MERCURY CONCENTRATIONS IN FISH FROM THE GUADALUPE RIVER, TEXAS: RELATIONSHIPS WITH BODY LENGTH AND TROPHIC POSITION

by

Taylor Rene Gold Quiros, B.S.

A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Master of Science
with a Major in Aquatic Resources
December 2018

Committee Members:

Jessica Dutton, Chair

Tim Bonner

Weston Nowlin

Clint Robertson

FAIR USE AND AUTHOR'S PERMISSION STATEMENT

DEDICATION

My Toby. Te amo. I miss you

ACKNOWLEDGEMENTS

I would first like to thank my thesis advisor Dr. Jessica Dutton; her office was always open whenever I ran into a trouble spot or had a question about my research or writing. Dr. Tim Bonner and Dr. Weston Nowlin were incredibly helpful in teaching me how to think like a scientist and their patience with my questions was without end. Gratitude is also due to Clint Robertson, without whom I wouldn't have fish to analyze in the first place nor would I know the differences between those pesky sunfish. I would also like to thank Texas State University for funding this study. Many people donated time and effort to help with both field and lab work and I am grateful to each and every one of you: Stacey Britton, Meaghan McCormack, Kristyn Cunningham, and Alexis Lambert. Nina Noreika, you were a wonderful sounding board for many of my more goofy ideas and I thank you for it. Lily - thanks for getting me outside and out of my own head. Lastly, I would like to thank my family who kept me sane, reminded me to eat, and supported me throughout this process. I could not have done this without you.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Figure **Page**

ABSTRACT

Mercury (Hg) is known to bioaccumulate over time in freshwater fish and biomagnify up freshwater food webs, so top predatory fish have the highest Hg body burden. Within Texas, Hg studies in freshwater fish have primarily focused on the northern half of the state and south Texas is relatively understudied. This study investigated the concentration of Hg in muscle tissue from 41 trophically diverse species ($n = 1,772$) in relation to body length and trophic position at five sites on the Guadalupe River in South Central Texas using a direct mercury analyzer and stable isotope analysis ($\delta^{13}C$, $\delta^{15}N$). The majority of fishes showed a positive relationship between body length and Hg concentration, indicating that Hg was bioaccumulating over time. Striped mullet was the only species that displayed an inverse relationship suggesting growth dilution is occurring. Mercury concentrations were higher in top predators including longnose gar, flathead catfish, and striped bass, and lower in moderate and low trophic level fishes, including Mexican tetra, threadfin shad, and suckermouth catfish. Within the five sites examined, the average Hg concentration in each species was higher in reservoir sites than riverine sites. There was a positive relationship ($p < 0.05$) between $\delta^{15}N$ and Hg concentration at 4 of the 5 sites, indicating that Hg biomagnification is occurring at these sites. The biomagnification factor differed between sites, however it was not positively correlated with food chain length. Among species, the estimated trophic level was the strongest predictor of Hg concentration and within species, total length was the strongest predictor of Hg concentration. These findings provide valuable insight into bioaccumulation and biomagnification of Hg in a relatively understudied freshwater system in South Central Texas. Four species (flathead catfish, white bass, striped bass and longnose gar) had at least one individual that exceeded the Texas Department of State Health Services (TDSHS) human health criterion for Hg (0.7 μg/g wet weight), with at least one species at Canyon Lake, Lake Dunlap, and Victoria exceeding the guideline. Based on this data, the current Hg advisory for Canyon Lake needs to be reevaluated and Victoria and Lake Dunlap may need to have Hg advisories issued.

I. MERCURY LEVELS IN TROPHICALLY DIVERSE FISH THROUGHOUT THE GUADALUPE RIVER, TEXAS

ABSTRACT

Mercury (Hg) is known to bioaccumulate in freshwater fish and biomagnify up aquatic food webs, so top predatory fish have the highest Hg body burden. Prior studies in Texas have focused on examining Hg concentrations in freshwater fish in the northern and eastern regions of the state, and the south-central region is understudied. This study collected 41 species of trophically diverse fish from five sites (3 reservoir and 2 riverine) on the Guadalupe River and determined the total Hg concentration in muscle tissue using a Direct Mercury Analyzer. There was a positive relationship between Hg concentration and body length in 58.3% of investigated species, and species at higher trophic levels were more likely to display this positive relationship. The Hg concentration varied significantly between sites for six species and there was a trend of higher Hg levels in reservoir sites, in particular Canyon Lake. Individuals from four species (3 game species: flathead catfish, white bass, and striped bass, 1 non-game fish: longnose gar) exceeded the Texas health based standard of 0.7 μg/g wet weight and three of the five sites had at least one species that exceeded this criterion. This study supports a continued Hg advisory for longnose gar in Canyon Lake (with the possible addition of white bass and flathead catfish) as well as consideration of an advisory for longnose gar in Victoria and striped bass in Lake Dunlap.

i. INTRODUCTION

1.1 Mercury as a global pollutant

Mercury (Hg) is a nonessential trace element that is of ecological and public health concern because it is toxic to aquatic life and humans at low concentrations (Mason et al., 2012; Driscoll et al., 2007). Mercury exists in the environment in three forms: elemental [Hg(0)], inorganic [Hg(II)], and organic [methylmercury (MeHg), CH_3Hg]. Hg(0) and some forms of Hg(II) are released into the atmosphere by natural emissions (e.g., erosion of mineral deposits and volcanic eruptions; Mason, 2009) and from anthropogenic sources (e.g., gold mining and coal-fired power plants; Pacyna et al., 2006). Mercury is ubiquitously distributed throughout freshwater, estuarine, and marine environments (Merritt and Amirbahman, 2009; Hammerschmidt and Fitzgerald, 2006; Gavis and Ferguson, 1972), and due to its ability to be distributed around the planet via the wind belts, Hg can be found in high concentration in organisms in regions far away from sources, e.g., the Artic region (Hall et al., 2008). Elemental mercury [Hg(0)] can be photooxidized into Hg(II) in the atmosphere and then enter the terrestrial and aquatic ecosystems through wet and dry deposition (Weiner et al., 2003). Once in aquatic environments, Hg(II) can be converted into MeHg primarily via sulfate reducing bacteria in the sediment and water column (Gilmour et al., 1992). Methylmercury is the most bioavailable form of Hg that can be readily taken up by algae and transferred up the food web.

1.2 Mercury accumulation in fish

Mercury is well known to bioaccumulate over time in aquatic organisms, in particular fish, so for a given species, larger, older individuals have a higher body burden than smaller, younger individuals (Somers and Jackson, 2011; Chumchal et al., 2010; Adams and Onorato, 2005). Mercury also biomagnifies up aquatic food webs, so that top predators including fish such as spotted gar (*Lepisosteus oculatus*), striped bass (*Morone saxatilis*), and largemouth bass (*Micropterus salmoides*), and fish-eating birds such as, razorbill (*Alca torda*), great black-backed gull (*Larus marinus*), and glaucous gull (*Larus hyperboreus*) have the highest Hg concentration in their tissues (Chumchal et al., 2011; Lavoie et al, 2010; Chumchal and Hambright, 2009; Ward et al., 2006; Atwell et al. 1988).

Fish accumulate Hg, in particular MeHg, through their diet and the surrounding water, although laboratory studies using a biokinetic model have shown that the diet is the predominant exposure route (Dutton and Fisher 2014, 2010; Pickhardt et al., 2006). Of the three Hg species, MeHg is the most toxic to aquatic life; however, the majority of studies report the total Hg (THg) concentration in fish muscle tissue because > 85% of Hg in muscle tissue is present as MeHg (Bloom, 1992; May et al., 1987); therefore THg is a suitable proxy for MeHg. In addition, measuring the THg concentration is cheaper and quicker than speciating for MeHg.

Exposure to Hg can result in deleterious health effects and reduced reproductive potential in fish, which can ultimately impact their population numbers. Prior studies have shown that exposure to MeHg can result in reduced reproductive output including a lower spawning and hatching success rate (Crump and Vance, 2009; Drevnick and

Sandheinrich, 2003; Hammerschmidt et al., 2002). Early life stages are the most sensitive to Hg exposure due to rapid growth and development, and previous studies have shown that fish can have a reduced heart rate and developed physical deformities, including spinal curvature and craniofacial defects during the embryonic and larval developmental stages (Latif et al., 2001; Weis et al., 1981). The female parent can also offload some of her Hg body burden to the eggs through maternal transfer, thereby reducing her body burden. For example, a 2001 study determined that the MeHg concentration in the eggs of walleye (*Sander vitreus*) was 1.1-12% of that in the female's muscle tissue and represented between 0.2 and 2.1% of the female's MeHg total body burden (Johnston et al., 2001).

1.3. Mercury and human health

Humans are primarily exposed to Hg through fish and shellfish consumption. In the United States, over 90% of human exposure to Hg is through the consumption of freshwater and marine fish (Carrington and Bolger, 2002; U.S. EPA, 2002). Chronic exposure to Hg can result in deleterious effects on the central nervous system, including reduced motor skills, problems with sensory input, and muscle weakness (Rice et al., 2014; Tchounwou et al. 2003, Ryan and Terry, 1997), as well as the cardiovascular system including increased risk of hypertension, myocardial infarction, and stroke (Rice et al., 2014; Roman et al., 2011; Choi et al., 2009). Mercury can also be transferred from mother to child via the placenta and breast milk (Yang et al., 1997). Fetal exposure to elevated Hg can result in miscarriage, stillbirth and low birth weight (Rice et al., 2014). Children exposed to Hg while *in utero* and through breast milk have been diagnosed with cerebral palsy, craniofacial malformations, impaired thinking and problem-solving and motor skills, and delayed growth (Rice et al., 2014; Axelrad et al., 2007; National Research Council, 2000; Grandjean et al., 1997).

Due to these adverse health effects, the U.S. Food and Drug Administration (FDA) issues Hg advisories for commercially caught fish when the Hg concentration in muscle tissue exceeds 1 µg/g wet weight. The U.S. Environmental Protection Agency (EPA) recommends that state governments issue advisories regarding recreationally caught fish when the muscle Hg concentration exceeds the EPA's human health criterion of 0.3 µg/g wet weight. However, individual states have the authority to determine their own human health criterion, which can exceed the EPA guideline. This has resulted in disparity in the concentration at which advisories were issued among states; for example, the human health criterion for the south-central U.S. states ranges between 0.7 and 1 µg/g wet weight (Adams et al., 2016). As a result, the general public often receives conflicting messages when it comes to eating fish. From one perspective, they are encouraged to eat fish due to various health benefits including low levels of saturated fat and high protein, selenium, and omega-3 fatty acid content (Lund, 2013), but this is contradicted by advisories attempting to limit fish intake in order to reduce their Hg exposure.

1.4 Mercury in Texas

Within Texas, atmospheric Hg deposition increases with proximity to anthropogenic sources, in particular coal-fired power plants (National Atmospheric Deposition Program [NADP], 2018; Menounou and Presley, 2002). The Mercury

Deposition Network (MDN), which is run by the NADP, currently has no active atmospheric Hg wet deposition monitoring sites in Texas; however, there were sites in Fort Worth and Longview, but these have been inactive since 2006 and early 2018, respectively (NADP, 2018). Since these sites were based in the northeastern half of the state, there is no deposition information for the south and western portions of Texas. The most recent total mercury wet deposition map for the United States released in 2017 showed that the highest amount of Hg deposition occurred in eastern Texas (NADP, 2018). Eastern Texas has both a higher precipitation rate and a higher concentration of coal-fired power plants compared to west Texas (U.S. Energy Information Administration, 2018; Ground and Groeger, 1994). This deposition pattern was echoed by fish Hg concentrations which increase from west-to-east as well as north-to-south in this region; however, sampling in the south central part of the state is limited (Drenner et al., 2013; 2011; Smith et al., 2010).

Prior studies that investigated Hg concentrations in freshwater fish have predominantly focused on the northeast and southwest regions of Texas. Elevated Hg concentrations (>1.0 μg/g wet weight) have been found in largemouth bass from areas with high Hg and sulfate deposition and low agricultural coverage in northeast Texas, and concentrations ranging from 0.2 - 0.4 μg/g wet weight have been found in largemouth bass from the Western Gulf Coastal Plain, East Central Texas Plain and Texas Blackland Prairies ecoregions (Drenner et al., 2013; 2011). Mercury concentrations have been shown to increase with trophic position [0.03 μg/g wet weight for gizzard shad (*Dorosoma cepedianum*) to 0.464 μg/g wet weight for spotted gar] in open water species in Caddo Lake (Chumchal and Hambright, 2009). Fluvial sediments revealed a decrease longitudinal amount of Hg from the upper portion of the Trinity River watershed, with a marked increase as the river passes through the Dallas - Fort Worth metropolitan area (Matsumoto et al., 2010). In the Southwestern portion of the state, the lower Rio Grande/Rio Bravo del Norte drainage has also been the subject of Hg investigation, finding spatial variation in Hg levels in fishes grouped by trophic guilds as well as positive relationships between environmental factors such as dissolved organic carbon (DOC) and sediment Hg concentrations and Hg concentrations found in sampled fish (Smith et al., 2010). Additionally, MeHg concentrations in largemouth bass from the Amistad reservoir in southwest Texas have been correlated with higher MeHg in the sediment, as well as with DOC and porewater sulfate levels (Becker et al., 2011).

The Texas Department of State Health Services (TDSHS) issues consumption bans and advisories when sampled marine and freshwater fish exceed the 0.7 µg/g wet weight human health based standard for Hg. Currently, there are numerous Hg advisories for marine and freshwater fish species throughout Texas, including blue marlin (*Makaira nigricans*), blackfin tuna (*Thunnus atlanticus*), and all shark species caught along the Texas Gulf coast, and 19 freshwater bodies located throughout Texas (TDSHS, 2018; TPWD, 2018a), a summary of which is provided in Appendix A.

1.5 The Guadalupe River as a case study

Originating in Kerr County, the Guadalupe River flows along a longitudinal gradient southeast towards San Antonio Bay, which is connected to the Gulf of Mexico. Because several locations along the river's continuum are dammed, it provides both riverine and reservoir habitats for a broad variety of fish families (Thomas et al., 2007).

This also contributes to its recreational fishing popularity, in particular for Guadalupe bass (*Micropterus treculii*), striped bass, largemouth bass, blue catfish (*Ictalurus furcatus*), and channel catfish (*Ictalurus punctatus*), making the consumption of fish with Hg levels a pertinent health issue to South Central Texas citizens. Additionally, a study assessing Hg in trophically diverse fishes will provide a better understanding of how feeding guild and trophic position impact Hg accumulation in aquatic food webs. Investigating bioaccumulation in freshwater fish in a river ecosystem expands current knowledge of the drivers behind variation in Hg accumulation between systems.

Reservoir and riverine studies allow for an evaluation of Hg content in sites where environmental parameters (e.g., temperature, dissolved organic carbon, pH) that affect the bioavailability of Hg vary (Becker et al., 2011; Smith et al., 2010). In addition, the Guadalupe River flows through four ecoregions: Edwards Plateau, Texas Blackland Prairies, East Central Texas Plains, and Western Gulf Coastal Plains (Griffith et al., 2007), each of which has variations in vegetation type, soil type, and ecological communities. Ecoregions and environmental gradients within river systems are linked to Hg accumulation patterns in Northeast and Southwest Texas, with higher Hg levels in the South Central Plains and other forested-wetland habitats and in areas with high dissolved organic carbon and high sediment Hg concentrations (Drenner, et al., 2011; Chumchal et al., 2010; Smith et al., 2010).

Fishes located in reservoirs have higher Hg concentration than those from bordering riverine sites (Dong et al., 2016; Abernathy and Crombie, 1977), a trend that is linked to higher Hg methylation rates in more anoxic lacustrine sediment (Gilmour et al., 1992). This can also be seen in the enlargement of natural lakes into hydroelectric reservoirs, where newly submerged terrestrial organic matter stimulates methylating microbes, leading to an increased Hg bioavailability for fish (Jackson, 1991). In addition, water has a much longer residence time in reservoirs compared to riverine sites, allowing Hg to accumulate to higher concentration in the water column and therefore biota. Higher Hg concentrations can therefore be expected in reservoir sites.

There are numerous Hg advisories for freshwater fish currently in effect throughout Texas (Appendix A); only one Hg advisory has been issued in Central Texas. Canyon Lake in Comal County (Appendix A; TDSHS Advisory 30) is also the only site on the Guadalupe River where an Hg advisory has been issued. This advisory states that for striped bass and longnose gar (*Lepisosteus osseus*), adults and children greater than 12 years in age are advised to eat no more than two 8 oz. servings per month (4 oz. for children under 12 year), and pregnant women, women who are breastfeeding, and women who may become pregnant are advised to not eat any striped bass or longnose gar from the lake. This advisory was issued based on a survey that included six fish (two gar and four striped bass) that exceeded the limit of 0.7 μg/g wet weight (with an average concentration of 0.772 and 1.149 μg/g wet weight respectively) when the initial survey was conducted in 2005 (Ward et al., 2006). Due to the limited sample size and lack of retesting in the last 13 years, a more in depth study is warranted. A larger survey will provide current Hg values for a variety of fish species allowing for the reevaluation of current advisories such as those for Canyon Lake as well as other sites on the Guadalupe River where recreational fishing is a popular pastime but in depth studies on the accumulation of Hg in fish have yet to be conducted (GBRA, 2018).

1.6 Objectives

This study investigated the concentration of Hg in muscle tissue from a variety of trophically diverse fish species from five sites (3 reservoirs and 2 riverine) along the Guadalupe River in Texas.

The objectives for this study were:

1. Determine the relationship between Hg concentration and body length for each species with the prediction that Hg concentration will increase with increase in body length
2. Investigate whether the concentration of Hg in each species differs among the sites with the prediction that for each species, the Hg concentration will be greater in individuals collected from reservoir sites.
3. Identify which species and sites exceed the Texas health based standard of 0.7 μg/g wet weight with the prediction that species at the highest trophic level (e.g., longnose gar, channel catfish, and striped bass) are most likely to exceed the criterion, and for a given species this is most likely to occur in larger individuals and at reservoir sites.

ii. METHODS

2.1 Field sites

Originating in Kerr County where springs from the Edwards Aquifer form the North and South Forks (Reeves, 1969), the Guadalupe River flows to the east until the forks converge, and then flows southeast towards San Antonio Bay, which is connected to the Gulf of Mexico; the total length of the river is approximately 400 km (TPWD, 2018b). The river has one major reservoir (Canyon Lake), and six smaller reservoirs (Lake McQueeney, Lake Dunlap, Lake Placid, Lake Gonzales, Lake Wood, and Meadow Lake). The five sites chosen for this study (Fig. 1) are examples of the type of riverine and reservoir sites found along the Guadalupe River and allowed for sampling of trophically diverse fish. From upstream to downstream, the sites are as follows:

1. Flat Rock Lake: This manmade lake is located on the upper reaches of the Guadalupe River, below the confluence of the North and South Forks. Upstream of the Kerrville Lake Dam and downstream of one of the eight small, low water dams located between Kerrville and Comfort, Flat Rock Lake is approximately 2.4 km long. The city of Kerrville, which surrounds the lake, has a population of 23,434 (US Census Bureau, 2016a). The upper Guadalupe has vegetation and geological formations typical of the Edwards Plateau. Flat Rock Park allows for recreational swimming, kayaking, and fishing.
2. Canyon Lake: Located above Canyon Dam, Canyon Lake covers 33.3 km^2 with a maximum depth of 38 m. The Lake is undergoing increasing urban development and is used recreationally for fishing, swimming, boating and water sports. With

no true city, Canyon Lake census designated place (CDP) has a population of 21,262 (US Census Bureau, 2015).

3. Lake Dunlap: Located in New Braunfels, this lake is a narrow 6.4 km long body of water. Highly developed, it is open for recreational activities year round and is heavily utilized in summer months. Above the lake, the Comal River joins the Guadalupe River, adding substantially to the flow of the Guadalupe. New Braunfels has a population of 73,959 (US Census Bureau, 2016b).
4. Gonzales: Downstream from New Braunfels, the Guadalupe becomes a meandering, low gradient coastal river, devoid of the limestone bluffs characteristic of the upper reaches. The area surrounding the river is used for ranching, and agriculture. The city of Gonzales has a population of 7,660 (US Census Bureau, 2016c). The study site sampled was upstream from the Independence Park dam and therefore under it's influence.
5. Victoria: The city of Victoria has a population of 67,670 (US Census Bureau, 2016d). Located upstream of the river mouth, the river maintains its low gradient as it flows past the city. The area surrounding the river is used for manufacturing, ranching, and agriculture. Salinity increases downstream of the city due to its close proximity to San Antonio Bay, which is connected to the Gulf of Mexico.

2.2 Fish Collection

All fish analyzed in this study were collected between August 2016 and November 2017. Fishes were collected by boat-mounted high- (e.g., catfish) and low-voltage (e.g., sunfish, bass, and sucker fish) electrofishing, gill nets (e.g., gar) and seining (e.g., minnows). For fish caught by electrofishing, the pulse range was 60 pulses per second DC in the low voltage (50 - 500 volts) range and 30 pulses per second DC in the high voltage (50 - 1000 volts) range. Gill nets were 38.1 m x 2.4 m experimental monofilament nets with five 7.62 m panels of 2.54 cm, 3.81 cm, 5.08 cm, 6.35 cm, and 7.62 cm mesh. Seine nets were 4.57 m x 1.82 m with 0.476 cm mesh. All fish were collected under TPWD collection authorization # 165 and IACUC protocol # 201473646. Fish were sorted upon returning to the lab and stored at -20°C until processing. A list of species collected at each site and their corresponding sample size are shown in Table 1.

2.3 Sample processing and Hg analysis

Fish were thawed and the total length (TL) and wet weight recorded. An axial muscle sample was taken from the left side of each fish (or both sides in small fish) using a ceramic knife, the skin removed, and the wet weight recorded. The muscle was then desiccated in a drying oven at 60°C for 48 hours and dry weight was recorded. To allow for the conversion between dry weight and wet weight, the average percentage water content in muscle tissue for each species is shown in Table 1. All fish were analyzed individually with the exception of smaller minnow species; due to their small size, muscle from several fish of similar size had to be pooled together to obtain enough mass. Prior to Hg analysis, all muscle samples were ground into a fine powder using a pestle and Ziploc bags. All sample processing and subsequent Hg analysis followed a trace metal clean technique to avoid sample contamination.

The total Hg concentration in each muscle sample was determined using a Direct Mercury Analyzer (DMA-80; Milestone Inc. Shelton, CT), which uses thermal combustion, gold amalgamation, and atomic absorption spectrometry (U.S. EPA, 1998). Total Hg was measured in this study because it is a suitable proxy for the concentration of MeHg, since > 85% of total Hg is present as MeHg in fish muscle tissue (May et al., 1987; Bloom, 1992; Chumchal et al., 2011). Depending on the predicted Hg concentration for each species, between 15 and 50 mg of ground sample was weighed into a quartz boat and analyzed for Hg. All data was reported as µg/g dry weight.

The DMA-80 was calibrated at least every 4 weeks using three certified reference materials (CRM) from the National Research Council Canada (NRCC): MESS-4, marine sediment, certified Hg concentration = 0.08 ± 0.06 µg/g; TORT-3, lobster hepatopancreas, certified Hg concentration = 0.292 ± 0.022 µg/g; and PACS-3, marine sediment, certified Hg concentration = 2.98 ± 0.36 µg/g).

Boat blanks (empty quartz boat with no sample), duplicate samples, and CRMs or standard reference materials (SRM) [DORM-4 fish protein (NRCC) and ERM-CE464 tuna (European Reference Materials)] were included with every 15 samples for quality assurance/quality control (QA/QC). Blanks (n = 181) were below detection (<0.0000 µg/g) and the relative difference between duplicate samples (n = 213) was < 2%. The recovery (mean ± standard deviation) of the CRM/SRM was 97 ± 0.038% for DORM-4 (range = 89 - 105%; certified Hg concentration = 0.412 ± 0.036 µg/g; n = 146) and 99 ± 0.049% for ERM CE-464 (range = 90 - 110%; certified Hg concentration = 5.24 ± 0.10 µg/g; n = 34).

2.4 Statistical Analysis

All statistical analysis was carried out using Rstudio (Rstudio Inc., Boston, MA, USA), SigmaPlot 13 (Systat Software, Inc. San Jose, CA), and SPSS version 25 (IBM Corp., Armonk, NY). For each species with an $n \geq 10$ for at least 3 sites, in order to identify any significant ($p < 0.05$) differences in Hg concentration between sites, either an Analysis of Covariance [ANCOVA; total length (TL) as the covariate], analysis of variance (ANOVA), general linear model (GLM), or Kruskal-Wallis ANOVA on Ranks was run depending on if total length was a significant covariate and if the data passed the assumptions of constant variance, normality, and equal slopes. If the test was nonsignificant ($p > 0.05$), the Hg data for all sites for that species was combined for the subsequent linear regression analysis. 17 fishes were excluded from this analysis because of low sample size. Significance was set at $p < 0.05$ for all analyses.

Linear regressions were used to evaluate relationships between TL and Hg concentration. For species that did not meet assumptions of normality and constant variance, the Hg concentrations were natural log (ln) transformed prior to analysis. Following transformation, the majority of species then met assumptions; however, the test was still accepted if one assumption failed because the ANOVA is still robust if one assumption is violated (Glass et al, 1972). For species with a significant difference between sites, linear regression models were also run for each site individually.

