MERCURY MONITORING IN TEXAS ESTUARIES:

A CONCURRENT STUDY WITH THE

NATIONAL COASTAL CONDITION

ASSESSMENT

by

Nicole F. Morris, B.S.

THESIS

Presented to the faculty of

The University of Houston - Clear Lake

in Partial Fulfillment

of the Requirements

for the Degree

MASTER OF SCIENCE

UNIVERSITY OF HOUSTON - CLEAR LAKE
DECEMBER, 2016

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DEDICATION

This manuscript is dedicated to my sister, Fiona, who has selflessly

supported me throughout its writing. It is also dedicated to my parents, from

whom I've learned so much, especially about nature, science, math, and love.

ABSTRACT

MERCURY MONITORING IN TEXAS ESTUARIES:

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Nicole F. Morris, M.S.

The University of Houston - Clear Lake, 2016

Thesis Chair: George Guillen

During June through August of 2015 tissue sampling was conducted on fish from eight Texas estuaries as part of the USEPA's National Coastal Condition Assessment (NCCA). The goal of this study was to assess spatial and temporal trends in total mercury levels in fish muscle tissue and to compare these levels with state and federal human health criteria and screening guidelines. Mercury is a highly toxic heavy metal that can cause many deleterious effects in humans and wildlife. It can be found in the muscle tissue of most fish. Multiple species of fish were analyzed for mercury including Hardhead Catfish, Silver Perch, Gafftopsail Catfish, Gulf Menhaden, Spotted Seatrout, Pinfish, Spot, Atlantic Croaker, and Pigfish. Due to their ubiquitous distribution

the highest number of specimens collected per species during this study came from Atlantic Croaker. Since Atlantic Croaker exhibits many of the typical life history traits of most Gulf of Mexico demersal estuarine fish community it was used as an indicator species for inter-estuarine comparisons. In general, mercury levels in fish muscle tissue have declined since the 1970's in Texas estuaries, as evidenced by declines in mercury from Atlantic Croaker tissue from 0.80 mg Hg/kg ww in 1970 to less than 0.10 mg Hg/kg ww in the 2000's. Mercury in tissue data from fish captured during 2015 was compared with state and federal screening criteria. No fish from the 2015 data exceeded federal or state guidelines. Comparisons of tissue from fish captured in 2005 revealed that Sabine Lake specimens had the highest levels of mercury. It appears that based on the NCCA 2015 summer survey, most Texas bay fish should be safe for human consumption. However, this survey was not intended to replace a detailed health risk assessment used to advise fish consumption advisories. Additional periodic monitoring of mercury in finfish from Texas estuaries and in particular Sabine Lake is recommended.

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INTRODUCTION

Sources of Mercury

In the environment, natural sources account for ~10% of mercury emissions, with anthropogenic sources making up the other ~90% (UNEP 2013). Anthropogenic mercury primarily originates from coal fired power plants, industrial plants, or gold mining operations (Krabbenhoft, 2013). Many of these are classified as point-source inputs, meaning that pollutants from these sources have identifiable discharge points. Once mercury is released from these point sources it is transported through the environment both physically and through bioconcentration and biomagnification in the food chain (Eagles-Smith, 2008; Thera, 2014; Engle, 2008).

The element mercury has no known biological function and has properties that make it universally toxic to organisms at low concentrations (Mcclain, 2006; Mahmoud, 2012). Due to its inherent toxicity and high bioconcentration potential, scientists and the public have an active interest in understanding and preventing mercury contamination. In 2004 the National Science and Technology Council Committee on the Environment and Natural Resources Interagency Working Group on Methylmercury produced a report for the Executive Office of the President of the United States outlining the need for further scientific advancement investigating methylmercury in the Gulf of Mexico region

(Marburger, 2004). In particular, they called for: 1) a review and analysis of historic mercury emissions, 2) a method for active monitoring and measuring of dry deposition of mercury, 3) development of methods for active monitoring and measuring of total and dissolved mercury in streams and rivers including dissolved mercury, 4) increased atmospheric mercury monitoring, and 5) development of sampling and analytical techniques (Marburger 2004).

Point-Sources and Human Impacts

The most infamous case of mercury poisoning attributable to a point source occurred at an industrial facility located adjacent to Minamata Bay in Japan. This catastrophe began in 1953 when a severe outbreak of human methylmercury poisoning hit the Kumamoto Prefecture. Affected individuals suffered damage to the nervous system, especially the cerebral cortex. By the end of the outbreak at least 480 individuals showed signs of pathological changes associated with methylmercury (Eto et al. 2010).

In an effort to determine the source of the outbreak researchers associated with the Kumamoto University School of Medicine analyzed shellfish taken from Minamata Bay. They found that these shellfish contained methylmercury sulfide. The researchers also found that that sludge from the nearby acetaldehyde plant contained methylmercuric chloride, a precursor to the formation of the methylmercury sulfide found in the shellfish. Researchers had established a causal link between the source of mercury, a probable pathway, and the disease, now famously known as Minamata disease (Eto et al. 2010).

In another instance, in Texas, the most famous case of mercury pollution comes from Lavaca Bay, a sub-bay of the Matagorda bay system. Over a period of ~20 years several chemical plants operated from a site close to Lavaca bay near the City of Point Comfort, Texas (USEPA 2016). One plant in particular, the chlor-alkali plant "Alcoa, Inc", operated on this site from 1966 to 1979 (USEPA 2016). Between 1966 and 1970 Alcoa transported wastewater offshore and discharged it into the bay (USEPA 2016). This caused elevated levels of mercury in finfish and crabs. As a result, in 1988, the Texas Department of State Health Services, then the Texas Department of Health, closed ~1 sq mile of Lavaca Bay to fishing and crabbing (USEPA 2016).

In 1994 the site was confirmed as a National Priority (Superfund) and remediation efforts began in earnest. In 2000, after significant remediation, the Texas Department of State Health Services reopened parts of the bay (USEPA 2016c). Finally, in 2012 NOAA featured the restoration efforts in their 200 year celebration page, calling the results of the restoration project "a happy ending" (NOAA 2012).

The tragedy at Minamata and the discharge at Lavaca bay were extreme cases of pollution associated with a point-source mercury source. However, there are still many smaller point-source discharges of mercury in the world today. In Galveston Bay, Texas, it is estimated that 25% of mercury in the bay comes from point-source input (Armstrong and Ward 1992). Of that 25%, 75% or more comes from municipal wastewater discharges.

Non-point Sources

Point source inputs are one of two means through which pollutants can enter the environment. Pollutants can also enter the environment via non-point source input. Non-point sources, in contrast to point sources, are defined as those sources having no easily identifiable single location or origin. Some examples include small spills from ships and vehicles, rainwater runoff, wet deposition via rain, and dry deposition associated with dust particles via wind. However, the ultimate source of mercury transported by dry deposition in the wind may be one or more point sources such as gaseous and particulate air emissions from coal fired power plants (Mcclain, 2006; Cocca, 2001; Engle, 2008).

Indeed, atmospheric deposition of mercury under ideal steady-state conditions with no overwhelming point-source inputs may be the ultimate source of much of the mercury that enters estuarine food chains (Cocca 2001). In Galveston Bay >75% of the total estimated mercury load came from non-point sources and connected tributaries which discharge into the bay (Armstrong and Ward 1992).

Source Identification and Management

The sources of mercury and their detectable impact on an ecosystem have changed over time. Potential sources of mercury have changed as human populations increased, industries evolved, land use was altered, regulations changed, and new treatment technology was implemented. The following

narrative provides a brief history of important events related to mercury source identification, risk assessment and monitoring along the Texas coast.

Prior to the 1970's Galveston Bay was one of the most polluted bodies of water in the United States (Armstrong and Ward 1992). People attributed frequent and massive fish kills which occurred in 1969 to the poor environmental conditions within the bay (Armstrong and Ward 1992). In response to this and rising public interest, the USEPA received a large amount of funding to help solve the problem of Galveston Bay pollution by upgrading sewage treatment facilities in Texas, especially around the bays. Prior to this, many residences dumped raw sewage into the bay directly (Youngblood 2010).

Several laws resulted from this rising concern such as the Federal Water Pollution Control Act of 1948 and later the Clean Water Act of 1972. This important result of public support allowed the USEPA to require permits for wastewater and industry discharge that monitor and control pollutant loadings including mercury (USEPA 2015b). Several other federal laws were also passed around the same time to control mercury and other contaminants in air emissions (Clean Air Act 1970), hazardous waste (RCRA 1976), and illegal spills and contaminated sites (CERCLA 1980). In similar vein, in 1989 a variety of government and private interests established the Galveston Bay Estuary Program tasked with managing the conservation of Galveston Bay (GBEP 2003-2013). One of their priority issues was the control of contaminants in the environment and seafood.

One of the major important outcomes of rising awareness happened in 1990 when the USEPA established and collected data for the very first National Coastal Condition Assessment report. The USEPA published the report in 2001 (USEPA 2001). Today, as in 1990, the researchers on NCCA teams continue their work and produce a condition report on probability-based sampling of all United States estuaries and coastal waters every five years. The condition report provides information on the status of the United States' coasts including biota condition, pollution, and water quality. Mercury is one of the pollutants in fish tissue monitored by this program.

Mercury Analysis

During this same period when regulators were attempting to control mercury loading and impacts, researchers were developing better analytical methods. One of the first methods developed to measure mercury in various media was the Cold Vapor Atomic Absorption Spectrometery (CV-AAS) (Briscoe, 2015). However, the CV-AAS required a large sample volume and had a minimum detection limit (MDL) of only ~0.2 ug/L; very high compared to today's MDL's which are on the scale of nanograms (Briscoe 2015).

During the next ten years from 1980 to 1990 scientists and engineers made more advances to technology for mercury monitoring. Researchers developed two new methods of mercury detection: Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and Cold Vapor Atomic Fluorescence Spectrometry (CV-AFS). The former required much smaller sample volumes of

~5 mL and had a minimum detection limit of ~0.2ug/L. The latter could detect mercury levels as low as ~0.05 ng/L (Briscoe 2015).

By 1990 chemists had developed better analytical techniques and sampling procedures to analyze mercury in environmental media collected from estuaries and coastal areas. In addition, careful examination of historical data collected prior to 1991 by the United States Geological Survey determined that previous surveys collected by that agency and others may have generated exaggerated mercury levels (Armstrong, 1992). The primary cited cause of the inflated values was contamination of samples by use of inappropriate field and laboratory handling procedures (Armstrong and Ward 1992).

Adoption of newer analytical techniques and procedures since 1990 into national and state monitoring programs has made it possible for scientists and agencies to address critical information gaps associated with mercury fate and transport in the environment. For example, in 2011 the USEPA conducted a national-scale mercury risk assessment and promulgated new mercury and air toxics standards for power plants based on more recent environmental monitoring data (USEPA 2011). The 2015 National Coastal Condition Assessment is a part of this historic effort and represents the most current assessment of large-scale mercury monitoring programs.

Mercury Cycling

Atmospheric

The USEPA needed to conduct an atmospheric risk assessment because the atmosphere is a large contributor to total non-point source deposition. This is because mercury discharged into the atmosphere can travel long distances due to air currents before reaching its eventual destination (Marburger 2004, Krabbenhoft 2013). In the United States, the highest level of mercury along the Gulf coast occurs when air currents from the continent blow south to the coast. Lower mercury levels occur when air currents from the Gulf of Mexico blow north to the coast. Researchers speculate that the Gulf coast in particular has a high risk for mercury exposure because the mercury in the atmosphere binds to coarse particles in the air, such as sea-salt, and then falls to the ground with rain (Engle, 2008). The coast sees more of this wet-deposition than elsewhere because of both prevalence of sea-salt and high rainfall. They estimated that coastal areas experience wet deposition of ~7 ug $Hg/m^3/yr$ year versus inland amounts of < 15 ug $Hg/m^3/yr$ (Engle 2008).

Water

Although atmospheric deposition contributes greatly to an estuarine system's total mercury loads, there are a variety of other factors which can influence the amount of mercury in an individual area. In an estuary these factors include size of the drainage basin, anthropogenic influences, minerals present in sediment, atmospheric loading, redox environments, and the amount of methylating bacteria. Flat-plain estuaries located along the Gulf of Mexico coast

have very large drainage basins that capture a potentially large amount of waterborne and atmospheric inputs sources and can therefore concentrate large amounts of heavy metals and other pollutants (Teuchies 2013). The Galveston Bay watershed has a watershed that extends up to the Dallas Fort Worth area and its immediate drainage below Lake Livingston and Lake Houston encompasses ~600 sq. miles which is furthermore affected by shipping, the petrochemical industry, channel dredging, and urban development (Youngblood 2010). Pyrite in bottom sediment may also increase the amount of mercury in a system (Huerta-Diaz 1992).

Although these factors all influence the amount and availability of mercury in an area it appears that direct input, either via point-sources or non-point sources, may cause the greatest increase in mercury levels. In Ontario, researchers deposited mercury onto the surface of an experimental lake (Harris, 2007). They detected a spike in mercury levels in the bottom waters of the lake 3 days later. They also detected mercury spikes in the sediment at 2 to 4 weeks and spikes in the biota (fish) within 2 months (Harris 2007). When testing for mercury levels in runoff water, they found that the highest concentrations present were ~100 times less than ambient lake levels of mercury in, and much less than concentrations measured after direct deposition (Harris 2007).

All of the previously discussed factors taken together will influence the final overall distribution and concentration of mercury present in the system. Zapp-Sluis (2013) found that ambient mercury levels were more accurately

predicted by dividing research areas by geographical region rather than by focusing on the presence of potential sources such as oil rigs.

Methylation

Many of the factors that influence the amount of bioavailable mercury present in a system are linked to the exposure of bacteria to inorganic mercury (Hall et al. 2008). These bacteria are found in estuary soils and transform inorganic mercury into methylmercury, the form of mercury most commonly found in organisms (Benoit 1999). Anthropomorphic sources do not commonly emit methylmercury but rather the precursor to methylmercury, elemental mercury (Marburger 2004). The process of transformation starts with methylating bacteria in the sediment using the elemental mercury and producing the highly toxic and bioaccumulative methylmercury (Marburger 2004, Marvin-Dipasquale 2000). Estuaries are particularly important sources of methylmercury since associated wetlands normally contain high densities of methylating bacteria (Marburger 2004, Marvin-Dipasquale 2000).

Ambient conditions, including those affecting mercury input into the system as well as those linked to bacteria, may affect the amount of methylmercury found in any one estuary. For example, researchers found that sulfur plays a role in the methylation of mercury. In pore waters of estuarine soil, methylating bacteria accumulate mercury in the form of HgS^0, which they transform it into methylmercury (MeHg) which is the common form that bioconcentrates in the tissues and organs of fish (Benoit 1999). When pH levels are more basic (>7) and sulfur concentrations are low, methylmercury

degradation increases (Marvin-Dipasquale 2000). In Galveston Bay, greater than 95% of total dissolved mercury in surface water binds to dissolved organic material or sulfide under anoxic conditions (Han 2004). Submerged sediment in estuarine wetlands commonly possesses reduced, anoxic conditions, acidic pH levels, and high sulfur concentrations which create an ideal substrate for the formation of HgS^0 and eventual production of methylmercury by sediment bacteria. In contrast, conditions leading to high dissolved oxygen, basic pH, and low sulfur will result in higher degradation rates of methylmercury into the less toxic Hg(II) (Marvin-Dipasquale 2000).

Food Webs

The amount of mercury present in an organism, especially methylmercury, broadly determines the risk of harm to the organism (Thera and Rumbold 2014). Top-level predators, in particular, are at greatest risk due to the amount of mercury they may ingest (Thera and Rumbold 2014). Since methylmercury bioaccumulates any amount originating in the soil may enter into benthic organisms or plants which can then transfer through the food web into other bottom feeders, detritivores, or herbivores. Ultimately low-trophic level feeders are then eaten by predators. Therefore, mercury present in small amounts in lower trophic levels increases in concentration at higher trophic levels (Thera and Rumbold 2014). Due to biomagnification of mercury in aquatic and terrestrial food webs, problems have arisen in Gulf of Mexico species such as fish-eating birds, raccoons, alligators, and even endangered species such as the Florida panther (Marburger 2004). There also currently exists an advisory from the

Texas Department of State Health Services for Texas waters of the Gulf of Mexico limiting human consumption of a variety of top predators including Blackfin Tuna (*Thunnus atlanticus*), Blue Marlin (*Makaira nigricans*), Crevalle jack (*Caranx hippos*), and others (Lakey 2013).

There are many examples of mercury biomagnification occurring in estuarine ecosystems. In 2013 researchers conducted two studies, one on the Lower Chesapeake Bay, and another on the Florida coast (Xu, 2013; Thera, 2014). Both studies tested the amount of mercury in marine organisms based on trophic level. In Chesapeake Bay the researchers collected ~300 samples and determined that mercury increased 10-fold for each trophic level position (Xu et al. 2013). On the Florida coast they determined that mercury increased ~5-fold via biomagnification. In Florida researchers used 40 finfish samples as well as invertebrates (Thera and Rumbold 2014). Therefore, depending on location and route, a ~5-fold to a 10-fold increase in mercury concentrations can be expected with each increase in trophic level.

Researchers are interested in determining whether lower trophic level species can be used to predict mercury concentrations in higher tropic levels (Xu, 2013; Thera, 2014). The same researchers in 2013 and 2014 compared observed mercury levels in a low-trophic species, the Atlantic Croaker (*Micropogonias undulatus*), with predicted levels based on trophic bioconcentration models of mercury bioaccumulation (Xu, 2013; Thera, 2014). Xu et al (2013) concluded that Atlantic Croaker in Chesapeake Bay had comparatively high levels of mercury for their trophic position compared to

predicted expected values and observed environmental levels (Xu et al. 2013). In contrast, predicted values of mercury in tissue in Atlantic Croaker from Florida were very similar (high r^2) to observed levels of mercury for their respective trophic position (Thera and Rumbold 2014). These studies indicate that depending on location, season, salinity, and other factors, the bioconcentration of mercury in Atlantic Croaker is not constant and therefore may not always be a good predictor or correlated well with mercury levels in organisms or life stages inhabiting higher trophic levels.

Ecological Effects

In addition to affecting humans, mercury can also negatively affect fish and wildlife directly. Fish in particular may experience negative reproductive and population effects from exposure to mercury, although what effect this might have on commercial harvest and the fishing industry is poorly documented (Marburger 2004). Direct effects include increased mortality, lower population numbers, and less biomass. If a fish advisory is issued, various effects on the fishery may occur including reduced landings, consumer demand, and earnings (Mahmoud et al. 2012). Mercury has been shown to cause liver, kidney, and protein malfunction in the African catfish *Clarias gariepinus* (Mahmoud 2013).

In general, methylmercury will bioaccumulate to higher concentrations in fish possessing life histories that include larger sizes, slow growth, and who inhabit higher trophic levels (McClain 2006). As a result, for any given organism, bioaccumulating sufficient mercury to cause deleterious effects may fluctuate seasonally as food sources and abundance change (Loftus 2000). Recently, the

USEPA has become more interested in developing fish contaminant indicators in the form of fish tissue screening levels that will not only protect humans, but also provide protection to the target species of aquatic organism (Bowersox et al. 2015).

In order to assess the risk from mercury exposure in seafood public health officials and scientists have recognized the need to establish a systematic monitoring program to measure the bioaccumulation of methylmercury in a representative or "sentinel" or "indicator" fish species as well as a need to collect and archive existing data on Gulf of Mexico fish tissue and ambient mercury concentration data (Marburger, 2004). Comprehensive information on the distribution of mercury in Gulf fish assemblages is currently lacking (Marburger 2004). Marburger (2004) further recommended that until new monitoring programs are established analyzing currently available historical data may provide sufficient data to conduct early risk assessments.

Human Exposure

The greatest exposure and risk from mercury to human health occurs when humans consume mercury that has bioconcentrated in fish and shellfish (Thera and Rumbold 2014). Consuming mercury in excess of human health guidelines can potentially cause damage to the brain, similar to the Minamata incident (Loftus 2000).

Humans living in coastal areas and/or who consume large amounts of seafood are particularly susceptible to exposure to high levels of mercury. People most affected include individuals who eat local finfish, oysters, crabs and shrimp

in areas containing high levels of mercury in the environment. In the United States, adults consume on average 15lbs/yr of fish and shellfish (Marburger 2004). However, fishermen and traditional or subsistence groups may consume more than this (Marburger 2004). Women of child bearing age are at the greatest risk due to the developmental toxicity of mercury to the undeveloped child (Marburger 2004). The USEPA advises that individuals follow recommendations and advisories for their local waterbody (USEPA 2016a).

Comparison of Monitoring Data to Screening Levels and Criteria

USEPA creates recommendations based on historical mercury events, but there are many challenges to using historical data that they, and other researchers, must address (USEPA, 2015). One of the challenges to historical analysis of mercury is that researchers have reported the amount of mercury present in organisms and seafood using many different methods and have compared these values to a variety of different numerical standards. Fish can be processed for mercury either skin on or skin off, filets or plugs, muscle tissue or liver tissue. Researchers may analyze and report total mercury or methylmercury. The fish tissue can either be analyzed directly resulting in a wet weight (ww) or dried first resulting in a dry weight. Further complicating the process of collecting long-term mercury data, many different agencies have different standards for mercury. Agencies providing health risk standards that may apply to Texas include the U.S. Food and Drug Administration (FDA), U.S. Environmental Protection Agency (USEPA), Texas Department of State Health

Services (TDSHS), and the Agency for Toxic Substances and Disease Registry (ATSDR).

In order to understand how data is compared to these numerical standards it is important to review various terms used in mercury risk assessment. Mercury risk assessment includes the process of determining the level of risk of adverse effects or danger present to the health and wellbeing of humans, organisms or the environment. Often researchers complete a more comprehensive risk assessment after establishing a numerical criterion based on non-regulatory screening values associated with some threshold associated with deleterious effects in the target organism. Numerical criteria are values often including a magnitude and an exposure duration that support actual standards that are enforceable by a regulatory agency; in this case commonly the USEPA. States often adopt USEPA standards although some states may choose to develop their own criteria (USEPA, 2016d). For example, for water quality the USEPA has both narrative and numerical standards which may be adopted by states, territories, and authorized tribes (USEPA, 2016d). On the other hand, screening values are usually developed from case studies or from published literature (Ford and Beyer 2014; Plata et al. 2009; Salatas et al. 2004). Using these definitions, the USEPA daily consumption limit is a screening value, whereas the commercial limit – the limit at which an agency would take steps to remove a fish from the market – is a criteria (USEPA 2008) (Table 1). The reported marine fish toxicity level would also be considered a screening value

since it is derived from available scientific literature but is not legally enforceable

by any current agencies (Bowersox et al. 2015) (Table 1).

Table 1 Agency-reported limits to mercury.

Limits to Mercury Consumption (wet weight)	Concentration
Human Health Daily Consumption Limit	
USEPA	0.0001 mg/kg
ATSDR	0.0003 mg/kg
Commercial Human Health Limit	
USEPA standard	0.30 mg/kg
TDSHS standard	0.70 mg/kg
FDA action level	1.00 mg/kg
Marine Fish Health	
Marine Fish TRL	0.31 mg/kg

USEPA = United States Environmental Protection Agency (USEPA 2008), ATSDR = Agency for Toxic Substances and Disease Registry (GHASP 2004), TDSHS = Texas Department of State Health Services (TDSHS 2015), FDA = United States Food and Drug Administration (FDA 1979), TRL = Toxicity Reference Level (Bowersox et al. 2015)

The availability of various criteria and screening levels, types of mercury data (e.g. whole fillet, whole fish), and analytical methods, can become a major source of confusion when trying to assemble comparable data sets to evaluate trends in mercury in tissue and compliance with numerical standards . For example, when analytical detection limits are higher than current or future numerical criteria or screening levels, it is very difficult to assess risk from exposure to mercury since a large percentage of the data is reported as "less than" values. It is impossible to determine if the non-detect values are below the criteria or above it.

There are other major difficulties with compiling and comparing data for determining risks from exposure to mercury. First databases created by different monitoring programs do not necessarily contain all variables (species, location, date, type of mercury analysis) of interest that may be necessary to compare temporal time series. Second, when a database contains all necessary variables of interest there are often differences in reporting units (e.g. mg/kg wet weight mercury versus mg/kg dry weight mercury) that must be converted prior to comparison.

