ABSTRACT

THE IMPACT OF A BENTHIC OMNIVORE ON THE BIOMAGNIFICATION OF MERCURY IN TOP-PREDATOR FISH

by Anna Marie Bowling

Crayfish, as benthic omnivores, have the potential to be a link between the benthic and pelagic systems by feeding on fish carcasses and being prey to top-predator fish. The objective of this research was to investigate the impact of crayfish on the biomagnification of methylmercury (MeHg) in top-predator fish, by combining a field study, controlled laboratory studies, and a mathematical model. The results of the field study were inconclusive and this was mainly due to the fact that all sites had the same relative abundance of crayfish and that we were not able to collect all species of top-predator fish. Laboratory experiments supported our prediction that largemouth bass would assimilate MeHg more efficiently from MeHg-dosed crayfish than from MeHg-dosed artificial food. Model projections of laboratory results supported our prediction that MeHg biomagnification would be highest in the presence of a feedback cycle between crayfish and top-predator fish.
THE IMPACT OF A BENTHIC OMNIVORE ON THE BIOMAGNIFICATION OF
MERCURY IN TOP-PREDATOR FISH

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Introduction
Mercury (Hg) is a contaminant of global concern for both human and environmental health. The Department of Health and Human Services Agency for Toxic Substances & Disease Registry ranks mercury as third on the list of the top 50 priority pollutants (http://www.atsdr.cdc.gov/cercla/05list.html) in the United States. It is the long history of industrial release of Hg, into the atmosphere, mostly as a by-product of coal combustion, that is the major source of contamination to the environment (Wiener et al. 2003). Aquatic ecosystems are particularly sensitive to atmospheric deposition of Hg because it can be converted from the inorganic forms (Hg$^0$, HgS), which are generally less toxic and less bioavailable to aquatic organisms, to methylmercury (MeHg), a process mediated by sulfate-reducing bacteria in lake sediments (Gilmour et al. 1992). Methylmercury is a lipophilic and proteophilic substance that bioaccumulates in aquatic organisms through their diet (Bloom 1992; Hall et al. 1997). Methylmercury is not easily eliminated or metabolized by aquatic organisms, including fish (Trudel & Rasmussen 1997) and therefore concentrations increase up the food chain (i.e. trophic transfer) with the highest concentrations found in top-predator fish, a process known as biomagnification (Watras & Bloom 1992). MeHg becomes a health problem for humans when they consume top-predator fish such as tuna, sharks, and bass. Currently, there are 48 states that have fish consumption advisories that are a result of Hg contamination (U.S. EPA 2007).

Since the consumption of fish is the main source of MeHg exposure to humans (Fitzgerald & Clarkson 1991), much of the research conducted in recent years has focused on determining the primary mechanisms that drive the large variation that exists in MeHg concentrations among top-predator fish. There is strong agreement in the scientific literature that trophic transfer is responsible for the biomagnification of MeHg in aquatic systems; that is, the longer the food chain, the higher the concentrations of MeHg in top-predator fish (Cabana et al. 1994; Cabana & Rasmussen 1994; Watras et al. 1998; Da Silva 2005). However, the large variation in MeHg concentrations among lakes, even in those that have similar geography, watersheds, and limnological parameters, is not well understood. Factors such as presence of wetlands, pH, alkalinity, dissolved organic carbon, primary productivity, and atmospheric Hg deposition can affect the degree of Hg biomagnification (Wiener & Stokes 1990; St. Louis et al. 1994; Scheuhammer & Graham 1999; Pickhardt & Fisher 2007; Pickhardt et al. 2002; Hammerschmidt & Fitzgerald 2006a), but there is disagreement in the level of importance of
these factors and whether they are positively or negatively correlated with Hg in biota (Chen et al. 2005). Still others have suggested biological factors play a key role in driving the variability in MeHg concentrations among top-predator fish (Wren et al. 1983; Skurdal et al. 1985; Wren & MacCrimmon 1986; Lange et al. 1993; Cabana & Rasmussen 1994; Eagles-Smith et al. 2008a, b).

Recent studies have highlighted the importance of understanding differences in life history traits when trying to understand variations in MeHg concentrations among fish species. Typically, Hg levels are highest in very large, long-lived fish species; and there has been a lot of research that has documented a strong positive correlation between Hg concentration and fish size (Wren et al. 1983; Skurdal et al. 1985; Wren & MacCrimmon 1986; Lange et al. 1993), which is a proxy for fish age. However, some studies have found that fast-growing species of fish tend to have lower than predicted body burdens of MeHg (Schindler et al. 1995; Simoneau et al. 2005; Desta et al. 2007; Piraino & Taylor 2009). In these cases, fish grow fast enough to offset the uptake of MeHg from their diet, which leads to a lower amount of MeHg per unit of tissue, a concept described as growth dilution. The correlation between growth rate and MeHg concentration in fish is further obscured when taking into account the wide variation in diet among fish.

Many fish experience significant dietary shifts as they transition between juvenile and adult life stages. For example, fish are often planktivorous as juveniles and transition to a piscivorous diet as they become adults. This shift to prey occupying a higher trophic level can lead to an increase in uptake of MeHg (Cabana et al. 1994; Lepak et al. 2009). Dietary shifts can also occur in response to changes in the quantity and quality of prey species available, particularly for fish that are omnivorous.

Omnivory by aquatic organisms can serve as a link between benthic and pelagic systems, both for energy flow (Vander Zanden & Vadeboncoeur 2002) and the transfer of contaminants, such as MeHg (Cabana & Rasmussen 1994; Eagles-Smith et al. 2008a,b). A well-known example would be a fish, such as largemouth bass (Micropterus salmoides), that feed both on pelagic prey, such as small fish, as well as on benthic organisms, such as crayfish (Garcia-Berhou 2002). In turn, crayfish feed on benthic items such as periphyton and macrophytes (Momot et al. 1978), but will also feed on pelagic-derived items such as fish carcasses (Minckley & Craddock 1961). A study by Vander Zanden & Rasmussen (1996) found that by using trophic
position which incorporates omnivory, instead of discrete trophic levels, greatly improved model predictions of polychlorinated biphenyl concentrations in lake trout, a contaminant that also biomagnifies in aquatic systems. They also found that estimates of biomagnification factors, a metric often used to assess the uptake of bioaccumulating contaminants in aquatic organisms, were usually higher when omnivory was considered.

The introduction of invasive species has also been shown to have dramatic effects on fish diets and has the potential to affect the trophic transfer of Hg (Vander Zanden & Rasmussen 1996; Vander Zanden et al. 1999; Hogan et al. 2007; Eagles-Smith et al. 2008; Lepak et al. 2009). Invasive species can create dietary shifts in resident fish species through direct or indirect competition, or they themselves can provide a new source of prey for top-predator fish. A few studies have examined the effect of non-native species introductions on the Hg in resident fish populations, some of which found an increase in Hg concentrations in top-predator fish (Eagles-Smith et al. 2008b; Lepak et al. 2009), while others found minimal change in Hg concentrations for both lower or higher trophic level fish (Johnston et al. 2003; Swanson et al. 2006; Hogan et al. 2007). Again, there is very little consensus as to how individual factors, such as diet, growth rate, lake chemistry and geographic location affect the MeHg levels in top predator fish. The goal of this study was to try to determine what role a benthic omnivore has in the bioaccumulation of MeHg in top predator fish by combining a field study, controlled laboratory studies, and mathematical models.

