IMPACT OF ENVIRONMENTAL CONTAMINANTS ON THE THREERIDGE MUSSEL (*AMBLEMA PLICATA*) IN THE GUADALUPE RIVER BASIN, TEXAS

by

Stacey Lea Britton, B.S.

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

Committee Members:

Jessica Dutton, Co-Chair

Astrid Schwalb, Co-Chair

Clint Robertson

FAIR USE AND AUTHOR'S PERMISSION STATEMENT

Fair Use

Duplication Permission

DEDICATION

This thesis is dedicated to every nontraditional student who strives for more despite where they started and every first-generation college student who so often must be their own light on the path to higher education.

ACKNOWLEDGEMENTS

I would like to thank the Texas Parks and Wildlife Department (TPWD) and the United States Fish and Wildlife Service (USFWS) for funding for this project. I extend my deep gratitude to my co-advisors, Dr. Jessica Dutton and Dr. Astrid Schwalb, for their guidance, thoughtful feedback, and their role in broadening a true reverence for environmental toxicology and freshwater ecology in myself. Heartfelt thanks is also extended to my committee member Clint Robertson for providing transportation, patient guidance, and for imparting indispensable knowledge of the rivers during many hours of musseling adventures. I would like to thank Dr. Weston Nowlin for use of his laboratory, Gabrielle Timmins for her expertise and assistance in water quality analysis, and Joe Guerrero at the Edwards Aquifer Research and Data Center (EARDC) for processing the *E. coli* samples for this project. A special thank you to Dr. Brian Jackson for performing the ICP-MS analysis, Dr. Steven Wiseman for completing the biomarker analysis, and Ashley Seagroves for assistance in gamete sampling and mussel identification. I would like to recognize and thank my field collection and laboratory processing helpers Taylor Gold Quiros, Kristyn Cunningham, Meaghan McCormack, and Nina Noreika for your cheerful assistance and unforgettable comradery that made this whole experience so much richer. I would also like to thank my family for supporting me in this enormous endeavor and providing the extra hands and support that made going to school with a young family a possibility. Most of all, to my incredible husband Josh: your love, patience, and partnership have been vital to the cultivation of this dream and all the

dreams to come. Finally, a very special thank you to my brilliant young daughters, Juniper and Ruby, who give me the courage and drive to do big things; I love you always and I hope I make you as proud as you make me. Thank you all.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

ABSTRACT

Aquatic pollution has contributed to the significant decline of unionid mussel populations throughout North America. In this study, adult Threeridge (*Amblema plicata*) were collected from 8 sites within the Guadalupe River basin and the concentration of 8 essential (Co, Cu, Fe, Mn, Mo, Ni, Se, Zn) and 8 nonessential (Ag, As, Cd, Cr, Hg, Pb, Sn, V) trace elements were determined in gill and foot tissue using microwave acid digestion and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis. Water samples were also collected at each site and analyzed for nutrient concentrations including total nitrogen, total phosphorus, and ammonium, as well as the presence of fecal coliform bacteria (*Escherichia coli*) to better understand local water quality. The physiological response of *A. plicata* to contaminants was quantified through biomarker analysis (lipid peroxidation, antioxidant capacity against peroxyl radicals, and protein concentration) of gill tissue. Overall, the concentration of both essential and nonessential elements was higher in the gills than in the foot at all sites; however, Hg was higher in foot tissue at upstream sites and not significantly different from gills at downstream sites. The two uppermost study sites on the San Marcos and Guadalupe Rivers were characterized by higher Hg and As, respectively and the lower four sites (UC, LC, UV, and LV) were characterized by higher Cd concentrations. No relationship was evident between the spatial variation in trace element concentrations in gill tissue and the investigated biomarkers. Higher nutrient concentrations at sites upstream of Upper Cuero correlated with a) higher lipid peroxidation and lower antioxidant capacity against free

radicals, indicating greater oxidative stress, and b) lower protein concentration indicating reduced overall health. This is the first in-depth study on the impact of environmental contaminants on unionid mussels in Texas rivers and the findings suggest that mussels are exposed to a mixture of urban, industrial, and agriculturally-derived contaminants; however, future cage transplant studies are needed to further understand the impact of environmental contaminants on unionid mussels in the Guadalupe River Basin.

I. INTRODUCTION

Freshwater unionid mussels are an important part of a healthy aquatic ecosystem because they 1) filter particulate matter, plankton, and pathogens from the water column increasing both water clarity and health, 2) are a source of food for small mammals (e.g., otters, muskrats, and raccoons) some fish species, and water birds (e.g., herons and egrets) (Williams et al. 1993, Tyrrell and Hornbach 1998, Haag 2012), 3) influence sediment geochemistry by adding oxygen through bioturbation and nutrients through excretion, and 4) act to stabilize sediment when occurring in high densities (Vaughn and Hakencamp 2001, Howard and Cuffey 2006, Spooner and Vaughn 2006, Kim et al. 2011, Chowdhury et al. 2016, Ismail et al. 2016). Furthermore, mussel shells provide important substrate for periphyton growth in environments that lack hard substrate, thus increasing habitat for other benthic invertebrates such as caddisflies and mayflies that feed on the periphyton (Spooner & Vaughn 2006, Vaughn et al. 2008). Unionids respond negatively to habitat degradation due to their sensitivity to water quality (e.g. elevation in ammonia, trace metals), complex life history, low reproductive success rate and juvenile survival rates, and low growth rates (Vaughn & Taylor 1999). The presence of mussels in rivers is a strong predictor of the biodiversity of other benthic invertebrates (Lawler et al. 2003) and overall river health (Aldridge et al. 2007) making the evaluation of mussel population density and factors that impact their survival critical to river health (Aldridge et al. 2007). Utilization of sedentary filter feeders as a bioindicator of ecosystem health can provide an assessment of water quality over time that intermittent water sampling cannot duplicate, making them a model organism for studies investigating the bioaccumulation of contaminants such as trace elements, pesticides, and pharmaceuticals; for example,

freshwater mussels have been used to monitor industrial contaminants and pesticides in in the St. Lawrence River (Metcalfe and Charlton 1990). Many studies have demonstrated the utility of bivalves as a biomonitoring tool to investigate contaminant exposure around the world as reviewed by Zuykov et al. (2013). Since 1986, ongoing biomonitoring studies by the National Oceanic and Atmospheric Administration (NOAA) National Status and Trends (NS&T) Mussel Watch Program in coastal ecosystems worldwide as well as freshwater environments within the Great Lakes have successfully utilized mussels to determine long term changes in the accumulation of contaminants (Wade et al. 1998, Roditi et al. 2000, Jaruga et al. 2017).

1.1 Freshwater mussels in North America

North America has the highest diversity of unionid mussel species in the world (Haag 2012); however, 74% of the estimated 300 species endemic to North America are in significant decline (FMCS, 2016). Excluding catastrophic losses from zebra mussel (*Dreissena polymorpha*) invasion (Karatayev et al. 2015), the extinction of freshwater mussels was projected at 6.4% of species per decade by Ricciardi and Rasmussen (1999). Widespread decline in abundance has continued to be documented in North American mussel species (Strayer 2008, FMCS 2016) and conservative extinction rate projections likely underestimate the current rate of species loss. As of 2017, the International Union for Conservation of Nature (IUCN) listed 245 species in the family Unionidae, 46% of which are extinct, critically endangered or endangered, 7% are considered vulnerable to population decline, and 6 of the 245 listed species are considered data deficient so conservation status can't be determined (IUCN 2017). It is important to note that

although the IUCN Red List represents a comprehensive source of up-to-date species status information, data on invertebrate species, especially those found in freshwater, are significantly lacking in comparison to vertebrate species. Surveys and estimates of density, distribution, and species richness are time and funding intensive. To further complicate interpretations of population viability, freshwater mussels are often long lived with some species reaching 100 years old (Anthony et al. 2008), leaving stranded relic populations that are at the end of their lifespan to be counted along with viable adult mussel beds (Strayer 2008). The true extinction rate and threatened status of freshwater mussel species is assumed far greater than reported by the IUCN underscoring the importance of continued study and conservation efforts (Régnier et al. 2009).

1.2 Aquatic pollution and freshwater mussels

Anthropogenic threats to freshwater mussel populations result from habitat disturbance and degradation through waterway modification, reduction in stream flow, and aquatic pollution (Burlakova et al. 2011, Haag 2012, Nobles & Zhang 2015). Aquatic pollution in lotic systems predominantly enter streams as surface run-off from precipitation events (e.g. urban run-off from impervious surfaces and agricultural run-off), direct atmospheric deposition, ground water infiltration of a stream, or point source pollution (e.g. wastewater treatment plants; Pimentel 2004).

Point source pollution is defined by the U.S. Environmental Protection Agency (U.S. EPA) 1972 Clean Water Act (CWA) section 502 as “any discernible, confined and discrete conveyance… from which pollutants are or may be discharged. This term does not include agricultural stormwater discharges and return flows from irrigated

agriculture." Human use of surface water to dispose of waste products results in point source pollution consisting of various contaminants including, but not limited to, nutrients, suspended solids, trace elements, pesticides, industrial waste, pharmaceuticals, and personal care products and has been documented as an increasingly ubiquitous threat to stream health through eutrophication and lower concentration of dissolved oxygen, and decreased immune function and overall health in mussels (Williams et al. 1993, Burlakova et al. 2011, Gillis 2012, Jiann et al. 2013, Gillis et al. 2014a,b, Machado et al. 2014, Nobles and Zhang 2015). The sensitivity of juvenile mussels to elevated ammonium, a contaminant associated with wastewater effluent and agricultural runoff, is well known and the resulting effects of exposure on population survival is of wide concern (Augspurger et al. 2003). The U.S. EPA standards for acute and chronic concentrations of ammonia in the 2013 Ambient Water Quality Criteria for Ammonia were updated from previously published standards set in 1999 based on the survival and growth of less sensitive early life stages of salmonid fishes as well as reproductive success of benthic invertebrates (*Hyalella azteca*) to lower ammonia levels that are considered more protective of sensitive freshwater mussel species (EPA 2013).

Non-point source pollution is characterized as diffuse pollution originating from many sources over a wide area such as agricultural runoff and vehicle emissions and is difficult to regulate under the Clean Water Act. For example, nutrient runoff from land is the largest source of contamination in the Mississippi River, resulting in oxygen depletion and a dead zone in the Gulf of Mexico (Howarth et al. 2002). Pollution from agriculture (crops and livestock) is generally non-point source, entering aquatic systems through surface water run-off and/or infiltration of ground water. The water

contamination most often associated with intensive agricultural practices that affect freshwater mussel health are the introduction of pesticides, sediment via erosion, nutrient pollution from fertilizer run-off, irrigation salts, and fecal coliform bacteria. Agricultural pollution can result in eutrophication of streams, which can increase the presence of toxic algal blooms and suspended solids that are linked to reproductive failure in mussels (Gascho-Landis et al. 2013).

Urban run-off is another supply of pervasive and non-point source pollution ubiquitous to urbanized areas. Run-off from roadways contains significant quantities of trace elements such as Cd, Cr, Cu, Fe, Pb, and Zn as well as polycyclic aromatic hydrocarbons (PAHs) originating from both motor vehicles and the roadway itself; examples of which include roadway paint, vehicle emissions, brake and tire wear, and oil leakage which enter watersheds resulting in elevated toxicity affecting benthic invertebrates (Kouji and Tainosho 2004, Grapentine et al. 2008, Davis et al. 2011).

Sources of trace element to streams include wastewater effluent (da Silva Oliveira et al. 2007), mining of precious metals, e.g., gold (Malm 1998), coal-fired power plants (Donahue et al. 2006), industrial waste (Desenfant et al. 2004), phosphate fertilizers (Giuffré de López Camelo et al. 1997), motor vehicle wear (e.g. brake and tire) and emissions (Lough et al. 2005), and antifouling and anticorrosion paints used on boats and in marinas (Singh and Turner 2009, Sørensen et al. 2009). Essential elements are required for normal biological function; for example, as cofactors in enzymes and respiratory pigments (White and Rainbow 1985, Depledge and Rainbow 1990) but may be toxic at high concentration [e.g. Cu (Gillis et al. 2008, Clearwater et al. 2014), Zn (Wang et al. 2010, Clearwater et al. 2014)]. Nonessential elements, e.g., Cd and Pb, can be lethal to

early life stages of freshwater mussels at low environmentally relevant concentrations (Wang et al. 2010). Additionally, Pb and Cd may act as biological analogues for the essential element Ca causing sublethal effects in adult mussels such as brittle shell structure (Markich and Jeffree 1994) and reduced filtration rate and valve movement has been demonstrated after exposure to Cd, Cu, and Zn (Loayza-Muro and Elías-Letts 2007).

Exposure to complex urban and agricultural contamination in rivers has been shown to have a significant role in the decline of mussel populations and understanding physiological response to contaminant stressors is imperative to developing appropriate conservation strategies.

Biomarker analysis is a tool that can be utilized to determine the physiological impact of exogenic stressors on indicator organisms such as freshwater mussels. Biomarker response indicating oxidative stress in juvenile and adult mussels impacted by trace elements originating from urban runoff and municipal wastewater effluent has been well documented in wild populations (Couillard et al. 1995a,b, Gillis 2012, Gillis et al. 2014b, Falfushynska et al. 2014, Abel et al. 2014), demonstrating value as a biological measure of environmental contamination by trace elements and other anthropogenic sources of pollution (Viarengo et al. 2008, Machado et al. 2014). Gill tissue, the primary site of uptake of metals from the dissolved phase (Wang et al. 1996, Wang and Fisher 1996), is in continuous contact with both dissolved and particulate-bound contaminants in the aquatic environment; thus, this tissue is ideal for biomarker analysis. Due to trace element bioaccumulation in tissues, the use of freshwater mussels as bioindicator species often reveals contamination present in concentrations or phases not detected using

traditional water or sediment analysis (Ravera et al. 2003). Biomarker analysis of metallothioneins, lipid peroxidation, total antioxidant capacity against peroxyl radicals, and total protein and total lipid in mussel gill tissue may be used comparatively to assess relative physiological response in subpopulations of freshwater mussels. A multi-biomarker analysis approach best reflects effects of complex chronic environmental contamination independent of temporal effects of temperature and feeding rate (Falfushynska et al. 2014).

Metallothioneins bind with high affinity to metals and play a crucial role in regulation and detoxification of trace metals (Viarengo et al. 1999). Metallothionein concentration is used as a biomarker of trace metal accumulation due to unique metal-binding properties (Falfushynska et al. 2014) and concentrations are expected to be higher in mussels downstream of urban areas than those found upstream due to greater metal exposure from anthropogenic activity (Gillis et al. 2014b). Similarly, oxidative stress due to municipal wastewater effluent exposure can be quantified by proportional increases in lipid peroxidation which signals cellular membrane damage in response to exposure (Machado et al. 2014). Total antioxidant capacity against peroxyl radicals has been demonstrated as a measure of immunological stress in mussel gill tissue in the presence of wastewater effluent (Machado et al. 2014, Gillis et al. 2014b). Total protein and lipid concentration can be used as an indicator of general health because physiological stress causes changes in expected tissue compositions of these macromolecules. Furthermore, total protein in the mussel gills may be analyzed and used to normalize biomarker concentration data for this tissue (Gillis et al. 2014b). Overall, a chronic exposure to complex mixtures of contaminants results in quantifiable changes in

biomarker concentrations and negatively impacts the health and longevity of freshwater mussels by requiring immune and detoxification response across multiple organ systems (Gillis 2012).

1.3 Mussels in Texas Rivers

Texas is home to approximately 50 species of unionid mussels, 15 of which were listed as state threatened by the Texas Parks and Wildlife Department (TPWD) in 2009. Five of the state listed species are candidates for federal protection status under the Endangered Species Act (ESA) and one, the Texas Hornshell (*Popenaias popeii)*, was recently listed in February 2018 as the first federally protected freshwater mussel in Texas under the ESA (USFWS 2018; Table 1). A freshwater mussel survey across Texas found a significant negative correlation ($r = -0.69$, $p = 0.018$) between the number of extant species located relative to human population densities within Texas watersheds (Burlakova et al. 2011). Currently, little is known about the toxicological status specific to Texas freshwater mussels. At present, conservation efforts rely on mussel toxicology studies which have been performed elsewhere in North America.