McClain et.al. (2006) published a paper addressing the differences in the United States Environmental Protection Agency standards for mercury versus the Texas Health Department standards. They found that the Texas Health Department (TDH, now TDSHS) required tissue samples from three fish per species. If the value exceeds a mean of 0.7 mg/kg wet weight total mean mercury an advisory, regardless of fish ages or lengths, is issued (McCain 2006). If the specimens are small this may generate a biased estimate of the amount of mercury in the target fish species. This is due to smaller fish having less mercury than larger fish within a species. In contrast, the USEPA recommends fish be divided into size classes and that the smallest individual should be no smaller than 75% total length of the largest individual prior to generating mercury values. The USEPA criteria requires that each size class not exceed 0.3 mg Hg/kg wet weight. Furthermore, USEPA uses only one species of fish as the representative predator and one as representative detritivore (McClain 2006). The USEPA does allow variation between species due to average life span and growth rate

(McClain et al. 2006). Based on their analysis of 267 fish from 7 freshwater species they found that many sport fish exceeded USEPA guidelines for mercury but failed to exceed Texas Health Department guidelines (McClain 2006).

Cocca (2001) attempted to estimate total mercury loading and tissue concentrations as well as provide access to data that could be used for environmental prioritization and future legislation. Cocca (2001) created maps depicting mercury contamination across the United States in order to better understand areas in need of immediate management intervention and remediation. He also noted that two most common types of historical mercury in fish data were: whole fish and fish fillets, either with skin-on or off. For his analysis he chose to use both skin on and skin off fillets because most human beings consume some skin and tissue and the determination of how much mercury was actually consumed was the primary research question (Cocca 2001).

Significance

This study attempted to overcome these obstacles in part by using both skin-on and skin-off fillets and transforming numerical values into one unit of measure. It then used this to assemble a database of diverse data sets and current study results by analyzing currently available historical data along with new data collected in 2015 as part of the Texas portion of the National Coastal Assessment. The compiled database will be useful in the future to scientists and managers interested in long-term spatial and temporal trends and the management of mercury pollution and potential impacts to human health and

ecological resources. The objective of the study was to only compare data that is, "consistent, comparable, and collected using accepted methods" to provide a greater view of the trends and status of mercury in the bays and estuaries of Texas and the Gulf of Mexico (Kaough 1998).

The Texas coastline extends 3,359 miles and supports multiple industries contributing $121.3 billion to the state's annual gross domestic product (NOAA 2016). As of August, 2016, there are 14 different seafood consumption advisories for mercury in Texas waterways - these include all coastal Texas waters as well as thirteen different freshwater sites in Central Texas, East Texas, the Panhandle, and Houston-Galveston areas (TPWD 2015-2016). The most recent assessment of mercury in Texas finfish indicates levels exceed USEPA standards 19% of the time (Harvey et al. 2008). This work will examine a variety of species of fish from Texas coastal waters to fill in gaps in our knowledge of current and historical mercury in these important areas.

Indicator Species: Atlantic croaker

This study includes other species of fish but my analysis of historical data will focus on Atlantic Croaker because of the abundance of historically available data from this species. Since collection of the sufficient number of specimens of any particular species is often difficult, the use of common ubiquitous "indicator species" that represent major species trophic guilds and/or are utilized by human beings is often employed.

The Atlantic Croaker, *Micropogonias undulatus*, is a lower-trophic level, small sciaenid found over sandy or mud bottoms in estuaries of the Gulf of

Mexico up to the northeastern coast in Delaware and Chesapeake Bays. It gets

its name from the "drumming" or "croaking" sound it produces using specialized

muscles that beat against the fish's swim bladder (Gannon 2007). The largest

Croaker are ~55.0 cm total length, and up to 2.6 kg (IGFA 2001). The average

Croaker are less than one foot, or about 30.0 cm (Chao 1978). Researchers

debate the maximum age for Croakers, but many agree on lifespans up to 5

years (Hugg 1996). In the estuaries where they are found they are opportunistic

feeders and eat a variety of items including other small fish, decapods,

polychaetes, and non-decapod crustaceans (Akin and Winemiller 2012; Nye et

al. 2011; Willis et al. 2014). Croaker spawn offshore on the continental shelf and

the young are brought into estuaries and seagrass beds by inshore currents

(Akin and Winemiller 2012; Willis et al. 2014). These influxes of Croaker occur

annually during the winter months, mainly December (Akin and Winemiller 2012).

Researchers have found evidence to suggest that the numbers of Croaker are

also influenced by a decadal pattern of abundance driven by climate variability.

Global climate change may further influence these decadal patterns (Nye et al.

2011). Due to commonality and ease of collection (e.g. hook and line, trawl,

seine) this species is readily available for collection and testing for mercury in

their tissue. In addition, Atlantic Croaker are commercially harvested along the

Gulf and Atlantic coasts of the United States. In 2014 a total of 77,724 pounds of

Atlantic Croaker was commercially harvested in Texas (NMFS 2014).

The Atlantic Croaker, along with other fish such as Spot (*Leiostomus

xanthurus*), Gafftopsail Catfish (*Bagre marinus*), Hardhead Catfish (*Ariopsis*

felis), Pinfish (*Lagodon rhomboides*), Silver Perch (*Bairdiella chrysoura*), Sand

Seatrout (*Cynoscion arenarius*), Spotted Seatrout (*Cynoscion nebulosus*), Red

Drum (*Sciaenops ocellatus*), and Southern Flounder (*Paralichthys lethostigma*),

were also recommended target fish species for the 2015 National Coastal

Assessment (NCCA 2010).

OBJECTIVES

Objective 1: Sample tissue and determine mercury concentration in all fish species collected as part of the 2015 May to August Texas portion of the National Coastal Condition Assessment project in Texas bays.

Objective 2: Assemble a database of verifiable, quality controlled, reproducible historical and present mercury data from all species of fish from the 1970's to 2010.

Objective 3: Determine trends from 1970 to 2010 in levels of mercury in Atlantic Croaker from Texas bays.

Objective 4: Determine frequency of exceedance of existing state and federal mercury fish consumption levels and ecological thresholds for target species collected.

METHODS

Site Overview and Field Sampling

Fish were collected during the months of May to August 2015 as part of the National Coastal Condition Assessment project conducted by the students and staff of University of Houston - Clear Lake's Environmental Institute of Houston (UHCL) and sponsored by the United States Environmental Protection Agency (USEPA) and the Texas Commission on Environmental Quality (TCEQ). All fish were collected according to the Institutional Animal Care and Use Committee's (IACUC) guidelines and regulations (Appendix 1). Sample sites included those from several bays and estuaries of Texas (Figure 1 and 2).
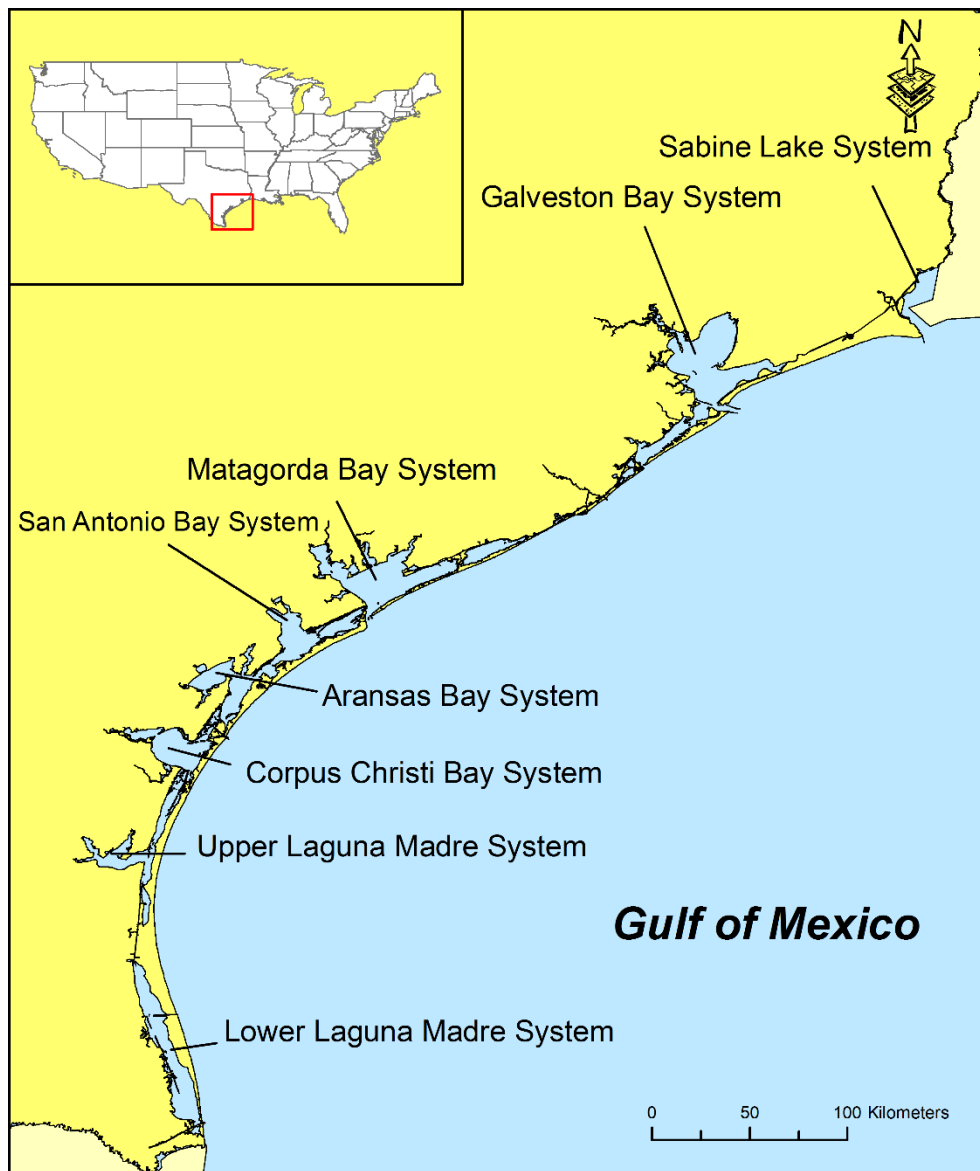
Figure 1 Sampled estuaries in Texas sampled during June-August 2015.
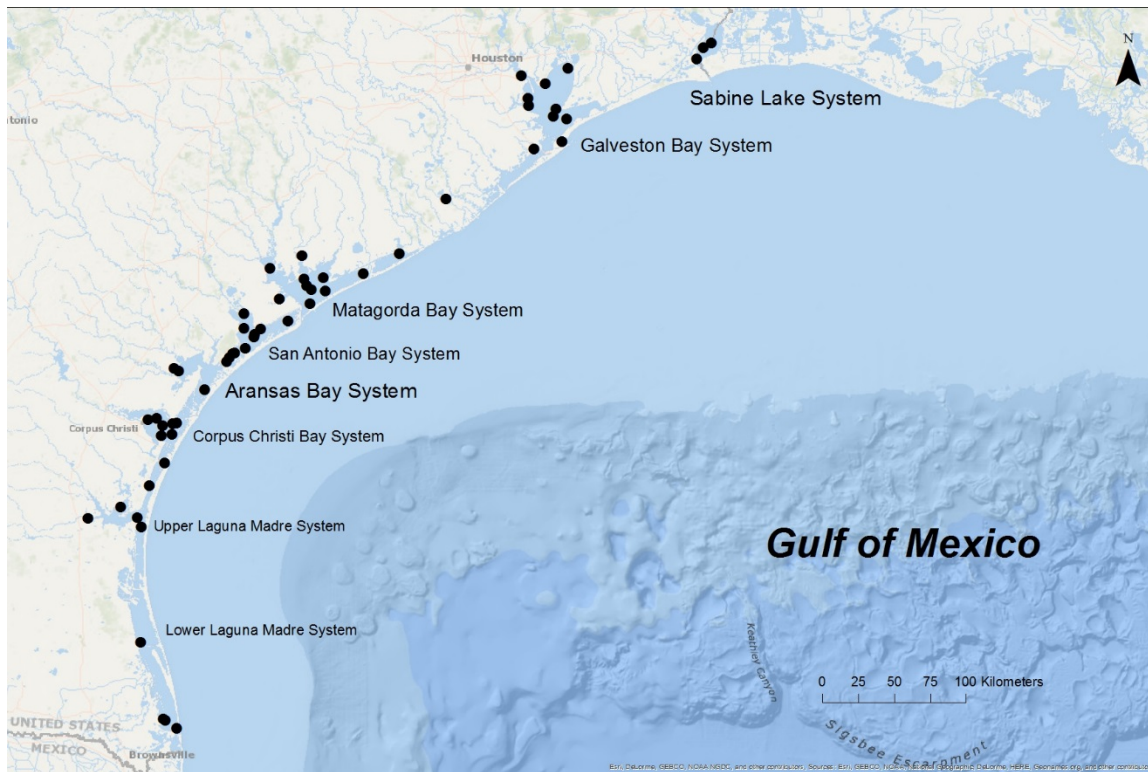
Figure 2 Sampled sites for fish for mercury analysis; June-August 2015.

Fish were mainly collected using an otter trawl and occasionally collected using a rod-and-reel method. Fish collected by otter trawl were caught from a 22 foot catamaran-type vessel using an otter trawl with mesh size of 3.2 cm and opening size of approximately 3.0m by 0.3m. Trawls were pulled for 5 to 15 minutes at ~2-3 knots. Replicate tows were conducted until a sufficient number of fish had been caught to satisfy National Coastal Condition Assessment target requirements (Appendix 2). Once fish were collected they were placed into a sorting basket (Figure 3). The largest fish of several species were collected and placed into zip-lock bags and placed on dry ice, cooler space permitting, or placed on wet ice (Table 2). Species were chosen and ranked based on the

NCCA recommended target fish species list as well as availability of historical

data. Where available, multiple species from this list were taken for sampling.

Table 2 Species ranking list, species ranked based on the NCCA recommended
target fish species list as well as availability of historical data (Espinosa-Pérez,
2013).

	Fish Priority List for Mercury Project	Scientific Name
1	Atlantic Croaker	*Micropogonias undulatus*
2	Spotted Seatrout	*Cynoscion nebulosus*
3	Sand Seatrout	*Cynoscion arenarius*
4	Red Drum	*Sciaenops ocellatus*
5	Black Drum	*Pogonias cromis*
6	Spot	*Leiostomus xanthurus*
7	Silver Perch	*Bairdiella chrysoura*
8	Hardhead Catfish	*Ariopsis felis*
9	Gafftopsail Catfish	*Bagre marinus*
10	Pinfish	*Lagodon rhomboides*
11	Gulf Menhaden	*Brevoortia patronus*
12	Pigfish	*Orthopristis chrysoptera*

Figure 3 2015 June-August NCCA sampling; example of work on the boat sampling fish; counting and determining species.

Once placed on either wet or dry ice in the field, samples were taken back to the laboratory and placed in a freezer, still in the original plastic zip-locks. Once fish were ready to be processed they were defrosted. The defrosted fish were weighed to the nearest hundredth gram and measured to the nearest millimeter standard length (SL). Five individuals of each species were selected, if available; larger specimens were preferentially selected for easier plugging. Fish not being presently handled were covered with a damp cloth to prevent drying out. When at least two people were available the USEPA standard "clean hands/dirty hands" method was used (Appendix 3). Where only one person was processing samples a similar method which involved one hand being the "clean hand" and one hand being the "dirty hand" was used. When there was one

person gloved hands were washed with distilled water after opening sterile packages and prior to handling fish.

Fish with scales had scales removed, scales were wiped off of the sterile scalpel blade by the "dirty hand". When fish were large enough an 8mm biopsy punch was used to take a sample from the left epaxial muscle of the fish (head pointing left). Two punches were taken whenever possible to allow for replicates. Biopsies were carefully taken so as to exclude blood or bones. Skin-on fillets were taken from smaller fishes. For catfish, biopsies included the skin; fillets either included the skin or did not. Biopsy plugs and scalpels were discarded and new ones used after every species of fish processed. Gloves were discarded after every sample site and rinsed with distilled water after every species. Every effort was made to prevent contamination of either scalpel or biopsy plug; if the biopsy plug or scalpel became contaminated a new one was used. Ideally, nothing but the fish muscle and skin to be sampled touched the biopsy plug. Occasionally a scalpel was necessary to remove tissue from the plug. When this was necessary care was taken to use only the tip of the scalpel so as to limit any potential contamination left by the scales on the scalpel. All tissue taken by biopsy was stored in plastic sterile cryogenic vials and placed in a -80 freezer that was monitored weekly until samples were ready for submittal for total mercury analysis.

Laboratory Processing

Samples were removed from the -80 freezer and dried, still in vials, in a 60 degree Celsius drying oven for at least 48 hours. These dry samples were then

processed using a DMA - 80, Direct Mercury Analyzer at Texas Christian

University under Dr. Matt Chumchal (Figure 4).

Figure 4 DMA-80, aka Direct Mercury Analyzer, photo taken March 2016.

This process involves a series of thermal decompositions, then absorption

by a catalyst, and finally atomic absorption spectrometry to detect trace amounts

in mg/kg of total mercury in dry samples (Figure 5).
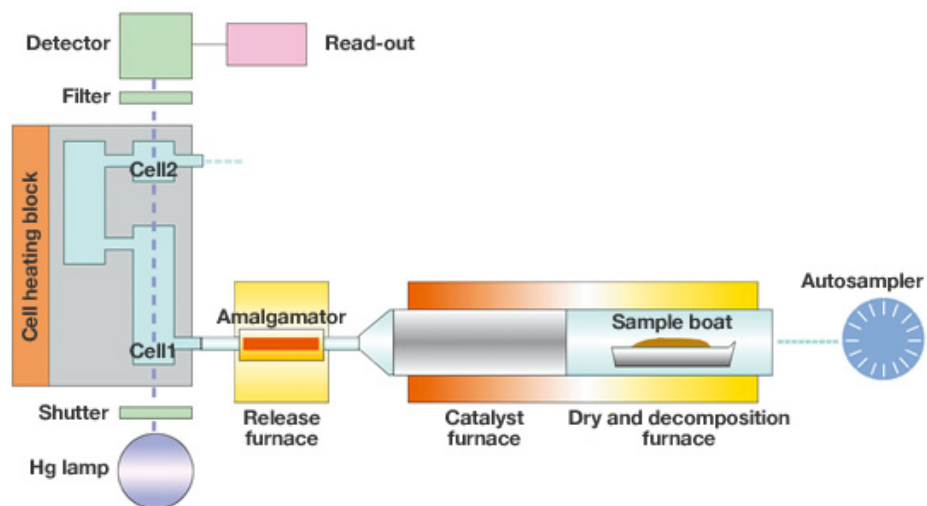
Figure 5 Direct Mercury Analyzer procedures (Milestone, 2014).

To load the samples into the DMA – 80 sterile techniques were used

including laying out aluminum foil to prevent contamination of sample boats from

laboratory counters and using sterile forceps cleaned with ethanol to transfer sample between the vial and boats. Foil was replaced after every sample and forceps were cleaned between every sample.

Several methods were used to determine instrument precision including duplicate samples and blanks run at regular intervals. To determine accuracy two standards were used in regular intervals; DORM-2, a fish protein standard for the low level of detects, and PACS-2, a marine sediment standard for the high levels of detects.

Data Analysis

Total mercury was used as a proxy for methylmercury following standards set by the USEPA and reported in Wiener in 2003 (USEPA 2015a; Wiener et al. 2003). Dry weight of mercury was converted to wet weight of mercury by dividing by 4.7 (Fry and Chumchal 2012). Mercury content was averaged for samples in which duplicates were run. Calculated from the returned percent recovery of standards, the mean percentage of recovery was 92%, with a standard deviation of 0.06 for PACS and 0.03 for DORM. The mean relative percentage of difference between duplicates was 96% similar, ± 6% (mean ± 95% CI) (Appendix 4. DMA-80 Raw Data).

Any Atlantic Croaker lengths reported in total length were converted to standard length using equations published in Matlock et. al. (1975).

All data were statistically and graphically analyzed using Minitab (Minitab 17 Statistical Software 2010, State College, PA; Minitab Inc.). Historical data

included only data for Atlantic Croaker collected from Galveston and Matagorda bays. For this historical data the Mann-Kendall nonparametric test was used to test for monotonic trends over time for mercury in Atlantic Croaker tissue in Galveston and Matagorda bays (Kendall 1948; Mann 1945). This test is a ranked test that plots actual values. Readings take on the same date are plotted alongside one another in rank order.

For the 2015 data values below the detection limit (non-detects) accounted for 15 out of 395 data points and were analyzed using two approaches including substituting ½ the detection limit and using a method outlined in Kruskal-Wallis (Kruskal and Wallis 1952). Little difference was found in the final results between the two methods so the simpler ½ detection limit replacement method was used. The Kruskal-Wallis non-parametric test was used to test for overall differences in median mercury tissue levels by bay system, location on coast, species, trophic level. When an overall statistical differences between categories were found the Dunn's multiple comparison test was also run to determine which groups differed (Dunn 1961). Mercury content was reported in units of mg/kg total mercury dry weight and each fish was individually compared to published human and ecological criteria, screening values and thresholds.

Fish trophic levels were estimated from a variety of studies reported in Fishbase, an international database containing species-specific information (Froese et al. 1992). Fishbase uses trophic equations to assign species to specific trophic categories commonly used by fisheries ecologists (Eagles-Smith

et al. 2008; Froese et al. 1992). Trophic categories were assigned based on

trophic level with 2-3 as herbivores, 3-4 as omnivores, and 4+ as carnivores

(Table 3).

Table 3: Species trophic levels, adapted from Fishbase (Froese et al. 1992) and
categorized by trophic level.

Species Trophic Levels	
Species	Trophic Level
Herbivores	
Gulf Menhaden	2
Pinfish	2.9
Omnivores	
Spot	3.2
Hardhead Catfish	3.3
Atlantic Croaker	3.4
Pigfish	3.4
Gafftopsail Catfish	3.5
Silver Perch	3.6
Carnivores	
Sand Seatrout	4.1

RESULTS

Trends over Time

Current consumption limits as set by a variety of agencies are depicted in Table 1. Historically, there were only two instances of Atlantic Croaker exceeding today's FDA commercial limits and USEPA limits (Figure 6). No fish tissue sampled during the summer of 2015 exceeded any FDA commercial limits and USEPA limits.

Overall, the USEPA has the most stringent levels at 0.0001 mg Hg/kg ww daily average based on human weight. They also set the most stringent commercial limits at 0.30 mg Hg/kg ww. The most lenient commercial limit was the FDA action level of 1.00 mg Hg/kg ww at which the FDA would take steps to remove the fish from market (FDA 1979). These human health exposure limits were used as reference points in evaluating trends in mercury risk. Bowersox et al. (2015) estimated an ecological threshold of 0.31 mg Hg/kg ww above which mercury would begin to effect fish health and survival.

