) were based on biweekly means. The mean temperature in the enclosure on each 15th day was used in the TR model (Table A.3). Temperature loggers inside the enclosure provided these measurements up to mid-April 2005. Escaped yellow perch were free to experience different depths of the water

column, though yellow perch are generally found in littoral zones and shallow areas (Scott and Crossman 1973). To represent the temperatures experienced by the escaped yellow perch in May and early June, the monthly mean temperatures recorded in the top 6 m in the lake were input into the model. These temperatures were recorded at each 1 m depth using a temperature probe (YSI Inc.) at the location of maximum depth in the lake on May 5, 9, 17, 31, 2005 and June 14, 2005. From July 5, 2005 to August 29, 2005, loggers placed at 2-m depth intervals at the location of maximum depth in the lake recorded the temperature every 30 min.

Chapter 3

Mean water temperatures were determined in both Lake 979 and Lake 240 from June to September 2005 using temperature loggers within the enclosures (HB model: Table A.4; Wisconsin model Table A.5; TR model: Table A.6).

Chapter 4

I used the maximum and minimum water temperatures experienced by pike to model fast and slow elimination scenarios respectively. The temperatures used were based on those reported by the temperature-sensing transmitters during manual tracking of pike. The maximum water temperatures were based on the highest measurements recorded within a 2 month time period, while the minimum water temperatures were based on the lowest temperature recorded during this time (HB model: Table A.7; Wisconsin model: Table A.8; TR model: Table A.9).

Caloric density and mercury content of prey

Chapter 2

The caloric density of the diet of yellow perch was entered in the HB model and Wisconsin models, which determines how much food the fish are required to consume to sustain the observed growth rates. Yellow perch were assumed to eat equal masses of zooplankton and benthos while in the enclosure and the larger escaped perch were assumed to exclusively consume benthos. At the time of sacrifice, the escaped fish captured on days 415 and 440 were large enough to consume young-of-the-year (YOY) fish; however, no fish were found in their gut when the stomach contents were removed, and therefore their diet was considered to have the caloric density of benthos (Tables A.1, A.2). Mercury content of prey was assumed to be zero, as the model simulations were for spike mercury.

Chapter 3

Determinations of caloric density in the diet were assumed in a similar way as for Chapter 2. Fish grew faster in Lake 979 than in Lake 240, and therefore were assumed to have a higher proportion of benthos in their diet at an earlier date. Ambient mercury was also modeled in Chapter 3, using different scenarios to explain the elevated ambient mercury concentrations in fish (Table A.10). A scenario (scenario 2) was tested using the Wisconsin model where large perch in Lake 979 had 10% of their diet consisting of YOY fish between days 60 and 90 (Table A.10). The ambient mercury concentrations of prey fish used in this scenario were based on those measured in finescale dace held in Lake

979 (Bodaly and Fudge 1999) and estimated relationships between mercury concentrations in age 1 and YOY perch (P. Blanchfield, unpublished data).

Chapter 4

Because northern pike are piscivores and the dominant prey species in Lake 240 is yellow perch, the input caloric density of prey was consistently that of yellow perch. The mercury content of the prey was set to zero as only spike mercury simulations were run for this chapter (Tables A.7 and A.8).

Mercury elimination

HB model

The excretion of mercury in the model depends on the ratio of methylmercury concentration in urine and in the fish ($[MeHg]_{urine}$ / $[MeHg]_{tissue}$) and was set to be 0.75 for yellow perch (Chapter 2: Table A.1; Chapter 3: Table A.4). This value was based on earlier calibrations of the model to field data of mercury concentrations in the muscle of yellow perch (Harris and Bodaly 1998). The ratio used for northern pike in Chapter 4 was 0.30 based on the earlier calibrations of the model to field data for walleye (Harris and Bodaly 1998) (Table A.7).