Fishes spanned several feeding classifications (Table 2). For analysis, species were grouped into one of three categories: low trophic level, moderate trophic level, and high trophic level, based on published (Hendrickson and Cohen, 2015; Bean, 2012; Folb, 2010; Bean and Bonner, 2008; Simon, 1999; Gu et al., 1997; Hensley and Courtenay, 1980) and unpublished data (Timothy Bonner, personal communication). The low trophic level was defined as species with classifications of planktivore, herbivore, detritivore, and planktivore/detritivore; moderate trophic level included invertivore, invertivore/herbivore, and invertivore/detritivore; and the high trophic level included all species classified as carnivore and invertivore/carnivore. A Welch's ANOVA and Games-Howell post hoc test was used to determine whether there was a significant difference in Hg concentration between low, moderate, and high trophic positions when all of the five investigated sites were combined. The number of fishes that bioaccumulate over time in each trophic position were compared using and ANOVA and Tukey HSD. An ANOVA followed by a Dunn's pairwise comparison was used to determine whether there was a significant difference in the average Hg concentration in low, moderate, and high trophic position fishes between sites. At each site and ANOVA with Tukey HSD was used to determine the differences in Hg concentration at each trophic position within each site. Water content was used to convert the TDSHS and FDA Hg advisories from wet weight to dry weight for comparison to the present study (Appendix B).

iii. RESULTS

In total, 1,772 samples from 41 species were collected from the 5 sites (Table 1). The number of species collected averaged 23 among sites (range 20- 26); however, the number of individuals collected at each site varied 2.6-fold between the highest (Canyon Lake) and lowest site (Gonzales). Lake Dunlap had the largest number of investigated species (26 species, 334 individuals), followed by Canyon Lake (24 species, 569 individuals), Flat Rock Lake (24 species, 411 individuals), Gonzales (21 species, 216 individuals), and Victoria (20 species, 229 individuals).

Twenty-four of the investigated species had a sample size of $n \geq 10$. Of these 23 species, 16 species had no significant difference in Hg concentration in muscle tissue between sites. For 7 species (common carp, redear sunfish, longnose gar, blue catfish, flathead catfish, green sunfish and largemouth bass) there was no site difference when body length was included as the covariate (ANCOVA, $p > 0.05$); therefore fish at all sites were subsequently pooled together when investigating the relationship between body length and Hg concentration. For 4 species, body length overlapped at each site, therefore an ANOVA or Kruskal-Wallis was used to determine whether there was a site difference [gizzard shad, bullhead minnow, and redbreast sunfish (ANOVA; $p > 0.05$) and red shiner (Kruskal-Wallis; $p > 0.05$)] and the sites were also pooled together. For 5 species (Mexican tetra, bluegill sunfish, channel catfish, warmouth sunfish, and smallmouth bass), the n was much higher at one site in relation to the others (Appendix B); therefore all sites were also pooled together to investigate the Hg concentration and body length relationship.

For the 6 fishes that had differences in muscle tissue Hg concentration among sites [threadfin shad ($F = 5.399$, $p = 0.025$), blacktail shiner ($F = 17.326$, $p < 0.001$), smallmouth buffalo ($F= 29.6$, $p < 0.001$), gray redhorse ($F = 6.351$, $p < 0.001$), longear sunfish ($p < 0.001$), and spotted bass ($F (3,31) = 9.58$, $p < 0.001$)]. A general linear model with body length as a covariate was used to examine this relationship for spotted bass, for smallmouth buffalo and gray redhorse using an ANCOVA, for threadfin shad and blacktail shiner an ANOVA was used as body length was not a significant covariate, and a Kruskal-Wallis was used for longear sunfish. Site differences were not examined for the remaining 2 species (suckermouth catfish, and striped mullet) because they were only found at one site.

3.1 Relationship between Hg concentration and body length

For the 24 species that had a sample size ≥ 10, the relationship between body length and Hg concentration is shown in Fig 2 - 6 and the corresponding linear regression results in Table 3. For the 18 species that had no site difference or were only found at one site, 10 species (common carp, redbreast sunfish, bluegill sunfish, redear sunfish, blue catfish, channel catfish, flathead catfish, warmouth sunfish, smallmouth bass, and largemouth bass) had positive relationships between body length and Hg concentration, whereas 7 species (gizzard shad, suckermouth catfish, red shiner, bullhead minnow, Mexican tetra, longnose gar, and green sunfish) had no relationship. While striped mullet (Fig. 3) had an overall inverse relationship ($p < 0.05$), Hg concentration decreased with increase in body length up to 300 mm, after which the Hg concentration appeared to increase again.

For the species that had a site difference in Hg concentration, there was a positive relationship between body length and Hg concentration at all sites for smallmouth buffalo and spotted bass, and no consistent relationship at all sites for threadfin shad, blacktail shiner, gray redhorse, and longear sunfish (Fig. 2, 3, 4, and 6; Table 3). For the 16 species without site differences and 2 species that were only found at one site, the fishes that had a significant positive relationship between body length and Hg concentration increased with increase in trophic group from 0% in low trophic level species to 62.5% in moderate trophic level species and 85.7% in high trophic level species. This increase is significant between sites (ANOVA, $F = 397$, $df = 1272$, $p < 0.001$; Tukey HSD, $p < 0.001$ for all pairwise comparisons).

3.3 Interspecific differences in Hg accumulation

For all 5 sites combined, the average dry weight Hg concentration in low (0.128 ± 0.101 μg/g), moderate (0.414 ± 0.309 μg/g), and high (0.771 ± 0.666 μg/g) trophic levels is shown in Figure 7. Mercury concentration significantly increased with trophic level designation (ANOVA; Welch's statistic = 478.47, df=1004.02, $p < 0.05$) and differences existed among all trophic level comparisons (Games-Howell, $p < 0.05$).

Figure 8 shows the average Hg concentration at low, moderate, and high trophic positions at each site. On average, the Hg concentration in low trophic level fish was highest in Gonzales, and for moderate and high trophic level fish was highest at Canyon Lake. Within each trophic level, Kruskal-Wallis ANOVA on Ranks was run with a Dunn's post hoc test to investigate differences in Hg concentration among sites. At the low trophic level differences between sites were significant between Flat Rock Lake and

Lake Dunlap ($p < 0.001$), Gonzales and Lake Dunlap ($p < 0.001$), and Gonzales and Canyon Lake ($p < 0.001$). The moderate trophic level also had significant differences between sites, with Canyon Lake different from all other sites ($p < 0.001$) and Flat Rock Lake different from Lake Dunlap ($p < 0.05$). At the high trophic level, differences were found between Canyon Lake and all other sites ($p <0.05$). All sites had significant differences between trophic levels [Flat Rock Lake (Kruskal-wallis; $p < 0.001$), Canyon Lake (Kruskal-Wallis; $p < 0.001$), Lake Dunlap (ANOVA; $F = 161.5$, $p < 0.001$), Gonzales (Kruskal-Wallis; $p < 0.001$), Victoria (ANOVA; $F = 12.03$, $p < 0.001$)] and the trophic levels were significantly different from each other at all sites and levels except for Victoria where moderate and low trophic levels were not significantly different (Tukey's HSD; $p = 0.281$).

The highest individual dry weight Hg concentrations were found in longnose gar (4.474 µg/g, TL = 760 mm, Victoria), flathead catfish (5.08 µg/g, TL = 886 mm, Canyon Lake), and white bass (3.391 µg/g, 340 mm, Canyon Lake). The lowest individual Hg values were found in striped mullet (0.015 µg/g, 364 mm), threadfin shad (0.016 µg/g, 64 mm), and blue tilapia (0.035 µg/g, 156 mm) (Appendix B).

Among all species and among all sites the highest mean Hg concentration was found in striped bass (3.379 µg/g), white bass (2.714 µg/g), and longnose gar (1.274 µg/g), all of which are classified as high trophic level species. The lowest mean Hg concentration was found in Mexican tetra (0.04 µg/g), threadfin shad (0.063 µg/g), and suckermouth catfish (0.107 µg/g). Both threadfin shad and suckermouth catfish are lower trophic level species, however the Mexican tetra, as an invertivore, was classified in the moderate trophic level.

For game fishes with a low sample size, the average Hg concentration at each site is shown in Figure 9. The Hg concentration was lowest in Guadalupe bass (0.541 µg/g at Flat Rock Lake) and highest in striped bass (3.38 µg/g at Lake Dunlap). Additionally, for all fishes the Hg concentration increased with distance downstream; all fishes were sampled from reservoirs with Lake Dunlap having the highest concentrations, followed by Canyon Lake and Flat Rock Lake.

3.4 Species exceeding state and federal Hg advisory levels

The species that exceeded the TDSHS and FDA Hg advisory levels and the corresponding body lengths at which they start to exceed these levels are shown in Table 4. Individuals from four high trophic level species exceeded the TDSHS advisory of 0.7 ppm wet weight: white bass (Canyon Lake), striped bass (Lake Dunlap), longnose gar (Canyon Lake, Victoria), and flathead catfish (Canyon Lake). Canyon Lake had the largest number of species that exceeded the advisory level. For each species, there was a minimum total length at which individuals begin to exceed the criterion. Longnose gar from Victoria, flathead catfish from Canyon Lake, and striped bass from Lake Dunlap also had individuals that exceeded the FDA Hg advisory of 1.0 µg/g wet weight.

iv. DISCUSSION

This study investigated the concentration of Hg in trophically diverse fishes from five sites within the Guadalupe River basin. The majority of species examined displayed a significant positive relationship between body length and Hg concentration at at least one site, indicating that Hg is bioaccumulating over time in these species. Mercury concentration biomagnified with trophic level; fish designated in the high trophic level had a higher Hg concentration than those in the moderate and low trophic levels. Of the 24 investigated species, the Hg concentration in muscle tissue was influenced by site in only 25% of the species. Overall, fishes from reservoirs generally had higher concentrations than those from riverine sites and of these, Canyon Lake had the highest Hg values. Individuals from four species exceeded the TDSHS advisory level of 0.07 μg/g wet weight and two species exceeded the FDA 1 μg/g wet weight advisory level at at least one site.

4.1 Relationship between body length and Hg concentration

The bioaccumulation of Hg occurs as the result of a balance between three mechanisms: uptake rate from the diet, uptake rate from the surrounding water, and the physiological turnover rate following exposure (Luoma and Rainbow, 2005). Previous studies have shown Hg bioaccumulation over time in freshwater fish (Chumchal et al., 2009; Gorski et al., 2003; Mason et al., 2000) as a result of the uptake rate of Hg from diet and surrounding water into the body being much higher than the loss rate (Dutton and Fisher, 2010; Pickhardt et al., 2006). Seventy-five percent of species in the current study supported this finding, showing bioaccumulation of Hg over time.

Additionally, as fish age an ontogenetic switchover in diet can occur, often within the first year of life, as body growth allows for the consumption of increasingly larger prey (Mittelbach and Persson, 1998). The effect of one such ontogenetic switch can be seen in smallmouth bass, which switches to piscivory between 40 - 100 mm in total length (Scott and Crossman, 1979; Carlander, 1977); in the present study, samples < 150 mm in length have an average Hg concentration of 0.243 μg/g (SD = 0.123) across all sites combined, whereas all samples > 200 mm average 1.5 μg/g (SD = 0.657) across all sites. The dietary switch to piscivory increases the average Hg level 6.17-fold in this species.

Striped mullet was the only species with an inverse relationship between body length and Hg concentration. Striped mullet experiences an ontogenetic switch of diet, from primarily carnivorous as a juvenile < 30 mm in length (consuming plankton, microcrustaceans, shrimp larvae, and zooplankton; Eggold and Motta, 1992; Blaber and Whitfield, 1977) to a diet of detritus, diatoms, sand grains, crustaceans, algae, and decomposed organic matter as adults > 30 mm standard length (Das and Chowdhury, 1983; Hiatt, 1944). They also may experience an ontogenetic habitat shift in conjunction with their dietary shift, as juveniles spawned in the Gulf of Mexico may migrate into riverine habitats (Ibáñez and Benítez, 2004). These ontogenetic shifts are likely to correspond to high Hg accumulation for a juvenile fish; these relatively high Hg levels then decrease swiftly with growth dilution as the rate of growth increases faster than the rate of Hg dietary uptake (Ward et al., 2010; Simoneau et al., 2005). Salinity differences between the Gulf and riverine sites may affect the uptake of Hg from the aqueous phase, as has previously been shown in killifish (*Fundulus heteroclitus*) (Dutton and Fisher, 2011). Striped mullet reach maturity at approximately 3 years of age, with corresponding lengths of 200-300 mm and it is at this point that growth slows and bioaccumulation of Hg can be seen in the positive relationship between Hg and total length (Gonzáles Castro et al., 2009; Jacot, 1920). Previous studies in northwest Mexico and southwest Taiwan support low Hg values in adult mullet (0.129 μg/g dry wt with a total length of 240-270 mm, Ruelas-Inzunza et al 2017; <0.0125 μg/g dry wt with a total length of 322 ± 180 mm, Chen et al., 2004) however, to my knowledge, this is the first study to evaluate Hg in striped mullet < 200 mm in length. An inverse relationship between total length and Hg concentration has been shown in juveniles of other species including Atlantic salmon (*Salmo salar*), Atlantic herring (*Clupea harengus harengus*) and freshwater tilapia (*Oreochromis niloticus*) (Wang and Wang, 2012; Ward et al., 2010; Braune, 1987)

Of the 24 fishes found at one site or with no site differences, 38.8% did not exhibit a significant relationship between total length and Hg concentration which can be attributed to several factors. Limited sample size, like that of suckermouth catfish (n = 16) can cause difficulty in estimating a relationship. Heavily weighted sample sizes, either by size or site, can also impact regressions, such as in longnose gar where > 50% of samples fell within a 200 mm size range (700-900 mm total length) or Mexican tetra where the sample size is 14 at Victoria and 2 at Flat Rock Lake.

The percentage of fishes in this study with a positive relationship between Hg concentration and body length increased with increase in trophic position, from 0% for the low trophic level, to 62.5% for the moderate trophic level, and 85.7% for the high trophic level; however, the reason for this observation is not clear. Gizzard shad, for example, are a low trophic level species that lives for 7 to 10 years; this planktivorous species feeds on prey low in Hg and the percentage of total Hg in the prey that is comprised of MeHg is < 25% (Almajed Butayban and Preston, 2006) – even though the prey's Hg concentration is low, a positive relationship between the muscle Hg concentration and body length would still be expected due to the long life span.

4.2 Site differences

For the six species with a difference in Hg concentration between sites, Canyon Lake had consistently higher Hg levels than all other sites while Lake Dunlap and Flat Rock Lake were more varied in Hg concentration in comparison to riverine sites. Overall, reservoir sites had higher concentrations than riverine sites, with Flat Rock Lake and Canyon Lake having consistently higher concentrations than Lake Dunlap, which had similar Hg levels to that of Gonzales and Victoria. Though Lake Dunlap is a dammed reservoir, its lower residency time compared to Canyon Lake and Flat Rock Lake, as well as the input of the Comal River may contribute to its lower overall Hg levels. These findings therefore support previous studies that indicate fish from reservoirs should have higher Hg concentrations than those from riverine sites (Willacker et al., 2016; Abernathy and Crombie, 1977). This may be due to the higher methylation rate in reservoirs, which can be attributed to the stronger presence of sulfate reducing bacteria in anoxic waters and sediments, generally found in lacustrine environments (Lou et al., 2017; Gilmour et al., 1992; Gilmour and Henry, 1991).

Additionally Canyon Lake is a stratified lake while Lake Dunlap and Flatrock Lake are not, and temperature may have an effect on the depuration rate of MeHg as well as a stimulating effect on methylating bacteria in warmer environments (although this relationship varies substantially among systems; Plourde et al., 1997; Trudel and Rasmussen, 1997; Bodaly et al., 1993; Park et al., 1989). Canyon Lake is also defined as a eutrophic reservoir by the Texas Commission on Environmental Quality (TCEQ), a condition that is linked to an enrichment of MeHg compared to less anoxic sites (TCEQ, 2011; He et al., 2008; Ullrich et al., 2001). However, the location of the reservoir sites (upstream) and riverine sites (downstream) provides another gradient that may be impacting mercury uptake among sites. For example, pH tends to be higher upstream in the Edwards Plateau (with a range of 7.46 - 8.05 over the whole river), which would lead to a decrease in Hg uptake (GBRA, 2018; Jiann et al., 2013; Driscoll et al., 1995). Within the two riverine sites, Victoria had higher Hg concentrations than Gonzales (e.g., smallmouth buffalo: Gonzales = 0.550 ± 0.369 µg/g, Victoria = 0.967 ± 0.780 µg/g; spotted bass: Gonzales = 0.508 ± 0.165 µg/g, Victoria = 0.638 ± 0.239 µg/g; mean ± 1 SD). It has previously been shown that fish from downstream riverine sites have higher Hg concentrations than those from upstream sites; a phenomenon linked to downstream transport and increased Hg bioavailability (Carrasco et al., 2011).

The variability of Hg levels between sites may be due to the impacts of ecological parameters such as the dissolved organic carbon (DOC) concentration in the water, pH of the water, and ecoregion, which have been shown to impact Hg bioavailability at different sites (Drenner et al., 2011; Smith et al., 2010; Mason et al., 2000). As the concentration of DOC in the water increases, the uptake of Hg into fish decreases as the Hg has a stronger binding efficiency for DOC than the gills (Dutton and Fisher, 2012). Autochthonous dissolved organic matter (DOM) has recently been linked to the productivity of sulfate reducing bacteria, with increased methylation stimulated by increase in DOM (Jiang et al., 2018). The environmental differences in ecoregion may also impact Hg levels in fish and higher Hg accumulation is linked to forested wetland environments when compared to open water (Chumchal et al., 2009; Drenner et al., 2011). As the five investigated sites in this study span 4 different ecoregions (Fig 10), environmental parameter shifts between ecoregions may account for some site differences and warrant further study.

4.3 Differences among species

It is well known that the concentration of Hg in muscle tissue is higher in fish from higher trophic levels, and the likelihood of Hg biomagnification is inversely related to dietary exposure concentration, particularly at low exposure concentrations (De Forest et al., 2007). Our findings support this, with larger, piscivorous species showing the highest Hg concentrations at all sites, suggesting a trophic transfer factor > 1, making biomagnification more likely for these species. These findings are supported by many studies that have shown that trophic position is the best predictor of Hg concentration as Hg biomagnifies up the aquatic food chain (LaVoie et al., 2010; Chumchal et al., 2011; 2010; Chumchal and Hambright, 2009). Fish in low feeding guilds (and correspondingly low trophic levels) ingest less MeHg in their diet, leading to low Hg levels overall. Gizzard shad, for example, is omnivorous, primarily digesting detritus with the addition of zooplankton when available (Yako et al., 1996). Total Hg in zooplankton ranges from 0.004 - 0.035 μg/g however MeHg represents < 25% of the total Hg at this low trophic level (Al-Majed Butayban and Preston, 2000) and the other 75% is made up of primarily Hg(II), which has an assimilation efficiency (AE) of ≤ 10% in its predators, with some exceptions such as the western mosquitofish which has reached a recorded AE of 51% (Wang et al., 2010; Pickhardt et al., 2006; Riisgård and Hansen, 1990). Thus, very little MeHg is available for uptake from zooplankton, leading to low Hg concentrations in species that feed at a similar trophic level to gizzard shad (Lescord et al., 2018). Additionally, the MeHg assimilation efficiency (AE; 94.4 - 97.1%) in freshwater fish is not impacted by diet whereas other species of mercury such as Hg(II) have diet specific AEs that vary by up to 3.5 times (8.6 - 28.7%) among diets (Wang and Wang, 2017).

Mercury concentration also varied between sites within closely related fish. Thirteen species were sampled from Centrarchidae family, and although the majority of species were only found in reservoir sites, some fishes like longear sunfish and bluegill sunfish were found in high numbers at all sites. The lowest mean Hg concentration within the Centrarchidae family was found in redspotted sunfish at Lake Dunlap (0.476 μg/g) and the highest mean Hg concentration was found in smallmouth bass from Canyon Lake (1.525 μg/g), representing a 3.2-fold increase in Hg concentration. Because trophic ecology and family are highly related, although fishes within a family may vary in Hg concentration, they generally have more closely comparable values to each other than with those in other families. Within species, the largest increase in Hg between sites was found in longear sunfish, which increased 4.6-fold between Lake Dunlap (1.5 μg/g) and Canyon Lake (0.686 μg/g). Largemouth bass had the smallest difference between sites, only increasing 1.3-fold between Flat Rock Lake (0.538 μg/g) and Canyon Lake (0.68 μg/g). Total length variability between sampled sites contributes these differences, as percent difference in mean TL was much higher in longear sunfish (37% between Canyon Lake and Lake Dunlap) than largemouth bass (3% between Flat Rock Lake and Canyon Lake). Differences in bioavailable Hg at the base of the food chain between sites may also contribute to higher Hg levels in fish from some sites over others.

Mercury concentrations found in this study were lower or comparable to previous Hg levels found in the same species from Northeast Texas and comparable to those from Canyon Lake and Southwest Texas (Table 5). The majority of species studied in both the northeast region and the present study had comparable Hg values with only a few species showing differences between regions. None of the compared species had significantly higher Hg concentrations in the present study than those from North Texas, and those that were lower in Hg concentration were generally moderate and high trophic level species (e.g. redear sunfish and largemouth bass). In redear sunfish, these differences are likely due to differences in size range investigated (present study: 81-131mm; Chumchal et al., 2010: 178-193 mm), however in largemouth bass there is far greater overlap in size range (present study: 46-477mm; Chumchal et al., 2010; Drenner et al., 2011: 260-460mm), suggesting that other factors such as higher Hg deposition, methylation rate, bass feeding at different sites, and water quality in the Northeast portion of the state may be affecting the Hg concentration of largemouth bass.

4.4 Species exceeding state and federal advisory levels

All investigated sites are fished recreationally, with the highest frequency at reservoir sites; however, the Hg concentration in muscle tissue was higher at the reservoir sites (TPWD, 2018b). Given that the only data available on Hg in the Guadalupe River is limited to Canyon Lake and was conducted over 10 years ago (Ward et al., 2006), this study provided valuable additions to knowledge of Hg within the Guadalupe River, with Hg concentrations in fish species from multiple sites on the river, both reservoir and riverine. This creates an opportunity to reexamine current advisories using the most current data. Our results support the current Hg advisory for longnose gar in Canyon Lake and suggested that white bass and flathead catfish may also need an advisory issued. However, there is currently a Hg advisory for striped bass in Canyon Lake and our sampling concluded that they did not exceed the State of Texas 0.7 µg/g human health based standard. Furthermore, this study suggests that an advisory may need to be issued for longnose gar of any size in Victoria and possibly for striped bass in Lake Dunlap. However, it should be noted that prior to issuing advisories for flathead catfish, white bass, and striped bass, more fish should be analyzed due to the low sample size in this study; the current advisory for longnose gar in Canyon Lake is based on 2 samples.

White bass had a higher average Hg concentration at Canyon Lake in the present study (0.654 ± 0.08 µg/g wet wt) compared to Ward et al., 2006 (0.316 µg/g wet wt), whereas striped bass (present study: 0.634 ± 0.039 µg/g wet wt) was much lower in concentration than that of Ward et al. (1.149 wet wt), despite having similar size ranges [white: 306-340 mm compared to 346 mm, striped: 544 - 564 mm compared to 541 -

604 mm (Ward et al., 2006)]. A larger sample size in future studies will help identify if this is linked to an overall increase in Hg level for these species over time or may be attributed to variation caused by a low sample size.

These results further indicate that higher trophic level, larger fish are more likely to have Hg concentrations above the TDSHS limit. The lengths at which longnose gar began to exceed the TDSHS advisory varied between sites (Canyon Lake = 732 mm, Victoria = 760 mm). If each species that exceeded the 0.7 μg/g wet weight criterion must to reach a minimum body size before Hg concentrations begin to exceed the limit, smaller fish of this species may still be acceptable for human consumption This suggests that although piscivores from different species of similar length also tend to consume prey of similar size, if not species (Mittelbach and Persson, 1998), an upper catch limit based on body size may be a valuable consideration for future advisories at each site of concern rather than a species-wide ban. Harvest slot length limits, which restrict harvest to intermediate lengths is linked to a greater number of fish harvested while retaining efficient reproductive biomass (Gwinn et al., 2015). For stocked fish like striped bass, this is less likely to cause an issue for the population as a whole but for native populations, the long-term impacts should be considered prior to any addition of a maximum catch limit. Additionally, while not fished commercially, two species (channel catfish and longnose gar) at Canyon Lake and Victoria also exceeded the FDA action limit of 1.0 μg/g wet weight, indicating very high Hg levels in these fish.

4.5 Conclusions

This is the first in-depth study on the concentration of Hg in a wide range of trophically diverse fish collected from multiple sites in the Guadalupe River and will significantly increase the understanding of Hg concentrations in fish in South Central Texas. Ongoing bioaccumulation of Hg was found in the majority of the investigated species, and species in higher trophic levels were more likely to show a significant positive relationship between body length and Hg concentration. Hg concentration significantly varied between sites for six species and there was a trend of higher Hg levels in reservoir sites, particularly in Canyon Lake. Four species (longnose gar, flathead catfish, white bass, striped bass) exceeded the TDSHS advisory of 0.7 μg/g in some way and three of the five sites had at least one species that exceeded the criterion. This study concludes that Canyon Lake still needs to have Hg advisories regarding fish consumption and an advisory may possibly be needed for Lake Dunlap and Victoria.