Historical Trends in Mercury in Atlantic croaker by Bay System

Overall, only two bay systems contained sufficient historical data from other agencies and sources to observe changes in mercury concentrations in Atlantic Croaker muscle tissue over time. These bay systems with agency

historical data were Matagorda Bay and Galveston Bay. Both bay systems displayed a statistically significant downward trend (Figure 6). For Matagorda Bay, Atlantic Croaker exceeded the USEPA commercial limit on two occasions; in 1970 and 1971 with mercury levels of 0.85 mg Hg/kg ww and 0.35 mg Hg/kg ww respectively (Figure 6). 0.85 mg Hg/kg ww also exceeds the TDSHS commercial limit. No Galveston Bay Croaker exceeded commercial limits (Figure 6). The peak in Galveston Bay in 2004 represents a Croaker caught off Smith Point, TX, with a mercury concentration of 0.162010 mg Hg/kg ww (Figure 6).
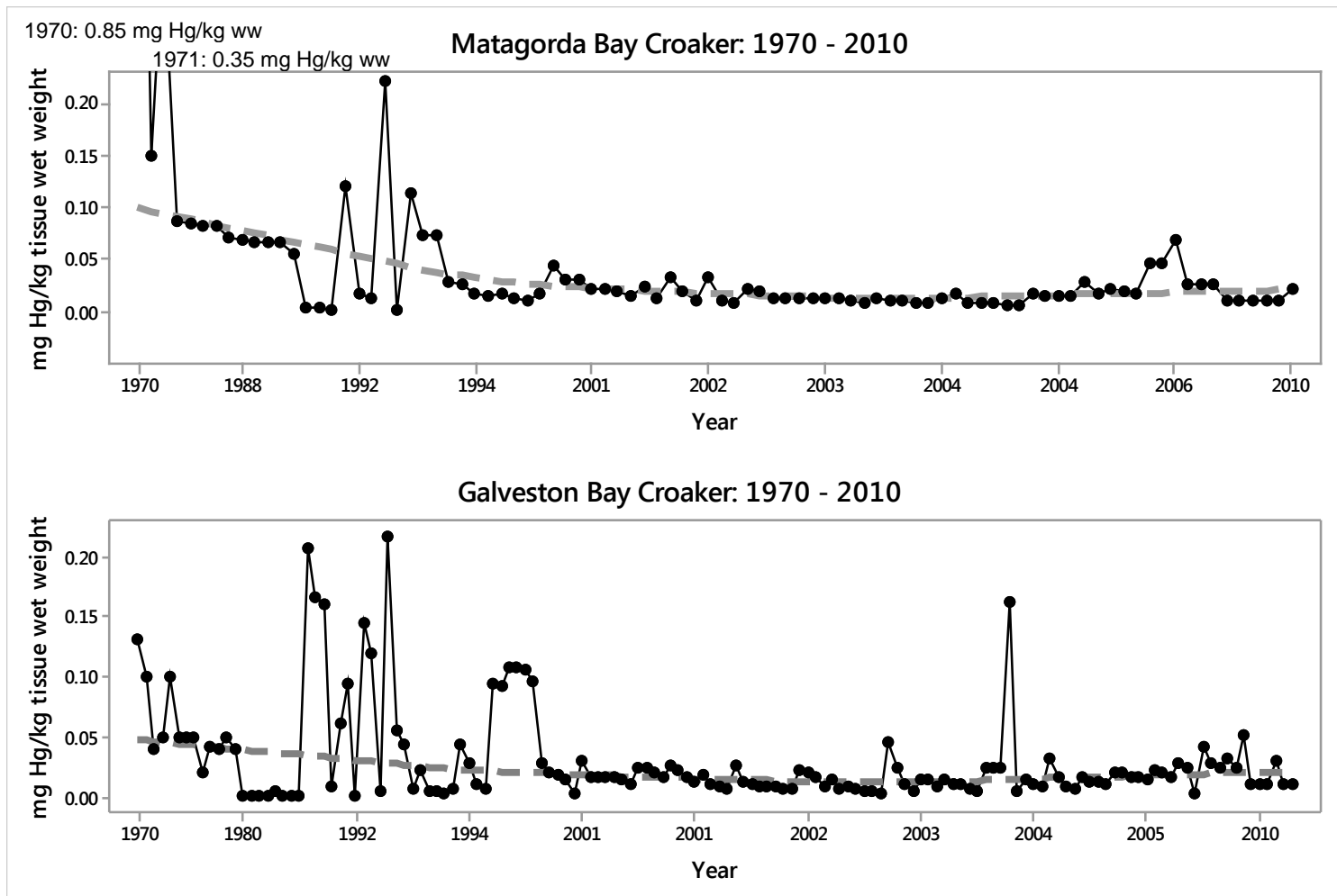
Figure 6: Matagorda Bay Croaker 1970-2010 and Galveston Bay Croaker 1970-2010; Mann-Kendall nonparametric ranked trend test showing trend line (dashed) for Matagorda (p<0.000), and Galveston Bay (p<0.025).

Comparison of Bays: All Species Combined

Statistically significant differences in overall median mercury levels were detected between bay systems and in subsequent pair-wise comparisons (Table 4). The lowest number of fish caught and sampled came from Sabine Lake whereas the most fish caught and sampled came from Matagorda Bay system (Figure 7). The highest measured single mercury level was found in tissue from Gafftopsail Catfish from the Galveston bay system (~0.29 mg/kg wet weight) (Figure 7). Fish from the Sabine Lake system has a statistically higher median amount of mercury in their tissues (Figure 7 and 8). Although not statistically significant, fish caught from the Aransas Bay system had the lowest amount of mercury in their tissue (Figure 7 and 8). No fish exceeded any commercial limits.
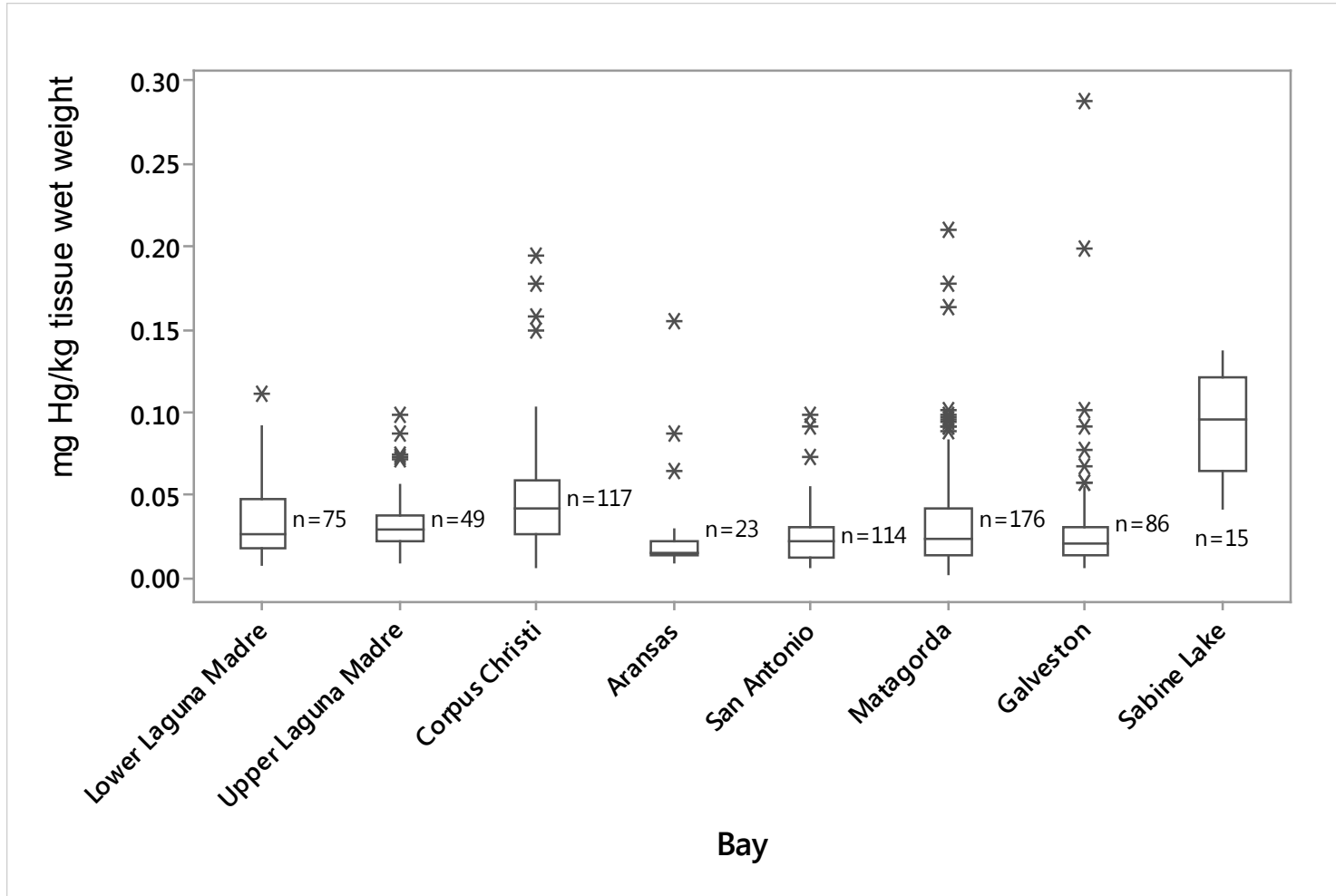
Figure 7: Boxplot of mercury in tissue plugs from all species combined by bay. Based on data from June-August 2015.

Table 4: Kruskal-Wallis with Dunn's Multiple Comparison test results; listed bay groups are significantly different at target confidence of 95%; Llag=Lower Laguna Madre, Ulag=Upper Laguna Madre, CorC=Corpus Christi, Aran=Aransas, SanB=San Antonio, Mata=Matagorda, Galv=Galveston, SabL=Sabine. Based on data from June-August 2015

Kruskal-Wallis Bays; Bonferroni Individual Alpha 0.007		
Groups	Z vs. Critical Value	p-Value
CorC vs. SanB	7.48885 >= 2.69	< 0.0001
SanB vs. SabL	6.81793 >= 2.69	< 0.0001
Galv vs. SabL	6.27638 >= 2.69	< 0.0001
CorC vs. Galv	6.11896 >= 2.69	< 0.0001
Aran vs. SabL	5.93938 >= 2.69	< 0.0001
Mata vs. SabL	5.77336 >= 2.69	< 0.0001
CorC vs. Mata	5.58181 >= 2.69	< 0.0001
Llag vs. SabL	4.87183 >= 2.69	< 0.0001
CorC vs. Aran	4.7529 >= 2.69	< 0.0001
Ulag vs. SabL	4.46829 >= 2.69	< 0.0001
Llag vs. SanB	3.32704 >= 2.69	0.0009
Llag vs. CorC	3.31859 >= 2.69	0.0009
Ulag vs. SanB	3.24371 >= 2.69	0.0012
CorC vs. SabL	3.23454 >= 2.69	0.0012

Figure 8: Sign confidence interval of median mercury in tissue plugs for all species by bay system and associated pairwise; Dunn's Multiple Comparison results for median mercury; Llag=Lower Laguna Madre, Ulag=Upper Laguna Madre, CorC=Corpus Christi, Aran=Aransas, SanB=San Antonio, Mata=Matagorda, Galv=Galveston, SabL=Sabine; similar letters are not significantly different. Based on data from June-August 2015.

Comparison of Locations on Texas Gulf Coast: All Species Combined

Fish tissue obtained from fish caught in Texas coastal bend estuaries (Aransas bay and Corpus Christi) had significantly greater median mercury levels than those captured from other locations along the coast (Figure 9 and 10). In addition, Dunn's Multiple Comparison test detected significantly higher median mercury values in fish tissue taken from fish captured in lower coast estuaries when compared to upper coast estuaries (Figure 10). No fish exceeded any commercial limits.
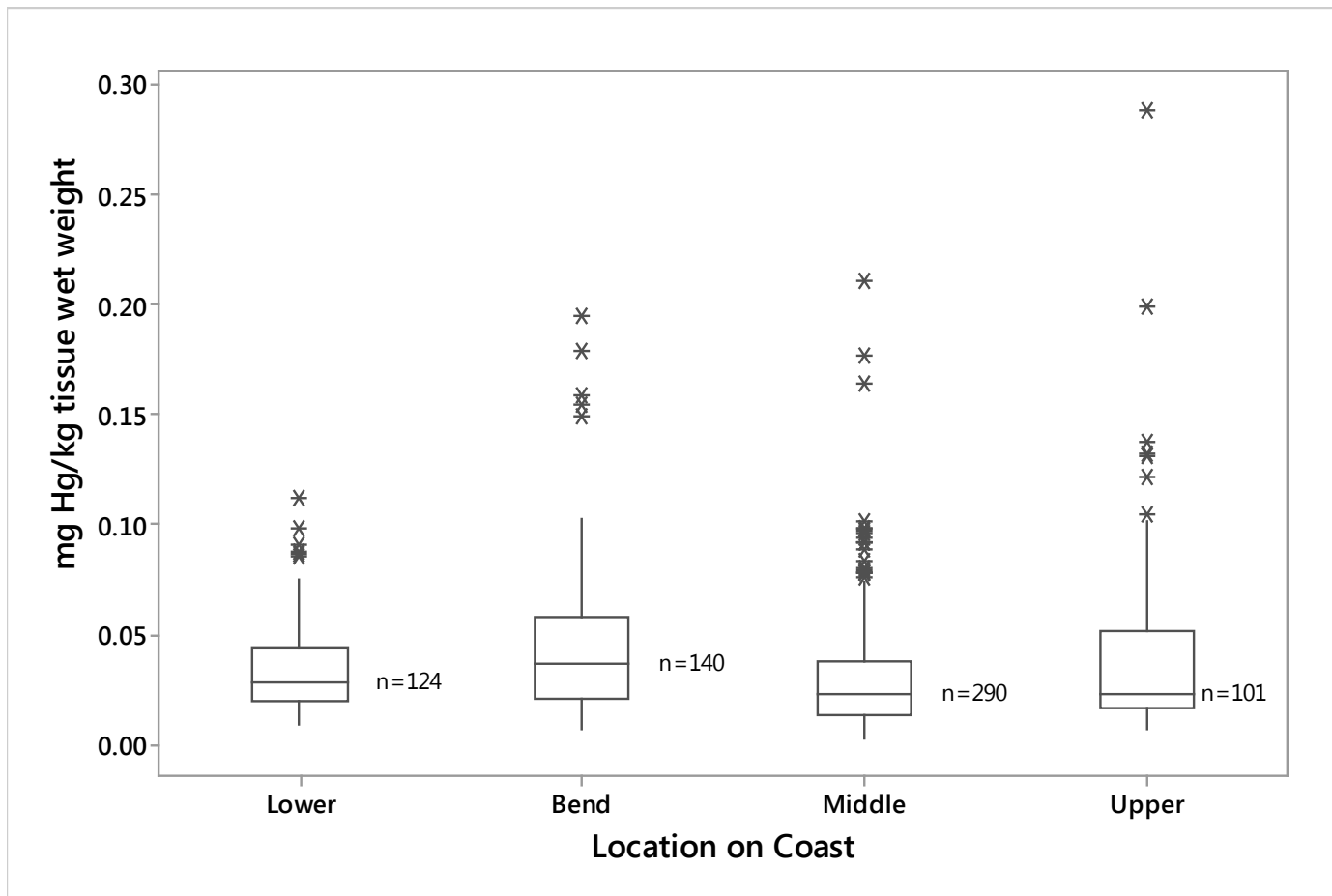
Figure 9: Boxplot of median mercury in tissue plugs from all species by location on the Texas gulf coast; Lower=Lower Laguna Madre and Upper Laguna Madre, Bend=Aransas and Corpus Christi, Middle=Matagorda and San Antonio, Upper=Galveston and Sabine. Based on data from June-August 2015.
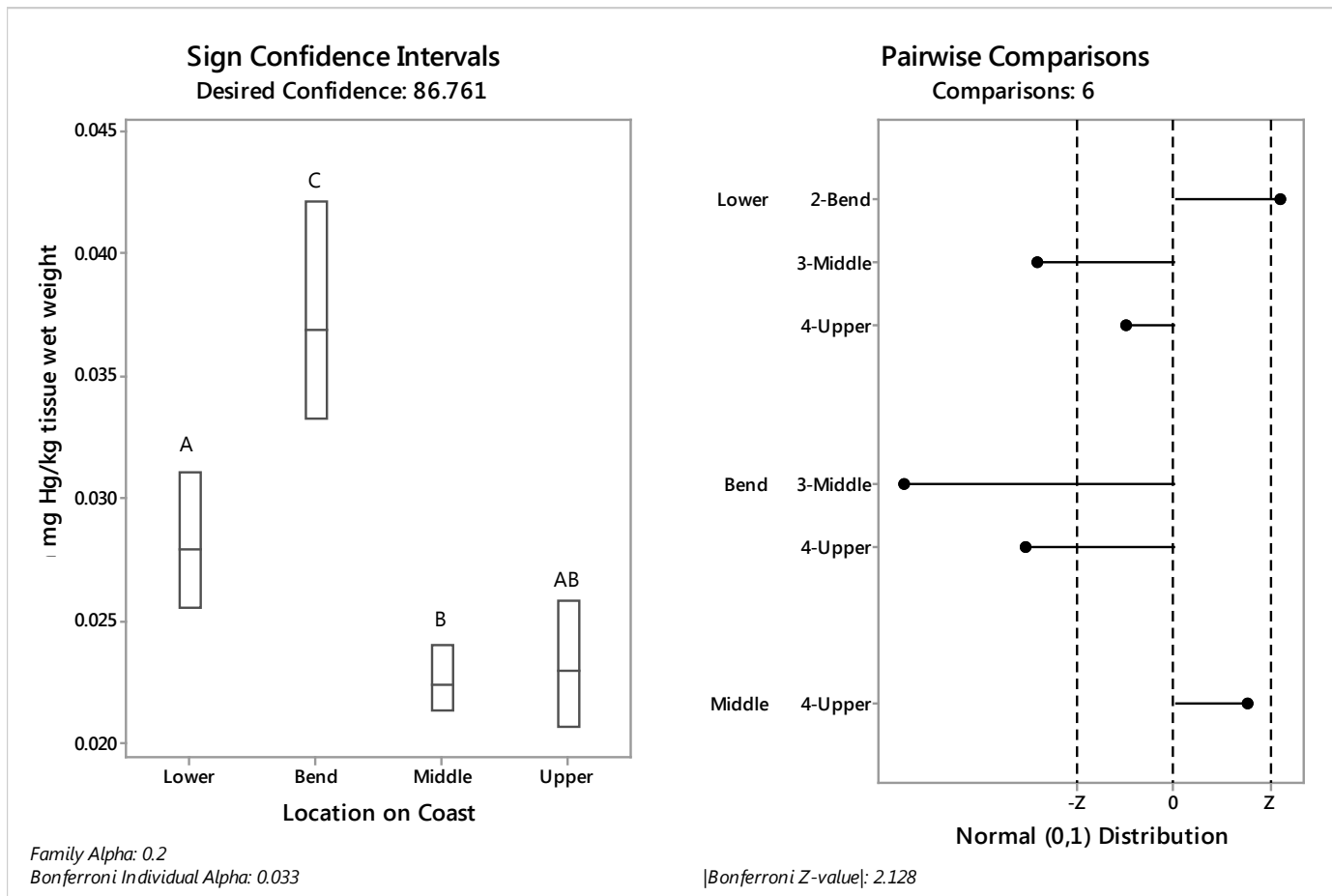
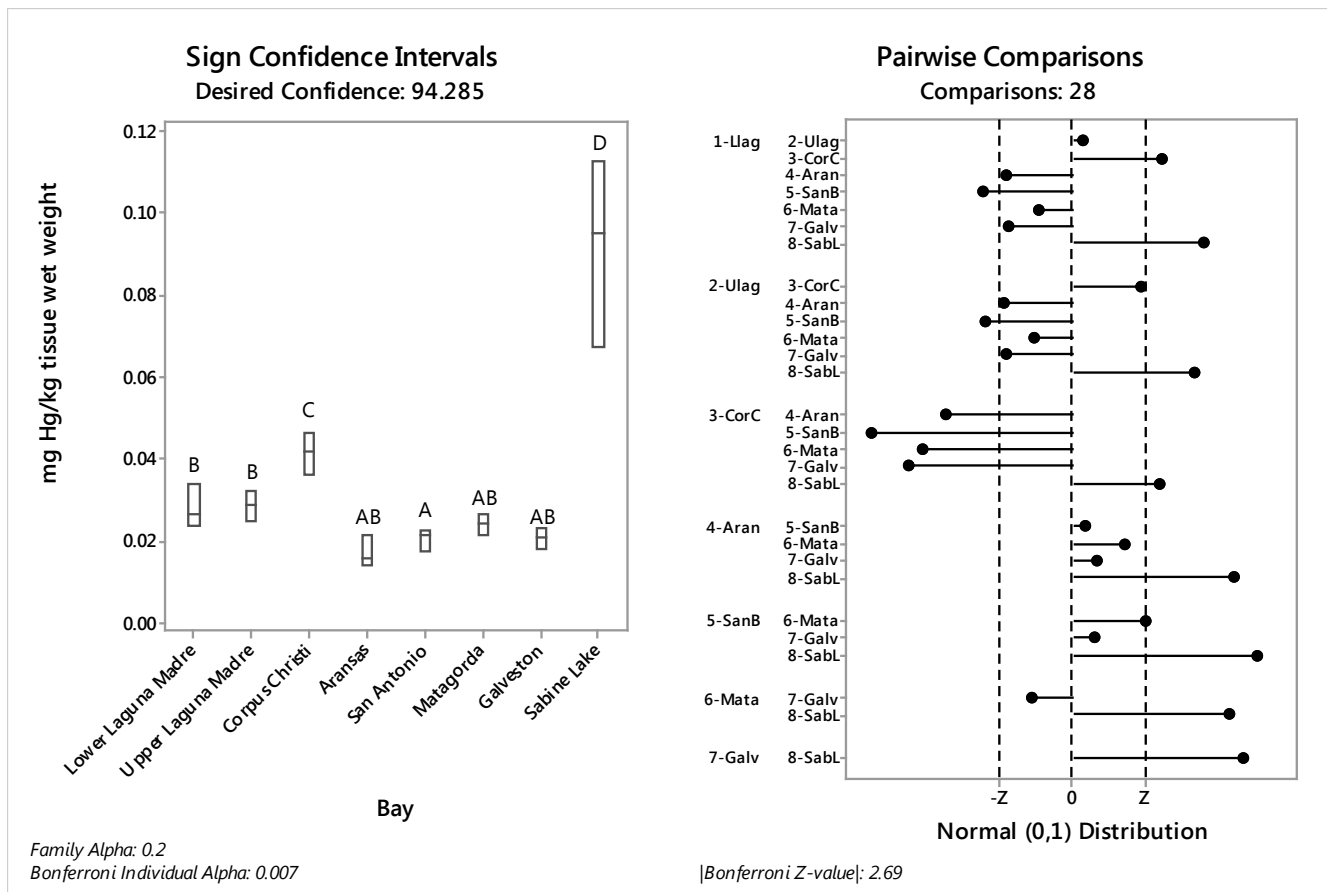Figure 10: Sign confidence interval of median mercury in tissue plugs for all locations and associated pairwise; Dunn's Multiple Comparison results for median mercury; Lower=Lower Laguna Madre and Upper Laguna Madre, Bend=Aransas and Corpus Christi, Middle=Matagorda and San Antonio, Upper=Galveston and Sabine; similar letters are not significantly different. Based on data from June-August 2015.

