SPATIAL VARIABILITY IN THE SPECIATION AND BIOACCUMULATION OF MERCURY IN A SUBTROPICAL RESERVOIR SYSTEM: AMISTAD INTERNATIONAL RESERVOIR, TEXAS, USA.

THESIS

Presented to the Graduate Council of Texas State University-San Marcos in Partial Fulfillment of the Requirements for the Degree Master of SCIENCE by

Jesse C. Becker, B.S.

San Marcos, Texas August 2008
SPATIAL VARIABILITY IN THE SPECIATION AND BIOACCUMULATION OF MERCURY IN A SUBTROPICAL RESERVOIR SYSTEM: AMISTAD INTERNATIONAL RESERVOIR, TEXAS, USA.

Committee Members Approved:

______________________________
Alan Groeger, Chair

______________________________
Dittmar Hahn

______________________________
Weston Nowlin

Approved:

______________________________
J. Michael Willoughby
Dean of the Graduate College
ACKNOWLEDGEMENTS

I owe a debt of gratitude to my major advisor, Dr. Alan Groeger, and to my esteemed committee members, Dr. Dittmar Hahn and Dr. Weston Nowlin, for their expert advice and encouragement. I thank Dr. Matthew Chumchal, Dr. Glen Longley and the staff of the Edwards Aquifer Research and Data Center, and Dr. Chris Shade for their assistance with the analyses. Without their help I would not have been able to finish this project. I am indebted to Randy Meyers, inland fisheries director for the Texas Parks and Wildlife Department, for the donation of fish to this project, adding a very important component to the work. Additionally, Kristen Epp, Gilles Tremblay, and Dr. Tim Bonner were a great help with the statistical analyses, and Allana Welsh was a patient teacher for this neophyte microbiologist. I also thank my lab-mates involved with the Amistad project: Tom Ryan, Jessica Strickand, and Liz Fulcher. Major funding for this project was provided by the U.S. Department of Agriculture through the Sustainable Agricultural Water Conservation (SAWC) research programs established by Congress in 2004. Additional support was provided by a Chuck Nash Aquatic Studies Scholarship, and a Howard D. Schulze Biology scholarship.

This manuscript was submitted on May 7, 2008.
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS ........................................................................................................ iv

LIST OF TABLES .................................................................................................................. vi

LIST OF FIGURES ............................................................................................................... vii

ABSTRACT ................................................................................................................................. ix

CHAPTER

1. Introduction ....................................................................................................................... 1

2. Materials and Methods ................................................................................................. 7

   Study area
   Sediment and water collection
   Fish collection
   Laboratory analyses
   Statistical methods

3. Results and Discussion ................................................................................................. 16

   Sediments and Water
   Multivariate Analyses
   Fish

4. Conclusions ..................................................................................................................... 34

APPENDIX ............................................................................................................................. 36

   Photographs of PCR amplifications using SRB family and genus specific 16S rRNA primer sets.

REFERENCES ......................................................................................................................... 41
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PCR primer sets, target organisms, and references for primers used in this study.</td>
<td>14</td>
</tr>
<tr>
<td>2. Summary of results for “Direct” PCR amplification of 16S rRNA in DNA extracted from Amistad International Reservoir using SRB general and group specific primers.</td>
<td>28</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Location of sampling sites in Amistad International Reservoir. The inset shows the location of Amistad International Reservoir in Texas.</td>
<td>8</td>
</tr>
<tr>
<td>2. Location of fish collection areas during November of 2007.</td>
<td>10</td>
</tr>
<tr>
<td>3. Spatial and temporal distribution of sediment total Hg in AIR.</td>
<td>17</td>
</tr>
<tr>
<td>4. Spatial distribution of sediment MeHg at 12 locations in AIR.</td>
<td>19</td>
</tr>
<tr>
<td>5. Temporal sediment MeHg concentrations at 4 locations in AIR.</td>
<td>20</td>
</tr>
<tr>
<td>6. Spatial and temporal distribution of sediment percent water.</td>
<td>21</td>
</tr>
<tr>
<td>7. Spatial and temporal distribution of sediment percent organic matter.</td>
<td>21</td>
</tr>
<tr>
<td>8. Spatial and temporal distribution of sediment percent carbonates.</td>
<td>21</td>
</tr>
<tr>
<td>9. Spatial and temporal distribution of sediment porewater SO$_4^{2-}$.</td>
<td>22</td>
</tr>
<tr>
<td>10. Spatial and temporal distribution of sediment porewater DOC.</td>
<td>23</td>
</tr>
<tr>
<td>11. Deep water temperature.</td>
<td>25</td>
</tr>
<tr>
<td>12. Deep water dissolved oxygen.</td>
<td>25</td>
</tr>
<tr>
<td>13. Deep water SO$_4^{2-}$.</td>
<td>25</td>
</tr>
<tr>
<td>14. Deep water DOC.</td>
<td>26</td>
</tr>
<tr>
<td>15. Principal components analysis, showing relationships along PCA axes I - III.</td>
<td>30</td>
</tr>
<tr>
<td>16. Concentration of Hg in large fish, showing USEPA, TxDSHS, and FDA screening values for Hg.</td>
<td>32</td>
</tr>
</tbody>
</table>
17. Concentration of Hg in large fish, showing updated USEPA/FDA risk based consumption recommendation levels .......................................................... 32

18. Mean muscle Hg in fish normalized to 18.5 cm from five different regions of AIR. ........................................................................................................... 34
ABSTRACT

SPATIAL VARIABILITY IN THE SPECIATION AND BIOACCUMULATION OF MERCURY IN A SUBTROPICAL RESERVOIR SYSTEM: AMISTAD INTERNATIONAL RESERVOIR, TEXAS, USA.

by

Jesse Corey Becker, B.S.

Texas State University-San Marcos

August 2008

SUPERVISING PROFESSOR: DR. ALAN GROEGER

Mercury (Hg) is highly toxic and organic forms are known to bioaccumulate in aquatic organisms. Although Hg is a global pollutant there is a paucity of data on the behavior of Hg in subtropical reservoirs. I conducted a study examining spatial variation in concentration of Hg in Amistad International Reservoir, a large subtropical water body in the Rio Grande drainage. Sediments and muscle tissue of largemouth bass (Micropterus salmoides) were analyzed for concentrations of total Hg, methylmercury (MeHg), and environmental and biological factors known to influence the production
(and bioaccumulation) of MeHg. The Rio Grande arm of the reservoir had the highest sediment concentrations of total mercury, but was below the TCEQ 100 ng/g sediment screening level. However, the concentration of MeHg was highest at sites in the Pecos River and Devils River arm (5.02 and 3.90 ng/g, respectively). Conditions in the sediments of the Pecos and Devils Rivers were likely more favorable to the production of MeHg, with higher sediment porewater dissolved organic carbon, porewater sulfate levels in the optimum range for methylation, and a higher number of detections for sulfate reducing bacteria, the microbial group believed to be associated with MeHg production.

