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Mercury concentrations in muscle tissue from sportfish in Lake Mead, Nevada

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MERCURY CONCENTRATIONS IN MUSCLE

TISSUE FROM SPORTFISH IN

LAKE MEAD, NEVADA

by

Joanna L. Kramer

Bachelor of Science
Ursinus College 2007

A thesis submitted in partial fulfillment
of the requirements for the

Master of Public Health Degree
Department of Environmental and Occupational Health
School of Community Health Sciences
Division of Health Sciences

Graduate College
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MASTER OF PUBLIC HEALTH

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Dean of the Graduate College

Examination Committee Member

Examination Committee Member

Graduate College Faculty Representative

ABSTRACT

Mercury Concentrations in Muscle Tissue from Sportfish in Lake Mead, Nevada

by

Joanna L. Kramer

Dr. Shawn L. Gerstenberger, Committee Chair
Professor, Department of Environmental and Occupational Health
School of Community Health Sciences
University of Nevada, Las Vegas

Lake Mead is the largest reservoir by volume in the United States and provides fishing opportunities for numerous anglers. Considerable attention has been given to the bioaccumulation of methylmercury in fish tissues, however, no formal study utilizing approved USEPA methodology has been conducted to quantify the amount of mercury present in fish tissue from Lake Mead. The purpose of this study is to determine the concentrations of mercury present in the most commonly consumed fish from Lake Mead and to identify if any of the 4 major basins contain fish with elevated concentrations of mercury. Largemouth bass (n=49), striped bass (n=94), and channel catfish (n=78) were collected from selected sites in Boulder Basin, Overton Arm, Virgin Basin, and Gregg Basin of Lake Mead by gill netting or electrofishing. Muscle tissue was homogenized, digested, and analyzed for mercury in accordance with USEPA Method 245.6 which must be used to construct human health based fish consumption advisories. Mean mercury concentrations were ($\bar{x}$ ±SD) 0.089± 0.065 ppm, 0.154±0.127 ppm, and 0.098± 0.080 ppm in largemouth bass, striped bass, and channel catfish, respectively. An

analysis of covariance (ANCOVA) indicated a significant difference between mercury concentrations among the three species ($F_{2,208}$= 22.448, p< 0.001). Contrasts revealed that each species differed significantly from each other (p<0.050).

There was a significant overall difference in mean mercury concentration between fish from the four major basins of Lake Mead ($F_{3,208}$= 20.541, p<0.001). The mean mercury concentration in Boulder Basin was significantly lower than that of Gregg Basin (p<0.001), Virgin Basin (p<0.001), and Overton Arm (p<0.001). Out of 221 samples analyzed, 2 samples (both striped bass) were found to have mean mercury concentrations above the Environmental Protection Agency's action level of 0.5 ppm. There were no samples found containing concentrations above the Food and Drug Administration's maximum allowable mercury concentration in fish and food products (1.0 ppm).

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ACKNOWLEDGEMENTS

In completing this project, I have had the honor and privilege to work with some of the most talented individuals in the fields of public health and aquatic research. This work would have been impossible without the knowledge, support, and guidance that they have provided. I would like to extend to these individuals my sincerest gratitude for the opportunities and experiences I have had at the University of Nevada, Las Vegas, for these have truly been the highlight of my education thus far.

Dr. Shawn Gerstenberger served as the chair of my thesis committee and graduate advisor. The prior research involved in constructing a protocol for contaminants monitoring in fish from Lake Mead is attributed to him. His guidance and knowledge greatly contributed to the development of this study including methodology and implementation. His generous allowance of the use of his environmental health laboratory and provision of all equipment, consumables, and support made this study possible. Finally, he provided the opportunity for this research to be completed through a graduate assistantship such that the time and effort required for a study such as this were possible.

I would like to thank the other members of my committee, including Dr. Chad Cross, the statistician on my committee. He generously donated his time to provide me with a basic foundation for biostatistics and guided me through method development, results, and interpretation for this study. Thanks also to Dr. Michelle Chino, who instilled in me a great love for environmental health and provided a different perspective from which to

CHAPTER 1

INTRODUCTION

Lake Mead, Nevada is currently the largest man-made reservoir in the United States. It is formed by water impounded from the Hoover Dam and spans approximately 110 miles over the states of Nevada and Arizona on the Colorado River. Inflows to the lake include the Colorado, Virgin, and Muddy Rivers, as well as the Las Vegas Wash which carries discharge from municipal wastewater treatment plants and storm- and rainwater runoff from the Las Vegas Valley (Gerstenberger and Eccleston 2002; LaBounty and Horn 1997). Lake Mead distributes water to communities in Southern California and Nevada and is used for a variety of recreational activities including sportfishing. According to a survey conducted by Gerstenberger and Eccleston (2002), an average of 22.7 ± 3.6 fish meals per year are consumed from Lake Mead by sport fishermen and their families, although the maximum number reported reached over 300 meals per year.

Sportfishing at Lake Mead provides both recreational and health benefits to residents and visitors alike. The health benefits of consuming fish are great: fish are relatively low in fat and cholesterol, high in protein, and are one of the best natural sources for omega 3 fatty acids which can protect against cardiovascular disease, improve cognitive development in children, and slow cognitive decline in the elderly (Kris-Etherton et al. 2002; Mozaffarian and Rimm 2006; Silver et al 2007). However, these benefits can be negated by the health risks posed by the toxic effects of mercury (Hg) and other

contaminants found in fish tissues. All bodies of water contain at least small amounts of mercury from both natural and anthropogenic sources. An increase in human activities that contribute to the environmental burden of mercury has raised public concern as to the safety of fish caught in these waters.

Previous studies have been conducted on the presence of mercury in fish in Lake Mead (Cizdziel et al. 2002), however, the methods of these studies have not been in accordance with the federal guidelines for quantifying mercury in edible tissues. This study is proposed in response to the protocol developed by Gerstenberger and Eccleston (2002) for a fish contaminant monitoring program on Lake Mead. It will assess and evaluate the concentrations of mercury present in four species of fish commonly caught and consumed from Lake Mead: largemouth bass, striped bass, channel catfish, and blue tilapia. Samples will be taken from each of the four major basins of the Lake: Boulder, Virgin, Gregg, and Overton Arm and will be analyzed for mercury content in relation to K factor, trophic level, and location.

CHAPTER 2

REVIEW OF RELATED LITERATURE

The Origin and Nature of Mercury

Mercury is present in three forms in the environment: the metallic element, inorganic

salts, and organic compounds (Inskip and Piotrowski 1985; Morel et al. 1998; Trasande

et al. 2005. Inorganic mercury exists in three valence states Hg^0, Hg^{2+}, and Hg^+ which are

present in equilibrium by chemical dismutation (Robinson and Tuovinen 1984). Hg^0 is

unique in that it exists in liquid form at room temperature. This and other properties

including: volatility, the ability to adsorb to surfaces, and the ability to form complexes

contribute to its wide distribution in the earth, air, and water.

Mercury occurs both naturally and anthropogenically in the environment. Geologic

sources include minerals such as cinnabar, metacinnabar, livingstonite, and tetrahydrite.

The degassing of the earth's crust releases a significant amount of elemental mercury

vapor into the environment (Gerstenberger et al. 1993). However, nearly eighty seven

percent of mercury present in the environment today is due to anthropogenic sources

(USEPA 2001). The environmental burden of mercury has increased dramatically in the

last century due to the industrial revolution (USEPA 1997; Jakus et al. 2002; Shimshack

et al. 2007). Coal fired electrical plants are currently the largest source of mercury

emissions into the atmosphere (Shimshack et al. 2007). These and other industrial

activities such as chlor-alkali production and waste incineration account for more than

seventy percent of the mercury released into the environment annually (Trasande et al. 2005). Mercury binds with sulfuric compounds found in coal and the burning of this material releases mercury into the atmosphere (Shimshack et al. 2007). These atmospheric releases are deposited into terrestrial and aquatic system and then reemitted into the atmosphere as illustrated in Figure 1. Other anthropogenic sources include industries which utilize mercury in production such as agriculture, paint, paper, pulp, and pharamaceutical industries. Past uses, such as the application of mercury containing fungicides to crops also contribute to the current environmental burden of mercury (USEPA 1997).

After its release into the environment, inorganic mercury settles into the ground and surface water where bacteria convert it to its organic form, methylmercury (Shimshack et al. 2007). Methylmercury, with a half-life of 72 days is taken up by microorganisms such as plankton and algae and remains, eventually being transferred through the food chain to larger aquatic organisms. Mercury also enters the bloodstream of fish via water passing over the gills and eventually accumulates in tissues such as the liver, blood, and muscle (Bloom 1992, Cizdziel et al. 2003) It is estimated that about ninety to ninety-five percent of mercury in fish is in the form of methylmercury (Jakus et al. 2002). Inorganic mercury is less readily absorbed and usually secreted from the body and so does not bioaccumulate (USEPA 1997).
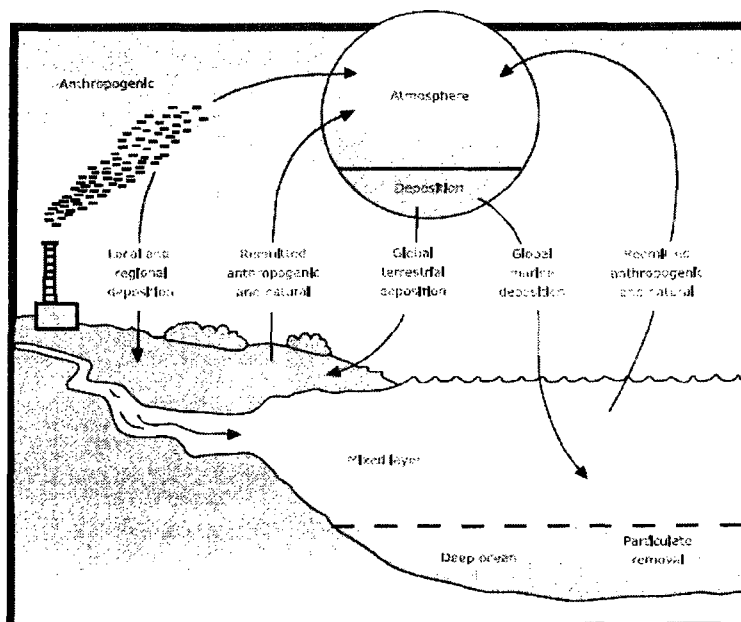
Figure 1. The Mercury Cycle (Source: USEPA 2005)