Comparison of Species: All Bays Combined

Statistically significant differences in mercury levels in tissue were detected between species (Table 5). The Dunn's test shows significant differences between 18 species pairs with Hardhead Catfish having significantly different mercury levels in tissue from all other fish species (Table 5). Tissue from Hardhead Catfish had greater median mercury levels compared to other species (Figure 11 and 12). Tissue from Silver Perch contained the second greatest median mercury amount, though not significantly different from other species (Figure 12). Low levels of tissue mercury were observed in Spot and Atlantic Croaker (Figure 11 and 12).
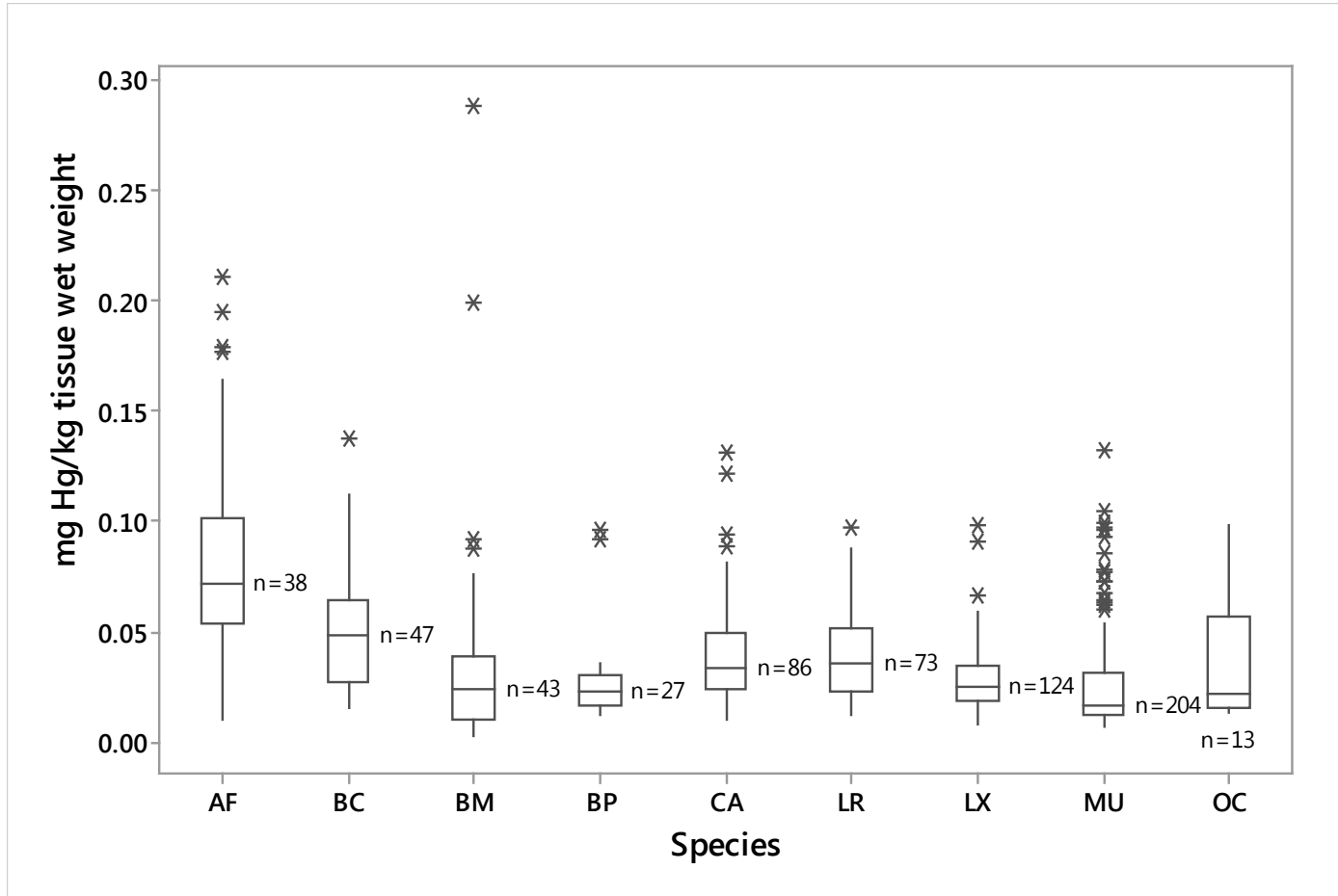
Figure 11: Boxplot of mercury in tissue plugs from all bays combined by species; boxplot with outliers; AF=*Ariopsis felis* (Hardhead Catfish), BC=*Bairdiella chrysoura* (Silver Perch), BM=*Bagre marinus* (Gafftopsail Catfish), BP=*Brevoortia patronus* (Gulf Menhaden), CA=*Cynoscion arenarius* (Sand Seatrout), LR=*Lagodon rhomboides* (Pinfish), LX=*Leistomus xanthurus* (Spot), MU=*Micropogonias undulatus* (Atlantic Croaker), OC=*Orthopristis chrysoptera* (Pigfish). Based on data from June-August 2015.

Table 5: Kruskal-Wallis with Dunn's Multiple Comparison results; listed species are significantly different at target confidence of 95%; AF=*Ariopsis felis* (Hardhead Catfish), BC=*Bairdiella chrysoura* (Silver Perch), BM=*Bagre marinus* (Gafftopsail Catfish), BP=*Brevoortia patronus* (Gulf Menhaden), CA=*Cynoscion arenarius* (Sand Seatrout), LR=*Lagodon rhomboides* (Pinfish), LX=*Leistomus xanthurus* (Spot), MU=*Micropogonias undulatus* (Atlantic Croaker), OC=*Orthopristis chrysoptera* (Pigfish); June-August 2015

| Kruskal-Wallis Species Groups	Z vs. Critical value	P-value
AF vs. MU	9.31174 >= 2.773	< 0.0001
BC vs. MU	7.24568 >= 2.773	< 0.0001
CA vs. MU	7.08007 >= 2.773	< 0.0001
AF vs. LX	6.69975 >= 2.773	< 0.0001
LR vs. MU	6.66297 >= 2.773	< 0.0001
AF vs. BM	6.14880 >= 2.773	< 0.0001
AF vs. BP	5.11349 >= 2.773	< 0.0001
BC vs. LX	4.49144 >= 2.773	< 0.0001
BC vs. BM	4.24637 >= 2.773	< 0.0001
AF vs. CA	3.77313 >= 2.773	0.0002
AF vs. LR	3.68197 >= 2.773	0.0002
CA vs. LX	3.61499 >= 2.773	0.0003
LX vs. MU	3.53898 >= 2.773	0.0004
LR vs. LX	3.42819 >= 2.773	0.0006
BM vs. CA	3.39472 >= 2.773	0.0007
BC vs. BP	3.37148 >= 2.773	0.0007
BM vs. LR	3.29016 >= 2.773	0.0010
AF vs. OC	3.25981 >= 2.773	0.0011
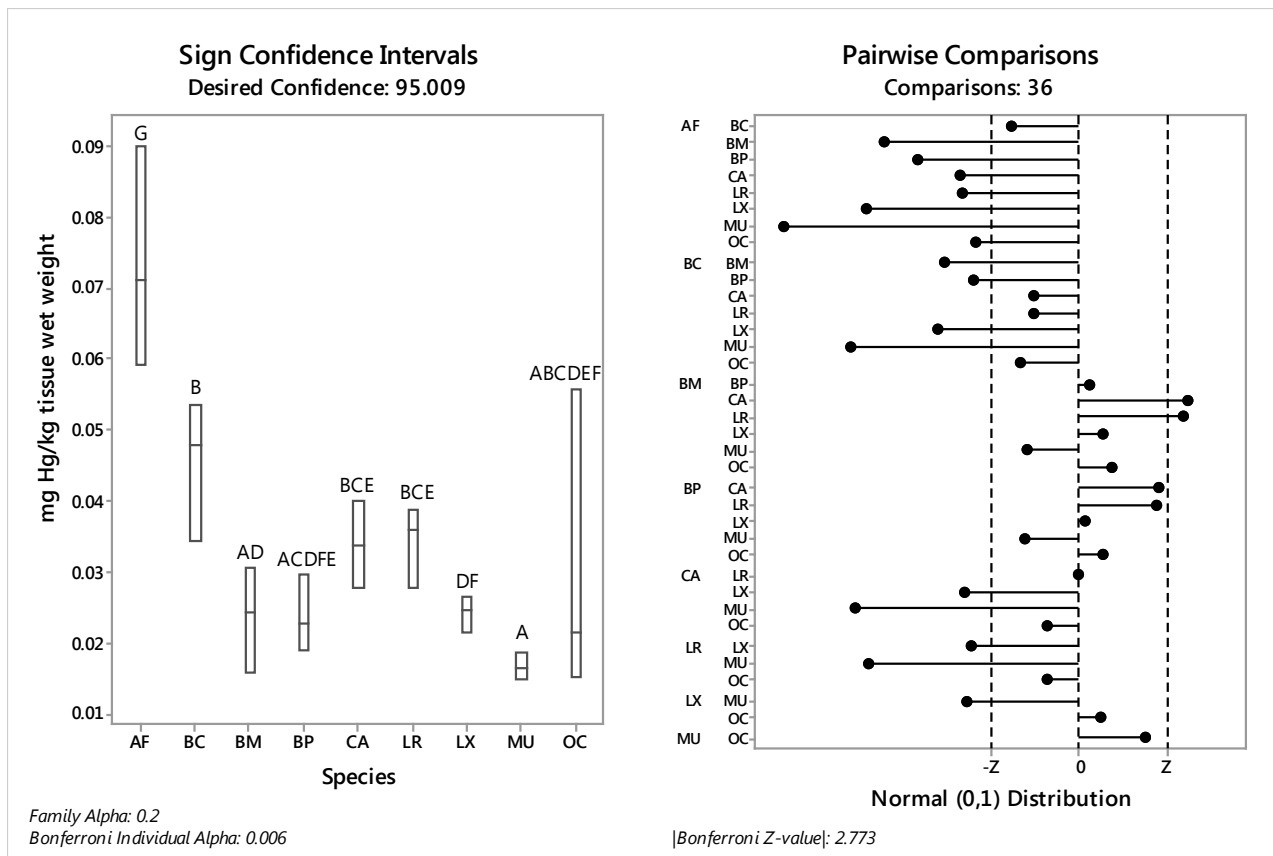
Figure 12: Sign confidence interval of median mercury in tissue plugs for all species by species and associated pairwise; Dunn's Multiple Comparison results for median mercury; AF=*Ariopsis felis* (Hardhead Catfish), BC=*Bairdiella chrysoura* (Silver Perch), BM=*Bagre marinus* (Gafftopsail Catfish), BP=*Brevoortia patronus* (Gulf Menhaden), CA=*Cynoscion arenarius* (Sand Seatrout), LR=*Lagodon rhomboides* (Pinfish), LX=*Leistomus xanthurus* (Spot), MU=*Micropogonias undulatus* (Atlantic Croaker), OC=*Orthopristis chrysoptera* (Pigfish); similar letters are not significantly different. Based on data from June-August 2015.

Comparison of Combined Species and Estuary Categories

Although we detected significant differences in mercury in tissue between bays and between species of fish there is also evidence of interaction between these variables. In all estuaries where the species occurred, Hardhead Catfish generally exhibited the highest or second highest calculated average mercury level in tissue with the exception of Sabine Lake (Figure 13). In the Sabine Lake system Silver Perch exhibited the highest calculated average mercury level in tissue; elsewhere it exhibited much lower mercury levels (Figure 13).
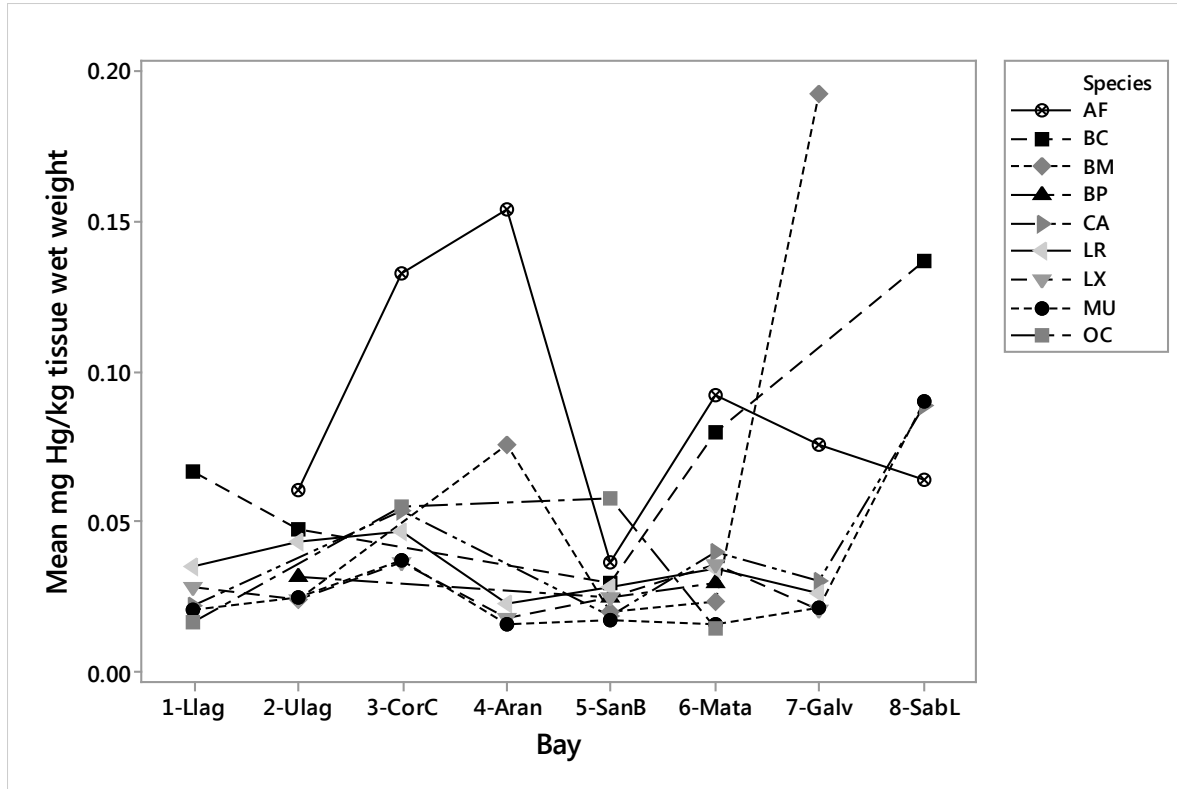
Figure 13: Interaction plot of mean mercury in tissue plugs all species combined by bay:
AF=*Ariopsis felis* (Hardhead Catfish), BC=*Bairdiella chrysoura* (Silver Perch), BM=*Bagre marinus*
(Gafftopsail Catfish), BP=*Brevoortia patronus* (Gulf Menhaden), CA=*Cynoscion arenarius* (Sand
Seatrout), LR=*Lagodon rhomboides* (Pinfish), LX=*Leistomus xanthurus* (Spot),
MU=*Micropogonias undulatus* (Atlantic Croaker), OC=*Orthopristis chrysoptera* (Pigfish); 1-
Llag=Lower Laguna Madre, 2-Ulag=Upper Laguna Madre, 3-CorC=Corpus Christi, 4-
Aran=Aransas, 5-SanB=San Antonio, 6-Mata=Matagorda, 7-Galv=Galveston, 8-SabL=Sabine.
Based on data from June-August 2015.

According to Figure 14, the species exhibiting the highest reported median concentration of mercury varied by estuary. The largest reported median level of mercury in fish tissue was associated with Gafftopsail Catfish captured in Galveston Bay. Individual Gafftopsail Catfish however exhibited a very large range of values and the resulting confidence interval of the median was also the widest observed in the study. Gulf Menhaden showed similar levels of mercury in tissue from all bays in which they were caught. The highest median concentration of mercury in fish tissue in Aransas Bay was Hardhead Catfish. The species exhibiting the highest median concentration of mercury in Laguna Madre and Matagorda Bay was Silver Perch. The largest reported median level of mercury in Sand Seatrout were from fish caught in Sabine Lake. Pinfish showed similar levels of mercury in tissue from all bays in which they were caught. Spot showed similar levels of mercury in tissue from all bays in which they were caught. The largest median mercury level Atlantic Croaker tissue were from fish caught in Sabine Lake. Pigfish showed similar levels of mercury in tissue from all bays in which they were caught. No fish exceeded any commercial limits.
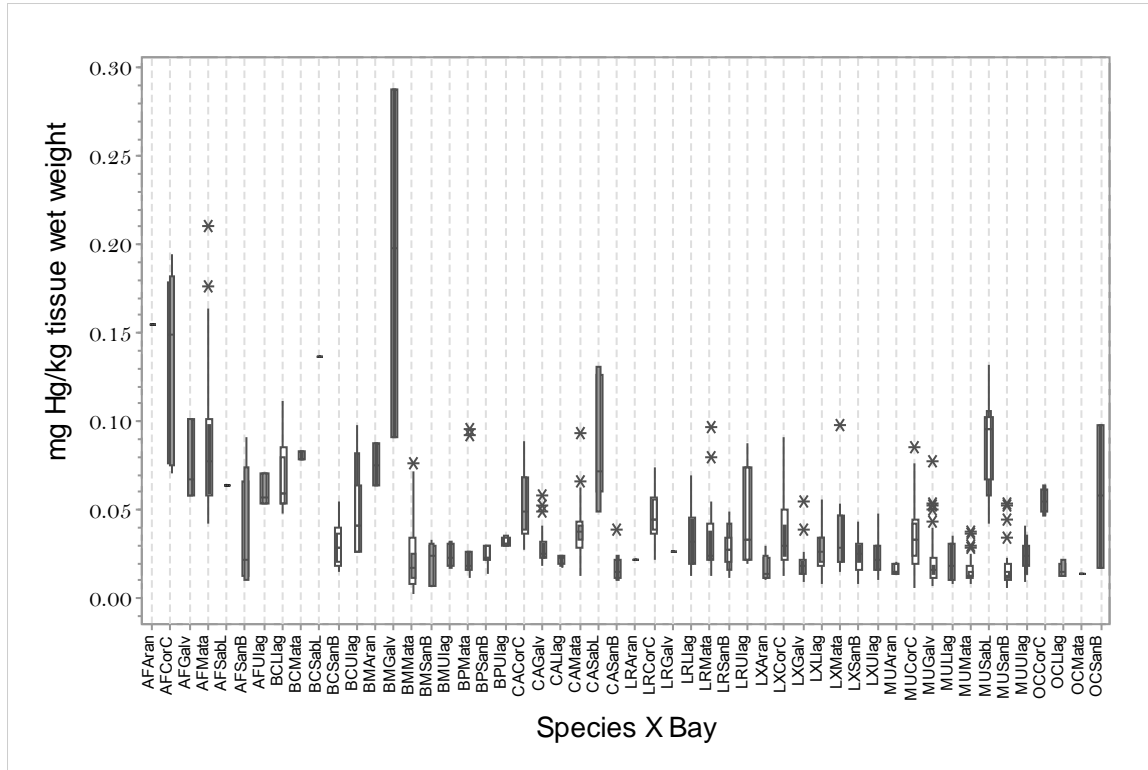
Figure 14: Boxplot with shaded 95% confidence interval of the median mercury in tissue plugs by species and bay system; AF=*Ariopsis felis* (Hardhead Catfish), BC=*Bairdiella chrysoura* (Silver Perch), BM=*Bagre marinus* (Gafftopsail Catfish), BP=*Brevoortia patronus* (Gulf Menhaden), CA=*Cynoscion arenarius* (Sand Seatrout), LR=*Lagodon rhomboides* (Pinfish), LX=*Leistomus xanthurus* (Spot), MU=*Micropogonias undulatus* (Atlantic Croaker), OC=*Orthopristis chrysoptera* (Pigfish); Llag=Lower Laguna Madre, Ulag=Upper Laguna Madre, CorC=Corpus Christi, Aran=Aransas, SanB=San Antonio, Mata=Matagorda, Galv=Galveston, SabL=Sabine. Based on data from June-August 2015.

Mercury vs Morphometrics: All Species Combined

Linear regression analysis was used to explore the relationship of mercury levels and fish morphometrics. Overall, when data from all species were combined, the standard length of a fish accounted for 26.9% of variation in the concentration of mercury in wet weight fish tissue (Figure 15). Body weight, by contrast, only accounted for 17.4% of variation in mercury in tissue levels (Figure 16). When data were analyzed by species, length predicted less than 26.9% of variation for all species except Silver Perch and Pigfish (Figure 17). For Silver Perch length predicted 41.4% of variation in mercury levels (Figure 17). For Pigfish length predicted 32.6% of variation in mercury levels (Figure 17). Weight was a poor predictor of mercury variation in all species except Pigfish for which it predicted 49% of total variation in mercury (Figure 18).

