MONITORING CHANGES IN TRACE ELEMENT CONCENTRATIONS IN AMBLEMA PLICATA IN THE GUADALUPE RIVER BASIN (TEXAS, USA) USING A CAGED TRANSPLANT EXPERIMENT

by

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ABSTRACT

Exposure to contaminants, including trace elements, can be one of the reasons for unionid mussel population declines throughout North America. Texas has over 50 species of native mussels, including 16 species that are state threatened; however, little is known about the impact of contaminants on Texas mussels. This study investigated the accumulation of seven essential (Co, Cu, Fe, Mn, Ni, and Zn) and eight non-essential (Ag, As, Bi, Cd, Cr, Hg, Pb, and U) trace elements in gill tissue of Threeridge (*Amblema plicata*) at six sites in the Guadalupe River Basin, Texas, using a caged transplant experiment. Mussels were collected from a reference site (Lake Wood) and transplanted to five different sites. After 3 and 12 weeks, mussels were collected and the concentration of trace elements in gill tissue was determined using microwave acid digestion and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis. The concentration of trace elements in gill tissue and calculated Biota Sediment Accumulation Factors (BSAF) were compared within and among sites and between week 0, 3 and 12. Overall, mean gill tissue concentrations for essential trace elements were greater than non-essential trace elements. For all sites and time points combined, mean gill concentrations (µg/g dry weight) were greatest for Mn (4641), Fe (838), Zn (433), and As (8.81), and lowest for Cd (0.248), U (0.107), Hg (0.075), and Ag (0.051). Bismuth (Bi) was the only element that was below the detection limit (0.0280 µg/g) at all sites and time points. Changes in trace element concentrations varied by element and location, and there was no clear accumulation patterns over time. This could be due to heavy rain events that occurred

during the experiment which resulted in either trace elements being added to the river due to urban and agricultural runoff or the dilution of trace elements at a given site. The mean Se:Hg molar ratio in gill tissue was > 1:1 at each site, indicating that Se may have a protective effect against Hg toxicity in freshwater mussels. *A. plicata* at each site had a mean BSAF > 1 for all trace elements except Co, Cr, Fe, Ni, Pb and U indicating that mussels are generally more enriched in trace elements than sediment at each location. Previous caged transplant experiments have been successful in identifying accumulation patterns in mussels placed in smaller riverine systems; however, the findings of this study identified the difficulties associated with conducting the same experiment in a large river system with a flashy regime.

I. INTRODUCTION

Importance of freshwater mussels

Unionid mussels play a critical role in maintaining healthy freshwater ecosystems because they perform a variety of important functions (Vaughn & Hakenkamp 2001; Howard & Cuffey 2006; Vaughn et al. 2008; Chowdhury et al. 2016; Vaughn 2018). In lakes and rivers, mussels purify water through biofiltration, aide in nutrient cycling, provide and improve habitat for local biota, and serve as an important food source for birds [e.g., hooded crow (*Corvus cornix*) and tufted duck (*Aythya fuligula*)] and mammals [e.g., muskrats (*Ondatra zibethicus*) and river otters (*Lontra canadensis*)] (Stanczykowska et al. 1990; Wekler & Waltz 1998; Vaughn & Hakenkamp 2001; Gutierrez et al. 2003; Howard & Cuffey 2006; Zimmerman & De-Szalay 2007; Strayer 2014; Chowdhury et al. 2016; Goldyn et al. 2016; Scordino et al. 2016; Mistry & Ackerman 2018). Mussels filter particles such as phytoplankton, bacteria, and pathogens from water, and at high densities alter freshwater environments by improving water clarity (Welker & Walz 1998; Ismail et al. 2016; Mistry & Ackerman 2018). Additionally, freshwater mussels stabilize the riverbed and their shells provide habitat for benthic algae and macroinvertebrates such as mayflies and caddisflies (Stewart & Haynes 1994; Horvath et al. 1999; Howard & Cuffey 2006; Zimmerman & Szalay 2007). Lastly, mussels serve as important biomonitoring species because they bioaccumulate contaminants such as trace elements, pesticides, polycyclic aromatic hydrocarbons (PAHs), and pharmaceuticals, providing an assessment of water quality over time that intermittent water sampling cannot duplicate. (Blaise et al. 2002; Damasio et al. 2010;

Gillis 2012; Gillis et al 2014a; Olveira et al. 2016; Archambalt et al. 2018).