The objective of this research was to investigate the impact of a benthic omnivore on the Hg concentrations in top-predator fish. The omnivore chosen for this study is the crayfish because it is a common prey item for top-predator fish (Garcia-Berhou 2002) and is known to consume fish carcasses (Minckley & Craddock 1961). Crayfish, as opportunistic feeders, have the potential to be a link between the benthic and pelagic systems by feeding on fish carcasses and being prey for top-predator fish. For an organism that also feeds on fish carcasses, leeches were found to have significantly higher concentrations of Hg and MeHg after feeding on yellow perch carcasses that had been labeled with $^{202}$Hg (Sarica et al. 2005). This study supports the idea that organisms that feed on fish carcasses can directly assimilate Hg in those carcasses, conserving Hg in higher trophic levels as opposed to being redistributed to the benthic microbial community upon carcass decomposition. This conserved Hg can be transferred back to the top of the food chain as these organisms are eaten by top-predator fish, potentially increasing Hg
biomagnification. For the focus of this study, I investigated how the process of crayfish and top-predator fish feeding on one another, referred to here as a ‘feedback cycle, affects Hg biomagnification.

To investigate the impact of crayfish on the biomagnification of Hg in top-predator fish 1) field studies were conducted in Lake Tahoe, a lake where a non-native, invasive crayfish (*Pacifiastacus leniusculus*; signal crayfish) was introduced over 50 years ago but is thought to vary in abundance throughout the lake; 2) laboratory studies were conducted to document the potential feedback cycle between crayfish and a top predator fish (i.e. largemouth bass) and quantify the uptake of MeHg in both of these organisms; and 3) mathematical modeling, parameterized from our laboratory results, was used to predict how such a feedback cycle would affect Hg biomagnification in natural systems. For the field study, I predicted that Hg concentrations in fish, especially those that feed on crayfish, would be higher in areas of Lake Tahoe where crayfish were the most abundant. For the laboratory studies, I predicted that MeHg uptake, as measured by assimilation efficiency, would be higher in largemouth bass fed MeHg-dosed crayfish versus fish fed MeHg-dosed artificial food. Furthermore, I predicted that the uptake of MeHg would be highest for largemouth bass from the feedback cycle (fed MeHg-dosed crayfish that have fed on MeHg-dosed, dead largemouth bass). I also expected that model predictions of steady state MeHg concentrations and biomagnification factors would be highest for the feedback cycle. The results of this study will show whether the uptake of MeHg from an artificial diet is representative of MeHg uptake from a natural food source. Furthermore, this study will provide much needed insight into how food web processes, such as the coupling of benthic and pelagic systems via a benthic omnivore like crayfish, can influence Hg concentrations in top-predator fish.

**Methods**

*Field Collection*

Lake Tahoe is a large, deep, oligotrophic, subalpine lake that is located on the border of California and Nevada (39.1°N, 120.1°W). It was selected as the study site for field studies because a non-native, invasive species of crayfish (signal crayfish; SCF) was introduced to the lake to promote introduced game fish, but believed to be in much greater abundance in the southern tip of Lake Tahoe, known as Tahoe Keys. This area is characterized by intense
shoreline development that has produced warm, eutrophic conditions that provide favorable habitat for SCF. Therefore, a gradient of density was thought to exist with SCF being low or even absent in parts of the northern reaches of the lake, restricted to areas in which Eurasian milfoil (*Myriophyllum spicatum*), and *Chara (Chara delicatula)* are present, while more abundant in the southern portion of the lake. Furthermore, SCF abundance has increased throughout the lake because the shoreline is becoming more eutrophic along with the establishment of the invasive macrophyte, Eurasian milfoil (Reuter & Miller 2000). A gradient of SCF density throughout the lake allows for in-lake comparisons of food web MeHg concentrations among sites with varying SCF abundance.

Six sites, representing a gradient of SCF density, were sampled within Lake Tahoe: Tahoe Keys Marina (TK), Carnelian Bay (CB), and Sand Harbor (SH) are sites know to have high densities of CF, Meek’s Bay (MB) is thought to have a moderate density of SCF, and Sunnyside Marina (SM) and Emerald Bay (EB) are thought to have relatively low densities of SCF (Figure 1). Signal crayfish (n=3 - 5) and minnows (*Rhinichthys osculus*, speckled dace and *Richardsonius egregius*, lahontan redside) were collected from the littoral zone at all sites, and I also collected a composite sample of macrophytes (n=1) and periphyton (n=1). Rainbow trout (*Oncorhynchus mykiss*) were found only in the SH littoral zone. Lake trout (*Salvelinus namaycush*) and kokanee salmon (*Oncorhynchus nerka*) were collected from CB and EB pelagic areas. Largemouth bass (*Micropterus salmoides*), tui chub (*Gila bicolor*), brown bullhead catfish (*Ameiurus nebulosus*), bluegill (*Lepomis macrochirus*), and tahoe sucker (*Catostomus tahoensis*) were found only in the TK littoral zone.

Periphyton was collected by scraping a sample from either rocks or boat docks, using a razor blade, and stored in an acid-washed scintillation vial. Macrophytes were collected by pulling them from lake sediments and were stored in re-sealable, plastic bags. Signal crayfish and minnows were collected with baited minnow traps. Lake trout, rainbow trout and kokanee salmon were collected by hook and line, and all other fish were collected by boat electroshocking. Total length (mm) and wet weight (g) were recorded for each fish collected, with the exception of minnow species for which only wet weight was recorded. Qualitative analysis of gut contents was performed on lake trout and rainbow trout only and major constituents were identified and recorded. Plugs of dorsal muscle (n=4) were taken from all fish except minnows (for which whole body samples were used) for MeHg analysis, using a
disposable, 10mm Acuderm® biopsy punch (Acuderm, Inc., Fort Lauderdale, FL), and put into acid-washed, scintillation vials. All samples were kept on ice until they were returned to the lab (Tahoe Environmental Research Center, Incline Village, NV), where they were stored at -20 °C before being shipped to Miami University where they were lyophilized, homogenized (SCF and minnows only) and stored at -20 °C until analyzed for MeHg (see Methods – MeHg Analysis).

Lab Experiments - Experimental Organisms & System Design

Largemouth bass (LMB) were selected as the top-predator fish species to use in all laboratory experiments because 1) they often co-occur with crayfish in lakes, 2) it has been well documented that LMB feed on crayfish, 3) they are ubiquitous in North America lakes, ponds and rivers, 4) they are the most sought after freshwater game fish in North America (Garcia-Berhou 2002), 5) are a recently introduced, non-native fish in Lake Tahoe (Reuter & Miller 2000), and 6) they are a species that can be reared under laboratory conditions. Juvenile LMB (length: 127 – 178 mm) were purchased from a commercial supplier (Jones Fish Hatchery, Cincinnati, OH).

Crayfish (CF) was chosen as the benthic omnivore because 1) is an important prey item for LMB (Garcia-Berhou 2002), 2) commonly feeds on fish carcasses (Minckley & Craddock 1961), and 3) and can be reared under laboratory conditions. Feeder crayfish (*Procambarus clarkii*; length: 25 - 76 mm) were purchased from Carolina Biological Supply (Burlington, NC). A study by Hothem et al. (2007) found no difference in MeHg concentration between natural populations of signal crayfish (*Pacifiastacus leniusculus*) and red swamp crayfish (*Procambarus clarkii*), therefore *Procambarus clarkii* was considered a good surrogate to use in laboratory studies that could be compared to signal crayfish collected in Lake Tahoe. Largemouth bass and CF were kept in flow-through holding tanks at 16 °C (+/- 2°C) and were maintained on unmanipulated, artificial fish food (extruded catfish feed: 5.5mm; 35-36 % protein, 7-8% moisture; Nelson and Sons, Inc., Murray, UT) until experiments were initiated.

For all experiments, LMB were placed in 54-L flow-through tanks that were divided by perforated, Plexiglas® dividers. Holes (< 5 mm) were large enough to allow for water flow, but too small for food to pass through. The turnover rate for experimental tank water was approximately 1.5 X per day. Tanks were siphoned every other day to remove feces. Crayfish were placed in 2-L, polycarbonate flow-through tanks (Marine Biotech Inc., Beverly, MA). The
turnover rate for experimental tank water was approximately 7 X per day. Tanks were siphoned every other day to remove feces and molts. All tanks received dechlorinated tap water (hardness: ~ 180 mg/L; pH: ~ 7.3) that had a temperature of 16 °C (+/- 2°C), and both LMB and CF were exposed to a 16:8 h light:dark photoperiod, typical of summer light conditions.