In 55 bass of legal sport fishing size 11% had concentrations over the TxDSHS screening value of 0.7 mg/kg, yet over 84% exceeded the 0.3 mg/kg US EPA screening value. Additionally, fish at a standardized length of 18.5 cm from the Devils River and San Pedro Canyon areas of the reservoir had higher muscle Hg concentrations than those collected in the Rio Grande arm, suggesting higher rates of bioaccumulation in the Devil’s River arm. This study adds to a growing body of evidence that spatial variation in Hg concentration of fish exists within lakes and reservoirs, and is potentially related to variation in Hg methylation.
1. Introduction

Mercury (Hg) is a pollutant of concern worldwide. Methylmercury (MeHg), an organic form of Hg is a potent neurotoxin and has been linked to health epidemics in Japan (i.e. Minamata disease), Iraq, Pakistan, and Guatemala (Peakall & Lovett, 1972; Bakir et al., 1973). While the root cause of these epidemics was exposure to Hg through industrial and agricultural emission, Hg is naturally present in the environment at low levels and can be transformed into highly toxic MeHg through natural biological processes. Additionally, over the past 150 years there has been a 2- to 5- fold increase in the background level of Hg in the atmosphere (Morel et al., 1998; Munthe et al., 2007). Most of this increase is tied to worldwide increases in usage in industry, such as in chlor-alkali production and gold mining, as well as to unintentional release through the burning of fossil fuels which is currently the primary source of Hg to the atmosphere (Pacyna & Pacyna, 2002). Even sites far removed from emission sources can be impacted (Fitzgerald et al., 1998; Morel et al., 1998; St. Louis et al., 2005; Munthe et al., 2007) and locally affected sites can show levels representing much greater increases (Richerson et al., 2000; Munthe et al., 2007). Even though use of Hg in industry and emissions in North American and Europe are currently decreasing, there has been no discernable change in the size of the global atmospheric Hg pool since the 1970s (Lindberg et al., 2007). As of 2006, 48 US states had issued fish consumption advisories due to elevated levels of Hg in tissue, and 23 of these were statewide (US EPA, 2007).

Mercury in the environment exists mostly as inorganic elemental Hg\(^0\) or divalent Hg(II), which often complexes with chloride, sulfide, or organic matter (Wang et al., 2004). Despite their predominance, Hg\(^0\) and Hg(II) are not bioaccumulated (Morel et al., 1998). King et al. (2000) estimated that more than 90% of the inorganic forms of
Hg are associated with sediments of aquatic systems, and that less than 1% is associated with biota. However, the dominant form of organic Hg, MeHg, is the form which will bioaccumulate (Cleckner et al., 1998; Morel et al., 1998; King et al., 2000; St. Louis et al., 2004). Direct deposition of MeHg appears to be very limited as atmospheric Hg exists almost entirely as either Hg⁰ or reactive gaseous mercury (RGM) (Ortiz et al., 2002; Lindberg et al., 2007). Organic MeHg is substantially more toxic than inorganic Hg (Wang et al., 2004) and only 1 – 10% of MeHg is associated with sediments, with the remaining 90 – 99% associated with biota (King et al., 2000). In large bodied piscivorous fish at the top of aquatic food webs 95 – 99% of the body burden of Hg is present as MeHg (Bloom, 1992). For humans, the most common route for MeHg exposure is through consumption of contaminated fish (US EPA, 1997).

While there is widespread agreement that MeHg is produced mainly in aquatic ecosystems, under anoxic conditions through biologically mediated processes by sulfate-reducing bacteria (SRB; Compeau & Bartha, 1985; Gilmour et al., 1992; King et al., 2000; Goulet et al., 2007), the exact biogeochemical processes and controls of Hg - MeHg transformations remain unclear (Munthe et al., 2007). Methylmercury is produced mainly in the sediments of aquatic ecosystems, although there is evidence for production in both water and some terrestrial soils (Compeau & Bartha, 1984; Gilmour et al., 1998; Canavan et al., 2000; Gray et al., 2006; Goulet et al., 2007). It appears that the transition zone between oxic and anoxic environments is the region of highest methylation (Watras et al., 1995; Bowles et al., 2003), and high levels of inorganic Hg in the water or sediments are not required for the production and bioaccumulation of substantial amounts of MeHg (Morel et al., 1998) on a local scale. On a broad regional scale there can be a relationship between atmospheric Hg loading and increased MeHg, but interpreting the relationship in individual systems has proven difficult (Munthe et al., 2007).

*Desulfovibrio desulfuricans* was one of the first SRB species to be linked to Hg methylation (Compeau & Bartha, 1985), and much of the early work on bacterial
methylation of Hg concentrated on this species (Gilmour et al., 1992; Choi & Bartha, 1993; Choi et al., 1994; Pak & Bartha, 1998). Since then, the number of identified sulfate reducing organisms has been expanded to over 19 genera of SRBs, including both gram-positive and gram-negative members, as well as to some Archaea (King et al., 2000). A number of other SRB taxa have been identified as potential methylators in freshwater and marine systems, with the families Desulfovibrionaceae and Desulfobacteriaceae implicated as often having high potential for Hg methylation (King et al., 2000; Macalady et al., 2000; Batten & Scow, 2003). Because both sulfate reduction and Hg methylation are thought to be enzymatic processes, each group of SRB has the potential to methylate mercury at different rates, depending on environmental conditions (King et al., 2000). Of the six major groups of SRB delineated by Daly et al. (2000) five have members implicated in Hg methylation and are in either the Desulfovibrionaceae or Desulfobacteriaceae families. The relationship of the sixth group, gram positive spore forming members of the genus Desulfotomaculum, to Hg methylation remains unknown. The reason that SRB produce MeHg is unclear. Some have suggested that it is an enzymatic “accident” in which Hg$^{2+}$ receives the methyl group from methylcobalamin (Ullrich et al., 2001) or an active detoxification mechanism against inorganic Hg, as MeHg is slightly more volatile than Hg$^{2+}$ (Batten & Scow, 2003). Further complicating our understanding of the process, some SRB in groups thought to methylate Hg under natural conditions fail to do so when in pure culture (Munthe et al., 2007), and a few iron reducing bacteria have recently been implicated in Hg methylation (Fleming et al., 2006; Kerin et al., 2006).

Once produced, MeHg can bioaccumulate through both benthic (Suchanek et al., 1995; Tremblay & Lucotte, 1997; Bloom et al., 1999; Bodaly & Fudge, 1999; Fischer & Gustin, 2002; Gorski et al., 2003; Haines et al., 2003) and pelagic food-webs (Bloom et al., 1999; Bodaly & Fudge, 1999; Bowles et al., 2001; Gorski et al., 2003; Chen et al., 2005). Bioaccumulation is a process dependent on both the uptake within a trophic
level and retention through successive trophic levels. It is thought that the main reason that MeHg \((\text{CH}_3\text{Hg}^+)\) accumulates is that it is reactive, while other species of Hg such as dimethylHg \([\text{CH}_3]_2\text{Hg}\) and Hg\(^0\) are not (Morel et al., 1998). Divalent Hg(II) is also reactive, but at the lower trophic levels (e.g. in diatoms) it most often complexes with cellular components (e.g. with membranes) which are excreted after consumption, and thus Hg(II) is not effectively retained. However, MeHg complexes with components of the soluble fraction of unicellular organisms, in particular the thiols of proteins and amino acids, and is thus more efficiently assimilated into the consumer organism (Morel et al., 1998; Houck & Cech, 2004; Ravichandran, 2004). Also, MeHg is more lipid soluble and less water soluble than inorganic Hg, which allows it to be retained in tissue rich in fat (Morel et al., 1998; Houck & Cech, 2004). In fish it appears that intestines preferentially take up MeHg compared to inorganic forms of Hg (Morel et al., 1998). The combination of these factors results in the accumulation of MeHg through trophic position as indicated by a shift of the MeHg:Total Hg ratio in biota from approximately 15% in phytoplankton to over 95% in top trophic level fish (Watras & Bloom, 1992).

