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Inductivley coupled plasma optical emission spectroscopy analysis of heavy metal concentrations in gulf menhaden (Brevoortia patronus) populations in the northern Gulf of Mexico from 2011 to 2012

Hannah Paula Rockett
Louisiana State University and Agricultural and Mechanical College

INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROSCOPY ANAYLSIS OF HEAVY METAL CONCENTRATIONS IN GULF MENHADEN (BREVOORTIA PATRONUS) POPULATIONS IN THE NORTHERN GULF OF MEXICO FROM 2011 TO 2012

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ABSTRACT

In 2010, the Deepwater Horizon (DWH) oil spill, released 4.9 million barrels of oil into the Gulf of Mexico, creating the largest marine oil spill in the history of the U.S. petroleum industry. Trace metals, including those from crude oil, were dispersed in the water column and bound to suspended particulates. As obligate filter-feeding omnivores and a predominate fishery in the Gulf of Mexico, Gulf menhaden (*Brevoortia patronus*) are susceptible to trace metal accumulation. Samples of menhaden were collected at two locations in coastal Louisiana, Grand Isle (GI) and Vermillion Bay (VB), with VB serving as the non-impacted DWH oil spill site and GI as the impacted DWH oil spill site. The analysis of trace metals was performed using inductively coupled plasma-optic emission spectroscopy (ICP-OES) methods. Eleven metals (As, Ba, Cd, Co, Cr, Cu, Fe, Ni, Pb, Zn, V) were chosen and observed by four variables: place (VB or GI), size (small or large), month (July, August or September), and year (2011 or 2012) and the interactions between the variables. Metal concentrations in the current study followed the sequence: Fe > Zn > Ba > As > V > Cr > Cu > Ni > Pb > Co > Cd. Results showed that in 2011, VB had statistically higher concentrations than GI for 9 of the 11 metals. Elevated discharge rates during the Mississippi River Flood of 2011 produced a dilution effect, decreasing the concentration of trace metals in the water column at GI. Size was also significant, with small fish having statistically higher concentrations for 8 of the 11 metals. In fish, younger/smaller fish have higher metabolic activities than older/larger fish, which leads to a higher metal accumulation in smaller fish. Arsenic was the only metal that had higher concentrations in 2011 and in large fish. Differences in arsenic trends may be attributed to the DWH oil spill, which may have caused reduced

adsorption of arsenic by the mineral goethite and increased concentrations of arsenic in the water. Iron and zinc in the current study exceeded the FAO/WHO maximum permissible limit.

1. INTRODUCTION

On April 20, 2010, the Mobile Offshore Drilling Unit Deepwater Horizon, located 45 miles southeast of Venice, Louisiana, exploded and caught fire (OSHA, 2011; US Coast Guard, 2011). NOAA experts estimated a flow rate of 5,000 barrels per day (BPD) leaked from the well, while other scientists estimated as high as 100,000 BPD (US Coast Guard, 2011). For 87 days, over 4.9 million (4,928,100) barrels of oil were released into the Gulf of Mexico (GOM) (Haycox, 2012; US Coast Guard, 2011). After various failed attempts and many response factors, the Macondo well was capped and closed on July 15, 2010 (US Coast Guard, 2011). The Deepwater Horizon (DWH) oil spill incident created the largest marine oil spill in the history of the petroleum industry and the worst man-made environmental disaster in the United States (Haycox, 2012; Kornfeld, 2011; McNutt et al., 2012).

The major constituents of crude oils are organic but also include trace concentrations of inorganics or metals. Analyses have shown that arsenic, cobalt, chromium, copper, nickel, lead, iron, vanadium, and zinc are consistently present in crude oil, with quantities of each metal varying greatly (Erickson et al., 1954; Petroleum HPV, 2003) Trace metals are significant sources of environmental pollution. They are abundant, easily dissolved in and moved by water, and quickly absorbed by aquatic organisms (Hodson, 1988; Jezierska et al., 2009). Some trace metals are beneficial and essential to fish; trace metals like copper, zinc and iron are required for fish metabolism and must be taken up from water, food, or sediment. However, non-essential trace metals, such as cadmium, lead, and mercury are also taken up and can accumulate in tissues (Hodson, 1988; Canli and Atli, 2003). Accumulation of the metals depends on several

factors including, the form of the metal, exposure route, environmental conditions (water temperature, pH, hardness, salinity), intrinsic factors (fish age, feeding habits), metal concentration, and exposure period (Dallinger et al., 1987; Jezierska and Witeska, 2006). Accumulation of trace metals in fish tissues is mainly dependent on water concentrations of metals and exposure periods, but all factors play an important role (Canli and Atli, 2003).

The DWH spill affected living organisms, entire ecosystems, fisheries, and livelihoods of fishermen (Upton, 2011; Kornfeld, 2011). Areas used for spawning, nurseries, and growth were destroyed or covered with oil. Oil in estuaries where many fish including menhaden spend their early life stages, can result in declined health, reduced growth and reproduction, and changes in migration patterns. Similarly, oil suspended in the water column can cause mortality in developing menhaden eggs as well as death in plankton, the main food source for menhaden (Upton, 2011). In the United States, the GOM is host to the largest amount of seafood by volume other than Alaska (Upton, 2011). In 2008, the Gulf commercial fishery landings totaled 1.273 billion pounds with a value of $697 million (NMFS, July 2010). The second largest commercial species by value was menhaden at $64 million, with $45 million of that revenue coming from the state of Louisiana (NOAA, 2010). The DWH oil spill negatively affected the menhaden fishery in 2010, when menhaden landings in Louisiana decreased by 171 million pounds or 17 percent when compared to the same time period in 2009 (Upton, 2011).

Gulf menhaden, *Brevoortia patronus*, commonly known as bunker, mossback, or pogy, are small fish found throughout the GOM, specifically from Sable, Florida to

Veracruz, Mexico (Lassuy, 1983; Reintjes, 1969; Franklin, 2007). Menhaden are obligate, filter-feeding omnivores who swim with their mouths open while filtering particulates within the water, including phytoplankton (Franklin, 2007; Vaughan et al., 2007). Gulf menhaden do not migrate long distances but travel inshore during early spring and offshore in late fall (Pristas et al., 1976; Ahrenholz, 1981). Spawning occurs from October to March and peaks in December and January in offshore waters (Lewis and Roithmayr, 1981). By way of currents, 3 to 5 week old larvae make their way to estuaries and grow rapidly, transforming into juveniles (Christmas et al., 1982; Vaughan et al., 2007). Juvenile gulf menhaden spend their first summer in deeper estuarine waters before journeying into offshore waters in late fall and winter (Christmas et al., 1982). By the spring months, gulf menhaden travel back to inshore waters where they swim in dense schools near the surface (Vaughan et al., 2007). Menhaden oil is rich in Omega-3 fatty acids, an essential fatty acid that humans and all mammals must obtain through diet (Simopoulous, 1991). Omega-3 fatty acids are beneficial to human health and have been shown to reduce the risk of cardiovascular disease and hypertension, decrease stiffness and joint pain associated with arthritis, reduce inflammation, help defend against Alzheimer's Disease and dementia, autoimmune disorders, and cancer (Simopoulos, 1991; Franklin, 2007).

Gulf menhaden are a fundamental part of Louisiana's economy and human health. In 2007, Louisiana led the harvest with 92 percent of the total commercial catch in the United States (Vaughan et al., 2007). Gulf menhaden are also vital prey for several commercial and recreational species of fish (Lassuy, 1983). Therefore, the monitoring of gulf menhaden and their life cycle is essential. As filter feeders and a prey species, gulf

menhaden are an ideal species to evaluate the health of aquatic ecosystems. They have been proven to be useful in monitoring the build-up of pollutants in the aquatic food chain that can lead to adverse effects and death of aquatic organisms (Yousuf and El-Shahawi, 1999; Farkas et al., 2002).

2. LITERATURE REVIEW

2.1 Gulf Menhaden (*Brevoortia patronus*)

Gulf menhaden, *Brevoortia patronus*, commonly known as bunker, mossback, or pogy are small fish that belong to the Clupeidae family (Franklin, 2007). Gulf menhaden are found throughout the Gulf of Mexico, but specifically from Sable, Florida to Veracruz, Mexico (Lassuy, 1983; Reintjes, 1969). Menhaden are obligate filter-feeding omnivores, meaning they swim with their mouths open and filter anything in the water in front of them, such as phytoplankton, through gill rakers (Franklin, 2007; Vaughan et al., 2007). The coloration of the gulf menhaden is excellent camouflage for the forage fish. From above, gulf menhaden have a green-blue tint to help blend the gulf menhaden with ocean waters (Franklin, 2007). From the side, their bodies appear silver with iridescent scales, while their bellies are a lighter shade to help them fade with lighter tones of the sky (Franklin, 2007; Lassuy, 1983). Like other members of the Clupeidae family, gulf menhaden have a deeply forked tail and single dorsal and anal fin (Franklin, 2007). However, there are several features that differentiate gulf menhaden from other fish. One distinct identification feature is a black spot directly behind the gill covering, with smaller spots following (Lassuy, 1983). Other characteristics unique to gulf menhaden include a large head and the absence of teeth in juveniles and adults as stated by Reintjes in 1969.

While gulf menhaden do not necessarily migrate long distances, they do travel inshore during early spring and offshore in late fall (Pristas et al., 1976; Ahrenholz, 1981). Spawning occurs from October to March, but peaks in December and January in offshore waters (Lewis and Roithmayr, 1981). Once laid, the eggs float near the surface

and drift defenselessly with currents (Lassuy, 1983; Vaughan et al., 2007). Within 48 hours, at 15°C, the eggs hatch (Lassuy, 1983). By way of currents, 3 to 5 week old larvae make their way to estuaries and grow rapidly transforming into juveniles (Christmas et al., 1982; Vaughan et al., 2007) (Figure 2.1).

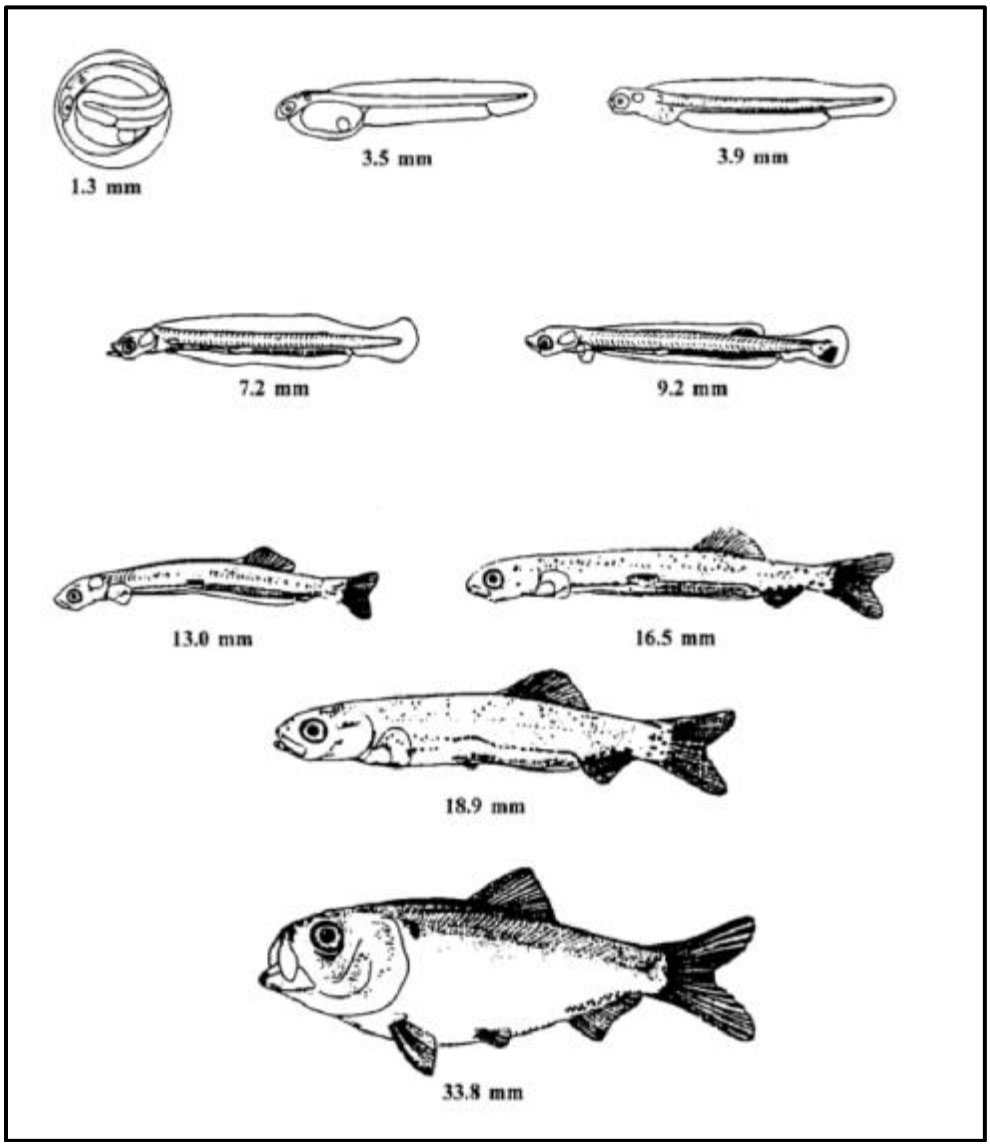

Figure 2.1 Developmental stages of gulf menhaden at specified lengths
Source: Hettler (1984)

Juvenile gulf menhaden spend their first summer in deeper estuarine waters before journeying into offshore waters in late fall and winter (Christmas et al., 1982). By the

spring months gulf menhaden travel back to inshore waters, where they swim in dense schools near the surface (Vaughan et al., 2007) (Figure 2.2).

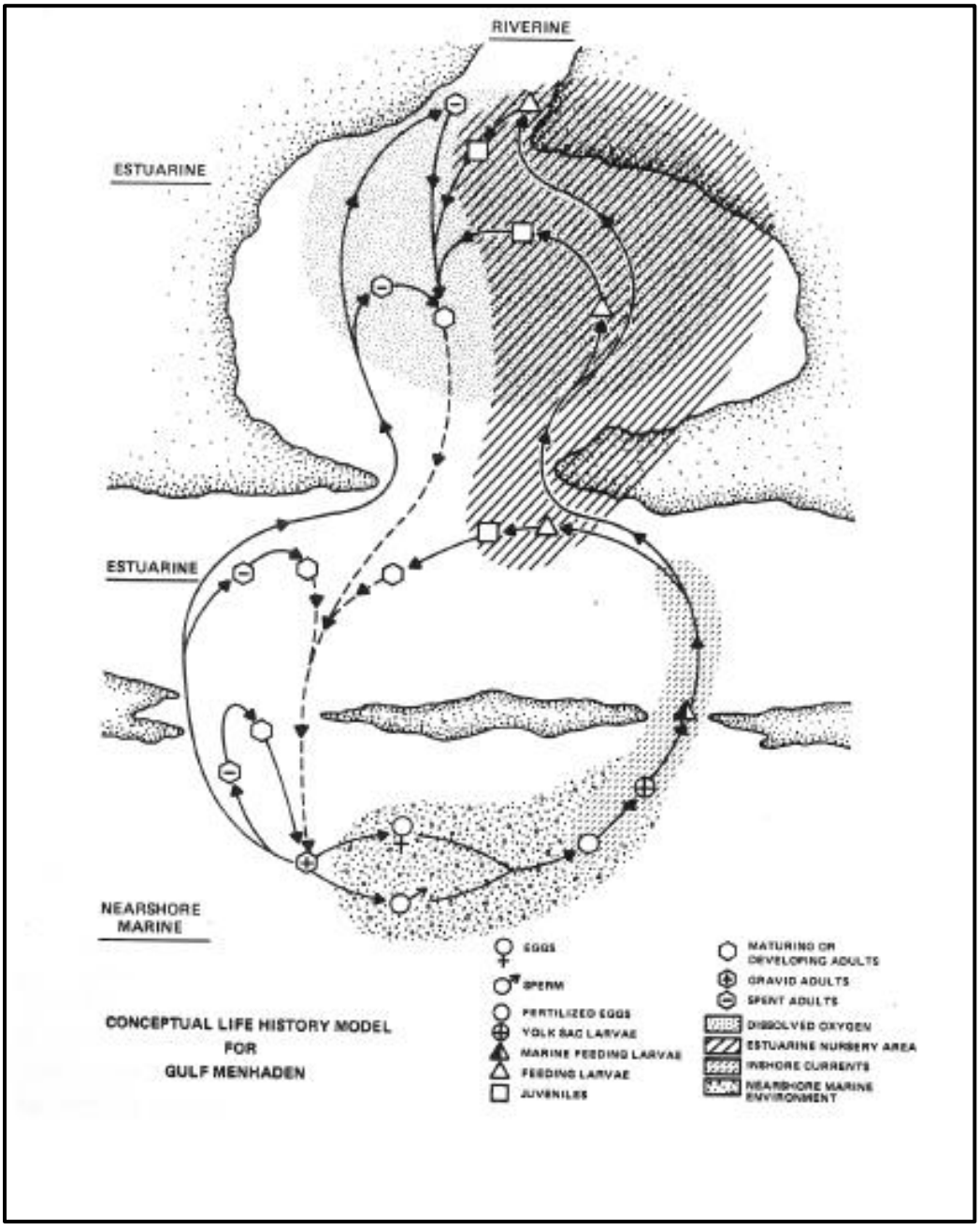

Figure 2.2 Conceptual life history model for gulf menhaden
Source: Christmas et al., (1982)

While gulf menhaden can live up to 5 years of age, most only make it to 3 years of age with an average length of 207 millimeters and weight of 190 grams (Nicholson, 1978;).

The gulf menhaden fishery began in the 1800's, but records were not kept until World War II (Nicholson, 1978; VanderKooy and Smith, 2002). Peak landings occurred during the mid-1980s, with 982,000 metric tons of gulf menhaden being landed in 1984 (VanderKooy and Smith, 2002; NMFS, unpublished data) (Appendix A). In 2004, the gulf menhaden fishery was the second largest, by weight, in the United States, totaling 468,736 metric tons landed annually (NMFS, 2005; Vaughan et al., 2007). In 2007, Vaughan, Shertzer, & Smith determined that Louisiana lead the harvest with 92 percent of the total catch coming from their coastline. The National Marine Fisheries Service's (2012) most recent forecast for gulf menhaden show that in 2011, 613,261 metric tons were landed, a 62 percent increase from 2010 (Figure 2.3). Smaller landings are attributed to the decrease in effort, vessels, and plants operating in the Gulf of Mexico (VanderKooy and Smith, 2002).

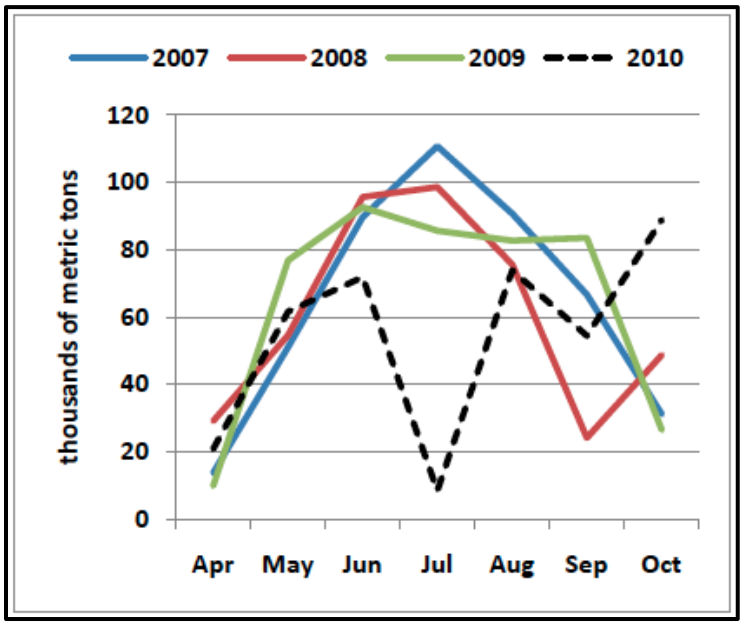

Figure 2.3 Gulf menhaden landings by month from 2007 to 2011
Source: National Marine Fisheries Service (2012)

The gulf menhaden fishery is one of the highest monitored fisheries, regulated by interstate agreement through the Gulf States Marine Fisheries Commission (VanderKooy and Smith, 2002). The gulf menhaden fishery season is specifically outlined to a 28-week period, beginning the third Monday in April through November 1 (Vaughan et al., 2007). In several states, boats using purse-seine nets dominate the gulf menhaden landing. The process begins with the help of a spotter pilot who sights a school of menhaden and directs the vessels toward the fish (NMFS, 2012). Once near the fish, two boats that are sharing a purse seine net begin to move in opposite directions. While moving, they throw out the net and surround the menhaden (Franklin, 2007). The top of the net has floats to keep it near the surface, whereas the bottom has weights to keep it beneath the school of menhaden (Franklin, 2007). The net contains rings along the bottom with a line running through them called a purse-line. Once a circle has been formed, the purse-line is pulled and all rings pull toward one another, trapping the fish (Franklin, 2007). The use of purse-seine nets for menhaden landing have been banned since 1995 and 2003 in Florida and Alabama, respectively; however, the nets are still legal and currently used in Mississippi, Louisiana and Texas (VanderKooy and Smith, 2002).

After the fish are confined and brought onboard, the vessels return to menhaden processing factories (Franklin, 2007). Today, four menhaden reduction factories are active along the Gulf coast with one in Moss Point, Mississippi and three in Louisiana - Empire, Abbeville, and Cameron (NMFS, 2012). There are two options when the menhaden arrive at the processing facility: reduction or bait (VanderKooy and Smith, 2002). In 2002, VanderKooy and Smith stated that, "the reduction fishery greatly overshadowed bait landing with highest totals of 982,000 metric tons (1984) for

reduction compared to 17.3 metric tons for bait (1987)." However, menhaden are still an important bait fish for fishermen (Hale et al., 1991). Reduction of menhaden will generate three products: fishmeal, fish oil and condensed fish solubles (VanderKooy and Smith, 2002). Fishmeal is an important ingredient in pet food, animal food, and aquaculture (Lassuy, 1983). In animal food, fishmeal is valuable due to its high protein content and is used to yield maximum growth rates and increase feed effectiveness in the poultry industry (VanderKooy and Smith, 2002).

Aquaculture feeds represent a developing market for fishmeal (Hale et al., 1991). Other uses of aquaculture feed include: 1) the potential use of menhaden hydrolysate as a milk replacer for calf feeding (Hale and Bauersfeld, 1978), 2) the application of menhaden hydrolysates as fish peptones for the culture of microorganisms (Green et al., 1973), 3) use of menhaden as a component in intermediate-moisture pet foods (Rasekh et al., 1976), and 4) diluted menhaden solubles for use as an emulsion fertilizer for house plants and agricultural crops (Aung et al., 1984).

Marine oils constitute just over 2 percent of the world production of fats and oils (Hale et al., 1991). In 1989, menhaden oil production was 0.10 million tons or $23.2 million in value (NMFS, 1990). Menhaden oil accounted for 97 percent of total fish oil production in the United States. The amount of menhaden oil produced each year varies due to natural variations in the abundance of stocks and the oil content of menhaden. The average annual menhaden oil production for the 10-year period of 1980-89 was 0.132 million tons (Hale et al., 1991; VanderKooy and Smith, 2002; NMFS, unpublished data) (Appendix B). Almost all menhaden oil is exported and competes in the internal marketplace, due to the past ban of menhaden oil being used for general use in foods in

the U.S. by the FDA (VanderKooy and Smith, 2002). In 1998, the United States exported 88 percent of total production of fish oil, with four countries receiving 91 percent of the total exports: Netherlands, Canada, Japan, and Norway (USDOC, 1999; VanderKooy and Smith, 2002) (Appendix C).

Menhaden oil has been incorporated into edible products in Europe for years and has recently been approved for use in the United States (VanderKooy and Smith, 2002). In 1997, the FDA affirmed that menhaden oil was generally recognized as safe (GRAS) as a direct human food ingredient with specific limitations (Substances Affirmed as GRAS: Menhaden Oil, 1997; VanderKooy and Smith, 2002). To produce menhaden oil, the entire fish is cooked at 96˚C for 8 to 10 minutes in a steam cooker. This allows the protein to coagulate and rupture the fat cells. Next, the cooked fish is pressed and the liquid is centrifuged to divide the oil and aqueous phases (Substances Affirmed as GRAS: Menhaden Oil, 1997; VanderKooy and Smith, 2002; Menhaden Oil, 2009). The crude oil must then be refined further for human consumption. The process begins when the crude oil is chilled and the solid fraction is filtered, a step called winterization. Following winterization, the free fatty acids are neutralized and removed by alkalai refining. The final steps include bleaching to reduce the color and deodorization to remove the odor causing bodies (Substances Affirmed as GRAS: Menhaden Oil, 1997; ENVIRON, 1999; Menhaden Oil, 2009) (Appendix D). Once refined, the oil is blended with other fats for cooking oils, shortening, margarine and other products (Dubrow et al., 1976; VanderKooy and Smith, 2002; Menhaden Oil, 2009) (Table 2.1). Menhaden oil is also used in other products such as paints, plastics, resins, cosmetics and fertilizers (VanderKooy and Smith, 2002). Because of its special properties, some menhaden oil is

still sold in the United States for certain industrial applications. Some of the industrial products containing menhaden oil include: protective coatings, lubricants, printing inks, carriers of insecticides, caulks and sealants, surfactants, plasticizers, and leather treatment agents (VanderKooy and Smith, 2002).

Table 2.1 Maximum level of menhaden oil in food as served
Source: modified from Menhaden Oil (2009)

Category of Food	Maximum Level of Use in Food (as Served)
Baked goods, baking mixes	5.0 percent
Cereals	4.0 percent
Cheese products	5.0 percent
Chewing gums	3.0 percent
Condiments	5.0 percent
Confections, frostings	5.0 percent
Dairy product analogs	5.0 percent
Egg products	5.0 percent
Fats, oils, but not in infant formula	12.0 percent
Fish products	5.0 percent
Frozen dairy desserts	5.0 percent
Gelatins, puddings	1.0 percent
Gravies, sauces	5.0 percent
Hard candy	10.0 percent
Jams, jellies	7.0 percent
Meat products	5.0 percent
Milk products	5.0 percent
Nonalcoholic beverages	0.5 percent
Nut products	5.0 percent
Pastas	2.0 percent
Plant protein	5.0 percent
Poultry products	3.0 percent
Processed fruit juices	1.0 percent
Processed vegetable juices	1.0 percent
Snack foods	5.0 percent
Soft candy	4.0 percent
Soup mixes	3.0 percent
Sugar substitutes	10.0 percent
Sweet sauces, toppings, syrups	5.0 percent
White granulated sugar	4.0 percent

Menhaden oil consists mainly of triglycerides, which are esters of glycerol and fatty acids with chains of 14 to 22 carbon atoms and small amounts of monoglycerides

and diglycerides (ENVIRON, 1999). Menhaden oil is different than edible vegetable oils and animals fats due to its high proportion of polyunsaturated fatty acids with 4, 5 and 6 double bonds (about 25 percent by weight). These polyunsaturated fatty acids in menhaden are C18:4 (2.3 percent), C20:4 (2.0 percent), C20:5 (13.1 percent), C22:5 (2.5 percent) and C22:6 (6.7 percent). (The first number refers to the total number of carbon atoms in the fatty acid; the second number refers to the total number of double bonds) (Substances Affirmed as GRAS: Menhaden Oil, 1997). Menhaden oil is also comprised of about 33 percent saturated fatty acids and 31 percent monounsaturated fatty acids (Substances Affirmed as GRAS: Menhaden Oil, 1997; ENVIRON, 1999). Eicosapentaenoic acid or EPA (C20:5) and docosahexaenoic acid or DHA (C22:6) are the major sources of omega-3 (ω3) fatty acids in menhaden oil. Menhaden oil is rich in ω3 fatty acids, an essential fatty acid that humans, and all mammals, cannot make and must obtain through diet (Simopoulous, 1991).

Omega-3 fatty acids are beneficial in many ways to human health and have been shown to reduce the risk of cardiovascular disease (CVD) and hypertension, decrease stiffness and joint pain associated with arthritis, reduce inflammation, help defend against Alzheimer's Disease and dementia, autoimmune disorders, and cancer (Simopoulos, 1991; Franklin, 2007; Glick and Fischer, 2013) . Evidence from the National Heart, Lung and Blood Institute study, suggests that the daily dietary intake of 0.5 to 1.0 grams of long chain ω3 fatty acids per day reduces the risk of cardiovascular death in middle-aged American men by about 40 percent. Atherosclerosis, which is associated with cardiovascular disease, is a condition where artery walls become clogged (Sperling et al., 1987; Jump et al., 2012). Omega-3 fatty acids have been shown to aid with

atherosclerosis by inhibiting the production of platelet activating factor (PAF). PAF activates platelets, which in turn leads to atherosclerosis (Sperling et al., 1987; Simopoulous, 1991) (Table 2.2). In clinical trials, EPA and DHA, in the form of fish oil along with antirheumatic drugs was shown to improve joint pain in patients with rheumatoid arthritis (Kremer et al., 1989; Robinson and Kremer, 1991; Goldberg and Katz, 2007).