Methylation of Mercury

Methylmercury concentrations in aquatic organisms are dependent upon

concentrations in their surrounding waters. This gradient is primarily controlled by the

processes of methylation and demethylation. The most common forms of mercury in

surface waters are $HgCl_2$ and $Hg(OH)_2$. In sediment, HgS is most abundant (Robinson

and Tuovinen 1984). Three pathways are recognized for the methylation of mercury:

abiotic or photochemical methylation of Hg^{2+}, methylation of Hg^{2+} in sediments by

bacteria which produce methylcobalamin (Vitamin B12), and the methylation of mercury

by flora in the intestinal tract of aquatic organisms, perhaps also utilizing

methycobalamin (Robinson and Tuovinen 1984). Methylation can occur both under

anaerobic conditions by bacteria in river and lake sediments and aerobically in

soil/sediment organisms as well as by bacteria found in the intestinal tract. The

mechanism of methylation is thought to involve the nonenzymatic transfer of methyl groups from methylcobalamin to Hg^{2+} via electrophilic attack (Robinson and Tuovinen 1984). The products of this reaction are hydroxycobalamin and methylmercury (Bertilisson and Neujahr 1971; Robinson and Tuovinen 1984).

Although the effect varies across different bodies of water, increased acidity has been shown to account for a net increase in methylation in natural lake water (Gilmour and Henry 1990). Xun et al. (1987) reported an increase in methylation and concurrent decrease in demethylation in acidified waters. However, acidification of sediments depressed methylation, presumably due to a decrease in bacterial metabolic activity (Gilmour and Henry 1990). Addition of either sulfate or organic substances to lakes also results in a net increase in methylation due to the resultant increase in the activity of sulfate-reducing bacteria. In fresh water, the addition of dissolved organic carbon (DOC) results in a decreased rate of methylation presumably due to ligand formation between DOC and dissolved mercury, making it unavailable for methylation by bacteria (Gilmour and Henry 1990). In sediments, however, the addition of DOC has the opposite effect, increasing both bacterial activity and methylation (Gilmour and Henry 1990).

Bioaccumulation of Mercury in Fish

For methylmercury to reach high concentrations in fish, it must be taken up and retained by the organisms at the bottom of the aquatic food web. Methylmercury is retained in both fatty and muscle tissues due to its liposolubility and association with proteins, respectively (Bahnick et al. 1994). Methylmercury is also absorbed by the intestinal wall which contributes to its concentration in tissues. The accumulation of

methylmercury in higher organisms is primarily controlled by ingestion of methylmercury-containing food rather than by uptake from the surrounding waters (Morel et al. 1998). With each successive stage of the food chain, mercury becomes more concentrated in tissues such that the highest level predators contain the highest concentrations of mercury in their tissues. Several studies have demonstrated this phenomenon, finding extremely high concentrations of mercury in predatory species such as swordfish, tuna, king mackerel, and shark (Shimshack et al. 2007; Trasande et al. 2005). In fact, some fish species have been found to have methylmercury concentrations 1,000 to 10,000 times greater that their surrounding waters (Jakus et al. 2002).

K Factor Effects

The condition factor (K) is calculated using the formula $(W/L^3)*100$ and is a measure of the relative robustness, nutritional status, and general well-being of a fish (Williams 2000). Lengths and weights of fish have generally been found to be positively correlated with mercury concentration (Dellinger et al. 1995; Gerstenberger et al. 1993; Morel et al. 1995; Shimshack et al. 2007; NDOW 2006). However, Cizdziel et al. (2003) identified an increased "starvation concentration" of mercury present in striped bass from Lake Mead which were emaciated in condition.

Trophic Level Effects

Due to the tendency of methylmercury to bioaccumulate, it follows that the highest trophic levels in aquatic food webs would contain the highest concentrations of mercury. Watras et al. (1998) applied this principle in a study and found that methylmercury concentrations at higher trophic levels reflected the supply of methylmercury available to lower trophic levels along with diet and other factors. Further, trophic levels have been

shown to correlate positively with mercury concentrations in a number of studies (Burger and Gochfield 2007; Jackson 1991; Watras et al. 1998).

<u>Location Effects</u>

Mercury is present in all bodies of water, however, certain aquatic factors have the potential to affect the amount of mercury which bioaccumulates in fish. Conditions which affect the rates of methylation and demethylation such as pH, DOC, and presence of sulfites can have a significant impact on the concentrations of mercury present in fish (Gilmour and Henry 1990). Additionally, the previously identified industrial activities which contribute to the mercury burden can contribute to differences in mercury concentration among and within bodies of water (Schmitt and Brumbaugh 1990).

Lake Mead is divided into four basins containing relatively separate populations of fish and varying aquatic conditions. The reservoir spans two states and is utilized by several different human populations in Nevada and Arizona. Increased concentrations of mercury in a particular basin may disproportionately affect those who consume fish from that basin. One of the objectives of this study is to determine if any of the basins contain fish with elevated concentrations of mercury.

Human Exposure to Methylmercury

For the general public, consumption of fish muscle tissue is the primary route of exposure to mercury (Dellinger et al. 1995; USEPA 2006; Gerstenberger et al. 1993; Inskip et al. 1985). The extent of toxicological effects depends on the type of fish consumed, the amount consumed, and how frequently fish are consumed (USEPA 1997;

Shimshack et al. 2007). The neurotoxicity of methylmercury coupled with its propensity to bioaccumulate up the food chain raise significant human health concerns.

Armbruster et al. (1988) demonstrated that cooking by various methods (baking, broiling, frying, microwaving, poaching, steaming) had no significant effect on concentrations of mercury within fish tissue and that cooking does not diminish the amount of mercury present in the fish. However, studies have found that mercury concentrations were higher in fillets without skin than in those with skin (Gutenmann and Lisk, 1991; Dellinger et al. 1995). Gutenmann and Lisk (1991) suggest that this tendency may be due to the fact that skin and fat removal of a fillet would result in higher protein content hence, higher mercury content.

Health Effects of Methylmercury Exposure

Methylmercury is neurotoxic in humans and is characterized by rapid absorption through the gastrointestinal tract and concentration in all tissues. It is found in highest concentration in the kidneys and easily crosses blood/brain and placental barriers (USNRC 2000). The estimated lethal dose of methylmercury is 10-60 mg/kg or parts per million (ppm) (Jakus et al. 2002). High concentrations are rare in adults but even low concentrations can cause damage in developing fetuses, infants, and children (Shimshack et al. 2007).

The effects of methylmercury were first observed in Minamata, Japan when consumption of fish high in mercury by pregnant women resulted in an elevated incidence of cerebral palsy in children and again in Iraq when fungicide containing mercury compounds led to the poisoning of thousands. Infants and children were the

most susceptible in both instances, reflecting methylmercury's readiness to pass through placenta, become concentrated in umbilical tissues, and be secreted in breast milk (Shimshack et al. 2007; Trasande et al. 2005). It is estimated that six percent of women in childbearing age in the US have methylmercury levels of concern (Silver et al. 2007). In an effort to reduce the deleterious effects of mercury, the USFDA and USEPA have issued consumption advisories which recommend the species and amounts of fish which are considered safe to be consumed (Silver et al. 2007). These regulations are more stringent for children and women of child-bearing age.

Several studies have been conducted to determine the public health impact of mercury consumption, most of these focusing on pregnant women and their children. A cohort study in New Zealand determined that there was a three-point deficiency in IQ scores of children whose mothers had greater than 6 micrograms of mercury/gram of hair sample (Trasande et al. 2005). A prospective study taking place in Denmark followed a subset of children for 14 years and found a significant dose-response relationship between prenatal mercury exposure and adverse effects on developmental aspects such as memory, attention, and visual-spatial perception tests (Trasande et al. 2005). Trasande et al. (2005) attempted to quantify the economic and public health costs of methylmercury toxicity by comparing the concentrations of mercury in cord blood to the loss of intelligence in children. They estimated that the loss of productivity amounts to about 8.7 billion dollars annually and urge stronger controls of mercury emissions into the environment. Although the toxicological effects of methylmercury are not yet completely understood, studies from the International Agency for Research on Cancer (IARC) and the USEPA have classified methylmercury as a possible human carcinogen. Studies on animals have

shown an inverse correlation between exposure to mercury and abundance of immune cells and autoimmune effects have been associated with exposure to mercury (USNRC 2000). In a study by Cordier et al. (1991), wives of mercury exposed men were shown to have double the incidence of spontaneous abortion than wives of non-exposed men. In addition, this study demonstrated that the children of exposed women exhibited abnormally high rates of congenital defects as compared to those of non-exposed women.

Fish Consumption Advisories

Consumption advisories are one method utilized by government to reduce mercury exposure in bodies of water determined to have elevated fish mercury concentrations. Advisories are issued by location stating the maximum amount of fish that can be safely consumed according to species. Safe concentrations are different for at-risk groups such as women and children. Current consumption recommendations from the USEPA for methylmercury noncarconogenic health endpoints are given in Table 1.

