Accumulation of dietary and waterborne mercury by fish - experimental and whole-ecosystem approaches using enriched stable isotopes

by

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ABSTRACT

To improve our understanding of how environmental mercury (Hg) concentrations influence Hg in fish, I conducted a field experiment to quantify the relative contributions of dietary and aqueous exposure to Hg levels in fish. To further assess the importance of water as a source of Hg to fish, a long-term dataset from the Mercury Experiment to Assess Atmospheric Loading in Canada and the United States was used as input data for two Hg bioaccumulation models: OneFish (accumulation from food and water) and Wisconsin (accumulation from food). Both approaches used enriched stable isotopes of Hg. Yellow perch accumulated 10-21% of their Hg directly from water. Wisconsin model predictions were significantly lower than observed fish Hg concentrations and OneFish predictions. These results suggest that waterborne Hg is an important contributor to Hg in fish and that the exclusion of water in bioaccumulation models may produce underestimates of fish Hg concentrations.

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Chapter 1. Introduction

1.1 Historical problem of mercury in fish

Methylmercury (MeHg) is a toxic form of mercury (Hg) and is by far the most prevalent contaminant in North American freshwater fish (Bloom 1992; Rennie 2003; Hammerschmidt and Fitzgerald 2006; Munthe *et al*. 2007; Bhavsar *et al*. 2010). Consumption of fish is the primary route of MeHg exposure for humans (Mahaffey 1998), and fish consumptions advisories are in place to limit human intake of MeHg as it is a neurotoxin (Mergler *et al*. 2007). MeHg binds to sulfhydryl groups, which disrupts the function of proteins and may lead to impairment of normal cellular processes (Galli and Restani 1993). The toxic effects of MeHg in humans resulting from fish consumption were first recognized in Minamata Bay, Japan in 1956 (Minamata City Planning Division 2000). Symptoms of MeHg poisoning (Minamata disease) include distal sensory disturbances, constriction of visual fields, ataxia, tremor, suppressed immune function, and cardiovascular disease (Mergler *et al*. 2007). Children and infants are particularly sensitive to high MeHg levels, and MeHg passed to fetuses from their mothers can be damaging (Mergler *et al*. 2007). Today, risk of MeHg toxicity in the general population results from chronic, low-dose exposure to dietary MeHg, mainly stemming from the consumption of contaminated fish and fish products (Mahaffey 1998). Populations who consume large quantities of fish or marine mammals are considered to be the most at risk (United Nations Environmental Programme 2009).

Atmospheric deposition of anthropogenically-emitted mercury is considered to be the primary source of MeHg contamination to lakes and their biota (Hammerschmidt and Fitzgerald 2006; Lindberg *et al*. 2007; Bhavsar *et al*. 2010). It is estimated that the global release of mercury from anthropogenic sources is approximately 4000 tonnes/year (Pacyna and Pacyna 2002; Environment Canada 2008), which is higher than the annual global release from natural sources such as volcanoes and other tectonic activity (Mason 2009). The majority of this mercury is released as elemental mercury (Hg^0) into the atmosphere and onto the surrounding terrestrial environment through activities such as metal-smelting (Li *et al*. 2008), sewage incineration, and fossil fuel combustion (Pacyna and Pacyna 2002; Canadian Council of Ministers of the Environment 2003). Hg^0 has a mean atmospheric residence time of 1-1.5 years (Environment Canada 2008), and although some of it settles out of the atmosphere in close proximity to the point of emission, the rest of the Hg^0 remains aloft and can travel hundreds of kilometers in air currents before it is oxidized to inorganic mercury (Hg^{2+}), a highly soluble form of mercury, and is deposited (Jackson 1997; Fitzgerald *et al*. 1998). As a result, pristine lakes and terrestrial ecosystems located far from point-sources of pollution can receive large annual inputs of mercury (Fitzgerald *et al*. 1998).

Inorganic mercury deposited to lakes is transformed to MeHg by methylating bacteria under anaerobic conditions (Rudd 1995; Jackson 1997). The resulting MeHg is highly bioavailable and is not readily excreted, leading to bioaccumulation and biomagnification (Cabana *et al*. 1994; Bowles *et al*. 2001). Organisms at the highest trophic levels such as game fish, which are the mainstay of subsistence, commercial, and recreational fisheries,

typically exhibit the highest concentrations of MeHg in the aquatic food web (Fleming *et al*. 1995). Although MeHg produces toxic effects in fish at high tissue concentrations (McKim *et al*. 1976; Reid and McDonald 1991; Heath 1995; Wiener and Spry 1996; Niyogi and Wood 2003; Webber and Haines 2003), fish are able to withstand high levels of MeHg much more effectively than other animals (i.e., mammals) (Giblin and Massaro 1973; Scheuhammer *et al*. 2007). Sub-lethal impacts of MeHg have been described for some fish species, including reproductive impairment (Friedmann *et al*. 1996; Matta *et al*. 2001; Drevnick and Sandheinrich 2003; Drevnick *et al*. 2006), reductions in startle response (Carvan and Weber 2009), and changes in predator-avoidance behaviours (Webber and Haines 2003), but these impacts are not often evident at the population level.

Fish tissue MeHg levels that are higher than those considered fit for human consumption are a common cause of fish consumption advisories in North America. (Giblin and Massaro 1973). The current Canadian market limit for MeHg in fish flesh sold for human consumption is 0.5 $\mu g \cdot g^{-1}$ (Health Canada 2007), however, sport fish may contain much higher levels (Manitoba Water Stewardship 2007). Consumers are advised not to eat fish muscle containing 0.2 $\mu g \cdot g^{-1}$ MeHg more than 19 times per month, fish containing 0.2-0.5 μg/g MeHg more than 8 times per month, fish containing 0.5-1.0 $\mu g \cdot g^{-1}$ MeHg more than 4 times per month, and fish containing 1.0-1.5 $\mu g \cdot g^{-1}$ MeHg more than 3 times per month. Consumers are advised not to eat fish muscle containing more than 1.5 $\mu g \cdot g^{-1}$ MeHg (Manitoba Water Stewardship 2007). Pregnant women and children under 12 years of age have stricter consumption limits. Currently, MeHg in fish is the cause of

a large proportion of fish consumption advisories in Ontario (Cole *et al*. 2004), and is the primary reason for 80% of the fish consumption advisories issued by 48 states in the United States in 2006 (USEPA 2006).

Past studies of mercury cycling have suggested that to restore MeHg-contaminated fisheries to levels safe for human-consumption, it will be important to reduce global anthropogenic mercury emissions (Jackson 1997; Harris *et al*. 2007; Lindberg *et al*. 2007). A number of strategies to reduce anthropogenic Hg emissions have been implemented over the last two decades (Bhavsar *et al*. 2010). The key problem is to understand how fish will respond following decreased atmospheric inputs of mercury. It is unclear whether it will be years or decades until contaminated fisheries are again useable as food sources. Armstrong and Scott (1979) found decreases in northern pike (*Esox lucius*), walleye (*Sander vitreus*), and lake whitefish (*Coregonus clupeaformis*) tissue MeHg levels following the closure of a nearby chlor-alkali plant, and argued that this was due to a reduction in concentrations of mercury in river water. Similarly, Chalmers *et al*. (2010) report that strict regulatory controls on Hg emissions introduced in the 1970s led to a general decline in fish MeHg concentrations. This research suggests that fish Hg levels are most influenced by 'new' Hg inputs (i.e. from the atmosphere) rather than 'old' Hg (i.e. stored in sediments and watershed), and that fish in contaminated systems may therefore recover relatively quickly following the cessation of atmospheric inputs. Conversely, the mercury-polluted waters in Minamata Bay, Japan, were closed to fishing for 34 years following the outbreak of Minamata disease in 1956

due to persistent high levels of MeHg in fish tissues (Minamata City Planning Division 2000).

Overall it is clear that fish will respond to changes in Hg emissions, but the magnitude and timing of this response is not clear (Kamman and Engstrom 2002; Chalmers *et al*. 2010). If rates of mercury deposition are linked to water mercury levels, which are in turn related to fish MeHg levels, decreased inputs of mercury may lead to decreases in fish tissue MeHg. Studies examining these relationships have shown that fish mercury levels respond quickly to changes in mercury deposition (Harris *et al*. 2007; Orihel *et al*. 2007; Orihel *et al*. 2008), and that fish MeHg levels may be moderately (Hammerschmidt *et al*. 2006) or strongly (Orihel *et al*. 2007) correlated with atmospheric mercury loading rates. Although these studies provide evidence of a link between atmospheric deposition of Hg and MeHg in fish, the specific connections between fish MeHg concentrations and Hg levels in their environment, and the relative importance of respiration and feeding to fish MeHg accumulation remain poorly understood. To determine how fish MeHg levels may change following a reduction in atmospheric inputs, it is important to examine how fish accumulate MeHg from their natural environment. Diet is generally considered to be the main route of mercury uptake by fish, with uptake from solution contributing much less to fish MeHg levels (Hall *et al*. 1997; Wang and Wong 2003). However, past field estimates of fish mercury uptake have not been able to clearly distinguish between waterborne and dietary pathways because of the fast dynamics between these compartments (Hall *et al*. 1997). Quantifying the relative importance of dietary and aqueous pathways to fish MeHg levels under natural conditions is an important step

towards predicting how fish mercury concentrations will respond to decreased atmospheric inputs (Parks 1994), and is the focus of my thesis research.

The studies presented in this thesis aim to quantify the relative importance of the two main mercury exposure pathways – water and food – to levels of MeHg in fish. This is an area that remains poorly studied in the field of mercury research, yet is critical for predicting the response (magnitude and duration) of Hg-contaminated fisheries following changes in atmospheric Hg deposition. It has become commonplace in the scientific literature to assume that Hg levels in water do not contribute to MeHg levels in fish, and is often ignored (MacRury *et al*. 2002; Rennie 2003; Lepak 2009). In my thesis, I quantify the relative importance of mercury uptake from diet and water by yellow perch (*Perca flavescens*), an important forage fish in boreal lake food webs. I use two different approaches to assess Hg exposure and uptake by yellow perch. The first is a short-term experiment designed to separate the sources of Hg to fish to directly quantify the importance of Hg accumulation from water. The second compares two Hg bioaccumulation models – one that includes Hg uptake from water and one that does not - using a long-term aquatic food web Hg data set and evaluates the models for accuracy in predicting MeHg levels in fish. Underlying these two approaches is the use of enriched stable isotopes of Hg, an emerging tool that allows for quantification of newly-deposited Hg in nature (Hintelmann and Evans 1997; Harris *et al*. 2007; Babaev *et al*. 2010; see Section 1.5). The results presented in this thesis are valuable for understanding the processes of Hg accumulation in fish.

This introductory chapter will briefly describe the pathways of mercury into and within lakes, exposure of fish to mercury, mercury uptake dynamics, an introduction to modelling contaminant transfer, seasonal patterns in fish mercury uptake, and a description of the chosen study species, yellow perch. Finally, the approach and objectives of this thesis are presented.

1.2 Movement of Hg in aquatic ecosystems

Mercury accumulation and movement in aquatic ecosystems has been increasingly studied over the past 40 years (Mierle 1990; Rudd 1995; Harris *et al*. 2007; Munthe *et al*. 2007; Orihel *et al*. 2007; Van Walleghem *et al*. 2007). In aquatic environments located far from point-sources, such as coal-fired power plants, chlor-alkali plants, cement manufacturing plants, and landfills, anthropogenically-emitted Hg^{2+} may enter through atmospheric deposition (Mierle 1990; Mason *et al*. 1994; Rudd 1995; Jackson 1997; Pacyna and Pacyna 2002; Canadian Council of Ministers of the Environment 2003; Lindberg *et al*. 2007; Swain *et al*. 2007). Natural sources of mercury also contribute to the global atmospheric mercury load, releasing approximately 1850 tonnes of mercury annually from mercury-laden ore bodies, forest fires, and volcanoes (Jackson 1997; Canadian Council of Ministers of the Environment 2003; Environment Canada 2008; Mason 2009). These natural emissions represent a combination of primary emissions and recycled Hg originally emitted from anthropogenic sources (Mason 2009). Runoff from surrounding uplands and wetlands, which often contributes a mix of natural and anthropogenic Hg (St. Louis *et al*. 1994; Kelly *et al*. 1997; Hall *et al*. 2008; Selvendiran *et al*. 2008), and the flooding of reservoirs (Bodaly *et al*. 1984; Jackson *et al*. 1991; Kelly

et al. 1997; Plourde *et al*. 1997; Paterson *et al*. 1998; Bodaly and Fudge 1999) also increase mercury levels in lakes.

Following deposition, Hg^{2+} cycles between three distinct environmental compartments: water, sediment, and aquatic biota (Watras 1994; Jackson 1997). Initially, some of the Hg^{2+} in the surface waters can be converted back to Hg^0 and lost to the atmosphere through evasion (Poulain *et al*. 2006; Southworth *et al*. 2007). For example, Southworth *et al*. (2007) found that 45% of the Hg^{2+} applied to the epilimnion of a small boreal lake over a four month period was lost to the atmosphere through evasion.

Secondly, some of the deposited Hg^{2+} can adsorb to particles in the water column and sink to the bottom where it is incorporated into sediments (Watras 1994). Luoma (1983) indicates that sediments are the most concentrated physical pool of metals in the aquatic environment, and that they represent both a permanent repository for metals (deep sediments) and a reservoir for metal cycling (surface sediments). Hg^{2+} that enters the sediment pool may remain bound in this state, or may be modified by methylating bacteria and incorporated into the food web as MeHg (Jackson 1997; Lawson and Mason 1998; Orihel *et al*. 2007; Coelho *et al*. 2008). Methylation of Hg^{2+} to MeHg is thought to occur primarily in surface sediments (Winfrey and Rudd 1990; Gilmour *et al*. 1998), but may also occur in the water column (Armstrong and Scott 1979; Xun *et al*. 1987; Eckley and Hintelmann 2006), as well as in the external slime layer of fish and in the fish intestinal tract (Rudd 1995).

Hg^{2+} and MeHg are both taken up by aquatic organisms, either directly from the water (Phillips and Buhler 1978; Boudou and Ribeyre 1985; Hall *et al*. 1997; Klinck *et al*. 2005) or from their food (Wang and Wong 2003; Pickhardt *et al*. 2006). Methylmercury bioaccumulates in the aquatic food web, with top predators (piscivorous fish) showing the highest concentrations (Cabana *et al*. 1994; Bowles *et al*. 2001). Predatory fish may have tissue MeHg concentrations 10^4-10^6 times those of the surrounding waters (Bloom 1992). Correspondingly, the ratio between MeHg and Hg^{2+} in aquatic organisms also increases with trophic level, with MeHg making up approximately 1% of the total mercury present in aquatic plants (Bowles *et al*. 2001), 40-50% of the total mercury in zooplankton (Paterson *et al*. 1998) and over 95% of the total mercury in fish muscle (Bloom 1992; Bowles *et al*. 2001; Rennie 2003). Hg^{2+} is accumulated less-efficiently than MeHg and does not appear to bioaccumulate (Tsui and Wang 2004).

1.3 Methylmercury levels in fish

The mercury concentration of an environmental compartment (e.g. a fish) is the total mass of mercury contained within that compartment divided by the weight of the compartment (Watras 1994). Fish MeHg concentrations increase over time because fish accumulate MeHg faster than it is eliminated from the body (Huckabee *et al*. 1975; de Boer *et al*. 1994). Fish are exposed to many factors that collectively determine their body concentrations of MeHg (McKone *et al*. 1971; de Boer *et al*. 1994; Kennedy 2003). These factors may be based on the physical processes that govern the passage of mercury across lipid membranes (Luoma 1983). They may be biological, including body size, metabolism, condition, and age (McKone *et al*. 1971; de Boer *et al*. 1994; Cizdziel *et al*.

2002), or environmental, including trophic position (Cabana *et al*. 1994; Cizdziel *et al*. 2002; Bowles *et al*. 2001), water and prey concentrations of mercury (Lockhart *et al*. 1972; Bodaly *et al*. 1984; Kelly *et al*. 1997; Kennedy *et al*. 2003; Madenjian and O'Connor 2008), pH (Mason *et al*. 2000), and temperature (Jackson 1997; Foulkes 2000). The transfer of mercury across biological membranes and the factors that influence mercury uptake by fish are described below.

1.3.1 Transfer of mercury across biological membranes

Mercury can be taken up by fish directly from the water through their gills (Phillips and Buhler 1978; Boddington *et al*. 1979; Post *et al*. 1996; Kennedy 2003), and from their food through the wall of their gut (Giblin and Massaro 1973; Hall *et al*. 1997; Wang and Wong 2003; Pickhardt *et al*. 2006). The mucous layer that coats the skin of the fish is also able to bind metals but is not considered a major point of entry for mercury into the fish (McKone *et al*. 1971; Handy and Eddy 1990).

The environmental interface of an organism (e.g., gills, lining of intestinal tract) is a lipid membrane studded with carrier molecules that facilitate that transport of polar substances, such as trace metals, from one side of the membrane to the other (Luoma 1983). These carrier molecules are negatively charged and attract metals that exist in the surrounding environment (Boddington *et al*. 1979; Kennedy 2003). Although the exact transport mechanisms are unknown, it is generally accepted that both MeHg and Hg^{2+} are able to cross branchial and intestinal membranes through a combination of passive and active transport processes (reviewed in Luoma 1983; Andres *et al*. 2002). The

prevalence of sulfhydryl-containing proteins in the blood also enhances mercury uptake as the mercury is attracted to, and forms strong bonds with these molecules (Jackson 1997).

Despite uncertainties regarding the precise mechanisms of mercury uptake in fish, the characteristics of uptake by the intestine and gills are more well-understood. When fish are exposed to mercury in their diet, the amount of mercury transferred from prey to predator depends on several factors. First, the mercury content of a prey item dictates the total amount of mercury available to a consumer (Beamish *et al*. 1974; Watras and Bloom 1992; Harris and Bodaly 1998). It is intuitive that prey species that carry low mercury concentrations provide less mercury to their predators than prey species that contain large amounts of mercury. Secondly, the chemical form of mercury in an organism will affect the amount of mercury that can be derived from it because MeHg is more biologically available than Hg^{2+} (Boudou and Ribeyre 1985). Methylmercury is rapidly absorbed by the digestive tract of many animals, including fish (Sharpe *et al*. 1977; Wang and Wong 2003), mice (Nose 1969), and humans (Aberg 1969). Inorganic mercury, on the other hand, is preferentially accumulated by the gut and then excreted, reducing the transfer of Hg^{2+} to other organs (Boudou and Ribeyre 1985). However, Rudd (1995) indicates that Hg^{2+} can be methylated in the gut to form MeHg, which may enhance uptake from Hg^{2+}-rich prey items.

The digestive efficiency of a prey item also dictates the amount of mercury that can be stripped from it by the gut (Luoma 1983; Reinfelder and Fisher 1994). Soft-bodied prey

species with few undigestible parts will yield more mercury per weight than prey species with body parts that are less easily digested, such as chitinous structures (Reinfelder and Fisher 1994). Any mercury bound in the undigested parts will be excreted in feces, rendering it unavailable for uptake (Beamish *et al*. 1974; Luoma 1983). Luoma (1983) suggests that the most important characteristic influencing dietary mercury uptake may be stomach pH. Highly acidic intestinal fluids strip available metals from the prey items more efficiently than fluids with higher pHs, meaning that more of the total mercury present in the food may be mobilized and absorbed. Assimilation efficiencies (AE_f) may be calculated for predators to represent the mean percentage of available dietary mercury that is taken up from prey items. Assimilation efficiencies have been calculated for many fish species, including yellow perch (AE_f= 80%) (Rodgers 1994), goldfish (*Carassius auratus*) (AE_f = 70-90%) (Sharpe *et al*. 1977), mosquito fish (AE_f = 86-94%) (Pickhardt *et al*. 2006), and rainbow trout (AE_f >73%) (Giblin and Massaro 1973).

Fish take in mercury from solution as water passes across their gills (McKone *et al*. 1971; Boddington *et al*. 1979; Luoma 1983; Klinck *et al*. 2005). Boddington *et al*. (1979) and Rodgers and Beamish (1981) suggest that the mercury exposure level is the most important factor in determining uptake from solution. This exposure depends on i) the concentration and chemical forms of mercury ions in the water (Luoma 1983) and ii) the total amount of water that passes across the gills of the fish (Boddington *et al*. 1979). The ventilation volume is the rate at which water moves across the gills and is measured as volume/time (Boddington *et al*. 1979). Heightened ventilation volumes may be caused by increased respiration rates and swimming velocities, and can enhance the

amounts of contaminants a fish is exposed to. Rodgers and Beamish (1981) also indicate that MeHg uptake is positively correlated with oxygen consumption. As with trophic contamination, mercury assimilation efficiencies can be calculated for gill membranes (AE_w). Rainbow trout have been studied most intensively for this purpose, and researchers have reported AE_w of MeHg from solution of 7% (Boddington *et al*. 1979), 8% (Rodgers and Beamish 1981), 10% (Phillips and Buhler 1978), and 36% (Harris and Bodaly 1998).

In addition to the kinetics of fish mercury uptake, many biological and environmental factors dictate the amount of mercury a fish is exposed to and the amount it accumulates. These factors are discussed below.

1.3.2 Biological factors affecting fish mercury uptake

Fish MeHg concentrations are generally positively correlated with age because of the tendency of MeHg to bioaccumulate (McKone *et al*. 1971; Mathers and Johansen 1985). Accordingly, since fish continue to grow throughout their lives, body concentrations of MeHg are often shown to increase with body size (de Boer *et al*. 1994 ; Cizdziel *et al*. 2002; Ethier *et al*. 2008; Kehrig *et al*. 2008; Simonin *et al*. 2008). These increases may be augmented if the larger body size allows a fish to consume progressively larger prey (e.g. forage fish) that have higher burdens of mercury than smaller prey (Harris and Snodgrass 1993; de Boer *et al*. 1994; Harris and Bodaly 1998). Alternately, the high metabolic rates exhibited by smaller fish can lead to increased rates of digestion and respiration (Post 1990), meaning that more water and food passes through the fish

overall, increasing exposure (de Boer *et al*. 1994). Intuitively, it would be expected that this increased exposure would heighten MeHg concentrations in the fish. However, it appears that in addition to enhancing uptake, the rapid metabolism of smaller fishes also allows them to excrete contaminants more quickly, resulting in low body concentrations of contaminants overall (de Boer *et al*. 1994).

Body size is not always an accurate indicator of tissue mercury levels and has been shown to be inversely correlated with tissue MeHg levels (Greenfield *et al*. 2001; Cizdziel *et al*. 2002). Fast growth initiated by a surplus of resources can reduce the concentration of MeHg within an organism; a phenomenon termed growth dilution (Cizdziel *et al*. 2002; Karimi *et al*. 2007). When a fish experiences rapid tissue growth (e.g. due to an ontogenetic dietary shift to larger prey items) the MeHg already present in its body becomes dispersed throughout a greater amount of tissue, causing the overall MeHg concentration to decrease (Essington and Houser 2003; Simoneau *et al*. 2005). For example, Simoneau *et al*. (2005) found growth rate to be the best predictor of walleye THg concentrations in 12 lakes in Québec, Canada, with the fastest growing fish exhibiting the lowest THg concentrations. In contrast to growth dilution is bioconcentration, which can occur if a fish is undernourished and begins to catabolize its muscle tissue for energy. In this situation, the muscle mass of the fish is reduced more quickly than the MeHg is excreted, leading to an increased tissue concentration of MeHg (Gorski *et al*. 1999; Cizdziel *et al*. 2002).

1.3.3 Environmental factors affecting fish mercury uptake

Methylmercury concentrations increase with trophic level in aquatic food webs (Cabana *et al*. 1994; Becker and Bigham 1995; Bowles *et al*. 2001; Cizdiziel *et al*. 2002; Gantner *et al*. 2009). As discussed above, this biomagnification can lead to differences in mercury concentrations of several orders of magnitude among trophic levels, with top predators exhibiting the highest concentrations overall (Ramade 1987; Cabana *et al*. 1994; Mason *et al*. 2000). Bowles *et al*. (2001) examined bioaccumulation of mercury within the food web. They found that the greatest MeHg concentration increases occurred between the water column (6.7×10^{-8} $\mu g \cdot g^{-1}$) and seston (1.5×10^{-2} $\mu g \cdot g^{-1}$), and that distinct increases (approximately ten-fold) could be seen between subsequent trophic level (planktivorous fish = 0.026 $\mu g \cdot g^{-1}$, piscivorous fish = 0.28-0.46 $\mu g \cdot g^{-1}$). Similarly, the length of the food chain has been shown to be positively correlated with organismal mercury concentrations (Cabana *et al*. 1994; Stemberger and Chen 1998). Cabana *et al*. (1994) found that top predator fish in lakes with food chains that contained both forage fish species and the freshwater crustacean *Mysis relicta* had tissue MeHg concentrations that were 3.6 times higher than those in lakes that did not contain *M. relicta*. Additionally, Cizdziel *et al*. (2002) found that piscivorous fish (striped bass, *Morone saxatilis*) had higher MeHg levels than herbivores (blue tilapia, *Oreochromis aureus*) inhabiting the same system.

Lake productivity has been shown to influence Hg levels in fish. Fish in eutrophic systems consistently exhibit lower MeHg concentrations than fish in systems with lower productivity (Larsson *et al*. 1992; Essington and Houser 2003; Chen and Folt 2005).

Essington and Houser (2003) found that age-1 yellow perch in eutrophic lakes in Wisconsin were 4-5 times larger and had 50% lower THg levels than fish from oligotrophic systems in the same area. Similarly, Chen and Folt (2005) noted strong negative correlations between zooplankton density and fish MeHg levels in a study of 20 lakes across northeastern United States. These studies suggest that the low fish Hg concentrations associated with high productivity result from a combination of growth dilution (Larsson *et al*. 1992; Essington and Houser 2003; Chen and Folt 2005), dietary shifts (Essington and Houser 2003), and increased sedimentation and turnover rates of phytoplankton in the water column (Larsson *et al*. 1992).

Fish are exposed to mercury from their diet and from the water they inhabit (Phillips and Buhler 1978; Post *et al*. 1996). Trophic position, food chain length, lake productivity, and prey mercury content play major roles in determining the amount of mercury a fish receives from its diet, while the uptake of mercury from solution is governed by water mercury concentrations and the chemical and physical properties of the water (McKone *et al*. 1971; Lockhart *et al*. 1972 ; Bodaly *et al*.1984; Francesconi *et al*. 1996; Kennedy 2003; Orihel *et al*. 2006; Choy *et al*. 2008). The uptake of trace metals from solution is determined by exposure level, suggesting that the more concentrated a contaminant is in a system, the higher fish tissue concentrations are likely to be (McKone *et al*. 1971; Luoma 1983; Kennedy 2003). For example, Bodaly *et al*. (1984) found that the impoundment of Southern Indian Lake, MB caused a rapid increase in MeHg production in the lake. These elevated levels of MeHg in water coincided with increases in the tissue MeHg concentrations of the large-bodied fish species in the lake, including northern pike,

walleye, and lake whitefish. The researchers did not see similar increases in fish MeHg levels in nearby lakes that were not flooded, and therefore concluded that the enhanced MeHg concentrations in fish were the result of increased methylation caused by the creation of the reservoir. Kelly *et al*. (1997) noted similar results in their study of a flooded wetland, where MeHg levels increased in water, vegetation, peat, the lower food web, and fish following flooding.

Alkalinity and temperature also appear to influence fish MeHg levels (Luoma 1983; Mason *et al*. 2000). The high degree of lipophilicity created by an acidic environment appears to enhance the ability of MeHg to pass across biological membranes (Luoma 1983; Winfrey and Rudd 1990; Watras and Bloom 1992; Mason *et al*. 2000; Greenfield *et al*. 2001; Ethier *et al*. 2008; Simonin *et al*. 2008). Greenfield *et al*. (2001) compared 24 lake traits with tissue mercury levels of yellow perch in 43 lakes in Wisconsin, USA. They found pH to be the strongest predictor of fish mercury levels, with the highest mercury levels occurring at the lowest pH values ($r^2 = -0.65$). Similarly, Ethier *et al*. (2008) found that pH explained 75% of the variability between mean yellow perch mercury levels in 10 lakes in ON, Canada. The increased membrane fluidity caused by warm temperatures also increases the transfer of trace metals across the lipid bilayer (Jackson 1997; Foulkes 2000). The accumulation of mercury is a first-order process with a Q_{10} value of 2, meaning that the uptake rate doubles with every 10 ºC increase in temperature (Luoma 1983). Boudou and Ribeyre (1985) noted that rainbow trout (*Oncorhynchus mykiss*) assimilated more MeHg in water maintained at 26 ºC than in waters at 18 ºC or 10 ºC.

1.4 Redistribution and elimination of mercury by fish

Mercury accumulated by a fish is absorbed by the bloodstream and distributed to organs and tissues for storage (McKim *et al*. 1976; Schultz and Newman 1997; Leaner and Mason 2004). Giblin and Massaro (1973) used the radioisotope ^{203}MeHg to follow the movement of dietary mercury through the body of rainbow trout for 100 days. Shortly after administration of the ^{203}MeHg-laden food, ^{203}MeHg concentrations were highest in the blood, heart, liver, and spleen. After 100 days, however, 71% of the mercury that had been taken in by the fish was bound in muscle tissue. Redistribution of this nature is thought to occur to protect critical areas such as the nervous system and reproductive organs from the potentially toxic MeHg (Giblin and Massaro 1973; Wiener and Spry 1996). By sequestering the majority of the contaminant in a long-term storage site (muscle), the fish ensures that important body systems are able to continue to operate without compromising function. These redistribution patterns are similar to those seen for branchially-derived mercury (Boudou and Ribeyre 1985).

Methylmercury stored in fish muscle is tightly bound to proteins in the tissue, however, some MeHg can be released and excreted (Huckabee *et al*. 1975; Galli and Restani 1993; Oliveira Ribeiro 1999; Cizdziel *et al*. 2002). This movement of MeHg out of the body is termed elimination, and is a first-order process in which the total body content of MeHg acts as a single homogeneous compartment (Sharpe *et al*. 1977). Elimination occurs in two major stages, the first being a rapid (weeks) elimination of mercury from the viscera, and the second being a slow (years) discharge of stored mercury from the muscle (Lockhart *et al*. 1972; Huckabee *et al*. 1975; Trudel and Rasmussen 1997; Van

Walleghem *et al*. 2007). Fecal matter appears to be the main route of mercury elimination in fish (Giblin and Massaro 1973) although there is evidence that females expel a limited amount of mercury from their bodies in eggs (McKim *et al*. 1976; Birge *et al*. 1979; Hammerschmidt *et al*. 1999).

Studies on the biological half-life of MeHg (time it takes for the burden of MeHg to decrease by half) suggest that the elimination of MeHg by fish is a very slow process (Laarman *et al*. 1976; McKim *et al*. 1976; Bodaly *et al*. 1984; Galli and Restani 1993; Van Walleghem *et al*. 2007; Madenjian and O'Connor 2008). Estimates of the half-life of MeHg in yellow perch range from 30 days (deFreitas *et al*. 1974) to 489 days (Van Walleghem *et al*. 2007). This slow elimination means that the recovery of mercury-contaminated fisheries is likely to be slow as well. Van Walleghem *et al*. (2007) conducted a field study using enriched stable mercury isotopes to examine Hg elimination in yellow perch and emphasized the importance of having accurate elimination rates for estimating fishery recovery times. Similarly, precise understanding of uptake rates and pathways is important in order to project mercury accumulation patterns in fish following decreased atmospheric inputs.

In summary, it is evident that physiological and environmental factors affect the exposure of fish to mercury, and the resulting burden of MeHg in a fish is determined by rates of uptake and elimination (Norstrom *et al*. 1976). Accurate estimates of the relative amounts of mercury fish receive from food and from water are critical to further understand the factors that govern fish MeHg levels (Post *et al*. 1996; Kennedy 2003).

This information will be key to producing accurate estimates of fish MeHg concentrations with proposed reductions in atmospheric emissions.

1.5 Past research on relative importance of Hg exposure pathways to fish

Although it is understood that fish can take Hg in from water as it moves across their gills (Phillips and Buhler 1978; Boudou and Ribeyre 1985; Post *et al*. 1996; Klinck *et al*. 2005) and from ingested material across their intestinal membrane (Leaner and Mason 2002; Wang and Wong 2003; Klinck *et al*. 2005), the relative contributions of these two exposure pathways to fish MeHg concentrations remain unclear. Table 1.1 provides comparisons of research regarding uptake routes completed to date. Hall *et al*. (1997) indicate that finescale dace (*Phoxinus neogaeus*) receive 15% of their mercury from aqueous sources, whereas Rodgers and Qadri (1982) report that waterborne mercury uptake accounts for 38% of tissue MeHg in yellow perch. Pickhardt *et al*. (2006) indicate that both aqueous and dietary exposure routes provide considerable amounts of mercury to fish, whereas Wang and Wong (2003) suggest that food is the main uptake pathway. Boudou and Ribeyre (1983) conducted a 30 day experiment with steelhead trout (*Oncorhynchus mykiss*) and found that the fish took up 8 times more mercury from food than from water. Alternately, Huckabee *et al*. (1975) suggest that mosquito fish assimilate mercury from food and from water in equal proportions. Post *et al*. (1996) studied age-0 yellow perch and proposed that mercury uptake is seasonal, indicating that dietary sources are likely the most important contributors to fish MeHg levels in the summer, and that aqueous uptake is more dominant in the spring and fall when consumption is reduced. Phillips and Buhler (1978) also conclude that fish MeHg comes

both from food and water, indicating that mercury accumulation from food and water is additive. McKim *et al*. (1976) noted rapid uptake of MeHg from solution by brook trout (*Salvelinus fontinalis*), and found that the amount accumulated was directly proportional to water MeHg concentrations. Similar studies have been performed with other aquatic organisms, including mussels (King and Davies 1987), crayfish (Parks *et al*. 1988), and blue crabs (Andres *et al*. 2002). Mussels and blue crabs appear to take mercury in both through their gills and their digestive organs, whereas the majority of crayfish mercury originates in their diet.

It is evident that aquatic organisms take in mercury from water and from their diet, although the contributions made by each compartment have yet to be verified. The variation in results seen among studies (Table 1.1) stems in part from the difficulty in separating the two exposure pathways in field and laboratory experiments. The rapid exchange of mercury that occurs between water and prey organisms (zooplankton) means that experiments designed to expose fish to mercury in only one pathway (food or water) are susceptible to contamination, as seen in Hall *et al*. (1997). To avoid losing mercury from ^{203}Hg-labeled prey, Pentreath (1976) incorporated the contaminant into food pellets that were fed to plaice (*Pleuronectes platessa*) rather than using live prey. This study examined mercury uptake from food only, however, and does not report whether mercury diffused from food into water. Reliable separation of the two uptake routes is integral to experimental manipulations that attempt to examine exposure pathways. Any uncertainty regarding the source of mercury to fish created by

contamination of one compartment by the other would make it difficult to quantify the contributions of each source accurately.

The use of non-labelled mercury in uptake experiments can also give rise to uncertain results due to the difficulty of establishing baseline concentrations of mercury in small fish where lethal sampling is required for analyses. Ambient MeHg concentrations present in fish tissues vary within ecosystems (Kudo and Mortimer 1979; Ethier *et al*. 2008) and it is important that recorded time-zero concentrations be precise in order to accurately track changes in tissue MeHg levels (Hintelmann *et al*. 2002). The use of enriched stable isotopes of mercury is an alternative that allows researchers to accurately follow the movement of mercury through a system and has been employed with success using both the radioisotope ^{203}Hg (Pentreath 1976; Oliveira Ribeiro *et al*. 2000; Wang and Wong 2003; Pickhardt *et al*. 2006) and enriched stable isotopes of mercury (Paterson *et al*. 2006; Van Walleghem *et al*. 2007; Orihel *et al*. 2008). Although the use of radioactive mercury can be useful to track uptake in fish, it is often administered to study organisms in single-pulse doses that are much larger than concentrations seen in natural situations (Pickhardt *et al*. 2006). This short time period and concentrated exposure regime makes it difficult to apply these results to wild populations.

Enriched stable isotopes of mercury are an emerging new tool that provides a non-radioactive alternative to ^{203}Hg that may be tracked accurately through a system. There are seven naturally-occurring stable mercury isotopes (^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, and ^{204}Hg) which exist in fixed ratios in the environment (Figure 1.1a). When a

sample of mercury is enriched with one particular isotope, the ratios change, with the "enriched" isotope becoming dominant (Hintelmann and Evans 1997) (Figure 1.1b). Enrichment is achieved through atomic engineering, including photochemical, electromagnetic, and centrifugal isotope separation methods, and does not occur naturally in the environment (Babaev *et al*. 2010). Stable isotope ratios can be determined in samples from experimental systems that have been subjected to enriched stable isotope additions using Inductively Coupled Plasma Mass Spectrometry (Hintelmann and Evans 1997). To determine the amount of "added" mercury present in the sample, one must calculate the amount of isotope in the sample that exceeds the amount that would occur naturally (based on the fixed ratios). Enriched stable isotopes of mercury are powerful tools for mercury transfer studies because they may be tracked through an ecosystem separately from ambient Hg (Hintelmann and Evans 1997). A series of large-scale mesocosm experiments (Paterson *et al*. 2006; Orihel *et al*. 2007) and a recent whole-ecosystem loading study (Harris *et al*. 2007) at the Experimental Lakes Area (ELA), ON, have used stable isotopes of mercury to examine the uptake and elimination of mercury by fish in nature. These projects are outlined below.

1.6 Mercury research at the Experimental Lakes Area, ON

The Mercury Experiment To Assess Atmospheric Loading In Canada and the United States (METAALICUS) at the Experimental Lakes Area is a whole-ecosystem mercury loading study designed to link changes in atmospheric deposition of mercury to MeHg levels in fish (Harris *et al*. 2007). It is the first whole-lake experiment to use stable isotopes of mercury to track the movement of mercury through an entire terrestrial and

aquatic ecosystem (Harris *et al*. 2007). Researchers applied separate enriched stable isotopes ("spikes") to the lake water of Lake 658 (L658) (^{202}Hg; "lake spike"), the surrounding upland areas (^{200}Hg; "upland spike"), and a wetland that drains into the lake (^{198}Hg; "wetland spike") during the open-water season for 7 y (2001-07) following 2 years of background data collection (Sandilands *et al*. 2005; Sandilands *et al*. 2008). Regular summer sampling of water, zooplankton, and planktivorous and piscivorous fish for mercury has occurred since the commencement of the experiment, creating a rich and complete data set. Results following the initial three years of loading show added isotopic mercury detectable (detection limit = 0.5% of ambient Hg) in the zooplankton community 1 month following the initial application and in fish muscle after only 2 months (Harris *et al*. 2007). This rapid uptake of mercury in fish is suggestive of potential uptake of isotopic mercury from the water because trophic uptake requires the Hg to move through the food web after deposition, whereas uptake from water can occur sooner after deposition.

Two mesocosm experiments completed at the ELA also used isotopic mercury and noted similarly that added mercury was quickly taken up by resident organisms (Paterson *et al*. 2006; Orihel *et al*. 2007). They indicate that mercury deposited to the surface of lakes is rapidly incorporated into the food web, which may be due, in part, to direct uptake of mercury from the water. Additionally, ELA researchers have recorded substantial increases in age-0 yellow perch spike MeHg in L658 over the winter, typically a time of little feeding, suggesting that the water uptake pathway may be important during this time (Dr. P. J. Blanchfield, Freshwater Institute, Winnipeg, MB, unpublished data).

Most recently, a fish-transfer study at the ELA moved yellow perch from Lake 658 to a clean lake to track the depuration of isotopic MeHg that had accumulated in the fish in Lake 658 (Van Walleghem *et al*. 2007). This study provided evidence that fish MeHg elimination rates are much slower than previously thought, and suggested that mercury elimination models be adjusted to reflect this new information. The use of enriched stable isotopes was integral to this study as it allowed researchers to separate the mercury that had been accumulated in Lake 658 from mercury accumulated in the clean lake. These studies illustrate the advantages of using enriched stable isotopes of mercury to examine the movement of mercury in an ecosystem.

1.7 Modelling mercury bioaccumulation

Modelling uptake and elimination of mercury by fish is one approach used to predict MeHg concentrations in fish tissues based on environmental information (Harris and Snodgrass 1993; Korhonen *et al*. 1995; Trudel and Rasmussen 1997; Trudel and Rasmussen 2001; Rennie 2003). Bioenergetics models incorporate parameters such as fish growth per day, consumption, respiration, and water and prey Hg concentrations into equations designed to predict fish MeHg levels (Norstrom *et al*. 1976; Harris and Snodgrass 1993; Rodgers 1994; Rennie 2003). The equations created are often based on laboratory studies with various field validations performed to test for model accuracy (Post *et al*. 1996; Trudel and Rasmussen 1997). Some mercury bioaccumulation models include waterborne mercury as a source of fish MeHg (e.g., Harris and Bodaly 1998),

while one of the most widely-used models assumes that all fish MeHg is derived from their food (Hanson *et al*. 1997).

When coupled with field-based studies, bioaccumulation models are useful tools for predicting contaminant accumulation. These models are easily modified to project multiple scenarios and may be adapted for site-specific characteristics of many ecosystems (Hanson *et al*. 1997). They are particularly useful for identifying linkages and relationships in a system that are the strongest drivers of fish mercury concentrations (as illustrated by MacRury *et al*. 2002). Accumulation models have been used with success to explore mercury transfer and flow in fish in both laboratory and natural systems (e.g., Post *et al*. 1996; Harris and Bodaly 1998; Van Walleghem *et al*. 2007; Wang *et al*. 2010), and to predict consumption rates in fish (Trudel *et al*. 2000). Mercury accumulation models will be relied on heavily to predict fish mercury concentrations under hypothetical exposure regimes based on projected reductions in atmospheric mercury emissions (Knightes *et al*. 2009).

Although the use of models has contributed greatly to the general understanding of mercury dynamics in aquatic ecosystems, it is evident that these tools require refining if they are to be used to accurately estimate future fish MeHg concentrations (Van Walleghem *et al*. 2007; Madenjian and O'Connor 2008). Rennie (2003) emphasizes that the accuracy of a model's output depends on its construction and the strength of the input data. Failing to include water as a source of mercury to fish may lead to underestimates of fish mercury levels. Biases in input data may be created by sampling protocol, for

example by collecting fish samples at only one time during the season, resulting in an under- or overestimate of fish Hg (Rennie 2003). Similarly, Luoma (1983) noted that models that attempt to estimate trace metal concentrations in biota are often based on uncertain assimilation efficiencies derived from lab experiments and limited knowledge of feeding rates and sources of food. These uncertainties can result in unreliable model predictions.

Ecological models are often evaluated for accuracy by comparing the results of test simulations to laboratory- or field-collected data (Jusup *et al*. 2009). These evaluations are generally conducted with data collected over a short period of time and may include some estimated rather than measured parameters (e.g., Post *et al*. 1996; Trudel and Rasmussen 1997). These types of evaluation are not the most rigorous forms of testing possible, however, it is essential than a model undergoes some testing and validation prior to broad-scale use (Post *et al*. 1996; Ryaboshapko *et al*. 2002; Bajer *et al*. 2003; Van Walleghem *et al*. 2007). In an evaluation of the Wisconsin Fish Bioenergetics 3.0 model (Hanson *et al*. 1997) and the Trudel and Rasmussen (1997) mass balance model for accuracy in depicting mercury elimination, Van Walleghem *et al*. (2007) found that both overestimated the rate of loss in age-1 yellow perch. They suggested that both models be refined prior to use as management tools as they do not accurately represent Hg elimination in a natural setting. Given this finding it is possible that mercury models also depict mercury uptake inaccurately.

The METAALICUS project at the ELA provides a unique opportunity to test mercury bioaccumulation models. Unlike many data sets that have been used previously in model validation exercises, the METAALICUS data set is long-term (2 years of background data collection followed by 7 years of experimental manipulation), detailed (monthly collections of water, zooplankton, and fish samples), and uses enriched stable isotopes of mercury which behave like ambient mercury (Harris *et al*. 2007), but may be followed through the ecosystem independently. The combination of these traits yields a stronger data set than any that has been used previously in mercury model validation, presenting an unparalleled opportunity to evaluate two existing fish mercury bioaccumulation models.

1.8 Yellow Perch

The proposed research project will focus Hg accumulation in young-of-year yellow perch. Yellow perch are a small forage fish found in many water bodies across North America (Craig 1987; Scott and Crossman 1998), and are an important commercial and recreational sport fish (Craig 1987). They have been studied extensively due to their wide distribution and the ease with which they can be caught, and have been the subjects of long-term monitoring projects (Laarman *et al*. 1976; Cope *et al*. 1990; Harris *et al*. 2007). Yellow perch feed opportunistically and their consumption is limited only by the size of their gape (Craig 1987). Initially perch feed on small zooplankton, but as they grow they experience ontogenetic diet shifts, consuming larger prey items such as benthic invertebrates and small fish (including young perch if available) (Graeb *et al*. 2006; Urbatzka *et al*. 2008). It has been suggested that these shifts in diet may lead to

changes in mercury uptake as larger prey items often contain higher concentrations of the contaminant (MacCrimmon *et al*. 1983). Yellow perch are commonly used in bioenergetics and contaminant models because of the detailed information available about their ecology (Harris and Snodgrass 1993; Hanson *et al*. 1997; Trudel and Rasmussen 1997; Rennie 2003; Iles and Rasmussen 2005). Examining the results of simulations modeled on yellow perch data may aid in increasing the understanding of general teleost ecology and contaminant transfer (Cope *et al*. 1990). Additionally, as yellow perch commonly exist as prey for larger sport fishes such as northern pike and lake trout (Craig 1987), unraveling the dynamics of perch mercury uptake will enhance our ability to predict mercury concentrations in these and other predatory species at both current and projected mercury deposition rates.

1.9 Experimental approach and objectives

Mercury concentrations in aquatic ecosystems have increased over the past 100 years as a direct result of enhanced anthropogenic Hg emissions. Humans release Hg into the environment largely as a product of power generation, incineration of wastes, and metal smelting. As Hg tends to bioaccumulate and biomagnify, top predator fish species typically exhibit the highest Hg concentrations the food web. MeHg levels in these fish can be so high that their muscle is considered hazardous for human-consumption because of its ability to cause neurological and cardiovascular dysfunction. High MeHg levels in fish have led to the implementation of fish consumption advisories in nearly all states in the United States, and are the main cause of fish consumption advisories in Canada. Canada, the United States, and Mexico have made it a priority to reduce Hg emissions

with the establishment of the North American Implementation Task Force on Mercury (2000). The establishment of this program, and others that aim to reduce global Hg emissions, should lead to reductions in Hg levels in the aquatic environment. Unfortunately, the linkages between environmental Hg levels and MeHg concentrations in fish are poorly understood.

Environmental sources of mercury to fish and the relative contributions of each source to final fish MeHg concentrations are key components of models that aim to predict mercury bioaccumulation. As models are used as ecosystem management tools (e.g., Delta Tributaries Mercury Council 2000; Chipps 2009) and will be integral to planning and implementing emission-reduction strategies, it is important that they represent Hg dynamics in nature. Past studies of the relative contributions of diet and water to MeHg levels in fish tissues have yielded conflicting results. The problems associated with these studies include contamination of "clean" compartments, the use of ambient mercury, and lab settings that do not reflect natural conditions (Mann 1978). A reliable estimate of fish mercury uptake in nature is needed to improve the structure of bioaccumulation models and thus to increase the predictability of fish MeHg in natural populations.

The data set provided by METAALICUS presents a rare opportunity to quantify mercury uptake by fish in a field setting. The use of enriched stable isotopes of mercury has allowed researchers to track the movement of newly-deposited mercury in an aquatic ecosystem. Findings from this study (Engstrom *et al*. 2007; Harris *et al*. 2007) and a series of mesocosm studies (Paterson *et al*. 2006; Orihel *et al*. 2007) show that fish

respond rapidly to mercury additions, which suggests that uptake of mercury from the water column may contribute more to MeHg loads in fish than previously thought.

The objectives of this thesis are first, to perform a controlled field experiment to quantify the relative proportions of yellow perch MeHg derived from dietary and aqueous sources. Second, to model yellow perch mercury exposure in Lake 658 at the ELA using historical data, and, in doing so, to evaluate the accuracy of two freshwater mercury bioaccumulation models. Overall, I will combine a field experiment with the examination of long-term food web, mercury, and limnological data sets to quantify relative contributions of dietary and water mercury exposure to fish MeHg levels in a natural situation. In doing so, I hope to provide information to increase the accuracy of uptake and elimination models for mercury cycling in aquatic food webs, which will enhance our ability to predict mercury concentrations in fish tissues following decreases in mercury pollution to ecosystems and under other projected environmental scenarios.

Table 1.1 Summary of previous research on mercury uptake from water by fish.

Source	Species	Type of study	Hg type	Duration (days)	Total uptake from water
Rodgers and Qadri 1982	yellow perch (*Perca flavescens)*	field	Hg^{2+} MeHg	90	38%
Post *et al.* 1996	yellow perch (YOY)	literature search field study	THg	n/a	varies seasonally
Hall *et al.* 1997	finescale dace (*Phoxinus neogaeus*)	field	MeHg	32	15%
Lock 1975	rainbow trout (*Oncorhynchus mykiss)*	laboratory	MeHg	7 to 84	10-20%
Phillips and Buhler 1978	rainbow trout	laboratory	Hg^{2+} MeHg	24	10% of Hg that passes across gills is assimilated
Boudou and Ribeyre 1983	steelhead trout (*Oncorhynchus mykiss*)	laboratory	Hg^{2+} MeHg	30	12.5%
Trudel and Rasmussen 2006	many	literature search modelling	MeHg	n/a	<0.1%
Pickhardt *et al.* 2006	mosquitofish (*Gambusia affinis)* redear sunfish (*Lepomis microlophus*)	laboratory	$^{203}Hg^{2+}$ ^{203}MeHg	4 h (water) 2 h (food)	12-27% ($^{203}Hg^{2+}$) 0% (^{203}MeHg)
Wang and Wong 2003	sweetlips (*Plectorhinchus gibbosus)*	laboratory	$^{203}Hg^{2+}$ ^{203}MeHg	1.5	negligible
Huckabee *et al.* 1975	mosquitofish	laboratory	$^{203}Hg^{2+}$	7 (water) 10 (food)	50%
Parks *et al.* 1988	Crayfish (*Orconectes* spp.)	field	THg	68	negligible
Andres *et al.* 2002	blue crab (*Callinectes sapidus*)	laboratory	Hg^{2+} MeHg	1	50%
King and Davies 1987	Mussels (*Mytilus edulis*)	laboratory	Hg^{2+}	21	33%

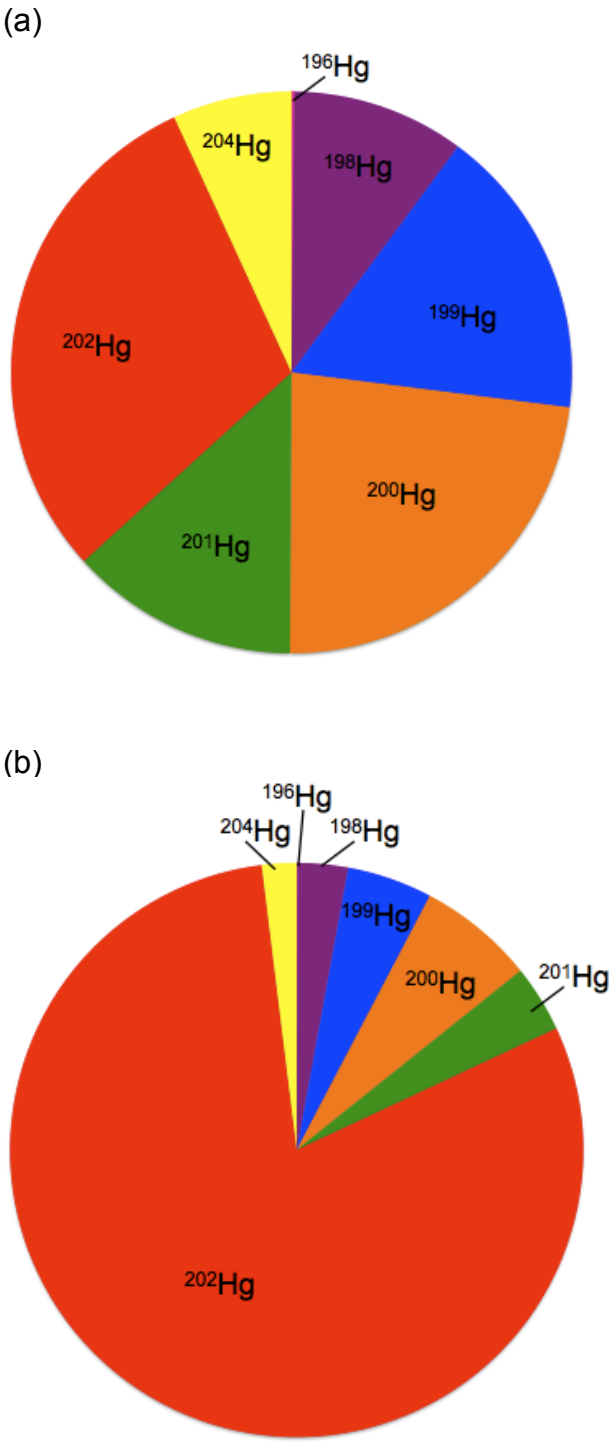

Figure 1.1 Mercury has seven naturally-occurring stable isotopes which exist in fixed ratios in nature (a) (Firestone 2000). Mercury in this form is termed "ambient" mercury. When a sample of Hg is enriched in one of the stable isotopes, the ratios shift to be dominated by that isotope. Enrichment does not occur naturally, but is achieved manually through atomic engineering methods, including photochemical and electromagnetic separation, and centrifugation (Babaev *et al*. 2010). The sample in (b) has been enriched in the stable isotope ^{202}Hg. Enriched stable isotopes applied to a natural system can be quantified relative to background (ambient) Hg and may therefore be tracked separately.