Texas rivers flowing through large population centers such as the Trinity River in Dallas-Fort Worth, the Colorado River in Austin, and the San Antonio River in San Antonio have been shown to exhibit significant water quality degradation and elevated trace element concentration (e.g., Cd, Cu, Ni, Pb, Zn) associated with urbanization in comparison with other Texas rivers such as the Sabine and Neches rivers which occur in eastern Texas in areas that are relatively less impacted by human development and more forested (Jiann et al. 2013). In addition, Texas often experiences both drought and

flooding; the impacts of a flashy hydrologic regime in Texas are intensified by anthropogenic activities that alters water chemistry, sediment composition, temperature, flow, concentrations of wastewater effluent in streams, and interactions with host fish species necessary for reproduction (Winemiller et al. 2010, Allen et al. 2013). Significant rainfall events and increased impervious cover in riparian areas has resulted in the amplification of intensity and frequency of flooding events for many Texas rivers including the proposed study area, the Guadalupe River basin. These frequent high flow conditions compromise the stability of sediments and contribute substantially to mussel mortality events threatening at-risk species (Mabe & Kennedy 2014).

Studies on the impact of contaminants on native mussels in Texas rivers are limited to two recent studies that focused on pharmaceutical uptake in mussels in the North Bosque River in the Brazos River Basin (Du et al. 2014) and the effects of contaminant and nutrient exposure on the survival, growth, and condition of mussels in Willbarger Creek, an effluent dominated 3rd order tributary of the Colorado River (Nobles & Zhang 2015). Both studies found a negative relationship between exposure to wastewater and mussel health indicators. In addition, no studies focusing on threatened species of Texas mussels could be found. Greater understanding of the impact of complex contaminants on mussel health in Texas rivers will better inform mussel recovery and conservation efforts that target threatened and endangered unionids native to the region.

1.4 The Guadalupe River basin as a study area

The Guadalupe River (Fig. 1) begins in Kerr County and flows 230 miles to San Antonio Bay in the Gulf of Mexico and has a basin drainage area of approximately 6,070

square miles. More than 50 permitted wastewater outfalls contribute varying volumes and chemistry of effluent with both municipal and industrial origins representing significant point source pollution in the basin (Fig. 1). The river is also impacted by non-point source pollution originating from significant human population and in Kerrville, New Braunfels, Seguin, Gonzales, Cuero, and Victoria (Table 2) and land development which dominates the upper basin and intersperses the more agriculturally and industrially influenced lower basin. The San Marcos River, a major tributary of the Guadalupe River, rises from the Edwards Aquifer at Spring Lake in San Marcos and flows approximately 75 miles to the confluence with the Guadalupe River upstream of Gonzales. The San Marcos River is impacted by urban development and anthropogenic activity in San Marcos and Luling which contribute to aquatic contamination in both the San Marcos River and Guadalupe River. The total number of mussel species reported in the Guadalupe River Basin in 2011 was 16; 14 of which were found live or recently dead during field surveys (Burlakova et al. 2011). Additionally, a field survey in 2012 discovered a small population of False Spike (*Quadrula mitchelli*) near Gonzales that was previously considered extinct (Randklev et al. 2012).

1.5 Threeridge mussel (*Amblema plicata*) as a study organism

Of the native unionid species found in the lower Guadalupe River, the Threeridge (*Amblema plicata*) was chosen as the investigated study species for this study. *A. plicata* is endemic to North America, is widely distributed and abundant throughout its range spanning from Canada to the Gulf states (Cordeiro and Bogan 2012), and has been used in biomonitoring and toxicological studies at all life stages (Naimo 1995, Fritts et al.

2013, Nobles and Zhang 2015). The IUCN Red List categorizes *A. plicata* as a species of least concern, making it ideal for toxicology studies requiring the sacrifice of a large numbers of individuals. *A. plicata* is a habitat generalist, occupying relatively diverse substrates within a variety of climates allowing for their widespread distribution. Finally, they are a good bioindicator species which could potentially be used to understand the impact of environmental contaminants in less common and rare unionid species as a similar physiological response would be expected.

1.6 Objectives of the proposed study

The main objective of this study was to examine the impact of environmental contamination on *A. plicata* in the Guadalupe River basin by 1) examining the difference in trace element concentrations in gill and foot tissue with the prediction that (a) higher concentrations would be found in gill tissue and (b) for each trace element there would be greater concentration range in gill than foot tissue; 2) determining how the concentration of trace elements in gill tissue differs with distance downstream (a) between the two sites above the confluence of the Guadalupe and San Marco Rivers, (b) upstream and downstream of Cuero, Gonzales, and Victoria, and (c) along the continuum of the river with the prediction that trace element concentrations would increase with distance downstream; and 3) determining differences in the physiological response in mussels between sites by investigating correlations between environmental contaminants (trace elements, nutrients, and *E. coli*) and the biomarker response in gill tissue.

The chosen trace elements included 8 essential (Co, Cu, Fe, Mn, Mo, Ni, Se, Zn) and 8 nonessential (Ag, As, Cd, Cr, Hg, Pb, Sn, V) elements. Of these elements, Ag, As, Cd, Cr, Cu, Hg, Ni, Pb, Se, Zn are listed as priority pollutants by the EPA under the Clean Water Act 40 CFR Part 423, Appendix A (EPA, 2014). Sources of trace elements to streams include wastewater effluent (da Silva Oliveira et al. 2007), mining of precious metals, e.g., gold (Malm 1998), coal-fired power plants (Donahue et al. 2006), industrial waste (Desenfant et al. 2004), phosphate fertilizers (Giuffré de López Camelo et al. 1997), motor vehicle wear (e.g. brake and tire) and emissions (Lough et al. 2005), and antifouling and anticorrosion paints used on boats and in marinas (Singh and Turner 2009, Sørensen et al. 2009). Essential elements are required for normal biological function; for example, as cofactors in enzymes and respiratory pigments (White and Rainbow 1985, Depledge and Rainbow 1990) but may be toxic at high concentration [e.g. Cu (Gillis et al. 2008, Clearwater et al. 2014), Zn (Wang et al. 2010, Clearwater et al. 2014)]. Nonessential elements, e.g., Cd and Pb, can be lethal to early life stages of freshwater mussels at low environmentally relevant concentrations (Wang et al. 2010). Additionally, Pb and Cd may act as biological analogues for essential elements like Ca causing sublethal effects in adult mussels such as brittle shell structure (Markich and Jeffree 1994) or reduced filtration rate and valve movement after exposure to Cd, Cu, and Zn (Loayza-Muro and Elías-Letts 2007).

Biomarker response indicating oxidative stress in juvenile and adult mussels impacted by trace elements originating from urban runoff and municipal wastewater effluent have been well documented in wild populations (Couillard et al. 1995a,b, Gillis 2012, Gillis et al. 2014b, Falfushynska et al. 2014, Abel et al. 2014). Exposure to

complex urban and agricultural contamination in rivers has been shown to have a significant role in the decline of mussel populations and understanding physiological response to contaminant stressors is imperative to developing appropriate conservation strategies.

II. METHODS

2.1 Study Sites

In the present study, an upstream site on the Guadalupe River at Lake Wood Recreational Area (LKW) approximately 13 river km above the confluence with the San Marcos River and an upstream site on the San Marcos River at Palmetto State Park (PSP) approximately 33 river km above the confluence with the Guadalupe River were chosen to account for the difference in urban influence between these rivers on sites downstream of the river confluence. The six additional sites downstream of the confluence were identified upstream (U) and downstream (L) of the urban population centers and major wastewater treatment outfalls of Gonzales (UG and LG), Cuero (UC and LC), and Victoria (UV and LV; Fig. 2; Table 3). It is important to note that wastewater management practices and effluent discharge rates vary significantly between cities and facilities contributing to increased complexity and variations of the environmental impact within river systems; however, due to the continuous nature of the river environment, downstream sites may experience additive impact of aquatic pollution originating upstream. While contaminants are often presumed to be largely additive in a stream, dilution from groundwater infiltration, precipitation events, and bioremediation may confound the expected cumulative effects of contaminant input between cities. The significant presence and growth of urban and suburban development within the Guadalupe River basin (Table 2) continues to be rapid and associated impacts to the aquatic environment on imperiled species can be expected (Winemiller et al. 2010).

2.2 Sample Collection

Mussels, sediment, water samples, and in-situ environmental measurements were collected at each site. Fifteen *A. plicata* were collected at each site using tactile and visual search methods (n = 120 total). Live mussels were held in aerated site water in 5 gallon buckets for a maximum of 4 hours while being transported to the lab. Upon arrival, mussels were removed from the water and immediately euthanized by freezing at -80°C for 15 minutes.

Current velocity and water depth at the primary mussel bed location were determined using a standard flow meter. River water pH, temperature, dissolved oxygen concentration, conductivity, and turbidity were measured using a Manta +30 water probe (Eureka Water Probes, Austin, Texas) and are shown in Appendix Table A.

Two sets of water samples were collected at each site. Water for nutrients, ions, dissolved organic carbon (DOC), chlorophyll-a, and suspended solids analysis were collected using two 1 L brown Nalgene bottles which were rinsed 3 times in site water prior to collection and then stored in a cooler during transportation to the lab. Water for fecal coliform analysis (*Escherichia coli*) was collected using a 125 ml sterile water sample bottle (Microtech Scientific) and stored in a cooler; duplicate samples were collected at two sites for quality control.

Surface sediment was collected at each site using a plastic shovel, stored in a 1-gallon Ziploc bag in a cooler, and refrigerated on arrival at the lab.

2.3 Mussel Processing

Mussel shells were rinsed and scrubbed of debris using deionized water in preparation for biological measurements and dissection. Length, width, and height were recorded using calipers and whole wet weight mass was recorded after mussel valves were opened and drained of water (Appendix table B). Condition factor (CF), used to evaluate growth and nutritive status as described by Crosby and Gale (1990) was calculated using the following equation:

$$CF = \text{whole mussel mass (g)} / \text{shell length (mm)}$$

Gonad tissue was extracted using a 20-gauge hypodermic needle and syringe by insertion into the foot and entering the soft tissue cavity. Gamete samples were preserved with 0.5 ml of a 10% formalin solution and stained with 0.5 ml of a 0.01% methylene blue solution and sex was determined by light microscopy.

Gill and foot tissue were carefully separated from the rest of the mussel using forceps and scissors and rinsed with deionized water to remove any debris. Gills were checked for the presence of glochidia prior to rinsing; if glochidia was present, they were removed from the gills, with the exception of upstream and downstream of Gonzales. Approximately, 120 mg of gill tissue was subsampled for biomarker analysis and frozen at -80°C and the remainder was used for trace element analysis. All of the foot tissue from each mussel was used for trace element analysis.

2.4 Trace Element Analysis of Mussel Tissue

The wet weight of the gill and foot tissue from each mussel was recorded, after which they were freeze dried at -54°C for 48 h, the dry weight recorded, and then

homogenized into a fine powder. The percentage water content in the gills and foot was 84 ± 2% and 83 ± 2% [mean ± 1 standard deviation (SD)], respectively.

All samples were acid digested prior to trace element analysis; in summary, approximately 0.25 g of homogenized tissue was added to a quartz vial with 5 ml of acid (9:1 HNO_3:HCl) and digested in a high temperature, high pressure microwave (Ethos-UP; Milestone, Shelton, CT) for 75 min. Once cooled to room temperature, the digested samples were diluted with 25 ml of Milli-Q water to obtain a total sample volume of 30 ml. All samples were then sent to the Trace Element Analysis Core Lab at Dartmouth College (Hanover, NH) for ICP-MS (Inductively Coupled Plasma Mass Spectrometry) analysis of 8 essential (Co, Cu, Fe, Mn, Mo, Ni, Se, Zn) and 8 nonessential (Ag, As, Cd, Cr, Hg, Pb, Sn, V) elements using an Agilent 7900 ICP-MS (Agilent Technologies Inc., Santa Clara, CA). All of the data is reported as µg/g dry weight.

All sample processing and subsequent analysis used a trace metal clean technique to avoid contaminating the samples. Quality Assurance/Quality Control (QA/QC) included blanks (n = 13), standard reference material (SRM; NIST 1566b oyster tissue; n = 14), duplicate samples (n = 14), and spiked samples (n = 13). For all investigated elements, the blanks were below detection, the SRM recovery ranged between 88 and 112%, the relative % difference between duplicate samples was < 10% for As, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Se and V, and between 11 and 17% for Ag, Co, Cr, Mo, Sn, and Zn, and the % recovery of spiked samples was between 82 and 120%.

2.5 Biomarker Analysis of Gill Tissue

Frozen gill tissue was shipped to the University of Lethbridge (Alberta, Canada) for biomarker analysis. Lipid peroxidation, total antioxidant capacity against peroxyl radicals, and protein content were determined following the methods described in Gillis et al. (2012, 2014b). Attempts to analyze gill tissue for metallothioneins and total lipid were unsuccessful.

2.6 Water Quality Analysis

All water quality analysis (Appendix table A and C) followed methods described in the American Public Health Association (APHA) Standard Methods for the Examination of Water and Wastewater (2005). The concentration of *E.coli* in each water sample was determined at the Edwards Aquifer Research and Data Center (EARDC) following APHA method 9223 B. Other water quality parameters were determined using the following methods: total nitrogen (APHA method 4500-NO3 C), nitrate (APHA method 4500-NO3 B-2000), ammonium (APHA method 4500-NH3 F), total phosphorus (APHA method 4500-P E), soluble reactive phosphate (SRP; APHA, method 4500-P E), ions (APHA method 300.0), dissolved organic carbon (DOC; APHA method 5310 B-2000), chlorophyll a (APHA method 10200 H 3), total suspended solids (TSS; APHA method 2540 D), and non-volatile suspended solids (NVSS; APHA method 2540 E).

2.7 Sediment Analysis

For each site, the wet weight of 300 g of sediment (n = 4) was recorded, after which the sediment was dried in an oven at 60°C for 48 h, and the dry weight recorded;

the % water content is shown in Appendix table D. Replicates were then pooled and subsampled to determine the % coarse sediment (> 63 μm; Appendix table D), % organic carbon content (Appendix table D), and concentration of trace elements.

To determine the % coarse sediment (n = 3 per site), 30 g of sediment was rehydrated and passed through a 63 μm sieve; sediment > 63 μm was dried at 60°C for 24 hours and the % coarse sediment determined.

To determine % organic carbon content (n = 3 per site), ~4 g of sediment was combusted in a furnace at 450°C for 4 hours, cooled, and the weight recorded. The % organic carbon was calculated as the % difference between the before and after combustions weights.

For trace element analysis, 10 g of sediment from each site (grain size < 2 mm) were sent to Dartmouth College for microwave acid digestion and ICP-MS analysis. For QA/QC (n = 1), the blank was below detection, the SRM (NIST 2709a, San Joaquin soil) recovery ranged between 83 and 105%, and the relative % difference between duplicate samples ranged between 4 to 300% (due to difference in grain size between samples).

2.8 Statistical Analysis

All results are expressed as mean ± 1 SD unless otherwise stated. Following the methods of Hopkins et al. (2006) and Glass and Gray (2001), trace element concentrations that were below detection limit (BDL) in < 50% of samples at a study site were replaced by a value of 50% of the detection limit for that element. For study sites in which > 50% of samples were below detection limit for that element, the site was

eliminated from further analysis. Data were $\log_{10}$ transformed as appropriate to meet parametric assumptions of normality and homoscedasticity.

Overall differences in trace element concentrations in gill and foot tissue between sites, as well as biomarker levels in gill tissue were first examined using a PERMANOVA. A PERMANOVA was chosen because data were not normally distributed. Redundancy Analysis (RDA) biplots were used to examine patterns in trace element concentrations in mussel gills in relation to sampling sites. Comparison of gill and foot tissue trace element concentrations were done for each element at each site using paired *t*-tests or Wilcoxon Signed Rank test when data did not meet assumptions of normality with a Bonferroni correction ($p < 0.006$) for multiple comparisons. When parametric assumptions were met, differences in trace element and biomarker concentrations between sites were determined with an ANOVA and if a significant difference was found ($p < 0.05$), a Tukey post hoc test was applied. When data did not meet parametric assumptions, a Kruskal Wallis test and Dunn post hoc test was used. A Spearman's rank-order correlation was used to determine the correlation between biomarker concentrations in gill tissue and either trace element concentrations in gill tissue or nutrient concentrations in water as well as the correlation between trace element concentration in gill tissue and trace element concentrations in sediment.

Analyses were performed using Program R version 3.5.0 and Sigmaplot 13 software (Systat Software Inc., San Jose, CA, USA). Significance was determined at the $p < 0.05$ and $p < 0.01$ confidence level.