The findings of this study will be of interest to recreational fishermen, state agencies (e.g., Texas Commission on Environmental Quality, TPWD), doctors, other scientists, environmental groups and the TDSHS, who issue Hg advisories. The survey will additionally provide sufficient data to allow reevaluation of advisories for Canyon Lake and potential creation of advisories at other sites along the Guadalupe River.

4.5 Future directions

Future studies should increase the sample size of species that were sampled in low number in this study, especially those that were found to exceed the TDSHS advisory of 0.7 μg/g wet weight. This will aid in stronger statistical analysis and further elucidate total lengths at which these species begin to exceed the limit for future advisory evaluation.

Previous studies have shown that environmental parameters can change between ecoregion, which could therefore impact Hg uptake in fish. As the Guadalupe River passes through four ecoregions (Edwards Plateau, Texas Blackland Prairies, East Central Texas Plains, Western Gulf Coastal Plain), a long-term evaluation of the environmental parameter differences between sites (e.g. riparian vegetation, dissolved organic carbon concentration in the water, pH of the water) may further explain bioaccumulation patterns among sites. A useful addition to this study would be the inclusion of an in-depth evaluation of water chemistry, testing water quality at each of the sites as well as baseline Hg values in the water column over two years in order to determine how water chemistry varies and how it influences Hg uptake in the aquatic food web.

Previous studies have shown that selenium (Se) has an antagonistic relationship with Hg and if present in molar excess it has been proposed that Se may have a protective role against Hg toxicity (Raymond and Ralston, 2009; Kaneko and Ralston, 2007). Selenium preferentially binds to Hg in fish muscle tissue, thereby moderating Hg toxicity in consumed fish. Current Hg advisories are based on the Hg concentration in muscle tissue alone, however, studies are now supporting the use of Se:Hg in risk assessment. The Se:Hg ratio at which Se begins to have a protective effect from Hg is still being investigated and may differ between species therefore an examination of Se:Hg ratios for these species, particularly in commonly consumed fish that are a concern for human health, would help evaluate advisory viability and decision making for consumers.

The use of stable isotope analysis would allow for quantitative comparison between and amongst trophic levels at different sites. $\delta^{13}C$ and $\delta^{15}N$ can determine feeding ecology and trophic level respectively for each species and site individually. Using $\delta^{15}N$ values to determine trophic level for each site makes it possible to compare Hg concentration within a trophic level across sites. Predictions for which trophic levels are most likely to exceed TSHS advisories can be made based on current data. Additionally analyzing the Hg concentration relative to $\delta^{15}N$ values can determine a biomagnification factor for fish within the same trophic level. This is the focus of Chapter 2.

Table 1. Sampling locations for each investigated species (separated by family) with corresponding sample size (n; total for all five sites) and percentage water content in muscle tissue (mean ± 1 standard deviation). F = Flat Rock Lake, C = Canyon Lake, D = Lake Dunlap, G = Gonzales, V = Victoria.

Family	**Species**	**Common Name**	**n**	**F**	**C**	**D**	**G**	**V**	**% water**
Lepisosteidae	*Lepisosteus oculatus*	Spotted Gar	5				x	x	79 ± 2
	Lepisosteus osseus	Longnose Gar	90	x	x	x	x	x	76 ± 2
Clupeidae	*Dorosoma cepedianum*	Gizzard Shad	231	x	x	x	x	x	78 ± 6
	Dorosoma petenense	Threadfin Shad	43		x	x			79 ± 1
Cyprinidae	*Cyprinella lutrensis*	Red Shiner	31				x	x	78 ± 1
	Cyprinella venusta	Blacktail Shiner	48	x	x	x	x		76 ± 3
	Cyprinus carpio	Common Carp	49	x	x	x	x	x	77 ± 3
	Notropis volucellus	Mimic Shiner	4			x			77 ± 1
	Opsopoeodus emiliae	Pugnose Minnow	1			x			76
	Pimephales vigilax	Bullhead Minnow	33	x	x	x	x	x	77 ± 2
Catostomidae	*Carpiodes carpio*	River Carpsucker	1				x		80
	Ictiobus bubalus	Smallmouth Buffalo	44				x	x	78 ± 5
	Moxostoma congestum	Gray Redhorse	74	x	x	x	x		79 ± 2
Characidae	*Astyanax mexicanus*	Mexican Tetra	16	x				x	78 ± 1
Ictaluridae	*Ameiurus natalis*	Yellow Bullhead	4	x					79 ± 1
	Ictalurus furcatus	Blue Catfish	30		x		x	x	81 ± 1
	Ictalurus punctatus	Channel Catfish	98	x	x	x	x	x	81 ± 1
	Pylodictis olivaris	Flathead Catfish	23	x	x	x	x	x	80 ± 2

Table 1. Continued

Family	Species	Common Name	n	F	C	D	G	V	% water
Mugilidae	*Mugil cephalus*	Striped Mullet	17					x	77 ± 1
Loricariidae	*Hypostomus plecostomus*	Suckermouth Catfish	16			x			78 ± 1
Poeciliidae	*Gambusia affinis*	Western Mosquitofish	1		x				82
Moronidae	*Morone chrysops*	White Bass	5	x	x	x			78 ± 1
	Morone saxatilis	Striped Bass	3	x	x				80 ± 1
Centrarchidae	*Lepomis auritus*	Redbreast Sunfish	293	x	x	x			80 ± 1
	Lepomis cyanellus	Green Sunfish	30	x	x	x	x	x	81 ± 1
	Lepomis gulosus	Warmouth Sunfish	26	x	x	x		x	81 ± 1
	Lepomis macrochirus	Bluegill Sunfish	179	x	x	x	x	x	80 ± 1
	Lepomis megalotis	Longear Sunfish	77	x	x	x	x	x	80 ± 2
	Lepomis microlophus	Redear Sunfish	53	x	x	x		x	79 ± 1
	Lepomis miniatus	Redspotted Sunfish	5	x		x			82 ± 2
	Micropterus dolomieu	Smallmouth Bass	4		x	x			78 ± 1
	Micropterus punctulatus	Spotted Bass	35				x	x	79 ± 1
	Micropterus salmoides	Largemouth Bass	140	x	x	x			79 ± 2
	Micropterus treculii	Guadalupe Bass	3	x					80 ± 0.5
	Pomoxis annularis	White Crappie	1					x	80
	Etheostoma lepidum	Greenthroat Darter	1	x					79
Percidae	*Percina carbonaria*	Texas Logperch	3	x	x	x			82 ± 6
	Percina macrolepida	Bigscale Logperch	6	x	x				76 ± 1

Table 1. Continued

Family	Species	Common Name	n	F	C	D	G	V	% water
Sciaenidae	*Aplodinotus grunniens*	Freshwater Drum	5				x	x	81 ± 5
Cichlidae	*Herichthys cyanoguttatus*	Rio Grande Cichlid	10	x	x		x	x	80 ± 1
	Oreochromis aureus	Blue Tilapia	5		x	x			79 ± 1

Table 2. Feeding guild allocation for each investigated species. Detrit. = detritovore, Plank. = plankivore, Herb. = herbivore, Invert. = invertivore, Carn. = carnivore.

Family	**Species**	**Detrit.**	**Plank.**	**Herb.**	**Invert.**	**Carn.**
Lepisosteidae	Spotted Gar					x
	Longnose Gar					x
Clupeidae	Gizzard Shad			x		
	Threadfin Shad		x			
Cyprinidae	Red Shiner			x	x	
	Blacktail Shiner				x	
	Common carp	x			x	
	Mimic Shiner			x	x	
	Pugnose Minnow	x				
	Bullhead Minnow			x	x	
Catostomidae	River Carpsucker	x	x			
	Smallmouth Buffalo			x	x	
	Gray Redhorse				x	
Characidae	Mexican Tetra				x	
Ictaluridae	Yellow Bullhead				x	x
	Blue Catfish				x	x
	Channel Catfish				x	x
	Flathead Catfish				x	x
Mugilidae	Striped Mullet	x			x	
Loricariidae	Suckermouth Catfish	x	x			
Poeciliidae	Western Mosquitofish				x	
Moronidae	White Bass				x	x
	Striped Bass				x	x
Centrarchidae	Redbreast Sunfish				x	
	Green Sunfish				x	x
	Warmouth Sunfish				x	x
	Bluegill Sunfish				x	
	Longear Sunfish				x	
	Redear Sunfish				x	
	Redspotted Sunfish				x	
	Smallmouth Bass				x	x

Table 2. Continued

Family	**Species**	**Detrit.**	**Plank.**	**Herb.**	**Invert.**	**Carn.**
	Spotted Bass				x	x
	Largemouth Bass				x	x
	Guadalupe Bass				x	x
	White Crappie				x	x
	Greenthroat Darter				x	
Percidae	Texas Logperch				x	
	Bigscale Logperch				x	
Sciaenidae	Freshwater Drum				x	x
Cichlidae	Rio Grande Cichlid	x			x	
	Blue Tilapia	x	x			

Table 3. Linear regression results describing the relationship between Hg concentration in muscle tissue and total length for species shown in Figures 2 – 6.

Common Name	Equation	DF	F	r^2	p
Low Trophic Position					
Gizzard Shad	Hg = 0.149 - (3.11E-05*TL)	236	0.171	7.27E-04	0.68
Threadfin Shad					
Canyon Lake	Hg = -0.697 + (0.018*TL)	10	7.32	0.449	0.024
Lake Dunlap	ln(Hg) = -3.38 - (0.007*TL)	31	1.41	0.045	0.244
Suckermouth Catfish	Hg = 0.062 + (8.32E-05*TL)	15	0.078	0.005	0.783
Moderate Trophic Position					
Red Shiner	Hg = 0.427 + (1.86 E-03*TL)	30	0.542	0.018	0.467
Common Carp	ln(Hg) = -2.62 + (3.8 E-03*TL)	48	23.4	0.333	< 0.001
Blacktail Shiner					
Flat Rock Lake	ln(Hg) = -2.71 + (0.022*TL)	20	20.8	0.524	< 0.001
Canyon Lake	Hg = 0.491 + (0.001*TL)	4	0.063	0.020	0.817
Lake Dunlap	Hg = -0.101 + (0.005*TL)	19	6.15	0.255	0.023
Bullhead Minnow	Hg = 0.223 - (1.84 E-03*TL)	31	0.883	0.028	0.355
Smallmouth Buffalo	ln(Hg) = -3.00 + (5.83 E-03*TL)	43	29.4	0.412	< 0.001
Gonzales	Hg = -0.529 + (0.002*TL)	21	15.1	0.432	< 0.001
Victoria	ln(Hg) = -3.33 + (0.006*TL)	21	14.1	0.415	0.001
Gray Redhorse	ln(Hg) = -2.89 + (5.93 E-03*TL)	73	59.7	0.454	< 0.001
Flat Rock Lake	Hg = -0.268 + (0.002*TL)	31	13.2	0.307	0.001
Canyon Lake	Hg = -1.00 + (0.004*TL)	17	1.31	0.075	0.269
Lake Dunlap	Hg = -1.80 + (0.007*TL)	14	36.9	0.740	< 0.001
Gonzales	ln(Hg) = -2.00 + (0.002*TL)	8	0.480	0.064	0.511
Mexican Tetra	ln(Hg) = -2.66 - (5.75 E-04*TL)	15	0.235	0.016	0.635
Striped Mullet	Hg = 0.069 - (1.09 E-04*TL)	16	4.79	0.242	0.045
Redbreast Sunfish	Hg = 0.129 + (0.002*TL)	292	32.9	0.102	< 0.001
Bluegill Sunfish	ln(Hg) = -1.91 + (9.49 E-03*TL)	178	36.2	0.170	< 0.001
Longear Sunfish	ln(Hg) = -1.94 + (8.40 E-03*TL)	76	11.4	0.132	0.001
Flat Rock Lake	ln(Hg) = -0.973 - (0.002*TL)	11	0.169	0.016	0.690
Canyon Lake	ln(Hg) = -0.313 - (0.001*TL)	13	0.126	0.010	0.729
Lake Dunlap	Hg = -0.061 + (0.002*TL)	6	26.8	0.843	0.004
Gonzales	ln(Hg) = -1.68 + (0.002*TL)	28	1.06	0.038	0.311
Victoria	ln(Hg) = -2.37 + (0.014*TL)	14	14.3	0.524	0.002
Redear Sunfish	ln(Hg) = -1.77 + (4.41 E-03*TL)	53	9.19	0.150	0.004
High Trophic Position					
Longnose Gar	ln(Hg) = 0.040 - (7.39 E-06*TL)	89	2.72E-04	3.09E-06	0.987
Blue Catfish	Hg = -0.534 +(2.22 E-3*TL)	29	28.3	0.503	< 0.001
Channel Catfish	ln(Hg) = -1.28 + (1.71 E-03*TL)	97	2.87E+01	0.231	< 0.001
Flathead Catfish	ln(Hg) = -1.47 + (2.86 E-03*TL)	22	68.9	0.767	< 0.001
Green Sunfish	ln(Hg) = -1.02 + (2.48 E-03*TL)	25	0.731	0.029	0.401
Warmouth Sunfish	ln(Hg) = -1.35 + (5.01 E-03*TL)	30	11.6	0.287	< 0.001
Smallmouth Bass	Hg = -0.318 + (6.06 E-03*TL)	14	41.1	0.76	< 0.001

Table 3. Continued.

Common Name	**Equation**	**DF**	**F**	r^2	**p**
Spotted Bass	Hg = 0.15 + (2.7 E-03*TL)	34	15.1	0.315	< 0.001
Gonzales	Hg = 0.214 + (0.002*TL)	23	11.0	0.334	0.003
Victoria	Hg = -0.577 + (0.008*TL)	10	16.2	0.644	0.003
Largemouth Bass	ln(Hg) = -1.12 + (3.35 E-03*TL)	139	89.5	0.393	< 0.001

Table 4. Percentage of individuals that exceeded the TDSHS Hg advisory of 0.7 µg/g wet wt and the FDA Hg advisory of 1.0 µg/g wet wt, with the corresponding total length (TL) at which the Hg concentration began to exceed these advisory limits.

Species	Common name	Site	n	State % Exceed	TL (mm)	FDA % Exceed	TL (mm)
Lepisosteus osseus	Longnose Gar	Canyon Lake	21	19	732	0	-
		Victoria	39	2.5	760	2.5	760
Pylodictis olivaris	Flathead Catfish	Canyon Lake	12	8.3	886	8.3	886
Morone chrysops	White Bass	Canyon Lake	3	33	340	0	-
Morone saxatilis	Striped Bass	Lake Dunlap	1	100	482	100	482

Table 5. Comparison in Hg concentrations in the present study to literature values from Northeast and South Texas. All Hg values are presented as minimum and maximum concentration recorded from all studies in the region and as μg/g wet wt.

Species	**Current Study**	**South Texas**	**North Texas**	**Rio Grande/ Pecos**	**Source**
Spotted gar	0.102 - 0.380	-	0.474 - 0.833	-	E, G
Longnose gar	0.066 - 1.075	0.749-0.795	-	-	F
Gizzard shad	0.009 - 0.197	-	0.0264-0.120	-	D
Threadfin shad	0.003 - 0.147	-	0.0404	-	G
Common carp	0.025 - 0.505	-	0.3	-	G
Rivercarp sucker	0.108	-	0.251	-	G
Blue catfish	0.020 - 0.300	0.199-0.321	-	-	F, G
Channel catfish	0.021 - 0.576	-	0.075 - 0.139	0.309	E
Flathead catfish	0.080 - 1.016	0.167-0.564	0.150 - 1.065	-	A, B, C, E, F
White bass	0.334 - 0.746	0.316	0.100 - 0.554	-	E
Striped bass	0.606 - 0.759	0.955-1.418		-	E, F, G
Bluegill sunfish	0.018 - 0.297	-	0.0814 - 0.18	-	E
Redear sunfish	0.001 - 0.176	-	0.127 - 0.234	-	D
Largemouth bass	0.019 - 0.455	0.188-0.844	0.193 - 1.30	0.15 - 1.2	F
Freshwater drum	0.114 - 0.391	-	0.319 - 0.6	-	E

References: A = Drenner et al., 2013; B = Becker et al., 2011; C = Drenner et al., 2011; D = Chumchal et al., 2010; E = Chumchal and Hambright, 2009; F = Ward et al., 2006; G = McClain et al., 2006

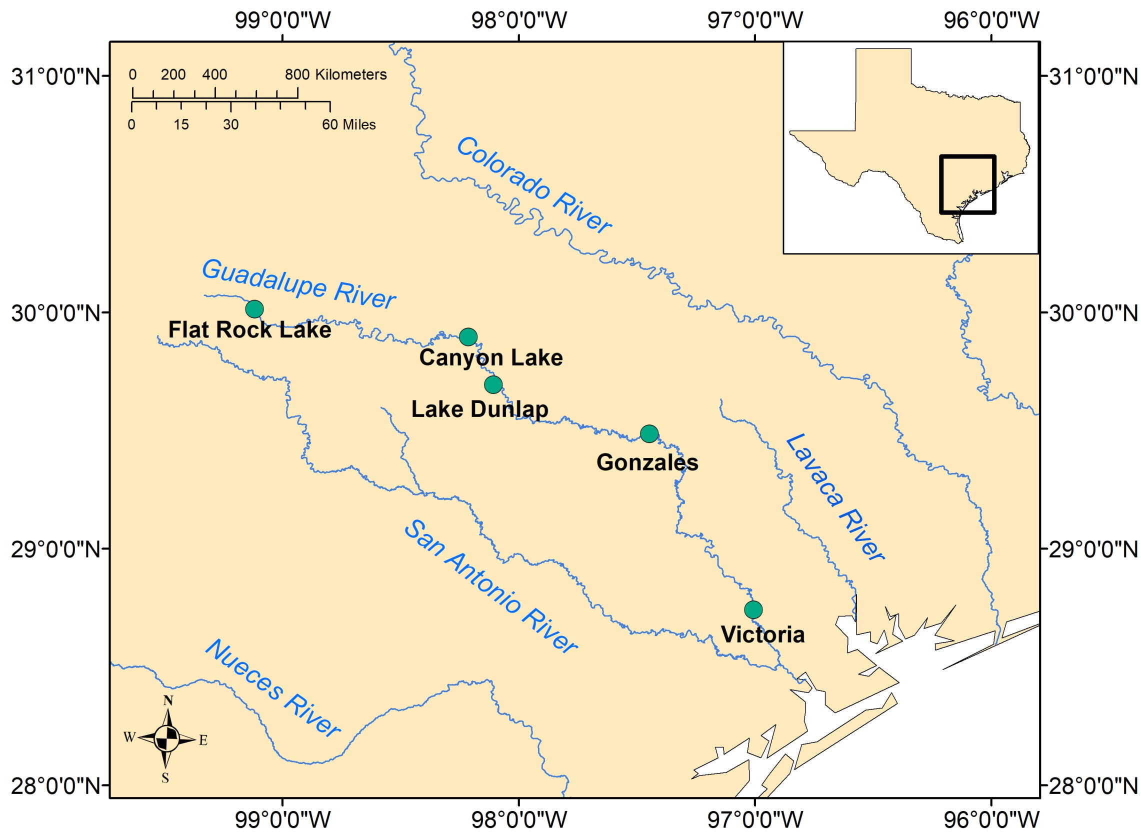

Figure 1. Fish collection sites on the Guadalupe River.

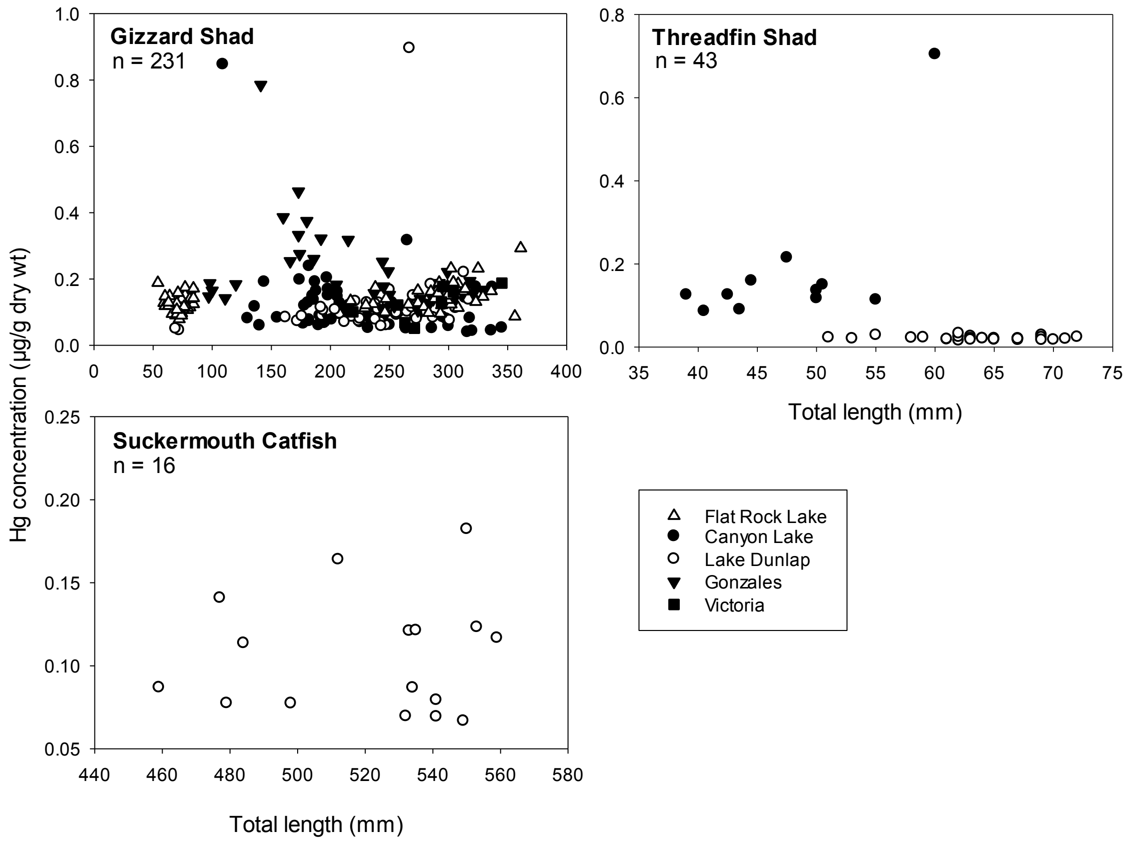

Figure 2. Relationship between total length and Hg concentration in muscle tissue for low trophic level (planktivore, herbivore, detritivore, and planktivore/detritivore) species. The corresponding linear regression results are shown in Table 3.

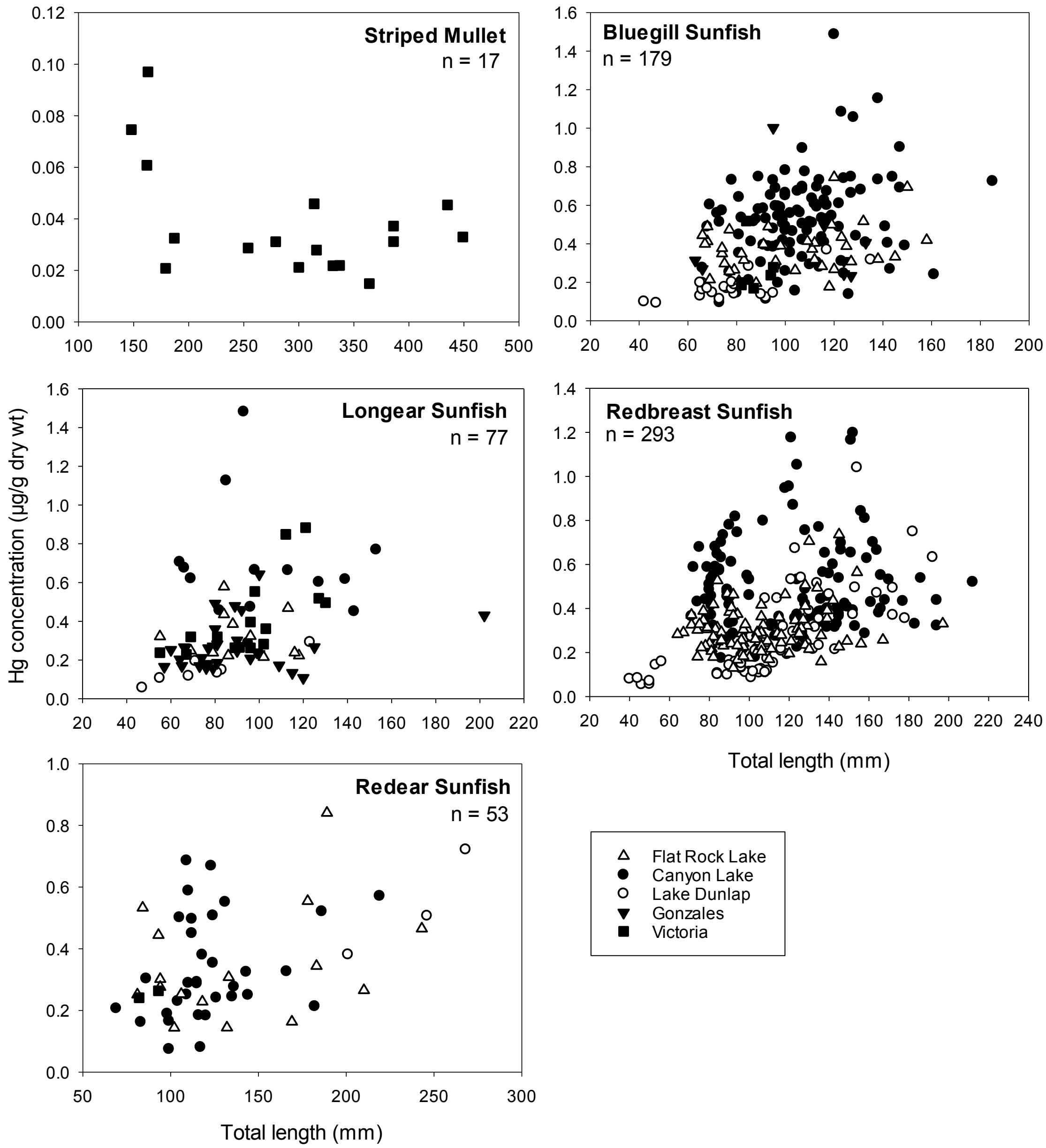

Figure 3. Relationship between total length and Hg concentration in muscle tissue for moderate trophic level (invertivore, invertivore/herbivore, and invertivore/detritivore) species in the Mugilidae and Centrarchidae families. The corresponding linear regression results are shown in Table 3.