Water quality was monitored in both the LMB and CF tanks twice a week for Experiment #1. Dissolved oxygen was maintained at healthy levels (> 5 mg/L) for warm-water fish. Nitrate (NO$_3^-$) and ammonia (NH$_3$) never exceeded 0.80 mg N/L (usually ≤ 0.50 mg N/L) and 0.25 mg N/L (typically ≤ 0.10 mg N/L), respectively. Therefore, NO$_3^-$ and NH$_3$ were only monitored at the beginning and end of Experiments #2 and #3, in which concentrations never exceeded those reported above.

**Experiment #1**

All laboratory experiments were designed to use maintenance diets, in order to minimize growth and reduce the potential effect of growth dilution. Experiment #1 was designed to measure the rate of MeHg uptake and assimilation efficiency (AE) from dosed, artificial food, for both LMB and CF (Figure 2). The results of this experiment were used to determine the optimal length of time to dose LMB and CF with MeHg, from food, for laboratory Experiments #2 and 3.

Artificial diets were prepared from the same artificial fish food described above. Two diets were prepared as follows (adapted from Hammerschmidt et al. 2002): 400 g of pelleted food was placed in a glass pan and soaked in 400 mL of a 95% ethanol solution (control diet) and an additional 400 g of pelleted food was placed in a separate glass pan and soaked in 400 mL of a 5 ppm methylmercury chloride/95% ethanol solution (MeHg diet). Both diets were placed in a fume hood for three days to allow for all of the ethanol to evaporate, after which sub-samples (n=5) were taken from each diet to determine MeHg content. Diets were stored in separate, acid-washed, plastic containers at -20 °C for the duration of Experiments #1 and 2 (< 3 months). Ribeiro et al. (2008) found that concentrations of MeHg in artificial fish food, prepared in the same manner, did not change significantly over a period of approximately three months. Therefore, one batch of each diet was prepared and used for the experiments that required artificial food, Experiments #1 and 2.

Initial wet weights (g) of both LMB and CF were measured with a top-loading balance and by placing organisms in a tared, 2-L tank of dechlorinated tap water to reduce stress.
Largemouth bass (n=30) were then placed in 54-L experimental tanks, with two per tank that were separated by perforated Plexiglas®, and randomly assigned to a treatment. Control LMB (n=15) and MeHg-dosed LMB (n=15) were fed the control diet and the MeHg diet, respectively, every 2 days. Feeding rate was determined as a percentage of body weight (bw), on a daily basis, using the dry weight (dw) of the food and estimated total dry weight of the LMB or CF. Mean daily feeding rate for LMB was 0.89% bw (range: 0.68 - 1.10% bw). Crayfish (n=30) were placed into experimental tanks, 1 CF per tank, and were randomly assigned to a treatment. Control CF (n=15) and MeHg CF (n=15) were fed the control diet and the MeHg diet, respectively, every three days. The mean daily feeding rate for CF, calculated as described above, was 5.74% bw (range: 3.48 - 8.00% bw). In an effort to not compromise the MeHg content in the food, each CF was fed a whole pellet at each feeding, instead of a partial pellet. For many CF, a whole pellet represented a considerable percent of body weight and that is why CF were fed a diet that was much higher and more variable than the diet for LMB.

Initial samples of both LMB (n=3) and CF (n=3) were taken before the start of the experiment (week 0) to determine initial MeHg concentrations. To determine the uptake rate of MeHg from dosed artificial food, LMB (n=5) and CF (n=5) from each treatment were sacrificed at weeks 1, 3, and 6 for MeHg analysis. Largemouth bass were euthanized with an overdose of MS-222 (tricaine methanesulfonate), weighed (g), and measured for total length (mm). Plugs of dorsal muscle (n=4) were taken from all LMB, using a 10mm Acuderm® biopsy punch, and stored in acid-washed, scintillation vials. Frozen plugs were lyophilized and stored at -20 °C until analyzed for MeHg. The remaining portions of LMB carcasses were kept frozen (-20 °C) until used as CF food in experiment #2. CF were weighed (g) and stored at -20 °C, until lyophilized and homogenized whole. Homogenates were then stored at -20 °C until analyzed for MeHg.

The results of experiment #1 were used to determine an optimal time for dosing LMB and CF with MeHg through a more natural food source, CF, in Experiments #2 and 3. This optimal time was determined as the time at which MeHg concentrations in MeHg-dosed LMB and CF were significantly different from that of initial and control LMB and CF (p-value < 0.05).
Experiment #2

This experiment was designed to investigate the AE of MeHg from a natural food source (Figure 3) and to prepare LMB to be used in Experiment #3. For this experiment, two sets of CF were prepared and initial wet weight (g) was recorded for individuals in both sets, using the same methods described for Experiment #1. The first set of CF were prepared as food for MeHg-dosed LMB and were fed either control pellet food (n=60) or MeHg-dosed pellet food (n=60) every three days for three weeks, the optimal time determined from the results of experiment #1. Mean daily feeding rate for CF was 5.69% bw (range: 2.52 - 8.85%). After three weeks, a subset of CF was sacrificed from the control (n=15) and MeHg-dosed (n=15) food treatments to determine MeHg concentration. The rest of the CF (n=45 Control and n=45 MeHg-dosed) were used to feed the LMB in this experiment as described below.

The second set of CF (n=10) was fed muscle tissue from either a control LMB (n=5) or a MeHg-dosed LMB (n=5), every three days for three weeks. This is the same feeding regime used for the CF fed control and MeHg-dosed artificial food. Mean daily feeding rate was 4.17% bw (range: 4.16 - 4.17%). The muscle tissue fed to these CF was excised from partially thawed LMB, using a disposable 5mm Acuderm® biopsy punch and acid-washed, plastic forceps, weighed (g), stored in acid-washed scintillation vials at -20 °C until fed to LMB.

The procedure used to measure initial wet weights of LMB in Experiment #1 appeared to be stressful and appeared to be due to the small size of the water container used. In Experiment #2, and in an effort to reduce stress, initial wet weights were measured with a standard fish scale (oz) and by placing the LMB in a tared, 4-L bucket filled with dechlorinated tap water. Weights were converted to grams using a conversion factor of 28.35 g/oz. The LMB were then placed in experimental tanks similar to those described for Experiment #1. Largemouth bass in Experiment #2 were fed either control CF (n=3) or MeHg CF (n=3). Largemouth bass in Experiment #2 had a difficulty transitioning to live CF food from pellet food, which was fed to them in the holding tanks prior to the experiment. Therefore, LMB were fed until the number of successful feedings was equal to a feeding frequency of every two days for three weeks (total feedings = 11). The mean daily feeding rate was 0.85% bw (range: 0.54 - 1.39%). After 11 successful feedings, LMB were sacrificed, weighed, measured, and muscle plugs were taken for MeHg analysis. The remaining portions of LMB carcasses were kept frozen (-20 °C) until used as CF food in Experiment #3.
Experiment #3

This experiment was designed to investigate the feedback cycle between CF and LMB that was hypothesized to increase MeHg biomagnification in top-predator fish (Figure 4). A large sample size of CF was needed to conduct this experiment, therefore two CF were placed in each tank and separated by a perforated, Plexiglas® divider. The holes (< 5 mm) were big enough to allow for water to flow through, but not food. Crayfish (n=110) were fed muscle tissue from either control LMB (n=55) or MeHg-dosed LMB (n=55) from Experiment #2, every three days for three weeks, with the same feeding methods described for Experiment #2. The mean daily feeding rate was 3.30% bw (range: 2.38 - 4.43%). After three weeks, a subset of CF was sacrificed from the control (n=15) and MeHg-dosed (n=15) food treatments to determine MeHg concentration.