Chapter 2. Quantifying the uptake of dietary and waterborne mercury by yellow perch: a field experiment

2.1 INTRODUCTION

Methylmercury (MeHg) is a neurotoxin for many animals (Scheuhammer *et al*. 2007), including humans (Mergler *et al*. 2007), and is a common cause of fish consumption advisories in North America (United States Environmental Protection Agency 2010). Organisms at the highest trophic levels such as game fish, which are the mainstay of subsistence, commercial, and recreational fisheries, typically exhibit the highest concentrations of MeHg (Fleming *et al*. 1995).

The main routes of MeHg exposure to fish are respiration and consumption. Fish accumulate Hg from water as it moves across their gills (McKim *et al*. 1976; Phillips and Buhler 1978; Boudou and Ribeyre 1985; Post *et al*. 1996; Klinck *et al*. 2005) and from ingested material across their intestinal membrane (Leaner and Mason 2002; Wang and Wong 2003; Klinck *et al*. 2005). Although the exact transport mechanisms are unknown, MeHg and Hg^{2+} appear to be able to cross branchial and intestinal membranes through a combination of passive and active transport mechanisms (reviewed in Luoma 1983; Andres *et al*. 2002). The amount of MeHg taken in by a fish is largely dictated by concentrations of MeHg in prey (Watras and Bloom 1992; Harris and Bodaly 1998) and lake water to which it is exposed (Luoma 1983; Wang *et al*. 2010). Other factors such as

prey type (Reinfelder and Fisher 1994), respiration rate (Boddington *et al*. 1979), and lake temperature and pH (Jackson 1997) also influence fish Hg uptake.

It has been suggested that reducing global anthropogenic mercury emissions will lead to reductions in fish MeHg concentrations to levels that are safe for human consumption (Jackson 1997; Harris *et al*. 2007; Lindberg *et al*. 2007). Understanding how fish accumulate MeHg in their natural environment is an important component of predicting how fish will respond to reduced atmospheric inputs. Specifically, the extent to which fish accumulate Hg from water versus food is a key question to resolve in order to better understand and predict fish Hg concentrations.

It is generally accepted that food is the dominant route of MeHg exposure to fish, with the acknowledgement that fish mercury levels represent the sum of water and food Hg uptake pathways (Phillips and Buhler 1978). However, the relative contributions of waterborne and dietary Hg to fish MeHg levels remain unclear. Many laboratory and field-based studies that report fish Hg concentrations disregard water as a source of Hg to fish, simply stating that all Hg uptake can be attributed to diet (e.g., Gorski *et al*. 1999; Bowles *et al*. 2001; Crump and Trudeau 2009; Dittman and Driscoll 2009). To date, there have been only two field studies that have attempted to determine aqueous Hg uptake by fish under natural conditions. Rodgers and Qadri (1982) studied yellow perch and Hall *et al*. (1997) studied finescale dace (*Phoxinus neogaeus*). Although both studies concluded that fish accumulate Hg from water, the proportions of uptake from water differed, with Hall *et al*. (1997) suggesting 15%, and Rodgers and Qadri (1982)

estimating 38%. Laboratory studies that have attempted to answer this question have found similar estimates of waterborne Hg uptake, ranging from negligible (Wang and Wong 2003) to 12.5% (Boudou and Ribeyre 1983) to 50% (Huckabee *et al*. 1975) (Table 1.1). Wang *et al*. (2010) show that fish accumulate Hg from the dissolved phase, but indicate that food is the dominant source. The variation in experimental results stems from two key design issues encountered by these studies: contamination of clean food or water by the Hg-spiked food or water, and estimated baseline ambient Hg concentrations that are not representative of Hg levels in all individual fish in a population.

Previous Hg accumulation studies have had difficulty preventing Hg transfer between live prey and water in field and laboratory experiments (e.g., Hall *et al*. 1997). Zooplankton (e.g., *Daphnia* spp.) exchange Hg with water very rapidly (Hirota *et al*. 1983; Tsui and Wang 2004). Tsui and Wang (2004) and Hirota *et al*. (1983) both suggest that zooplankton can accumulate and eliminate Hg in less than 24 hours. In fact, Tsui and Wang (2004) noted a 4-fold increase in *Daphnia magna* Hg^{2+} and MeHg levels after only 8 hours of exposure to Hg-spiked water. Although the use of live prey provides experimental conditions that are similar to those encountered by the fish in nature, it can lead to contamination, making it difficult to expose the fish to Hg from only one source and subsequently discern the relative amounts of Hg accumulated from the water and from food by the fish. Hall *et al*. (1997) encountered this problem in their study of Hg uptake by finescale dace (*Phoxinus neogaeus*). This study exposed fish to one of four treatments: low MeHg water + low MeHg food; low MeHg water + high MeHg food; high MeHg water + low MeHg food; high MeHg water + high MeHg food. After the

initiation of the study, MeHg concentrations in the water increased in mesocosms with high MeHg zooplankton due to direct release of MeHg to the water from the zooplankton (Hall *et al*. 1997). Golding (2009) encountered similar contamination between cadmium-spiked water and live periphyton in a study designed to examine the uptake of cadmium from food and water by *Hyalella azteca*. Cross-contamination has limited the amount of data available on the relative importance of sources of MeHg to fish in nature.

The use of ambient Hg can also cause problems in bioaccumulation studies because it is indistinguishable from background Hg accumulated by a fish prior to the initiation of an experiment. In studies involving small fish (e.g. yellow perch), lethal sampling is often required to provide the amount of tissue necessary for Hg analyses (Dr. H. Hintelmann, Department of Chemistry, Trent University, Peterborough, ON). To establish baseline (t_0) Hg concentrations in a population, it is common practice to determine the mean ambient Hg level in a group of fish at the start of the experiment, and to use this value as the t_0 concentration for all fish instead of determining individual t_0 Hg concentrations (e.g., Phillips and Buhler 1978; Boudou and Ribeyre 1983; Hall *et al*. 1997; Simon and Boudou 2001; Leaner and Mason 2004; Klinck *et al*. 2005). However, as fish Hg concentrations vary considerably in nature, even among fish of similar sizes within the same cohort of a population (Boudou and Ribeyre 1985; Hall *et al*. 1997; Simon and Boudou 2001; Rennie *et al*. 2005), it is likely that estimated t_0 concentrations will not be representative of Hg levels of all fish. In order to determine accurately the amount of Hg that has been accumulated by a fish during an experiment, it is essential to quantify

individual t_0 Hg concentrations, or to provide experimental fish with a form of Hg that may be distinguished from background ambient Hg.

The use of enriched stable isotopes of Hg is an emerging tool that has just recently been applied to ecological questions and is providing new insights into the fate of newly deposited Hg to aquatic ecosystems (Paterson *et al*. 2006; Orihel *et al*. 2007; Van Walleghem *et al*. 2007; Orihel *et al*. 2008). Hg in the environment exists in fixed ratios of seven naturally-occurring stable isotopes (Figure 1.1a). Enrichment, which is achieved through atomic engineering processes and does not occur naturally, results in Hg dominated by a single stable isotope (e.g. ^{202}Hg; Figure 1.1b). Enriched stable isotopes of Hg appear to behave like ambient Hg (Hintelmann and Evans 1997; Southworth *et al*. 2007), but can be distinguished analytically from background Hg already present in the system (Hintelmann and Evans 1997). The ability to measure enriched stable isotopes of Hg separately from ambient Hg provides an opportunity to overcome some of the challenges faced by previous researchers when attempting to isolate mercury accumulation pathways such as having well-defined t_0 Hg concentrations (zero enriched stable isotope).

Mercury bioaccumulation modelling has been used to determine pathways of Hg exposure to fish as an alternate approach to accumulation experiments. Bioaccumulation models attempt to mimic natural chemical and ecological relationships to estimate the uptake of a contaminant over time. Fish mercury accumulation models have been used by researchers and ecosystem managers to provide quantitative predictions of fish Hg

levels based on environmental input data (Norstrom *et al*. 1976; Hewett and Johnson 1992; Hanson *et al*. 1997; Harris and Bodaly 1998; Trudel and Rasmussen 2006). Previous research that has used bioenergetics modelling to predict Hg accumulation in fish has, like field and laboratory studies, yielded variable results. Model predictions of Hg uptake from water range from <0.1% (Trudel and Rasmussen 2006) to 10% (Harris and Bodaly 1998). The main difference between various Hg accumulation models lies in the treatment of waterborne Hg uptake. Some models include water as a source of Hg to fish (e.g., Norstrom *et al*. 1976; Rodgers 1994; Harris and Bodaly 1998; Knightes *et al*. 2009), while others do not (Hanson *et al*. 1997; Trudel and Rasmussen 2001). It is possible that the exclusion of water as a source of Hg to fish in accumulation models may yield underestimates of fish Hg concentrations. Predicting levels of Hg in fish following decreases in atmospheric emissions will likely rely heavily on published bioaccumulation models; as such, it is important that they provide an accurate representation of fish Hg uptake in nature.

I conducted a field experiment to estimate the relative contributions of dietary and waterborne mercury to young-of-year (YOY) yellow perch MeHg levels in a semi-natural setting (Figure 2.1). I housed yellow perch in large tanks situated onshore between a pristine boreal lake ("clean") and an experimental lake (Lake 658) that had received additions of Hg enriched with the stable isotope ^{202}Hg ("spike Hg") (Figures 2.2, 2.3). I exposed fish to one of four treatments: clean water + clean food; clean water + spike Hg food; spike Hg water + clean food; spike Hg water + spike Hg food. This two-factorial experimental design was employed by Hall *et al*. (1997) to study uptake of Hg from food

and water by finescale dace, Niyogi *et al*. (2007) to examine uptake of zinc from food and water by adult yellow perch and Kraemer *et al*. (2006) to examine cadmium uptake in age-1 yellow perch. I fed the fish with a pelletized food made from zooplankton collected from clean and spike lakes and filled the tanks with water from Lake 658 and a nearby clean lake (Lake 660). The use of natural lake water and zooplankton allowed me to expose fish to spike Hg that was naturally accumulated and methylated for both the water and food treatments. Together these experimental conditions provided a semi-natural environment in which to raise YOY yellow perch and to monitor their Hg uptake.

The design of this experiment allowed me to overcome many of the challenges faced by previous laboratory and field attempts to study the relative contributions of water and diet as sources of MeHg to fish. Specifically, the study design allowed for (i) reliable separation of the two Hg exposure pathways by using pellet food that did not exchange spike Hg with the water, (ii) time zero yellow perch spike MeHg concentrations of zero, made possible through the use of spike Hg in experimental food and water, and (iii) environmentally-relevant (i.e. low) concentrations of spike MeHg in fish food and water.

In addition to quantifying Hg uptake by experimental fish, I used two bioenergetics-based Hg bioaccumulation models to predict fish Hg uptake in the experiment. The Wisconsin Fish Bioenergetics v.3.0 model (Hanson *et al*. 1997) assumes that all fish Hg uptake comes from food, while the OneFish model (Harris and Bodaly 1998) accounts for Hg uptake from both food and water. By entering environmental information collected during my experiment into the models, I generated predictions of spike Hg concentrations

in fish for each treatment, which I then compared to measured fish spike Hg levels. This exercise allowed me to evaluate the ability of the models to accurately predict fish Hg accumulation and to compare the predictive abilities of the two models to determine whether it is important for accumulation models to include water as a source of Hg to fish.

The two objectives of this study were (i) to quantify accumulation of Hg through aqueous and dietary pathways by yellow perch under summer conditions in a semi-natural setting and (ii) to compare the abilities of two bioenergetics-based mercury accumulation models, one that assumes all fish Hg uptake is from food (Wisconsin model) and one that accounts for uptake from both food and water (OneFish model), to predict the uptake of Hg by YOY perch. I hypothesized that fish would accumulate Hg from both their food and water, that uptake from the two compartments would be additive, and that the OneFish model (uptake of Hg from water and food) would predict fish Hg concentrations more accurately than the Wisconsin model (uptake of Hg from food only).

2.2 METHODS

2.2.1 Study location

This experiment took place at Lakes 658 and 660 (49° 39′ 14″ N, 93° 43′ 18″ W) at the Experimental Lakes Area (ELA) in northwestern Ontario, Canada (Figure 2.2). Lake 658 is the focus lake for the Mercury Experiment to Assess Atmospheric Loading in Canada and the United States (METAALICUS). Lake 658 is an 8.4 ha, circumneutral, headwater

lake located in the Precambrian Shield. The lake drains a 96.2 ha upland forest and a 3.8 ha wetland. Lake 658 contains six main fish species: northern pike (*Esox lucius*), yellow perch, lake whitefish (*Coregonus clupeaformis*), white sucker (*Catostomus commersoni*), blacknose shiner (*Notropis heterolepis*), and fathead minnow (*Pimphales promelas*). From 2001 to 2007, inorganic Hg enriched in the stable isotope ^{202}Hg (to 90.8%; as $HgCl_2$ diluted with 5% nitric acid) was mixed into the surface waters of Lake 658 by boat every two weeks during the open water season (Sandilands *et al*. 2005; Sandilands *et al*. 2008). Isotopic Hg was added to achieve an annual loading of 22 $\mu g \cdot m^{-2} \cdot yr^{-1}$, which is approximately 6-fold higher than local ambient (background) Hg deposition (Harris *et al*. 2007).

2.2.2 Study species

Yellow perch are a small forage fish found in many water bodies across North America (Craig 1987; Scott and Crossman 1998), and are an important commercial and recreational sport fish (Mills and Forney 1981; Craig 1987; Power and van den Heuvel 1999; Essington and Houser 2003). Adult yellow perch spawn in the littoral zones of lakes when water temperatures are 8-12 ºC, typically from April to June (Guma'a 1978; Kayes and Calbert 1979; Ciereszko *et al*. 1997). Yellow perch larvae hatch from egg masses in the littoral zone 10-20 d after spawning (Scott and Crossman 1998) and remain in the littoral zone for 1-2 weeks until the yolk sac has been fully absorbed (Post and McQueen 1988). Larvae migrate to the pelagic zone where they remain as plankton until they have fully metamorphosed and have attained fork lengths of 20-30 mm (4-8 weeks) (Lin 1975; Guma'a 1978; Mills and Forney 1981; Post and McQueen 1988). Following

metamorphosis YOY yellow perch migrate back to the littoral zone where they live in schools for the remainder of the summer season (Coles 1981). When the lake mixes in the fall, YOY perch move to deeper, offshore areas to over-winter (Guma'a 1978; Coles 1981; Post and McQueen 1988). Movements of larval perch in Lake 658 in the early summer are unknown, but YOY yellow perch appear in the littoral zone of Lake 658 in July or August at fork lengths of approximately 30-40 mm and remain there in schools until late fall (personal observation).

Perch are generalist, gape-limited predators that experience ontogenetic dietary shifts (Keast 1977; Graeb *et al*. 2006). The diet of YOY yellow perch consists mainly of zooplankton including copepods (Schael *et al*. 1991) and cladocerans (Mills and Forney 1981). As perch grow, they may ingest larger prey items such as chironomid larvae and pupae and *Chaoborus* spp (Mills and Forney 1981). The diet composition of YOY yellow perch in Lake 658 is consistent with the observations made for other YOY perch populations, with zooplankton constituting over 80% of their diet (see Section 3.2..2).

Yellow perch have been used in many contaminant transfer experiments and bioaccumulation modelling exercises (e.g., Rogers and Qadri 1982; Post *et al*. 1996; Harris and Bodaly 1998; Van Walleghem *et al*. 2007). Yellow perch are ideally suited to these studies because they are abundant and are widely dispersed in many North American water bodies, their biology is well-understood, they are an important commercial and game fish, and they are consumed by many larger sport fish species (Power and van den Heuvel 1999; Essington and Houser 2003). Young-of-year (YOY)

fish were selected for this study instead of an older age-class because they exhibit low ambient Hg levels, having had only 2-3 mo to accumulate Hg (Dr. P.J. Blanchfield, Freshwater Institute, Winnipeg, MB, unpublished data). As spike Hg is detected as a percent of ambient Hg and cannot be detected below a certain percentage (0.5%), low t_0 ambient Hg concentrations allow spike Hg accumulated from food and water to be detected in the fish sooner than it would be in fish that had higher t_0 ambient Hg levels (e.g. older yellow perch). This early-detection allowed for a short (1 mo) experimental period in this study. Additionally, low t_0 ambient Hg concentrations made it possible to use food and water with low spike Hg concentrations, which are representative of a natural situation, rather than having to use unrealistically high spike Hg concentrations. The use of high doses of Hg is common in accumulation experiments (e.g., Phillips and Buhler 1978; Simon and Boudou 2001), but their results may not be applicable to fish in the wild.

YOY yellow perch were also selected because they exist in high numbers in Lake 240 and are one of the focus age-classes for the METAALICUS project in Lake 658. Additionally, YOY perch in the same cohort maintain similar weights for the first few months of life (Mills and Forney 1981), which lead to low variability in baseline (t_0) weights among fish. The small size of YOY fish was advantageous as it allowed me to stock fish at a higher density than would be possible with larger (older) fish.

2.2.3 Experimental design

YOY yellow perch were collected from a clean lake (Lake 240) and held in 160 L (1.08 m long × 0.42 m high × 0.44 m wide with rounded corners) insulated fiberglass tanks (Figures 2.2, 2.3). These fish had no previous isotope exposure, giving them t_0 spike Hg concentrations of zero. Fish were exposed to one of four treatments designed to separate the sources of spike Hg: clean water + clean food (CWCF); clean water + spike Hg food (CWSF); spike Hg water + clean food (SWCF); spike Hg water + spike Hg food (SWSF) (Figure 2.1). Each treatment was replicated three times for a total of 12 tanks. Tanks were arranged onshore between Lake 658 and Lake 660 (Figure 2.2) and were randomly assigned one treatment (Figures 2.1, 2.3). Each tank was covered completely with a plywood lid that had a 40 cm × 40 cm window at one end. The windows were covered with 1 cm × 1 cm plastic mesh to allow air and light into the tanks, but to prevent predation. Spike Hg water was drawn from Lake 658 and clean water from Lake 660 (Figure 2.3). All water was filtered through 160 μm mesh and held overnight in 500 L reservoir tanks prior to being used in fish tanks. Although Lake 658 did not receive spike additions in 2008, spike Hg was present in the water column for the duration of this study (Dr. H. Hintelmann, Department of Chemistry, Trent University, Peterborough, ON, Canada, unpublished data). I created clean and spike fish food pellets by incorporating freeze-dried zooplankton from Lake 658 (spike) and Lake 240 (clean). The food preparation method is described in detail below.

2.2.4 Preparation of fish food

To avoid contamination of clean compartments that might result from the exchange of spike Hg between water and live zooplankton prey, I created a pelletized fish food using freeze-dried zooplankton according to methods modified from Tomy *et al*. (2004). The resulting fish food combined the benefits of live prey (which contain naturally-methylated, naturally-accumulated spike Hg), with the benefits of using a pelletized food (easy to transport, no need to collect zooplankton each day, easy to measure). Most importantly, pelletized food allowed for consistent dietary spike Hg exposure to fish throughout the study, while preventing loss of spike Hg to water.

I collected zooplankton from Lakes 240 and 658 by towing a 160 μm mesh, 0.5 m diameter round plankton net (Limno Tech Enterprises, Winnipeg, MB) horizontally through the water behind a boat. I filtered as much water as possible from the collected samples through 160 μm mesh, transferred the zooplankton to 4 oz WhirlPak® bags (Nasco, Fort Atkinson, WI, USA), and froze the samples (-20 °C). I freeze-dried the zooplankton in a Lyph-lock 12-L freeze dry system (Model 77545, Labconco, Kansas City, MO) until a constant weight was achieved (approximately 96 h), keeping clean and spike bags separate. Freeze-dried zooplankton samples were stored double-bagged in Ziploc bags (SC Johnson & Son, Inc., Brantford, ON, Canada).

Identical protocols were followed for making both clean and spike food pellets. All tools were acid cleaned for 24 h (if Teflon) or washed with hot soapy water and rinsed with 95% ethanol before use. To make the pellet mixture, I mixed 250 g of Silver Cup fish

food base (Sterling Silver Cup Fish Food; Nelson and Sons, Murray, UT) and 22 g of freeze-dried zooplankton in a food processor (KitchenAid, Canada) on medium speed for 2 min. I heated 65 mL of milli-Q water on a hot plate until bubbles formed, then removed it from the hot plate and added 6.5 g of gelatin (Knox, Kraft Foods Global Inc., Northfield, IL, USA). When the gelatin had dissolved I poured it gradually into the zooplankton mixture. With the food processor on medium speed, I added enough additional milliQ water to make a slightly runny dough. When this mixture appeared homogeneous, I stopped the food processor and let the dough stand for 1 h covered with KimWipes (Kimberly Clark Professional, Roswell, GA, USA). Once the dough had set, I extruded it in lines onto parchment paper cleaned with 95% ethanol with a 60 cc acid-rinsed Monoject syringe (Covidien, Mansfield, MA, USA). Separate syringes were used for clean and spike food types. I covered the extruded dough lightly with KimWipes and air-dried it for 24 h. Once dry, I broke the food into small pieces and store it double-bagged in Ziplocs sealed in acid-washed 1 L mason jars.

2.2.5 Transfer of fish and maintenance of tanks

Tanks at Lake 658 were filled with water on August 10, 2008 in preparation for receiving fish the following day. YOY yellow perch were collected from Lake 240 on August 11, 2008 using a beach seine net (16.8 m × 2.4 m). Ten of these fish were euthanized immediately in an overdose bath of 0.25 $g \cdot L^{-1}$ tricaine methanesulfonate (TMS; Argent Chemical Laboratories, Inc., Redmond, WA, USA) to determine baseline (t_0) fish MeHg concentrations. Fish were left in the bath for 10 min following cessation of opercular movements. Live fish were placed into 12 L polyethylene bags (Allied Pioneer

Industries, Ltd., Winnipeg, MB, Canada) filled ⅓ full of Lake 240 water at a density of 26 fish per bag (total = 182 fish). The remaining ⅔ of each bag was filled with oxygen. Sealed bags were placed on ice in coolers to maintain water temperatures and were transported to Lake 658. Upon arrival at the lake, bags were placed in the tanks for 45 min to allow the fish to acclimate to the tank water temperatures. Following acclimation, fish were stocked at a density of 15 fish per tank.

The experiment ran for a total of 27 d. The same series of activities was carried out daily for the duration of the experiment. Prior to departing, I crushed the food into pellets of appropriate size for consumption by YOY perch (1-3 mm long) and weighed the pellets for each tank into a dedicated food vial. The amount of food placed in each vial was based on the number of fish alive in each tank the previous sampling day. Vials were filled with a dry weight amount of food equivalent to 5% of the total wet weight of the fish in each tank. The equivalent wet weight of food would be approximately 15% of fish weight, which is essentially an *ad libitum* ration for YOY yellow perch (Craig 1987; Rinchard *et al* 2008). This feeding regime was designed to satiate fish and promote growth, and has been used in previous pellet food-based studies with yellow perch (Rinchard *et al*. 2008) and fathead minnows (Hammerschmidt *et al*. 2002; Klaper *et al*. 2006; Sandheinrich and Miller 2006). To reduce stress, fish were not weighed during the experiment to determine the ration, but were assumed to have an average mass of 0.8 g.

Upon arrival at Lake 658, maximum, minimum, and current temperatures were recorded for each tank from max-min thermometers (Sper Scientific, Scottsdale, AZ) suspended

mid-way in the tanks (reset each day following reading). Carcasses of fish that had died during the preceding night were removed during these temperature checks. Pellet food was added to each tank gradually over a 2 h period. Following feeding, uneaten food pellets and feces were siphoned out of the tanks and discarded (as in Sandheinrich and Miller 2006). All tanks were drained to half and then re-filled with water from the reservoir tanks. Drainage water from all tanks was directed into Lake 658 through a system of hoses to prevent any spike Hg from entering Lake 660. Due to low initial consumption levels, beginning on day 7 (17 August) fish were fed a second time for 1 h after the tanks had been filled. Any remaining food was siphoned from the tanks at the end of the hour. At the end of each day, reservoir tanks (clean and spike) were filled with water pumped from the two lakes. Separate pump hoses were used for each water type, but the same pump was used for both lakes. To prevent cross-contamination, water was pumped from Lake 660 first. Following exposure to Lake 658 water, the pump was rinsed for 5 min with clean water from Lake 660 to flush out any residual spike Hg.

At the end of the 4 week period, fish were euthanized in the field with an overdose of TMS (0.25 $g \cdot L^{-1}$) and placed in WhirlPak® bags according to tank number. All bags were immediately placed on ice in a cooler and brought back to the ELA field station. After returning from Lake 658, fish were processed in the lab for fresh length and weight and frozen (-20 °C) in individual WhirlPak® bags.

2.2.6 Water sampling

Water was sampled from each reservoir tank and from one tank of each treatment once per week before the first feeding period using ultra-clean sampling techniques (United States Environmental Protection Agency 1996). Water was collected using a battery-operated pump (CanSun Electronics, Winnipeg, MB, Canada) and Teflon tubing. In the lab prior to sampling, the pump was cleaned by running a 10% HCl solution through it for 10 min, followed by 10 min of pumping milli-Q water. All sample vials were acid cleaned for 72 h in 10% HCl prior to use and stored individually, double-bagged in Ziploc bags. Tanks were sampled in the following order: clean reservoir tank, CWCF treatment tank, CWSF tank, SWCF tank, SWSF tank, spike reservoir tank. Clean-hands, dirty-hands protocol (St. Louis *et al*. 1994) was followed for all samples using powder-free Nitrile gloves (Best Glove, Inc., Menlo, GA, USA). The pump was allowed to discharge for 15 sec before each sample was collected. Vials were rinsed with pumped water three times prior to being filled. Approximately 5 mL of headspace was left in each vial. Filtered and unfiltered THg (40 mL) and MeHg (250 mL) samples were collected from each tank (total = 4 samples per tank) and immediately placed on ice in a cooler for transport back to the lab. Filtered samples were pumped through 0.22 μm nylon membrane filters (GE Osmonics, Trevose, PA) housed in a 47 mm in-line filter holder (Cole Parmer, Inc., Montreal, QC). The filters and the in-line filter holder were both acid cleaned for 72 h in 10% HCl prior to each water sampling day. Filters were stored in milli-Q water in an acid-cleaned mason jar, and the filter holder was stored double-bagged in Ziploc bags. Upon returning to the lab, water samples were acidified immediately with concentrated HCl to 0.4% HCl. To achieve this concentration, I added

1.0 mL HCl to the 250 mL MeHg bottles and 0.16 mL HCl to the 40 mL THg vials. Following acidification, vials were stored in a refrigerator at 4 °C until they could be shipped for spike and ambient Hg analyses.

To determine whether spike food pellets released any spike Hg to the water during feeding periods, I conducted an independent trial after completing the main experiment. I filled two clean tanks (CWCF; no previous spike Hg exposure), with water from Lake 660 and added a typical ration of spike food pellets. I sampled water from each tank 1 h, 2 h, and 24 h after adding the food to simulate the typical length of time pellets would be in the tanks (1 h and 2 h) and the maximum length of time a pellet would be in the water if it was overlooked during siphoning (24 h). I collected and acidified the water samples as above. As spike Hg levels were so low in Lake 658 water, I did not test whether clean pellets absorbed spike Hg from the water.

2.2.7 Processing of fish tissues

All fish collected at the end of the experiment were analyzed for THg and MeHg. Samples were handled using mercury clean techniques with Teflon or stainless steel tools (Bloom 1992; Van Walleghem *et al*. 2007). Tools and surfaces were cleaned with 95% ethanol (Commercial Alcohols Inc., Brampton, ON, Canada) and KimWipes between each sample. I dissected the gut contents out of each fish carcass so that any Hg present in undigested food would not influence the analysis. Each fish was placed whole into a 22 mL acid-washed glass vial (National Scientific Company, Rockwood, TN, USA) and freeze-dried in a Lyph-lock 12-L freeze dry system until a constant weight was achieved

(approximately 72 h). Dried fish were ground individually with an acid-washed glass mortar and pestle until the tissue was a fine powder. The mortar and pestle were cleaned with 95% ethanol between fish from the same experimental treatment, and in a 10% HCl acid bath for a minimum of 4 h between fish from different treatments. Approximately 0.08 g of the ground tissue from each fish was weighed and transferred into a new acid-washed vial with a Teflon-lined lid for Hg analysis.

All water and fish tissue samples were analyzed for spike THg and MeHg species at Trent University, Peterborough, ON (Dr. Holger Hintelmann, Department of Chemistry). THg was measured in samples after digestion with HNO_3/H_2SO_4 (7:3 v/v) and heating at 80 °C until brown NOx gases no longer formed. THg of sample digests was reduced by $SnCl_2$ and determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (Thermo-Finnigan Element2) using a continuous flow cold vapor generation technique. MeHg in samples was solubilized by treatment with 20% (w/v) KOH/MeOH solution at 50 °C and measured after aqueous phase ethylation using $NaBEt_4$. Volatile Hg species were purged and trapped onto Tenax and MeHg was measured after thermodesorption and GC separation using ICP-MS detection (Micromass Platform).

2.2.8 Model simulations

I used two bioenergetics-based contaminant accumulation models to predict the uptake of Hg by experimental fish. The Wisconsin Fish Bioenergetics version 3.0 model (Hanson *et al*. 1997), which has been widely-used to examine contaminant transfer in fish (e.g., MacRury *et al*. 2002; Van Walleghem *et al*. 2007; Drouillard *et al*. 2009; Lepak *et al*.

2009) and the OneFish model developed by Harris and Bodaly (1998) were chosen because they are based on identical bioenergetics equations but make contrasting assumptions regarding fish Hg uptake. The Wisconsin model assumes that all mercury accumulated by a fish originated in its food, while the OneFish model accounts for uptake of Hg from both dietary and aqueous sources.

The models allow users to input assimilation efficiency values for food (AE_f; Wisconsin and OneFish) and for water (AE_w; OneFish). The assimilation efficiency of Hg from food by fish has been thoroughly researched, and is commonly suggested to centre around 80% (Rodgers 1994; Pickhardt *et al*. 2006). The assimilation efficiency of Hg from water (AE_w) by fish is less clear, with values ranging from 7% (Boddington *et al*. 1979) to 36% (Harris and Bodaly 1998). The rate of Hg elimination is another input. Elimination of Hg by yellow perch has been shown to have a half life >400 d (VanWalleghem *et al*. 2007; Madenjian and O'Connor 2008). Due to the short duration of this experiment, elimination was set at zero in both models. Other input parameters used in model simulations include initial fish conditions, water temperatures, fish diet, food and water mercury concentrations, and food energy density (Appendix 1, Tables A.1, A.2). The Wisconsin model calculates Hg concentration in fish muscle, while OneFish may be set to calculate Hg in fish muscle or whole fish. Concentrations of Hg in fish muscle appear to be approximately 1.3× concentrations in whole fish bodies (J.L. Van Walleghem, Ontario Ministry of Natural Resources, unpublished data). In this study, all Wisconsin model predictions were converted from muscle to whole body Hg concentrations using this ratio.

The Wisconsin model assumes all fish Hg is derived from food, and therefore most closely resembles uptake by fish in the CWSF treatment. As the Wisconsin model is commonly used to estimate fish Hg levels in nature, I also compared these results to the treatment where spike Hg water and food sources were both present (SWSF). The OneFish model, on the other hand, accounts for both food and water Hg uptake and may be tailored to represent any of the SWSF, CWSF, or SWCF treatments. The Wisconsin model allows a user to input fish start and end weights and computes consumption within the model based on the amount of food necessary to achieve a given end weight. The OneFish model allows for input of the start weight, and relies instead on a growth constant that may be adjusted by the user to fit observed fish growth. It is important that growth predicted by the model match observed growth for the predicted Hg concentrations to be valid (Hanson *et al*. 1997).

I ran four simulations in each model for each treatment type giving a total of 4 Wisconsin simulations and 12 OneFish simulations (4 for each of the SWCF, CWSF, and SWSF treatments). For the Wisconsin model, the simulations represented a suite of start and end weights intended to span the range of possible growth patterns: smallest observed start weight (0.7 g) to smallest observed end weight (0.6 g); smallest observed start weight to largest observed end weight (1.3 g); largest observed start weight (0.9 g) to largest observed end weight; largest observed start weight to smallest observed end weight. For the OneFish model, I ran simulations beginning with each of the start weights observed in t_0 fish (0.6, 0.7, 0.8, and 0.9 g) and adjusted the growth rate constant (kt) so that

simulated end weights fell in line with end weights used in the Wisconsin simulations. Predicted Hg concentrations generated by the models were pooled by treatment for each model to generate mean values (with sample size of n=4 for each) to compare to mean observed values.

2.2.9 Calculations

Body condition

Body condition (K) is commonly used to describe the health of a population of fish (Ricker 1975; Cone 1989). Condition describes the relationship between fish length and weight, with higher condition factors observed in fish that have greater weight per unit length. Condition factor is calculated with this equation (Ricker 1975):

$$(2.1) \quad K = (W \times 100) \div L^3$$

Where K is condition factor, W is weight of the fish in g, and L is length of the fish in cm.

Mercury concentration conversions

Wet weight Hg concentrations ($ng \cdot g^{-1}$) were calculated for both THg and MeHg as follows:

$$(2.2) \quad [Hg]_{wet} = [Hg]_{dry} \times sample_{dw} \div sample_{ww}$$

Where $[Hg]_{dry}$ is the dry weight Hg concentration ($ng \cdot g^{-1}$) in a sample, $sample_{dw}$ is the dry weight of the sample (g) and $sample_{ww}$ is the wet weight of the sample (g). Wet weight Hg concentrations are primarily used throughout this chapter.

Percent uptake from water

I estimated the percent of total body mercury fish accumulated from water using two different methods. The first is modified from Hall *et al*. (1997), and involves examining the difference in spike Hg concentrations between fish exposed to spike Hg in both their food and water and fish exposed to spike Hg only in their food. These values were calculated using the following equation:

$$(2.3) \quad \% \text{ Hg from water} = (([Hg]_{SWSF} - [Hg]_{CWSF}) \div [Hg]_{SWSF}) \times 100\%$$

Where $[Hg]_{SWSF}$ is the mean wet weight Hg concentration ($ng \cdot g^{-1}$) of fish from the spike water + spike food treatment, and $[Hg]_{CWSF}$ is the mean wet weight Hg concentration ($ng \cdot g^{-1}$) of fish from the clean water + spike food treatment.

The second method examines the relationship between spike Hg concentrations in fish exposed to spike Hg only through water and fish exposed to spike Hg through both pathways. This calculation is outlined below:

$$(2.4) \quad \% \text{ Hg from water} = ([Hg]_{SWCF} \div [Hg]_{SWSF}) \times 100\%$$

Where $[Hg]_{SWSF}$ is as above and $[Hg]_{SWCF}$ is the mean wet weight Hg concentration ($ng \cdot g^{-1}$) of fish from the spike water + clean food treatment.

2.2.10 Data analysis

Statistical tests were performed using Statistica v.5.5 (StatSoft, Inc.) and JMP Student Edition v.6.0.3 (SAS Institute Inc.). Assumptions of normality and homogeneity of variance were tested. I conducted one-way analysis of variance (ANOVA) to test for daily differences in water temperatures among treatments. Relationships between tank

water temperatures and air and surface water temperatures collected at Lake 658 were investigated with linear regression. Relationships between fish weight and whole-body spike mercury concentrations, and between mortality levels and tank water temperatures were also tested with linear regression. Due to high levels of mortality in all treatments, sample numbers were too low to analyze growth and fish mercury data solely by tank. I calculated mean and standard error of Hg concentrations and growth for fish in each tank (n varied from 2 to 5 per tank). I then pooled these means to calculate mean of means (treatment means) for THg, MeHg, and growth in each treatment. I used ANOVA to examine differences in fish growth and mercury concentrations among treatment means. Significant differences were investigated with Tukey's honestly significant difference post-hoc test. *t*-tests were also used to compare model predictions of Hg values with observed values for each treatment.

2.3 RESULTS

2.3.1 Experimental conditions

2.3.1.1 Tank water temperatures

Temperatures in all tanks tended to be warmer at the start of the experiment in early August, and declined as the month went on. Average 24 h minimum and 24 h maximum water temperatures were not significantly different among treatments (p=0.08 for mean minimum temperatures; p=0.18 for mean maximum temperatures). Overall, the mean daily maximum and minimum temperatures in tanks receiving spike water were not significantly different from those receiving clean water (Figure 2.4). Only 6 of 27 d

showed significant differences between spike and clean tank mean maximum temperatures, and only 4 d had significant differences between the spike and clean tank mean minimum temperatures ($p<0.05$ for all). Although temperatures on these days were significantly different, the maximum difference between mean spike and clean maximum temperatures was 2.3 °C on August 11, and 1.7 °C on August 11 for mean minimum temperatures. Generally, temperatures in tanks receiving spike and clean water were not different.

Experimental tank temperatures were lower and more variable than Lake 658 water temperatures (Figure 2.4), likely due to water being held in reservoir tanks overnight prior to use in the fish tanks the following day. The maximum change in Lake 658 water temperatures over a 24 h period was 3.2 °C, while the tanks experienced water temperature swings of up to 6.9 °C in 24 h. Air temperatures influenced tank temperatures. Minimum air temperatures, with a 1 d lag, explained 61.4% and 59.8% of the variability in the minimum spike and clean tank temperatures respectively ($p<0.001$ for both), while the maximum air temperatures explained 63.4% of the variability in the maximum spike tank temperatures ($p<0.001$) and 70.5% of the variability in the maximum clean tank temperatures ($p<0.001$).

2.3.1.2 Mortality and tank water temperatures

Fish mortality was observed in all tanks, with pulses of high mortality in the first and final weeks of the study (total of 6 to 21 fish per day), and low mortality during interim weeks (Figure 2.5). No mortality was observed in any tanks between August 17 and 23,

and low mortality (<3 fish per day) was observed between August 24 and 29. Mortality was not strongly related to tank water temperatures overall, with maximum spike water temperature explaining only 2.4% of the variability observed in daily number of mortalities ($p<0.46$), and minimum spike water temperature explaining 0.5% of the variability ($p<0.73$). Additionally, mortality does not appear to correspond to days with the greatest 24 h water temperatures swings ($r^2=0.17$). Although mortality appeared to roughly follow the patterns observed in tank temperatures between August 30 and September 4, the overall relationship between mortality and temperature was not strong. Body condition of fish that died prior to the completion of the experiment (mean=0.78; SEM=0.021; n=45) was significantly lower ($t=1.99$; $df=84$; $p<0.001$) than fish that were sacrificed on the final day of the study (mean=0.96; SEM=0.020; n=40). Although there was high mortality overall during the experiment, there were enough fish alive at the end of the study to conduct statistical analyses for all results.

2.3.1.3 Water and food spike mercury levels

Spike Hg was not added to Lake 658 in 2008, but was detectable in the water column at low levels on each METAALICUS water sampling date during the 2008 open water season (9 dates between April 3 and November 11) (Dr. H. Hintelmann, Department of Chemistry, Trent University, Peterborough, ON, Canada, unpublished data). Spike THg was present in the spike reservoir tank on each water sampling date at an average concentration of 0.030 $ng \cdot L^{-1}$ (SEM=0.014; n=4), and spike MeHg was present at an average concentration of 0.026 $ng \cdot L^{-1}$ (SEM=0.001; n=4) (Table 2.1). Approximately half (49%) of the spike THg was present as MeHg in the food pellets made from L658

zooplankton. Spike THg was present in food pellets at an average concentration of 4.39 $ng \cdot g^{-1}$ (SEM=0.20; n=2), and the mean spike MeHg concentration in food was 2.15 $ng \cdot g^{-1}$ (SEM=0.13; n=2) (Table 2.1). Spike Hg did not appear to transfer from the spike food pellets to the water. Water sampled 1 h, 2 h, and 24 h after adding pellets to a full tank of clean water did not contain detectable levels of spike Hg. I did not test whether spike Hg from the water transferred to the food pellets, but as spike Hg concentrations in the water were so low, it is likely any transfer that did occur would not have contributed significantly to fish Hg levels.

2.3.2 Experimental results

2.3.2.1 Fish growth

Fish collected from Lake 240 at t_0 had a mean weight of 0.76 g. Little growth was seen over the course of the experiment (Figure 2.6). Final weights of fish did not differ among tanks in each treatment (CWCF $p<0.14$; SWCF $p<0.41$; CWSF $p<0.11$; SWSF $p<0.52$). Means calculated from the means of the three tanks in each treatment did not differ significantly from t_0 fish weights or from one another ($p<0.16$). In comparison, YOY yellow perch in Lake 240 increased in body mass by 43% over this same time period. Perch did not consume all of the food they were provided with on any days of the study, meaning that their actual ration was less than the provided 15% of body wet weight per day. The fish appeared to consume approximately one-third of this ration, on average (personal observation), with some individuals consistently consuming more than others.

2.3.2.2 Fish spike mercury concentrations

YOY yellow perch collected from Lake 240 at t_0 did not contain detectable levels of spike Hg (data not shown); therefore, all spike Hg present in fish was accumulated either from water or food during the experiment. Spike THg and MeHg was detected in all fish from all treatments except for CWCF (Table 2.2). Spike MeHg constituted a mean of 93.7% (SEM=5.6; n=30) of the spike THg present in the fish from the CWSF, SWCF, and SWSF tanks. This ratio was consistent among treatments.

Mean whole body THg and MeHg concentrations in YOY yellow perch did not differ significantly among tanks within treatments ($p>0.1$ for all treatments; Table 2.2). Mean concentrations observed in each tank were averaged by treatment (treatment means in Table 2.2). Using these treatment means in one-way ANOVA calculations, I observed significant differences in the amount of spike mercury accumulated by fish among treatments (THg: $p<0.001$; MeHg: $p<0.001$; Table 2.2 and Figure 2.7). Fish that were exposed to spike only in their food (CWSF) had mean concentrations of THg and MeHg that were higher than fish from the SWCF treatment (THg: $p<0.001$; MeHg: $p<0.001$). Fish from the SWSF treatment exhibited the highest spike Hg concentrations overall, showing levels that were significantly higher than fish in in all other treatments (THg: $p<0.007$; MeHg: $p<0.045$).

The presence of spike Hg in fish from the SWCF treatment tanks indicates that these fish accumulated spike Hg directly from water (Figure 2.7). Uptake of spike Hg from the two compartments (food and water) was additive, with both uptake vectors contributing to

final fish spike Hg levels. When mean treatment concentrations of spike Hg in fish from the SWCF and CWSF treatments were added together (THg: 0.06+0.29=0.35 ng · g^{-1}; MeHg: 0.06+0.26=0.32 ng · g^{-1}), they approximated the treatment mean concentrations observed in SWSF fish (THg: 0.34 ng · g^{-1}; MeHg: 0.29 ng · g^{-1}).

Individual fish spike Hg levels were not significantly related to body weight. Body weight explained only 8.2% ($p<0.42$) of the variability in fish spike THg levels in the CWSF treatment, 3.3% ($p<0.61$) of the variability in the SWCF treatment, and 5.4% ($p<0.52$) of the variability in the SWSF treatment. Comparisons between fish spike MeHg concentrations and individual fish weights yielded similar results, with $r^2<0.08$ and $p>0.40$ for each treatment.

2.3.2.3 Mercury derived from water

Fish from the SWSF treatment accumulated more THg and MeHg than fish from the CWSF treatment. SWSF fish had higher THg and MeHg levels than CWSF fish by 14.6% for spike THg and 10.3% for spike MeHg (calculated with equation 3) (Table 2.3). Fish that received spike Hg from water only (SWCF) had mean spike THg and MeHg concentrations equal to 18.2% (spike THg) and 20.7% (spike MeHg) of the mean concentrations of fish that received spike Hg from both sources (calculated with equation 4). Overall, the proportion Hg accumulated directly from the water by YOY yellow perch averaged of 16.0% in this 1-month study.

2.3.7 Model predictions

The Wisconsin model assumes that all fish Hg comes from diet and simulates the CWSF treatment in this study. The OneFish model accounts for Hg uptake from both water and food by fish, allowing for predictions of YOY yellow perch spike Hg concentrations in CWSF, SWCF, and SWSF treatments. Fish weights predicted by the models for the final day of the experiment were identical to the observed weights. Mean spike Hg concentrations produced by the models for the treatments they most closely resemble were not significantly different from concentrations observed in yellow perch in those treatments (SWCF: mean=0.06 $ng \cdot g^{-1}$, $p<0.67$ for OneFish; CWSF: mean=0.25 $ng \cdot g^{-1}$, $p<0.87$ for OneFish and mean=0.27 $ng \cdot g^{-1}$, $p<0.87$ for Wisconsin; SWSF: mean=0.31 $ng \cdot g^{-1}$, $p<0.83$ for OneFish) (Table 2.4, Figure 2.8). OneFish model predictions were lower than the mean observed concentrations by 7.9% (SWCF), 12.2% (SWCF), and 6.4% (SWSF). The Wisconsin model resembles the CWSF treatment most closely as it does not account for the uptake of Hg from water. However, as the model is commonly used to estimate fish Hg levels in nature where fish receive Hg from both uptake vectors, it should be tested for accuracy in predicting concentrations observed in the SWSF treatment. The Wisconsin model prediction was lower than the mean concentration observed in the SWSF treatment by 18.6% and lower than the OneFish model prediction for spike Hg in fish in the SWSF treatment by 13%, although these differences were not significant ($p=0.77$).

2.4 DISCUSSION

Fish accumulate Hg directly from the water and from food, but the relative importance of the two sources to fish Hg concentrations is unresolved. Many studies assume that fish take in Hg only from food, and ignore water as an important source. I conducted a field experiment to quantify the uptake of Hg from food and water by yellow perch under semi-natural conditions. I used the data from the experiment to assess the accuracy of two published Hg bioaccumulation models and to determine whether the exclusion of water as a source of Hg to fish in one of the models (Wisconsin) led to lower predictions of fish Hg concentrations than those generated by the model that included both food and water as sources of Hg (OneFish). I found that uptake of spike Hg from water was not negligible, as has been suggested previously, but accounts for between 10.3% and 20.7% of the total Hg accumulated by fish. I also found that the uptake of spike Hg from food and water was additive. The Wisconsin model (no water Hg uptake) predictions were not significantly different from the concentrations observed in the CWSF or SWSF treatments, but were lower than the concentrations observed in the SWSF treatment by 18.6%. The OneFish model (water and food uptake) accurately predicted fish Hg levels in individuals from the SWCF, CWSF, and SWSF treatments when presented with different scenarios to mimic each treatment type. Overall, OneFish model predictions were higher than Wisconsin model predictions when presented with identical data, although this difference was not significant.

2.4.1 Spike Hg in fish

Mean treatment spike Hg concentrations were significantly different among all treatments (Table 2.4, Figure 2.7). As fish growth and tank environmental conditions (e.g., water temperature) did not differ among treatments, the differences seen in spike Hg concentrations among treatments should be attributed to treatment type, not to another confounding factor.

The presence of spike Hg in YOY yellow perch from the SWCF treatment indicates clearly that the fish accumulated spike Hg directly from the water. This result is in agreement with previous research that has suggested that fish take in Hg from the dissolved phase as water passes across their gills (Lock 1975; Phillips and Buhler 1978; Rogers and Qadri 1982; Boudou and Ribeyre 1983; Klinck *et al*. 2005; Pickhardt *et al*. 2006). Similar research has suggested that fish also take in other metals from the water, including cadmium (Kraemer *et al*. 2006) and zinc (Niyogi *et al*. 2007). Although some studies acknowledge water as a potential source of Hg to fish, water-derived Hg has generally been dismissed as an important contributor to fish Hg levels (e.g., Gorski *et al*. 1999; Bowles *et al*. 2001; Trudel and Rasmussen 2001; Hammerschmidt *et al*. 2002; Rennie 2003; Trudel and Rasmussen 2006; Dittman and Driscoll 2009). The use of spike Hg in this study provides convincing evidence that water is an important direct source of Hg to fish.

In this study it was determined that water accounts for between 10% and 21% of Hg in fish tissues. Previous Hg accumulation studies have used a range of fish species,

experimental conditions (field, lab), and mercury types (Table 1.1) and, as a result, have produced a wide range of estimates of the importance of waterborne Hg accumulation by fish. Rodgers and Qadri (1982) completed a field survey of yellow perch and concluded that uptake of waterborne Hg accounted for 38% of the total accumulation. Hall *et al*. 1997 studied Hg uptake by finescale dace housed in mesocosms in Lake 240 at the ELA over a period of 31 d, and despite problems with contamination of "low Hg water" treatments with Hg from "high Hg" live zooplankton prey, they estimated that fish accumulate approximately 15% of their Hg directly from water. Both studies used natural levels of Hg in food and water and did not dose fish artificially. Although the studies do not agree in their estimates of Hg accumulation, they suggest that uptake of Hg from the water by fish is not negligible. Post *et al*. (1996) also conducted a field experiment to assess waterborne Hg accumulation by yellow perch and suggest that accumulation of Hg from water is less important than Hg derived from diet in the warm summer months, but that it becomes more dominant in the fall and winter as consumption and activity rates decline. Results of my study are applicable to YOY yellow perch under summer conditions and are in general agreement with all previous field studies in which uptake of Hg from food is dominant, but waterborne Hg is also an important source.