.

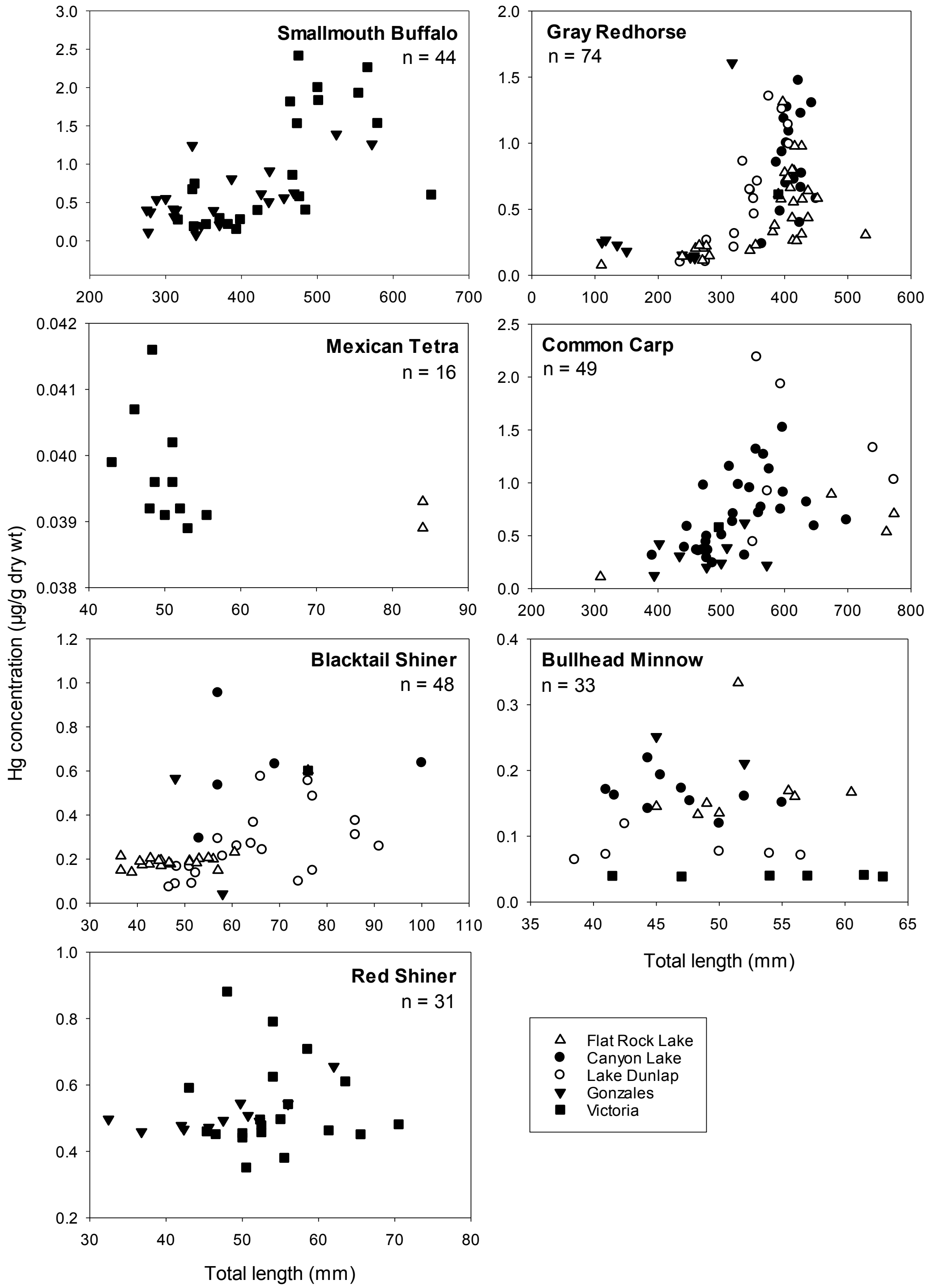

Figure 4. Relationship between total length and Hg concentration in muscle tissue for moderate trophic level (invertivore, invertivore/herbivore, and invertivore/detritivore) species in the Cyprinidae, Characidae, and Castomidae families. The corresponding linear regression results are shown in Table 3.

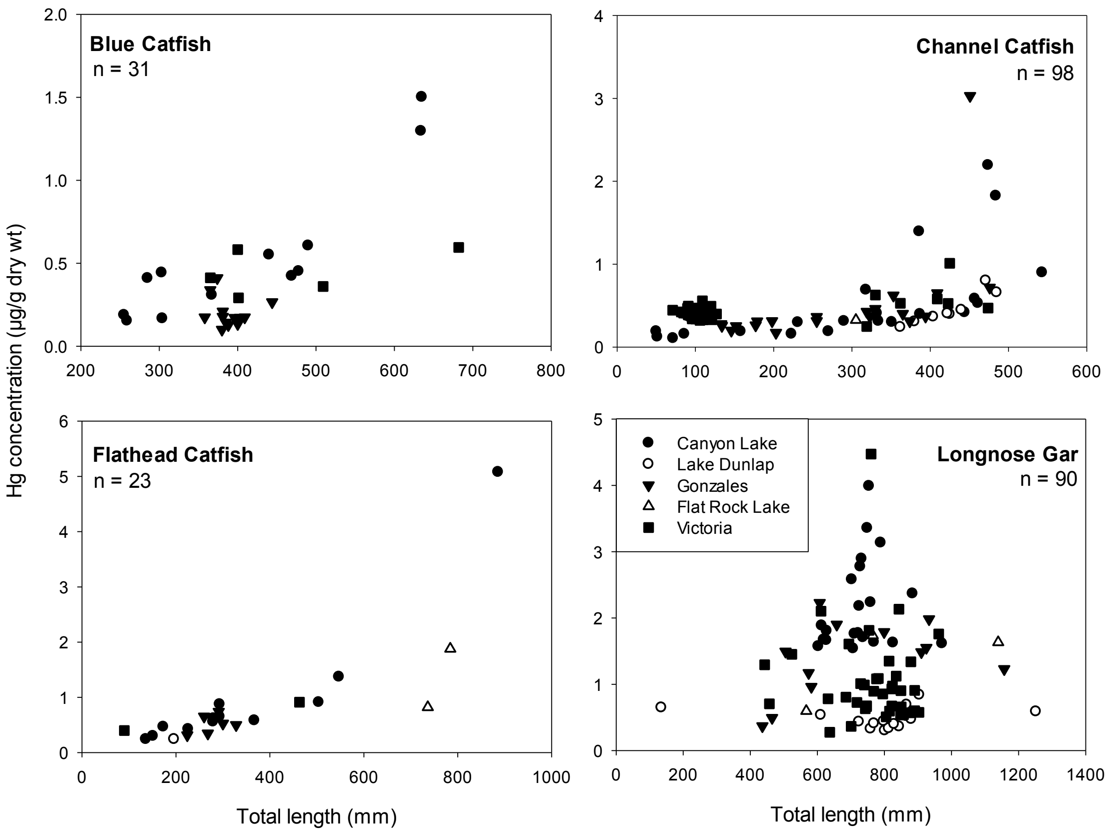

Figure 5. Relationship between total length and Hg concentration in muscle tissue for high trophic level (carnivore and invertivore/carnivore) species in the Ictaluridae and Lepisosteidae families. The corresponding linear regression results are shown in Table 3.

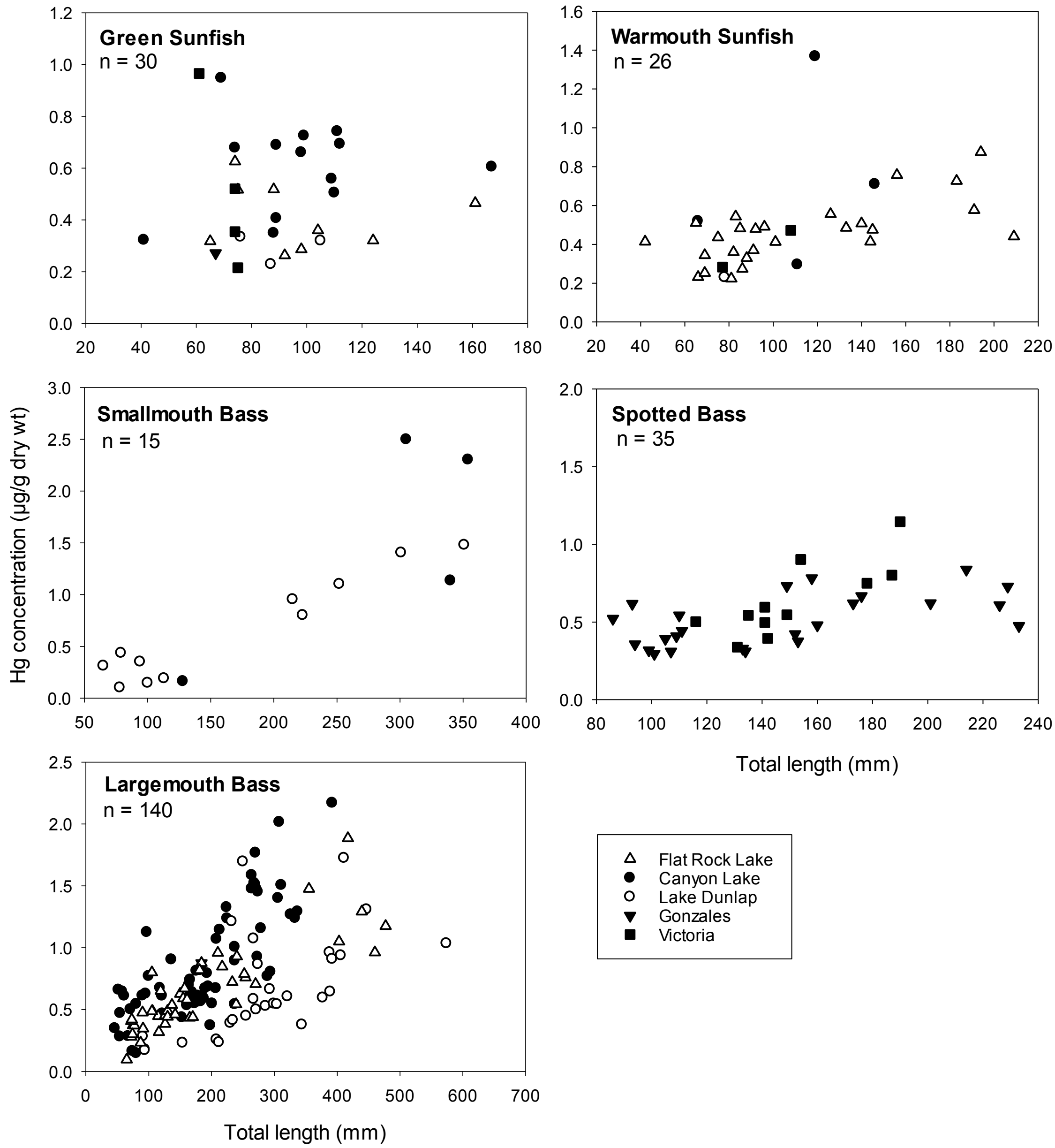

Figure 6. Relationship between total length and Hg concentration in muscle tissue for high trophic level (carnivore and invertivore/carnivore) species in the Centrarchidae family. The corresponding linear regression results are shown in Table 3.

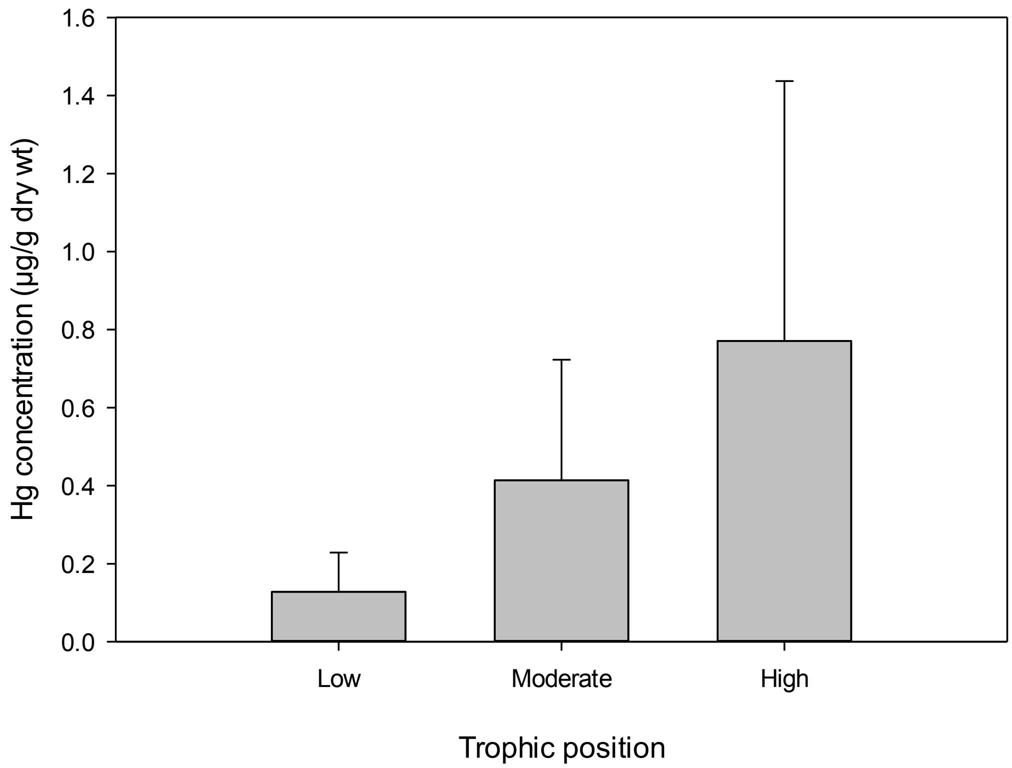

Figure 7. Mean Hg concentration in muscle tissue for all species designated as low, moderate, and high trophic levels for all five Guadalupe River sites combined. Error bars are 1 standard deviation.

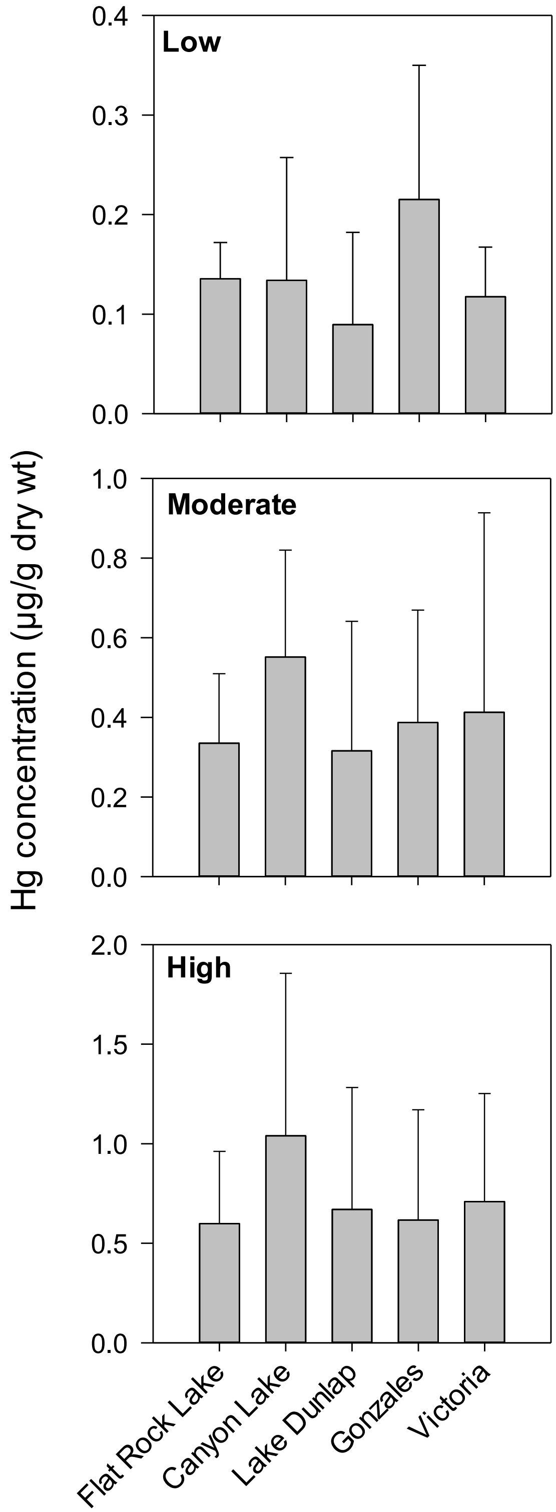

Figure 8. Mean Hg concentration in low, moderate, and high trophic level species at the five investigated sites. Error bars ±1 standard deviation.

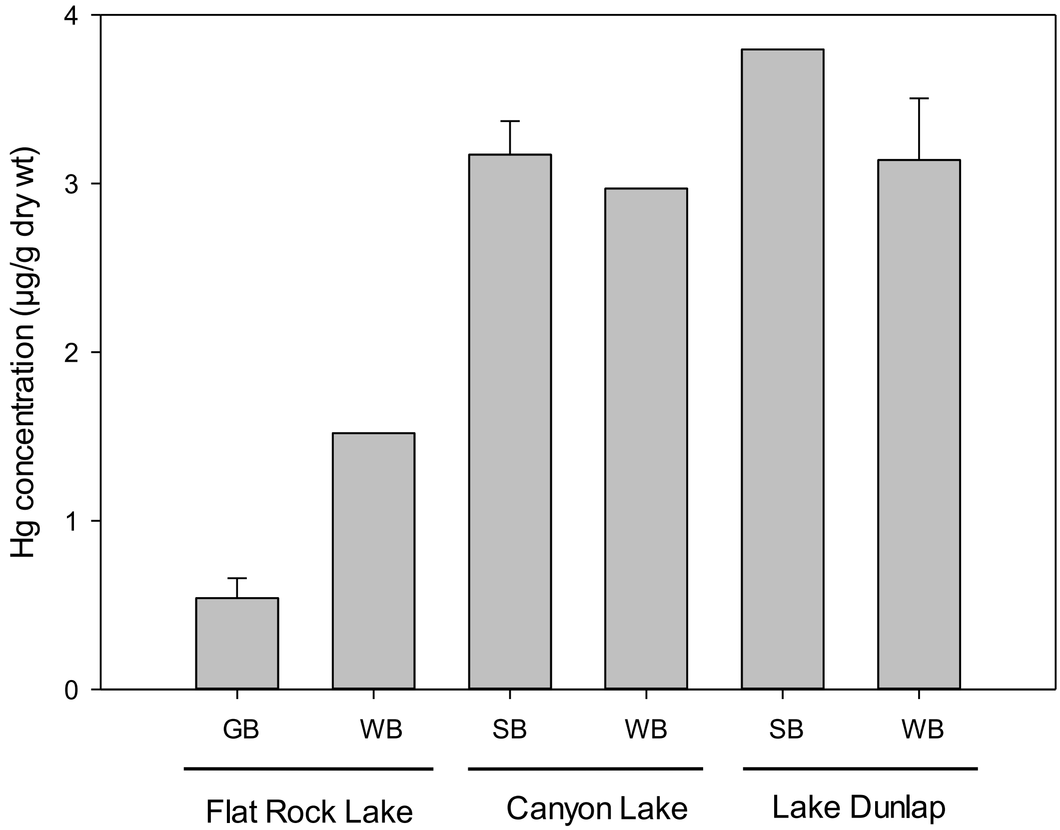

Figure 9. Mean Hg concentration in three commonly consumed bass species with n ≤ 5: Guadalupe bass (GB), white bass (WB), and striped bass (SB). Error bars are 1 standard deviation. Sample sizes are shown in Appendix B.

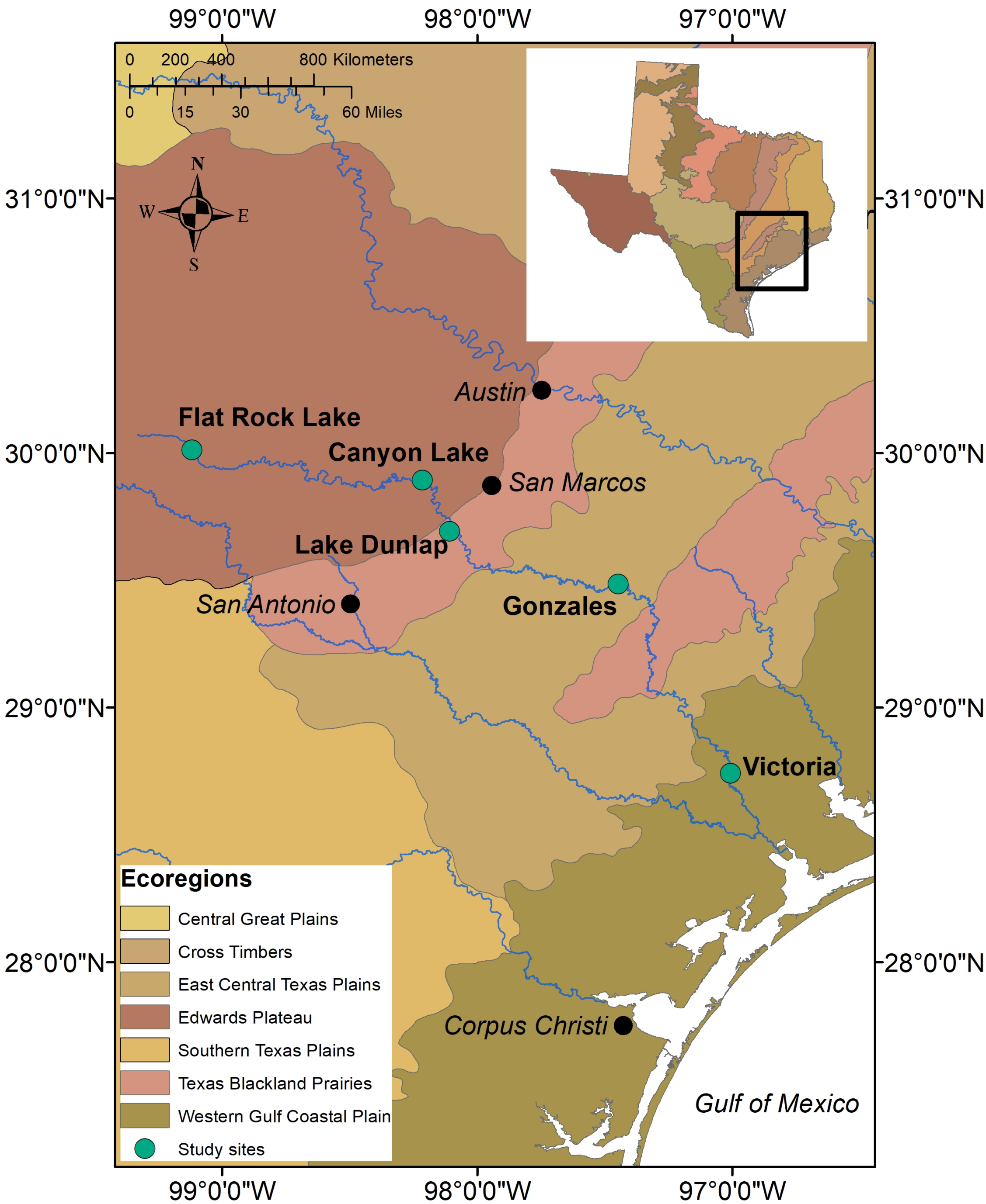

Figure 10. Map showing the location of the investigated study sites in relation to ecoregion.