Wisconsin model

The elimination of mercury in the model is based on the following equation:

$$\text{Loss of burden per day} = -kcl*B*W^{\zeta}$$

Where B is mercury burden, W is weight, and kcl (which changes with temperature) (Rodgers 1994) and ζ are elimination values entered by the model user. The values for the elimination constants are based on the values used by Rodgers (1994).

TR model

The TR model allows the elimination rate to change in relation to changing water temperatures and changing weights. The model is shown below:

$$\ln k = 0.066\ T - 0.20 \ln W + 0.73\ E - 6.56$$

Where k is the elimination rate, T is water temperature, W is fish weight, and E is entered as 1 for chronic exposure and 0 for acute exposure. The water temperature and fish weights entered into the model changed every 15 days (Chapter 2: Table A.3; Chapter 3: Table A.6; Chapter 4: Table A.9). The resulting k values for each 15 days were used to predict the changes in spike burden.

Table A.1. Inputs used for the HB model simulations of spike mercury body burdens of yellow perch (*Perca flavescens*) over 440 days after transfer from Lake 658 to Lake 240. Two starting concentrations were used: body burdens method 1, which corresponds to concentration in muscle, and body burdens method 2, which corresponds to the concentration in the whole fish.

		Method 1		Method 2	
Parameter	Description	< day 365	> day 365	< day 365	> day 365
Wmax	max. weight possible (g)	25	300	25	300
kt	growth rate	0.32	0.4	0.32	0.4
Q10	relates growth to water temp	2.3	2.3	2.3	2.3
b	growth related input	2.4	2.4	2.4	2.4
neta	length to weight relationship	3.02	3.02	3.02	3.02
lambda	length to weight relationship	0.017	0.017	0.017	0.017
Water temperatures					
June/July	°C	18.6	23.8	18.6	23.8
July/Aug.	°C	20.8	21.5	20.8	21.5
Aug./Sept.	°C	16.8	18.6	16.8	18.6
Sept./Oct.	°C	13.8		13.8	
Oct./Nov.	°C	6.6		6.6	
Nov./Dec.	°C	2.5		2.5	
Dec./Jan.	°C	1.8		1.8	
Jan./Feb.	°C	1.4		1.4	
Feb./Mar.	°C	1.0		1.0	
Mar./Apr.	°C	1.1		1.1	
Apr./May	°C	9.0		9.0	
May/June	°C	14.4		14.4	
MeHg exposure					
MeHg water	[MeHg] in water (ng/L)	10^{-7}	10^{-7}	10^{-7}	10^{-7}
MeHg food	[MeHg] in food (µg/g w.w.)	10^{-7}	10^{-7}	10^{-7}	10^{-7}
MeHg rate constants					
Epf	ratio absorbed from food	0.80	0.80	0.80	0.80
Epw	ratio absorbed from water	0.12	0.12	0.12	0.12
Excrete factor	relates MeHg loss to wastes	0.75	0.75	0.75	0.75
Bioenergetics					
Cal dens food	food energy (kCal/g)	0.57	0.65	0.57	0.65
Cal dens fish	fish energy (kCal/g)	1.0	1.0	1.0	1.0
Act	activity multiplier	1.0	1.0	1.0	1.0
Initial fish conditions					
agenot (days)	starting age of fish	1	365	1	365
Wnot	starting weight of fish (g)	3.7	5.1	3.7	5.1
Cnot	starting [MeHg] (µg/g w.w.)	0.08	0.039	0.057	0.027

Table A.2. Inputs used for the Wisconsin model simulations of spike mercury body burdens of yellow perch (*Perca flavescens*) over 440 days after transfer from Lake 658 to Lake 240. Two starting concentrations were used: body burdens method 1, which corresponds to concentration in muscle, and body burdens method 2, which corresponds to the concentration in the whole fish.