III. RESULTS

Concentration rankings across sites for essential and nonessential trace elements varied between the two investigated tissues; with a greater rearrangement in ranking order observed for the nonessential elements. The ranking of average concentration in gill tissue across sites was Mn (4,400 µg/g dry wt) >Fe>Zn>Cu>Se>Ni>Co>Mo (0.388 µg/g dry wt) for essential elements and As (6.90 µg/g dry wt) >Cr>V>Pb>Cd>Hg>Ag>Sn (<0.004 µg/g dry wt) for nonessential elements (Fig. 3, 4; Appendix table E and F). For essential elements, the average concentration ranking between sites was Fe (120 µg/g dry wt) >Zn>Mn>Cu>Se>Ni>Co>Mo (0.106 µg/g dry wt) and for nonessential elements the ranking was As (2.98 µg/g dry wt) >Hg>Pb>V>Ag>Cr>Cd>Sn (< 0.004 µg/g dry wt) in foot tissue (Fig. 3, 4; Appendix table E and F). Overall, the concentration of essential elements exceeded those of nonessential elements in both gill and foot tissue. All essential elements were above the detection level for both tissues in all samples at all sites. However, for the nonessential elements, Sn was BDL (< 0.004 µg/g) in >50% of samples for both foot and gill at all sites except gill tissue from mussels sampled at the Lower Gonzales (LG) for which the 93% of mussels sampled had a measurable concentration of Sn in their gill tissue [0.052 ± 0.022 µg/g dry wt (n = 14)]. V was BDL in 60% of foot tissue at the PSP site and was therefore excluded from further analysis; however, for mussels that did have a measurable level of V the mean ± SD V concentration was 0.060 ± 0.022 µg/g dry wt (n = 6).

Overall, there were significant differences in trace element concentrations between sites in gill tissue (PERMANOVA, $F_{7,118} = 4.55$, $p = 0.01$, $R^2 = 0.22$, Fig. 3, 4) and foot tissue (PERMANOVA, $F_{7,99} = 3.31$, $p = 0.01$, $R^2 = 0.18$, Fig 3, 4). Mussel size as

measured by shell length explained only 3.3% of the variation in trace element concentrations for gill tissue (PERMANOVA, $F_{1,118} = 4.79$, $p = 0.01$, $R^2 = 0.03$) and was not a significant predictor of trace element concentration in foot tissue ($p = 0.65$) or biomarkers in gill tissue ($p = 0.47$). Differences between sites explained more of the variation in trace element concentrations for nonessential elements compared to essential elements for both gill tissue (PERMANOVA, $R^2 = 0.37$ and 0.22, respectively, $p = 0.01$ in both cases) and to a lesser extent for foot tissue (PERMANOVA, $R^2 = 0.24$ and 0.18, respectively, $p = 0.01$).

Trace element concentrations in the gills were significantly higher compared to foot tissue of *A. plicata* as predicted, except for Hg concentrations, which were greater in foot tissue at upstream sites LKW, PSP and LG; (paired t-test, $p < 0.002$), but not significantly different at all other sites (paired t-test, $p > 0.006$, Bonferroni corrected $\alpha = 0.006$). Furthermore, significant differences between sites were observed in Se:Hg molar ratios (Kruskal-Wallis, $p = 0.001$, Fig. 5). In both gill and foot tissue, Se:Hg molar ratios were highest in mussels at LKW (23.9 and 11.1, respectively) and lowest in mussels at PSP (7.97 and 3.64, respectively) with higher ratios in gill than foot in 98% of mussels. Concentrations of trace elements in sediment (Appendix Table G) did not correlate with trace elements found in gill tissue (Spearman's rank-order correlation, $p > 0.05$).

A redundancy analysis (RDA) showed that trace element concentrations in gill tissue differed significantly between sites in different areas of the Guadalupe basin (Fig. 6A) and these differences were associated with certain trace elements (Fig. 6B). Trace element concentrations did not generally increase downstream, and were not necessarily higher at sites downstream of cities compared to upstream; for examples: 1) The San

Marcos River site (PSP, Fig. 1, Fig. 6A) was characterized by significantly higher Hg concentration in the gill tissues compared to the lowest concentrations which were found in the uppermost site on the Guadalupe River (LKW, Fig. 4, 6). 2) As concentrations were highest in the Guadalupe River upstream of the confluence with the San Marcos River (site: LKW, Fig. 1, 4, 6) and significantly higher than all other sites except for the upper Victoria site (Fig. 6); however, the lowest mean As concentration was found at the upper Gonzales (UG) site directly below the confluence (Fig. 6). 3) Higher concentrations of Cd were found at sites in the lower Guadalupe downstream of Gonzales and concentrations in the gill tissue at UC, LC, and UV were significantly higher compared to LKW, UG, and LG (Fig. 4). Not all trace elements showed significant differences between sites, i.e., two of the essential elements (Ni and Zn, Fig. 3) and two of the nonessential elements (Ag, and V, Fig. 4) showed no significant difference in concentrations in gill tissue (ANOVA, $p > 0.05$, Fig. 6).

Nutrient concentrations were highest at upstream sites (LKW, PSP, UG, and LG) for total nitrogen, nitrate, total phosphorus, and soluble reactive phosphorus (Fig. 7). The highest concentration of total nitrogen, nitrate, total phosphorus, soluble reactive phosphorus, and coliform bacteria levels was found at PSP on the San Marcos River. There was no observed difference between sites for ammonium with concentrations ranging between 0.132- 0.231 mg/L (Fig. 7).

As with trace element concentrations and nutrient concentrations, significant differences between sites were also observed for biomarker concentrations in gill tissue (PERMANOVA, $F_{7,119} = 9.23$, $p = 0.01$, $R^2 = 0.36$, Fig. 8); however, trace element concentrations in gill tissue showed no correlation with the analyzed biomarker suite. An

exception to this finding was observed for Co (Fig. 3) and Cd (Fig. 4) which both showed a positive correlation with total antioxidant capacity against peroxyl radicals, protein content, and condition factor ($\rho > 0.211$, $p < 0.02$) but high negative correlation with lower lipid peroxidation ($\rho < -0.354$, $p < 0.0002$).

Overall, lipid peroxidation tended to be lower and total capacity against peroxyl radicals, protein content and condition factor tended to be higher at sites downstream of Gonzales than at upstream sites (Fig. 8). No significant differences in biomarker concentrations were found at sites above the river confluence LKW, PSP, Fig. 8) and sites directly below the confluence (UG and LG) were similar to LKW and PSP for all but condition factor which was found to be significantly lower than at upper sites (Kruskal- Wallis, $p < 0.003$, Fig. 8). Condition factor was not statistically different in the two upstream sites (LKW and PSP), was lowest at the UG site directly below the river confluence and progressively increased to the most downstream site (Fig. 8). At sites below Gonzales, lipid peroxidation in gill tissue was significantly lower than the upper four sites (Kruskal-Wallis, $p < 0.01$, Fig. 8) with the exception of the last study site (LV, Fig. 7). Total antioxidant capacity against peroxyl radicals was significantly higher in gill tissue at downstream sites UC, LC, UV, and LV than the lower Gonzales site (Kruskal-Wallis, $p > 0.01$, Fig. 8). Protein content in gill tissue was highly similar at all sites downstream of Gonzales (UC, LC, UV, LV, Fig. 8). Furthermore, both total capacity against peroxyl radicals and protein content in gill tissue trended higher in mussels at downstream sites (UC, LC, UV, and LV) than upstream sites (LKW, PSP, UG, LG) although differences were not all significant (Fig. 8).

As expected, higher lipid peroxidation correlated to lower mussel condition (ρ = -0.71, p = 0.05) and protein content in gills showed positive correlation with total antioxidant capacity against peroxyl radicals (ρ = 0.74, p = 0.03) and negative correlation with lipid peroxidation (ρ = -0.83, p = 0.005, Fig. 8). Positive correlations between lipid peroxidation in gill tissue and nutrient content and *E. coli* contamination in river water samples were observed (Table 4, Fig. 7, Fig. 8). Conversely, negative correlations between the remaining biomarkers in the suite (antioxidant capacity against peroxyl radicals, protein content, and condition factor) and nutrients and *E. coli* contamination were observed in which higher biomarkers were associated with lower nutrient levels (Table 4, Fig.7, Fig. 8).

IV. DISCUSSION

This is the first in-depth study on the impact of environmental contaminants on unionid mussels in the Guadalupe River basin. Recent studies by Du et al. (2014) examining the bioaccumulation of ionizable pharmaceuticals such as diphenhydramine and carbamazepine in a tributary of the Brazos River and another study by Nobles and Zhang (2015) describing reduced survival, growth, and condition of mussels exposed to municipal wastewater effluent in a tributary of the Colorado River have recently been published; however, this is the most comprehensive study investigating the effects of environmental contaminants on unionid mussels in Texas rivers and confirms the use of *A. plicata* as a suitable bioindicator in Texas as well as a potential biomonitoring species for long term studies.

In the present study, spatial variation in trace element accumulation was best characterized by differences in nonessential elements such as As, Hg, and Cd in gill tissue. Although significant spatial variation in trace element concentrations in gills was observed, no stress response determined by an increase in lipid peroxidation and a decrease in total antioxidant capacity against peroxyl radicals was detected in the biomarker suite that correlated with increased concentration of trace elements. Significant spatial differences in biomarker response was, however, observed and correlated strongly with the nutrient concentrations of water samples.

4.1 Trace element concentrations in gill and foot tissue

Overall, trace element concentration and the range of variability in concentration was reduced in foot compared to gill (Fig. 3 and 4) as predicted reflecting remobilization of elements in the body after uptake as well as greater regulation of trace elements after remobilization. Reduction in the range of variability in trace elements is visualized by shorter error bars for foot tissue concentrations (Fig. 3 and 4). The exception to the finding was Hg, in which concentrations in foot tissue were greater or were not significantly different to concentrations in gill confirming high remobilization of Hg into foot tissue after uptake and diet as the primary exposure route (Roditi and Fisher 1999, Shi and Wang 2005).

Se has a known antagonist relationship with Hg due to its strong binding affinity and in molar excess Se has been proposed to offer some protection from Hg toxicity (Tran et al. 2007, Ralston et al. 2007, 2008). Additionally, a 5:1 ratio suggested in a 2013 study by Burger and Gochfeld more realistically accounts for the role of free Se as an essential element required for normal biological function. Se:Hg molar ratios in this study were all above the 1:1 ratio in both gill and foot and only lower than the 5:1 proposed ratio in foot tissue at the PSP site (Fig. 5). Se:Hg molar ratios were most influenced by variation in Hg (Fig. 4) concentrations and similar Se concentrations (Fig. 3) across sites. Thus, Se:Hg molar ratios were highest at LKW were Hg concentrations were lowest, and lowest at PSP where Hg concentrations were highest.

All essential elements were found in greater concentration than nonessential elements with the exception of As which was found in higher concentrations than Co, Cu, Mo, Ni, and Se in gills and foot. Greater biological regulation of essential elements was

reflected in the minimized variability in concentration ranking between gill and foot which only affected elements found in greatest concentration (Fe, Mn, and Zn); whereas greater rearrangement in concentration ranking for nonessential elements affected the ranking of all elements except As and Sn (Ag, Cd, Cr, Hg, Pb, V).

The few studies found in the literature involving trace element analysis of gill tissue in wild unionid mussels show concentrations comparable to the present study of Cu, Ag, and Se (3-7, 0-1.8, and 2-2.5 µg/g dry wt, respectively) and higher Cr and Pb (1-7 and 2.5-13 µg/g dry wt, respectively) in wastewater exposed mussels in Ontario, Canada (Gillis et al. 2014b), comparable Zn (407 µg/g dry wt) and higher Cd (1.8 µg/g dry wt) in a relatively uncontaminated river in Indiana (Adams et al. 1981), and higher Hg (0.256- 0.368 µg/g dry wt) in the Experimental Lakes Area of Ontario (Malley et al.1995). Comparison to these prior studies suggests trace element contamination at sites in the present study within the Guadalupe River basin is lower or comparable to other low to moderately impacted freshwater systems in North America.

Concentrations of trace elements in sediment (Appendix Table G) did not correlate with gill or foot tissue concentrations for any element. Trace elements in sediment are known to bind readily to organic carbon, to be found in greater concentration in sediment with smaller grain size due to a greater surface area to volume ratio (Horowitz and Elrick 1987), and metals associated with organic carbon have been shown to be absorbed by mussels at a higher rate (Gagnon and Fisher 1997). Thus, the lack of correlation between the trace elements in sediment and mussel tissues likely reflects the low organic carbon content and large grain size (> 63 µm) of sediment across sites (Appendix Table C and G). Furthermore, recent flooding events may have resulted

in disturbance of river sediment composition and thus, may not be reflective of long-term exposure trace element exposure to mussels. In water, trace elements concentrations are typically very dilute, experience greater temporal variation in concentration in comparison to sediment, and there is an increased risk of secondary contamination during analysis; therefore, trace element analysis of water was not included.

4.2 Spatial differences in trace element accumulation in gill tissue and nutrient and coliform bacteria in water

A prediction of higher accumulation of trace elements in gill tissue of mussels downstream was based on a simplistic model of additive contributions of point and nonpoint source contaminants as flow moves downstream through urban, industrial, and agricultural regions; however, environmental concentrations of contaminants are highly influenced by dilution from groundwater and precipitation events and results did not reflect compounding exposure in many cases. On the San Marcos River near the PSP site, the median discharge is 221 ft^3/sec, on the Guadalupe River above the confluence it is 411 ft^3/sec, and at the lower sites above and below Victoria the median discharge is 974 ft^3/sec illustrating increasing discharge rates along the continuum as well as differences in the contribution of each river to downstream water volume (USGS, 2018).

Furthermore, differences in effluent chemistry, quality of wastewater treatment, as well as the volume of effluent released into the aquatic environment can be expected to result in wide variation of complex contamination originating from wastewater outfalls in the Guadalupe River basin. The influence of nonpoint source pollution entering the water column such as urban runoff and agricultural runoff may also vary seasonally and

significantly between sites in both volume and composition. Overall, predictions that trace element accumulation would increase in mussels with distance downstream were not found to be true; however, significant differences between sites were found and best characterized by the accumulation of As, Hg, and Cd in gill tissue.

As is present both naturally and by human activity in the environment with high spatial and temporal variation of environmental concentrations (Garelick et al. 2008, Ng 2005, Gong et al. 2014); however, As concentrations are generally low (<10 µg/L) within the Guadalupe River basin and aquifers contributing groundwater to the surface flow such as the Edwards Aquifer have low arsenic concentrations (Gong et al. 2014). Uptake of As is primarily through dietary exposure (Zhang and Wang 2018) and may explain some uptake but analysis of chlorophyll a, an indicator of algal concentration in water samples which showed the lowest concentration of chlorophyll a, was found at the LKW site where the highest tissue concentrations of As in *A. plicata* were recorded. As is primarily speciated as arsenate [As(V)] in oxic freshwater systems and is taken up in competition with phosphate (PO_4) by phytoplankton and other aquatic microorganisms important to mussel diet (Rahman et al. 2012). In addition to exposure through diet, burrowing behavior in mussels may expose them to arsenite [As(III)], the dominant As species under anoxic conditions such as those found in sediment that is in biological competition with sulfur uptake (Rahman et al. 2012). Thus, the relative environmental abundance of As, trophic transfer from microorganisms in the diet, and exposure to As in the sediment may contribute to elevated As in mussels in comparison to other nonessential elements.

Long-term water data is required to determine the relative influence of temporal variation in algal presence at all study sites however a search of the GBRA water quality data for monitoring sites suggests that the relative concentrations of chlorophyll a found in this study (Appendix Table A) are similar those found in long term monitoring by GBRA. Leaching of chromated copper arsenate (CCA) from treated wood, disposal of arsenic and arsenic laden products, erosion of land laden with arsenic based insecticides and pesticides, and burning of fossil fuels contribute to As pollution in aquatic systems (Garelick et al. 2008). The relatively elevated concentrations (9.65 µg/g dry wt, Fig. 4) in gill tissue at the LKW appear to be relatively low to moderate in comparison to concentrations of As in marine mussel gills which ranged between 5.35-19.6 µg/g dry wt in mussels around the world (Clowes and Francesconi 2004, Chandurvelan et al. 2015) and comparative study between marine and freshwater mussels by Soeroes et al. (2005) suggests little difference in the overall As uptake rates between freshwater and marine bivalves.

Hg concentration in gill tissue differed the most between sites above the river confluence (LKW and PSP) with higher concentrations at PSP suggesting that the San Marcos River contributes significantly more Hg than the upper Guadalupe River to sites below the confluence where median concentrations in mussels appear to reflect mixing (Fig. 4).