II. EVALUATING THE RELATIONSHIP BETWEEN $\delta^{15}N$ AND MERCURY CONCENTRATIONS IN FRESHWATER FISH FROM THE GUADALUPE RIVER

ABSTRACT

Stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) is a well-established method of tracing Hg pathways through an aquatic ecosystem. This study determined the relationship between $\delta^{13}C$, $\delta^{15}N$ and muscle tissue Hg concentrations in trophically diverse fish ($n = 472$) from five sites (3 reservoir, 2 riverine) along the Guadalupe River using a direct mercury analyzer and elemental analyzer with IRMS. Zooplankton, benthic invertebrates and zebra mussels used to estimate the baseline trophic level at each site. Estimated trophic levels (ETL) for each investigated species varied between sites with the percentage of individuals in higher trophic levels greater in reservoir sites. In addition, food chain length was higher on average in reservoir sites. Further investigation of six species (longear sunfish, longnose gar, bluegill sunfish, gizzard shad, largemouth bass, and channel catfish) determined that Hg differences between sites were not due to a significant difference in the biomagnifications factor (BMF; $p > 0.05$), and controlling for ETL, sites were significantly different ($p < 0.05$) in 5 of the investigated species (excluding channel catfish). Between species, ETL was the strongest predictor of Hg concentration and within species, total length was the strongest predictor of Hg concentration. This study increases the understanding of how biomagnification differs between reservoir and riverine sites, as well as provides insight into the behavior of Hg in a relatively understudied South Central Texas freshwater systems.

i. INTRODUCTION

1.1 Use of stable isotopes to understand food web dynamics

Carbon and nitrogen stable isotopes ($\delta^{13}C$, $\delta^{15}N$) are utilized in freshwater ecosystems to identify food sources and estimate trophic position (Clayden et al., 2015; Al-Reasi et al., 2007). $\delta^{13}C$ concentrations in aquatic organisms vary based on the source of dietary carbon from the base of the foodweb (e.g. macroalgae, phytoplankton). When excretion of carbon is subtracted from uptake, $\delta^{13}C$ can serve as a time dependent measure of an organism's average diet (Fry, 2006). However $\delta^{15}N$ is the main measure of trophic division, widely used in estimating food webs based on both human and natural based sources of nitrogen (Anderson and Cabana, 2007; Vander Zanden et al., 1997; Cabana and Rasmussen, 1996). Together $\delta^{13}C$ and $\delta^{15}N$ ratios create isotopic profiles, which can be used to divide organisms into trophic groups within a food web. Isotopic profiles are variable within trophic positions, however top piscivores have less variable $\delta^{13}C$ and $\delta^{15}N$ profiles than other trophic groups e.g., herbivores and omnivores (Jepsen and Winemiller, 2002).

Consumers become enriched in $\delta\,^{15}N$ relative to their prey, allowing for a continuous estimation of trophic position for aquatic consumers based on a baseline $\delta^{15}N$ (Minagawa and Wada, 1984). In order to determine trophic position using $\delta^{15}N$, organisms such as benthic invertebrates and zooplankton are analyzed to determine the lowest $\delta^{15}N$ values amongst primary consumers. This serves as the established baseline $\delta^{15}N$ indicator from which estimates of trophic position in food webs can be calculated. Site-specific samples are necessary for riverine studies as differences often found between $\delta^{15}N$ values for the same species between sites within the same watershed (Anderson and Cabana, 2007). Additionally, longer-lived species such as freshwater Unionid mussels are preferable for such estimation over zooplankton as their isotopic signatures include a level of temporal integration lacking in the short-lived zooplankton and are therefore less variable (Matthews and Mazumder, 2003; Post, 2002). Spatial variation in $\delta^{15}N$ signatures at the base of the food chain is expected in such systems (Cabana and Rasmussen, 1996); however, it is currently unknown if significant variation exists between the riverine and reservoir systems in the same river system. If significant variation does exist between ecosystems, food webs must be calculated separately for each site, based on the lowest $\delta^{15}N$ found in each. Previous studies in gizzard shad (*Dorosoma cepedianum*) in the Brazos River have found that the isotopic signature of the fish varies with movement between the river channel and oxbow lakes, with significantly heavier nitrogen and carbon ratios in the river channel relative to the lakes (Zeug et al., 2009).

1.2 Relationship between $\delta^{15}N$ and Hg concentration in freshwater food webs

Analysis of both Hg concentration in fish muscle tissue and $\delta^{15}N$ allows for the tracing of dietary Hg throughout a food web, where Hg concentration should have a positive relationship with trophic level (derived from $\delta^{15}N$) to provide evidence of Hg biomagnification (Power et al., 2002). This is due to the stepwise enrichment of $\delta^{15}N$ allowing food chain biomagnification to be traced using $\delta^{15}N$ ratios (Ouédraogo et al., 2015; Rasmussen et al., 1990). In aquatic food webs, mean trophic fractionation of $\delta^{15}N$ has been previously quantified as 3.4 ‰ (Post, 2002; Cabana et al., 1994). This can further be quantified with a biomagnification factor (BMF), which calculates the likelihood Hg will biomagnify as trophic level increases (Lavoie et al., 2010); a BMF > 1 indicates biomagnification of Hg is occurring between two trophic levels, while a BMF < 1 indicates biomagnification of Hg is unlikely to occur (Arnot and Gobas, 2006). Biomagnification factors calculated for seven fish species in Caddo Lake, Texas indicated that BMFs for MeHg (4.3) and total Hg (THg; 4.3) were not significantly different, allowing THg BMFs to serve as a predictor for MeHg BMFs (Chumchal et al., 2011).

Additionally, $\delta^{15}N$ values can be used to calculate food chain length (FCL); considered a fundamental ecosystem attribute, FCLs have previously been linked to trophic position variation with a correlation between an increased FCL and an increase in $\delta^{15}N$ in lake trout (*Salvelinus namaycush*) (Cabana and Rasmussen, 1994). Food chain length often correlates with Hg concentration as higher Hg levels can be found in systems with longer food chains (Cabana et al., 1994; Ouédraogo et al., 2015). The baseline levels of Hg available may vary between given sites based on atmospheric deposition, erosion, pollution from natural and anthropogenic sources, and site history (Ullrich et al., 2010). This means that fish of different ETLs at different sites can have the same Hg concentration because the baseline Hg available at a given site differs. Gizzard shad have been shown to have different ETLs between oxbow lakes and the main river channel in the Brazos River, due to basal resource shifts (Zeug et al., 2009), but their Hg concentrations may still be comparable between river channel and oxbow sites.

The use of $\delta^{15}N$ values is a well-known method to investigate the relationship between trophic level and Hg concentration in fish in many freshwater and marine environments (Sluis et al., 2013; Chumchal et al., 2011). In Texas, isotope analysis is used in conjunction with Hg concentration primarily in North and Southwest Texas (Chumchal et al., 2011; Smith et al., 2010; Chumchal and Hambright, 2009). Trophic level is the best predictor of Hg concentration between freshwater species and total length/age the best predictor within species in Caddo Lake (Chumchal and Hambright, 2009). Spatial and environmental gradients [i.e., dissolved organic carbon (DOC) in the water and sediment Hg concentrations] can serve as predictors of Hg concentration, as shown in the Lower Rio Grande/Rio Brave del Norte drainage and the Amistad Reservoir (Becker et al., 2011; Smith et al., 2010). There is a lack of knowledge regarding the relationship between Hg concentration and trophic level for freshwater fish in South Central Texas, which will be investigated in this study. Additionally, these results will allow for a more nuanced understanding of the relationships explored in Chapter 1.

1.3 Objectives

This study determined the $\delta^{13}C$ and $\delta^{15}N$ values and measured the Hg concentration in muscle tissue from a variety of trophically diverse fish species from five sites (3 reservoirs and 2 riverine) along the Guadalupe River in Texas.

The study can be broken down into two main objectives:

1. Investigate differences in estimated trophic level (ETL) for each investigated species between sites with the prediction that individuals sampled from reservoir sites will have higher ETLs than those of the same species from riverine sites.
2. Determine the relationship between Hg concentration and trophic level and calculate the BMF at each site, with the prediction that there will be a positive

relationship, indicating biomagnification of Hg as trophic level increases at each site.

ii. METHODS

2.1 Field sites, fish collections, and Hg analysis

Investigated species, sites, collection methodology, and Hg analysis are described in Chapter 1. A subsample of individuals from 22 fish species (spotted gar, longnose gar, gizzard shad, red shiner, blacktail shiner, common carp, bullhead minnow, smallmouth buffalo, gray redhorse, channel catfish, flathead catfish, striped mullet, redbreast sunfish, green sunfish, warmouth sunfish, bluegill sunfish, longear sunfish, redear sunfish, smallmouth bass, spotted bass, largemouth bass, striped bass) described in Chapter 1 was analyzed for $\delta^{13}C$ and $\delta^{15}N$. The sample size of each species at each site is shown in Appendix C.

2.2 Invertebrates sample collection

Benthic invertebrates and zooplankton were collected at each site. A Wisconsin sampler was used for zooplankton collection and a Hess sampler as well as a D-frame kick net for invertebrate collection. Benthic invertebrates were stored in ethanol prior to sorting into family and zooplankton was filtered to a < 64 μm seston sample. Zebra mussels (*Dreissena polymorpha*) were also collected at Canyon Lake, and stored at -20°C until processing.

2.3 Stable isotope analysis

To obtain $\delta^{13}C$ and $\delta^{15}N$ values from a subsample of different fish and invertebrate species at each site, approximately 1 mg of homogenized sample, packaged in a tin capsule, was sent to the UC Davis Stable Isotope Facility (Davis, California), for analysis using an elemental analyzer interfaced to a continuous flow isotope ratio mass spectrometer (IRMS). Replicates of laboratory standards are interspersed between samples during analysis and all laboratory standards have been previously calibrated against NIST Standard Reference Materials (SRM). The recovery (mean ± SD) of the SRM was -21.73 ± 0.119 $\delta^{13}C$, 7.69 ± 0.075 $\delta^{15}N$ for bovine liver (expected concentration: $\delta^{13}C$ = -21.69; $\delta^{15}N$ = 7.72; *n* = 11); -16.66 ± 0.129 $\delta^{13}C$, -6.87 ± 0.164 $\delta^{15}N$ for glutamic acid (expected concentration: $\delta^{13}C$ = -16.65; $\delta^{15}N$ = -6.8; *n* = 46); 43.02 ± 0.072 $\delta^{13}C$, 41.13 ± 0.042 $\delta^{15}N$ for enriched alanine (expected concentration: $\delta^{13}C$ = 43.02; $\delta^{15}N$ = 41.13; *n* = 24); and -27.76 ± 0.083 $\delta^{13}C$, -10.54 ± 0.079 $\delta^{15}N$ for nylon 6 (expected concentration: $\delta^{13}C$ = -27.76; $\delta^{15}N$ = -10.54; *n* = 143). The relative difference between duplicate samples (*n* = 22) was < 1.5 %.

2.4 Estimation of baseline $\delta^{15}N$ and statistical analysis

Zebra mussels were used as the baseline indicator for Canyon Lake, due to its low $\delta^{15}N$ values and status as a filter feeder, placing it firmly in trophic level 2 as a primary consumer. The average $\delta^{15}N$ value (10.89 ± 0.011; mean ± standard error) was used as the $\delta^{15}N_{baseline}$ to determine trophic position in a modification of the Hobson and Welch (1992) equation:

(A) $$\text{Trophic position}_{consumer} = ([\delta^{15}N_{consumer} - \delta^{15}N_{baseline}] / 3.4‰) + ETL$$

where $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ value of the consumer for which the trophic position is being estimated; 3.4 ‰ is the fractionation value between trophic levels for most consumers and corresponds to ~1 trophic level observed by Post (2002); and ETL is the estimated trophic level of the organism used as the $\delta^{15}N$ baseline (e.g. primary consumer) (Vander Zanden and Rasmussen, 1999).

Zooplankton (< 64 µm seston) samples were collected from all reservoir sites (Flat Rock Lake, Canyon Lake, and Lake Dunlap), however mussels were only available at Canyon Lake. Therefore, a hypothetical filter feeder was created at both Flat Rock Lake and Lake Dunlap to use as the $\delta^{15}N$ baseline as zooplankton is less reliable as a baseline due to their short lifespans (Cabana and Rasmussen, 1996; Gu et al., 1994). The established difference in $\delta^{15}N$ values between zooplankton (< 64 µm seston) and filter feeders (zebra mussels) collected from Canyon Lake was 2.02 ‰. This value was added to the mean $\delta^{15}N$ values for zooplankton collected at Flat Rock Lake (1.41 ± 0.106, mean± standard error) and Lake Dunlap (1.41 ± 0.090) in order to estimate the $\delta^{15}N$ of the hypothetical filter feeder. This estimate was then used as the $\delta^{15}N$ baseline for Flat Rock Lake (8.68 $\delta^{15}N$) and Lake Dunlap (7.22 $\delta^{15}N$), using equation (A) with an ETL of 2 (primary consumer).

For the two riverine sites, Gonzales and Victoria, benthic macroinvertebrates were used as the $\delta^{15}N$ baseline due to their low $\delta^{15}N$ values. The $\delta^{15}N$ baseline was established separately at each site using the lowest mean $\delta^{15}N$ values macroinvertebrate family (or tribe) collected [Gonzales - (Chironominae), 11.15 ± 0.025; Victoria (Ephemeroptera), 10.01 ± 0.036; (mean ± standard error)]. The estimated trophic level used in the equation is 2, primary consumer.

Biomagnification factor of Hg was then determined for all samples combined by site, using the slope (m) of a simple linear regression (SLR):

$$\text{(B)} \qquad \mathrm{Log}_{10}[\mathrm{Hg}] = m\,(\delta^{15}\mathrm{N}) + b$$

where [Hg] is the concentration of Hg of all organisms within that site and b is the intercept. Victoria was the only site that failed the assumption of normality; however, it was still accepted for analysis due to the strength of the test (Glass et al., 1972). Biomagnification factor (BMF) was then calculated using the following equation:

$$\text{(C)} \qquad \mathrm{BMF} = 10^{m}$$

where m is the simple linear regression slope. An ANCOVA was then run between all significant SLR slopes, to determine if biomagnification rate differs between sites (controlling for estimated trophic position). Food chain length (FCL) was calculated at each site according to Vander Zanden and Fetzer (2007) using the following equation:

$$\text{(D)} \qquad \mathrm{FCL} = (\delta^{15}\mathrm{N}_{\text{top predator}} - \delta^{15}\mathrm{N}_{\text{baseline}})/3.4\ \text{‰} + \mathrm{ETL}$$

In order to identify relationships between Hg concentration and ecological characteristics, Pearson's correlation coefficient was calculated for Hg, total length, trophic position, and δ^{13}C for 4 - 6 species common at each site (longnose gar, channel catfish, largemouth bass, gizzard shad, bluegill sunfish, and longear sunfish). An

ANCOVA or ANOVA (depending on if total length was a significant covariate and if the data passed the assumptions of constant variance, normality, and equal slopes) was also run for each individual species to determine if Hg concentration differed between sites.

iii. RESULTS

This study analyzed 472 fish for $\delta^{13}C$, $\delta^{15}N$, and Hg from five sites on the Guadalupe River (Appendix C). The number of species sampled at each site averaged 14 (range 12 - 16); however the number of samples varied 1.3-fold between highest and lowest site. Canyon Lake had the highest number of species investigated (16 species, 118 individuals), followed by Lake Dunlap (15 species, 93 individuals), Victoria (15 species, 85 individuals), Flat Rock Lake (13 species, 88 individuals) and Gonzales (12 species, 88 individuals) (Appendix C). Additionally, $\delta^{13}C$ and $\delta^{15}N$ was analyzed in 5 zebra mussel samples (Canyon Lake) and a representative sampling of macroinvertebrates at Gonzales [5 families (Ephemeroptera, Baetidae, Chironomidae, Perlidae, Hydropsychidae), 24 samples] and Victoria [4 families (Ephemeroptera, Chironomidae, Perlidae, Hydropsychidae), 14 samples].

3.1 Estimated Trophic Level

Significant differences in Hg concentration among sites were found in ETL 3 - 3.99 (ANOVA, $p < 0.001$) and ETL 4 + (ANOVA, $p < 0.001$, excluding Gonzales where $n = 2$) (Fig. 11). ETL 3 - 3.99 showed significant differences between sites in a Tukey's HSD post hoc multiple comparison procedure, between Canyon Lake and Flat Rock Lake ($p < 0.001$), Canyon Lake and Lake Dunlap ($p < 0.001$), and Canyon Lake and Victoria ($p < 0.001$). These differences were also found in ETL 4 + ($p < 0.001$ for each comparison).

3.2 Predictors of Hg concentration in fish

Relationships between Hg and trophic level are shown in Fig. 12. Linear regressions revealed significant positive relationships between trophic level ($p < 0.05$) and $\log_{10}$ [Hg] at all sites except Victoria (Fig. 12). Significant differences (ANCOVA, df(3,381), $p < 0.05$) were found between slopes for Canyon Lake and both Lake Dunlap and Flat Rock Lake, controlling for ETL. Biomagnification factor decreased from upstream to downstream (Flat Rock Lake: 2.09, Canyon Lake: 2.03, Dunlap: 1.59, Gonzales: 1.50). FCL was highest in Lake Dunlap (5.07) followed by Flat Rock Lake (4.65), Victoria (4.36), Canyon Lake (4.11) and Gonzales (3.77). There was no correlation between BMF and FCL (df = 7, F = 0.022, $p > 0.05$).

In order to investigate the relationship between Hg concentration and ecological characteristics associated with six common fish species (longnose gar, channel catfish, largemouth bass, gizzard shad, bluegill sunfish, and longear sunfish) a Pearson's correlation coefficient analysis was conducted (Table 6). This analysis determined that the ecological characteristics most likely to significantly correlate were total length and Hg, followed by total length and $\delta^{13}C$, with all other ecological characteristics showing low levels of correlation. Mercury concentration and total length were correlated for largemouth bass at Flat Rock Lake, gizzard shad at Flat Rock Lake, Canyon Lake, and Lake Dunlap and channel catfish at Canyon Lake, Lake Dunlap, and Gonzales. Therefore, the differences in Hg concentration at each site with total length as a covariate was further investigated for these species (Fig. 13). All of the six investigated species, except channel catfish, had significant differences in Hg concentration between sites (longnose gar - ANCOVA, $F_{4,29} = 11.2$, $p < 0.001$; largemouth bass - ANCOVA, $F_{2,17} =$

24.8, $p < 0.001$; gizzard shad - ANCOVA, $F_{4,30} = 5.04$, $p = 0.003$; bluegill sunfish - ANOVA, df (32), $F = 9.7$, $p < 0.001$; longear sunfish - ANCOVA, $F_{4,32} = 222$, $p < 0.001$). Differences in Hg concentration were more likely to be found between reservoir sites and of these, Canyon Lake had the most significant differences from other sites across all five species investigated (Table 7). The lowest ETL species (gizzard shad) had relatively few significant differences between sites compared to the other 4 species, however there was no relationship between ETL and likelihood of site-specific differences in Hg concentration.

iv. DISCUSSION

This study determined the $\delta^{13}C$ and $\delta^{15}N$ values tissue from zooplankton, benthic invertebrates, zebra mussels, and trophically diverse fish (n = 527) from five sites along the Guadalupe River. Muscle Hg concentration was also determined for all fish samples. Trophic levels were estimated on a by-site basis for each sample and the percentage of individuals in higher trophic levels was greater in reservoir sites than riverine. Mercury concentration in high ETL individuals differed between sites, and biomagnification rate also differed between reservoirs over all species examined. Between species, ETL was the best predictor of Hg concentration and within species, total length was the best predictor. Biomagnification factor was not positively correlated with food chain length, possibly due to variation in the organisms used to set the $\delta^{15}N$ baseline.

4.1 Site differences in estimated trophic level

The $\delta^{15}N$ baseline for Canyon Lake was set at trophic level 2 using zebra mussels, an invasive species that was first identified in the lake in 2017. The use of unionid mussels as a $\delta^{15}N$ baseline is well documented (Post, 2002; Vander Zanden and Rasmussen, 1999) and preferable to zooplankton whose temporal variation in $\delta^{15}N$ values due to their relatively short lifespan makes them less reliable as a primary consumer (Cabana and Rasmussen, 1996; Gu et al., 1994). However, we were unable to collect mussels from Flat Rock Lake and Lake Dunlap, leaving zooplankton as the only appropriate organism with which to set the baseline; in order to compensate for the disparity in $\delta^{15}N$ values between the zooplankton and mussel $\delta^{15}N$ values, a hypothetical long-lived filter feeder was created using the $\delta^{15}N$ enrichment between zooplankton and zebra mussels found at Canyon Lake. This produced trophic level estimates that were less variable between the reservoir sites, but still likely accounts for most of the variability between ETLs at the three sites, as fish assemblage differed very little. It has also been suggested that variation in $\delta^{15}N$ baseline at one site can produce trophic estimates that place organisms in similar feeding guilds into different trophic levels (Vander Zanden and Rasmussen, 1999).

Interestingly, significant differences between Hg concentrations at different sites were only found in upper ETL (3 - 4+) species, likely because these were also the species with the highest Hg concentrations and were therefore more likely to show any differences. Canyon Lake was the only site that had a significant difference in Hg from all other sites exempting Victoria, suggesting that the Hg values at that site are much higher than those in the rest of the river. Canyon Lake is also the largest reservoir of those studied, with longer residency time for Hg in the water column for uptake into the food web and a longer residency time of the fish themselves, which spend more time in this high Hg system.

4.2 Hg biomagnification and food chain length

The BMF decreased in downstream sites with an overall average of 1.8. This is significantly lower than previously recorded values of 4.3 in Caddo Lake, Texas and 4.8 in the Lancaster Sound, Canada (Chumchal et al., 2011; Atwell et al., 1998). Further investigation of BMF in freshwater food webs is necessary to determine whether this variability is due to the lack of low-level primary consumers in the data set or a result of the overall lower levels of Hg when compared to these previously recorded values.

Food chain length in the present study was generally higher in reservoir systems and ranged from 3.77 - 5.07; this trend is supported by a number of studies showing that FCL is longest in reservoir ecosystems and shortest in rivers due to the addition of intermediate predators and/or an increase in omnivory in the reservoir systems (Hoeinghaus et al., 2008). Omnivory in lower trophic levels leads to longer FCLs in reservoirs as top predators generally increase in trophic position in lacustrine systems (Post, 2000). Our findings also did not support higher Hg bioaccumulation in systems with longer food chains as out FCLs were negatively correlated with mean Hg concentration at each site, however given that the species sampled at each site varied by necessity, this is likely due to study limitations (Cabana et al., 1994; Ouédraogo et al., 2015).

4.3 Intra- and interspecies comparisons of Hg concentration

Six widely distributed species (longnose gar, channel catfish, largemouth bass, gizzard shad, bluegill sunfish, and longear sunfish) were further investigated in order to determine what ecological characteristics had the strongest correlation with Hg muscle concentration. Between species, ETL was the best predictor of Hg concentration, with an increase in Hg at higher ETLs. A positive relationship between ETL and Hg concentration indicates that biomagnification is occurring and is used as an indicator of biomagnification in marine, freshwater, and Artic ecosystems, (Lavoie et al., 2013; Chen et al., 2008; Atwell et al., 1998).

Within a species, total length was the best indicator of Hg concentration, a result that is supported by many previous studies as Hg is well known to bioaccumulate over time in freshwater fish (Somers and Jackson, 2011; Chumchal et al., 2010; Adams and Onorato, 2005). For five of the six investigated species (excluding channel catfish) variation in ETL within each species between sites did not contribute to differences Hg concentration, suggesting that differences in Hg levels between sites are due to environmental characteristics such as water chemistry and methylation rate that varies between sites.

Overall, rate of biomagnification among all species at each site (as measured by linear regression slopes, Fig.12) were statistically different between Canyon Lake and the two other reservoir sites, controlling for ETL. This suggests that the rate of biomagnification for Hg at Canyon Lake is different from that of Flat Rock Lake and Lake Dunlap, even though these differences are not clearly reflected in the calculated BMFs. Because this difference in rate is only present at one site, the resulting differences in Hg concentration between sites can more likely be tied to variation in baseline Hg levels in the sediments and higher methylation of Hg by sulfate reducing bacteria -- which have a stronger presence in anoxic waters (Lou et al., 2017; Gilmour et al., 1992). Variation of Hg levels in sediment can be due to a number of factors including atmospheric deposition, natural and anthropogenic pollution, and site history (Ullrich et al., 2010). Fish located in reservoir systems have been shown to have higher Hg levels than those from the accompanying tributaries, a trend that is also linked to high reservoir Hg sediment concentrations (Dong et al., 2016; Abernathy and Crombie, 1977). Additionally, erosion and leaching during flooding facilitates Hg accumulation in northern pike (*Esox lucius*) and walleye (*Sander vitreus*) likely due to terrestrial sediment stimulating methylation in sulfate reducing bacteria (Jiang et al., 2018; Jackson, 1991). In reservoirs up to 60 years of age, enhancement of microbial methylation leads to higher Hg concentrations, which supports Canyon Lake, which was built in 1964 having higher Hg levels than Lake Dunlap, which was impounded in 1928 (TPWD, 2018c; U.S. Army Corps of Engineers, 2018; Heckey et al., 1991). Large runoff events can also bring terrestrial sediment that is high in Hg into reservoirs through corresponding tributaries. Water chemistry can also contribute to differences in Hg bioavailability between sites; and increase in Hg uptake is linked to a corresponding increase in DOC and a decrease in pH (Driscoll et al., 1995). Any combination of the above factors as well as the individual histories of the investigated sites may contribute to the differences in the amount of Hg available for uptake into the aquatic food web. Further investigation is necessary to determine where the site-specific differences in Hg originate and by which mechanisms they affect Hg concentration in the food web.

4.4 Conclusions

This is the first study to investigate Hg concentrations in conjunction with trophic position in fish from multiple sites on the Guadalupe River. Estimated trophic levels varied between sites, potentially due to variation in organisms used to set the $\delta^{15}N$ baseline at Flat Rock Lake and Lake Dunlap. Nevertheless, reservoirs overall had the highest percentage of organisms in the upper ETLs (3 - 4+); differences in Hg concentration between sites were found only in the high ETLs.