Status of freshwater mussels and threats to their survival

Globally, unionid mussels are one of the most imperiled groups of organisms (Lopes-Lima et al. 2014). There are approximately 298 species endemic to North America with 74% of those species in significant decline (Williams et al. 2017). The International Union for Conservation of Nature (IUCN) states that 46% of the species in the superfamily Unionidae are critically endangered or endangered (IUCN 2017). Ricciardi & Ramussen (1999) predicted that 6.4% of freshwater mussel species will go extinct each decade and that 50% of the remaining species will go extinct by the end of the 21st century if the quality of their aquatic environment continues to decline. Current research shows that extinction rates will be far greater than predicted because not all habitats have been evaluated and data on some mussel populations has not been collected (Strayer 2008; Régnier et al. 2009). Therefore, it is important to note that the data submitted to the IUCN is severely lacking in comparison to vertebrate species and that more research on freshwater mussels is critical in developing conservation and recovery plans.

Threats to freshwater mussels include natural system modification through dam impoundments and hydrologic alteration, aquatic pollution, introduction of invasive species, and urban development (Richter et al. 1997; Lydeard et al. 2004). These threats can result in deleterious health effects in mussels such as lower reproductive success rates, slower growth rates, and are the primary cause of mussel deaths across North America (Vaughn & Taylor 1999; Gascho-Landis et al. 2013). Of these, human-derived

pollution can be one of the main causes of mussel mortality (Conners & Black 2004; Bringolf et al. 2007; Wang et al. 2007).

Impacts of contaminants on freshwater mussels

Aquatic pollution incorporates a wide array of contaminants which include, but are not limited to, trace elements, organic contaminants [e.g., polychlorinated biphenyls (PCBs)], nutrients, pesticides, suspended solids, pharmaceuticals, and personal care products (Williams et al. 1993; Burlakova et al. 2011; Gillis 2012, 2014a; Jiann et al. 2013; Gascho-Landis et al. 2013; Rasmussen et al. 2015). Aquatic pollution predominantly enters rivers through urban and agricultural runoff, atmospheric deposition, groundwater infiltration and direct inputs from point sources. Point source pollutants can be traced back to an identifiable source that emits the pollutants into the environment. The source of these pollutants includes, but are not limited to, coal-fired power plants, mining operations, industry (e.g., chemical, electronic, and automobile manufacturers), oil refineries, and wastewater treatment plants (Nance 1991; Menounou & Presley 2003; Gray et al. 2015). Non-point source pollution originates from numerous sources over a widespread area including agricultural runoff, urban runoff, and atmospheric deposition. Both point and non-point source pollution has increased because of human population growth and have resulted in more stressful conditions for freshwater mussels.

Exposure to contaminants can lead to negative health impacts on mussels such as reduction in growth, decrease in enzyme efficiency, decrease in filtration rate, interference with ion regulation, decreased recruitment, behavioral changes, and

increased mortality (Kraak et al. 1994; Naimo 1995; Strayer & Malcom 2012; Nogueira et al. 2013; Jorge et al. 2018). For example, Gascho-Landis et al. (2013) found that an increase in total suspended solids (TSS) resulted in decreased reproduction and reduced growth rates in freshwater mussels. Additionally, chronic, and acute exposure to trace elements such as lead (Pb), cadmium (Cd), zinc (Zn), and aluminum (Al) have been proven to alter growth, filtration rates, and enzyme activity (Naimo 1995; Wang et al. 2010; Wang et al. 2018). Among the aquatic pollutants, ammonia (NH_3) is one of the most detrimental contaminants to freshwater mussel survival and growth rates and is especially toxic to mussels during their early life stages. For example, laboratory studies have shown the sensitivity of mussels to ammonia (e.g., Augspurger et al. 2003; Mummert et al. 2003; Newton 2003; Newton & Bartsch 2007; Wang et al. 2007, 2008) and as a result The U.S. Environmental Protection Agency (EPA) criteria maximum concentrations (CMC) for NH_3 have been lowered (EPA 2013).