Due to difficulties in transitioning from pellet to live CF food in Experiment #2, LMB in Experiment #3 were conditioned to feed on live CF for two weeks prior to the start of the experiment. After the two-week conditioning period, initial LMB wet weights were measured with the same methods as in Experiment #2. Largemouth bass were fed either control CF (n=5 LMB) or MeHg-dosed CF (n=5 LMB), every two days for three weeks, with a mean daily feeding rate of 0.86% bw (range: 0.84 - 0.88%). After three weeks, all LMB were sacrificed, weighed (g and oz), measured and muscle plugs were taken for MeHg analysis. A regression of final wet weight (ounces) versus final wet weight (g) was used as a more accurate method for converting initial wet weight from ounces to grams, in comparison to a general conversion factor. To determine whole body MeHg concentrations, LMB carcasses were lyophilized, weighed for dry weight (g), homogenized using a Reutsch grinder, and stored at -20 °C until analyzed for MeHg.

MeHg Analysis

Sub-samples (80 - 150 mg) of lyophilized, dorsal muscle (for all fish except minnows) or whole-body homogenates (CF and minnows) were weighed (± 0.1 mg) into 15-mL polypropylene centrifuge tubes. Samples were digested in 7-mL of 4.57 M HNO₃ in a warm water bath (60° C) for 12–14 h (Hammerschmidt & Fitzgerald 2006a). Digestates were analyzed for MeHg with gas chromatographic cold-vapor atomic fluorescence spectroscopy (CVAFS) in the laboratory of
Dr. C. Hammerschmidt, Wright State University (Dayton, OH), following methods detailed in Bloom (1989) & Tseng et al. (2004).

Quality assurance measures were taken to ensure the accuracy of the MeHg determinations. Each batch of samples included analysis of the following: blanks and standards taken through the digestion procedure, a certified reference material from the National Research Council of Canada (TORT-2 lobster hepatopancreas), and analytical and procedural sample replicates. Linear calibration regressions were used to estimate MeHg in samples. Coefficients of determination ($r^2$) were always $\geq 0.995$. Methylmercury standards were calibrated periodically versus Hg$^0$ gas and an inorganic Hg standard traceable to the U.S. National Institute of Science and Technology. All measurements of MeHg in TORT-2 were within the certified range (139 - 165 ng/g) with a mean measured MeHg concentration of 148 ng/g. Analytical sample replicates, in which the digestate of the sample was analyzed in duplicate, were analyzed routinely. The mean relative standard deviation for analytical replicates was 2%. Procedural sample replicates, in which 2 separate sub-samples were digested and analyzed for a particular sample, were also analyzed in each analysis. The mean relative standard deviation of procedural replicates was 4%.

Uptake of MeHg by LMB and CF was calculated as whole-body MeHg burden (ng MeHg, dw). Whole-body MeHg concentration was estimated for each individual LMB based on a comparison of MeHg in dorsal muscle and whole bodies of a sub-set of five LMB. This analysis yielded the following correlation: whole-body MeHg concentration (ng/g, dw) = 0.6254 * dorsal muscle MeHg concentration (ng/g, dw) – 3.5106 ($r^2 = 0.98$). Accordingly, whole-body MeHg burden (ng, dw) was estimated as the product of whole-body MeHg concentration (ng/g, dw) and whole-body dry weight (g, dw). Whole-body dry weight was estimated for LMB based on the following relationship determined from seven individual LMB: whole-body dry weight (g) = 0.3585 * whole-body wet weight (g) – 10.067 ($r^2 = 0.98$). Percent assimilation efficiency of MeHg for LMB was calculated as: whole-body MeHg (ng, dw)/total MeHg fed (ng, dw) * 100.

Whole-body MeHg burden of CF was calculated as the product of whole-body MeHg concentration (measured) and the whole-body dry weight estimated from the following relationship determined from 35 individual CF: whole-body dry weight (g) = 0.2681 * whole-
body wet weight (g) - 0.1799 ($r^2 = 0.90$). Percent assimilation efficiency for CF was calculated using the same methods described for LMB.

**Modeling Steady State MeHg Concentration & Biomagnification Factor (BMF)**

The laboratory experiments in this study were designed to investigate the potential feedback mechanism between fish and CF that may contribute to the large variation in MeHg concentrations found in top-predator fish among aquatic ecosystems. To determine if the feedback mechanism occurs in nature, it would be useful to compare MeHg concentrations in LMB from our laboratory experiments to those of LMB in lakes that contain CF. An issue that makes this comparison difficult is that the duration of experiments conducted in this study (three weeks) was too short for LMB to have achieved a steady state, where the MeHg concentration in the organism remains relatively constant despite continued intake of food that contains MeHg. This is important because MeHg concentrations that are reported for top-predator fish in nature are for adults that are assumed to be at steady state with regard to MeHg uptake. Furthermore, biomagnification factors (BMF) are commonly used to assess the amount of MeHg uptake by top-predator fish, which also requires knowledge of steady state MeHg concentrations. Therefore a modeling approach was used to project steady state MeHg concentrations and BMF values for LMB in this study, using the results from Experiments #1, 2, and 3.

A deterministic model was developed using fixed, mean values for the following parameters measured in each experiment: initial weight (g), growth (g/wk), MeHg food dose/wk (ng/wk), MeHg food concentration (ng/g), and AE (proportion). Growth (i.e. weight gain) of bass over time was modeled as a constant rate of growth, based on growth rates achieved during laboratory Experiments #1 - 3 and was assumed to be indeterminate. Additional assumptions were that all parameters remained constant and there was no elimination of MeHg. In each model iteration (1 iteration = 1 week), the concentration of MeHg in LMB was calculated as:

\[
[MeHg]_{LMB} = \frac{[MeHg]_0 + (D*t)}{W_t + (G*t)}
\]

Where $[MeHg]_{LMB} =$ concentration of MeHg in LMB at time = t, $[Hg]_0 =$ initial concentration of MeHg in LMB, $D =$ dose of MeHg (ng/week) = dose of MeHg * assimilation efficiency, t = time
(weeks), \( W_0 = \) initial weight of LMB, \( G = \) growth (g/week). As \( t \) becomes very large, the initial MeHg concentration and weight for LMB become less important and the equation becomes dominated by dose of MeHg and growth. The concentration of MeHg in LMB converges to the ratio of the weekly dose rate to the weekly growth rate:

\[
\text{Equation 2: } [\text{MeHg}]_{\text{LMBs}} = \frac{D}{G}
\]

To illustrate the bioaccumulation process, STELLA\textsuperscript{®} modeling software (version 9.0.2; isee systems, Lebanon, NH) was used to simulate the increase in MeHg concentrations in fish from the initial experimental conditions to the steady state. The model was run for 5,000 iterations, which provided sufficient simulation time to reach steady state conditions. Biomagnification factors were then determined as:

\[
\text{Equation 3: } BMF = \frac{[\text{MeHg}]_{\text{LMBs}}}{[\text{MeHg}]_{\text{FOOD}}}
\]

Where \([\text{MeHg}]_{\text{LMBs}} = \) steady state MeHg concentration in LMB, \([\text{MeHg}]_{\text{FOOD}} = \) mean MeHg concentration in LMB food.

To determine which model parameter had the greatest influence on the predicted steady-state BMF value, a sensitivity analysis was employed by varying one model parameter (e.g., growth, AE) while holding all other parameters constant and comparing the change in predicted BMF values, as a percentage, between model simulations.

An uncertainty analysis was also performed on the model to determine how steady state MeHg concentrations and BMF values would be affected by incorporating variation in model parameters, which will be referred to here as the stochastic model. Instead of fixed, mean values, a normal distribution of the mean and standard deviation values was used for initial weight (g, \( \text{ww} \)), growth (g/wk, \( \text{ww} \)), MeHg food dose/wk (ng/wk, \( \text{dw} \)), MeHg food concentration (ng/g, \( \text{dw} \)), and AE. For each of these model parameters, a random value was selected from the given distribution in each model iteration. The sample size for determining the mean and standard deviation values for model parameters was too small (\( n \leq 5 \)) to determine how the values were
distributed, therefore the distribution was assumed to be normal. The model was run for 5,000 iterations (steady state conditions were achieved after 3,000 iterations in all cases) and the last 1,000 iterations of the simulation were used to calculate the mean and standard deviation of steady state MeHg concentrations and BMF values.

Statistical Analyses
Significant differences between all treatments were determined by a one-way ANOVA and Tukey’s multiple comparison of means, conducted with the software package R (version 2.9.0 GUI 1.28). Values were considered significantly different at a $p$-value $< 0.05$ and if zero was not included in the upper or lower quantile of the Tukey results.

Results
Field Collection
All samples were collected in July 2008. Signal crayfish were collected at all six sites in Lake Tahoe and relative abundance did not differ among sites. The top-predator fish species that were collected included largemouth bass (LMB) collected only at Tahoe Keys, rainbow trout (RT) collected only at Sand Harbor, and lake trout (LT) collected at Carnelian Bay and Emerald Bay. Summary statistics for size and MeHg content of samples collected from Lake Tahoe are presented in Table 1. The largest fish species collected, both in terms of length and weight, was the lake trout (LT) and the smallest fish species collected was the speckled-dace minnow (SDM). All signal crayfish (SCF), minnows (SDM, LRSM), and top-predator fish had relatively low MeHg concentrations, with the exception of the tui chub (TC; Table 1). The lowest concentration of MeHg was found in speckled-dace minnow (SDM) and the highest concentration was found in the tui chub (TC). Fish size (length or weight) for top-predator fish species was not correlated with MeHg concentration.

Laboratory Diet and Assimilation Efficiency
The mean MeHg concentration of the unmanipulated (i.e. control) and MeHg-dosed artificial food was 20 ng/g, dw and 5100 ng/g, dw respectively. Trace amounts of MeHg in the artificial control diet necessitated that I take initial samples at the start of each experiment to account for differences in starting concentration of MeHg for both CF and LMB. These initial samples
allowed me to account for any temporal biases among experiments. Furthermore, by subtracting the mean initial MeHg concentration (i.e. week 0) from MeHg-dosed samples in each experiment, I was able to more accurately calculate assimilation efficiency (AE), which was used to compare the results both within and among experiments.

Assimilation efficiency was used to 1) better understand how the transfer of MeHg among biological food sources (i.e. CF and fish tissue) compares to artificial food sources (i.e. pelleted fish food), and 2) examine how CF, as an omnivore that can feed on fish carcasses, affects the accumulation of MeHg in top-predator fish, such as the LMB. Due to inherent variation in organism growth, and MeHg food concentration and dose (see Table 2) associated with these types of feeding experiments, AE was used as my response variable because it integrates these variables among experiments and allowed me to compare MeHg uptake between and within experiments for both CF and LMB. However, no comparisons were made between CF and LMB because that was outside the scope of this study and no efforts were made to standardize the daily feeding rate between these two organisms.

**Laboratory Experiment #1**
Mean whole-body burdens of MeHg in LMB fed MeHg-dosed artificial food increased over the course of Experiment #1 (Table 3; Figure 5a). Significant differences were found for all possible comparisons of whole-body burdens of MeHg in LMB among weeks 0, 1, 3, and 6 (p-values < 0.001). While the amount of MeHg accumulated by LMB increased over the course of the experiment, the rate of uptake did not remain constant between sample events. The mean MeHg concentration for LMB in week 1 was 96% greater compared to week 0, 77% greater in week 3 compared to week 1, and only 37% greater in week 6 compared to week 3 (Table 2; Figure 4a).

Mean whole-body burdens of MeHg in CF fed MeHg-dosed artificial food also increased dramatically during Experiment #1 (Table 2; Figure 5b). Similar to the results for LMB, all comparisons of whole-body burdens of MeHg in CF among weeks 0, 1, 3, and 6 were significantly different (p-values <0.001). CF uptake rates also changed over the course of the experiment, similar to LMB; however, the rate of MeHg uptake appeared to approach steady state more rapidly for CF compared to LMB (Figure 4b). The mean MeHg concentration for CF in week 1 was 89% greater than week 0, but only 50% greater in week 3 versus week 1 and week 6 versus week 3 (Figure 4b).
While there was significant uptake of MeHg by week 1 for both LMB and CF, there was higher variability in uptake compared to week 3. Therefore, three weeks was chosen as a more optimal time for dosing organisms in Experiments #2 and 3.

The mean AE for MeHg-dosed LMB was 55% (n=3) for week 1, 60% (n=3) for week 3, and 54% (n=5) for week 6 (Figure 6a). The mean AE for MeHg-dosed CF was 108% (n=3) for week 1, 75% (n=4) for week 3, and 85% (n=4) for week 6 (Figure 6b). There were no significant differences in AE for MeHg, for LMB or CF, among sample events in Experiment #1 (p-value = 0.55, 0.35, respectively).

Laboratory Experiment #2

Initial whole-body burdens of MeHg, in both LMB and CF were not significantly different from whole-body burdens of MeHg in control organisms (p-value = 0.84 for LMB, 0.63 for CF). This suggests that the small amount of MeHg in the control food was negligible.

The mean whole-body MeHg burden for LMB that were fed CF that had been dosed with MeHg via MeHg-dosed artificial food for three weeks was 13200 ng, dw (n=3; Table 2). This was significantly greater than the mean whole-body MeHg burden for control LMB that were fed CF that had been maintained on a control artificial food for three weeks (1480 ng, dw; n=3; p-value = 0.013). The mean AE for MeHg-dosed LMB was 78.7% (n=2; Figure 7a), which was ~18% greater than the AE for LMB fed MeHg-dosed artificial food for three weeks in Experiment #1 (60.24%; Figure 7a). Due to the fact that the sample size was so low for MeHg-dosed LMB in Experiment #2 (n=2), the results for AE could not be statistically compared to the other experiments.

The mean whole-body MeHg burden for CF that were fed tissue from LMB that had been dosed with MeHg via MeHg-dosed artificial food for three weeks was 1,900 ng, dw (Table 2; n=4), which was significantly greater than that of control CF that were fed tissue from LMB that had been maintained on a control artificial food for three weeks on (87 ng, dw; n=4; p-value < 0.001). The mean AE for MeHg-dosed CF was 72% (n=4; Figure 7b), which was not significantly different from the AE for CF fed MeHg-dosed artificial food for three weeks in Experiment #1 (75%; p-value = 0.83; Figure 7b).
Laboratory Experiment #3

Similar to the results of Experiment #2, initial whole-body burdens of MeHg in LMB and CF were not significantly different from whole-body burdens of MeHg in control organisms (P-value = 0.84 for LMB, 0.63 for CF).

Whole-body MeHg concentrations were highly correlated with \( r^2 = 0.98 \) and significantly less (\( p\)-value < 0.001) than dorsal muscle concentrations. The mean whole-body MeHg burden for LMB that were fed CF that had been dosed with MeHg via MeHg-dosed LMB tissue for three weeks was 4,780 ng, dw (n=5; Table 2). This amount was significantly greater than the mean whole-body MeHg burden for control LMB that were fed CF that had been fed control LMB tissue for three weeks (2,040 ng, dw; n=5; \( p\)-value < 0.01; Table 2). The mean AE for MeHg-dosed LMB was 94\% (n=4; Figure 7a), which was ~15\% greater than the AE for LMB fed MeHg-dosed CF for three weeks in Experiment #2 (79\%; Figure 7a). Again, due to the small sample size of MeHg-dosed LMB in Experiment #2, results could not be statistically compared for significant difference. However, AE of LMB in Experiment #3 (94\%) was significantly greater than the AE of LMB fed MeHg-dosed artificial food for three weeks in Experiment #1 (60\%; \( p\)-value = 0.03; Figure 7a).

The mean whole-body MeHg burden for CF that were fed tissue from LMB dosed with MeHg via MeHg-dosed CF for three weeks was 271 ng, dw (n=5; Table 2), which was significantly greater than that of control CF that were fed tissue from LMB that were fed control CF for three weeks (58 ng, dw; n=6; \( p\)-value < 0.001). The mean AE for MeHg-dosed CF (77\%; n=4; Figure 7b) in Experiment #3 was not significantly different from the AE for MeHg-dosed CF in either Experiment #1 or 2 (\( p\)-value = 0.83; Figure 7b).

Modeling Results

Parameters and results for the deterministic model are presented in Table 3. The predicted steady state MeHg concentrations (dry-weight basis) for LMB in Experiments #1, 2, and 3 were as follows: 19200 ng/g, 2880 ng/g, and 3360 ng/g. The predicted BMF values were 3.79, 3.06, and 13.2, for Experiments #1, 2, and 3, respectively (Figure 8). Growth was the model parameter that had the greatest influence on predicted BMF values, followed by AE. Predicted BMF values increased by an average of 202\% when the growth for each experiment was reduced by ~50\% (Table 3 – Simulation 2) and decreased by an average of 52\% when the growth for
each experiment was increased by ~100% (Table 3 – Simulation 3). When AE was increased to 100%, predicted BMF values increased by an average of 133% (Table 3 – Simulation 4) and decreased by an average of 48% when AE was reduced by ~50% (Table 3 – Simulation 5).

The parameters and results for the stochastic model are presented in Table 4. The mean (StDev) predicted steady state MeHg concentrations (dry-weight basis) for LMB in Experiments #1, 2, and 3 were as follows: 19235 (66), 2875 (1.1), and 3389 (2.3) ng/g, respectively. The mean (StDev) predicted steady state BMF values were 2.80 (0.07), 2.05 (1.08), and 3.66 (0.35) for Experiments #1, 2, and 3, respectively. All pair-wise comparisons of predicted BMF values were significantly different (P-value < 0.001).

**Discussion**

*Field Study*

The objective for the field study in Lake Tahoe was to validate the laboratory and model findings of a possible Hg feedback cycle between crayfish and top-predator fish, in a natural system that had a gradient of crayfish abundance. The results of the field study were inconclusive due to 1) the fact that SCF were present at all sites sampled, 2) relative abundance of SCF did not vary among sites, and 3) not all species of top-predator fish were collected at each site. Of those species collected, only a few had the sample size required to address our objective. In addition, MeHg concentrations were relatively similar and low among all fish species collected (including those found in the SCF), with the exception of the tui chub (TC; Table 1).

The fact that SCF abundance did not differ among our sampling sites in Lake Tahoe eliminates the ability to make in-lake, food web comparisons of MeHg concentrations and prevented me from validating my laboratory and model results in a natural ecosystem. Further research is needed in a system or multiple systems where crayfish abundance varies in order to verify our laboratory results that the presence of crayfish results in greater biomagnification of MeHg in top-predator fish.

One interesting result of this study that also deserves further investigation was the relatively high MeHg concentration in the tui chub. Only two samples of tui chub were successfully collected, but of all the fish collected in Lake Tahoe they had the highest concentrations of MeHg. What is more interesting is the fact that the tui chub is one of the few native fish species that still exist in Lake Tahoe. Tui chubs are omnivorous and their diet
includes a wide variety of items, ranging from detritus to benthic invertebrates. They are also slow-growing, long-lived fish that can live up to 30+ years, particularly in large lakes. They can be a source of prey to top-predator fish, such as largemouth bass and trout (Moyle 2002).

Unexpected high mercury concentrations have also been found in another lower trophic level fish in the Long Island Sound, the tautog, which was also attributed to a long life-span similar to that of the tui chub (up to 34 years; Hammerschimdt & Fitzgerald 2006b). The life history traits, coupled with the elevated concentrations of MeHg measured in the samples collected, may make the tui chub a more useful species to study in Lake Tahoe for the potential Hg feedback cycle with top-predator fish.

**Laboratory Experiments**

The main objectives of the laboratory experiments were to 1) document the potential feedback cycle of MeHg between a benthic omnivore, crayfish, and a top predator fish, largemouth bass, and 2) to quantify the uptake of MeHg in both of these organisms, fed either a MeHg-dosed artificial food source (i.e. pelleted fish food) or a MeHg-dosed biologically relevant food source (i.e. CF or fish tissue). The results of my experiments supported my predictions that 1) LMB would assimilate MeHg more efficiently from MeHg-dosed CF (Experiment #2; Figure 3) than from MeHg-dosed artificial food (Experiment #1; Figure 2) and 2) the AE of MeHg would be the greatest for LMB fed MeHg-dosed CF that had fed on MeHg-dosed LMB tissue (Experiment #3; Figure 4).

To my knowledge, there have been no other studies that have measured AE of MeHg in LMB, using either an artificial or natural diet, although similar studies have been conducted with other fish species. For example, Rodgers and Beamish (1982) reported a MeHg AE of 70–80% for rainbow trout fed MeHg-dosed artificial food. The MeHg AE for LMB fed artificial food in our study was slightly less (55–60%; Figure 7a) than those reported by Rodgers and Beamish (1982). This difference in AE could be attributed to the fact that they used an artificial diet that was produced specifically for trout, as opposed to the artificial diet used in this study that was produced for catfish, not LMB. Therefore, the AE of MeHg in LMB fed artificial food may have been greater in this study if a diet closer to the nutritional needs of LMB was used. However, the MeHg AE for LMB fed CF in our study is comparable to MeHg AE’s that have been reported for killifish (90%) and mosquitofish (90-94%) that were fed Me$^{203}$Hg-labeled Daphnia, a natural
prey item for these fish species (Mathews & Fisher 2008; Pickhardt et al. 2006), as well as for sweetlips (95.4%), a marine fish that was fed Me$^{203}$Hg-labeled marine copepods (Wang & Wong 2003).

The significantly greater AE of MeHg from a natural food source, as compared to an artificial food source such as pelleted fish food, is a significant finding because some studies (e.g., Rodgers & Beamish 1982) have used only Hg-dosed artificial fish food in laboratory experiments to measure the rate of Hg uptake and AE for fish, with the assumption that the results are representative of uptake by fish in natural systems. Our findings suggest that the uptake of MeHg from dosed artificial food may under-represent the assimilation of MeHg for fish feeding on common prey items in natural systems. An increased AE of MeHg in LMB feeding on a more natural food source could be due to the fact that CF may meet the nutritional needs of LMB better than artificial food. If this is the case, it may be that CF are easier for LMB to digest, enhancing the assimilation of both nutrients and MeHg. Furthermore, Leaner & Mason (2002) point to digestive processes playing a key role in determining the bioavailability of MeHg from food sources in fish. They found that more MeHg was solubilized in the gastric fluids of channel catfish and Atlantic sturgeon from a more natural food source, bloodworms, in comparison to sediment. They related the enhanced MeHg solubilization from bloodworms to the higher digestibility of tissue, in comparison to sediment. They also found differences in the amount of MeHg solubilized between catfish and sturgeon, which they attribute to species-specific differences in gastric fluid composition. Increased digestibility may also explain the increase in AE for LMB from Experiment #1 to #2 to #3. As the food source for LMB in our study became further removed from the artificial food, the MeHg may have become more complexed with proteins that were easier for LMB to digest, thereby enhancing MeHg uptake (Wiener et al. 2003).

Finding the highest AE of MeHg was for LMB in Experiment #3 (94%; Figure 7a), via the feedback cycle between crayfish and largemouth bass, suggests that as MeHg continues to cycle through biological organisms it becomes more bioavailable. As this cycle continues, increasingly more MeHg is conserved in the upper trophic levels of the food web. This supports the idea that omnivores like crayfish can play a critical role in the high concentrations of MeHg found in natural populations of top-predator fish. This cycle may not be limited to just crayfish and largemouth bass, as there are other benthic omnivores such as leeches, amphipods, and crabs.
that also feed on fish carcasses (Sarica et al. 2005; Ramsay et al. 1997; Dahl 1979) and are prey for other top-predator fish such as pike, smallmouth bass, and sharks (Venturelli & Tonn 2005; Clady 1974; Maia et al. 2006). However in freshwater systems, crayfish may play more of a key role in generating a Hg feedback cycle with top-predator fish as a result of growing larger and living longer (Momot et al. 1978) than leeches and amphipods, and it has been documented that crayfish can accumulate high levels of Hg (Hothem et al. 2007; Parkman & Meili 1993; Mueller & Serdar 2002). This feedback cycle could also extend to terrestrial communities when animals such as raccoons and birds feed on crayfish (Wiener 2003). However, in systems that lack benthic organisms that could generate this feedback cycle, MeHg in fish carcasses would be redistributed in the benthos through decomposition by microbial communities and other microinvertebrates (Wiener et al. 2003), where it would be available for uptake at the base of the food web. Theoretically, this would lead to decreased Hg biomagnification compared to systems where the feedback cycle is possible.

Model Predictions
To better understand how our laboratory results would represent MeHg concentrations for top-predator fish in natural systems, we developed a model to project steady state MeHg concentrations and BMF values. Model projections supported our prediction that Hg biomagnification would be greatest in the presence of a feedback cycle between crayfish and top-predator fish. This was evident in the predicted deterministic BMF value for Experiment #3 (BMF=13.2), where LMB were fed CF that were fed tissue from LMB, compared to Experiments #1 (BMF=3.79) and #2 (BMF=3.06) (Table 3). The stochastic model also predicted the highest BMF values for Experiment #3 (BMF=3.66). The magnitude of difference between Experiments #1 (BMF=2.80) and 2 (BMF=2.05) was much smaller compared to the deterministic model (Table 4), but the differences were still significant. The change in the predicted BMF values between the deterministic and stochastic model was most prominent for Experiment #3 (13.2 versus 3.66, respectively) and was likely due to the incorporation of variation in the growth parameter. Even though Experiment #3 had the lowest measured growth, it also had the largest variation in measured growth. The results of the sensitivity analysis also support this idea, as growth was the model parameter that had the largest influence on the predicted steady state BMF value. The variation in growth rate within and among experiments
was most likely due to the result of complications in obtaining accurate initial wet weights on LMB prior to the start of experiments. Another explanation for differences in growth rates among experiments may be related to differences in diet composition. The amount of food that was fed to LMB, as a percentage of body weight, was similar across experiments (0.86 – 0.89%) and kept low to prevent considerable growth. While the caloric content was not measured for any of the food sources, it can be speculated that if the caloric content of the artificial food was higher than live crayfish, it could explain why growth was the highest in Experiment #1. Similar to the idea of caloric content, the nutritional quality of food has been found to produce growth dilution of Hg in zooplankton. Daphnia that were fed high nutrient algae grew faster (~ 3.5x) and had lower body burdens of MeHg (~ ½x) than Daphnia that were fed low nutrient algae (Karimi et al. 2007).

For comparing the results of model predictions to natural systems, the predicted BMF value from the stochastic model is the most appropriate value to use because it incorporates variation in growth, MeHg food dose and concentration, and assimilation efficiency, providing a more representative simulation of these factors in naturals systems. The predicted BMF value for LMB from the feedback cycle (3.66; Table 4) is comparable to BMF values published for LMB in natural systems, 3.5 – 7.1 (Grieb et al. 1990; Suchanek et al. 1993; U.S. EPA 1997), and it is also similar to the national average BMF value for piscivorous fish of 4.9 (U.S. EPA 1997). Biomagnification factor values as high 14 have been reported for walleye and pike feeding on yellow perch and spottail shiners (Jackson 1991; U.S. EPA 1997), suggesting that Hg biomagnification can be as high as that predicted for the feedback cycle in the deterministic model (13.2; Table 3). Most of the published BMF values for LMB, as well as most top-predator fish, are calculated as the concentration of Hg in LMB versus the concentration of Hg in prey fish species. To my knowledge there are no published BMF values that compare Hg concentrations in LMB, or any other top-predator fish, to concentrations in benthic omnivores such as crayfish. However, a study by Allard and Stokes (1989) found a significant and positive correlation between Hg concentrations in smallmouth bass and crayfish. This suggests that crayfish should be considered as a key prey species when calculating Hg BMF values for top-predator fish and it gives further support to the idea that crayfish play an important role in influencing Hg concentrations in top-predator fish.
In conclusion, the results of my laboratory studies and modeling projections provide support to the ideas that feedback cycles between benthic omnivores, in particular crayfish, and top-predator fish can occur and can lead to an increase in Hg biomagnification by conserving Hg in higher trophic levels; therefore, feedback cycles are a very plausible mechanism that can contribute to the variation in Hg concentrations of top-predator fish among aquatic ecosystems. Much attention has been given to the process of omnivory, in terms of how it affects the transfer of energy and nutrients in aquatics systems, but less attention has been given to how it affects the movement of contaminants such as Hg, especially in benthic communities. The results of this research support the need for further investigation to how benthic omnivores can affect the biomagnification of Hg in top-predator fish in natural systems.
References


Bloom, N.S. 1989. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. Canadian Journal of Fisheries and Aquatic Sciences 46(7):1131-1140.


Table 1. Summary statistics for fish species collected from Lake Tahoe. Species ID: crayfish (CF), speckled dace minnow (SDM), lahontan red-sided minnow (LRSM), brown bullhead catfish (BBC), taho sucker (TS), tui chub (TC), bluegill (BG), largemouth bass (LMB), kokanee salmon (KS), lake trout (LT), rainbow trout (RT).

<table>
<thead>
<tr>
<th>Species ID</th>
<th>n</th>
<th>Length (mm)</th>
<th>Wet wt (g)</th>
<th>MeHg (ng/g, dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDM</td>
<td>3</td>
<td>ND</td>
<td>3.3 (2.1 - 4.6)</td>
<td>47 (29 - 68)</td>
</tr>
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<td>3</td>
<td>266 (260 - 320)</td>
<td>ND</td>
<td>71 (54 - 85)</td>
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<tr>
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<td>13</td>
<td>47 (37 - 54)</td>
<td>32 (11 - 95)</td>
<td>96 (14 - 238)</td>
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<td>3</td>
<td>ND</td>
<td>5.1 (3.8 - 4.8)</td>
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<tr>
<td>RT</td>
<td>2</td>
<td>377 (300 - 455)</td>
<td>539 (198 - 879)</td>
<td>175 (147 - 202)</td>
</tr>
<tr>
<td>LT</td>
<td>6</td>
<td>535 (476 - 635)</td>
<td>1309 (709 - 2126)</td>
<td>218 (161 - 321)</td>
</tr>
<tr>
<td>BBC</td>
<td>4</td>
<td>212 (185 - 241)</td>
<td>78 (102 - 206)</td>
<td>236 (218 - 261)</td>
</tr>
<tr>
<td>LMB</td>
<td>4</td>
<td>170 (147 - 224)</td>
<td>78 (37 - 183)</td>
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</tr>
<tr>
<td>TS</td>
<td>3</td>
<td>185 (173 - 206)</td>
<td>73 (59 - 100)</td>
<td>355 (249 - 529)</td>
</tr>
<tr>
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<td>4</td>
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<td>78 (67 - 94)</td>
<td>408 (273 - 537)</td>
</tr>
<tr>
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<td>2</td>
<td>241 (216 - 267)</td>
<td>152 (100 - 205)</td>
<td>1370 (972 - 1760)</td>
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</table>

ND = not determined
Table 2. Mean values, with ranges in parentheses, for measurements of length, weight, and whole-body MeHg burdens for largemouth bass (LMB) and crayfish (CF) in Experiments #1, 2, and 3.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Species</th>
<th>Sample Period (wks)</th>
<th>Initial Weight (g, ww)</th>
<th>Final Weight (g, ww)</th>
<th>Growth (g/wk, ww)</th>
<th>Final MeHg burden (ng, dw)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>LMB</td>
<td>1</td>
<td>110 (96 - 124)</td>
<td>120 (110 - 130)</td>
<td>8.5 (-3.2 - 13.6)</td>
<td>9860 (7550 - 12000)</td>
</tr>
<tr>
<td>1</td>
<td>LMB</td>
<td>3</td>
<td>99 (86 - 112)</td>
<td>110 (96 - 120)</td>
<td>2.7 (1.3 - 3.3)</td>
<td>41400 (40100 - 46100)</td>
</tr>
<tr>
<td>1</td>
<td>LMB</td>
<td>6</td>
<td>99 (79 - 128)</td>
<td>110 (82 - 140)</td>
<td>1.8 (0.55 - 2.3)</td>
<td>67800 (63900 - 71100)</td>
</tr>
<tr>
<td>1</td>
<td>CF</td>
<td>1</td>
<td>6.1 (4.2 - 6.9)</td>
<td>7.4 (6.6 - 8.4)</td>
<td>1.3 (0.40 - 2.4)</td>
<td>1490 (1140 - 1830)</td>
</tr>
<tr>
<td>1</td>
<td>CF</td>
<td>3</td>
<td>5.1 (3.0 - 6.7)</td>
<td>6.8 (3.9 - 9.5)</td>
<td>0.59 (0.28 - 0.93)</td>
<td>2800 (1990 - 3400)</td>
</tr>
<tr>
<td>1</td>
<td>CF</td>
<td>6</td>
<td>6.0 (5.6 - 6.3)</td>
<td>9.8 (8.1 - 13)</td>
<td>0.64 (0.39 - 1.1)</td>
<td>6150 (5680 - 7240)</td>
</tr>
<tr>
<td>2</td>
<td>LMB</td>
<td>3(^a)</td>
<td>113.39(^b)</td>
<td>130 (120 - 150)</td>
<td>1.6 (0.24 - 3.5)</td>
<td>13200 (9160 - 18400)</td>
</tr>
<tr>
<td>2</td>
<td>CF</td>
<td>3</td>
<td>5.1 (4.8 - 5.4)</td>
<td>6.1 (5.0 - 7.9)</td>
<td>0.35 (-0.09 - 0.94)</td>
<td>1900 (1630 - 2340)</td>
</tr>
<tr>
<td>3</td>
<td>LMB</td>
<td>3</td>
<td>190 (170 - 190)</td>
<td>190 (130 - 220)</td>
<td>0.71 (-13 - 9.0)</td>
<td>4780 (4100 - 6030)</td>
</tr>
<tr>
<td>3</td>
<td>CF</td>
<td>3</td>
<td>4.2 (3.5 - 5.8)</td>
<td>4.8 (4.0 - 7.1)</td>
<td>0.19 (-0.16 - 0.43)</td>
<td>271 (203 - 326)</td>
</tr>
</tbody>
</table>

\(^a\) actual sample period was between 5 - 8 weeks, with an average of 6 weeks

\(^b\) all LMB initial weights were the same, in ounces, so there was no range for initial weights when converted to grams
Table 3. Parameters for the deterministic model used to predict steady state MeHg (SS MeHg) concentrations and biomagnification factors (BMFs) for largemouth bass (LMB). Definitions of model parameters: growth rate (g/week, ww); AE = assimilation efficiency (%); food dose = MeHg food dose (ng/week, dw); food conc. = concentration of MeHg in food (ng/g, dw). Simulation 1 was run using fixed, mean values for model parameters measured in each experiment. Simulations 2 - 5 represent the parameters and results of the sensitivity analysis conducted on the deterministic model.

<table>
<thead>
<tr>
<th>Simulation</th>
<th>Experiment</th>
<th>Growth (g/wk)</th>
<th>AE (%)</th>
<th>Food Dose (ng/wk)</th>
<th>Food Conc. (ng/g)</th>
<th>SS MeHg (ng/g)</th>
<th>BMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>2.02</td>
<td>60.24</td>
<td>23261</td>
<td>5080</td>
<td>19235.48</td>
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<td>1.58</td>
<td>78.76</td>
<td>2037</td>
<td>940</td>
<td>2875.11</td>
<td>3.06</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.71</td>
<td>93.77</td>
<td>940</td>
<td>256</td>
<td>3389.02</td>
<td>13.24</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1.00</td>
<td>60.24</td>
<td>23261</td>
<td>5080</td>
<td>38532.5</td>
<td>7.59</td>
</tr>
<tr>
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<td>0.80</td>
<td>78.76</td>
<td>2037</td>
<td>940</td>
<td>5921.63</td>
<td>6.30</td>
</tr>
<tr>
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<td>0.35</td>
<td>93.77</td>
<td>940</td>
<td>256</td>
<td>6806.13</td>
<td>26.59</td>
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<tr>
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<tr>
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<td>93.77</td>
<td>940</td>
<td>256</td>
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<td>6.62</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
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<td>100.00</td>
<td>23261</td>
<td>5080</td>
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</tr>
<tr>
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<td>100.00</td>
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<td>3.86</td>
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<tr>
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<td>940</td>
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<td>45.00</td>
<td>940</td>
<td>256</td>
<td>1664.35</td>
<td>6.50</td>
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</tbody>
</table>
Table 4. Parameters and results of uncertainty analysis Definitions of model parameters: growth = LMB growth rate (g/week, ww); AE = assimilation efficiency (proportion); food dose = MeHg food dose (ng/week, dw); food conc. = concentration of MeHg. Analysis was conducted using a normal distribution of the mean and standard deviation values (presented in parentheses) for model parameters measured in each experiment.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Growth (g/wk)</th>
<th>AE (%)</th>
<th>Food Dose (ng/wk)</th>
<th>Food Conc. (ng/g)</th>
<th>SS MeHg (ng/g)</th>
<th>BMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.02 (0.90)</td>
<td>60.24 (3.40)</td>
<td>23261 (1652)</td>
<td>5080 (130)</td>
<td>19235.48 (66.12)</td>
<td>2.80 (0.07)</td>
</tr>
<tr>
<td>2</td>
<td>1.58 (1.68)</td>
<td>78.76 (0.50)</td>
<td>2037 (1118)</td>
<td>940 (331)</td>
<td>2875.11 (6.72)</td>
<td>2.05 (1.08)</td>
</tr>
<tr>
<td>3</td>
<td>0.71 (6.21)</td>
<td>93.77 (16.98)</td>
<td>940 (55)</td>
<td>256 (24)</td>
<td>3389.02 (2.33)</td>
<td>3.66 (0.35)</td>
</tr>
</tbody>
</table>
Figure 1. Map illustrating sites sampled at Lake Tahoe and the distribution of signal crayfish (SCF) at each site.
Figure 2. Diagram tracing the food source for largemouth bass (LMB) and crayfish (CF) in Experiment #1.
Figure 3. Diagram tracing the food sources for largemouth bass (LMB) and crayfish (CF) in Experiment #2.
Figure 4. Diagram tracing the food sources for largemouth bass (LMB) and crayfish (CF) in Experiment #3 (feedback cycle).
(a) Largemouth bass (LMB)

(b) Crayfish (CF)

Figure 5. Whole-body MeHg burden (dry weight (dw)) for (a) LMB and (b) CF in week 1, 3 and 6 of experiment #1. Data points are jittered for visual clarity.
Figure 6. The mean assimilation efficiency (AE) for (a) LMB and (b) CF in week 1, 3 and 6 of experiment #1. Bars represent standard deviation.
Figure 7. The mean assimilation efficiency (AE) for (a) LMB and (b) CF in experiments #1, 2 and 3. Bars represent standard deviation.
Figure 8. Deterministic model projections of biomagnification factor (BMF) values for Experiments #1 – 3.