Although my experiment mimicked field conditions as much as possible, the use of tanks makes the experimental design reminiscent of lab-based Hg accumulation studies as well. Various estimates of the importance of waterborne Hg to fish Hg levels have been produced in lab studies. These studies range in duration (2 h in Pickhardt *et al*. (2006) to 84 d in Lock (1975)) and some use unrealistically high doses of Hg in food and water, but

the majority suggest that at least some Hg in fish is derived from water. Boudou and Ribeyre (1983) studied steelhead trout and suggest that uptake of waterborne Hg accounts for 12.5% of total fish Hg. Pickhardt *et al*. (2006) used the radioisotope ^{203}Hg to study uptake in redear sunfish and mosquitofish, and indicate that waterborne Hg uptake accounts for 12-27% of ^{203}Hg accumulation. Lock (1975) suggests that although dietary Hg uptake is dominant, 10-20% of fish Hg is derived from water. Wang and Wong (2003) used ^{203}Hg to study accumulation in sweetlips, and suggest that uptake of Hg from water is negligible. Although the use of ^{203}Hg gives strength to their conclusions because it may be measured separately from ambient Hg, this study was conducted with a single pulse dose of ^{203}Hg instead of gradual uptake over time. This method may reduce the applicability of the results to wild populations. Overall, laboratory accumulation studies estimate that uptake of Hg from water contributes approximately 10-25% of Hg in fish. Despite the use of different methods, this estimate is echoed in my results.

It is possible that total mercury accumulation from water was lower for YOY yellow perch in the tanks than it would be in the wild. The limited size of the tanks (160 L) may have constrained fish activity levels, resulting in reduced respiration rates. Fish held in tanks are not exposed to the same pressures as those in the wild (Mann 1978). Wild fish must exert energy performing predator-avoidance and foraging behaviours, but these actions are not required for fish housed in tanks. Combined with the small size of the tanks compared to lake habitat area, these factors may reduce experimental fish activity levels compared to those in nature (Trudel and Rasmussen 2001). Fish activity rates influence respiration and oxygen consumption rates, which in turn influence the volume

of water that passes across the gills (ventilation volume) (Boddington *et al*. 1979). As waterborne Hg exposure and assimilation increases as ventilation volume and oxygen consumption increase (Boddington *et al*. 1979; Rodgers and Beamish 1982; MacCrimmon *et al*. 1993), low activity rates of experimental fish may reduce their exposure to waterborne Hg compared to fish in nature. In this study, it is possible that the activity levels of the fish are lower than those in natural yellow perch populations and that, as a result, the amount of Hg taken in from water was also lower than it would be in the wild.

In summary, the range of 10.3% to 20.7% Hg uptake from water predicted in this study is consistent with preceding field- and lab-based research. Although this range likely varies among individual fish, populations, species, fish size and age, and with seasonal changes, it is important to acknowledge that water is an important source of Hg to fish and should not be ignored. The use of pellet food with field-collected zooplankton, enriched stable isotopes of Hg, and environmentally-relevant levels of Hg in my experiment provide several advantages over all previous studies and give strength to these conclusions.

2.4.2 Additive nature of food- and water-derived spike Hg

In this study, spike Hg taken in from food and from water were additive and together contributed to fish Hg concentrations. When mean concentrations of spike Hg from the SWCF and CWSF treatments were summed (sum for THg = 0.35; sum for MeHg = 0.32) they closely approximated the mean spike THg and MeHg concentrations in the SWSF treatment (THg = 0.34 $ng \cdot g^{-1}$; MeHg = 0.29 $ng \cdot g^{-1}$). The idea of the two sources of Hg

contributing additively to fish Hg was originally suggested by Phillips and Buhler (1978), and has been further supported by more recent research (Braune 1987; Post *et al*. 1996). Selenium accumulation from food and water by fish has also been shown to be additive (Bertram and Brooks 1986). These studies indicate that food and water Hg uptake pathways do not interact, but instead contribute individually to fish Hg levels. This additivity is supported by evidence that Hg present in the body of a fish follows first order kinetics, acting as a single mass of Hg rather than in compartments (Trudel and Rasmussen 1997). The clear Hg uptake patterns demonstrated with the use of enriched stable isotopes of Hg in this study strongly support the idea of additive uptake. The main implication of this finding is that the two uptake vectors may be treated independently from one another in mercury bioaccumulation models (Post *et al*. 1996). This independence simplifies internal model structure by alleviating the need for terms to describe interactions between the two.

2.4.3 Total and methylmercury

Spike MeHg concentrations observed in whole yellow perch in this study accounted for almost all of the mercury in fish in all treatments (mean of 93.7% of spike THg). The high MeHg:THg ratio seen in whole fish in this study is consistent with results from other studies (Bowles *et al*. 2001; Bloom 1992; Becker and Bigham 1995; Kannan *et al*. 1998). It has been well-established that >90% of Hg in muscle tissue of predatory fish tends to be in the organic form (Bloom 1992; Bowles *et al*. 2001; Rennie *et al*. 2003). This value can be lower for whole-bodies of fish as some organs (e.g., liver) tend to contain less MeHg (Boudou and Ribeyre 1983; Riisgard and Hansen 1990). Planktivorous fish may

also have MeHg:THg ratios that are less than 90% because of their low trophic status (Bodaly and Fudge 1999; Bowles *et al*. 2001). However, other studies have reported MeHg:THg ratios of 96-99% in planktivorous fish (Bloom 1992) and 90% (Watras and Bloom 1992). As most spike THg appears to be in the form of spike MeHg, conclusions regarding MeHg accumulation in the fish can be drawn from both THg and MeHg results. The consistency of these results with those from other research provides further evidence that spike Hg behaves like ambient Hg and that data collected using spike Hg may be used to make inferences about ambient Hg (as suggested by Hintelmann and Evans 1997; Paterson *et al*. 2006; Harris *et al*. 2007; Southworth *et al*. 2007).

2.4.4 Baseline mercury concentrations

Yellow perch collected from Lake 240 at t_0 did not contain any spike Hg. Any spike Hg present in fish at the conclusion of the experiment was taken up from water or food in the tanks. The establishment of accurate t_0 ambient Hg concentrations in fish has proven difficult in uptake studies that have used ambient Hg because of the considerable variation seen in fish ambient Hg concentrations in nature (Boudou and Ribeyre 1985; Hall *et al*. 1997; Simon and Boudou 2001; Rennie *et al*. 2005). This variation stems from differences in many physiological parameters, including activity levels (Rennie *et al*. 2005), consumption rates and fish growth (Rogers 1992). Since ambient Hg taken up by a fish before and after the start of a study is impossible to distinguish, estimating uptake is complicated without accurate measurements or estimates of t_0 ambient Hg levels for individual fish. Calculating average t_0 ambient Hg concentrations is common in studies that focus on small-bodied organisms which must be sampled lethally for Hg analysis.

This approach has been used by Hall *et al*. (1997) for finescale dace, Boudou and Ribeyre (1985) for rainbow trout (*Oncorhynchus* mykiss*),* Parks *et al*. (1988) for crayfish (*Oronectes* spp.), and King and Davies (1987) for mussels (*Mytilus edulis*). The main disadvantage of using this approach is that estimates of uptake derived from comparing final Hg concentrations with the mean t_0 value are not sufficiently constrained, especially when working with environmentally-relevant contaminant concentrations. For example, the highest t_0 ambient THg concentration in this experiment was 1.6 times higher than the lowest t_0 concentration (data not shown). A mean baseline ambient Hg concentration estimated using these data would likely be far higher than actual baseline Hg concentrations in some experimental fish, and far lower than concentrations in others. As a result, changes in fish Hg concentrations from the estimated baseline level would not represent actual accumulation patterns, and might dampen the water Hg uptake signal entirely.

An alternate solution to the problem of distinguishing newly-deposited Hg from pre-existing Hg involves exposing fish to unrealistically high levels of Hg in water and food to ensure that the natural signal is drowned in the influx of new Hg. This approach has been used previously by Simon and Boudou (2001), Boudou and Ribeyre (1983) and others. The problem with this strategy is that it is unclear whether the results are applicable to natural fish populations where food and water Hg levels are drastically lower than the experimental conditions. Studies that use enriched isotopes of Hg avoid the obstacles associated with tracking the movement of ambient Hg. Enriched stable isotopes act as labelled forms of Hg that are easily distinguished from pre-existing

ambient Hg and are useful at low concentrations. The advantages of using spike Hg are evident in this study and have been illustrated in previous studies that use stable isotopes of Hg (Paterson *et al*. 2006; Harris *et al*. 2007; Orihel *et al*. 2007; Orihel *et al*. 2008).

2.4.5 Fish mortality

Fish mortality was observed in all tanks during the first and final weeks of the study (Figure 2.6). It is likely that mortality observed during the first week resulted from stress caused by the transfer of fish from Lake 240 to the tanks at Lake 658. Mortality of yellow perch following transport has been observed in other ELA studies (e.g., Van Walleghem *et al*. 2007). Water temperatures do not appear to have influenced mortality. Optimal temperatures for yellow perch are between 18 °C and 23 °C, with mortality rates in increasing with higher temperatures (Craig 1987; Hanson *et al*. 1987). Tank temperatures were within the optimal range for the majority of the study, straying higher on only a few occasions, and always below the upper lethal temperature of 32 °C (Hanson *et al*. 1997). The maximum temperature observed in any tank in this experiment was 25 °C. Additionally, although levels of mortality observed between Aug 30 and September 4 appear to mimic the patterns in tank water temperatures, number of mortalities was not strongly related to water temperature or to the magnitude of water temperature swings experienced during a 24 h period.

The high levels of mortality observed in the latter part of the experiment were likely caused by weight loss resulting from low consumption. Body condition is a widely-used indicator of the health of fish populations (Cone 1989; Sutton *et al*. 2000). In this study,

condition factor may be used as a surrogate measure of consumption, assuming that fish that ate more had higher condition factors (as suggested by Pothoven *et al*. 2001). Fish that died during the experiment had significantly lower body condition than those that survived until the end of the study, suggesting that low consumption was the cause of the mortality. Previous research by Letcher *et al*. (1996) indicates that the time to 50% mortality in a population of larval yellow perch that does not have access to food is 8-10 days. It is possible that the fish in this experiment survived longer than this period because they consumed some food, but did not consume enough to survive the 27 days of the full experiment. The mortality observed in this experiment is a limitation of the study as it reduced the design from a replicated experiment to a 4-treatment ANOVA design. Despite the mortality, however, at least two fish were recovered from each tank for Hg analysis.

2.4.6 Fish growth

Although the experiment took place under optimal temperatures for growth and YOY yellow perch were essentially allowed to feed *ad libitum*, mean weights observed at the end of the experiment in all treatments were not significantly different from starting weights. The negligible growth observed in this experiment can likely be attributed limited feeding by yellow perch. At the start of the experiment, fish were fed once daily for a period of 2 h. Without exception yellow perch in the tanks did not consume all the food provided over the time in which they were fed. After observing limited feeding during the early part of the experiment (days 1-6), I altered feeding practices. I noted that the fish were more interested in eating food from the water column, rather than picking it

up from the bottom of the tank. As the main diet of YOY yellow perch is zooplankton (Mills and Forney 1981; Schael *et al*. 1991), it is likely that the fish were used to eating out of the water column, rather than off the bottom of the lake. To encourage greater consumption, I doubled the feeding time and delivered the food in small amounts frequently throughout the feeding period rather than in one large amount at the beginning of the period. These changes provided the fish with additional opportunities to ingest pellets directly from the water column, and increased the amount of food consumed by fish overall. Many studies that use pelletized food begin the study with an acclimation period, in which the fish are fed a non-experimental pellet diet (Hammerschmidt *et al*. 2002; Rinchard *et al*. 2008). It is possible that the fish would have grown more during the experiment if they had been given a chance to become familiar with pellet food prior to the initiation of the study.

It is probable that the low overall rates of consumption contributed to the observed high mortality and low growth rates in the experimental fish. Although this may be viewed as a limitation of this study, it was consistent among treatments. If the fish were consuming substantially less that they would in nature, it is possible that the contribution of waterborne Hg to fish Hg levels would be exaggerated. However, as all fish that survived to the end of the experiment appeared to consume food each day, it is unlikely that this is the case. Hall *et al*. (1997) observed limited growth and many cases of weight loss over a 32 day experiment designed to quantify ambient Hg uptake by finescale dace and Simon and Boudou (2001) reported limited growth in a 30 day study of Hg accumulation from

food and water by carp (*Ctenopharyngodon idella*). Neither study suggests that lack of growth influenced their estimates of Hg accumulation.

2.4.7 Food and water spike mercury levels

Pellet food and water used in this study both had concentrations of spike Hg that would be considered at the low end of ambient Hg levels in the natural environment. The Experimental Lakes Area is considered a "pristine" region, receiving less than 4 μg THg $m^{-2} \cdot yr^{-1}$ ambient deposition (Harris *et al*. 1997). At the ELA, Harris *et al*. (2007) report Lake 658 ambient water THg concentrations of 1.73 $ng \cdot L^{-1}$, water MeHg concentrations of 0.2-0.6 $ng \cdot L^{-1}$, and dry weight zooplankton concentrations of 100-500 ng MeHg $\cdot g^{-1}$. Similarly, Simonin *et al*. 2008 give mean Hg concentrations for a group of uncontaminated New York State lakes, reporting THg values of 0.25-7.71 $ng \cdot L^{-1}$ and MeHg values of 0.03-3.6 $ng \cdot L^{-1}$. Concentrations of spike Hg in food (means of 2.15 $ng \cdot g^{-1}$ MeHg and 4.39 $ng \cdot g^{-1}$ THg) and water (means of 0.026 $ng \cdot L^{-1}$ MeHg and 0.031 $ng \cdot L^{-1}$ THg) from this study were similar to or slightly lower than those observed in pristine locations. The use of environmentally-relevant levels of Hg in this study sets it apart from previous Hg transfer studies that have used much elevated Hg concentrations. For example, Phillips and Buhler (1978) exposed rainbow trout to MeHg concentrations of 220-1380 $ng \cdot L^{-1}$ in water and 120-3080 $ng \cdot g^{-1}$ in food, Boudou and Ribeyre (1983) exposed rainbow trout to 1 $ng \cdot g^{-1}$ Hg $\cdot L^{-1}$ in water and 830 ng Hg $\cdot g^{-1}$ in food, and Simon and Boudou (2001) exposed herbivorous carp (*Ctenopharyngodon idella*) to 300 ng $Hg^{2+} \cdot L^{-1}$ and 30 ng MeHg $\cdot L^{-1}$ in water and 1000-2000 ng $Hg^{2+} \cdot g^{-1}$ and 500-700 ng

MeHg · g^{-1} in food. As these levels are orders of magnitude higher than concentrations observed in many natural systems, they may give rise to results that are not applicable to fish in lakes with lower water and prey Hg concentrations.

Pelletized forms of fish food have been used previously to study Hg in fish (Pentreath 1976; Lock 1975; Klaper *et al*. 2006; Sandheinrich and Miller 2006). The pellets allow for consistent dietary Hg exposure while limiting the contamination of the clean water (Sandheinrich and Miller 2006). The use of pelletized food containing zooplankton collected from clean and spike lakes in this study sets it apart from previous Hg transfer studies that have used live prey (e.g., Hall *et al*. 1997) or pellets spiked directly with the contaminant (e.g., Hammerschmidt *et al*. 2002; Sandheinrich and Miller 2006). The pellets in this study acted as a source of naturally-accumulated, naturally-methylated spike Hg to the fish, while preventing transfer to the clean water.

Spike Hg did not leach from the pellet food into the water. This finding is consistent with results from previous research using Hg-spiked pellet food. Drevnick and Sandheinrich (2003) and Hammerschmidt *et al*. (2002) used pellet food that had been soaked in a solution of alcohol and methylmercuric chloride and then dried in a fume hood. Both studies found negligible dissociation of MeHg from food to water. It is reasonable to suggest that there would be even less transfer of spike Hg to water from the pellets used in this study because the spike Hg source was incorporated directly into the food as freeze dried zooplankton. The methods employed by Drevnick and Sandheinrich (2003), on the other hand, would only have allowed the MeHg to adsorb to the pellets

superficially. As fish wastes and uneaten food pellets were siphoned out of the tanks after each feeding period, and as there does not appear to be any substantial transfer of spike Hg from the pellets to the water, it is reasonable to assume that the food and water uptake pathways were successfully separated in this study. Although clean pellets placed in the spike water tanks were not tested for spike Hg contamination, the short duration the pellets were in the tanks and the low concentrations of spike Hg in the water make it unlikely that enough (if any) spike Hg from the water adsorbed to the clean pellets to impact fish Hg levels.

The ratio of spike MeHg to spike THg ratio in the pellet food was approximately 49%. Zooplankton THg:MeHg ratios in natural populations vary greatly, with previous research reporting MeHg values from 30-90% (Watras and Bloom 1992), 33-80% (Plourde *et al*. 1997), 24-54% (Paterson *et al*. 2006) and 40% (Becker and Bigham 1995). Considering this variability among studies, a value of 49% MeHg is well within the range observed for zooplankton and suggests that the pellet food fed to yellow perch in this study is a realistic approximation of what these fish would experience in their natural food source. By exposing the fish in this study to pellet food and lake water containing natural ratios of spike THg and MeHg, I have replicated conditions observed in the wild as closely as possible, thus increasing the applicability of my results to existing yellow perch populations.

When examining the uptake of any contaminant by an organism, it is important to know the rate of assimilation of the contaminant from the source. The AE of MeHg by yellow

perch from zooplankton prey has been clearly established at 80% (Weiner and Spry 1996; Rodgers 1994; Pickhardt *et al*. 2006). One might think that since pellet food may be more fully digested than zooplankton prey, the AE of MeHg from pellet food would be greater than 80%. However, it is important to remember that the spike MeHg is present in the food pellets bound in whole dried zooplankton and that, for this reason, the AE of spike MeHg from the pellets is likely comparable to the AE of MeHg from zooplankton in nature. Pentreath (1976) observed an AE of Hg of 70% by plaice (*Pleuronectes platessa*) from a gelatin and starch-based fish food, which is slightly lower than the published AE of Hg from zooplankton by fish.

2.4.8 Model predictions

The OneFish model accounts for a combination of uptake from food and water (Harris and Bodaly 1998) and was employed in this study to predict Hg uptake in the SWCF, CWSF, and SWSF treatments. The Wisconsin model assumes that all fish Hg is derived from food (Hanson *et al*. 1997) and was used in this study to represent the CWSF treatment. Despite the exclusion of a water uptake component, however, the Wisconsin model is frequently used to predict fish Hg concentrations in nature. For this reason, estimates of fish Hg generated by this model were also compared to concentrations observed in the SWSF treatment.

When modelling bioaccumulation, it is essential that fish weight predicted at the end of the simulation matches the weight observed in the study population (Hanson *et al*. 1997; Rennie 2003; Van Walleghem *et al*. 2007). As Hg concentrations are calculated by

weight, any discrepancy between predicted and observed weights will lead to incorrect estimates of fish Hg levels. The model simulations conducted in this study were designed to represent the extreme range of possible growth patterns. As mean observed weights ±SEM for each treatment overlap values generated by the models, it is clear that the models fit the growth data well. It is relatively simple to accurately simulate observed weights in the Wisconsin model, as the weights of fish at the end of a simulation are used as input data to estimate consumption (Hanson *et al*. 1997), but it is not as easy to achieve in the OneFish model. When using the OneFish model, it is of particular importance that users observe and adjust the growth constant to mirror observed growth in order to reflect natural Hg uptake patterns.

Hg concentrations predicted by both models fit observed data well overall. The OneFish model was able to predict fish Hg concentrations that were not significantly different from those observed in SWCF, CWSF, and SWSF treatments when using input data from those treatments. Estimates generated by the Wisconsin model were not significantly different from mean concentrations observed in CWSF or SWSF treatments. Although the difference was not significant, the Wisconsin estimate was 18.6% lower than the mean SWSF concentration. Additionally, the mean OneFish model prediction for the SWSF treatment was 13.0% higher than that generated by the Wisconsin model, despite the use of identical input data. These data suggest that the OneFish model could be used with confidence to estimate fish Hg concentrations in natural populations, but that users of the Wisconsin model should interpret their results with caution as estimates of fish Hg concentrations produced by the model may be lower than actual concentrations.

The difference between the two models may be attributed to the exclusion of a water uptake component in the Wisconsin model. Bioenergetics and contaminant accumulation relationships in the Wisconsin model appear to mimic those found in natural populations, thus allowing the model to represent the concentrations observed in CWSF fish accurately, however, the predictions fall short of Hg levels observed in fish exposed to spike Hg in both water and food. As experimental conditions were consistent among treatments and all parameters required as model input were known, the discrepancy suggests that the model is missing something intrinsically. This idea is strengthened by the 13% difference between the Wisconsin and OneFish model predictions. The two models are nearly identical except for their treatment of Hg uptake from water, suggesting that the disparity observed between the predictions may stem from this difference. Several other existing bioaccumulation models include water as a source of Hg to fish, indicating that although food is a more important source, uptake of Hg from the dissolved phase should not be discounted (e.g., Norstrom *et al*. 1976; Kitchell *et al*. 1977; Rodgers 1994; Knightes *et al*. 2009). The Wisconsin model is a widely-used accumulation model (e.g., Stafford and Haines 2001; MacRury *et al*. 2002; Van Walleghem *et al*. 2006; Chipps *et al*. 2009; Lepak *et al*. 2009). Users should be aware that predictions generated by the Wisconsin model will be lower than actual values in nature and adjust their predicted values accordingly. Overall, it is evident that uptake of Hg through water Hg should not be discounted in fish Hg bioaccumulation models.

2.4.9 Conclusions and recommendations

This study used enriched stable isotopes of Hg to monitor the uptake of Hg by yellow perch from food and water under semi-natural summer conditions. The main conclusions to be drawn from this study are that (i) yellow perch accumulated spike THg and MeHg directly from the water, (ii) 10-21% of the spike mercury present in fish tissues following exposure resulted from direct uptake from the water, (iii) uptake of mercury from food and water by yellow perch is additive, and (iv) models that attempt to predict mercury uptake by fish based on environmental information should consider waterborne mercury an important source of mercury to fish to avoid producing underestimates of fish mercury concentrations.

Table 2.1 Concentrations of the stable isotope ^{202}Hg ("spike Hg") in pellet food and water samples collected during the experiment. Water samples were taken weekly from the spike water reservoir tank.

sample type	sample date	spike THg ($ng \cdot L^{-1}$ for water; $ng \cdot g^{-1}$ for food)	spike MeHg ($ng \cdot L^{-1}$ for water; $ng \cdot g^{-1}$ for food)
spike water	12-Aug-08	0.023	0.024
spike water	19-Aug-08	0.073	0.027
spike water	26-Aug-08	0.016	0.025
spike water	4-Sep-08	0.010	0.026
spike food	-	4.588	2.286
spike food	-	4.192	2.019

Table 2.2 Mean wet weights of fish in each tank and treatment (calculated as mean of means); mean wet weight concentrations of spike total mercury (THg) and spike methylmercury (MeHg) in whole-bodies of yellow perch in each tank and treatment (calculated as mean of means).

treatment	tank	n	tank mean weight (g)	treatment mean weight (g)	tank mean spike THg (ng · g^{-1} w.w.)	treatment mean spike THg (ng · g^{-1} w.w.)	tank mean spike MeHg (ng · g^{-1} w.w.)	treatment mean spike MeHg (ng · g^{-1} w.w.)
CWCF	5	3	0.87	0.92	0.00	0.00	0.00	0.00
	8	5	0.98		0.00		0.00	
	11	2	0.90		0.00		0.00	
SWCF	2	5	1.08	0.97	0.05	0.06	0.05	0.06
	4	2	0.85		0.06		0.06	
	10	3	0.97		0.07		0.07	
CWSF	3	5	0.84	0.94	0.29	0.29	0.27	0.26
	6	2	0.85		0.25		0.24	
	12	3	1.13		0.28		0.27	
SWSF	1	3	0.83	0.84	0.35	0.34	0.30	0.29
	7	2	0.78		0.31		0.28	
	9	5	0.90		0.34		0.29	

Table 2.3 Mean estimates of whole-body YOY yellow perch spike total mercury (THg) concentrations predicted by the Wisconsin and OneFish models and mean levels observed in the experiment (n=10 fish per treatment) for the spike water + clean food, clean water + spike food, and spike water + spike food treatments. Each model prediction is based on four separate modelling simulations designed to span the range of possible fish growth patterns in the experiment. Results of oneway ANOVA analyses among observed and model results are presented for each treatment (α=0.05). The percent difference between modelled output and mean observed concentrations are also reported. Note: when Wisconsin output was compared to the concentrations observed in the SWSF treatment, the ANOVA result was p=0.77 and the difference was -18.6%.

source	treatment	mean THg ($ng \cdot g^{-1}$)	SEM	n	oneway ANOVA by treatment	model % difference from experiment concentration
Experiment	SWCF	0.06	0.007	10	p<0.67	
OneFish	SWCF	0.06	0.001	4		-7.9%
Experiment	CWSF	0.29	0.045	10	p<0.87	
OneFish	CWSF	0.25	0.004	4		-12.2%
Wisconsin	CWSF	0.27	0.01	4		-4.7%
Experiment	SWSF	0.34	0.059	10	p<0.83	
OneFish	SWSF	0.31	0.005	4		-6.4%

Table 2.4 Percent of spike Hg derived from water by YOY yellow perch during a 1 month experiment calculated with equation 2.3 and equation 2.4. Equation 2.3 calculates the difference in spike Hg concentration between fish that received spike Hg in both food and water (SWSF) and fish that only received spike Hg in their food (CWSF). Equation 2.4 examines the relationship between spike Hg concentrations in fish exposed to spike Hg only in their water (SWCF) and fish exposed to spike Hg through both pathways (SWSF).

type of Hg	equation 2.3	equation 2.4
THg	14.6%	18.2%
MeHg	10.3%	20.7%

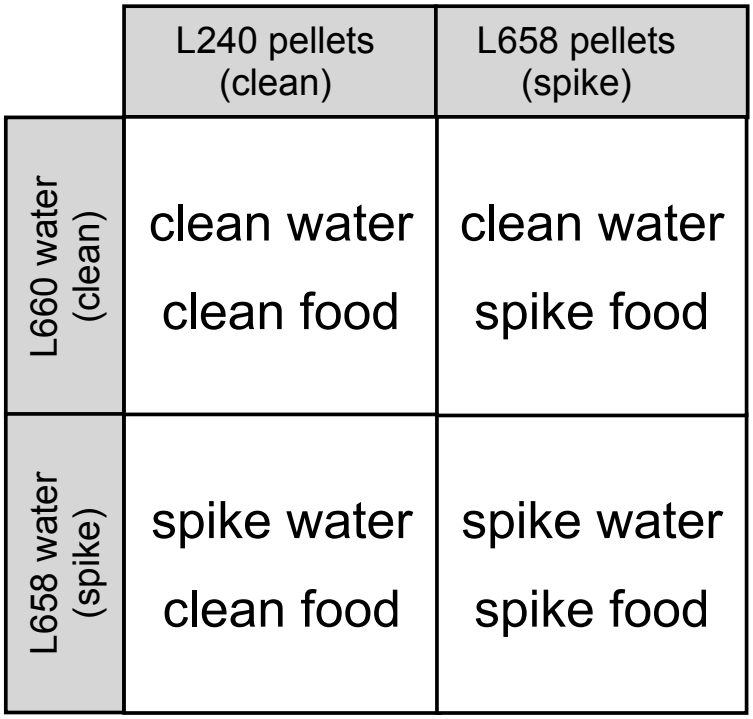

Figure 2.1 The 2-factorial experimental design. The treatments were designed to separate the sources of spike mercury to fish (food and water). Water was drawn from both clean (Lake 660) and spike (Lake 658) sources. Food pellets were made from zooplankton collected from clean (Lake 240) and spike (Lake 658) lakes. Each treatment was replicated three times, for a total of twelve 160 L tanks.

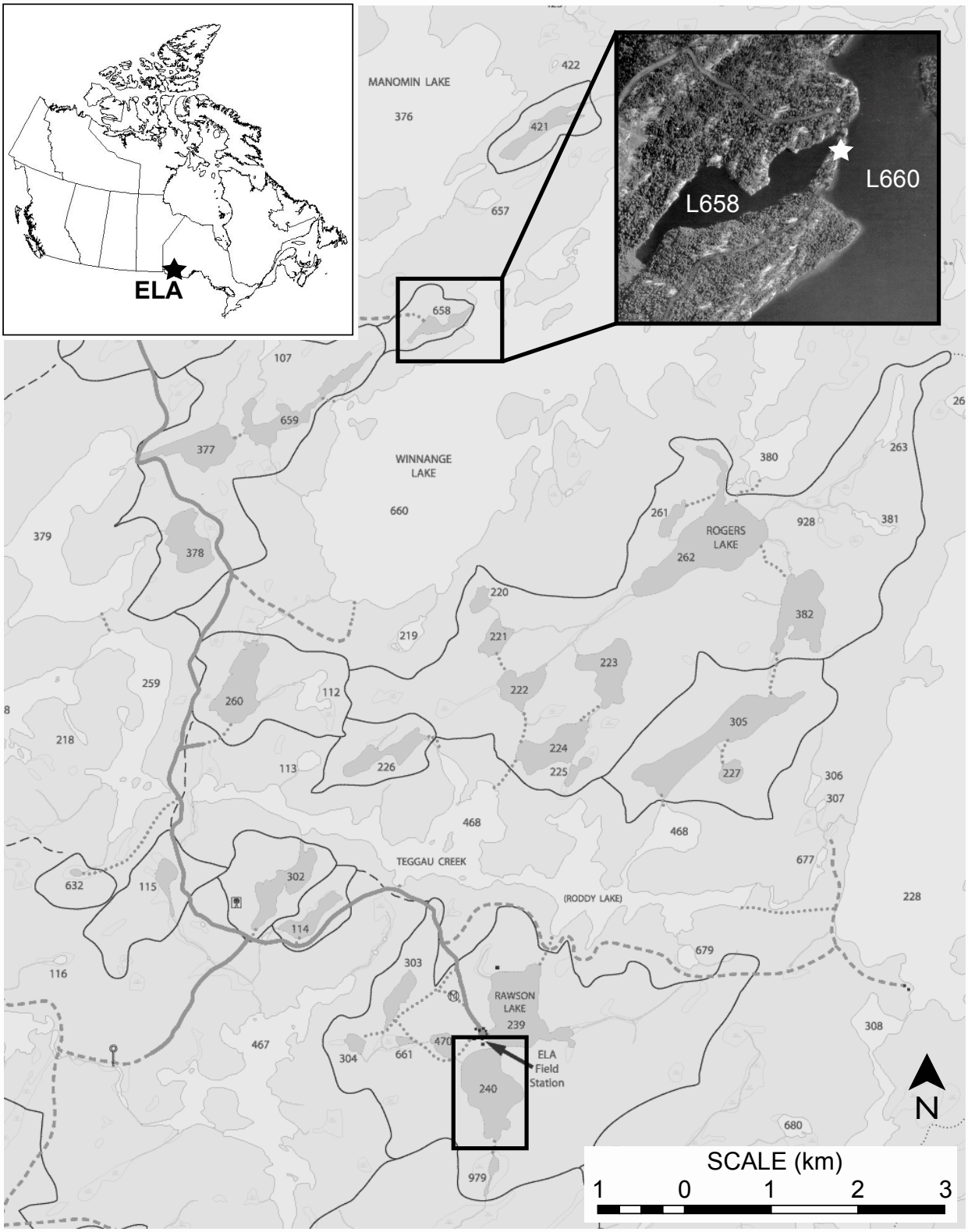

Figure 2.2 Map of the Experimental Lakes Area (ELA), ON, Canada. Location of the ELA in relation to the rest of Canada is shown on the upper left. Lake 658 (L658) and Lake 240 are indicated by black boxes. The white star on the aerial photograph of Lakes 658 and 660 (L660) denotes the location of the fish tanks.

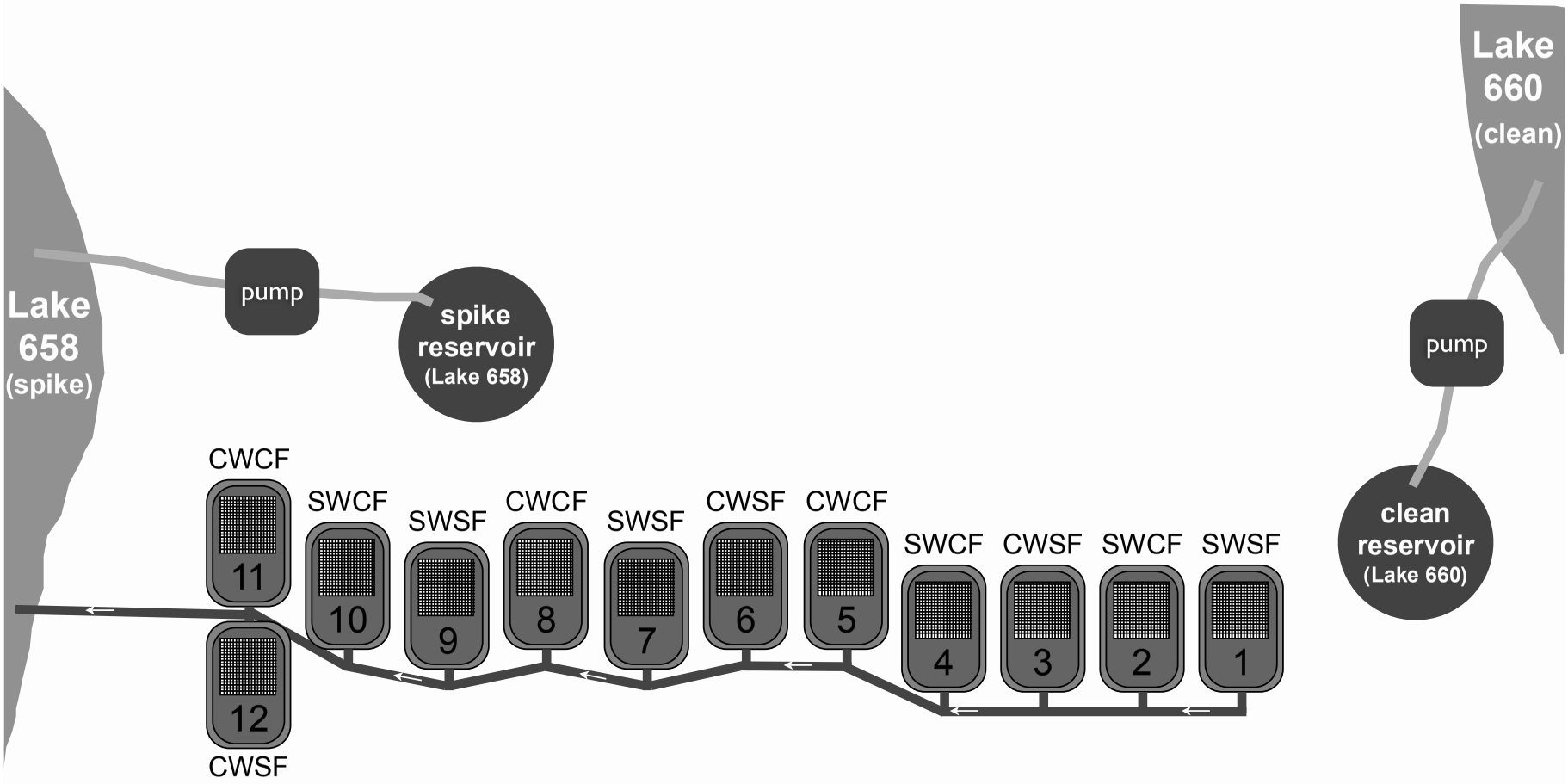

Figure 2.3 The location and setup of fish tanks between Lake 658 (spike water source) and Lake 660 (clean water source). Tanks were randomly assigned one treatment. All fish tanks were covered with plywood lids with mesh-covered windows to prevent predation. Fish tanks and reservoir tanks were insulated to moderate temperature fluctuations. Tanks were connected to a single drainage system that directed all waste water into Lake 658 to prevent any spike Hg from entering Lake 660.

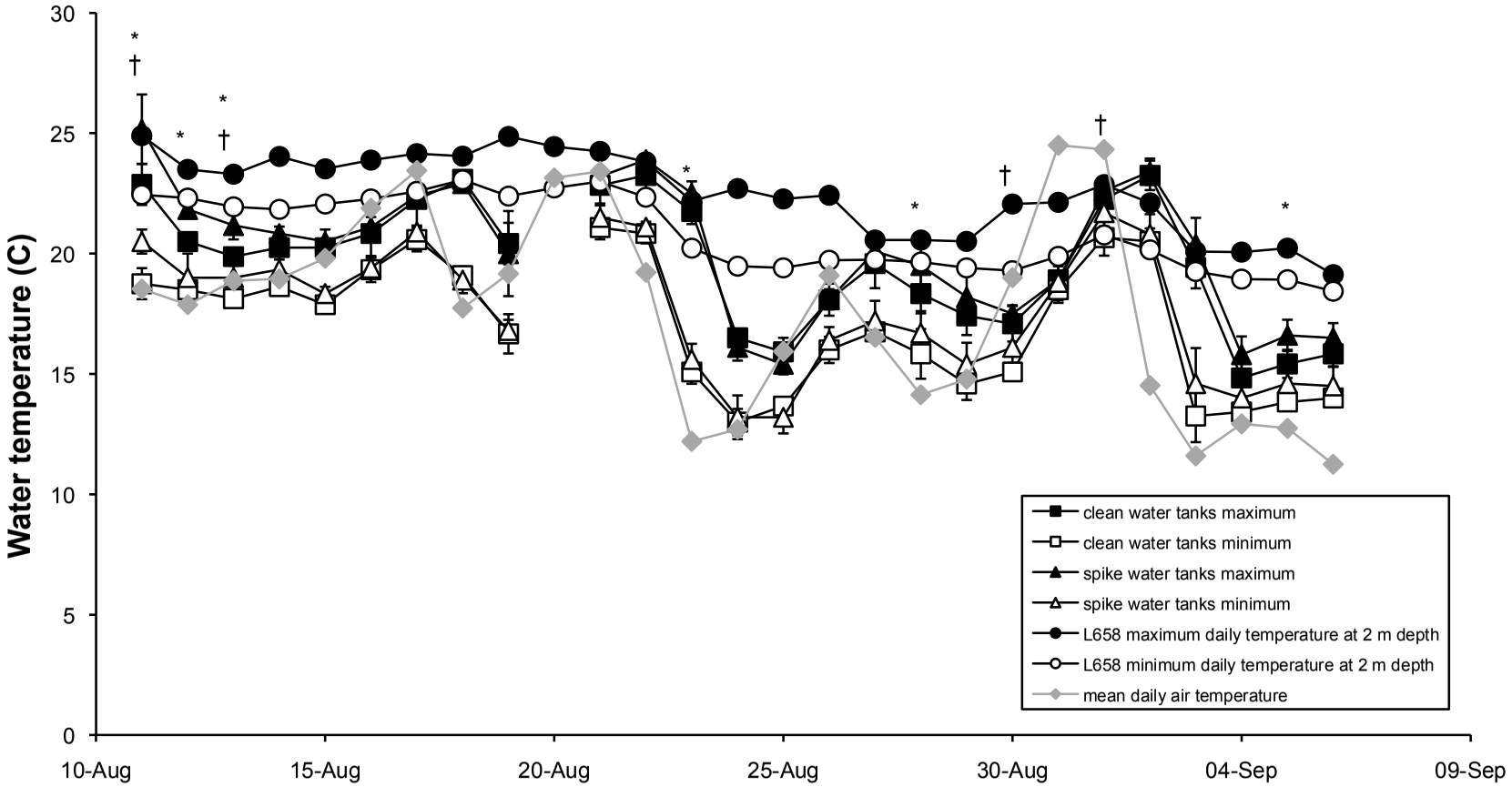

Figure 2.4 Mean daily maximum (closed circles) and minimum (open circles) water temperatures in tanks receiving spike (triangles) and clean (squares) water. Error bars indicate SEM. Mean temperatures for August 11-15 were calculated based on n=4 tanks for each water type (8 of 12 tanks had thermometers during this time). Mean temperatures for August 16 - September 6 were calculated based on n=5 tanks (spike) and n=6 tanks (clean). Water temperatures were never measured in tank 10 (spike water). Water temperatures were not available on August 20. Circles indicate daily maximum (closed circles) and minimum (open circles) Lake 658 surface water temperatures measured 2 m below the surface at the centre of Lake 658. Grey diamonds indicate mean daily air temperature collected at a raft anchored near the centre of Lake 658.

* significant difference between mean spike and clean maximum water temperatures

† significant difference between mean spike and clean minimum water temperatures

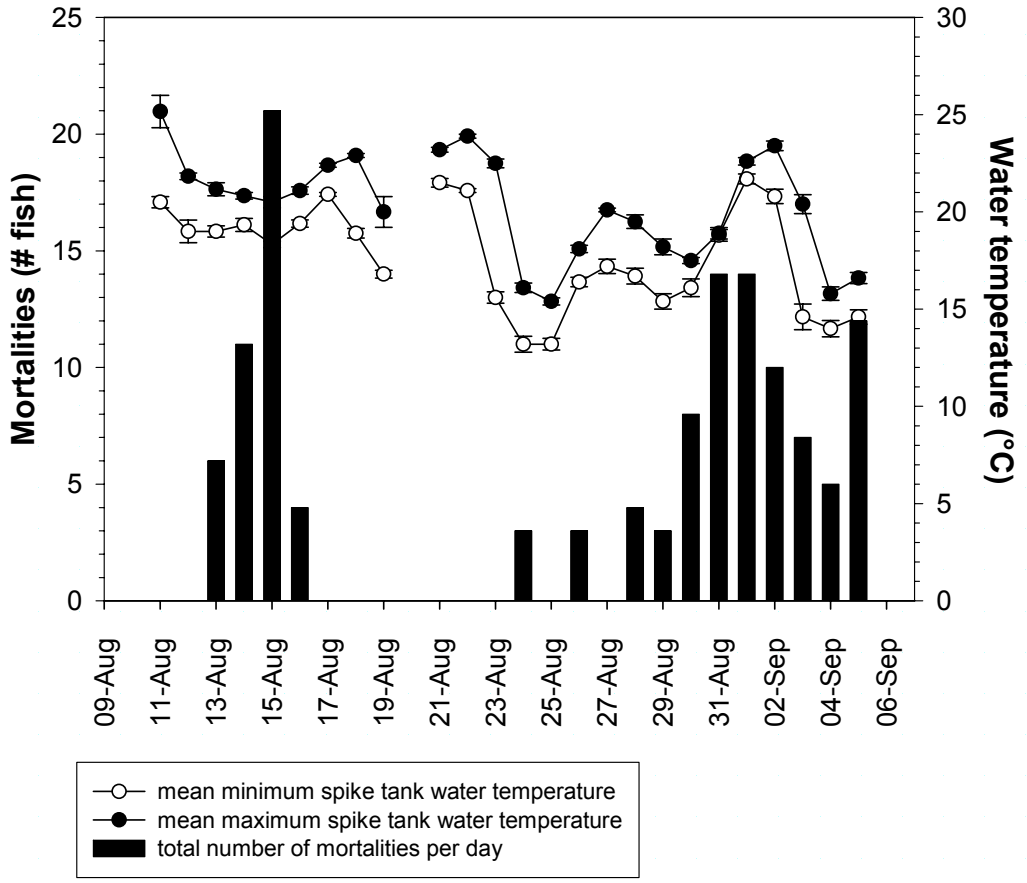

Figure 2.5 Total number of mortalities in all tanks on each day of the experiment. Fish were stocked in tanks on August 11 and were sacrificed on September 6. Mean spike water tank minimum and maximum temperatures are also shown (n=5 tanks; tank 10 did not have a thermometer). Clean water tank minimum and maximum temperatures did not differ significantly from mean spike tank water temperatures. Error bars represent SEM.

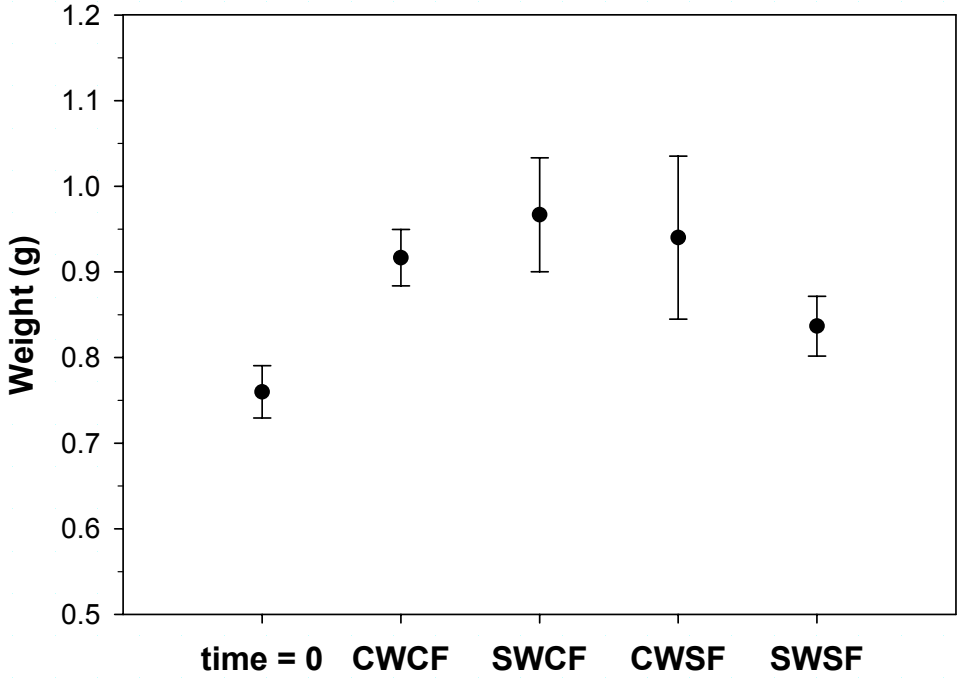

Figure 2.6 Treatment means of fresh weights of YOY yellow perch collected from Lake 240 at the start of the study (time = 0) and after 27 days in the experimental treatment tanks: clean water + clean food (CWCF), spike water + clean food (SWCF), clean water + spike food (CWSF), spike water + spike food (SWSF). Error bars represent SEM.

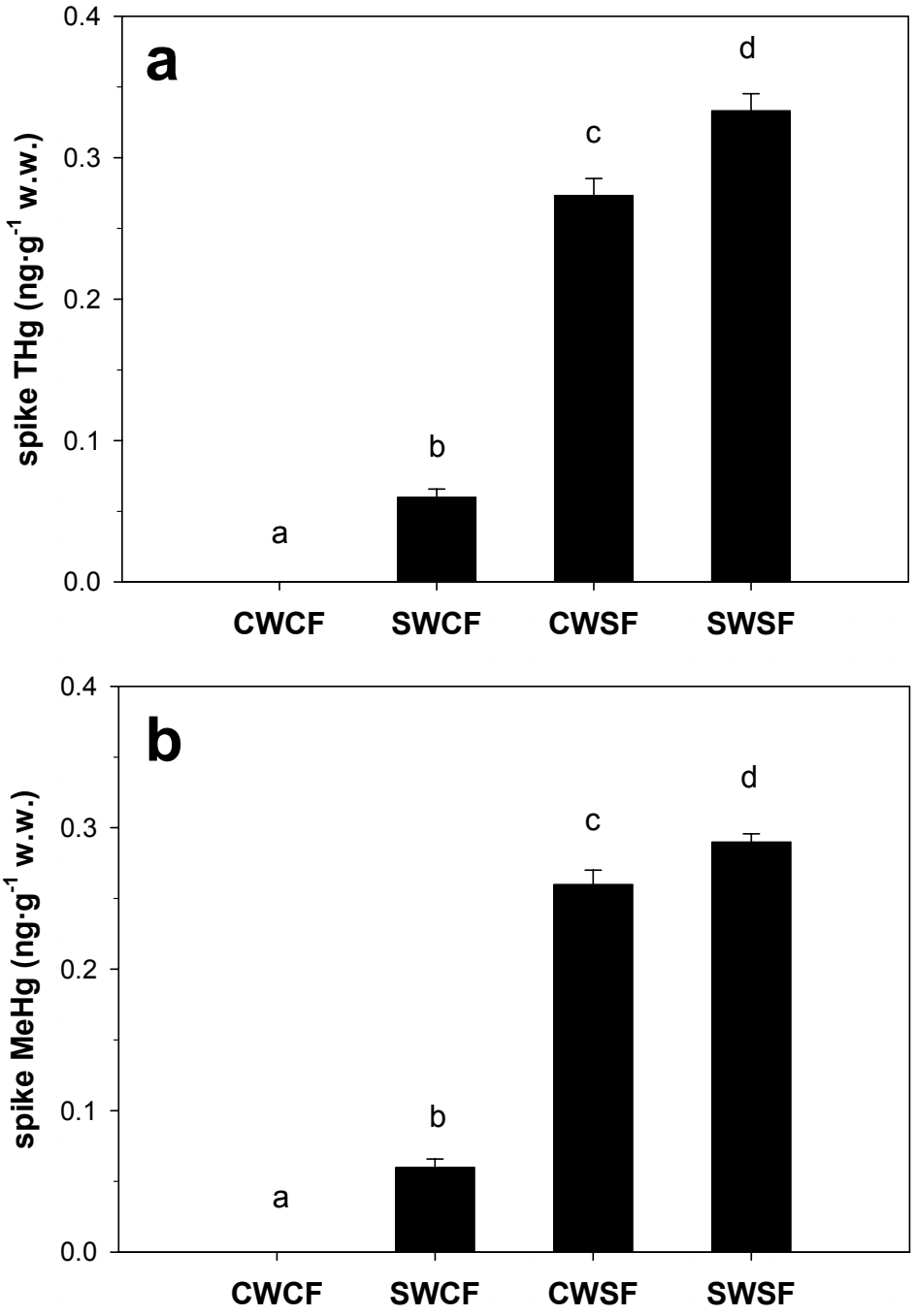

Figure 2.7 Mean of means concentrations of (a) spike total mercury (THg) and (b) spike methylmercury (MeHg) in whole YOY yellow perch from clean water + clean food (CWCF), spike water + clean food (SWCF), clean water + spike food (CWSF), and spike water + spike food (SWSF) treatments (n=10 for all treatments; see Table 2.2 for mean concentrations and n for each tank). Spike Hg was not detected in any fish from the clean water + clean food treatment. Error bars indicate SEM.