Table 1. Monthly Fish Consumption Limits for Noncarcinogenic Health Endpoint-Methylmercury (Source: USEPA 2000)

Risk Based Consumption Limit[a] Fish Meals/Month	Noncancer Health Endpoints[b] Fish Tissue Concentrations (ppm, wet weight)
Unrestricted (>16)	0-0.029
16	>0.029-0.059
12	>0.059-0.078
8	>0.078-0.12
4	>0.12-0.23
3	>0.23-0.31
2	>0.31-0.47
1	>0.47-0.94
0.5	>0.94-1.9
None (<0.5)	>1.9

[a]The assumed meal size is 8 oz (0.227 kg). The ranges of chemical concentrations are conservative, e.g. the 12-meal-per month levels represent the concentrations associated with 12-15.9 meals.
[b]Chronic, systemic effects.
Notes:
1. Consumption limits are based on an adult body weight of 70 kg and an interim RfD of 1×10^4 mg/kg-d.
2. None= No consumption recommended.
3. Monthly limits are based on the total dose allowable over a 1-month period (based on the RfD). When the monthly limit is consumed in less than 1 month (e.g. in a few large meals), the daily dose may exceed the RfD.

While governmental agencies may issue consumption advisories to at-risk populations, the advisories are not always followed and may not even be common knowledge. A study by Silver et al. (2007) demonstrated that among low-income women in the Sacramento-San Joaquin Delta twenty nine percent exceeded federal advisory limits and only fifty five percent were aware of the advisory. These data clearly suggest the need for more culturally and linguistically appropriate information to be readily available. Low-income and non-English speakers, particularly Hispanics and Native Americans, make up a significant portion of the population in Southern Nevada and

Arizona. Advisories may need to be modified or translated if necessary in these subpopulations.

Costs and Benefits

The presence of mercury in fish poses a threat to the welfare of those who consume it. Reducing the consumption of species known to have high concentrations of mercury will result in a direct benefit to one's health. Benefits may include but are not limited to: increase of IQ in children, increase in reproductive capabilities of both women and men, fewer congenital abnormalities, fewer spontaneous abortions, decreased cancer rates, and decreased neurotoxicity. Women of child-bearing age and children should be especially prudent because of the fetus's increased susceptibility to methylmercury. Anglers should be aware of local mercury advisories that may exist in various bodies of water and abide by recommended consumption rates.

The intent of consumption advisories is to keep populations safe from mercury toxicity, however, they can be harmful to the industry. Shimshack et al. (2007) found that mercury advisories can have unintended spillover effects in that populations not considered at risk may also choose to limit consumption which results in a negative economic impact in the fishing industry. Issuing fish consumption advisories will likely result in recreational loss to many anglers and financial loss for the recreational and commercial fishing industries (Jakus et al. 2002). Costs may also be incurred for industries due to governmental regulations for reducing mercury emissions in the form of technological expenses and waste disposal costs. These costs should be considered along with benefits to build state-specific advisories which maximize benefit for all parties.

Current Policy

In addition to issuing consumption advisories, state and national governments have issued legislation to reduce mercury emissions. In 1988, a ban on the industrial use of mercury as an additive in paint and pesticides was enstated. Additionally, the Clean Water Act disallows any person to deposit contaminants, including mercury, into the water without a permit (USEPA 2005). In 2005 the USEPA issued a cap on emissions from coal fired power plants (the largest contributor to mercury emissions) in the form of the Clean Air Mercury Rule (USEPA 2005). These policies are just some of the efforts to control mercury, but they are difficult and expensive to monitor and not always 100% effective, thus it is necessary to continue to exercise caution when consuming fish from affected water bodies.

Presently, the U.S. Food and Drug Administration (USFDA) sets the action level for safe consumption of fish at 1.0 ppm on a fresh wet weight basis (USFDA 1977, Gutenmann and Lisk 1991). This value represents the concentration at or above which the USFDA may take legal action to remove products from the marketplace. This value is used to regulate commercially bought fish, but there is also a need to monitor the consumption of non-commercial sportfish. The U.S. Environmental Protection Agency (USEPA), however, sets a much lower screening value of 0.50 ppm which indicates that the mercury concentration in a particular area warrants further investigation (USEPA 1997). A standard reference dose (RfD) of 0.0001 ppm/day is used along with an average consumption rate (CR) to calculate screening value (Ball 2002).

Need for Monitoring on Lake Mead

Gerstenberger and Eccleston (2002) constructed a contaminant sampling protocol for Lake Mead, Nevada and determined that striped bass, largemouth bass, and channel catfish between thirteen and eighteen inches in length (33 and 45.7 centimeters) were the most commonly consumed fish in the area. Among the study participants (n=150), approximately 23 fish meals per year were consumed, although the maximum number exceeded 300 fish meals per year. Anglers reported that they often share their catch with their families (Gerstenberger and Eccleston 2002). Based on this information, the authors established that there is a need for a study of mercury concentrations in the tissues of fish in Lake Mead using USEPA approved analytical methods targeted at assessing consumption. The benefits of this method include low detection limits, small required sample size, and concordance with USEPA approved methodology for quantifying mercury in edible tissues (Gerstenberger and Eccleston 2002).

The aim of this study is to address this need and evaluate mercury concentrations in popular sportfish from each basin of Lake Mead using approved methods. Lake Mead will be divided into 4 major sampling areas: Boulder Basin, Overton Arm, Virgin/Temple Basin, and Gregg Basin. These sampling locations were chosen because they contain separate fish populations with minimal overlap (Gerstenberger and Eccleston 2002, Cizdziel et al. 2002). Four species: largemouth bass, striped bass, channel catfish, and blue tilapia, will be evaluated based on the catch rates reported by Gerstenberger and Eccleston (2002). Mercury concentration will be determined and the data will be complied and analyzed to determine if there is a need for a fish consumption advisory within Lake Mead.

CHAPTER 3

QUESTIONS, OBJECTIVES, AND HYPOTHESES

Questions

• What is the average Hg concentration in muscle tissue of each of 4 popular sport fish

found in Lake Mead (largemouth bass, striped bass, channel catfish, blue tilapia)?

• Is there a significant correlation between mercury concentration and K factor in fish?

• What relationship, if any, exists between mercury concentration and trophic levels of

fish?

• Do the four basins (Boulder, Virgin, Gregg, and Overton Arm) differ significantly with

respect to the concentration of mercury present in fish?

• Are mercury concentrations in fish high enough to warrant a consumption advisory in

Lake Mead? If so, which species, location, and what governmental guideline should be

followed for the maximum allowable amount of Hg in edible fish tissue (USEPA,

USFDA)?

Objectives

• Utilize USEPA approved methodology (FIMS 100 Cold Vapor Hg Analyzer) to

determine amount of Hg present in edible tissues of fish from Lake Mead.

• Compare Hg concentrations between fish species based on K factor.

• Determine the relationship between two independent variables, basin and species, and mercury while controlling for the effects of K factor.

• Determine which, if any, of 4 popular sport fishing species from Lake Mead, largemouth bass, striped bass, channel catfish, and blue tilapia contain unsafe Hg concentrations based on consumption guidelines.

Hypotheses

Hypothesis One: K Factor

Hg concentration will correlate positively with K factor in fish except in extremely emaciated fish. In general, fish with greater lengths and weights will have higher concentrations of Hg than smaller fish. Fish that are extremely emaciated (K factors of 0.8 or below) may exhibit a higher starvation concentration of Hg in tissues as discussed by Cizdziel et al. (2003).

   • H_o: K factor and mercury concentrations are not significantly correlated.

   • H_a: K factor and mercury concentrations are significantly correlated.

Hypothesis Two: Trophic Level

Largemouth and striped bass will have highest Hg concentrations out of the 4 species because they are high trophic level predators and Hg is known to bioaccumulate. Hg concentration will correlate positively with trophic levels of fish. Piscivores such as largemouth and striped bass will have higher concentrations of Hg than herbivores, such as blue tilapia. Channel catfish are insectivores/piscivores and will have intermediate Hg concentrations.

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- H_o: There will not be significant difference in mercury concentrations among species.

- H_a: There will be a significant difference in mercury concentrations among species.

Hypothesis Three: Location

Fish collected from Boulder Basin will contain the highest concentrations of Hg overall, because it is the drainage site of the Las Vegas Wash, which receives all of the wastewater effluent from the highly populated city of Las Vegas.

- H_o: There will not be a significant difference in mercury concentrations among locations.

- H_a: There will be a significant difference in mercury concentrations among locations.

CHAPTER 4

METHODOLOGY AND DATA DESCRIPTION

Sample Collection

Four species of sportfish were collected from Lake Mead during October of 2007 and 2008 including: 49 largemouth bass (*Micropterus salmoides*), 94 striped bass (*Morone saxatilis*), 78 channel catfish (*Ictalurus punctatus*), and 31 blue tilapia (*Oreochromis aurea*). Samples of fish from each species were taken at selected sites from the Nevada Division of Wildlife (NDOW) annual fish survey within the four major basins of Lake Mead: Boulder Basin, Virgin Basin, Overton Arm, and Gregg Basin (Figure 2). Exact numbers and species of fish collected from each basin can be found in Table 2.

Figure 2. Map of Lake Mead with sampling locations

Table 2. Species and locations of fish collected for study

Location	Largemouth bass	Striped bass	Channel catfish	Blue tilapia
Gregg Basin 10/12/07, 10/8/08	17	21	19	2
Boulder Basin 10/16/07, 10/16/08	12	22	19	22
Virgin Basin 10/23/07, 10/22/08	11	28	17	0
Overton Arm 10/24/07, 10/23/08	9	23	23	7

Fish were collected by one of two methods established by NDOW and Arizona Game and Fish (AZGF). Method one involved the suspension of vertical gill nets in 20-40 feet of water overnight. The gill nets measured 2.5 m x 75 m and consisted of mesh netting with graduated openings which increased in size from one end to the other. Nets were pulled the following morning and fish were extracted and processed. Fish were weighed to the nearest gram and measured to the nearest 0.1 cm (total length). The data were entered into a logbook and samples were placed in a labeled plastic bag or aluminum foil and stored on ice until return to the laboratory. At selected collection sites, the secondary method electrofishing, was performed using a stun boat which sends an electric current through the water. The current momentarily stuns fish which are then collected with a dip net and processed. At each electrofishing site, pulsed DC current was applied for a total of 900 seconds alternating power on and off.