Historically, a majority of research on Hg in lentic systems has been performed on lakes and reservoirs in temperate (Slotton et al., 1995; Suchanek et al., 1997; Bowles et al., 2003) and boreal zones (Watras & Bloom, 1992; St Louis et al., 2004; Goulet et al., 2007), with very different water chemistry and environmental conditions than those in the arid West and Southwest of the United States. Some research has been conducted on subtropical wetland and estuarine systems (Cleckner et al., 1998; Gilmour et al., 1998; Bloom et al., 2004), and a large amount of work has been done on polluted systems of a diversity of types, including lakes, reservoirs, wetland, and riverine systems (Slotton et al., 1995; Suchanek et al., 1997; Diamond et al., 2000; Waldron et al., 2000; Haines et al., 2003; Domagalski et al., 2004); however, there has been very little work performed in tropical and subtropical lakes or reservoirs (Canavan et al., 2000; Bowles et al., 2001; Cizdziel et al., 2002a).
Reservoirs in Texas are classified as subtropical (Groeger et al., 2005) and tend to have high specific conductance, alkalinity, calcium, sulfate, pH, chloride, and sodium (Ground & Groeger, 1994). Recent findings of elevated levels of MeHg in two species of sport fish in Canyon Lake, Comal County, TX (Ward et al., 2006), a reservoir located on the southeastern edge of the Edwards Plateau and presumably isolated from direct point source inputs of elemental Hg, further demonstrate that even in lakes and reservoirs with low total Hg input, concentrations of MeHg in fish species consumed by humans may reach levels that present health concerns.

Like Canyon Lake, Amistad International Reservoir (AIR) is spatially isolated from point source inputs of Hg and thus, presents an interesting opportunity for study. Sources of Hg to the reservoir are not well understood. VanMetre et al. (1997) suggested that a substantial amount of Hg input was through atmospheric deposition. There are two large coal fired power plants (Carbón I and II) in Mexico approximately 100 km to the south of the reservoir, which account for approximately 700 kg of atmospheric Hg emissions annually (Miller & Van Atten, 2004). Mercury mining in the watershed occurred in the Terlingua district, approximately 250 km upstream on the Rio Grande. The Terlingua district was the third largest mercury mining district in the US, but the mines have been inactive since 1973 (Gray et al., 2006).

In the Rio Grande watershed, Borunda (1997), Canavan (1998), and Canavan et al. (2000) have studied Elephant Butte and Caballo Reservoirs in New Mexico, which are approximately 550 km upstream of AIR and upstream of both the Terlingua mining district and the El Paso-Ciudad Juárez urban area. Fish from Elephant Butte Reservoir have shown elevated levels of Hg in muscle tissue (Schmitt et al., 2005). In their evaluation of Hg methylation in the Terlingua mining district, Gray et al. (2006) postulated that transport was limited to a few km downstream from the abandoned mines, due to the arid climate and lack of precipitation and run-off. Lee & Wilson (1997) have shown sediment levels of Hg upstream of AIR which occasionally surpass the Texas
Commission on Environmental Quality screening level of 100 ng/g. In that survey, the highest average sediment levels of Hg in that data set were found in the Pecos River near Red Bluff Reservoir, on the Texas-New Mexico border, approximately 300 km upstream from AIR.

Because of their size and location on the landscape, reservoirs can exhibit spatial complexity not seen in natural lakes (Thornton et al., 1990; Straškraba, 1998). In AIR large differences in the physical and chemical qualities of the three main rivers flowing into the reservoir add to this complexity (Groeger et al., in press). The Rio Grande arm is characterized by higher SO$_4^{2-}$, Cl$^-$, and turbidity levels as well as a presumably higher sedimentation rate due to the much larger watersheds and more intensive land-use of the Rio Grande and Pecos River as compared to the Devils River watershed (Becker & Groeger, in review). It is estimated that as much as 24 m of sediment have been deposited at the confluence of the Rio Grande and Pecos Rivers since the reservoir was filled in 1969, and that the riverine zone of the Rio Grande arm of the reservoir has approximately 12 m of sediment (TCEQ, 2004). When AIR was flooded, two smaller reservoirs on the Devils River were submerged. Water upstream from the upper dam, which impounded Devils Lake (at 29°34’ N; 100°59’ W), exhibits hypolimnetic anoxia earlier in the year and with apparently higher accumulation of H$_2$S than other sites (A. Groeger, unpublished data). These factors are can be important influences on the production of MeHg in sediments (Compeau & Bartha, 1984, 1985; Slotton et al., 1995; St. Louis et al., 2004).

The first goal of this project was to examine the spatial distribution and temporal trends of both total Hg and MeHg in the sediments of AIR, as well as the contribution of several of the control factor on the production of MeHg. Second, I wanted to determine if there are areas of the reservoir bioaccumulating more or less MeHg, using a top predator sport fish as an indicator. On a broad scale, the complexity and gradients present in reservoirs in general (Thornton et al., 1990; Straškraba, 1998) and AIR in particular allow
Cizdziel et al. (2002a) found spatial variation in the muscle concentration of Hg in multiple species of fish from Lake Mead, NV, suggesting that testing fish from only one or two locations in a reservoir may not be enough to understand the dynamics of Hg bioaccumulation and risk to humans in large reservoirs. Amistad International Reservoir supports a large recreational fishery on the U.S. side of the reservoir, and a commercial fishery on the Mexican side. Developing a better understanding of the spatial patterns of Hg bioaccumulation in large reservoirs is important, as sport fishing is one of the major uses of these systems.

2. Materials and methods

2.1. Study area

Amistad International Reservoir (Figure 1) is a large subtropical reservoir located on the border between Texas, USA and Coahuila, Mexico (29°27’N; 101°03’W) with three main riverine water sources: the Rio Grande, Pecos, and Devils River. Each river has its own chemical characteristics and levels of environmental impact (Groeger et al., in press). At conservation elevation (340.5 m a.s.l.) the reservoir covers approximately 263 km², has a mean depth of 16.5 m, and the watershed encompasses 324,000 km² (Ground & Groeger, 1994). The reservoir is oligotrophic, has high alkalinity, high pH, low dissolved organic carbon (DOC), and has a small percentage of littoral zone and wetland area (Ground & Groeger, 1994; A. Groeger, unpublished data). These characteristics are not usually linked to elevated Hg levels in biota (Driscoll et al., 1995; Sonesten, 2003; Wiener et al., 2006).

2.2. Study Design and Sampling Plan

Fourteen sites, located in the thalweg of the Rio Grande (RG) and Devils River (DR) arms of the reservoir, were sampled. Additionally, two sites in the Pecos River (PR), approximately 1 and 9 km upstream from the confluence with the Rio Grande were
sampled (Figure 1). At each location I collected sediment and water samples from the reservoir monthly for four months (May-August 2007). Approximately 10% of samples were collected as field replicate or split samples. Where values were within 10%, the initial sample is presented, where values differed greater than 10% an average between the two values is presented. Sites are identified as follows: A two letter abbreviation for river channel (RG = Rio Grande; DR = Devils River; PR = Pecos River), followed by a number corresponding to the approximate river channel distance, in km, from the dam. In this and other studies conducted by this group, site RG1 is the near dam reference station for the reservoir. Two collections of largemouth bass (*Micropterus salmoides*) were made by the Texas Parks and Wildlife Department (TPWD), and donated

Figure 1. Location of sampling sites in Amistad International Reservoir. The inset shows the location of Amistad International Reservoir in Texas. Map modified from TCEQ (2004).
to this project (April and November, 2007); these were analyzed for fish muscle Hg concentration.