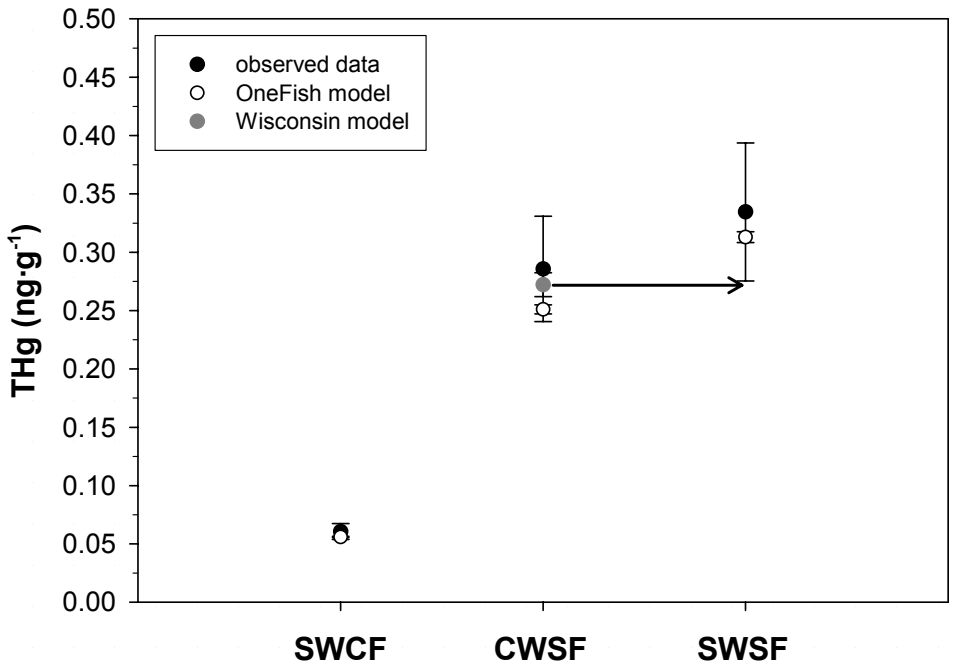

Figure 2.8 Mean YOY yellow perch spike total mercury (THg) concentrations predicted by the Wisconsin model (n=4; gray circle) and OneFish model (n=4 for each; open circles) and mean levels observed in the experiment (n=10 for each; black circles) for the spike water + clean food, clean water + spike food, and spike water + spike food treatments. Error bars represent SEM.

Chapter 3. Modelling the accumulation of mercury by yellow perch in a boreal aquatic food web

3.1 INTRODUCTION

Atmospheric deposition of anthropogenically-emitted mercury is the primary source of MeHg contamination to lakes located far from point-sources of Hg pollution (Hammerschmidt and Fitzgerald 2006; Lindberg *et al*. 2007). Inorganic mercury deposited to lakes from the atmosphere is transformed to MeHg by methylating bacteria in lake sediments and water (Rudd 1995; Jackson 1997) and is subsequently taken up by the food web (Cabana *et al*. 1994; Bowles *et al*. 2001). Organisms at the highest trophic levels such as sport fish typically exhibit the highest concentrations of MeHg in the food web (Fleming *et al*. 1995), which may exceed tissue MeHg levels considered fit for human consumption. The current Canadian market limit for MeHg in fish flesh is 0.5 μg/g (Health Canada 2007), and consumers are advised not to eat fish muscle containing more than 1.5 μg/g MeHg (Manitoba Water Stewardship 2007). Mercury contamination is the most common cause of fish consumption advisories in Ontario (Minstry of the Environment 2009).

Fish Hg levels have been shown to increase rapidly when subjected to enhanced atmospheric Hg deposition (Harris *et al*. 2007; Orihel *et al*. 2007; Orihel *et al*. 2008), and may be moderately (Hammerschmidt *et al*. 2006) or strongly (Orihel *et al*. 2007) correlated with atmospheric Hg loading rates. The United Nations Environment

Programme (UNEP) has proposed restrictions for industry and power generation facilities that aim to reduce global anthropogenic mercury emissions by 2013 (UNEP 2009), and should lead to the successful restoration of MeHg-contaminated fisheries (Jackson 1997; Harris *et al*. 2007; Lindberg *et al*. 2007). Being able to anticipate how quickly fish Hg levels will decline following reductions in anthropogenic emissions will be integral to emissions reduction strategies and will rely heavily on our understanding of how fish Hg concentrations are influenced by environmental Hg levels.

Mercury bioaccumulation models are commonly employed by managers and researchers to examine patterns of Hg accumulation in existing fish populations and to predict future Hg concentrations in fish based on environmental data. They are useful for identifying processes that contribute to elevated Hg levels in fish and may be used to estimate the effects of environmental disturbances (Rogers 1994). The theory behind bioaccumulation models assumes that fish Hg levels are governed by a set of environmental variables and that the linkages between these variables and resulting fish Hg concentrations may be quantified (Harris and Snodgrass 1993; Korhonen *et al*. 1995; Trudel and Rasmussen 1997; Trudel and Rasmussen 2000; Rennie 2003). Bioaccumulation models use laboratory- and field-derived mathematical equivalents of these linkages to predict Hg levels in fish based on inputs of real environmental data (Post *et al*. 1996; Trudel and Rasmussen 1997). The equations within the models are based on fish bioenergetics and Hg kinetics principles and are designed to mimic consumption, growth, and contaminant accumulation in natural fish populations (Hanson *et al*. 1997). The general bioenergetics

equation of existing Hg bioaccumulation models is (Hewett and Johnson 1987; Hanson *et al*. 1997):

consumption = respiration + waste + growth

Consumption and respiration rates are influenced by various environmental and physiological parameters, such as water temperature and oxygen concentrations, and together determine the amount of Hg a fish is exposed to. By combining consumption and respiration rates with assimilation efficiencies and Hg concentrations in prey and water, models predict fish Hg accumulation. The general form of the equation for mercury kinetics is (Harris and Bodaly 1998):

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

Some mercury bioaccumulation models include waterborne mercury as a source of Hg to fish (e.g., Norstrom *et al*. 1976; Post *et al*. 1996; Harris and Bodaly 1998), while others assume that all fish Hg is derived from their food (e.g., Hanson *et al*. 1997; Trudel and Rasmussen 2006). In the latter case, water Hg concentrations and assimilation efficiencies are not required inputs for the models.

The use of Hg bioaccumulation models has contributed greatly to the general understanding of mercury dynamics in aquatic ecosystems (Rodgers 1994). However, it is important to verify that models accurately represent natural populations and processes if they are to be used as management tools (Van Walleghem *et al*. 2007). Norstrom *et al*. (1976) stress that our understanding of fish energetics and Hg kinetics determine a model's accuracy. Luoma (1983) noted that relationships in models that attempt to estimate trace metal concentrations in biota are often based data from lab experiments,

which may have limited applicability to the natural environment. Rennie (2003) emphasizes that the accuracy of a model's output depends on the strength of the input data, noting that biases in input data may result in under- or overestimates of fish Hg levels. In an evaluation of the Wisconsin Fish Bioenergetics 3.0 model (Hanson *et al*. 1997) and the Trudel and Rasmussen (1997) mercury mass balance model, Van Walleghem *et al*. (2007) found that both overestimated the rate of Hg loss in age-1 yellow perch. It was suggested that both models be refined as they did not accurately represent Hg elimination in a natural setting. Given this finding, it is possible that Hg models also depict Hg uptake inaccurately.

Previous studies have concluded that water is not an important source of Hg to fish in lakes with low water Hg concentrations (Becker and Bigham 1995; Rennie 2003; Wang and Wong 2003; Trudel and Rasmussen 2006). Laboratory- and field-based studies of Hg in fish frequently dismiss water as a source of Hg (e.g. Bowles *et al*. 2001, Rennie 2003, Dittman and Driscoll 2009), and several prominent Hg bioaccumulation models account for Hg uptake only from food (e.g. Hanson *et al*. 1997, Trudel and Rasmussen 2001). As discussed in Chapter 2, it is possible that models that exclude water as a source of Hg to fish may underestimate fish Hg levels by 10-21%. Additionally, when modelling accumulation of Hg, a user may set elimination to zero, or may use elimination rate constants to predict Hg depuration. Previous research has indicated that several models designed to predict Hg accumulation in fish overestimate elimination (including the Wisconsin model), which may result in inaccurate estimates of fish Hg levels (Van Walleghem et al. 2007; Madenjian and O'Connor 2008). If models are to be used by

industry and ecosystem managers to assist in making decisions regarding Hg emissions to ecosystems, it is essential that they reflect uptake and elimination in the natural environment. Determining the importance of including water as a source of Hg in bioaccumulation models and assessing model performance under a variety of elimination scenarios will provide commentary on the ability of existing accumulation models to predict Hg levels in fish, and will yield data to improve the accuracy of these models.

Lake 658 at the Experimental Lakes Area, ON, is an ideal location to test fish Hg bioaccumulation models. As discussed in Chapter 2, Lake 658 is the site of the Mercury Experiment To Assess Atmospheric Loading In Canada and the United States (METAALICUS). METAALICUS used enriched stable isotopes of Hg to track the movement of newly-deposited Hg through terrestrial and aquatic ecosystems (Harris *et al*. 2007). Separate enriched stable-isotopes of Hg ("spikes") were applied to the water of Lake 658, the surrounding upland areas, and a wetland, during the open-water season for 7 years (2001-07) (Sandilands *et al*. 2008). Water, zooplankton, and yellow perch were sampled regularly throughout the summer of each year, creating a complete picture of spike and ambient Hg concentrations in fish, their underlying food web, and water. Combined with detailed environmental records for parameters such as water temperatures and oxygen concentrations, these data provide a complete, long-term data set that is robust for the testing of Hg bioaccumulation models.

In the METAALICUS project, spike Hg represents a pool of recently-added Hg which was deposited to the system over a seven-year period. Ambient Hg, on the other hand,

represents all of the Hg that has entered the system naturally over time, a portion of which may be new, and much of which is bound in lake sediments and may be slowly released over time (Luoma 1983). Reductions in atmospheric emissions of Hg will reduce new additions of Hg to ecosystems (represented in this study by spike Hg), but will not affect Hg already present in the system (represented by ambient Hg). Newly-deposited Hg appears to be the source of the majority of Hg available to aquatic food webs (Hrabik *et al*. 2002; Hammerschmidt *et al*. 2006). By tracking the behaviour of spike Hg in Lake 658, it will be possible to assess how food web Hg concentrations may respond to changes in atmospheric deposition rates. Additionally, the examination of trends in ambient Hg may provide commentary about the availability of older Hg to food web cycling.

I used the 7-year METAALICUS data set to compare the predictive abilities of two fish Hg bioaccumulation models, Wisconsin Fish Bioenergetics 3.0 (Hanson *et al*. 1997) and OneFish (Harris and Bodaly 1998), focusing on young-of-year (YOY) yellow perch. YOY yellow perch have been used extensively in bioenergetics and Hg accumulation studies, making them ideal candidates for this research (e.g., Rogers and Qadri 1982; Post *et al*. 1996; Harris and Bodaly 1998). Yellow perch are abundant and widely dispersed in many North American water bodies, their biology is well-understood, they are an important commercial and game fish, and they contribute to Hg accumulation in higher trophic levels as they are consumed by many larger sport fish species (Power and van den Heuvel 1999; Essington and Houser 2003). In this study, YOY fish are an ideal target group because they accumulate all of their Hg during the year they are collected. By

sampling YOY perch from their first appearance in the littoral zone until the end of the season it is possible to generate Hg profiles that nearly span the whole life of fish in the cohort. This would not be possible if older fish were used.

The Wisconsin and OneFish models were chosen for this study because they are based on identical bioenergetics equations but make differing assumptions regarding Hg accumulation. The Wisconsin model assumes all Hg uptake comes from food, while the OneFish model allows for uptake from both food and water. Both models are discussed in detail in Appendix 1 and Chapter 2. The Wisconsin model was originally created strictly as a bioenergetics model but had a contaminant accumulation option added to version 3 of the software. It has been used widely to examine both bioenergetics and contaminant accumulation in many fish species, including yellow perch (Bajer *et al*. 2003; Van Walleghem 2007a; Drouillard *et al*. 2009), largemouth bass (MacRury *et al*. 2002), northern pike (MacRury *et al*. 2002), lake whitefish (Madenjian *et al*. 2006), smallmouth bass and lake trout (Lepak *et al*. 2009b). The contaminant accumulation equations in the Wisconsin model are not specifically designed for Hg accumulation, but instead predict concentrations of a number of bioaccumulative contaminants, including MeHg and polychlorinated biphenyls (PCBs) (Hanson *et al*. 1997). The OneFish model, on the other hand, was designed specifically as a Hg accumulation model, and has been used to predict Hg concentrations in yellow perch (Harris and Bodaly 1998; Van Walleghem 2006), walleye (Harris and Bodaly 1998), and northern pike (Van Walleghem 2006).

In this chapter I use the Wisconsin and OneFish bioenergetics models to predict mercury accumulation by YOY yellow perch in Lake 658 during the open water seasons of 2001-07. The main strength of this study lies in the data set provided by METAALICUS. The use of both enriched-stable isotopes of Hg and ambient Hg, the long-term nature of the experiment, and the richness of the data set are unmatched in previous bioaccumulation modelling exercises (e.g., Kitchell *et al*. 1977; Post *et al*. 1996; MacRury *et al*. 2002; Madenjian *et al*. 2006). The objectives of this study are i) to compare fish Hg estimates generated by the two models to determine whether it is important to include waterborne Hg as a source of Hg to fish in bioaccumulation models, ii) to determine whether existing bioaccumulation models can predict the levels of Hg observed in Lake 658 under a variety of dietary and Hg elimination conditions, and iii) to determine which model parameters have the most influence over model output.

3.2 METHODS

3.2.1 Study location

This study took place at Lake 658 (49° 39′ 14″ N, 93° 43′ 18″ W) at the Experimental Lakes Area (ELA) in northwestern Ontario, Canada (Figures 2.1 and 3.1). The ELA is isolated from point sources of Hg emissions and has a low annual ambient Hg deposition rate of 3-4 μg m^{-2} yr^{-1} (Harris *et al*. 2007). As discussed in Chapter 2, Lake 658 is the site of the METAALICUS) project, in which inorganic "spike" Hg enriched in the stable isotope ^{202}Hg was mixed into the surface waters of Lake 658 every two weeks during the open water seasons of 2001-07 at an annual loading rate of 22 μg m^{-2} yr^{-1} (Sandilands *et*

al. 2008). Previous studies examining Hg cycling and accumulation in aquatic systems have used enriched stable isotopes of Hg successfully to distinguish between experimentally-applied and naturally-deposited Hg (ambient Hg) (e.g., Pickhardt *et al*. 2006; Paterson *et al*. 2006; Harris *et al*. 2007; Orihel *et al*. 2007).

3.2.2 Water temperatures

Lake 658 water temperature data was collected for the METAALICUS study with a thermistor suspended 2 m below the surface of the water (Flett Research, Winnipeg, MB) (Figure 3.2). Temperature was assumed to shift in a linear fashion between sampling periods, so all data points were connected with straight lines to interpolate water temperatures between sampling days. It would have been advantageous to have more frequently collected temperature data; however, these data are what was available to me at the time. Yellow perch appear to spawn when water temperatures reach 12 °C, so I estimated yellow perch spawning dates by determining the dates on which water temperatures in Lake 658 reached this temperature. Incubation times for yellow perch are dependent on overall water temperatures and on having a certain number of days that reach a given water temperature (degree days; e.g. 90.9-104.2 degree days above 4.9 °C) (Hokanson and Kleiner 1974). As water temperatures in this study were collected on a bi-weekly basis, it was not possible to examine degree-days in Lake 658. For consistency among simulations, hatch dates were estimated by adding 12 days to each spawning date (Table A.3).

3.2.3 Collection and processing of zooplankton, Chaoborus, and water samples

The MeHg concentrations in zooplankton and *Chaoborus* used as model inputs in this study were from samples collected in 2001-07 in the pelagic zone of Lake 658 as part of routine METAALICUS sampling as described in Harris *et al*. (2007). Briefly, a 0.5 m plankton net (150 μm mesh) was hauled vertically through the whole water column at centre buoy in Lake 658 (Figure 3.1) to create a composite sample, which was placed on ice in the field and frozen (-20 °C) upon return to the field station. As YOY perch were captured in the littoral zone in this study, it is likely that they also feed on littoral zooplankton and benthic invertebrate species which would not be represented in the samples collected at the centre of Lake 658. I sampled zooplankton in the pelagic and littoral zones to determine whether concentrations of Hg in zooplankton differed among locations. Zooplankton were collected from the centre of the lake as described above, and from the littoral zone by completing horizontal tows approximately 1 m below the surface of the water, 1-2 m from shore in three separate areas where yellow perch had been observed. These samples were placed in WhirkPak bags on ice immediately after collection and were frozen (-20 °C). Zooplankton samples were freeze-dried in a Lyph-lock 12-L freeze dry system until a constant weight was achieved (approximately 96 h). Two replicates from each sampling location were analyzed for THg and MeHg species by inductively coupled plasma mass spectrometry (ICP-MS) at Trent University, Peterborough, ON (Dr. H. Hintelmann, Department of Chemistry).

Water samples were collected from Lake 658 every two weeks during the open water seasons of 2001-07 using a similar sampling protocol to that described in Chapter 2 for

water samples. See Harris *et al*. (2007) for detailed sample collection and processing protocols. Water samples were analyzed for THg and MeHg species by ICP-MS at Trent University (Dr. H. Hintelmann, Department of Chemistry).

3.2.4 Collection and processing of yellow perch samples

Yellow perch are a common focus species for bioenergetics and contaminant transfer research as they are widely dispersed in lakes and rivers in North America, their biology is well understood, and they are prey for many game fish species (Post et al. 1996; Power and van den Heuvel 1999; Essington and Houser 2003). Yellow perch spawn in the littoral zones of lakes in the spring at water temperatures of approximately 12 °C (Guma'a 1978; Kayes and Calbert 1979; Ciereszko *et al*. 1997). The time between spawn and hatch varies depending on water temperatures and degree days, but appears to centre around 12 days. Warmer water temperatures may trigger shorter development times, while cooler temperatures prolong the period. Following hatch, yellow perch larvae migrate to the pelagic zone where they remain until they have fully metamorphosed (Post and McQueen 1988). Yellow perch are gape-limited predators (Keast 1977; Graeb et al. 2006), subsisting on a diet of zooplankton when they are small (<50 mm fork length), and shifting to larger prey items such as benthic invertebrates and small fishes as they grow (Mills and Forney 1981; Schael *et al*. 1991). The biology and ecology of yellow perch are discussed thoroughly in Chapters 1 and 2.

YOY yellow perch were collected from Lake 658 monthly during the open water seasons of 2001-07 (2001: n=11; 2002: n=15; 2003: n=9; 2004-07: n=10 per year). Perch were

captured with 6.25 and 8 mm mesh gillnets (1 m × 15 m, Lundgrens Fiskredskap, Sweden) set for short durations (<20 min) in the littoral zone (<3 m depth; Figure 3.1). Immediately upon removal from the net, perch were euthanized in an overdose bath of 0.25 $g \cdot L^{-1}$ tricaine methanesulfonate (TMS; Argent Chemical Laboratories, Inc., Redmond, WA, USA). Fish were left in the bath for 10 min following the cessation of opercular movements. Euthanized perch were transported back to the ELA in WhirlPak® bags buried in ice in a cooler. Upon arrival, fish were processed for basic biological information, including fork length and weight. Stomachs were removed from 10 individuals on each sampling day and preserved in 95% ethanol (Commercial Alcohols Inc., Brampton, ON, Canada) in 15 mL glass vials sealed with rubber-stoppers (Fisher Scientific Company, Ottawa, ON). All dissecting tools were cleaned with 95% ethanol and KimWipes (Kimberly Clark Professional, Roswell, GA, USA) between each sample. Fish were frozen in individual, labelled WhirlPak® bags at -20 °C.

Perch were prepared for THg analyses in the fall of each year following the field season. Ten fish were selected by weight for each sampling date, with chosen individuals spanning the range of wet weights observed in fish collected on that date. Samples were handled using mercury clean techniques with Teflon or stainless steel tools (Bloom 1992; Van Walleghem 2006). Prior to use, Teflon tools were acid-washed for 24 h in a 10% HCl bath (12.6 L milliQ water with 1.4 L concentrated HCl, Fisher Scientific), and stainless steel tools were washed in hot soapy water and then rinsed with 95% ethanol. Tools and surfaces were cleaned with 95% ethanol and KimWipes between each sample. Care was taken to ensure that the fish remained as frozen as as possible during processing

to avoid inconsistent wet weights among samples. Approximately 0.2 g of muscle tissue was removed from each fish and placed in a 22 mL acid-washed glass vial with a Teflon-lined lid (National Scientific Company, Rockwood, TN). Skin, bones and parasites were removed from the muscle samples prior to being placed in the vials. Samples were sealed and frozen in the vials immediately following processing.

All muscle samples were analyzed for THg content (ambient and spike Hg) at Trent University, Peterborough, ON (Dr. Holger Hintelmann, Department of Chemistry). Concentrations of spike and ambient mercury were measured with ICP-MS (Chapter 2, section 2.2.7).

3.2.5 Analysis of yellow perch diet composition

I analyzed the stomach contents of 10 YOY yellow perch collected in August of each year (60 full stomachs, 10 empty stomachs) and of 10 YOY yellow perch collected on each sampling date in 2001 (57 full stomachs, 3 empty stomachs). The multi-year analysis allowed me to track differences in consumption among years, while the 2001 samples identified dietary shifts that occurred during the summer season as the fish grew. All gut content analyses were performed using a Leica MZ8 binocular dissecting microscope at 16x magnification (Leica Microsystems, Inc., Richmond Hill, ON).

For gut content analysis, I removed each stomach from the vial and placed it in a shallow glass dish under the microscope. Using two pairs of fine-pointed forceps (Dumont, Switzerland), I separated the stomach from the rest of the tissue by severing the intestine

just posterior to the pyloric sphincter. I analyzed all material found within the stomach and esophagus and discarded the remainder of the intestine (George and Hadley 1979; Little *et al*. 1998). I opened the stomach and esophagus with forceps, removed all material from inside, and added several drops of 95% ethanol. I separated all prey items according to class (Copepods, Ostracoda, Insecta (miscellaneous)), order (Cladocera, Amphipoda), suborder (Hydracarina), or family (Chironomidae, Chaoboridae), and then counted the number of individuals present for each type. After recording the count number for a group, I transferred all individuals of that group to a pre-weighed 44 mm aluminum weigh boat (Fisher Scientific Company) labelled with the fish identification number and prey type. This procedure was repeated for each prey type. Glass dishes and forceps were cleaned with 95% ethanol and KimWipes between samples.

The prey items in weigh boats were dried in an oven at 60 °C for 24 h until a constant weight was achieved. When samples were dry, the boats and samples were weighed together to give a dish + sample weight. Sample weights of each prey type for individual fish were determined by subtracting dish weight (pre-weighed) from dish + sample weight. Prey weights, counts, and number of occurrences were used to calculate the Relative Importance index (RI) of prey types for fish from August of each year and for fish in four weight categories throughout the 2001 season. These calculations were made using equation 3.1 and are described below.

3.2.6 Mercury bioaccumulation modelling

I compared mercury accumulation observed in YOY yellow perch in Lake 658 to predictions of fish Hg concentrations generated by the Wisconsin Fish Bioenergetics 3.0 model (Hanson *et al*. 1997) and the OneFish model (Harris and Bodaly 1998). The Wisconsin model assumes that all fish Hg is derived from food, while the OneFish model accounts for uptake from both food and water. These models are described fully in Appendix 1 and Chapter 2. I used environmental information from Lake 658 as input data for the two models, including zooplankton MeHg concentrations, water temperatures, and water Hg and oxygen concentrations. I completed model simulations for each year individually, with each simulation designed to represent the life of the perch from an estimated hatch date until the end of the summer season (Figure 3.2 and Table A.3).

I ran both models under two sets of conditions: (1) zooplankton diet, no elimination; (2) zooplankton + *Chaoborus* diet ("combination" diet), elimination based on different elimination constants. Type 1 simulations were designed to examine the differences between the two models with particular focus on the inclusion or exclusion of water as a source of Hg to fish. Type 2 simulations were designed with a set of inputs that were more representative of how the models would be used by an ecosystem manager, and explored the effects of changing the diet and introducing elimination into the models. Type 1 and 2 simulations were conducted for all sampling years and are outlined below.

3.2.6.1 Type 1 simulations

Type 1 simulations were conducted with a diet of entirely zooplankton and with no elimination of Hg from the fish. I completed 5 separate simulations for each year (2001-2007) for each mercury type (spike and ambient) using both models, giving a total of 140 simulations (70 per model). I completed a range of simulations for each year so that I had a sample of predicted values to compare statistically to the observed data. The 5 simulations were designed to achieve a variety of end weights that spanned the range of weights observed in perch on the last sampling day of each year. I achieved this in the Wisconsin model by assigning different end weights to each simulation and in the OneFish model by adjusting the kt value (growth rate) (Table A.4). Inputs used in the Wisconsin model are presented in Table A.5, and the inputs for the OneFish model are shown in Table A.6.

3.2.6.2 Type 2 simulations

To examine the impacts of Hg elimination and to introduce benthic invertebrates to the diet of simulated fish, I completed a second set of model simulations (type 2). Elimination of Hg by fish has been shown to be limited, with recent estimates of the half-life of MeHg in fish of 398 d (Madenjian *et al*. 2008) and 489 d (Van Walleghem *et al*. 2007). Since both models include an elimination option, however, it is important to assess the effects of introducing elimination into a model simulation. Since both models provide suggestions for Hg elimination rates and because the Wisconsin model allows for inputs of multiple types of prey, a model user following model setup instructions would likely incorporate both a mixed diet and some Hg elimination in their simulations. As

such, type 2 simulations were designed to emulate "standard" inputs that were representative of real-world model use and to provide commentary on the ability of models to predict fish Hg concentrations accurately under these standard conditions. Type 2 simulations were run with a combination diet of 75% zooplankton and 25% *Chaoborus* spp., and incorporated published Hg elimination rates. Preliminary dietary analyses of Lake 658 YOY yellow perch suggested that zooplankton constituted approximately 70-80% of the diet, and the rest consisted of benthic invertebrates. Hg concentrations were collected for *Chaoborus* in Lake 658 as part of the METAALICUS project (Dr. M. J. Paterson, Freshwater Institute, Winnipeg, MB), so they were used to represent the benthic invertebrate portion (25%) of the combination diet. I used the median end weights and kt values from type 1 modelling scenarios as the growth input for these simulations, completing only one simulation per year for each scenario. I completed three type 2 simulations in both models for each year, each representing a different scenario: (i) combined diet, no elimination; (ii) combined diet, elimination rates as suggested by Madenjian and O'Connor (2008); (iii) combined diet, elimination rates as suggested by the models. The calculations used in these simulations are described in Appendix 1 and the inputs used for all scenarios are listed in Tables A.7 (Wisconsin) and A.8 (OneFish).

3.2.7 Sensitivity analysis of models

I completed sensitivity analyses for both models to determine the magnitude of influence each input parameter has on the predicted fish Hg concentrations (Salacinska *et al*. 2010; Thogmartin 2010). I selected a set of "base" input values to represent a typical scenario

and used these values to run a "base" simulation in each model. I then ran additional simulations by manipulating the values of each input parameter one at a time. I compared fish Hg concentrations generated in the base simulation with those from subsequent simulations to determine the relationships between magnitude shifts of each input parameter and changes in model output. The parameters that I manipulated for the Wisconsin model are: water temperature, prey Hg concentration, prey energy density, and AE_f. For the OneFish model, I manipulated water temperature, prey Hg concentration, prey energy density, AE_f, water Hg concentration, and AE_w. All inputs remained constant over time for each simulation, except for temperature, which fluctuated every 30 days for the base simulation and two of the manipulations (Tables A.10, A.11).

3.2.8 Calculations

Relative Importance Index

The Relative Importance index (RI) was used to determine overall yellow perch diet composition. This calculation was first described by George and Hadley (1979) and takes into account the number of individual diet items of each prey type present in the stomachs, the weights of these items, and the percent of analyzed stomachs in which each prey type occurs. Many estimates of fish diet composition rely solely on the total weight of prey items or the number of prey items present. RI combines both of these parameters with the percent of analyzed stomachs in which the prey types occur to produce a stronger representation of fish diet composition than any one parameter can generate

alone (Wallace 1981; Little *et al*. 1998). RI uses the absolute importance (AI) of each prey type:

$$(3.1) \quad AI_i = \%O_i + \%N_i + \%W_i$$

Where $\%O_i$ (% occurrence) is the percentage of stomachs that contain prey type *i*; %N (% number) is the number of stomachs that contain prey type *i*; $\%W_i$ (% weight) is the percentage that the total mass of prey type *i* contributed to the total mass of food in all stomachs.

RI of prey type *i* is then calculated with the following equation:

$$(3.2) \quad RI_i = 100\ AI_i / \sum_{i=1}^{n} AI_i$$

Combination diet

The combination diet used in type 2 modelling simulations consisted of 75% zooplankton and 25% *Chaoborus*. Zooplankton and *Chaoborus* MeHg concentrations were input directly into the Wisconsin model. As the OneFish model only allows for one input of prey energy density and one of prey Hg concentration, I combined the energy densities and Hg concentrations of zooplankton and *Chaoborus* to yield single input values for the combination diet (Table A.8). These calculations are discussed in detail in Appendix 1.

3.2.9 Data analysis

Statistical tests were performed using Statistica v.5.5 (StatSoft, Inc.) and Sigma Plot v.8.0 (Systat Software Inc.). I used one-way analysis of variance (ANOVA) to examine differences in mercury concentrations observed in Lake 658 and predicted by the two models for each year (objective 1), and to compare C and N stable isotope levels among

organisms in the Lake 658 food web. Significant differences were investigated with Tukey's honestly significant difference post-hoc test. I used linear regression to compare concentrations predicted by the two models (objective 1) and to compare modelled concentrations to observed concentrations (objective 2), and investigated these comparisons further by testing regression lines against a 1:1 line. Linear regression was also used to relate yellow perch weight to muscle Hg concentration, to relate $\log_{10}$-transformed spike Hg concentrations to $\log_{10}$-transformed ambient Hg concentrations in perch and zooplankton, and to explore the relationships between various input variables and resulting changes in fish Hg concentrations in the sensitivity analyses of the two models (objective 3).

F-tests were performed to test the significance of all regressions. I rejected the null hypothesis (H_0: there is no relationship between two variables) if the *F* statistic was significant at α=0.05. Two-tailed *t*-tests were performed to test whether the slopes (b_1) of regression lines were significantly different from 1. A slope of 1 signifies that the relationship between the variables is linear and directly proportional. If the *t* statistic was not significant, the slope of the relationship was assumed to be equal to 1, indicating that i) the relationship between log-transformed variables was proportional and linear (e.g., spike versus ambient Hg concentrations), and ii) pairs of values in populations compared on axes that cross at (0,0) were not different from one another (e.g., predicted versus observed Hg concentrations). Bonferroni corrections were performed to account for multiple comparisons among predicted and observed data. Observed spike and ambient Hg datasets were each compared to modelled data 8 times (outlined in Table 3.3), so *t*-

tests were considered significant at $\alpha/8$ where $\alpha=0.05$. The new significance level was 0.006. All plots of $\log_{10}$-transformed variables were scaled so that a slope of 1 would have an angle of 45 degrees, while graphs of untransformed variables were prepared so that x- and y-axes met at (0,0) and a slope of 1 had an angle of 45 degrees.

3.3 RESULTS

3.3.1 Spawn and hatch dates

Spawn and hatch dates were estimated for YOY yellow perch in Lake 658 for each sampling year based on water temperatures (Figure 3.2, Table A.3). May and June water temperatures varied greatly among years, resulting in estimated hatch dates as early as May 17 and as late as June 11. Yellow perch hatched in May of years that had warm spring water temperatures, including 2007 (May 17), 2006 (May 21), 2003 (May 23), and 2001 (May 24). Perch hatched in June in years with cooler springs, including 2005 (June 2), 2002 (June 7), and 2004 (June 11). Estimated hatch dates were used as starting points for modelling simulations each year to simulate fish Hg accumulation during the first few months of life.

3.3.2 Diet

Fish diet composition is an important input parameter in Hg bioaccumulation models. The diet of YOY yellow perch in Lake 658 is dominated by zooplankton species (cladocerans, copepods), with amphipods, chironomids, chaoboridae, ostracods, hydracarina, and miscellaneous insecta making up the remainder of the diet. For yellow

perch analyzed in August of each sampling year, zooplankton made up 79.5% of the diet, chironomids 12.8%, amphipods 6.1%, and miscellaneous insecta, *Chaoborus*, and ostracods constituted less that 1% each (Figure 3.3). Yellow perch collected on all sampling dates in 2001 were divided into whole-body categories of 1.0-1.9 g; 2.0-2.4 g; 2.5-2.9 g; 3.0-3.4 g to examine size-related ontogenetic dietary shifts. Length groupings of the same individuals were 48-58 mm; 58-62 mm; 63-68 mm; 65-69 mm. As with the fish collected in August of each year, diets of all weight classes were dominated by zooplankton species (Figure 3.4). Zooplankton was less important (52.8%) in the diet of fish in the 2.0-2.4 g weight category, which showed an increased importance of chironomid larvae (23.1%). This trend is not mirrored in the larger size classes, however, which show RI for zooplankton of 80.0% (2.5-2.9 g) and 68.9% (>3.0 g). Stable isotope analyses of carbon and nitrogen in the Lake 658 food web confirm these feeding relationships (P. Blanchfield and M. Paterson, Fisheries & Oceans Canada, unpublished data).

Due to the overwhelming dominance of zooplankton in yellow perch diet (Figures 3.3, 3.4) all type 1 model simulations were conducted with a diet of 100% zooplankton. To explore the effects of a mixed diet on projected fish mercury concentrations, and because benthic invertebrates made up a portion of the yellow perch diet in Lake 658 (Figures 3.3, 3.4), the type 2 modelling simulations were conducted with a diet of 25% *Chaoborus* and 75% zooplankton.

Concentrations of spike and ambient Hg in zooplankton collected from the pelagic and littoral zones in Lake 658 were not different (ambient Hg: $t=1.5$, $df=6$ $p=0.19$; spike Hg: $t=1.7$, $df=6$, $p=0.14$). As a result, all model simulations were completed using Hg values for zooplankton collected in the pelagic zone of Lake 658 during routine METAALICUS sampling.

3.3.3 Mercury in yellow perch

Ambient THg was present in all fish on all sampling dates (Figure 3.6). Spike Hg was first detected in fish muscle on July 30, 2001, 41 d after the first addition of spike Hg to the lake. Overall, concentrations of THg in fish muscle tended to increase throughout the season, consistent with the the accumulation of Hg from food and water continuously over time. THg concentrations in perch were variable within sampling dates, with some fish showing THg concentrations that were double those of other fish caught on the same sampling dates. When analyzed by year, fish weight showed positive relationships with fish ambient THg concentrations in 2001 ($r^2 = 0.43$, $p<0.0001$) and 2007 ($r^2 = 0.60$; $p<0.0001$). Spike THg concentrations were moderately related to fish weight in 2001 ($r^2 = 0.33$; $p<0.0001$) and 2005 ($r^2 = 0.17$; $p<0.025$).

Ambient and spike THg concentrations were significantly correlated in all fish muscle samples except for fish collected on August 14, 2007 ($p=0.61$) (Table 3.1). When analyzed by year, slopes of regressions of $\log_{10}$-transformed ambient and spike Hg concentrations were only significantly different from 1 for fish samples collected in 2002 ($p=0.02$) (Table 3.1). Zooplankton spike and ambient Hg concentrations were also

proportional in 2001 (p=0.96), 2004 (p=0.21), and 2007 (p=0.27), however, the regressions for other years had slopes significantly different from 1 (2002: p=0.01; 2003: p<0.0001; 2005: p<0.0001; 2006: p=0.0006) (Table 3.2).

3.3.4 Model predictions of fish growth

Fish weights observed in Lake 658 on the last sample date varied from year to year, ranging from a mean of 0.66 g (SEM=0.037) in 2005 to 3.11 g (SEM=0.185) in 2003 (Figures 3.5 and 3.6). When predicted (models) and observed (Lake 658 yellow perch) weights on the last sampling dates were plotted against one another for each model, the variables were significantly correlated (p<0.0001 for both models) (Figure 3.7), and slopes of the regression lines were not significantly different from 1 (Wisconsin: p=0.75, OneFish: p=0.68) (Table 3.3).

3.3.5 Model predictions of fish mercury concentrations

Estimates of mercury accumulation by YOY yellow perch during their first summer season were compared to concentrations observed in Lake 658 perch. The Wisconsin model assumes that fish only take in Hg from their food, while the OneFish model allows for uptake from both food and water.

3.3.5.1 Type 1 simulations

Five type 1 model simulations were completed for each year for each Hg type (ambient and spike) to encompass a range of growth scenarios (Figures 3.8, 3.9). Mean concentrations of Hg in Lake 658 fish collected on the last sampling day were compared

to mean concentrations generated by the models for that day. Predicted and observed concentrations were significantly correlated in both models for both spike and ambient Hg (Wisconsin: ambient Hg $p=0.02$, spike Hg $p=0.003$; OneFish: ambient Hg $p=0.012$, spike Hg $p=0.004$) (Figure 3.10).

The OneFish type 1 model predictions tended to fit more closely with observed concentrations than the Wisconsin model estimates (Tables 3.4 and 3.5, Figure 3.10). However, mean Hg concentrations predicted by both models were, on the whole, not significantly different from observed concentrations, suggesting good fits for both models. One-way ANOVA comparisons indicate that mean observed ambient Hg concentrations in perch were not significantly different from Wisconsin or OneFish model predictions for 5 of the 7 years (Table 3.4). In 2002, concentrations predicted by the Wisconsin model were significantly different from OneFish predictions ($p=0.03$). In 2003, observed ambient Hg concentrations were higher than predictions generated by both models (Wisconsin: $p=0.0002$; OneFish: $p=0.005$). Mean observed spike Hg levels were not different from levels predicted by both models in 2005 ($p<0.30$) (Table 3.5). Spike Hg model predictions were significantly different from observed values but not from each other in 2001 ($p<0.01$ for observed; $p<0.99$ for models), 2003 ($p<0.0001$ for observed; $p<0.69$ for models), and 2007 ($p<0.003$ for observed; $p<0.001$ for models). In 2004 and 2006, the mean observed spike Hg levels were significantly different from the mean Wisconsin model predictions (2004: $p<0.04$; 2006: $p<0.04$) but not from the mean OneFish model prediction (2004: $p<0.68$; 2006: $p<0.97$). Wisconsin predictions were lower than observed concentrations by an averages of 18.5% (spike) and 17.0%

(ambient), while OneFish predictions were lower than observed concentrations by an averages of 4.9% (spike) and 8.9% (ambient).

Slopes of the relationships between observed concentrations and model predictions (Figure 3.10) were not significantly different from 1 for either model (Wisconsin: ambient p=0.08, spike: p=0.19; OneFish: ambient p=0.07, spike Hg p=0.27), suggesting that the predicted concentrations were not different from observed (Table 3.3). The abilities of both models to predict accurate Hg concentrations tended to decrease as Hg concentrations in fish increased, illustrated in Figure 3.10 by the shallow slopes of the trend lines compared to the 1:1 lines.

Wisconsin model predictions of fish Hg concentrations were lower overall than OneFish predictions for both spike and ambient Hg (Tables 3.4 and 3.5, Figure 3.11). Although slopes of the relationships between spike and ambient Hg concentrations predicted by the two models were not significantly different from 1 (ambient: p=0.61, spike: p=0.48) (Table 3.3), Wisconsin model predicted concentrations were lower than OneFish predictions by an average of 14.3% (SEM=3.2%) for ambient Hg and 10.0% (SEM=4.8%) for spike Hg.

3.3.5.2 Type 2 simulations

Type 2 model simulations were designed to examine the impacts of introducing Hg elimination and a combination diet (zooplankton and *Chaoborus*) and a variety of Hg elimination rates to the Wisconsin and OneFish models. All type 2 scenarios produced

estimates of fish Hg concentration that were lower than the mean concentrations observed in Lake 658 fish for each year (Tables 3.4 and 3.5, Figure 3.12). Type 2 simulations conducted with no elimination were identical to type 1 simulations except for fish diet composition. Type 2 (no elimination) predictions were lower than mean concentrations predicted in type 1 simulations by averages of 23.2% (spike Hg; SEM=5.5) and 21.4% (ambient Hg; SEM=2.9) for the Wisconsin model and 10.2% (spike Hg; SEM=2.5) and 6.0% (ambient Hg; SEM=3.1) for the OneFish model.

Wisconsin model estimates were lower that OneFish predictions in all scenarios. In both models, the "no elimination" scenarios produced the highest estimates, followed by scenarios that used Madenjian and O'Connor (2008) elimination rates, with the simulations that used the elimination constants suggested by the models producing the lowest estimates of fish Hg. Slopes of regressions comparing concentrations observed in Lake 658 YOY yellow perch to values predicted by the Wisconsin model were significantly different from 1 for both spike and ambient Hg under all spike Hg modelling scenarios, and for ambient Hg with suggested elimination rates (Table 3.4). When the same comparisons were made between OneFish predictions and observed concentrations, the slopes were not significantly different from 1 (Table 3.3).

The no elimination scenario in the Wisconsin model produced results that were lower than observed values by 36% (SEM=3.9) for ambient and 39% (SEM=4.1) for spike Hg. In this same scenario, the OneFish model results were lower than the observed results by 11% (SEM=3.2) for ambient and 18% (SEM=5.8) for spike Hg. The results of the

simulations that used Madenjian and O'Connor (2008) elimination rates were lower than observed concentrations in the Wisconsin (ambient: 56%, SEM=3.6; spike: 57%, SEM=3.6) and OneFish models (ambient: 15%, SEM=3.2; spike: 23%, SEM=5.8). Simulations that used elimination rates suggested by the models produced the lowest estimates of fish Hg in both models. In this simulation, Wisconsin model estimates were lower than observed values by 72% (SEM=2.8) for ambient and 71% (SEM=2.8) for spike Hg, and OneFish model predictions were lower than observed concentrations by 22% (SEM=3.1) for ambient Hg and 32% (SEM=5.5) for spike Hg.

The Wisconsin model consistently predicted lower concentrations than the OneFish model in type 2 simulations. Slopes of regressions between Wisconsin and OneFish type 2 predictions were compared to a slope of 1 with *t*-tests (Table 3.3). These results indicate that Wisconsin predictions were significantly lower than OneFish predictions for all scenarios and both Hg types, except for ambient Hg with no elimination. Estimates produced by the Wisconsin model in the scenario with no elimination were 29% (SEM=2.1) and 25% (SEM=2.6) lower than OneFish predictions for ambient and spike Hg, respectively. For scenarios using Madenjian and O'Connor (2008) elimination rates, Wisconsin model predictions were lower than OneFish estimates by 49% (SEM=2.6) for ambient Hg and 44% (SEM=3.7) for spike Hg. Finally, simulations using suggested elimination rates were lower in the Wisconsin model by 64% (SEM=2.5) for ambient and 57% (SEM=4.6) for spike Hg. The range of values generated for the three scenarios by the OneFish model was much smaller than the range of values produced by the Wisconsin

model for each year, suggesting the Wisconsin model has greater sensitivity to changes in input.

Similar to the type 1 model simulations, as Hg concentrations increased, the ability of the models to mimic the observed values decreased. This phenomenon is evident in Figure 3.14 in which the 1:1 lines have steeper slopes than the regression lines.

3.3.6 Sensitivity analyses of models

I conducted sensitivity analyses of both models to determine the influence of several input variables on predictions generated by the models. With identical "base" input variables, the OneFish model estimate of yellow perch Hg concentration (136.5 ng/g) was 8.5% higher than the Wisconsin model prediction (125.8 ng/g).

I compared the percent change of the input variables to the percent change of model output (yellow perch Hg concentrations) from the base model simulation (modified from Pannell 1997) (Figure 3.13). Percent change in prey Hg concentration and % change in AE_f had 1:1 relationships with % change in the Wisconsin model output ($p<0.0001$; $r^2 = 1$ for both) and nearly 1:1 relationships with OneFish model output ($p<0.0001$; $r^2 = 1$ for both). In the OneFish model, % change in water Hg concentration and % change in AE_w both had linear relationships with % change in fish Hg concentration ($p=0.0002$ for water Hg; $p=0.002$ for AE_w; $r^2=1$ for both). Change in prey energy density had negative exponential relationships with % change in fish Hg in both models ($p=0.014$ for Wisconsin; $p=0.015$ for OneFish; $r^2=0.995$ for both). Temperature was influential in the

Wisconsin model, but did not have a definable relationship (Figure 3.14a), and had minor influence in the OneFish model (Figure 3.14b). Although increased water temperatures appear to promote fish Hg accumulation in both models, concentrations predicted on the final sampling days were within 16% of the base simulation prediction for the OneFish model, and within 17% of the base simulation concentrations for all temperature regimes in the Wisconsin model, except for the simulation in which all temperatures were set at 25 °C (74% higher than base).

3.4 DISCUSSION

Mercury bioaccumulation models exist to predict concentrations of Hg in fish based on environmental information. It is important that these models reflect Hg accumulation in nature because they are widely used by managers to make decisions about Hg emissions standards. I previously established that water can be an important source of Hg to fish in a field experiment (Chapter 2). Here I examined the ability of Hg bioaccumulation models to predict Hg levels in fish using models that did and did not include exposure to waterborne Hg. I also assessed the ability of the models to generate accurate predictions of fish Hg concentrations under a variety of diet and Hg elimination scenarios, and determined which parameters have the most influence over model output. I modelled the accumulation of ambient and spike MeHg by YOY yellow perch in Lake 658 at the Experimental Lakes during the 7 year loading phase of the METAALICUS study. The rich detail of the METAALICUS dataset makes it the ideal system with which to test the models. The availability of complete, long-term records for Lake 658 water, zooplankton

and fish Hg levels, water temperatures, and other environmental parameters sets this study apart from previous model validation attempts.

Prior to examining the main results of the study, this discussion will focus on observed concentrations of spike and ambient Hg in Lake 658 YOY yellow perch, which were used to measure model accuracy for all model simulations. This section will also discuss model input data because the accuracy of any model simulation is dependent in part on the strength of input data. Estimated spawn and hatch dates, yellow perch diet, and Lake 658 food web C and N isotopes, are important input parameters that were analyzed for this study prior to the completion of all model simulations.

3.4.1 Ambient and spike mercury in Lake 658 yellow perch

This study does not focus on specific patterns of Hg accumulation in YOY yellow perch or on differences in accumulation among years, but since observed concentrations were compared to predictions made in all model simulations, the Hg concentrations require consideration. Yellow perch in Lake 658 accumulated ambient and spike Hg in their muscle tissue during each sampling year. Concentrations of both Hg types increased throughout the season as a result of the bioaccumulative nature of MeHg (McKone *et al*. 1971; Mathers and Johansen 1985; Cabana *et al*. 1994; Bowles *et al*. 2001). The variability seen among samples collected on the same date may stem from the many factors that influence fish Hg uptake, particularly water temperatures (Jackson 1997), fish activity levels (Rennie et al. 2005), consumption rates (Rogers 1992), diet composition (Beamish *et al*. 1974; Watras and Bloom 1992; Harris and Bodaly 1998), and growth rate

(Ethier *et al*. 2008; Kehrig *et al*. 2008; Simonin *et al*. 2008). The variability in these factors, and others, among individual fish promotes varied tissue Hg concentrations within a cohort. Variable Hg concentrations in fish of the same age class have been observed previously (Phillips and Buhler 1978; Boudou and Ribeyre 1985; Hall et al. 1997; Hammerschmidt et al. 2002; Lepak *et al*. 2009; Sackett *et al*. 2009).

The variability observed in fish ambient and spike Hg concentrations could not be explained by fish size within years. Previous research has suggested that Hg concentrations in fish tissues may be related to the weight of the fish, with higher levels of Hg observed in larger individuals (Stafford and Haines 2001; Ethier *et al*. 2008; Kehrig *et al*. 2008; Simonin *et al*. 2008). This idea goes hand in hand with the observation that Hg concentration often increases with age, as the largest fish are often the oldest (McKone *et al*. 1971; Mathers and Johansen 1985). The lack of a strong relationships between fish weight and Hg content likely arises from the small sizes and young ages of the fish. Although size may be a good predictor of Hg levels in large fish such as walleye and northern pike (Mathers and Johansen 1985), this relationship may break down for smaller, planktivorous fish (Greenfield *et al*. 2001; Cizdziel *et al*. 2002). Eagles-Smith *et al*. (2009) suggest that MeHg levels in small fish may vary substantially even among individuals of similar weights. It is possible that the variability in fish Hg levels introduced by factors such as water temperature, activity, consumption, and prey Hg concentrations outweighs the influence of body size for small fish.

Although ambient and spike Hg levels were variable among individuals, spike Hg concentrations tracked ambient concentrations well in all samples (Table 3.1), indicating that spike and ambient Hg behave in the same manner. This finding is congruent with previous research that has highlighted the ability of enriched-stable isotopes of Hg to mimic the behaviour of ambient Hg (Hintelmann *et al*. 1997; Harris *et al*. 2007). The relationship between spike and ambient Hg in fish collected in 2002 had a slope significantly different from 1 (Table 3.1), despite the slope (b_1=1.17) being closer to 1 than the slope of the relationship for 2001 fish (b_1=1.77), which was not significantly different. Low variability in Hg concentrations (SE of slope = 0.07) compared to other years may have caused the significant difference. Despite the significance, the relationship between spike and ambient Hg in 2002 is very strong ($p<0.0001$) and the slope is close to 1, suggesting similar behaviour of spike and ambient Hg.

Concentrations of spike Hg observed in fish in 2001 and 2002 were lower than those observed in subsequent years. It is likely that the gradual buildup of spike Hg in the system caused low spike Hg:ambient Hg ratios in the first two years before an adequate pool of spike had been deposited and was available for uptake. Inorganic spike Hg was added to the lake for the first time in 2001 and would have taken time to begin to accumulate in fish. Spike Hg was detected in YOY yellow perch 41 days after the first addition, however, levels remained low throughout the open water season, having a mean concentration of only 5.5 ng/g (wet weight) on the final sampling day in 2001 (Table 3.5). Concentrations of Hg in fish collected in 2002 were higher than those observed in 2001, suggesting that spike Hg levels in 2002 fish resulted from continued methylation and

uptake of the 2001 spike and uptake of the newly-deposited 2002 spike. The combined influence of Hg deposited over multiple years on fish Hg levels was observed previously in yellow perch at the ELA by Paterson *et al*. (2006), emphasizing the long-term availability of Hg to the food web following deposition.

Overall, relationships between spike and ambient Hg in fish were significant for all years, and spike Hg increased in direct proportion to ambient Hg for each year except for 2002. These results suggest that spike Hg behaves in the same manner as ambient Hg. Since all fish populations in nature are subject to ambient Hg accumulation, the similar behaviour of spike and ambient Hg in this study enhances the applicability of spike Hg results to wild populations. By modelling the two Hg types separately, it is possible to look for patterns that exist for both types, thereby strengthening the conclusions.

3.4.2 Model input data

3.4.2.1 Spawn and hatch dates

I estimated spawn and hatch dates for yellow perch in Lake 658 based on water temperatures. Estimated hatch dates provide potential sources of error for the models because they are based solely on lake water temperatures and do not reflect actual hatch observations. Yellow perch spawning dates appear to be influenced by water temperatures more than other contributing factors such as photoperiod, making temperature a good estimator of spawn dates (Hokanson 1977; Kayes and Calbert 1979; Ciereszko *et al*. 1997). Based on these data, estimated hatch dates in Lake 658 ranged from May 17 (2003) to June 11 (2004) (Figure 3.1), with spawn and hatch times

occurring later in years with cooler springs. Hatch date does not appear to impact overall Hg accumulation levels, as fish from the latest hatch date (June 11, 2003) had similar Hg concentrations to fish with the earliest hatch date (May 17, 2004) (Tables 3.4 and 3.5). However, fish that hatched later in the year tend to be smaller at the end of the season than fish with early hatch dates (Figures 3.5 and 3.6), which is possibly the result of a reduced growing season caused by delayed spawning (Post and McQueen 1988).