Table 2.2 The effects dietary ω3 fatty acids have on the factors and mechanisms involved in the development of inflammation, atherosclerosis, and immune diseases
Source: modified from Simopoulous (1991); Simopoulous et al. (1991)

Reduce or inhibit risk and/or precipitating factors
Arachidonic acid
Platelet aggregation
Thromboxane A_2 formation
Monocyte and/or macrophage function
Leukotriene formation (LTB_4)
Formation of platelet activating factor (PAF)
Toxic oxygen metabolites
Interleukin 1 formation (IL-1)
Formation of tumor necrosis factor (TNF)
Platelet-derived growth factor-like protein (PDGF)
Intimal hyperplasia
Blood pressure and/or blood pressure response
Very-low density and low-density lipoproteins (VLDL, LDL)
Triglycerides
Lipoprotein (a) [Lp(a)]
Fibrinogen
Blood viscosity
Increase beneficial and/or protective factors
Prostacyclin formation (PGI_1 + PGI_3)
Leukotriene B_3 (LTB_3)
Interleukin 2 (IL-2)
Endothelial-derived relaxing factor (EDRF)
Fibrinolytic activity
Red-cell deformability
High-density lipoprotein (HDL)

The anti-inflammatory effects of ω3 fatty acids function by inhibiting the 5-lipoxygenase pathway, the source for proinflammatory leukotrienes, in neutrophils and

monocytes and inhibiting the leukotriene B_4 (LTB_4), whose function is to promote inflammation (Lee et al., 1985; Kremer et al., 1987; Afman and Müller, 2012). For diseases associated with inflammation, such as asthma and ulcerative colitis, ω3 fatty acids are potential therapeutic agents (Simopoulos, 1991; Goldberg and Katz, 2007). Omega-3 fatty acids were also shown to successfully benefit patients with psoriasis. When used in combination with etretinates, it effectively lowered the hyperlipidemia caused by that drug and decreased the nephrotoxicity of cyclosporine, a side effect (Allen, 1991). Analysis of brain cortical regions displayed that patients with Alzheimer disease or mild cognitive impairment displayed 14 percent lower DHA in the mid-frontal cortex and 12 percent lower in superior temporal cortex (Tan et al., 2012). It was found that DHA (900 mg/day) resulted in improved-verbal recognition-memory scores; however not on working memory or executive function (Yurko-Mauro et al., 2010). In cancer studies, results have consistently shown that ω3 fatty acids delayed tumor appearance and decreased both the rate of growth and the size and number of tumors (Fernandes and Venkatraman, 1991; Cave, 1991; Greene et al., 2011). Furthermore, it was shown that ω3 fatty acids decreased PGE_2 production, which is overproduced in tumors and aids in the progression of cancer by promoting angiogenesis and metastasis (Karmali, 1989). In other studies containing human breast-cancer cells in nude mice, it was shown that mice fed ω3 fatty acids had less pulmonary metastases, reduced serum estrogen and prolactin concentrations, less PGE_2 in the tumor, and reduced *c-myc* oncogene mRNA concentrations in the tumor-tissue cells (Fernandes and Venkatraman, 1991). Based upon clear evidence, it is evident that DHA is essential for the normal functional development of the retina and brain, predominantly in premature infants

(Bazan, 1989; Martinez, 1989). A new area for ω3 fatty acids is developing as scientists look at the effects of adding ω3 fatty acids to drug treatments, which may have a synergistic effect (increasing the effects of drugs) or decreasing their toxicity (Simopoulos, 1991).

Omega-3 fatty acids are an important part of the human diet and health. For this reason, the following recommendations were made by scientists: 1) omega-3 fatty acids should be included in all infant formula and diets of pregnant women, premature infants, full-term infants, children, young adults and elderly adults for normal growth, development and overall human health (Martinez, 1989; Simopoulous, 1991; Brenna and Diau, 2007), 2) increased intake of fish or fish oils may be necessary over and above the amount determined for their essentiality, particularly in those who have a family history or other evidence of susceptibility to coronary heart disease, hypertension, arthritis, psoriasis, and cancer (Simopoulous, 1991; Mozaffarian and Wu, 2012), and 3) omega-3 fatty acids are potentially valuable as additional treatment to some diseases (Simopoulous, 1991).

Overall, gulf menhaden are a fundamental part of Louisiana's economy and human health. The gulf menhaden fishery is largest in the Gulf of Mexico with 420,706 metric tons being brought in annually (VanderKooy and Smith, 2002). This fishery provides many jobs and brings commerce into Louisiana. Gulf menhaden are also vital prey for several commercial and recreational species of fish (Lassuy, 1983). Furthermore, gulf menhaden oil is beneficial to human health. Therefore, the monitoring of gulf menhaden and their life cycle is essential.

2.2 Deepwater Horizon Oil Spill

On the evening of April 20, 2010, the Mobile Offshore Drilling Unit Deepwater Horizon, located 45 miles southeast of Venice, Louisiana, exploded and caught fire (OSHA, 2011; US Coast Guard, 2011). The fire continued to burn fiercely for 36 hours, until the Deepwater Horizon rig sank on April 22, 2010 (US Coast Guard, 2011). As the rig sank, it dragged the underwater riser, a pipe that carries oil from the well head to the rig, 5,000 feet below the ocean's surface to the ocean floor (US Coast Guard, 2011). The riser bent and twisted as it fell, and resulted in subsea leaks from three locations in the bent riser (OSHA, 2011). NOAA experts estimated a flow rate of 5,000 barrels per day (BPD) leaked from the well, while other scientists estimated as high as 100,000 BPD (US Coast Guard, 2011). For 87 days, over 4.9 million (4,928,100) barrels of oil were released into the Gulf of Mexico (Haycox, 2012; US Coast Guard, 2011). After various failed attempts and many response factors, the Macondo well was capped and closed on July 15, 2010 (US Coast Guard, 2011). Analysts and experts maintain that the Deepwater Horizon (DWH) oil spill was avoidable, with the National Commission stating, "The Deepwater Horizon blowout, explosion and oil spill did not have to happen," in the final report to the president on January 11, 2011 (Haycox, 2012). This incident created the largest marine oil spill in the history of the petroleum industry and the worst man-made environmental disaster in the United States (Haycox, 2012; Kornfeld, 2011; McNutt et al., 2012).

The DWH spill affected living organisms, entire ecosystems, fisheries and livelihoods of fishermen (Upton, 2011; Kornfeld, 2011). Areas used for spawning, nurseries, and growth were destroyed or covered with oil. Having oil in estuaries, where

many fish, including menhaden, spend their early life stages, can result in declined overall health, reduced growth and reproduction and changes in migration pattern. Similarly, having oil suspended in the water column can cause mortality of plankton, a food source for menhaden, and eggs (Upton, 2011). The DWH spill not only affected individual fish species, but also other species due to ecological interactions. Immediate damages occurred when 12 days following the explosion and spill, NOAA closed 6,817 square miles of federal and state waters to fishing to ensure seafood safety (Upton, 2011). However, greater damages were yet to come when 88,522 square miles or nearly 37 percent of federal waters in the Gulf of Mexico were closed to fishing by June 2, 2012 (Upton, 2011). Teams of analysts also found that 1,053 total linear miles of shoreline were oiled (Upton, 2011). In the United States, the Gulf area produces the greatest amount of seafood by volume, other than Alaska (Upton, 2011). In 2008, the Gulf commercial fishery landings totaled 1,273 million pounds with a value of $697 million (NMFS, July 2010). The second largest commercial species by value was menhaden at $64 million, with $45 million of that revenue coming from Louisiana (NOAA, 2010) (Table 2.3). The DWH spill has negatively affected the menhaden fishery of 2010, when menhaden landings in Louisiana decreased by 171 million pounds or 17 percent when compared to the exact same time period in 2009 (Upton, 2011).

Other than fisheries, livelihoods were also affected. Upton states that, "The Gulf states supported over 213,000 full and part-time jobs with related income impacts of $5.5 billion" (NMFS, April 2010). Without commercial fishing waters open, fishermen were unable to harvest seafood. Not only did fishermen lose earnings, processors, distributors and buyers also suffered. Many distributors were forced to sell alternative products from

other areas. Many suppliers from the Gulf region became worried that recovering markets would be challenging to restore (Upton, 2011).

Table 2.3 Commercial landings and revenue (in thousands) for major species in the Gulf of Mexico region by state
Source: modified from NOAA, 2010; NMFS, July 2010

State	Total Landings and Revenue		Revenue for Major Commercial Species			
	Landings	Revenue	Shrimp	Menhaden	Oysters	Blue Crab
West	58,643	$ 162,182	$ 23,265	$ 15	$ 5,473	$ 3,300
Alabama	24,534	$ 44,234	$ 38,355	$ 59	$ 243	$ 1,533
Mississippi	201,822	$ 43,697	$ 17,146	$ 18,534	$ 6,869	$ 447
Louisiana	915,956	$ 272,857	$ 130,623	$ 45,768	$ 38,852	$ 32,185
Texas	72,469	$ 174,621	$ 157,182	0	$ 8,835	$ 2,341
Totals	1,273,424	$ 697,591	$ 366,571	$ 64,376	$ 60,272	$ 39,806

Another major concern was consumer trust in Gulf seafood. A study, conducted by the marketing research company MRops, found that of the people polled 70 percent of buyers had some level of apprehension about the safety of seafood from the Gulf of Mexico. Furthermore, 23 percent decreased their intake following the DWH spill (Upton 2011; McGill 2011). A protocol was put in place by the FDA, NOAA and coastal states to determine if areas were able to be re-opened to fishing. The protocol included samples of species passing sensory and chemical analyses to guarantee the seafood was safe for human consumption (Upton, 2011; Ylitalo et al., 2012). The sensory portion entailed an expert panel inspecting the edible portions of the samples for oil and dispersants by odor and taste (Upton, 2011). Once all the samples for a given area passed the sensory test, chemical analysis was performed on additional samples to check for polycyclic aromatic hydrocarbons (PAHs) and dispersants. If a sample from an area passed both tests, the area was deemed safe for fishing and could be re-opened. However, some have critiqued the protocol by saying the number of toxic substances being tested was too small and the

setting of PAH levels of concern should have included further factors (Marshall, 2010). In a congressional testimony on August 19, 2010 FDA officials said:

> To date all samples have passed sensory testing for oil or dispersants and, as with the surveillance sampling, the results of all chemical analyses have shown PAH levels well below the levels of concern, usually by a factor of 100 to 1,000 below those levels, essentially at the same level as were seen before the spill (U.S. Congress, House Committee on Energy and Commerce, Subcommittee on Energy and Environment, 2010).

While immediate damages and injuries were alarming, perhaps long-term harm was of greater concern. Another alarming factor was that while seafood samples were being tested for PAHs, they were not being tested for trace metals.

2.3 Crude Oil and Trace Metals

The petroleum industry dates back to the 1800s when crude oil and tar were first used for waterproofing and medical purposes. Then in the 1850s, when it was discovered that crude petroleum could be distilled to make kerosene for lighting, the industry began to expand. The requirement for gasoline and diesel fuel, thanks to the creation of the internal combustion engine, helped to establish the petroleum business (Petroleum HPV, 2003). Crude oils are formed over millions of years when the remains of tiny marine plants and animals descend to the sea floor, and become submerged with mud and silt. Layers upon layers build upon one another, resulting in high pressures and temperature on the remains. This causes a chemical transformation to hydrocarbons and other crude oil components (Petroleum HPV, 2003).

Crude oil is a combination of several compounds, typically four key hydrocarbon groups; saturates, aromatics, asphlatenes and NSO compounds. Saturates are hydrocarbons made up of straight chains of carbon atoms. Aromatics are hydrocarbons made up of rings of carbon. Asphlatenes are compound polycyclic hydrocarbons that

encompass complex carbon rings; while NSO compounds are generally nitrogen, sulfur and oxygen (Hardaway et al., 2004). The saturate fraction is the largest for most crude oils and is composed of two subgroups: paraffins, simple straight-chain hydrocarbon groups, and isoprenoids, hydrocarbon chains with branches (Hardaway et al., 2004). While the major constituents of crude oils are organic, there are trace concentrations of inorganics or metals. These metals and inorganic elements range from subparts per billion (ppb) to tens and hundreds of parts per million (ppm) (Hardaway et al., 2004). Analyses have shown that cobalt, chromium, copper, nickel, lead, iron, vanadium and zinc are consistently present in crude oil, with quantities of each metal varying greatly (Erickson et al., 1954; Petroleum HPV, 2003). However, studies have shown that nickel and vanadium are the most abundant (Erickson et al., 1954; Hardaway et al., 2004). A study performed by Marathon Ashland Petroleum LLC in 1994, showed levels of nickel and vanadium were 7.7 and 11.0 ppm, respectively, in a high quality Light Louisiana Sweet Crude (Petroleum HPV, 2003). Even though some of these metals are removed during the refining process, these metals can enter the environment through accidental spills, such as the DWH spill (Petroleum HPV, 2003; Hardaway et al., 2004).

2.4 Trace Metals in Fish

Trace metals are significant sources of environmental pollution. They are abundant, easily dissolved in and moved by water, quickly absorbed by aquatic organisms, and tightly bound by sulfhydryl groups of proteins (Hodson, 1988; Jezierska et al., 2009). Some trace metals are beneficial and essential to fish. For example, trace metals like copper, zinc and iron are required for fish metabolism and must be taken up from water, food or sediment. However, non-essential trace metals, such as cadmium,

lead and mercury are also taken up and can accumulate in their tissues (Hodson, 1988; Canli and Atli, 2003). Accumulation of the metals depends on several factors: form of metal, exposure route, environmental conditions (water temperature, pH, hardness, salinity), intrinsic factors (fish age, feeding habits), metal concentration, and exposure period (Dallinger et al., 1987; Jezierska and Witeska, 2006). While some studies have shown that accumulation of trace metals in fish tissues is mainly dependent on water concentrations of metals and exposure periods, all factors play an important role (Canli and Atli, 2003). In fish tissue metal concentrations follow the ranking: Fe > Zn > Pb > Cu > Cd > Hg. Levels of zinc can be very high with over 300 µg/g d. w., while cadmium is accumulated in low amounts, below 1 µg/g d. w. (Jezierska and Witeska, 2006).

The form of the metal during exposure can greatly alter where metals accumulate and how quickly or harmful the metal will be to the fish (Dallinger et al., 1987; Jerzierska and Witeska, 2006). Metals exist in water as one of two forms, particulate or soluble. The soluble form consists of unbound and bound fractions. The unbound metal compounds are the most toxic to fish and contain several ionic forms of different accessibility to fish (Jezierska and Witeska, 2006). For metal concentration, generally the higher the metal concentration in the water, the more metals are taken up and accumulated by the fish. The relationship between body metal level and waterborne concentration is only related if the metal is taken up by the fish from the water. This relationship is not certain if the source of metal is from food (Jezierska and Witeska, 2006).

Metals can enter a fish's body in three routes: the body surface, the gills and the digestive tract (Dallinger et al., 1987; Jezierska and Witeska, 2006). The uptake of trace metals through the skin needs more examination; however, it is expected that the body

surface of the fish is resistant to harmful substances in the surrounding water (Dallinger et al., 1987). Several studies have reported that mucus secretion may inhibit trace metals from entering the body of fish (Varanasi and Markey, 1978; Lock and Van Overbeeke, 1981; Eddy and Fraser, 1982; Pärt and Lock, 1983). The gills of fish play a key role in the uptake of trace metals. Gills are the main organs of gas exchange and are a vital site for the uptake of essential and non-essential metal ions from the water (Chartier, 1974; Fenwick and So, 1974; Dallinger et al., 1987). In an experiment performed by Pärt and Svanberg (1981), it was revealed that cadmium is taken up by perfused gills of rainbow trout and that a substantial uptake happens immediately after exposure. Once absorbed in the gills, the metals are spread throughout the whole body, accumulating in specific organs (Dallinger et al., 1987). Trace metals have also been shown to induce harmful changes in gill morphology (Baker, 1969; Skidmore, 1970; Van der Putte et al., 1981; Karlsson-Norrgren et al., 1985). Metals enter the digestive tract of fish when suspended matter, sediments and organisms serving as food sources are contaminated with trace metals (Dallinger et al., 1987). Pollution of aquatic systems has led to metal contamination of the food for several fish species (Hardistry et al., 1974; Prosi, 1983; Anderson et al., 1978; Heyraud and Cherry, 1979; Van Hassel et al., 1980).

There are several environmental conditions that can modify the uptake and accumulation of trace metals in fish. Water temperature has shown to increase the rate of uptake of certain metals with increasing water temperature (Jezierska and Witeska, 2006). Kock et al. (1996) indicated that *Salvelinus alpinus*, or Arctic char, higher uptake rates of cadmium and lead occurred during the summer when water temperature was higher. Furthermore, it was presented by Douben (1989b) that the rate of uptake and

elimination of cadmium by *Noemacheilus barbatulus* increased with water temperature, with a stronger effect on absorption than on elimination. Water temperature can also cause differences in where the metals accumulate in the fish (Jezierska and Witeska, 2006). Yang and Chen (1996) demonstrated that higher water temperatures stimulate accumulation of cadmium in the kidneys and liver. The reason for increased accumulation of metals by fish at higher temperatures is most likely due to a higher metabolic rate, resulting in a higher rate of metal uptake and binding (Jezierska and Witeska, 2006).

Water acidification or pH is another environmental condition that affects metal accumulation rates in fish (Jezierska and Witeska, 2006). Data comparing concentrations of cadmium, lead and zinc in fish from various lakes, indicated that fish from acidified lakes had significantly higher levels of cadmium and lead, but not zinc (Haines and Brumbaugh, 1994). Jezierska and Witeska (2006) stated, "we may conclude that water acidification affects bioaccumulation of metals by the fish in an indirect way, by changing solubility of metal compounds or directly, due to damage of epithelia which become more permeable to metals, and on the other hand, competitive uptake of H^+ ions may inhibit metal absorption."

Water hardness (primarily calcium concentration) affects the uptake of metals in fish, mainly in the gill epithelium (Jezierska and Witeska, 2006). Several studies have reported that water enhanced with calcium led to a reduced copper accumulation in the gills of fish (Playle et al., 1992). Baldisserotto et al. (2005) indicated that fish were protected against dietary and waterborne cadmium uptake due to an elevated diet in Ca^{2+}. In 2000, Barron and Albeke reported that zinc uptake in *Oncorhynchus mykiss*, or

rainbow trout, was reduced by calcium. Calcium competing with other metals for binding sites on the gill surface may be an explanation as to why calcium reduces the uptake of metals in fish and was presented by Pagenkopf (1983).

Like water hardness, salinity also decreases the uptake and accumulation of metals by fish (Jezierska and Witeska, 2006). A study performed with *Platichthys flessus*, showed that the fish that adapted to seawater displayed lower copper concentration than those adapted to freshwater (Stagg and Shuttleworth, 1982). Consistent with that study, Somero et al. (1977) reported that the rate of lead accumulation by *Gillichthys mirabilis* was inversely proportional to the salinity of the medium.

Intrinsic factors such as age of the fish and feeding habits can considerably change the amount of metal that is accumulated or taken up by the fish (Jezierska and Witeska, 2006). For example, predatory fish species were shown to accumulate more mercury than benthivores. Kidwell et al. (1995) reported that benthivores contained more cadmium and zinc (Kidwell et al., 1995; Voigt 2004). Similarly, Ney and Van Hassel (1983) found that lead and zinc levels were higher in benthic fish. Metal accumulation can differ between species in the same water body due to living and feeding habits (Jezierska and Witeska, 2006).

Age and size of the fish also play an important role in accumulation and tissue concentrations (Jezierska and Witeska, 2006). Tissue concentrations for most metals (except mercury) are typically inversely related to the age and size of the fish (Jezierska and Witeska, 2006). According to De Wet et al. (1994) measurements of bioaccumulation of iron, manganese, zinc, copper, nickel and lead by *Pseudocrenilabrus philander* exhibited an inverse relationship between metal concentrations and body mass of the fish.

An inverse relationship between the age of the fish and metal concentrations were found in two similar studies, with lead by Allen-Gill and Martynov (1995) and with zinc, lead, cadmium and nickel by Ney and Van Hassel (1983). The youngest fish displayed the highest concentrations of metals, with zinc having the greatest differences (Jezierska and Witeska, 2006). Furthermore, Canli and Atli (2003) indicated negative relationships between fish length and metal concentrations for chromium, lead and copper.

2.5 Trace Metal Effects on Early Development

Waters that are polluted with trace metals can affect physiological processes in fish, including breeding and development, which can cause a reduction of offspring quantity and quality. Early developmental stages, such as the formation of eggs, hatching, larval development and juvenile growth are especially sensitive to water pollution (Heath, 1987; Jezierska et al., 2009). Exposure to metals during spawning has been shown to result in contamination of eggs and sperm, and negatively affect fish fertility and embryonic development (Jezierska et al., 2009) (Table 2.4). Miller et al. (1992) observed that white sucker, *Catostomus commersoni,* from polluted water exhibited higher concentrations of copper and zinc in the testes and ovaries than fish from non-polluted sites. In a laboratory study, Allen (1995) exposed *Oreochromis aureus* to cadmium and lead for a week. The results showed metal deposition in the testes and ovaries, with the ovaries having higher concentrations of metals, specifically cadmium. Other studies have indicated that metals may also affect spermatozoa motility time, which is crucial for successful fertilization (Jezierska et al., 2009). Sarnowska et al. (1997) reported a concentration-related decrease in motility time of *Ctenopharyngodon idella* spermatozoa when exposed to copper and lead.

Table 2.4 The effects of metals on early stages of fish development
Source: modified from Jezierska et al. (2009)

The effects of trace metals during various stages of fish embryonic development
I. Swelling, cleavage, blastula, gastrulation • Reduced swelling (reduced space for developing embryo) • Abnormal cleavage, blastula malformation • Malformation of embryos • Death of embryos
II. Organogenesis • Reduced metabolic rate, and development rate • Disturbed organogenesis • Malformation of embryos • Death of embryos
III. Hatching • Inhibited hatching gland development • Altered hatching rate (premature or delayed hatching) • Malformation of embryos • Death of unhatched embryos • Death of newly hatched larvae
Consequences • **Reduced hatchability** • **Increased in abnormality of newly hatched larvae** • **Reduced body size of larvae** • **Reduced survival of larvae**

Trace metals affect various stages during embryonic development, including swelling, cleavage, blastula, and gastrulation (Jezierska et al., 2009). During embryonic development, fish eggs swell because the perivitelline space, containing a colloidal suspension of protein secreted by the vitelline membrane, causes water absorption (Peterson and Martin-Robichaud, 1982). Metal ions are able to enter into the egg and change chorion structure and permeability when the egg shell is still highly permeable (Jezierska et al., 2009). Studies performed by Jezierska et al. (2009) on *Cyprinus carpio* eggs found that copper, cadmium, and lead reduced swelling compared to control groups. Eggs that are properly swollen allow the embryo to change its position every 5 to 10 seconds, while eggs that do not swell enough are too small. This does not allow the

embryos to move and may result in hatching of abnormal larvae (Korwin-Kossakowski, 1996). Abnormal cleavage was indicated in *Cyprinus carpio* eggs treated with lead, copper or cadmium by Jezierska et al. (2009). Other results showed uneven or irregularly distributed blastomeres, sometimes with the entire blastula deformed. Furthermore, Chow and Cheng (2003) concluded that the critical period of cadmium exposure of *Danio rerio* was the gastrulation period. They determined that the developmental defect that alters axial curvature results from defects in myotomes of the somites and the gastrulation period precedes the formation of the somites. In a study with the same species and metal, embryo malformations were seen as blastodermal lesions and exogastrulation in the first 48 hours (Hallare et al., 2005). The highest mortality of embryos occurred during the first 24 hours after fertilization, with the maximum occurring at the stage of blastula formation (Slomińska, 1998; Ługowska, 2005). Studies performed by Ługowska (2005) indicated that after exposure to highly toxic copper, embryos died mostly at the stage of blastula (>25%) and body segmentation (>15%).

Another stage of embryonic development that trace metals may affect is organogenesis, the period from body segmentation to hatching. This may include reduced metabolic rate and development rate, disturbed organogenesis, malformation of embryos, and even death of embryos (Jezierska et al., 2009). Studies performed with *Cyprinus carpio* embryos showed developmental retardation at the stage of eye pigmentation, when exposed to copper or lead (0.2 mg dm^{-3} and 2 mg dm^{-3}, respectively) (Ługowska and Jezierska, 2000; Ługowska, 2005). Futhermore, Ługowska and Jezierska (2000) found that lead shortened the entire embryonic development time. Malformations of embryos were observed by Jezierska et al. (2009) in *Cyprinus carpio* treated with lead, copper or

cadmium. The most common malformations perceived were craniofacial anomalies, yolk sac malformation, vertebral shortening and curvatures, and cardiac malformations (Jezierska et al., 2009). Embryo mortality is much lower during organogenesis, with Ługowska (2005) showing that during organogenesis most embryos (>5%) die before the stage of eye pigmentation.

The final stage of embryonic development that trace metals disrupt is hatching (Jezierska et al., 2009). Prior to hatching, fish embryos develop hatching glands located on the head. The glands generate chorionase, the enzyme required to breakdown the egg shell during hatching. Waterborne metals have been shown to affect the development and functioning of these glands (Jezierska et al., 2009). Kapur and Yadav (1982) observed disturbances of transcription and translation from metals, which resulted in reduced synthesis of proteins, including chorionase. Furthermore, Witeska et al. (1995) found that when common carp embryos were incubated in water containing 0.05 mg dm^{-3} of cadmium, the effect of metals was most prominent during the hatching process, where hatching was inhibited or accelerated by trace metals. Upon hatching, Hallare et al. (2005) demonstrated that exposure to cadmium caused *Danio rerio* embryos numerous malformations including: acute heart and head edema, weak pigmentation, helical bodies, hooked tail, tail degeneration, blistering of fins, immobilization and abnormal body posture. Structural and functional disturbances during embryonic development led to a reduced number of hatched larvae, with Słomińska (1998) revealing that lead and copper caused significantly elevated (8%) mortality of newly hatched larvae. Similarly, Cleveland et al. (1986) observed that the embryos of *Salvelinus fontinalis* incubated in acidified, Al-containing water were unable to hatch, with 50 percent of them "incomplete

hatch" and no chance of survival.

Intoxication with trace metals during embryonic development can have some severe consequences on fish. Some of those include: reduced hatchability, increased abnormality of newly hatched larvae, reduced body size of larvae, and reduced survival of larvae. The first period of embryonic development, just after fertilization and most likely the period of hatching, are the most susceptible to metal intoxication. Therefore, several disturbances induced by trace metals during early development of fish cause a reduced number and quality of larvae (Jezierska et al., 2009).

2.6 Trace Metal Toxic Mechanisms in Fish

The key mechanisms of toxic action of trace metals are correlated to the osmotic disturbances and alterations of enzyme synthesis and activity (Jezierska et al., 2009). Cadmium causes a reduced level of calcium in the organism (Sauer and Watabe, 1988; Verbost et al., 1989) by means of reducing Ca^{2+}-ATPase activity and disturbing calcium uptake (Reddy et al., 1988; Wong and Wong, 2000). Whereas, copper affects sodium and chloride actions and concentrations (Pelgrom et al., 1995; Sloman, 2003) by altering the Na^{+}/K^{+}-ATPase activity, causing osmoregulatory failure (Grosell et al., 2004). Another mechanism of action that metals use is binding to the sulfur groups (-SH) of proteins, cysteine and glutathione. This causes a change in the structure and enzymatic activities of the proteins, leading to an inhibition in the function of these biomolecules (Hodson, 1988; Jezierska et al., 2009). An example of sulfhydryl binding is when cadmium and lead bind to calmodulin, a sensor protein of free calcium, which affects many different cellular functions (Behra, 1993). There are several trace metals that are known to disrupt the activity of various enzymes (Jezierska et al., 2009). For instance, cadmium reduces

the activity of various enzymes of oxidative metabolism: citrate synthase (Couture and Kumar, 2003), succinate dehydrogenase (SDH), glucose-6-phosphate dehydrogenase (G6PDH) (Gargiulos et al., 1996), and lactate dehydrogenase (LDH) (Hilmy et al., 1985). Lead is also known to cause a decline in the activity of three significant metabolic enzymes: G6PDH, LDH, and pyruvate kinase (PK) (Osman et al., 2007). In addition, both cadmium and lead disrupt hemoglobin synthesis by inhibition of two enzymes, ferrochelatase and gamma levulinic acid dehydrogenase (ALA-D) (Nakagawa et al., 1995; Caldwell and Phillips, 1998). Another course of action that trace metals have been known to take is to cause endocrine disruption in fish (Jezierska et al., 2009). Cadmium has been reported to diminish thyroid hormone levels (Hontela et al., 1996), inhibit estrogen receptors (Le Guével etl al., 2000), and interrupt growth hormone expression (Jones et al., 2005). Likewise, Chaurasia et al. (1996) showed that by affecting the iodine metabolism, lead inhibits thyroid hormone synthesis. Additionally, cadmium, copper, and lead have been reported to exert a genotoxic effect on fish (Cavas et al., 2005; Bagdonas and Vosyliene, 2006).

It is common that fish from metal-contaminated water have low metal accumulation in the muscle tissue (except mercury) or the part consumed by humans; therefore, they are safe for human consumption. Although these fish may not be a threat for human consumption, they may be a potential threat for predatory fishes, birds and mammals feeding on these fish (Jezierska and Witeska, 2006). The transfer of trace metals through food chains is an important issue in metal assimilation by fish (Dallinger et al., 1987). Many trace metals are detrimental even at very low concentrations;

consequently, low absorption rates are enough to attain biologically significant or damaging concentrations in tissues (Jackim et al, 1970; Murai et al., 1981).