Hardhead and Gafftopsail were the longest species recorded (Figure 17). ~21% of Hardhead and Gafftopsail Catfish over 160 mm SL came from Galveston Bay (Figure 17).
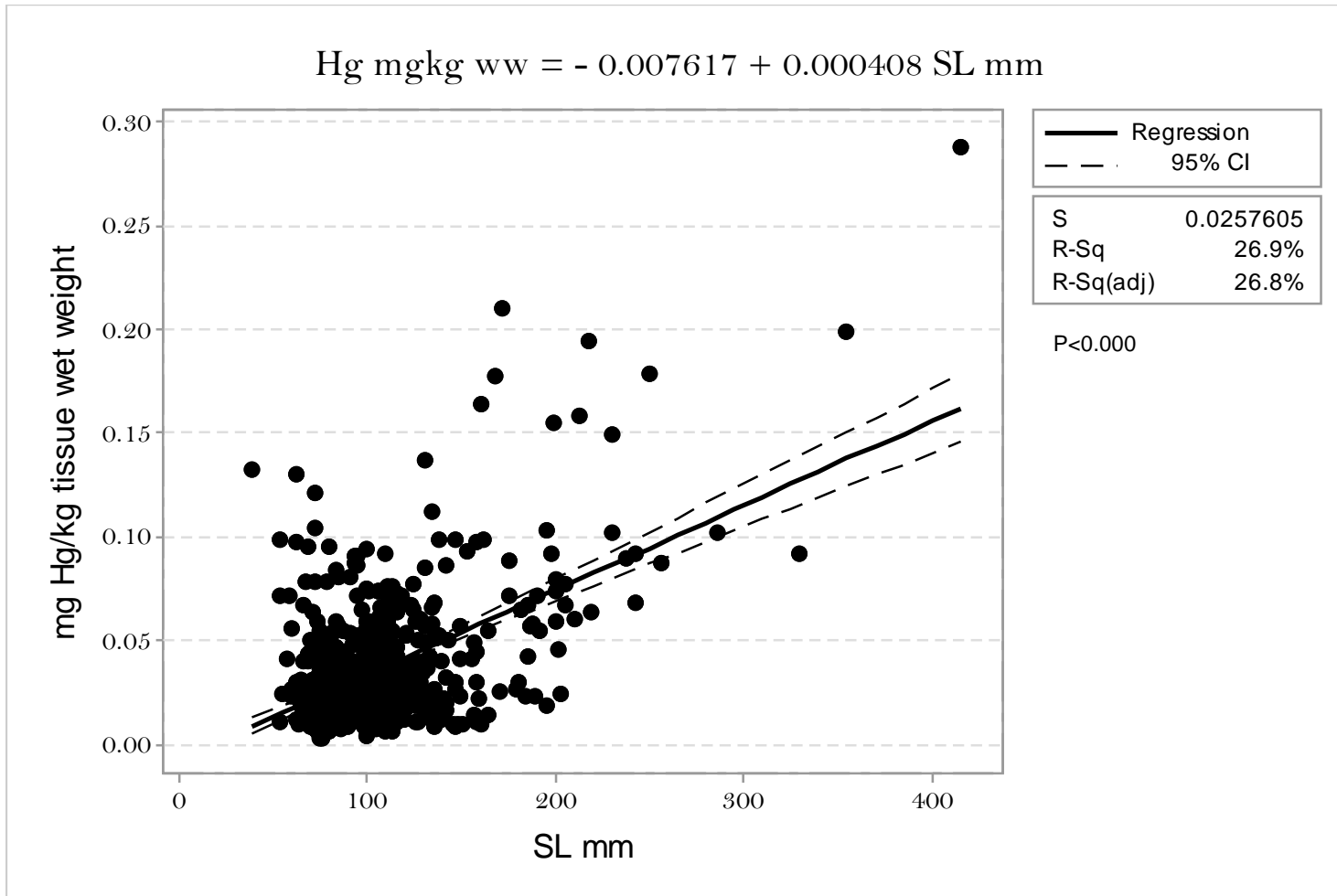
Figure 15: Mercury in tissue plugs versus standard length: all species combined; linear fitted line plot with 95% confidence interval shown. Based on data from June-August 2015.
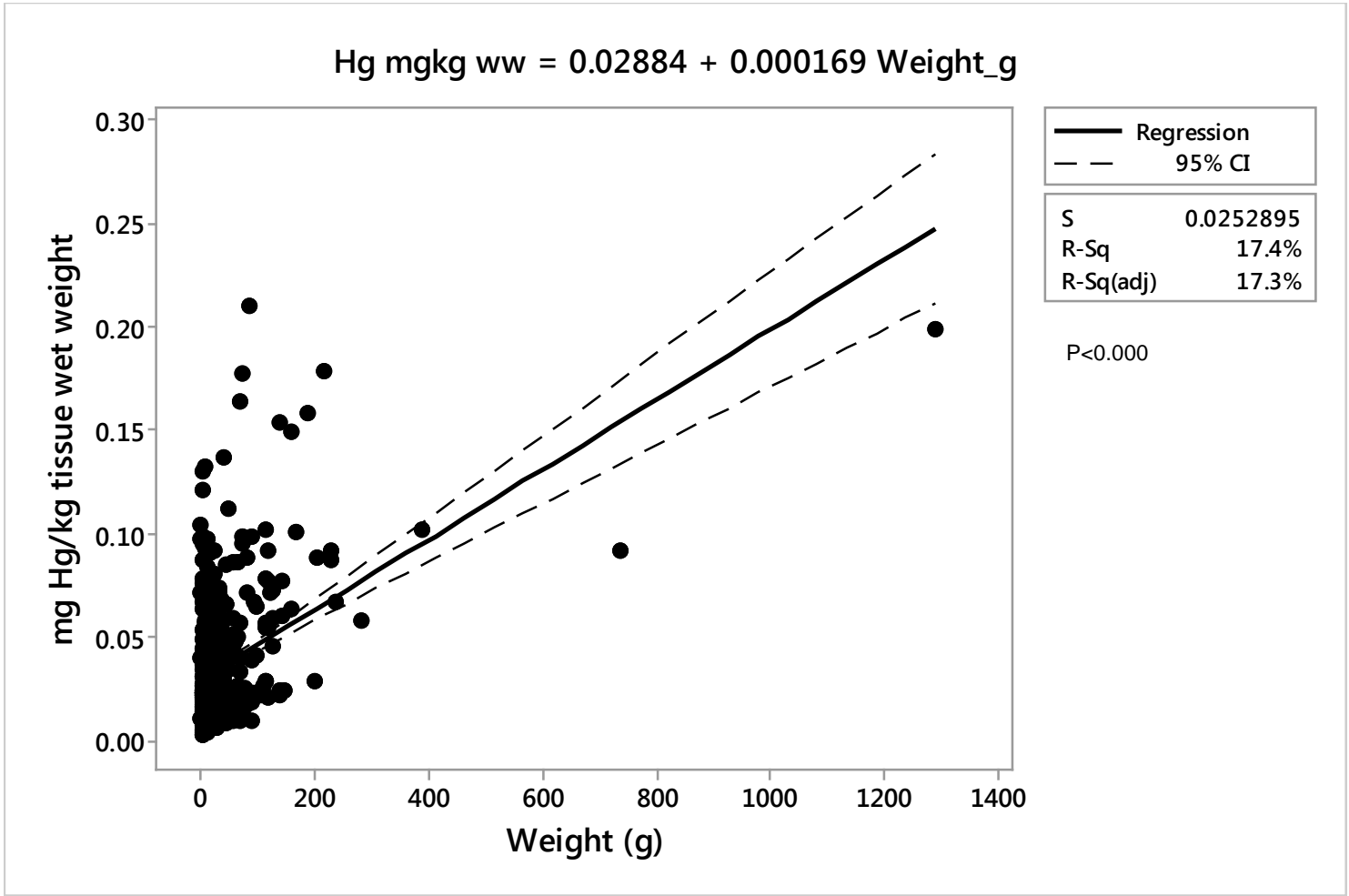
Figure 16: Mercury in tissue plugs versus weight: all species combined; linear fitted line plot with 95% confidence interval shown. Based on data from June-August 2015.
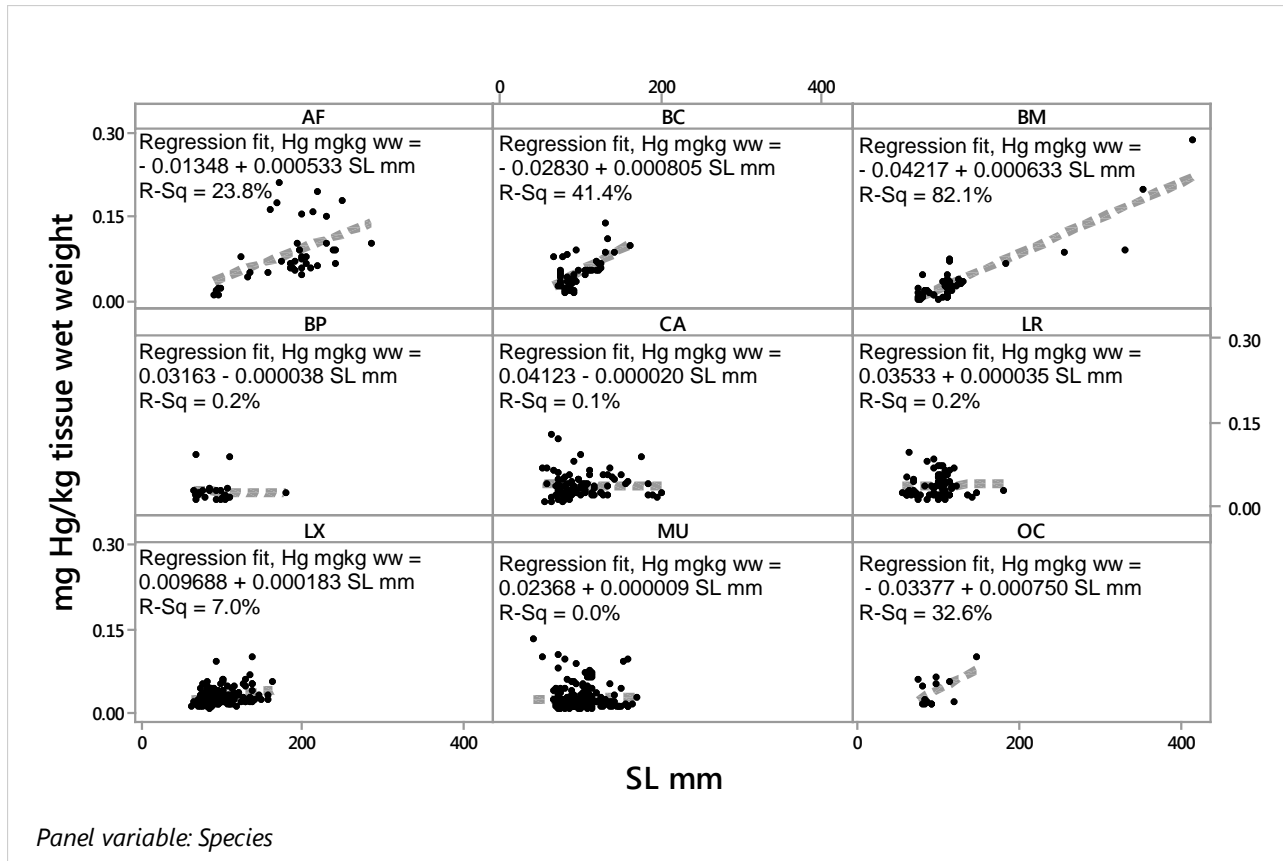
Figure 17: Mercury in tissue plugs versus standard length: all species separate with bays combined; Scatterplots with simple linear regression; AF=*Ariopsis felis* (Hardhead Catfish), BC=*Bairdiella chrysoura* (Silver Perch), BM=*Bagre marinus* (Gafftopsail Catfish), BP=*Brevoortia patronus* (Gulf Menhaden), CA=*Cynoscion arenarius* (Sand Seatrout), LR=*Lagodon rhomboides* (Pinfish), LX=*Leistomus xanthurus* (Spot), MU=*Micropogonias undulatus* (Atlantic Croaker), OC=*Orthopristis chrysoptera* (Pigfish). Based on data from June-August 2015.
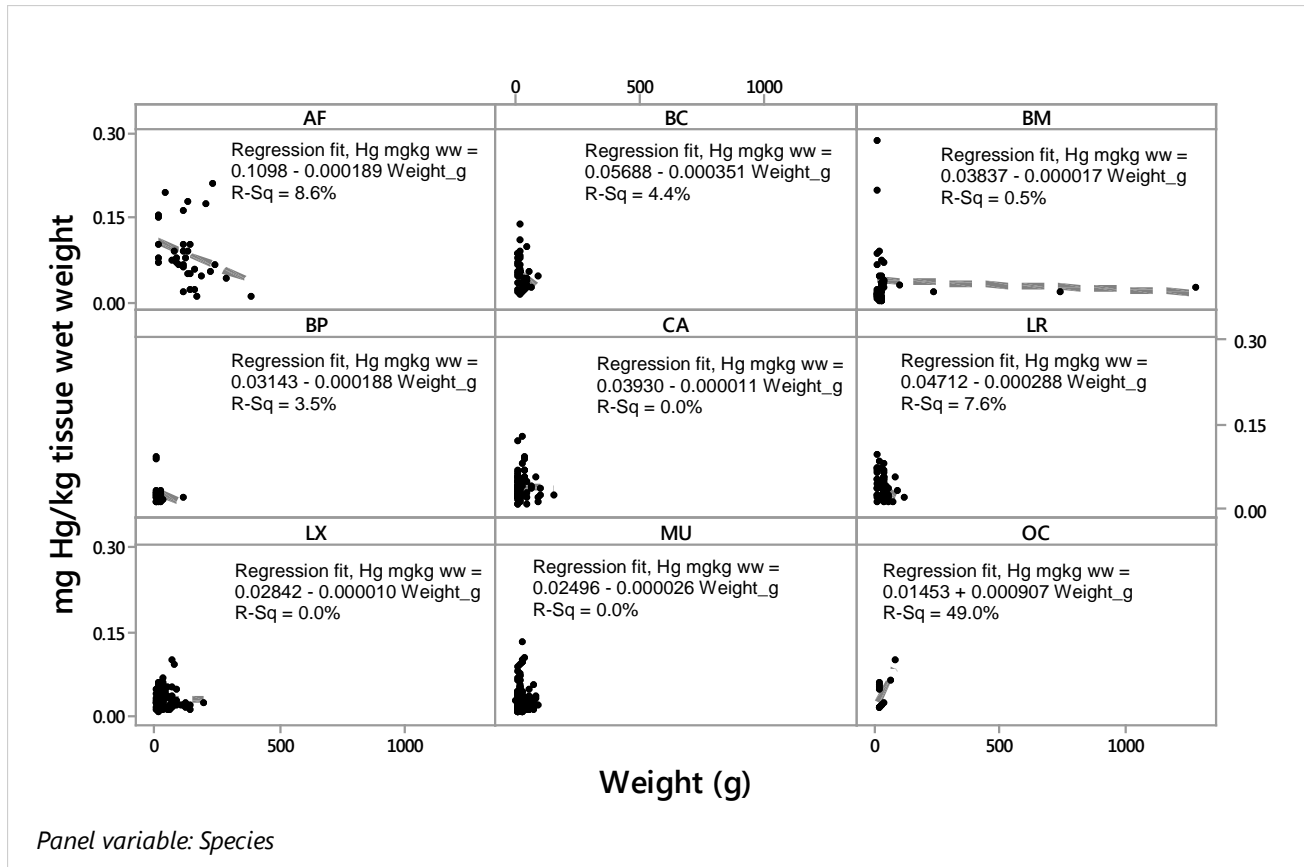
Figure 18: Mercury in tissue plugs versus weight: all species separate with bays combined; Scatterplots with simple linear regression; AF=*Ariopsis felis* (Hardhead Catfish), BC=*Bairdiella chrysoura* (Silver Perch), BM=*Bagre marinus* (Gafftopsail Catfish), BP=*Brevoortia patronus* (Gulf Menhaden), CA=*Cynoscion arenarius* (Sand Seatrout), LR=*Lagodon rhomboides* (Pinfish), LX=*Leistomus xanthurus* (Spot), MU=*Micropogonias undulatus* (Atlantic Croaker), OC=*Orthopristis chrysoptera* (Pigfish). Based on data from June-August 2015.

Comparison by Trophic Level

Species classified as herbivorous had a trophic value of less than 3 and were represented by a total 100 specimens of Gulf Menhaden and Pinfish (Figure 19). Species classified as omnivorous had a trophic value of between 3 and 4 (non-inclusive) and included a total of 469 specimens of Spot, Hardhead Catfish, Atlantic Croaker, Pigfish, Gafftopsail Catfish, and Silver Perch (Figure 19). Species classified as carnivorous had a trophic value of 4 or more and were represented by 86 specimens of Sand Seatrout (Figure 19).

For omnivores the standard length of the fish accounted for 38.5% of total variation in mercury from tissue (Figure 20). In the other categories length was a poor predictor of mercury (Figure 20). There were no strong relationships at any trophic level between mercury and weight (Figure 21). The median amount of mercury in fish tissue from omnivores was significantly different from the other two trophic groups based on the results of Kruskal-Wallis and Dunn's test results (Figure 22).
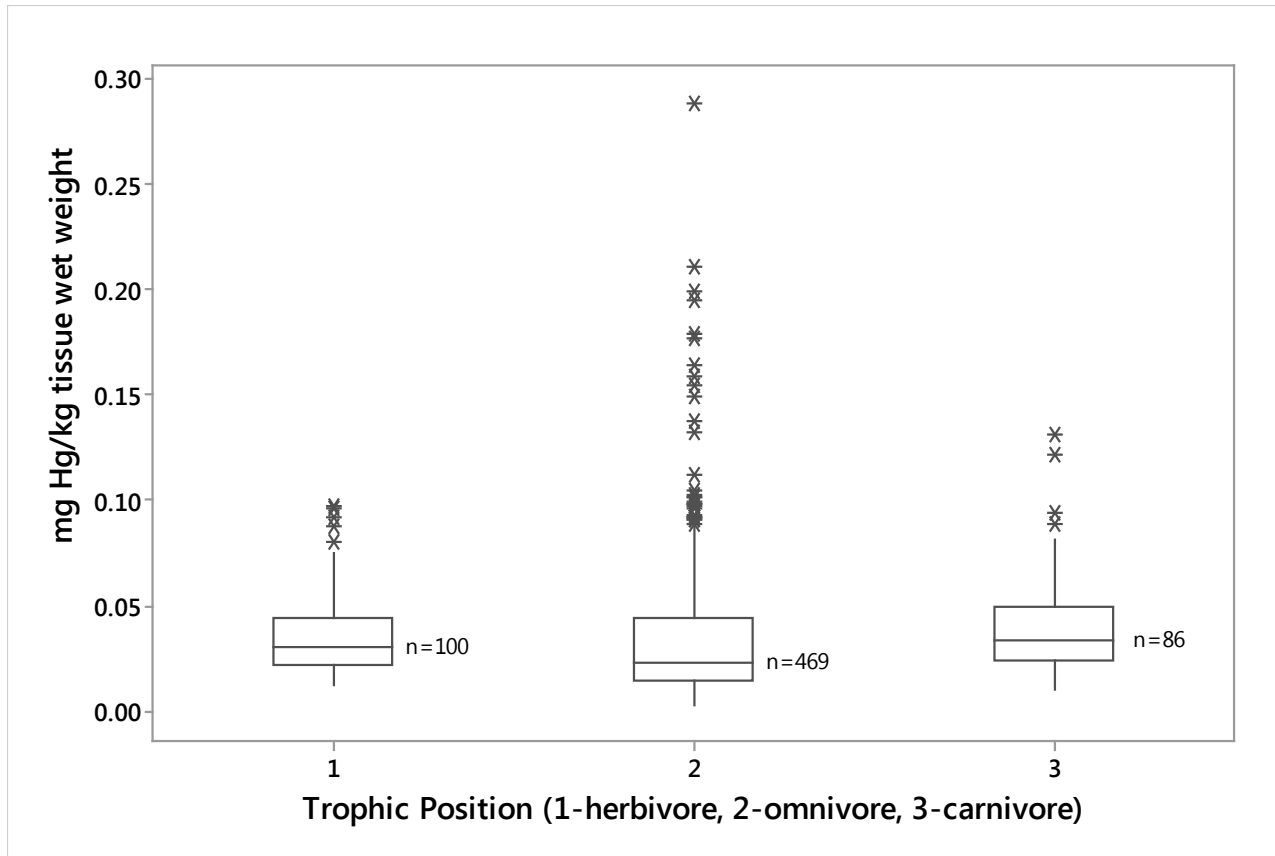
Figure 19: Boxplot of mercury in tissue plugs from all bays and species sorted by trophic level; herbivore=*Brevoortia patronus* (Gulf Menhaden) and *Lagodon rhomboides* (Pinfish), omnivore=*Leistomus xanthurus* (Spot), *Ariopsis felis* (Hardhead Catfish), *Micropogonias undulatus* (Atlantic Croaker), *Orthopristis chrysoptera* (Pigfish), *Bagre marinus* (Gafftopsail Catfish), and *Bairdella chrysoura* (Silver Perch), carnivore=*Cynoscion arenarius* (Sand Seatrout). Based on data from June-August 2015.
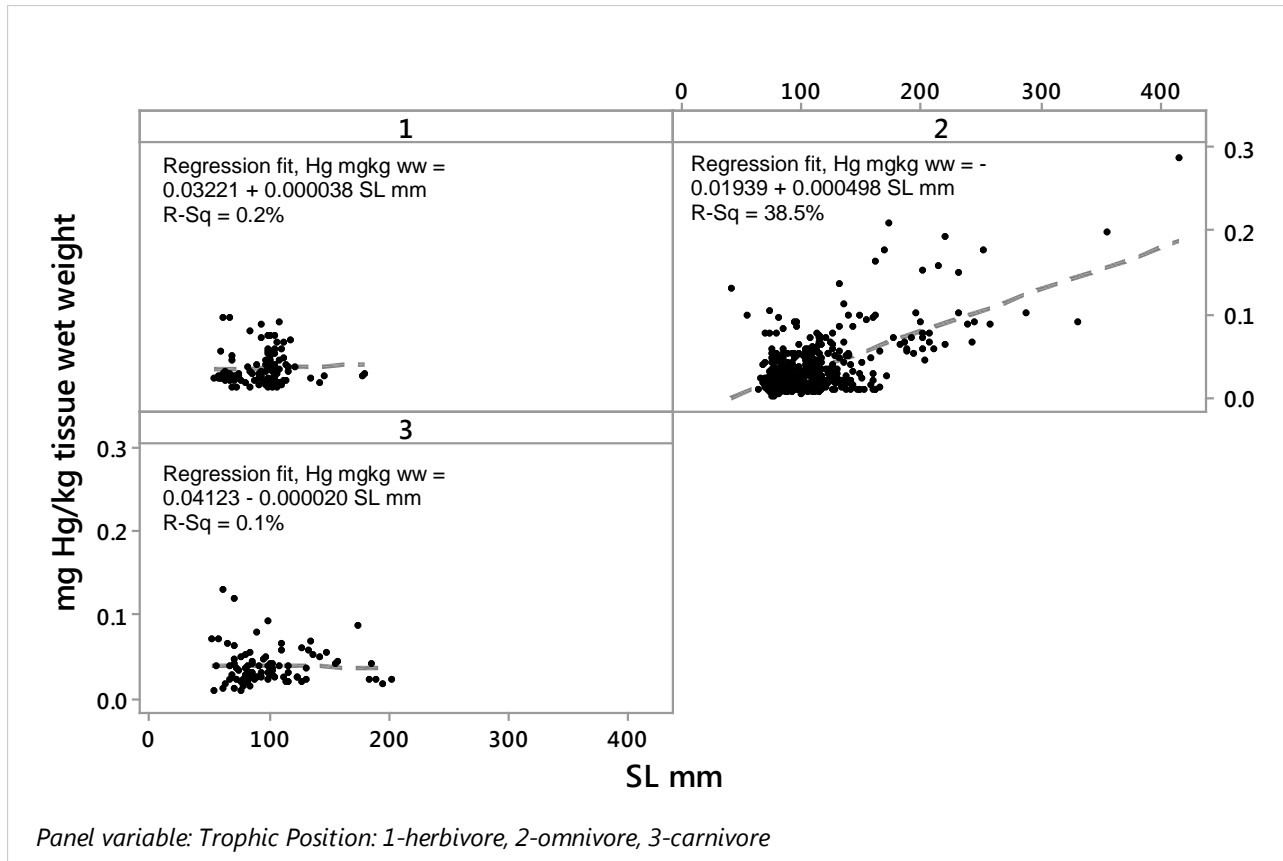
Figure 20: Mercury in tissue plugs versus length: sorted by trophic level; Scatterplots with simple linear regression; herbivore (1)=*Brevoortia patronus* (Gulf Menhaden) and *Lagodon rhomboides* (Pinfish), omnivore (2)=*Leistomus xanthurus* (Spot), *Ariopsis felis* (Hardhead Catfish), *Micropogonias undulatus* (Atlantic Croaker), *Orthopristis chrysoptera* (Pigfish), *Bagre marinus* (Gafftopsail Catfish), and *Bairdella chrysoura* (Silver Perch), carnivore (3)=*Cynoscion arenarius* (Sand Seatrout). Based on data from June-August 2015.
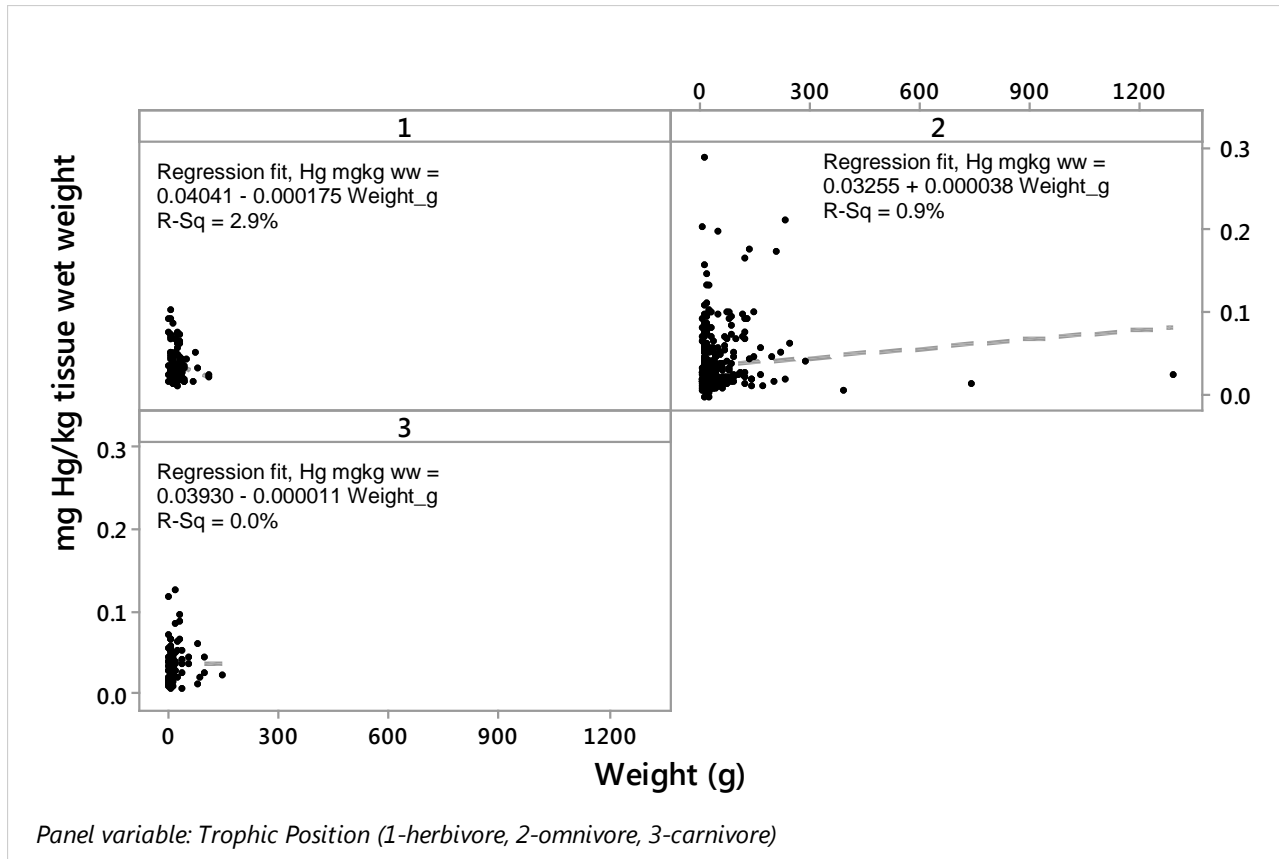
Figure 21: Mercury in tissue plugs versus weight: sorted by trophic level; Scatterplots with simple linear regression; herbivore (1)=*Brevoortia patronus* (Gulf Menhaden) and *Lagodon rhomboides* (Pinfish), omnivore (2)=*Leistomus xanthurus* (Spot), *Ariopsis felis* (Hardhead Catfish), *Micropogonias undulatus* (Atlantic Croaker), *Orthopristis chrysoptera* (Pigfish), *Bagre marinus* (Gafftopsail Catfish), and *Bairdella chrysoura* (Silver Perch), carnivore (3)=*Cynoscion arenarius* (Sand Seatrout). Based on data from June-August 2015.
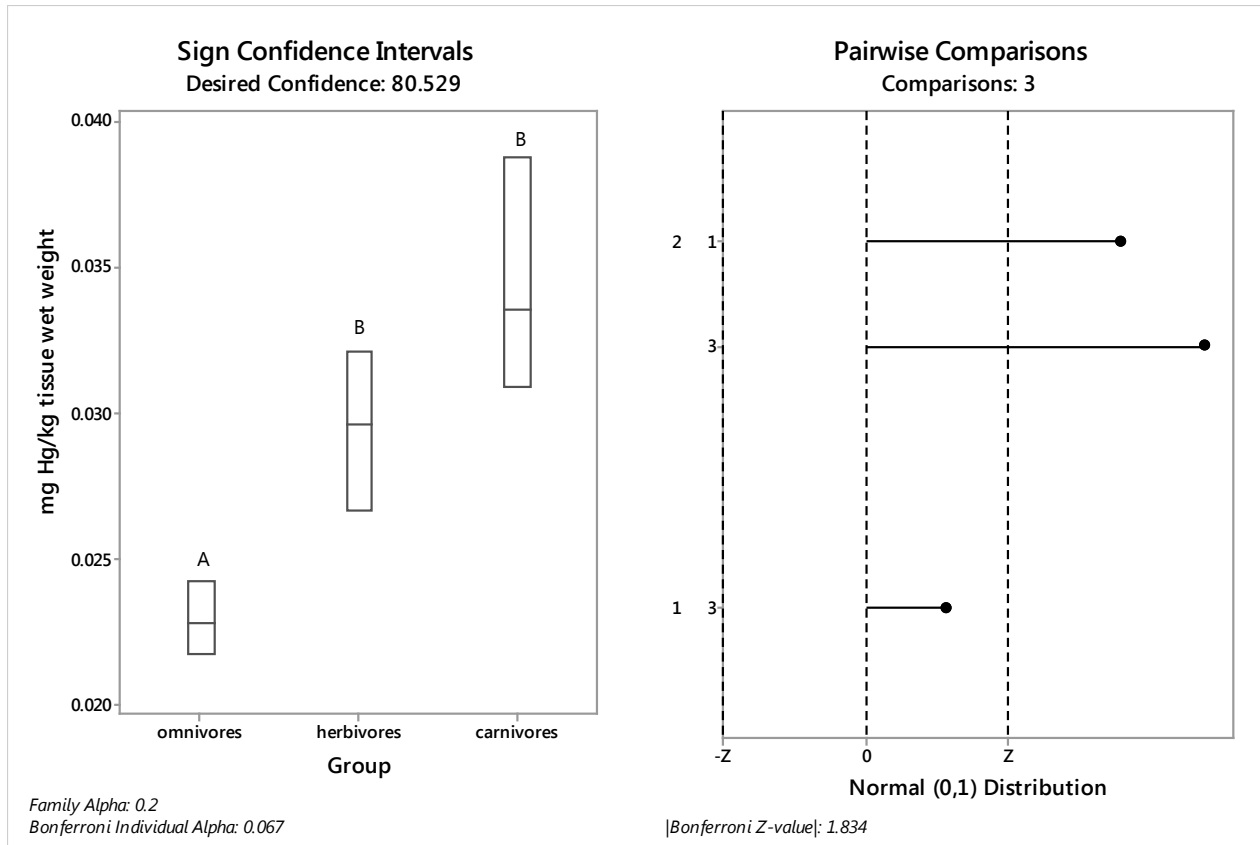
Figure 22: Sign confidence interval of median mercury in tissue plugs for all species by trophic level and associated pairwise; Dunn's Multiple Comparison results for median mercury; herbivore (1)=*Brevoortia patronus* (Gulf Menhaden) and *Lagodon rhomboides* (Pinfish), omnivore (2)=*Leistomus xanthurus* (Spot), *Ariopsis felis* (Hardhead Catfish), *Micropogonias undulatus* (Atlantic Croaker), *Orthopristis chrysoptera* (Pigfish), *Bagre marinus* (Gafftopsail Catfish), and *Bairdella chrysoura* (Silver Perch), carnivore (3)=*Cynoscion arenarius* (Sand Seatrout).; similar letters are not significantly different. Based on data from June-August 2015.