3.4.2.2 Diet

The diet of YOY yellow perch in Lake 658 was dominated by zooplankton both within and among years, with zooplankton making up approximately 75% of the diet in most cases (Figures 3.3 and 3.4). This finding is consistent with Mills and Forney (1981) who found yellow perch diet to consist mainly of cladoceran zooplankton species. Schael *et al*. (1991) also noted the dominance of zooplankton in YOY yellow perch diet, citing copepod zooplankton as the main prey group. Although yellow perch have been identified as gape-limited, visual predators that experience ontogenetic dietary shifts as they grow (Keast 1977; Schael *et al*. 1991), these shifts were not observed in Lake 658 YOY yellow perch. Three of four YOY yellow perch size classes in 2001 (1.0-1.9 g; 2.5-2.9 g; >3.0 g) had similar dietary compositions with zooplankton as the main food source. Fish in the mid-range size class 2.0-2.4 g showed a higher affinity for chironomid larvae, but as this trend was not continued in the larger fish it cannot be identified as a definite ontogenetic shift. Graeb *et al*. (2006) indicate that the shift to a diet dominated by benthic invertebrates generally occurs in perch at total lengths of approximately 80 mm. As the maximum length of fish collected in 2001 was 69 mm, it is possible that

YOY yellow perch in Lake 658 did not reach large enough sizes to shift to a diet of benthic invertebrates.

In summary, YOY yellow perch diet was composed mainly of littoral and pelagic zooplankton, with other prey types such as *Chaoborus* and chironomids making up a portion of total consumption. As such, the majority of model simulations were run with a diet of 100% zooplankton (type 1). This approach was also chosen because the OneFish model accepts a single input of prey Hg concentration which remains constant throughout the simulation. The approach of using a single prey type has been used previously in both the OneFish model (Harris and Bodaly 1998) and the Wisconsin model (Bajer *et al*. 2003; Madenjian *et al*. 2006). To explore the effects of a combined diet on the model results, type 2 simulations were completed with a diet of 25% *Chaoborus* spp. and 75% zooplankton. The use of a combination diet in the modelling simulations may be more representative of YOY yellow perch in nature because of the presence of benthic invertebrates in dietary analyses. A combination of prey types has been used previously to examine fish Hg uptake in both the OneFish (Van Walleghem 2006) and Wisconsin models (MacRury *et al*. 2002; Van Walleghem *et al*. 2007). The two types of YOY yellow perch dietary inputs produced different predictions of fish Hg concentrations which are discussed in section 3.4.3.3 below.

3.4.3 Model simulations and predictions

Bioenergetics-based Hg accumulation models combine basic fish energy requirements with environmental Hg exposure data to predict Hg concentrations in fish (Norstrom *et*

al. 1976; Post *et al*. 1996; Hanson *et al*. 1997; Harris and Bodaly 1998; Knightes *et al*. 2009). Many existing models are based on comparable equations that examine processes like uptake of Hg from food and water, Hg elimination, and growth. Although many models show similarities in structure, the inclusion or exclusion of water as a direct source of Hg to fish produces two distinct modelling approaches. Models that do not include water as a source of Hg suggest that direct uptake is negligible and can therefore be ignored (Hanson *et al*. 1997; Rennie *et al*. 2003; Trudel and Rasmussen 2006), while models that account for uptake from both sources acknowledge that although water contributes substantially less Hg to fish than dietary uptake, direct uptake of aqueous Hg is an important source (Norstrom *et al*. 1976; Rodgers 1994; Post *et al*. 1996; Harris and Bodaly 1998; Knightes *et al*. 2009).

In this study, I evaluated the ability of the Wisconsin (dietary Hg uptake) and OneFish (water and dietary Hg uptake) models to predict ambient and spike Hg accumulation in YOY yellow perch. I completed two types of modelling simulations, the first with no elimination and a diet of 100% zooplankton (type 1), and the second with some elimination of Hg from the body of the fish and a combined diet of zooplankton and *Chaoborus* (type 2). Type 1 simulations were completed to examine the importance of including water as a source of Hg to fish (objective 1), and type 2 simulations were run to assess the accuracy of model predictions under a variety of dietary and elimination scenarios (objective 2).

I conducted simulations of YOY yellow perch Hg accumulation from estimated hatch dates until the last day of the sampling season for each year that spike Hg was added to Lake 658 (2001-07). Modelling Hg accumulation in the fish for one whole season beginning at an estimated hatch date is representative of the type of modelling an ecosystem manager might perform. Starting a simulation at hatch allows a modeller to track Hg accumulation for the entire lifespan of a fish, and is useful because body weights are small and similar for all individuals at hatch, and maternal transfer of Hg to fish embryos is low (Hammerschmidt *et al*. 1999b). It is also possible to execute modelling scenarios on shorter time-scales, but whole-season scenarios are more applicable to real-world modelling practices.

3.4.3.1 Growth predictions

It is crucial that growth patterns in model simulations match growth of the target population precisely because Hg concentrations are reported in the models on a per weight basis (Norstrom *et al*. 1976; Trudel and Rasmussen 2006). I ran type 1 simulations in both models for a variety of fish growth rates (n=5 for each year) that spanned the range of weights observed in Lake 658 each year (Figures 3.5, 3.6). End weights predicted by both models matched weights observed in Lake 658 yellow perch for all sampling years (Table 3.3, Figure 3.7). Within-year fish weights were consistent, with individuals from the same cohort exhibiting similar weights on each sampling date (Figures 3.5 and 3.6). YOY individuals of the same age may maintain similar weights during the first few months of life as they begin life at similar sizes, and are exposed to similar environmental conditions (Craig 1987). Observed weights were highly variable

among years (Figure 3.7), likely resulting from variations in environmental factors such as water temperatures, nutrient inputs, lake productivity, and consumption (Boisclair and Leggett 1989a; Boisclair and Leggett 1989b). Despite the variability among years, predicted growth mimicked observed growth consistently. As such, it can be assumed that fish Hg concentration estimates are strong and have not been biased by incorrect weight estimates.

3.4.3.2 Type 1 model predictions of Hg concentrations in fish

Fish Hg concentration estimates produced by both models for type 1 scenarios were within the range of values observed in Lake 658 fish for all years (Figures 3.8 and 3.9). As both models were subject to testing and calibration prior to their publication, it was expected that they would generate passable estimates of Hg in fish. The differences between the two models, however, are evident in the overall trends. Type 1 Wisconsin model predictions were lower than observed concentrations of spike Hg by an average of 17.0% and ambient Hg by an average of 18.5%. Comparisons of model concentrations versus observed concentrations were often not significantly different (Tables 3.4, 3.5), and the slopes of lines comparing predicted to observed concentrations were not significantly different from 1 (Table 3.3), but the trend of underestimation for both spike and ambient Hg is apparent in the predicted vs observed plots (Figure 3.10) where most points fall below the 1:1 comparison line. The OneFish model generated estimates of fish Hg concentrations that fell more closely in line with those observed in Lake 658 yellow perch. Differences between the spike and ambient Hg concentrations in the model were not significantly different from levels observed in the Lake 658 fish. Additionally, when

predicted and observed values were plotted together, the slopes of the regression lines were not significantly different from 1. Together, these results suggest that the OneFish model represents YOY yellow perch in Lake 658 well.

Hansen *et al*. (1993) suggest that poor agreement between model output and field data does not invalidate either method, but simply suggests that the model does not fully represent the quantity being studied. Accordingly, to resolve these discrepancies one must adjust the structure of the model to more fully represent the natural situation. The Wisconsin and OneFish models are based on similar equations for bioenergetics and Hg kinetics. The main difference between the two is the assumption they make regarding Hg uptake from water. The Hg kinetics equations used in the Wisconsin model are essentially equations derived in previous Hg accumulation research (Norstrom *et al*. 1976; Rodgers *et al*. 1994; Post *et al*. 1996) with the water uptake components removed. It is possible that the deficiency of the model is caused by the omission of the Hg taken in directly from the water. Chapter 2 details the importance of waterborne Hg uptake to fish and suggests that yellow perch derive 10-21% of their Hg directly from the water. The differences between Wisconsin model predictions and observed concentrations fall within this 10-20% range, suggesting that if Wisconsin model predictions were increased to reflect additional uptake from water, they would more closely match both the OneFish model predictions and Hg levels observed in Lake 658 fish. Many other existing aquatic Hg accumulation models account for Hg uptake from both sources (Norstrom *et al*. 1976; Rodgers *et al*. 1994; Post *et al*. 1996; Knightes *et al*. 2009). The researchers stress the importance of representing natural and ecosystem processes as accurately as possible,

suggesting that although uptake of Hg from water is low, it is nevertheless important (Rodgers 1994). Other modelling exercises have ignored water as a source of Hg to fish, indicating that the impact of water uptake is negligible (Trudel and Rasmussen 1997; Trudel and Rasmussen 2001; Rennie 2003). While I cannot comment on these models, it is apparent that uptake of Hg from water has some influence over fish Hg concentrations, and that accurate representation of Hg exposure, Hg uptake, and fish Hg levels may only be generated by models that incorporate a water uptake component.

3.4.3.3 Type 2 model predictions of Hg concentrations in fish

In an evaluation of the Wisconsin model, Van Walleghem *et al*. (2007) found that it produced underestimates of fish Hg elimination over time. They indicated that inaccurate (i.e. too fast) elimination rates were the cause of the results, suggesting that the half-life of MeHg in the model should be increased to 489 d. Similarly, Madenjian and O'Connor (2008) suggested that the half-life of MeHg in fish is approximately 398 d. In type 1 model scenarios I set elimination to zero because of the long half-life of MeHg and the comparatively short durations of the model simulations. This approach was also employed by MacRury *et al*. (2002) in Wisconsin model simulations of largemouth bass and northern pike. Despite the given elimination rate of zero in this study, estimates produced by the models for both spike and ambient Hg tended to be lower than those observed in the lake, with greater discrepancies observed for the Wisconsin model.

To examine the impacts of incorporating Hg elimination into the scenarios and to introduce benthic invertebrates to the diet of simulated fish, I completed type 2 model

simulations. These simulations were designed to represent "standard" inputs that represent real-world model use and provide commentary on the ability of the models to predict fish Hg concentrations accurately under "standard" conditions.

By comparing type 2 simulations that used a combination diet but did not include any Hg elimination to type 1 simulations, the effects of the combined diet become clear. Model type 2 spike and ambient Hg predictions were lower than type 1 predictions for both models (Tables 3.4, 3.5, Figure 3.12). The Wisconsin model showed greater sensitivity to benthic invertebrate prey and produced estimates of fish Hg that were 21.4-23.2% lower than type 1 predictions. The OneFish model was not as affected by the mixed diet, perhaps because the model does not allow input of more than one prey type. The mixed diet input was achieved in the OneFish model by combining zooplankton and *Chaoborus* Hg concentrations and energy densities (Section A.3.2). It is possible that the finer resolution of Wisconsin model input data (allows daily inputs) may make it more sensitive than the OneFish model. It is not surprising that the introduction of *Chaoborus* into yellow perch diet reduced fish Hg accumulation in both models because *Chaoborus* spike and ambient Hg concentrations are much lower than those observed in zooplankton (M. Paterson, Fisheries & Oceans Canada, unpublished data). Prey Hg concentration has been suggested to be one of the strongest drivers of fish Hg concentrations, and subtle changes in dietary Hg can lead to shifts in fish Hg levels (Lockhart *et al*. 1972; Bodaly *et al*. 1984; Kennedy *et al*. 2003). Overall, type 2 simulations with no elimination produced Hg estimates that were lower than observed concentrations and concentrations predicted in type 1 simulations. Although a mixed diet may be more representative of a yellow

perch diet in the wild, the models did not perform as well with the introduction of benthic invertebrate prey.

When elimination was also introduced into the models, predicted concentrations were lower than those in type 2 simulations with no elimination (Tables 3.4, 3.5, Figure 3.12). Elimination rates suggested by Madenjian and O'Connor (2008) produced moderate reductions in fish Hg concentrations, while simulations that used elimination rates suggested by the models showed more most drastic differences. The Wisconsin model again proved more sensitive than the OneFish model, producing estimates of fish Hg that were lower than observed concentrations by averages of 71% (spike Hg) and 72% (ambient Hg) when run with elimination rates suggested in the model. Although the use of Madenjian and O'Connor (2008) elimination rates produced higher fish Hg concentrations in the Wisconsin model, the estimates were still lower than observed levels by 57% (spike Hg) and 56% (ambient Hg). OneFish type 2 predictions were generally not significantly different from observed concentrations (Table 3.3), but simulations including elimination rate suggested by the model were averages of 22% (ambient Hg) and 32% (spike Hg) lower than observed.

Wisconsin and OneFish models treat Hg elimination differently (Appendix 1). The Wisconsin model calculates elimination according to fish weight, and OneFish measures elimination according to a fixed ratio of Hg in the body of a fish to Hg in urine. The allometric scaling of elimination in the Wisconsin model may cause greater fluctuations in elimination rate as the fish change size, and it is likely that the high sensitivity of the

Wisconsin model to Hg elimination stems from this allometry. In contrast, elimination in the OneFish model may be more constant because of the fixed elimination ratio, thus reducing the magnitude of impact caused by shifts in Hg elimination rate.

Overall the OneFish model was able to predict fish Hg concentrations more accurately than the Wisconsin model under a variety of scenarios. However, although OneFish model predictions were not significantly different from observed concentrations, neither model performed particularly well under type 2 scenarios. These scenarios were designed to represent real-world modelling exercises more closely than type 1 simulations, but the discrepancies between model output and observed data make it apparent that both models should be used with caution. The Wisconsin model is widely used by researchers and ecosystem managers because it is available as a computer program with a simple user-interface and has been used with success in the past to model Hg accumulation in fish (e.g. Stafford and Haines 2001; MacRury *et al*. 2002; Lepak *et al*. 2009a). Although previous uses of the model have reported good fits, even with the inclusion of Hg elimination, past research (Van Walleghem *et al*. 2007) and the results presented in this study suggest that the model needs refining before it can be used with confidence as a tool for assessing Hg bioaccumulation.

It is interesting that the accuracy of predictions decreased as Hg concentrations increased for both models and for both types of model scenarios. 2003 and 2004 stand out as the years with the highest observed Hg concentrations (both spike and ambient). The predictions generated for these two years by both models are lower than observed

concentrations, causing the trend lines for the plots of predicted and observed data to have slopes that are less than 1 (Figures 3.10, 3.12). Growth trends exhibited in each of these years are not similar, with 2003 end weights (mean=3.11 g) triple those of 2004 (mean=1.03 g). Temperature patterns were also different for the two years. 2004 was one of the coolest years, and 2003 was one of the warmest of the seven study years (Figure 3.2).

Patterns in modelled growth of fish in 2003 show 2 growth rates, the first phase (approximately 90 d) exhibiting moderate growth, and the second phase (approximately 60 d) showing much more rapid growth. The first phase appears to correspond with warm water temperatures, which may inhibit growth because they are above the optimal growing range. The second phase corresponds with a decline in water temperatures, which appears to have enhanced growth rate. It is possible that the rapid growth in the latter half of the study caused modelled fish Hg concentrations to decrease due to growth dilution (de Freitas *et al*. 1974; Cizdziel *et al*. 2002; Karimi *et al*. 2007) or increased Hg elimination rates (Rodgers and Beamish 1982). Rapid growth has been shown to correspond with decreases in fish Hg concentrations perhaps because the growth efficiency of the fish is increased. Although modelled fish may have exhibited growth dilution, this trend was not observed in Lake 658 fish. As such, estimates of Hg concentrations in both type 1 and 2 simulations were much lower than observed.

Growth dilution could be a cause of the discrepancy between predicted and observed for 2003 fish, but not for the smaller 2004 fish. The YOY yellow perch in Lake 658 grew

very little in 2004, achieving end weights of only 1.03 g. It is possible that the low growth rate exhibited by the 2004 fish caused low consumption estimated, and therefore low overall Hg exposure for the fish. Although it is possible that the fish were small because their consumption was low (Boisclair and Leggett 1989a), their Hg concentrations suggest otherwise. Recent research has highlighted the importance of including activity levels in estimates of consumption (Boisclair and Leggett 1989c; Rennie *et al*. 2005). A small fish that is very active may actually consume more than a fish that is larger but less active (Rennie *et al*. 2005). This high consumption rate would expose the smaller fish to more Hg than the larger fish, thus increasing its Hg concentration. Both models suggest the same activity multiplier for YOY yellow perch. I did not change the activity level among years in either model as I did not have data to test for differences in activity among years. It is possible that the 2004 fish were simply more active than the model predicted, increasing their consumption and therefore their Hg exposure while maintaining low growth rates. Future uses of these models should include measurements of fish activity levels rather than assuming an average activity level is appropriate for all fish. 2003 and 2004 water and zooplankton Hg concentrations do not stand out from the other years, suggesting that these parameters did not cause the inaccurate predictions of fish Hg at high concentrations.

3.4.4 Sensitivity analysis

Sensitivity analysis is used in modelling to determine the distribution of changes in response variables with changes of the input variable values (Salacinska et al. 2010; Thogmartin 2010). It is intended to describe the relationships between input parameters

and response values by identifying which input parameters have the largest influence on the model output (Kitchell *et al*. 1977; Salacinska *et al*. 2010). The amount of influence each parameter has over fish Hg concentrations in a model should accurately reflect the factors that drive fish Hg concentrations in nature (Rodgers 1994; Hanson *et al*. 1997; Harris and Bodaly 1998). Sensitivity analyses are designed to enhance model accuracy and streamline field data collection efforts.

Sensitivity analyses of the Wisconsin and OneFish models identified prey Hg concentration, assimilation efficiency of Hg from prey (AE_f), prey energy density, and temperature as the most influential input parameters. As the majority of the Hg taken in by the fish comes from their food (Chapter 2; Post *et al*. 1996; Hall *et al*. 1997), it is intuitive that parameters that guide consumption rates (prey energy, temperature) and dietary Hg exposure (prey Hg, AE_f) would have the most impact on model output. MacRury *et al*. (2002) used the Wisconsin model to examine how shifts in various input parameters influenced model output in an exercise designed to highlight the influence of various environmental factors on fish Hg exposure in nature. They found prey Hg concentrations and temperature to be the most influential factors. Similarly, the model developed by Rodgers (1994) was most sensitive to factors influencing the uptake of dietary Hg, including prey Hg concentration, AE_f, and prey caloric content. Other studies have found comparable results, with consumption inputs being most influential (Norstrom *et al*. 1976; Kitchell *et al*. 1977; Post 1990).

Changes in temperature influenced fish Hg concentrations in both models. The Wisconsin model showed greater sensitivity to increases in temperature, exhibiting increases and decreases in fish Hg concentrations as temperatures increased and decreased (Figure 3.14a). Despite the changes in fish Hg during the simulation, final predictions of fish Hg concentration were generally close to base estimates (16% for OneFish and 17% for Wisconsin). The only simulation to predict a fish Hg concentration that was very different than the base simulation was Wisconsin model simulation where all temperatures were set at 25 °C (fish Hg estimate was 74% higher than base prediction). The main difference between Wisconsin and OneFish temperature manipulations was not in final fish Hg concentration estimates, but in Hg accumulation patterns observed during the simulations. The Wisconsin model will accept daily inputs of water temperatures and the OneFish model will only allow one temperature input per month. However, differences in Hg accumulation patterns cannot be attributed to differing inputs because temperatures were entered into both models on a monthly basis for this analysis (Table A.7 and A.8). Fish growth is highly influenced by temperature and is determined differently in the models. The Wisconsin model calculates the total consumption required to achieve a user-defined end weight, while the OneFish model calculates growth according to several growth rate constants: kt (growth rate constant), Q_{10} (relates growth to temperature), and b (growth-related constant). It is possible that fish Hg estimates in the Wisconsin model would be more tightly linked to temperature changes because consumption, which drives growth, is highly influenced by water temperature. In the OneFish model, on the other hand, growth is more defined by constants than by consumption, which may dampen the influence of temperature on fish

Hg concentration. Overall, although the models exhibited different patterns of Hg accumulation over time, final predictions of fish Hg concentration were slightly higher (with warmer temperatures) or slightly lower (with cooler temperatures) than base estimates, suggesting that water temperatures do not have great influence over final fish mercury concentrations.

The linear relationships observed in this study for prey and water Hg content, AE_f and AE_w, and the negative exponential relationship observed for prey energy content make the impacts of changes in these input values predictable. The influence of temperature is more variable. Overall, the most important data to collect in detail for running simulations in these models are prey Hg concentrations and water temperatures. Although temperatures has minimal influence over final fish Hg concentrations, it is important to have a general idea of the thermal environment of the fish. The assimilation efficiency of Hg from prey by fish has been well-established at 80% and may be used with confidence in modelling simulations (Rodgers *et al*. 1994; Pickhardt et al. 2006). Dietary and prey energy density analyses are also useful for ensuring accurate predictions.

3.4.5 Model limitations

There are limitations associated with all ecological modelling exercises (Jorgensen 2008). By definition models are generalizations of complex interactions and cannot be expected to function in exactly the same ways as the relationships they are designed to explore (Walters 1986). The main limitations in this study are associated with model design and

input data. The OneFish model has a simpler design than the Wisconsin model in that it will not accept more than one prey type, uses one prey Hg concentration for the whole simulation, and requires water temperatures to be input on a monthly basis. The Wisconsin model will accept daily inputs of prey Hg and water temperatures, and allows input of more than one prey type. It would be intuitive that performance of the OneFish model would be poor compared to the Wisconsin model because of the limited input data, but despite the simpler form, the OneFish model consistently produced the most accurate estimates of fish Hg concentrations. Overall, although the simple form of the OneFish model may appear to be a limitation, it does not appear to have impacted model performance.

Another limitation of this study is that prey energy densities were estimated based on literature values. Since prey energy is a strong driver of consumption, which in turn influences fish Hg concentration, estimated prey energy values provide a potential source of error. It would be advantageous for future modelling exercises to measure prey energy content. Estimated spawn and hatch dates provide a potential source of error in the modelling exercises presented in this study. The water temperature data used to estimate yellow perch spawning dates was collected every two weeks from Lake 658, and water temperatures between sampling days were interpolated with straight lines between data points. It would have been advantageous to estimate spawn and hatch dates by counting degree days each year (Craig 1987), but since temperatures were only collected biweekly, it was not possible to examine daily trends or count degree days. Future modelling

exercises could improve upon this method by collecting daily water temperatures to estimate spawn and hatch dates.

Other limitations of this study include the small sample size (n=7 years) and the multiple comparisons using observed data. A Bonferroni correction was applied to compensate for the multiple comparisons. Although important when completing multiple comparisons using a family of data, Bonferroni corrections increase the probability of making a type II error (accepting a false H_0) (Kutner *et al*. (2005). In model validation, accepting a false H_0 because of a Bonferroni correction could give support to a model that in fact does not provide accurate results.

3.4.6 Applicability of models to wild populations

The METAALICUS study at the ELA has produced a complete, long-term dataset of fish, lower food web, and water Hg concentrations. The presence of both spike and ambient Hg in the lake provides a unique opportunity to model accumulation of both newly-deposited Hg (spike) and Hg that has accumulated in the system over time (ambient). The METAALICUS dataset was used in this study to examine the ability of two Hg bioaccumulation models to predict Hg concentrations in YOY yellow perch.

Type 1 Wisconsin and OneFish model simulations predicted ambient and spike Hg levels in fish that were close to the levels observed in YOY yellow perch collected from Lake 658. Although not significantly different, Wisconsin predictions were lower than OneFish predictions and observed concentrations. It is possible that the omission of

water as a source of Hg to fish in the Wisconsin model limits its ability to generate accurate predictions. The models did not perform well under type 2 simulations that were designed to represent examples of real-world model use, although OneFish type 2 predictions were closer to observed concentrations than Wisconsin estimates in all type 2 simulations, suggesting a better fit of the OneFish model. Overall, the introduction of a mixed-prey diet and Hg elimination did not produce accurate estimates of fish Hg in nature.

Anthropogenic Hg emissions are expected to decrease over the next decade with the introduction of strict regulations for emissions from industry and power generation facilities (UNEP 2009). It is not known how these reductions will impact concentrations of MeHg in fish, but it would be advantageous for ecosystem managers to be able to use bioaccumulation models to predict the responses of fish populations. Although both the Wisconsin and OneFish models have been used with success to model fish Hg accumulation in the past, it is apparent that both models require refining. The OneFish model performed better overall, partly due to the inclusion of water as a source of Hg to fish. This model could be used as it is to predict Hg accumulation in wild fish populations, but with elimination of Hg should be set to zero. The Wisconsin model is more widely available, but should be used with less confidence because it does not include water as a source of Hg to fish. MacRury *et al*. (2002) identified the Wisconsin model as an under-used tool, suggesting that it should be promoted among managers and researchers alike. I support this idea, but would caution users of the Wisconsin model that they may produce underestimates of fish Hg concentrations because of the omission

of direct uptake of Hg from water. I would also suggest that elimination be set to zero in the Wisconsin model.

Table 3.1 Proportionality of the relationship between spike and ambient THg in yellow perch collected from Lake 658 in all sampling years. All variables were $\log_{10}$-transformed. This table presents the slopes (b_1) of the relationships, with the standard errors of the slopes (SE) and the results of the two-tailed t-tests (df, t, p) testing the null hypothesis that the slope of the line is not different from 1. A slope of 1 suggests that the relationship between the variables in their untransformed state is proportional and linear. Significant results are indicated by *.

Year	**Slope**		**H_0: $b_1 = 1$**		
	b_1	**SE**	**df**	***t***	***p***
2001	1.77	0.45	28	1.73	0.096
2002	1.17	0.07	15	2.55	0.022*
2003	1.02	0.02	25	1.05	0.304
2004	0.97	0.04	33	-0.86	0.398
2005	0.89	0.07	28	-1.56	0.129
2006	0.96	0.02	18	-1.75	0.097
17 July, 2007	1.17	0.10	8	1.72	0.124
14 August, 2007	0.84	0.10	8	-1.59	0.150
12 September, 2007	0.20	0.39	8	-1.17	0.278

Table 3.2 Proportionality of the relationship between spike and ambient MeHg in zooplankton collected from Lake 658 in all sampling years. All variables were $\log_{10}$-transformed. This table presents the slopes (b_1) of the relationships, with the standard errors of the slopes (SE) and the results of the two-tailed t-tests (df, t, p) testing the null hypothesis that the slope of the line is not different from 1. A slope of 1 suggests that the relationship between the variables in their untransformed state is proportional and linear. Significant results are indicated by *.

Year	**Slope**		**H_0: slope = 1**		
	b_1	**SE**	**df**	***t***	***p***
2001	0.92	1.42	5	-0.056	0.958
2002	1.29	0.10	11	2.90	0.014*
2003	1.57	0.09	11	6.33	<0.0001*
2004	0.72	0.21	11	-1.33	0.210
2005	1.50	0.08	9	6.25	<0.0001*
2006	1.60	0.13	12	4.62	0.0006*
2007	1.48	0.41	10	1.17	0.269

Table 3.3 Results of two-tailed *t*-tests performed to determine whether the slopes of regression lines in figures 3.7, 3.10, 3.11, and 3.12 were significantly different from a slope of 1. Bonferroni correction for multiple comparisons gave a significance level of 0.006. Simple linear regression models for relationships between predicted and observed results and between predictions made by the two models are illustrated in Figures 3.7 (model growth predictions compared to observed), 3.10 (Wisconsin and OneFish model type 1 Hg predictions compared to observed), 3.11 (type 1 Wisconsin Hg predictions compared to OneFish predictions), and 3.12 (Wisconsin and OneFish type 2 Hg predictions compared to observed). This table presents the slopes (b_1) of the relationships, with the standard errors of the slopes (SE) and the results of the two-tailed t-tests (df, t, p) testing the null hypothesis that the slope of the line is not different from 1. Significant results are indicated by *.

Description	**Slope**		**H_0: slope = 1**		
	b_1	**SE**	**df**	***t***	***p***
Figure 3.7 (growth predictions vs. observed)					
Wisconsin	0.95	0.06	5	-0.83	0.444
OneFish	0.95	0.08	5	-0.63	0.556
Figure 3.10 (type 1 model simulations vs. observed)					
Wisconsin ambient THg	0.61	0.18	5	-2.17	0.082
OneFish ambient THg	0.70	0.13	5	-2.31	0.069
Wisconsin spike THg	0.71	0.19	5	-1.53	0.187
OneFish spike THg	0.80	0.16	5	-1.25	0.267
Figure 3.11 (type 1 model simulations; Wisconsin vs. OneFish)					
ambient THg	0.92	0.15	5	-0.53	0.619
spike THg	0.90	0.13	5	-0.77	0.476
Figure 3.12 (type 2 model simulations vs. observed)					
Wisconsin ambient THg (no elimination)	0.56	0.17	5	-2.59	0.049
Wisconsin ambient THg (Madenjian elimination)	0.35	0.16	5	-4.06	0.01
Wisconsin ambient THg (suggested elimination)	0.2	0.12	5	-6.67	0.001*
OneFish ambient THg (no elimination)	0.83	0.15	5	-1.13	0.31
OneFish ambient THg (Madenjian elimination)	0.79	0.15	5	-1.4	0.22
OneFish ambient THg (suggested elimination)	0.71	0.14	5	-2.07	0.093
Wisconsin spike THg (no elimination)	0.54	0.09	5	-5.11	0.004*
Wisconsin spike THg (Madenjian elimination)	0.35	0.08	5	-8.13	0.0005*
Wisconsin spike THg (suggested elimination)	0.22	0.05	5	-15.6	<0.0001*
OneFish spike THg (no elimination)	0.78	0.14	5	-1.57	0.177
OneFish spike THg (Madenjian elimination)	0.73	0.15	5	-1.8	0.132
OneFish spike THg (suggested elimination)	0.68	0.13	5	-2.46	0.057
(type 2 model simulations; Wisconsin vs. OneFish)					
ambient THg (no elimination)	0.75	0.08	5	-3.13	0.026
ambient THg (Madenjian elimination)	0.53	0.1	5	-4.7	0.005*
ambient THg (suggested elimination)	0.36	0.1	5	-6.4	0.001*
spike THg (no elimination)	0.68	0.04	5	-8	0.0005*
spike THg (Madenjian elimination)	0.47	0.04	5	-13.25	<0.0001*
spike THg (suggested elimination)	0.32	0.05	5	-13.6	<0.0001*

Table 3.4 Mean yellow perch ambient total Hg (THg) concentrations observed in Lake 658 (±SEM) and predicted in the type 1 (zooplankton diet) and 2 (combination diet of zooplankton and *Chaoborus)* modelling simulations. Type 1 simulations were conducted for 5 growth patterns per year to represent the range of observed growth in the lake. Type 2 simulations were conducted for the median growth pattern (end weight or growth rate constant) from the type 1 simulations (one simulation per year). When ANOVA analyses were significant, Tukey honestly significant difference tests were performed (see text).

Type	Year	Scenario	Observed Hg ($ng \cdot g^{-1}$ w.w.)	Wisconsin Hg ($ng \cdot g^{-1}$ w.w.)	OneFish Hg ($ng \cdot g^{-1}$ w.w.)	ANOVA results
1	2001	no elimination	169.5 ± 12.6	138.6 ± 2.5	164.7 ±2.4	p=0.193
	2002	no elimination	129.0 ± 2.8	117.0 ± 4.2	133.1 ± 4.2	p=0.027*
	2003	no elimination	166.6 ± 7.0	96.9 ± 2.0	129.7 ± 1.1	p<0.0001*
	2004	no elimination	176.0 ± 14.1	132.6 ± 5.7	154.4 ± 1.9	p=0.064
	2005	no elimination	161.2 ± 6.9	136.13 ± 4.0	138.06 ± 2.6	p=0.102
	2006	no elimination	64.5 ± 4.7	52.9 ± 1.7	69.4 ± 1.2	p=0.102
	2007	no elimination	108.7 ± 1.1	106.7 ± 3.5	115.4 ± 1.5	p=0.214
2	2001	no elimination	169.5 ± 12.6	119.0	161.3	
	2001	Madenjian elimination	169.5 ± 12.6	79.2	155.1	
	2001	suggested elimination	169.5 ± 12.6	46.7	141.4	
	2002	no elimination	129.0 ± 2.8	100.4	128.7	
	2002	Madenjian elimination	129.0 ± 2.8	76.3	123.3	
	2002	suggested elimination	129.0 ± 2.8	53.8	110.3	
	2003	no elimination	166.6 ± 7.0	71.4	119.1	
	2003	Madenjian elimination	166.6 ± 7.0	43.0	113.0	
	2003	suggested elimination	166.6 ± 7.0	25.8	99.1	
	2004	no elimination	176.0 ± 14.1	112.8	156.5	
	2004	Madenjian elimination	176.0 ± 14.1	76.4	149.2	
	2004	suggested elimination	176.0 ± 14.1	49.1	134.7	
	2005	no elimination	161.2 ± 6.9	103.4	143.4	
	2005	Madenjian elimination	161.2 ± 6.9	70.0	136.7	
	2005	suggested elimination	161.2 ± 6.9	45.0	123.4	
	2006	no elimination	64.5 ± 4.7	41.4	57.4	
	2006	Madenjian elimination	64.5 ± 4.7	28.0	54.7	
	2006	suggested elimination	64.5 ± 4.7	18.0	49.4	
	2007	no elimination	108.7 ± 1.1	69.7	96.7	
	2007	Madenjian elimination	108.7 ± 1.1	47.2	92.1	
	2007	suggested elimination	108.7 ± 1.1	30.3	83.2	

Table 3.5 Mean yellow perch spike total Hg (THg) concentrations observed in Lake 658 (±SEM) and predicted in the type 1 (zooplankton diet) and 2 (combination diet of zooplankton and *Chaoborus*) modelling simulations. Type 1 simulations were conducted for 5 growth patterns per year to represent the range of observed growth in the lake. Type 2 simulations were conducted for the median growth pattern (end weight or growth rate constant) from the type 1 simulations (one simulation per year). When ANOVA analyses were significant, Tukey honestly significant difference tests were performed (see text).

Type	Year	Scenario	Observed Hg ($ng \cdot g^{-1}$ w.w.)	Wisconsin Hg ($ng \cdot g^{-1}$ w.w.)	OneFish Hg ($ng \cdot g^{-1}$ w.w.)	ANOVA results
1	2001	no elimination	5.3 ± 0.6	2.9 ± 0.04	3.3 ± 0.04	p=0.010*
	2002	no elimination	25.8 ± 0.7	24.1 ± 1.0	26.8 ± 1.0	p=0.204
	2003	no elimination	59.9 ± 2.2	36.9 ± 0.8	39.2 ± 0.4	p<0.0001*
	2004	no elimination	67.1 ± 5.3	49.7 ± 2.2	61.6 ± 0.7	p=0.049*
	2005	no elimination	47.4 ± 1.8	51.4 ± 1.5	47.5 ± 1.5	p=0.300
	2006	no elimination	30.1 ± 2.1	21.9 ± 0.7	31.7 ± 0.6	p=0.014*
	2007	no elimination	36.5 ± 1.1	42.0 ± 1.6	42.2 ± 0.5	p=0.003*
2	2001	no elimination	5.3 ± 0.6	2.6	3.1	
	2001	Madenjian elimination	5.3 ± 0.6	2.0	2.9	
	2001	suggested elimination	5.3 ± 0.6	1.4	2.4	
	2002	no elimination	25.8 ± 0.7	20.4	23.7	
	2002	Madenjian elimination	25.8 ± 0.7	15.9	22.2	
	2002	suggested elimination	25.8 ± 0.7	11.5	18.2	
	2003	no elimination	59.9 ± 2.2	26.6	34.8	
	2003	Madenjian elimination	59.9 ± 2.2	17.1	31.9	
	2003	suggested elimination	59.9 ± 2.2	11.2	29.0	
	2004	no elimination	67.1 ± 5.3	43.0	61.0	
	2004	Madenjian elimination	67.1 ± 5.3	29.1	58.3	
	2004	suggested elimination	67.1 ± 5.3	18.7	52.7	
	2005	no elimination	47.4 ± 1.8	30.4	43.1	
	2005	Madenjian elimination	47.4 ± 1.8	20.6	41.2	
	2005	suggested elimination	47.4 ± 1.8	13.2	37.2	
	2006	no elimination	30.1 ± 2.1	19.3	27.4	
	2006	Madenjian elimination	30.1 ± 2.1	13.1	26.2	
	2006	suggested elimination	30.1 ± 2.1	8.4	23.7	
	2007	no elimination	36.5 ± 1.1	23.4	33.2	
	2007	Madenjian elimination	36.5 ± 1.1	15.8	31.7	
	2007	suggested elimination	36.5 ± 1.1	10.2	28.7	

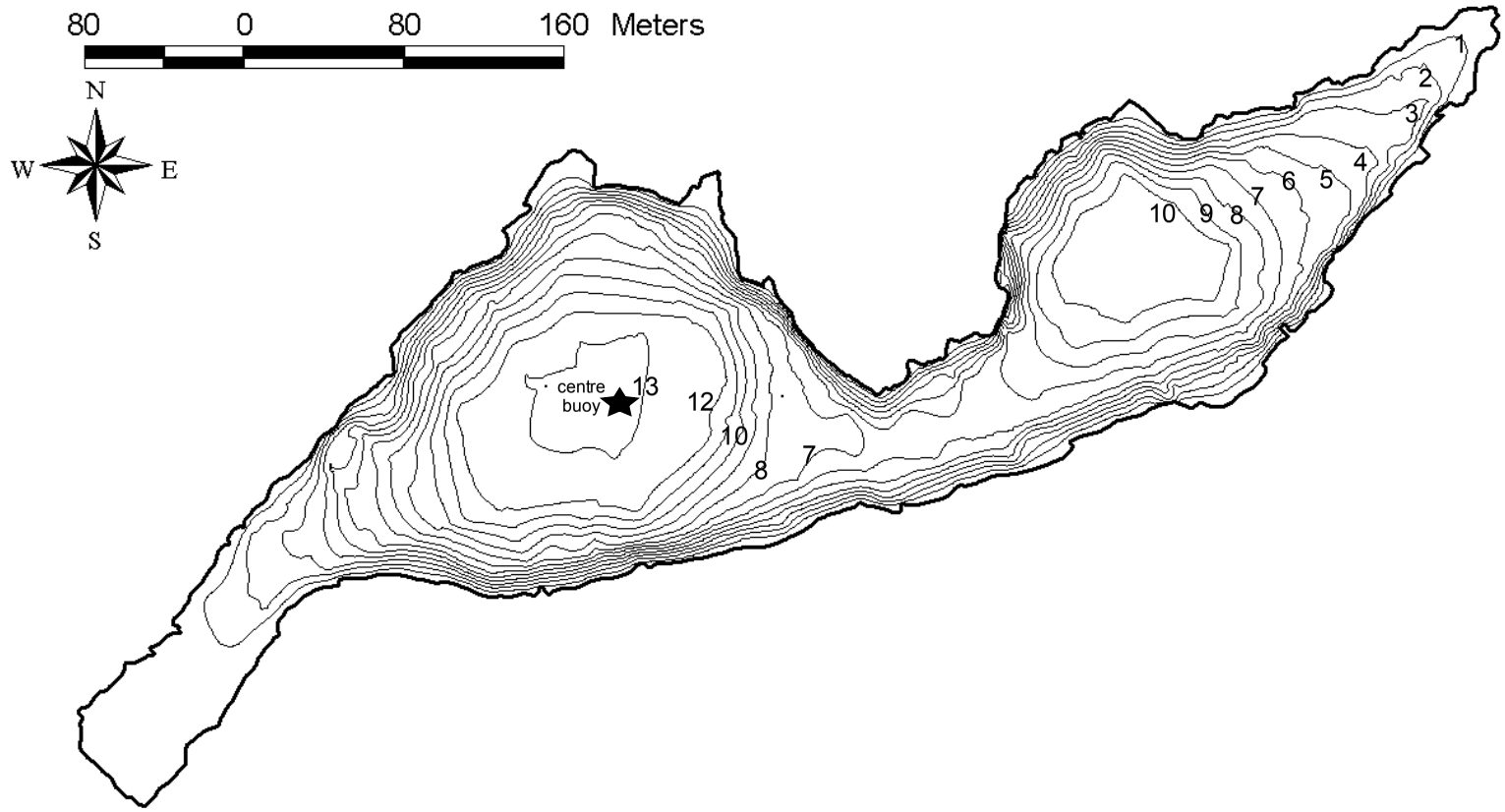

Figure 3.1 Bathymetry of Lake 658 at the Experimental Lakes Area, ON as in Sandilands *et al*. (2005). Bathymetry was surveyed on September 8, 2003. Depth contours are labelled in 1 m increments. Maximum lake depth is 13.2 m.

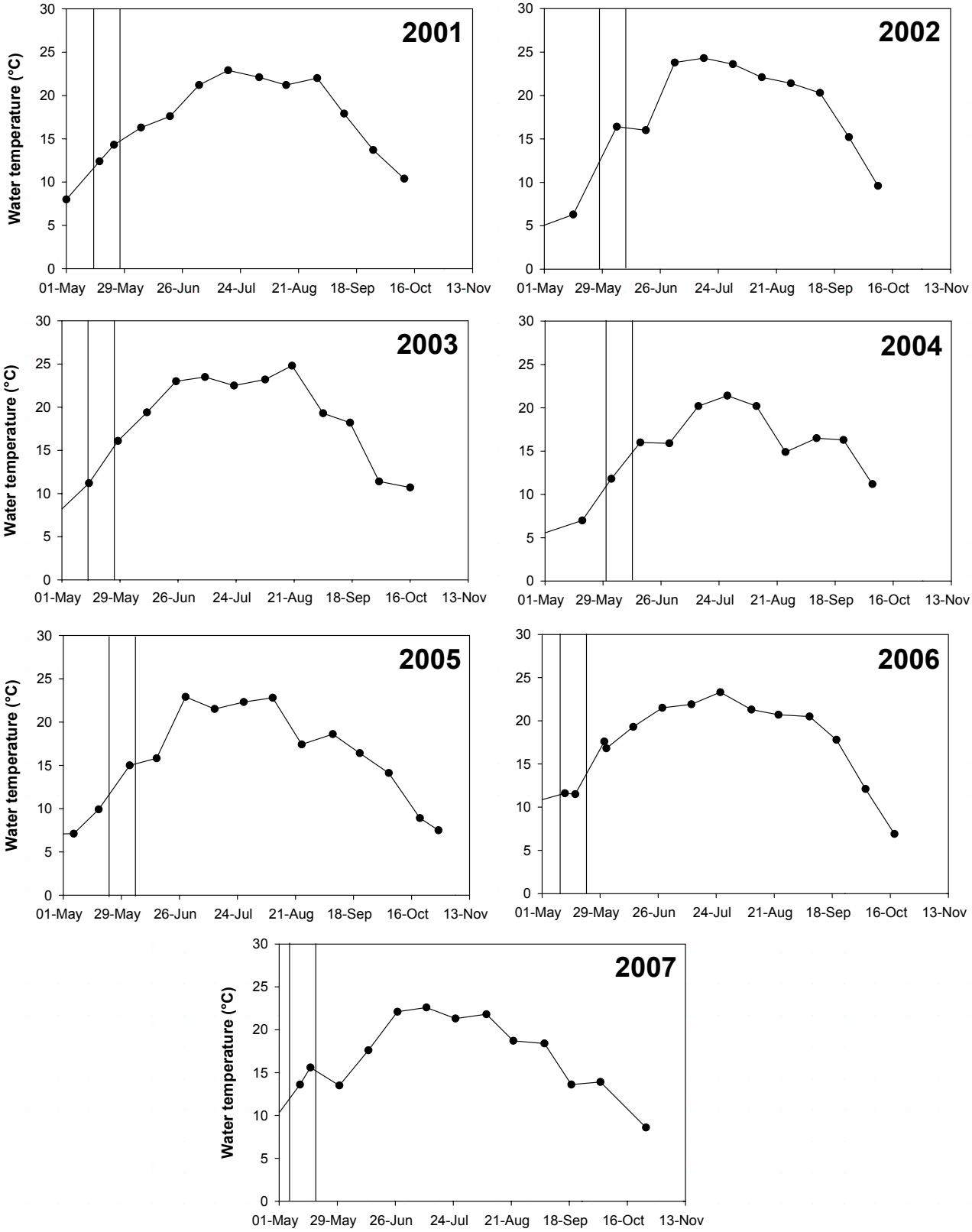

Figure 3.2 Water temperatures taken at 2 m below the surface at centre buoy in Lake 658 (Figure 3.1) in 2001-07 (black circles). Vertical lines indicate estimated yellow perch spawn (left lines) and hatch (right lines) dates. Spawn dates were based on 12°C water temperature, to which 12 days were added to estimate hatch date. All model simulations were conducted from the hatch date until the last sampling day of the season for each year.

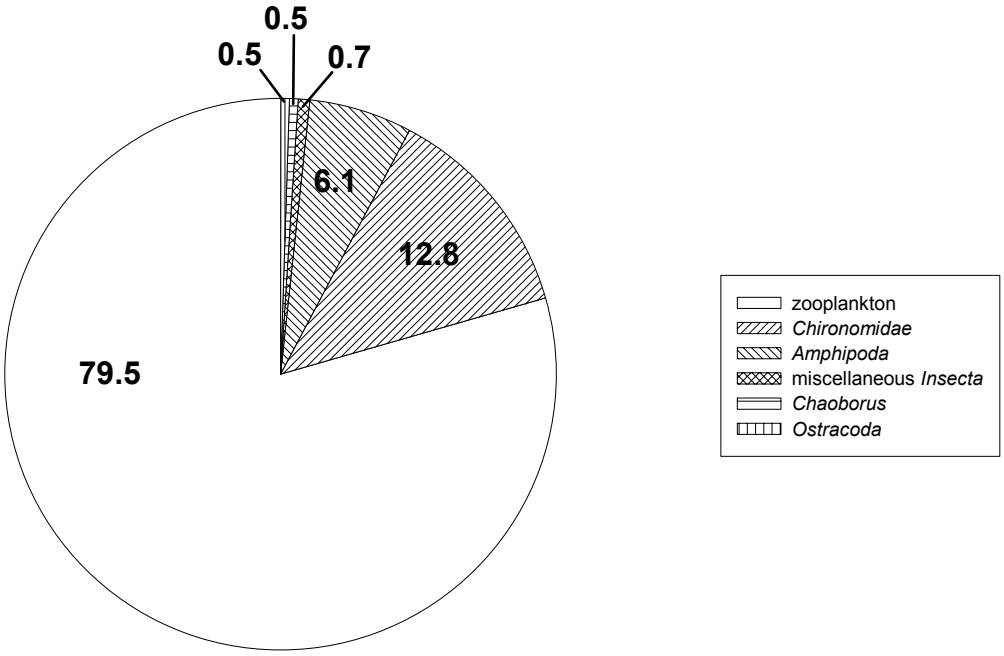

Figure 3.3 Diet composition of YOY yellow perch collected from Lake 658 in August of each sampling year (total of 70 stomachs collected; n=60 contained prey items). Prey items were counted, dried, and weighed and then compared according to the Relative Importance index (RI) (George and Hadley 1979) (see text).

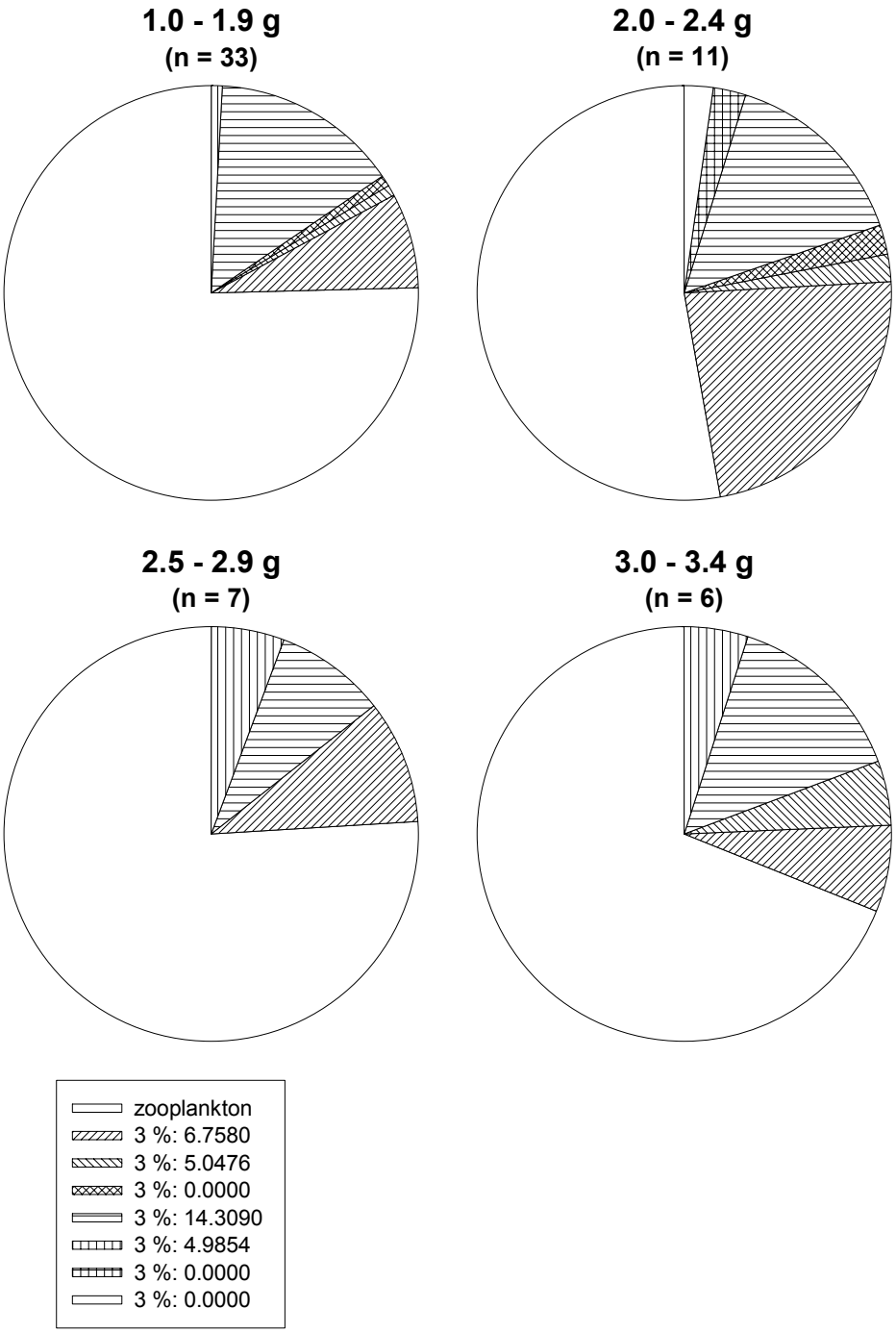

Figure 3.4 Diet composition of YOY yellow perch collected from Lake 658 in the summer season of 2001. Relative Importance index for prey was calculated for 4 fish weight categories: 1.0 to 1.9 g, 2.0 to 2.4 g, 2.5 to 2.9 g, and 3.0 to 3.4 g.