Parameter	Units	Method 1	Method 2
Growth			
day 0 weight	g	3.7	3.7
day 365 weight	g	4.8	4.8
day 440 weight	g	11.1	11.1
Proportions of diet			
Zooplankton			
day 1		0.60	0.60
day 30		0.35	0.35
day 60		0.25	0.25
day 90		0.25	0.25
day 365		0.05	0.05
day 440		0.00	0.00
Benthos			
day 1		0.40	0.40
day 30		0.65	0.65
day 60		0.75	0.75
day 90		0.75	0.75
day 365		0.95	0.95
day 440		1.00	1.00
MeHg rate constants			
Epf		0.8	0.8
ζ		-0.58	-0.58
Kcl		0.029	0.029
Bioenergetics			
caloric density of zooplankton	joules	2093	2093
caloric density of benthos	joules	2720	2720
Act		1	1
Initial fish conditions			
initial [MeHg] concentration	µg/g w.w	0.08	0.057

Note: Epf = assimilation efficiency of MeHg from food, ζ = allometric exponent for elimination, Kcl = clearance coefficient

water temperatures used were similar to those listed in Table A.3 for the TR model.

Table A.3. Weights and temperatures input into the TR model to predict spike mercury body burdens of yellow perch (*Perca flavescens*) over 440 days after transfer from Lake 658 to Lake 240.

Day	Weight (g)	Temperature (°C)
0	3.7	16.3
15	3.2	20.1
30	3.7	22.2
45	3.9	21.0
60	4.1	18.7
75	3.8	16.1
90	3.5	16.2
105	3.7	14.8
120	3.8	10.5
135	4.1	7.6
151	4.5	3.3
166	4.9	2.4
181	5.4	2.1
196	4.9	1.8
211	4.4	1.6
226	3.8	1.4
241	3.3	1.2
256	3.5	1.1
269	3.7	1.0
284	3.9	1.0
300	4.1	2.5
315	4.2	7.6
330	4.4	9.9
345	4.6	13.8
364	4.8	14.9
376	5.7	19.5
391	6.5	25.5
406	7.4	20.2
416	8.2	21.3
436	9.7	18.7

Table A.4. Inputs used for the HB model simulations of spike mercury burden in yellow perch (*Perca flavescens*) over 90 days after transfer from Lake 658 to Lake 240 or Lake 979.

Parameter	Description	Lake 240	Lake 979
Wmax	maximum possible weight (g)	275	325
kt	growth rate	0.37	0.4
Q10	relates growth to water temp	2.3	2.3
b	growth related input	2.2	2.2
neta	length to weight relationship	3.02	3.02
lambda	length to weight relationship	0.017	0.017
Water temperatures			
mid-June to mid-July	°C	20.7	20.8
mid-July to mid-Aug.	°C	23.7	21.0
mid-Aug. to mid-Sept.	°C	19.2	18.0
MeHg exposure			
MeHg water	[MeHg] in water (ng/L)	10^{-7}	10^{-7}
MeHg food	[MeHg] in food (μg/g)	10^{-7}	10^{-7}
MeHg rate constants			
Epf	proportion absorbed from food	0.80	0.80
Epw	proportion absorbed from water	0.12	0.12
Excrete factor	relates MeHg loss to wastes	0.75	0.75
Bioenergetics			
Cal dens food	caloric density of food (kCal/g)	0.65	0.7
Cal dens fish	caloric density of fish (kCal/g)	1	1
Act	activity multiplier	1	1
Initial fish conditions			
agenot (days)	starting age of fish	1	1
Wnot	starting weight of fish (g)	1.1	1.1
Cnot	starting [MeHg] (μg/g)	0.039	0.039

Table A.5. Inputs used for the Wisconsin model simulations of spike mercury burden in yellow perch (*Perca flavescens*) over 90 days after transfer from Lake 658 to Lake 240 or Lake 979.