Sample Preparation

Fish were filleted and skin was removed from each sample using an electric fillet knife. Fillets were rinsed with deionized water and frozen individually in labeled plastic freezer bags. Fillets were thawed and transferred to a clean glass beaker. Fillets were homogenized using a Kinematica® Polytron PT 6100 (Lucerne, Switzerland) homogenizer for approximately 2 minutes or until sufficiently homogenized. Alternatively, larger samples were homogenized by passing the fillet through a Cabela's® Pro450 professional meat grinder (Sidney, NE) and collecting in a clean beaker. This process was repeated approximately 3 times for each fillet or until sufficiently homogenized. Individual homogenized fillets were stored in clean Whirl-packs® at -20

degrees C. All labware used in the homogenization procedures were cleaned using the Cleaning Procedure for Cold Vapor Mercury Analysis Labware (Appendix A).

Individual samples of fish tissue were digested in an Anton-Parr Multiwave 3000 microwave digestion system using the Fish Tissue Digestion Procedure for Cold Vapor Mercury Analysis (Appendix B). For analysis, a 1 mL aliquot of the raw digested material was transferred into a separate clean and labeled centrifuge tube containing 5 mL 3% HCl. This 1:6 solution was used in most cases for analysis using the PerkinElmer® Flow-Injection Mercury System 100 (FIMS 100) (Sheldon, CT). The ratio of digestate to HCl was adjusted as necessary for the concentration of mercury in the sample to fall within the range of the calibration curve.

Sample Analysis

Total mercury was analyzed in accordance with USEPA Method 245.6 using a Perkin-Elmer FIMS 100 equipped with an AS-91 autosampler using the flow-injection mercury cold-vapor technique. The software program WinLab 32 for AA was used in conjunction. The instrument detection limit is reported to be 0.2 parts per billion (ppb). The method detection limit was calculated to be 0.010 ppm.

Calibration

Calibration standard solutions of 0.0 ppb, 0.5 ppb, 1.0 ppb, 2.5 ppb, 5.0 ppb, and 10.0 ppb were prepared from 1000ug/mL Hg in 5% HNO3 JT Baker® stock reference solution (Phillipsburg, NJ) by serial dilution. Calibration methods are described in detail in Appendix C.

FIMS Operating Procedure

Samples were placed in the autosampler tray. The following parameters were entered into a sample information file: autosampler location, sample ID, initial sample weight, sample prep volume (25 mL), aliquot volume (1 mL), diluted to volume (in most cases: 6 mL), and nominal sample weight (1 g).

Argon was used as the carrier gas with an inlet pressure of 350 kPa. The carrier solution was 3% hydrochloric acid (v/v) and 5.5% stannous chloride (w/v) was the reducing agent. The FIMS program used included a prefill time of 15 s. and a sampling time of 10 s. in step 1, during which the sample was injected into the carrier stream. The sample was mixed with the carrier gas and the reaction products entered into a gas liquid separator. In step 2, the gas phase was transferred into a glass cell in which absorption of mercury vapor was measured over 20 s. The absorbance was plotted vs. time and peak height was measured. Peak height of the sample was compared to the initial calibration through which the sample concentration of Hg was measured. Three replicates were performed and an average of the three measurements was reported.

Quality Assurance/ Quality Control

Quality Assurance and Quality Control were ensured by performing a calibration with calibration blank each day prior to analysis. A 0.995 or higher correlation coefficient was considered acceptable for the calibration curve. Further assurance was gained through the use of certified reference materials and spiked replicate samples. Each microwave digestion tray contained 16 samples including a reagent blank, two certified standard reference materials: National Research Council Canada DORM-3 dogfish muscle tissue (Ontario, Canada) and National Institute of Standards and Technology Standard

Reference Material® 1946 Lake Superior Fish Tissue (Gaithersburg, MD), and a replicate sample of fish muscle tissue spiked with a known amount of liquid Hg. A recovery between 80% and 120% of expected value was accepted for the SRMs and spiked samples. Samples were not recovery corrected.

Statistical Approach

A power analysis was conducted by Gerstenberger and Eccleston (2002) to determine sufficient sample size. Sample size was determined to be 20 fish from each species in each of the four basins. Unfortunately, we were not able to acquire 20 fish of each species for all locations. While complete samplings of striped bass were obtained from all four locations, only 19 channel catfish were collected from Boulder and Gregg Basin and only 17 were collected from Virgin Basin. Complete samples of largemouth bass were not obtained from any of the locations. Only 9 largemouth bass were collected from Boulder Basin, 11 from Virgin Basin, 12 from Boulder Basin, and 17 from Overton Arm. These groups remained in the statistical model, but caution should be used when interpreting results containing these groups.

K Factor

A bivariate correlation will be performed to test the null hypothesis that there is no correlation between K factor and mercury concentration. If a significant correlation exists at the $p < 0.05$ level, an analysis of covariance (ANCOVA) will be performed comparing two factors, location and species, with mercury concentration while controlling for the effect of the covariate, K factor. If no correlation exists, K factor will be disregarded and an analysis of variance (ANOVA) will be performed.

Trophic Level and Location

The data will be tested for normality using a Shapiro-Wilks test with the null hypothesis that the data are normal. If the data are normal an ANOVA with a Bonferonni post hoc test or ANCOVA with contrasts will be performed comparing trophic level and location with mercury concentration while controlling for the effect of K factor, if necessary. If the data are not normal, they will be log transformed and again checked for normality. If normality exists among the data at this point, an ANOVA or ANCOVA will be performed and any trends between mercury concentration, location, and species will be recorded. Two null hypotheses will be tested by the analysis of variance/covariance: first, there is no difference in mercury concentration among different trophic levels and second, there is no difference in mercury concentrations among different locations. If normality does not exist, a Kruskal-Wallace test with a Nemenyi post-hoc test will be performed to observe trends in the data. The data will be examined to determine if variability is sufficient to discount pseudoreplication.

CHAPTER 5

STUDY RESULTS

Measurements and Statistics

In total, 49 largemouth bass, 94 striped bass, 78 channel catfish, and 31 blue tilapia were analyzed from all four locations. Largemouth bass ranged in length from 20.0 cm to 71.2 cm with a mean length of 34.4 cm. Weights ranged from 98.0 g to 4180.0 g (mean 606.0 g). Striped bass ranged in length from 25.2 cm to 62.5 cm (mean 37.6 cm). Weights of striped bass ranged from 95.0 g to 1900.0 g (mean 466.4 g). Channel catfish ranged in length from 26.1 cm to 65.0 cm with a mean length of 42.1 cm. Weights ranged from 119.0 g to 3110.0 g (mean 710.9 g). Blue tilapia ranged in length from 15.6 cm to 39.6 cm with a mean length of 23.2 cm. Weights of blue tilapia ranged from 78 g to 2500 g with a mean of 416.5 g. Mercury concentrations in all blue tilapia were below the limit of detection for this method (0.010 ppm) and therefore were not included in the statistical model.

All data were analyzed using SPSS® version 16.0 (Chicago, IL). Normality and homogeneity of variance assumptions were tested using the Shapiro-Wilks and Levine tests. Untransformed mercury values were not normal ($W = 0.785$, p<0.001) and variances were not homogenous ($t= 3.755$, p=0.012). The data were transformed using a $\log_{10}$ transformation. After transformation, mercury values met the assumptions of normality ($W = 0.997$, p = 0.968) and homogeneity of variance ($t= 1.604$, p=0.189).

An Analysis of Covariance (ANCOVA) with Type IV sum of squares error was completed to observe trends between mean mercury concentration in muscle tissue, trophic level, and location while removing for the effect of length. The $\log_{10}$ of the mean mercury concentration was the dependent variable and trophic level and location were the independent variables. The effect of length was controlled for through its use as a covariate.

<u>K Factor</u>

K factors were calculated by species using the formula $(W/L^3)*100$ (Williams 2000). K factors for largemouth bass, striped bass, and channel catfish from all locations are summarized in Table 3. Overall, largemouth bass had the highest mean K factor among the three species ($\bar{x} \pm SD$) (1.27±0.15) and channel catfish had the lowest mean K factor (0.79±0.17). Striped bass had an intermediate mean K factor (0.81±0.17) (Table 3).