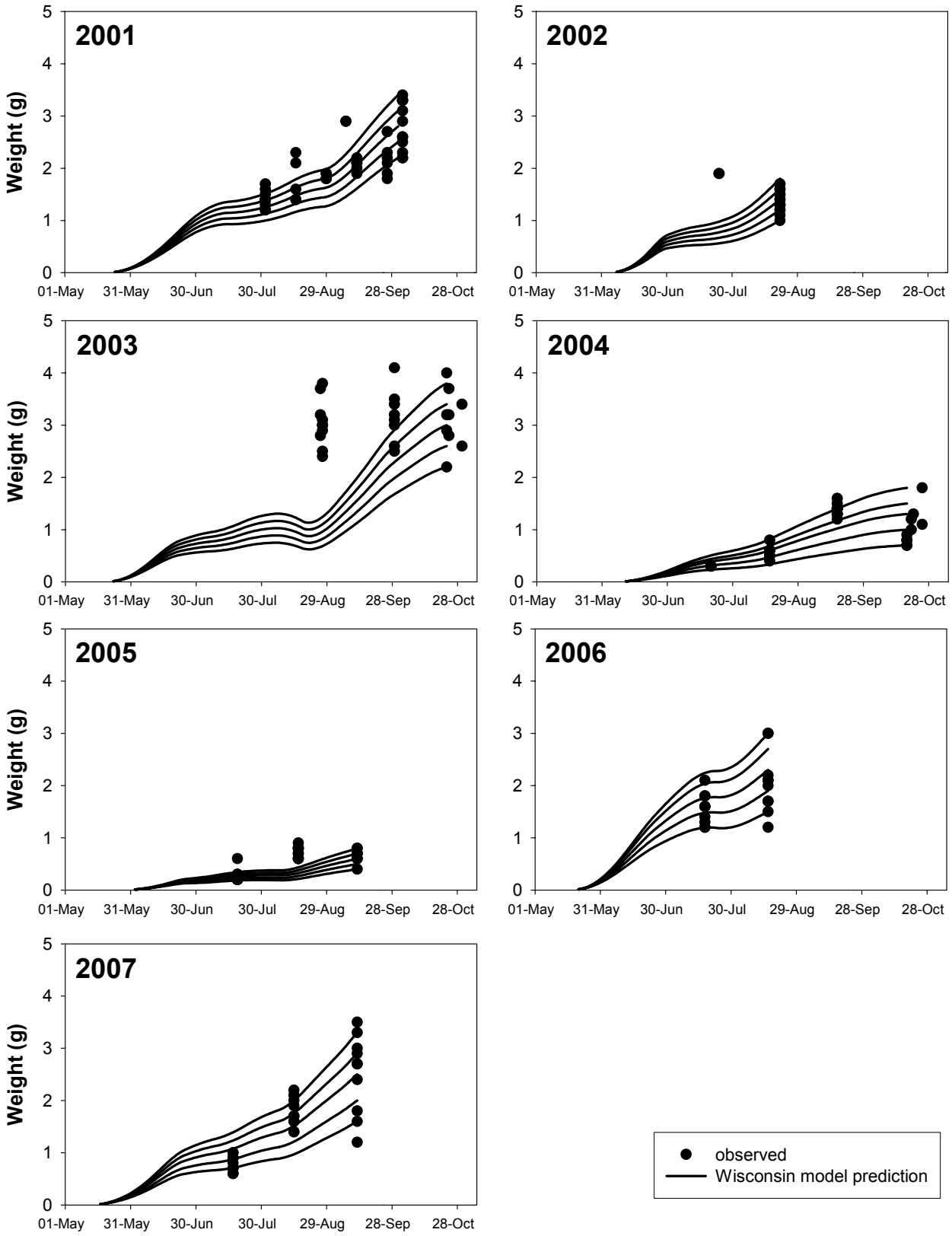

Figure 3.5 Predictions of fish weight generated by the Wisconsin model and observed weights of YOY yellow perch in Lake 658 for each sampling year. Five type 1 simulations were completed for each year, yielding 5 separate growth curves. Model growth rates were calibrated to match the range of weights achieved by the fish in Lake 658 on the last sample day for each year.

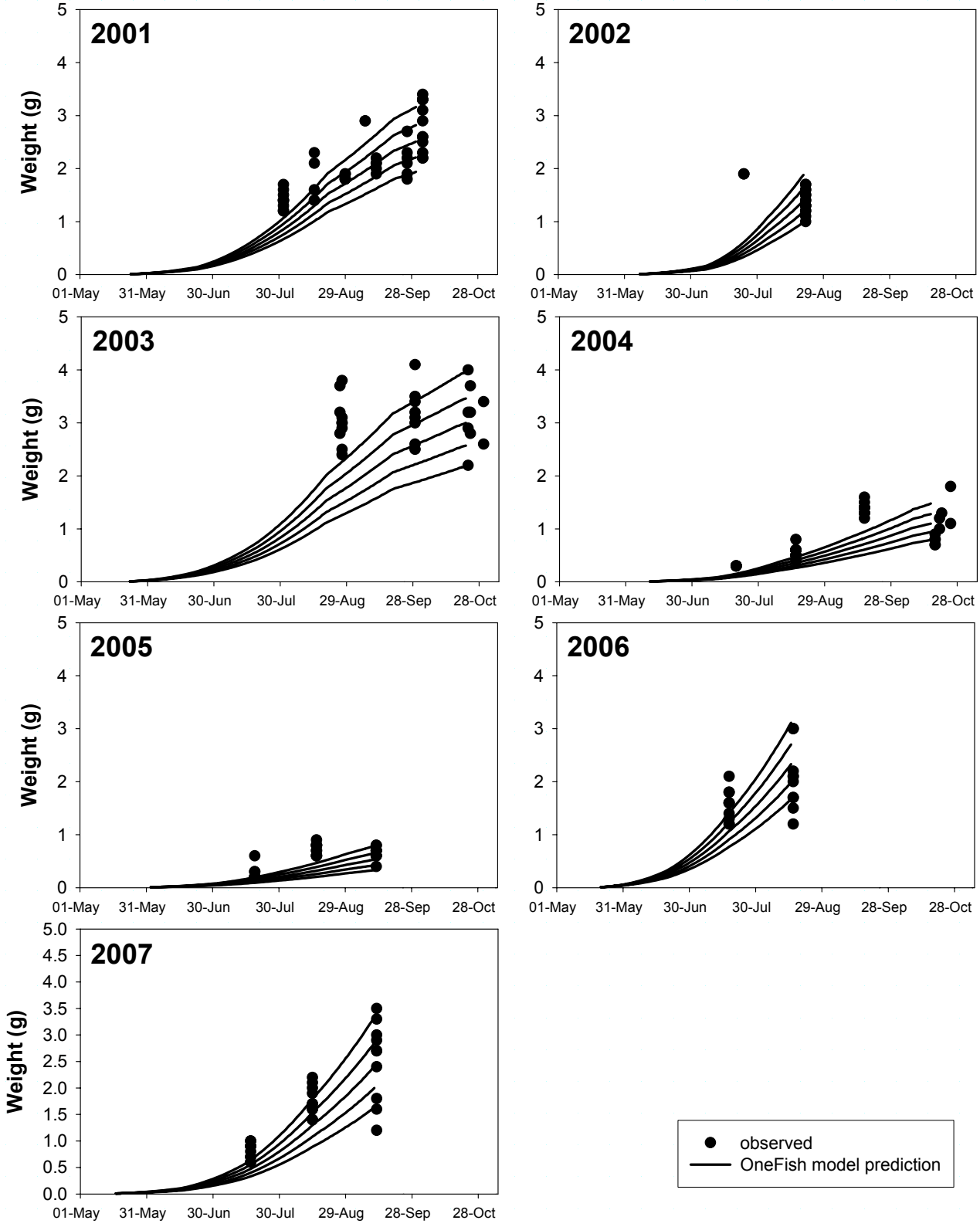

Figure 3.6 Predictions of fish weight generated by the OneFish model and observed weights of YOY yellow perch in Lake 658 for each sampling year. Five type 1 simulations were completed for each year, yielding 5 separate growth curves. Model growth rates were calibrated to match the range of weights achieved by the fish in Lake 658 on the last sample day for each year.

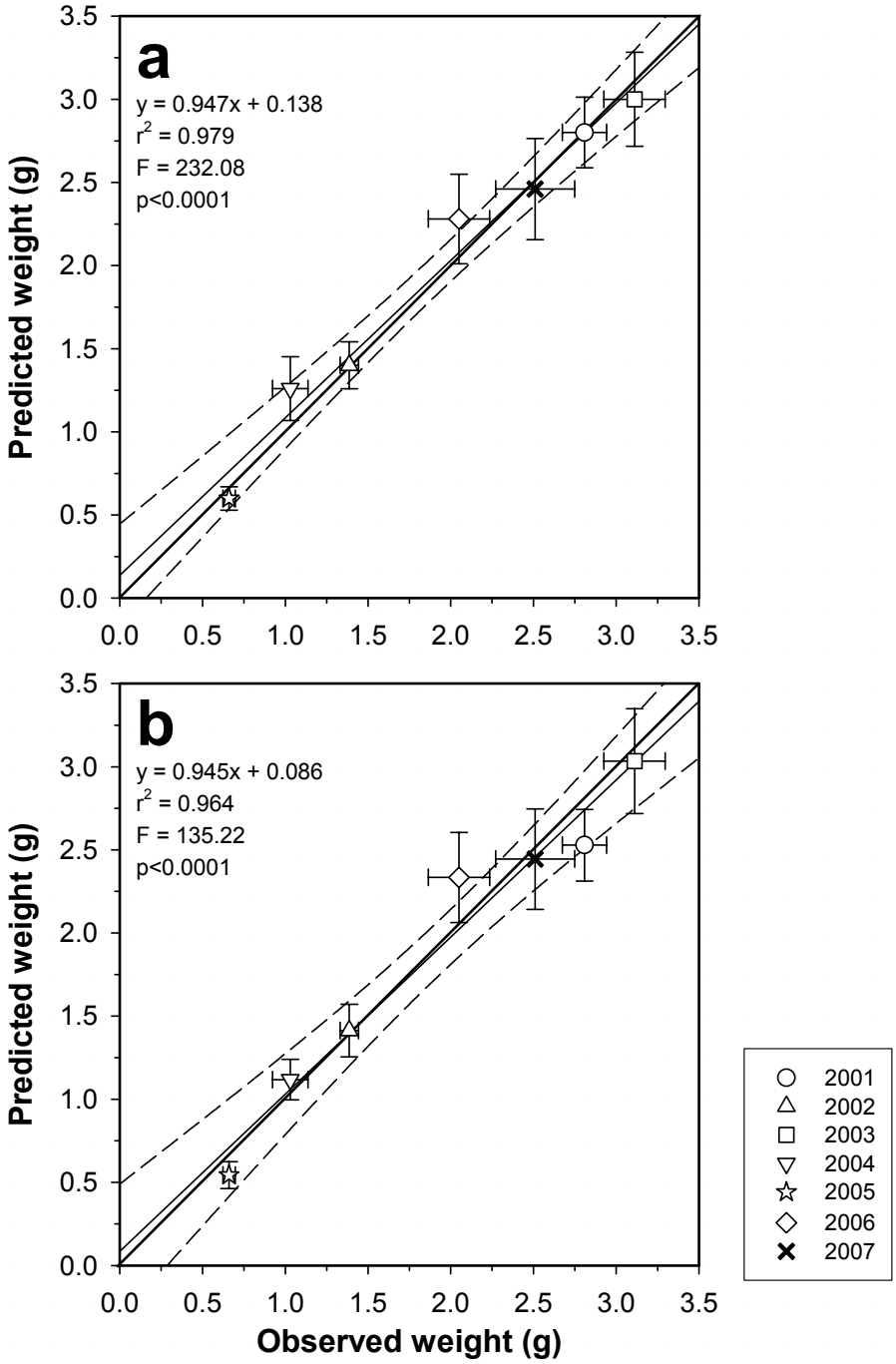

Figure 3.7 Observed weights of YOY yellow perch collected in Lake 658 on the last sampling day of each year compared to the weights predicted by the Wisconsin (a) and OneFish (b) models. Axes are scaled so that a slope of 1 has an angle of 45 degrees, and regression lines are shown with 95% confidence bands. Each plot shows the results of the *F*-test (α=0.05) which was performed to determine whether the relationship between the variables was significant. The line representing a 1:1 ratio between the two variables is presented. Error bars represent SEM.

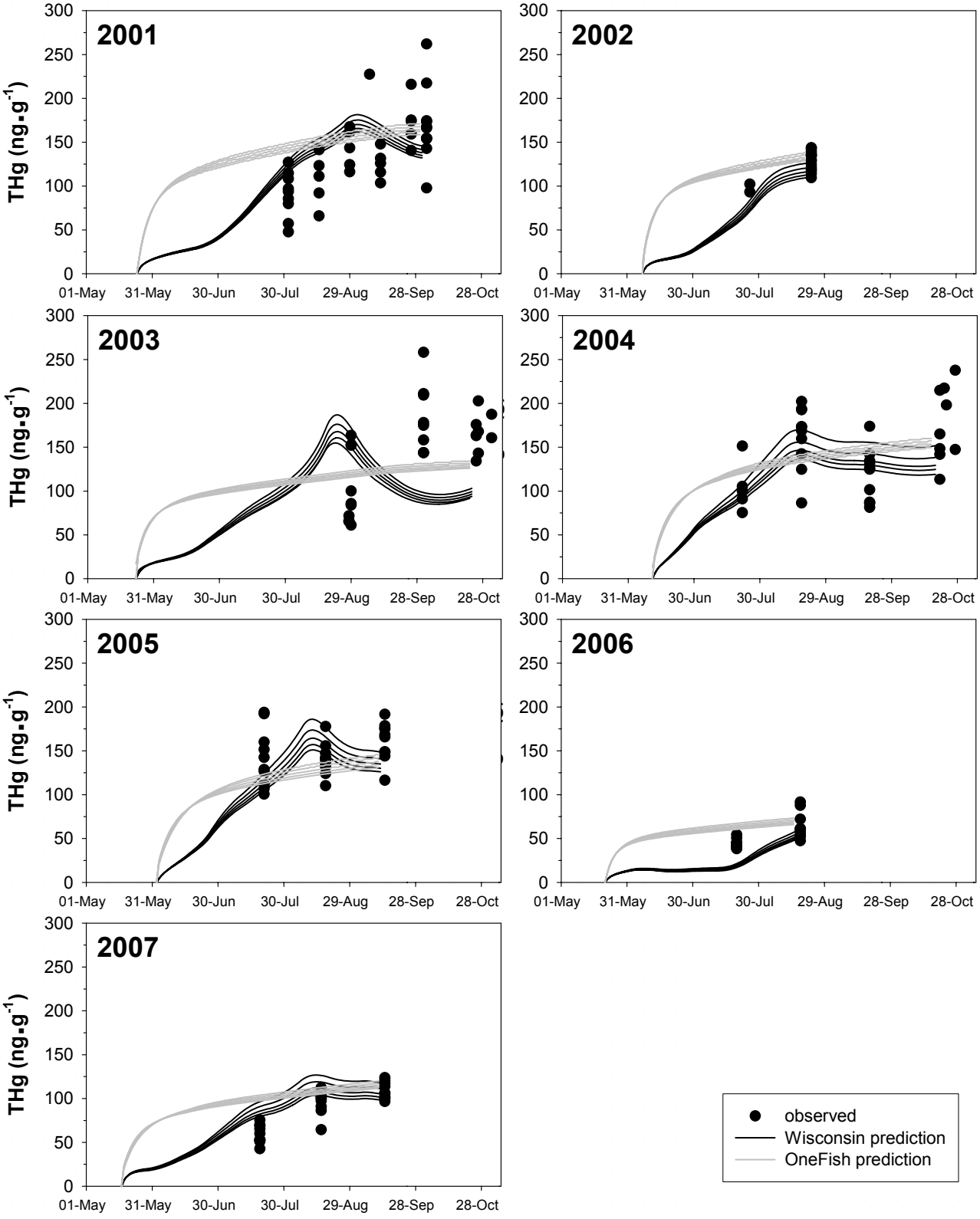

Figure 3.8 Type 1 predictions of fish ambient total mercury (THg) concentrations generated by the Wisconsin and OneFish models and observed concentrations of YOY yellow perch in Lake 658 for each sampling year. Five simulations were completed for each year, yielding 5 separate curves for each model.

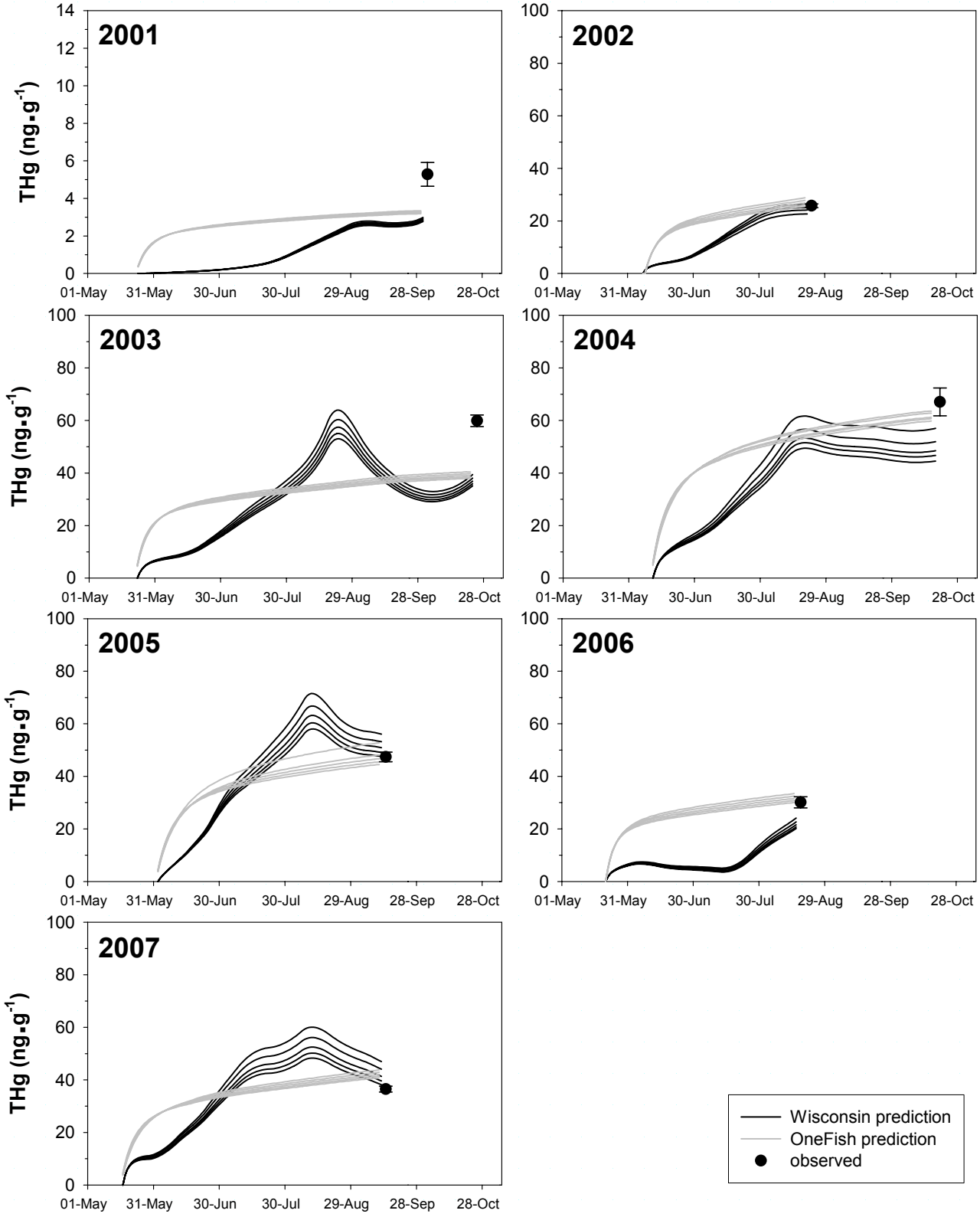

Figure 3.9 Type 1 predictions of fish spike total mercury (THg) concentrations generated by the Wisconsin and OneFish models and mean observed concentrations of YOY yellow perch in Lake 658 for each sampling year. Five simulations were completed for each year, yielding 5 separate curves for each model. Note different y-axis scale on 2001 plot. Error bars represent SEM.

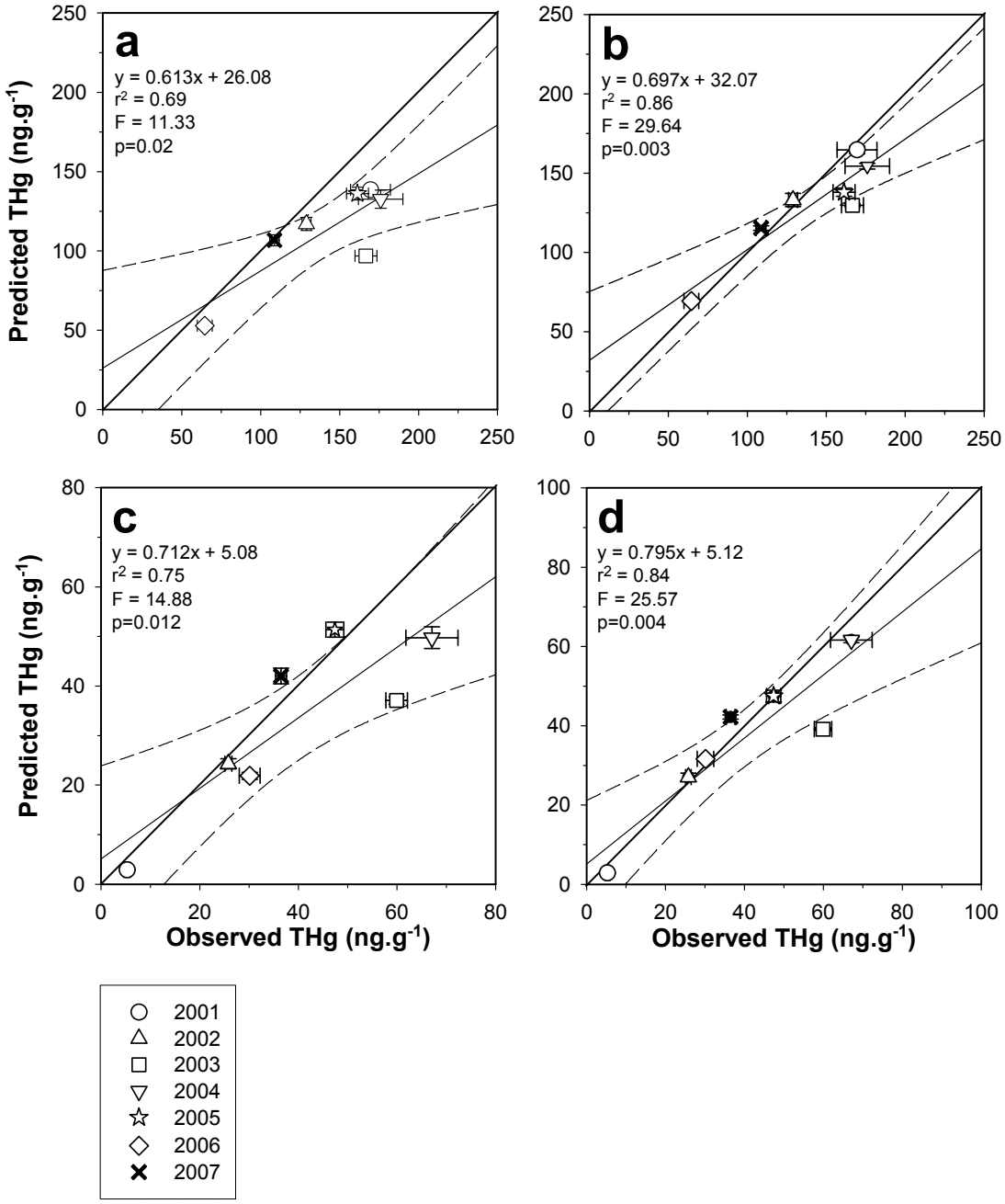

Figure 3.10 Observed total mercury (THg) concentrations of YOY yellow perch in Lake 658 on the last sampling day of each year compared to the concentrations predicted by the Wisconsin and OneFish models in type 1 model simulations. Data are presented for both ambient THg (Wisconsin (a) and OneFish (b)) and spike THg (Wisconsin (c) and OneFish (d)). Axes are scaled so that a slope of 1 has an angle of 45 degrees, and regression lines are shown with 95% confidence bands. Each plot presents the results of the *F*-test (α=0.05) that was performed to determine whether the relationship between the variables was significant. Diagonal lines representing 1:1 ratios are presented. Error bars represent SEM.

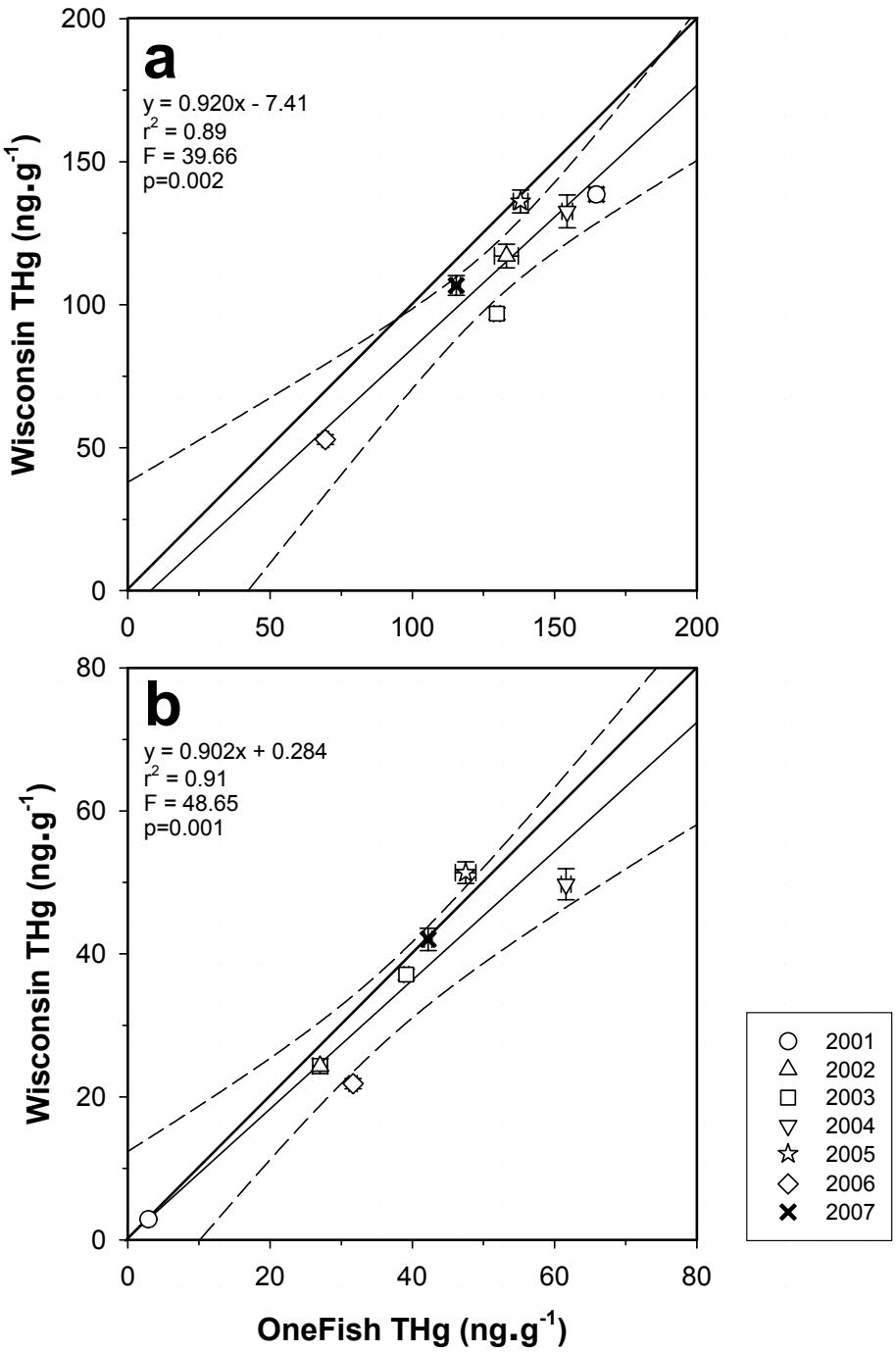

Figure 3.11 Comparison of Wisconsin and OneFish type 1 model predictions for ambient total mercury (THg) (a) and spike THg (b). Axes are scaled so that a slope of 1 has an angle of 45 degrees, and regression lines are shown with 95% confidence bands. Each plot shows the results of the *F*-test (α=0.05) which was performed to determine whether the relationship between the variables was significant. The line representing a 1:1 ratio between the two variables is presented. Error bars represent SEM.

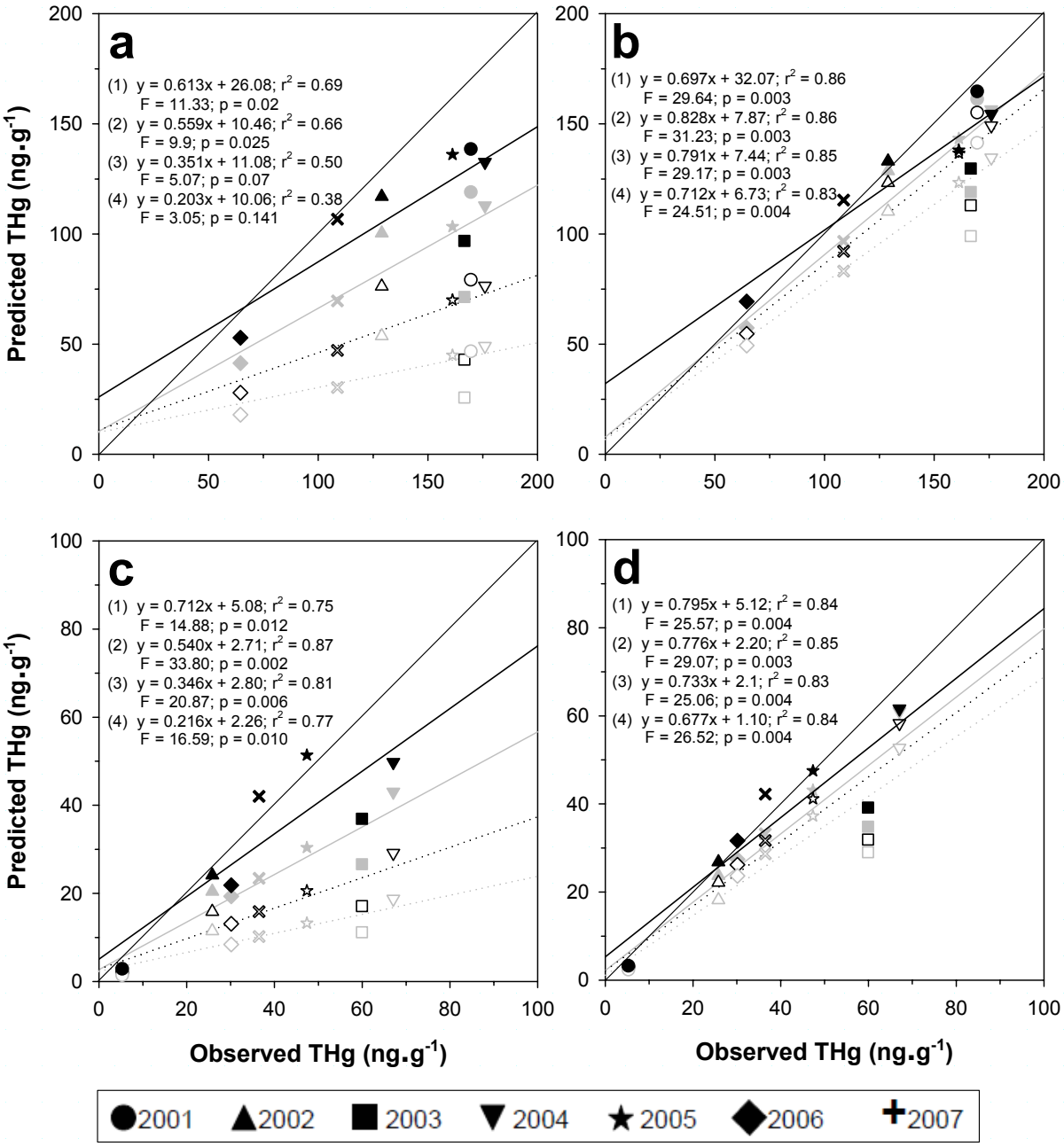

Figure 3.12 Observed total mercury (THg) concentrations of YOY perch collected from Lake 658 on the last sampling day of each year compared to the concentrations predicted in type 1 and 2 simulations by the Wisconsin and OneFish models (type 1: closed black symbols; no elimination: closed grey symbols; Madenjian and O'Connor (2008): open black symbols; suggested elimination: open grey symbols). Data are presented for both ambient THg (Wisconsin (a) and OneFish (b)) and spike THg (Wisconsin (c) and OneFish (d)). Axes are scaled so that a slope of 1 has an angle of 45 degrees, and black diagonal lines represent the 1:1 ratio. Regression lines are shown for each simulation type: (1) type 1 simulations (solid black); (2) no elimination (solid grey); (3) Madenjian and O'Connor (2008) elimination (dotted black); (4) suggested elimination (dotted grey). Each plot presents the results of the *F*-test (α=0.05) which was performed to determine whether the relationship between the variables was significant.

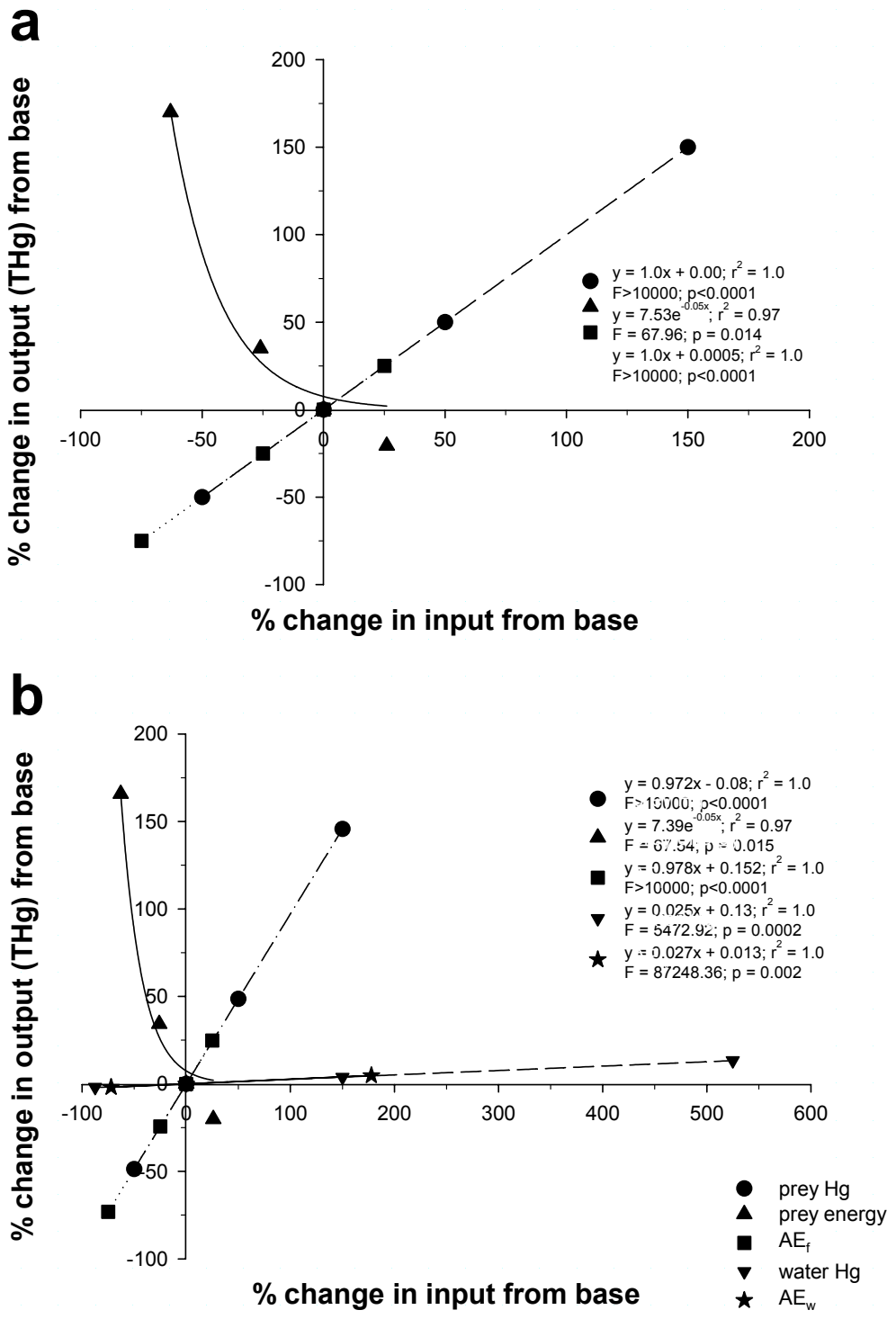

Figure 3.13 Sensitivity analyses for the Wisconsin (a) and OneFish (b) models. The data points located at (0,0) represent the base simulation (manipulation 1). All other points show the % deviation from the base simulation for the input variable and the resulting total mercury (THg) concentration prediction. All simulations were conducted with identical inputs to the base simulation except for the target variable, which was manipulated to represent a range of possible values. Results from regression analyses are presented for each type of manipulation, and regression lines are plotted. Results of *F*-tests (α=0.05) performed to test the significance of the regressions are presented.

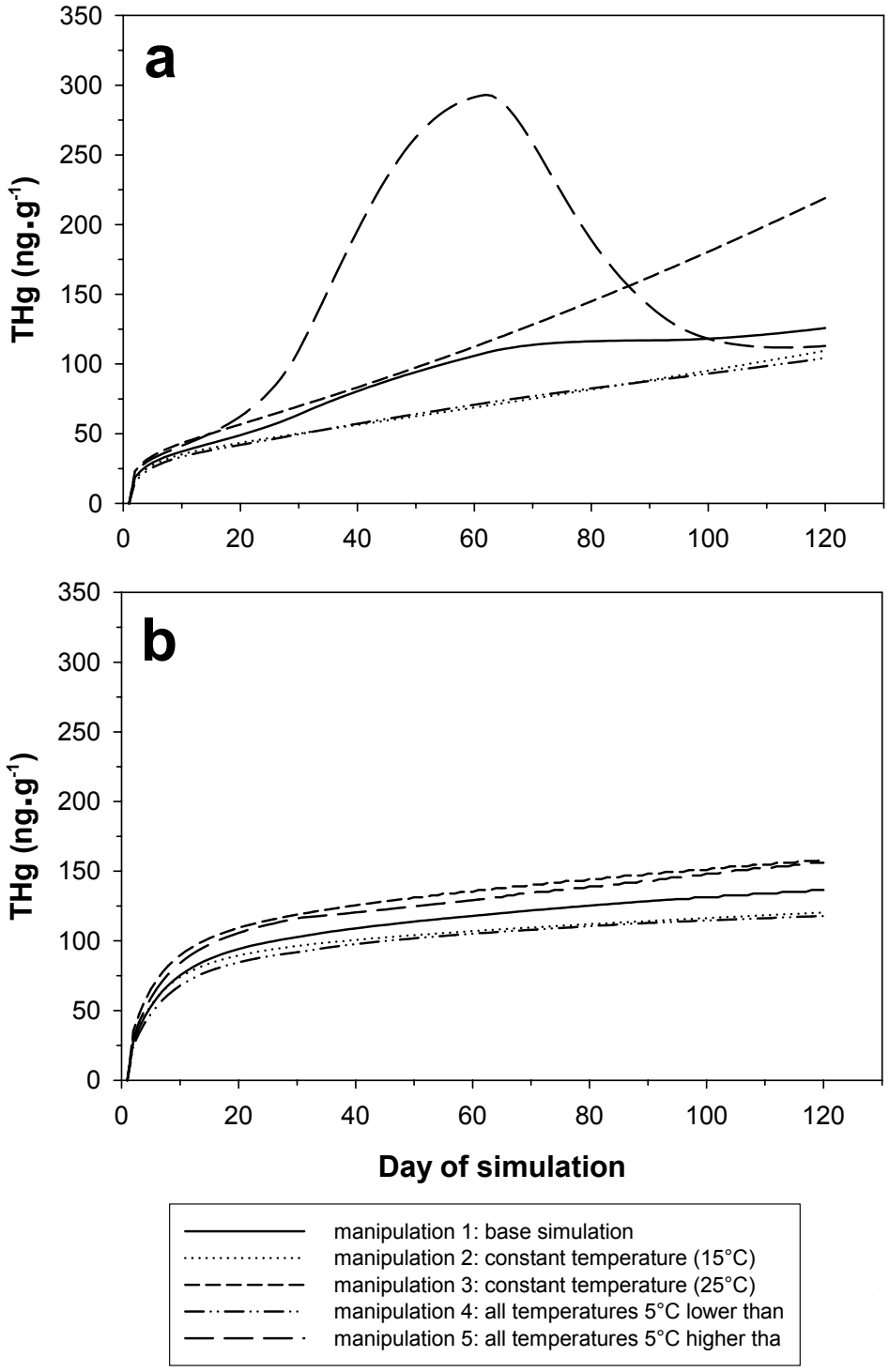

Figure 3.14 Results of temperature manipulations in sensitivity analyses of the Wisconsin (a) and OneFish (b) models. Predictions of yellow perch total mercury (THg) concentrations are presented for each model for the base simulation, and four simulations in which the water temperature was manipulated: (i) all temperatures set at 15°C; (ii) all temperatures set at 25°C; (iii) 5°C lower than temperatures in base simulation; (iv) 5°C higher than temperatures in base simulation (outlined in Tables A.10 and A.11).

Chapter 4. Synthesis

Mercury (Hg) contamination in aquatic ecosystems is a global problem that is detrimental to the health of humans, fish, and wildlife (Bloom 1992; Mergler *et al*. 2007; Munthe *et al*. 1997), and has arisen as the result of human influence on the global mercury cycle (Mason *et al*. 2005; Pacyna *et al*. 2010). Inorganic Hg (Hg^{2+}) released to the atmosphere through anthropogenic activities is deposited to aquatic ecosystems primarily through precipitation and may be converted to methylmercury (MeHg) by microbial activity following deposition (Rudd 1995; Jackson 1997). MeHg is highly toxic and is not easily eliminated by organisms, leading to bioaccumulation and biomagnification in the food web (Fleming *et al*. 1995). As a result, organisms at the highest trophic levels (e.g., predatory fish) frequently exhibit concentrations of MeHg that are considered unsafe for human-consumption (USEPA 2008; Hinck *et al*. 2009; Bhavsar *et al*. 2010). Human exposure to MeHg results primarily from consumption of contaminated fish flesh (Mahaffey 1998), and many consumption advisories are in place in lakes and rivers across North America to limit MeHg intake (USEPA 2008). The majority of anthropogenic Hg emissions arise from activities which release volatiles and particulates to the atmosphere, including the burning of coal, metal smelting, and waste incineration (Bhavsar *et al*. 2010; Pacyna *et al*. 2010).

Strategies to reduce global anthropogenic Hg emissions have been proposed in an effort to control fish MeHg levels (Mason *et al*. 2005; UNEP 2009; Lubick 2009). A global treaty established by the United Nations Environment Programme (UNEP) aims to

drastically reduce global Hg emissions by 2013 (Lubick 2009; Pacyna *et al*. 2010). Unfortunately, many questions about the potential effectiveness of these reductions remain unanswered (Mason *et al*. 2005). The processes that link atmospheric deposition of Hg to MeHg accumulation in fish are numerous and complex (Mason *et al*. 2005), often depending on location-specific controls (Lepak *et al*. 2009; Sackett *et al*. 2009). Previous research has stressed that the critical knowledge gap lies in our lack of comprehensive understanding of how fish accumulate Hg from their environment (Mason *et al*. 2005; Lepak *et al*. 2009b; Sackett *et al*. 2009). Many studies dismiss waterborne Hg (MeHg and Hg^{2+}) as a source of MeHg to fish (e.g. Gorski *et al*. 1999; Bowles *et al*. 2001; Rennie 2003; Lepak *et al*. 2009b), while others suggest that although diet is the main source, direct uptake from the water also contributes to MeHg in fish (Rodgers and Qadri 1982; Post *et al*. 1996; Hall *et al*. 1997; Pickhardt *et al*. 2006). Without a clear understanding of how environmental Hg concentrations in diet and water impact MeHg accumulation in fish, it will be difficult to determine how emissions reduction strategies may remediate contaminated fish stocks, and to plan and implement effective monitoring programs.

Fish MeHg bioaccumulation models are used today by researchers and managers to examine trends in fish MeHg accumulation (e.g. Delta Tributaries Mercury Coucil 2000; MacRury *et al*. 2002; Imhoff *et al*. 2004; Van Walleghem *et al*. 2007) and will be used extensively as management tools, in conjunction with broader ecosystem Hg cycling and global Hg transport models (e.g. Ryaboshapko *et al*. 2002; Knightes *et al*. 2009), to simulate the effects of decreased environmental Hg concentrations on fish MeHg levels

(Imhoff *et al*. 2004; USEPA 2006). Many published bioaccumulation models are based on similar energetics equations but make differing assumptions regarding MeHg uptake, some assuming that all Hg accumulation is from food (e.g. Hanson *et al*. 1997; Trudel and Rasmussen 2001; Rennie 2003), and others accounting for Hg uptake from both food and water (e.g. Rogers 1994; Post *et al*. 1996; Harris and Bodaly 1998). As there is conflicting evidence surrounding the importance of waterborne Hg uptake to fish MeHg levels, it is unclear which models will provide more accurate predictions. Bioaccumulation models must best represent natural fish Hg uptake if model predictions are to be used to make policy and ecosystem management decisions. Additionally, as some models have simple user interfaces and are available to the public for download or purchase (e.g. Hudson *et al*. 1993; Hanson *et al*. 1997; Knightes *et al*. 2009), they are used widely and with confidence (Hansen *et al*. 1993; Imhoff *et al*. 2004). Hansen *et al*. (1993) suggest that "some [model] users will accept on faith that a model is well-constructed simply because it exists as a canned computer program, and they will use it without understanding the potential risks and assumptions." The ready availability of these models makes it even more important that model output be accurate. To achieve this accuracy, and to enhance their validity as predictive tools, it is integral that models be properly evaluated using real-world data (Ryaboshapko *et al*. 2002; Rennie 2003; Van Walleghem *et al*. 2007). Unfortunately, the detailed, long-term data sets required to effectively test these models are not common (Luoma 1983; Mason *et al*. 2005).

The purpose of this study was to examine the accumulation of MeHg by fish in nature with a particular focus on uptake of Hg directly from water. I combined a field

experiment with the examination of rich, long-term food web and limnological data sets (from the Mercury Experiment to Assess Atmospheric Loading in Canada and the United States (METAALICUS) at the Experimental Lakes Area (ELA), ON, Canada) to quantify the relative contributions of dietary and water Hg exposure to fish MeHg levels, and to determine the importance of exclusion of waterborne Hg in fish Hg bioaccumulation models. For both the field experiment and bioaccumulation modeling I examined Hg levels in young-of-year (YOY) yellow perch, an important forage and game fish species across North America (Craig 1987).

Both approaches used the enriched stable isotope ^{202}Hg (spike Hg), which is distinguished from background (ambient) Hg, to examine fish MeHg accumulation. Enriched stable isotopes of Hg have been used in bioaccumulation and Hg transfer studies with increasing frequency over the last decade (Hintelmann and Evans 1997; Paterson *et al*. 2006; Harris *et al*. 2007). Enriched stable isotopes behave in the same manner as ambient Hg but may be tracked separately after they are experimentally-applied to an ecosystem. This separation allows researchers to make a distinction between newly-deposited Hg (spike) and ambient Hg that is a combination of new Hg and Hg that has been present in the system for long time periods. It has been suggested that newly-deposited Hg influences Hg concentrations in biota far more than sediment-bound reservoirs of Hg, and that as a result, decreases in atmospheric deposition may lead to proportional decreases in fish Hg (Hammerschmidt *et al*. 2006; Orihel *et al*. 2007). By following spike Hg through the ecosystem in this study, it was possible to examine transfer and accumulation of 'new' Hg by yellow perch.

The field experiment used a replicated 2-factorial design to separate the sources of spike Hg (food and water) to YOY yellow perch. The clarity of the response observed in this study surpasses that of previous accumulation experiments, and resulted from the use of spike Hg in food and water sources. This experiment provided the following observations about the accumulation of Hg by YOY yellow perch under summer conditions:

i) YOY yellow perch accumulated spike Hg (THg and MeHg) directly from the water;

ii) 10-21% of spike Hg present in YOY yellow perch whole-body tissue samples following exposure resulted from direct uptake from water;

iii) Accumulation of spike Hg from food and water by YOY yellow perch was additive;

iv) Mean spike Hg concentration predicted by the Wisconsin model (no uptake from water) was lower than the mean spike Hg concentration observed in fish from the spike water + spike food (SWSF) treatment by 18.6% and lower than the OneFish model (uptake from water) prediction by 13.0%.

In the second part of this study, I used two published fish Hg bioaccumulation models to examine the seasonal accumulation of spike and ambient Hg in YOY perch from the METAALICUS study for each of the 7 years that spike Hg was added to the lake (2001-07). One model assumes that all fish Hg comes from diet (Wisconsin Fish Bioenergetics v.3.0), while the other includes uptake from both water and food

(OneFish). The Wisconsin model (Hanson *et al*. 1997) is available for purchase and is widely used by researchers and managers (e.g. MacRury *et al*. 2002; Van Walleghem *et al*. 2007; Vieira 2007; Chipps 2009; Lepak *et al*. 2009b). The equations used in the OneFish model (Harris and Bodaly 1998) are presented in the paper, and the computer program may be available from the authors. OneFish has been used previously to examine fish Hg cycling at the ELA (Van Walleghem 2006). I used observed Hg concentration data in YOY perch diet and water from the METAALICUS project as inputs. Comparisons of model predictions to spike and ambient Hg concentrations observed in METAALICUS fish, yielded the following conclusions:

i) Wisconsin predictions were lower than observed concentrations by 17.0% (ambient Hg) and 18.5% (spike Hg) using observed inputs (type 1);

ii) Type 1 OneFish predictions were slightly lower than observed concentrations (8.9% for ambient Hg; 4.9% for spike Hg);

iii) The inclusion of a combination diet and some Hg elimination (type 2 simulations) in the Wisconsin model yielded predictions that were significantly lower than observed concentrations. Simulations that used the elimination rate suggested by the model produced the lowest estimates of fish Hg, underestimating perch Hg concentrations by up to 72%;

iv) When subjected to a combination diet and some elimination, the OneFish model predicted fish Hg concentrations that were not significantly lower than observed values;

v) The most influential parameters in both models are prey energy content, prey Hg concentration, assimilation efficiency of Hg from prey, and water temperature.

Together, these observations suggest that waterborne Hg is an important contributor to overall fish MeHg levels during the summer months, and that the exclusion of water as a source of Hg to fish in bioaccumulation models may produce underestimates of fish MeHg concentrations. Furthermore, these results highlight the need for caution when interpreting model results if Hg elimination is included, and emphasize that the most sensitive parameters in bioaccumulation models are water temperature and inputs related to consumption. The main strengths of this study stem from the use of enriched stable Hg isotopes, and from the richness of the METAALICUS dataset. The results reinforce the benefits of using enriched stable isotopes to track the movement of Hg through the food web, and emphasize the advantages of combining field data with bioaccumulation modelling. The main implication of these findings is that studies that attempt to predict the response of fish Hg concentrations to decreases in global anthropogenic Hg emissions and deposition to aquatic ecosystems should not ignore waterborne Hg as a source of Hg to fish.