Parameter	Units	Lake 240	Lake 979
Growth			
day 0 weight	g	1.1	1.1
day 90 weight	g	4.4	12.0
[MeHg] in prey	ng/g w.w.	0	0
Proportions of diet			
Zooplankton			
day 1		0.60	0.60
day 30		0.35	0.35
day 60		0.25	0.10
day 90		0.25	0.00
Benthos			
day 1		0.40	0.40
day 30		0.65	0.65
day 60		0.75	0.90
day 90		0.75	1.00
Water temperatures			
day 1	°C	20.4	20.8
day 15	°C	20.7	20.5
day 30	°C	24.9	21.9
day 45	°C	22.3	20.4
day 60	°C	20.4	18.9
day 75	°C	18.4	17.4
day 90	°C	18.3	17.7
MeHg rate constants			
Epf		0.8	0.8
ζ		-0.58	-0.58
Kcl		0.029	0.029
Bioenergetics			
caloric density of zooplankton	joules	2093	2093
caloric density of benthos	joules	2720	2720
activity multiplier		1	1
Initial fish conditions			
initial fish [MeHg]	ng/g w.w.	39	39

Note: Epf = assimilation efficiency of MeHg from food, ζ = allometric exponent for elimination, Kcl = clearance coefficient

Table A.6. Weights and temperatures input into the TR model to predict spike mercury body burdens of yellow perch (*Perca flavescens*) over 90 days after transfer from Lake 658 to Lake 240 or Lake 979.

	Lake 240		Lake 979	
Day	Weight (g)	Temperature (°C)	Weight (g)	Temperature (°C)
0	1.1	16.6	1.1	17.2
15	1.1	22.9	1.3	22.4
30	2.5	23.9	3.0	24.5
45	3.0	23.7	3.5	21.5
60	3.6	23.6	3.9	21.0
75	4.0	19.1	8.0	17.3
90	4.4	18.3	12.0	17.7

Table A.7. Inputs used for the HB model simulations of spike mercury body burdens of northern pike (*Esox lucius*) over 650 days after being transferred from Lake 658 to Lake 240. Both slow and fast elimination scenarios are shown.

Parameter	Description	Slow elimination	Fast elimination
Wmax	maximum possible weight (g)	6100	4100
kt	growth rate	0.45	0.37
Q10	relates growth to water temp	2.3	2.3
b	growth related input	2.2	2.2
neta	length to weight relationship	3.02	3.02
lambda	length to weight relationship	0.017	0.017
Water temperatures			
June/Aug.	°C	10	24
July/Sept.	°C	10	24
Aug./Oct.	°C	9	22
Sept./Nov.	°C	8	12
Oct./Dec.	°C	4	8
Nov./Jan.	°C	3	4
Dec./Feb.	°C	2	3
Jan./Mar.	°C	1	2
Feb./Apr.	°C	3	4
Mar./May	°C	5	12
Apr./June	°C	7	19
May/July	°C	9	22
MeHg exposure			
MeHg water	[MeHg] in water (ng/L)	10^{-7}	10^{-7}
MeHg food	[MeHg] in food (µg/g)	10^{-7}	10^{-7}
MeHg rate constants			
Epf	proportion absorbed from food	0.80	0.80
Epw	proportion absorbed from water	0.12	0.12
Excrete factor	relates MeHg loss to wastes	0.30	0.30
Bioenergetics			
Cal dens food	caloric density of food (kCal/g)	1.1	1.1
Cal dens fish	caloric density of fish (kCal/g)	1.2	1.2
Act	activity multiplier	1	1
Initial fish conditions			
Wnot	starting weight of fish (g)	1233	754
Cnot	starting [MeHg] (µg/g)	0.061	0.100

Table A.8. Inputs used for the Wisconsin model for both fast and slow elimination scenarios to predict spike mercury loss by northern pike (*Esox lucius*) over 650 days after being transferred from Lake 658 to Lake 240.