Dallinger et al. (1987) refer to the relationship of predators and consumers obtaining trace metals from other organisms they consume as the food chain effect. Two factors should be considered to determine to what degree the food chain effect in fish is affected by ecological conditions. The first factor is associated with the amount of contamination in the food supply (Dallinger et al., 1987). Many studies have suggested that trace metal pollution in aquatic ecosystems is evidently reflected by high levels of metals in sediments, macrophytes, and benthic animals rather than by elevated concentrations in water (Enk and Mathis, 1977; McIntosh et al., 1978; Mathis et al., 1979; Van Hassel et al., 1980). Studies presented by Delisle et al. (1975) and Ney and Van Hassel (1983) have shown that bottom-dwelling fish species acquire trace metals because of their association with metal-containing sediments. Furthermore, consumption of sediment and sediment-dwelling invertebrates is a vital source of metal uptake by fish (Czarnezki, 1985; Loring and Prosi, 1986). As a result, the food chain effect is magnified in aquatic environments where metal loaded food, like macrophytes or invertebrates, are a large portion of the diet of fish (Hardisty et al., 1974; Murphy et al., 1978). The second influence on the food chain effect in fish is the diminishment of species diversity (Dallinger et al., 1987). Rygg (1985) and Roch et al. (1985) have presented trace metal pollution leading to the elimination of susceptible species, therefore increasing the dominance of a few tolerant and opportunistic species (Lang and Lang-Dobler, 1979). Subsequently, trophic relationships become simple: food chains are reduced and predatory fish are obligated to feed on fewer or one kind of metal-tolerant food organism

(Dallinger and Kautzky, 1985). Metal tolerance of food organisms is established on two contradictory effects: detoxification of metals by cellular inclusion and metal exclusion (Dallinger et al., 1987). There are certain food organisms that are capable of storing exceptionally large amounts of trace metals, such as isopods, snails, and sludge worms (Enk and Mathis, 1977; Mathis et al., 1979; Dallinger and Kautzky, 1985; Rainbow, 1985; Prosi and Back, 1985). These animals possess effective detoxification mechanisms whereby trace metals are bound to metal-binding proteins or stored in cellular structures like vacuoles and lysosomes (Brown, 1977; Brown, 1978; Prosi, 1983; Simkiss and Mason, 1983; Bouquegneau et al., 1984; Dallinger and Prosi, 1986). The choice of such tolerant species in polluted habitats would result in a positive feedback mechanism, whereby the food chain effect would be intensified (Dallinger et al., 1987). However, the opposite effect has also been reported: Gächter and Geiger (1979) found that metal pollution of aquatic environments may favor the growth of metal-tolerant phytoplankton species that are represented by decreasing the uptake of trace metals per unit of biomass. Tolerance is accomplished by the exclusion of trace metals. This negative feedback mechanism plays a significant part in ecosystems by decreasing the availability of metals for organisms belonging to higher trophic levels, causing the food chain effect to be weakened (Gächter and Geiger, 1979; Dallinger et al., 1987). Thus, it is important to monitor the levels of trace metals in gulf menhaden and determine if the DWH spill has affected the accumulation of metals.

3. DATA AND METHODS

3.1 Study Site

The two locations, Vermillion Bay (VB) and Grand Isle (GI), were chosen because one was able to serve as a control or non-impacted, while the other as an experimental location or impacted site from the DWH spill. It was determined, that Vermillion Bay, Louisiana would serve as the control or non-impacted site; Grand Isle, Louisiana was and currently remains an impacted site from the DWH spill.

3.2 Sample Collection

A five-panel gill net, approximately 700 feet in length, was used to catch the samples. Once menhaden were onboard, they were separated by length, placed in plastic freezer bags, and placed on ice. At the lab, menhaden samples were taken off the ice and placed in a freezer. The collections of samples were accomplished with the help from the Louisiana Department of Wildlife and Fisheries (LDWF). The sampling protocols that were followed were chosen by the LDWF agents.

3.3 Preparation of Samples

For each metal analysis per month and year, six menhaden from Vermillion Bay and six from Grand Isle were removed from the deepfreeze. Of the six menhaden, there were three small and three large menhaden. The terms small and large were defined by their fork length; small samples have fork lengths of less than 16 cm, and large samples have fork lengths of 16 cm or larger. Once separated by size, the samples were cut into smaller portions. The small samples were then placed in 150 or 200 mL beakers, while large samples, because of size, were placed in 400 or 500 mL beakers. Beforehand, each beaker was prewashed and weighed. Some samples were compacted further into the beaker using a clean pestle to ensure that the entire sample was inside the beaker.

Samples were covered with aluminum foil with two to three holes cut in the top, and placed in a -86˚C deep freezer to freeze solid. The next step was to remove the samples and place them in a freeze dryer. The freeze dryer removed any liquid in the fish by first freezing the sample to -60˚C and then reducing the pressure to remove the water by transitioning it to a gas. For 24 to 36 hours and 36 to 48 hours, small and large samples were freeze dried respectively. Samples were then removed from the freeze dryer and placed in a dessicator to finish drying. After 24+ hours in the dessicator, samples were weighed in the beakers to get a final dry weight.

Upon removal from the dessicator, samples were ground to get a homogenous sample. About 1 g of dry fish tissue was weighed to four decimal places and placed in a 55 mL glass digestion tube. Next, 5 mL of concentrated trace-metal-grade nitric acid was added to the digestion tubes and allowed to sit for 12 hours. Samples were then placed in a digestion block for 8 hours at 120˚C. After completely digested the mixture was evaporated down approximately 1.5 mL. Once cool, the mixture was diluted to 50 mL with deionized water, covered with plastic paraffin film and vigorously shaken. Samples settled for a minimum of 14 hours until the supernatant was clear. Next, 14 to 15 mL of supernatant was transferred to 15mL glass inductively coupled plasma (ICP) tubes. Digestion and ICP tubes were washed in a 5% nitric acid bath for 14 hours and rinsed six times with deionized water before use. Metal analyses were performed on the samples and two blanks using a Vista-MPX CCD Simultaneous inductively coupled plasma-optical emission spectrometry (ICP-OES). The method used is referenced Hou et al., 2006.

3.4 ICP-OES Instrumentation

Menhaden were analyzed for metals using ICP-OES. Plasma is formed when argon gas flows through a fluctuating elctro- magnetic field, which is produced by a radiofrequency of 0.5-2kW power at 27 or 41 MHz. This field forms a state of partial ionization that produces ohmic heating and temperatures up to 10,000˚C. This high temperature results in the elements emitting light of characteristic wavelengths of visible or ultraviolet light specific for different elements, the intensity of which can be measured and used to determine concentration. Samples are presented to the plasma as a fine droplet aerosol. Then the light from the different elements is divided into different wavelengths by grating, and captured by light-sensitive detectors. Each metal is observed at one dominant wavelength; however other wavelengths are available (Table 3.1).

Table 3.1 Wavelength (nm) for each metal analyzed with ICP-OES

Metal	**Wavelength**
Arsenic	193.696
Barium	455.403
Cadmium	228.802
Cobalt	228.615
Chromium	267.716
Copper	324.754
Iron	238.204
Nickel	231.604
Lead	220.353
Zinc	213.857
Vanadium	292.401

The ICP-OES system can analyze 40 elements or more at one time and can detect at the µg/L level. The Vista-MPX CCD Simultaneous ICP-OES was operated at a power level of 1200 Watts and had an axial torch orientation with a CCD detector (Hardaway et al., 2004).

3.5 Quality Assurance

Quality assurance methods were outlined by Hou et al., 2006.

4. RESULTS AND DISCUSSION

4.1 Statistical Analysis

A total of 23 metals were analyzed for each tissue sample. From those, 11 metals were chosen based on previous studies done on metal analyses in fish and metals in crude oil: arsenic, barium, cadmium, cobalt, chromium, copper, iron, nickel, lead, zinc and vanadium.

Concentrations of metals were given in mg/L and converted to µg/g with the following equation:

$$\mu g/g = \frac{\left(\left(mg/L - \left(\frac{(blank\ 1 + blank\ 2)}{2}\right)\right) \times .05L\right)}{mass\ of\ sample\ in\ g} \times \frac{1000\ \mu g}{1\ mg}$$

Once converted to µg/g, metals were divided by four variables: place (Vermillion Bay or Grand Isle), size (small or large), month (July, August or September), and year (2011 or 2012) (Table 4.1). SAS® version 9.1.3 was used for all statistical analyses using the Tukey-Kramer Method to run pair-wise comparisons (Saxton, 1998; SAS Institute Inc., 2004). A confidence interval of $p < 0.05$ was used for all statistical analysis. The four variables and the interactions between the variables were observed for each metal.

4.2 Metals in *Brevoortia patronus*

4.2.1 Arsenic

Arsenic demonstrated a statistical difference for the single variables: place, month, size, and year (Table 4.1). The variable size ($F_{1,48} = 88.28$, P = <.0001) had the lowest P-value, with large fish having a statistically higher concentration of arsenic than small fish, at 5.97 ± 1.16 µg/g and 3.87 ± 0.06 µg/g respectively. The next variable, month ($F_{2,48} = 10.13$, P = 0.0002), had the highest concentrations of arsenic during the months of September, at 5.37 ± 1.34 µg/g, and August, at 5.03 ± 1.51 µg/g. When

compared to July, with a concentration at 4.36 ± 1.02 µg/g, August and September were statistically greater; although when compared to one another there was no statistical difference. The variable year ($F_{1,48}$ =7.33, P = 0.0094) had a significant difference between 2011 and 2012, with 2011 having a higher concentration of arsenic at 5.35 ± 2.31 µg/g than 2012 at 4.49 ± 1.17 µg/g. The last single variable, place ($F_{1,48}$ =6.93, P = 0.0113), had statistically greater levels of arsenic at VB with a concentration at 5.17 ± 0.64 µg/g as compared to GI at 4.67 ± 0.58 µg/g. All mean concentration values for arsenic are indicated in Table 4.2.

The interactions place*month, place*size, month*year, place*month*year, and size*year were significant for arsenic (Table 4.1). The interaction month*year ($F_{2,48}$ = 33.84, P = <.0001) had the second lowest P-value of all single or multiple factor models. When comparing the same month between years, all months statistically differed between years. July*2012 at 5.08 ± 1.35 µg/g was statistically higher than July*2011, at 3.64 ± 2.03 µg/g. While during August, 2011 was statistically higher than 2012 at 6.10 ± 2.02 µg/g and 3.96 ± 0.90 µg/g, respectively; For September, 2011 at 6.32 ± 1.97 µg/g was also significantly higher than 2012 at 4.42 ± 1.01 µg/g. When comparing different months within the same year, August and September of 2011 did not statistically differ from one another, but did statistically differ from July; with July having the lowest concentration among all three months at 3.64 ± 2.03 µg/g. In 2012, July at 5.08 ± 1.35 µg/g was significantly higher than August at 3.96 ± 0.90 µg/g; while September was not statistically different from either month.

For the interaction size*year ($F_{1,48}$ =9.46, P = 0.0035) there was a statistical difference when comparing different sizes within the same year. In 2011, large fish had a

higher concentration of arsenic at 6.79 ± 2.19 µg/g than small fish at 3.91 ± 1.34 µg/g. In 2012, large fish also had higher concentrations at 5.15 ± 1.24 µg/g when compared to small fish 3.82 ± 0.58 µg/g. When comparing the same size between years, large*2011 had statistically higher concentrations than large*2012 at 6.79 ± 2.19 µg/g and 5.15 ± 1.24 µg/g, respectively. For small fish there was no statistical difference between years.

The interaction place*size ($F_{1,48}$ =5.85, P = 0.0194) was significant when comparing different sizes within the same location. VB*large at 6.49 ± 1.53 was significantly higher than VB*small at 3.85 ± 0.84. For GI, large fish were also statistically higher than small fish at 5.45 ± 2.19 and 3.89 ± 1.19, respectively. When comparing the same size between locations, VB at 6.49 ± 1.53 had statistically higher concentrations of arsenic than GI at 5.45 ± 2.19 for large fish; while there was no statistical difference for small fish between locations.

The three way interaction: place*month*year ($F_{2,48}$ =5.48 , P = 0.0072) was significant for arsenic. When comparing the interaction of different places (VB to GI), same month (July to July), and same year (2011 to 2011) the interaction, VB*July*2011 at 4.78 ± 2.32 µg/g was statistically higher than GI*July*2011 at 2.49 ± 0.74 µg/g. Other interactions were statistically significant.

The last interaction for arsenic that was significant was place*month ($F_{2,48}$ = 5.41, P = 0.0076). When comparing the same month between locations, only VB*July was statistically higher than GI*July at 5.07 ± 1.93 µg/g and 3.64 ± 1.49 µg/g, respectively; August and September did not differ statistically between the two locations. When comparing different months within the same location, there was no statistical difference

between the three months for VB. For GI, August and September were not statistically different from one another, but were statistically greater than July at 3.64 ± 1.49 µg/g.

Table 4.1 Numerator DF, Denominator DF, F-Value and P-Value of variables and interactions of variables for arsenic

ARSENIC				
Effect	**Numerator DF**	**Denominator DF**	**F Value**	**Pr > F**
Place	1	48	6.93	0.0113
Month	2	48	10.13	0.0002
Place*Month	2	48	5.41	0.0076
Size	1	48	88.28	< .0001
Place*Size	1	48	5.85	0.0194
Month*Size	2	48	1.07	0.3497
Place*Month*Size	2	48	1.45	0.2452
Year	1	48	7.33	0.0094
Place*Year	1	48	0.27	0.6085
Month*Year	2	48	33.84	< .0001
Place*Month*Year	2	48	5.48	0.0072
Size*Year	1	48	9.46	0.0035
Place*Size*Year	1	48	0.00	0.9932
Month*Size*Year	2	48	2.30	0.1108
Place*Month*Size*Year	2	48	1.18	0.3154

Table 4.2 Average arsenic concentration (µg/g) for each variable

	VB	**GI**	**Small**	**Large**	**July**	**Aug**	**Sept**	**2011**	**2012**
Arsenic	5.17 ± 0.64	4.67 ± 0.58	3.87 ± 0.06	5.97 ± 1.16	4.36 ± 1.02	5.03 ± 1.51	5.37 ± 1.34	5.35 ± 2.31	4.49 ± 1.17

4.2.2 Barium

Barium demonstrated a statistical difference for the single variables: month, size, and year (Table 4.3). The variable size ($F_{1,48}$ = 152.08, P = <.0001) had the lowest P-value, with small fish having a higher concentration of barium at 9.88 ± 0.13 µg/g than large fish at 5.55 ± 2.09 µg/g. The variable year ($F_{1,48}$ =36.69, P = <.0001) had the second lowest P-value, with 2012 having a higher concentration of barium than 2011 at 8.50 ± 2.57 µg/g and 6.93 ± 3.95 µg/g, respectively. The next variable month ($F_{2,48}$ =34.44, P = <.0001), had the highest concentrations during the months of August, at 9.09 ± 1.39 µg/g, and September, at 8.17 ± 0.11 µg/g. When compared to July, with a concentration at 5.88 ± 1.83 µg/g, August and September were statistically greater,

although when compared to one another there was no statistical difference. All mean concentration values for barium are indicated in Table 4.4.

The interactions month*size, place*year, month*year, place*month*year, size*year, place*size*year, and place*month*size*year were all significant for barium (Table 4.3). The interaction size*year ($F_{1,48}$ = 24.76, P = <.0001) was significant when comparing different sizes within the same year. In 2011, small fish had a higher concentration of barium at 9.79 ± 3.23 µg/g than large fish at 4.07 ± 2.10 µg/g. In 2012, small fish also had higher concentrations at 9.97± 1.85 µg/g when compared to large fish 7.03 ± 2.36 µg/g. When comparing the same size between years, large*2012 had statistically higher concentrations than large*2011 at 4.07 ± 2.10 µg/g and 7.03 ± 2.36 µg/g, respectively. For small fish there was no statistical difference between years.

The interaction place*year ($F_{1,48}$ =16.71, P = 0.0002) was statistically significant for barium. When comparing different locations within the same year, VB*2011 had significantly higher concentrations of barium at 7.56 ± 3.68 µg/g than GI*2011 at 6.30 ± 4.22 µg/g. For 2012, there was no statistical difference in barium concentrations between the two locations. When comparing the same location between years, there was no statistical difference between 2011 and 2012 for VB. While for GI, 2012 was statistically higher than 2011 at 9.01 ± 2.24 µg/g and 6.30 ± 4.22 µg/g, respectively.

The three way interaction: place*size*year ($F_{1,48}$ =9.14, P = 0.0040) was significant when comparing the interaction of the same place (VB to VB), different sizes (small to large), and same year (2011 to 2012) for: VB*small*2011 at 10.25 ± 2.96 µg/g compared to VB*large*2011 at 4.86 ± 1.92 µg/g, GI*small*2011 at 9.33 ± 3.60 µg/g compared to GI*large*2011 at 3.27 ± 2.06 µg/g, and VB*small*2012 at 10.00 ± 1.84

µg/g compared to VB*large*2012 at 5.99 ± 2.15 µg/g. When comparing the interaction of different places (VB to GI), same size (small to small), and same year (2011 to 2011) there was a statistical difference for: VB*large*2011 at 4.86 ± 1.92 µg/g compared to GI*large*2011 at 3.27 ± 2.06 µg/g. Other interactions were statistically significant.

Another three way interaction that was significant for barium was: place*month*year ($F_{2,48}$ =7.29 , P = 0.0017). When comparing the interaction of different places (VB to GI), same month (July to July), and same year (2011 to 2011) there was a statistical difference for: VB*July*2011 at 5.95 ± 3.25 µg/g compared to GI*July*2011 at 3.22 ± 2.42 µg/g. Other interactions were statistically significant.

The next interaction for barium that had a significant interaction was month*year ($F_{2,48}$ =6.53 , P = 0.0031). When comparing the same month between years, July*2012, at 7.18 ± 2.57 µg/g, was statistically higher than July*2011, at 4.59 ± 3.08 µg/g. For August, 2012 also had a statistically higher concentration than 2011 at 10.07 ± 1.78 µg/g and 8.11 ± 3.86 µg/g, respectively; September did not statistically differ between 2011 and 2012. When comparing different months within the same year, August and September of 2011 did not statistically differ from one another, but did statistically differ from July; with July having the lowest concentration among all three months at 4.59 ±3.08 µg/g. In 2012, August, at 10.07 ± 1.78 µg/g, was statistically higher than July, at 7.18 ± 2.57 µg/g; while September*2012 was not statistically different from July or August.

The interaction month*size ($F_{2,48}$ =4.83, P = 0.0122) was significant for barium when comparing different sizes within the same month. For July, small fish had statistically higher concentrations of barium than large fish at 8.06 ± 1.94 µg/g and 3.71 ±

2.27 µg/g, respectively; August*small, at 10.86 ± 2.33 µg/g, was also statistically higher than August*large at 7.32 ± 2.81 µg/g; and for September, small fish at 10.72 ± 2.51 µg/g were significantly higher than large fish at 5.62 ± 1.55 µg/g. When comparing the same size between months, August*small and September*small did not statistically differ from one another, but were significantly higher than July*small; with July*small having the lowest concentration at 8.06 ± 1.94 µg/g. Similarly in large fish, August and September did not statistically differ from one another, but were statistically greater than July; with July*large having the lowest concentration at 3.71 ± 2.27 µg/g.

The four way interaction place*month*size*year, ($F_{2,48}$ = 3.57, P = 0.0360), had several significant interactions for barium.

Table 4.3 Numerator DF, Denominator DF, F-Value and P-Value of variables and interactions of variables for barium

BARIUM				
Effect	**Numerator DF**	**Denominator DF**	**F Value**	**Pr > F**
Place	1	48	1.31	0.2588
Month	2	48	34.44	< .0001
Place*Month	2	48	0.95	0.3944
Size	1	48	152.08	< .0001
Place*Size	1	48	0.01	0.9233
Month*Size	2	48	4.83	0.0122
Place*Month*Size	2	48	1.95	0.1540
Year	1	48	36.69	< .0001
Place*Year	1	48	16.71	0.0002
Month*Year	2	48	6.53	0.0031
Place*Month*Year	2	48	7.29	0.0017
Size*Year	1	48	24.76	< .0001
Place*Size*Year	1	48	9.14	0.0040
Month*Size*Year	2	48	0.07	0.9284
Place*Month*Size*Year	2	48	3.57	0.0360

Table 4.4 Average barium concentration (µg/g) for each variable

	VB	**GI**	**Small**	**Large**	**July**	**Aug**	**Sept**	**2011**	**2012**
Barium	7.77 ± 0.31	7.66 ± 1.91	9.88 ± 0.13	5.55 ± 2.09	5.88 ± 1.83	9.09 ± 1.39	8.17 ± 0.11	6.93 ± 3.95	8.50 ± 2.57

4.2.3 Cadmium

Cadmium demonstrated a statistical difference for the single variables: month and year (Table 4.5). The variable year ($F_{1,48}$ =11.77, P = 0.0012) was significant with, 2012 having a significantly higher concentration than 2011 at 0.18 ± 0.07 µg/g and 0.15 ± 0.04 µg/g, respectively. The variable, month ($F_{2,48}$ =8.70, P = 0.0006) had the highest concentrations of cadmium during August with a concentration at 0.19 ± 0.05 µg/g. August was statistically greater than September, at 0.15 ± 0.01 µg/g, and July, at 0.14 ± 0.04 µg/g; however there was no statistical different between September and July. All mean concentration values for cadmium are indicated in Table 4.6.

The interactions place*month, place*year and month*year were all significant for cadmium (Table 4.5). The interaction place*year ($F_{1,48}$ = 25.35, P = <.0001) had the lowest P-value and was significant when comparing different locations within the same year. In 2011, VB had significantly higher concentrations of cadmium at 0.17 ± 0.04 µg/g when compared to GI at 0.13 ± 0.04 µg/g. In 2012, GI had significantly higher concentrations than VB at 0.21 ± 0.09 µg/g and 0.15 ± 0.02 µg/g, respectively. When comparing the same location between years, there was no statistical difference between 2011 and 2012 for VB. While for GI, 2012 was statistically higher than 2011 at 0.21 ± 0.09 µg/g and 0.13 ± 0.04 µg/g, respectively.

The interaction place*month ($F_{2,48}$ = 10.15, P = 0.0002) was significant for cadmium. When comparing the two locations to the same month, only GI*August was statistically higher than VB*August at 0.22 ± 0.10 µg/g and 0.16 ± 0.04 µg/g, respectively; July and September did not differ statistically between the two locations. When comparing the same location to different months, there was no statistical difference

between the three months for VB. While for GI, July and September were not statistically different from one another, but were statistically lower than GI*August at 0.22 ± 0.10 µg/g.

The last interaction that was significant for cadmium was month*year ($F_{2,48}$ =6.98, P = 0.0022). When comparing the same month between years, July*2012, at 0.17 ± 0.03 µg/g, was statistically higher than July*2011, at 0.12 ± 0.05 µg/g. For August, 2012 also had a statistically higher concentration than 2011 at 0.22 ± 0.10 µg/g and 0.16 ± 0.04 µg/g, respectively; September did not statistically differ between 2011 and 2012. When comparing different months in the same year, September*2011 at 0.16 ± 0.02 µg/g was statistically greater than July*2011 at 0.12 ± 0.05 µg/g; while August*2011 was not statistically different from either month. In 2012, July and September did not statistically differ from one another, but did statistically differ from August; with August having the greatest concentration among all three months at 0.22 ± 0.10 µg/g.

Table 4.5 Numerator DF, Denominator DF, F-Value and P-Value of variables and interactions of variables for cadmium

CADMIUM				
Effect	**Numerator DF**	**Denominator DF**	**F Value**	**Pr > F**
Place	1	48	0.49	0.4870
Month	2	48	8.70	0.0006
Place*Month	2	48	10.15	0.0002
Size	1	48	0.06	0.8117
Place*Size	1	48	0.51	0.4788
Month*Size	2	48	0.21	0.8086
Place*Month*Size	2	48	0.88	0.4214
Year	1	48	11.77	0.0012
Place*Year	1	48	25.35	< .0001
Month*Year	2	48	6.98	0.0022
Place*Month*Year	2	48	2.45	0.0969
Size*Year	1	48	0.01	0.9314
Place*Size*Year	1	48	0.21	0.6475
Month*Size*Year	2	48	1.11	0.3383
Place*Month*Size*Year	2	48	0.37	0.6934

Table 4.6 Average cadmium concentration (µg/g) for each variable

	VB	GI	Small	Large	July	Aug	Sept	2011	2012
Cadmium	0.16 ± 0.01	0.17 ± 0.06	0.16 ± 0.02	0.16 ± 0.02	0.14 ± 0.04	0.19 ± 0.05	0.15 ± 0.01	0.15 ± 0.04	0.18 ± 0.07

4.2.4 Cobalt

Cobalt demonstrated a statistical difference for the single variables: place, month, and size (Table 4.7). The variable month ($F_{2,48}$ =12.00, P = <.0001) had highest concentrations of cobalt during September, at 0.98 ± 0.49 µg/g, and August, at 0.68 ± 0.16 µg/g. When compared to July, with a concentration at 0.49 ± 0.15 µg/g, August and September were statistically greater; although when compared to one another there was no statistical difference. The next variable place ($F_{1,48}$ = 7.23, P = 0.0098) was also significant with VB having higher concentrations of cobalt than GI at 0.84 ± 0.28 µg/g and 0.59 ± 0.16 µg/g, respectively. The last single variable, size ($F_{1,48}$ = 6.08, P = 0.0173), was significant with small fish having higher concentrations at 0.74 ± 0.05 µg/g than large fish at 0.69 ± 0.17 µg/g. All mean concentration values for cobalt are indicated in Table 4.8.

The interactions month*size, place*year, month*year, place*month*year, and month*size*year were all significant for cobalt (Table 4.7). The interaction place*year ($F_{1,48}$ =14.27, P = 0.0004) was significant when comparing different locations within the same year. In 2011, VB had significantly higher concentrations of cobalt at 1.04± 0.36 µg/g as compared to GI at 0.47 ± 0.25 µg/g. While, in 2012 there was no statistical difference in cobalt concentrations between the two locations. When comparing the same locations between years, there was no statistical difference between 2011 and 2012 for VB. For GI, 2012 was statistically higher than 2011 at 0.70 ± 0.14 µg/g and 0.47 ± 0.25 µg/g, respectively.

The next interaction that had a significant interaction was month*year ($F_{2,48}$ =12.58 , P = <.0001). When comparing the same month between years, September*2011, at 1.33 ± 1.60 µg/g, was statistically higher than September*2012, at 0.63 ± 0.19 µg/g. However, July and August did not statistically differ between 2011 and 2012. When comparing different months within the same year, July*2011 and August*2011 did not statistically differ from one another, but did statistically differ from September*2011; with September having the largest concentration at 0.63 ± 0.19 µg/g. In 2012, there was no statistical difference among the three months.

The three way interaction: place*month*year ($F_{2,48}$ =4.67, P = 0.0141) was significant for cobalt. When comparing the interaction of different places (VB to GI), same month (July to July), and same year (2011 to 2011) there was a statistical difference for: VB*September*2011 at 2.00 ± 2.14 µg/g compared to GI*September*2011 at 0.67 ± 0.17 µg/g. Other interactions were statistically significant.

The three way interaction: month*size*year ($F_{1,48}$ =3.96, P = 0.0255) was not significant for cobalt when comparing the interaction of the same month (July to July), different sizes (small to large), and same year (2011 to 2011). However, other interactions were statistically.

The interaction month*size ($F_{2,48}$ =3.91, P = 0.0268) was significant for cobalt when comparing different sizes within the same month. For July, small fish at 0.64 ± 0.16 µg/g had statistically higher concentrations than large fish at 0.34 ± 0.16 µg/g; while August and September did not statistically differ between sizes. When comparing the same size between months, for small fish there was no statistical difference among the three months. For large fish, July and August did not statistically differ from one another,

but did statistically differ from September*large; with September having the largest concentration at 1.16 ± 1.66 µg/g.

Table 4.7 Numerator DF, Denominator DF, F-Value and P-Value of variables and interactions of variables for cobalt

COBALT				
Effect	**Numerator DF**	**Denominator DF**	**F Value**	**Pr > F**
Place	1	48	7.23	0.0098
Month	2	48	12.00	< .0001
Place*Month	2	48	0.94	0.3963
Size	1	48	6.08	0.0173
Place*Size	1	48	0.78	0.3805
Month*Size	2	48	3.91	0.0268
Place*Month*Size	2	48	1.72	0.1900
Year	1	48	0.16	0.6929
Place*Year	1	48	14.27	0.0004
Month*Year	2	48	12.58	< .0001
Place*Month*Year	2	48	4.67	0.0141
Size*Year	1	48	0.64	0.4261
Place*Size*Year	1	48	3.40	0.0714
Month*Size*Year	2	48	3.96	0.0255
Place*Month*Size*Year	2	48	0.88	0.4199

Table 4.8 Average cobalt concentration (µg/g) for each variable

	VB	**GI**	**Small**	**Large**	**July**	**Aug**	**Sept**	**2011**	**2012**
Cobalt	0.84 ± 0.28	0.59 ± 0.16	0.74 ± 0.05	0.69 ± 0.17	0.49 ± 0.15	0.68 ± 0.16	0.98 ± 0.49	0.76 ± 1.01	0.67 ± 0.18

4.2.5 Chromium

Chromium demonstrated a statistical difference for the single variables: place, month, size, and year (Table 4.9). The variable size ($F_{1,48}$ = 84.51, P = <.0001) had the lowest P-value of all single or multiple factor models. Small fish had statistically higher concentrations, at 2.92 ± 0.09 µg/g, than large fish, at 1.75 ± 0.58 µg/g. The next variable month ($F_{2,48}$ =26.97, P = <.0001), had the highest concentration of chromium during August, at 2.83 ± 0.96 µg/g. September and July followed with concentrations at 2.47 ± 0.47 µg/g and 1.70 ± 0.51 µg/g, respectively. All months were statistically different from one another. The variable year ($F_{1,48}$ = 25.66, P = <.0001) was also significant, with 2012 having a higher concentration of chromium at 2.57 ± 0.98 µg/g than 2011 at 2.10 ± 1.43

µg/g. The last single variable, place ($F_{1,48}$ =10.89, P = 0.0018), had statistically greater levels of chromium at VB with a concentration at 2.50 ± 0.19 µg/g as compared to GI at 2.17 ± 0.86 µg/g. All mean concentration values for chromium are indicated in Table 4.10.