2.2.1. Sediment and water collection

Sediment samples were taken using either a Petite Ponar or Ekman Dredge. From each dredge, the top 5 cm of sediments was sub-sampled into pre-cleaned glass jars (for total Hg and MeHg analysis) or pre-cleaned 50 ml centrifuge tubes (all other sediment analyses). All glassware and spoons used in sampling were washed in 0.15N HCl and put into clean plastic bags. Samples for the analysis of MeHg were the taken from the middle of the dredge, using a new clean spoon at each site. Samples were stored on ice in the field, and frozen at -70 °C immediately upon return. Bottom water at each of these sites was also collected during ongoing monitoring at the lake, and was analyzed for $\text{SO}_4^{2-}$ and DOC, in addition to the regular monitoring parameters of temperature, dissolved oxygen (DO), and pH. Water was collected using an acrylic 4 L Kemmerer bottle ~1 m above the sediment-water interface. This water sampling scheme was part of a four year monitoring program at the reservoir. The remaining monitoring parameters were taken using a Hydrolab™ H2O or DS5 multiprobe sonde, which was calibrated prior to each sampling trip. Sonde data were taken within 1 m above the sediment-water interface.

2.2.2. Fish collection

Fish for this project were collected by TPWD under agency permits and regulations, and donated, post-mortem, after use in TPWD projects. Two separate collections of $M. \text{salmoides}$ from the reservoir were utilized. The first collection by TPWD was conducted in April 2007, using hook and line methods. From this collection, a subset of 55 individuals was analyzed for muscle Hg concentration. All fish were $\geq 33.5$ cm total length (TL) with ages estimated to be $>3$ years (TPWD, unpublished data). Selection of individual fish for analysis was done to cover the full size range of fish. The second set was collected by electrofishing in November 2007 for TPWD monitoring of the largemouth bass population at AIR; this second set includes rough location data. For
this collection, TPWD blocked the reservoir into five areas and I used them to assess the differences in the rates of bioaccumulation in different areas of the reservoir. Two areas are on the Rio Grande arm, one is in the main Devils River arm, and two are side channels in the Devils River arm (Figure 2). It is assumed that all fish were resident of the area in which they were collected, as multiple studies have concluded that *M. salmoides* have a <5 ha home range, even when there is an abundance of habitat (Warden & Lorio, 1975; Mesing & Wicker, 1986; Sammons & Maceina, 2005). All *M. salmoides* in this second collection were year 0 - 3 fish (TPWD, *unpublished data*), less than 30 cm TL, and from the U.S. side of the reservoir. This second collection of fish had otoliths removed in the field by TPWD personnel. For both collections, fish were placed on ice and transported to Texas State University where they were weighed to the nearest gram.

Figure 2. Location of fish collection areas during November of 2007. Map modified from TCEQ (2004).
and total length (TL) recorded to the nearest 0.5 cm. Fish were then frozen until further processing.

2.2.3. Laboratory analyses

Total Hg for both fish tissue and sediment was determined using combustion atomic-absorption spectrometry (US EPA, 1998; Cizdziel et al., 2002b) on a Milestone DMA-80 (Milestone Inc., Monroe, CT USA). Concentrations are presented on a wet weight (w.w.) basis for fish and on a dry weight (d.w.) basis for sediments. All tissue samples and May sediment samples were oven dried at 55 °C for 48 hrs. All other sediment total Hg samples were freeze-dried using a LABCONCO Freezone 6. All fish muscle samples were taken from fillets, and where possible, anteriodorsally. After drying, homogenization was performed with a ceramic mortar and pestle which was washed with reagent grade acetone between samples. Order of homogenization was done haphazardly among sediment or fish samples. Calibration curves were generated using reference material from the National Research Council of Canada Institute for National Measurement Standards: PACS-2 (marine sediment, certified value = 3,040 ± 200 ng/g total Hg d.w.), MESS-3 (marine sediment, certified value = 91 ± 9 ng/g total Hg d.w.), and DORM-2 (dogfish muscle, certified value = 4,460 ± 260 ng/g total Hg d.w.). Reference samples were analyzed every 10 samples. Duplicate samples were analyzed every 20 samples. Runs were accepted when reference samples were within 10% of certified values. Percent recovery on reference samples was 103 ± 4% (n = 37) and mean percent difference on duplicates was 1 ± 3% (n = 20).

Methylmercury analysis on sediments was performed using Hg-thiourea complex ion chromatography with on-line cold vapor generation and atomic fluorescence spectrometric detection (Shade & Hudson, 2005) at Quicksilver Scientific, LLC, Lafayette, CO. Twelve of the sites sampled in August 2007 were selected to assess spatial patterns of MeHg concentrations in the reservoir. Of the 12 sites selected, four were additionally selected for temporal evaluation and samples for all months of
collection (May – August 2007) were analyzed. For analysis, samples were thawed and then put on a magnetic mixer with a PTFE-coated stir-bar and mixed to a homogeneous slurry. A sub-sample was quickly withdrawn with a transfer pipette while stirring and dispensed into a tared 40 mL I-Chem vial and mass recorded. Ten mL of extraction solution (8 mL KBr/H$_2$SO$_4$ (18% m/v in 5% v/v acid) plus 2 mL of 1M CuSO$_4$) was then added and the sample was allowed to leach overnight. After leaching, samples were filtered through acid-rinsed 0.45 μm PVDF syringe filters. Approximately 10% of samples were run as laboratory duplicates and spikes. Spikes were added to aliquoted slurry and allowed to equilibrate for 15 minutes before extraction. All containers and implements were rigorously acid-washed. The stir bars used for homogenization were cleaned with extraction solution between uses. Spike recovery was 96 - 99%, and laboratory duplicates were within 4%. Recovery on reference material (BCR 463) was 97%. Method detection limit was approximately 0.12 ng/g (d.w.).

Percent water and loss-on-ignition for organic matter and carbonates were determined using the sequential combustion method of Heiri et al. (2001) with the following modifications: initial drying of approximately 4 g wet sample was done at 55 °C for 48 hrs and the first sequential combustion was done at 500 °C for 4 hours. After reweighing, the second combustion was done at 900 °C. Loss-on-ignition at 500 °C allows for the estimation of organic carbon and organic matter. Percent organic carbon is approximately half of the percent organic matter lost at 500 °C (Dean, 1999). Loss-on-ignition at 900 °C allows for the estimation of sediment carbonates.

Sediment porewater and deep water SO$_4^{2-}$ concentration was determined by ion chromatography (APHA, 1999a) by the Edwards Aquifer Resource and Data Center (EARDC) laboratory on a Lachat QuickChem 8500 ion chromatograph. To extract sediment porewater, 50 ml sediment samples were centrifuged at 3600 rpm for 1 hr at 4 °C. Eight ml of supernatant liquid was removed and centrifuged for an additional 30 min at 3600 rpm to remove any particulate matter. Four ml of the sample was transferred
to clean 13 mm borosilicate test tubes for analysis. For deep water samples, 250 ml of raw water collected as described in *Sediment and water collection* was filtered through Whatman GF/F filters, with approximately 5 ml used for analyses, as above. Method detection limit was 5 mg/L. For statistical analysis, a value of half the detection limit (2.5 mg/L) was used when samples were below detection.

Sediment porewater and deep water DOC concentration was determined using high temperature catalytic combustion (APHA, 1999b) on a Shimadzu TOC-V cSh Total Organic Carbon Analyzer. To extract sediment porewater, 50 ml sediment samples were centrifuged at 3600 rpm for 1 hr at 4 °C. Deep water for DOC analysis was collected as described in *Sediment and water collection*. Centrifuged supernatant or deep water was filtered through pre-combusted and Milli-Q rinsed Whatman GF/F filters, and transferred to pre-combusted 48 ml vials with acid-cleaned PTFE lined caps. Filtrate from sediment porewater was diluted 1:1 with Milli-Q water for analysis. Method detection limit was 0.88 mg/L. For statistical analysis, a value of half the detection limit (0.44 mg/L) was used when samples were below detection.