Although the upstream reservoir on the Guadalupe River at Canyon Lake is under advisory for Hg in fish (DSHS 2006), mussels at the LKW site below this impoundment had the lowest Hg concentrations of any site in the present study. The relationship between increased Hg concentrations in fish and hydroelectric dams is known (Hecky et

al. 1991, Mailman et al. 2006); however, by increasing residence time of water in the reservoir and allowing for settling of Hg bound to organic matter (Rueda et al. 2006), river impoundment on the upper Guadalupe River acts may reduce downstream Hg concentrations in comparison to the San Marcos River where impoundment is largely absent.

Another possible explanation may involve the influence of different ecoregions on Hg in the Guadalupe River basin which covers 4 different ecoregions. Land cover differences in ecoregions is known to influence the methylation rate and bioavailability of Hg locally (Allen-Gill et al. 1995, Drenner et al. 2013). Nonetheless, the source of Hg at the investigated sites is unknown and beyond the scope of the study. Despite significant differences in Hg concentrations between sites, concentrations of Hg found in mussels in this study were relatively low in comparison to reported total Hg concentrations of 0.256-.368 µg/g dry wt in gill tissue of mussels in the relatively unimpacted Experimental Lakes Area in Ontario, Canada (Malley et al. 1995). Local water chemistry including chloride, dissolved organic carbon, and well as the speciation of Hg present in the environment also act to determine toxicity and bioavailability of Hg in mussels and other aquatic organisms (Boening 2000, Pan and Wang 2004, Dutton and Fisher 2012); however, difference in chloride and DOC appeared to be minimal between sites and Hg was not speciated in this study.

Significantly higher concentrations of Cd in gill tissue at UC, LC, UV, and LV (Fig. 3 and 4) coincide with a shift in land use between Gonzales and Cuero that includes intensive oil and gas activity in the Eagle-Ford shale. Hydraulic fracturing fluid and associated wastewater have been demonstrated to contain Cd (EPA 2012, Elliot et al.

2016), and findings in the Barnett Shale formation in North Texas support high volume hydraulic fracturing (HVHF) activity as a source of groundwater pollution in Texas (Fontenot et al. 2013). Due to the proximity of many HVHF sites to the Guadalupe River, hydrofracking in the Eagle-Ford Shale has been identified as concern to surface water and ground water quality warranting further investigation (GBRA, 2018). Additionally, other industries known to contribute to Cd pollution such as cement production (Ogunbileje et al. 2013), metal alloys, Cd plating effluent, and Cd containing products (Tchounwou et al. 2014) were found to be present or historically present within the Guadalupe River basin in this area as identified in an online search.

It is important to note that although numeric standards for trace element concentrations in surface water have been established in Texas (TCEQ, 2018), water quality criteria for trace element contamination of aquatic systems is based only on concentrations in the dissolved phase that are likely to be inadequate for protecting mussels and other aquatic life from elevated trace element concentrations such as Se, Cr, and Co which have been demonstrated to primarily be obtained from dietary exposure (Wang and Fisher 1997).

Spatial differences in nutrients were also observed with higher concentrations of total nitrogen, nitrate, total phosphorus, and soluble reactive phosphorus at upstream sites LKW, PSP, UG, and LG likely reflecting multiple sources such as increased wastewater effluent due to rising population in the upper Guadalupe River Basin, failing septic systems resulting in groundwater contamination, as well as a fertilizer runoff from agricultural land use documented in the region (GBRA, 2018). In addition, cattle operations which extend to the river as a source of water for livestock and may be

significant sources of total nitrogen, nitrate, total phosphorous, and *E. coli* (Fig. 7) found in river water samples at upper sites as well. The highest concentrations of nitrate, total phosphorus, soluble reactive phosphorus, and *E. coli* were observed at PSP on the San Marcos River. PSP is downstream of growing urban development in Kyle and San Marcos as well as the Plum Creek watershed that drains to the San Marcos River approximately 8 km upstream of PSP and has been listed in the Water Quality Inventory and 303(d) List of Impaired Waterbodies due to high bacterial levels and nutrient enrichment as a result of wastewater effluent dominance and failing septic systems in the area (TCEQ 2008, GBRA 2018). Although efforts have been made to mitigate impairment of the Plum Creek watershed, further study is needed to determine the impact of continued impairment on downstream sites.

Ammonium concentration which ranged between 0.132- 0.241 mg/L were well below the 2013 EPA criteria continuous concentration (CCC) of 1.24 mg/L for conditions relevant to the study area, below protective concentrations of 0.3 mg/L proposed by Augspurger et al. (2003), but at or above the 0.2 mg/L threshold for sensitive populations proposed by Strayer and Malcom (2012) at PSP, UG, LG, UC, and LV (Fig. 7). The lower thresholds (0.2 and 0.3 mg/L) are primarily concerned with the sensitivity of glochidia and juvenile mussels to ammonia toxicity resulting in population decline through recruitment failure. Furthermore, nitrate concentrations ranging between 0.385-0.816 mg/L were below 25mg/L in surface waters suggested for the protection of all freshwater invertebrates (Camargo et al. 2005); however this estimate does not consider the sensitivity of glochidia and juvenile mussels either. Numeric data informing protective nitrate and other nutrient criteria in surface water with regard to mussel health

continues to be limited although. Presently, water quality criteria in Texas is primarily informed by aesthetic value associated with suitability for recreation (TCEQ 2018).

4.3 Physiological stress response of *A. plicata* to contaminant exposure

There was no indication of physiological stress from trace element exposure in *A. plicata* with the remaining biomarker suite; however, metallothionein data is necessary to understand physiological response to trace elements. In the present study, metallothioneins and total lipid could not be examined for *A. plicata* due to failure of the assays.

The recorded concentrations of nitrate in this study are in range of known concentrations found in the Edwards Aquifer (0.22-1.86 mg/L) which contributes ground water in a decreasing gradient moving downstream in the Guadalupe River basin (Musgrove et al. 2016); however, it has been proposed that the presence of invasive water plants such as *Hydrilla verticillata* in the Guadalupe River could be acting to significantly mask the presence of wastewater by reducing nutrient concentrations (GBRA 2018). Correlations observed between biomarkers of physiological stress in *A. plicata* and the nutrient content of water could be due to factors closely associated with nutrient concentrations such as other constituents of wastewater and agricultural pollution (Gillis 2014a, b, Machado 2014) which were not measured (e.g. pesticides, pharmaceuticals, siltation) or due to a combination of factors including food availability or stress from recent flooding events.

As a measure of general health, condition factor (CF) was compared between sites but was not in agreement with patterns in biomarker response indicating higher

physiological stress at upper sites LKW and PSP. While CF gives information about relative long-term condition status in mussels, biomarkers account for more sensitive subcellular response showing measurable response to changes in low urban and metal related influences documented in as little as 2 weeks (Gagné et al. 2007, Farcy et al. 2011, Gillis et al. 2014a).

It should be stressed that any lack of physiological response in adult mussels does not preclude the known detriment to freshwater mussel populations when sensitive early life stages of unionid mussels are subjected both acute and chronic exposures of trace elements (i.e. Cu, Zn, Pb, and Cd) as well as ammonia and nitrate (Augspurger 2003).

Limitations of analysis prevent conclusive statements as this study was intended to provide a baseline of knowledge regarding the physiological response of *A. plicata* to environmental contaminants. Further investigation will be necessary to better understand specific influences of environmental stressors on mussel health.

4.4 Future directions

Caged transplant studies should be utilized to better understand the rate of uptake and elimination of trace elements over time and the resulting physiological response to exposure of a mixture of contaminants. This will allow for placement of mussels close to known sources of point source pollution and in impacted locations where mussel have been extirpated to better understand the role of effluent contamination on mussel health.

Future studies should also investigate the impact of environmental contaminants on glochidia and juvenile mussels within the Guadalupe River basin as they are much more sensitive to contaminants. Gravid *A. plicata* containing large quantities of glochidia

were found in the present study indicating that all life stages can be investigated using one species.

From a conservation perspective, *A. plicata* is a suitable bioindicator species which can potentially be used in long term biomonitoring studies throughout its range in Texas. A. plicata can also be used as a proxy for threatened or endangered unionid species in Texas since a similar physiological response is expected. This will provide valuable data which can be incorporated into conservation and recovery plans for Texas mussels.

Table 1. State and federal status of imperiled unionid mussels in Texas.

Name	State Listing Status	Federal Status
False Spike (*Quadrula mitchelli*)	Threatened	
Golden Orb (*Quadrula aurea*)	Threatened	Candidate
Louisiana Pigtoe (*Pleurobema riddellii*)	Threatened	
Mexican Fawnsfoot (*Truncilla cognata*)	Threatened	
Salina Mucket (Potamilus *metnecktayi*)	Threatened	
Sandbank Pocketbook (*Lampsilis satura*)	Threatened	
Smooth Pimpleback (*Quadrula houstonensis*)	Threatened	Candidate
Southern Hickorynut (*Obovaria jacksoniana*)	Threatened	
Texas Fatmucket (*Lampsilis bracteata*)	Threatened	Candidate
Texas Fawnsfoot (*Truncilla macrodon*)	Threatened	Candidate
Texas Heelsplitter (*Potamilus amphichaenus*)	Threatened	
Texas Hornshell (*Popenaias popeii*)	Threatened	Listed
Texas Pigtoe (*Fusconaia askewi*)	Threatened	
Texas Pimpleback (*Quadrula petrina*)	Threatened	Candidate
Triangle Pigtoe (*Fusconaia lananensis*)	Threatened	

Table 2. 2017 US Census Bureau population estimates of major Texas cities on the San Marcos and Guadalupe Rivers. Cities are listed from upstream to downstream beginning with tributaries of the Guadalupe River. The city of Kyle on the Blanco River is included as a significant area of urban development on a tributary of the San Marcos River. (U.S. Census Bureau, 2018)

City	River	Population Estimate
Kyle	Blanco	43,480
San Marcos	San Marcos	63,071
Luling	San Marcos	5,903
Kerrville	Guadalupe	23,386
New Braunfels	Guadalupe	79,152
Seguin	Guadalupe	28,983
Gonzales	Guadalupe	7,628
Cuero	Guadalupe	8,292
Victoria	Guadalupe	67,106

Table 3. Geographic location of study sites.

Study Site	Latitude	Longitude
Lakewood Recreational Area (LKW)	29.4694	-97.4906
Palmetto State Park (PSP)	29.5885	-97.5860
Upper Gonzales (UG)	29.4839	-97.4486
Lower Gonzales (LG)	29.4942	-97.4314
Upper Cuero (UC)	29.1517	-97.3157
Lower Cuero (LC)	29.0531	-97.2638
Upper Victoria (UV)	28.8487	-97.0667
Lower Victoria (LV)	28.7532	-97.0092

Table 4. Spearman's Rank Order Correlations between physiological stress biomarkers and water quality indicators. Only significant relationships between biomarker response and water quality analytes are presented.

Biological Response Indicator	Water Quality Analyte	Correlation Coefficient (ρ)	*p*-value
Lipid peroxidation	Total phosphorus	0.81	0.01
	Total nitrogen	0.79	0.01
	E. coli	0.714	0.04
TACAPR	Total phosphorus	-0.83	0.005
	Total nitrogen	-0.762	0.02
	Nitrate	-0.71	0.04
	E. coli	-0.74	0.03
Protein content	Total nitrogen	-0.69	0.05
Condition factor	Total nitrogen	-0.79	0.02

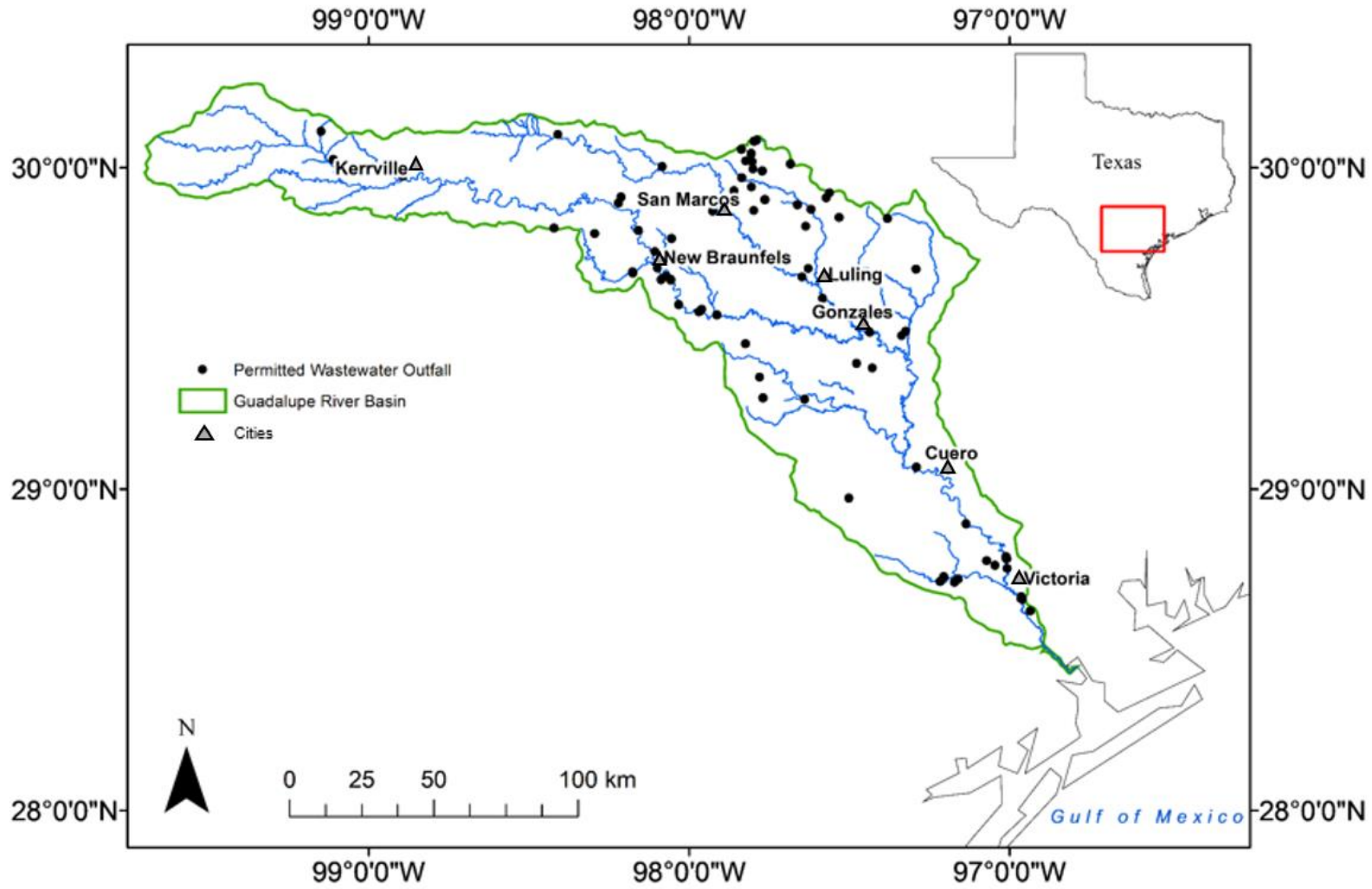

Figure 1. Location of permitted wastewater outfalls within the Guadalupe River basin.