Parameter	Units	Slow elimination	Fast elimination
Growth			
day 0 weight	g	1233	754
day 650 weight	g	1935	1423
[MeHg] in prey			
fish	μg/g w.w.	0	0
Proportions of diet			
fish			
day 1		1.00	1.00
day 650		1.00	1.00
MeHg rate constants			
Epf		0.8	0.8
ζ		-0.58	-0.58
Kcl		0.029	0.029
Bioenergetics			
caloric density of prey fish	joules	5201	5201
activity multiplier		1	1
Initial fish conditions			
initial fish [MeHg]	μg/g w.w.	0.061	0.100

Note: Epf = assimilation efficiency of MeHg from food, ζ = allometric exponent for elimination, Kcl = clearance coefficient
water temperatures used were similar to those listed in Table A.9 for the TR model.

Table A.9. Weights and temperatures input into the TR model for both fast and slow elimination scenarios to predict spike mercury loss by northern pike (*Esox lucius*) over 650 days after being transferred from Lake 658 to Lake 240.

	Slow elimination		Fast elimination	
Day	Weight (g)	Temperature (°C)	Weight (g)	Temperature (°C)
1	1233	8	754	24
15	1266	11	780	24
30	1299	10	807	24
45	1329	10	834	23
60	1352	10	856	22
75	1374	9	879	16
90	1385	8	889	9
105	1395	8	900	8
120	1406	8	910	8
135	1416	6	921	6
150	1427	3	931	4
165	1437	2	942	4
180	1448	2	952	3
195	1458	2	963	2
210	1469	2	973	2
225	1479	1	984	2
240	1490	1	994	2
255	1500	3	1005	3
270	1511	4	1015	6
285	1521	6	1026	12
300	1535	6	1036	15
315	1550	7	1051	17
330	1578	8	1077	19
345	1608	10	1102	22
360	1641	13	1128	24
375	1671	15	1158	24
390	1701	14	1185	24
405	1724	12	1207	22
420	1746	11	1230	19
435	1769	9	1252	15
450	1791	8	1275	14
465	1814	7	1297	9
480	1832	6	1315	5
495	1842	4	1326	4
510	1853	2	1336	4
525	1863	2	1347	3
540	1872	1	1356	3
555	1881	1	1365	2
570	1890	1	1374	2
585	1899	2	1383	3
600	1908	2	1392	4
615	1919	3	1402	9
630	1929	4	1413	10
650	1935	5	1423	12

Table A.10. Inputs used for the Wisconsin model simulations of ambient mercury burden in yellow perch (*Perca flavescens*) over 90 days after being removed from Lake 658. The models were run separately for fish held in Lake 240 and for 2 scenarios in Lake 979.

Parameter	Units	Lake 240		Lake 979	
				scenario 1	scenario 2
Growth					
day 0 weight	g	1.1		1.1	1.1
day 90 weight	g	4.4		12.0	12.0
[MeHg] in prey					
Zooplankton					
day 1	ng/g w.w.	3.1	day 1	9.0	9.0
day 21	ng/g w.w.	6.7	day 25	10.6	10.6
day 64	ng/g w.w.	14.1	day 47	16.5	16.5
day 78-89	ng/g w.w.	11.3	day 90	6.1	6.1
Benthos					
day 1	ng/g w.w.	3.1	day 1	31.5	9.0
day 21	ng/g w.w.	6.7	day 25	37.1	10.6
day 64	ng/g w.w.	14.1	day 47	57.8	16.5
day 78-89	ng/g w.w.	11.3	day 90	21.3	16.5
Fish					
day 1-89	ng/g w.w.	-	day 1-89	-	230
Proportions of diet					
Zooplankton					
day 1		0.60		0.60	0.60
day 30		0.35		0.35	0.35
day 60		0.25		0.10	0.1
day 90		0.25		0.00	0
Benthos					
day 1		0.40		0.40	0.40
day 30		0.65		0.65	0.65
day 60		0.75		0.90	0.80
day 90		0.75		1.00	0.90
Larval fish					
day 1		0.00		0.00	0.00
day 60-90		0.00		0.00	0.10
Water temperatures					
day 1	°C	20.4		20.8	20.8
day 15	°C	20.7		20.5	20.5
day 30	°C	24.9		21.9	21.9
day 45	°C	22.3		20.4	20.4
day 60	°C	20.4		18.9	18.9
day 75	°C	18.4		17.4	17.4
day 90	°C	18.3		17.7	17.7
MeHg rate constants					
Epf		0.8		0.8	0.8
ζ		-0.58		-0.58	-0.58
Kcl		0		0	0
Bioenergetics					
calories in zooplankton	joules	2093		2093	2093
calories in benthos	joules	2720		2720	2720
calories in larval fish	joules	-		-	3698
Act		1		1	1
Initial fish conditions					
initial fish [MeHg]	ng/g w.w.	96		96	96