Table 3. Mean, minimum, and maximum K factor values of fish from Lake Mead by species

Species	Mean	Minimum	Maximum	Standard Deviation
Largemouth bass	1.27	0.94	1.67	0.15
Striped bass	0.81	0.40	1.21	0.17
Channel catfish	0.79	0.40	1.52	0.17

The data showed a consistent pattern with respect to K factor among locations. Fish of all three species had the highest K factor in Boulder Basin followed by Gregg Basin, Overton Arm, and Virgin Basin (Figure 3).
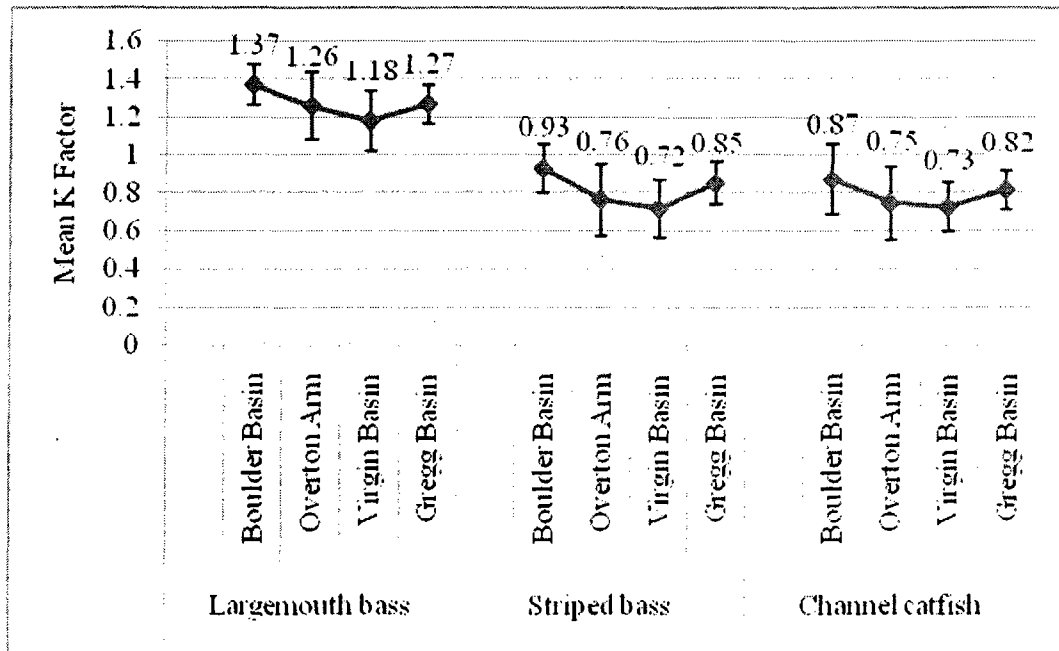
Figure 3. Mean K factors of fish from Lake Mead by species and location
Error bars indicate standard deviation of the mean.

A bivariate correlation was used to evaluate K factor as a possible covariate. Based

on the Pearson Correlation Coefficient (r) of -0.272, p<0.001, the overall K factor was

found to be significantly correlated at the 0.01 level (one-tailed) with the $\log_{10}$ of mercury

concentration. An ANCOVA using K factor as the covariate did not reduce the

interaction (F= 2.171, p=0.047) between the independent variables species and location.

A species-specific analysis revealed that a significant correlation between K factor and

$\log_{10}$ of mercury concentration only existed for striped bass (r = -0.536, p<0.001). No

significant correlation was found for largemouth bass or channel catfish. Therefore, K

factor was found not to be a suitable covariate for these data.

Length

Length was also evaluated as a possible covariate and found to be positively

correlated overall at the 0.01 level (one-tailed) with the $\log_{10}$ of mercury concentration (r

=0.234, p<0.001). A simple linear regression was performed between the 5% trimmed

mercury concentration (ppm) and length (cm) for each species as well as for all species

combined (Figure 4). Length accounted for approximately 10% of the variability in

mercury concentrations overall. In largemouth bass, striped bass, and channel catfish

length accounted for about 35%, 35%, and 0.05% of the variability in mercury

concentrations, respectively. An ANCOVA utilizing length as a covariate reduced

interaction between the independent variables species and location so that it was no

longer significant (F=1.261, p=0.277). For these reasons, length was determined to be a

useful covariate for these data. Mean lengths of fish collected for this study by species are

shown Table 4.

Table 4. Mean, minimum, and maximum lengths of fish (in centimeters) from Lake Mead
by species

Species	Mean	Minimum	Maximum	Standard Deviation
Largemouth bass	34.4	20.0	71.2	7.9
Striped bass	37.6	25.3	62.5	7.7
Channel catfish	42.1	26.1	65.0	9.0
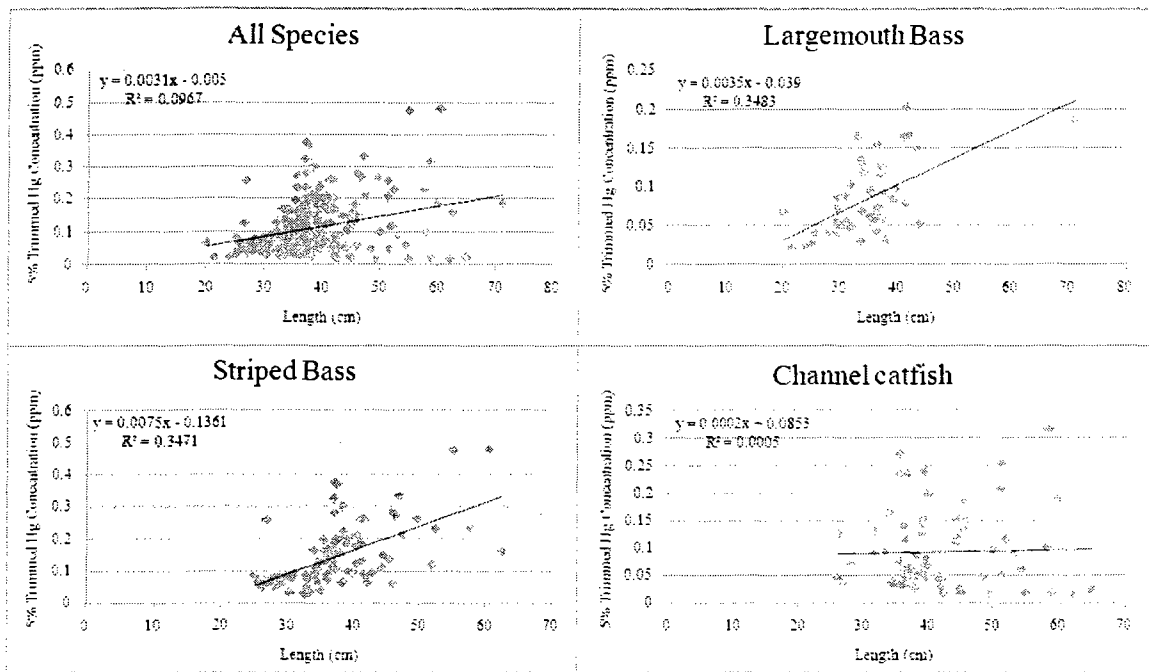
Figure 4. Simple linear regression plots of length (cm) vs. 5 % trimmed mercury concentration (ppm) for largemouth bass, striped bass, channel catfish, and all species combined

Trophic Level

Mean mercury concentrations of largemouth bass, striped bass, and channel catfish prior to length adjustment are as follows: ($\bar{x}$ ±SD) 0.089± 0.065 ppm, 0.154±0.127 ppm, and 0.098± 0.080 ppm. Blue tilapia (n=31) were also collected, however, all mean mercury concentrations in tilapia were below our method limit of detection of 0.010 ppm and therefore were not included in the statistical model.

Figure 5. Length-adjusted mean mercury concentrations (ppm) in fish muscle
tissue by species (all locations combined)
Error bars indicate 95% confidence intervals. * The mean mercury concentration
in striped bass differed significantly from the mean in largemouth bass (p=0.001)
and channel catfish(p<0.001). The mean mercury concentration in largemouth
bass differed significantly from the mean in channel catfish (p=0.025).

The ANCOVA indicated a significant difference between mercury concentrations

among the three species ($F_{2,208}$= 22.488, p< 0.001). The use of contrasts revealed that the

mean mercury concentrations of all three species differed significantly from each other at

the p=0.05 level, so significance was evaluated at the p=0.01 level. Contrasts revealed

that mean mercury concentration of largemouth bass differed significantly (p=0.001)

from that of striped bass and that of channel catfish (p=0.025). The means of striped bass

and channel catfish also differed significantly (p<0.001) from each other (Figure 5). If

blue tilapia are included in the model with all mercury concentrations reported at the

method detection limit of 0.010 ppm, there is a significant difference (p<0.001) between

the mean mercury concentration in blue tilapia and the means of all other species.

Location

Mean mercury concentrations among the four basins prior to length adjustment were as follows ($\bar{x}$ ±SD): Boulder Basin: 0.066±0.059, Overton Arm: 0.140± 0.104, Virgin Basin: 0.128±0.125, Gregg Basin: 0.141±0.100. The ANCOVA revealed a significant overall difference in mean mercury concentrations between the four major basins of Lake Mead ($F_{3,208}$= 20.541, p<0.001). Contrasts showed that there was a significant difference between the mean of Boulder Basin and that of the other three locations: Gregg Basin (p<0.001), Virgin Basin (p<0.001), and Overton Arm (p<0.001) (Figure 6). No significant difference was found between the mean mercury concentrations in Gregg Basin, Virgin Basin, and Overton Arm.
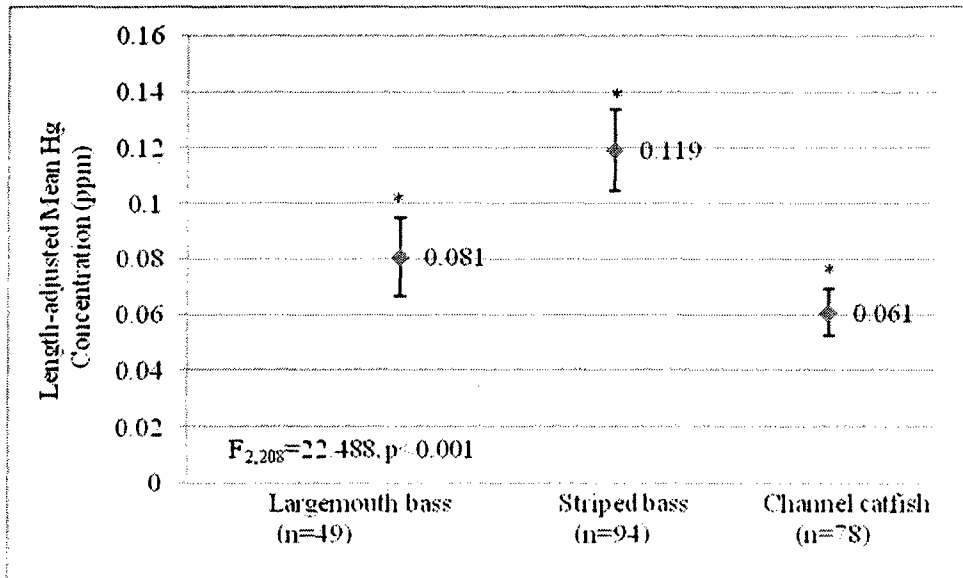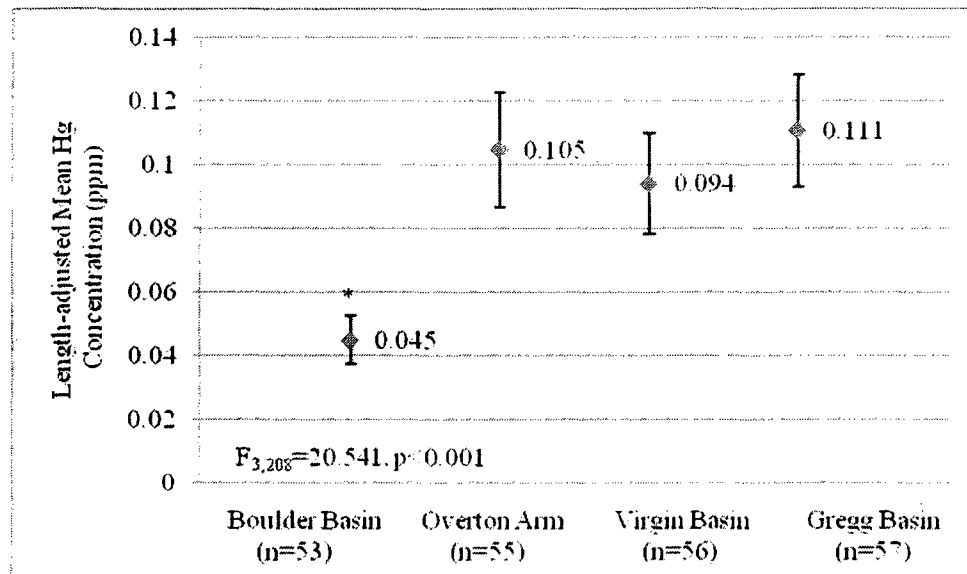