The interactions month*size, place*year, month*year, size*year, and month*size*year were all significant for chromium (Table 4.9). The interaction place*year ($F_{1,48}$ =37.28, P = <.0001), had the second lowest P-value for chromium. When comparing different locations within the same year, VB*2011 had significantly higher concentrations of chromium at 2.63 ± 1.49 µg/g as compared to GI*2011 at 1.57 ± 1.17 µg/g. Conversely, in 2012 there was no statistical difference in chromium concentrations between the two locations. When comparing the same location between years, there was no statistical difference between 2011 and 2012 for VB. For GI, 2012 was statistically higher than 2011 at 2.78 ± 1.11 µg/g and 1.57 ± 1.17 µg/g, respectively.

The next interaction that had a significant interaction was month*year ($F_{2,48}$ = 15.58, P = <.0001). When comparing the same month between years, July*2012, at 2.06 ± 0.47 µg/g, was statistically higher than July*2011, at 1.34 ± 0.66 µg/g. August*2012 also had statistically higher concentrations than August*2011 at 3.51 ± 0.88 µg/g and 2.15 ± 0.97 µg/g, respectively; September did not statistically differ between 2011 and 2012. When comparing different months within the same year, August and September of 2011 did not statistically differ from one another, but did statistically differ from July*2011; with July having the lowest concentration at 1.34 ± 0.66 µg/g. In 2012, September and July did not statistically differ from one another, but did have significantly lower concentrations of chromium than August*2012 at 3.51 ± 0.88 µg/g.

For the interaction size*year ($F_{1,48}$ =8.57, P = 0.0052) there was a statistical difference. When comparing different sizes within the same year, small*2011 had higher concentrations of chromium at 2.86 ± 1.62 µg/g than large*2011 at 1.34 ± 0.60 µg/g. In 2012, small fish also had higher concentrations than large fish at 2.99 ± 0.96 µg/g and 2.16 ± 0.83 µg/g, respectively. When comparing the same size between years, there was no statistical difference between 2011 and 2012 for small fish. For large fish, 2012 was statistically higher, at 2.16 ± 0.83 µg/g, than 2011, at 1.34 ± 0.60 µg/g.

Another interaction that was significant for chromium was month*size ($F_{2,48}$ =8.02, P = 0.0010). When comparing different sizes within the same month, August*small, at 3.38 ± 1.02 µg/g, was statistically higher than August*large, at 2.28 ± 1.01 µg/g. For the month of September, small fish also had significantly higher levels of chromium than large fish at 3.47 ± 1.56 µg/g and 1.48 ± 0.43 µg/g, respectively; while there was no statistical difference between July*small and July*large. When comparing the same size between months, August*small and September*small did not statistically differ among one another, but did statistically differ from July*small; with July having the lowest concentration among all three months at 1.92 ± 0.56 µg/g. For large fish, September and July did not statistically differ from one another, but did have significantly lower concentrations of chromium than August at 2.28 ± 1.01 µg/g.

The last interaction for chromium that was significant was the three way interaction: month*size*year ($F_{2,48}$ =3.91, P = 0.0267). When comparing the interaction of the same month (July to July), different sizes (small to large), and same year (2011 to 2011) there was a statistical difference for: August*small* 2011 at 2.83 ± 0.83 µg/g compared to August*large*2011 at 1.47 ± 0.54 µg/g, September*small*2011 at 4.28 ±

1.80 µg/g compared to September*large*2011 at 1.33± 0.43 µg/g, and July*small*2012 at 2.37 ± 0.24 µg/g compared to July*large*2012 at 1.75 ± 0.45 µg/g. Other interactions were statistically significant.

Table 4.9 Numerator DF, Denominator DF, F-Value and P-Value of variables and interactions of variables for chromium

CHROMIUM				
Effect	**Numerator DF**	**Denominator DF**	**F Value**	**Pr > F**
Place	1	48	10.89	0.0018
Month	2	48	26.97	< .0001
Place*Month	2	48	1.76	0.1822
Size	1	48	84.51	< .0001
Place*Size	1	48	0.09	0.7654
Month*Size	2	48	8.02	0.0010
Place*Month*Size	2	48	2.12	0.1314
Year	1	48	25.66	< .0001
Place*Year	1	48	37.28	< .0001
Month*Year	2	48	15.58	< .0001
Place*Month*Year	2	48	0.16	0.8505
Size*Year	1	48	8.57	0.0052
Place*Size*Year	1	48	0.62	0.4350
Month*Size*Year	2	48	3.91	0.0267
Place*Month*Size*Year	2	48	2.72	0.0758

Table 4.10 Average chromium concentration (µg/g) for each variable

	VB	**GI**	**Small**	**Large**	**July**	**Aug**	**Sept**	**2011**	**2012**
Chromium	2.50 ± 0.19	2.17 ± 0.86	2.92 ± 0.09	1.75 ± 0.58	1.70 ± 0.51	2.83 ± 0.96	2.47 ± 0.47	2.10 ± 1.43	2.57 ± 0.98

4.2.6 Copper

Copper demonstrated a statistical difference for the single variables: place, month, size, and year (Table 4.11). The variable size ($F_{1,48}$ = 29.98, P = <.0001) had the lowest P-value of all single or multiple factor models with small fish having statistically higher concentrations, at 2.84 ± 0.07 µg/g, then large fish, at 2.06 ± 0.36 µg/g. The next variable place ($F_{1,48}$ =18.30, P = <.0001) had the second lowest P-value with statistically greater concentrations at VB as compared to GI at 2.74 ± 0.11 µg/g and 2.15 ± 0.39 µg/g, respectively. The variable year ($F_{1,48}$ = 8.76, P = 0.0048) was also significant with 2012

having greater concentrations at 2.55 ± 0.51 µg/g than 2011 at 2.35 ± 1.45 µg/g. The last variable month ($F_{2,48}$ = 6.10, P = 0.0043), had the highest concentrations of copper during the months of September, at 2.59 ± 0.01 µg/g, and August, at 2.45 ± 0.27 µg/g. When compared to July, with a concentration at 2.30 ± 0.15 µg/g, September and August were statistically greater; although when compared to one another there was no statistical difference. All mean concentration values for copper are indicated in Table 4.12.

The interactions place*month, place*year, place*month*year, and size*year were all significant for copper (Table 4.11). The three way interaction place*month*year ($F_{2,48}$ =1309, P = <.0001) was significant for copper. When comparing the interaction of different places (VB to GI), same month (July to July), and same year (2011 to 2011) there was a statistical difference for: VB*July*2011 at 3.50 ± 2.95 µg/g compared to GI*July*2011 at 0.88 ± 0.39 µg/g. Other interactions were statistically significant.

The interaction place*month ($F_{2,48}$ =9.89, P = 0.0003) was significant for copper. When comparing the same month between locations, only VB*July was statistically higher than GI*July at 2.98 ± 2.12 µg/g and 1.62 ± 0.84 µg/g, respectively; August and September did not differ statistically between the two locations. When comparing different months within the same location, for VB there was no statistical difference between the three months. For GI, September and August were not statistically different from one another, but were statistically greater than GI*July at 1.62 ± 0.84 µg/g.

The interaction place*year ($F_{1,48}$ =7.65, P = 0.0080) was significant for copper when comparing different locations within the same year. VB*2011 had significantly higher concentrations of copper, at 2.82 ± 1.75 µg/g, as compared to GI*2011, at 1.87 ± 0.90 µg/g. Conversely, for 2012 there was no statistical difference in copper

concentration between the two locations. When comparing the same location between years, there was no statistical difference between 2011 and 2012 for VB. For GI, 2012 had statistically higher concentrations than 2011 at 2.43 ± 0.38 µg/g and 1.87 ± 0.90 µg/g, respectively.

The last interaction size*year ($F_{1,48}$ =18.55, P = <.0001) was statistically significant for copper. When comparing different sizes within the same year, small*2011 had higher concentrations of copper at 2.89 ± 1.79 µg/g than large*2011 at 1.80 ± 0.72 µg/g. While in 2012, there was no statistical difference between small and large fish. When comparing the same size between years, small*2012 did not statistically differ from small*2011; while large*2012 at 2.31 ± 0.35 µg/g was statistically higher than large*2011 at 1.80 ± 0.72 µg/g.

Table 4.11 Numerator DF, Denominator DF, F-Value and P-Value of variables and interactions of variables for copper

COPPER				
Effect	**Numerator DF**	**Denominator DF**	**F Value**	**Pr > F**
Place	1	48	18.30	< .0001
Month	2	48	6.10	0.0043
Place*Month	2	48	9.89	0.0003
Size	1	48	29.98	< .0001
Place*Size	1	48	0.44	0.5126
Month*Size	2	48	2.83	0.691
Place*Month*Size	2	48	2.74	0.0747
Year	1	48	8.76	0.0048
Place*Year	1	48	7.65	0.0080
Month*Year	2	48	2.58	0.0862
Place*Month*Year	2	48	13.09	< .0001
Size*Year	1	48	4.40	0.0413
Place*Size*Year	1	48	1.73	0.1942
Month*Size*Year	2	48	1.23	0.3023
Place*Month*Size*Year	2	48	0.10	0.9033

Table 4.12 Average copper concentration (µg/g) for each variable

	VB	**GI**	**Small**	**Large**	**July**	**Aug**	**Sept**	**2011**	**2012**
Copper	2.74 ± 0.11	2.15 ± 0.39	2.84 ± 0.07	2.06 ± 0.36	2.30 ± 0.15	2.45 ± 0.27	2.59 ± 0.01	2.35 ± 1.45	2.55 ± 0.51

4.2.7 Iron

Iron demonstrated a statistical difference for the single variables: place, month, size, and year (Table 4.13). The variable size ($F_{1,48}$ = 135.68, P = <.0001) had the lowest P-value of all single and multiple factor models, with small fish having statistically higher concentrations at 1011.42 ± 61.81µg/g than large fish at 582.16 ± 215.68 µg/g. The variable year ($F_{1,48}$ = 42.95, P = <.0001) had a significant difference between 2011 and 2012, with 2012 having higher concentrations of iron at894.90 ± 335.63 µg/g than 2011 at 698.68 ± 404.91 µg/g. The next variable, month ($F_{2,48}$ =38.83, P = <.0001), had the highest concentrations of iron during the months of August, at 923.22 ± 246.11 µg/g, and September, at 876.32 ± 12.33 µg/g. When compared to July, at 590.80 ± 182.47 µg/g, August and September were statistically greater; although when compared to one another there was no statistical difference. The last single variable place ($F_{1,48}$ =10.08, P = 0.0026) had statistically greater levels at VB, with a concentration at 841.94 ± 0.85 µg/g, as compared to GI, at 751.64 ± 276.64 µg/g. All mean concentration values for iron are indicated in Table 4.14.

The interactions place*month, month*size, place*year, month*year, place*month*year, size*year, place*size*year, and place*month*size*year were significant for iron (Table 4.13). The interaction place*year ($F_{1,48}$ =44.35, P = <.0001) had the second lowest P-value for iron and was significant when comparing different locations within the same year. In 2011, VB had significantly higher concentrations of iron, at 841.34 ± 408.01 µg/g, when compared to GI, at 556.02 ± 357.74 µg/g. Conversely, in 2012 there was no statistical difference between the two locations. When comparing the same location between years, there was no statistical difference between

2011 and 2012 for VB. For GI, 2012 was statistically higher than 2011 at 947.25 ± 264.96 µg/g and 556.02 ± 357.74 µg/g, respectively.

For the interaction size*year ($F_{1,48}$ =18.55, P = <.0001) there was a statistical difference when comparing different sizes within the same year. In 2011, small fish had higher concentrations of iron at 967.71 ± 370.66 µg/g than large fish at 429.65 ± 216.54 µg/g. In 2012, small fish also had higher concentrations at 1055.12 ± 294.70 µg/g when compared to large fish at 734.67 ± 301.17 µg/g. When comparing the same size between years, small*2012 did not statistically differ from small*2011. For large fish, 2012 was statistically higher, at 734.67± 301.17 µg/g, than 2011, at 429.65 ± 216.65 µg/g.

The three way interaction: place*size*year ($F_{1,48}$ =11.75, P = 0.0013) was significant when comparing the interaction of the same place (VB to VB), different size (small to large), and same year (2011 to 2012) for: VB*small*2011 at 1126.21 ± 381.33 µg/g compared to VB*large*2011 at 556.46 ± 160.46 µg/g, GI*small*2011 at 809.21 ± 300.02 µg/g compared to GI*large*2011 at 302.83± 194.18 µg/g, and VB*small*2012 at 1041.23 ± 315.53 µg/g compared to VB*large*2012 at 643.85 ± 378.06 µg/g. When comparing the interaction of different places (VB to GI), same size (small to small), and same year (2011 to 2011) there was a statistical difference for: VB*large*2011 at 556.46 ± 160.46 µg/g compared to GI*large*2011 at 302.83 ± 194.18 µg/g, GI*large*2012 at 825.49 ± 176.81 µg/g compared to VB*large*2012 at 643.85 ± 378.06 µg/g. Other interactions were statistically significant.

The next interaction that had a significant interaction was month*year ($F_{2,48}$ = 9.79, P = 0.0003). When comparing the same month between years, July*2012, at 719.84 ± 281.31 µg/g, was statistically higher than July*2011, at 461.80 ± 302.02 µg/g.

August*2012 also had statistically higher concentrations than August*2011 at 1097.25 ± 257.96 µg/g and 749.20 ± 342.74 µg/g, respectively; September did not statistically differ between 2011 and 2012. When comparing different months within the same year, August*2011 and September*2011 did not statistically differ from one another, but did statistically differ from July*2011; with July having the lowest concentration among all three months at 461.80 ± 302.02 µg/g. In 2012, September and July did not statistically differ from one another, but did have significantly lower concentrations of iron than August*2012 at 1097.25 ± 257.96 µg/g.

Another three way interaction that was significant for iron was: place*month*year ($F_{2,48}$ =8.45, P = 0.0007). When comparing the interaction of different places (VB to GI), same month (July to July), and same year (2011 to 2011) there was a statistical difference for: VB*July*2011 at 626.59 ± 290.10 µg/g compared to GI*July*2011 at 297.00 ± 226.59 µg/g, and GI*September*2012 at 1076.78 ± 356.53 µg/g compared to VB*September*2012 at 658.41 ± 249.57 µg/g. Other interactions were statistically significant.

The interaction month*size ($F_{2,48}$ =7.55, P = 0.0014) was significant for iron when comparing different sizes within the same month. For July, small fish at 812.57 ± 214.58 µg/g had statistically higher concentrations than large fish at 369.07 ± 218.98 µg/g; August*small, with concentrations at 1087.89 ± 296.01 µg/g, was also statistically higher than August*large, at 758.56 ± 321.43 µg/g; and for September, small fish were significantly higher than large fish at 1133.79 ± 384.56 µg/g and 618.84 ± 229.24 µg/g, respectively. When comparing the same size between months, August*small and September*small did not statistically differ from one another, but did statistically differ

from July*small; with July having the lowest concentration at 812.57 ± 214.58 µg/g. Similarly in large fish, August and September did not statistically differ from one another, but were statistically different from July; with July having the lowest concentrations of iron at 369.07 ± 218.98 µg/g.

The interaction place*month ($F_{2,48}$ = 4.04, P = 0.0230) was significant for iron. When comparing the same month between locations, only VB*July was statistically higher than GI*July at 648.18 ± 312.95 µg/g and 533.46 ± 318.68 µg/g, respectively; August and September did not differ statistically between the two locations. When comparing different months within the same year, VB*August, at 1010.71 ± 369.59 µg/g, was significantly higher than VB*July, at 648.18 ± 312.95 µg/g; while VB*September did not statistically differ from either month. For GI, September and August were not statistically different from one another, but were statistically greater than GI*July at 533.46 ± 318.68 µg/g.

Table 4.13 Numerator DF, Denominator DF, F-Value and P-Value of variables and interactions of variables for iron

IRON				
Effect	**Numerator DF**	**Denominator DF**	**F Value**	**Pr > F**
Place	1	48	10.08	0.0026
Month	2	48	38.83	< .0001
Place*Month	2	48	4.04	0.0239
Size	1	48	135.68	< .0001
Place*Size	1	48	0.24	0.6260
Month*Size	2	48	7.55	0.0014
Place*Month*Size	2	48	2.24	0.1178
Year	1	48	42.95	< .0001
Place*Year	1	48	44.35	< .0001
Month*Year	2	48	9.79	0.0003
Place*Month*Year	2	48	8.45	0.0007
Size*Year	1	48	18.55	< .0001
Place*Size*Year	1	48	11.75	0.0013
Month*Size*Year	2	48	0.28	0.7585
Place*Month*Size*Year	2	48	3.22	0.0487

Table 4.14 Average iron concentration (µg/g) for each variable

	VB	GI	Small	Large	July	Aug	Sept	2011	2012
Iron	841.94 ± 0.85	751.64 ± 276.64	1011.42 ± 61.81	582.16 ± 215.68	590.82 ± 182.47	923.22 ± 246.11	876.32 ± 12.33	698.68 ± 404.91	894.90 ± 335.63

The four way interaction place*month*size*year, ($F_{2,48}$ = 3.22, P = 0.0487), had several significant interactions for iron.

4.2.8 Nickel

Nickel demonstrated a statistical difference for the single variables: place, month, size, and year (Table 4.15). The variable size ($F_{1,48}$ = 82.12, P = <.0001) had the lowest P-value of all single and multiple factor models, with small fish having statistically higher concentrations than large fish at 1.79 ± 0.07 µg/g and 1.05 ± 0.32 µg/g, respectively. The variable place ($F_{1,48}$ =19.96, P = <.0001), had statistically higher levels of nickel at VB, with a concentration at 1.58 ± 0.09 µg/g, as compared to GI, at 1.25 ± 0.48 µg/g. The next variable, month ($F_{2,48}$ =19.94, P = <.0001), had the highest concentrations of nickel during the months of August, at 1.59 ± 0.46 µg/g, and September, at 1.60 ± 0.06 µg/g. When compared to July, at 1.06 ± 0.18 µg/g, August and September were statistically greater; although when compared to one another there was no statistical difference. The last single variable year ($F_{1,48}$ = 17.93, P = 0.0001), had a significant difference between 2011 and 2012, with 2012 having higher concentrations of nickel at 1.55 ± 0.58 µg/g than 2011 at 1.28 ± 0.79 µg/g. All mean concentration values for nickel are indicated in Table 4.16.

The interactions month*size, place*year, month*year, and size*year were all significant for nickel (Table 4.15). The interaction place*year ($F_{1,48}$ =32.36, P = <.0001), had the second lowest P-value and was significant when comparing different locations within the same year. In 2011, VB had significantly higher concentrations of nickel at

1.65 ± 0.75 µg/g as compared to GI at 0.92 ± 0.66 µg/g. Conversely, in 2012 there was no statistical difference in nickel concentrations between the two locations. When comparing the same location between years, there was no statistical difference between 2011 and 2012 for VB. For GI, 2012 was statistically higher than 2011 at 1.59 ± 0.53 µg/g and 0.92 ± 0.66 µg/g, respectively.

For the interaction size*year ($F_{1,48}$ =6.32, P = 0.0153) there was a statistical difference when comparing different sizes within the same year. In 2011, small fish had higher concentrations of nickel, at 1.74 ± 0.83 µg/g, than large fish, at 0.82 ± 0.37 µg/g. In 2012, small fish also had higher concentrations at 1.83 ± 0.57 µg/g when compared to large fish 1.28 ± 0.44 µg/g. When comparing the same size between years, small*2012 did not statistically differ from small*2011. For large fish, 2012 was statistically higher, at 1.28 ± 0.44 µg/g, than large*2011, at 0.82 ± 0.37 µg/g.

The interaction month*size ($F_{2,48}$ =5.23, P = 0.0088) was significant for nickel when comparing different sizes within the same month. For July, small fish at 1.26 ± 0.40 µg/g had statistically higher concentrations of nickel than large fish at 0.86 ± 0.42 µg/g; August*small, with concentrations at 1.89 ± 0.44 µg/g, were also statistically higher than August*large at 1.29 ± 0.56 µg/g; and for September, small fish were significantly higher than large fish at 2.21 ± 0.84 µg/g and 1.00 ± 0.29 µg/g, respectively. When comparing the same size between months, August*small and September*small did not statistically differ from one another, but did statistically differ from July*small; with July having the lowest concentration at 1.26 ± 0.40 µg/g. In large fish, August was statistically higher than July at 1.29 ± 0.56 µg/g and 0.86 ± 0.42 µg/g, respectively; while September*large did not statistically differ from either month.

The next interaction that had a significant interaction was month*year ($F_{2,48}$ = 4.98, P = 0.0109). When comparing the same month between years, August*2012 was statistically higher than August*2011 at 1.92 ± 0.45 µg/g and 1.27 ± 0.52 µg/g, respectively; while July and September did not statistically differ between 2011 and 2012. When comparing different months within the same year, August and September of 2011 did not statistically differ from one another, but did statistically differ from July*2011; with July having the lowest concentration at 0.93 ± 0.55 µg/g. In 2012, August was statistically higher than July at 1.92 ± 0.45 µg/g and 1.18 ± 0.32 µg/g, respectively; while September*2012 did not statistically differ from either month. Other interactions were statistically significant.

Table 4.15 Numerator DF, Denominator DF, F-Value and P-Value of variables and interactions of variables for nickel

NICKEL				
Effect	**Numerator DF**	**Denominator DF**	**F Value**	**Pr > F**
Place	1	48	19.96	< .0001
Month	2	48	19.94	< .0001
Place*Month	2	48	1.69	0.1959
Size	1	48	82.12	< .0001
Place*Size	1	48	0.47	0.4959
Month*Size	2	48	5.23	0.0088
Place*Month*Size	2	48	2.39	0.1022
Year	1	48	17.93	0.0001
Place*Year	1	48	32.36	< .0001
Month*Year	2	48	4.98	0.0109
Place*Month*Year	2	48	1.27	0.2895
Size*Year	1	48	6.32	0.0153
Place*Size*Year	1	48	1.27	0.2650
Month*Size*Year	2	48	0.96	0.3900
Place*Month*Size*Year	2	48	0.71	0.4953

Table 4.16 Average nickel concentration (µg/g) for each variable

	VB	**GI**	**Small**	**Large**	**July**	**Aug**	**Sept**	**2011**	**2012**
Nickel	1.58 ± 0.09	1.25 ± 0.48	1.79 ± 0.07	1.05 ± 0.32	1.06 ± 0.18	1.59 ± 0.46	1.60 ± 0.06	1.28 ± 0.79	1.55 ± 0.58

4.2.9 Lead

Lead demonstrated a statistical difference for the single variables: place and size (Table 4.17). The variable size ($F_{1,48}$ = 32.51, P = <.0001) had the lowest P-value of all single and multiple factor models, with small fish having statistically higher concentrations, at 1.44 ± 0.10 µg/g, than large fish, at 1.05 ± 0.20 µg/g. The variable place ($F_{1,48}$ =16.80, P = 0.0002), was also significant with VB having higher concentrations than GI at 1.34 ± 0.02 and 1.15 ± 0.12 µg/g, respectively. All mean concentration values for lead are indicated in Table 4.18.

The interactions place*month, place*year, month*year, place*month*year, size*year, and place*size*year were all significant for lead (Table 4.17). The interaction month*year ($F_{2,48}$ = 17.98, P = <.0001) was significant and had the second lowest P-value. When comparing the same month between years, July*2012, at 1.44 ± 0.34 µg/g, was statistically higher than July*2011, at 0.89 ± 0.53 µg/g. September*2011 was also statistically higher than September*2012 at 1.47 ± 0.45 µg/g and 1.07 ± 0.35 µg/g, respectively; August did not statistically differ between 2011 and 2012. When comparing different months within the same year, August*2011 and September* 2011 did not statistically differ from one another, but did statistically differ from July*2011; with July having the lowest concentration at 0.89 ± 0.53 µg/g. In 2012, July was statistically higher than September at 1.44 ± 0.34 µg/g and 1.07 ± 0.35 µg/g, respectively; while August*2012 did not statistically differ from either month.

The three way interaction: place*month*year ($F_{2,48}$ =9.50, P = 0.0003) was significant for lead. When comparing the interaction of different places (VB to GI), same month (July to July), and same year (2011 to 2011) there was a statistical difference for:

VB*July*2011 at 1.33 ± 0.29 µg/g when compared to GI*July*2011 at 0.45 ± 0.27 µg/g. Other interactions were statistically significant.

For the interaction size*year ($F_{1,48}$ =9.39, P = 0.0036) there was a statistical difference when comparing different sizes within the same year. Small*2011 had statistically larger concentrations of lead than large*2011 at 1.52 ± 0.52 µg/g and 0.91 ± 0.37 µg/g, respectively. In 2012, there was no statistical difference between small and large fish. When comparing the same size between years, small*2011 did not statistically differ from small*2012. For large fish, 2012 was statistically higher, at 1.19 ± 0.35 µg/g, than 2011, at 0.91 ± 0.37 µg/g.

The interaction place*month ($F_{2,48}$ = 9.02, P = 0.0005) was significant for lead. When comparing the same month between locations, only VB*July was statistically higher than GI*July at 1.48 ± 0.32 µg/g and 0.90 ± 0.55 µg/g, respectively; August and September did not differ statistically between the two locations. When comparing different months within the same location, GI*September and GI*August did not statistically differ from one another, but were statistically greater than GI*July at 0.90 ± 0.55 µg/g; while VB did not statistically differ between any of the three months.

The three way interaction: place*size*year ($F_{1,48}$ =7.26, P = 0.0097) was significant when comparing the interaction of the same place (VB to VB), different size (small to large), and same year (2011 to 2012) for: GI*small*2011 at 1.44 ± 0.62 µg/g compared to GI*large*2011 at 0.70 ± 0.40 µg/g. When comparing the interaction of different places (VB to GI), same size (small to small), and same year (2011 to 2011) there was a statistical difference for: VB*large*2011 at 1.12 ± 0.18 µg/g compared to GI*large*2011 at 0.70 ± 0.40 µg/g. Other interactions were statistically significant.

The interaction place*year ($F_{1,48}$ =4.32, P = 0.0430), was significant when comparing different locations within the same year. In 2011, VB had significantly higher concentrations of lead, at 1.35 ± 0.40 µg/g, as compared to GI, at 1.07 ± 0.63 µg/g. Conversely, in 2012 there was no statistical difference in lead concentrations between the two locations. When comparing the same location between years, there was no statistical difference for VB or GI between the two years.

Table 4.17 Numerator DF, Denominator DF, F-Value and P-Value of variables and interactions of variables for lead

LEAD				
Effect	**Numerator DF**	**Denominator DF**	**F Value**	**Pr > F**
Place	1	48	9.11	0.0041
Month	2	48	2.34	0.1071
Place*Month	2	48	9.02	0.0005
Size	1	48	32.51	< .0001
Place*Size	1	48	0.00	0.9936
Month*Size	2	48	0.37	0.6940
Place*Month*Size	2	48	0.74	0.4814
Year	1	48	2.33	0.1338
Place*Year	1	48	4.32	0.0430
Month*Year	2	48	17.98	< .0001
Place*Month*Year	2	48	9.50	0.0003
Size*Year	1	48	9.39	0.0036
Place*Size*Year	1	48	7.26	0.0097
Month*Size*Year	2	48	0.17	0.8405
Place*Month*Size*Year	2	48	0.47	0.6275

Table 4.18 Average lead concentration (µg/g) for each variable

	VB	**GI**	**Small**	**Large**	**July**	**Aug**	**Sept**	**2011**	**2012**
Lead	1.34 ± 0.02	1.15 ± 0.12	1.44 ± 0.10	1.05 ± 0.20	1.16 ± 0.39	1.30 ± 0.03	1.27 ± 0.28	1.21 ± 0.54	1.28 ± 0.37

4.2.10 Zinc

Zinc demonstrated a statistical difference for the single variables: place, month, and year (Table 4.19). The variable year ($F_{1,48}$ = 52,27, P = <.0001) had the lowest P-value and had a significant difference between 2011 and 2012, with 2012 having significantly higher concentrations of zinc at 115.75 ± 169.55 µg/g than 2011 at 59.01 ± 18.01 µg/g. The variable place ($F_{1,48}$ =16.80, P = 0.0002) was also significant, with VB,

at 108.18 ± 64.16 µg/g, having higher concentrations of zinc than GI, at 66.59 ± 16.09 µg/g. For the last single variable, month ($F_{2,48}$ =7.85, P = 0.0011), September was statistically higher than July at 121.52 ± 73.32 µg/g and 68.16 ± 32.35 µg/g, respectively; while August did not statistically differ from either month. All mean concentration values for zinc are indicated in Table 4.20.

The interactions place*month, month*year, place*month*year, and place*size*year were significant for zinc (Table 4.19). The three way interaction: place*month*year ($F_{2,48}$ =10.31, P = 0.0002) was significant when comparing the interaction of different places (VB to GI), same month (July to July), and same year (2011 to 2011) for: VB*July*2011 at 62.57± 7.58 µg/g compared to GI*July*2011 at 28.01 ± 5.32 µg/g, and VB*August*2012 at 92.48 ± 10.91 µg/g compared to GI*August*2012 at 73.24 ± 10.03 µg/g. Other interactions were statistically significant.

The three way interaction: place*size*year ($F_{1,48}$ =4.50, P = 0.0390) was not significant when comparing the interaction of the same place (VB to VB), different size (small to large), and same year (2011 to 2011). When comparing the interaction of different places (VB to GI), same size (small to small), and same year (2011 to 2011) there was a statistical difference for: VB*small*2012 at 211.92 ± 333.44 µg/g compared to GI*small*2012 at 72.46 ± 10.58 µg/g. Other interactions were statistically significant.

The interaction month*year ($F_{2,48}$ = 4.01, P = 0.0245) was significant for zinc. When comparing the same month between years, July*2012, at 91.04 ± 13.00 µg/g, was statistically higher than July*2011, at 45.29 ± 19.09 µg/g. September*2012 was also statistically higher than September*2011 at 173.36 ± 292.62 µg/g and 69.68 ± 10.46 µg/g, respectively; while August did not statistically differ between 2011 and 2012.