Biomarkers and their response to contaminant exposure

Biomarkers are used to determine the physiological impact of contaminants on freshwater mussels. Gill tissue is ideal for biomarker analysis because it is in constant contact with contaminants in the aquatic environment (Wang & Fisher 1996; Wang et al. 1996). Biomarkers that have been proven successful in toxicological studies focused on oxidative stress (lipid peroxidation and antioxidant capacity against peroxyl radicals), trace element exposure (metallothioneins), and general health (total protein and total lipid). A multi-biomarker approach is required to best reflect the physiological response of mussels to a suite of environmental contaminants (Gillis et al. 2014a). Including a suite

of biomarkers in a research study increases the likelihood of determining whether contaminants are affecting an organism in a negative way and if the biomarker is contaminant-specific (e.g., metallothioneins).

Metallothioneins provide information on the physiological response of the mussels to trace element exposure. They are involved in the homeostasis of essential trace elements (those which have a biological function) within the body [e.g., copper (Cu) and zinc (Zn)] and detoxification of non-essential trace elements [e.g., mercury (Hg) and Cd] (Giguere et al. 2003; Geret et al. 2003; Bebianno et al. 2005). An increase in metallothionein proteins can indicate that an organism has been exposed to increased concentrations of trace elements (Blaise et al. 2002; Falfushynska et al. 2014; Olveira et al. 2016). For example, Gillis et al. (2014a) found that metallothionein concentrations increased in the gill tissue of mussels downstream of a wastewater treatment plant in response to exposure to a higher concentrations of silver (Ag), chromium (Cr), Pb, and Zn.

Oxidative stress occurs in mussels when antioxidant defense mechanisms can no longer keep up with the production of reactive oxygen species (ROS) or if there is a decrease in the capacity of antioxidant defenses (Gillis et al. 2014a). Lipid peroxidation occurs when lipids undergo oxidative stress and results in damage to cell membranes. The measurement of lipid peroxidation can indicate the amount of oxidative stress occurring in mussels. Conversely, the measurement of antioxidant capacity against peroxyl radicals (ACAP) assesses the ability for mussels to respond to oxidative stress; a decrease in ACAP can indicate higher susceptibility to oxidative stress. Previous research has found an increase in lipid peroxidation in freshwater mussels exposed to trace

elements [e.g., Pb and Cd] and organic contaminants (e.g., PCBs) (Cossu et al. 2000; Giguere et al. 2003; Gillis et al. 2014a). Gillis et al. (2014a) found that mussels collected from polluted sites had lower ACAP concentrations and that ACAP concentrations were significantly correlated with Cu, Pb, Ag, Zn, Cr, and bismuth (Bi) concentrations in gill tissue.

Total protein and total lipid biomarkers are used to indicate the general health of mussels because they are required for proper physiological function (Basen et al. 2011; Gillis et al. 2014a). A decrease in the concentration of these macromolecules can indicate reduced protein synthesis and lipid synthesis resulting in alterations to metabolic activity and poor health (Geret et al. 2003; Pytharopoulou et al. 2006). For example, Ali et al. (2019) found a significant decrease in total protein concentrations in mussels exposed to pesticides and Bergen et al. (2001) found higher total lipid concentrations at sites with higher contaminants such as PCB's.