DISCUSSION

Comparison of Mercury in Tissue to Screening Levels

No fish exceeded commercial limits. For the FDA this means that none of the fish from the summer of 2015 would be taken off the market due to mercury in tissue. Mercury from fish tissue may appear to exceed the USEPA and ATSDR daily limit screening level. However, this does not necessarily mean the fish are unsafe for consumption. These screening levels or criteria are used to calculate risk based on human weight and based on the recommended daily human ingestion limits. These limits are calculated by the USEPA and ATSDR by using hair concentrations of those known to be affected by methymercury and back-calculating to blood concentrations then to daily dietary intake level. A body weight of 60 kg was used in the original calculation. Both organizations used a historical mercury poisoning event which involved 83 Iraq women in 1960. The difference in values between the two guidelines comes from using a blood volume of 5.0L (USEPA) versus a blood volume of 4.1L (ATSDR) (USEPA 1997). This has informed the current recommendation, which is applicable to the general public, on USEPA's official "Guidelines for Eating Fish that Contain Mercury" website which suggests an intake of one meal per week of locally caught fish and recommends eating no fish from areas with advisories against those fish (USEPA 2016b). Therefore, if the official USEPA guidelines are

followed and since none of the fish caught in the summer of 2015 contained mercury in muscle tissue that exceeded guidelines, the fish should be safe for human consumption.

In both Matagorda Bay and Galveston Bay mercury levels in Atlantic Croaker have decreased since the earliest recorded mercury levels in 1970 (Figure 6). By 1980 the USEPA had asked for and received a large amount of funding to address wastewater treatment needs for Galveston Bay sewage (Youngblood 2010). It appears that this likely had an impact on resulting ambient and fish tissue mercury levels, since mercury levels after the 1980's gradually decreased until in 2001, when they reached a consistently low value of less than 0.05 mg Hg/kg wet weight. This suggests that regulations may have had led to a reduction in mercury loading and subsequent levels of mercury in fish tissue within estuarine systems. This hypothesis is also supported by recent data reported by Harris et.al. (2007) which presented evidence that mercury levels in fish were more correlated with atmospheric deposition of mercury applied directly to the surface of a lake, rather than the watershed. The researchers also presented evidence suggesting that reducing amounts of discharged mercury would reduce the amounts of mercury in the fish in a watershed on the scale of years They discussed the potential of mercury emissions reduction to rapidly reduce the methymercury concentration in fish (Harris et al. 2007).

Differences between Texas Estuaries

Besides Sabine Lake, there appeared to be no significant trend between Texas estuaries. Fish caught from Sabine Lake that were used for mercury

analysis ranged between 39-219 mm SL; fish from other bays were also commonly within this range. This data suggest that neither the size of fish nor a greater number of high-mercury containing species likely accounts for the greater mercury content reported in fish from Sabine Lake. If this is the case, it also implies that we can probably eliminate age, weight, size, and species from possible contributing causes since age is related to size, and weight did not predict mercury content well in the bay fish species evaluated. Since a ranked test using medians was used to compare estuaries it is also unlikely that a single, high mercury specimen is affecting the results strongly. The variability (SD = 0.03067) exhibited by specimens within Sabine Lake is nearly identical to the measures of overall variability (SD = 0.03010) across all estuaries and species Therefore, some other factor is likely responsible for the higher amount of mercury in fish tissue observed in Sabine Lake in comparison to other estuaries.

Mercury maps generated by Cocca (2001) indicated, by providing data, that the upstream Sabine Lake watersheds have air deposition as a primary source of mercury loading. The maps also stated that the current actual average fish mercury concentration in Sabine Lake is 0 to 0.14 mg/kg (Cocca 2001). Data from this study also ranged from 0.04 to 0.14 mg/kg. Unfortunately, the mercury maps do not provide data for other Texas estuaries so direct comparison between recent data and their study results is not possible (Cocca 2001). A recent study conducted at Texas Christian University suggest that there may exist some correlation between evergreen trees and mercury contamination in fish, which may contribute to mercury in Sabine Lake since the bay lies near

the heavily wooded Sabine National Wildlife Refuge (Jones 2012). More data on emission sources, atmospheric currents, deposition patterns, and local conditions would greatly assist in explaining potential reasons for Sabine Lake's higher fish tissue mercury compared to other Texas bays. Further monitoring and research in regards to mercury loading sources and biological effects within Sabine Lake is needed. Further research documenting and comparing the total mercury loads entering bay systems in Texas from air, water, and point-source input is greatly needed to better assess the cause of observed variation in mercury in fish tissue between estuaries.

Interspecies Differences in Mercury

During this study tissue obtained from Hardhead Catfish in 2015 had the greatest amount of mercury by median, followed by tissue from Gafftopsail Catfish. These are both omnivorus species of catfish at trophic levels 3.3 and 3.5 respectively. The other omnivores which had trophic ranks between, above, and below these catfish did not exhibit the same concentration of mercury. These two species of catfish reach maturity at 5 years or later, at a standard length of 12cm by age 1 (Classen, 1988). Unpublished data from Florida Fish and Wildlife suggests that they may live up to 25 years although past studies indicate a more conservative maximum of 8 years (FWC 2014). In contrast, Atlantic Croaker, which has a similar trophic level of 3.4 has a maximum lifespan in the wild of only 5 years (IGFA, 2001). Another species within the same trophic level, Pigfish, only lives up to 4 years (IGFA, 2001). Both Atlantic Croaker and Pigfish reach approximately 46-55cm maximum length (IGFA, 2001). Hardhead Catfish and

Gafftopsail Catfish can reach up to 69-70cm maximum (IGFA 2001). The fact that the sea catfishes, although they feed on similar trophic levels as Atlantic Croaker and Pigfish, are both longer-lived and larger fish probably explains the greater amount of mercury found in these fish compared to the others. Indeed, many of the catfish specimens used in this study exceeded 190 mm (19 cm) standard length (~3 years old); whereas most of the largest Croaker in this study did not exceed 160 mm (16 cm) (~1 year old) (Matlock, 1975; FWC, 2014; FWC, 2014b). The wide variation in mercury amounts in Pigfish most likely suggests that the small sample size (n=13) is not enough to explain changes in tissue mercury completely within the same trophic level.

Interactions between Species, Bay, and Trophic Levels

There are many potential interactions between variables that could influence the observed mercury in fish tissue. Attempts to utilize broader categories of fish, such as trophic groupings, may introduce additional error. This is because the species of fish analyzed significantly differed in mercury concentrations; most likely because each species inhabits different trophic levels and eat different foods at various sizes which may contain different mercury concentrations. In addition, each species has different life spans and will attain different sizes which reflects other important metabolic differences. These differences would be less noticeable when comparing bays or species, since many of the same species were collected at each bay.The location of where fish were captured, i.e. the specific estuary, is also important, since each estuary has a distinct associated air shed and watershed with unique combinations of

mercury loading from various sources which would ultimately affect the concentration of mercury in fish tissues. For example, Sabine Lake has higher mercury concentrations in fish than other bays.

Due to the limitations of evaluating species and bay separately, as evidenced by examination of the interaction chart, species and bay categories were combined to create separate species-bay categories (Figure 13). From this analysis it appears that sea catfish from Aransas and Galveston exhibited the highest concentration of mercury in tissue (Figure 14). For Galveston, this may be a result of catching fewer, larger specimens of sea catfish in comparison to other Texas estuaries.

One more interesting trend that emerged from the evaluation of interactions of species and estuary is that those species within the lowest trophic levels, Gulf Menhaden (2), Pinfish (2.9), and Spot (3.2), showed the least variation in mercury levels between bays. Since these species vary in morphology and life span –Pinfish lives up to 7 years whereas Spot lives up to 4 years– their uniting characteristic is most likely their trophic level (Hugg, 1996; Ohs, 2011). This suggests that either food sources for these three species do not vary greatly between bays or that the food sources avaliable, diverse though they may be, have similar levels of mercury in them. All three species consume zoobenthos, Pinfish and Gulf Menhaden consume plants, and Spot consumes detritus (Bowman et al. 2000; Hansen 1970; Matlock and Garcia 1983). Since they share some but not all food sources, both hypotheses may possibly

explainthe overall low variation between bays by mercury in the tissues of these fish.

Morphology

Different fish species grow at vastly different rates and have many different maximum lengths, so it is no surprise that when all species are combined and analyzed using a regression analysis linear trends explained only ~30% of variation in mercury tissue levels. However, it is clear that, in general, as fish grow larger the amount of mercury present in fish tissue can be expected to increase according to prior literature (Mcclain et al. 2006). One hypothesis is that fish feed on higher trophic levels as size increases (Mcclain et al. 2006). Furthermore, larger fish, even within a species, eat larger prey. The literature also suggests that fish with slower growth rates exhibit size-mercury concentration relationships with steeper slopes, which may partially explain the steeper relationships seen in Hardhead Catfish and Gafftopsail Catfish, the two long-lived catfish species (Mcclain et al. 2006).

In contrast to length, weight was not a good predictor of mercury; linear trends only explained 17.4% of variation. Availability of food may have played a role in this. If food was scarce, the same age fish would weigh significantly less than a well-fed counterpart although the two fish may have the same length.

Trophic Levels

Interestingly enough, during our study tissue from omnivorous fish contained lower median mercury concentrations compared to herbivorous or carnivorous fish; whereas median mercury levels were not significantly different

between herbivores and carnivores (Figure 22). This pattern is different than what was expected based on current literature documenting positive correlations between trophic levels and mercury content (Eagles-Smith et al. 2008; Mcclain et al. 2006; Wang 2002).

However, results from this study do suggest that lower trophic levels exhibited less variability in mercury tissue levels compared to higher trophic levels. A wider range of prey items available to higher trophic levels might explain the variation in observed mercury tissue concentrations. The one apparent exception to this trend was Spot which exhibited low variability in mercury tissue levels despite its comparatively high trophic level.

Thus, if mercury levels and variation within a species is an indicator of expressed trophic level the placement of Spot in the omnivore category may be influencing the outcome of the analysis by lowering the overall median mercury tissue level of omnivores. If the test were run again with Spot classified as an herbivore, both herbivores and carnivores mercury tissue levels would be significantly different (p=0.0061) and herbivores and omnivores would be barely significant (p=0.0552). Therefore, the trophic placement of Spot may be key to understanding differences in mercury concentration by trophic position. To this end, stomach content studies to determine the feeding patterns of local populations of Spot would greatly assist in future studies.

Fish Residency and Usability of Data

Another factor that influences the amount of mercury in fish is whether or not the sampled fish are resident or transient. A study by Fry and Chumchal

(2012) compared mercury levels in Louisiana coastal areas based on differences in the residency pattern of fish species. The study examined isotopes in the local fish and found that they provided evidence that many estuarine fishes showed a high level of residency (Fry and Chumchal 2012). High levels of residency means that patterns observed during this study between bays in which estuarine fish were caught infers fundamental differences between each estuary. In contrast low residency would obscure any possible inferences that could be made about the influence of a particular estuary where a fish was captured since a majority of life span and potential exposure to mercury may have occurred in another geographic area(s).

An analysis of mercury speciation in fish tissue could also potentially support published literature which claims that most mercury in fish tissue is methylmercury and assist this study.

Overall, further investment in both trophic analysis including stomach content analysis, as well as stable isotope analysis would help explain spatial patterns in mercury in tissue between species and estuaries This study provides an excellent reference point in which to begin or continue exploring spatial and temporal trends in mercury and related contaminants among the most common estuarine fish inhabiting Texas and Gulf coast estuaries and their potential risk to humans.

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APPENDIX 1. IACUC PROTOCOL.

v.110323
Protocol # _11.001. R1

University of Houston-Clear Lake
Institutional Animal Care and Use Protocol

Federal animal welfare regulations require that the Institutional Animal Care and Use Committee (IACUC) must review and approve all activities involving the use of vertebrate animals prior to initiation of such use. Once approved by the IACUC, any change(s) to the following protocol must be submitted in a written amendment for review and approval of the IACUC prior to implementation of the change(s).

1. Title of Project
Long term changes in freshwater, estuarine and marine fish populations in Texas due to biological competition and alteration of habitat and water quality.

2. Principal Investigator:

Name:	George Guillen		
Title:	Associate Professor	Program:	EIH
Phone:	281-283-3950	Emergency Phone:	281-804-5607
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Secondary Contact Person involved in the study:

Name:	Jenny Oakley		
Email:	Oakley@uhcl.edu	Emergency Phone:	713-392-7975

3. Project Type

☐ New Protocol ☒ Renewal ☐ Addendum Previous Protocol # 11.001		
If renewal: ☒ Annual Renewal ☐ Protocol Renewal **see Appendix E**		
If addendum: ☒ Personnel Change ☒ Minor Revision ☐ Major Revision **see Appendix E**		
Number of years project is expecting to continue: ☐ 1 year ☒ 2 years ☐ 3 years		
This protocol is for: ☐ Teaching ☒ Research ☐ Breeding		
If teaching:		
Course name and number:		
Frequency course is offered:		
If research:		
How will this project be funded:	NOAA, HGAC, Harris County Flood Control District, TPWD	
If grant, this project is:	☐ Pending ☒ Funded – Federal ☒ Funded – Other	
Grant title and/or contract number (if available):		
Has this project already received an independent scientific peer-review?	☒ Yes	☐ No
If yes, by whom?	NOAA	

4. Location

Where will animals be housed?	☐ UHCL Animal Research Facility ☒ Field Study	
☐ Other:*		
Where will animal use take place?	Texas (Freshwater and Marine Ecosystems).	
Will animals be kept for over 12 hours outside of housing area?	☐ Yes*	☒ No
If yes, give location and reason:		

*A standard operating procedure (SOP) to ensure proper welfare and housing of animals must be attached to this protocol in Appendix F. This does not apply to animals housed at other AAALAC accredited animal facilities (e.g., UH or NASA).

Page **1** of **11**

5. Lay Summary:
Describe the goals and intended benefits of the project in terms that can be understood by a non-scientist. Include the species and the number of animals to be used. This description should be no more than 250 words. Avoid the use of technical jargon and abbreviations.

This ongoing research project will investigate the relative role that habitat, invasive species and water quality plays in the structuring fish populations that inhabit freshwater, estuarine and marine ecosystems in, south central and southeast Texas, and the upper coast of the Gulf of Mexico. Fish communities, water quality and habitat will be sampled in streams, reservoirs, marshes, bayous, open bays and the near-shore Gulf of Mexico to determine the relationships between fish diversity, population parameters, and the abundance of alien species, habitat and water quality. This research project will incorporate the collection of fish in the field for taxonomic identification and population assessment. Sampling methods will include the collection of fish using standardized seines nets, plankton nets, traps, gillnets, and electrofishing gear (Murphy and Willis 1996. Fisheries Techniques). It is difficult to accurately estimate the total number of fish that will be collected. In fact that is one of our primary study objectives. However, based on historical data we expect to collect 15 to 65 species (average 21) per site. In general, we expect that on average fewer than 1000 fish per species will be collected at each site during the study period each year. For approximately 50% of the species sampled, we expect to collect less than 30 specimens per site. Depending on ultimate funding and the resulting number of sites monitored, the maximum total number of fish that could be collected annually from 38 sites would be between 650,000 – 2,470,000 fish. Due to the high fecundity of fish and small sample size relative to the waterbodies studied (Southeast Texas estuaries and nearshore Gulf of Mexico); these samples would however represent a minute fraction of the total population of any given species (Martinez-Andrade et al. 2005; Donaldson et al. 1996). Invertebrate species will also be collected. Approximately 95% of all individuals of all fish collected for the study will be euthanized. These fish will be sacrificed with MS222 immediately after collection. The remaining fish (5%) will be released back into the water body unharmed. It is necessary to retain these specimens for identification due to inherent difficulty in identifying this taxonomically complex group and their small size, and need to critically examine physical features necessary for correct species identification. A small number (< 50 year) of larger fish (sharks, gar, blue catfish, tarpon) may be tagged with acoustic tags and/or dart tags to track movement within riverine systems. This would be in cooperation with a Gulf wide shark monitoring program sponsored by NOAA. A few live specimens of stingrays may be transported for use by other researchers under their approved animal care protocol.

State or federally listed protected species will not be retained. The principal investigator has all required government collecting permits. The long range objective of my study is to develop a predictive habitat/fish model useful for management of fisheries resources and water quality including development of biocriteria (e.g. state and EPA biological surface water regulatory criteria).

6. Animal Use:
Provide the specifications for all of the animals requested for use in this protocol. List each strain separately.

Species (common name)	Breed/Strain	Vender/Source	Number Requested			
			Year 1	Year 2	Year 3	Total

☒ Not Applicable (this is a population field study)
If a population field study, check all vertebrate animals that are <u>planned</u> to be studied:
☒ Fish ☐ Amphibians ☐ Reptiles ☐ Birds ☐ Mammals / ☐ Cetaceans

7. Personnel:
List all personnel having contact with animals, the species proposed and the years of experience the individual has with the species. List the specific roles the individual will have in the project and the date of last training received.

Name, Degree, Title	Species and Years of Experience	Specific Role in Project*	TRAINING DATE
George Guillen, Assoc. Professor Biology, Ph.D.	Fish, 30	Principal Investigator	5/4/2011
Greg Knothe, B.S.Grad student	Fish, 4	Field Monitoring	5/6/2011
Jenny Wrast, M.S.,Senior Research Assoc.	Fish, 10	Field Coordinator	5/6/2011
Colby Lawrence, B.S. Res. Assoc.	Fish 10	Field Monitoring	5/6/2011
Emma Clarkson, B.S. Grad Student	Fish 4	Field Monitoring	5/6/2011
Abby Marlow, B.S. Grad Student	Fish 4	Field Monitoring	5/6/2011
Alex Miller, B.S. Grad Student	Fish 4	Field Monitoring	5/6/2011
Khem Paudel, B.S.Grad Student	Fish 1	Field Monitoring	5/6/2011

Misty Shepard, B.S. Research Assoc.	Fish 1	Field Monitoring	5/6/2011
Kristen Vale, B.S. Research Assoc.	Fish 2	Field Monitoring	NA
Sybil Glenos	Fish 2	Field Monitoring	NA
Laila Melendez	Fish 2	Field Monitoring	NA
Up to 4 new students - future	Fish 1-2	Field Monitoring	NA

* Examples include: supervision, care/handling, anesthesia, surgery, monitoring, post-procedural care, euthanasia in the stated species

☒ Student visitors will/may participate in this protocol and will be supervised by: George Guillen

8a. Literature Search

Using at least two different databases, perform literature searches to determine alternatives to procedures that may cause more than a momentary or slight pain or distress to the animals, and unnecessary duplication of research.

Search Database (e.g., Agricola)	Date of Search MM/DD/YYYY	Years Covered (e.g., 1980 – 2010)	Keywords or Search Strategy Used in Search
* UHCL One Search	3-29-12	pre-2012 47,785 references	Alternative approaches to site specific fish population sampling
* *Google Scholar	3-29-12	pre-2012 60,400 references	Alternative approaches to site specific fish population sampling

* No articles found providing alternative methods.

** No articles found providing alternative methods.

8b. Rationale and purpose of animal use

State the overall rationale, purpose, and significance of this project.

Native fish communities are at risk due to changing climate, habitat degradation and ongoing water quality impairment and invasive species. Recent studies have shown that alteration of native fish communities in Texas may be occurring due to an interaction of various factors including long term changes in habitat quality reduced freshwater inflow, global warming and introductions of invasive species. Management agencies are considering closure of certain areas to fishing to reduce damage to habitat from fishing and trawling. In order to assess the extent of this degradation and responses to ongoing management actions it is necessary to conduct analyses of time series data from before and after management actions or alterations in the environment. Fortunately, data sets exist that can be used to compare current population data with previous data collected during the 1970-1990's. My research will focus on the quantification of habitat and water quality and their affect on marine, estuarine and freshwater fishes. Fish communities, water quality and habitat will be sampled in streams, reservoirs, marshes, bayous, open bays and the near-shore Gulf of Mexico to determine the relationship of diversity, population parameters, and these variables. In addition, changes in these environmental variables and fishing pressure will be used to develop predictive models of fish abundance over a wide salinity regime.