When comparing different months within the same year, August*2011 and September*2011 did not statistically differ from one another, but did statistically differ from July*2011; with July having the lowest concentration at 45.29 ± 19.09 µg/g. In 2012, there was no statistical difference among the three months.

The interaction place*month ($F_{2,48}$ = 3.22, P = 0.0486) was significant for zinc when comparing the same month between locations. VB*July was statistically higher than GI*July at 78.83 ± 18.17 µg/g and 57.50 ± 33.12 µg/g, respectively. For September, VB, at 170.64 ± 293.61 µg/g, was also statistically higher than GI, at 72.40 ± 11.57 µg/g; while August did not differ statistically between the two locations. When comparing different months within the same location, GI*September and GI*August did not statistically differ from one another, but were statistically greater than GI*July at 57.50 ± 33.12 µg/g; while there was no statistical difference for VB between any of the months.

Table 4.19 Numerator DF, Denominator DF, F-Value and P-Value of variables and interactions of variables for zinc

ZINC				
Effect	**Numerator DF**	**Denominator DF**	**F Value**	**Pr > F**
Place	1	48	16.80	0.0002
Month	2	48	7.85	0.0011
Place*Month	2	48	3.22	0.0486
Size	1	48	3.91	0.0539
Place*Size	1	48	1.18	0.2837
Month*Size	2	48	2.76	0.0734
Place*Month*Size	2	48	1.97	0.1505
Year	1	48	52.27	< .0001
Place*Year	1	48	1.39	0.2449
Month*Year	2	48	4.01	0.0245
Place*Month*Year	2	48	10.31	0.0002
Size*Year	1	48	0.50	0.4808
Place*Size*Year	1	48	4.50	0.0390
Month*Size*Year	2	48	2.30	0.1116
Place*Month*Size*Year	2	48	1.62	0.2094

Table 4.20 Average zinc concentration (µg/g) for each variable

	VB	GI	Small	Large	July	Aug	Sept	2011	2012
Zinc	108.18 ± 64.16	66.59 ± 16.09	103.19 ± 55.16	71.58 ± 25.09	68.16 ± 32.35	72.46 ± 14.71	121.52 ± 73.32	59.01 ± 18.01	115.75 ± 169.55

4.2.11 Vanadium

Vanadium demonstrated a statistical difference for the single variables: place, month, size, and year (Table 4.21). The variable year ($F_{1,48}$ = 31.21, P = <.0001) had the lowest P-value of all single and multiple factor models, with 2012 having statistically higher concentrations of vanadium than 2011 at 3.51 ± 1.13 µg/g and 2.64 ± 1.23 µg/g, respectively. The variable size ($F_{1,48}$ = 18.09, P = <.0001) was also significant with small fish having higher concentrations at 3.38 ± 0.11µg/g than large fish at 2.77 ± 1.11 µg/g. The variable, place ($F_{1,48}$ =8.48, P = 0.0054), had statistically greater levels of vanadium at VB, with a concentration at 3.29 ± 0.41 µg/g, as compared to GI, at 2.86 ± 0.81 µg/g. The last variable, month ($F_{2,48}$ =7.78, P = 0.0012), had the highest concentrations of vanadium during the months of August, at 3.35 ± 0.78 µg/g, and September, at 3.21 ± 0.07 µg/g. When compared to July, with a concentration at 2.67 ± 1.12 µg/g, August and September were statistically greater; although when compared to one another there was no statistical difference. All mean concentration values for vanadium are indicated in Table 4.22.

The interactions place*month, place*month*size, place*year, month*year, place*month*year, size*year, and place*size*year were all significant for vanadium (Table 4.21). For the interaction size*year ($F_{1,48}$ =18.53, P = <.0001) there was a statistical difference when comparing different sizes within the same year. In 2011, small fish had higher concentrations of vanadium, at 3.30 ± 1.18 µg/g, than large fish, at 1.99 ± 0.90 µg/g. However in 2012, large fish had higher concentrations than small fish at 3.56

± 1.34 µg/g and 3.46 ± 0.91 µg/g, respectively. When comparing the same size between years, small*2012 did not statistically differ from small*2011; while large*2012 were statistically higher, at 3.56 ± 1.34 µg/g, than large*2011 at 1.99 ± 0.90 µg/g.

The next interaction that had a significant interaction was month*year ($F_{2,48}$ = 10.23, P = 0.0002). When comparing the same month between years, July*2012, at 3.46 ± 0.97 µg/g, was statistically higher than July*2011, at 1.88 ± 0.98 µg/g. August*2012 also had statistically higher concentrations than August*2011 at 3.91 ± 1.30 µg/g and at 2.80 ± 0.93 µg/g, respectively; September did not statistically differ between 2011 and 2012. When comparing different months within the same year, September*2011 and August*2011 did not statistically differ from one another, but were statistically greater from July*2011; with July having the lowest concentration at 1.88 ± 0.98 µg/g. In 2012, there was no statistical difference between the three months.

The three way interaction: place*month*year ($F_{2,48}$ =9.87, P = 0.0003) was significant for vanadium. When comparing the interaction of different places (VB to GI), same month (July to July), and same year (2011 to 2011) there was a statistical difference for: VB*July*2011 at 2.61 ± 0.59 µg/g compared to GI*July*2011 at 1.15 ± 0.70 µg/g, and VB*August*2012 at 4.90 ± 1.11 µg/g compared to GI*August*2012 at 2.91 ± 0.30 µg/g. Other interactions were statistically significant.

The interaction place*month ($F_{2,48}$ = 8.20, P = 0.0009) was also significant for vanadium. When comparing the month between locations, VB*July was statistically higher than GI*July at 2.99 ± 0.83 µg/g and 2.35 ± 1.54 µg/g, respectively; For August, VB, at 3.92 ± 1.40 µg/g, was statistically higher than GI, at 2.78 ± 0.75 µg/g; while September did not differ statistically between the two locations. When comparing

different months within the same location, GI*September, at 3.44 ± 1.14 µg/g, was significantly higher than GI*July, at 2.35 ± 1.54 µg/g; while GI*August did not statistically differ from either month. For VB, there was no statistical difference between the three months.

The interaction place*year ($F_{1,48}$ =6.94, P = 0.0113), was significant when comparing different locations within the same year. In 2011, VB had significantly higher concentrations of vanadium, at 3.00 ± 1.08 µg/g, as compared to GI, at 2.29 ± 1.29 µg/g. Conversely, in 2012 there was no statistical difference in vanadium concentrations between the two locations. When comparing the same location between years, there was no statistical difference between 2011 and 2012 for VB. For GI, 2012 was statistically higher than 2011 at 3.43 ± 0.89 µg/g and 2.29 ± 1.29 µg/g, respectively.

Table 4.21 Numerator DF, Denominator DF, F-Value and P-Value of variables and interactions of variables for vanadium

VANADIUM				
Effect	**Numerator DF**	**Denominator DF**	**F Value**	**Pr > F**
Place	1	48	8.48	0.0054
Month	2	48	7.78	0.0012
Place*Month	2	48	8.20	0.0009
Size	1	48	18.09	< .0001
Place*Size	1	48	0.64	0.4280
Month*Size	2	48	2.52	0.0913
Place*Month*Size	2	48	3.28	0.0462
Year	1	48	31.21	< .0001
Place*Year	1	48	6.94	0.0113
Month*Year	2	48	10.23	0.0002
Place*Month*Year	2	48	9.87	0.0003
Size*Year	1	48	18.53	< .0001
Place*Size*Year	1	48	5.61	0.0219
Month*Size*Year	2	48	0.08	0.9205
Place*Month*Size*Year	2	48	2.40	0.1017

Table 4.22 Average vanadium concentration (µg/g) for each variable

	VB	**GI**	**Small**	**Large**	**July**	**Aug**	**Sept**	**2011**	**2012**
Vanadium	3.29 ± 0.41	2.86 ± 0.81	3.38 ± 0.11	2.77 ± 1.11	2.67 ± 1.12	3.35 ± 0.78	3.21 ± 0.07	2.64 ± 1.23	3.51 ± 1.13

The three way interaction: place*size*year ($F_{1,48}$ =5.61, P = 0.0219) was significant when comparing the interaction of the same place (VB to VB), different size (small to large), and same year (2011 to 2012) for: GI*small*2011 at 3.07 ± 1.14 µg/g compared to GI*large*2011 at 1.50 ± 0.94 µg/g. When comparing the interaction of different places (VB to GI), same size (small to small), and same year (2011 to 2011) there was a statistical difference for: VB*large*2011at 2.48 ± 0.54 µg/g compared to GI*large*2011 at 1.50 ± 0.94 µg/g. Other interactions were statistically significant.

4.3 Discussion

4.3.1 Place

For VB the mean concentration of trace metals followed a sequence of:

Fe > Zn > Ba > As > V > Cu > Cr > Ni > Pb > Co > Cd.

For GI the mean concentration of trace metals followed a sequence of:

Fe > Zn > Ba > As > V > Cr > Cu > Ni > Pb > Co > Cd.

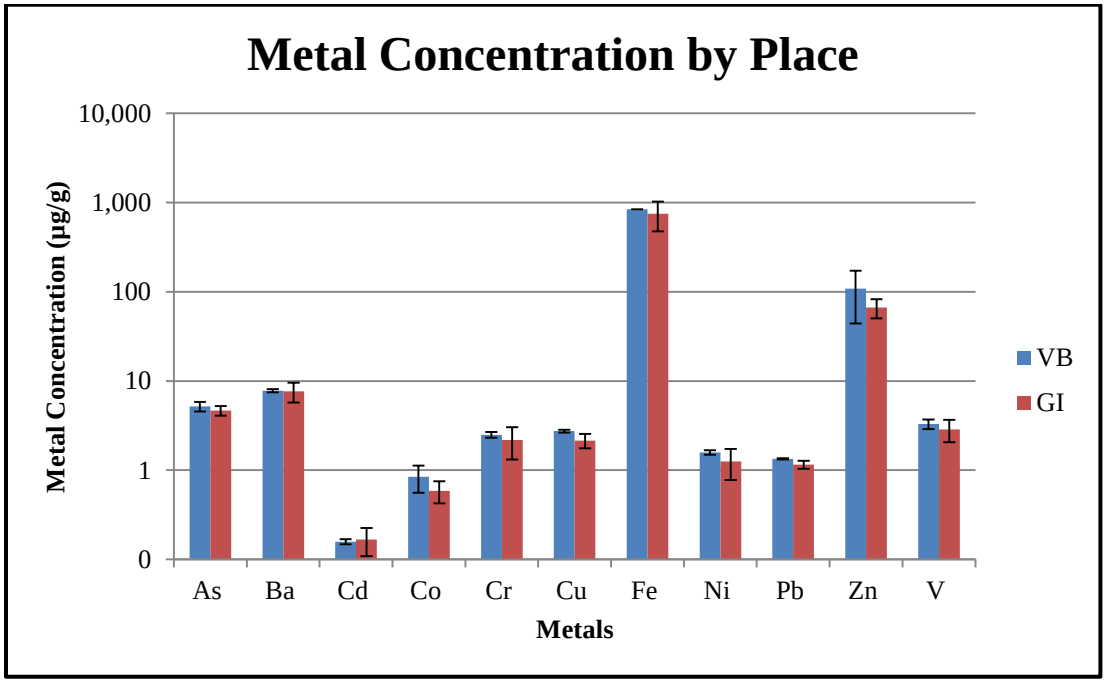

Figure 4.1 Mean metal concentrations (µg/g) at Vermillion Bay and Grand Isle for both years

Both locations followed the same sequence for metal concentrations, with the exception of chromium and copper exchanging order between the two sampling sites. Overall, VB had significantly higher concentrations for all metals, except for barium and cadmium which had no statistical difference (Figure 4.1).

4.3.2 Year

For 2011 the mean concentration of trace metals followed a sequence of:

Fe > Zn > Ba > As > V > Cu > Cr > Ni > Pb > Co > Cd.

For 2012 the mean concentration of trace metals followed a sequence of:

Fe > Zn > Ba > As > V > Cr > Cu > Ni > Pb > Co > Cd.

Both years had a similar sequence for metal concentrations, with copper and chromium differing. 2012 had statistically higher concentrations for all metals, except arsenic, cobalt, and lead. Cobalt and lead had no statistical differences between years; arsenic had significantly higher concentrations in 2011 fish than 2012 (Figure 4.2).

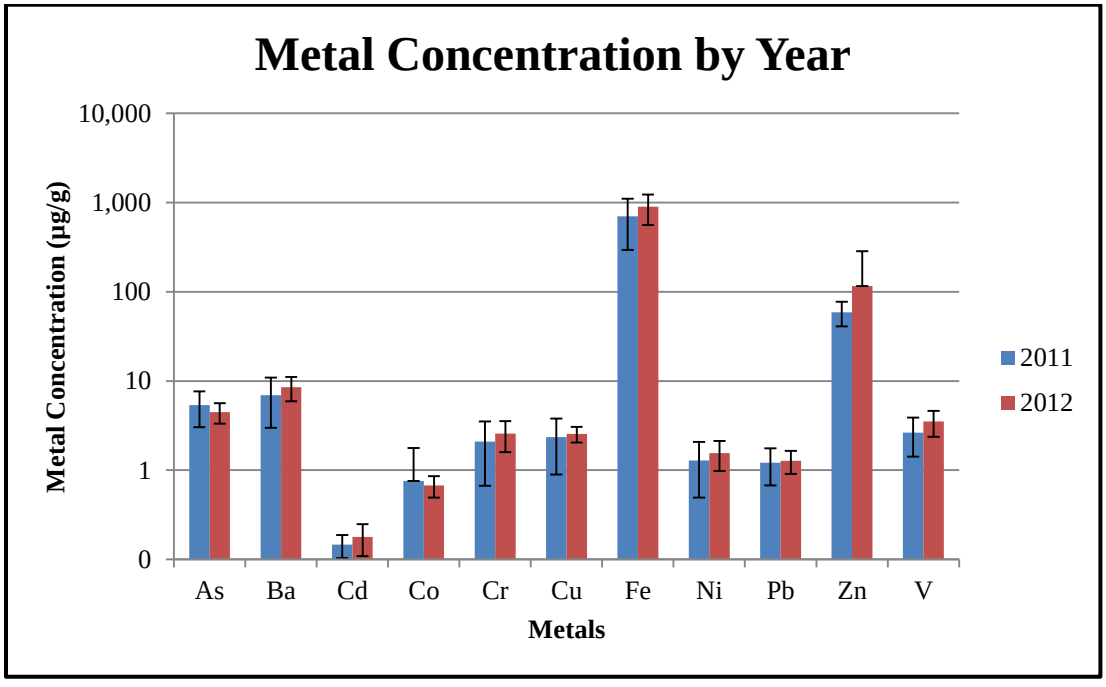

Figure 4.2 Mean metal concentrations (µg/g) in 2011 and 2012 for both locations

Arsenic was the only metal with higher concentrations in 2011 rather than 2012. Arsenic is naturally found in seawater and concentrations of arsenic are controlled by minerals found in ocean sediments (Smedley and Kinniburgh, 2002; Wainipee et al., 2010). The mineral goethite, an iron bearing oxide, filters arsenic and promotes regulation of low arsenic levels (Wainipee et al., 2010). In a study performed by Wainipee et al. (2010) the effects of adsorption of arsenic on goethite in the presence and absence of oil coatings was investigated. It was found that in the presence of oil, adsorption of arsenic by goethite was reduced by at least half when compared to oil-free conditions. When oil coats the goethite mineral it causes a physical barrier and reduces the surface area by approximately four times, which inhibits the arsenic from binding. The oil also alters the chemistry of the goethite, which in turn weakens the attraction between the arsenic and goethite. In addition, oil contains additional arsenic that contributes to the increase of concentration in the water (Wainipee et al., 2010). Another factor that may play an important role is the chemistry of the metal in the water. Metals can undergo structural changes, altering its binding affinity for its role in adsorption. Therefore, the DWH oil spill may have resulted in less arsenic being adsorbed by goethite, possibly leading to increased arsenic concentrations in the water. This may have resulted in higher arsenic concentrations in 2011 because concentrations were still high from the DWH oil spill.

4.3.3 Place*Year Interaction

When monitoring metal concentrations for both years, VB had significantly higher concentrations of trace metals than GI. However, when observing the interaction place*year and comparing different locations within the same year, the trend differed. In

2011, VB had statistically greater concentrations of barium, cadmium, cobalt, chromium, copper, iron, nickel, lead and vanadium than GI (Figure 4.3). In 2012, the only metal that differed was cadmium with higher concentrations at GI than VB (Figure 4.4)

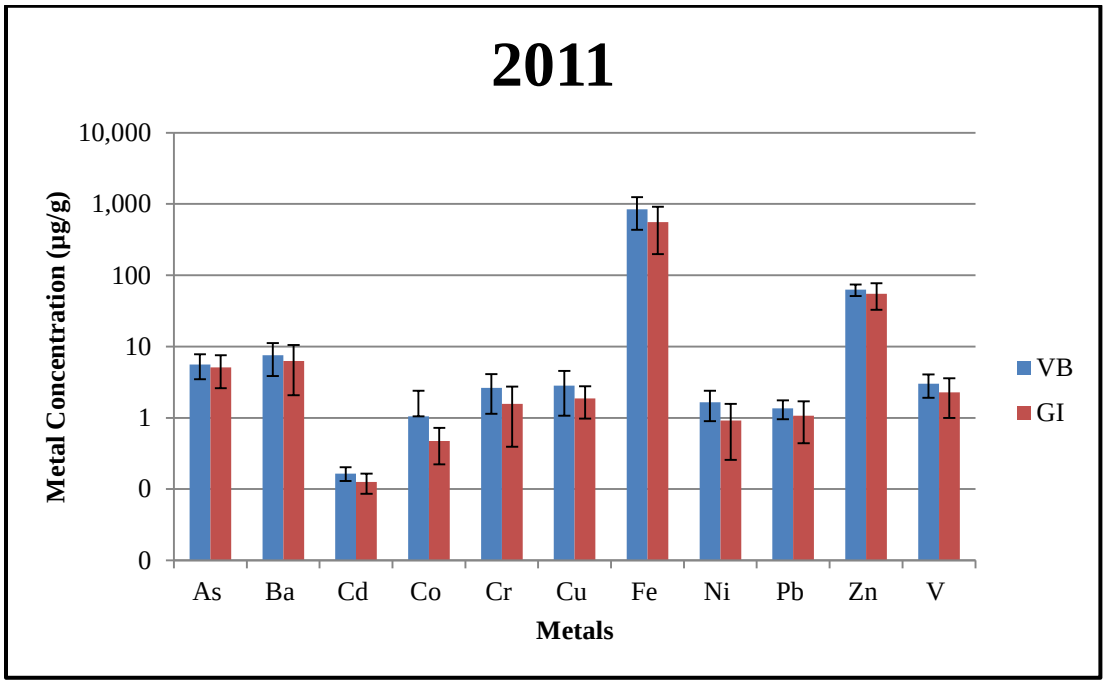

Figure 4.3 Mean metal concentrations (µg/g) at Vermillion Bay and Grand Isle for 2011

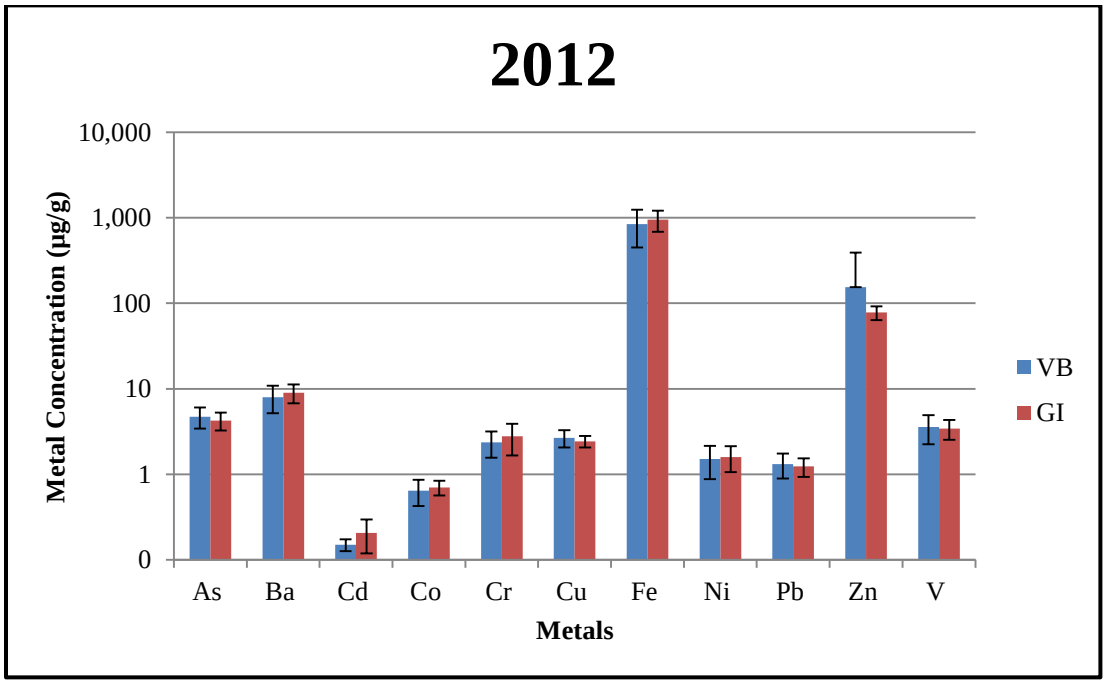

Figure 4.4 Mean metal concentrations (µg/g) at Vermillion Bay and Grand Isle for 2012

Higher trace metal concentrations in VB fish from 2011 may be the result of a high flood year where the Mississippi River discharged extreme amounts of freshwater to GI and diluted the metal concentrations. In 2011, the Mississippi River Flood broke several stage records, which resulted in the highest discharge recorded from Cairo, Illinois to the Morganza Floodway in Louisiana. Numerous meteorological factors led to the flood including, higher than normal snowfall over the Upper Mississippi Valley, raised river levels from heavy rain events from February to April, and extremely trace rain at the end of April/beginning of May. River stages and discharge rates from the Mississippi River Flood of 2011 were comparable to the major floods of 1927 and 1937.

Heavy snow in December 2010/early January 2011 and once more at the end of February/beginning of March began the Mississippi River Flood of 2011. By February 12th and 13th above average temperatures caused snow south of Rock Island, Illinois to melt. At the same time, river ice coverage of 70 to 100 percent north of St. Louis began to break-up, producing ice jam flooding. From the snowmelt and ice break-up, several tributaries in Iowa, Missouri, and Illinois experienced flooding by the third week of February. Additionally from snowmelt, on March 1st the Ohio River at Cairo, Illinois increased above flood stage (40.0 feet) to 44.3 feet and continued to a height of 50.7 feet by March 10th. In the last week of March, 150 to 300 percent of normal snow water equivalent was on the ground over Minnesota and Wisconsin, causing flooding along the main stem Mississippi River.

Rain also contributed to flood of 2011 when several cities reached historically high river crests. Heavy rains over the Lower Ohio Valley near Cairo, Illinois on March 18th caused the 14th highest historical Mississippi River crest at 53.41 feet; by May 2nd,

Cairo reached 61.0 feet with a projected crest of 63.0 feet on May 5th. On March 29th the Mississippi River at St. Paul, Minnesota surpassed major flood stage and produced its 8th highest crest at 19.01 feet. The U.S. Army Corps of Engineers (2012) determined two week totals from April 19th to May 4th over three extents along the Mississippi River;8 to 16 inches of rain fell over the Mississippi watershed from Arkansas City, Kansas to Caruthersville, Missouri and 12 to 22 inches occurred over the watershed from Caruthersville to Chester, Illinois and over the Lower Ohio Valley. Past records for that time period indicated that the totals were 600 to 1000 percent of normal rainfall (DeHaan et al., 2012). Gage height or river stage for the Mississippi River at Baton Rouge, Louisiana rose beyond flood stage (35.0 feet) from March 30th to April 7th and then fell below flood stage for the rest of April. River stages rose again during May and remained above flood stage until June 21st, with a maximum height of 45.48 feet on May 18th and 19th (USGS Current Conditions for Louisiana) (Figure 4.5).

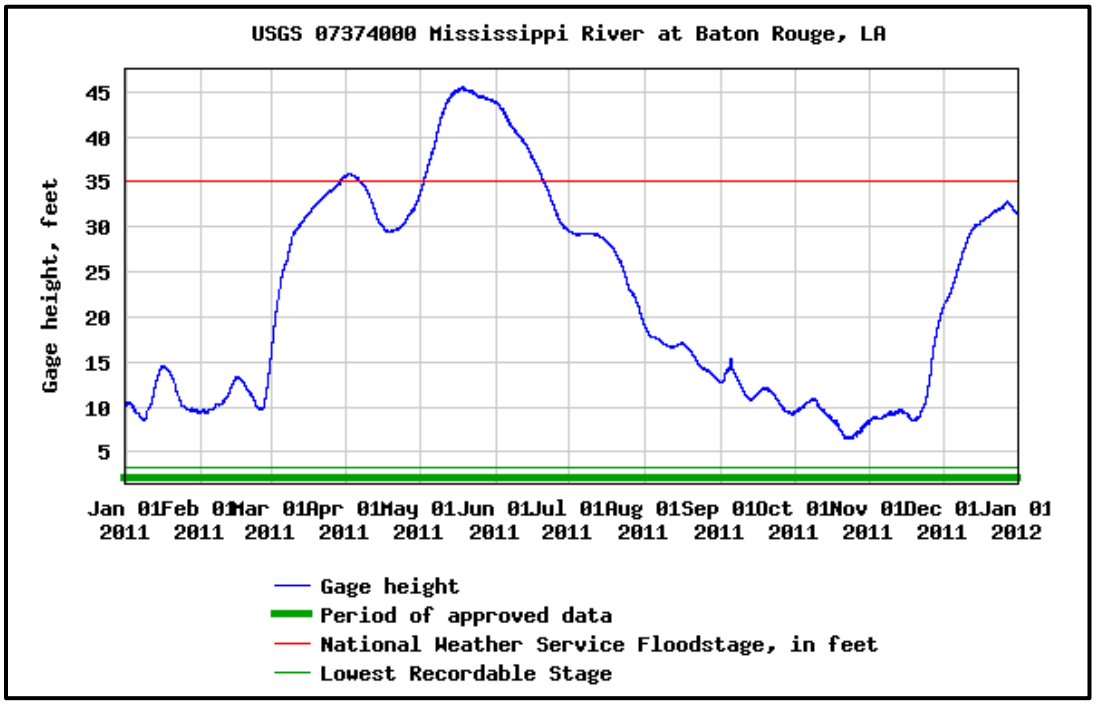

Figure 4.5 Monthly gage height (ft) for the Mississippi River at Baton Rouge, LA in 2011
Source: USGS Current Conditions for Louisiana

Furthermore, in 2011 the months March, April, May, June, July, August, September, and October had higher river stages than those in 2012 (USGS Surface-Water Monthly Statistics for Louisiana) (Table 4.23 and 4.24).

Table 4.23 Monthly mean gage height (ft) for the Mississippi River at Baton Rouge, LA in 2011
Source: modified from USGS Surface-Water Monthly Statistics for Louisiana

2011	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mean of Gage height	10.9	11.1	29.6	32.2	42.4	37.3	26.8	16.1	11.7	8.7	10.7	29.0
Incomplete data has been used for statistical calculation												

Table 4.24 Monthly mean gage height (ft) for the Mississippi River at Baton Rouge, LA in 2012
Source: modified from USGS Surface-Water Monthly Statistics for Louisiana

2012	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Mean of Gage height	25.50	28.05	25.01	23.75	16.27	7.98	5.26	4.68	4.74	5.37
Incomplete data has been used for statistical calculation										

Heavy rainfall, elevated river levels and greater than average snowfall were the significant factors that led to the 2011 Flood. Discharge rates in 2011 were higher than those in 2012 (USGS Current Conditions for Louisiana) (Figure 4.6 and 4.7). In 2011, the months March, April, May, June, July, August, and September had higher discharge rates than those months in 2012; May and June had the largest variation between years and highest mean discharge rates (USGS Surface-Water Monthly Statistics for Louisiana) (Table 4.25 & 4.26). In 2011, the mean discharge rate for May was 1,290,000 cfs (cubic feet per second) as compared to May of 2012 at 425,000 cfs. Additionally, on May 18 and 19,of 2011, discharge rates reached a peak of 1,436,000 cfs (USGS Current Conditions for Louisiana) (Figure 4.7).