The final month of samples (August 2007) was additionally sub-sampled to evaluate the presence and make-up of the SRB community. DNA was extracted from 0.5 g sediment sub-samples through bead beating and sequential phenol, phenol:chloroform, chloroform extraction, followed by PEG treatment and isopropanol precipitation, as in Welsh et al. (2007), except that only DNA was extracted. Confirmation of DNA yield was done electrophoretically on 1% (w/v) agarose gels. “Direct” PCR amplification was performed on the DNA extracts from each site, using primer sets outlined in Amann et al. (1992) and Daly et al. (2000). Table 1 outlines primer sets and PCR annealing temperatures. Reactions were carried out as follows: Denaturing at 96 °C for 30 sec, annealing for 1 min 20 sec, and extension at 72 °C for 1 min 15 sec for 35 cycles. Amplifications were carried out using a hot-start PCR protocol, where the reaction was heated to 96 °C for 5 min, without *Taq* polymerase, to completely denature the template.
<table>
<thead>
<tr>
<th>Primer set</th>
<th>Target group</th>
<th>Target organism</th>
<th>Expected size of product (bp)</th>
<th>Annealing Temp. (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRB385F</td>
<td></td>
<td>Desulfovibrionaceae/Desulfobacteriae</td>
<td>1100</td>
<td>54</td>
<td>Amann et al. 1992</td>
</tr>
<tr>
<td>UNIV1492R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFM 140</td>
<td>1</td>
<td>Desulfotomaculum</td>
<td>700</td>
<td>58</td>
<td>Daly et al. 2000</td>
</tr>
<tr>
<td>DFM 842</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBB121</td>
<td>2</td>
<td>Desulfobulbus</td>
<td>1120</td>
<td>66</td>
<td>Daly et al. 2000</td>
</tr>
<tr>
<td>DBB1237</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBM169</td>
<td>3</td>
<td>Desulfobacterium</td>
<td>840</td>
<td>64</td>
<td>Daly et al. 2000</td>
</tr>
<tr>
<td>DBM1006</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSB127</td>
<td>4</td>
<td>Desulfobacter</td>
<td>1150</td>
<td>60</td>
<td>Daly et al. 2000</td>
</tr>
<tr>
<td>DSB1273</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCC305</td>
<td>5</td>
<td>Desulfovoccus-Desulfonema Desulfosarcina</td>
<td>860</td>
<td>65</td>
<td>Daly et al. 2000</td>
</tr>
<tr>
<td>DCC1165</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSV230</td>
<td>6</td>
<td>Desulfovibrio-Desulfomicrobium</td>
<td>610</td>
<td>61</td>
<td>Daly et al. 2000</td>
</tr>
<tr>
<td>DSV838</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Tubes were then cooled to 80 °C while the enzyme was added. Presence of amplification product for each reaction was determined electrophoretically on 2% (w/v) agarose gels. Negative controls were run on all gels. Sediment DNA extract from Harrier Meadow, which was confirmed to have SRB DNA (Welsh et al., 2007), was used as a positive control for the main SRB reactions. No positive controls were used in the group reactions.

2.2.4. Statistical methods

In addition to basic descriptive statistics used to investigate differences between the sediments of the two main arms of the reservoir (the Devils River arm and the Rio Grande arm, using both RG and PR sites), the multivariate method of principal components analysis (PCA) was used as a descriptive tool on a reduced data set (due to missing data points). PCA is an ordination form of multiple linear regression (Macalady et al., 2000) and was used to assess spatial differences and patterns in the physiochemical and microbiological parameters among sites.

The first batch of fish, lacking any spatial data, was analyzed using length:muscle Hg correlation analysis to assess the likelihood of human exposure to Hg from fish in the reservoir from the consumption of legal-sized sportfish. The focus of the statistical analyses was on the second batch of fish muscle data, which was analyzed using the polynomial regression analysis method of Tremblay et al. (1996, 1998). Data were checked for normality and homoscedasticity using scatter and residual plots. A Ladder of Powers analysis determined that a square root transformation was the most appropriate transformation for this data set. A Shapiro-Wilk Goodness of Fit test was run on the transformed data. Although the data are not normally distributed, and box-plot analysis (Barnett & Lewis, 1984) revealed two outliers, the W value is high (W = 0.9783; p = 0.0145) and the regression technique used here is robust to minor violations of normality (Tremblay et al., 1998). Analysis was initially run on both sets of data (with and without outliers), but results for the full data set are presented here, as there was no difference in
the conclusions. Because of an inability to meet the assumption of common slopes in the length:Hg regressions between lake areas, conventional analysis of covariance methods (ANCOVA) were not appropriate. The Tremblay et al. (1996, 1998) method allows for the comparison of differing relationships between sites using dummy variables to code for the different sites and adding polynomial terms to allow for curvilinear relationships. Length data in any of the model polynomial terms was centered by subtracting the mean length. This reduces the effect of colinearity between the length terms in the model (Tremblay et al., 1996). A backwards elimination regression analysis with dummy variables for the intercept, slope, quadratic, and cubic terms at each location was run on the transformed data. Coefficients with $p < 0.05$ were retained in the model. The resulting model was used to predict Hg concentrations (mean $\pm$ 95% confidence intervals) at a standard length of 18.5 cm for each lake area. The 18.5 cm length was chosen as it is close to the mean length of the entire November 2007 collection. Although data were transformed for analysis, all figures use untransformed data to ease visual interpretation and comparison.

Descriptive statistics were performed using the Data Analysis Toolpak in Excel (Microsoft Corporation, WA). All other statistical analyses were performed using the JMP statistical package (version 6.0, SAS Institute) or R (version 2.6.2, The R Foundation for Statistical Computing).

3. Results and Discussion

3.1. Sediments and water

For any descriptive statistics, site RG1 is not included in the averages for each arm. This site is at the thalweg confluence of both arms of the reservoir and is influenced by conditions in both arms. For this study and others which this group has done, it is considered our lake reference site and almost universally represents an average between the two arms. It is included in the multivariate analyses.
3.1.1. Sediment Total Mercury

All sediment samples analyzed for total Hg were above the method detection limit. Figure 3 shows the spatial distribution of total Hg (ng/g d.w.) in the sediments of AIR. Rio Grande arm sites have higher and more variable values than Devils River arm sites (46.37 ± 2.48 ng/g and 30.65 ± 4.01 ng/g respectively. Confidence limits for the Devils River are expanded by the inclusion of the two extreme low values for site DR43, a scoured riverine site with no permanent sediment). There is a distinct trend of decreasing Hg concentrations as the site location moves upstream on the Devils River, but no temporal trend for the time period sampled. The total Hg levels at site RG1 are in-between the two arms, and the 95% confidence limits overlaps with the values for both arms (mean = 41.21 ± 8.2 ng/g).

The spatial patterns in total Hg concentrations strongly suggest that there is higher loading to the Rio Grande arm of the reservoir. The watershed for the Rio Grande arm above AIR is 300,200 km$^2$ (including the 91,100 km$^2$ Pecos River drainage), while the drainage basin for the Devils River is 10,250 km$^2$ (USGS, 2001). If loading to each arm was similar, as is likely the case for atmospheric input, the higher sedimentation rates in the Rio Grande arm should substantially dilute the total Hg concentrations. Instead,

![Figure 3. Spatial and temporal distribution of sediment total Hg.](image-url)
concentrations in the riverine zone of the Rio Grande arm are substantially higher than those in the Devil’s River. Whether this is atmospherically deposited Hg moving through the watersheds or movement of naturally contaminated sediments from the Terlingua mining district is unknown.

These concentrations are not substantially different than those found in IBWC (1997) or VanMetre et al. (1997), where samples were collected in 1991 and 1992. The most complete assessment previous to this study was presented in TCEQ (2004), where samples were collected in 1996. Rio Grande arm samples appear to be higher in TCEQ (2004) (67 ± 29 ng/g, n = 4) but as only one site overlaps both studies (RG1), it is impossible to truly compare the data sets.