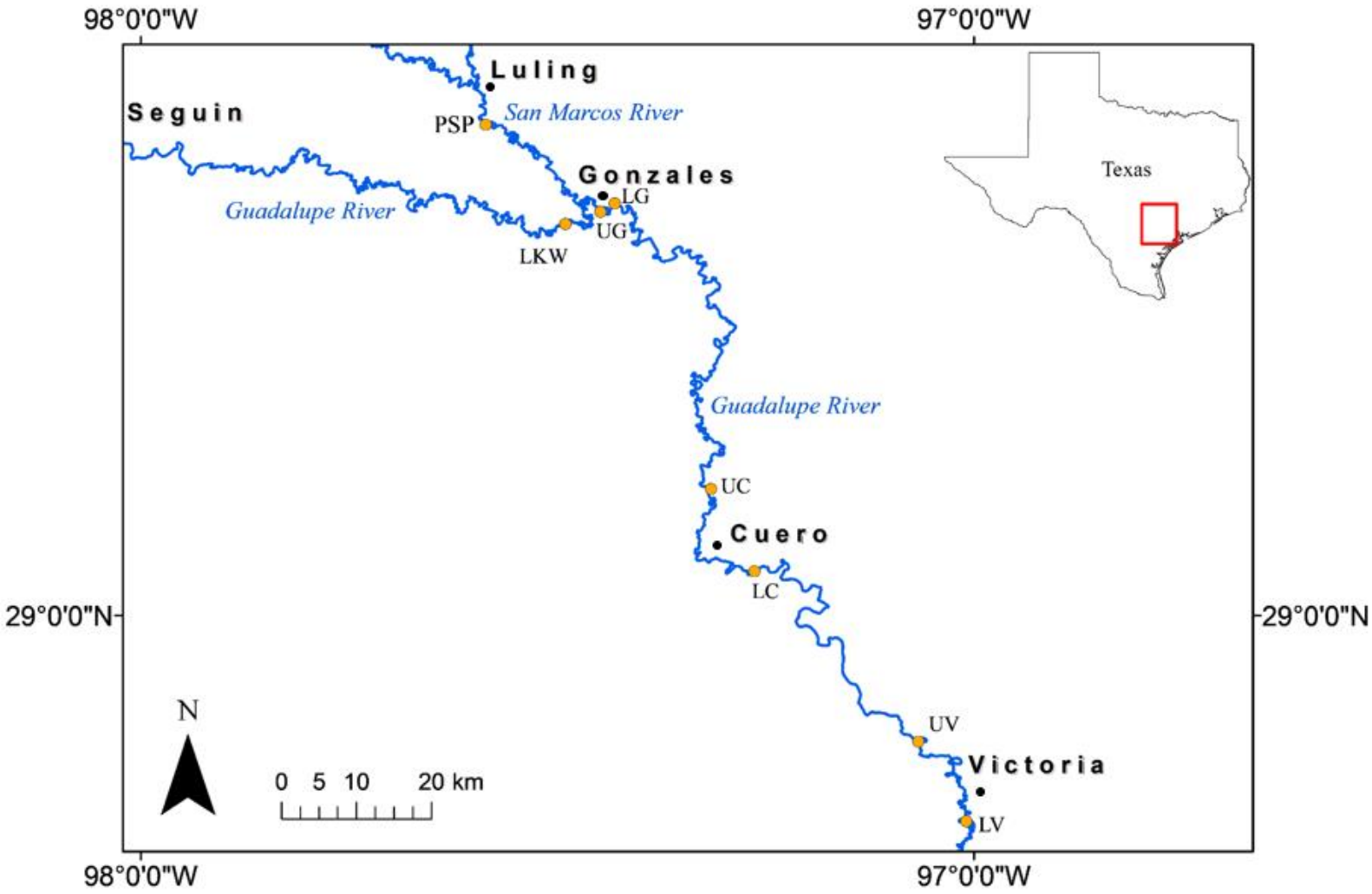

Figure 2. Location of study sites within the Guadalupe River basin. LKW = Lake Wood Recreational Area; PSP = Palmetto State Park; UG = Upper Gonzales; LG = Lower Gonzales; UC = Upper Cuero; LC = Lower Cuero; UV = Upper Victoria; LV = Lower Victoria

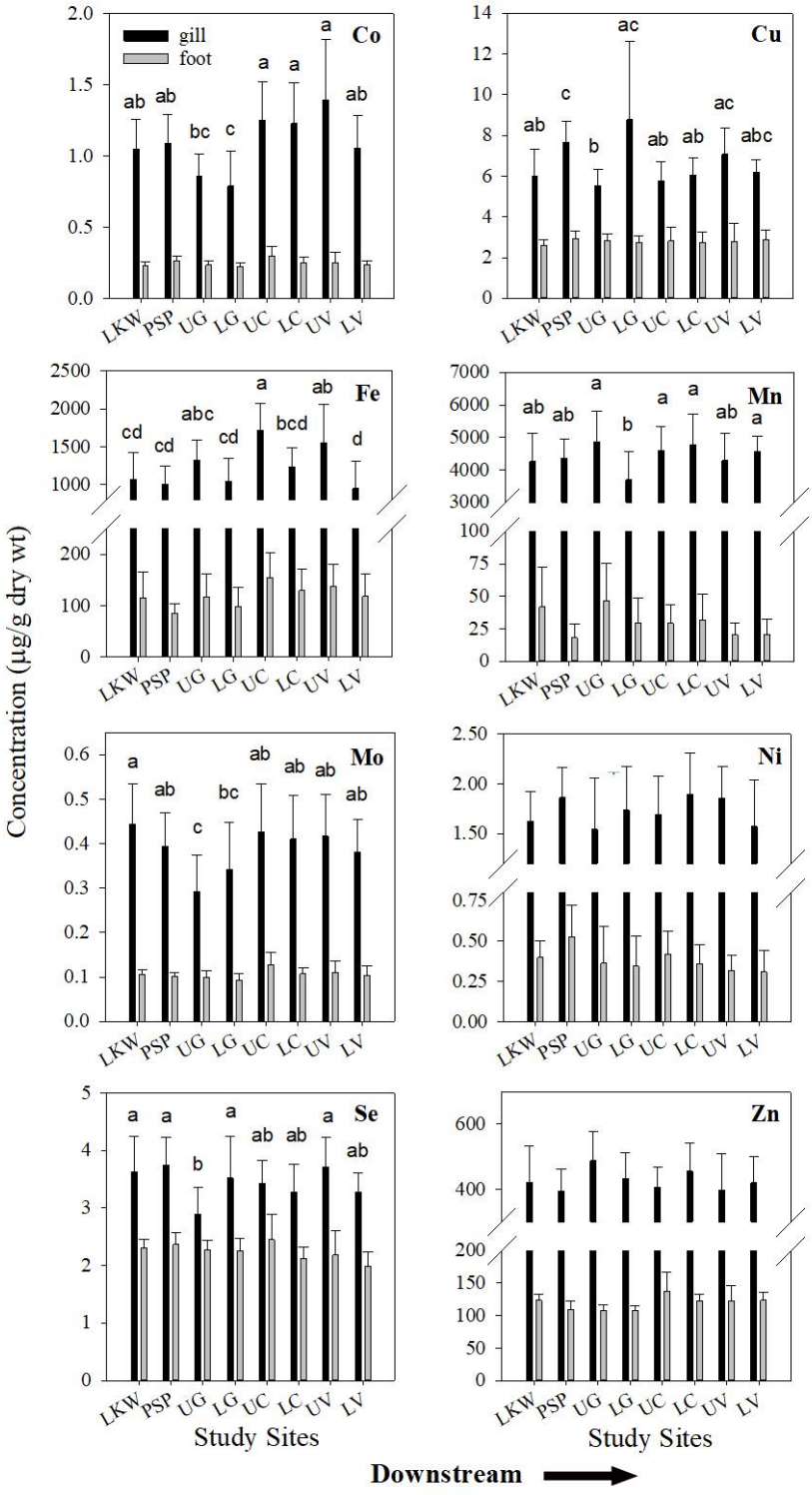

Figure 3. Concentration of essential trace elements in gill and foot tissue of *Amblema plicata* at each site. Lowercase letters indicate site groupings by similar trace element concentrations in gill tissue. No significant differences were detected between sites for Ni and Zn.

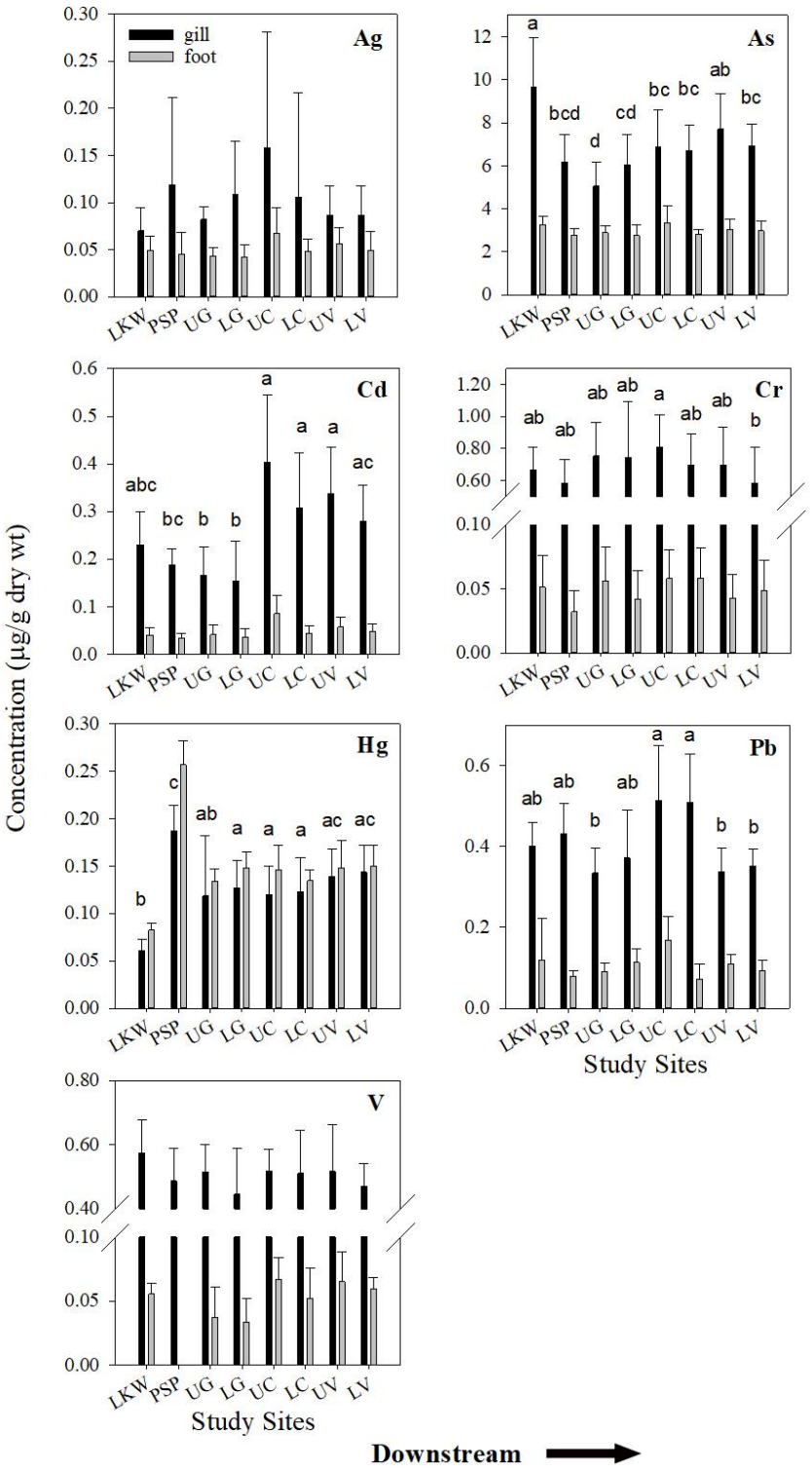

Figure 4. Concentration of nonessential trace elements in gill and foot tissue of *Amblema plicata* at each site. Lowercase letters indicate site groupings by similar trace element concentrations in gill tissue. No significant differences were detected between sites for Ag and V.

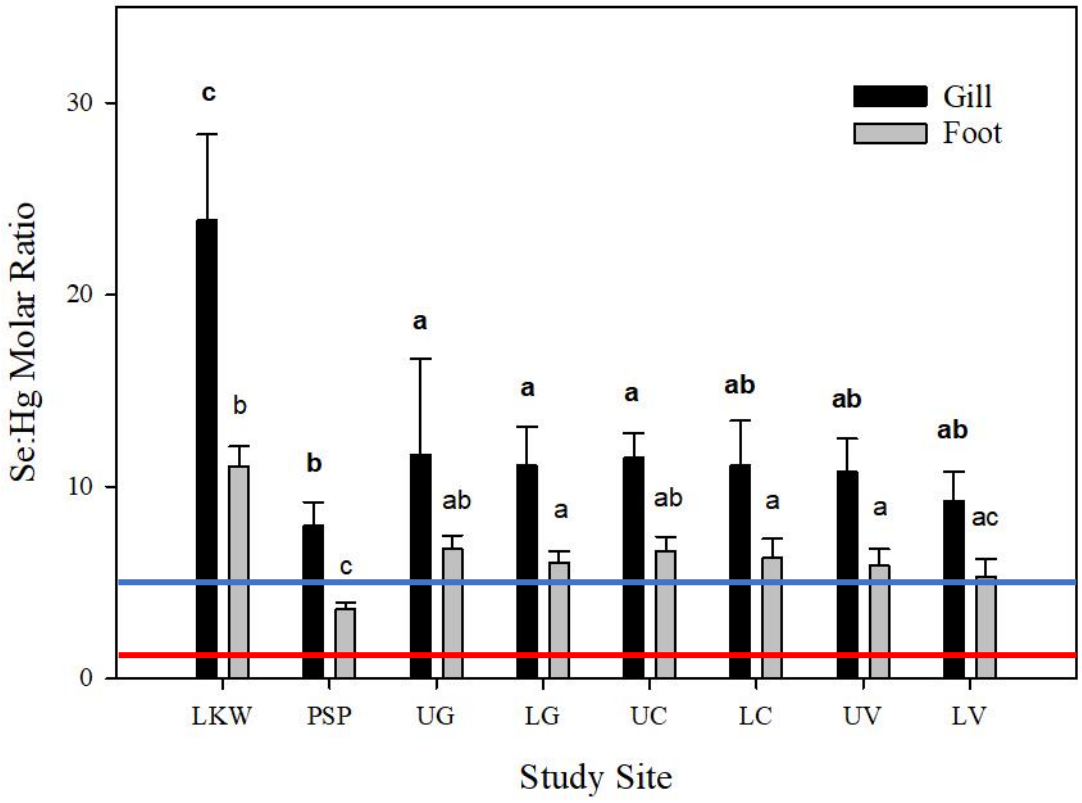

Figure 5. Se:Hg molar ratios in gill and foot tissue of *Amblema plicata* at each site. Lowercase letters indicate groupings of sites by similar Se:Hg ratios in gill tissue (bold) and foot tissue. Lines represent Se:Hg molar ratio of 1:1 (red) and 5:1 (blue).

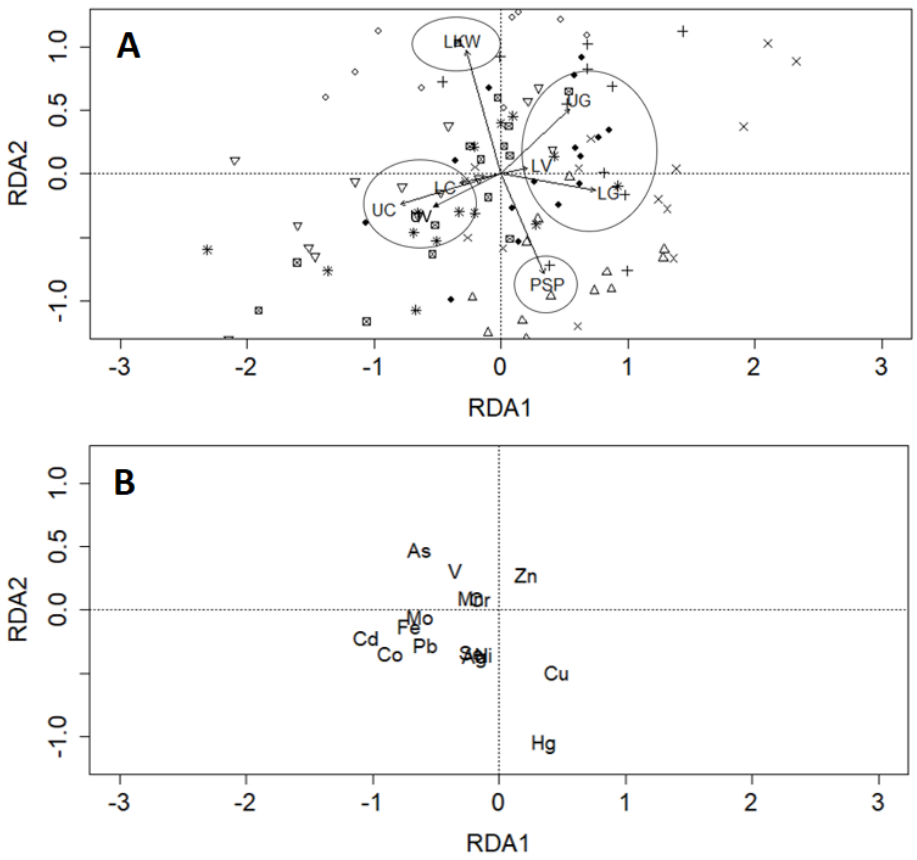

Figure 6. Redundancy analysis (RDA) biplots of trace element concentrations in gill tissue at all sites. Site data presented as (A) symbols representing individual mussels at different sites and sites with similar patterns in trace element accumulation are grouped, and (B) trace element distribution patterns.