The Wisconsin model is widely used to predict Hg accumulation by fish. Reports of these studies often indicate that the model successfully predicted Hg concentrations that match those observed in the target population (e.g. MacRury *et al*. 2002; Lepak *et al*. 2009b). In this study, despite the use of robust diet and water Hg data and the inclusion

of published Hg elimination rates, Wisconsin model predictions fell far short of concentrations observed in Lake 658 fish. Type 2 model simulations were completed to represent how the model might be used in a real-world Hg monitoring program. Given that the model predicted growth accurately, it is disconcerting that the predictions were so far from accurate in these 'standard' scenarios. The physiological parameters for each type of fish may be altered by a user in the Wisconsin model. These parameters are based on laboratory and field studies, and together, in response to input data, define consumption, respiration, growth, Hg accumulation, and all other model functions. Alteration of these parameters causes changes in model output. It is possible that these parameters do not reflect yellow perch accurately, which could be a source of part of the inaccuracies of the model. Clearly, although the Wisconsin model is widely-used either as a standalone program or as a base structure for other models, if used with current model inputs fish Hg concentrations predicted by the model will be lower than concentrations in nature and should be interpreted with caution.

In a broader sense, these results emphasize the challenges faced by researchers when using bioaccumulation models. Model success depends on many factors, including the equations and setup of the model itself, input data, assumptions made by the model, and how the model captures location-specific effects, among others. Jorgensen (2008) emphasizes that ecotoxicological models inherently have a high degree of uncertainty due to our lack of understanding of the specific processes that drive contaminant transfer and accumulation. The use of robust, detailed input data is a step toward accurate model predictions, but, as highlighted in this study by the accuracy of the OneFish model

predictions which used simpler, less-detailed input data, the performance of a model appears to hinge more on its structure and assumptions. The majority of existing Hg bioaccumulation models are based on a few early versions that were designed as tentative explorations into coupling Hg bioaccumulation with fish bioenergetics. Although these models have performed adequately in the past, it is possible that the basic relationships governing Hg transfer and accumulation within these models require revision, particularly with respect to accumulation of Hg from water.

Recently, there has been a call to develop cohesive North American or global Hg monitoring programs to predict and track the effects of reduced Hg emissions on aquatic and marine systems (Mason *et al*. 2005; USEPA 2006; Evers *et al*. 2008; Keeler *et al*. 2009; Lepak *et al*. 2009). It is assumed that proposed reductions in global Hg emissions will lead to decreased Hg concentrations in aquatic biota, but the timing and magnitude of this response is unclear. Many existing and proposed strategies use bioaccumulation modelling to attempt to predict Hg in fish under future emissions regimes. Our ability to build and use these models depends on concrete knowledge of the linkages between Hg emissions, atmospheric deposition, and Hg in fish.

Previous research has suggested that decreases in emissions lead to immediate declines in water Hg concentrations (Hrabik and Watras 2002; Evers *et al*. 2007). For example, Hrabik and Watras (2002) report that a 10% decline in atmospheric deposition of Hg lead to 5% decreases in water Hg levels in a seepage lake in Wisconsin. Other research has suggested that Hg in fish is proportionally related to local Hg deposition rates

(Hammerschmidt and Fitzgerald 2006; Orihel *et al*. 2007). The common theme in these studies is that in lakes where precipitation provides the largest inputs of Hg to the system (i.e. seepage lakes with small watersheds), newly-deposited Hg from the atmosphere is the most important determinant of waterborne and biotic Hg concentrations, and recycling of old Hg from upland and sediment reservoirs contributes little to Hg in water and biota. In lakes with large watersheds that receive most of their Hg from runoff, long-term deposits of Hg (in uplands, wetlands) may be more influential over aquatic Hg levels. The results of this study suggest that direct accumulation of newly-deposited Hg from water is an important source of Hg to fish. It is intuitive that substantial declines in atmospheric deposition would lead to immediate declines in water Hg, which would trigger declines in fish Hg because of reduced accumulation from water. If fish Hg bioaccumulation models do not include uptake from water, it is possible that predictions of estimated changes will miss this phase of reduced water uptake.

Additionally, although atmospheric deposition of Hg will be reduced under new emissions policies, deposition will not be cut off entirely. It is also likely that there will be a certain lag-time of ecosystem response to decreased emissions because the pool of Hg in the atmosphere is large. In fact, Mason *et al*. (1994) suggest that elimination of the anthropogenic load of Hg in the atmosphere and ocean would take 15-20 years if all anthropogenic Hg emissions were terminated. As such, 'new' Hg will continue to be deposited to ecosystems and will be available for uptake from water for the foreseeable future, again promoting the value of including water as a source of Hg to fish in bioaccumulation models.

Overall, the results of this study fit well with proposed modelling strategies designed to predict and monitor the response of fish Hg levels to decreased atmospheric Hg emissions. It has been suggested that the combination of accurate models with field data collection programs "gives maximum power to the deductive engine of research and to its use in management" (Walters 1986). Many researchers also stress the importance of maintaining a "healthy skepticism" (Hansen *et al*. 1993) when interpreting model output, acknowledging the many sources of error that are inherent in all model simulations. The results of this study agree with these views and suggest that models that include a water uptake component will be useful tools for predicting fish Hg levels. I encourage the use of Hg bioaccumulation models as part of local and global strategies to predict and monitor Hg in fish. I believe that with some basic adjustments to model structure, these and other bioaccumulation models would be particularly useful at the local level. If the models were somewhat standardized and promoted among ecosystem managers and researchers, monitoring efforts and data synthesis could be streamlined. It is important that models be subjected to frequent testing and revision, particularly as new studies clarify details of Hg transfer and accumulation. Many existing accumulation models are based on a limited number of laboratory studies, and it would be advantageous for models to be updated with new information as it arises. Refining existing models is an important aspect modelling, and will enhance the ability of the models to mimic processes in the natural environment.

This study has made strides toward addressing the knowledge gap that exists in our understanding of how environmental Hg influences MeHg concentrations in small fish.

Small-bodied fish species, such as YOY yellow perch, have been identified as important biosentinels for monitoring environmental Hg levels because they are sensitive to short term change in Hg deposition (Exponent 2003; Mason *et al*. 2005; Orihel *et al*. 2007; Eagles-Smith *et al*. 2009), they are abundant in many aquatic systems (Power and van den Huevel 1999; Essington and Houser 2003), and they are the principal conduit of MeHg transfer to organisms at higher trophic levels, such as predatory fish and, ultimately, humans (Exponent 2003; Eagles-Smith *et al*. 2009; Lepak *et al*. 2009b). As yellow perch are commonly used in Hg monitoring studies (e.g. Post *et al*. 1996; Harris *et al*. 2007; Orihel *et al*. 2007), and will likely be used in the future as biosentinels for gauging the effectiveness of Hg emissions reduction strategies, the results of this study are highly relevant to real-world Hg monitoring practices. It is important to note that large inter-annual variations in fish Hg levels are common, particularly in small fish. This phenomena was observed in the METAALICUS study, with fish from some years exhibiting much higher concentrations of spike and ambient Hg than in other years (Dr. P. J. Blanchfield, Fisheries and Oceans Canada, unpublished data). It is possible that even though water appears to account for 10-20% of fish Hg uptake, inter-annual variability in fish Hg levels may dampen this signal in multi-year simulations of fish Hg accumulation.

This study has provided new insights into patterns of Hg uptake by fish, providing convincing evidence that fish accumulate Hg directly from water, and that bioaccumulation models should include water as a source of Hg to fish. It has been suggested that seasonal environmental changes alter fish Hg uptake patterns (Post *et al*. 1996). To enhance our understanding of overall fish Hg uptake, future research efforts

should focus on examining accumulation during time periods when water uptake may play a larger role, such as lake turnover in autumn, and during winter when lakes are covered with ice. MeHg accumulates in hypolimnetic waters of stratified lakes during spring and summer months where anoxic conditions promote Hg methylation (Gilmour *et al*. 1998; Eckley 2005). Fish are exposed to large releases of MeHg during destratification in autumn when the hypolimnion mixes with the rest of the lake (Herrin *et al*. 1998). These pulse increases of waterborne MeHg may enhance uptake of Hg from water by fish, and future studies could be directed toward examining the impacts of fall turnover on fish Hg accumulation from water. Furthermore, it has been suggested that fish Hg uptake from water becomes more dominant in winter as consumption decreases (Post *et al*. 1996). During this time, it is likely that food and water uptake would remain additive, but that proportions of Hg accumulated from each source would shift toward equality. As many Canadian lakes and rivers are frozen for several months each year, future research could focus on accumulation of Hg by fish under winter conditions, and on how bioaccumulation models may be adjusted to accommodate these changing patterns.

Appendix 1. Model input parameters and equations

This appendix details the equations and input parameters for two mercury bioaccumulation models. The Wisconsin Fish Bioenergetics v.3.0 model (Hanson *et al*. 1997) assumes that fish accumulate all of their mercury from dietary sources, while the OneFish model (Harris and Bodaly 1998) accounts for uptake of mercury from both food and water. Both models are based on identical bioenergetics equations.

A.1 Growth

It is important that model predictions of fish weight on the final day of a simulation reflect weights observed in a target population. As fish mercury concentration is reported by weight, incorrect predictions of fish weight will yield inaccurate predictions of mercury levels. The Wisconsin model requires an "end weight" input to represent the weight of the fish on the final day of the simulation. Using this end weight in combination with water temperatures and prey caloric content, the Wisconsin model determines appropriate growth for the simulation. The OneFish model does not require an end weight input but instead has an adjustable growth rate input parameter (kt) that may be scaled by a user to achieve the desired end weight.

A.1.1 Chapter 2

Fish in experimental tanks were not individually tagged so it was not possible to follow the growth of individuals during the experiment. To account for a variety of growth scenarios, I completed four simulations in both models that spanned the range of possible

growth patterns. These simulations crossed the smallest and largest observed start weights (day 1) with the smallest and largest weights observed at the end of the experiment (day 27) to produce four simulations, each with a different growth pattern (Wisconsin: Table A.1; OneFish: Table A.2).

A.1.2 Chapter 3

The start dates for all model simulations represented hatch dates that were estimated for each year based on water temperatures (Table A.3). The start weight (at hatch) of the simulated larval yellow perch on was 0.008 g for all model scenarios (Babiak *et al*. 2004 (Eurasian perch, *Perca fluviatalis*); Tables A.5 and A.7). The end weight (Wisconsin) and kt (OneFish) inputs chosen for each year were selected to reflect weights observed in the fish collected from Lake 658 on the final sampling dates. Five weights were selected for each year and were entered directly into the Wisconsin model (Table A.4). For the OneFish model, kt values were selected on a trial-and-error basis until weights on the final days of the simulations reflected end weights used in the Wisconsin model (Table A.4).

A.2 Water temperature

The OneFish model allows input of one water temperature per month (i.e. monthly means). The Wisconsin model will accept as many temperature inputs are there are days in the simulation, however, as the model will interpolate between data points it is not essential for each day to have an input value associated with it.

A.2.1 Chapter 2

As the experiment was shorter than one month in duration, it was only possible to expose fish to a single water temperature in the OneFish simulations (20 °C; Table A.2). To ensure consistency among the two models, all Wisconsin simulations were also conducted with a constant temperature of 20 °C (Table A.1).

A.2.2 Chapter 3

Although yellow perch may occupy many depths of the water column, YOY perch tend to spend the majority of their time in shallow water (Guma'a 1978; Mills and Forney 1981; Post and McQueen 1988). All water temperatures used in this chapter were determined with a thermistor (Flett Research, Winnipeg, MB) every two weeks at a depth of 2 m in the centre of Lake 658. All temperatures collected between 2001 and 2007 were used directly as input for the Wisconsin model (Figure 3.2). Monthly means were calculated for these data and were used as input for the OneFish model (Table A.6).

A.3 Prey energy density and mercury content

A.3.1 Chapter 2

Both models use the energy density (caloric content) of prey to determine consumption rates. The energy density of the fish food base (Silver Cup) used to create the pellet food was entered into both models (Tables A.1 and A.2) . The spike mercury content of the pellets was determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Dr. H. Hintelmann, Department of Chemistry, Trent University, Peterborough, ON) and was assumed to be constant for the duration of the experiment (Tables A.1 and A.2).

A.3.2 Chapter 3

The energy density of zooplankton is reported as ranging from 2000 $J \cdot g^{-1}$ to 3400 $J \cdot g^{-1}$ (Hanson *et al*. 1997). For this study, I assumed that the energy density of zooplankton was 2700 $J \cdot g^{-1}$ (Wisconsin model) or 0.6459 $kcal \cdot g^{-1}$ (OneFish model) (Tables A.5 and A.7). Determining the caloric content of prey accurately would be an asset to future modelling exercises, but was not possible for this study. The caloric content of *Chaoborus* was assumed to be 3000 $J \cdot g^{-1}$ in the Wisconsin model. As the OneFish model only allows for the input of one prey type, the energy content of a diet comprised of 75% zooplankton and 25% *Chaoborus* was determined by combining the two energy densities in the equation below.

(A.1) $$\text{energy density (kcal g}^{-1}) = 0.75(E_{zoop}) + 0.25(E_{Chaoborus})$$

Where E_{zoop} is the energy density of zooplankton (0.6495 $J \cdot g^{-1}$) and $E_{Chaoborus}$ is the energy density of *Chaoborus* (0.7177 $J \cdot g^{-1}$). Tables A.8 (Wisconsin) and A.9 (OneFish) outline the simulations that used this combined energy density.

Zooplankton and *Chaoborus* were collected monthly from Lake 658 during the open water season, and their spike and ambient mercury concentrations were determined with ICP-MS. Zooplankton and *Chaoborus* spike and ambient mercury concentrations for each sampling date were used as input for the Wisconsin model (M. Paterson, Fisheries & Oceans Canada, unpublished data). Samples collected in July, August, and September of each year were averaged to produce mean summer zooplankton and *Chaoborus* spike and ambient mercury concentrations. These concentrations were combined using the below equation and were then used as input for the OneFish model (Table A.6).

(A.3) $[Hg]_{combined}$ ($ng \cdot g^{-1}$ w.w.) = 0.75(mean $[Hg]_{zoop}$) + 0.25(mean $[Hg]_{\textit{Chaoborus}}$)

Where $[Hg]_{zoop}$ is the mean summer mercury content of zooplankton, and $[Hg]_{\textit{Chaoborus}}$ is the mean summer mercury concentration of *Chaoborus*.

A.4 Mercury uptake

A.4.1 Wisconsin model

The Wisconsin model assumes that all mercury taken in by a fish comes from diet, and that the uptake of waterborne mercury is negligible. Two approaches were used to predict mercury concentrations in yellow perch with the Wisconsin model in this study. Type 1 simulations assumed that all mercury taken in by a fish is retained and that none is lost to elimination. Using this principle, the change in fish mercury concentration over some unit of time can be described with this equation:

(A.3) Hg from food = $C \times [Hg]_f \times AE_f$

Where C is the mass of prey consumed, $[Hg]_f$ is the prey mercury concentration, and AE_f is the assimilation efficiency of mercury from the prey. This mercury accumulation approach was used in chapter 2 (Table A.1) and chapter 3 (Tables A.5 and A.9).

Type 2 simulations assumed that some mercury is eliminated from the fish over time.

(A.4) Hg from food = $C \times [Hg]_f \times AE_f$ – elimination

Where all parameters are the same as in equation A.3 and elimination is a function of fish mass. Elimination of mercury in the models is discussed below. This approach was not used in chapter 2, and was used for type 2 simulations in chapter 3 (Table A.7).

A.4.2 OneFish model

The OneFish model assumes that fish take in mercury from food and water. The general form of the equation describing this uptake is:

(A.5) mercury burden = mercury from food + mercury from water – elimination

All simulations completed in chapter 2 omitted the elimination parameter, assuming the fish eliminated no mercury from their bodies (Table A.2). Type 1 simulations in chapter 3 were completed assuming no elimination (Tables A.6 and A.10) and type 2 simulations were completed with elimination included (Table A.8).

The equation describing the uptake of mercury from food in the OneFish model is identical to equation A.3. The uptake of mercury from water by fish in the OneFish model is based on the following equation:

(A.6) $Hg\ from\ water = AE_w \times [Hg]_w \times (R + S) \times W \times (E_{ox} \times C_{ox} \times Q_{ox})^{-1}$

Where AE_w is the assimilation efficiency of mercury from water, $[Hg]_w$ is the concentration of mercury in water ($\mu g \cdot L^{-1}$), R is respiration rate ($kcal \cdot g\ fish^{-1} \cdot day^{-1}$), S is the rate of specific dynamic action ($kcal \cdot g\ fish^{-1} \cdot day^{-1}$), W is fish weight (g), E_{ox} is the efficiency of uptake of oxygen from the water (dimensionless), C_{ox} is the concentration of oxygen in the water ($g\ O_2 \cdot m^{-3}$), and Q_{ox} is the caloric value of oxygen ($kcal \cdot g\ O_2^{-1}$).

A.5 Mercury elimination

A.5.1 Wisconsin

Type 2 simulations completed in chapter 3 included elimination of mercury from fish. The rate of mercury elimination in this model is allometrically scaled to fish weight and

is based on the equation presented below.

(A.7) $\text{elimination} = W^{\zeta} \times [Hg]_{YP} \times K_{cl}$

Where W is the weight of the fish (g), ζ is an exponent that describes the effect of allometry on contaminant elimination (dimensionless), $[Hg]_{YP}$ is the concentration of mercury in the fish, and K_{cl} is the clearance coefficient ($g^{-\zeta} \cdot day^{-1}$). The allometric constant (ζ) and clearance coefficient (K_{cl}) have not been well-researched, but recent research has provided insight into the rates of mercury elimination by fish (e.g. Van Walleghem *et al*. 2007, Madenjian and O'Connor 2008). In chapter 3, several combinations of ζ and K_{cl} were used to describe the elimination rate. These values are based on suggestions given in the Wisconsin model (Hanson *et al*. 1997), and on rates suggested by Madenjian and O'Connor (2008) (Table A.7).

A.5.2 OneFish

The elimination of mercury in this model depends on the ratio of mercury in urine and in fish tissue ($[Hg]_{urine}$ /s $[Hg]_{fish}$). Harris and Bodaly (1998) suggest a ratio of 0.75 for yellow perch. This value was used for the simulations that incorporated elimination in chapter 3 (Table A.8).

Table A.1 Inputs for the Wisconsin model simulations of whole body spike Hg accumulation from food by yellow perch over 27 days in experimental fish tanks at Lake 658 (Chapter 2). Fish were transferred to the tanks from Lake 240 where they had no previous spike Hg exposure.

Parameter	Units	Simulation 1	Simulation 2	Simulation 3	Simulation 4
species		larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch
simulation start day		1	1	1	1
simulation end day		27	27	27	27
maintenance temperature	°C	0	0	0	0
Temperatures					
day 1	°C	20	20	20	20
day 27	°C	20	20	20	20
Growth					
start weight	g	0.7	0.7	0.9	0.9
end weight	g	0.6	1.3	1.3	0.6
Diet proportions					
pellets		1	1	1	1
Diet [spike Hg]					
pellets	$ng \cdot g^{-1}$ d.w.	2.15	2.15	2.15	2.15
MeHg rate constants					
AE_f		0.8	0.8	0.8	0.8
Bioenergetics					
zooplankton energy density	$J \cdot g^{-1}$	16000	16000	16000	16000
Initial fish conditions					
start [spike MeHg]	$ng \cdot g^{-1}$ w.w.	0	0	0	0

Note: AE_f = assimilation efficiency of MeHg from food

Table A.2 Inputs for the OneFish model simulations of whole body spike Hg accumulation from food by yellow perch over 27 days in experimental fish tanks at Lake 658 (Chapter 2). Fish were transferred to the tanks from Lake 240 where they had no previous spike Hg exposure.

		Simulation			
Parameter	**Description**	**1**	**2**	**3**	**4**
species		yellow perch	yellow perch	yellow perch	yellow perch
Growth and spawning					
W_{max}	max. weight possible	5	5	5	5
kt	growth rate	0.2	0.2	0.2	0.2
Q_{10}	relates growth to temperature	2.3	2.3	2.3	2.3
b	growth-related	2.4	2.4	2.4	2.4
Neta	length vs. weight relationship	3.02	3.02	3.02	3.02
Lambda	length vs. weight relationship	0.017	0.017	0.017	0.017
Water temperatures					
August	°C	20	20	20	20
MeHg exposure					
[spike MeHg] water	$ng \cdot L^{-1}$	0.025	0.025	0.025	0.025
[spike MeHg] food	$\mu g \cdot g^{-1}$ w.w.	0.00215	0.00215	0.00215	0.00215
Cox	oxygen concentration in water ($mg \cdot L^{-1}$)	8	8	8	8
MeHg rate constants					
AE_w	percent absorbed from water	0.36	0.36	0.36	0.36
AE_f	percent absorbed from food	0.8	0.8	0.8	0.8
Bioenergetics					
Cal dens food	$kcal \cdot g^{-1}$	4	4	4	4
Numeric Solution and Outputs					
Run_Years	length of simulation (years)	0.074	0.074	0.074	0.074
Initial fish conditions					
Agenot	start age (days)	60	60	60	60
Wnot	start weight (g)	0.8	0.8	0.8	0.8
Cnot	start [MeHg] of fish ($\mu g \cdot g^{-1}$ w.w.)	0	0	0	0

Note: AE_f = assimilation efficiency of Hg from food; AE_w = assimilation efficiency of Hg from water

Table A.3 Estimated spawn and hatch dates of yellow perch in Lake 658 from 2001-2007 (Chapter 3). Yellow perch spawn at water temperatures of approximately 12°C and hatch approximately 12 days after spawning. Spawn dates were estimated for each year based on water temperatures, and 12 days were added to those dates to estimate hatch date.

	spawn		hatch	
year	**date**	**day (Wisconsin)**	**date**	**day (Wisconsin)**
2001	13 May	369	24 May	380
2002	27 May	748	7 June	759
2003	12 May	1098	23 May	1109
2004	31 May	1483	11 June	1494
2005	22 May	1843	2 June	1850
2006	10 May	2192	21 May	2203
2007	6 May	2553	17 May	2564

Table A.4 Growth inputs for simulations in the Wisconsin and OneFish models (Chapter 3). The Wisconsin model requires an end weight value to calculate growth rate. The OneFish model uses a growth rate (kt) to estimate end weight.

	end weights (g) - Wisconsin model					**kt values - OneFish model**				
Year	**Sim. 1**	**Sim. 2**	**Sim. 3**	**Sim. 4**	**Sim. 5**	**Sim. 1**	**Sim. 2**	**Sim. 3**	**Sim. 4**	**Sim. 5**
2001	2.2	2.5	2.8	3.1	3.4	0.28	0.3	0.32	0.34	0.36
2002	1	1.2	1.4	1.6	1.8	0.32	0.35	0.38	0.41	0.44
2003	2.2	2.6	3	3.4	3.8	0.22	0.24	0.26	0.28	0.3
2004	0.7	1	1.3	1.5	1.8	0.22	0.24	0.26	0.28	0.3
2005	0.4	0.5	0.6	0.7	0.8	0.14	0.16	0.18	0.2	0.22
2006	1.5	1.9	2.3	2.7	3	0.37	0.4	0.43	0.46	0.49
2007	1.6	2	2.5	2.9	3.3	0.29	0.32	0.35	0.38	0.41

Table A.5 Inputs for the Wisconsin type 1 model simulations (no elimination) of spike and ambient Hg accumulation from food by yellow perch in Lake 658.

Parameter	Units	2001	2002	2003	2004	2005	2006	2007
species		larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch
simulation start day		380	759	1109	1494	1850	2203	2564
simulation end day		510	834	1262	1623	1952	2290	2682
maintenance temperature	°C	0	0	0	0	0	0	0
Temperatures	°C	see Figure 3.2						
Growth								
start weight	g	0.008	0.008	0.008	0.008	0.008	0.008	0.008
end weight	g	see Table A.4						
Diet proportions								
zooplankton		1	1	1	1	1	1	1
Diet [spike Hg]	$ng \cdot g^{-1}$	M. Paterson, Fisheries & Oceans Canada, unpublished data						
MeHg rate constants								
AE_f		0.8	0.8	0.8	0.8	0.8	0.8	0.8
Bioenergetics								
zooplankton energy density	$J \cdot g^{-1}$	2700	2700	2700	2700	2700	2700	2700
Initial fish conditions								
start [MeHg]	$ng \cdot g^{-1}$	0	0	0	0	0	0	0

Note: AE_f = assimilation efficiency of MeHg from food

Table A.6 Inputs for the OneFish type 1 model simulations (no elimination) of spike and ambient Hg accumulation from food and water by yellow perch in Lake 658.

Parameter	Description	2001	2002	2003	2004	2005	2006	2007
Growth and spawning								
W_{max}	max. weight possible	5	5	5	5	5	5	5
kt	growth rate	See Table A.4						
Q_{10}	relates growth to temperature	2.3	2.3	2.3	2.3	2.3	2.3	2.3
b	growth-related	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Neta	length vs. weight relationship	3.02	3.02	3.02	3.02	3.02	3.02	3.02
Lambda	length vs. weight relationship	0.017	0.017	0.017	0.017	0.017	0.017	0.017
Water temperatures								
May	°C	13.3	6.3	13.7	9.4	10.7	14.4	14.2
June	°C	17.0	16.2	21.2	15.9	19.4	20.4	19.9
July	°C	22.1	23.9	23.0	20.8	21.9	22.6	22.0
August	°C	21.6	21.8	24.0	17.6	20.1	21.0	20.3
September	°C	15.8	17.8	18.8	16.4	17.5	19.2	16.0
October	°C	8.8	6.5	11.1	11.2	10.2	9.5	7.9
November	°C	5.2	5.2	4.2	6.4	5.6	4.5	5.2
MeHg exposure								
[spike MeHg] water	$ng \cdot L^{-1}$	0.0027	0.0250	0.0187	0.0283	0.0190	0.0193	0.0310
[amb MeHg] water	$ng \cdot L^{-1}$	0.0807	0.1633	0.0913	0.0667	0.0697	0.0433	0.0873
[spike MeHg] food	$\mu g \cdot g^{-1}$ w.w.	0.0005	0.0083	0.0052	0.0087	0.0066	0.0062	0.0063
[amb MeHg] food	$\mu g \cdot g^{-1}$ w.w.	0.0275	0.0399	0.0170	0.0220	0.0180	0.0136	0.0173
Cox	Oxygen in water ($mg \cdot L^{-1}$)	6.25	8.47	6.40	9.30	8.53	7.67	7.50
MeHg rate constants								
AE_w	percent absorbed from water	0.36	0.36	0.36	0.36	0.36	0.36	0.36
AE_f	percent absorbed from food	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Bioenergetics								
Cal dens food	$kKcal \cdot g^{-1}$	0.6459	0.6459	0.6459	0.6459	0.6459	0.6459	0.6459
Numeric Outputs								
Run_Years	length of simulation (years)	0.3562	0.2055	0.4192	0.3534	0.2795	0.2384	0.3233
Initial fish conditions								
Agenot	start age (days)	0	0	0	0	0	0	0
Wnot	start weight (g)	0.008	0.008	0.008	0.008	0.008	0.008	0.008
Cnot	start [MeHg] ($\mu g \cdot g^{-1}$ w.w.)	0	0	0	0	0	0	0

Table A.7 Inputs for the Wisconsin type 2 model simulations of spike and ambient Hg accumulation from food by yellow perch in Lake 658.

Parameter	**Units**	**2001**			**2002**		
scenario		no elimination	Madenjian elimination	suggested elimination	no elimination	Madenjian elimination	suggested elimination
species		larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch
simulation start day		380	380	380	759	759	759
simulation end day		510	510	510	834	834	834
maintenance temperature	°C	0	0	0	0	0	0
Temperatures	°C	see Figure 3.2					
Growth							
start weight	g	0.008	0.008	0.008	0.008	0.008	0.008
end weight	g	see Table A.4					
Diet proportions							
zooplankton		0.75	0.75	0.75	0.75	0.75	0.75
Chaoborus		0.25	0.25	0.25	0.25	0.25	0.25
Diet [spike Hg]	$ng \cdot g^{-1}$	M. Paterson, Fisheries & Oceans Canada, unpublished data					
AE_f		0.8	0.8	0.8	0.8	0.8	0.8
Elimination constants							
δ		n/a	-0.58	-0.58	n/a	-0.58	-0.58
K_{cl}	$g_{\delta} \cdot d^{-1}$	n/a	0.0108	0.029	n/a	0.0108	0.029
Bioenergetics							
zooplankton energy density	$J \cdot g^{-1}$	2700	2700	2700	2700	2700	2700
Chaoborus energy density	$J \cdot g^{-1}$	3000	3000	3000	3000	3000	3000
Initial fish conditions							
start [MeHg]	$ng \cdot g^{-1}$	0	0	0	0	0	0

Table A.7 (continued) Inputs for the Wisconsin type 2 model simulations of spike and ambient Hg accumulation from food by yellow perch in Lake 658.

Parameter	Units	2003			2004		
scenario		no elimination	Madenjian elimination	suggested elimination	no elimination	Madenjian elimination	suggested elimination
species		larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch
simulation start day		1109	1109	1109	1494	1494	1494
simulation end day		1262	1262	1262	1623	1623	1623
maintenance temperature	°C	0	0	0	0	0	0
Temperatures	°C	see Figure 3.2					
Growth							
start weight	g	0.008	0.008	0.008	0.008	0.008	0.008
end weight	g	see Table A.4					
Diet proportions							
zooplankton		0.75	0.75	0.75	0.75	0.75	0.75
Chaoborus		0.25	0.25	0.25	0.25	0.25	0.25
Diet [spike Hg]	$ng \cdot g^{-1}$	M. Paterson, Fisheries & Oceans Canada, unpublished data					
AE_f		0.8	0.8	0.8	0.8	0.8	0.8
Elimination constants							
δ		n/a	-0.58	-0.58	n/a	-0.58	-0.58
K_{cl}	$g^{-\delta} \cdot d^{-1}$	n/a	0.0108	0.029	n/a	0.0108	0.029
Bioenergetics							
zooplankton energy density	$J \cdot g^{-1}$	2700	2700	2700	2700	2700	2700
Chaoborus energy density	$J \cdot g^{-1}$	3000	3000	3000	3000	3000	3000
Initial fish conditions							
start [MeHg]	$ng \cdot g^{-1}$	0	0	0	0	0	0

Table A.7 (continued) Inputs for the Wisconsin type 2 model simulations of spike and ambient Hg accumulation from food by yellow perch in Lake 658.

Parameter	Units	2005			2006		
scenario		no elimination	Madenjian elimination	suggested elimination	no elimination	Madenjian elimination	suggested elimination
species		larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch
simulation start day		1850	1850	1850	2203	2203	2203
simulation end day		1952	1952	1952	2290	2290	2290
maintenance temperature	°C	0	0	0	0	0	0
Temperatures	°C	see Figure 3.2					
Growth							
start weight	g	0.008	0.008	0.008	0.008	0.008	0.008
end weight	g	see Table A.4					
Diet proportions							
zooplankton		0.75	0.75	0.75	0.75	0.75	0.75
Chaoborus		0.25	0.25	0.25	0.25	0.25	0.25
Diet [spike Hg]	$ng \cdot g^{-1}$	M. Paterson, Fisheries & Oceans Canada, unpublished data					
AE_f		0.8	0.8	0.8	0.8	0.8	0.8
Elimination constants							
δ		n/a	-0.58	-0.58	n/a	-0.58	-0.58
K_{cl}	$g^{-\delta} \cdot d^{-1}$	n/a	0.0108	0.029	n/a	0.0108	0.029
Bioenergetics							
zooplankton energy density	$J \cdot g^{-1}$	2700	2700	2700	2700	2700	2700
Chaoborus energy density	$J \cdot g^{-1}$	3000	3000	3000	3000	3000	3000
Initial fish conditions							
start [MeHg]	$ng \cdot g^{-1}$	0	0	0	0	0	0

Table A.7 (continued) Inputs for the Wisconsin type 2 model simulations of spike and ambient Hg accumulation from food by yellow perch in Lake 658.

Parameter	Units	2007		
scenario		no elimination	Madenjian *et al*. 2008 elimination	suggested elimination
species		larval yellow perch	larval yellow perch	larval yellow perch
simulation start day		2564	2564	2564
simulation end day		2682	2682	2682
maintenance temperature	°C	0	0	0
Temperatures	°C	see Figure 3.2		
Growth				
start weight	g	0.008	0.008	0.008
end weight	g	see Table A.4		
Diet proportions				
zooplankton		0.75	0.75	0.75
Chaoborus		0.25	0.25	0.25
Diet [spike Hg]	$ng \cdot g^{-1}$	M. Paterson, Fisheries & Oceans Canada, unpublished data		
AE_f		0.8	0.8	0.8
Elimination constants				
δ		n/a	-0.58	-0.58
K_{cl}	$g^{-\delta} \cdot d^{-1}$	n/a	0.0108	0.029
Bioenergetics				
zooplankton energy density	$J \cdot g^{-1}$	2700	2700	2700
Chaoborus energy density	$J \cdot g^{-1}$	3000	3000	3000
Initial fish conditions				
start [MeHg]	$ng \cdot g^{-1}$	0	0	0

Table A.8 Inputs for OneFish type 2 model simulations of spike and ambient Hg accumulation from food and water by yellow perch in Lake 658. Growth and spawning inputs are identical to Table A.7.

Parameter	**Description**	**2001**			**2002**		
simulation		no elimination	Madenjian *et al*. 2008 elimination	suggested elimination	no elimination	Madenjian *et al*. 2008 elimination	suggested elimination
Water temperatures							
May	°C	13.3	13.3	13.3	6.3	6.3	6.3
June	°C	17.0	17.0	17.0	16.2	16.2	16.2
July	°C	22.1	22.1	22.1	23.9	23.9	23.9
August	°C	21.6	21.6	21.6	21.8	21.8	21.8
September	°C	15.8	15.8	15.8	17.8	17.8	17.8
October	°C	8.8	8.8	8.8	6.5	6.5	6.5
November	°C	5.2	5.2	5.2	5.2	5.2	5.2
MeHg exposure							
[spike MeHg] water	$ng \cdot L^{-1}$	0.0027	0.0027	0.0027	0.0250	0.0250	0.0250
[amb MeHg] water	$ng \cdot L^{-1}$	0.0807	0.0807	0.0807	0.1633	0.1633	0.1633
[spike MeHg] food	$\mu g \cdot g^{-1}$ w.w.	0.0005	0.0005	0.0005	0.0072	0.0072	0.0072
[amb MeHg] food	$\mu g \cdot g^{-1}$ w.w.	0.0222	0.0222	0.0222	0.0343	0.0343	0.0343
Cox	O_2 in water ($mg \cdot L^{-1}$)	6.25	6.25	6.25	8.47	8.47	8.47
AE_w		0.36	0.36	0.36	0.36	0.36	0.36
AE_f		0.8	0.8	0.8	0.8	0.8	0.8
excrete_factor	urine [MeHg]: body [MeHg]	0.01	0.24	0.75	0.01	0.24	0.75
Bioenergetics							
Cal dens food	$kKcal \cdot g^{-1}$	0.6639	0.6639	0.6639	0.6639	0.6639	0.6639
Numeric Outputs							
Run_Years	simulation length (y)	0.3562	0.3562	0.3562	0.2055	0.2055	0.2055
Initial fish conditions							
Agenot	age (d)	0	0	0	0	0	0
Wnot	weight (g)	0.008	0.008	0.008	0.008	0.008	0.008
Cnot	[MeHg] ($\mu g \cdot g^{-1}$ w.w.)	0	0	0	0	0	0

Table A.8 Inputs for OneFish type 2 model simulations of spike and ambient Hg accumulation from food and water by yellow perch in Lake 658. Growth and spawning inputs are identical to those in Table A.7.

Parameter	**Description**	**2003**			**2004**		
simulation		no elimination	Madenjian *et al*. 2008 elimination	suggested elimination	no elimination	Madenjian *et al*. 2008 elimination	suggested elimination
Water temperatures							
May	°C	13.7	13.7	13.7	9.4	9.4	9.4
June	°C	21.2	21.2	21.2	15.9	15.9	15.9
July	°C	23.0	23.0	23.0	20.8	20.8	20.8
August	°C	24.0	24.0	24.0	17.6	17.6	17.6
September	°C	18.8	18.8	18.8	16.4	16.4	16.4
October	°C	11.1	11.1	11.1	11.2	11.2	11.2
November	°C	4.2	4.2	4.2	6.4	6.4	6.4
MeHg exposure							
[spike MeHg] water	$ng \cdot L^{-1}$	0.0187	0.0187	0.0187	0.0283	0.0283	0.0283
[amb MeHg] water	$ng \cdot L^{-1}$	0.0913	0.0913	0.0913	0.0667	0.0667	0.0667
[spike MeHg] food	$\mu g \cdot g^{-1}$ w.w.	0.0045	0.0045	0.0045	0.0084	0.0084	0.0084
[amb MeHg] food	$\mu g \cdot g^{-1}$ w.w.	0.0147	0.0147	0.0147	0.0205	0.0205	0.0205
Cox	O_2 in water ($mg \cdot L^{-1}$)	6.40	6.40	6.40	9.30	9.30	9.30
AE_w		0.36	0.36	0.36	0.36	0.36	0.36
AE_f		0.8	0.8	0.8	0.8	0.8	0.8
excrete_factor	urine [MeHg]: body [MeHg]	0.01	0.24	0.75	0.01	0.24	0.75
Bioenergetics							
Cal dens food	$kKcal \cdot g^{-1}$	0.6639	0.6639	0.6639	0.6639	0.6639	0.6639
Numeric Outputs							
Run_Years	simulation length (y)	0.4192	0.4192	0.4192	0.3534	0.3534	0.3534
Initial fish conditions							
Agenot	age (d)	0	0	0	0	0	0
Wnot	weight (g)	0.008	0.008	0.008	0.008	0.008	0.008
Cnot	[MeHg] ($\mu g \cdot g^{-1}$ w.w.)	0	0	0	0	0	0

Table A.8 Inputs for OneFish type 2 model simulations of spike and ambient Hg accumulation from food and water by yellow perch in Lake 658. Growth and spawning inputs are identical to those in Table A.7.

Parameter	**Description**	**2005**			**2006**		
simulation		no elimination	Madenjian *et al*. 2008 elimination	suggested elimination	no elimination	Madenjian *et al*. 2008 elimination	suggested elimination
Water temperatures							
May	°C	10.7	10.7	10.7	14.4	14.4	14.4
June	°C	19.4	19.4	19.4	20.4	20.4	20.4
July	°C	21.9	21.9	21.9	22.6	22.6	22.6
August	°C	20.1	20.1	20.1	21.0	21.0	21.0
September	°C	17.5	17.5	17.5	19.2	19.2	19.2
October	°C	10.2	10.2	10.2	9.5	9.5	9.5
November	°C	5.6	5.6	5.6	4.5	4.5	4.5
MeHg exposure							
[spike MeHg] water	$ng \cdot L^{-1}$	0.0190	0.0190	0.0190	0.0193	0.0193	0.0193
[amb MeHg] water	$ng \cdot L^{-1}$	0.0697	0.0697	0.0697	0.0433	0.0433	0.0433
[spike MeHg] food	$\mu g \cdot g^{-1}$ w.w.	0.0070	0.0070	0.0070	0.0077	0.0077	0.0077
[amb MeHg] food	$\mu g \cdot g^{-1}$ w.w.	0.0186	0.0186	0.0186	0.0154	0.0154	0.0154
Cox	O_2 in water ($mg \cdot L^{-1}$)	8.53	8.53	8.53	7.67	7.67	7.67
AE_w		0.36	0.36	0.36	0.36	0.36	0.36
AE_f		0.8	0.8	0.8	0.8	0.8	0.8
excrete_factor	urine [MeHg]: body [MeHg]	0.01	0.24	0.75	0.01	0.24	0.75
Bioenergetics							
Cal dens food	$kKcal \cdot g^{-1}$	0.6639	0.6639	0.6639	0.6639	0.6639	0.6639
Numeric Outputs							
Run_Years	simulation length (y)	0.2795	0.2795	0.2795	0.2384	0.2384	0.2384
Initial fish conditions							
Agenot	age (d)	0	0	0	0	0	0
Wnot	weight (g)	0.008	0.008	0.008	0.008	0.008	0.008
Cnot	[MeHg] ($\mu g \cdot g^{-1}$ w.w.)	0	0	0	0	0	0

Table A.8 Inputs for OneFish type 2 model simulations of spike and ambient Hg accumulation from food and water by yellow perch in Lake 658. Growth and spawning inputs are identical to those in Table A.7.

Parameter	Description	2007		
simulation		no elimination	Madenjian *et al*. 2008 elimination	suggested elimination
Water temperatures				
May	°C	14.2	14.2	14.2
June	°C	19.9	19.9	19.9
July	°C	22.0	22.0	22.0
August	°C	20.3	20.3	20.3
September	°C	16.0	16.0	16.0
October	°C	7.9	7.9	7.9
November	°C	5.2	5.2	5.2
MeHg exposure				
[spike MeHg] water	$ng \cdot L^{-1}$	0.0310	0.0310	0.0310
[amb MeHg] water	$ng \cdot L^{-1}$	0.0873	0.0873	0.0873
[spike MeHg] food	$\mu g \cdot g^{-1}$ w.w.	0.0054	0.0054	0.0054
[amb MeHg] food	$\mu g \cdot g^{-1}$ w.w.	0.0150	0.0150	0.0150
Cox	O_2 in water ($mg \cdot L^{-1}$)	7.50	7.50	7.50
AE_w		0.36	0.36	0.36
AE_f		0.8	0.8	0.8
excrete_factor	urine [MeHg]: body [MeHg]	0.01	0.24	0.75
Bioenergetics				
Cal dens food	$kKcal \cdot g^{-1}$	0.6639	0.6639	0.6639
Numeric Outputs				
Run_Years	simulation length (y)	0.3233	0.3233	0.3233
Initial fish conditions				
Agenot	age (d)	0	0	0
Wnot	weight (g)	0.008	0.008	0.008
Cnot	[MeHg] ($\mu g \cdot g^{-1}$ w.w.)	0	0	0

Table A.9 Inputs for the Wisconsin model sensitivity analysis.

		Standard	Temperature manipulations			
Parameter	**Units**	**1**	**2**	**3**	**4**	**5**
species		larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch
simulation start day		1	1	1	1	1
simulation end day		120	120	120	120	120
maintenance temperature	°C	0	0	0	0	0
Temperatures						
day 1	°C	17	15	25	12	22
day 31	°C	22	15	25	17	27
day 62	°C	21	15	25	16	26
day 93	°C	17	15	25	12	22
day 120	°C	17	15	25	12	22
Growth						
start weight	g	0.008	0.008	0.008	0.008	0.008
end weight	g	2	2	2	2	2
Diet proportions						
zooplankton		1	1	1	1	1
Diet [spike Hg]	$ng \cdot g^{-1}$ w.w.	0.02	0.02	0.02	0.02	0.02
MeHg rate constants						
AE_f		0.8	0.8	0.8	0.8	0.8
Bioenergetics						
zooplankton energy density	$J \cdot g^{-1}$	2700	2700	2700	2700	2700
Initial fish conditions						
start [spike MeHg]	$ng \cdot g^{-1}$ w.w.	0	0	0	0	0

Note: AE_f = assimilation efficiency of MeHg from food

Table A.9 (continued) Inputs for the Wisconsin model sensitivity analysis.

		Prey [Hg] manipulations			Prey energy density manipulations		
Parameter	**Units**	**6**	**7**	**8**	**9**	**10**	**11**
species		larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch	larval yellow perch
simulation start day		1	1	1	1	1	1
simulation end day		120	120	120	120	120	120
maintenance temperature	°C	0	0	0	0	0	0
Temperatures							
day 1	°C	17	17	17	17	17	17
day 31	°C	22	22	22	22	22	22
day 62	°C	21	21	21	21	21	21
day 93	°C	17	17	17	17	17	17
day 120	°C	17	17	17	17	17	17
Growth							
start weight	g	0.008	0.008	0.008	0.008	0.008	0.008
end weight	g	2	2	2	2	2	2
Diet proportions							
zooplankton		1	1	1	1	1	1
Diet [spike Hg]	$ng \cdot g^{-1}$ w.w.	0.01	0.03	0.05	0.02	0.02	0.02
MeHg rate constants							
AE_f		0.8	0.8	0.8	0.8	0.8	0.8
Bioenergetics							
zooplankton energy density	$J \cdot g^{-1}$	2700	2700	2700	1000	2000	3400
Initial fish conditions							
start [spike MeHg]	$ng \cdot g^{-1}$ w.w.	0	0	0	0	0	0

Note: AE_f = assimilation efficiency of MeHg from food

Table A.9 (continued) Inputs for the Wisconsin model sensitivity analysis.

		AE_f manipulations		
Parameter	**Units**	**12**	**13**	**14**
species		larval yellow perch	larval yellow perch	larval yellow perch
simulation start day		1	1	1
simulation end day		120	120	120
maintenance temperature	°C	0	0	0
Temperatures				
day 1	°C	17	17	17
day 31	°C	22	22	22
day 62	°C	21	21	21
day 93	°C	17	17	17
day 120	°C	17	17	17
Growth				
start weight	g	0.008	0.008	0.008
end weight	g	2	2	2
Diet proportions				
zooplankton		1	1	1
Diet [spike Hg]	$ng \cdot g^{-1}$ w.w.	0.02	0.02	0.02
MeHg rate constants				
AE_f		0.2	0.6	1
Bioenergetics				
zooplankton energy density	$J \cdot g^{-1}$	2700	2700	2700
Initial fish conditions				
start [spike MeHg]	$ng \cdot g^{-1}$ w.w.	0	0	0

Note: AE_f = assimilation efficiency of MeHg from food

Table A.10 Inputs for the OneFish model sensitivity analysis.

		Standard simulation	Temperature manipulations			
Parameter	**Description**	**1**	**2**	**3**	**4**	**5**
Growth and spawning						
W_{max}	max. weight possible	5	5	5	5	5
kt	growth rate	0.32	0.45	0.2	0.48	0.21
Q_{10}	relates growth to temperature	2.3	2.3	2.3	2.3	2.3
b	growth-related	2.4	2.4	2.4	2.4	2.4
Neta	length vs. weight relationship	3.02	3.02	3.02	3.02	3.02
Lambda	length vs. weight relationship	0.017	0.017	0.017	0.017	0.017
Water temperatures						
June	°C	17.0	15.0	25.0	12.0	22.0
July	°C	22.0	15.0	25.0	17.0	27.0
August	°C	21.0	15.0	25.0	16.0	26.0
September	°C	17.0	15.0	25.0	12.0	22.0
MeHg exposure						
[MeHg] water	$ng \cdot L^{-1}$	0.08	0.08	0.08	0.08	0.08
[MeHg] food	$\mu g \cdot g^{-1}$ w.w.	0.02	0.02	0.02	0.02	0.02
Cox	oxygen concentration in water ($mg \cdot L^{-1}$)					
AE_w	percent absorbed from water	0.36	0.36	0.36	0.36	0.36
AE_f	percent absorbed from food	0.8	0.8	0.8	0.8	0.8
Bioenergetics						
Cal dens food	$kcal \cdot g^{-1}$	0.6459	0.6459	0.6459	0.6459	0.6459
Numeric Solution and Outputs						
Run_Years	length of simulation (years)	0.3288	0.3288	0.3288	0.3288	0.3288
Initial fish conditions						
Agenot	start age (days)	0	0	0	0	0
Wnot	start weight (g)	0.008	0.008	0.008	0.008	0.008
Cnot	start [MeHg] of fish ($\mu g \cdot g^{-1}$ w.w.)	0	0	0	0	0

Table A.10 (continued) Inputs for the OneFish model sensitivity analysis.

		Prey [Hg] manipulation			Prey energy density manipulation		
Parameter	**Description**	**6**	**7**	**8**	**9**	**10**	**11**
Growth and spawning							
W_{max}	max. weight possible	5	5	5	5	5	5
kt	growth rate	0.32	0.32	0.32	0.32	0.32	0.32
Q_{10}	relates growth to temperature	2.3	2.3	2.3	2.3	2.3	2.3
b	growth-related	2.4	2.4	2.4	2.4	2.4	2.4
Neta	length vs. weight relationship	3.02	3.02	3.02	3.02	3.02	3.02
Lambda	length vs. weight relationship	0.017	0.017	0.017	0.017	0.017	0.017
Water temperatures							
June	°C	17.0	17.0	17.0	17.0	17.0	17.0
July	°C	22.0	22.0	22.0	22.0	22.0	22.0
August	°C	21.0	21.0	21.0	21.0	21.0	21.0
September	°C	17.0	17.0	17.0	17.0	17.0	17.0
MeHg exposure							
[MeHg] water	$ng \cdot L^{-1}$	0.08	0.08	0.08	0.08	0.08	0.08
[MeHg] food	$\mu g \cdot g^{-1}$ w.w.	0.01	0.03	0.05	0.02	0.02	0.02
Cox	oxygen concentration in water ($mg \cdot L^{-1}$)						
AE_w	percent absorbed from water	0.36	0.36	0.36	0.36	0.36	0.36
AE_f	percent absorbed from food	0.8	0.8	0.8	0.8	0.8	0.8
Bioenergetics							
Cal dens food	$kcal \cdot g^{-1}$	0.6459	0.6459	0.6459	0.2392	0.4785	0.8134
Numeric Solution and Outputs							
Run_Years	length of simulation (years)	0.3288	0.3288	0.3288	0.3288	0.3288	0.3288
Initial fish conditions							
Agenot	start age (days)	0	0	0	0	0	0
Wnot	start weight (g)	0.008	0.008	0.008	0.008	0.008	0.008
Cnot	start [MeHg] of fish ($\mu g \cdot g^{-1}$ w.w.)	0	0	0	0	0	0

Table A.10 (continued) Inputs for the OneFish model sensitivity analysis.

		Water [Hg] manipulations			AE_w manipulations	
Parameter	**Description**	**12**	**13**	**14**	**15**	**16**
Growth and spawning						
W_{max}	max. weight possible	5	5	5	5	5
kt	growth rate	0.32	0.32	0.32	0.32	0.32
Q_{10}	relates growth to temperature	2.3	2.3	2.3	2.3	2.3
b	growth-related	2.4	2.4	2.4	2.4	2.4
Neta	length vs. weight relationship	3.02	3.02	3.02	3.02	3.02
Lambda	length vs. weight relationship	0.017	0.017	0.017	0.017	0.017
Water temperatures						
June	°C	17.0	17.0	17.0	17.0	17.0
July	°C	22.0	22.0	22.0	22.0	22.0
August	°C	21.0	21.0	21.0	21.0	21.0
September	°C	17.0	17.0	17.0	17.0	17.0
MeHg exposure						
[MeHg] water	$ng \cdot L^{-1}$	0.01	0.20	0.50	0.08	0.08
[MeHg] food	$\mu g \cdot g^{-1}$ w.w.	0.02	0.02	0.02	0.02	0.02
Cox	oxygen concentration in water ($mg \cdot L^{-1}$)					
AE_w	percent absorbed from water	0.36	0.36	0.36	0.1	1
AE_f	percent absorbed from food	0.8	0.8	0.8	0.8	0.8
Bioenergetics						
Cal dens food	$kcal \cdot g^{-1}$	0.6459	0.6459	0.6459	0.6459	0.6459
Numeric Solution and Outputs						
Run_Years	length of simulation (years)	0.3288	0.3288	0.3288	0.3288	0.3288
Initial fish conditions						
Agenot	start age (days)	0	0	0	0	0
Wnot	start weight (g)	0.008	0.008	0.008	0.008	0.008
Cnot	start [MeHg] of fish ($\mu g \cdot g^{-1}$ w.w.)	0	0	0	0	0

Table A.10 (continued) Inputs for the OneFish model sensitivity analysis.