Note: Epf = assimilation efficiency of MeHg from food, ζ = allometric exponent for elimination, Kcl = clearance coefficient

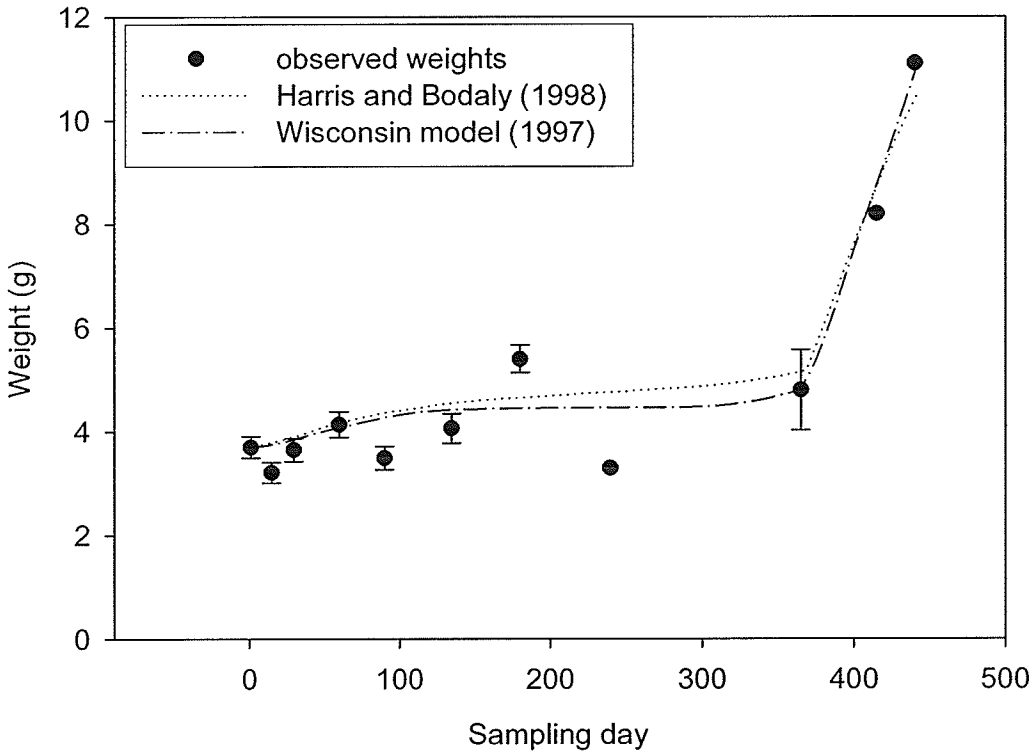

Fig. A.1. Mean (+/- 1 standard error of the mean) weight of yellow perch (*Perca flavescens*) over 440 days after transfer from Lake 658 to Lake 240 (closed circles). Growth curves used in the HB model and the Wisconsin model for predicting mercury concentrations are shown.

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