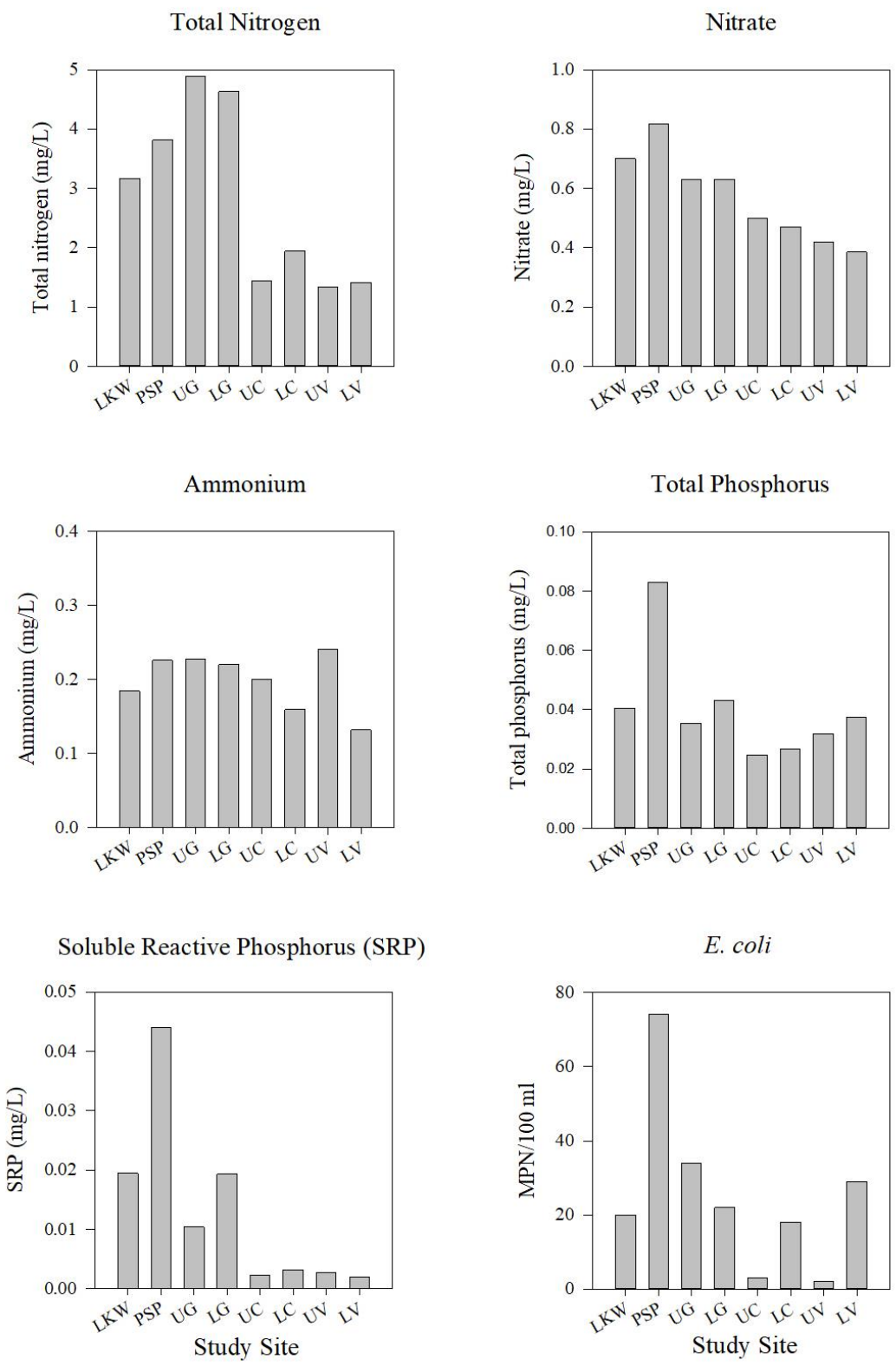

Figure 7. Nutrient concentration and coliform bacteria (*E. coli*) counts in water samples from mussel collection sites.

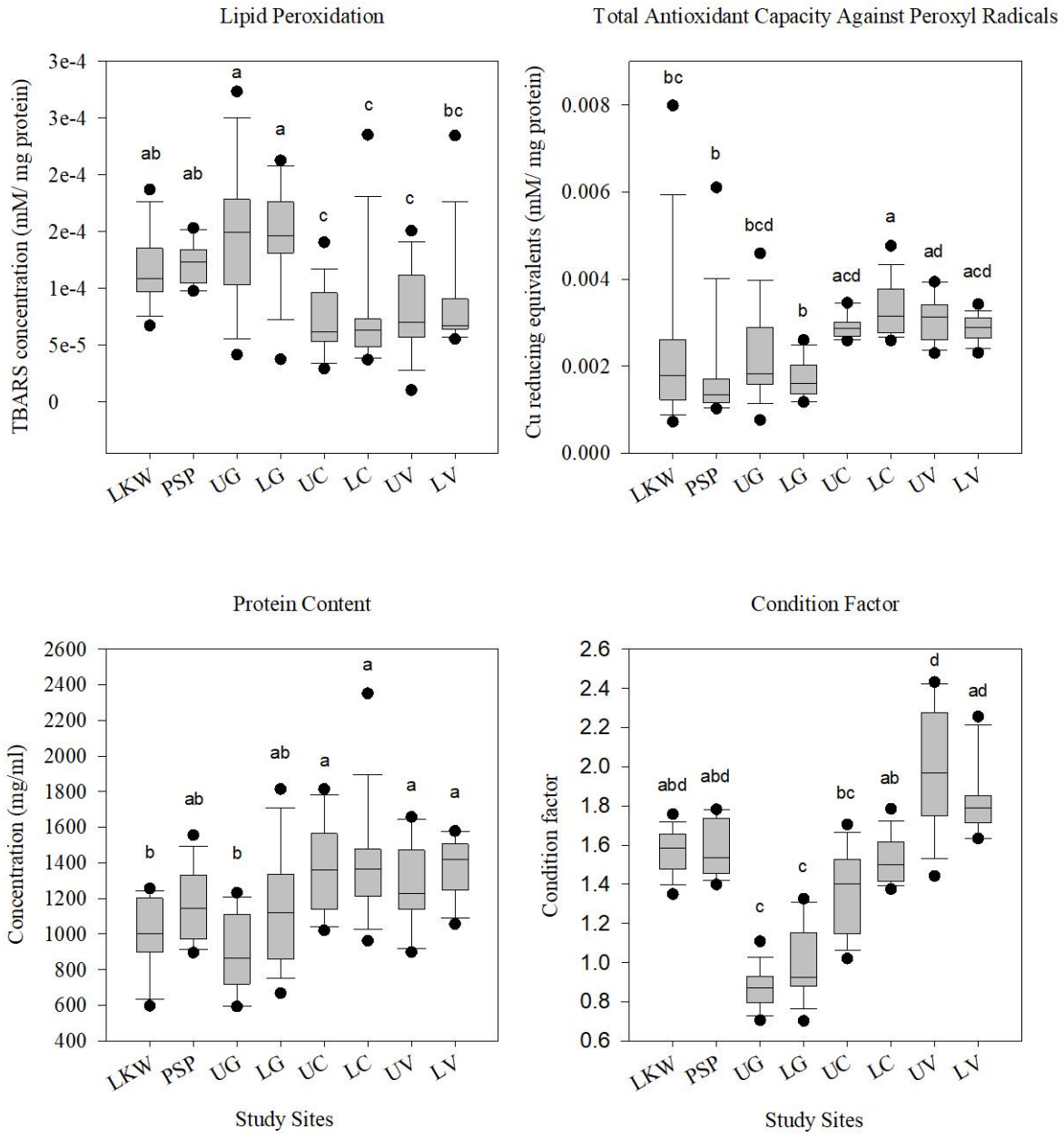

Figure 8. Biomarkers of physiological stress (lipid peroxidation, total antioxidant capacity against free radicals, total protein) in gill tissue from *A. plicata* and the calculated condition factor of mussel health at each site. Lipid peroxidation in gill tissue resulting in cellular membrane disruption is measured by levels of thiobarbituric acid reactive substances (TBARS), a byproduct of lipid peroxidation. Total antioxidant capacity against free radicals as measured in copper (Cu) reducing equivalents.

APPENDIX SECTION

Table A. Physical and chemical water parameters.

Analyte	LKW	PSP	UG	LG	UC	LC	UV	LV
Temperature (°C)	25.8	23	25.9	24.3	26.5	26	26.2	26.3
pH	ND	8.42	7	8.5	ND	ND	8.44	8.34
Dissolved Oxygen (mg/L)	7.48	8.37	8.26	8.52	9.58	8.61	8.3	7.83
Dissolved Oxygen %	92.6	100.6	104.4	105	119.1	105.7	106.3	99.9
Conductivity (µmS/cm)	516	620.9	526	573	535	514	528	546
TSS (mgSS/L)	0.056	0.078	0.063	0.07	0.055	0.055	0.058	0.06
NVSS (mgSS/L)	0.88	0.899	0.886	0.89	0.879	0.874	0.88	0.883
DOC (mg/L)	1.63	1.51	1.6	1.83	1.73	1.82	1.89	2
Chlorophyll-A (µg/L)	0.103	0.277	0.263	0.31	0.778	1.01	1.3	0.946

Table B. Biological measurements of *Amblema plicata* (mean ± SD; *n* = 15 per site).

Site	Length (mm)	Width (mm)	Height (mm)	Whole wet weight (g)	% Female	% Gravid
LKW	92 ± 2.70	36 ± 1.8	67 ± 2.2	145.19 ± 12.69	26.7	100
PSP	95 ± 3.7	35 ± 1.2	70 ± 2.9	149.56 ± 17.00	60	44
UG	65 ± 4.0	26 ± 2.4	50 ± 2.0	57.13 ± 8.98	53.3	0
LG	76 ± 5.9	29 ± 2.6	56 ± 3.3	76.19 ± 18.22	60	44
UC	85 ±6.2	33 ± 2.1	63 ± 3.3	116.35 ± 23.79	20	100
LC	90 ± 3.1	36 ± 1.7	67 ± 2.1	136.36 ± 15.10	66.7	30
UV	100 ± 6.8	40 ± 3.1	74 ± 4.2	199.77 ± 43.08	33.3	40
LV	98 ± 4.6	40 ± 2.1	74 ± 2.8	179.68 ± 25.35	26.7	50

Table C. Water ion concentrations (ppm) at each site.

Analyte	LKW	PSP	UG	LG	UC	LC	UV	LV
Ba	1.22	1.35	2.63	2.68	2.36	2.30	2.19	2.24
Br	BDL	0.333	0.273	0.276	0.279	0.297	0.289	0.303
Ca	70.6	72.6	67.5	67.5	58.2	55.3	53.7	54.2
Cl	23.7	33.6	28.7	28.8	29.3	31.0	32.0	35.1
F	1.33	1.36	1.29	1.31	1.28	1.27	1.26	1.28
Mg	17.4	17.9	17.6	17.7	17.7	17.6	17.5	17.6
K	2.20	2.36	2.49	2.54	2.47	2.55	2.49	2.58
Na	16.9	23.9	19.9	20.5	20.6	21.7	22.9	25.3
SO^{2-}_4	30.8	33.7	32.6	32.3	33.4	33.9	33.5	35.0

Table D: Water content, organic carbon content, and grain size distribution as determined by % of coarse sediment (> 63 μm) for sediment at each site. Values represent mean ± 1 SD.

Site	Water content (%)	Organic carbon (%)	Coarse sediment (%)
LKW	25 ± 0.4	2.4 ± 0.1	68 ± 2.0
PSP	22 ± 0.4	0.7 ± 0.0	98 ± 0.1
UG	35 ± 2.1	4.1 ± 0.6	97 ± 0.4
LG	29 ± 0.6	3.4 ± 0.1	53 ± 0.4
UC	12 ± 0.4	1.6 ± 1.7	70 ± 1.9
LC	24 ± 0.5	2.0 ± 0.5	62 ± 1.4
UV	19 ± 2.3	1.9 ± 0.8	89 ± 1.2
LV	19 ± 4.2	2.5 ± 0.1	79 ± 0.9

Table E. Concentration of essential trace elements (µg/g dry wt) in gill and foot tissue of *Amblema plicata* (mean ± SD).

Essential Elements	Tissue	LKW	PSP	UG	LG	UC	LC	UV	LV
Co	Gill	1.05 ± 0.206	1.09 ± 0.199	0.860 ± 0.155	0.791 ± 0.244	1.25 ± 0.268	1.23 ± 0.286	0.557 ± 0.405	1.06 ± 0.233
	Foot	0.230 ± 0.025	0.266 ± 0.030	0.236 ± 0.026	0.225 ± 0.026	0.299 ± 0.066	0.253 ± 0.036	0.135 ± 0.119	0.239 ± 0.026
Cu	Gill	5.98 ± 1.36	7.66 ± 1.03	5.55 ± 0.759	8.77 ± 3.83	5.78 ± 0.938	6.07 ± 0.851	4.60 ± 3.34	6.20 ± 0.606
	Foot	2.61 ± 0.271	2.94 ± 0.363	2.83 ± 0.342	2.74 ± 0.327	2.81 ± 0.696	2.76 ± 0.492	1.59 ± 1.37	2.88 ± 0.466
Fe	Gill	1073 ± 355	1005 ± 237	1327 ± 258	1040 ± 313	1722 ± 352	1235 ± 254	697 ± 482	952 ± 361
	Foot	116 ± 50.3	85.6 ± 18.1	117 ± 44.8	98.6 ± 37.1	155 ± 47.1	130 ± 41.0	66.8 ± 38.9	118 ± 44.8
Mn	Gill	4265 ± 864	4370 ± 589	4877 ± 941	3703 ± 878	4612 ± 734	4772 ± 964	2560 ± 1964	4563 ± 486
	Foot	41.8 ± 31	18.4 ± 10.4	46.9 ± 28.7	29.5 ± 19.2	29.3 ± 14.0	31.6 ± 19.9	25.5 ± 12.7	21.0 ± 11.8
Mo	Gill	0.444 ± 0.090	0.395 ± 0.076	0.292 ± 0.081	0.341 ± 0.106	0.425 ± 0.108	0.410 ± 0.099	0.215 ± 0.144	0.381 ± 0.074
	Foot	0.105 ± 0.010	0.102 ± 0.009	0.099 ± 0.015	0.093 ± 0.014	0.128 ± 0.028	0.108 ± 0.013	0.055 ± 0.047	0.104 ± 0.021
Ni	Gill	1.62 ± 0.303	1.86 ± 0.303	1.54 ± 0.515	1.74 ± 0.438	1.69 ± 0.394	1.89 ± 0.415	1.07 ± 0.720	1.57 ± 0.462
	Foot	0.397 ± 0.103	0.525 ± 0.197	0.362 ± 0.225	0.343 ± 0.187	0.416 ± 0.144	0.359 ± 0.119	0.306 ± 0.130	0.308 ± 0.133
Se	Gill	3.63 ± 0.612	3.75 ± 0.482	2.89 ± 0.459	3.52 ± 0.721	3.42 ± 0.407	3.28 ± 0.480	1.97 ± 1.58	3.28 ± 0.322
	Foot	2.30 ± 0.612	2.36 ± 0.203	2.28 ± 0.163	2.25 ± 0.225	2.45 ± 0.435	2.13 ± 0.190	1.25 ± 1.151	1.99 ± 0.250
Zn	Gill	421 ± 112	395 ± 68.6	488 ± 88.8	434 ± 77.1	406 ± 61.2	456 ± 87.6	259 ± 200	420 ± 79.8
	Foot	123 ± 10.2	109 ± 13.5	108 ± 9.26	108 ± 6.10	138 ± 29.4	123 ± 9.65	59.0 ± 54.2	123 ± 12.8

54

Table F. Concentration of nonessential trace elements (µg/g dry wt) in gill and foot tissue of *Amblema plicata* (mean ± SD).