3.1.2. Sediment Methylmercury

All sediment samples analyzed for MeHg (ng/g d.w.) were above the method detection limit. Of the 12 sites analyzed for spatial trends (Figure 4), sites DR31 and PR74, on opposite ends of the reservoir, had sediment concentrations an order-of-magnitude higher than elsewhere in the reservoir (3.90 ng/g and 5.02 ng/g respectively). The Devils River arm has slightly higher average MeHg concentrations than the Rio Grande arm (1.26 ng/g and 1.19 ng/g respectively) but the difference is not statistically significant (t-test). For consistency, site RG1 is not included in the average for either arm for the same reason discussed at the beginning of this section. Because sediment MeHg concentrations are dependent on in situ conditions and production, and not regional deposition, it may be reasonable to include it with the Rio Grande arm sites. Both sites PR74 and DR31 are upstream of physical features which have the potential to occasionally isolate them from downstream waters and conditions. A high sedimentation rate at the Pecos River – Rio Grande confluence has built up approximately 4 - 5 m of additional sediment at sites PR66 and RG65 (J. Becker, personal observation). Site DR31 is upstream of the submerged Devils Lake dam, which regularly isolates a mass of anoxic water (J. Becker, personal observation). It should also be noted that 2007
was not a hydraulically typical year. Large storms in May and July shifted the seasonal limnological progression and stratification back 1-2 months (A. Groeger, *unpublished data*).

The four sites analyzed for temporal trends (DR25, DR14, RG1, and RG33) all show a June or July peak in MeHg concentrations (Figure 5). The Devils River arm sites had both the highest concentrations for all four months and largest amount of increase in MeHg from the May baseline to June/July peak (2.5 ng/g in June for site DR25 and 1.4 ng/g in July for site DR14). By August, both of the Devils River sites had MeHg concentrations which were approximately the same as the May levels, while sites RG1 and RG33 both had concentrations which were still slightly higher than the May levels. A spring/early summer peak in MeHg is consistent with Cleckner et al. (1998), Bloom et al. (1999), and Bloom et al. (2004) in subtropical wetlands and estuaries. Other authors have found peaks in sediments and water at later times in boreal and temperate systems (Regnell et al., 1997; Canavan et al., 2000; St Louis et al., 2004; Heim et al., 2007), with water generally showing a peak after sediments. It is reasonable to expect the warmer water temperatures of subtropical systems would shift this peak earlier in the season.
relative to temperate and boreal zone systems.

3.1.3. Other sediment parameters

Sediment percent water (Figure 6) generally has the highest values in the lower Rio Grande arm, from site RG33 to site RG1, with the upstream portions of both arms having downward trends. There is no consistent temporal trend for the time period sampled. Sediment percent organic matter (Figure 7) as measured by loss on ignition at 500 °C, have a distinct decreasing temporal trend through most of the reservoir over the period sampled. There is a slight increase spatially down the Rio Grande arm and back up the Devils River arm. Sediment percent carbonates (Figure 8), as measured by loss on ignition at 900 °C, have a distinct upward trend for most of the reservoir over the time period sampled, as well as an upward trend downstream in the Rio Grande arm and back upstream in the Devils River arm. Given the oligotrophic nature of AIR it is possible that there is a large amount of organic matter mineralization occurring over the summer months (den Heyer & Kalff, 1998) which explains the reduction in organic matter over the season sampled. The increase in percent carbonates in the sediments of site PR74 and the upper Devils River sites is consistent with their water sources being

Figure 5. Temporal sediment MeHg concentrations at 4 locations in AIR.
Figure 6. Spatial and temporal distribution of sediment percent water.

Figure 7. Spatial and temporal distribution of sediment percent organic matter.

Figure 8. Spatial and temporal distribution of sediment percent carbonates.
heavily influenced by Edwards Plateau water. The Pecos River, while still having a high ion concentration, gets significantly diluted before it reaches the reservoir from springs which drain the Edwards Plateau. Conductivity drops from levels reaching 25,000 µS/cm 100 km upstream of the reservoir to approximately 1500 µS/cm at the confluence (A. Groeger, *unpublished data*). Much of the Devils River base flow is from springs draining the Edwards Plateau. Additionally Site DR31 is approximately 100 m from the largest surface spring on the reservoir. Although there is some spatial variation, Edwards Plateau aquifer water is dominated by Ca, Mg, and alkalinity, and surface waters in the region are at or near supersaturation with respect to CaCO$_3$ (Groeger and Gustafson, 1994).

Sediment porewater SO$_4^{2-}$ (Figure 9) has higher concentrations and variability in the Rio Grande arm as compared to the Devils River arm of the reservoir. There is no consistent temporal trend in the bulk of the reservoir, although late summer (July and August) levels in the upper Devils River arm are mostly at or below detection limit (5 mg/L). Porewater SO$_4^{2-}$ concentrations are predominantly driven by sediment conditions, but they are certainly influenced by conditions in the overlying water, which has high concentrations in the Rio Grande and Pecos Rivers (A. Groeger, *unpublished data*; TCEQ, 2004). Because SO$_4^{2-}$ levels can be greatly affected by sediment reduction rates,

![Figure 9. Spatial and temporal distribution of sediment porewater SO$_4^{2-}$.](image-url)
which were not measured in this study, some care should be used in inferring availability of \( \text{SO}_4^{2-} \) to SRB. Sites PR74 and sites above DR14 do have \( \text{SO}_4^{2-} \) levels in the optimum range for Hg methylation (1 - 29 mg/L; Munthe et al., 2007). The reasons for the low porewater \( \text{SO}_4^{2-} \) concentrations at site PR74 in August are unknown. While overlying water was not collected at this site, water from sites above and below this was collected. Water collected 5 km upstream had a \( \text{SO}_4^{2-} \) concentration of 312 mg/L, and water collected at site PR66, 8 km downstream had a concentration of 216 mg/L. Porewater at site PR66 had a \( \text{SO}_4^{2-} \) concentration of 262 mg/L. The amount of \( \text{SO}_4^{2-} \) reduction required to reach these levels should have produced enough sulfide to shut down methylation, yet this site had the highest MeHg found in any of the analyzed samples.

Sediment porewater DOC levels (Figure 10) were measured from June – August of 2007. Except for site PR74, which was only sampled in August 2007, the Devils River arm sites have generally higher, but variable concentrations of porewater DOC. Site PR74 has the highest concentrations in this study (35 mg/L). Site DR31 in June has the highest concentration in the Devil’s River arm with 22 mg/L. The relationship of DOC to Hg methylation is complicated and there is much conflicting data (Ullrich et al., 2001). High levels of DOC can bind Hg and MeHg compounds, and make them

![Figure 10. Spatial and temporal distribution of sediment porewater DOC.](image-url)
unavailable for either methylation or uptake. Low levels of DOC reduce the activity of the microbial communities, and thus reduce methylation. The “high” levels found in the sediment porewater in AIR are not particularly high, and may give the microbial communities enough carbon to be active, but not enough to reduce the availability of Hg for methylation. Generally there is a positive correlation between DOC concentrations and sediment MeHg concentrations under anoxic conditions (Ullrich et al., 2001).

3.1.4. Deep water parameters

While the conditions in the water immediately overlying the sediments cannot be directly related to the conditions in the sediments, it does have some influence, and is therefore presented here. As expected, the temperature of the deep water rose as the summer season progressed (Figure 11). Variability of the temperature at the upstream sites is likely due to large storm flows which occurred immediately prior to sampling in May and July. As temperature rose seasonally, the DO decreased (Figure 12). Again, at the upstream sites, variability is likely due to storm flows. The minimum temperature of the deep water in riverine zones of AIR is approximately 10 °C, while in the summer temperatures can exceed 25 °C. Together, the increased summer temperatures and low DO can increase SRB activity and MeHg release from sediments, and higher temperatures have been shown to favor methylation over demethylation (Ullrich et al., 2001).