Figure 6. Length-adjusted mean mercury concentrations (ppm) in fish muscle tissue by location (all species combined)
Error bars indicate 95% confidence intervals. *The mean mercury concentration in Boulder Basin differed significantly from the means in Overton Arm (p<0.001), Virgin Basin (p=0.002), and Gregg Basin (p<0.001).

For largemouth bass and striped bass, mean mercury concentrations are lowest in
Boulder Basin followed by Overton Arm, Virgin Basin, and highest in Gregg Basin
(Figure 7). Channel catfish display a slightly different pattern with the lowest
concentration again in Boulder Basin, intermediate concentrations in Virgin and Gregg
Basins, and the highest concentration in Overton Arm (Figure 7).
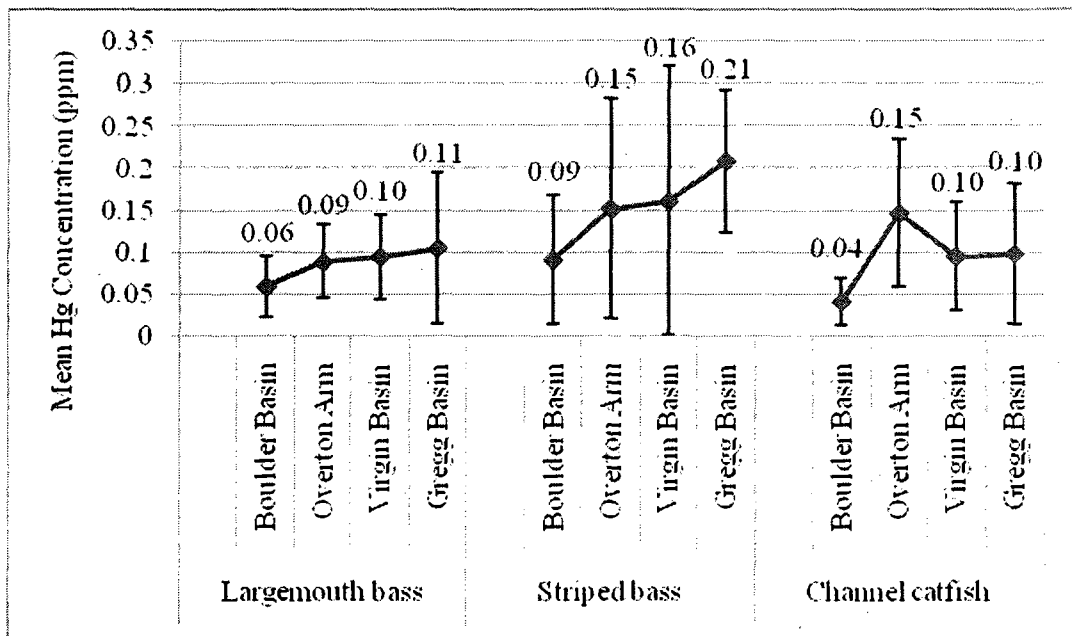
Figure 7. Mean mercury concentrations (ppm) in fish muscle tissue by species and
location in Lake Mead
Error bars indicate standard deviation of the mean.

<u>Collection Year</u>

Mean mercury concentrations from each species were compared by year of collection

to determine if the means differed by collection year. The means for each species did not

significantly differ from October of 2007 to October of 2008 (Figure 8).
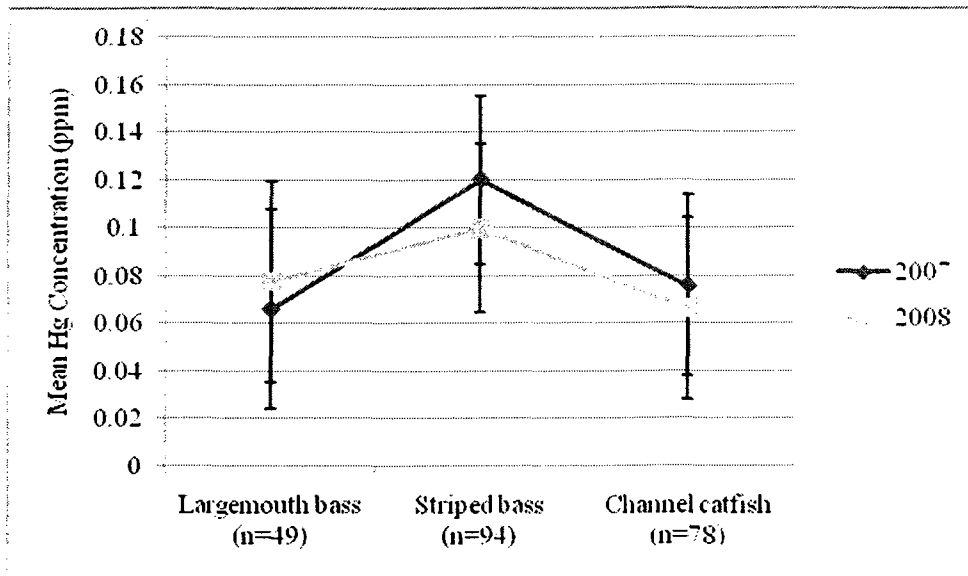
Figure 8. Mean mercury concentrations (ppm) in fish muscle tissue by species and
year (all locations combined)
Error bars indicate 95% confidence intervals. There was no significant yearly
variation in Hg concentrations within species.

CHAPTER 6

DISCUSSION

Fish Condition and Body Size

The condition factor (K) in fish is a measure of relative robustness and is primarily influenced by nourishment (Williams 2000). A distinct trend was noted in our data when evaluating K factor with respect to location. Fish from all three species collected from Boulder Basin contained the highest K factors followed by those collected from Gregg Basin, Overton Arm, and Boulder Basin (Figure 3). Because Boulder Basin is the major site of nutrient loading in the reservoir, it seems plausible that fish from this location would have greater access to food and therefore possess greater mass and length. Conversely, Virgin Basin has no unique inflows and very little nutrient loading. The Colorado River enters at Gregg Basin through Grand Wash contributing nutrients to this location and Overton Arm receives inflow from the Virgin and Muddy Rivers.

Multiple studies have found a positive correlation between muscle mercury concentration and fish length and/or body size (Dellinger et al. 1995; Lange et al. 1993; Scott 1974; Scott and Armstrong 1972; Watras et al. 1998). There are two main explanations for this observation. The first is that as fish age, they increase in length. Because bioaccumulation of methylmercury occurs over time and is not readily removed from tissues, it follows that greater concentrations of mercury are found in older fish. Secondly, an increase in fish body size is generally associated with greater intake of food.

As fish consume more mercury containing food, the mercury becomes more greatly concentrated in tissue. Mercury's high affinity for sulfur results in a preferential accumulation in mitochondria-rich tissues like muscle.

The data from this study were in partial agreement with previous studies in that length was positively correlated with mercury concentrations overall (r =0.234, p<0.001). When species were evaluated independently, the positive correlation remained for largemouth and striped bass. There was not a significant correlation between length and mercury concentration for channel catfish.

The data from this study showed an overall negative correlation between K factor and mercury muscle concentration (r= -0.272, p <0.001). This was contrary to the hypothesis presented earlier that there would be a positive correlation between K factor and mercury muscle concentration. This finding was not in agreement with prior studies which found fish condition to be positively correlated with mercury concentration. Upon division of species, the significant negative correlation only remained for striped bass. Channel catfish and largemouth bass showed no significant correlation between K factor and $\log_{10}$ of mercury concentration. In striped bass, there was no mercury concentration above which the correlation became positive.

Cizdziel et al. (2002) also reported an inverse correlation between fish condition and mercury concentration in striped bass collected during fall of 1998. The authors suggested that because fall in Lake Mead is a lean-food season, the striped bass may have been experiencing a starvation period during which organs shrank and mercury became more concentrated in tissues (Cizdziel et al. 2002). The samples for this study were also collected during the fall. If the resources were available to collect fish year round, it

would be interesting to determine if the correlation was reversed during seasons of high food availability.