Nonessential Elements	Tissue	LKW	PSP	UG	LG	UC	LC	UV	LV
Ag	gill	0.070 ± 0.025	0.119 ± 0.093	0.082 ± 0.014	0.109 ± 0.056	0.158 ± 0.123	0.106 ± 0.111	0.079 ± 0.039	0.087 ± 0.031
	foot	0.049 ± 0.015	0.045 ± 0.023	0.043 ± 0.009	0.043 ± 0.012	0.068 ± 0.027	0.048 ± 0.013	0.029 ± 0.016	0.050 ± 0.020
As	gill	9.65 ± 2.30	6.18 ± 1.27	5.04 ± 1.11	6.05 ± 1.39	6.88 ± 1.72	6.71 ± 1.17	3.51 ± 2.50	6.95 ± 1.01
	foot	3.25 ± 0.394	2.76 ± 0.300	2.88 ± 0.322	2.75 ± 0.497	3.35 ± 0.787	2.81 ± 0.218	1.58 ± 1.33	2.98 ± 0.459
Cd	gill	0.231 ± 0.068	0.188 ± 0.034	0.167 ± 0.060	0.153 ± 0.084	0.404 ± 0.142	0.308 ± 0.115	0.114 ± 0.063	0.280 ± 0.075
	foot	0.040 ± 0.015	0.035 ± 0.010	0.042 ± 0.019	0.036 ± 0.017	0.085 ± 0.038	0.045 ± 0.014	0.027 ± 0.013	0.049 ± 0.015
Cr	gill	0.668 ± 0.143	0.586 ± 0.148	0.753 ± 0.211	0.745 ± 0.347	0.809 ± 0.203	0.699 ± 0.190	0.465 ± 0.266	0.581 ± 0.230
	foot	0.052 ± 0.024	0.032 ± 0.017	0.056 ± 0.027	0.042 ± 0.022	0.058 ± 0.022	0.059 ± 0.023	0.033 ± 0.015	0.049 ± 0.023
Hg	gill	0.061 ± 0.012	0.187 ± 0.027	0.118 ± 0.064	0.127 ± 0.029	0.120 ± 0.029	0.123 ± 0.036	0.092 ± 0.063	0.144 ± 0.028
	foot	0.082 ± 0.007	0.257 ± 0.025	0.134 ± 0.013	0.148 ± 0.017	0.146 ± 0.026	0.134 ± 0.012	0.099 ± 0.098	0.150 ± 0.021
Mo	gill	0.444 ± 0.090	0.395 ± 0.076	0.292 ± 0.081	0.341 ± 0.106	0.425 ± 0.108	0.410 ± 0.099	0.215 ± 0.144	0.381 ± 0.074
	foot	0.105 ± 0.010	0.102 ± 0.009	0.099 ± 0.015	0.093 ± 0.014	0.128 ± 0.028	0.108 ± 0.013	0.055 ± 0.047	0.104 ± 0.021
Pb	gill	0.400 ± 0.058	0.431 ± 0.075	0.335 ± 0.062	0.372 ± 0.118	0.513 ± 0.137	0.509 ± 0.120	0.232 ± 0.165	0.352 ± 0.041
	foot	0.119 ± 0.102	0.078 ± 0.015	0.091 ± 0.021	0.113 ± 0.034	0.169 ± 0.058	0.073 ± 0.037	0.059 ± 0.041	0.093 ± 0.026
Sn	gill	ND	ND	ND	0.052 ± 0.022	ND	ND	ND	ND
	foot	ND	ND	ND	ND	ND	ND	ND	ND
V	gill	0.575 ± 0.104	0.487 ± 0.101	0.514 ± 0.086	0.445 ± 0.144	0.517 ± 0.069	0.510 ± 0.134	0.296 ± 0.206	0.470 ± 0.071
	foot	0.056 ± 0.008	ND	0.038 ± 0.023	0.034 ± 0.018	0.067 ± 0.017	0.052 ± 0.023	0.033 ± 0.015	0.060 ± 0.009

55

Table G. Trace element concentrations (µg/g dry wt) in sediment at each study site.

Element	LKW	PSP	UG	LG	UC	LC	UV	LV
Ag	0.019	0.012	0.019	0.025	0.003	0.016	0.009	0.016
As	2.21	2.16	1.86	2.70	0.79	2.11	0.80	1.27
Cd	0.122	0.106	0.119	0.119	0.097	0.080	0.039	0.113
Co	3.25	2.17	2.95	3.08	0.647	2.26	1.13	2.19
Cr	4.51	3.39	5.50	5.82	0.607	4.05	1.56	3.29
Cu	2.47	0.946	4.86	3.65	0.340	2.24	0.828	4.68
Fe	5221	4365	5456	6243	997	4724	1696	3546
Hg	0.036	0.017	0.056	0.017	0.044	0.047	0.057	0.031
Mn	136	137	73.2	143	51.1	124	51.5	58.3
Mo	0.131	0.078	0.221	0.137	0.030	0.101	0.052	0.089
Ni	4.51	2.27	5.31	5.04	0.74	3.62	1.29	3.88
Pb	4.61	3.18	5.68	5.97	0.914	4.54	1.71	4.58
Sb	0.064	0.057	0.078	0.078	0.016	0.063	0.028	0.075
Se	1.30	0.86	1.74	1.27	0.37	1.18	0.59	1.56
Sn	0.175	0.081	0.224	0.279	0.039	0.205	0.058	0.169
V	9.10	7.32	12.1	11.8	2.51	8.16	37.1	9.45
Zn	13.6	10.8	19.2	18.7	1.88	12.0	3.73	12.9

LITERATURE CITED

Adams TG, Atchison GJ, Vetter RJ (1981) The Use of Three-ridge Clam (*Amblema perplicata*) to Monitor Trace Metal Contamination. *Hydrobiologia* 83(1):67-72.

Allen DC, Galbraith HS, Vaughn CC, Spooner DE (2013) A Tale of Two Rivers: Implications of Water Management Practices for Mussel Biodiversity Outcomes During Droughts. *Ambio* 42(7):881-891.

Allen-Gil SM, Gilroy DJ, Curtis LR (1995) An Ecoregion Approach to Mercury Bioaccumulation by Fish in Reservoirs. *Archives of Environmental Contamination and Toxicology* 28(1):61-68.

Aldridge DC, Fayle TM, Jackson N (2007) Freshwater Mussel Abundance Predicts Biodiversity in UK Lowland Rivers. *Aquatic Conservation: Marine and Freshwater Ecosystems* 17(6):554-564.

American Public Health Association (2005) Standard Methods for the Examination of Water and Wastewater. 21st Edn. *American Public Health Association*, Washington, DC.

Anthony JL, Kesler DH, Downing WL, Downing JA (2008) Length-specific Growth Rates in Freshwater Mussels (Bivalvia: Unionidae): Extreme Longevity or Generalized Growth Cessation? *Freshwater Biology* 46(10)1349-1359.

Augspurger T, Keller AE, Black MC, Cope WG, Dwyer FJ (2003) Water Quality Guidance for Protection of Freshwater Mussels (Unionidae) from Ammonia Exposure. *Environmental Toxicology and Chemistry* 22(11):2569-2575.

Boening DW (2000) Ecological Effects, Transport, and Fate of Mercury: A General Review. *Chemosphere* 40:1335-1351.

Burger J, Gochfeld M (2013) Selenium and Mercury Molar Ratios in Commercial Fish from New Jersey and Illinois: Variation within Species and Relevance to Risk Communication. *Food and Chemical Toxicology* 57: 235-245.

Burlakova LE, Karatayev AY, Karatayev VA, May ME, Bennet DL, Cook MJ (2011) Biogeography and Conservation of Freshwater Mussels (Bivalvia: Unionidae) in Texas: Patterns of Diversity and Threats. *Diversity and Distributions*, 17(3):393-407.

Camargo JA, Alonso A, Salamanca A (2005) Nitrate Toxicity to Aquatic Animals: A Review with New Data for Freshwater Invertebrates. *Chemosphere* 58:1255–1267.

Chandurvelan R, Marsden ID, Glover CN, Gaw S (2015) Assessment of a Mussel as a Metal Bioindicator of Coastal Contamination: Relationships between Metal Bioaccumulation and Multiple Biomarker Responses. *Science of the Total Environment* 511:663-675.

Chowdhury GW, Zieritz A, Aldridge DC (2016) Ecosystem Engineering by Mussels Supports Biodiversity and Water Clarity in a Heavily Polluted Lake in Dhaka, Bangladesh. *Freshwater Science* 35(1):188-199.

Clearwater SJ, Thompson KJ (2014) Acute Toxicity of Copper, Zinc, and Ammonia to Larvae (Glochidia) of a Native Freshwater Mussel *Echyridella menziesii* in New Zealand. *Archives of Environmental Contamination and Toxicology* 66:213-226.

Clowes LA, Francesconi KA (2004) Uptake and Elimination of Arsenobetaine by the Mussel Mytilus edulis is Related to Salinity. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* 137(1): 35-42.

Couillard Y, Campbell PGC, Tessier A, Pellerin-Massicotte J, Auclair JC (1995a) Field Transplantation of a Freshwater Bivalve, *Pyganodon grandis*, Across a Metal Contamination Gradient. I. Temporal Changes in Metallothionein and Metal (Cd, Cu, and Zn) Concentrations in Soft Tissues. *Canadian Journal of Fisheries and Aquatic Sciences* 52:690-702.

Couillard Y, Campbell PGC, Pellerin-Massicotte J, Auclair JC (1995b) Field Transplantation of a Freshwater Bivalve, *Pyganodon grandis*, Across a Metal Contamination Gradient. II. Metallothionein Response to Cd and Zn Exposure, Evidence for Cytotoxicity, and Links to Effects at Higher Levels of Biological Organization. *Canadian Journal of Fisheries and Aquatic Sciences* 52:703-715.

Cordeiro J, Bogan A (2012) *Amblema plicata*. The IUCN Red List of Threatened Species 2012:e.T203724A277056. http://dx.doi.org/10.2305/IUCN.UK.2012-1.RLTS.T203724A2770567.en.

Crosby MP and Gale LD (1990) A Review and Evaluation of Bivalve Condition Index Methodologies with a Suggested Standard Method. *Journal of Shellfish Research* 9:233-237.

da Silva Oliveira A, Bocio A, Beltramini Trevilato TM, Magosso Takayanagui AM, Domingo JL, Segura-Muńoz SI (2007) Heavy Metals in Untreated/Treated Urban Effluent and Sludge from a Biological Wastewater Treatment Plant. *Environmental Science and Pollution Research* 14(7):483-489.

Davis AP, Shokouhian M, Ni Shubei (2001) Loading Estimates of Lead, Copper, Cadmium, and Zinc in Urban Runoff from Specific Sources. *Chemosphere* 44(5):997-1009.

Depledge MH, Rainbow PS (1990) Models of Regulation and Accumulation of Trace Metals in Marine Invertebrates. *Comparative Biochemistry and Physiology* 97C(1):1-7.

Desenfant F, Petrovský E, Rochette P (2004). Magnetic Signature of industrial Pollution of Stream Sediments and Correlation with Heavy Metals: Case Study from South France. *Water, Air, and Soil Pollution* 152:297-312.

Donahue WF, Allen EW, Schindler DW (2006). Impacts of Coal-fired Power Plants on Trace Metals and Polycyclic Aromatic Hydrocarbons (PAHs) in Lake Sediment in Central Alberta, Canada. *Journal of Paleolimnology* 35:111-128.

Drenner RW, Chumchai MM, Jones CM, Lehmann CM, Gay DA, Donato DI (2013) Effects of Mercury Deposition and Coniferous Forests on the Mercury Contamination of Fish in the South Central United States. *Environmental Science and Technology* 47(3):1274-1279.

Du B, Haddad SP, Luek A, Cassan Scott W, Saari GN, Kristofco LA, Connors KA, Rash C, Rasmussen JB, Chambliss CK, Brooks BW (2014) Bioaccumulation and Trophic Dilution of Human Pharmaceuticals Across Trophic Positions of an Effluent-Dependent Wadeable Stream. *Philosophical Transactions of the Royal Society B* 369:20140058.

Dutton J, Fisher NS (2012) Influence of Humic Acid on the Uptake of Aqueous Metals by the Killifish *Fundulus heteroclitus*. *Environmental Toxicology and Chemistry* 31(10):2225-2232.

Elliot EG, Ettinger AS, Leaderer BP, Bracken MB, Deziel NC (2016) A Systematic Evaluation of Chemicals in Hydraulic-Fracturing Fluids and Wastewater for Reproductive and Developmental Toxicity. *Journal of Exposure Science and Environmental Epidemiology* 27:90-99.

Environmental Protection Agency (EPA) (2013) Final Aquatic Life Ambient Water Quality Criteria for Ammonia- Freshwater 2013. *Environmental Protection Agency Federal Register* 78(163): 52192-52194.

Falfushynska HI, Gnatyshyna LL, Gyori J, Stoliar OB (2014). Metallothioneins, Caspase-3 and Oxidative Stress Response in the Multi-marker Study of Freshwater Mussel Inhabiting Sites of Various Human Impact. *Turkish Journal of Fisheries and Aquatic Sciences* 14:591-603.

Farcy E, Gagné F, Martel L, Fortier M, Trépanier S, Brousseau P, Fournier M (2011) Short-term Physiological Effects of a Xenobiotic Mixture on the Freshwater Mussel *Elliptio complanata* Exposed to Municipal Effluents. *Environmental Research* 111:1096-1106.

Fontenot BE, Hunt LR, Hildenbrand ZL, Carlton DD Jr., Oka H, Walton JL, Hopkins D, Osorio A, Bjorndal B, Hu QH, Schug KA (2013) An Evaluation of Water Quality in Private Drinking Water Wells Near Natural Gas Extraction Sites in the Barnett Shale Formation. *Environmental Science and Technology* 47(17):10032-10040.

Freshwater Mollusk Conservation Society (2016) A National Strategy for the Conservation of Native Freshwater Mollusks. *Freshwater Mollusk Biology and Conservation* 19:1–21.

Fritts AK, Barnhart MC, Bradley M, Liu N, Cope WG, Hammer E, Bringolf RB (2013) Assessment of Toxicity Test endpoints for Freshwater Mussel Larvae (Glochidia). *Environmental Toxicology and Chemistry* 33(1):199-207.

Gagné F, Blaise C, André C (2007). Time Course Study on the Manifestation of lethal effects in *Elliptio complanata* Mussels Exposed to Aeration Lagoons: A Biomarker Study. *Environmental Research* 1:65-92.

Gagnon and Fisher (1997) Bioavailability of Sediment-Bound Methyl and Inorganic Mercury to a Marine Bivalve. *Environmental Science and Technology* 31(4):993-998.

Garelick H, Jones H, Dybowska A, Valsami-Jones E (2008) Arsenic Pollution Sources *Reviews of Environmental Contamination* 197:17-60.

Gascho Landis AM, Haag WR, Stoeckel JA (2013) High Suspended Solids as a factor in Reproductive Failure of a Freshwater Mussel. *Freshwater Science* 32(1):70-81.

Gillis PL, Mitchell RJ, Schwalb AN, McNichols KA, Mackie GL, Wood CM, Ackerman JD (2008) Sensitivity of the Glochidia (Larvae) of Freshwater Mussels to Copper: Assessing the Effect of Water Hardness and Dissolved Organic Carbon on the Sensitivity of Endangered Species. *Aquatic Toxicology* 88:137-145.

Gillis PL (2012) Cumulative Impacts of Urban Runoff and Municipal Wastewater Effluents on Wild Freshwater Mussels (*Lasmigona costata*). *Science of the Total Environment*, 431:348-356.

Gillis PL, Gagne F, McInnis R, Hooey TM, Choy ES, Andre C, Hoque E, Metcalfe CD (2014a) The Impact of Municipal Wastewater Effluent on Field-deployed Freshwater Mussels in the Grand River (Ontario, Canada). *Environmental Toxicology and Chemistry* 33(1):134-143.

Gillis PL, Higgins SK, Jorge MB (2014b) Evidence of Oxidative Stress in Wild Freshwater Mussels (*Lasmigona costata*) Exposed to Urban-derived Contaminants. *Ecotoxicology and Environmental Safety* 102:62-69.

Giuffré de López Camelo L, Ratto de Miguez S, Marbán L (1997) Heavy Metals Input with Phosphate Fertilizers used in Argentina. *Science of the Total Environment* 204:245-250.

Glass DC, Gray CN (2001) Estimating Mean Exposures from Censored Data: Exposure to Benzene in the Australian Petroleum Industry. *The Annals of Occupational Hygiene* 45(4):275-282.

Gong G, Mattevada S, O'Bryant SE (2014) Comparison of the Accuracy of Kriging and IDW Interpolations in Estimating Groundwater Arsenic Concentrations in Texas. *Environmental Research* 130:59-69.