Concentrations of \( \text{SO}_4^{2-} \) in the overlying water (Figure 13) were assessed in July and August 2007, and were highest in the upper Rio Grande arm and Pecos River, with generally decreasing levels downstream and back up the Devils River arm. Three sites were not sampled (PR74, DR18 and DR21). Spatial trends are similar to that found in the sediment porewater with higher levels in the upper Rio Grande arm with decreasing concentrations in the Devils River arm.

In August 2007 additional samples were taken to assess the DOC in the overlying water (Figure 14). DOC concentrations for most of the sites were at or below detection
Figure 11. Deep water temperature.

Figure 12. Deep water dissolved oxygen.

Figure 13. Deep water $\text{SO}_4^{2-}$. 
(0.88 mg/L). Only 3 sites had DOC concentrations above detection, and all of these are below 3 mg/L. Driscoll et al. (1995) showed that DOC over approximately 8 mg/L decreased MeHg concentrations in fish and Gorski et al. (2008) have shown a decrease of bioconcentration into algae at levels above 5 mg/L. While sediment porewater DOC has been shown to increase MeHg concentrations, low concentrations of DOC in the water column have been shown to increase bioaccumulation, with some variation depending on the quality of the DOC (Ullrich et al., 2001). Filterable MeHg has been shown to preferentially bind to colloidal organic carbon, reducing its bioavailability (Gorski et al., 2003), thus low DOC in water can result in more MeHg binding to algae and diatoms and entering the food chain.

3.1.5. Bacterial communities

Direct PCR amplification of DNA extracted from the sediments of AIR at each site sampled in August 2007 was attempted with the primers presented in Table 1. Subsamples from site RG45 were split and analysis was run on both splits. Field replicates were taken at site DR25. For all analyses except that for Desulfo bacter, results between the splits and replicates were identical. At site DR25 there was a weak signal.

Figure 14. Deep water DOC.
in the first sample, but no detection in the replicate sample. Table 2 outlines the results of the general and group amplifications, photographs of the gels are in the Appendix. Amplification products for the *Desulfovibrionaceae* and *Desulfobacteriaceae* families were found at all sites except DR43. *Desulfobulbus* (DBB) group organisms were only detected at site PR74. *Desulfobacter* (DSB) group organisms were detected at site PR 74 as well as three Devils River sites (DR21, DR25, and DR31). *Desulfovibrio-Desulfomicrobium* (DSV) group organisms were detected at sites RG1, RG12, and RG19 as well as all of the Devils River sites except DR43. Using direct PCR techniques I was unable to detect product for the *Desulfotomaculum*, *Desulfobacterium*, and *Desulfococcus-Desulfonema-Desulfosarcina* groups of SRB at any of the sites in the reservoir. It is important to note that the techniques used here are mostly qualitative, and not quantitative. Even groups which were not detected may be present, but at low enough numbers to not be considered a substantial part of the SRB community. Using PLFA markers, Macalady et al. (2000) found that *Desulfobacter* group organisms were more abundant than *Desulfovibrio* at all sites in Clear Lake, CA. At those sites with higher measured Hg methylation potentials, the difference was even more pronounced. While the methods used here are not quantitative, *Desulfobacter* was detected at the sites with the highest MeHg concentrations. Whether the variation of MeHg concentrations is due to the presence of the different bacterial groups, or the presence of favorable environmental conditions for methylation by whichever group is present is unknown.

3.2. Multivariate analyses

A reduced data set was used for the multivariate analysis of the sediments. Modifications to the data set included using only sites with a complete suite of data points, except in the case of DOC and SRB. Dissolved organic carbon was analyzed in June – August 2007. Because there was no distinct seasonal trend in the concentrations, the values used for May in the matrix consisted of the site average for the other three months at each site. This allowed me to use the rest of the data
Table 2. Summary of results for “Direct” PCR amplification of 16S rRNA in DNA extracted from Amistad International Reservoir using SRB general and group specific primers.

\[+ = \text{positive detection of amplification product; } - = \text{no visible band of PCR product on agarose gel}
\]

*Site RG1 is closest to the dam at the confluence of the Devils River and Rio Grande arms of the reservoir.

<table>
<thead>
<tr>
<th>Bacterial Group</th>
<th>PR74</th>
<th>PR66</th>
<th>RG65</th>
<th>RG53</th>
<th>RG45</th>
<th>RG33</th>
<th>RG26</th>
<th>RG19</th>
<th>RG12</th>
<th>RG1*</th>
<th>DR14</th>
<th>DR18</th>
<th>DR21</th>
<th>DR25</th>
<th>DR31</th>
<th>DR43</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRB</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1. DFM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2. DBB</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3. DBM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4. DSB</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5. DCC-DNM-DSS</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6. DSV-DMB</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
with a reasonable estimation of the May 2007 DOC values. The detection matrix for SRB from August 2007 was applied to May – June. PCA run without SRB included did not have substantially different outcome from analysis run including it, so it is included as descriptive tool. The number of predictors in the PCA model was limited to the nine because of the sample size of this data set (n = 24) and are the most likely according to the literature to have an influence on MeHg levels. Because of different variances between the predictors, all data was z-score transformed and PCA was run on a correlation matrix. Figure 15 shows the results of PCA I – III. Data points are size weighted by MeHg concentration. Small points have concentrations < 1 ng/g d.w., medium points have concentrations of 1 – 3 ng/g d.w., and large points have concentrations > 3 ng/g d.w. The PCA model explained 75% of the variance with the first three axes. Along PCA I and II there is spatial separation of the Devils River arm, upper Rio Grande arm, and lower Rio Grande arm. Along PCA III there is no clear spatial pattern. The main components of PCA I are porewater DOC (0.48), sediment DSB (0.45), sediment DBB (0.40), sediment total Hg (-0.44), and porewater SO$_4^{2-}$ (-0.35). The main components of PCA II are deep water temperature (0.50), porewater SO$_4^{2-}$ (0.34), sediment DSV (-0.55), and sediment percent organic matter (-0.40). The main components of PCA III are sediment percent organic matter (0.61) and deep water DO (0.58).

In Swedish estuarine sediments, Lambertsson & Nilsson (2006) found a strong correlation between MeHg and sediment organic matter, yet in the sediments of AIR, MeHg was most strongly correlated with increased concentrations of porewater DOC, and presence of Desulfobulbus and Desulfo bacter group bacteria. Sediment organic matter does not become significant until PCA III, which doesn’t show any substantial spatial trends. The areas of highest sediment MeHg concentrations are in the areas of the reservoir with the lowest sediment total Hg concentrations. The lack of relationship between sediment total Hg and MeHg is consistent with what Kelly et al. (1995) found
Figure 15. Principal components analysis, showing relationships along PCA axes I - III.
in boreal freshwater systems and what Lambertsson & Nilsson (2006) found in estuarine sediments. For August 2007, the ratio of MeHg:total Hg is 14% for site DR31 and 16% for site PR74, well above the typical 1% level for sediments, suggesting high rates of MeHg production in these areas. Concentrations of MeHg in these sites, separated by over 100 km, were similar to the low end of those in Clear Lake, CA (Suchanek et al., 1998), Venice Lagoon, IT and Lavaca Bay TX (Bloom et al., 2004) and the Carson River, NV (Fischer and Gustin, 2002), all sites with industrial or mining impact. The conditions in AIR are not what are typically considered at risk for elevated Hg levels in the biota: the reservoir has low wetland area, low sediment organic levels, high pH and buffering capacity, and low inorganic Hg levels in the sediments. Yet, conditions in the Devils River arm and upper Pecos River appear to be favorable to more active SRB communities, and thus more Hg methylation, even though the total Hg is low at these sites.