Between species, ETL served as a good predictor of Hg concentration and within species body length was the best predictor. Mercury was higher overall in reservoir sites as was food chain length whereas BMF was variable throughout the investigated sites. Site differences in Hg at upper ETLs was not due to differences in biomagnification, suggesting that there are environmental parameters that vary significantly between sites to create differences in baseline Hg concentration available. Methylation rates, historic Hg deposition and longer food chains are likely mechanisms by which the Hg baseline differs between sites.

This study provides a valuable insight in to the biomagnifications of Hg in a relatively understudied South Central Texas freshwater ecosystem. The findings of this study will be of interest to future researchers as biomagnification of Hg is studied in more freshwater systems with lower Hg values relative to marine and Artic food webs. Additionally, this study will significantly add to the body of knowledge of Hg levels in freshwater fish in Texas.

4.5 Future directions

Future studies on the Guadalupe River would benefit from a higher sample size, intentionally including fish of similar size length from all species in order to remove total length from consideration as a source of Hg variation between sites. As the strongest differences between sites were seen in the 3 and 4 + ETLs, a focused study on site differentiation must include a large sample size of top predators, as well as a variety of primary consumers to create a consistent baseline between sites.

Previous biomagnification studies have included terrestrial consumers both in their food chain estimates and Hg analyses (Chumchal et al., 2011; Campbell et al., 2005; Atwell et al., 1998). Canyon Lake, which had the highest overall values of Hg, would benefit from the evaluation of Hg in terrestrial consumers that live near the lake to determine if the level of Hg in these organisms is high relative to the high ETL fish of the lake and investigate Hg biomagnification in a larger food web.

Finally, a long-term study evaluating environmental factors such as riparian vegetation, water chemistry, and sediment Hg load at each of the investigated sites would be a valuable addition to this data. A two-year study would offer an in-depth look at how environmental parameters affect Hg uptake in the aquatic food web in these systems over time. Additionally, this would provide an opportunity to evaluate ecoregion-based differences in Hg bioavailability as the Guadalupe River passes through four different ecoregions.

Table 6. Pearson's correlation coefficient (r) for relationships between Hg concentration and body length (BL), $\delta^{13}C$, $\delta^{15}N$, and estimated trophic level (ETL). * indicates significance. To account for repeated tests with Hg as the dependent variable, significance was determined at $p < 0.0125$ (0.05/4) when Hg was the dependent variable. In all other tests, significance was set at $p < 0.05$.

Site, species	**n**	**Hg vs BL**	**Hg vs ETL**	**Hg vs $\delta^{13}C$**	**BL vs ETL**	**BL vs $\delta^{13}C$**	**ETL vs $\delta^{13}C$**
Flat Rock Lake							
Largemouth bass	8	0.864*	0.184	-0.373	-0.139	-0.559	0.372
Gizzard shad	8	0.832*	0.599	0.549	0.330	0.858*	-0.138
Bluegill sunfish	8	0.196	0.497	-0.146	-0.391	0.689	-0.433
Longear sunfish	8	-0.234	0.131	-0.386	-0.680	0.775*	-0.614
Canyon Lake							
Longnose gar	8	0.076	0.271	0.0915	-0.087	0.666	0.244
Channel catfish	12	0.731*	-0.245	0.672	-0.129	0.554	-0.206
Largemouth bass	8	0.874*	0.113	0.915*	0.473	0.824*	0.306
Gizzard shad	8	-0.395	0.275	0.490	-0.640	-0.621	0.389
Bluegill sunfish	8	-0.240	0.199	-0.440	0.324	0.900*	0.240
Longear sunfish	8	-0.052	-0.507	0.474	0.182	0.670	-0.115
Dunlap							
Longnose gar	8	-0.077	0.318	-0.110	0.370	0.061	0.230
Channel catfish	8	0.916*	0.113	0.419	0.053	0.100	0.326
Largemouth bass	8	0.905*	-0.621	0.676	-0.691	.0845*	-0.926*
Gizzard shad	8	0.866*	-0.819*	-0.906*	-0.864*	-0.684	0.687
Bluegill sunfish	8	0.793*	0.723	-0.406	0.532	-0.533	-0.650
Longear sunfish	6	0.888*	-0.214	0.900*	-0.611	0.935*	-0.560
Gonzales							
Longnose gar	8	0.273	0.636	-0.215	-0.158	0.345	-0.621
Channel catfish	8	0.832*	-0.250	0.795*	0.184	0.789*	-0.237

Table 6. Continued

Site, species	n	Hg vs BL	Hg vs ETL	Hg vs $\delta^{13}C$	BL vs ETL	BL vs $\delta^{13}C$	ETL vs $\delta^{13}C$
Bluegill sunfish	8	-0.905	0.030	-0.536	0.007	0.818*	-0.225
Longear sunfish	8	0.629	-0.393	-0.44	-0.763*	-0.0006	-0.415
Victoria							
Longnose gar	8	-0.563	-0.936*	-0.729	0.722*	0.285	0.799*
Channel catfish	12	-0.542	-0.076	-0.278	-0.721*	0.460	-0.821*
Gizzard shad	8	0.690	0.536	-0.520	0.345	-0.061	0.281
Bluegill sunfish	6	-0.032	0.881	-0.285	0.146	-0.034	-0.498
Longear sunfish	8	0.581	0.527	-0.140	0.194	-0.200	0.284

Table 7. Results of Tukey's HSD post hoc for differences in Hg concentration between sites. F = Flat Rock Lake, C = Canyon Lake, D = Lake Dunlap, G = Gonzales, V = Victoria.

Longear Sunfish

	F	C	D	G	V
F	-	< 0.001	0.007	0.089	0.436
C	-	-	< 0.001	< 0.001	< 0.001
D	-	-	-	0.345	0.001
G	-	-	-	-	0.017
V	-	-	-	-	-

Gizzard Shad

	F	C	D	G	V
F	-	0.055	0.999	0.718	0.855
C	-	-	0.047	0.002	0.361
D	-	-	-	0.759	0.82
G	-	-	-	-	0.188
V	-	-	-	-	-

Largemouth Bass

	F	C	D
F	-	0.006	0.082
C	-	-	< 0.001
D	-	-	-
G	-	-	-
V	-	-	-

Bluegill Sunfish

	F	C	D	G	V
F	-	0.129	0.025	0.995	0.254
C	-	-	< 0.001	0.342	0.001
D	-	-	-	0.018	0.895
G	-	-	-	-	0.172
V	-	-	-	-	-

Longnose gar

	F	C	D	G	V
F	-	0.353	0.037	0.817	0.62
C	-	-	< 0.001	0.374	0.03
D	-	-	-	< 0.001	0.022
G	-	-	-	-	0.392
V	-	-	-	-	-

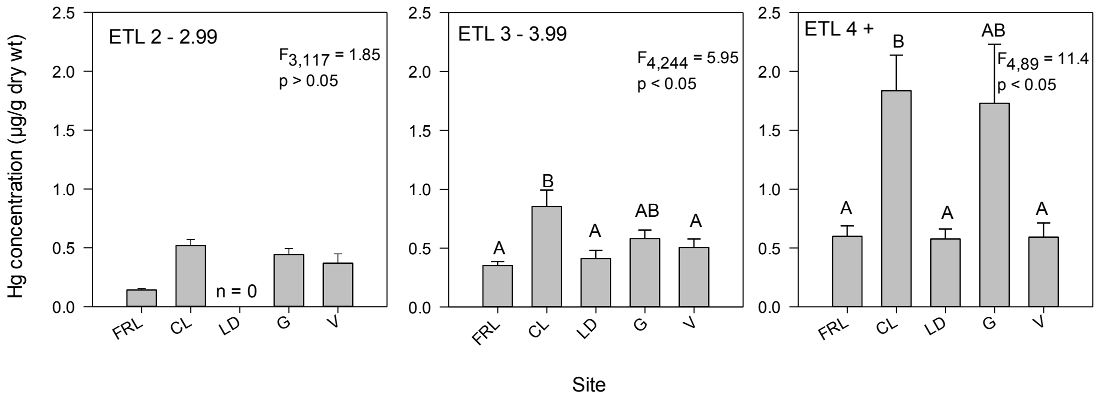

Figure 11. Mean muscle Hg concentration among sites at estimated trophic levels (ETLs) 2 - 2.99, 3 - 3.99, 4 +. A - B = sites not different from each other ($p > 0.05$). FRL = Flat Rock Lake, CL = Canyon Lake, LD = Lake Dunlap, G = Gonzales, V = Victoria.

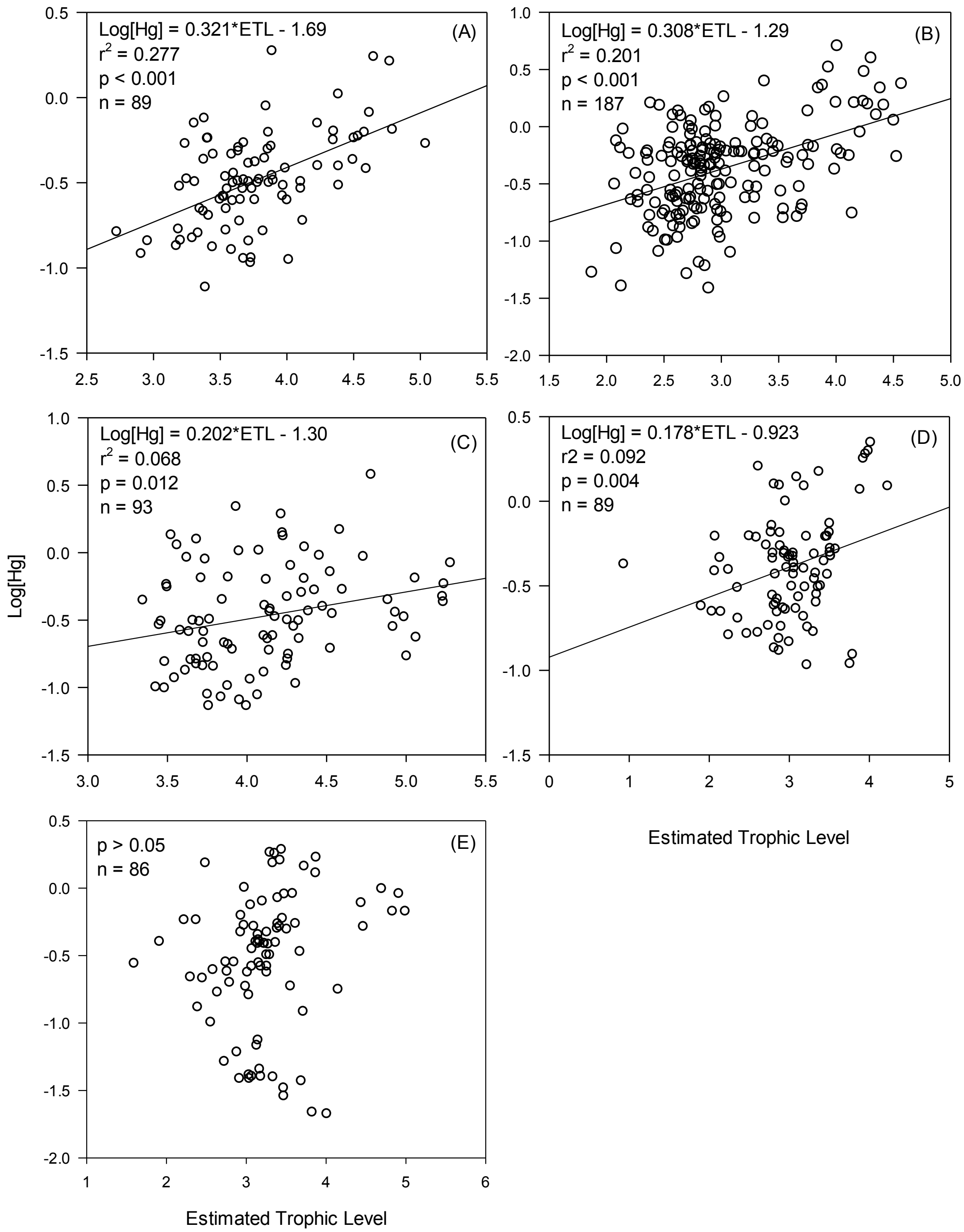

Figure 12. Relationship between $\log_{10}$ [Hg] and estimated trophic level at each site. Linear regression equations are provided except for Victoria (p > 0.05). A = Flat Rock Lake, B = Canyon Lake, C = Lake Dunlap, D = Gonzales, E = Victoria

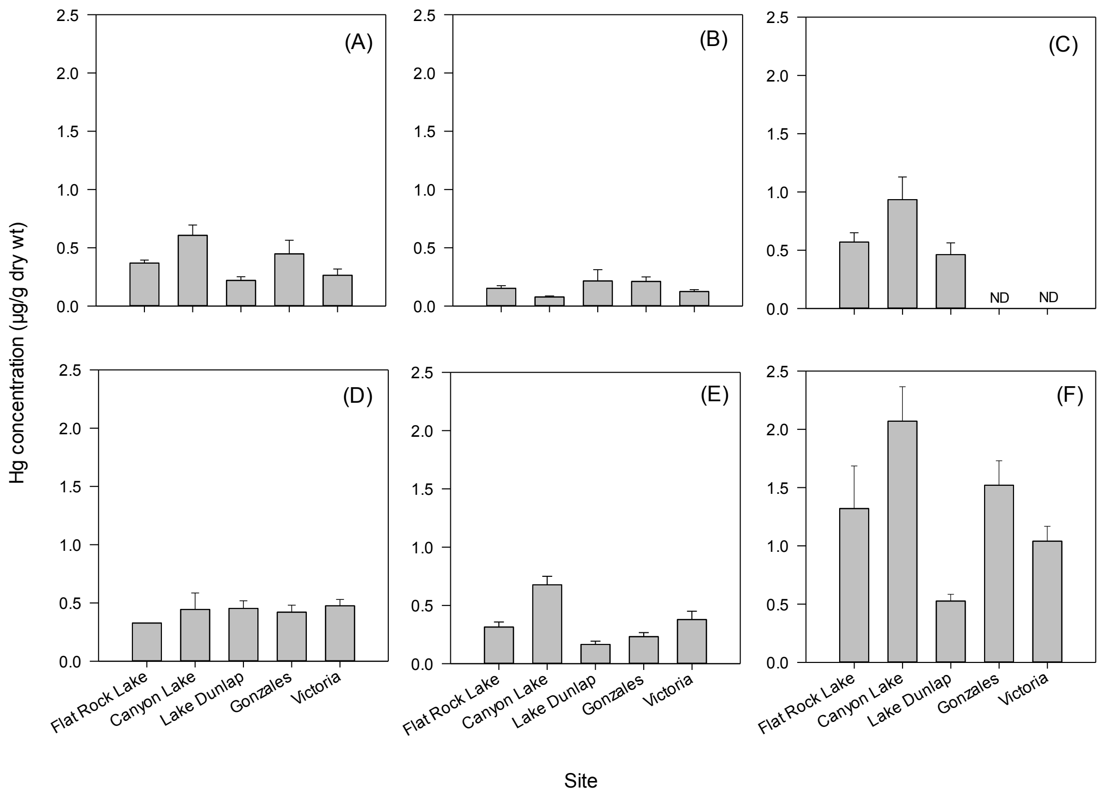

Figure 13. Mean Hg muscle concentration at each site for six fish species. (A) = bluegill sunfish, (B) = gizzard shad, (C) = largemouth bass, (D) = channel catfish, (E) = longear sunfish, (F) = longnose gar. ND = not determined.

Appendix A. Consumption advisories and bans issued by TDSHS for freshwater fish in Texas (TPWD, 2018a). DNE = Do not eat; ≥ length (in.) = fish greater than or equal to this length should not be consumed; A = adults and children over 12 years should eat no more than two 8-oz. servings per month; B = adults and children over 12 years should eat no more than one 8-oz. serving per month; C = children under 12 years old should eat no more than two 4-oz. servings per month; D = Pregnant women, women attempting to become pregnant, and breastfeeding mothers should not eat; and E = Women listed in 'D' and children under the age of 12 should not eat.

Region, Site	**County**	**Species**	**Consumption Advisory**
Valley (Harlingen/McAllen Area)			
Arroyo Colorado	Cameron and Hidalgo	Longnose gar	DNE
		Smallmouth buffalo	A, E
Llano Grande Lake	Cameron and Hidalgo	Longnose gar	DNE
		Smallmouth buffalo	A, E
Main Floodway (upstream of the Port of Harlingen)	Cameron and Hidalgo	Longnose gar	DNE
		Smallmouth buffalo	A, E
Central Texas			
Canyon Lake	Comal	Striped bass	A, C, D
		Longnose gar	A, C, D

Appendix A. Continued

Region, Site	County	Species	Consumption Advisory
Northeast/Southeast Texas			
Neches River and all contiguous waters (SH 7 bridge west of Lufkin downstream to US 96 bridge near Evadale, including B.A. Steinhagen and Sam Rayburn reservoirs)	Angelina, Hardin, Houston, Jasper, Nacagdoches, Polk, Sabine, San Augustine, Trinity, Tyler	Smallmouth buffalo	DNE
		Flathead catfish	A
		Longnose gar	A
		Blue catfish	30 inches
		Largemouth bass	16 inches
		Spotted bass	16 inches
Lake Madisonville	Madison	Largemouth bass	A, C, D
Clear Lake	Panola	Largemouth bass	A, C, D
		Freshwater drum	A, C, D
		Bowfin	A, C, D
Hills Lake	Panola	Largemouth bass	A, C, D
		Freshwater drum	A, C, D
Big Cypress Creek	Marion	Largemouth bass	A, C
		Freshwater drum	A, C
Caddo Lake	Harrison, Marion	Largemouth bass	A, C
		Freshwater drum	A, C
Toledo Bend Reservoir	Newton, Panola, Sabine, Shelby	Largemouth bass	A, C

Appendix A. Continued

Region, Site	**County**	**Species**	**Consumption Advisory**
Toledo Bend Reservoir	Newton, Panola, Sabine, Shelby	Freshwater drum	A, C
Village Creek upstream of Neches River	Hardin	Crappie	A, C, D
		Gar	A, C, D
		Largemouth bass	A, C, D
Lake Kimball	Hardin, Tyler	All species	A, C
Lake Pruitt (Black Cypress Creek)	Cass	All species	A, C
Lake Daingerfield	Morris	Largemouth bass	A, C
Lake Ratcliff	Houston	Largemouth bass	A, C
Panhandle			
Lake Alan Henry	Garza, Kent	Largemouth bass	A, E
		Spotted bass	A, E
		Blue catfish	A, E
		Flathead catfish	A, E
		Crappie	A, E
Lake Meredith	Hutchinson, Moore, Potter	Walleye	A, C
Houston/Galveston Area			
Lake Isabell	Harris	Largemouth bass	A, C, D

Appendix B. Mercury concentrations [median, mean, standard deviation (SD), minimum and maximum concentration; µg/g dry wt] and size range for each investigated species at each site.

Family	Common Name	Site	n	TL (mm)	Median	Mean	SD	Min	Max
Lepisosteidae	Spotted Gar	Gonzales	2	418-594	ND	0.859	ND	0.488	1.23
		Victoria	3	440-549	1.69	1.341	0.715	0.518	1.81
	Longnose Gar	Flat Rock Lake	3	566-1139	1.633	1.321	0.633	0.593	1.737
		Canyon Lake	21	603-972	1.806	2.18	0.695	1.541	3.99
		Lake Dunlap	15	135-1252	0.441	0.486	0.152	0.299	0.84
		Gonzales	12	505-1157	1.491	1.39	0.575	0.369	2.23
		Victoria	39	443-962	0.896	1.051	0.722	0.275	4.474
Clupeidae	Gizzard Shad	Flat Rock Lake	75	54-361	0.131	0.137	0.035	0.079	0.293
		Canyon Lake	55	109-345	0.102	0.125	0.114	0.04	0.847
		Lake Dunlap	53	69-328	0.101	0.122	0.113	0.046	0.896
		Gonzales	40	83-329	0.081	0.209	0.129	0.082	0.785
		Victoria	8	219-345	0.126	0.125	0.05	0.052	0.188
	Threadfin Shad	Canyon Lake	11	38-60	0.126	0.184	0.176	0.087	0.704
		Lake Dunlap	32	51-87	0.02	0.021	0.004	0.016	0.033
Cyprinidae	Red Shiner	Gonzales	11	29-64	0.493	0.51	0.056	0.459	0.656
		Victoria	20	42-71	0.479	0.531	0.135	0.351	0.881
	Blacktail Shiner	Flat Rock Lake	21	35-76	0.189	0.205	0.093	0.14	0.602
		Canyon Lake	5	53-100	0.631	0.611	0.237	0.295	0.955
		Lake Dunlap	20	46-91	0.25	0.258	0.151	0.073	0.575
		Gonzales	2	48-58	ND	0.304	ND	0.0423	0.566
	Common Carp	Flat Rock Lake	4	309-773	0.621	0.562	0.333	0.112	0.892

Appendix B. Continued

Family	Common Name	Site	n	TL (mm)	Median	Mean	SD	Min	Max
	Common Carp	Canyon Lake	30	391-698	0.641	0.697	0.346	0.244	1.525
		Lake Dunlap	6	550-773	1.182	1.31	0.655	0.444	2.191
		Gonzales	8	394-572	0.274	0.317	0.158	0.124	0.621
		Victoria	1	496	ND	0.581	ND	ND	ND
	Mimic Shiner	Lake Dunlap	4	34-50	0.106	0.172	0.141	0.091	0.383
	Pugnose Minnow	Lake Dunlap	1	55	ND	0.171	ND	ND	ND
	Bullhead Minnow	Flat Rock Lake	8	44-62	0.155	0.174	0.066	0.133	0.333
		Canyon Lake	10	41-55	0.161	0.165	0.027	0.12	0.219
		Lake Dunlap	6	37-57	0.073	0.079	0.02	0.064	0.119
		Gonzales	2	45-52	ND	0.231	ND	0.211	0.252
		Victoria	6	41-63	ND	0.04	0.001	0.039	0.041
Catostomidae	River Carpsucker	Gonzales	1	387	ND	0.541	ND	ND	ND
	Smallmouth Buffalo	Gonzales	22	275-572	0.461	0.55	0.369	0.078	1.392
		Victoria	22	316-650	0.637	0.967	0.78	0.155	2.417
	Gray Redhorse	Flat Rock Lake	32	110-528	0.406	0.47	0.3	0.077	1.313
		Canyon Lake	18	364-450	0.813	0.868	0.342	0.239	1.475
		Lake Dunlap	15	235-407	0.644	0.644	0.411	0.101	1.356
		Gonzales	9	111-317	0.182	0.345	0.476	0.131	1.608
Characidae	Mexican Tetra	Flat Rock Lake	2	50-54	0.144	0.15	0.028	0.1243	0.18
		Victoria	13	43-84	0.039	0.04	0.001	0.039	0.042
Ictaluridae	Yellow Bullhead	Flat Rock Lake	4	108-294	0.388	0.408	0.136	0.287	0.565

Appendix B. Continued

Family	Common Name	Site	n	TL (mm)	Median	Mean	SD	Min	Max
	Blue Catfish	Canyon Lake	12	255-635	0.433	0.542	0.428	0.154	1.502
		Gonzales	13	358-444	0.175	0.201	0.089	0.101	0.412
		Victoria	5	365-682	0.413	0.449	0.135	0.292	0.596
	Channel Catfish	Flat Rock Lake	1	305	ND	0.327	ND	ND	ND
		Canyon	21	49-543	0.315	0.557	0.572	0.108	2.194
		Dunlap	8	362-485	0.402	0.454	0.186	0.242	0.86
		Gonzales	20	133-476	0.34	0.505	0.612	0.174	3.03
		Victoria	48	71-474	0.416	0.435	0.109	0.249	1.011
	Flathead Catfish	Flat Rock Lake	2	736-784	ND	1.35	ND	0.818	1.88
		Canyon Lake	12	136-886	0.599	1.006	1.319	0.241	5.08
		Lake Dunlap	1	196	ND	0.241	ND	ND	ND
		Gonzales	6	224-328	0.512	0.514	0.166	0.3164	0.7398
		Victoria	2	90-463	ND	0.655	ND	0.4	0.911
Mugilidae	Striped Mullet	Victoria	17	148-449	0.031	0.038	0.022	0.015	0.097
Loricariidae	Suckermouth Catfish	Lake Dunlap	16	459-559	0.1	0.106	0.035	0.067	0.1823
Poeciliidae	Western Mosquitofish	Canyon Lake	1	53.5	ND	0.022	ND	ND	ND
Moronidae	White Bass	Flat Rock Lake	1	336	ND	1.519	ND	ND	ND
		Canyon Lake	3	306-340	2.79	2.971	0.365	2.732	3.391
		Lake Dunlap	1	366	ND	3.14	ND	ND	ND
	Striped Bass	Canyon Lake	2	544-564	ND	3.172	ND	3.031	3.313
		Lake Dunlap	1	482	ND	3.38	ND	ND	ND