Grapentine L, Rochfort Q, Marsalek J (2008) Assessing Urban Stormwater Toxicity: Methodology Evolution from Point Observations to Longitudinal Profiling. *Water Science & Technology* 57:1375-1381.

Guadalupe Blanco River Authority (GBRA) (2018) 2018 Clean Rivers Program Basin Summary Report. *Guadalupe Blanco River Authority* Seguin, Texas pp. 1-132.

Haag WR. 2012. North American Freshwater Mussels: Natural History, Ecology, and Conservation, 1st edition. *Cambridge University Press*, Cambridge, MA pp. 538.

Hecky RE, Bodaly RA, Ramsey DJ, Strange NE (1987) Enhancement of Mercury Bioaccumulation in Fish by Flooded Terrestrial Materials in Experimental Ecosystems. A Summary Report for the Canada-Manitoba Agreement on the Study and Monitoring of Mercury in the Churchill River Diversion: Project 2.1 Experimental Ecosystems. *Department of Fisheries and Oceans*, Canada.

Hopkins WA, DuRant SE, Staub BP, Rowe CL, Jackson BP (2006). Reproduction, Embryonic Development, and Maternal Transfer of Contaminants in the Amphibian *Gastrophyrne carolinensis*. *Environmental Health Perspectives* 114:661-666.

Horowitz AJ, Elrick KA (1987) The Relation of Stream Sediment Surface Area, Grain Size, and Composition to Trace Element Chemistry. *Applied Geochemistry* 2:437-451.

Howard JK, Cuffey KM (2006) The Functional Role of Native Freshwater Mussels in the Fluvial Benthic Environment. *Freshwater Biology* 51(3):460-474.

Howarth RW, Sharpley A, Walker D (2002) Sources of Nutrient Pollution to Coastal Waters in the United States: Implications for Achieving Coastal Water Quality Goals. *Estuaries* 25(4)656-676.

IUCN 2017. *The IUCN Red List of Threatened Species. Version 2017-3*. <http://www.iucnredlist.org>. Downloaded on 21 June 2018.

Ismail NS, Tommerdahl JP, Boehm AB, Luthy RG (2016) Escherichia coli Reduction by Bivalves in an Impaired River Impacted by Agricultural Land Use. *Environmental Science & Technology* 50(20):11025-11033.

Jaruga P, Coskun E, Kimbrough K, Jacob A, Johnson WE, Dizdaroglu M (2017) Biomarkers of Oxidatively Induced DNA Damage in Dreissenid Mussels: A Genotoxicity Assessment Tool for the Laurentian Great Lakes. *Environmental Toxicology* 32(9): 2144-2153.

Jiann KT, Santschi PH, Presley BJ (2013) Relationships Between Geochemical Parameters (pH, DOC, SPM, EDTA Concentrations) and Trace Metal (Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn) Concentrations in River Waters of Texas (USA). *Aquatic Geochemistry* 19:173-193.

Karatayev AY, Burlakova LE, Padilla DK (2015) Zebra Versus Quagga Mussels: A Review of Their Spread, Population Dynamics, and Ecosystem Impacts. *Hydrobiologia* 746(1):97-112.

Kim BH, Lee JH, Hwang SJ (2011) Inter- and Intra-specific Differences in Filtering Activities Between Two Unionids, *Anodonta woodiana* and *Unio douglasiae*, in Ambient Eutrophic Lake Waters. *Ecological Engineering* 37(12):1957-1967.

Kouji A, Tainosho Y (2004) Characterization of Heavy Metal Particles Embedded in Tire Dust. *Environment International* 30(8):1009-1017.

Lawler JJ, White D, Sifneos JC, Master LL (2003) Rare Species and the Use of Indicator Groups for Conservation Planning. *Conservation Biology* 17(3):875-882.

Loayza-Muro R, Elías-Letts R (2007) Responses of the Mussel *Anodontites trapesialis* (Unionidae) to Environmental Stressors: Effects of pH, Temperature, and Metals on Filtration Rate. *Environmental Pollution* 149(2):209-215.

Lough GC, Schauer JJ, Park JS, Shafer MM, Deminter JT, Weinstein JP (2005) Emissions of Metals Associated with Motor Vehicle Roadways. *Environmental Science & Technology* 39(3):826-836.

Mabe JA, Kennedy J (2014) Habitat Conditions Associated with a Reproducing Population of the Critically Endangered Freshwater Mussel *Quadrula mitchelli* in Central Texas. *The Southwestern Naturalist* 59(2):297-300.

Machado AA, Wood CM, Bianchini A, Gillis (2014) Response of Biomarkers in Wild Freshwater Mussels Chronically Exposed to Complex Contaminant Mixtures. *Ecotoxicology*, 23:1345-1358.

Mailman M, Stepnuk L, Cicek N, Bodaly RA (2006) Strategies to Lower Methyl Mercury Concentrations in Hydroelectric Reservoirs and Lakes: A Review. *Science of the Total Environment*. 368(1): 224-235.

Malley D, Stewart AR, Hall BD (1995) Uptake of Methyl Mercury by the Floater Mussel, *Pyganodon grandis* (Bivalvia, Unionidae), Caged in a Flooded Wetland. *Environmental Toxicology and Chemistry* 15(6):928-936.

Malm O (1998) Gold Mining as a Source of Mercury Exposure in the Brazilian Amazon. *Environmental Research* 77:73-78.

Markich SJ, Jeffree RA (1994) Absorption of Divalent Trace Metals as Analogues of Calcium by Australian Freshwater Bivalves: An Explanation of How Water Hardness Reduces Metal Toxicity. *Aquatic Toxicology* 29(3-4):257-290.

Metcalfe JL, Charlton MN (1990) Freshwater Mussels as Biomonitors for Organic Industrial Contaminants and Pesticides in the St. Lawrence River. *Science of the Total Environment* 97/98: 595-615.

Musgrove M, Opsahl SP, Mahler BJ, Herrington C, Sample TL, Banta JR (2016) Source, Variability, and Transformation of Nitrate in a Regional Karst Aquifer: Edwards Aquifer, Central Texas. *Science of the Total Environment* 568:457-469.

Naimo TJ (1995) A Review of the Effects of Heavy Metals on Freshwater Mussels. *Ecotoxicology* 4(6):341-362.

Ng, JC (2005) Environmental Contamination of Arsenic and its Toxicological Impact on Humans. *Environmental Chemistry* 2(3):146-160.

Nobles T, Zhang Y (2015) Survival, Growth, and Condition of Freshwater Mussels: Effects of Municipal Wastewater Effluent. *PLoS ONE* 10(6): e0128488.

Ogunbileje JO, Sadagoparamanujam VM, Anetor JI, Farombi EO, Akinosun OM, Okorodudu AO (2013) Lead, Mercury, Cadmium, Chromium (VI) Levels in Nigeria and United States of America Cement Dust. *Chemosphere* 90:2743-2749.

Pan JF, Wang WX (2004) Uptake of Hg(II) and Methylmercury by the Green Mussel Perna viridis Under Different Organic Carbon Conditions. *Marine Ecology Progress Series* 276:215-136.

Pimentel D, Berger B, Filiberto D, Newton M, Wolfe B, Karabinakis E, Clark S, Poon E, Abbett E, Nandagopal S (2004) Water Resources: Agricultural and Environmental Issues. *BioScience* 54 (10): 909-91.

Ralston NV, Blackwell JL III, Raymond LJ (2007) Importance of Molar Ratios in Selenium-dependent Protection Against Methylmercury Toxicity. *Biological Trace Element Research* 119(3):255-265.

Ralston NVC, Ralston CR, Blackwell III JL, Raymond LJ (2008) Dietary and tissue selenium in relation to methylmercury toxicity. *Neurotoxicology* 29:802–11.

Rahman MA, Hasegaw H, Lim RP (2012) Bioaccumulation, Biotransformation, and Trophic Transfer of Arsenic in the Aquatic Food Chain. *Environmental Research* 116:118-135.

Randklev CR, Johnson MS, Tsakiris ET, Rogers-Oetker S, Roe KJ, Harris JL, McMurray SE, Robertson C, Groce J, Wilkins N (2012) False Spike, *Quadrula mitchelli* (Bivalvia: Unionidae), is Not Extinct: First Account of a Live Population in Over 30 Years. *American Malacological Bulletin* 30(2):327-328.

Ravera O, Cenci R, Beone GM, Dantas M, Lodigiani P (2003) Trace Element Concentrations in Freshwater Mussels and Macrophytes as Related to Those in Their Environment. *Journal of Limnology* 62(1):61-70.

Régnier C, Fontaine B, Bouchet P (2009) Not Knowing, Not Recording, Not Listing: Numerous Unnoticed Mollusk Extinctions. *Conservation Biology, 23*(5):1214-1221

Ricciardi A, Rasmussen JB (1999) Extinction Rates of North American Freshwater Fauna. *Conservation Biology* 13(5):1220-1222.

Roditi HA, Fisher NS (1999) Rates and Routes of Trace Element Uptake in Zebra Mussels. *Limnology and Oceanography* 44(7):1730-1749.

Roditi HA, Fisher NS, Sanudo-Wilhelmy SA (2000) Field Testing a Metal Bioaccumulation Model for Zebra Mussels. *Environmental Science in Technology* 34(13):2817-2825.

Rueda F, Moreno-Ostos E, Armengo J (2006) The Residence Time of River Water in Reservoirs. *Ecological Modelling* 191:260-274.

Shi D, Wang WX (2005) Uptake of Aqueous and Dietary Metals by Mussel Perna viridis with Different Cd Exposure Histories. *Environmental Science and Technology* 39(23):9363-9369.

Singh N, Turner A (2009) Trace Metals in Antifouling Paint Particles and Their Heterogeneous Contamination of Coastal Sediments. *Marine Pollution Bulletin* 58:559-564.

Sørensen PA, Kiil S, Dam-Johansen K, Weinell CE (2009). Anticorrosive coatings: A Review. *Journal of Coatings Technology and Research* 6(2):135-176.

Spooner DE, Vaughn CC (2006) Context-dependent Effects of Freshwater Mussels on Stream Benthic Communities. *Freshwater Biology* 51:1016-1026.

Strayer, D (2008) Freshwater Mussel Ecology: A Multifactor Approach to Distribution and Abundance. *University of California Press*. Berkeley, CA pp. 1-216.

Strayer DL, Malcom HM (2012) Causes of Recruitment Failure in Freshwater Mussel Populations in Southeastern New York. *Ecological Applications* 22(6):1780-1790.

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012) Heavy Metals Toxicity and the Environment. *Molecular, Clinical and Environmental Toxicology* 101:133-164.

Texas Commission on Environmental Quality (TCEQ) (2008) 2008 Texas 303(d) List. *Texas Commission on Environmental Quality*. Austin, Texas. https://www.tceq.texas.gov/assets/public/waterquality/swqm/assess/08twqi/2008_303d.pdf

Texas Commission on Environmental Quality (TCEQ) (2018) Chapter 307- Texas Surface Water Quality Standards. *Texas Commission on Environmental Quality*. Austin, Texas. https://www.tceq.texas.gov/assets/public/waterquality/standards/tswqs2018/2018swqs_allsections_nopreamble.pdf

Texas Department of State Health Services (DSHS) (2006) Characterization of Potential Adverse Health Effects from Consuming Fish from Canyon Reservoir. *Policy, Standards, and Quality Assurance Unit Seafood and Aquatic Life Group and Division for Regulatory Services and Environmental and Injury Epidemiology and Toxicology Branch*, Austin, Texas.

Tran D, Moody J, Fisher AS, Foulkes ME, Jha AN (2007) Protective Effects of Selenium on Mercury-Induced DNA Damage in Mussel Hemocytes. *Aquatic Toxicology* 84(1):11-18.

Tyrrell M, Hornbach DJ (1998) Selective Predation by Muskrats on Freshwater Mussels in 2 Minnesota Rivers. *Freshwater Science* 17(3):301-310.

United States Census Bureau (2018) Annual Estimates of the Resident Population in Texas: April 1, 2010 to July 1, 2017. *U.S. Census Bureau, Population Division, Room 2313-3*. Washington, DC.

United States Environmental Protection Agency (1972) Clean Water Act Section 502(14). *U.S. Environmental Protection Agency (EPA)*. Washington, D.C.

United States Environmental Protection Agency (2012) Study of the Potential Impacts of Hydraulic Fracturing on Drinking Water Resources: Progress Report. EPA/601/ R-12/011. Washington, DC. http://www2.epa.gov/hfstudy/study-potentialimpacts-hydraulic-fracturing-drinking-water-resources-progress-report-0.

United States Environmental Protection Agency (2013) Aquatic Life Ambient Water Quality Criteria for Ammonia- Freshwater. *Office of Science and Technology*. Washington, DC.

United States Environmental Protection Agency (2014) Appendix A to Part 423- 126 Priority Pollutants. *U.S. Environmental Protection Agency (EPA)*. Washington, D.C. https://www.epa.gov/eg/toxic-and-priority-pollutants-under-clean-water-act#priority.

United States Fish and Wildlife Service (USFWS) (2018) 50 CFR Part 17 Endangered and Threatened Wildlife and Plants; Endangered Species Status for Texas Hornshell. *Federal Register* 83(28):5720-5735.

United State Geological Survey (2018) Water Dashboard United States Department of the Interior, Washington, DC. https://txpub.usgs.gov/txwaterdashboard/index.html

Vaughn CC, Taylor CM (1999) Impoundments and the Decline of Freshwater Mussels: A Case Study of an Extinction Gradient. *Conservation Biology* 13(4):912-920.

Vaughn CC, Hakencamp CC (2001) The Functional Role of Burrowing Bivalves in Freshwater Ecosystems. *Freshwater Biology* 46(11):1431-1446.

Vaughn CC, Nichols SJ, Spooner DE (2008) Community and Foodweb Ecology of Freshwater Mussels. *Journal of the North American Benthological Society* 27(2):409-423.

Viarengo A, Burlando B, Cavaletto M, Marchi B, Ponzano E, Blasco J (1999) Role of Metallothionein Against Oxidative Stress in the Mussel *Mytilus galloprovincialis. American Journal of Physiology* 277(6):1612-1619.

Viarengo A, Burlando B, Dondero F, Marro A, Fabbri R (2008). Metallothionein as a Tool in Biomonitoring Programmes. *Biomarkers* 4(6):455-466.

Wade TL, Sericano JL, Gardinali PR, Wolff G, Chambers L (1998) NOAA's 'Mussel Watch' Project: Current Use Organic Compounds in Bivalves. *Marine Pollution Bulletin* 37(1-2):20-26.

Wang N, Ingersoll CG, Ivey CD, Hardesty DK, May TW, Augspurger T, Roberts AD, van Genderen E, Barnhart MC (2010) Sensitivity of Early Life Stages of Freshwater Mussels (Unionidae) to Acute and Chronic Toxicity of Lead, Cadmium, and Zinc in Water. *Environmental Toxicology and Chemistry* 29(9):2053-2063.

Wang WX, Fisher NS, Luoma SN (1996) Kinetic Determinations of Trace Element Bioaccumulation in the Mussel *Mytilus edulis. Marine Ecology Progress Series* 140:91-113.

Wang WX, Fisher NS (1996) Assimilation of Trace Elements and Carbon by the Mussel *Mytilus edulis*: Effects of Food Composition. *Limnology and Oceanography* 41(2): 197-207.

Wang WX, Fisher NS (1997) Modeling Metal Bioavailability of Marine Mussels. *Reviews of Environmental Contamination and Toxicology* 151:39-65.

White SL, Rainbow PS (1985) On the Metabolic Requirements for Copper and Zinc in Molluscs and Crustaceans. *Marine Environmental Research* 16:215-229.

Williams JD, Warren ML, Cummings KS, Harris JL, Neves RJ (1993) Conservation Status of Freshwater Mussels of the United States and Canada. *Fisheries* 18(9):6-22.

Winemiller K, Lujan N, Wilkins RN, Snelgrove RT, Dube AM, Skow KL, Snelgrove AG (2010). Status of freshwater mussels in Texas. *Texas A&M Department of Wildlife and Fisheries Sciences and Texas A&M Institute of Renewable Natural Resources*. College Station, Texas pp 1-62.

Zhang W, Wang WX (2018) Arsenic Biokinetics and Bioavailability in Deposit-Feeding Clams and Polychaetes. *Science of the Total Environment* 616:594-601.

Zuykov M, Pelletier E, Harpe DAT (2013) Bivalve Mollusks in Metal Pollution Studies: From Bioaccumulation to Biomonitoring. *Chemosphere* 93(2):201-208.