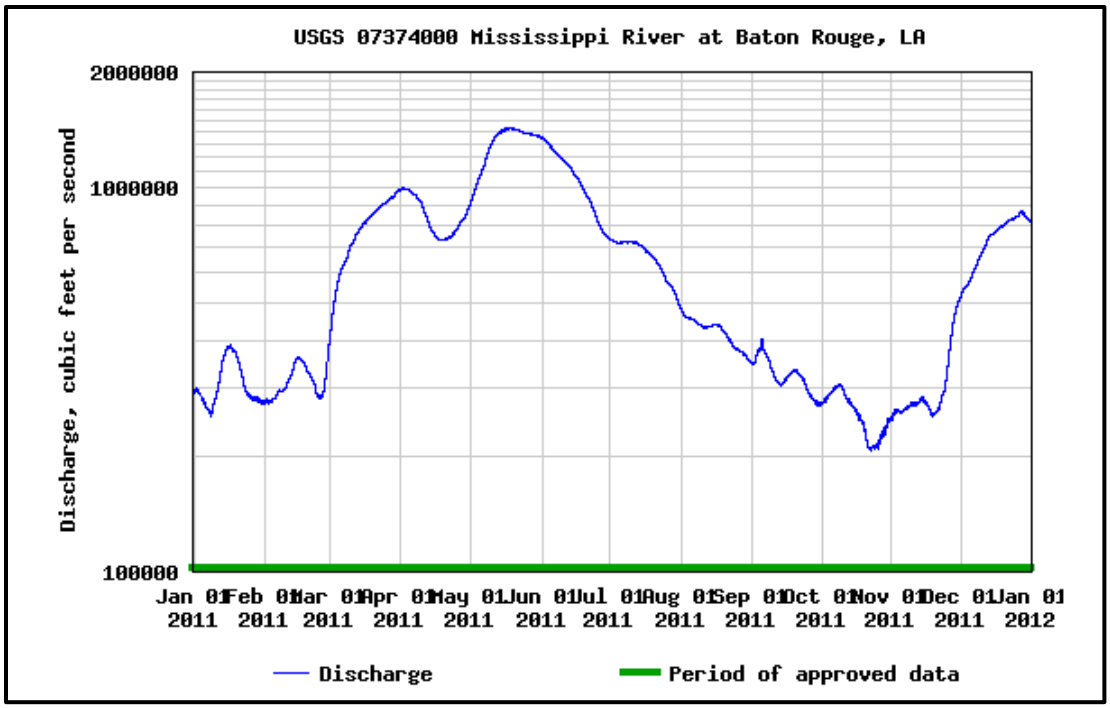

Figure 4.6 Monthly discharge (cfs)for the Mississippi River at Baton Rouge, LA in 2011
Source: USGS Current Conditions for Louisiana

The additional water and increase in discharge rates may be linked to the difference in metal concentrations seen in menhaden tissue between years and locations. Absorption of trace metals is affected by the metal concentration in water. The higher the metal concentration in the water, the more metals available to be taken up and accumulated by the fish (Jezierska and Witeska, 2006). When river stages and discharge rates are lower, as in droughts and winter months, metal concentrations are higher. Water volume is decreased and the dilution effect diminishes. During periods when river stages are higher and discharge rates are greater, as during a flood, metal concentrations are lower; trace metal concentrations are diluted with the greater volumes of water (Garbarino et al., 1995; Papafilippaki et al., 2008). During the Mississippi River Flood of 2011, there was a significant increase in water volume, which may have resulted in the dilution of trace metal concentrations. Furthermore, the dilution of the trace metals occurred specifically

at GI, since GI is closer to the Mississippi River than VB. There was an increase in water volume entering GI, which had a dilution effect on the concentration of trace metals. As a result, GI had lower metal concentrations than VB in 2011but the concentrations were not significantly different by location in 2012.

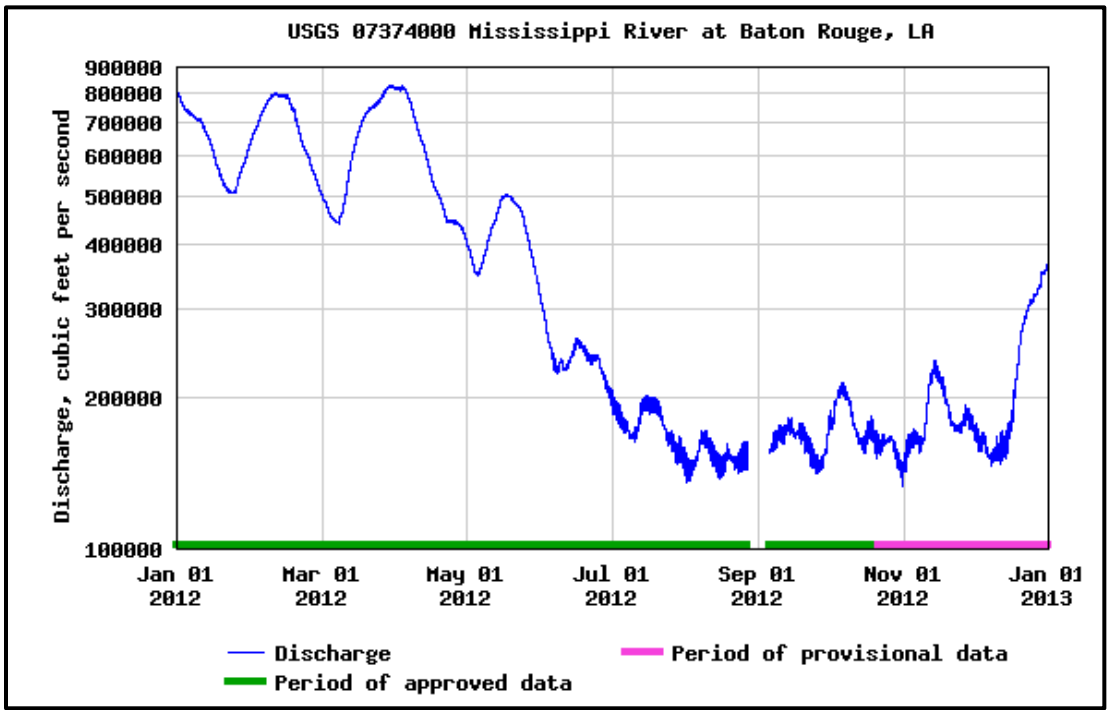

Figure 4.7 Monthly discharge (cfs) for the Mississippi River at Baton Rouge, LA in 2012
Source: USGS Current Conditions for Louisiana

Table 4.25 Monthly mean discharge (cfs) for the Mississippi River at Baton Rouge, LA in 2011
Source: modified from USGS Surface-Water Monthly Statistics for Louisiana

2011	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mean of Discharge	309	312	768	845	1,290	1,060	661	421	326	259	304	736
** In hundreds of thousands												
No Incomplete data has been used for statistical calculation												

Table 4.26 Monthly mean discharge (cfs) for the Mississippi River at Baton Rouge, LA in 2012
Source: modified from USGS Surface-Water Monthly Statistics for Louisiana

2012	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Mean of Discharge	633	701	631	602	425	244	177	152	162
** In hundreds of thousands									
No Incomplete data has been used for statistical calculation									

Suspended sediment also plays a key role in metal absorption as trace metals bind and are transported with suspended sediment in the water column. In warmer months or floods, water volume increases and as a result suspended sediment concentrations increase. When suspended sediment concentrations increase, dissolved metal concentrations decrease through increased scavenging processes. Conversely, for droughts and winter months, suspended sediment concentrations decrease, reducing metal scavenging processes and dissolved metal concentrations (Garbarino et al., 1995). During the Mississippi River Flood of 2011, suspended sediment concentrations increased due to extreme river discharge (DeHaan et al., 2012). Trace metals enter the digestive tract of fish when suspended matter, sediments, and organisms serving as food sources are contaminated with trace metals (Dallinger et al., 1987). Gulf menhaden are obligate filter-feeding omnivores and they swim with their mouths open to filter particulates in the water including phytoplankton and suspended sediment (Franklin, 2007; Vaughan et al., 2007). Since GI is closer to the Mississippi River and there was an increase in suspended sediment, menhaden from GI in 2011 would be expected to display higher levels of metals than those from VB. However, the results demonstrated the opposite trend with VB having higher concentrations of metals than GI in 2011. As stated earlier, the higher the metal concentration in the water, the more metals are taken up and accumulated by

the fish. This relationship between body metal level and waterborne concentration is only related if the metal is taken up by the fish from the water. This relationship is not certain if the source of metal is from food (Jezierska and Witeska, 2006). Therefore, data from the current study suggests that the relationship between metal absorption and food is not as effective as absorption from dissolved metal concentrations. Another possible explanation is that the dilution effect had a greater impact than the effect of suspended sediment.

Water temperature also plays an important role in metal absorption by increasing in the rate of uptake of certain metals as water temperature increases (Jezierska and Witeska, 2006). Studies have shown that an increased water temperature, as during summer months, leads to a higher uptake of metals (Kock et al., 1996; Douben, 1989b). Increased temperatures can cause higher metabolic rates, which in turn result in a higher rate of metal uptake and binding (Jezierska and Witeska, 2006; Papafilippaki et al., 2008). In 2011, water temperatures were lower than those in 2012 (USGS Current Conditions for Louisiana) (Figure 4.8 and 4.9); specifically, temperatures were lower from January to July in 2011 than those in 2012 (USGS Surface-Water Monthly Statistics for Louisiana) (Table 4.27 and 4.28). This decrease in water temperature can be attributed to the Mississippi River Flood of 2011, where extreme drops in river water temperature occurred because of large rainstorms (DeHaan et al., 2012). Lower water temperatures may have affected metal absorption in menhaden from GI, where concentrations were lower than menhaden from VB in 2011.

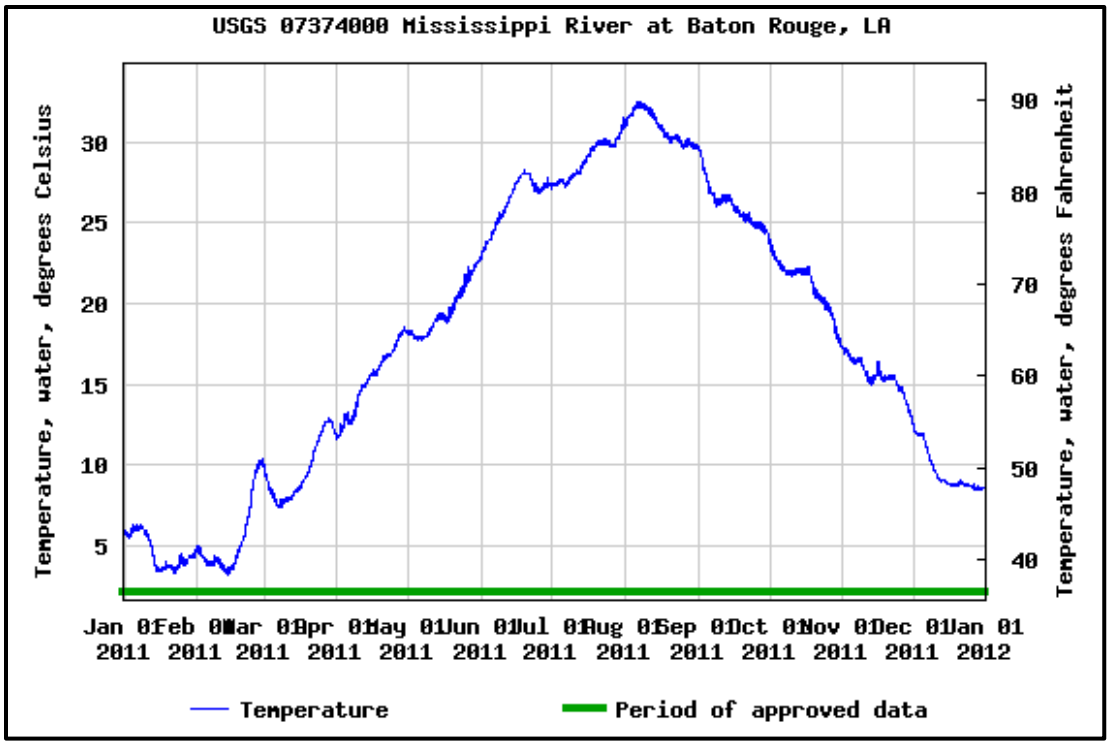

Figure 4.8 Monthly water temperature for the Mississippi River at Baton Rouge, LA in 2011

Source: USGS Current Conditions for Louisiana

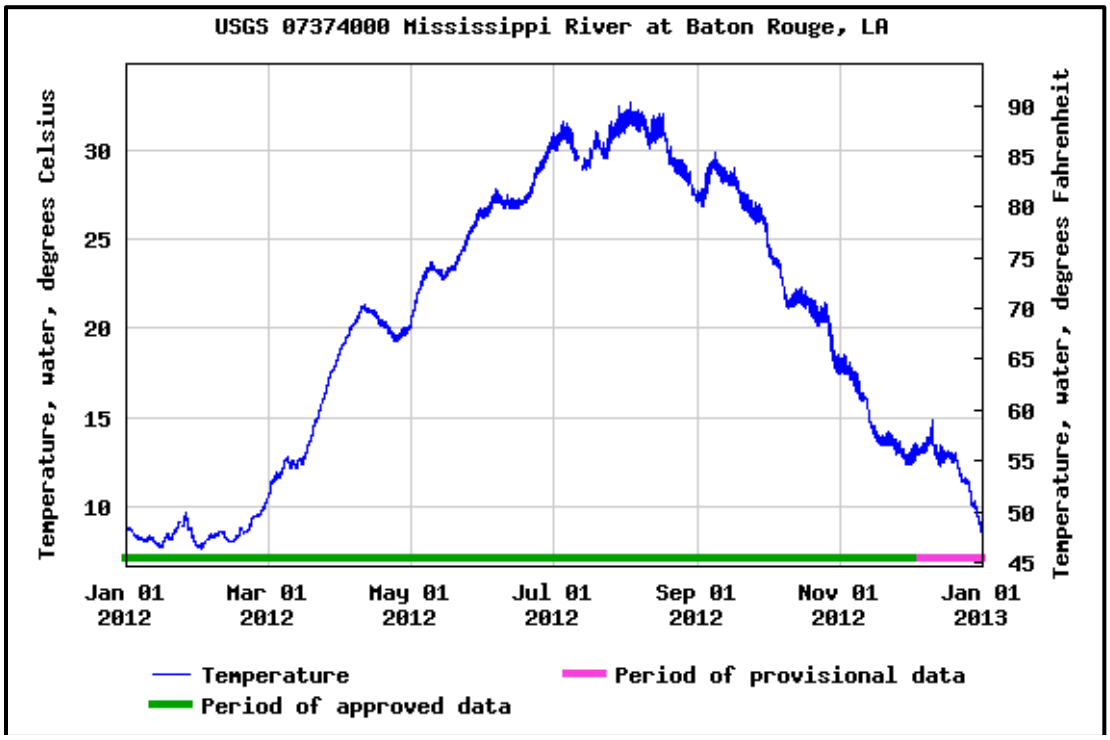

Figure 4.9 Monthly water temperature for the Mississippi River at Baton Rouge, LA in 2012

Source: USGS Current Conditions for Louisiana

Table 4.27 Monthly mean water temperature (ºC) for the Mississippi River at Baton Rouge, LA in 2011
Source: modified from USGS Surface-Water Monthly Statistics for Louisiana

2011	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mean of Temp in Water	4.6	5.3	9.6	15.2	19.6	26.3	28.9	31.0	26.2	21.2	15.5	9.6
No Incomplete data has been used for statistical calculation												

Table 4.28 Monthly mean water temperature (ºC) for the Mississippi River at Baton Rouge, LA in 2012
Source: modified from USGS Surface-Water Monthly Statistics for Louisiana

2012	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mean of Temp in Water	8.4	8.6	13.8	20.1	23.6	27.7	30.4	30.2	27.6	21.3	14.9	13.0
Incomplete data has been used for statistical calculation												

Several other factors such as pH, water hardness, and salinity have also been shown to be important in metal uptake and accumulation (Dallinger et al., 1987; Jezierska and Witeska, 2006). While these environmental factors are important there was not enough variance between location, year, or month for each factor. Therefore, it can be concluded that for the current study pH, water hardness, and salinity did not play a significant role in the rate of absorption for metals. While it has been determined that the Mississippi River flood led to a dilution of metal concentrations, further results of the current study cannot be attributed to the DWH oil spill. Since pre-spill baselines of trace metals in gulf menhaden tissues are not known, there is no basis for which comparison can be made to the 2011 and 2012 data.

4.3.4 Fish Size

For small fish the mean concentration of trace metals followed a sequence of:

Fe > Zn > Ba > As > V > Cr > Cu > Ni > Pb > Co > Cd.

For large fish the mean concentration of trace metals followed a sequence of:

Fe > Zn > As > Ba > V > Cu > Cr > Ni > Pb > Co > Cd.

Both fish sizes had similar trends for metal concentration sequence with some variation. Small fish demonstrated significantly greater concentrations for all metals, except cadmium, zinc, and arsenic. Cadmium and zinc showed no statistical differences in concentration between sizes of fish; arsenic had significantly larger concentrations in large fish than small fish (Figure 4.10). Fish size and metal concentration demonstrated an inverse relationship, as younger fish had higher concentrations of metals and large fish had lower metal concentrations.

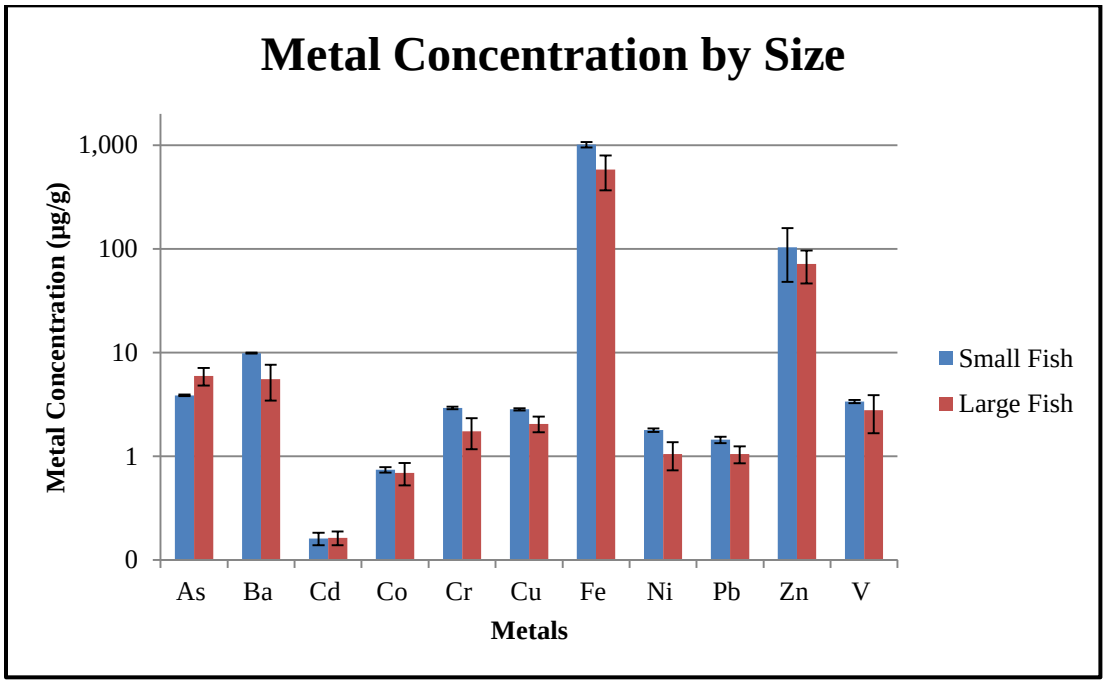

Figure 4.10 Mean metal concentrations (µg/g) for small and large fish for both years and locations

Metabolic activity is one of the most important factors in trace metal accumulation in fish, as young fish normally have higher metabolic activity than older fish (Heath, 1987; Langston, 1990; Roesijadi and Ribinson, 1994; Canli and Atli, 2003). Consequently, this leads to higher rates of metal accumulation in younger fish than older

ones (Douben, 1989a; Nussey et al., 2000; Widianarko et al., 2000). A study by Canli and Atli (2003) supports the negative relationships between metal concentrations and fish size due to a difference in metabolic activity between the younger and older fish. The net accumulation of trace metals in an organism is a consequence of the variance between uptake and excretion, the most important factor in metal accumulation.

4.3.5 Place*Size Interaction

For the interaction place*size only arsenic had a statistical difference, with large fish demonstrating significantly higher concentrations than small fish for both locations (Figure 4.11).

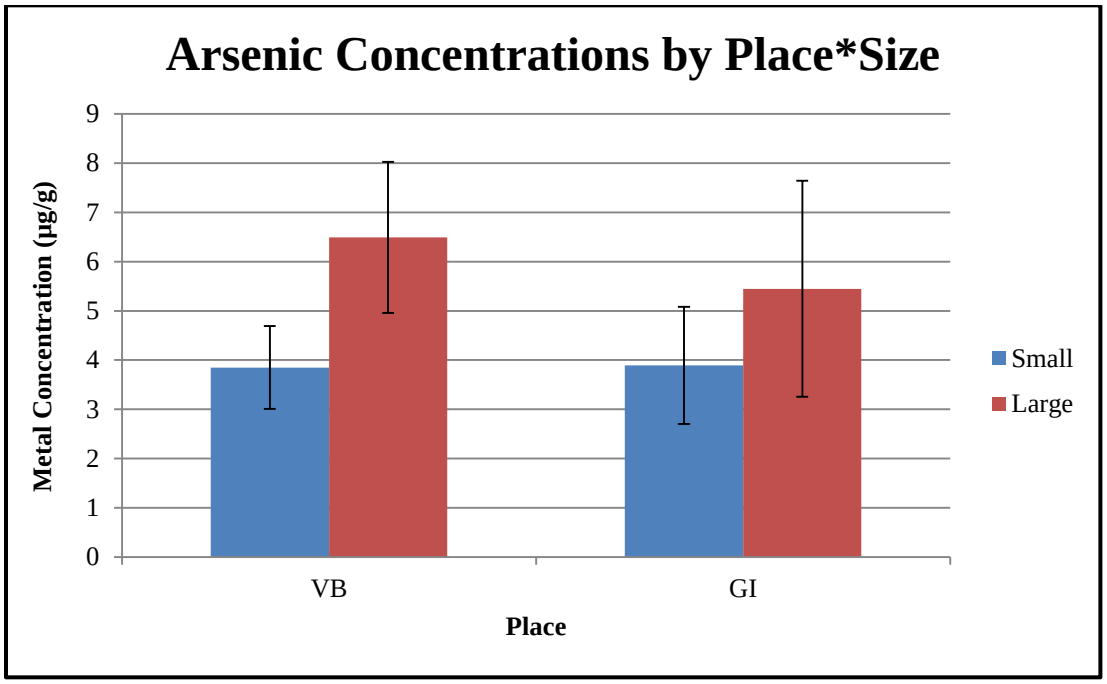

Figure 4.11 Mean arsenic concentrations (µg/g) at Vermillion Bay and Grand Isle for small and large fish

Higher concentrations of arsenic in large fish rather than small fish may be linked to higher concentrations of arsenic in the water. This may be linked to a concept by Canli and Atli (2003), where a positive relationship between animal size and metal concentrations in tissues will result. If metal concentrations in water are higher than the

capacity of dilution of tissue metal concentrations from growth and/or lowered metabolic activity in older individuals, accumulation of metals may continue (Canli and Atli, 2003). As previously discussed, arsenic concentrations in seawater are controlled by the mineral goethite (Smedley and Kinniburgh, 2002; Wainipee et al., 2010). The goethite filters arsenic and keeps arsenic levels suitable for marine life (Wainipee et al., 2010). Wainipee et al. (2010) demonstrated the negative effects of adsorption of arsenic on goethite in the presence oil. Adsorption of arsenic by goethite was reduced because the oil creates a physical barrier, which inhibits the arsenic from binding. The chemistry of the goethite is also altered from the oil and weakens the attraction between the arsenic and goethite. Additionally, oil contains more arsenic that leads to higher levels in the water (Wainipee et al., 2010). The chemistry of the metal in the water may also play a role. Metals can undergo structural changes, altering its binding affinity for its role in adsorption. Results from the current study suggest that the DWH oil spill may have resulted in less adsorption of arsenic by goethite, causing increased arsenic concentrations in the water. As a result, the concentrations of arsenic in the water would be greater than the dilution of tissue metal concentrations from growth and/or metabolic activity in large fish.

4.3.6 Month

For July the mean concentration of trace metals followed a sequence of:

Fe > Zn > Ba > As > V > Cu > Cr > Pb > Ni > Co > Cd.

For August the mean concentration of trace metals followed a sequence of:

Fe > Zn > Ba > As > V > Cr > Cu > Ni > Pb > Co > Cd.

For September the mean concentration of trace metals followed a sequence of:

Fe > Zn > Ba > As > V > Cu > Cr > Ni > Pb > Co > Cd.

All three months had similar sequences for metal concentrations, with copper, chromium, lead and nickel varying. For most metals, August and September did not statistically differ; however for all metals except lead, July had the lowest concentration statistically. There was no significant difference in lead concentrations between months (Figure 4.12).

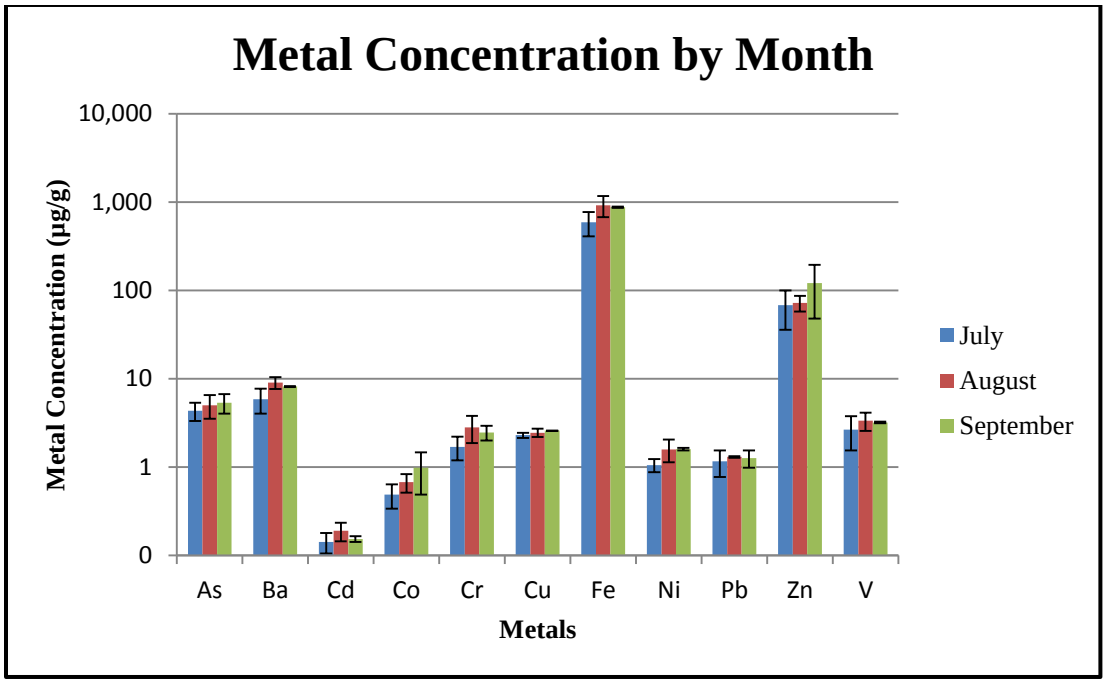

Figure 4.12 Mean metal concentrations (µg/g) for July, August, and September for both years and locations

4.3.7 Month*Year Interaction

For the interaction month*year all metals had a significant interaction, except copper. When comparing the same month between years, all metals that were significantly different had higher concentrations during July*2012 than July*2011. Similarly, for the month of August 2012 had significantly higher concentrations than 2011 (Figure 4.13 & 4.14). Arsenic also displayed a different trend than other metals for August. Arsenic was the only metal that had statistically higher concentrations during August*2011 than August*2012. For most metals, September did not statistically differ

between the two years. However, for arsenic, cobalt, and lead September*2011 had significantly higher concentrations than September*2012. Zinc was the only metal with significantly higher concentrations during September*2012 than September*2011.

When comparing different months within the same year, the trend varied. In 2011, most metals displayed no statistical difference between the months August and September; July was statistically lower than both months. Cadmium differed from this trend, with September having statistically higher concentrations than July; while August did not statistically differ from either month. Cobalt also differed, with September having statistically higher concentrations than July and August, while July and August did not differ statistically. In 2012, the trend followed that most metals had significantly higher concentrations during the month of August.

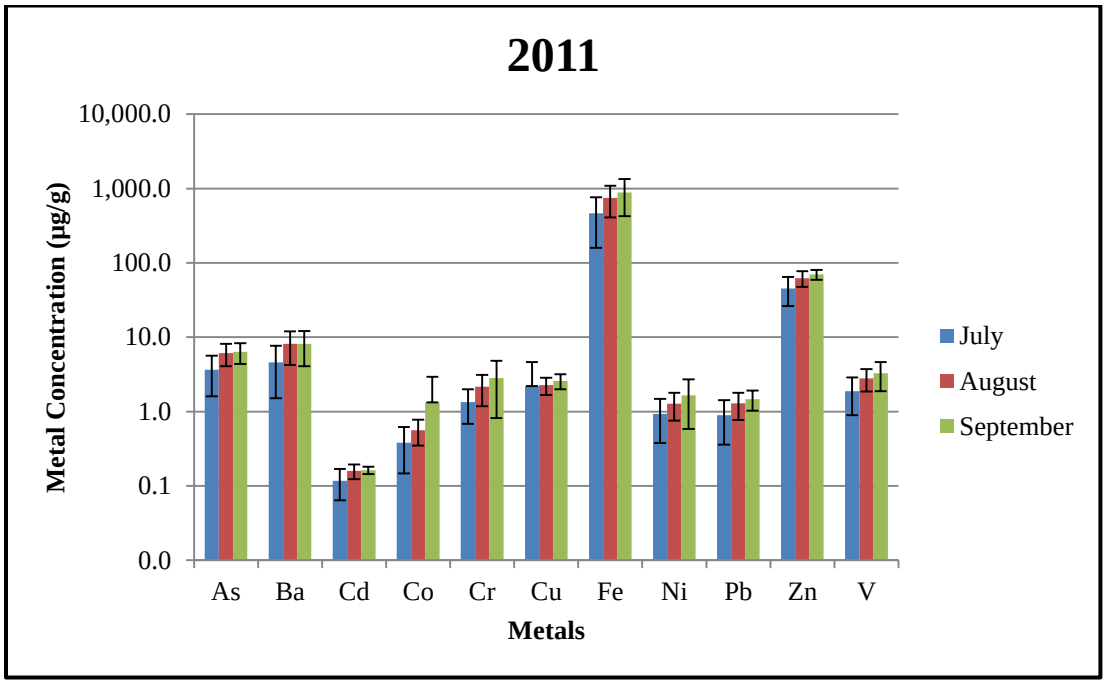

Figure 4.13 Mean metal concentrations (µg/g) for July, August, and September in 2011

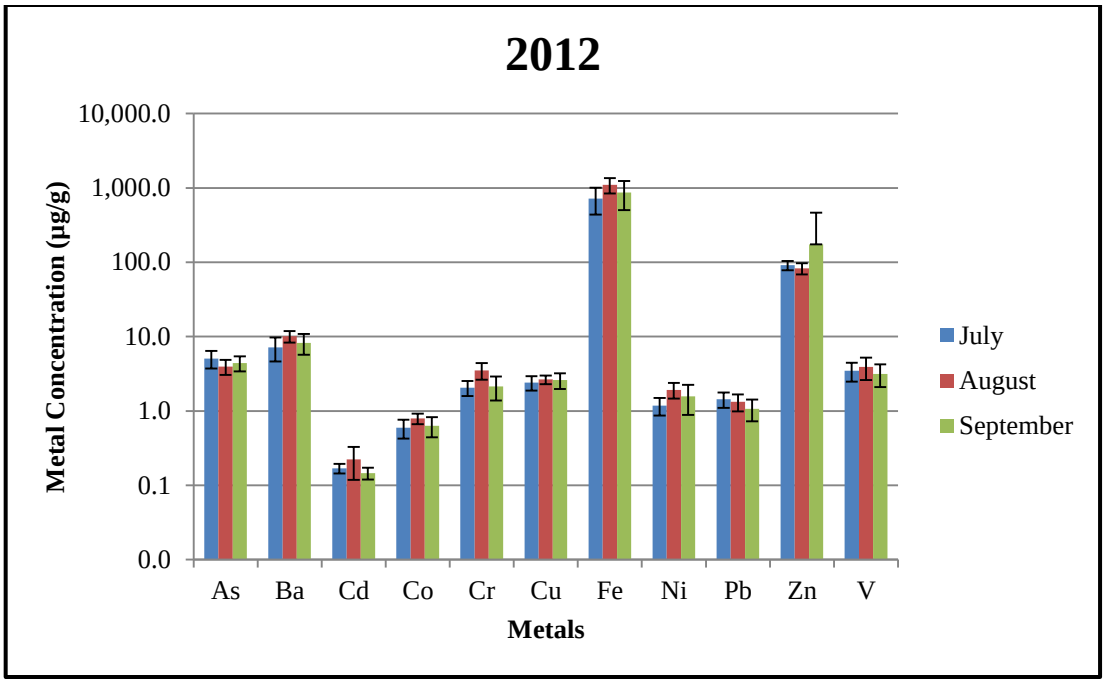

Figure 4.14 Mean metal concentrations (µg/g) for July, August, and September in 2012

Water temperature is important for metal absorption, with increasing water temperatures causing increases in the rate of uptake of metals (Jezierska and Witeska, 2006). In 2011, July had the lowest concentrations of all metals that were significantly different, while in 2012 the lowest metal concentrations were not always in July. When comparing the same month between years, metals that were significant in July and August 2011 had lower concentrations than those of 2012. All of these trends can be attributed to water temperature drops during the Mississippi River Flood of 2011, where great drops in river water temperature occurred due to large rainstorms (DeHaan et al., 2012). The lower water temperatures in 2011 and July*2011 may have affected metal absorption in menhaden resulting in lower metal absorption in 2011 and July*2011.

5. CONCLUSIONS

5.1 Summary of the Results

The FAO/WHO set maximum permissible limits of trace metals in tissue (FAO, 1983; FAO/WHO, 1984) (Table 5.1). Iron and zinc in this study exceeded the FAO/WHO maximum permissible limit while arsenic, cadmium, chromium, copper, nickel and lead were within the FAO/WHO set limits.

Table 5.1 Mean concentrations (µg/g) of trace metals in tissues of *B. patronus* compared with maximum permissible limits (µg/g)
Source: FAO (1983); FAO/WHO (1984)

	As	Cd	Cr	Cu	Fe	Ni	Pb	Zn
FAO/WHO	86.0	0.5-1.0	13.0	20.0-30.0	300.0	80.0	0.5-1.5	5.0-30
VB	5.17	0.16	2.50	2.74	841.94	1.58	1.34	108.18
GI	4.67	0.17	2.17	2.15	751.64	1.25	1.15	66.59
Small	3.87	0.16	2.92	2.84	1011.42	1.79	1.44	103.19
Large	5.97	0.16	1.75	2.06	582.16	1.05	1.05	71.58
2011	5.35	0.15	2.10	2.35	698.68	1.28	1.21	59.01
2012	4.49	0.18	2.57	2.55	894.90	1.55	1.28	115.75
VB*2011	5.62	0.17	2.63	2.82	841.34	1.65	1.35	62.81
GI*2011	5.08	0.13	1.57	1.87	556.02	0.92	1.07	55.21
VB*2012	4.72	0.15	2.37	2.67	842.54	1.52	1.32	153.54
GI*2012	4.26	0.21	2.78	2.43	947.25	1.59	1.24	77.96

Metal concentrations for all four variables (place, year, size and month) followed the sequence: Fe > Zn > Ba > As > V > Cr > Cu > Ni > Pb > Co > Cd. However, there was a variation in sequence for all four variables between Cu and Cr and a variation between Ba and As for fish size. Metal concentrations varied between all variables and all interactions between variables. For location, there was a difference in metal concentrations between VB and GI. Overall, VB had significantly higher concentrations than GI for all metals, excluding barium and cadmium which had no statistical difference. For metal concentrations between years, 2012 had significantly higher concentrations than 2011 for all metals, except arsenic, cobalt, and lead. There was no statistical

difference between cobalt and lead, while arsenic was significantly higher in 2011. Increased arsenic levels may be attributed to decreased adsorption of arsenic by the mineral goethite due to the DWH oil spill.

Metal concentrations differed by location and year. In 2011, VB had statistically higher concentrations than GI for 9 of the 11 metals (barium, cadmium, cobalt, chromium, copper, iron, nickel, lead and vanadium). However, in 2012 none of the metals were statistically higher at VB. In 2012, Cd was the only metal that was statistically higher at GI. In 2011, the Mississippi River broke several stage records and some of the highest discharge rates were recorded. Several meteorological factors led to the flood, including higher than normal snowfall over the Upper Mississippi Valley, raised river levels from heavy rain events from February to April, and extremely heavy rain at the end of April/beginning of May. All of these events caused a significant increase in water volume, which may have resulted in a dilution effect on the trace metal concentrations (USGS Current Conditions for Louisiana; USGS Surface-Water Monthly Statistics for Louisiana; Garbarino et al., 1995; Papafilippaki et al., 2008; DeHaan et al., 2012). Furthermore, because GI is closer to the output of Mississippi River than VB, metal concentrations were lower for GI in 2011. For 2012, there was not as much water volume output from the Mississippi River and GI had higher concentrations for many metals.

Size was also an important variable with small fish having higher concentrations for 8 metals (barium, cobalt, chromium, copper, iron, nickel, lead, and vanadium) having statistically higher concentrations. For large fish arsenic and cadmium had higher concentrations than small fish, however only arsenhic was statistically higher. An

inverse relationship between fish size and metal concentration is common and was demonstrated in the current study; younger fish had higher concentrations of metals, while larger fish had lower metal concentrations. Metabolic activity is significant in trace metal accumulation in fish. Younger/smaller fish have higher metabolic activities than older/larger fish, which leads to a higher metal accumulation in smaller fish (Heath, 1987; Douben, 1989a; Langston, 1990; Roesijadi and Ribinson, 1994; Nussey et al., 2000; Widianarko et al., 2000; Canli and Atli, 2003). A higher concentration of arsenic in large fish rather than small fish was linked to higher concentrations of arsenic in the water. Canli and Atli (2003) determined a positive relationship between animal size and metal concentrations in tissues would result if metal concentrations in water are higher than the capacity of dilution of tissue metal concentrations from growth and/or lowered metabolic activity in older individuals, accumulation of metals may continue (Canli and Atli, 2003). Arsenic concentrations in seawater are controlled by the mineral goethite, which adsorbs arsenic and keeps concentrations suitable for marine life (Smedley and Kinniburgh, 2002; Wainipee et al., 2010). However, in the presence of oil the goethite surface becomes covered with oil, which reduces the adsorption of arsenic by creating a physical barrier and altering the chemistry, weakening the attraction between the arsenic and goethite. Additionally, oil contains more arsenic that leads to higher levels in the water (Wainipee et al., 2010). Another factor that may play an important role is the chemistry of the metal in the water. Metals can undergo structural changes, altering its binding affinity for its role in adsorption. Therefore, results from the current study suggest that the DWH oil spill may have resulted in less adsorption of arsenic by goethite, causing increased arsenic concentrations in the water. Consequently, the

concentrations of arsenic in the water may have been greater than the dilution of tissue metal concentrations from growth and/or metabolic activity in large fish.

For monthly temporal changes, little variation among the three months was observed. July had the lowest concentrations among the three months for all of the metals except lead, which was not statistically significant. When observing the months by years, July*2011 had statistically lower concentrations than July*2012 for 8 metals (arsenic, barium, cadmium, chromium, iron, lead, zinc and vanadium). For the month of August, 2011 also had statistically lower concentrations than August*2012 for 6 metals (barium, cadmium, chromium, iron, nickel, and vanadium). Conversely in the month of September, 2011 had statistically higher concentrations for 3 metals (arsenic, cobalt, and lead) than 2012. Similarly to the variables place and year, July and August may have been affected by water temperature. As water temperature increases, the rate of uptake of metals also increases (Jezierska and Witeska, 2006). During the Mississippi River Flood of 2011, several large rainstorms caused river temperatures to drastically drop, especially in July (DeHaan et al., 2012). Lower temperatures may have led to lower metal absorption in 2011 for all three months, with the lowest temperatures in July.

5.2 Future Research

Trace metal analysis is an important area of toxicology, however research pertaining to metal bioaccumulation in marine fish is limited. Even at low levels, the essential metals required for metabolism and growth (zinc, iron, etc.) can become toxic (Garbarino et al., 1995). Therefore, it is important to monitor trace metal concentrations in the aquatic environment. While the FAO/WHO have maximum permissible limits of trace metals in fish, the limits are difficult to find and not publicly known. The limits also

varied from each document and did not specify limits between species or fresh and salt water fish. The shortage of published reference database for fish tissue quality in near-coastal areas, specifically in the Gulf of Mexico, limits an evaluation of the environmental significance of these results.

Several studies have revealed that metal concentrations differ significantly in organ-specific fish tissue.(Dallinger et al., 1987; Canli and Atli, 2003; Jezierska and Witeska, 2006). The differences in metal concentrations of the tissues may be due to their affinity to specific organs. Essential metals such as iron, zinc, copper, or cobalt show accumulation affinity in the organ where their main metabolic roles occur (Jezierska and Witeska, 2006). For example, a study by Dallinger et al. (1987) found that copper shows a distinct affinity to the liver, while zinc accumulates in the gonads. Toxic metals such as cadmium is found mainly in the kidney and liver; however it may also reach great levels in the gills, digestive tract and spleen (Jezierska and Witeska, 2006). Lead concentrates in numerous organs including the liver, kidneys, spleen, digestive tract, gills and bone (Dallinger et al., 1987). Fish muscles generally contain the lowest concentrations of metals (Jezierska and Witeska, 2006). Also, preliminary research in gulf menhaden has indicated structural lesions and functional disturbances; similar studies have shown that these disturbances are due to accumulation of metals in various organs (Jezierska and Witeska, 2006). Therefore, further research on gulf menhaden should focus on trace metals in specific organs.

Additionally, increased arsenic concentrations in the water may have resulted from the DWH oil spill. Arsenic is toxic at high concentrations and even chronic exposure to low concentrations is damaging. Accumulation of arsenic in fish and other

aquatic life can result in developmental and behavioral changes. Additionally, higher trophic animals are at risk by eating contaminated food, resulting in a food chain effect.

Furthermore, results from the current study included two years of data. Longer time periods are required to determine if the DWH spill has affected gulf menhaden chronically. The Mississippi River Flood of 2011 may have diluted the concentrations of trace metals. However, provisional data of 2013 monthly discharge from the Mississippi River at Baton Rouge, LA displays rates similar to those in 2012. Therefore, if the Flood did play a role in metal accumulation, concentrations in 2013 should be similar to 2012 (USGS Current Conditions for Louisiana) (Figure 5.1). Additionally, environmental conditions and water concentrations should be taken at each location to determine the role that conditions play.

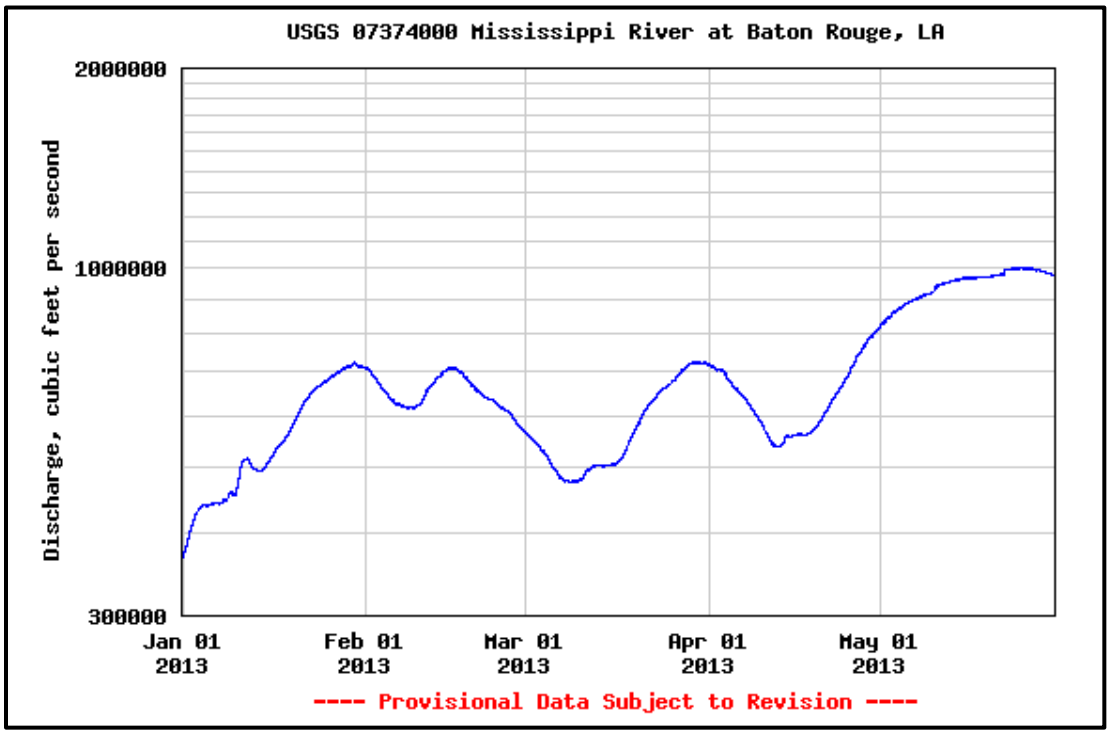

Figure 5.1 Monthly discharge (cfs) for the Mississippi River at Baton Rouge, LA in 2013
Source: USGS Current Conditions for Louisiana

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APPENDIX A: TOTAL GULF OF MEXICO MENHADEN LANDINGS (ALL FISHERIES) AND REDUCTION FISHERY EFFORT, 1963-1998

Year	Total Gulf Landings	Fishing Effort (1000 vessel-ton-weeks)
1963	438,939	277.3
1964	410,093	272.9
1965	463,952	335.6
1966	359,654	381.3
1967	317,555	404.7
1968	373,337	382.8
1969	523,991	411.0
1970	548,605	400.0
1971	728,868	472.9
1972	502,184	447.5
1973	486,655	426.2
1974	587,801	485.5
1975	542,940	538.0
1976	561,448	575.8
1977	447,458	532.7
1978	820,344	574.3
1979	779,383	533.9
1980	702,067	627.6
1981	552,562	623.0
1982	854,328	653.8
1983	923,571	655.8
1984	982,874	645.9
1985	883,520	560.6
1986	828,509	606.5
1987	907,109	604.2
1988	638,722	594.1
1989	519,587	555.3
1990	519,590	563.1
1991	550,718	472.3
1992	432,718	408.0
1993	551,822	455.2
1994	767,448	472.0
1995	472,039	417.0
1996	491,612	451.7
1997	621,943	430.2
1998	497,461	409.3

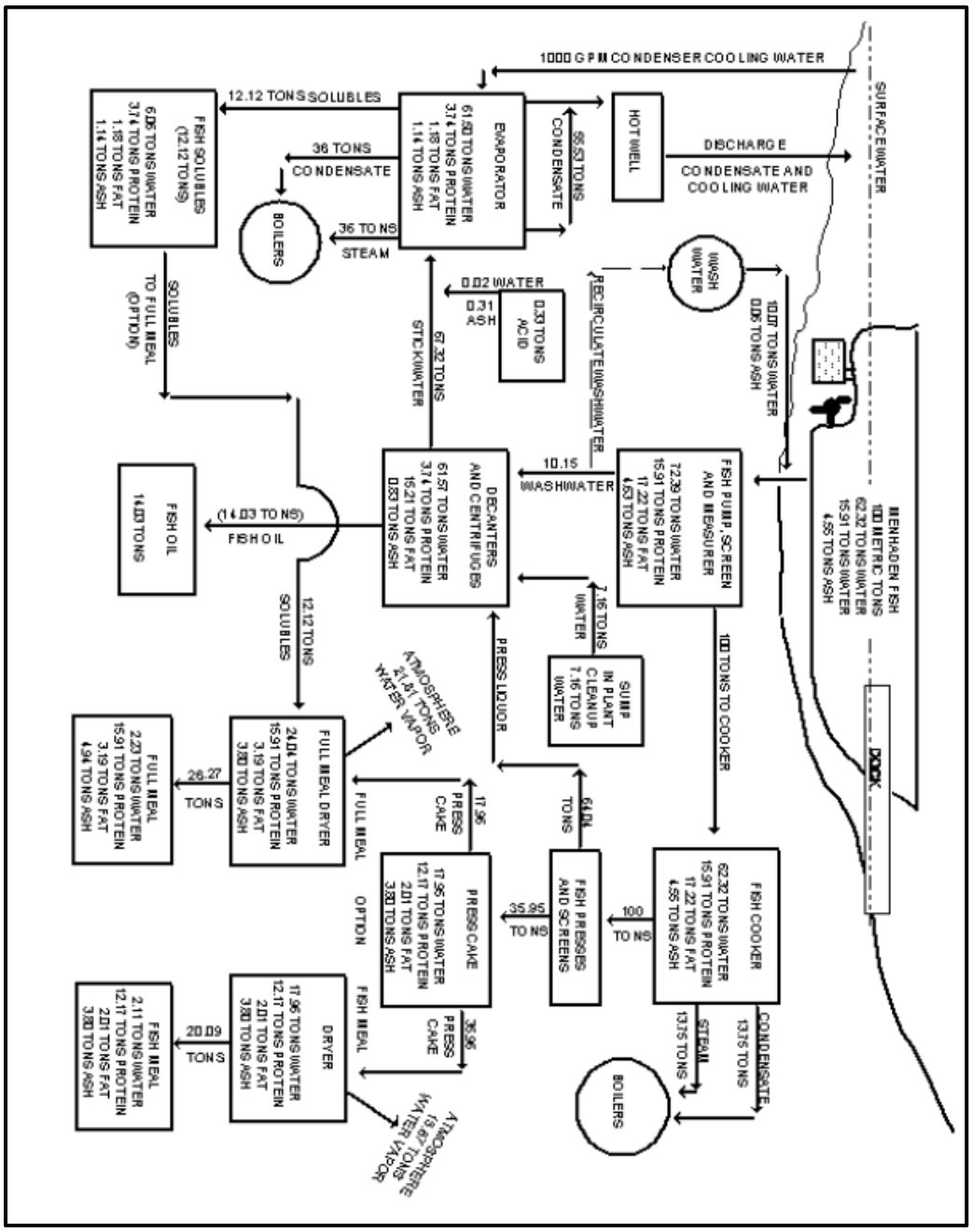
APPENDIX B: LANDINGS AND EX-VESSEL VALUE OF THE GULF MENHADEN REDUCTION FISHERY, 1980-1993

Year	Landings (1000 mt)	Value (x1000)
1980	701.3	69,100
1981	552.6	47,700
1982	853.9	72,300
1983	923.5	82,500
1984	982.8	88,000
1985	881.1	67,300
1986	822.1	67,000
1987	894.2	69,900
1988	623.7	71,300
1989	569.6	52,000
1990	528.3	55,600
1991	544.3	57,700
1992	421.4	50,200
1993	539.2	57,800
1994	761.6	_____
1995	463.9	_____
1996	479.4	_____
1997	611.2	_____
1998	486.2	_____

APPENDIX C: U.S. PRODUCTION, EXPORTS, AND IMPORTS OF FISH OIL IN LBS (x1000) FOR 1987-1998

Year	Domestic Production	Exports	Imports
1987	298,496	249,246	30,509
1988	224,733	150,002	27,667
1989	225,478	198,009	25,449
1990	281,949	236,589	36,702
1991	267,345	254,525	21,828
1992	180,899	177,446	23,772
1993	293,452	184,488	26,052
1994	291,189	242,788	40,642
1995	241,941	260,394	23,913
1996	248,399	187,294	35,622
1997	283,379	215,255	25,622
1998	223,149	196,664	24,213

APPENDIX D: THE PROCESSING OF 100 METRIC TONS OF RAW MENHADEN THROUGH A MODERN PLANT



APPENDIX E: MEAN CONCENTRATION VALUES FOR INTERACTIONS (µg/g)

Place*Month						
	As	**Ba**	**Cd**	**Co**	**Cr**	**Cu**
VB*July	5.07 ± 1.93	6.04 ± 2.88	0.16 ± 0.03	0.55 ± 0.21	1.95 ± 0.47	2.98 ± 2.12
VB*August	5.00 ± 1.72	9.22 ± 2.88	0.16 ± 0.04	0.71 ± 0.22	2.93 ± 0.74	2.41 ± 0.61
VB*September	5.44 ± 1.91	8.07 ± 3.35	0.16 ± 0.02	1.27 ± 1.63	2.62 ± 1.78	2.84 ± 0.50
GI*July	3.64 ± 1.49	5.73 ± 3.37	0.12 ± 0.06	0.43 ± 0.24	1.45 ± 0.76	1.62 ± 0.84
GI*August	5.06 ± 2.10	8.96 ± 3.44	0.22 ± 0.10	0.64 ± 0.20	2.73 ± 1.47	2.50 ± 0.43
GI*September	5.30 ± 1.80	8.28 ± 3.42	0.15 ± 0.03	0.70 ± 0.17	2.33 ± 1.25	2.34 ± 0.59
	Fe	**Ni**	**Pb**	**Zn**	**V**	
VB*July	648.18 ± 312.95	1.27 ± 0.39	1.43 ± 0.32	78.83 ± 18.17	2.99 ± 0.83	
VB*August	1010.71 ± 369.59	1.65 ± 0.43	1.35 ± 0.41	75.06 ± 22.29	3.92 ± 1.40	
VB*September	866.91 ± 437.02	1.83 ± 0.99	1.24 ± 0.49	170.64 ± 293.61	2.97 ± 1.27	
GI*July	533.46 ± 318.68	0.85 ± 0.44	0.90 ± 0.55	57.50 ± 33.12	2.35 ± 1.54	
GI*August	835.74 ± 311.12	1.54 ± 0.71	1.26 ± 0.45	69.86 ± 11.94	2.78 ± 0.75	
GI*September	885.72 ± 392.30	1.38 ± 0.71	1.30 ± 0.41	72.40 ± 11.57	3.44 ± 1.14	

Place*Size						
	As	**Ba**	**Cd**	**Co**	**Cr**	**Cu**
VB*Small	3.85 ± 0.84	10.12 ± 2.39	0.15 ± 0.04	0.80 ± 0.20	3.11 ± 1.29	3.26 ± 1.64
GI*Small	3.89 ± 1.19	9.64 ± 2.83	0.17 ± 0.07	0.68 ± 0.20	2.73 ± 1.34	2.42 ± 0.67
VB*Large	6.49 ± 1.53	5.43 ± 2.06	0.16 ± 0.02	0.89 ± 1.39	1.88 ± 0.65	2.23 ± 0.45
GI*Large	5.45 ± 2.19	5.67 ± 3.21	0.17 ± 0.09	0.49 ± 0.23	1.61 ± 0.97	1.88 ± 0.71
	Fe	**Ni**	**Pb**	**Zn**	**V**	
VB*Small	1083.72 ± 342.33	2.01 ± 0.67	1.55 ± 0.39	139.30 ± 240.79	3.58 ± 1.09	
GI*Small	939.12 ± 316.26	1.57 ± 0.69	1.34 ± 0.49	67.07 ± 18.03	3.17 ± 0.99	
VB*Large	600.16 ± 285.30	1.16 ± 0.37	1.13 ± 0.31	77.05 ± 20.44	3.00 ± 1.34	
GI*Large	564.16 ± 323.68	0.94 ± 0.53	0.97 ± 0.44	66.10 ± 25.55	2.54 ± 1.41	

Month*Size						
	As	**Ba**	**Cd**	**Co**	**Cr**	**Cu**
July*Small	3.22 ± 0.96	8.06 ± 1.94	0.14 ± 0.04	0.64 ± 0.16	1.92 ± 0.56	2.93 ± 2.23
August*Small	4.02 ± 0.78	10.86 ± 2.33	0.19 ± 0.07	0.78 ± 0.18	3.38 ± 1.02	2.65 ± 0.55
September* Small	4.37 ± 0.96	10.72 ± 2.51	0.16 ± 0.03	0.81 ± 0.22	3.47 ± 1.56	2.94 ± 0.51
July*Large	5.49 ± 1.81	3.71 ± 2.27	0.15 ± 0.05	0.34 ± 0.16	1.48 ± 0.70	1.67 ± 0.76
August*Large	6.04 ± 2.13	7.32 ± 2.81	0.19 ± 0.10	0.58 ± 0.19	2.28 ± 1.01	2.26 ± 0.41
September* Large	6.38 ± 1.93	5.62 ± 1.55	0.15 ± 0.02	1.16 ± 1.66	1.48 ± 0.43	2.24 ± 0.46
	Fe	**Ni**	**Pb**	**Zn**	**V**	
July*Small	812.57 ± 214.58	1.26 ± 0.40	1.34 ± 0.43	66.25 ± 23.65	2.73 ± 0.74	
August*Small	1087.89 ± 296.01	1.89 ± 0.44	1.48 ± 0.48	72.86 ± 13.11	3.53 ± 0.95	
September* Small	1133.79 ± 384.56	2.21 ± 0.84	1.51 ± 0.44	170.46 ± 293.55	3.87 ± 1.10	
July*Large	369.07 ± 218.98	0.86 ± 0.42	0.99 ± 0.54	70.08 ± 32.56	2.61 ± 1.63	
August*Large	758.56 ± 321.43	1.29 ± 0.56	1.13 ± 0.28	72.07 ± 21.96	3.18 ± 1.50	
September* Large	618.84 ± 229.24	1.00 ± 0.29	1.03 ± 0.29	72.58 ± 14.39	2.54 ± 0.93	

Place*Month*Size						
	As	**Ba**	**Cd**	**Co**	**Cr**	**Cu**
VB*July*Small	3.34 ± 0.69	8.66 ± 1.22	0.14 ± 0.03	0.75 ± 0.07	2.14 ± 0.48	4.08 ± 2.63
VB*August* Small	3.96 ± 1.02	10.76 ± 3.32	0.16 ± 0.05	0.80 ± 0.26	3.37 ± 0.55	2.56 ± 0.73
VB*September* Small	4.24 ± 0.62	10.94 ± 1.77	0.16 ± 0.03	0.85 ± 0.24	3.83 ± 1.82	3.13 ± 0.48
VB*July*Large	6.79 ± 0.77	3.41 ± 0.47	0.18 ± 0.03	0.36 ± 0.05	1.76 ± 0.42	1.88 ± 0.28
VB*August* Large	6.03 ± 1.69	7.67 ± 1.20	0.15 ± 0.02	0.63 ± 0.15	2.48 ± 0.67	2.26 ± 0.48
VB*September* Large	6.64 ± 2.04	5.19 ± 1.31	0.15 ± 0.02	1.68 ± 2.32	1.40 ± 0.27	2.55 ± 0.33
GI*July*Small	3.10 ± 1.29	7.45 ± 2.65	0.13 ± 0.06	0.53 ± 0.20	1.70 ± 0.63	1.78 ± 0.64
GI*August* Small	4.08 ± 0.53	10.96 ± 0.96	0.22 ± 0.08	0.76 ± 0.07	3.39 ± 1.41	2.74 ± 0.34
GI*September* Small	4.49 ± 1.28	10.50 ± 3.26	0.15 ± 0.03	0.76 ± 0.21	3.10 ± 1.31	2.74 ± 0.51
GI*July*Large	4.19 ± 1.58	4.01 ± 3.30	0.12 ± 0.05	0.32 ± 0.24	1.20 ± 0.84	1.46 ± 1.04
GI*August* Large	6.05 ± 2.67	6.96 ± 3.95	0.23 ± 0.13	0.52 ± 0.22	2.08 ± 1.31	2.26 ± 0.38
GI*September* Large	6.11 ± 1.97	6.05 ± 1.77	0.15 ± 0.02	0.64 ± 0.12	1.56 ± 0.56	1.93 ± 0.35
	Fe	**Ni**	**Pb**	**Zn**	**V**	
VB*July*Small	928.37 ± 147.97	1.54 ± 0.29	1.66 ± 0.19	79.50 ± 19.19	3.06 ± 0.69	
VB*August* Small	1142.37 ± 414.60	1.87 ± 0.29	1.47 ± 0.49	73.69 ± 18.65	3.81 ± 1.26	
VB*September* Small	1180.42 ± 400.44	2.61 ± 0.80	1.52 ± 0.47	264.71 ± 409.99	3.87 ± 1.21	
VB*July*Large	368.00 ± 71.79	1.00 ± 0.28	1.20 ± 0.26	78.16 ± 18.90	2.92 ± 1.01	
VB*August* Large	879.06 ± 294.99	1.43 ± 0.45	1.23 ± 0.30	76.43 ± 27.22	4.04 ± 1.63	
VB*September* Large	553.41 ± 154.71	1.04 ± 0.21	0.96 ± 0.35	76.56 ± 17.91	2.06 ± 0.35	
GI*July*Small	696.77 ± 243.07	0.97 ± 0.34	1.02 ± 0.39	52.99 ± 23.37	2.40 ± 0.78	
GI*August* Small	1033.41 ± 117.27	1.92 ± 0.58	1.49 ± 0.52	72.02 ± 5.34	3.25 ± 0.46	
GI*September* Small	1087.17 ± 399.72	1.81 ± 0.73	1.50 ± 0.46	76.20 ± 12.77	3.87 ± 1.09	
GI*July*Large	370.14 ± 316.76	0.72 ± 0.51	0.78 ± 0.69	62.00 ± 42.64	2.30 ± 2.14	
GI*August* Large	638.06 ± 324.69	1.16 ± 0.66	1.03 ± 0.24	67.71 ± 16.55	2.32 ± 0.72	
GI*September* Large	684.27 ± 285.31	0.96 ± 0.37	1.10 ± 0.22	68.60 ± 9.83	3.02 ± 1.11	