Appendix B. Continued

Family	Common Name	Site	n	TL (mm)	Median	Mean	SD	Min	Max
Centrarchidae	Redbreast Sunfish	Flat Rock Lake	102	64-197	0.286	0.303	0.103	0.13	0.736
		Canyon Lake	119	71-212	0.443	0.498	0.211	0.175	1.2
		Lake Dunlap	72	40-192	0.182	0.255	0.186	0.056	1.041
		Flat Rock Lake	9	65-161	0.359	0.408	0.127	0.263	0.625
		Canyon Lake	13	41-167	0.661	0.607	0.176	0.323	0.948
		Lake Dunlap	3	76-105	0.32	0.295	0.057	0.229	0.335
		Gonzales	1	67	ND	0.272	ND	ND	ND
	Green Sunfish	Victoria	4	61-75	0.437	0.514	0.326	0.215	0.965
	Warmouth Sunfish	Flat Rock Lake	26	42-209	0.457	0.459	0.156	0.223	0.874
		Canyon Lake	4	66-146	0.614	0.723	0.462	0.296	1.368
		Lake Dunlap	1	78	ND	0.23	ND	ND	ND
		Victoria	2	77-108	ND	0.377	0.133	0.283	0.472
	Bluegill Sunfish	Flat Rock Lake	37	66-158	0.372	0.372	1.222	1.176	0.745
		Canyon Lake	107	66-185	0.528	0.531	0.221	0.096	1.487
		Lake Dunlap	21	42-135	0.165	0.178	0.07	0.093	0.369
		Gonzales	8	63-133	0.312	0.409	0.255	0.234	1.002
		Victoria	6	82-95	0.218	0.265	0.13	0.169	0.518
	Longear Sunfish	Flat Rock Lake	12	55-118	0.284	0.325	0.118	0.215	0.578
		Canyon Lake	14	64-153	0.642	0.686	0.299	0.289	1.48
		Lake Dunlap	7	47-123	0.134	0.15	0.075	0.057	0.292
		Gonzales	29	64-202	0.24	0.266	0.126	0.108	0.643

Appendix B. Continued

Family	Common Name	Site	n	TL (mm)	Median	Mean	SD	Min	Max
	Longear Sunfish	Victoria	15	55-130	0.319	0.416	0.21	0.229	0.883
	Redear Sunfish	Flat Rock Lake	16	81-243	0.288	0.344	0.184	0.143	0.84
		Canyon Lake	33	69-219	0.288	0.335	0.166	0.075	0.686
		Lake Dunlap	4	201-268	0.488	0.536	0.172	0.382	0.722
	Redspotted Sunfish	Flat Rock Lake	3	30.5-72	0.54	0.508	0.063	0.436	0.548
		Lake Dunlap	2	87-131	ND	0.476	ND	0.315	0.636
	Smallmouth Bass	Canyon Lake	4	128-354	1.719	1.525	1.089	0.163	2.499
		Lake Dunlap	11	65-351	0.436	0.662	0.509	0.102	1.481
	Spotted Bass	Gonzales	24	86-233	0.477	0.508	0.165	0.296	0.838
		Victoria	11	116-190	0.546	0.638	0.239	0.339	1.146
	Largemouth Bass	Flat Rock Lake	44	65-477	0.538	0.644	0.347	0.095	1.884
		Canyon Lake	68	46-392	0.68	0.849	0.443	0.147	2.17
		Lake Dunlap	28	91-447	0.59	0.693	0.42	0.171	1.724
	Guadalupe Bass	Flat Rock Lake	3	143-218	0.547	0.541	0.118	0.4187	0.655
	White Crappie	Victoria	1	106	ND	0.731	ND	ND	ND
	Greenthroat Darter	Flat Rock Lake	1	45	ND	0.368	ND	ND	ND
Percidae	Texas Logperch	Flat Rock Lake	1	101	ND	0.191	ND	ND	ND
		Canyon Lake	1	103	ND	0.45	ND	ND	ND
	Texas Logperch	Lake Dunlap	1	90	ND	0.159	ND	ND	ND
	Bigscale Logperch	Flat Rock Lake	1	97	ND	0.246	ND	0.246	ND
		Canyon Lake	5	75-108	0.668	0.64	0.223	0.305	0.932
Sciaenidae	Freshwater Drum	Gonzales	2	331-527	ND	1.326	ND	0.601	2.051

Appendix B. Continued

Family	Common Name	Site	n	TL (mm)	Median	Mean	SD	Min	Max
	Freshwater Drum	Victoria	3	293-540	0.919	1.219	0.737	0.678	2.059
Cichlidae	Rio Grande Cichlid	Flat Rock Lake	2	63-215	ND	0.232	ND	0.207	0.257
		Lake Dunlap	2	241-243	ND	0.208	ND	0.1636	0.2518
		Gonzales	3	52-61	0.218	0.2266	0.031	0.201	0.261
		Victoria	3	59-117	0.347	0.386	0.114	0.297	0.515
	Blue Tilapia	Canyon Lake	2	95-156	ND	0.102	ND	0.035	0.169
		Lake Dunlap	3	408-454	0.123	0.128	0.038	0.092	0.168

Appendix C. Mean $\delta^{13}C$, $\delta^{15}N$, and Hg concentration (µg/g dry weight) values for each species at each site examined. All values are reported as mean ± standard error. ETL = estimated trophic level. ND = not determined. * = order. $^{+}$ = tribe.

Family	Species	Common Name	Site	n	$\delta^{13}C$	$\delta^{15}N$	Hg	ETL
Zooplankton			FR Lake	3	-16.87 ± 0.982	6.66 ± 0.361	-	1.41 ± 0.106
			Canyon Lake	3	-26.82 ± 0.256	8.79 ± 1.84	-	1.38 ± 0.542
			Lake Dunlap	3	-18.86 ± 1.08	5.20 ± 0.308	-	1.41 ± 0.090
Ephemeroptera		Leptohyphidae*	Gonzales	5	-30.01 ± 0.057	11.29 ± 0.049	-	2.04 ± 0.014
			Victoria	2	-28.45 ± 0.168	10.01 ± 0.036	-	2.00 ± 0.011
Baetidae		Leptohyphidae*	Gonzales	5	-29.20 ± 0.047	11.43 ± 0.046	-	2.08 ± 0.013
Chironominae^{+}		Diptera*	Gonzales	4	-27.85 ± 0.249	11.15 ± 0.025	-	2.00 ± 0.007
			Victoria	2	-27.59 ± 0.523	10.45 ± 0.059	-	2.13 ± 0.017
Perlidae		Plecoptera*	Gonzales	5	-29.88 ± 0.137	12.73 ± 0.053	-	2.46 ± 0.015
			Victoria	5	-28.58 ± 0.039	11.15 ± 0.041	-	2.33 ± 0.012
Hydropsychidae		Tricoptera*	Gonzales	5	-29.16 ± 0.064	11.93 ± 0.111	-	2.23 ± 0.033
			Victoria	5	-30.35 ± 0.069	10.08 ± 0.045	-	2.02 ± 0.013
Dressenidae	*Dreissena polymorpha*	Zebra mussel	Canyon Lake	5	-30.05 ± 0.023	10.89 ± 0.011	-	2.00 ± 0.003
Lepisosteidae	*Lepisosteus oculatus*	Spotted Gar	Gonzales	2	-25.81 ± 0.258	17.17 ± 1.55	0.859 ± 0.371	3.77 ± 0.459
			Victoria	3	-26.24 ± 0.957	16.46 ± 1.09	1.341 ± 0.413	3.90 ± 0.321
	Lepisosteus osseus	Longnose Gar	FR Lake	3	-29.11 ± 0.700	17.70 ± 0.233	1.321 ± 0.365	4.65 ± 0.068
			Canyon Lake	8	-28.66 ± 0.273	18.08 ± 0.207	2.070 ± 0.295	4.12 ± 0.061
			Lake Dunlap	8	-30.27 ± 0.286	17.65 ± 0.276	0.526 ± 0.058	5.07 ± 0.081
			Gonzales	8	-25.81 ± 0.412	17.01 ± 0.393	1.522 ± 0.210	3.72 ± 0.116
			Victoria	8	-26.83 ± 0.776	18.04 ± 0.727	1.048 ± 0.128	4.36 ± 0.214

Appendix C. Continued

Family	Species	Common Name	Site	n	$\delta^{13}C$	$\delta^{15}N$	Hg	ETL
Clupeidae	*Dorosoma cepedianum*	Gizzard Shad	FR Lake	8	-30.40 ± 0.448	14.49 ± 0.454	0.154 ± 0.022	3.71 ± 0.133
			Canyon Lake	8	-29.24 ± 0.728	11.71 ± 0.475	0.079 ± 0.009	2.24 ± 0.140
			Lake Dunlap	8	-31.02 ± 0.459	13.37 ± 0.249	0.217 ± 0.098	3.81 ± 0.073
			Gonzales	8	-25.30 ± 0.856	13.40 ± 0.613	0.219 ± 0.034	2.66 ± 0.180
			Victoria	8	-26.79 ± 0.876	13.94 ± 0.734	0.125 ± 0.018	3.16 ± 0.216
Cyprinidae	*Cyprinella lutrensis*	Red Shiner	Gonzales	8	-26.44 ± 0.125	13.78 ± 0.118	0.656 ± 0.022	2.77 ± 0.034
	Cyprinella venusta	Blacktail Shiner	Lake Dunlap	8	-28.51 ± 0.370	12.83 ± 0.183	0.221 ± 0.056	3.65 ± 0.054
	Cyprinus carpio	Common carp	FR Lake	4	-29.14 ± 0.313	14.12 ± 0.657	0.562 ± 0.167	3.60 ± 0.193
			Canyon Lake	8	-28.81 ± 0.441	11.13 ± 0.214	0.613 ± 0.093	2.07 ± 0.063
			Lake Dunlap	6	-28.41 ± 0.400	13.62 ± 0.478	1.310 ± 0.267	3.88 ± 0.140
			Gonzales	8	-26.57 ± 1.25	11.84 ± 0.944	0.317 ± 0.056	2.20 ± 0.278
			Victoria	1	-28.59 ± ND	11.27 ± ND	0.581 ± ND	2.37 ± ND
	Pimephales vigilax	Bullhead Minnow	FR Lake	8	-31.12 ± 0.181	13.11 ± 0.159	0.174 ± 0.023	3.30 ± 0.047
			Canyon Lake	8	-29.32 ± 0.323	12.94 ± 0.249	0.159 ± 0.007	2.60 ± 0.073
			Victoria	6	-27.60 ± 0.564	13.73 ± 0.201	0.040 ± 4E-4	3.09 ± 0.059
Catostomidae	*Ictiobus bubalus*	Smallmouth Buffalo	Gonzales	8	-27.56 ± 0.494	13.70 ± 0.373	0.810 ± 0.147	2.75 ± 0.110
			Victoria	8	-26.56 ± 0.354	11.96 ± 0.844	1.04 ± 0.260	2.57 ± 0.248
	Moxostoma congestum	Gray Redhorse	FR Lake	8	-28.98 ± 0.380	14.61 ± 0.491	0.290 ± 0.064	3.74 ± 0.144

Appendix C. Continued

Family	Species	Common Name	Site	n	$\delta^{13}C$	$\delta^{15}N$	Hg	ETL
	Moxostoma congestum	Gray Redhorse	Canyon Lake	8	-28.30 ± 0.242	14.40 ± 0.330	0.823 ± 0.143	3.03 ± 0.097
			Lake Dunlap	8	-29.11 ± 0.233	12.59 ± 0.119	0.708 ± 0.172	3.58 ± 0.035
			Gonzales	8	-28.37 ± 0.097	14.06 ± 0.139	0.355 ± 0.180	2.86 ± 0.041
Ictaluridae	*Ictalurus punctatus*	Channel Catfish	FR Lake	1	-30.65 ± ND	15.13 ± ND	0.327 ± ND	3.90 ± ND
			Canyon Lake	12	-27.83 ± 0.635	14.17 ± 0.326	0.318 ± 0.141	2.95 ± 0.095
			Lake Dunlap	8	-29.32 ± 0.226	14.27 ± 0.302	0.454 ± 0.065	4.07 ± 0.089
			Gonzales	8	-26.02 ± 0.363	14.24 ± 0.143	0.421 ± 0.060	2.91 ± 0.042
			Victoria	12	-27.61 ± 0.418	13.61 ± 0.189	0.475 ± 0.055	3.06 ± 0.056
	Pylodictis olivaris	Flathead Catfish	FR Lake	2	-26.31 ± 1.11	16.34 ± 1.23	1.34 ± 0.530	4.25 ± 0.364
			Canyon Lake	8	-27.39 ± 0.381	16.72 ± 0.195	1.18 ± 0.569	3.72 ± 0.057
			Lake Dunlap	1	-29.58 ± ND	14.38 ± ND	0.241 ± ND	4.11 ± ND
			Gonzales	6	-26.64 ± 0.052	16.10 ± 0.115	0.514 ± 0.068	3.46 ± 0.034
	Plyodictis olivaris	Flathead Catfish	Victoria	2	-27.20 ± 1.21	14.60 ± 0.783	0.655 ± 0.255	3.35 ± 0.230
Mugilidae	*Mugil cephalus*	Striped Mullet	Victoria	8	-24.92 ± 0.376	14.96 ± 0.455	0.040 ± 0.007	3.46 ± 0.134
Centrarchidae	*Lepomis auritus*	Redbreast Sunfish	FR Lake	8	-28.46 ± 0.335	15.18 ± 0.332	0.379 ± 0.058	3.91 ± 0.098
			Canyon Lake	8	-27.97 ± 0.415	14.42 ± 0.627	0.482 ± 0.060	3.04 ± 0.184
			Lake Dunlap	8	-28.92 ± 0.164	13.59 ± 0.193	0.340 ± 0.116	3.87 ± 0.056
	Lepomis cyanellus	Green Sunfish	FR Lake	7	-26.77 ± 0.450	14.29 ± 0.265	0.410 ± 0.049	3.65 ± 0.078
			Canyon Lake	8	-27.46 ± 0.350	13.77 ± 0.447	0.653 ± 0.062	2.85 ± 0.131
			Lake Dunlap	3	-26.40 ± 0.910	14.10 ± 0.442	0.295 ± 0.033	4.02 ± 0.130
			Gonzales	1	-27.75 ± ND	14.94 ± ND	0.272 ± ND	3.11 ± ND

Appendix C. Continued

Family	Species	Common Name	Site	n	$\delta^{13}C$	$\delta^{15}N$	Hg	ETL
	Lepomis cyanellus	Green Sunfish	Victoria	3	-26.71 ± 0.469	12.97 ± 0.716	0.364 ± 0.088	2.87 ± 0.210
	Lepomis gulosus	Warmouth	FR Lake	8	-27.98 ± 0.307	14.18 ± 0.183	0.497 ± 0.051	3.62 ± 0.054
			Canyon Lake	4	-28.78 ± 0.766	13.53 ± 0.541	0.723 ± 0.231	2.78 ± 0.159
			Lake Dunlap	1	-30.77 ± ND	15.13 ± ND	0.230 ± ND	4.33 ± ND
			Victoria	2	-26.08 ± 3.44	13.41 ± 0.874	0.377 ± 0.094	3.00 ± 0.257
	Lepomis macrochirus	Bluegill	FR Lake	8	-28.33 ± 0.336	15.04 ± 0.359	0.370 ± 0.026	3.87 ± 0.106
			Canyon Lake	8	-28.10 ± 0.279	14.66 ± 0.124	0.608 ± 0.089	3.11 ± 0.037
			Lake Dunlap	8	-28.92 ± 0.243	14.85 ± 0.104	0.221 ± 0.031	4.24 ± 0.030
			Gonzales	8	-26.96 ± 0.373	14.49 ± 0.180	0.448 ± 0.118	2.98 ± 0.053
			Victoria	6	-27.62 ± 0.616	13.41 ± 0.931	0.265 ± 0.130	3.00 ± 0.274
	Lepomis megalotis	Longear Sunfish	FR Lake	8	-27.52 ± 0.456	14.20 ± 0.338	0.315 ± 0.043	3.62 ± 0.100
			Canyon Lake	8	-27.62 ± 0.586	13.41 ± 0.160	0.677 ± 0.074	2.75 ± 0.047
			Lake Dunlap	6	-27.05 ± 0.727	13.52 ± 0.621	0.166 ± 0.028	3.85 ± 0.183
			Gonzales	8	-27.52 ± 0.273	15.29 ± 0.131	0.233 ± 0.034	3.22 ± 0.038
			Victoria	8	-27.72 ± 0.462	14.21 ± 0.242	0.379 ± 0.073	3.23 ± 0.071
	Lepomis microlophus	Redear Sunfish	FR Lake	8	-28.30 ± 0.500	13.39 ± 0.493	0.273 ± 0.036	3.39 ± 0.145
			Canyon Lake	8	-28.64 ± 0.223	12.98 ± 0.625	0.399 ± 0.060	2.61 ± 0.184
			Lake Dunlap	4	-29.04 ± 0.119	15.10 ± 0.271	0.520 ± 0.072	4.32 ± 0.079
			Victoria	2	-26.83 ± 2.12	13.30 ± 0.718	0.253 ± 0.011	2.97 ± 0.211
	Micropterus dolomieu	Smallmouth Bass	Canyon Lake	4	-27.31 ± 0.541	16.59 ± 0.813	1.525 ± 0.544	3.68 ± 0.239

Appendix C. Continued

Family	Species	Common Name	Site	n	δ^{13}C	δ^{15}N	Hg	ETL
	Micropterus dolomieu	Smallmouth Bass	Lake Dunlap	8	-28.58 ± 0.422	14.98 ± 0.320	0.738 ± 0.198	4.28 ± 0.094
	Micropterus punctulatus	Spotted Bass	Gonzales	8	-25.99 ± 0.190	15.98 ± 0.127	0.498 ± 0.037	3.42 ± 0.037
			Victoria	8	-27.70 ± 0.398	14.78 ± 0.244	0.604 ± 0.070	3.40 ± 0.072
	Micropterus salmoides	Largemouth Bass	FR Lake	8	-29.01 ± 0.604	17.29 ± 0.406	0.571 ± 0.079	4.53 ± 0.119
			Canyon Lake	8	-28.61 ± 0.366	16.76 ± 0.579	0.935 ± 0.194	3.73 ± 0.170
			Lake Dunlap	8	-27.86 ± 0.253	16.63 ± 0.357	0.464 ± 0.101	4.77 ± 0.105
Moronidae	*Morone saxatilis*	Striped Bass	Canyon Lake	2	-29.07 ±0.444	17.39 ± 0.530	3.172 ± 0.141	3.91 ± 0.156
			Lake Dunlap	1	-27.87 ± ND	16.67 ± ND	3.796 ± ND	4.78 ± ND

LITERATURE CITED

Abernathy, A.R., and Cumbie, P.M. (1977). Mercury accumulation by largemouth bass (*Micropterus salmoides*) in recently impounded reservoirs. *Bulletin of Environmental Contamination & Toxicology 17*(5):595-602.

Adams, K.J., Drenner, R.W., Chumchal, M.M., Donato, D.I. (2016). Disparity between state fish consumption advisory systems for methylmercury and U.S. environmental protection agency recommendations: A case study of the South Central United States. *Environmental Toxicology and Chemistry 35*(1):247-251.

Adams, D.H., Onorato, G.V. (2005). Mercury concentrations in red drum, *Sciaenops ocellatus*, from estuarine and offshore waters of Florida. *Marine Pollution Bulletin, 50*:291-300.

Al-Majed Butayban, N., Preston, M. (2005). The distribution and inventory of total and methylmercury in Kuwait Bay. *Marine Pollution Bulletin 49*:930-937.

Al-Reasi, H.A., Ababneh, F.A., Lean, D.R. (2007). Evaluating mercury biomagnification in fish from a tropical marine environment using stable isotopes ($\delta^{13}C$ and $\delta^{15}N$). *Environmental Toxicology and Chemistry 26*(8):1572-1581.

Anderson, C., Cabana, G. (2007). Estimating the trophic position of aquatic consumers in river food webs using stable nitrogen isotopes. *Journal of the North American Benthological Society 26*(2):273-285.

Arnot, J.A., Gobas, F.A.P.C. (2006). A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. *Environmental Reviews 14*:257-297.

Atwell, L., Hobson, K.A., Welch, H.E. (1998). Biomagnification and bioaccumulation of mercury in an arctic marine food web: Insights from stable nitrogen isotope analysis. *Canadian Journal of Fisheries and Aquatic Sciences, 55*:1114-1121.

Axelrad D.A., Bellinger D.C., Ryan L.M., Woodruff T.J. (2007). Dose-response relationship of prenatal mercury exposure and I.Q.: An integrative analysis of epidemiologic data. *Environmental Health Perspectives 115*(4): 609-615.

Bean, P.T. (2012). Introgressive status, population genetic structure, phylogeographic history and individual-level resource specialization of the Guadalupe bass *Micropterus treculii*. (Unpublished Ph.D. Dissertation). Texas State University, San Marcos TX.

Bean, P.T., Bonner, T.H. (2008). Diet and reproduction of the Gray Redhorse (*Moxostoma congestum*) in a Texas Hill Country stream and reservoir. *Journal of Freshwater Ecology 23*(3):397-403.

Becker, J.C., Groeger, A.W., Nowlin, W.H., Chumchal, M.M., Hahn, D. (2011). Spatial variability in the speciation and bioaccumulation of mercury in an arid subtropical reservoir ecosystem. *Environmental Toxicology and Chemistry 30*(10):2300-2311.

Blaber, S.J.M., Whitfield, A.K. (1977). The feeding ecology of juvenile mullet (Mugilidae) in south-east African estuaries. *Biological Journal of the Linnean Society, 9*(3): 277-284.

Bloom N.S. (1992). On the chemical form of mercury in edible fish and marine invertebrate tissue. *Canadian Journal of Fisheries and Aquatic Sciences* 49(5): 1010-1017.

Bodaly, R.A., Rudd, JW.M., Fudge, R.J.P., Kelly, C.A. (1993). Mercury concentrations in fish related to size of remote Canadian shield lakes. *Canadian Journal of Fisheries and Aquatic Sciences 50*(5):980-987.

Bonner, T.H., Wilde, G.R. (2000). Changes in the Canadian river fish assemblage associated with reservoir construction. *Journal of Freshwater Ecology 15*(2):189-198.

Braune, B.M. (1987). Mercury accumulation in relation to size and age of Atlantic herring (*Clupea harengus harengus*) from the Southwestern Bay of Fundy, Canada. *Archives of Environmental Contamination and Toxicology 16*:311-320.

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

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

Campbell, L., Verburg, P., Dixon, D.G., Hecky, R.E. (2008). Mercury biomagnification in the food web of Lake Tanganyika (Tanzania, East Africa). *Science of the Total Environment 402*:184-191.

Carlander, K.D. (1977). *Handbook of freshwater fishery biology, vol. 2*. Ames, Iowa: Iowa State University Press.

Carrasco, L., Barata, C., García-Berthou, E., Tobias, A., Bayona, J.M., Díez, S. (2011). Patterns of mercury and methylmercury bioaccumulation in fish species downstream of a long-term mercury-contaminated site in the lower Ebro River (NE Spain). *Chemosphere 84*:1642-1649.

Carrington, C.D., Bolger, M.P. (2002). An exposure assessment for methylmercury from seafood for consumers in the United States. *Risk Analysis 22*(4):689-699.

Chen, Y.-C., Chen, C.-Y., Hwang, H.J., Chang, W.-B., Yeh, W.-J., Chen, M.-H. (2004). Comparison of the metal concentrations in muscle and liver tissues of fishes from the Erren River, Southwestern Taiwan, after the restoration in 2000. *Journal of Food and Drug Analysis 12*(4):358-366.

Chen, C., Amirbahman, A., Fisher, N., Harding, G, Lamborg, C., Nacci, D., Taylor, D. (2008). Methylmercury in Marine ecosystems: Spatial patterns and processes of production, bioaccumulation, and biomagnification. *EcoHealth 5*:399-408.

Choi, A.L., Weihe P., Budtz-Jørgensen E., Jørgensen, P.J., Salonen J.T., Tuomainen T., Murata K., Nielsen H.P., Petersen M.S., Askham, J., Grandjean, P. (2009). Methylmercury exposure and adverse cardiovascular effects in Faroese whaling men. *Environmental Health Perspectives 117*(3):367-372.

Chumchal M.M., Hambright K.D. (2009). Ecological factors regulating mercury contamination of fish from Caddo Lake, Texas, USA. *Environmental Toxicology and Chemistry* 28(5): 962-972.

Chumchal M.M., Drenner R.W., Cross D.R., Hambright K.D. (2010). Factors influencing mercury accumulation in three species of forage fish from Caddo Lake, Texas, USA. *Journal of Environmental Sciences* 22(8):1158-1163.

Chumchal, M.M., Rainwater, T.R., Osborn, S.C., Roberts, A.P., Abel, M.T., Cobb, G.P., Smith, P.N., Bailey, F.C. (2011). Mercury speciation and biomagnification in the food web of Caddo Lake, Texas and Louisiana, USA, a subtropical freshwater ecosystem. *Environmental Toxicology and Chemistry 30*(5):1153-1162.

Clayden, M.G., Arsenault, L.M., Kidd, K.A., O'Driscoll, N.J., Mallory, M.L. (2015). Mercury bioaccumulation and biomagnification in a small Arctic polynya ecosystem. *Science of the Total Environment 509-510*:206-215.

Crump, K.L., Trudeau, V.L. (2009). Mercury-induced reproductive impairment in fish. *Environmental Toxicology and Chemistry 28*(5):895-907.

Das, N.G., Chowdhury, Z.A. (1983). Food and feeding habits of 3 grey mullet from the Matamuhury river estuary (Bangladesh). *Chittagung Universtiy Studies Part I1 Science 7*(2): 57-68.

DeForest, D., Brix, K., Adams, W. (2007). Assessing metal bioaccumulation in aquatic environments: The inverse relationship between bioaccumulation factors, trophic transfer factors and exposure concentration. *Aquatic Toxicology 84*(2):236-246.