		AE_f manipulation		
Parameter	**Description**	**17**	**18**	**19**
Growth and spawning				
W_{max}	max. weight possible	5	5	5
kt	Growth rate	0.32	0.32	0.32
Q_{10}	Relates growth to temperature	2.3	2.3	2.3
b	growth-related	2.4	2.4	2.4
Neta	length vs. weight relationship	3.02	3.02	3.02
Lambda	length vs. weight relationship	0.017	0.017	0.017
Water temperatures				
June	°C	17.0	17.0	17.0
July	°C	22.0	22.0	22.0
August	°C	21.0	21.0	21.0
September	°C	17.0	17.0	17.0
MeHg exposure				
[MeHg] water	$ng \cdot L^{-1}$	0.08	0.08	0.08
[MeHg] food	$\mu g \cdot g^{-1}$ w.w.	0.02	0.02	0.02
Cox	oxygen concentration in water ($mg \cdot L^{-1}$)			
AE_w	percent absorbed from water	0.36	0.36	0.36
AE_f	percent absorbed from food	0.2	0.6	1
Bioenergetics				
Cal dens food	$kcal \cdot g^{-1}$	0.6459	0.6459	0.6459
Numeric Solution and Outputs				
Run_Years	length of simulation (years)	0.3288	0.3288	0.3288
Initial fish conditions				
Agenot	start age (days)	0	0	0
Wnot	start weight (g)	0.008	0.008	0.008
Cnot	start [MeHg] of fish ($\mu g \cdot g^{-1}$ w.w.)	0	0	0

LITERATURE CITED

Aberg,B., Ekman,L., Falk,R., Greitz,U., Persson,G., and Snihs,J.-O. 2009. Metabolism of methylmercury (^{203}Hg) compounds in man. Archives of Environmental Health **19**: 478-484.

Andres,S., Laporte,J.M., and Mason,R.P. 2002. Mercury accumulation and flux across the gills and the intestine of the blue crab (Callinectes sapidus). Aquatic Toxicology **56**: 303-320.

Armstrong,F.A.J. and Scott,D.P. 1979. Decrease in mercury content of fishes in Ball Lake, Ontario, since imposition of controls on mercury discharges. Journal of the Fisheries Research Board of Canada **36**: 670-672.

Babaev,N.S., Cheltsov,A.N., Sazykin,A.A., Sosnin,L.Y., and Kuchelev,A.P. 2010. Centrifugal enrichment of mercury isotopes. Nuclear Instruments & Methods in Physics Research Section A-Accelerators Spectrometers Detectors and Associated Equipment **613**: 473-476.

Bajer,P.G., Whitledge,G.W., Hayward,R.S., and Zweifel,R.D. 2003. Laboratory evaluation of two bioenergetics models applied to yellow perch: Identification of a major source of sytematic error. Journal of Fish Biology **62**: 436-454.

Beamish, F. W. H., Niimi, A. J., and Lett, P. F. K. P. Bioenergetics of teleost fishes: enviromental influences. Proceedings of the international conference on comparative physiology , 187-209. 1974.

Becker,D.S. and Bigham,G.N. 1995. Distribution of mercury in the aquatic food web of Onondaga Lake, New York. Water, Air, and Soil Pollution **80**: 563-571.

Bertram,P.E. and Brooks,A.S. 1986. Kinetics of Accumulation of Selenium from Food and Water by Fathead Minnows. Water Research **20**: 877-884.

Bhavsar,S.P., Gewurtz,S.B., McGoldrick,D.J., Keir,M.J., and Backus,S.M. 2010. Changes in Mercury Levels in Great Lakes Fish Between 1970s and 2007. Environmental Science & Technology **44**: 3273-3279.

Birge,W.J., Black,I.A., Westerman,A.G., and Hudson,J.E. 1979. The effect of mercury on the reproduction of fish and amphibians. *In* The Biogeochemistry of Mercury in the Environment. Elsevier, New York pp. 629-655.

Bloom,N.S. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. Can.J.Fish.Aquat.Sci. **49**: 1010-1017.

Bodaly,R.A., Hecky,R.E., and Fudge,R.J.P. 1984. Increases in fish mercury levels in lakes flooded by the Churchill River diversion, northern Manitoba. Canadian Journal of Fisheries and Aquatic Sciences **41** : 682-691.

Bodaly,R.A. and Fudge,R.J.P. 1999. Uptake of mercury by fish in an experimental boreal reservoir. Archives of Environmental Contamination and Toxicology **37**: 103-109.

Boddington,M.J., Mackenzie,B.A., and deFreitas,A.S.W. 1979. A respirometer to measure the uptake efficiency of waterborne contaminants in fish. Ecotoxicology and Environmental Safety **3**: 383-393.

Boisclair,D. and Leggett,W.C. 1989. Among-Population Variability of Fish Growth. 1. Influence of the Quantity of Food Consumed. Canadian Journal of Fisheries and Aquatic Sciences **46**: 457-467.

Boisclair,D. and Leggett,W.C. 1989. Among-Population Variability of Fish Growth .2. Influence of Prey Type. Canadian Journal of Fisheries and Aquatic Sciences **46**: 468-482.

Boudou,A. and Ribeyre,F. 1983. Contamination of aquatic biocenoses by mercury compounds: an experimental ecotoxicological approach. *In* Aquatic Toxicology. *Edited by* J.O.Nriagu. John Wiley and Sons, New York pp. 73-116.

Boudou,A. and Ribeyre,F. 1985. Experimental study of trophic contamination of *Salmo gairdneri* by two mercury compounds - $HgCl_2$ and CH_3HgCl - analysis at the organism and organ levels. Water, Air, and Soil Pollution **26**: 137-148.

Bowles,K.C., Apte,S.C., Maher,W.A., Kawei,M., and Smith,R. 2001. Bioaccumulation and biomagnification of mercury in Lake Murray, Papua New Guinea. Canadian Journal of Fisheries and Aquatic Sciences **58**: 888-897.

Cabana,G., Tremblay,A., Kalff,J., and Rasmussen,J.B. 1994. Pelagic food chain structure in Ontario lakes: A determinant of mercury levels in lake trout (*Salvelinus namaycush*). Canadian Journal of Fisheries and Aquatic Sciences **51**: 381-389.

Cabana,G. and Rasmussen,J.B. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences of the United States of America **93**: 10844-10847.

Canadian Council of Ministers of the Environment 2003. Canadian water quality guidelines for the protection of aquatic life: inorganic mercury and methylmercury. Canadian Council of Ministers of the Environment.

Carvan, M. J. and Weber, D. Effects of developmental methylmercury exposure on adult behaviour and the implications for fish populations. Society of Environmental Toxicology and Chemistry. 2009.

Chalmers,A.T., Argue,D.M., Gay,D.A., Brigham,M.E., Schmitt,C.J., and Lorenz,D.L. 2010. Mercury trends in fish from rivers and lakes in the United States, 1969-2005., In press.

Chipps,S.R. and Wahl,D.H. 2008. Bioenergetics modeling in the 21st century: Reviewing new insights and revisiting old constraints. Transactions of the American Fisheries Society **137**: 298-313.

Choy,E.S., Hodson,P.V., Campbell,L.M., Fowlie,A.R., and Ridal,J. 2008. Spatial and temporal trends of mercury concentrations in young-of-the-year spottail shiners (*Notropis hudsonius*) in the St. Lawrence River at Cornwall, ON. Archives of Environmental Contamination and Toxicology **54** : 473-481.

Ciereszko,R.E., Dabrowski,K., Ciereszko,A., Ebeling,J., and Ottobre,J.S. 1997. Effects of temperature and photoperiod on reproduction of female yellow perch *Perca flavescens*: Plasma concentrations of steroid hormones, spontaneous and induced ovulation, and quality of eggs. Journal of the World Aquaculture Society **28**: 344-356.

Cizdziel,J.V., Hinners,T.A., Pollard,J.E., Heithmar,E.M., and Cross,C.L. 2002. Mercury concentrations in fish from Lake Mead, USA, related to fish size, condition, trophic level, location, and consumption risk. Archives of Environmental Contamination and Toxicology **43**: 309-317.

Coelho,J.P., Nunes,M., Dolbeth,M., Pereira,M.E., Duarte,A.C., and Pardal,M.A. 2008. The role of two sediment-dwelling invertebrates on the mercury transfer from sediments to the estuarine trophic web. Estuarine, Coastal and Shelf Science **78**: 505-512.

Cole,D.C., Kearney,J., Sanin,L.H., Leblanc,A., and Weber,J.P. 2004. Blood mercury levels among Ontario anglers and sport-fish eaters. Environmental Research **95**: 305-314.

Coles,T.F. 1981. The distribution of perch, *Perca fluviatalis* L. throughout their first year of life in Llyn Tegid, North Whales. Journal of Fish Biology **18**: 15-30.

Cope,W.G., Wiener,J.G., and Rada,R.G. 1990. Mercury accumulation in yellow perch in Wisconsin seepage lakes: Relation to lake characteristics. Environmental Toxicology and Chemistry **9**: 931-940.

Craig,J.F. 1987. The Biology of Perch and Related Fish. Croom Helm, London & Sydney. 333 p.

De Boer,J., Van Der Valk,F., Kerkhoff,M.A.T., Hagel,P., and Brinkman,U. 1994. 8-year study on the elimination of PCBs and other organochlorine compounds from Eel (*Anguilla anguilla*) under natural conditions. Environmental Science Technology **28**: 2242-2248.

Delta Tributaries Mercury Council 2000. Strategic Plan for Mercury Risk in the Sacramento Watershed. Accessed 10 April, 2010. http://www.sacriver.org/issues/mercury/dtmc/

Dittman,J.A. and Driscoll,C.T. 2009. Factors influencing changes in mercury concentrations in lake water and yellow perch (*Perca flavescens*) in Adirondack lakes. Biogeochemistry **93**: 179-196.

Drevnick,P.E. and Sandheinrich,M.B. 2003. Effects of dietary methylmercury on reproductive endocrinology of fathead minnows. Environmental Science and Technology **37**: 4390-4396.

Drevnick,P.E., Sandheinrich,M.B., and Oris,J.T. 2006. Increased ovarian follicular apoptosis in fathead minnows (*Pimephales promelas*) exposed to dietary methylmercury. Aquatic Toxicology **79**: 49-54.

Drouillard,K.G., Paterson,G., and Haffner,G.D. 2009. A combined food web toxicokinetic and species bioenergetic model for predicting seasonal PCB elimination by yellow perch (*Perca flavescens*). Environmental Science and Technology **43**: 2858-2864.

Eagles-Smith,C.A. and Ackerman,J.T. 2009. Rapid changes in small fish mercury concentrations in estuarine wetlands: Implications for wildlife risk and monitoring programs. Environmental Science and Technology **43**: 8658-8664.

Eckley,C.S. and Hintelmann,H. 2006. Determination of mercury methylation potentials in the water column of lakes across Canada. Science of the Total Environment **368**: 111-125.

Engstrom,D.R. 2007. Fish respond when the mercury rises. Proceedings of the National Academy of Sciences of the United States of America **104**: 16394-16395.

Environment Canada. 2008. Mercury in the environment. Accessed 13 November, 2008. http://www.ec.gc.ca/MERCURY/EH/EN/eh-i.cfm?SELECT=EH

Essington,T.E. and Houser,J.N. 2003. The effect of whole-lake nutrient enrichment on mercury concentration in age-1 yellow perch. Transactions of the American Fisheries Society **132**: 57-68.

Ethier,A.L.M., Scheuhammer,A.M., and Bond,D.E. 2008. Correlates of mercury in fish from lakes near Clyde Forks, Ontario, Canada. Environmental Pollution **154**: 89-97.

Evers,D.C., Mason,R.P., Kamman,N.C., Chen,C.Y., Bogomolni,A.L., Taylor,D.L., Hammerschmidt,C.R., Jones,S.H., Burgess,N.M., Munney,K., and Parsons,K.C. 2008. Integrated Mercury Monitoring Program for Temperate Estuarine and Marine Ecosystems on the North American Atlantic Coast. Ecohealth **5**: 426-441.

Firestone, R. B. Isotopes of Mercury. Berkely National Laboratory . 2000. 1-25-2010. Accessed 21 January, 2010. http://ie.lbl.gov/education/parent/Hg_iso.htm

Fitzgerald,W.F., Engstrom,D.R., Mason,R.P., and Nater,E.A. 1998. The case for atmospheric mercury contamination in remote areas. Environmental Science and Technology **32**: 1-7.

Fleming,L.E., Watkins,S., Kaderman,R., Levin,B., Ayyar,D.R., Bizzio,M., Stephens,D., and Bean,J.A. 1995. Mercury exposure in humans through food consumption from the Everglades of Florida. Water, Air, and Soil Pollution **80**: 41-48.

Foulkes,E.C. 2000. Transport of toxic heavy metals across cell membranes. Proceedings of the Society for Experimental Biology and Medicine **223**: 234-240.

Francesconi,K.A., Lenanton,R.C.J., Caputi,N., and Jones,S. 1996. Long-term study of mercury concentrations in fish following cessation of a mercury-containing discharge. Marine Environmental Research **43**: 27-40.

Friedmann,A.S., Watzin,M.C., Brinck-Johnsen,T., and Leiter,J.C. 1996. Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). Aquatic Toxicology **35**: 265-278.

Fry,B. 1991. Stable isotope diagrams of freshwater food webs. Ecology **72**: 2293-2297.

Galli,C.L. and Restani,P. 1993. Can methylmercury present in fish affect human health? Pharmacological Research **27**: 115-127.

Gantner,N., Power,M., Babaluk,J.A., Reist,J.D., K÷ck,G., Lockhart,L.W., Solomon,K.R., and Muir,D.C.G. 2009. Temporal trends of mercury, cesium, potassium, selenium, and thallium in Arctic char (*Salvelinus alpinus*) from Lake Hazen, Nunavut, Canada: Effects of trophic position, size, and age. Environmental Toxicology and Chemistry **28**: 254-263.

George,E.L. and Hadley,W.F. 1979. Food and habitat partitioning between rock bass (*Ambloplites rupestris*) and smallmouth bass (*Micropterus dolomieui*) young of the year. Transactions of the American Fisheries Society **108**: 253-261.

Giblin,F.J. and Massaro,E.J. 1973. Pharmacodynamics of methyl mercury in the rainbow trout (*Salmo gairdneri*): Tissue uptake, distribution and excretion. Toxicology and Applied Pharmacology **24**: 81-91.

Gilmour,C.C., Riedel,G.S., Ederington,M.C., Bell,J.T., Benoit,J.M., Gill,G.A., and Stordal,M.C. 1998. Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. Biogeochemistry **40**: 327-345.

Golding, L. Predicting toxicity of dietary cadmium from bioaccumulation in the freshwater amphipod *Hyalella azteca*. Society of Environmental Toxicology and Chemistry North America 30th Annual Meeting. 2009.

Gorski,P.R., Lathrop,R.C., Hill,S.D., and Herrin,R.T. 1999. Temporal mercury dynamics and diet composition in the mimic shiner. Transactions of the American Fisheries Society **128**: 701-712.

Graeb,B.D.S., Mangan,M.T., Jolley,J.C., Wahl,D.H., and Dettmers,J.M. 2006. Ontogenetic changes in prey preference and foraging ability of yellow perch: Insights based on relative energetic return of prey. Transactions of the American Fisheries Society **135**: 1493-1498.

Greenfield,B.K., Hrabik,T.R., Harvey,C.J., and Carpenter,S.R. 2001. Predicting mercury levels in yellow perch: Use of water chemistry, trophic ecology, and spatial traits. Canadian Journal of Fisheries and Aquatic Sciences **58**: 1419-1429.

Guma'a,S.A. 1978. On the early growth of 0+ perch, *Perca fluviatalis*, in Windermere. Freshwater Biology **8**: 213-220.

Hall,B.D., Bodaly,R.A., Fudge,R.J.P., Rudd,J.W.M., and Rosenberg,D.M. 1997. Food as the dominant pathway of methylmercury uptake by fish. Water, Air, and Soil Pollution **100**: 13-24.

Hall,B.D., Aiken,G.R., Krabbenhoft,D.P., Marvin-DiPasquale,M., and Swarzenski,C.M. 2008. Wetlands as principal zones of methylmercury production in southern Louisiana and the Gulf of Mexico region. Environmental Pollution **154**: 124-134.

Hammerschmidt,C.R., Wiener,J.G., Frazier,B.E., and Rada,R.G. 1999. Methylmercury content of eggs in yellow perch related to maternal exposure in four Wisconsin lakes. Environmental Science and Technology **33**: 999-1003.

Hammerschmidt,C.R., Sandheinrich,M.B., Wiener,J.G., and Rada,R.G. 2002. Effects of dietary methylmercury on reproduction of fathead minnows. Environmental Science and Technology **36**: 877-883.

Hammerschmidt,C.R. and Fitzgerald,W.F. 2006. Methylmercury in freshwater fish linked to atmospheric mercury deposition. Environmental Science and Technology **40**: 7764-7770.

Handy,R.D. and Eddy,F.B. 1990. The influence of starvation on waterborne zinc accumulation by rainbow trout, *Salmo gairdneri*, at the onset of episodic exposure in neutral soft water. Water Research **24**: 521-527.

Hansen,M.J., Boisclair,D., Brandt,S.B., Hewett,S.W., Kitchell,J.F., Lucas,M.C., and Ney,J.J. 1993. Applications of bioenergetics models to fish ecology and management: Where do we go from here? Transactions of the American Fisheries Society **122**: 1019-1030.

Hanson, P. C., Johnson, T. B., Schindler, D. E., and Kitchell, J. F. Wisconsin Fish Bioenergetics (3.0). 1997. University of Wisconsin-Madison Center for Limnology; University of Wisconsin Sea Grant Institute.

Hansson,S., Hobbie,J.E., Elmgren,R., Larsson,U., Fry,B., and Johansson,S. 1997. The stable nitrogen isotope ratio as a marker of food-web interactions and fish migration. Ecology **78**: 2249-2257.

Harris,R.C. and Snodgrass,W.J. 1993. Bioenergetic simulations of mercury uptake and retention in walleye (*Stizostedion vitreum*) and yellow perch (*Perca flavescens*). Water pollution research journal of Canada **28**: 217-236.

Harris,R.C. and Bodaly,R.A. 1998. Temperature, growth and dietary effects on fish mercury dynamics in two Ontario lakes. Biogeochemistry **40**: 175-187.

Harris,R.C., Rudd,J.W.M., Amyot,M., Babiarz,C.L., Beaty,K.G., Blanchfield,P.J., Bodaly,R.A., Branfireun,B.A., Gilmour,C.C., Graydon,J.A., Heyes,A., Hintelmann,H., Hurley,J.P., Kelly,C.A., Krabbenhoft,D.P., Lindberg,S.E., Mason,R.P., Paterson,M.J., Podemski,C.L., Robinson,A., Sandilands,K.A., Southworth,G.R., Louis,V.L., and Tate,M.T. 2007. Whole-ecosystem study shows rapid fish-mercury response to changes in mercury deposition. Proceedings of the National Academy of Sciences of the United States of America **104**: 16586-16591.

Health Canada. 2007. Information on mercury levels in fish. Health Canada. Accessed 17, March 2009. http://www.hc-sc.gc.ca/hl-vs/iyh-vsv/environ/merc-eng.php

Heath,A.G. 1995. Water pollution and fish physiology. Lewis, Boca Raton. 384 p.

Hesslein,R.H., Capel,M.J., Fox,D.E., and Hallard,K.A. 1991. Stable isotopes of sulfur, carbon, and nitrogen as indicators of trophic level and fish migration in the lower Mackenzie River basin, Canada. Canadian Journal of Fisheries and Aquatic Sciences **48**: 2258-2265.

Hinck,J.E., Schmitt,C.J., Chojnacki,K.A., and Tillitt,D.E. 2009. Environmental contaminants in freshwater fish and their risk to piscivorous wildlife based on a national monitoring program. Environmental Monitoring and Assessment **152**: 469-494.

Hintelmann,H. and Evans,R.D. 1997. Application of stable isotopes in environmental tracer studies - Measurement of monomethylmercury (CH_3Hg) by isotope dilution ICP-MS and detection of species transformation. Fresenius' Journal of Analytical Chemistry **358**: 378-385.

Hintelmann,H., Harris,R., Heyes,A., Hurley,J.P., Kelly,C.A., Krabbenhoft,D.P., Lindberg,S., Rudd,J.W.M., Scott,K.J., and Louis,V.L. 2002. Reactivity and mobility of new and old mercury deposition in a boreal forest ecosystem during the first year of the METAALICUS study. Environmental Science and Technology **36**: 5034-5040.

Hokanson,K.E.F. and Kleiner,C.F. 1974. Effects of constant and rising temperatures on survival and developmental rates of embryonic and larval yellow perch, *Perca flavescans*. *In* The Early Life History of Fish. *Edited by* J.H.S.Blaxter. Springer-Verlag, New York pp. 437-448.

Hokanson,K.E.F. 1977. Temperature requirements of some percids and adaptations to the seasonal temperature cycle. Journal of the Fisheries Research Board of Canada **34**: 1524-1550.

Huckabee,J.W., Goldstein,R.A., Janzen,S.A., and Woock,S.E. 1975. Methylmercury in a freshwater food chain. Proceedings, International Conference on Heavy Metals in the Environment 199-215.

Iles,A.C. and Rasmussen,J.B. 2005. Indirect effects of metal contamination on energetics of yellow perch (*Perca flavescens*) resulting from food web simplification. Freshwater Biology **50**: 976-992.

Imhoff, J. C, Clough, J., Park, R. A., and Stoddard, A. 2004. Evaluation of Chemical Bioaccumulation Models of Aquatic Ecosystems. Report No. 68-C-01-037.

Jackson,T.A. 1991. Biological and environmental control of mercury accumulation by fish in lakes and reservoirs of northern Manitoba, Canada. Canadian Journal of Fisheries and Aquatic Sciences **48**: 2449-2470.

Jackson,T.A. 1997. Long-range atmospheric transport of mercury to ecosystems, and the importance of anthropogenic emissions - A critical review and evaluation of the published evidence. Environmental Reviews **5**: 99-120.

Jacob,U., Mintenbeck,K., Brey,T., Knust,R., and Beyer,K. 2005. Stable isotope food web studies: A case for standardized sample treatment. Marine Ecology Progress Series **287**: 251-253.

Jardine, T. D., McGeachy, S. A., Paton, C. M., and Cunjak, R. A. 2003. Stable itotopes in aquatic systems: sample preparation, analysis, and interpretation. No. 2656.

Jardine,T.D., MacLatchy,D.L., Fairchild,W.L., Cunjak,R.A., and Brown,S.B. 2004. Rapid carbon turnover during growth of Atlantic salmon (*Salmo salar*) smolts in sea water, and evidence for reduced food consumption by growth-stunts. Hydrobiologia **527**: 63-75.

Jusup,M., Klanjek,J., Petricioli,D., and Legoviç,T. 2009. Predicting aquaculture-derived benthic organic enrichment: Model validation. Ecological Modelling **220**: 2407-2414.

Kamman,N.C. and Engstrom,D.R. 2002. Historical and present fluxes of mercury to Vermont and New Hampshire lakes inferred from Pb-210 dated sediment cores. Atmospheric Environment **36**: 1599-1609.

Kannan,K., Smith,J., Lee,R.F., Windom,H.L., Heitmuller,P.T., Macauley,J.M., and Summers,J.K. 1998. Distribution of total mercury and methyl mercury in water, sediment, and fish from South Florida estuaries. Archives of Environmental Contamination and Toxicology **34**: 109-118.

Karimi,R., Chen,C.Y., Pickhardt,P.C., Fisher,N.S., and Folt,C.L. 2007. Stoichiometric controls of mercury dilution by growth. Proceedings of the National Academy of Sciences of the United States of America **104**: 7477-7482.

Kayes, T. B. and Calbert, H. E. Effects of photoperiod and temperature on the spawning of yellow perch (*Perca flavescens*). Proceedings of the World Mariculture Society 10, 306-316. 1979.

Keast,A. 1977. Diet overlaps and feeding relationships between the year classes in the yellow perch (*Perca flavescens*). Environmental Biology of Fishes **2**: 53-70.

Keeler,G.J., Pirrone,N., Bullock,R., and Sillman,S. 2009. The need for a coordinated global mercury monitoring network for global and regional models validations. *In* Mercury Fate and Transport in the Global Atmosphere. *Edited by* N.Pirrone and R.P.Mason. Springer Science and Business Media, pp. 391-424.

Kehrig,H., Howard,B.M., and Malm,O. 2008. Methylmercury in a predatory fish (*Cichla* spp.) inhabiting the Brazilian Amazon. Environmental Pollution **154**: 68-76.

Kelly,C.A., Rudd,J.W.M., Bodaly,R.A., Roulet,N.P., Louis,V.L., Heyes,A., Moore,T.R., Schiff,S., Aravena,R., Scott,K.J., Dyck,B., Harris,R., Warner,B., and Edwards,G. 1997. Increases in fluxes of greenhouse gases and methyl mercury following flooding of an experimental reservoir. Environmental Science and Technology **31**: 1334-1344.

Kennedy,C.J. 2003. Uptake and accumulation of mercury from dental amalgam in the common goldfish, *Carassius auratus*. Environmental Pollution **121**: 321-326.

King,D.G. and Davies,I.M. 1987. Laboratory and field studies of the accumulation of inorganic mercury by the mussel *Mytilus edulis* (L.). Marine Pollution Bulletin **18**: 40-45.

Kitchell,J.F., Stewart,D.J., and Weininger,D. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). Journal of the Fisheries Research Board of Canada **34**: 1922-1935.

Klaper,R., Rees,C.B., Drevnick,P., Weber,D., Sandheinrich,M., and Carvan,M.J. 2006. Gene expression changes related to endocrine function and decline in reproduction in fathead minnow (*Pimephales promelas*) after dietary methylmercury exposure. Environmental Health Perspectives **114**: 1337-1343.

Klinck,J., Dunbar,M., Brown,S., Nichols,J., Winter,A., Hughes,C., and Playle,R.C. 2005. Influence of water chemistry and natural organic matter on active and passive uptake of inorganic mercury by gills of rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology **72**: 161-175.

Knightes,C.D., Sunderland,E.M., Barber,M.C., Johnston,J.M., and Ambrose,J. 2009. Application of ecosystem-scale fate and bioaccumulation models to predict fish mercury response times to changes in atmospheric deposition. Environmental Toxicology and Chemistry **28**: 881-893.

Korhonen,P., Virtanen,M., and Schultz,T. 1995. Bioenergetic calculation of mercury accumulation in fish. Water, Air, and Soil Pollution **80**: 901-904.

Kraemer,L.D., Campbell,P.G.C., Hare,L., and Auclair,J.C. 2006. A field study examining the relative importance of food and water as sources of cadmium for juvenile yellow perch (*Perca flavescens*). Canadian Journal of Fisheries and Aquatic Sciences **63**: 549-557.

Kudo,A. and Mortimer,D.C. 1979. Pathways for mercury uptake by fish from bed sediments. Environmental Pollution **19**: 239-245.

Laarman,P.W., Willford,W.A., and Olson,J.R. 1976. Retention of mercury in the muscle of yellow perch (*Perca flavescens*) and rock bass (*Ambloplites rupestris*). Transactions of the American Fisheries Society **105**: 296-300.

Larsson,P., Collvin,L., Okla,L., and Meyer,G. 1992. Lake Productivity and Water Chemistry As Governors of the Uptake of Persistent Pollutants in Fish. Environmental Science & Technology **26**: 346-352.

Lawson,N.M. and Mason,R.P. 1998. Accumulation of mercury in estuarine food chains. Biogeochemistry **40**: 235-247.

Leaner,J.J. and Mason,R.P. 2002. Factors controlling the bioavailability of ingested methylmercury to channel catfish and atlantic sturgeon. Environmental Science and Technology **36**: 5124-5129.

Leaner,J.J. and Mason,R.P. 2004. Methylmercury uptake and distribution kinetics in sheepshead minnows, *Cyprinodon variegatus*, after exposure to CH_3Hg-spiked food. Environmental Toxicology and Chemistry **23** : 2138-2146.

Lepak,J.M., Robinson,J.M., Kraft,C.E., and Josephson,D.C. 2009a. Changes in mercury bioaccumulation in an apex predator in response to removal of an introduced competitor. Ecotoxicology **18**: 488-498.

Lepak,J.M., Shayler,H.A., Kraft,C.E., and Knuth,B.A. 2009b. Mercury Contamination in Sport Fish in the Northeastern United States: Considerations for Future Data Collection. Bioscience **59**: 174-181.

Letcher,B.H., Rice,J.A., Crowder,L.B., and Binkowski,F.P. 1996. Size-dependent effects of continuous and intermittent feeding on starvation time and mass loss in starving yellow perch larvae and juveniles. Transactions of the American Fisheries Society **125**: 14-26.

Li,G., Feng,X., Qiu,G., Bi,X., Li,Z., Zhang,C., Wang,D., Shang,L., and Guo,Y. 2008. Environmental mercury contamination of an artisanal zinc smelting area in Weining County, Guizhou, China. Environmental Pollution **154**: 21-31.

Lindberg,S., Bullock,R., Ebinghaus,R., Engstrom,D., Feng,X., Fitzgerald,W., Pirrone,N., Prestbo,E., and Seigneur,C. 2007. A synthesis of progress and uncertainties in attributing the sources of mercury in deposition. Ambio **36**: 19-32.

Little,A.S., Tonn,W.M., Tollman,R.F., and Reist,J.D. 1998. Seasonal variation in diet and trophic relationships within the fish communities of the lower Slave River, Northwest Territories, Canada. Environmental Biology of Fishes **53**: 429-445.

Lock,R.A.C. 1975. Uptake of methylmercury by aquatic organisms from water and food. *In* Sublethal effects of toxic chemicals on aquatic animals. *Edited by* J.H.Koeman and J.J.T.W.A.Strik. Elsevier, Amsterdam pp. 61-79.

Lockhart,W.L., Uthe,J.F., Kenney,A.R., and Mehrle,P.M. 1972. Methylmercury in northern pike (*Esox lucius*): Distribution, elimination, and some biochemical characteristics of contaminated fish.Journal of the Fisheries Research Board of Canada. **29**: 1519-1523.

Lubick,N. 2009. Funding struggle for mercury monitoring. Nature **459**: 620-621.

Luoma,S.N. 1983. Bioavailability of trace metals to aquatic organisms - A review. Science of the Total Environment **Vol. 28**: 1-22.

MacCrimmon,H.R., Wren,C.D., and Gots,B.L. 1983. Mercury uptake by lake trout, *Salvelinus namaycush*, relative to age, growth, and diet in Tadenac Lake with comparative data from other PreCambrian Shield lakes. Canadian Journal of Fisheries and Aquatic Sciences **40**: 114-120.

MacRury,N.K., Graeb,B.D.S., Johnson,B.M., and Clements,W.H. 2002. Comparison of dietary mercury exposure in two sympatric top predator fishes, largemouth bass and northern pike: A bioenergetics modeling approach. Journal of Aquatic Ecosystem Stress and Recovery **9**: 137-147.

Madenjian,C.P., O'Connor,D.V., Pothoven,S.A., Schneeberger,P.J., Rediske,R.R., O'Keefe,J.P., Bergstedt,R.A., Argyle,R.L., and Brandt,S.B. 2006. Evaluation of a lake whitefish bioenergetics model. Transactions of the American Fisheries Society **135**: 61-75.

Madenjian,C.P. and O'Connor,D.V. 2008. Trophic transfer efficiency of mercury to lake whitefish *Coregonus clupeaformis* from its prey. Bulletin of Environmental Contamination and Toxicology **81**: 566-570.

Mahaffey,K.R. 1998. Methylmercury exposure and neurotoxicity. Journal of the American Medical Association **280**: 737-738.

Manitoba Water Stewardship. 2007. Mercury in fish & guidelines for the consumption of recreationally angled fish in Manitoba. Pamphlet.

Mann,K.H. 1978. Estimating the food consumption of fish in nature. *In* Ecology of freshwater fish production. *Edited by* S.D.Gerking. Blackwell Scientific Publications, Oxford pp. 250-273.

Mason,R.P., Fitzgerald,W.F., and Morel,F.M.M. 1994. The biogeochemical cycling of elemental mercury: Anthropogenic influences. Geochimica et Cosmochimica Acta **58**: 3191-3198.

Mason,R.P., Laporte,J.M., and Andres,S. 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. Archives of Environmental Contamination and Toxicology **38**: 283-297.

Mason,R.P. 2009. Mercury emissions from natural processes and their importance in the global mercury cycle. *In* Mercury Fate and Transport in the Global Atmosphere. *Edited by* N.Pirrone and R.P.Mason. Springer Science and Business Media, pp. 173-191.

Mason,R.R., Abbot,M.L., Bodaly,R.A., Bullock,O.R., Driscoll,C.T., Evers,D., Lindberg,S.E., Murray,M., and Swain,E.B. 2005. Monitoring the response to changing mercury deposition. Environmental Science & Technology **39**: 14A-22A.

Mathers,R.A. and Johansen,P.H. 1985. The effects of feeding ecology on mercury accumulation in walleye (*Stizostedion vitreum*) and pike (*Esox lucius*) in Lake Simcoe. Canadian Journal of Zoology **63**: 2006-2012.

Matta,M.B., Linse,J., Cairncross,C., Francendese,L., and Kocan,R.M. 2001. Reproductive and transgenerational effects of methylmercury or Aroclor 1268 on *Fundulus heteroclitus*. Environmental Toxicology and Chemistry **20**: 327-335.

McKim,J.M., Olson,G.F., Holcombe,G.W., and Hunt,E.P. 1976. Long term effects of methylmercuric chloride on three generations of brook trout (*Salvelinus fontinalis*): Toxicity, accumulation, distribution, and elimination. Journal of the Fisheries Research Board of Canada. **33**: 2726-2739.

McKone,C.E., Young,R.G., Bache,C.A., and Lisk,D.J. 1971. Rapid uptake of mercuric ion by goldfish. Environmental Science and Technology **5**: 1138-1139.

Mergler,D., Anderson,H.A., Chan,L.H.M., Mahaffey,K.R., Murray,M., Sakamoto,M., and Stern,A.H. 2007. Methylmercury exposure and health effects in humans: A worldwide concern. Ambio **36**: 3-11.

Mierle,G. 1990. Aqueous inputs of mercury to precambrian shield lakes in Ontario. Environmental Toxicology and Chemistry **9**: 843-851.

Mills,E.L. and Forney,J.L. 1983. Impact on *Daphnia-Pulex* of predation by young yellow perch in Oneida Lake, New-York. Transactions of the American Fisheries Society **112**: 154-161.

Minamata City Planning Division 2000. Minamata disease: its history and lessons.

Munthe,J., Bodaly,R.A., Branfireun,B.A., Driscoll,C.T., Gilmour,C.C., Harris,R., Horvat,M., Lucotte,M., and Malm,O. 2007. Recovery of mercury-contaminated fisheries. Ambio **36**: 33-44.

Niyogi,S. and Wood,C.M. 2003. Effects of chronic waterborne and dietary metal exposures on gill metal-binding: Implications for the biotic ligand model. Human and Ecological Risk Assessment **9**: 813-846.

Niyogi,S., Pyle,G.G., and Wood,C.M. 2007. Branchial versus intestinal zinc uptake in wild yellow perch (*Perca flavescens*) from reference and metal-contaminated aquatic ecosystems. Canadian Journal of Fisheries and Aquatic Sciences **64**: 1605-1613.

Norstrom,R.J., McKinnon,A.E., and DeFreitas,A.S.W. 1976. A bioenergetics based model for pollutant accumulation by fish. Simulation of PCB and methylmercury residue levels in Ottawa River yellow perch (*Perca flavescens*). Journal of the Fisheries Research Board of Canada**. 33**: 248-267.

Nose.K. 1969. Studies on the toxicity of low alkyl mercury compounds. Japanese Journal of Hygiene **24**: 17-24.

Oliveira Ribeiro,C.A., Rouleau,C., Pelletier,E., Audet,C., and Tjlve,H. 1999. Distribution kinetics of dietary methylmercury in the arctic charr (*Salvelinus alpinus*). Environmental Science and Technology **33**: 902-907.

Oliveira Ribeiro,C.A., Pelletier,E., Pfeiffer,W.C., and Rouleau,C. 2000. Comparative uptake, bioaccumulation, and gill damages of inorganic mercury in tropical and nordic freshwater fish. Environmental Research **83**: 286-292.

Orihel,D.M., Paterson,M.J., Gilmour,C.C., Bodaly,R.A., Blanchfield,P.J., Hintelmann,H., Harris,R.C., and Rudd,J.W.M. 2006. Effect of loading rate on the fate of mercury in littoral mesocosms. Environmental Science and Technology **40**: 5992-6000.

Orihel,D.M., Paterson,M.J., Blanchfield,P.J., Bodaly,R.A., and Hintelmann,H. 2007. Experimental evidence of a linear relationship between inorganic mercury loading and methylmercury accumulation by aquatic biota. Environmental Science and Technology **41**: 4952-4958.

Orihel,D.M., Paterson,M.J., Blanchfield,P.J., Bodaly,R.A., Gilmour,C.C., and Hintelmann,H. 2008. Temporal changes in the distribution, methylation, and bioaccumulation of newly deposited mercury in an aquatic ecosystem. Environmental Pollution. **154**: 77-88.

Pacyna,E.G. and Pacyna,J.M. 2002. Global emission of mercury from anthropogenic sources in 1995. Water, Air, and Soil Pollution **137**: 149-165.

Pacyna,E.G., Pacyna,J.M., Sundseth,K., Munthe,J., Kindbom,K., Wilson,S., Steenhuisen,F., and Maxson,P. 2010. Global emission of mercury to the atmosphere from anthropogenic sources in 2005 and projections to 2020. Atmospheric Environment **44**: 2487-2499.

Parks,J., Sutton,J., Hollinger,J., and Russell,D. 1988. Uptake of mercury by caged crayfish. Applied Organometallic Chemistry. **2**: 181-184.

Paterson,M.J., Rudd,J.W.M., and Louis,V. 1998. Increases in total and methylmercury in zooplankton following flooding of a peatland reservoir. Environmental Science and Technology **32**: 3868-3874.

Pentreath,R.J. 1976. The accumulation of mercury from food by the plaice, *Pleuronectes platessa* L. Journal of Experimental Marine Biology and Ecology **25**: 51-65.

Peterson,B.J. 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs: A review. Acta Oecologica **20**: 479-487.

Phillips,G.R. and Buhler,D.R. 1978. The relative contributions of methylmercury from food or water to rainbow trout (*Salmo gairdneri*) in a controlled laboratory environment. Transactions of the American Fisheries Society **107**: 853-861.

Pickhardt,P.C., Stepanova,M., and Fisher,N.S. 2006. Contrasting uptake routes and tissue distributions of inorganic and methylmercury in mosquitofish (*Gambusia affinis*) and redear sunfish (*Lepomis microlophus*). Environmental Toxicology and Chemistry **25**: 2132-2142.

Plourde,Y., Lucotte,M., and Pichet,P. 1997. Contribution of suspended particulate matter and zooplankton to MeHg contamination of the food chain in midnorthern Quebec (Canada) reservoirs. Canadian Journal of Fisheries and Aquatic Sciences **54**: 821-831.

Post,J.R. 1990. Metabolic allometry of larval and juvenile yellow perch (*Perca flavescens*): in situ estimates and bioenergetic models. Canadian Journal of Fisheries and Aquatic Sciences **47**: 554-560.

Post,J.R., Vandenbos,R., and McQueen,D.J. 1996. Uptake rates of food-chain and waterborne mercury by fish: Field measurements, a mechanistic model, and an assessment of uncertainties. Canadian Journal of Fisheries and Aquatic Sciences **53**: 395-407.

Pothoven,S.A., Nalepa,T.F., Schneeberger,P.J., and Brandt,S.B. 2001. Changes in diet and body condition of lake whitefish in southern Lake Michigan associated with changes in benthos. North American Journal of Fisheries Management **21**: 876-883.

Poulain,A.J., Orihel,D.M., Amyot,M., Paterson,M.J., Hintelmann,H., and Southworth,G.R. 2006. Relationship between the loading rate of inorganic mercury to aquatic ecosystems and dissolved gaseous mercury production and evasion. Chemosphere **65**: 2199-2207.

Power,M. and Van Den Heuvel,M.R. 1999. Age-0 yellow perch growth and its relationship to temperature. Transactions of the American Fisheries Society **128**: 687-700.

Ramade,F. 1987. Ecotoxicology. Wiley Press, Chichester, UK.

Reid,S.D. and McDonald,D.G. 1991. Metal binding activity of the gills of rainbow trout (*Oncorhynchus mykiss*). Canadian Journal of Fisheries and Aquatic Sciences **48**: 1061-1068.

Reinfelder,J.R. and Fisher,N.S. 1994. Retention of elements absorbed by juvenile fish (*Menidia menidia, Menidia beryllina*) from zooplankton prey. Limnology and Oceanography **39**: 1783-1789.

Rennie,M.D. Mercury in aquatic foodwebs: refining the use of mercury in energetics models of wild fish populations. M. Sc. thesis, University of Toronto.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. No. 191.

Rinchard,J., Becheret,R., and Dabrowski,K. 2008. Growth, survival, and body composition of yellow perch juveniles fed commercial and experimental diets. North American Journal of Aquaculture **70**: 74-79.

Rodgers,D.W. and Beamish,F.W.H. 1981. Uptake of waterborne methylmercury by rainbow trout (*Salmo gairdneri*) in relation to oxygen consumption and methylmercury concentration. Canadian Journal of Fisheries and Aquatic Sciences **38**: 1315.

Rodgers,D.W. and Qadri,S.U. 1982. Growth and mercury accumulation in yearling yellow perch, *Perca flavescens*, in the Ottawa River, Ontario. Environmental Biology of Fishes **7**: 377-383.

Rodgers,D.W. 1994. You are what you eat and a little bit more: bioenergetics-based models of methylmercury accumulation in fish revisited. pp. 427-439.

Rudd,J.W.M. 1995. Sources of methyl mercury to freshwater ecosystems: a review. Water, Air, and Soil Pollution **80**: 697-713.

Ryaboshapko,A., Bullock,R., Ebinghaus,R., Ilyin,I., Lohman,K., Munthe,J., Petersen,G., Seigneur,C., and Wangberg,I. 2002. Comparison of mercury chemistry models. Atmospheric Environment **36**: 3881-3898.

Sackett,D.K., Aday,D.D., Rice,J.A., and Cope,W.G. 2009. A Statewide Assessment of Mercury Dynamics in North Carolina Water Bodies and Fish. Transactions of the American Fisheries Society **138**: 1328-1341.

Salacinska,K., Serafy,G.Y., Los,F.J., and Blauw,A. 2010. Sensitivity analysis of the two dimensional application of the Generic Ecological Model (GEM) to algal bloom prediction in the North Sea. Ecological Modelling **221**: 178-190.

Sandheinrich,M.B. and Miller,K.M. 2006. Effects of dietary methylmercury on reproductive behavior of fathead minnows (*Pimephales promelas*). Environmental Toxicology and Chemistry **25**: 3053-3057.

Sandilands, K. A., Rudd, J. W. M., Kelly, C. A., Hintelmann, H., Gilmour, C. C., and Tate, M. T. 2005. Application of enriched stable mercury isotopes to the Lake 658 watershed for the METAALICUS project, at the Experimental Lakes Area, Northwestern Ontario, Canada. No. 2597.

Sandilands, K. A., Kelly, C. A., Rudd, J. W. M., Tate, M. T., Hintelmann, H., Dimock, B., and Harris, R. 2008. Application of enriched stable mercury isotopes to the Lake 658 watershed for the METAALICUS project, at the Experimental Lakes Area, northwestern Ontario, Canada, 2001-2007. Fisheries and Oceans Canada No. 2813.

Schael,D.M., Rudstam,L.G., and Post,J.R. 1991. Gape Limitation and Prey Selection in Larval Yellow Perch (*Perca-Flavescens*), Fresh-Water Drum (*Aplodinotus-Grunniens*), and Black Crappie (*Pomoxis-Nigromaculatus*). Canadian Journal of Fisheries and Aquatic Sciences **48**: 1919-1925.

Scheuhammer,A.M., Meyer,M.W., Sandheinrich,M.B., and Murray,M.W. 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. Ambio **36**: 12-18.

Schultz,I.R. and Newman,M.C. 1997. Methyl mercury toxicokinetics in channel catfish (*Ictalurus punctatus*) and largemouth bass (*Micropterus salmoides*) after intravascular administration. Environmental Toxicology and Chemistry **16**: 990-996.

Scott,W.B. and Crossman,E.J. 1998. Freshwater Fishes of Canada. Galt House Publications, Oakville, ON.

Selvendiran,P., Driscoll,C.T., Bushey,J.T., and Montesdeoca,M.R. 2008. Wetland influence on mercury fate and transport in a temperate forested watershed. Environmental Pollution **154**: 46-55.

Sharpe.M.A., DeFreitas,A.S.W., and McKinnon,A.E. 1977. The effect of body size on methylmercury clearance by goldfish (*Carassius auratus*). Environmental Biology of Fishes **2**: 177-183.

Simon,O. and Boudou,A. 2001. Direct and trophic contamination of the herbivorous carp *Ctenopharyngodon idella* by inorganic mercury and methylmercury. Ecotoxicology and Environmental Safety **50**: 48-59.

Simoneau,M., Lucotte,M., Garceau,S., and Laliberteü,D. 2005. Fish growth rates modulate mercury concentrations in walleye (*Sander vitreus*) from eastern Canadian lakes. Environmental Research **98**: 73-82.

Simonin,H.A., Loukmas,J.J., Skinner,L.C., and Roy,K.M. 2008. Lake variability: Key factors controlling mercury concentrations in New York State fish. Environmental Pollution **154**: 107-115.

Southworth,G., Lindberg,S., Hintelmann,H., Amyot,M., Poulain,A., Bogle,M., Peterson,M., Rudd,J., Harris,R., Sandilands,K., Krabbenhoft,D., and Olsen,M. 2007. Evasion of added isotopic mercury from a northern temperate lake. Environmental Toxicology and Chemistry **26**: 53-60.

St Louis,V.L., Rudd,J.W.M., Kelly,C.A., Beaty,K.G., Bloom,N.S., and Flett,R.J. 1994. Importance of wetlands as sources of methyl mercury to boreal forest ecosystems. Canadian Journal of Fisheries and Aquatic Sciences **51**: 1065-1076.

Stafford,C.P. and Haines,T.A. 2001. Mercury contamination and growth rate in two piscivore populations. Environmental Toxicology and Chemistry **20**: 2099-2101.

Stahl,L.L., Snyder,B.D., Olsen,A.R., and Pitt,J.L. 2009. Contaminants in fish tissue from US lakes and reservoirs: a national probabilistic study. Environmental Monitoring and Assessment **150**: 3-19.

Stemberger,R.S. and Chen,C.Y. 1998. Fish tissue metals and zooplankton assemblages of northeastern U.S. lakes. Canadian Journal of Fisheries and Aquatic Sciences **55**: 339-352.

Sutton,S.G., Bult,T.P., and Haedrich,R.L. 2000. Relationships among fat weight, body weight, water weight, and condition factors in wild Atlantic salmon parr. Transactions of the American Fisheries Society **129**: 527-538.

Swain,E.B., Jakus,P.M., Rice,G., Lupi,F., Maxson,P.A., Pacyna,J.M., Penn,A., Spiegel,S.J., and Veiga,M.M. 2007. Socioeconomic consequences of mercury use and pollution. Ambio **36**: 45-61.

Thogmartin,W.E. 2010. Sensitivity analysis if North America bird population estimates. Ecological Modelling **221**: 173-177.

Tomy,G.T., Palace,V.P., Halldorson,T., Braekevelt,E., Danell,R., Wautier,K., Evans,B., Brinkworth,L., and Fisk,A.T. 2004. Bioaccumulation, biotransformation, and biochemical effects of brominated diphenyl ethers in juvenile lake trout (*Salvelinus namaycush*). Environmental Science and Technology **38**: 1496-1504.

Trudel,M. and Rasmussen,J.B. 1997. Modeling the elimination of mercury by fish. Environmental Science and Technology **31**: 1716-1722.

Trudel,M., Tremblay,A., Schetagne,R., and Rasmussen,J.B. 2000. Estimating food consumption rates of fish using a mercury mass balance model. Canadian Journal of Fisheries and Aquatic Sciences **57**: 414-428.

Trudel,M. and Rasmussen,J.B. 2001. Predicting mercury concentration in fish using mass balance models. Ecological Applications **11**: 517-529.

Tsui,M.T.K. and Wang,W.X. 2004. Uptake and Elimination Routes of Inorganic Mercury and Methylmercury in *Daphnia magna*. Environmental Science and Technology **38**: 808-816.

United Nations Environmental Programme. 2009. Overarching Framework UNEP Global Mercury Partnership.

United States Environmental Protection Agency 2006. EPA's Roadmap for Mercury. Report No. EPA-HQ-OPPT-2005-0013.

United States Environmental Protection Agency. Fisheries Advisories Database. United States Environmental Protection Agency . 2010. Accessed 26 March, 2010. http://134.67.99.49/scripts/esrimap.dll?name=Listing&Cmd=Map

Urbatzka,R., Beeck,P., Van Der Velde,G., and Borcherding,J. 2008. Alternative use of food resources causes intra-cohort variation in the size distribution of young-of-the-year perch (*Perca fluviatilis*). Ecology of Freshwater Fish **17**: 475-480.

Van Walleghem,J.L.A. 2006. The elimination of mercury by fish in the wild. M.Sc. thesis, University of Manitoba.

Van Walleghem,J.L.A., Blanchfield,P.J., and Hintelmann,H. 2007. Elimination of mercury by yellow perch in the wild. Environmental Science and Technology **41**: 5895-5901.

Walters,C.J. 1986. Adaptive management of renewable resources. Macmillan, New York.

Wang,R., Wong,M.H., and Wang,W.X. 2010. Mercury exposure in the freshwater tilapia *Oreochromis niloticus*. Environmental Pollution **158**: 2694-2701.

Wang,W.X. and Wong,R.S.K. 2003. Bioaccumulation kinetics and exposure pathways of inorganic mercury and methylmercury in a marine fish, the sweetlips *Plectorhinchus gibbosus*. Marine Ecology Progress Series **261**: 257-268.

Watras,C.J. and Bloom,N.S. 1992. Mercury and methylmercury in individual zooplankton: implications for bioaccumulation. Limnology & Oceanography **37**: 1313-1318.

Watras,C.J., Bloom,N.S., Hudson,R.J.M., Gherini,S., Munson,R., Claas,S.A., Morrison,K.A., Hurley,J., Wiener,J.G., Fitzgerald,W.F., Mason,R., Vandal,G., Powell,D., Rada,R., Rislov,L., Winfrey,M., Elder,J., Krabbenhoft,D., Andren,A.W., Babiarz,C., Porcella,D.B., and Huckabee,J.W. 1994. Sources and fates of mercury and methylmercury in Wisconsin lakes. Mercury Pollution: Integration and Synthesis 153-177.

Webber,H.M. and Haines,T.A. 2003. Mercury effects on predator avoidance behavior of a forage fish, golden shiner (*Notemigonus crysoleucas*). Environmental Toxicology and Chemistry **22**: 1556-1561.

Wiener,J.G. and Spry,D.J. 1996. Toxicological significance of mercury in freshwater fish. Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations 297-339.

Winfrey,M.R. and Rudd,J.W.M. 1990. Environmental factors affecting the formation of methylmercury in low pH lakes. Environmental Toxicology and Chemistry **9**: 853-869.

Xun,L., Campbell,N.E.R., and Rudd,J.W.M. 1987. Measurements of specific rates of net methyl mercury production in the water column and surface sediments of acidified and circumneutral lakes. Canadian Journal of Fisheries and Aquatic Sciences **44**: 750-757.