	Fe	Ni	Pb	Zn	V
VB*2011	841.34 ± 408.01	1.65 ± 0.75	1.35 ± 0.40	62.81 ± 11.74	3.00 ± 1.08
GI*2011	556.02 ± 357.74	0.92 ± 0.66	1.07 ± 0.63	55.21 ± 22.34	2.29 ± 1.29
VB*2012	842.54 ± 394.86	1.52 ± 0.64	1.32 ± 0.43	153.54 ± 236.55	3.59 ± 1.35
GI*2012	947.25 ± 264.96	1.59 ± 0.53	1.24 ± 0.30	77.96 ± 14.33	3.43 ± 0.89
July*2011	461.80 ± 302.02	0.93 ± 0.55	0.89 ± 0.53	45.29 ± 19.09	1.88 ± 0.98
August*2011	749.20 ± 342.74	1.27 ± 0.52	1.28 ± 0.51	62.06 ± 14.76	2.80 ± 0.93
September*2011	885.04 ± 459.44	1.64 ± 1.06	1.47 ± 0.45	69.68 ± 10.46	3.25 ± 1.38
July*2012	719.84 ± 281.31	1.18 ± 0.32	1.44 ± 0.34	91.04 ± 13.00	3.46 ± 0.97
August*2012	1097.25 ± 257.96	1.92 ± 0.45	1.33 ± 0.34	82.86 ± 14.17	3.91 ± 1.30
September*2012	867.59 ± 365.82	1.57 ± 0.68	1.07 ± 0.35	173.36 ± 292.62	3.16 ± 1.06
VB*July*2011	626.59 ± 290.10	1.39 ± 0.35	1.33 ± 0.29	62.57 ± 7.58	2.61 ± 0.59
VB*August*2011	822.01 ± 326.73	1.51 ± 0.44	1.18 ± 0.42	57.64 ± 15.70	2.94 ± 0.87
VB*September* 2011	1075.41 ± 503.54	2.04 ± 1.14	1.56 ± 0.44	68.23 ± 9.88	3.45 ± 1.56
GI*July*2011	297.00 ± 226.59	0.48 ± 0.23	0.45 ± 0.27	28.01 ± 5.32	1.15 ± 0.70
GI*August*2011	676.39 ± 372.79	1.03 ± 0.50	1.38 ± 0.60	66.48 ± 13.63	2.65 ± 1.06
GI*September* 2011	694.66 ± 351.93	1.25 ± 0.89	1.38 ± 0.48	71.13 ± 11.75	3.06 ± 1.29
VB*July*2012	669.78 ± 360.81	1.15 ± 0.43	1.53 ± 0.35	95.10 ± 5.84	3.37 ± 0.90
VB*August*2012	1199.42 ± 329.08	1.79 ± 0.41	1.52 ± 0.35	92.48 ± 10.91	4.90 ± 1.11
VB*September* 2012	658.41 ± 249.57	1.61 ± 0.87	0.92 ± 0.31	273.05 ± 405.44	2.49 ± 0.75
GI*July*2012	769.91 ± 194.69	1.22 ± 0.18	1.35 ± 0.33	86.98 ± 17.27	3.55 ± 1.11
GI*August*2012	995.08 ± 114.24	2.05 ± 0.49	1.14 ± 0.23	73.24 ± 10.03	2.91 ± 0.30
GI*September* 2012	1076.78 ± 356.53	1.52 ± 0.51	1.22 ± 0.34	73.67 ± 12.35	3.82 ± 0.92

Place*Year						
	As	**Ba**	**Cd**	**Co**	**Cr**	**Cu**
VB*2011	5.62 ± 2.15	7.56 ± 3.68	0.17 ± 0.04	1.04 ± 1.36	2.63 ± 1.49	2.82 ± 1.75
GI*2011	5.08 ± 2.48	6.30 ± 4.22	0.13 ± 0.04	0.47 ± 0.25	1.57 ± 1.17	1.87 ± 0.90
VB*2012	4.72 ± 1.30	7.99 ± 2.83	0.15 ± 0.02	0.64 ± 0.22	2.37 ± 0.80	2.67 ± 0.60
GI*2012	4.26 ± 1.00	9.01 ± 2.24	0.21 ± 0.09	0.70 ± 0.14	2.78 ± 1.11	2.43 ± 0.38
	Fe	**Ni**	**Pb**	**Zn**	**V**	
VB*2011	841.34 ± 408.01	1.65 ± 0.75	1.35 ± 0.40	62.81 ± 11.74	3.00 ± 1.08	
GI*2011	556.02 ± 357.74	0.92 ± 0.66	1.07 ± 0.63	55.21 ± 22.34	2.29 ± 1.29	
VB*2012	842.54 ± 394.86	1.52 ± 0.64	1.32 ± 0.43	153.54 ± 236.55	3.59 ± 1.35	
GI*2012	947.25 ± 264.96	1.59 ± 0.53	1.24 ± 0.30	77.96 ± 14.33	3.43 ± 0.89	

Month*Year						
	As	**Ba**	**Cd**	**Co**	**Cr**	**Cu**
July*2011	3.64 ± 2.03	4.59 ± 3.08	0.12 ± 0.05	0.38 ± 0.24	1.34 ± 0.66	2.19 ± 2.43
August*2011	6.10 ± 2.02	8.11 ± 3.86	0.16 ± 0.04	0.56 ± 0.22	2.15 ± 0.97	2.27 ± 0.59
September*2011	6.32 ± 1.97	8.10 ± 4.05	0.16 ± 0.02	1.33 ± 1.60	2.80 ± 1.99	2.58 ± 0.60
July*2012	5.08 ± 1.35	7.18 ± 2.57	0.17 ± 0.03	0.60 ± 0.17	2.06 ± 0.47	2.41 ± 0.54
August*2012	3.96 ± 0.90	10.07 ± 1.78	0.22 ± 0.10	0.79 ± 0.13	3.51 ± 0.88	2.64 ± 0.36
September*2012	4.42 ± 1.01	8.25 ± 2.56	0.15 ± 0.03	0.63 ± 0.19	2.14 ± 0.77	2.59 ± 0.61
	Fe	**Ni**	**Pb**	**Zn**	**V**	
July*2011	461.80 ± 302.02	0.93 ± 0.55	0.89 ± 0.53	45.29 ± 19.09	1.88 ± 0.98	
August*2011	749.20 ± 342.74	1.27 ± 0.52	1.28 ± 0.51	62.06 ± 14.76	2.80 ± 0.93	
September*2011	885.04 ± 459.44	1.64 ± 1.06	1.47 ± 0.45	69.68 ± 10.46	3.25 ± 1.38	
July*2012	719.84 ± 281.31	1.18 ± 0.32	1.44 ± 0.34	91.04 ± 13.00	3.46 ± 0.97	
August*2012	1097.25 ± 257.96	1.92 ± 0.45	1.33 ± 0.34	82.86 ± 14.17	3.91 ± 1.30	
September*2012	867.59 ± 365.82	1.57 ± 0.68	1.07 ± 0.35	173.36 ± 292.62	3.16 ± 1.06	

Place*Month*Year						
	As	**Ba**	**Cd**	**Co**	**Cr**	**Cu**
VB*July*2011	4.78 ± 2.32	5.95 ± 3.25	0.16 ± 0.04	0.53 ± 0.22	1.86 ± 0.39	3.50 ± 2.95
VB*August*2011	5.91 ± 1.90	8.63 ± 3.54	0.16 ± 0.05	0.60 ± 0.22	2.70 ± 0.94	2.04 ± 0.54
VB*September* 2011	6.17 ± 2.34	8.09 ± 4.26	0.18 ± 0.01	2.00 ± 2.14	3.32 ± 2.29	2.92 ± 0.32
GI*July*2011	2.49 ± 0.74	3.22 ± 2.42	0.07 ± 0.01	0.23 ± 0.14	0.82 ± 0.38	0.88 ± 0.39
GI*August*2011	6.28 ± 2.30	7.58 ± 4.42	0.15 ± 0.02	0.52 ± 0.22	1.60 ± 0.69	2.49 ± 0.60
GI*September* 2011	6.48 ± 1.73	8.11 ± 4.23	0.15 ± 0.01	0.67 ± 0.17	2.29 ± 1.67	2.25 ± 0.64
VB*July*2012	5.36 ± 1.63	6.12 ± 2.77	0.16 ± 0.02	0.57 ± 0.22	2.04 ± 0.57	2.46 ± 0.74
VB*August*2012	4.08 ± 0.93	9.81 ± 2.19	0.15 ± 0.03	0.83 ± 0.17	3.16 ± 0.47	2.78 ± 0.44
VB*September* 2012	4.72 ± 1.13	8.05 ± 2.57	0.14 ± 0.01	0.54 ± 0.14	1.91 ± 0.70	2.76 ± 0.66
GI*July*2012	4.80 ± 1.08	8.24 ± 2.03	0.17 ± 0.03	0.62 ± 0.11	2.08 ± 0.40	2.36 ± 0.29
GI*August*2012	3.85 ± 0.94	10.34 ± 1.41	0.29 ± 0.10	0.76 ± 0.06	3.87 ± 1.08	2.51 ± 0.22
GI*September* 2012	4.13 ± 0.88	8.45 ± 2.78	0.15 ± 0.03	0.73 ± 0.19	2.38 ± 0.81	2.43 ± 0.58
	Fe	**Ni**	**Pb**	**Zn**	**V**	
VB*July*2011	626.59 ± 290.10	1.39 ± 0.35	1.33 ± 0.29	62.57 ± 7.58	2.61 ± 0.59	
VB*August*2011	822.01 ± 326.73	1.51 ± 0.44	1.18 ± 0.42	57.64 ± 15.70	2.94 ± 0.87	
VB*September* 2011	1075.41 ± 503.54	2.04 ± 1.14	1.56 ± 0.44	68.23 ± 9.88	3.45 ± 1.56	
GI*July*2011	297.00 ± 226.59	0.48 ± 0.23	0.45 ± 0.27	28.01 ± 5.32	1.15 ± 0.70	
GI*August*2011	676.39 ± 372.79	1.03 ± 0.50	1.38 ± 0.60	66.48 ± 13.63	2.65 ± 1.06	
GI*September* 2011	694.66 ± 351.93	1.25 ± 0.89	1.38 ± 0.48	71.13 ± 11.75	3.06 ± 1.29	
VB*July*2012	669.78 ± 360.81	1.15 ± 0.43	1.53 ± 0.35	95.10 ± 5.84	3.37 ± 0.90	
VB*August*2012	1199.42 ± 329.08	1.79 ± 0.41	1.52 ± 0.35	92.48 ± 10.91	4.90 ± 1.11	
VB*September* 2012	658.41 ± 249.57	1.61 ± 0.87	0.92 ± 0.31	273.05 ± 405.44	2.49 ± 0.75	
GI*July*2012	769.91 ± 194.69	1.22 ± 0.18	1.35 ± 0.33	86.98 ± 17.27	3.55 ± 1.11	
GI*August*2012	995.08 ± 114.24	2.05 ± 0.49	1.14 ± 0.23	73.24 ± 10.03	2.91 ± 0.30	
GI*September* 2012	1076.78 ± 356.53	1.52 ± 0.51	1.22 ± 0.34	73.67 ± 12.35	3.82 ± 0.92	

Size*Year						
	As	**Ba**	**Cd**	**Co**	**Cr**	**Cu**
Small*2011	3.91 ± 1.34	9.79 ± 3.23	0.15 ± 0.05	0.71 ± 0.25	2.86 ± 1.62	2.89 ± 1.79
Large*2011	6.79 ± 2.19	4.07 ± 2.10	0.15 ± 0.04	0.81 ± 1.42	1.34 ± 0.60	1.80 ± 0.72
Small*2012	3.82 ± 0.58	9.97 ± 1.85	0.18 ± 0.06	0.77 ± 0.15	2.99 ± 0.96	2.79 ± 0.54
Large*2012	5.15 ± 1.24	7.03 ± 2.36	0.18 ± 0.08	0.57 ± 0.16	2.16 ± 0.83	2.31 ± 0.35
	Fe	**Ni**	**Pb**	**Zn**	**V**	
Small*2011	967.71 ± 370.66	1.74 ± 0.83	1.52 ± 0.52	64.18 ± 18.03	3.30 ± 1.18	
Large*2011	429.65 ± 216.54	0.82 ± 0.37	0.91 ± 0.37	53.84 ± 16.91	1.99 ± 0.90	
Small*2012	1055.12 ± 294.70	1.83 ± 0.57	1.37 ± 0.36	142.19 ± 239.84	3.46 ± 0.91	
Large*2012	734.67 ± 301.17	1.28 ± 0.44	1.19 ± 0.35	89.32 ± 13.49	3.56 ± 1.34	

Place*Size*Year						
	As	**Ba**	**Cd**	**Co**	**Cr**	**Cu**
VB*Small*2011	3.90 ± 1.07	10.25 ± 2.96	0.16 ± 0.05	0.83 ± 0.24	3.48 ± 1.73	3.47 ± 2.31
VB*Large*2011	7.34 ± 1.43	4.86 ± 1.92	0.17 ± 0.02	1.26 ± 1.94	1.78 ± 0.36	2.17 ± 0.50
GI*Small*2011	3.93 ± 1.63	9.33 ± 3.60	0.13 ± 0.04	0.59 ± 0.21	2.24 ± 1.31	2.31 ± 0.85
GI*Large*2011	6.23 ± 2.73	3.27 ± 2.06	0.13 ± 0.04	0.36 ± 0.25	0.89 ± 0.43	1.43 ± 0.74
VB*Small*2012	3.80 ± 0.60	10.00 ± 1.84	0.14 ± 0.02	0.77 ± 0.16	2.75 ± 0.53	3.05 ± 0.52
VB*Large*2012	5.64 ± 1.15	5.99 ± 2.15	0.16 ± 0.03	0.52 ± 0.20	1.98 ± 0.86	2.28 ± 0.42
GI*Small*2012	3.85 ± 0.59	9.94 ± 1.98	0.21 ± 0.06	0.78 ± 0.14	3.22 ± 1.24	2.53 ± 0.44
GI*Large*2012	4.66 ± 1.19	8.07 ± 2.18	0.20 ± 0.11	0.63 ± 0.09	2.34 ± 0.80	2.33 ± 0.28
	Fe	**Ni**	**Pb**	**Zn**	**V**	
VB*Small*2011	1126.21 ± 381.33	2.16 ± 0.75	1.59 ± 0.43	66.68 ± 12.76	3.52 ± 1.25	
VB*Large*2011	556.46 ± 160.46	1.14 ± 0.16	1.12 ± 0.18	58.94 ± 9.83	2.48 ± 0.54	
GI*Small*2011	809.21 ± 300.02	1.32 ± 0.71	1.44 ± 0.62	61.68 ± 22.66	3.07 ± 1.14	
GI*Large*2011	302.83 ± 194.18	0.51 ± 0.21	0.70 ± 0.40	48.74 ± 21.27	1.50 ± 0.94	
VB*Small*2012	1041.23 ± 315.53	1.86 ± 0.58	1.51 ± 0.37	211.92 ± 333.44	3.64 ± 0.97	
VB*Large*2012	643.85 ± 378.06	1.18 ± 0.51	1.14 ± 0.42	95.16 ± 7.32	3.53 ± 1.71	
GI*Small*2012	1069.02 ± 290.78	1.81 ± 0.60	1.24 ± 0.32	72.46 ± 10.58	3.27 ± 0.87	
GI*Large*2012	825.49 ± 176.81	1.38 ± 0.36	1.24 ± 0.30	83.47 ± 16.00	3.58 ± 0.93	

Month*Size*Year						
	As	**Ba**	**Cd**	**Co**	**Cr**	**Cu**
July*Small*2011	2.35 ± 0.51	7.09 ± 2.14	0.10 ± 0.04	0.54 ± 0.22	1.47 ± 0.45	3.16 ± 3.18
August*Small* 2011	4.33 ± 0.79	10.82 ± 3.32	0.17 ± 0.05	0.71 ± 0.20	2.83 ± 0.83	2.57 ± 0.69
September* Small*2011	5.06 ± 0.68	11.46 ± 2.56	0.17 ± 0.02	0.88 ± 0.24	4.28 ± 1.80	2.94 ± 0.28
July*Large*2011	4.92 ± 2.20	2.08 ± 1.11	0.13 ± 0.06	0.23 ± 0.13	1.21 ± 0.84	1.22 ± 0.80
August*Large* 2011	7.87 ± 0.91	5.39 ± 2.00	0.15 ± 0.01	0.41 ± 0.10	1.47 ± 0.54	1.96 ± 0.27
September* Large*2011	7.58 ± 2.07	4.73 ± 1.54	0.16 ± 0.01	1.79 ± 2.26	1.33 ± 0.43	2.23 ± 0.64
July*Small*2012	4.09 ± 0.33	9.02 ± 1.59	0.17 ± 0.02	0.74 ± 0.04	2.37 ± 0.24	2.71 ± 0.53
August*Small* 2012	3.72 ± 0.70	10.89 ± 0.99	0.21 ± 0.09	0.84 ± 0.16	3.94 ± 0.93	2.73 ± 0.41
September* Small*2012	3.67 ± 0.64	9.99 ± 2.46	0.15 ± 0.03	0.74 ± 0.19	2.65 ± 0.72	2.93 ± 0.71
July*Large*2012	6.06 ± 1.25	5.34 ± 1.94	0.17 ± 0.03	0.45 ± 0.11	1.75 ± 0.45	2.11 ± 0.38
August*Large* 2012	4.21 ± 1.07	9.25 ± 2.09	0.23 ± 0.13	0.74 ± 0.07	3.09 ± 0.64	2.55 ± 0.30
September* Large*2012	5.18 ± 0.70	6.50 ± 1.03	0.14 ± 0.02	0.53 ± 0.12	1.64 ± 0.40	2.25 ± 0.23
	Fe	**Ni**	**Pb**	**Zn**	**V**	
July*Small*2011	681.48 ± 238.60	1.14 ± 0.56	1.13 ± 0.50	47.38 ± 16.48	2.19 ± 0.66	
August*Small* 2011	1000.83 ± 279.12	1.69 ± 0.29	1.60 ± 0.55	68.49 ± 15.69	3.34 ± 0.84	
September*Small*2011	1220.82 ± 394.68	2.39 ± 1.00	1.82 ± 0.27	76.68 ± 6.22	4.36 ± 0.89	
July*Large*2011	242.11 ± 167.10	0.73 ± 0.51	0.65 ± 0.48	43.19 ± 22.80	1.57 ± 1.21	
August*Large* 2011	497.57 ± 169.03	0.85 ± 0.30	0.96 ± 0.15	55.64 ± 11.57	2.25 ± 0.70	
September*Large*2011	549.26 ± 195.01	0.89 ± 0.32	1.12 ± 0.27	62.68 ± 9.20	2.15 ± 0.69	
July*Small*2012	943.66 ± 122.42	1.37 ± 0.20	1.55 ± 0.27	85.11 ± 14.52	3.27 ± 0.44	
August*Small* 2012	1174.95 ± 310.92	2.10 ± 0.48	1.36 ± 0.42	77.22 ± 9.28	3.71 ± 1.10	
September* Small*2012	1046.77 ± 389.10	2.02 ± 0.69	1.20 ± 0.36	264.24 ± 410.40	3.38 ± 1.13	
July*Large*2012	496.03 ± 197.21	0.99 ± 0.30	1.32 ± 0.38	96.97 ± 8.76	3.64 ± 1.34	
August*Large* 2012	1019.55 ± 187.73	1.73 ± 0.37	1.30 ± 0.29	88.50 ± 16.71	4.10 ± 1.55	
September* Large*2012	688.42 ± 256.84	1.11 ± 0.24	0.94 ± 0.30	82.49 ± 11.64	2.93 ± 1.03	

Place*Month*Size*Year						
	As	**Ba**	**Cd**	**Co**	**Cr**	**Cu**
VB*July*Small*2011	2.77 ± 0.28	8.83 ± 1.21	0.13 ± 0.04	0.72 ± 0.10	1.79 ± 0.44	5.09 ± 3.75
VB*August*Small*2011	4.36 ± 1..20	10.05 ± 5.03	0.18 ± 0.07	0.70 ± 0.30	3.44 ± 0.75	2.21 ± 0.80
VB*September*Small*2011	4.56 ± 0.39	11.87 ± 1.15	0.18 ± 0.01	1.06 ± 0.10	5.20 ± 1.55	3.10 ± 0.21
VB*July*Large*2011	6.78 ± 1.14	3.08 ± 0.28	0.19 ± 0.02	0.34 ± 0.04	1.94 ± 0.40	1.92 ± 0.41
VB*August*Large*2011	7.47 ± 0.56	7.21 ± 0.16	0.15 ± 0.01	0.50 ± 0.02	1.96 ± 0.07	1.87 ± 0.10
VB*September*Large*2011	7.77 ± 2.41	4.30 ± 1.04	0.17 ± 0.00	2.94 ± 2.96	1.44 ± 0.32	2.74 ± 0.33
GI*July*Small*2011	1.93 ± 0.18	5.36 ± 0.97	0.08 ± 0.01	0.35 ± 0.08	1.15 ± 0.13	1.23 ± 0.12
GI*August*Small*2011	4.30 ± 0.31	11.60 ± 0.60	0.15 ± 0.03	0.72 ± 0.08	2.21 ± 0.18	2.94 ± 0.38
GI*September*Small*2011	5.57 ± 0.50	11.05 ± 3.81	0.15 ± 0.01	0.69 ± 0.19	3.36 ± 1.77	2.77 ± 0.27
GI*July*Large*2011	3.06 ± 0.61	1.09 ± 0.16	0.07 ± 0.01	0.11 ± 0.01	0.48 ± 0.09	0.53 ± 0.09
GI*August*Large*2011	8.26 ± 1.14	3.57 ± 0.18	0.15 ± 0.01	0.32 ± 0.03	0.98 ± 0.05	2.05 ± 0.38
GI*September*Large*2011	7.38 ± 2.19	5.17 ± 2.07	0.15 ± 0.02	0.64 ± 0.17	1.21 ± 0.56	1.72 ± 0.36
VB*July*Small*2012	3.91 ± 0.38	8.50 ± 1.47	0.16 ± 0.00	0.77 ± 0.03	2.49 ± 0.09	3.07 ± 0.44
VB*August*Small*2012	3.57 ± 0.84	11.47 ± 0.86	0.14 ± 0.03	0.89 ± 0.23	3.31 ± 0.42	2.91 ± 0.56
VB*September*Small*2012	3.92 ± 0.70	10.02 ± 1.99	0.13 ± 0.01	0.65 ± 0.10	2.46 ± 0.50	3.16 ± 0.73
VB*July*Large*2012	6.81 ± 0.41	3.75 ± 0.37	0.17 ± 0.03	0.37 ± 0.07	1.58 ± 0.44	1.85 ± 0.17
VB*August*Large*2012	4.60 ± 0.82	8.14 ± 1.71	0.16 ± 0.03	0.76 ± 0.10	3.00 ± 0.54	2.64 ± 0.34
VB*September*Large*2012	5.52 ± 0.88	6.08 ± 0.94	0.14 ± 0.02	0.42 ± 0.05	1.35 ± 0.27	2.36 ± 0.25
GI*July*Small*2012	4.27 ± 0.16	9.55 ± 1.84	0.18 ± 0.02	0.71 ± 0.03	2.25 ± 0.29	2.34 ± 0.32
GI*August*Small*2012	3.86 ± 0.68	10.32 ± 0.84	0.28 ± 0.03	0.79 ± 0.06	4.57 ± 0.89	2.55 ± 0.15
GI*September*Small*2012	3.41± 0.58	9.96 ± 3.35	0.16 ± 0.05	0.83 ± 0.25	2.84 ± 0.96	2.71 ± 0.75
GI*July*Large*2012	5.32 ± 1.44	6.92 ± 1.31	0.16 ± 0.04	0.54 ± 0.07	1.92 ± 0.47	2.38 ± 0.34
GI*August*Large*2012	3.83 ± 1.32	10.36 ± 2.06	0.30 ± 0.16	0.72 ± 0.03	3.18 ± 0.83	2.46 ± 0.30
GI*September*Large*2012	4.84 ± 0.31	6.93 ± 1.09	0.15 ± 0.02	0.63 ± 0.06	1.92 ± 0.31	2.15 ± 0.19

	Fe	Ni	Pb	Zn	V
VB*July*Small* 2011	865.87 ± 179.83	1.60 ± 0.35	1.57 ± 0.13	62.36 ± 1.47	2.60 ± 0.73
VB*August* Small*2011	997.18 ± 416.05	1.91 ± 0.15	1.30 ± 0.62	63.51 ± 22.21	3.11 ± 1.21
VB*September* Small*2011	1515.57 ± 148.86	2.97 ± 0.76	1.90 ± 0.25	74.18 ± 5.29	4.85 ± 0.36
VB*July*Large* 2011	387.31 ± 79.31	1.18 ± 0.20	1.08 ± 0.13	62.77 ± 11.88	2.62 ± 0.59
VB*August* Large*2011	646.83 ± 41.51	1.12 ± 0.05	1.06 ± 0.13	51.77 ± 4.41	2.78 ± 0.58
VB*September* Large*2011	635.25 ± 174.63	1.11 ± 0.24	1.22 ± 0.26	62.27 ± 10.48	2.04 ±0.20
GI*July*Small* 2011	497.09 ± 89.39	0.67 ± 0.13	0.69 ± 0.14	32.40 ± 2.07	1.77 ± 0.24
GI*August* Small*2011	1004.48 ± 147.09	1.47 ± 0.23	1.90 ± 0.30	73.47 ± 6.90	3.58 ± 0.39
GI*September* Small*2011	926.0632 6.53	1.82 ± 0.97	1.74 ± 0.32	79.17 ± 7.07	3.86 ± 1.05
GI*July*Large* 2011	96.92 ± 16.39	0.28 ± 0.02	0.22 ± 0.07	23.62 ± 2.92	0.52 ± 0.04
GI*August* Large*2011	348.30 ± 53.50	0.58 ± 0.05	0.87 ± 0.12	59.50 ± 16.44	1.73 ± 0.24
GI*September* Large*2011	463.26 ± 205.88	0.67 ± 0.23	1.02 ± 0.28	63.09 ± 10.06	2.26 ± 1.06
VB*July*Small* 2012	990.87 ± 103.36	1.47 ± 0.26	1.74 ± 0.23	96.65 ± 6.09	3.52 ± 0.15
VB*August* Small*2012	1287.55 ± 439.77	1.84 ± 0.42	1.64 ± 0.36	83.88 ± 8.08	4.51 ± 1.04
VB*September* Small*2012	845.26 ± 204.27	2.25 ± 0.80	1.14 ± 0.24	455.24 ± 557.93	2.89 ± 0.80
VB*July*Large* 2012	348.69 ± 74.00	0.82 ± 0.26	1.31 ± 0.33	93.55 ± 6.41	3.21 ± 1.39
VB*August* Large*2012	1111.29 ± 232.46	1.73 ± 0.49	1.40 ± 0.35	101.08 ± 3.19	5.29 ± 1.25
VB*September* Large*2012	471.57 ± 96.17	0.97 ± 0.21	0.71 ± 0.21	90.86 ± 8.90	2.08 ± 0.51
GI*July*Small* 2012	896.45 ± 141.76	1.27 ± 0.07	1.36 ± 0.16	73.57 ± 9.52	3.03 ± 0.54
GI*August* Small*2012	1062.34 ± 101.16	2.37 ± 0.43	1.08 ± 0.26	70.57 ± 4.17	2.91 ± 0.24
GI*September* Small*2012	1248.28 ± 463.63	1.79 ± 0.62	1.27 ± 0.51	73.23 ± 18.21	3.87 ± 1.36
GI*July*Large* 2012	643.37 ± 163.18	1.16 ± 0.26	1.34 ± 0.50	100.39 ± 10.76	4.07 ± 1.41
GI*August* Large*2012	927.82 ± 93.92	1.73 ± 0.34	1.19 ± 0.23	75.91 ± 14.58	2.91 ± 0.41
GI*September* Large*2012	905.27 ± 120.80	1.24 ± 0.20	1.17 ± 0.16	74.12 ± 7.01	3.77 ± 0.48

VITA

Hannah Paula Rockett, the daughter of Gloria and Keith Rockett, was born in Eunice, Louisiana. She attended Louisiana State University and received a Bachelor of Science degree in biology in December 2010. Hannah hopes to complete the Louisiana State University requirements for the Master of Science degree in environmental sciences in August 2013. She intends to further her education with admittance into a doctoral program in environmental sciences.