Dong, Z., Lynch, R.A., Schaider, L.A. (2016). Key contributors to variations in fish mercury within and among freshwater reservoirs in Oklahoma, USA. *Environmental Science: Processes & Impacts 18*(2):222-236.

Drenner, R.W., Chumchal, M.M, Jones, C.M., Lehmann, C.M.B., Gay, D.A., Donato, D.I. (2013). Effects of mercury deposition and coniferous forests on the mercury contamination of fish in the South Central United States. *Environmental Science & Technology 47*:1274-1279.

Drenner, R.W., Chumchal M.M., Wente S.P., McGuire M., Drenner S.M. (2011). Landscape-level patterns of mercury contamination of fish in north Texas, USA. *Environmental Toxicology and Chemistry* 30(9):2041-2045.

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

Driscoll, C.T., Han Y., Chen C.Y., Evers D.C., Fallon Lambert K., Holsen T.M., Kamman N.C., Munson R.K. (2007). Mercury contamination in forest and freshwater ecosystems in the northeastern United States. *BioScience* 57:17-28.

Driscoll, C.T., Blette, V., Yan, C., Schofield, C.L., Munson, R., Holsapple, J. (1995). The role of dissolved organic carbon in the chemistry and bioavailability of mercury in remote Adirondack lakes. *Water, Air, and Soil Pollution 80*:499-508.

Dutton, J., Fisher N.S. (2010). Intraspecific comparisons of metal bioaccumulation in the juvenile Atlantic silverside *Menidia menidia*. *Aquatic Biology* 10:211-226.

Dutton J., Fisher, N.S. (2011). Salinity effects on the bioavailability of aqueous metals for the estuarine killifish *Fundulus heteroclitus*. *Environmental Toxicology and Chemistry 30*(9):2107-2114.

Dutton, J., Fisher, N.S. (2012). Influence of humic acid on the uptake of aqueous metals by the killifish *Fundulus heteroclitus 31*(10): 2225-2232.

Dutton, J., Fisher N.S. (2014). Modeling metal bioaccumulation and tissue distribution in killifish (*Fundulus heteroclitus*) in three contaminated estuaries. *Environmental Toxicology and Chemistry* 33(1):89-101.

Eggold, B.T. Motta, P.J. (1992). Ontogenetic dietary shifts and morphological correlates in striped mullet, *Mugil cephalus*. *Environmental biology of fishes*, *34*:139-158.

Estrada, M. 1999. The ecology and life history of the Mexican tetra, *Astyanax mexicanus*, (Teleostei: Characidae) in the lower Rio Grande Valley, Texas. Unpubl. M. S. thesis, The University of Texas-Pan American, Edinburg, TX, 53 pp.

Folb, C.E., (2010). Reproductive seasons and life histories of three Texas *Percina* (Actinopterygii). Unpublished M.S. thesis. Texas State University, San Marcos, TX.

Fry, B. (2007). Stable Isotope Ecology. New York, NY: Springer Science & Business Media, LLC.

Gavis J and Ferguson J.F. (1972). The cycling of mercury through the environment. *Water Research*. *6*:989-1008.

Gilmour, C.C., Henry, E.A., Mitchel, R. (1992). Sulfate stimulation of mercury methylation in freshwater sediments. *Environmental Science and Technology 26*(11):2281-2287.

Gilmour, C.C., Henry, E.A. (1991). Mercury methylation in aquatic systems affected by acid deposition. *Environmental Pollution 71*(2-4):131-169.

Glass, G.V., Peckham, P.D., Sanders, J.R. (1972). Consequences of failure to meet assumptions underlying the fixed effects analyses of variance and covariance. *American Educational Research Association 42*(3):237-288.

Gonzáles Castro, M., Abachian, V., Perrotta, R.G. (2009). Age and growth of the striped mullet, *Mugil platanus* (Actinopterygii, Mugilidae), in a southwestern Atlantic coastal lagoon (37°32'S-57°19'W): A proposal for a life-history model. *Journal of Applied Ichthyology 25*:61-66.

Gorski, P.R., Cleckner, L.B., Hurley, J.P., Sierxzen, M.E., Armstrong, D.E. (2003). Factors affecting enhanced mercury bioaccumulation in inland lakes of Isle Royale National Park, USA. *The Science of the Total Environment 304*(327-348.

Grandjean P., Weihe P., White R.F., Debes F., Araki S., Yokoyama K., Murata K., Sørensen N., Dahl R., Jørgensen P.J. (1997). Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicology and Teratology 19*(6) 417–428.

Griffith, G., Bryce, S., Omernik, J., Rogers, A. (2007). Ecoregions of Texas. Project Report to Texas Commission on Environmental Quality. Austin, Texas.

Ground, T.A., Groeger, A.W. (1994). Chemical classification and trophic characteristics of Texas reservoirs. *Lake and Reservoir Management 10*(2): 189-201.

Gu, B., Schelske, C.L., Hoyer, M.V. (1997). Intrapopulation feeding diversity in Blue Tilapia: Evidence from stable-isotope analyses. *Ecology 78*(7):2263-2266.

Gu, B., Schell, D.M., Alexander, V. (1994). Stable carbon and nitrogen isotopic analysis of the plankton food web in a subartic lake. *Canadian Journal of Fisheries and Aquatic Sciences 51*(6):1338-1344.

Guadalupe Blanco River Authority (2018). Basin summary report: Guadalupe River and Lavaca-Guadalupe coastal basins. *Clean Rivers Program.* Seguin, Texas: GBRA.

Gwinn, D.C., Allen, M.S., Johnston, F.D., Brown, P., Todd, C.R., Arlinghaus, R. (2015). Rethinking length-based fisheries regulations: the value of protecting old and large fish with harvest slots. *Fish and fisheries 16*:259.281.

Hall B.D., Aiken G.R., Krabbenhoft D.P., Marvin-DiPasquale M., Swarzenski C.M. (2008). Wetlands as principal zones of methylmercury production in southern Louisiana and the Gulf of Mexico region. *Environmental Pollution 154*(1): 124-134.

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

Hammerschmidt, C.R., Fitzgerald, W.F. (2006). Bioaccumulation and trophic transfer of methylmercury in Long Island Sound. *Archives of Environmental Contamination and Toxicology 51*:416-424.

He, T., Feng, X., Guo, Y., Qiu, G., Li, Z., Liang, L., Lu, J. (2008). The impact of eutrophication on the biogeochemical cycling of mercury species in a reservoir: A case study from the Hongfeng Reservoir, Guizhou, China. *Environmental Pollution 154*: 56-67.

Heckey, R.E., Ramsey, D.J., Bodaly, R.A., Strange, N.E. (1991). Increased methylmercury contamination in fish in newly formed freshwater reservoirs. In Suzuki, T., Imura, N., Clarkson, T.W. (eds.) Advances in Mercury Toxicology Springer, Boston, MA.

Hendrickson, D.A., Cohen, A.E. (2015). Fishes of Texas project database (version 2.0). Published by the Ichthyology Collection of the University of Texas at Austin.

Hensley, D. A., Courtenay, W. R. Jr. (1980). Tilapia mossambica (Peters). pp. 774 in D. S. Lee, C. R. Gilbert, C. H. Hocutt, R. E. Jenkins, D. E. McAllister, J. R. Stauffer Jr., eds. Atlas of North American Freshwater Fishes. North Carolina State Muse.

Hiatt, R.W. (1944) Food chains and the food cycle in Hawaiian fish ponds. Part 11. The food and feeding habits of mullet (Mugilcephalus,) milkfish (Chanoschanos) and the ten pounder (Elops machnata). *Transactions of the American Fisheries Society, 74*: 25 261.

Hobson, K.A., Welch, H.E. (1992). Determination of trophic relationships within a high Artic food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. *Marine Ecology Progress Series 84*:9-18.

Hoeinghaus, D.J., Winemiller, K.O., Agostinho, A.A. (2008). Hydrogeomorphology and river impoundment affect food-chain length of diverse neotropical food webs. *Oikos 117*:984-995.

Ibáñez, A.L., Benítez, G. (2004). Climate variables and spawning migrations of the striped mullet and white mullet in the north-western area of the Gulf of Mexico. *Journal of Fish Biology 65*:822-831.

Jackson, T.A. (1991). Biological and environmental control of mercury accumulation by fish in the lakes and reservoirs of Northern Manitoba, Canada. *Canadian Journal of Fisheries and Aquatic Sciences 48*(12):2449-2470.

Jacot, A.P. (1920). Age, growth and scale characters of the mullets, *Mugil cephalus* and *Mugil curema*. *Transactions of the American Microscopical Society 39*(3):199-229.

Jepsen, D.B., Winemiller, K.O. (2002). Structure of tropical river food webs revealed by stable isotope ratios.*Oikos 96:*46-55.

Jiang, T., Bravo, A.G., Skyllberg, U., Björn, E., Wang, D., Yan, H., Green, N.W. (2018). Influence of dissolved organic matter (DOM) characteristics on dissolved mercury (Hg) species composition in sediment porewater of lakes from southwest China. *Water Research 146*:146-158.

Jiann, K.T., Santschi, P.H., Presley, B.J. (2013). Relationships between geochemical parameters (pH, DOC, SPM, EDTA concentrations) and trace metal (Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn) concentrations in river waters of Texas (USA). *Aquatic Geochemistry 19*: 173-193.

Johnston, T.A., Bodaly, R.A., Latif, M.A., Fudge, R.J.P., Strange, N.E. (2001). Intra- and interpopulation variability in maternal transfer of mercury to eggs of walleye (*Stizostedion vitreum*). *Aquatic Toxicology 52*:73-85.

Kaneko, J.J., Ralston, N.V.C. (2007). Selenium and mercury in pelagic fish in the Central North Pacific near Hawii. *Biological Trace Element Research 119*:242-254.

Latif M.A., Bodaly R.A., Johnston T.A., Fudge R.J.P. (2001). Effects of environmental and maternally derived methylmercury on the embryonic and larval stages of walleye (*Stizostedion vitreum*). *Environmental Pollution* 111:139-148.

Lavoie, R.A., Herbert, C.E., Rail, J., Braune, B.M., Yumvihoze, E., Hill, L.G., Lean, D.R.S. (2010). Trophic structure and mercury distribution in a Gulf of St. Lawrence (Canada) food web using stable isotope analysis. *Science of the Total Environment, 408*:5529-5539.

Lescord., G.L., Johnston, T.A., Branfireun, B.A., Gunn, J.M. (2018). Percentage of methylmercury in the muscle tissue of freshwater fish varies with body size and age and among species. *Environmental Toxicology and Chemistry 37*(10):2682-2691.

Lou, H.-W., Yin, S., Jubb, A.M., Chen, H., Lu, X., Zhang, W., Lin, H., Yu., H.-Q., Liang, L., Sheng, G.-P., Gu, B. (2017). Photochemical reactions between mercury (Hg) and dissolved organic matter decrease Hg bioavailability and methylation. *Environmental Pollution 220*:1359-1365.

Louma, S.N., Rainbow, P.S. (2005). Why is metal bioaccumulation so variable? Biodynamic as a unifying concept. *Environmental Science Technology 39*:1921-1931.

Lund, E.K. (2013). Health benefits of seafood; is it just the fatty acids?. *Food Chemistry 140*:413-420.

Mason, R.P., Choi, A.L., Fitzgerald, W.F., Hammerschmidt, C.R., Lamborg, C.H., Soerensen, A.L., Sunderland, E.M. (2012). Mercury biogeochemical cycling in the ocean and policy implications. *Environmental Research, 119*:101-117.

Mason, R.P. (2009). Mercury emissions from natural processes and their importance in the global mercury cycle. In: Mason R., Pirrone N. (eds) Mercury fate and transport in the global atmosphere. Springer, Boston, MA.

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

Matsumoto I., Wolfee III June, Hoffman D., Ishiga H. (2010). Longitudinal distribution of heavy metals in fluvial sediments of the Trinity River, Texas. *Texas Journal of Science* 62(3):223-236.

Matthews, B., Mazumder, A. (2003). Compositional and interlake variability of zooplankton affect baseline stable isotope signatures. *Limnology and Oceanography 48*(5):1977-1987.

May K., Stoeppler M., Reisinger K. (1987). Studies of the ratio of total mercury/methylmercury in the aquatic food chain. *Toxicological and Environmental Chemistry* 13, 153–159.

Menounou, N., Presley, B.J. (2003). Mercury and other trace elements in sediment cores from Central Texas lakes. *Archives of Environmental Contamination and Toxicology 45*:11-29.

Merritt, K.A., Amirbahman, A. (2009). Mercury methylation dynamics in estuarine and coastal marine environments - A critical review. *Earth-Science Reviews, 96*:54-66.

Minagawa, M., Wada, E. (1984). Stepwise enrichment of δ^{15}N along food chains: Further evidence and the relation between δ^{15}N and animal age. *Geochimica et Cosmochimica Acta 48*:1135-1140.

Mittelbach, G.G., Persson, L. (1998). The ontogeny of piscivory and its ecological consequences. *Canadian Journal of Fisheries and Aquatic Sciences 55*:1454-1465.

Miranda L.E. (2001) A review of guidance and criteria for managing reservoirs and associated riverine environments to benefit fish and fisheries. In G. Marmulla, *Fish and Fisheries:Opportunities, Challenges and conflict resolution* (pp.91-138) Rome: Food and Agriculture Organization of the United Nations.

National Atmospheric Deposition Program (2018). Mercury atmospheric deposition program. *NADP Program Office, Wisconsin State Laboratory of Hygiene, 465 Henry Mall, Madison, WI 53706*. http://nadp.slh.wisc.edu/.

National Research Council (NRC) (2000). *Toxicological Effects of methylmercury*. Washington, D.C.: National Academy Press.

Ouédraogo, O., Chételat, J., Amyot, M. (2014). Bioaccumulation and trophic transfer of mercury and selenium in African sub-trophical fluvial reservoirs food webs (Burkina Faso). *PLOS ONE 10*(4): e0123048.

Pacyna, E.G., Pacyna, J.M., Steenhuisen, F., Wilson, S. (2006). Global anthropogenic mercury emission inventory for 2000. *Atmospheric environment 40*: 4048-4063.

Padfield, J. H., Jr. 1951. Age and growth differentiation between the sexes of the largemouth black bass, *Micropterus salmoides* (Lacepede). *Journal of the Tennessee Academy of Science 26*(1):42-54.

Park, J.D., Zheng, W. (2012). Human Exposure and Health Effects of Inorganic and Elemental Mercury. *Journal of Preventive Medicine and Public Health 45*(6), 344–352.

Parks, J.W., Lutz, A., Sutton, J.A. (1989). Water column methylmercury in the Wabigoon/English river-lake system: Factors controlling concentrations, speciation, and net production. *Canadian Journal of Fisheries and Aquatic Sciences 46*(12):2184-2202.

Pickhardt P.C., Stepanova M., Fisher N.S. (2006). Contrasting uptake routes and tissue distributions of inorganic and methylmercury in mosquitofish (*Gambusia affinis*) and redear sunfish (*Lepomis microlophus*). *Environmental Toxicology and Chemistry* 25(8):2132-2142.

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

Post, D.M. (2002) Using stable isotopes to estimate trophic position: Models, methods and assumptions. *Ecology 83*(3):703-718.

Post, D.M., Pace, M.L., Hairston, N.G. Jr. (2000). Ecosystem size determines food-chain length in lakes. *Nature 405*:1047-1049.

Power, M., Klein, G.M., Guiguer, K.R.R.A., Kwan, M.K.H. (2002). Mercury accumulation in the fish community of a sub-Artic lake in relation to trophic position and carbon sources. *Journal of Applied Ecology 39*:819-830.

Rasmussen , J.B., Rowan, D.J., Lean, D.R.S., Carey, J.H. (1990). Food chain structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*) and other pelagic fish. *Canadian Journal of Fisheries and Aquatic Science 47*:2030-2038.

Raymond, L.J., Ralston, N.V.C. (2009). Selenium's importance in regulatory issues regarding mercury. *Fuel Processing Technology 90*:1333-1338.

Reeves, R.D. (1969). Ground-water resources of Kerr County, Texas. Austin, Texas: Texas Water Development Board. Report 102.

Rice, K.M., Walker, E.M. Jr., Wu, M., Gillette, C., Blough, E.R. (2014). Environmental mercury and its toxic effects. *Journal of preventive medicine & public health 47I:*74-83.

Riisgård, H.U., Hansen, S. (1990). Biomagnification of mercury in a marine grazing food-chain: algal cells *Phaeodactylum tricornutum*, mussels *Mytilus edulis* and flounders *Platichthys flesus* studied by means of a stepwise-reduction-CVAA method. *Marine Ecology Progress Series 62*:259-270.

Roman, H.A., Walsh, T.L., Coull B.A., Dewailly E., Guallar E., Hattis, D., Mariën K,. Schwartz, J., Stern, A.H., Virtanen, J.K., Rice, G. (2009). Evaluation of the cardiovascular effects of methylmercury exposures: Current evidence supports development of a dose-response function for regulatory benefits analysis. *Environmental Health Perspectives 119*(5): 607-614.

Ruelas-Inzunza, J., Kohen-Sandoval, C.K., Ramos-Osuna, M.A., Sánchez-Osuna, K.G., Spanopoulos-Zarco, P., Delgado-Alvarez, C.G., Amezcua-Martínez, F. (2017). Total mercury concentrations in white and striped mullet (*Mugil curema* and *M. cephalus*) from a coastal lagoon in the SE Gulf of California. *Journal of Environmental Science and Health, Part A, 52*(5):459-465.

Ryan, R.P., & Terry, C.E. (Eds.). (1997). *Toxicology Desk Reference 4th Ed*. (Volumes 1-3). (pp.1577-1583). Washington, D.C.: Taylor & Francis,

Scott, W.B., and Crossman, E.J. (1973). *Freshwater fishes of Canada: Bulletin 184*. Ottawa, Canada: Fish Research Board of Canada.

Simon, T.P. (1999).Toward a united definition of guild structure for feeding ecology of North American freshwater fishes. In Goldstean, R.M. & Simon, T.P. (Eds.). Assessing the sustainability and biological integrity of water resources using fish communities (pp.124-139). Boca Raton, FL USA: CRC Press LLC.

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

Sluis, M.Z., Boswell, K.M., Chumchal, M.M., Wells, R.J.D., Soulen, B., Cowan, J.H. Jr. (2013). Regional variation in mercury and stable isotopes of red snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico, USA. *Environmental Toxicology and Chemistry 32*(2):434-441.

Smith A., Abuzeineh A.A., Chumchal M.M., Bonner T.H., Nowlin, W.H. (2010). Mercury contamination of the fish community of a semi-arid and arid river system: spatial variation and the influence of environmental gradients. *Environmental Toxicology and Chemistry* 29:1762-1772.

Somers, K.M., Jackson, D.A. (1993). Adjusting mercury concentration for fish-size covariation: A multivariate alternative to bivariate regression. *Canadian Journal of Fisheries and Aquatic Sciences 50*:2388-2396.

Texas Commission on Environmental Quality (2011). Trophic classification of Texas reservoirs. *2010 Texas Water Quality Inventory and 303(d) List*. pp 1-18.

Thomas CT, Bonner TH, Whiteside BG. 2007. Freshwater Fishes of Texas, A Field Guild. Texas A&M Press: College Station, Texas

Tchounwou, P.B., (2003). Review: Environmental exposure to mercury and its toxicopathologic implications for public health. *Environmental Toxicology, 18*(3):149-175.

Texas Department of State Health Services (2018). Fishing advisories, bans, and FAWs about bodies of water - seafood and aquatic life. Retrieved from http://dshs.texas.gov/seafood/advisories-bans.aspx

Texas Parks and Wildlife Department (2018a). Fish consumption bans and advisories. *Texas Department of State Health Services*. https://tpwd.texas.gov/regulations/outdoor-annual/fishing/general-rules-regulations/fish-consumption-bans-and-advisories.

Texas Parks and Wildlife Department (2018b). Major waterways of Central Texas: Guadalupe River. An analysis of Texas waterways: A report on the physical characteristics of rivers, streams, and bayous in Texas. https://tpwd.texas.gov/publications/pwdpubs/pwd_rp_t3200_1047/17_c_tx_guadalupe.phtml

Texas Parks and Wildlife Department (2018c). Lake Dunlap: Lake Characteristics. https://tpwd.texas.gov/fishboat/fish/recreational/lakes/dunlap/

Ullrich, S.M., Tanton, T.W., Abdrashitova, S.A. (2001). Mercury in the aquatic environment: A review of factors affecting methylation. *Critical Reviews in Environmental Science and Technology 31*(3):241-293.

U.S. Army Corps of Engineers (2018). Fort Worth District: History of Canyon Lake. http://www.swf-wc.usace.army.mil/canyon/Information/History.asp

U.S. Census Bureau (2016a). QuickFacts: City of Kerrville, Texas. U.S. Department of Commerce. https://www.census.gov/quickfacts/fact/table/kerrvillecitytexas/PST045217

U.S. Census Bureau (2016). QuickFacts: City of New Braunfels, Texas. U.S. Department of Commerce. https://www.census.gov/quickfacts/fact/table/newbraunfelscitytexas/PST045217

U.S. Census Bureau (2016b). QuickFacts: City of Gonzales, Texas. U.S. Department of Commerce. https://www.census.gov/quickfacts/gonzalescitytexas

U.S. Census Bureau (2016d). QuickFacts. City of Victoria, Texas. U.S. Department of Commerce. https://www.census.gov/quickfacts/fact/table/victoriacountytexas/PST045217

U.S. Census Bureau (2015). QuickFacts: Canyon Lake CDP, Texas. U.S. Department of Commerce. https://www.census.gov/quickfacts/fact/table/canyonlakecdptexas/AGE775217

U.S. Energy Information Administration (2018). [Texas: State profile and energy estimates]. U.S. Energy Information Administration: State Energy Data System.

U.S. Environmental Protection Agency (2002). Estimated per capita fish consumption in the United States. Washington D.C.: United States Environmental Protection Agency.

U.S. Environmental Protection Agency (1998). Method 7473 (SW-846): Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry, Revision 0. Washington, DC.

Vander Zanden, M.J., Fetzer, W.W. (2007). Global patterns of aquatic food chain length. *Oikos 116*(8):1378-1388.

Vander Zanden, M.J., Rasmussen, J.B. (1999). Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. *Ecology 80*(4):1395-1404.

Vander Zanden, M.J., Cabana, G., Rasmussen, J.B. (1997). Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios ($\delta^{15}N$) and literature dietary data. *Canadian Journal of Fisheries and Aquatic Sciences 54*:1142-1158.

Wang, R., Wang, W.X. (2017). Diet-specific trophic transfer of mercury in tilapia (*Oreochromis niloticus*): Biodynamic perspective. *Environmental Pollution 234*:288-296.

Wang, R., Wang, W.X. (2012). Contrasting mercury accumulation patterns in tilapia (*Oreochromis niloticus*) and implications on somatic growth dilution. *Aquatic Toxicology114-115*:23-30.

Wang, R., Wang, W.X. (2010). Importance of speciation in understanding mercury bioaccumulation in tilapia controlled by salinity and dissolved organic matter. *Environmental Science and Technology 44*(20): 7964-7969.

Ward, D.M., Nislow, K.H., Chen, C.Y., Folt, C.L. (2010). Rapid, efficient growth reduces mercury concentrations in stream-dwelling Atlantic salmon. *Transactions of the American Fisheries Society 139*(1): 1-10.

Ward J.A., Tennant M., Thomas Z., Wiles K., Heideman G. (2006). Characterization of potential adverse health effects from consuming fish from Canyon Reservoir, Comal County, Texas. Canyon Reservoir RC Report. *Texas Department of State Health Services, Policy, Standards, and Quality Assurance Unit Seafood and Aquatic Life Group* and *Division for Regulatory Services* and *Environmental and Injury Epidemiology and Toxicology Branch.*

Webb, J. F., Reeves, W.C. (1975) Age and growth of Alabama spotted bass and northern largemouth bass, pp. 204-215. *In:* Black bass biology and management. H. Clepper, ed. Sport Fishing Institute, Washington, D. C.

Weiner, J.G., Krabbemhoft, D.P., Heinz, G.H., Scheuhammer, A.M. (2003). Ecotoxicology of mercury. In: Hoffman, D.J., Rattner, B.A., Burton, G.A. Jr, Cairns, J. Jr (Eds). Handbook of ecotoxicology. 2nd edition. CRC Press, New York, pp409-463.

Weis J.S., Weis P., Heber M., Vaidya S. (1981) Methylmercury tolerance of killifish (*Fundulus heteroclitus*) embryos from a polluted vs non-polluted environment. *Marine Biology* 65:283-287.

Willacker, J.J., Eagles-Smith, C.A., Lutz, M.A., Tate, M.T., Lepak, J.M., Ackerman, J.T. (2016). Reservoirs and water management influence fish mercury concentrations in the western United States and Canada. *Science of the Total Environment 568*:739-748.

Yako, L.A., Dettmers, J.M., Stein, R.A. (1996). Feeding preferences of omnivorous gizzard shad as influenced by fish size and zooplankton density. *Transactions of the American Fisheries Society 125*:753-759.

Yang, J., Jiang, Z., Qureshi, I.A., Wu, X.D. (1997). Maternal-fetal transfer of metallic mercury via the placenta and milk. *Annals of Clinical and Laboratory Science 27*(2):135-141.

Zeug, S.C., Peretti, D., Winemiller, K.O. (2009). Movement into floodplain habitats by gizzard shad (*Dorosoma cepedianum*) revealed by dietary and stable isotope analyses. *Environmental Biology of Fishes 84*:307-314.