The collection and identification of fish is a necessary component of my research study. One of the primary objectives is to estimate the density and abundance of individual species populations and overall community structure. This data will be combined with historical data collected various researchers and agencies in the 1980's and 1990's have to evaluate temporal and spatial trends in diversity and community composition. This study will facilitate the investigation of the relative role of habitat and water quality in structuring of native fish populations that inhabit freshwater, estuarine and marine ecosystems across a salinity gradient.

All of these past studies, including those conducted by state and federal agencies, have used standardized gear and effort. Therefore we will use the same gear to facilitate comparison of our data with historical data. Some of our sampling will occur in boats or in remote isolated waterbodies accessible only by foot. We plan to monitor up to 38 sites in the Galveston and East Mataagorda Bay watershed and nearshore Gulf of Mexico over a three year period. Most of these sites will be monitored 1-2 times a year, while a few (10) may be monitored quarterly. Depending on gear used and time of year, a variety of species may be collected. It is difficult to accurately estimate the total number of fish that will be collected. In fact that is one of our primary study objectives.

However, based on historical data we expect to collect 15 to 65 species (average 21) per site. In general, we expect that on average fewer than 1000 fish per species will be collected at each site during the study period each year. For approximately 50% of the species sampled, we expect to collect less than 30 specimens per site. Depending on ultimate funding and the resulting number of sites monitored, the maximum total number of fish that could be collected annually from 38 sites would be between 650,000 – 2,470,000 fish. Due to the high fecundity of fish and small sample size relative to the waterbodies studied (Southeast Texas estuaries and nearshore Gulf of Mexico); these samples would however represent a minute fraction of the total population of any given species (Martinez-Andrade et al. 2005; Donaldson et al. 1996). Invertebrate species will also be collected. Approximately 95% of all individuals of all fish collected for the study will be euthanized. These fish will be sacrificed with MS222 immediately after collection. The remaining fish (5%) will be released back into the water body unharmed. Based on the primary sampling gear that we will be using (trawls, seines, plankton nets, and back-pack electroshockers), the majority of species that we will be collecting will be small (< 6 inches) or juvenile and larval fish. State or federally listed protected species will not be retained. The principal investigator has all required government collecting permits.

Due to extremely low water clarity, and the presence of density gradients and bottom obstructions, it is impossible to use visual or acoustic census techniques that would reduce the need to retain specimens. In most cases, due to the small size and/or taxonomic diversity of fish there is a need to utilize taxonomic keys that emphasize meristic (e.g. fin ray counts, morphological measurements, etc.) internal and external characters, to identify fish reliably. Due to the high diversity of fishes collected, their small size, and need to obtain in some cases hard structure samples (scales and otoliths) for aging, it is nearly impossible to analyze and release all fish back into the field. We must bring them back for identification and/or processing. In addition, large amounts of debris including submerged grass, mud and invertebrates (e.g., shrimp, crabs, etc.) are occasionally collected as well, increasing processing time. Finally, due to the patchy nature of fish distribution due to schooling and migration, we occasionally can be overwhelmed with a large catch, which of course reduces processing time. However, we plan to release whenever possible, larger more easily identified specimens. In all other cases, fish will be collected, euthanized, and subsequently preserved for later identification and/or removal of otoliths.

Population and community statistics that would be generated include density, catch per unit effort, absolute numbers, community diversity, size and age composition (commercially and recreationally important species), and by calculation growth and mortality of the population. Therefore primary variables like density must be estimated using catch statistics and/or mark recapture methods. Other variables are derived from measurements of annuli (annual growth rings) on scales, spines, otoliths (ear bones), or length and weight. In addition, we plan to extract tissue from a subsample of recently

euthanized dead fish. This tissue will be analyzed for one or all of the following condition biomarkers including length-weight condition factors, RNA/DNA ratios (larvae), liver condition/weight indices, hematocrit/leucocrit levels and level of parasitism.

A small number (< 50 year) of larger fish (sharks, gar, blue catfish, tarpon) may be tagged with acoustic tags and/or dart tags to track movement within riverine systems. This would be in cooperation with a Gulf wide shark monitoring program sponsored by NOAA Training on the proper care of animal specimens and safety will be provided to all participants using this protocol.

8c. Justification for animal use.
Explain why non-animal models such as isolated organ preparation, cell or tissue culture, or computer simulation cannot be used.

Since populations and communities of fish vary geographically based on known patterns of biogeography and land use it is necessary to evaluate the response of local populations to adjust for this source of variation when evaluating waterbodies. The central question of this study is how fish communities have varied both spatially and temporally within Texas coastal systems, including freshwater tributaries. Fish population analysis requires the evaluation of fish populations and vital statistics. This requires the collection of fish density data, growth information estimated from bony structures and length data, and spawning as estimated from gonad developmental stage, and overall condition (weight and length data). There are no other animal models in ecology that can substitute for this group, since we are trying to evaluate local endemic population trends.

8d. Justification for using this particular species.
Explain why the species and/or strain(s) requested is/are the most appropriate for this research. Statements that the planned species is traditionally used for the proposed research are not sufficient.

The purpose of the study is to evaluate local populations and community structure and their response to changing climate, habitat change and freshwater flow regime. Sponsoring agencies need site specific data.

8e. Alternatives to Potentially Distressful Procedures
Describe considerations of alternatives to procedures that may cause more than a momentary or slight pain or distress to the animals, and determination that alternatives were not available.
☒ Not Applicable (animals listed are only in USDA Category B or C)

8f. Assurance of Non-Duplication
☒ This experiment does not unnecessarily duplicate previous experiments.
Otherwise, provide justification of the necessity of experiments proposed.

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9. Justification of animal numbers.
Provide a detailed justification for the numbers of animals requested. Include number of animals per group and total number of animals. If power analysis was utilized, give appropriate details. If the determination was based on prior experience, please cite reference. If a population study in the field give justification of sampling method.

This is a population and community study. The research question requires we collect these organisms and identify them in the laboratory due to difficulty taxonomy. Based on past studies

we expect that on the average fewer than 250 fish per species will be collected at each site during the study period each year. For greater than 70% of the species sampled, less than 30 specimens will be collected. Approximately 95% of all individuals of each species collected will be euthanized for the study. These fish will be sacrificed with MS222 immediately after collection. The remaining fish (5%) will be released back into the water body unharmed. Based on past years a maximum number of 17,000 fish per /year may be collected study-wide. This number represents a minute percentage of the total number of fish present in regional waterbodies based on extrapolation of density of fish per stream distance. The methods used include seining and electrofishing which are identified in the TCEQ guidance manual as the standard protocol required for studies that will be used for regulatory purposes. Also, this method allows comparison with past data.

Animals sacrificed in the field will be used to estimate various population parameters including density, mortality, growth, and age. Density will be obtained from counting the number of organisms collected by unit area or volume. Mortality, growth and age data will be obtained from a variety of methods including, reductions in catch per unit effort (CPUE) over various age groups, length or weight frequency analysis (e.g., statistical mixture analysis), analysis of otolith (ear bones) and scale growth rings (ear bones), and examination of RNA/DNA ratios. These statistical methods are outlined in various fisheries population analysis texts including Pauly (1984), Hilborn and Walters (1991), and Quinn (1998). Due to the nature of ecological research it is difficult to project absolute numbers of fish that will be collected. In fact that is one of our primary objectives of our study. However, based on historical data, we expect to collect 15 to 65 species (average 21) per site. In general, we expect that on average fewer than 1000 fish per species will be collected at each site during the study period each year. For approximately 50% of the species sampled, we expect to collect less than 30 specimens. A small number (< 50 year) of larger fish (sharks, gar, blue catfish, tarpon) may be tagged with acoustic tags and/or dart tags to track movement within riverine systems. This would be in cooperation with a Gulf wide shark monitoring program sponsored by NOAA

Depending on future funding and the resulting number of sites monitored, the maximum total number of fish that could be collected annually from 38 sites would be between 650,000 – 2,470,000 fish. During the first year less than 1000 fish were collected. Due to the high fecundity of fish and small sample size relative to the waterbodies studied (Southeast Texas estuaries and nearshore Gulf of Mexico); these samples would however represent a minute fraction of the total population of any given species (Martinez-Andrade et al. 2005; Donaldson et al. 1996). . State or federally listed protected species will not be retained. The principal investigator has all required government collecting permits.

Martinez-Andrade, F., P. Campbell and B. Fuls. 2005. Trends in relative abundance and size of selected finfishes and shellfishes along the Texas coast: November 1975-December 2003. TPWD Management Data Series 232. Austin, Texas

Donaldson, D., N. Sanders Jr., R. Minkler and P. Thompson. 1996. SEAMAP Environmental and Biological Atlas of the Gulf of Mexico, 1993. Gulf States Maine Fisheries Commission. Ocaan Springs, MS.

10. Pain, Discomfort, and Distress.
a. USDA Pain/Distress Classification
Check the category that indicates the highest level of pain/distress the animals will experience during the course of these studies. (Refer to the Instructions, Section 10 for help.)
☐ Category B   ☒ Category C   ☐ Category D   ☐ Category E

b. If Category E is selected, provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

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11. What will happen with animals at the end of their roles in the project.
a. Check all that apply and provide explanation if necessary.
☐ Placed for adoption
☐ Released into wild (field study)
☒ Euthanasia:
Rodents:
  ☐ CO_2- followed by secondary method (e.g. bilateral thoracotomy, cervical dislocation)
    State secondary method:
  ☐ Injectable agent *(Specify Agent, Route, Dose):*
  ☐ Inhalant agent *(Specify Agent, Dose):*
  ☐ Cervical Dislocation (rodents < 200 gm) w/ anesthesia- *(Specify Agent, Route, Dose):*
  ☐ Decapitation/Guillotine w/ anesthesia- *(Specify Agent, Route, Dose):*
  ☐ Exsanguination w/ anesthesia *(Specify Agent, Route, Dose):*
  ☐ Anesthetic + Perfusion *(Specify Agent, Route, Dose):*
    Type of perfusion:
Amphibians, Fish, Reptiles:
  ☐ CO_2
  ☐ Injectable agent *(Specify Agent, Route, Dose):*
  ☐ External or topical agent *(Specify Agent, Route, Dose):*
  ☒ Inhalant agent *(Specify Agent, Dose):* MS-222, 1,000 mg/L
  ☐ Decapitation and pithing
  ☒ Stunning and decapitation/pithing

☐ Other:

b. Explanation / Justification
MS-222 will be used in a dip bath to euthanize smaller fish retained for species identification. Larger fish that are not identified in the field and are retained for i.d. in the lab will be euthanized by a sharp blow to the head. Death is confirmed by cessation of operculum movement.

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12. Additional Forms Attached
Check all that apply and attach appropriate forms. If a form is not needed, delete the page from the protocol.
☐ Appendix A: Laboratory Research or Classroom
☐ Appendix B: Surgical Procedures
☒ Appendix C: Wild Animal and/or Field Research
☐ Appendix D: Safety
☒ Appendix E: Renewal / Addendum
☐ Appendix F: Additional Information / Standard Operating Procedures

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13. Check the following:

☒ I certify that the use of all animals involved in this project will be carried out within the provisions of the Animal Welfare Act, the Guide for Care and Use of Laboratory Animals, the PHS Policy on Humane Care and Use of Animals, the University of Houston Policy on Care and Use of Animals and related animal welfare rules and regulations as issued by state and/or federal agencies.

☒ I am aware that the Institutional Animal Care and Use Committee (IACUC) may make periodic inspections of all labs in which animals are used. I will permit unannounced inspections and observations of my animals and surgical techniques by a UH veterinarian or other representative of the IACUC.

☒ I am aware that the IACUC is empowered to stop any objectionable procedure or project. Where procedures have caused severe distress to an animal which cannot be alleviated, UH staff veterinarians are authorized to humanely euthanize that animal. I understand that every attempt will be made to contact me before any action is taken.

☒ I understand that I cannot start this project until I have received approval from the IACUC.

☒ I understand that I will make written notification to the IACUC of any proposed changes to the project. I understand that I will not be able to implement such changes until approval is received from the IACUC.

☒ I certify that the above statements are true and that I will make written notification to the IACUC of any changes in the proposed project prior to proceeding with any animal experiment.

George Guillen   3/28/12
Signature of Principal Investigator or Instructor   Date
☒ Submitted Electronically: Instead of signature, protocol is emailed from the PI's UHCL email address

Appendix C: Wild Animal and/or Field Research
Delete page if not needed.

1. How will the animals be captured?

Seine, trawls, gillnets, long-line, and electrofishing. Seine distances will be less than 30 ft per haul to reduce stress and mortatlity. Trawls will be towed for less than 10 minutes to reduce mortality and stress. Electroshocking will be done using a Smith Root electroshocker designed for collection of fisheries data. Total electroshocking effort duration will be less than 90 seconds of effort. All collections methods will follow protocols outlined in:

Gillnets and long-lines will not be deployed longer than 2 hrs to reduce mortality. Larger fish will be released within 5 minutes. A small number (< 50 year) of larger fish (sharks, gar, blue catfish, tarpon) may be tagged with acoustic tags and/or dart tags to track movement within riverine systems.

Nickum, J.G., H. L. Bart, Jr, P. R. Bowser, I. E. Greer, C. Hubbs, J. A. Jenkins, J., R. MacMillan, J. W. Rachlin, J. D. Rose, P. W. Sorensen, and J. R. Tomasso. 2004. Guidelines for the Use of Fishes in Research. Published by: American Fisheries Society, American Institute of Fishery Research Biologists, American Society of Ichthyologists and Herpetologists.. American Fisheries Society. Bethesda, M.D.

2. Will animals be maintained for any length of time, where they will be maintained (field and/or animal facility), and for how long?

Most will be euthanized within 2-5 minutes. Those retained up to 10 minutes will be kept in a large bucket with aeration prior to identification, length and weight measurement and release. Larger fish will be released within 5-10 minutes to reduce stress.

3. Will animals be transported, and if so, how will stress be minimized?

No

4. How will the housing and nutritional needs of animals that are captured and detained for a research project be met?

Not applicable. All organisms will be euthanized unless resleased immediately

5. What criteria will be used in determining whether the animals can be released after they have been captured (even if the animals are part of a capture and release project, and they will not be maintained for any length of time)?

Normal gill operculum movement and response to tactile stimulus

6. How will pain and/or distress be monitored in these animals?

Primarily through observation of operculum respiration rate.

Appendix E: Renewal / Addendum
Delete page if not needed.

1. Please check all that apply:

☒ Renewal	☒ Addendum		
If renewal:	☐ Annual Renewal	☐ Protocol Renewal (3 yr)	☐ No Changes in Protocol
If addendum:	☐ Personnel Change	☐ Minor Revision	☐ Major Revision

2. Current Status
☒ Active   ☐ Temporarily Inactive   ☐ Never Started
If the study is temporarily inactive, or never started please explain why:

3. Personnel Change
Please list personnel added or removed from the protocol. Additionally, the personnel list along with experience, role and training should be updated on the original protocol to reflect current project personnel.

Add	Remove
Kristen Vale	Kevin Young
Sybil Glenos	Amanda Moss
Laila Melendez	Rosaleen March
	Jonathan Barton

4. Methodological Changes
Has there been, or do you anticipate in the next 12 months, any change in your protocol?
☒ Yes   ☒ No
This includes change of species, change in numbers of animals used, change of techniques, change of anesthesia or analgesia, change in drugs or test chemicals being used, changes in euthanasia methods, etc. **Briefly** summarize changes below. In addition, edit the original protocol submission and indicate any changes by underlining new text.

A small number (< 50 year) of larger fish (sharks, gar, blue catfish, tarpon) may be tagged with acoustic tags and/or dart tags to track movement within riverine and marine systems. This would be in cooperation with a Gulf wide shark monitoring program sponsored by NOAA.

5. Problems
Did any of the animals used have an unanticipated adverse reaction(s)?
☐ Yes   ☒ No
If yes, please describe:

6. Progress
Describe the progress and/or any significant findings relative to this project. Projects which have not realized any progress should provide an explanation. Please include citations of abstracts, publications, etc. (full text is not needed).
Project activities were completed through 2011. We are in process of completing interim report report. Students will be using part of this data for M.S. thesis projects. Project will continue 2 more years at least.

APPENDIX 2. NCCA LABORATORY PROCEDURES.

From the 2015 NCCA Laboratory Methods Manual (USEPA 2009):

4.3 MERCURY IN FISH TISSUE AND SEDIMENTS

4.3.1 Scope of Application

1. This method may be used for both saltwater and freshwater samples.
2. This procedure measures total mercury (organic v. inorganic) in fish tissue and sediments.
3. The range of the method is 0.2 - 5 µg/g. The range may be extended beyond the normal range by increasing or decreasing sample size or through instrument and recorder control.

4.3.2 Summary of Method

1. A weighed portion of the sample is digested in aqua regia for 2 minutes at 95°C, followed by oxidation with potassium permanganate. Mercury in the digested sample is then measured by the conventional cold vapor technique.
2. An alternate digestion involving the use of an autoclave is described in section 4.3.8.3.

4.3.3 Sample Handling and Preservation

1. Because of the extreme sensitivity of the procedure and the omnipresence of mercury, care must be taken to avoid extraneous contamination. Sampling devices and sample jars should be ascertained to be free of mercury; the sample should not be exposed to any condition in the laboratory that may result in contact or air-borne mercury contamination.
2. While the sample may be analyzed without drying, it has been found to be more convenient to analyze a dry sample. Moisture may be driven off in a drying oven at a temperature of 60°C. No mercury losses have been observed by using this drying step. The dry sample should be pulverized and thoroughly mixed before the aliquot is weighed.

4.3.4 Interferences

1. The same types of interferences that may occur in water samples are also possible with fish tissue and sediments, *i.e.*, sulfides, high copper, high chlorides, *etc.*
2. Volatile materials which absorb at 253.7 nm will cause a positive interference. In order to remove any interfering volatile materials, the dead air space in the BOD bottle should be purged before the addition of stannous sulfate.

4.3.5 Apparatus

1. Atomic Absorption Spectrophotometer. Any atomic absorption unit having an open sample presentation area in which to mount the absorption cell is suitable. Instrument settings recommended by the particular manufacturer should be followed.
2. Mercury Hollow Cathode Lamp. Westinghouse WL-22847, argon filled, or equivalent.
3. Any multi-range variable speed recorder that is compatible with the UV detection system.
4. Standard spectrophotometer cells 10 cm long, having quartz end windows may be used. Suitable cells may be constructed from plexiglass tubing, 1" O.D. X 4-1/2". The ends are ground perpendicular to the longitudinal axis and quartz windows (1" diameter X 1/16" thickness) are cemented in place. Gas inlet and outlet ports (also of plexiglass but 1/4" O.D.) are attached approximately 1/2" from each end. The cell is strapped to a burner for support and aligned in the light beam to give the maximum transmittance.

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Note. Two 2" X 2" cards with one inch diameter holes may be placed over each end of the cell to assist in positioning the cell for maximum transmittance.

1. Air Pump. Any peristaltic pump capable of delivering 1 liter of air per minute may be used. A Masterflex pump with electronic speed control has been found to be satisfactory. Regulated compressed air can be used in an open one-pass system.
2. Flowmeter. Flowmeter must be capable of measuring an air flow of 1 liter per minute.
3. Aeration Tubing. Tygon tubing is used for passage of the mercury vapor from the sample bottle to the absorption cell and return. Straight glass tubing terminating in a coarse porous frit is used for sparging air into the sample.
4. Drying Tube. 6" X 3/4" diameter tube containing 20 g of magnesium perchlorate.

Note. In place of the magnesium perchlorate drying tube, a small reading lamp

with 60W bulb may be used to prevent condensation of moisture inside the cell.

The lamp is positioned to shine on the absorption cell maintaining the air

temperature in the cell about 10°C above ambient.

4.3.6 Reagents

1. Aqua Regia. Prepare immediately before use by carefully adding three volumes of conc. HCl to one volume of conc. HNO3.
2. Sulfuric Acid, 0.5 N. Dilute 14.0 ml of conc. sulfuric acid to 1 liter.
3. Stannous Sulfate. Add 25 g stannous sulfate to 250 ml of the 0.5 N sulfuric acid. This mixture is a suspension and should be stirred continuously during use.
4. Sodium Chloride-Hydroxylamine Sulfate Solution. Dissolve 12 g of sodium chloride and 12 g of hydroxylamine sulfate in distilled water and dilute to 100 ml.

Note. A 10% solution of stannous chloride may be substituted for the stannous

sulfate and hydroxylamine hydrochloride may be used in place of hydroxylamine

sulfate in the sodium chloride-hydroxylamine sulfate solution.

1. Potassium Permanganate. 5% solution, w/v. Dissolve 5 g of potassium permanganate in 100 ml of distilled water.
2. Stock Mercury Solution. Dissolve 0.1354 g of mercuric chloride in 75 ml of distilled water. Add 10 ml of conc. nitric acid and adjust the volume to 100.0 ml. 1.0 ml = 1.0 mg Hg.
3. Working Mercury Solution. Make successive dilutions of the stock mercury solution to obtain a working standard containing 0.1 µg/ml. This working standard and the dilution of the stock mercury solutions should be prepared fresh daily. Acidity of the working standard should be maintained at 0.15% nitric acid. This acid should be added to the flask as needed before the addition of the aliquot.

4.3.7 Calibration

1. Transfer 0, 0..5, 1.0, 2.0, 5.0 and 10 ml aliquots of the working mercury solution containing 0 to 1.0 µg of mercury to a series of 300 ml BOD bottles. Add enough distilled water to each bottle to make a total volume of 10 ml. Add 5 ml of aqua regia and heat for 2 minutes in a water bath at 95°C.

2. Allow sample to cool; add 50 ml distilled water and 15 ml of KMnO4 solution to each bottle and return to water bath for 30 mins. Cool and add 6 ml of sodium chloride-hydroxylamine sulfate solution to reduce the excess permanganate. Add 50 ml distilled water.

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1. Treating each bottle individually, add 5 ml of stannous sulfate solution and immediately attach the bottle to the aeration apparatus. At this point, the sample is allowed to stand quietly without manual agitation. The circulating pump, which has previously been adjusted to rate of 1 liter per minute, is allowed to run continuously. The absorbance, as exhibited either on the spectrophotometer or the recorder, will increase and reach maximum within 30 seconds. As soon as the recorder pen levels off, approximately 1 minute, open the bypass value and continue the aeration until the absorbance returns to its minimum value. Close the bypass value, remove the fritted tubing from the BOD bottle and continue the aeration. Proceed with the standards and construct a standard curve by plotting peak height versus micrograms of mercury.

Note. Because of the toxic nature of mercury vapor precaution must be taken to avoid its inhalation. Therefore, a bypass has been included in the system to either vent the mercury vapor into an exhaust hood or pass the vapor through some absorbing media, such as:

a) equal volumes of 0.1 N KmnO4 and 10% H2SO4

b) 0.25% iodine in a 3% KI solution.

Specially treated charcoal that will absorb mercury vapor is also commercially available.

4.3.8 Procedure

1. Weigh triplicate 0.2 g portions of dry sample and place in bottom of a BOD bottle. Add 5 ml of distilled water and 5 ml of aqua regia. Heat for 2 mins in a water bath at 95°C. Cool, add 50 ml distilled water and 15 ml potassium permanganate solution to each sample bottle. Mix and place in the water bath for 30 mins at 95°C. Cool and add 6 ml of sodium chloride-hydroxylamine sulfate to reduce the excess permanganate. Add 55 ml of distilled water.

2. Treating each bottle individually, add 5 ml of stannous sulfate and immediately attach the bottle to the aeration apparatus. Continue as described under section 4.2.7.3 above.
3. An alternate digestion procedure employing an autoclave may also be used. In this method 5 ml of conc. H2SO4 and 2 ml of conc. HNO3 are added to the 0.2 g of sample. Add 5 ml of saturated KMnO4 solution and the bottle covered with a piece of aluminum foil. The samples are autoclaved at 121°C and 15 lbs. for 15 minutes. Cool, make up to a volume of 100 ml with distilled water and add 6 ml of sodium chloride hydroxylamine sulfate solution to reduce the excess permanganate. Purge the dead air space and continue as described under section 4.3.7.3.