Field study versus caged transplant experimental techniques

To understand the impact of environmental contaminants on freshwater mussels, data can be collected using field study methods or caged transplant methods. Field studies are used when mussels are collected directly from polluted and non-polluted sites and tissues undergo contaminant (e.g., trace elements) and biomarker analyses; however, this just provides tissue concentrations at the time of sample collection (e.g., Blaise et al. 2002; Gillis 2012; Falfushynska et al. 2014; Gillis et al. 2014a; Britton 2018). A caged transplant experiment is used when mussels are collected from a reference site and transplanted in cages to presumably polluted sites, where the uptake of contaminants can

be monitored over time. Caged transplant studies have been successfully used to investigate the change in contaminant concentrations, particularly trace elements, in freshwater mussels for a known period of time and the resulting physiological response over the same time period using biomarkers. (Blaise et al. 2003; Damasio et al. 2010; Gillis et al. 2014b; Maranho et al. 2015; Olveira et al. 2016; Taylor et al. 2017). Caged transplant experiments give a better understanding of how fast mussels respond to changes in environmental contaminant concentrations and allow mussels to be transferred to areas of high contaminant loading (e.g., nutrients and trace elements) where they normally would not be present. Both approaches are crucial to understand the effects of contaminants on freshwater mussels and provide important information that can assist in the development of conservation and recovery plans for freshwater mussels and other aquatic organisms.

Impact of contaminants on freshwater mussels in Texas

Texas is home to over 50 species of native freshwater mussels. Currently, 16 of these species are listed as threatened or endangered at the state level (TPWD 2020), three of those species are candidates, with seven additional species being petitioned for federal protection under the Endangered Species Act (ESA) and one of the species [Texas hornshell (*Popenaias popeii*)] is federally listed as endangered under the ESA (USFWS 2018). These species are distributed across 13 major river systems which are hydrologically diverse. Central and West Texas river systems experience a flashy hydrologic regime where large flood events and long drought events can affect mussel survival rates (Haag et al. 2008; Newton et al. 2015; Vaughn et al. 2015, Mitchell et al.

2018, Dascher et. al. 2018, Mitchell et al. 2019). Anthropogenic activities such as urbanization and impoundments make an even more challenging environment for mussels because they can alter important environmental conditions such as water chemistry, sediment composition, temperature, and flow (Winemiller et al. 2010; Burlakova et al. 2011; Allen et al. 2013). While it is known that anthropogenic activities negatively affect mussel abundance, little is known about the impact of contaminants on freshwater mussels in Texas.

There have been few studies on the uptake of contaminants in mussels located in Texas. Of those, one focused on the impact of trace elements and nutrients and the physiological response of freshwater mussels located in the Guadalupe River Basin (Britton 2018), two on pharmaceutical uptake in mussels in the North Bosque River Basin (Du et al. 2014) and Pecan Creek (Burket et al. 2019), one on the effect of trace element [e.g., aluminum (Al) and Cu] exposure and nutrient [e.g., nitrate/nitrite and total phosphorus] exposure on the survival, growth and condition of mussels in the Colorado River (Nobles & Zhang 2015), and one on trace element concentrations in mussel shells collected from the Brazos River (VanPlantinga & Grossman 2019). Overall, these studies found that exposure to contaminants negatively impacted mussel health. Britton (2018) found higher oxidative stress and lower total protein concentrations indicating reduced mussel health in areas with higher nutrient levels, however there was no correlation between trace elements and investigated biomarkers. Although several toxicological studies have focused on freshwater mussels in Texas, none have used caged transplant experiments and only one focused on trace element concentrations in the soft tissue (gill, foot) in mussels (Britton 2018). Caged transplant experiments can be used to

collect data required to better understand the potential impact of contaminants on mussel populations in Texas and ultimately the overall health of Texas rivers.

Study area

The Guadalupe River which begins in Kerr County and flows 230 miles to San Antonio Bay in the Gulf of Mexico has a drainage area of approximately 6,070 square miles (Figure 1). The Guadalupe River flows through six major cities (Kerrville, New Braunfels, Seguin, