Bioaccumulation of mercury (Hg) in the brain, muscle, and liver tissues of inshore pompano and offshore amberjack from the Gulf of Mexico

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Abstract

Mercury is a toxic global pollutant that poses a health threat to many organisms including fish and animals that eat fish. In this study, we examined the effects of diet and the trophic position of prey on the bioaccumulation of mercury (Hg) in fish. We also determined the distribution of Hg in various fish tissues and the change in Hg accumulation in these tissues over time. Over the course of 80 days, fish were raised in captivity and fed a diet of shrimp or tuna. At five separate time periods, a subset of the fish were sacrificed and dissected for the collection of liver, muscle, and brain tissue, and mercury concentrations were measured in these tissues. Species variations in Hg bioaccumulation were observed by comparing the results from two different species of the *Carangidae* family from different home ranges: *Trachinotus carolinus* (Florida pompano), *and Seriola dumerili* (greater amberjack). In both species we observed changes in body mass and tissue mercury concentration during the feeding experiments. The mercury concentrations in shrimp-fed pompano increased by 1.46, 1.33, and 0.31 fold in the brain, muscle, and liver tissues, respectively, and 5.29, 2.43, and 3.09 fold in shrimp-fed amberjack in these same tissues. The mercury concentrations in tuna-fed pompano increased by 46.5, 25.4, and 94.9 fold in the brain, muscle, and liver tissues, respectively, and 306, 41.9, and 52.1 fold in these tissues of tuna-fed amberjack,. Species, diet, and exposure time were found to play important roles in mercury bioaccumulation, distribution, and excretion over time.

Introduction

Mercury (Hg) is a trace element released by anthropogenic and natural processes. As a hazardous toxic global pollutant, it serves no biological function and poses a threat upon exposure to many organisms. Hg in its inorganic form undergoes a process of methylation in wetlands and sediments by sulfur (S) and iron (Fe) reducing bacteria (Choi and Bartha 1994) and in the pelagic water column (Sunderland et al. 2009). Methylmercury (MeHg) is a common organometallic compound of Hg and its most toxic form. The major source of organic Hg for uptake by organisms is food, while minor quantities are acquired from air, precipitation, sediments, and suspended particulate matter (Lindqvist et al. 1991).

As the consumption of marine fish is the primary cause of human exposure to Hg (Chen et al. 2008), greater knowledge of mercury bioaccumulation is of importance. Furthermore, marine species are also at risk due to the toxicity of Hg and its capacity for methylation followed by biomagnification in the food web (LaPorte et al. 1997). Specifically, MeHg is of greatest concern due to the threats it poses as a potent neurotoxin (Chen et al. 2008), as it can cross the blood-brain barrier (Boening 1999). Thus, to better understand human and environmental health risks, further research is needed on the dynamics of mercury in fishes and their environment.

Due to its nonpolar complexes, Hg differs from other metals in its accumulation and elimination patterns (Mason et al. 1996), and MeHg is bioaccumulated differently among fish species (Chen et al. 2008). The concentration of Hg in a fish depends on the balance between mercury uptake from food and water and mercury elimination from fish tissues. Generally, there is a higher assimilation efficiency of organic MeHg than inorganic Hg from food and a lower elimination rate of MeHg from the body (Trudel and Rasmussen 1997). Inorganic Hg is membrane bound and MeHg accumulates in cytoplasm, entering the base of the food web via phytoplankton and bioaccumulating at all trophic levels, including in fish (Mason et al. 1995). Bioaccumulation refers to the uptake of bioavailable Hg to organisms and biomagnification refers to the transfer of MeHg to a higher trophic level (Cabana and Rasmussen 1994). MeHg concentration in fish can be estimated based on their trophic position. In previous studies using stable nitrogen isotopes to estimate the trophic position, it was suggested that trophic position determines the extent of MeHg accumulation in the food web, and MeHg concentration increases with trophic position in the food chain. Additionally, top predatory species have displayed elevated mercury concentrations especially among the largest individuals (Campbell et al. 2008).

Once mercury is assimilated by fish, the contaminant is then distributed throughout the organism via the blood and stored in various tissues. Thus, blood plays a fundamental role in the bioaccumulation process since it provides the connection between storage organs such as the muscle and excretion organs such as the kidney. Additionally, transfers between "donor" and "receiver" organs take place during excretion, suggesting the possibility of varying concentrations of MeHg in specific organs over time (Boudou and Ribeyre 1983). Previous studies have shown MeHg accumulation in muscle tissue accounting for more than 90% of total Hg (Bloom et al. 1992; Senn et al. 2009). Giblin and Massaro (1975) observed that the blood had the greatest concentration of MeHg among all other tissues. It has been speculated that this is due to the fact that during detoxification, not all mercury can be excreted immediately, and much of it is recycled throughout the tissues and retained for long periods of time. Additionally, the liver is also an important site for contaminant storage, redistribution, detoxification, or transformation (Evans et al. 1993).

Previous studies determined few significant effects of pH or salinity on bioaccumulation (Wang and Wang 2010; LaPorte et al. 1997), while contamination route and temperature have been considered to have significant influences on bioaccumulation (Boudou and Ribeyre 1983). This is because higher temperatures lead to higher assimilation efficiency and thus require more feeding. Therefore, assuming all fish are kept under similar environmental conditions (salinity, pH, temperature), the largest species with the diet highest in Hg content should display the greatest MeHg concentration in muscle tissues and blood.

This study is distinct from previous studies concerning Hg concentrations that have mostly focused on freshwater fishes (Campbell et al. 2008; Simon and Boudou 2001). In addition, this study made use of natural food sources caught from the same habitats as the test species rather than commercial food pellets spiked with varying levels of Hg concentration (Trudel and Rasmussen 1997; Simon and Boudou 2001). The results of this study provide information on the extent of bioaccumulation in different tissues and the physiological distribution of Hg within the fish. This allows for a better understanding and ability to predict the effects of Hg exposure to organisms in a given ecosystem.

The goal of this experiment was to examine the effects of a fish's diet on bioaccumulation of Hg as it pertains to the trophic position of prey. This study also sought to determine the distribution of Hg in fish tissues and the change in Hg accumulation over time. Differences in the Hg concentrations due to diet, exposure time, and tissue sample were examined. In addition, species variations in Hg bioaccumulation were observed by comparing the results from two different species of the *Carangidae* ("jack") family, from different home ranges: *Trachinotus carolinus* (Florida pompano), *and Seriola dumerili* (greater amberjack). While both species used for this study are from the same family, *Carangidae*, their preferred habitats and diets vary. Pompano from the Gulf of Mexico surf zone grow to about 0.5 kg grams at maturity (Figure 1). Pompano are bottom-feeders, which feed mainly on small crustaceans such as shrimp and clams. Conversely, amberjack are found offshore at depths of up to 300 feet. At maturity, greater amberjack commonly weigh 10–23 kg but can reach up to 77 kg (figure 2). Amberjack are aggressive predators and eat a variety of fish, including small tuna (Rigs, Reefs, and Wrecks Inc. 2011). Both species are valued commercially as seafood.

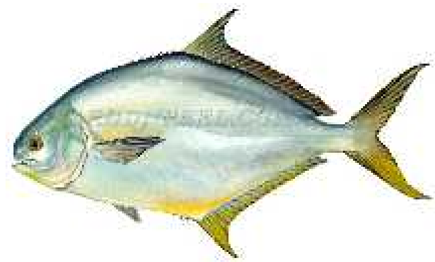

Figure 1. Florida pompano illustration (Duane Raver, rodnreel.com 2011). Pompano are bottom-feeders found in the Gulf of Mexico surf zone grow up to 500 grams at maturity (Rigs, Reefs, and Wrecks Inc. 2011).

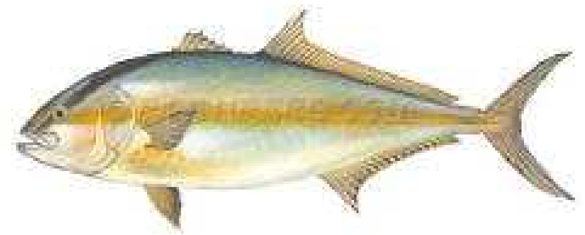

Figure 2. Greater amberjack illustration (Duane Raver, rodnreel.com 2011). This offshore species can reach 10–23 kg at maturity. These aggressive predators eat a variety of fish, including small tuna (Rigs, Reefs, and Wrecks Inc. 2011).

Methods

Four recirculating tank systems with biofilters and aeration were used to rear two species of juvenile fish (Figure 3, Figure 4, Figure 5). Two tanks of pompano and two tanks of amberjack each contained 25 fish. This sample size was established to account for the number of individuals needed for the study and allowed for some mortality. Pompano were collected by seining at Holly Beach in Cameron Parish, LA. Amberjacks were caught using a sabike rig around offshore weed lines and oil platforms in the Gulf of Mexico.

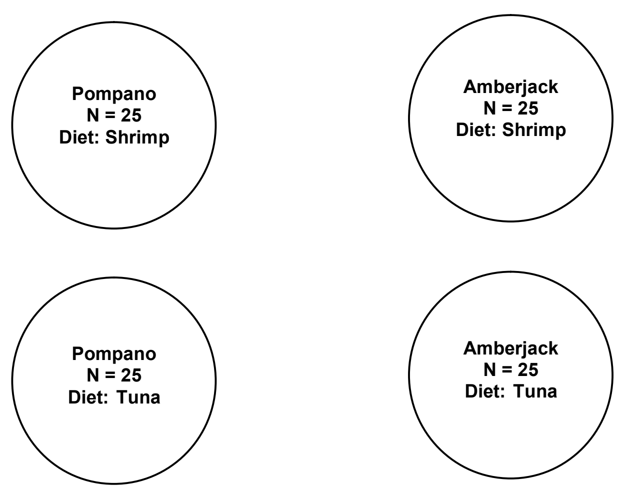

Figure 3. Experimental design. Four tanks with recirculating biofilters maintained a sample size of 25 fish, established to account for the individuals needed for the study and allow for some natural mortality. Tanks were kept to the appropriate temperature, pH, and salinity based on the conditions under which each species was caught and kept in the lab.

Figure 4. Pompano tanks

Figure 5. Amberjack tanks

Tanks were maintained within an appropriate range of temperature (22°C – 30°C), pH (8.0 – 8.8), and salinity (23 ppt – 30 ppt). One tank of each species was fed shrimp caught in the bayous of Terrebonne Parish, LA, within five miles of the Louisiana University Marine Consortium marine center (figure 6). The other tank of each species was fed tuna. Pompano were fed yellow fin tuna caught around the Brutus Oil Rig, Green Canyon Block in the Gulf of Mexico (27' 47.4286" N, 90' 38.5115" W). Amberjack were fed black fin tuna caught at the same location (figure 7). Shrimp were peeled and tuna were filleted. Food was cut into small pieces, frozen, and thawed before feedings. Fish were fed approximately 3% to 5% of body mass twice a day to apparent satiation. Consumption was weighed and recorded. Uneaten food on the bottom of the tanks was removed to avoid contamination to the water.

Figure 6. Wharf-mounted popier used for catching shrimp in the bayous (Donald W. Davis Slide Collection 1976).

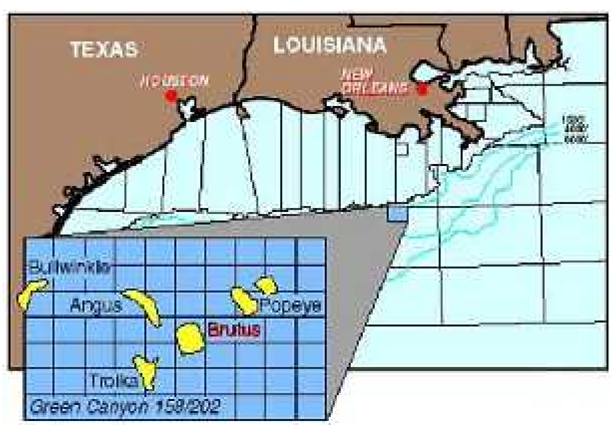

Figure 7. Brutus Oil Rig, located 165 miles southwest of New Orleans in the Gulf of Mexico (Offshore-technology.com 2011)

Water quality testing was conducted daily to ensure maintenance of the appropriate temperature, salinity, and pH. At day 0, five fish of each species were sacrificed as wild “control” samples. After time periods of 10, 30, 50, and 80 days of feeding, fish from each tank were sacrificed. Due to mortalities, varying numbers of fish were sacrificed at each time period (table 1).

Table 1. Experimental Design

Food Source (Treatment)	Species	Day 0	Day 10	Day 30	Day 50	Day 80	Total (# fish per diet)
Inshore shrimp	Juvenile Amberjack	5 Total	5	5	3	5	18
Offshore BF tuna	Juvenile Amberjack		5	5	3	0	13
Inshore shrimp	Juvenile Pompano	6 Total	5	5	4	10	24
Offshore YF tuna	Juvenile Pompano		5	5	3	0	13

Sacrificed fish were measured for total length (mm) and weight (g). Fish were anesthetized with 0.4 mL clove oil / liter of water to deeply anesthetize them but not kill them. The caudal fin was removed for blood collection and the fish euthanized. Fish were dissected for collection of the muscle, kidney, liver, and brain (figure 8). To avoid cross contamination, tools were cleaned with alcohol and distilled water before the removal of each tissue. Each organ or tissue was carefully weighed, placed in a whirlpack or glass vial and frozen in a –18°C freezer**.** Samples of the shrimp and tuna feed were also set aside for analysis.

Figure 8. Example of pompano dissection.

Frozen samples were placed on dry ice and delivered to the University of Michigan, Ann Arbor for mercury analyses. Samples of blood and kidney were archived in a freezer. Muscle, brain, and liver tissue samples were freeze-dried and homogenized, and composite samples were analyzed for total Hg concentrations (figure 9). Briefly, dried samples were combusted at 800^{o}C and total Hg was quantified by a Nippon Instruments MA-2000 Hg analyzer. Calibration was obtained by a standard solution of NIST SRM 3133 and was checked every three sample runs. The values were always within 5% of certified values. Two standard reference materials, ERM CE 464 (average measured THg = 4.71 µg/g, *n*=7) and NRCC DORM-2 (4.16 µg/g, *n*=7), were combusted along with samples, which agreed within ± 10% of the certified values.

Figure 9. Samples were homogenized by hand using a mortar and pestle.

Results

(See Appendix for data tables)

[Hg] in food

The mercury concentrations of each food source given to pompano and amberjack were as follows: shrimp = 28.6 ng/g, yellowfin tuna = 839 ng/g, blackfin tuna = 2670 ng/g (Figure 10).

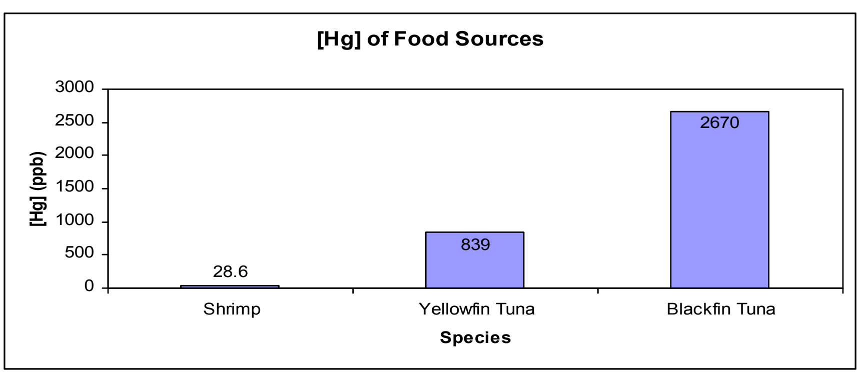

Figure 10. Mercury concentration (ppb) is highest in blackfin tuna, followed by yellowfin tuna, and shrimp.

Body mass and growth rate

The average body mass of all trials initially increased with feeding. Both species when fed with shrimp displayed slow growth from 0-10 days followed by more rapid and nearly linear growth in average body mass from day 10 to 80. The average body mass of tuna-fed amberjack increased from day 0 to day 10, decreased from day 10 to day 30, and increased again from day 30 and day 50. The average body mass of tuna-fed pompano decreased after day 10 (Figure 11, Figure 12).

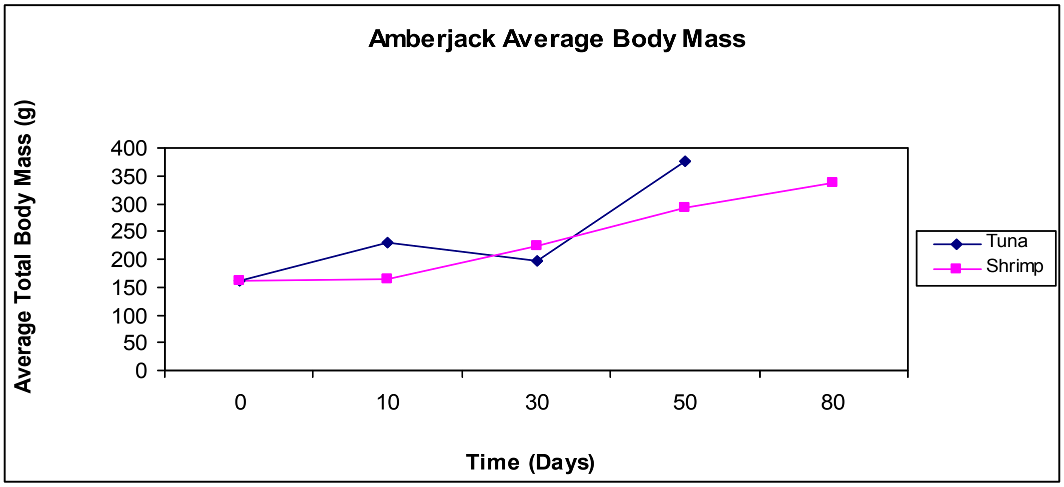

Figure 11. Variation in average wet weight of amberjack over time after the diet was switched to tuna or shrimp

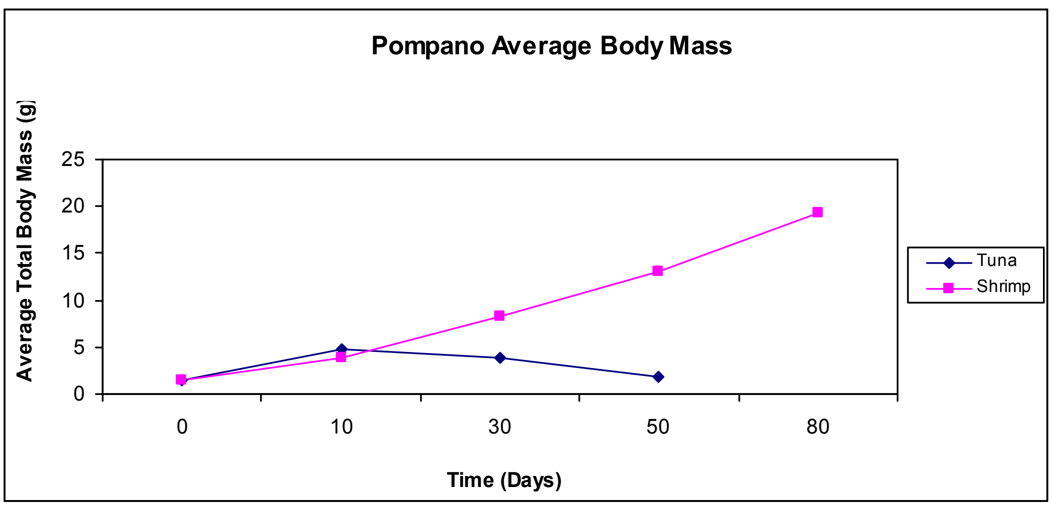

Figure 12. Variation in average wet weight of amberjack over time after the diet was switched to tuna or shrimp

For each trial, the percent body mass increase during the experiment was calculated. The percent body mass increase is equal to $[(W_f-W_i)/W_i]*100$ where W_f is the final weight of the fish and W_i is the initial weight of the fish: 1183% for shrimp-fed pompano, 25.7% for tuna-fed pompano, 108% for shrimp-fed amberjack, and 132% for tuna-fed amberjack.

The specific growth rate (G) was calculated using the equation ($G = [\ln(W_{50})-\ln(W_0)]/50$) where W_{50} is the weight of the fish at day 50 since all trials survived to at least this time period, and W_0 is the weight of the fish at day 0. All trials displayed increasing growth rates from day 0 to day 50. Specific growth rate measured from day 0 to day 50 was highest among shrimp-fed pompano (4.32 g/day), followed by tuna-fed amberjack (1.68 g/day), shrimp-fed amberjack (1.17 g/day), and tuna-fed pompano (0.46 g/day).

Mass and growth of individual organs

Values above weighing measurement uncertainty were averaged. In shrimp-fed amberjack the average weights of all organs displayed an increase followed by a plateau (Figure 13). Among tuna-fed amberjack, the average weight of the muscle paralleled that of the body mass, and the liver and brain showed minimal growth (Figure 14). For shrimp-fed pompano, the average weight of all organs increased, especially the muscle, which paralleled that of the total average body mass (Figure 15). Among the tuna-fed pompano, mass of the brain and muscle decreased after day 10, similar to that of the total average body mass; the liver did not lose mass until after day 30 (Figure 16).

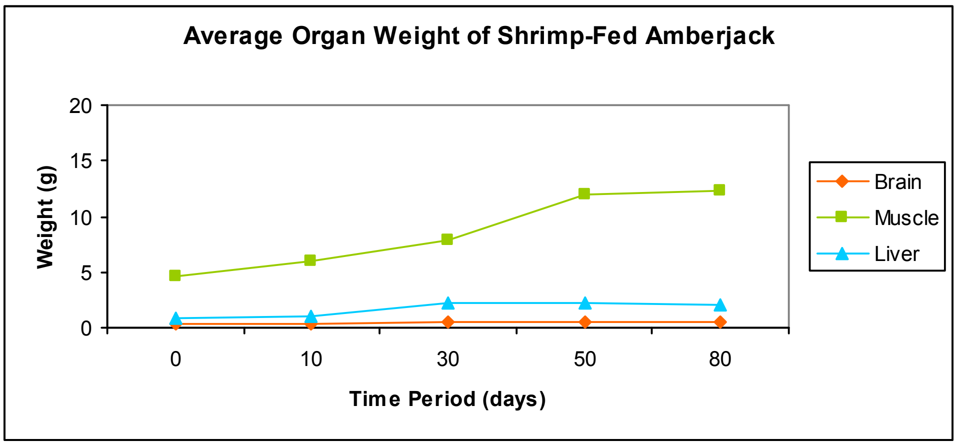

Figure 13. The average weights of the organs of shrimp-fed amberjack vary with time and growth.

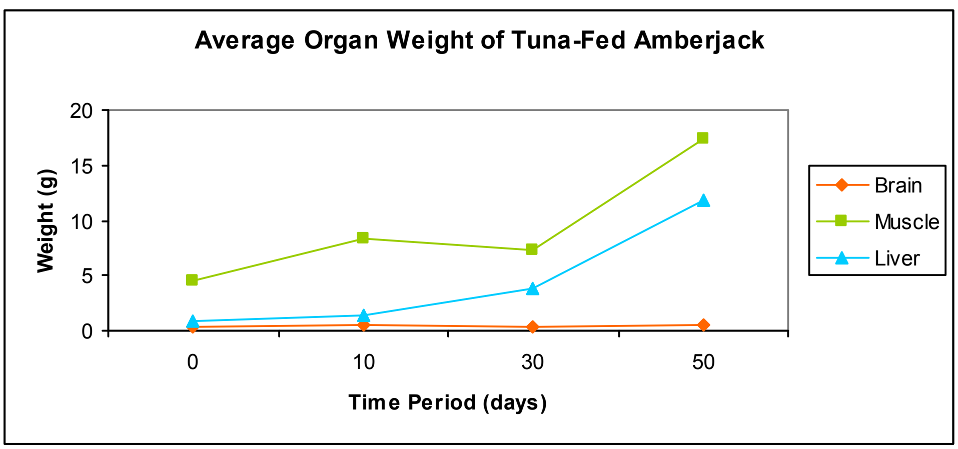

Figure 14. The average weights of the organs of tuna-fed amberjack vary with time and growth.

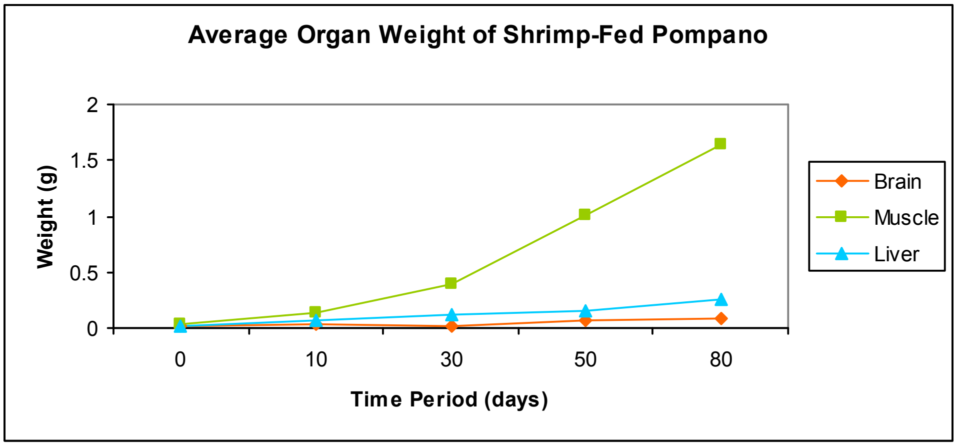

Figure 15. The average weights of the organs of shrimp-fed pompano vary with time and growth.

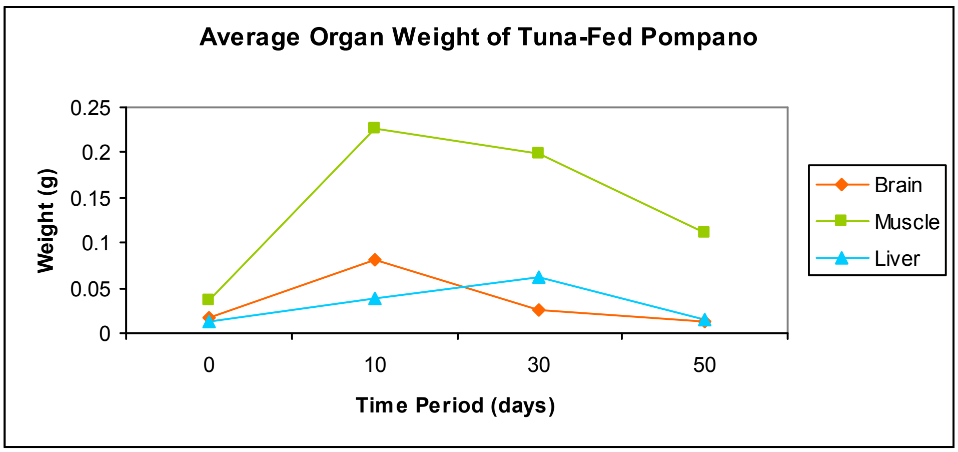

Figure 16. The average weights of the organs of tuna-fed pompano vary with time and growth.

Bioaccumulation Factor

The bioaccumulation factor (BAF) is equal to the Hg concentration in the tissue sample in proportion to the Hg concentration in the food sample (Table 2). Over time, the bioaccumulation factor increased until the organ reached an equilibrium and began to eliminate Hg. From day 0 to 50 the BAF increased in shrimp-fed amberjack brain, muscle, and liver, and decreased from day 50 to 80. The BAF in the tissues of tuna-fed amberjack increased from day 0 to 50, with the exception of the liver, which decreased from day 30 to 50. In shrimp-fed pompano, the BAF fluctuated over time but ultimately it became clear that the brain and muscle had not yet reached saturation whereas the liver decreased after day 50. BAF for tuna-fed pompano tissues increased from day 0 to 50.

Table 2. Bioaccumulation factor (BAF). *The day 30 shrimp-fed pompano brain sample was insufficient for accurate measurement within the calibration curve and thus may have greater uncertainty.

BAF = [tissue] / [food]						
AMBERJACK [s]=28.6 ppb [t]=2670 ppb						
Day	*Brain (S)*	*Brain (T)*	*Muscle (S)*	*Muscle (T)*	*Liver (S)*	*Liver (T)*
0	0.421	0.005	2.25	0.024	1.56	0.017
10	0.826	0.092	2.77	0.153	2.18	0.175
30	1.91	0.517	4.10	0.997	3.53	0.990
50	2.60	1.38	6.64	1.01	5.12	0.873
80	2.23		5.46		4.85	
POMPANO [s]=28.6 ppb [t]=839 ppb						
Day	*Brain (S)*	*Brain (T)*	*Muscle (S)*	*Muscle (T)*	*Liver (S)*	*Liver (T)*
0	1.61	0.055	3.92	0.133	3.63	0.124
10	2.82	0.644	3.99	0.834	4.32	1.77
30	*1.50	1.75	3.39	2.10	3.99	3.49
50	1.89	2.55	3.74	3.39	3.29	11.7
80	2.34		5.21		1.13	

Shrimp-fed amberjack

A total of 18 shrimp-fed amberjack were sacrificed over 80 days (table 1). The liver, brain, and muscle tissues all increased in mercury concentration from day 0 to day 50 by 6.13, 3.00, and 3.28 fold respectively, and decreased in mercury concentration from day 50 to day 80 by 1.17, 1.22, and 1.06 fold respectively (figure 17). The bioaccumulation factors (BAF), representing the ratio of Hg concentrations in the tissues to the food, were calculated to determine the extent of Hg bioaccumulation upon exposure to natural diet. The BAFs for shrimp-fed amberjack after day 80 were 5.29, 2.43, and 3.01 in the brain, muscle, and liver tissues respectively.

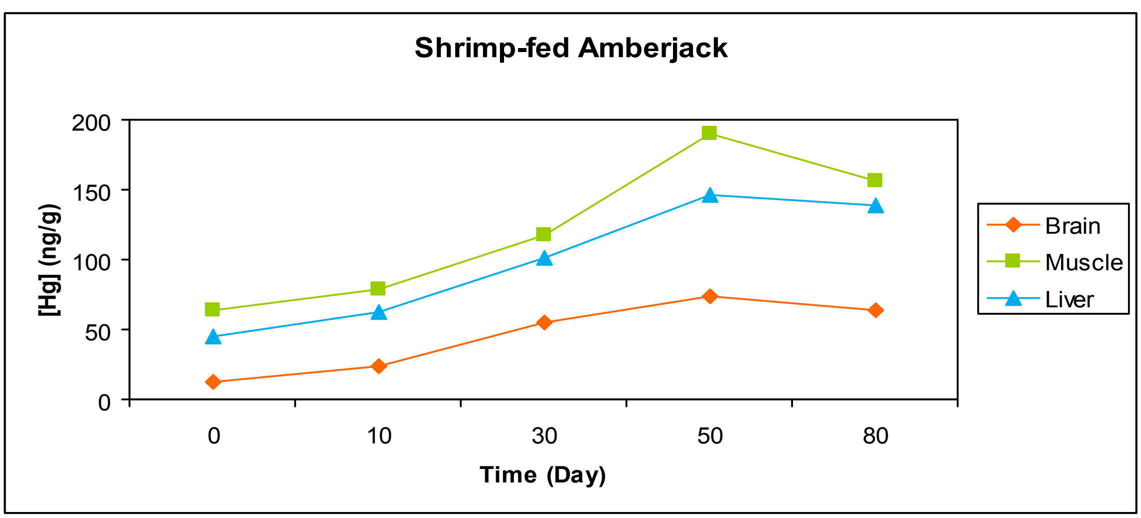

Figure 17. Mercury concentrations (ng/g) in shrimp-fed amberjack brain, muscle, and liver

Tuna-fed amberjack

A total of 13 tuna-fed amberjack were sacrificed over 50 days (table 1). Around day 30, many amberjacks began to die, resulting in small sample sizes for the remainder of the experiment. Dying fish were observed as discolored and slow moving and they became emaciated as they stopped feeding, which was perhaps due to an unnatural diet or the fact that gill parasites were found on several occasions throughout the experiment. The muscle and liver showed similar trends of increase until day 30 by 41.5 and 59.1 fold respectively. After day 30 the muscle reached a plateau, and the liver [Hg] decreased by 1.13 fold from day 30 to 50. The brain concentration increased exponentially from day 0 to 50 by 306 fold (Figure 18). The bioaccumulation factors (BAF), representing the ratio of Hg concentrations in the tissues to the food, were calculated to determine the extent of Hg bioaccumulation upon exposure to natural diet. The BAFs for tuna-fed amberjack after day 80 were 276, 42.0, and 51.4 in the brain, muscle, and liver tissues respectively.

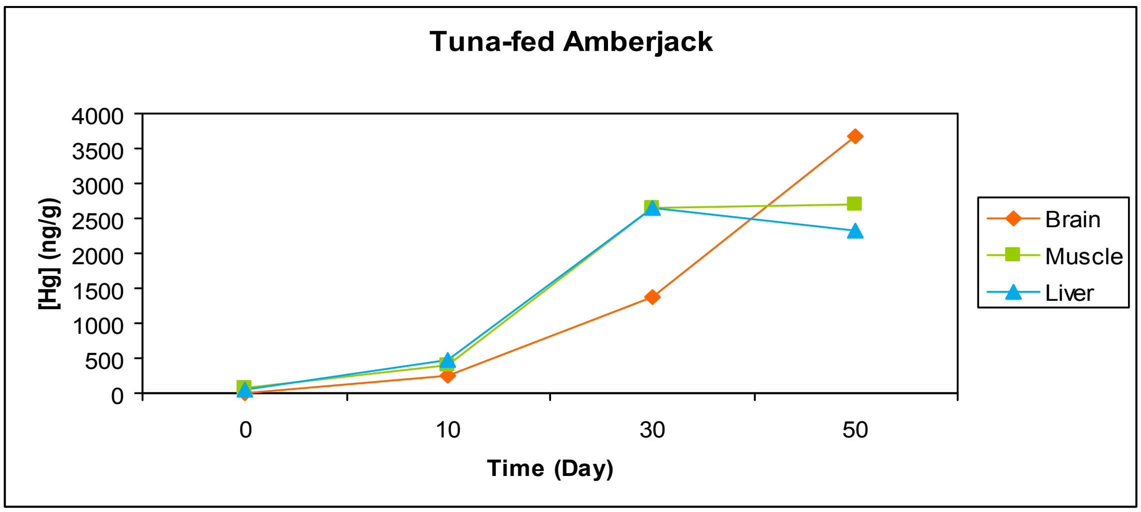

Figure 18. Mercury concentration (ng/g) in tuna-fed amberjack brain, muscle, and liver

Shrimp-fed pompano

A total of 24 shrimp-fed pompano were sacrificed over 80 days (table 1). The Hg concentrations in the brain and muscle tissues were variable over time but increased slowly by 1.46 and 1.33 fold respectively. The Hg concentrations in the liver showed a general decreasing trend from day 10 to 80 by 3.84 fold (Figure 19). The bioaccumulation factors (BAF), representing the ratio of Hg concentrations in the tissues to the food, were calculated to determine the extent of Hg bioaccumulation upon exposure to natural diet. The BAFs for shrimp-fed pompano after day 80 were 1.46, 1.33, and 0.31 in the brain, muscle, and liver tissues respectively.

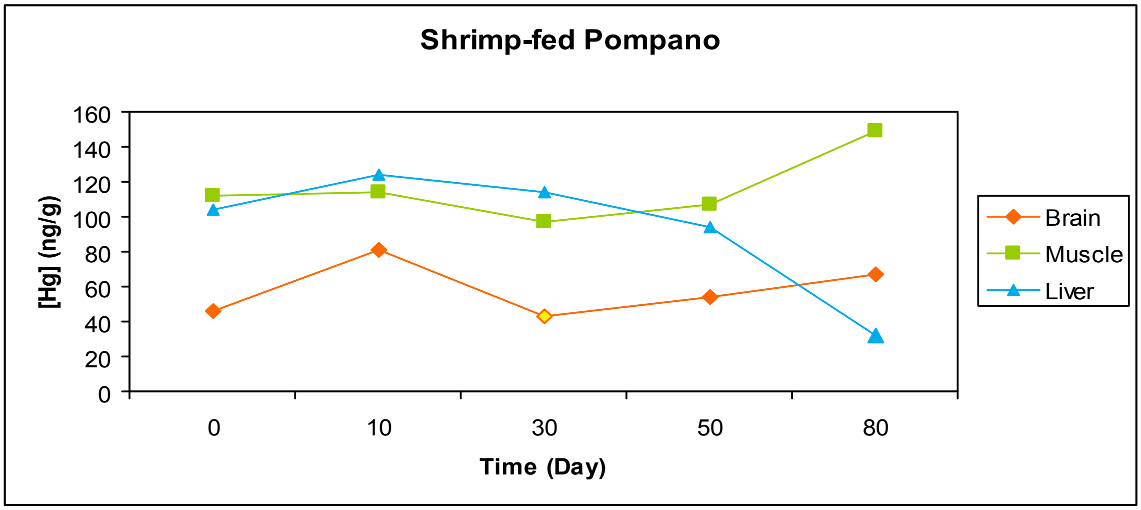

Figure 19. Mercury concentration (ng/g) in shrimp-fed pompano brain, muscle, and liver. ◆The day 30 brain sample was insufficient for accurate measurement within the calibration curve and thus may have greater uncertainty.

Tuna-fed pompano

A total of 13 tuna-fed pompano were sacrificed over 50 days (table 1). Around day 30, many pompano began to die, resulting in small sample sizes for the remainder of the experiment. Dying fish were observed as discolored and slow moving, and they became emaciated as they stopped feeding, perhaps due to an unnatural diet. The brain and muscle tissues of tuna-fed pompano exhibited a linear increase in mercury concentration from days 0 to 50 by 46.5 and 25.4 fold respectively, and the liver showed an accelerated increase in Hg concentration after day 30 by 3.36 fold (figure 20). The bioaccumulation factors (BAF), representing the ratio of Hg concentrations in the tissues to the food, were calculated to determine the extent of Hg bioaccumulation upon exposure to natural diet. The BAFs for tuna-fed pompano after day 80 were 46.3, 25.5, and 94.7 in the brain, muscle, and liver tissues respectively.

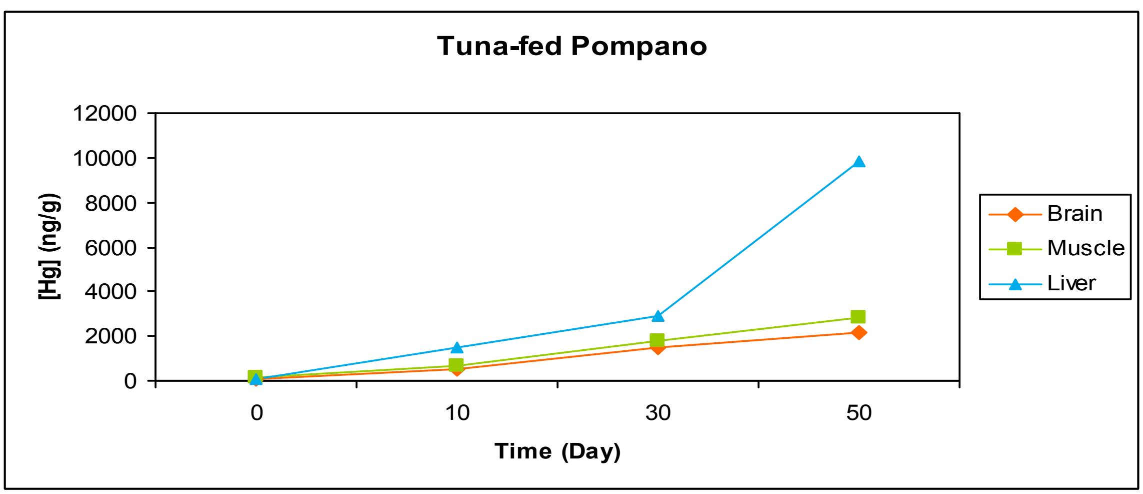

Figure 20. Mercury concentration (ng/g) in tuna-fed pompano brain, muscle, and liver

Statistical analyses

A Univariate Analysis of Variance was conducted using Predictive Analytics Software (PASW) to determine the significance (p-value < 0.05) of fixed factors (time, species, diet, and organ) and covariates (body mass) on the dependent variable, mercury concentration (table 3). Not all variables displayed a normal distribution, so natural log transformations were made for [Hg] and body mass. All factors were shown to significantly affect [Hg]. A test of between-subject effects was also conducted and determined no significant influence between independent variables (species, diet, organ, and day) and/or covariates (body mass).

Table 3. Factors such as species, diet, organ, day, and body mass significantly affect mercury concentration.

Tests of Between-Subjects Effects

Dependent Variable:LnHg

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (p-value)
Corrected Model	118.911[a]	10	11.891	21.784	<0.001
Intercept	52.063	1	52.063	95.377	<0.001
Species	6.653	1	6.653	12.187	0.001
Diet	44.036	2	22.018	40.336	<0.001
Organ	6.160	2	3.080	5.643	0.007
Day	44.550	4	11.137	20.403	<0.001
LnMass	7.700	1	7.700	14.106	0.001

a. R Squared = .835 (Adjusted R Squared = .797)

Discussion

The variation in bioaccumulation factors (BAF) over time represents the relative amount of Hg in the diet that is sequestered in the fish tissues. The general trend shows a decrease in the BAF over time. Tissue concentrations tend toward an equilibrium with the diet and in some cases transition to elimination of Hg from the tissue. Among shrimp-fed amberjack, this transition takes place between days 50 and 80. Tuna-fed amberjack BAFs for the brain and muscle continue to increase until the end of the trial at day 50. Between days 30 and 50 however, the BAF in the liver decreases, perhaps representing the liver's role as a tissue of redistribution and excretion. The BAFs of shrimp-fed pompano appear to fluctuate with the exception of the liver, which begins decreasing between days 10 and 30. Tuna-fed pompano Hg continually increases until the end of the trial at day 50. These results suggest the importance of diet and tissue type on trends in Hg concentration over time.

Roles of tissues: liver, muscle, brain

Compared to other metals, which concentrate in detoxifying organs before excretion, MeHg distributes across the body more evenly (Mason et al. 1996). Tissues are specific in their ability to accumulate metals and translocate to other tissues. In fish, the site of entry into the circulatory system influences tissue distribution of metals. Learner and Mason (2004) suggest that the pathway for MeHg distribution following digestion is uptake by the intestinal wall and transfer to the blood and liver, which distribute it to the rest of the body. The liver is an important site for contaminant storage, redistribution, detoxification, or transformation of MeHg methyl mercury (Evans et al. 1993). The liver's role is to excrete harmful compounds as bile for further detoxification. Once excreted to the small intestine, MeHg can be excreted in bile as methylmercury cytosine, reabsorbed by the gut, or accumulated by the kidney for further redistribution (Boening 1999; Boudou and Ribeyre 1985; Pentreath 1976). Studies by Branco et al. (2011) determined that the main site of demethylation and detoxification is the liver, and postulate that this is because it is where MeHg-Se complexes are formed. When exposed to both MeHg and Se, accumulation of Hg is observed to be lower in the liver, kidney, brain, and muscle tissues of fishes (Branco et al. 2011). The presence of Se may induce production of proteins that trap MeHg, may compete with MeHg for binding sites, or may bind with MeHg itself and enhance coexcretion. The results of this study are consistent with Branco et al. (2011), in which mercury accumulation in the brain and muscle proceeded throughout the entire experiment, whereas the liver displayed an elimination process. Inconsistencies still exist in liver [Hg] over time however, due to differences in fish species and size, as well as the duration of exposure to a diet high in Hg.

Redistribution was evident in this experiment among shrimp-fed pompano, in which the [Hg] decreased in the liver while increasing in the brain and muscle. However, shrimp-fed amberjack did not display signs of redistribution, as [Hg] in the liver, brain, and muscle all decrease around the same time (day 50). This may be due to the excretion of inorganic Hg, which reduced the total Hg concentrations, while some organic MeHg may have been retained in the muscle tissue. Amberjack did display redistribution from the liver to the muscle when fed a diet of tuna, however. As liver [Hg] decreased after day 30, muscle and brain [Hg] continued to increase. This trend in particular could be attributed to the higher amount of organic mercury in tuna compared to shrimp. It is also possible that the trend of increased [Hg] in the liver among tuna-fed pompano is the result of stress-induced mobilization of energy from lipid sources (liver) to meet the increased energy demands of a high-stress state (Montero et al. 1999; Barton and Iwama 1991).

Effects of size and growth rate on [Hg]

There is a distinct positive correlation between a fish's size and its mercury burden (Campbell et al. 2008; Senn et al. 2009). Thus, the fish that lived and were exposed to a given source of Hg for a longer period of time were expected to have higher MeHg concentrations than earlier sacrificed individuals due to growth and bioaccumulation over time. However we did not always see this pattern, as the growth trend in which average body mass decreased over intermediate periods of time was unexpected. Juvenile fish typically grow very fast, but as the experiments progressed, poor feeding behavior among tuna-fed amberjack and tuna-fed pompano was observed. Because chronic metal exposure can affect the whole animal, including its behavioral responses and appetite, the conditions under which an animal lives can affect its background levels of mercury and its responses to increased exposure of metal concentrations (McGreer 2004). Studies of mercury concentrations in other animals have displayed autoimmune consequences associated with exposure to MeHg (Scheuhammer et al. 2007). This is one possible explanation for the poor feeding behaviors of tuna-fed fish. The intestinal wall is permeable to methyl mercury (Boening 1999), and metals in the gut can alter digestive processes and nutrient uptake by disrupting enzyme activity, changing mucus secretion rate, and interfering with neuroendocrine functions and hormone secretion. This has been determined in studies of PAHs, PCBs, and heavy metals such as mercury, cadmium, copper, and silver which increase blood cortisol levels, indicating stress (Hontela et al. 1992; Scott et al. 2003; Grosell et al. 1997; McGreer et al. 2000).

The average body mass decrease among tuna-fed amberjack from day 10 to day 30 reflects reduction in the average by inclusion of the fish that stopped feeding and started to die before day 30. The samples taken after day 30 were those that did survive and continued to grow, thus increasing the average body mass of the sample. Similarly, pompano fed a diet of tuna also stopped feeding around day 10 and regular deaths occurred until there were none left after day 50. This caused the decreasing trend in average body mass of tuna-fed pompano after day 10. Shrimp-fed fish continued feeding throughout the experiment, growing and living until day 80. It is also speculated that MeHg causes a decline in growth efficiency, leading to an overall Hg accumulation with age. Thus, older, higher level predators, such as the tuna-fed amberjack, are expected to exhibit a decline in growth followed by higher [Hg] (McGreer 2004).

Conversely, Pentreath (1976) suggest that excretion and elimination are proportional to growth rate. While the amberjack displayed a relatively constant body mass until day 30 and a sharply increasing growth rate until day 50, there was no sign of growth dilution, as the mercury concentration in the brain continued to increase. Concentrations of Hg at the beginning of the experiment, days 0 to 30, were higher among the pompano. This may be explained by their metabolic rate, which is faster in smaller fish and results in faster uptake and transfer of MeHg between tissues faster (Learner and Mason 2004). Growth rates of individual tissues were also variable. While shrimp-fed species continued to feed and grow over time, tuna-fed species experienced large losses in muscle tissue. All shrimp-fed amberjack tissues increased slightly in weight while [Hg] decreased. In tuna-fed amberjack, brain and muscle mass increased and [Hg] increased to reach a plateau. Liver mass of tuna-fed amberjack increased as [Hg] decreased. In shrimp-fed pompano, brain and muscle [Hg] increased and liver [Hg] decreased as liver mass increased. Finally, all tissues in the tuna-fed pompano decreased in mass as the [Hg] increased. These results may be due to two different processes: potential growth dilution, and the remobilization and redistribution of Hg from muscle tissue. The variability of these trends and the unexpected results of this experiment emphasize the importance of growth rate on Hg concentrations.

Role of species, life stage, diet, and exposure

The expression of toxicity depends on the organism, its life stage, diet, and dosage (McGreer 2004). As this experiment included two different species, it was expected that mercury concentrations would vary because the mechanisms of reducing metal accumulation and toxicity vary with the organism's ability to detoxify, store, inhibit uptake, and increase elimination of a toxin (McGreer 2004). By the end of the experiment, the Hg concentrations were higher in amberjack than in pompano. While previous studies suggest this is likely due to age differences and background Hg, the amount of Hg that was already present in fish prior to capture, juvenile amberjack actually had lower background concentrations of Hg in the brain, muscle and liver (12.0 ng/g, 64.2 ng/g, and 44.7 ng/g respectively), whereas the tissues from young of year pompano had higher background concentrations of Hg (45.9 ng/g, 112 ng/g, and 104 ng/g respectively). Thus, the concentrations in amberjack tissues may have exceeded those of the pompano due to diet, as the amberjack were fed a blackfin tuna diet that was 3.18 times higher in [Hg] than the yellowfin tuna-fed pompano.

The shrimp or tuna that each species was fed represented a difference in inorganic (Hg) versus organic (MeHg) forms of mercury. In tuna, approximately 85% to 95% of the total mercury (THg) is MeHg, whereas MeHg comprises 3.2% to 4.5% of THg in shrimp (Bloom 1992). The trophic transfer efficiency of MeHg is approximately four times as great as that of inorganic Hg (Mason et al. 1996). Thus, the higher MeHg concentration of tuna compared to shrimp accounts for the constant increase of Hg in all organs of the tuna-fed pompano and generally increasing trend of tuna-fed amberjack despite its decreasing body mass. Shrimp-fed pompano grew consistently and were able to excrete Hg, as shrimp is higher in inorganic mercury. Furthermore, exposure time is an important factor to consider. A study of rainbow trout, *Salmo gairdneri,* by Giblin and Massaro (1973) showed that concentrations of MeHg in the brain and skeletal muscle increased and generally reached a maximum. As the length of observation increased however, so did the half-life of Hg. This is an important factor to consider as skeletal muscle makes up over 50% of the edible portion of fish, and the fish consumed by humans are often at least 100 days old.

Conclusion

The results of the four experiments conducted for this study cannot be compared without considering factors such as species, life stage, and diet. Shrimp-fed amberjack and pompano, blackfin tuna-fed juvenile amberjack and yellowfin tuna-fed young of year pompano exhibited varying concentrations of mercury in their tissues as well as varying trends over time. When diet was held constant and all fish were fed shrimp, amberjack exhibited higher concentrations of Hg. When species was held constant, mercury concentrations in the fish varied based on diet, as significant differences in mercury concentration were evident among shrimp fed and tuna fed fish. Trends in the tissues were evidence for the prevalence of more organic MeHg in tuna versus predominantly inorganic Hg in shrimp, of which MeHg has greater binding affinity and Hg is more easily excreted. In all scenarios, time was a factor as well. Initial increases in mercury concentration occurred, followed by decreases that could be attributed to change in mass, the excretion of inorganic mercury, or the roles of tissues. Specifically, the results of this study exemplify the importance of the liver and its role in detoxification and redistribution of MeHg to other tissues such as the brain and muscle. Additionally, examining the mass of individual organs over time relative to the trends in [Hg] displayed the effects of growth dilution and redistribution among the tissues for each trial.

Further studies should aim to incorporate larger sample sizes over a longer period of time so as to determine long-term trends in tissue mercury concentrations. This was difficult in this experiment as many fish did not eat and survive which was possibly due to the negative effects of MeHg on feeding behavior, or to the unnatural, unpalatable diet of tuna for these fish. Furthermore, comparisons among different species' methods of mercury distribution and elimination would be more effective if studied among species of the same age class. Analysis of blood and other organs could provide further insight as to the distribution and elimination of mercury concentration in fish over time.

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References

Barton, B.A., and Iwama, G.K. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Rev. of Fish Diseases*. 1991. 3–26.

Bloom, N. S. On the methylmercury content of fish tissue. *Can. J. Fish. Aquat. Sci.* 1992. 49: 1010–1017.

Boening, Dean W. Ecological effects, transport, and fate of mercury: a general review. *Chemosphere*. 1999. 40: 1335–1351.

Boudou, A. and Ribeyre, F. Contamination of Aquatic Biocenoses by Mercury Compounds: An Experimental Ecotoxicological Approach. *Advances in Environmental Science and Technology*. 1983. 13: 73–116.

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

Branco, Vasco., Canário, João., Lu, Jun., Holmgren, Arne., and Carvalho, Cristina. Mercury and selenium interaction in vivo: Effects on thioredoxin reductase and glutathione peroxidase. *Free Radical Biology and Medicine*. 2011. 52.4: 781–793.

Cabana G, Rasmussen JB. Modeling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Nature 1994; 372: 255-257.

Campbell L.; Verburg, Piet. ; Dixon, D.G. ; Hecky, R.E. Mercury biomagnifications in the food web of Lake Tanganyika (Tanzania, East Africa). *Science of the Total Environment*. 2008.

Chen, Celia; Amirbaham, Aria; Fisher, Nicolas; Harding, Garth; Lamborg, Carl; Nacci, Diane; Taylor, David. Methylmercury in Marine Ecosystems: Spatial Patterns and Processes of Production, Bioaccumulation, and Biomagnification. *EcoHealth*. 2008. 5: 399–408

Choi SC, Bartha R. Environmental factors affecting mercury methylation in estuarine sediments. Bull Environ Contam Toxicol 1994; 53: 805-812

Donald W. Davis Slide Collection. Wharf-mounted popier. *Louisiana Sea Grant Collection: Images*. 1976. <http://louisdl.louislibraries.org/cdm4/item_viewer.php?CISOROOT=/p15140coll21&CISOPTR=2102&CISOBOX=1&REC=5>.

Evans, David W., Dodoo, David K., and Hanson, Peter J. Trace element concentrations in fish livers: Implications of variations with fish size in pollution monitoring. *Marine Pollution Bulletin*. 1993. 26.6: 329–334.

Giblin, F.J. and Massaro, Edward J. The Erythrocyte Transport and Transfer of Methylmercury to the Tissues of the Rainbow Trout (*Salmo gairdneri*). *Toxicology*. 1975. 5: 243–254.

Giblin, F.J. and Massaro, Edward J. Pharmacodynamics of Methyl Mercury in the Rainbow Trout (Salmo Gairdneri): Tissue Uptake, Distribution, and Excretion. *Toxicology and Applied Pharmacology*. 1973. 24: 81–91.

Grosell, M.H., Hogstrand, C., and Wood, C.M. Cu uptake and turnover in both Cu-acclimated and non-acclimated rainbow trout (Oncorhynchus mykiss). *Aquatic Toxicology*. 1997. 38: 257–276.

Hontela, A., Rasmussen, J.B., Audet, C., and Chevalier, G. Impaired Cortisol Stress Response in Fish from Environments Polluted by PAH, PCB, and Mercury. *Arch. Environ. Contam. Toxicol*. 1992. 22: 278–283.

LaPorte, J.M.; Truchot, J.P.; Ribeyre, F.; Boudou, A. Combined Effects of Water pH and Salinity on the Bioaccumulation of Inorganic Mercury and Methylmercury in the Shore Crab *Carcinus maenas*. *Marine Pollution Bulletin*. 1997. 34.11: 880–893.

Learner, Joy L., and Mason, Robert P. Methylmercury uptake and distribution kinetics in sheepshead minnows, *Cyprinodon variegates*, after exposure to CH_3Hg-spiked food. *Environmental Toxicology and Chemistry*. 2004. 23.9: 2138–2146.

Lindqvist, O., Johansson, K., Aastrup,M., Andersson, A., Bringmark, L., Hovsenius, G., Hakanson, L., Iverfeldt, A. Meili, M., and Timm, B. Mercury in the Swedish environment – Recent research on causes, consequences and corrective methods. *Water, Air, and Soil Pollution*. 1991. 55: 1–261.

Mason, R.P., Reinfelder, J.R., and Morel, F.M.M. Bioaccumulation of mercury and methylmercury. *Water, Air, and Soil Pollution*. 1995. 80: 915–921.

Mason, Robert P., Reinfelder, John R., and Morel, Françoism M. Uptake, Toxicity, and Trophic Transfer of Mercury in a Coastal Diatom. *Environmental Science and Technology*. 1996. 30.6: 1835–1845.

McGreer, J., Henningsen, G., Lanno, R., Fisher, N., Sappington, K., and Drexler J. Issue paper on the bioavailability and bioaccumulation of metals. *U.S. Environmental Protection Agency Risk Assessment Forum*. 2004: 1–122.

McGeer, J.C., Playle, R.C., Wood, C.M., and Galvez, F. A physiologically based acute toxicity model for predicting the effects of waterborne silver on rainbow trout in fresh waters. *Environmental Science and Technology*. 2000. 34: 4199–4207.

Montero, D., Izquierdo, M.S., Tort, L., Robaina, L., and Vergara, J.M. High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabream, *Sparus aurata*, juveniles. *Fish Physiology and Biochemistry*. 1999. 20: 53–60.

Offshore-technology.com. Brutus, United States of America. 2011. <http://www.offshore-technology.com/projects/brutus/brutus1.html>.

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

Rigs, Reefs, and Wrecks Inc. 2011. <http://www.rodnreel.com/>.

Scheuhammer, Anton M.; Meyer, Michael W. ; Sandheinrich, Mark B.; Murray, Michael W. Effects of Environmental Methylmercury on the Health of Wild Birds, Mammals, and Fish. *Ambio*. 2007. 36.1: 12–18.

Scott, G.R., Sloman, K.A., Rouleau, C., and Wood, C.M. Cadmium disrupts behavioural and physiological responses to alarm substance in juvenile rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology*. 2003. 206.11: 1779–1790.

Senn, David B.; Chesney, Edward J.; Blum, Joel D.; Bank, Michael S.; Maage, Amund; Shine, James P. Stable Isotope (N,C,Hg) Study of Methylmercury Sources and Trophic Transfer in the Northern Gulf of Mexico. *Environmental Science & Technology*. 2009.

Simon, O., and Boudou, A. Direct and Trophic Contamination of the Herbivorous Carp *Ctenopharyngodon idella* by Inorganic Mercury and Methylmercury. *Ecotoxicology and Environmental Safety*. 2001. 50: 48–59.

Sunderland EM, Krabbenhoft DP, Moreau JW, Strode SA, Landing WM. Mercury sources, distribution and bioavailability in the North Pacific Ocean: Insights from data and models. Global Biogeochem Cy 2009; 23, GB2010, doi: 10.1029/2008GB003425.

Trudel, Marc and Rasmussen, Joseph B. Modeling the Elimination of Mercury by Fish. *Environmental Science & Technology*. 1997. 31.6: 1716–1722

Wang, Rui, and Wang, Wen-Xiong. Importance of Speciation in Understanding Mercury Bioaccumulation in Tilapia Controlled by Salinity and Dissolved Organic Matter. *Environmental Science and Technology*. 2010. 44: 7964–7969.

Appendix

I. Tissue data

DAY 0	Weight (g)	FL (cm)	Blood		BLOOD	Liver		LIVER	Kidney		KIDNEY	Muscle		MUSCLE	Brain		BRAIN
6/27/2011			Centrifuge tube	Blood Total (g)		L Whirlpack	L Total (g)		K Whirlpack	K Total (g)		M Whirlpack	M Total (g)		Br Whirlpack	Br Total (g)	
Pompano - FEED																	
1	152.95	23	1.1	2.84	1.74	1.88	2.97	1.09	1.88	2.41	0.53	1.9	4.67	2.77	1.89	1.95	0.06
2	197.64	20.4	1.09	2.15	1.06	1.91	2.79	0.88	1.92	2.99	1.07	1.89	7.58	5.69	1.89	2.15	0.26
3	194.5	22.2	1.11	1.94	0.83	1.89	3.04	1.15	1.86	2.46	0.6	1.91	8.2	6.29	1.89	1.99	0.1
4	233.11	22.3	1.13	2.82	1.69	1.92	2.84	0.92	1.91	2.73	0.82	1.87	8.69	6.82	1.92	2.23	0.31
5	109.73	16.5	1.08	2.33	1.25	1.92	2.72	0.8	1.9	2.29	0.39	1.91	5.3	3.39	1.9	2.04	0.14
6/30/2011																	
Pompano			(Whirlpack)														
1	1.23	3.5	1.59	1.91	0.32	1.81	1.8	-0.01	1.8	1.8	0	1.76	1.76	0	1.77	1.8	0.03
2	1.67	3.9	1.7416	1.788	0.047	1.7431	1.762	0.0189	1.7492	1.7541	0.0049	1.7502	1.789	0.0394	1.7555	1.773	0.0175
3	1.44	4.3	1.7613	1.849	0.088	1.7498	1.7648	0.015	1.7341	1.7405	0.0064	1.7256	1.7633	0.0377	1.7536	1.7654	0.0118
4	1.42	3.9	1.7628	1.773	0.010	1.766	1.7801	0.0141	1.7575	1.7636	0.0061	1.7733	1.8266	0.0533	1.7626	1.7733	0.0107
5	1.69	4.4	1.7682	1.8893	0.121	1.7417	1.7524	0.0107	1.7589	1.762	0.0031	1.7747	1.8212	0.0465	1.7515	1.7686	0.0171
6	1.6	4.6	1.756	2.2097	0.454	1.7571	1.764	0.0069	1.7678	1.776	0.0082	1.7548	1.7935	0.0387	1.7401	1.7621	0.022
AVG	***1.49***				***0.1175***			***0.01312***			***0.0041***			***0.03538***			***0.01742***
7/8/2011								* did not factor in negative values									
Amberjack			(Whirlpack)														
1	100.64	18.7	1.8581	3.808	1.9499	1.8618	2.5547	0.6929	1.8694	2.0131	0.1437	1.8653	4.6713	2.806	1.8622	2.1176	0.2554
2	167.88	22.1	1.8885	5.1276	3.2391	1.8759	2.7544	0.8785	1.8759	2.0889	0.213	1.8715	6.5142	4.6427	1.8549	2.3263	0.4714
3	218.3	24.3	1.8901	4.3652	2.4751	1.8769	2.597	0.7201	1.8801	2.1702	0.2901	1.8703	6.5671	4.6968	1.8742	2.3546	0.4804
4	79.86	17.8	1.8695	3.8572	1.9877	1.8653	2.2902	0.4249	1.8644	1.9459	0.0815	1.8657	3.9198	2.0541	1.8842	2.2184	0.3342
5	245.2	25.2	1.8752	6.5461	4.6709	1.8773	3.1752	1.2979	1.8791	2.2611	0.382	1.8729	10.3262	8.4533	1.8853	2.4721	0.5868
AVG	***162.376***				***2.8645***			***0.80286***			***0.2221***			***4.53058***			***0.42564***
DAY 10	**Weight (g)**	**FL (cm)**	**Blood**			**Liver**			**Kidney**			**Muscle**			**Brain**		
7/10/2011			(Whirlpack)	Blood Total (g)		L Whirlpack	L Total (g)		K Whirlpack	K Total (g)		M Whirlpack	M Total (g)		Br Whirlpack	Br Total (g)	
Pompano																	
P1.1	2.0303	4.4	1.8792	1.9123	0.0331	1.8821	1.9035	0.0214	1.9022	1.9069	0.0047	1.8645	1.9196	0.0551	1.8773	1.8978	0.0205
P1.2	5.5372	6.4	1.8657	2.0189	0.1532	1.8993	1.9906	0.0913	1.9163	1.9329	0.0166	1.8661	1.971	0.1049	1.8798	1.9064	0.0266
P1.3	3.4303	5.6	1.8736	1.9321	0.0585	1.9003	1.9494	0.0491	1.9031	1.9073	0.0042	1.8819	2.0122	0.1303	1.8866	1.9141	0.0275
P1.4	3.2609	5.2	1.8653	1.9412	0.0759	1.9058	2.0077	0.1019	1.9035	1.927	0.0235	1.8866	2.0214	0.1348	1.8915	1.9332	0.0417

P1.5	4.7719	5.7	1.8728	1.9256	0.0528	1.9067	1.9841	0.0774	1.8941	1.9122	0.0181	1.8794	2.0957	0.2163	1.8923	1.9316	0.0393
AVG	***3.80612***				***0.0747***			***0.06822***			***0.0134***			***0.12828***			***0.03112***
P2.1	4.8722	6	1.8753	1.9838	0.1085	1.887	1.9738	0.0868	1.874	1.9021	0.0281	1.8709	2.158	0.2871	1.8755	1.9194	0.0439
P2.2	4.2343	6	1.8731	1.9657	0.0926	1.8882	1.9543	0.0661	1.8857	1.8939	0.0082	1.8723	2.0459	0.1736	1.8928	1.919	0.0262
P2.3	4.3961	5.8	1.8806	2.0027	0.1221	1.8828	1.9799	0.0971	1.8872	1.9143	0.0271	1.8797	2.0976	0.2179	1.8777	1.9168	0.0391
P2.4	5.6742	6.3	1.8708	1.9487	0.0779	1.8946	1.9502	0.0556	1.8882	1.9225	0.0343	1.8841	2.1855	0.3014	1.8877	1.9411	0.0534
P2.5	4.4793	5.9	1.8639	2.0008	0.1369	1.8817	1.983	0.1013	1.8851	1.8996	0.0145	1.8949	2.044	0.1491	1.8945	1.9262	0.0317
AVG	***4.73122***				***0.1076***			***0.08138***			***0.0224***			***0.22582***			***0.03886***
7/18/2011																	
Amberjack			(Whirlpack)														
A1.1	90.9	17.6	1.8924	3.605	1.7126	1.8867	2.3445	0.4578	1.9013	2.0446	0.1433	1.8927	4.7225	2.8298	1.8979	1.8992	0.0013
A1.2	143.2	20.4	1.893	5.1614	3.2684	1.8767	2.7955	0.9188	1.883	2.1292	0.2462	1.8869	9.4294	7.5425	1.8801	2.2107	0.3306
A1.3	253.4	24.7	1.8636	6.0264	4.1628	1.876	3.442	1.566	1.8858	2.1618	0.276	1.868	10.8995	9.0315	1.8539	2.2303	0.3764
A1.4	182.8	22.6	1.8803	6.0385	4.1582	1.8797	2.943	1.0633	1.8645	2.0736	0.2091	1.8797	6.3711	4.4914	1.8654	2.4022	0.5368
A1.5	153.7	20.5	1.8809	4.9415	3.0606	1.8866	2.6515	0.7649	1.8908	1.9936	0.1028	1.8887	7.8132	5.9245	1.8897	2.2547	0.365
AVG	***164.8***				***3.2725***			***0.95416***			***0.1955***			***5.96394***			***0.32202***
A2.1	303.9	27.1	1.867	6.2629	4.3959	1.8911	3.474	1.5829	1.8694	2.4479	0.5785	1.8746	10.4225	8.5479	1.874	2.4187	0.5447
A2.2	266.2	26	1.8879	7.5492	5.6613	1.8909	3.6303	1.7394	1.881	2.3131	0.4321	1.8919	10.4907	8.5988	1.8782	2.3632	0.485
A2.3	229.2	24.5	1.8987	5.5874	3.6887	1.8748	2.9376	1.0628	1.8783	2.1937	0.3154	1.8757	10.1694	8.2937	1.8682	2.2976	0.4294
A2.4	153.6	20.2	1.898	5.2725	3.3745	1.8851	3.359	1.4739	1.8922	2.1011	0.2089	1.8963	10.0434	8.1471	1.8948	2.1356	0.2408
A2.5	193.8	22.5	1.8912	6.7955	4.9043	1.8913	3.3543	1.463	1.8917	2.0825	0.1908	1.8861	10.0819	8.1958	1.8884	2.369	0.4806
AVG	***229.34***				***4.4049***			***1.4644***			***0.3451***			***8.35666***			***0.4361***
DAY 30	**Weight (g)**	**FL (cm)**	**Blood**			**Liver**			**Kidney**			**Muscle**			**Brain**		
7/30/2011			(Whirlpack)	Blood Total (g)		L Whirlpack	L Total (g)		K Whirlpack	K Total (g)		M Whirlpack	M Total (g)		Br Whirlpack	Br Total (g)	
Pompano																	
P1.1	4.7154	5.6	1.9105	2.2841	0.3736	1.8948	1.966	0.0712	1.8953	1.911	0.0157	1.8943	2.1018	0.2075	1.905	1.934	0.029
P1.2	7.9595	6.9	1.9025	2.4816	0.5791	1.9078	1.9904	0.0826	1.8977	1.908	0.0103	1.8884	2.3345	0.4461	1.8918	1.928	0.0362
P1.3	11.0973	7.6	1.89	2.2534	0.3634	1.9007	2.0086	0.1079	1.9059	1.9182	0.0123	1.8943	2.5184	0.6241	1.8863	1.896	0.0097
P1.4	10.2757	7.6	1.8955	2.4994	0.6039	1.8951	2.0581	0.163	1.8941	1.9198	0.0257	1.8924	2.3981	0.5057	1.8868	1.9154	0.0286
P1.5	7.2141	6.2	1.8796	2.0742	0.1946	1.8826	2.0137	0.1311	1.8715	1.8914	0.0199	1.8693	2.0647	0.1954	1.8736	1.8894	0.0158
AVG	***8.2524***				***0.4229***			***0.11116***			***0.0168***			***0.39576***			***0.02386***
P2.1	3.8505	5.3	1.8931	2.0782	0.1851	1.8944	1.9344	0.04	1.8892	1.9143	0.0251	1.8949	2.0543	0.1594	1.8979	1.9326	0.0347
P2.2	3.5591	5.5	1.8906	2.2853	0.3947	1.8908	1.9312	0.0404	1.898	1.9084	0.0104	1.8937	2.0492	0.1555	1.882	1.9076	0.0256
P2.3	2.4002	5	1.8891	2.1246	0.2355	1.8792	1.9159	0.0367	1.8897	1.8943	0.0046	1.8821	1.9864	0.1043	1.8951	1.9145	0.0194
P2.4	6.7734	6.3	1.8716	2.1002	0.2286	1.875	2.0328	0.1578	1.876	1.8881	0.0121	1.8762	2.279	0.4028	1.8848	1.9115	0.0267
P2.5	2.8456	5.4	1.8778	2.2275	0.3497	1.8866	1.925	0.0384	1.8959	1.9063	0.0104	1.8814	2.0492	0.1678	1.8771	1.9008	0.0237

AVG	***3.88576***				***0.2787***			***0.06266***			***0.0125***			***0.19796***			***0.02602***
8/7/2011																	
Amberjack			(Vial)														
A1.1	174.3	21.5	25.5744	N/A		1.8941	4.7786	2.8845	1.8885	2.9581	1.0696	1.8839	12.1087	10.2248	1.8796	2.3316	0.452
A1.2	289.87	25	25.4948	30.856	5.3608	1.8979	5.7009	3.803	1.8854	2.6499	0.7645	1.8904	13.7897	11.8993	1.8884	2.4236	0.5352
A1.3	192.5	23.5	25.6302	26.836	1.2055	1.8819	2.4592	0.5773	1.8794	2.2497	0.3703	1.8781	5.5556	3.6775	1.8919	2.1829	0.291
A1.4	151.37	20.2	26.8361	29.607	2.7713	1.8817	2.8313	0.9496	1.8741	2.3908	0.5167	1.8779	4.8899	3.012	1.8631	2.2511	0.388
A1.5	307.14	24.3	26.8138	32.309	5.4949	1.8706	5.0396	3.169	1.8689	2.3969	0.528	1.8781	12.211	10.3329	1.8842	2.4604	0.5762
AVG	***223.036***				***3.7081***			***2.27668***			***0.6498***			***7.8293***			***0.44848***
A2.1	216.02	24.8	25.6454	29.265	3.62	1.8647	4.6344	2.7697	1.8718	2.2196	0.3478	1.8768	10.2317	8.3549	1.8849	2.2567	0.3718
A2.2	237.63	23.8	25.6105	29.804	4.1937	1.8706	5.2129	3.3423	1.8767	2.23	0.3533	1.8695	10.3917	8.5222	1.8836	2.4947	0.6111
A2.3	179.54	19.6	26.5631	30.129	3.5661	1.8776	4.2873	2.4097	1.8724	2.0991	0.2267	1.8781	8.7326	6.8545	1.8703	2.1957	0.3254
A2.4	185.2	20.2	26.7991	30.333	3.5342	1.8743	9.5493	7.675	1.8811	2.2023	0.3212	1.8718	9.0865	7.2147	1.8605	2.198	0.3375
A2.5	165.29	20.4	26.1305	28.906	2.7759	1.8874	5.2232	3.3358	1.8767	2.1215	0.2448	1.8705	7.766	5.8955	1.8765	2.2365	0.36
AVG	***196.736***				***3.538***			***3.9065***			***0.2988***			***7.36836***			***0.40116***
DAY 50	**Weight (g)**	**FL (cm)**	**Blood**			**Liver**			**Kidney**			**Muscle**			**Brain**		
8/19/2011			(Vial)	Blood Total (g)		L Vial	L Total (g)		K Vial	K Total (g)		M Vial	M Total (g)		Br Vial	Br Total (g)	
Pompano																	
P1.1	17.1204	8.6	1.1175	1.3603	0.2428	1.8858	2.1115	0.2257	1.8721	1.897	0.0249	1.8888	3.1271	1.2383	1.8986	1.9641	0.0655
P1.2	12.2548	7.9	1.1155	1.1996	0.0841	1.8774	2.0041	0.1267	1.6852	1.8803	0.1951	1.8891	2.665	0.7759	1.9004	1.9362	0.0358
P1.3	11.4426	7.6	1.1089	1.2032	0.0943	1.8856	2.0258	0.1402	1.8786	1.8722	-0.0064	1.9023	3.0315	1.1292	1.8887	1.9696	0.0809
P1.4	11.4941	7.4	1.0998	1.1209	0.0211	1.8942	2.0455	0.1513	1.7801	1.903	0.1229	1.8981	2.7771	0.879	1.8782	1.9814	0.1032
AVG	***13.077975***				***0.1106***			***0.160975***			***0.1143***			***1.0056***			***0.07135***
P2.1	2.5059	5	1.0791	1.1315	0.0524	1.8951	1.9118	0.0167	1.6751	1.8754	0.2003	1.8984	2.077	0.1786	1.9075	1.9034	-0.0041
P2.2	1.4995	4.2	1.081	1.0941	0.0131	1.9091	1.9038	-0.0053	1.8853	1.9053	0.02	1.8844	1.9338	0.0494	1.8738	1.8993	0.0255
P2.3	1.6841	4.4	1.099	1.1169	0.0179	1.8803	1.8913	0.011	1.8852	1.8937	0.0085	1.8966	2.0016	0.105	1.8936	1.8942	0.0006
AVG	***1.8965***				***0.0278***			***0.01385***			***0.0763***			***0.111***			***0.01305***
								*			*						*
8/27/2011																	
Amberjack			(Vial)														
A1.1	393.35	33.3	27.13	30.271	3.141	26.405	29.961	3.5564	26.618	27.22	0.6016	26.9192	41.5226	14.6034	26.8872	27.414	0.5271
A1.2	238.63	28.4	26.52	27.987	1.4672	26.76	28.643	1.8833	26.472	26.8717	0.3996	30.1898	41.7601	11.5703	25.5331	26.096	0.5627
A1.3	244.34	28.9	27.21	28.901	1.6912	26.301	27.556	1.255	27.189	27.5088	0.3195	25.6971	35.2715	9.5744	26.8852	27.237	0.352

AVG	***292.106666***				***2.0998***			***2.231567***			***0.4402***			***11.91603***			***0.4806***
A2.1	315	28.6	26.05	30.188	4.1379	26.598	36.359	9.7611	26.270	27.2146	0.9445	25.798	43.3038	17.5058	25.7674	26.274	0.5069
A2.2	446.95	31.9	25.93	29.622	3.6917	26.431	41.381	14.9505	26.694	27.3456	0.6514	26.3135	46.0909	19.7774	17.7641	18.179	0.4152
A2.3	368.8	30.5	26.42	28.118	1.6983	25.68	36.267	10.5877	25.648	26.3829	0.7345	26.6419	41.6499	15.008	17.8024	18.552	0.7494
AVG	***376.91667***				***3.176***			***11.76643***			***0.7768***			***17.4304***			***0.557167***
DAY 80			**Blood**			**Liver**			**Kidney**			**Muscle**			**Brain**		
9/18/2011	**Weight (g)**	**FL (cm)**	(Vial)	Blood Total (g)		L Vial	L Total (g)		K Vial	K Total (g)		M Vial	M Total (g)		Br Vial	Br Total (g)	
Pompano																	
P1.1	11.8736	7.8	1.0939	1.233	0.1391	1.8765	2.193	0.3165	1.8628	1.629	-.2338	1.8664	2.83	0.9636	1.8706	1.64	-0.2306
P1.2	16.0691	8.8	1.101	1.229	0.128	1.8694	2.1003	0.2309	1.8649	1.8005	-.0644	1.8659	3.095	1.2291	1.8876	1.5196	-0.368
P1.3	28.1975	10.8	1.0846	1.5931	0.5085	1.8654	2.1584	0.293	1.8716	1.5906	-0.281	1.8684	3.9006	2.0322	1.8826	1.86	-0.0226
P1.4	22.117	9.7	1.0657	1.347	0.2813	1.8674	2.0405	0.1731	1.8833	2.019	0.1357	1.8666	3.5184	1.6518	1.8721	1.9765	0.1044
P1.5	21.0331	9.5	1.0606	1.3802	0.3196	1.8598	2.1901	0.3303	1.8896	1.7	-.1896	1.8683	3.3307	1.4624	1.8702	1.559	-0.3112
P1.6	15.3569	8.5	1.0711	1.2844	0.2133	1.8572	1.697	-0.1602	1.8819	0.6309	-1.251	1.864	2.85	0.986	1.8775	1.584	-0.2935
P1.7	19.4461	9.4	1.0685	1.3425	0.274	1.8645	2.126	0.2615	1.9001	2.083	0.1829	1.8649	4.4337	2.5688	1.8866	1.868	-0.0186
P1.8	8.9587	7.2	1.0957	1.3139	0.2182	1.8746	2.127	0.2524	1.8672	2.05	0.1828	1.8693	2.5806	0.7113	1.8791	1.933	0.0539
P1.9	25.1697	10.1	1.0708	1.3018	0.231	1.8671	1.81	-0.0571	1.8817	2.033	0.1513	1.8756	3.6	1.7244	1.8793	2.02	0.1407
P1.10	25.2451	10.1	1.0871	1.1941	0.107	1.8664	2.249	0.3826	1.8815	1.97	0.0885	1.8656	5.0062	3.1406	1.8773	1.985	0.1077
AVG	***19.34668***				***0.242***			***0.248922***			***0.1235***			***1.64702***			***0.08134***
								*			*						*
9/26/2011																	
Amberjack																	
A1.1	428.07	28.7	25.8202	27.379	1.5589	26.97	30.485	3.5148	26.245	26.712	0.4667	26.6305	39.4714	12.8409	17.9044	18.207	0.3028
A1.2	453.33	29.4	26.846	30.202	3.3558	25.551	29.975	4.4234	25.625	27.5168	1.8916	26.573	46.3386	19.7656	17.807	18.969	1.162
A1.3 (EXTRA - Lesser)	179.26	22.5	25.7114	30.026	4.3149	25.725	26.604	0.8785	25.801	26.09	0.2888	25.5839	31.544	5.9601	17.6872	17.995	0.308
A1.4 (EXTRA)	335.15	27.6	26.9446	27.926	0.9816	26.371	26.708	0.3365	26.746	26.1707	-.5748	26.4733	42.02	15.5467	18.2104	18.435	0.2248
A1.5 (EXTRA)	289.29	26.3	26.331	26.627	0.2958	25.652	26.358	0.7066	26.499	26.9829	0.4837	25.7477	32.8015	7.0538	17.8264	18.192	0.3658
AVG	***337.02***				***2.1014***			***1.97196***			***0.7827***			***12.23342***			***0.47268***
											*						

II. Post freeze-dry weights

POMPANO *(P1 - shrimp; P2 - YFT)*

DAY 0	BRAIN	MUSCLE	LIVER	KIDNEY
P1	1.7723	1.7486	1.7615	
P2	1.7623	1.7596	1.7603	
P3	1.7652	1.7559	1.7652	
P4	1.7773	1.7946	1.7735	
P5	1.7863	1.7938	1.7604	
P6	1.759	1.7634	1.7933	
DAY 10				
P1.1	1.9141	1.928	1.9089	
P1.2	1.9371	1.9313	1.9378	
P1.3	1.9471	1.9246	1.9422	
P1.4	1.9443	1.9863	1.9438	
P1.5	1.9205	2.0015	1.935	
P2.1	1.9713	1.9466	1.9184	
P2.2	1.9194	1.929	1.9136	
P2.3	1.887	1.9419	1.936	
P2.4	1.8981	1.9787	1.9831	
P2.5	1.9182	1.9314	1.9271	
DAY 30				
P1.1	1.9118	1.9414	1.9559	
P1.2	1.9312	1.9907	1.6806	
P1.3	1.8976	2.0447	1.9397	
P1.4	1.8173	2.0329	1.9352	
P1.5	1.8764	1.9381	1.6358	
P2.1	1.9046	1.9484	1.9144	
P2.2	1.9087	1.9548	1.9266	
P2.3	1.9205	1.9409	1.8936	
P2.4	1.8992	1.8472	1.9505	
P2.5	1.9272	1.9362	1.9106	
DAY 50				
P1.1	1.9186	2.1282	1.9476	1.8911
P1.2	1.9255	2.0492	1.9346	1.8833
P1.3	1.9042	2.1238	1.9158	1.8761
P1.4	1.902	2.0681	1.9324	1.8703
P2.1	1.8768	1.9139	1.9013	1.8993
P2.2	1.872	1.897	1.9043	1.8858
P2.3	1.8815	1.9096	1.8891	1.8932
DAY 80				
P1.1	1.9426	2.1092	2.001	1.8791
P1.2	1.8952	2.121	1.9391	1.8933
P1.3	1.9372	2.3436	2.0274	1.8879
P1.4	1.9144	1.3252	1.923	1.9018
P1.5	1.9133	1.2597	1.9951	1.9035
P1.6	1.8966	2.3052	1.946	1.876
P1.7	1.9015	2.4014	1.9481	1.8785
P1.8	1.8878	2.0127	1.9377	1.9073
P1.9	1.8995	2.2985	1.9441	1.9011
P1.10	1.894	2.2516	1.9697	1.9057

AMBERJACK *(AJ1 - shrimp; AJ2 - BFT)*

DAY 0	BRAIN	MUSCLE	LIVER	KIDNEY
AJ1	1.9151	2.4348	2.0963	1.9227
AJ2	1.9474	2.8792	2.12	1.9234
AJ3	1.9747	2.9028	2.0748	1.9453
AJ4	1.9408	2.2715	1.96	1.882
AJ5	2.0017	3.5713	2.195	1.9605
DAY 10				
AJ1.1	1.9664	2.5071	2.0108	1.9317
AJ1.2	1.9501	3.5128	2.128	1.937
AJ1.3	1.93	3.8351	2.3001	1.9444
AJ1.4	1.9779	2.8314	2.1704	1.9089
AJ1.5	1.9632	3.1873	2.1024	1.9136
AJ2.1	1.9901	3.5627	2.2676	1.9848
AJ2.2	1.9727	3.5921	2.3798	1.9745
AJ2.3	1.9569	3.4752	2.1256	1.9428
AJ2.4	1.9581	3.6994	2.3151	1.9375
AJ2.5	1.9937	3.6673	2.3009	1.9376
DAY 30				
AJ1.1	2.003	4.3074	2.5626	2.1394
AJ1.2	2.0622	4.6373	2.7186	2.0335
AJ1.3	1.9598	2.6029	2.0153	1.9530
AJ1.4	1.9577	2.5762	2.0921	1.9733
AJ1.5	2.0072	4.3195	2.7563	1.9846
AJ2.1	1.9696	3.8078	2.4479	1.9468
AJ2.2	2.0102	3.5487	2.6622	1.9448
AJ2.3	1.9514	3.5257	2.4153	1.9258
AJ2.4	1.9365	3.5803	3.9181	1.9548
AJ2.5	1.9499	3.2006	2.8318	1.9265
DAY 50				
AJ1.1	26.9881	30.5581	26.7291	27.3799
AJ1.2	25.6382	32.4183	26.5356	27.2244
AJ1.3	26.9398	27.7105	27.2346	26.5571
AJ2.1	25.8616	29.9566	26.4451	28.7792
AJ2.2	17.8377	31.1429	26.8137	32.4284
AJ2.3	17.9405	30.092	25.7921	28.1784
DAY 80				
AJ1.1	17.956	29.2414	26.3289	27.8515
AJ1.2	18.3562	30.9362	26.8783	27.197
AJ1.3	17.7401	26.5036	25.8482	25.8846
AJ1.4	17.9191	29.1011	25.7054	25.7695
AJ1.5	17.8906	26.635	25.5601	25.7495

III. Average mass of organ at time period

	Day	Brain	Muscle	Liver
P(s)	0	0.0174	0.0354	0.0097
	10	0.031	0.1283	0.0682
	30	0.024	0.3958	0.1112
	50	0.0714	1.0056	0.1609
	80	0.081	1.647	0.2489
P(t)	0	0.0174	0.0354	0.013
	10	0.0814	0.2258	0.039
	30	0.026	0.198	0.0627
	50	0.013	0.111	0.014
A(s)	0	0.426	4.5306	0.8029
	10	0.322	5.9639	0.9542
	30	0.448	7.8293	2.2767
	50	0.4806	11.916	2.2316
	80	0.473	12.233	1.972
A(t)	0	0.426	4.5306	0.8029
	10	0.436	8.3567	1.4644
	30	0.401	7.3684	3.9065
	50	0.557	17.43	11.766

IV. Hg concentrations

Shrimp-fed Amberjack

Time	Brain	Muscle	Liver
0	12.0345	64.2327	44.709
10	23.6204	79.2683	62.398
30	54.495	117.2727	100.826
50	74.2353	189.762	146.429
80	63.7103	156	138.571

Tuna-fed Amberjack

Time	Brain	Muscle	Liver
0	12.0345	64.2327	44.709
10	244.9355	409.6721	468.091
30	1381	2662.424	2643.333
50	3682.4	2692.5	2330.526

Shrimp-fed Pompano

Time	Brain	Muscle	Liver
0	45.926	112.0023	103.788
10	80.56	113.99	123.68
30	42.909	96.882	114.044
50	54.068	106.965	94.184
80	66.976	148.961	32.243

Tuna-fed Pompano

Time	Brain	Muscle	Liver
0	45.926	112.0023	103.788
10	540.476	699.412	1487.5
30	1465	1758.919	2930.588
50	2135.714	2846.364	9848