Due to the nature of the data collected, K factor was not a suitable covariate for use in this study. K factor, however, is a measure of fitness of a fish and not size. To better understand the relationship between mercury concentration and size of fish, length was evaluated independently from weight as a possible covariate. Length was found to be positively correlated with mercury concentration ($r = 0.234$, $p<0.001$) for all species combined. There was not a significant correlation overall between fish weight and mercury concentrations. Linear regression indicated that length accounted for approximately 10% of the variation in mercury concentrations overall. When regressions were performed on each species independently, length accounted for 35% of the variation in striped bass, 35% in largemouth bass, and 0.05% in channel catfish (Figure 4). When utilized as a covariate in the statistical model, length negated the significant interaction between location and species. Therefore, length was determined to be an effective covariate for these data.

Although length was a predictor for mercury concentrations in largemouth bass and striped bass, there are other predictors present which were not evaluated for the purposes of this study and may have had a greater impact on mercury accumulation in channel catfish. Some variables of interest include environmental factors such as temperature, seasonality, and prey availability and physiological factors such as age, growth rate, metabolism, and diet. In a study of top level predators largemouth bass and northern pike MacRury et al. (2002) found that diet shift, growth rates, and water temperature influenced methylmercury uptake. A dietary analysis has been conducted to determine

the food sources of largemouth bass, striped bass, and channel catfish in the Colorado River Basin (Minckley 1973), however, many variables can impact the food that the fish consume. Post et al. (1996) indicated that seasonal variation in methylmercury uptake was present in a study of yellow perch. A seasonal effect may be experienced in that during lean seasons, the body composition of the fish is altered. Growth rates of fish may impact mercury concentrations by altering the uptake and storage of mercury in tissues. Because mercury is associated with mitochondria-rich muscle tissue and not fat, very lean fish may experience a higher concentration of mercury than fish with a higher fat content in their tissues. A muscle to fat ratio analysis of the fillets that were used for this study may help to explain this phenomenon. Another possibility for altered mercury concentrations is that predators do not have an abundant food supply during the fall and must look to alternative food sources to sustain themselves. When predators consume prey which are lower on the food chain, they are subject to a lesser degree of contaminant bioaccumulation. A stomach content analysis of the fish collected would be of interest if time and resources permit. Also, a comparison of our data to the shad trawl data collected during October of 2007 and 2008 may help to explain disparities in our data from the current body of literature.

Trophic Level

According to angler interviews conducted during 1999-2002, striped bass is the most commonly consumed fish species from Lake Mead constituting 70% of all fish consumption followed by largemouth bass (12%) and channel catfish (11%) (Gerstenberger and Eccleston 2002). These species represent varying trophic levels.

Striped bass are piscivores, their diet consisting almost exclusively of threadfin shad, the primary forage fish of the reservoir. Largemouth bass are carnivores, their diet consisting of a mixture of shad, crayfish, and certrarchids. Channel catfish are omnivores, feeding on a wide variety of fish, insects, and detritus, while blue tilapia are herbivores (Cizdziel el al. 2002, Minckley 1973). Striped bass had significantly higher mercury muscle concentrations than largemouth bass and channel catfish (Figure 5). As strict piscivores, one would expect mercury concentrations to accumulate at a higher rate in striped bass than in species which consume a variety of prey, especially prey at lower trophic levels than threadfin shad. The mean mercury concentrations in largemouth bass were also significantly higher than those in channel catfish (p= 0.025). This is consistent with trophic level expectations because higher level predators are subject to a greater risk of bioaccumulation through diet. Channel catfish consume a mixture of fish, insects, and other detritus and therefore do not accumulate mercury as rapidly. Muscle mercury concentrations in blue tilapia were below the limit of detection for this method, however, when included in the model at the method detection limit, mercury concentrations were still significantly lower than all other species. A diet consisting entirely of plants does not promote the bioaccumulation of mercury as quickly as a diet containing fish, so one would expect mercury concentrations to be relatively low. The results of this study with respect to trophic level were consistent with those of Cizdziel et al. (2002) who reported striped bass to have the highest mercury concentrations followed by channel catfish, largemouth bass, and blue tilapia.

Location

The sampling for this study was completed during October of 2007 and 2008 with NDOW and AZGF during their annual fish survey of Lake Mead. Sampling sites were selected by these agencies to give an accurate representation of the fish populations found throughout the reservoir. Lake Mead consists of four large basins: Boulder, Virgin, Gregg, and Overton Arm, separated by narrow canyons (LaBounty and Horn 1997) (Figure 2). Based on previous studies on the distribution of fish populations within the reservoir, the assumption is held that the 4 basins sampled contain relatively distinct fish populations (Mueller and Horn 2004). This division is also in concordance with a previous study performing inter-basin analysis on contaminants in Lake Mead (Cizdziel et al 2002).

The data from this study revealed that Boulder Basin contained significantly lower mercury concentrations than all other locations analyzed (Figure 5). There was no significant difference between the mean mercury concentrations found in Overton Arm, Virgin Basin, and Gregg Basin. The results of this study with respect to location are in concordance with those of Cizdziel et al. (2002) in that fish from Boulder Basin contained the lowest muscle mercury concentrations overall. The authors of the aforementioned study suggested that this could be due to a starvation concentration whereby fish with low K factors concentrated mercury to a higher degree due to starvation. This hypothesis is not fully supported by the data from this study. For the starvation concentration theory to be supported, we would expect to see an inverse correlation between K factor and mercury concentration with respect to location. That is, the location with the second highest K factor should have the second lowest mercury

concentrations. In the data presented in this study, this was not the case. In fact, Gregg Basin had the second highest K factor for all species (Figure 3) and the highest mercury concentrations for two of the three species, largemouth bass and striped bass (Figure 7). However, there are several other factors influencing the accumulation of mercury in fish tissue with respect to location. Some of these factors are explored below.

Boulder Basin is the westernmost and most downstream basin of Lake Mead. This region receives inflow from the two main arms of the reservoir as well as all drainage and effluent from the Las Vegas Valley via the Las Vegas Wash (LaBounty and Horn 1997). The Colorado River outflow is also located in Boulder Basin at the Hoover Dam. Due to the discharge from the Las Vegas Wash, Boulder Basin experiences the majority of the nutrient loading within the reservoir (Paulson and Baker 1981, Prentki and Paulson 1983, LaBounty and Horn 1997). Many of the suggestions that follow are based on the fact that the Las Vegas Wash empties into Boulder Basin. Boulder Basin also contains the source of Las Vegas Valley's municipal water supply and as such is the site of much water quality research. The same amount of data is not available for the other three locations in this study. Therefore, comparison between basins is difficult and any possible suggestions put forth would require significantly more research.

A complex relationship exists between DOC and methylation of mercury between water and sediment. Gilmour and Henry (1990) reported that an increase of DOC in water results in a decreased rate of methylation; however, in sediment, increased DOC results in a greater rate of methylation of mercury. Due to inflow from the Las Vegas Wash, waters from Boulder Basin contain considerably elevated DOC levels which decline with increasing distance from the Wash (LaBounty and Horn 1997). Elevated

DOC in waters from Boulder Basin with respect to all other locations could result in a

decrease in methylation and thus lower concentrations of mercury in fish; however, a

current calculation of DOC in water and sediment for each location would be necessary

to draw any conclusions. The effects of pH in lake water on methylation rates are also

multifaceted. In fresh water, acidification increases methylation and decreases

demethylation but in sediment, decreased pH results in decreased methylation Gilmour

and Henry 1990). A review of current literature did not reveal any inter-basin analysis of

pH in waters or sediments; however, a 2007 study did survey water quality parameters of

the major inflows into Lake Mead: the Las Vegas Wash, Muddy River, Virgin River, and

Colorado River (Rosario-Ortiz et al. 2007). Throughout each of 3 sampling periods, the

pH in water from the Las Vegas Wash was lower than each of the other inflows,

suggesting that perhaps the water in Boulder Basin may also have held a lower pH with

respect to other locations (Rosario-Ortiz et al. 2007).

Another possibility for the disparity between mercury concentrations in Boulder

Basin compared with all other locations is a possible increased concentration of selenium

in Boulder Basin compared with other locations. Studies in freshwater lakes have

demonstrated that the addition of selenium decreases the rate of methylmercury

bioaccumulation (Chen and Belzile 2001; Pelletier 1986; Turner and Swick 1986).

Because mercury and selenium experience a high affinity for one another, it is thought

that the formation of mercury-selenium complexes renders both compounds biologically

inactive (Moller-Madsen and Danscher 1991; Raymond and Ralston 2004). Selenium

concentrations have been calculated recently in Boulder Basin because of the concern of

selenium input from the Las Vegas Wash. Cizdziel and Zhou (2005) calculated selenium

concentrations during 2002 in the Las Vegas Wash which feeds directly into Boulder Basin and reported concentrations as high as 20.2 ug/L. The Southern Nevada Water Authority conducted sampling in the Las Vegas Bay of Boulder Basin during 2007 and 2008 and found that concentrations ranged from 1.7 ug/L and 4.2 ug/L (Blish 2008, unpublished data). The USEPA's maximum tolerance level for ambient selenium is 5.0 ug/L (USEPA 1987). Selenium data are not available for Gregg Basin, Virgin Basin, or Overton Arm. If these data were available, a comparison between average selenium concentrations in each of the basins may explain the decreased mercury concentrations in fish from Boulder Basin.