3.3. Fish

The large fish, collected in April 2007, best fit a linear length:Hg correlation without any transformation ($r^2 = 0.61$, $p < 0.0001$, $n = 55$). Since this is a composite sampling of the entire lake, it reflects the amount of Hg in fish likely to be consumed by humans. Figure 16 shows that only 16% of this collection are under the 0.3 mg/kg level recommended by USEPA to issue a consumption advisory. Fish at the legal sport fishing limit (35.5 cm) can exceed this level, with the mean for the collection being 0.51 mg/kg. Texas Department of State Health Services (TxDSHS) sets the advisory level at 0.7 mg/kg. At this level only 11% of the fish exceed the state recommended advisory level, and while most of these are over 55 cm, one individual exceeds even the USFDA level of 1.0 mg/kg at a length of 47.5 cm. In 2004, the USEPA and USFDA combined their recommendations into a consumption based set of guidelines (USEPA, 2004a; 2004b). These current guidelines have levels of 0.12 mg/kg, 0.31 mg/kg, 0.47 mg/kg corresponding to a maximum of 4, 2, and 1 meals per month. Over half of the fish in this
Figure 16. Concentrations of Hg in large fish, showing USEPA, TxDSHS, and FDA screening values.

Figure 17. Concentrations of Hg in large fish, showing updated USEPA/FDA risk based consumption recommendation levels.
collection exceed the 0.47 mg/kg threshold (Figure 17).

The whole regression model for the November 2007 collection fish had a cumulative $R^2$ of 0.48 ($p < 0.0001$, $n = 156$). In the backward stepwise procedure the Rio Grande – Dam area was chosen as the reference site (all dummy variables set to 0), and only the Castle Canyon area had any significant polynomial terms. Of the five areas sampled in November 2007, two showed no length:Hg correlation (Rio Grande – Dam, and San Pedro Canyon, $p = 0.8712$ and $p = 0.6251$ respectively), two (Rio Grande – Mid and Devils River) had similar curves, but the intercepts were significantly different ($p < 0.0001$), and the Castle Canyon area had a weakly non-significant quadratic term ($p = 0.0773$), but a significant cubic term ($p = 0.0417$). While the Rio Grande – Mid and San Pedro Canyon areas have no significant length:Hg correlation, for consistency they are compared using the same methods as the other sites. The size range of fish for these sites should still span two years of age, and it is unlikely that this lack of relationship would exist in fish either greatly smaller or larger than these, as the length:Hg correlation is well established for this species (Chumchal et al., 2008; Davis et al., 2008), and it is unwise to extend this model outside the size range collected. Figure 18 shows the mean muscle Hg concentrations (mg/kg w.w.) ± 95% confidence intervals at the normalized length of 18.5 cm. Significant differences are shown by non-overlapping confidence intervals. At this length, which should be mostly year 0-1 fish (TPWD, unpublished data), both Rio Grande arm areas are significantly lower (mean levels both 0.080 mg/kg) than either the San Pedro Canyon or Devils River area (mean levels 0.126 and 0.112 mg/kg, respectively). Castle Canyon is higher (mean = 0.102 mg/kg), but due to the overlapping confidence intervals, cannot be considered significantly different from any of the other areas.

Fish were not collected in the in the areas around the two highest sediment MeHg sites, but sites in the Devils River arm did show elevated levels of MeHg in the sediments, which correlates with an apparently higher level of Hg in fish at 18.5
cm. Determining whether this remains true across all size classes and into fish of legal
sportfishing size would require sampling not included in this study. It does suggest that
some areas of the reservoir may be more likely to have the potential for human health
effects, and should be considered in further studies.

4. Conclusions

Amistad International Reservoir has relatively low sediment total Hg
congentrations and should be considered unpolluted in that respect. All of the sediment
samples are well below the TCEQ screening level of 100 ng/g. However, as this study
shows, low sediment total Hg concentrations and input do not mean that sediment
MeHg production and concentrations are also low. Methyl:total Hg ratios at two sites
are approximately 15%, suggesting high production. The concentrations of MeHg at
these sites are comparable to other locations which have received significant amounts
of pollution from industrial or mining sources. Additionally, concentrations of Hg in
the muscle tissue of *M. salmoides* are of concern, and it does appear that concentrations
vary depending on the area of the reservoir. *Micropterus salmoides* from what should be the “pristine” part of the reservoir show higher levels of muscle Hg concentration in the size range sampled. Larger fish also have levels of muscle Hg which should be further investigated to assess the risk from human consumption in these different areas, and this should be expanded to include the full lake, other sport fish species, as well as species caught commercially in Mexico.

Most of the spatial studies of Hg pollution have involved regional assessment of a large number of smaller lakes and reservoirs, and while this is very important in understanding large scale trends, it has the potential to miss much of the variability in the environment. Large reservoirs are an environmental fact on the landscape, and are heavily utilized by humans. They are heterogeneous systems and often quite different from large natural lakes. Understanding the spatial and temporal trends present in these managed systems is important in determining what the true risks to humans are. Assessment of conditions at a limited number of locations in one reservoir can easily give an incomplete picture of what the spatio-temporal dynamics of Hg pollution are, and this should be taken into account in designing and prioritizing future studies. This study shows that reservoirs in the arid West and Southwest regions of the U.S. appear to have risk of elevated Hg levels in biota, even when the concentrations of inorganic Hg are low, they are distant from input sources, and the environmental conditions are not typically thought of as favorable to MeHg production.
APPENDIX

Photographs of PCR amplifications using SRB family and genus specific 16S rRNA primer sets.
Gels for SRB general PCR amplification. Sample DR25d is a replicate of DR25. Sample RG45s is a split of RG45. Sites C1 & C2 are comparison samples from Canyon Lake, and not discussed in this report.
Gels for DBB group PCR amplification. Sample DR25d is a replicate of DR25. Sample RG45s is a split of RG45. Sites C1 & C2 are comparison samples from Canyon Lake, and not discussed in this report.
Gels for DSB group PCR amplification. Sample DR25d is a replicate of DR25. Sample RG45s is a split of RG45. Sites C1 & C2 are comparison samples from Canyon Lake, and not discussed in this report.
Gels for DSV group PCR amplification. Sample DR25d is a replicate of DR25. Sample RG45s is a split of RG45. Sites C1 & C2 are comparison samples from Canyon Lake, and not discussed in this report.
References


Borunda, D. 1997. Total mercury concentration in aquatic food web components of Caballo and Elephant Butte Reservoirs (Sierra County, NM). MS Thesis, New Mexico State University, Las Cruces, NM.


Canavan, C.M. 1998. Spatial distribution of total and methylmercury concentrations in Elephant Butte and Caballo reservoirs. MS Thesis, New Mexico State University, Las Cruces, NM.


VITA

Jesse Corey Becker was born February 12, 1970 in Santa Cruz, CA, the son of Jeffrey William Becker and Gwendolyn May Sky. After graduating from St. Helena High School (CA), he attended the University of California at Davis, earning a B.S. in Environmental Biology and Management in 1993. After working for UC Davis and the Sawtooth National Recreation Area, he spent three years as a team member at the Clear Lake Environmental Research Center. After a move to Austin, TX he spent 6 years as the service manager for Bicycle Sport Shop. Feeling the need to return to scientific research, he entered graduate school at Texas State University-San Marcos in January of 2006. Jesse will continue his research in the Aquatic Resources Ph.D. program, starting in August of 2008.

Permanent Address: 104 Ridgeway Dr.
San Marcos, TX 78666

This thesis was typed by Jesse C. Becker