4.3.9 Calculation

1. Measure the peak height of the unknown from the chart and read the mercury value from the standard curve.
2. Calculate the mercury concentration in the sample by the formula:

µg Hg/g = ___µg Hg in the aliquot___ wt. of the aliquot (g)
1. Report mercury concentrations as follows:

Below 0.1 µg/g, <0.1; between 0.1 and 1 µg/g, to the nearest 0.01 µg;

Between 1 and 10 µg/g, to nearest 0.1µg;

Above 10 µg/g, to nearest µg.

National Coastal Condition Assessment Laboratory Methods Manual Date:

November 2010

From 2015 NCCA Field Manual:

13.2 FISH TISSUE PLUG [FPLG]
13.2.1 SUMMARY OF METHOD
Because many fish spend their entire life in a particular water body, they can be important indicators of water quality, especially for toxic pollutants (e.g., pesticides and trace elements). Toxic pollutants, which may be present in the water column or sediments at concentrations below our analytical detection limits, can be found in fish tissue above detection limits due to bioaccumulation.

Typical fish tissue collection methods require the fish to be sacrificed, whether it be a whole fish or a skin-on fillet tissue sample. This can be problematic when there is a need to collect large trophy-sized fish for contaminant analysis or when a large sample size is necessary for statistical analysis. The following method collects fish tissue plugs instead of a skin-on fillet. One fish tissue plug for mercury analysis will be collected from each of two fish of the same species (one plug per fish) from the target list (below) at every site. These fish are collected during the ecological fish tissue collection effort (**Sections 13.1** and **13.3**). In order of preference, fish tissue plugs should be collected from 1) an ecological fish specimen that will be sent to the lab (when size and species requirements overlap), or 2) a live fish that will be released after the plug has been collected. When possible, select larger individuals from which to collect the fish plugs. Do not collect fish plugs from specimens that are part of the human health fish tissue sample collection. A tissue plug sample is collected by inserting a biopsy punch into a de-scaled area of dorsal muscle section of a fish. After the plug has been collected, ecofish specimens are frozen according to the protocol in **Section 13.1**; if a plug is collected from a live fish, antibiotic salve is placed over the wound and the fish is released.

13.2.3 SAMPLING PROCEDURE

The fish tissue plug indicator samples will be collected using the same gear and procedures used to collect the ecological and/or human health fish tissue samples, and collection occurs within the same area as other fish collections. Samples should be taken from the species listed in the target list (primary and secondary species) found in Table 13.8 and Table 13.9. When ecofish specimens meet the size and species requirements for fish plug samples, the plugs should be taken from the ecofish prior to placing on ice. If ecofish specimens do not meet the size and species requirement for fish plugs, fish plugs should be taken from live fish and the fish are released with antibiotic salve on the wound, as in step 14 below. If the recommended primary and secondary species are unavailable, the fisheries biologist will select an alternative species (i.e., a species that is commonly consumed in the study area, with specimens of harvestable or consumable size) to obtain a sample from the species that are available. If a listed species is unavailable, aim to collect fish in the following order: 1) those that are consumed by humans; 2) predatory fish; and 3) other available fish species. In no instance should fish plugs be removed from specimens submitted for the human health fish tissue sample.

In order of preference, crews should try to submit species from 1) the Primary Target List; 2) the Secondary Target List; and 3) any other available fish. It is recognized that there are species not on these lists that may be culturally or regionally important food sources, essential to subsistence fishers or increasingly popular among food trends. For these reasons, the guidance for selecting species for fish plug samples is purposefully inclusive.

Please note: There are no invertebrate organisms on this list with the exception of sea urchins for Hawaii. Crab, shrimp, molluscs, lobsters, etc., will not be used

in assessment of mercury content in fish plugs. If invertebrate species are submitted for FPLG samples, those data will be reported as MISSING for the associated sites.

The procedures for collecting and processing fish plug samples are presented below.

1. Spread out a cooler liner bag on a flat surface for your workspace.

2. Prepare the FPLG sample label with Site ID, date collected, and visit number.

3. Attach the completed label to the 20 milliliter scintillation vial and cover with clear tape.

4. Put on clean nitrile gloves before handling the fish.

Note: Do not handle any food, drink, sunscreen, or insect repellant until after the plug samples have been collected (or implement measures to reduce contamination by such chemicals if applied such as washing, wearing long gloves, etc.).

5. Rinse potential target species/individuals in ambient water to remove any foreign material from the external surface and place in clean holding containers (e.g., livewells, buckets). Return non-target fishes or small specimens to the water.

6. Retain two individuals of the same target species from each site. The fish should be:

. large enough to collect a fish plug yielding ~ 0.5 grams (wet weight) of tissue,

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. on the recommended primary or secondary target list (if not available select an alternative species present),

. both the same species,

. both satisfy legal requirements of harvestable size (or weight) for the sampled water body, or at least be of consumable size and

. of similar size, so that the smaller individual is no less than 75% of the total length of the larger individual.

Note: Whenever possible, larger specimens should be selected over smaller specimens.

7. Remove one fish retained for analysis from the clean holding container(s) (e.g., livewell) using clean nitrile gloves.

8. Measure the fish to determine total body length. Measure total length of the specimen in millimeters from the anterior-most part of the fish to the tip of the

longest caudal fin ray (when the lobes of the caudal fin are depressed dorsoventrally).

9. Weigh the fish in grams using the fish weigh scale.

10. Note any anomalies (e.g., lesions, cuts, sores, tumors, fin erosion) observed on the fish.

11. Record sample ID, species, and specimen length and weight in the Fish Tissue Plug Samples section of the Eco Fish Collection (Back) form. Make sure the sample ID numbers and specimen numbers/lengths that are recorded on the collection form match those on the sample tracking form and labels, where applicable.

12. On a meaty portion of the left side, dorsal area of the fish between the dorsal fin and the lateral line, clear a small area of scales with a sterile disposable scalpel.

13. Wearing clean nitrile gloves, insert the 8 millimeter biopsy punch into the dorsal muscle of the fish through the scale-free area. The punch is inserted with a slight twisting motion cutting the skin and muscle tissue. Once full depth of the punch is achieved, a slight bending or tilting of the punch is needed to break off the end of the sample. Remove biopsy punch taking care to ensure sample remains in the punch.

Note: The full depth of the punch should be filled with muscle tissue, which should result in collecting a minimum of 0.25 to 0.35 grams of fish tissue for mercury analysis.

14. If the fish is to be released, apply a generous amount of antibiotic salve to the plug area and gently return the fish to the water. If the fish is part of the ecofish collection, return the fish to the ecofish holding area without the application of antibiotic.

15. Using an aspirator bulb placed on the end of the biopsy punch, give a quick squeeze, blowing the tissue sample into the 20 milliliter scintillation vial.

16. Place the vial with sample immediately on dry ice for temporary storage.

17. Repeat steps 2-15 for the second fish, to collect a second fish plug sample. Place the second plug in the same scintillation vial as the first. The two plugs

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should provide at least 0.5 grams of tissue. NOTE: If two qualifying fish cannot be caught, both plugs may be taken from the same fish.

18. Replace the lid and seal tightly with electrical tape, insert the vial into the "bubble bag" to protect it from breakage, and then place it into the 4 by 4 self-sealing bag. Place the sample in a cooler with dry ice

19. Dispose of gloves, scalpel, and biopsy punch.

13.2.4 SAMPLE STORAGE

1. Keep the samples frozen on dry ice or in a freezer at .-20 ☐‹C until shipme

2. Frozen samples will subsequently be packed on dry ice and shipped to the batched sample laboratory via priority overnight delivery service within 1 week. Please see Appendix C: Shipping and Tracking Guidelines for next steps.

APPENDIX 3. CLEAN HANDS/DIRTY HANDS PROCEDURES USED DURING

STUDY.

Taken from USEPA Method 1669:
https://www3.epa.gov/caddis/pdf/Metals_Sampling_USEPA_method_1669.pdf

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8.3.1
Set up the filtration system inside the glove bag, using the shortest piece of pump tubing as is practicable. Place the peristaltic pump immediately outside of the glove bag and poke a small hole in the glove bag for passage of the tubing. Also, attach a short length of tubing to the outlet of the capsule filter.
8.3.2
"Clean hands" removes the water sample from the inner storage bag using the technique described in Sections 8.2.5.2 through 8.2.5.4 and places the sample inside the glove bag. "Clean hands" also places two clean empty sample bottles, a bottle containing reagent water, and a bottle for waste in the glove bag.
8.3.3
"Clean hands" removes the lid of the reagent water bottle and places the end of the pump tubing in the bottle.
8.3.4
"Dirty hands" starts the pump and passes approximately 200 mL of reagent water through the tubing and filter into the waste bottle. "Clean hands" then moves the outlet tubing to a clean bottle and collects the remaining reagent water as a blank.
"Dirty hands" stops the pump.
8.3.5
"Clean hands" removes the lid of the sample bottle and places the intake end of the tubing in the bottle.
8.3.6
"Dirty hands" starts the pump and passes approximately 50 mL through the tubing and filter into the remaining clean sample bottle and then stops the pump. "Clean hands" uses the filtrate to rinse the bottle, discards the waste sample, and returns the outlet tube to the sample bottle.
8.3.7
"Dirty hands" starts the pump and the remaining sample is processed through the filter and collected in the sample bottle. If preservation is required, the sample is acidified at this point (Section 8.4).
8.3.8

"Clean hands" replaces the lid on the bottle, returns the bottle to the inside bag, and zips the bag. "Clean hands" then places the zipped bag into the outer bag held by "dirty hands."

8.3.9

"Dirty hands" zips the outer bag, and places the double-bagged sample bottle into

a clean, ice-filled cooler for immediate shipment to the laboratory.

NOTE:

It is not advisable to reclean and reuse filters. The difficulty and risk associated with

failing to properly clean these devices far outweighs the cost of purchasing a new filter.

8.4

Preservation

8.4.1

Field preservation is not necessary for dissolved metals, except for trivalent and hexavalent chromium, provided that the sample is preserved in the laboratory and allowed to stand for at least two days to allow the metals adsorbed to the container walls to redissolve. Field preservation is advised for hexavalent chromium in order to provide sample stability for up to 30 days. Mercury samples should be shipped by overnight courier and preserved when received at the laboratory.

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8.4.2

If

field preservation is required, preservation must be performed in the glove bag or in a designated clean area, with gloved hands, as rapidly as possible to preclude particulates from contaminating the sample. For preservation of trivalent chromium, the glove bag or designated clean area must be large enough to accommodate the vacuum filtration apparatus (Section 6.17.3), and an area should be available for setting up the wrist-action shaker (Section 6.17.5). It is also advisable to set up a work area that contains a "clean" cooler for storage of clean equipment, a "dirty" cooler for storage of "dirty" equipment, and a third cooler to store samples for shipment to the laboratory.

8.4.3

Preservation of aliquots for metals other than trivalent and hexavalent chromium—Using a disposable, precleaned, plastic pipet, add 5 mL of a 10% solution of ultrapure nitric acid in reagent water per liter of sample. This will be sufficient to preserve a neutral sample to pH <2.

8.4.4

Preservation of aliquots for trivalent chromium (References 8-9).

8.4.4.1 Decant 100 mL of the sample into a clean polyethylene bottle.

8.4.4.2 Clean an Eppendorf pipet by pipeting 1 mL of 10% HCl (Section (7.4.4) followed by 1 mL of reagent water into an acid waste container. Use the

rinsed pipet to add 1 mL of chromium (III) extraction solution (Section
7.4.3) to each sample and blank.

8.4.4.3 Cap each bottle tightly, place in a clean polyethylene bag, and shake on
a wrist action shaker (Section 6.17.5) for one hour.

8.4.4.4
Vacuum-filter the precipitate through a 0.4 µm pretreated filter membrane
(Section 6.17.2), using fluoropolymer forceps (Section 6.17.1) to handle the
membrane, and a 47 mm vacuum filtration apparatus with a precleaned
filter holder (Section 6.17.3). After all sample has filtered, rinse the inside
of the filter holder with approximately 15 mL of reagent water.

8.4.4.5 Using the fluoropolymer forceps, fold the membrane in half and then in
quarters, taking care to avoid touching the side containing the filtrate to
any surface. (Folding is done while the membrane is sitting on the filter
holder and allows easy placement of the membrane into the sample vial).
Transfer the filter to a 30 mL fluoropolymer vial. If the fluoropolymer vial
was not pre-equipped with the ultrapure nitric acid (Section 7.4.1), rinse
the pipet by drawing and discharging 1 mL of 10% HCl followed by 1 mL
of reagent water into a waste container, and add 1 mL of ultrapure nitric
acid to the sample vial.

8.4.4.6 Cap the vial and double-bag it for shipment to the laboratory.

8.4.4.7 Repeat Steps 8.4.4.4-8.4.4.6 for each sample, rinsing the fluoropolymer
forceps and the pipet with 10% high-purity HCl followed by reagent water
between samples.

8.4.5
Preservation of aliquots for hexavalent chromium (Reference 20).

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8.4.5.1 Decant 125 mL of sample into a clean polyethylene bottle.

8.4.5.2 Prepare an Eppendorf pipet by pipeting 1 mL of 10% HCl (Section 7.4.4)
followed by 1 mL of reagent water into an acid waste container. Use the
rinsed pipet to add 1 mL NaOH to each 125 mL sample and blank aliquot.

8.4.5.3 Cap the vial(s) and double-bag for shipment to the laboratory.

9.0
Quality Assurance/Quality Control

9.1
The sampling team shall employ a strict quality assurance/ quality control
(QA/QC)
program. The minimum requirements of this program include the collection of
equipment blanks, field blanks, and field replicates. It is also desirable to include
blind
QC samples as part of the program. If samples will be processed for trivalent
chromium
determinations, the sampling team shall also prepare method blank, OPR, and
MS/MSD
samples as described in Section 9.6.

9.2

The sampling team is permitted to modify the sampling techniques described in this

method to improve performance or reduce sampling costs, provided that reliable analyses

of samples are obtained and that samples and blanks are not contaminated. Each time

a modification is made to the procedures, the sampling team is required to demonstrate

that the modification does not result in contamination of field and equipment blanks.

The requirements for modification are given in Sections 9.3 and 9.4. Because the acceptability of a modification is based on the results obtained with the modification, the

sampling team must work with an analytical laboratory capable of making trace metals

determinations to demonstrate equivalence.

9.3

Equipment Blanks

9.3.1

Before using any sampling equipment at a given site, the laboratory or equipment cleaning contractor is required to generate equipment blanks to demonstrate that the equipment is free from contamination. Two types of equipment blanks are required: bottle blanks and sampling equipment blanks.

9.3.2

Equipment blanks must be run on all equipment that will be used in the field. If, for example, samples are to be collected using both a grab sampling device and

the jar sampling device, then an equipment blank must be run on both pieces of equipment.

9.3.3

Equipment blanks are generated in the laboratory or at the equipment cleaning contractor's facility by processing reagent water through the equipment using the same procedures that are used in the field (Section 8.0). Therefore, the "clean hands/dirty hands" technique used during field sampling should be followed when preparing equipment blanks at the laboratory or cleaning facility. In addition, training programs must require must require sampling personnel to collect a clean equipment blank before performing on-site field activities.

9.3.4

Detailed procedures for collecting equipment blanks are given in the analytical methods referenced in Table 1.

9.3.5

The equipment blank must be analyzed using the procedures detailed in the Method 1669

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referenced analytical method (see Table 1). If any metal(s) of interest or any potentially interfering substance is detected in the equipment blank at the minimum level specified in the referenced method, the source of contamination/interference must be identified and removed. The equipment must be demonstrated to be free from the metal(s) of interest before the equipment may be used in the field.

9.4

Field Blank

9.4.1

To demonstrate that sample contamination has not occurred during field sampling and sample processing, at least one field blank must be generated for every 10 samples that are collected at a given site. Field blanks are collected before sample collection.

9.4.2

Field blanks are generated by filling a large carboy or other appropriate container with reagent water (Section 7.1) in the laboratory, transporting the filled container to the sampling site, processing the water through each of the sample processing steps and equipment (e.g., tubing, sampling devices, filters, etc.) that will be used in the field, collecting the field blank in one of the sample bottles, and shipping the bottle to the laboratory for analysis in accordance with the method(s) referenced in Table 1. For example, manual grab sampler field blanks are collected by directly submerging a sample bottle into the water, filling the bottle, and capping. Subsurface sampler field blanks are collected by immersing the tubing into the water and pumping water into a sample container.

9.4.3

Filter the field blanks using the procedures described in Section 8.3.

9.4.4

If it is necessary to acid clean the sampling equipment between samples (Section 10.0), a field blank should be collected after the cleaning procedures but before the next sample is collected.

9.4.5

If trivalent chromium aliquots are processed, a separate field blank must be collected and processed through the sample preparation steps given in Sections 8.4.4.1 through 8.4.4.6.

9.5

Field Duplicate

9.5.1

To assess the precision of the field sampling and analytical processes, at least one field duplicate sample must be collected for every 10 samples that are collected at a given site.

9.5.2

The field duplicate is collected either by splitting a larger volume into two aliquots in the glove box, by using a sampler with dual inlets that allows simultaneous collection of two samples, or by collecting two samples in rapid succession.

9.5.3

Field duplicates for dissolved metals determinations must be processed using the procedures in Section 8.3. Field duplicates for trivalent chromium must be processed through the sample preparation steps given in Sections 8.4.4.1 through

8.4.4.6.

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9.6

Additional QC for Collection of Trivalent Chromium Aliquots

9.6.1

Method blank—The sampling team must prepare one method blank for every ten or fewer field samples. Each method blank is prepared using the steps in Sections

8.4.4.1 through 8.4.4.6 on a 100 mL aliquot of reagent water (Section 7.1). Do not

use the procedures in Section 8.3 to process the method blank through the 0.45 µm filter (Section 6.14.1), even if samples are being collected for dissolved metals

determinations.

9.6.2

Ongoing precision and recovery (OPR)—The sampling team must prepare one OPR for every ten or fewer field samples. The OPR is prepared using the steps in Sections 8.4.4.1 through 8.4.4.6 on the OPR standard (Section 7.4.7). Do not use

the procedures in Section 8.3 to process the OPR through the 0.45 µm filter (Section 6.14.1), even if samples are being collected for dissolved metals determinations.

9.6.3

MS/MSD—The sampling team must prepare one MS and one MSD for every ten or fewer field samples.

9.6.3.1

If, through historical data, the background concentration of the sample can be estimated, the MS and MSD samples should be spiked at a level of one to five times the background concentration.

9.6.3.2 For samples in which the background concentration is unknown, the MS and MSD samples should be spiked at a concentration of 25 µg/L.

9.6.3.3 Prepare the matrix spike sample by spiking a 100-mL aliquot of sample with 2.5 mL of the standard chromium spike solution (Section 7.4.6), and processing the MS through the steps in Sections 8.4.4.1 through 8.4.4.6.

9.6.3.4 Prepare the matrix spike duplicate sample by spiking a second 100-mL aliquot of the same sample with 2.5 mL of the standard chromium spike solution, and processing the MSD through the steps in Sections 8.4.4.1 through 8.4.4.6.

9.6.3.5 If field samples are collected for dissolved metals determinations, it is

necessary to process an MS and an MSD through the 0.45 µm filter as described in Section 8.3.

10.0

Recleaning the Apparatus Between Samples

10.1

Sampling activity should be planned so that samples known or suspected to contain the

lowest concentrations of trace metals are collected first with the samples known or

suspected to contain the highest concentrations of trace metals collected last. In this

manner, cleaning of the sampling equipment between samples in unnecessary. If it is not

possible to plan sampling activity in this manner, dedicated sampling equipment should

be provided for each sampling event.

10.2

If samples are collected from adjacent sites (e.g., immediately upstream or downstream),

rinsing of the sampling Apparatus with water that is to be sampled should be sufficient.

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10.3

If it is necessary to cross a gradient (i.e., going from a high-concentration sample to a

low-concentration sample), such as might occur when collecting at a second site, the

following procedure may be used to clean the sampling equipment between samples:

10.3.1 In the glove bag, and using the "clean hands/dirty hands" procedure in Section 8.2.5, process the dilute nitric acid solution (Section 7.2) through the Apparatus.

10.3.2 Dump the spent dilute acid in the waste carboy or in the waterbody away from

the sampling point.

10.3.3 Process 1 L of reagent water through the Apparatus to rinse the equipment and

discard the spent water.

10.3.4 Collect a field blank as described in Section 9.4.

10.3.5 Rinse the Apparatus with copious amounts of the ambient water sample and

proceed with sample collection.

10.4

Procedures for recleaning trivalent chromium preservation equipment between samples
are described in Section 8.4.4.

11.0
Method Performance
Samples were collected in the Great Lakes during September–October 1994 using the
procedures in this sampling method.

12.0
Pollution Prevention

12.1
The only materials used in this method that could be considered pollutants are the acids
used in the cleaning of the Apparatus, the boat, and related materials. These acids are
used in dilute solutions in small amounts and pose little threat to the environment when
managed properly.

12.2
Cleaning solutions containing acids should be prepared in volumes consistent with use
to minimize the disposal of excessive volumes of acid.

12.3
To the extent possible, the Apparatus used to collect samples should be cleaned and
reused to minimize the generation of solid waste.

APPENDIX 4. DMA-80 RAW DATA

Sample listing "Morris_3_29_2016.d80"
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Created by "Celeste"
29.03.2016 19:06:06

Pos Nr.	Samplename / Remark	Weight [g] / Date	Slate / Date	Height	Hg [ng]	Concentr. [µg/kg]	Σ	Cal-Factor
1	Blank	1.0000 / 29.03.16 14:12	29.03.16 16:16	0.0403	0.7551	0.7551		1.0000
2	250	0.0854 / 29.03.16 14:12	29.03.16 15:22	0.4035	9.1636	107.3017		1.0000
3	259	0.0526 / 29.03.16 14:12	29.03.16 15:30	0.3696	8.2819	157.4511		1.0000
4	263	0.0434 / 29.03.16 14:12	29.03.16 16:39	0.2272	4.8214	111.0915		1.0000
5	260	0.0461 / 29.03.16 14:12	29.03.16 16:48	0.5126	12.2094	264.8459		1.0000
6	Blank	1.0000 / 29.03.16 14:12	29.03.16 16:54	0.0012	0.0000	0.0000		1.0000
7	261	0.0241 / 29.03.16 14:12	29.03.16 17:02	0.1264	2.5718	106.7138		1.0000
8	252	0.1063 / 29.03.16 14:12	29.03.16 17:09	0.0363	42.9503	404.0478		1.0000
9	Blank	1.0000 / 29.03.16 14:12	29.03.16 17:17	0.0024	0.0000	0.0000		1.0000
10	DORM	0.0180 / 29.03.16 14:12	R 29.03.16 17:25	0.3061	7.4160	412.0000		1.1082
11	Dirty	1.0000 / 29.03.16 14:12	29.03.16 17:33	0.0009	0.0000	0.0000		1.0000
12	?	0.1034 / 29.03.16 14:12	29.03.16 17:41	0.9890	39.2000	379.1101		1.0000
13	257	0.0412 / 29.03.16 14:12	29.03.16 17:49	0.1266	2.6194	63.5780		1.0000
14	253	0.0587 / 29.03.16 14:12	29.03.16 17:57	0.6811	17.7393	302.2027		1.0000
15	350	0.0967 / 29.03.16 14:12	29.03.16 10:05	0.0487	54.6173	564.8115		1.0000
16	347	0.0842 / 29.03.16 14:12	29.03.16 18:13	0.0360	42.6137	506.1013		1.0000
17	277	0.0364 / 29.03.16 14:12	29.03.16 18:20	0.2329	4.9544	136.1112		1.0000
18	279	0.0307 / 29.03.16 14:12	29.03.16 18:28	0.6662	17.1921	560.0024		1.0000
19	(duplicate of 18: 279)	0.0448 / 29.03.16 14:12	29.03.16 18:36	0.7831	21.9449	489.8422		1.0000
20	PACS	0.0153 / 29.03.16 14:12	R 29.03.16 10:44	0.0376	46.5120	3040.000		1.1031
21	Dirty	1.0000 / 29.03.16 14:12	29.03.16 18:52	0.0039	0.0123	0.0123		1.0000
22	Blank	1.0000 / 29.03.16 14:12	29.03.16 19:00	0.0033	0.0000	0.0000		1.0000