The limnology of Lake Mead has been altered in the recent past with the introduction of the quagga mussel (*Dreissena bugensis*), a nonindigenous invasive species of mussel. Quagga mussels were discovered on January 6, 2007 in Boulder Basin of Lake Mead (LaBounty 2007). After their discovery, their spread was monitored closely throughout all of Lake Mead. The mussels are currently present in all basins of Lake Mead, with varying densities. *Dreissena* species are filter feeders, grazing on phytoplankton and seston in lakes and rivers. As such, Roditi and Fisher suggested that they might be effectively utilized as bioindicators of freshwater contamination (1999). Based on recent estimates of quagga mussel density, Wong (2008) estimated that quagga mussels would effectively filter the entire volume of Lake Mead in 148.2 days (unpublished data). Although quagga mussels were discovered in January 2007, it is uncertain as to when they were first introduced into the reservoir. It is possible that they had been present and filtering the water for some time before their discovery in Boulder Basin. If this were

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true, it might help to explain the disparity in mercury concentrations between Boulder Basin and the other locations within the reservoir.

Relevance to Public Health

Lake Mead National Recreation Area provides angling opportunities for residents and visitors to the Las Vegas Valley. Previous research has indicated that anglers consume approximately 23 fish meals per year on average and often share their catch with their families (Gerstenberger and Eccleston 2002). The results from this study do not indicate the need for a fish consumption advisory for any of the species or locations sampled within Lake Mead. The mean mercury concentrations for all species and locations analyzed in this study were well below the governmental standards set by the USFDA and USEPA (1.0 ppm and 0.5 ppm, respectively). There were 2 samples out of 221 which had mercury concentrations higher than the USEPA safety standard of 0.5 ppm and no samples tested above the USFDA safety standard of 1.0 ppm. Both samples were striped bass which measured over 20 inches in length. The samples with elevated mercury concentrations were collected from Overton Arm and Virgin Basin. Comparing the mean values of each species to Table 1, approximately 8 fish meals/month of channel catfish and largemouth bass and 4 meals/ month of striped bass can be safely consumed. Pregnant women, infants and children should always exercise caution when consuming fish because of the increased susceptibility of the developing nervous system to methylmercury. Because it is impossible to judge mercury content without the proper analytical equipment, caution should always be exercised when consuming large fish (greater than 20 inches). Limiting the amount of fish meals and portion size of such fish are common and effective ways to reduce methylmercury consumption.

Study Limitations and Future Research

This study was initiated to implement the fish contaminant monitoring protocol for Lake Mead developed in 2002 by Gerstenberger and Eccleston. The sampling design from this protocol suggested that 20 fish from each species and each basin be analyzed for statistical validity. Although much time and effort went into the collections, this study fell slightly short of the desired sample size in several categories. Twenty or more striped bass were collected from each location, therefore, striped bass data are asserted in the study with complete statistical validity. Complete samplings of channel catfish were collected from Overton Arm; however, we were short one sample from both Boulder Basin and Gregg Basin and three samples from Virgin Basin. In the largemouth bass category, each location was deficient in samples. Only 9 largemouth bass were collected from Boulder Basin, 11 from Virgin Basin, 12 from Boulder Basin, and 17 from Overton Arm. However, a back calculation was performed using the sample size, variance, and error of the actual largemouth data that were collected and determined that the data expressed a 96% confidence interval. Additional samples could increase the statistical power of the study to an even greater confidence interval. Although some of the sample sizes did not meet the desired number, the mercury concentrations found in muscle tissue were quite low with respect to both USEPA and USFDA guidelines. Therefore, these trends are reported as a general guideline for consumption because the species and locations examined for this study did not contain mercury concentrations high enough to warrant a consumption advisory.

Due to the large sample size required and limited resources for collection, it was necessary to collect the samples for this study over a two year period. Samples were

collected within the same month of each year to obtain fish of the same stage of life cycle and minimize variation. However, there are certainly limnological and ecological changes that occurred in between the two years. Most notable is the introduction of the quagga mussel into Lake Mead and subsequent spread throughout all four basins. The filter-feeding lifestyle and large biomass of quagga mussels have the potential to drastically change water conditions and possibly impact the presence of contaminants in the water. Our results did not show a significant difference between the mean mercury concentrations during the two years over which sampling took place (Figure 8). Future research should continue to evaluate mercury concentrations in both fish and quagga mussels to determine if quagga mussels are indeed impacting mercury concentrations in Lake Mead.

As mentioned previously, further research is necessary to fully understand the relationship between seasonal, food source, water, and sediment parameters and the bioaccumulation of mercury in fish. A seasonal comparison of mercury concentrations with respect to K factor would enhance our understanding of the data presented in this study. A stomach content analysis of the fish collected for this study could help to determine whether alternate food sources were being sought. A muscle to fat ratio in the tissues could determine if mercury was being more concentrated because of starvation. Concentrations of selenium in water and sediments should be calculated to determine if there is an interaction between mercury and selenium occurring in Boulder Basin. The samples collected from this study should be analyzed for selenium content to determine if a relationship is present in fish tissue. DOC and pH calculations in water and sediments

should extend beyond Boulder Basin to include Overton Arm, Virgin Basin, and Gregg Basin.

Summary and Conclusion

Lake Mead, Nevada is a widely utilized as a sportfishing location for residents of Nevada, Arizona, and visitors to these states. Fish caught from the four major basins of Lake Mead are often consumed by anglers and their families; averages of approximately 23 fish meals per year are consumed (Gerstenberger and Eccleston 2002). Fish are a source of healthful protein and omega-3 fatty acids but recent industrial and human activities have led to the pollution of many aquatic environments. One of the widely studied aquatic pollutants is methylmercury, a neurotoxin which accumulates in fish species and causes severe health effects when consumed in large amounts. To date, there has been no analysis of the fish from Lake Mead utilizing USEPA-approved methodologies for quantifying mercury concentrations in edible fish tissue. One aim of this study was to fill this knowledge gap and determine if a consumption advisory is necessary for any of the popular species of varying trophic levels caught in Lake Mead. Another aim was to compare mercury concentrations in fish among the four major basins of the reservoir to determine if mercury concentrations are higher in some areas than others. The mean mercury concentrations of fish from all trophic levels and all locations were well below USEPA and USFDA standards for issuing consumption advisories (0.5 ppm and 1.0 ppm, respectively). Two samples out of 221 collected contained mercury concentrations which were greater than the USEPA screening limit of 0.5 ppm. There were no samples which contained mercury concentrations greater than the USFDA action level of 1.0 ppm. Both of these samples were striped bass measuring over 20 inches in

length. Fish collected from Gregg Basin contained the highest collective mean mercury concentrations but this mean was still well below federal guidelines for mercury in fish. The conclusions drawn from these results are that largemouth bass, striped bass, channel catfish, and blue tilapia from Lake Mead can be safely consumed but consumers should always abide by certain guidelines when consuming fish from Lake Mead or any other location: consume moderately sized fish as larger fish tend to accumulate more mercury, do not consume extremely emaciated fish, and limit portion size of fish, especially for infants, children, and pregnant women.

APPENDIX

PROCEDURES FOR COLD VAPOR MERCURY ANALYSIS

Cleaning Procedure for Cold Vapor Mercury Analysis Labware

- Scrub labware thoroughly with scrub brush and 2% Citranox to remove all solid matter.

- Rinse the labware with tap water until there is no presence of soap.

- Rinse the inside and outside of the labware twice with deionized water.Place the labware in a 10% nitric acid bath, ensuring that it is completely covered in the solution.

- Allow labware to soak for a minimum of 1 hour. Remove the labware from the acid bath, emptying the acid back into the bath.

- Rinse the labware three times with deionized water.

- Place the labware upside down on a clean dish rack to dry.

- Store labware in a clean, dust free location until use.

- Change the 10% nitric bath every few months as needed.

- Discard waste into designated acid waste container.

Fish Tissue Digestion Procedure for Cold Vapor Mercury Analysis

- Thaw sample at room temperature for 1 hour.

- Place sample in whirlpack in a Seward® Stomacher 80 apparatus and mix for 60 seconds to ensure homogeneity after thawing.

- Weigh approximately 2 grams of fish tissue into a clean vessel liner.

- Add 4 mL trace metal grade nitric acid.

- Add 2 mL deionized water.

- Cap and seal liner within digestion vessel.

- Place 16 digestion vessels in rotor including a reagent blank, a spiked sample, and a standard reference material.

- Place rotor into the Anton-Parr Multiwave 3000 Microwave Reaction System.

- Program the system for 10 minutes of increasing power until 1200 W and a temperature of 160 degrees C are reached.

- Hold samples at this pressure and temperature for 5 minutes.

- After cooling, empty contents of each vessel into a clean, labeled, metal-free centrifuge tube.

- Add 4 mL 10% amidosulfonic acid dropwise to the digested material.

- Rinse vessels three times with approximately 5 mL dI water.

- Dilute samples to 25 mL with dI water.

- Vortex samples to ensure homogeneity.

FIMS 100 Calibration Procedure for Cold Vapor Mercury Analysis

- A calibration curve using freshly prepared standards must be prepared prior to each analysis.

- The calibration curve must include a standard at the method reporting limit (0.05 ug/mL)

- Purchase stock standard JT Baker InstraAnalyzed® Mercury (0.10% w/v) in 5% HNO3

- Dilute stock standard to 100 ppb by adding 1 uL 1000ug/Ml and 5 uL KMnO4 5% w/v to 9.994 mL 2%HNO3

- Make serial dilutions of the 100 ppb intermediate stock standard by transferring 0.5 mL, 1 mL, 2.5 mL, 5 mL, and 10 mL of intermediate standard to a series of 100 mL volumetric flasks. Dilute to volume with reagent water. These flasks contain 0.5 ppb, 1 ppb, 2.5 ppb, 5 ppb, and 10 ppb of Hg, respectively.

- Place a clean stir bar in each flask and stir for 1 minute on a magnetic stir plate.

- Transfer each calibration standard to a labeled, clean, metal-free, centrifuge tube.

- Place calibration standards in ascending order in the AS-91 autosampler tray.

- In the Win Lab 32 for AA® software, open the automated analysis window and click Calibrate.

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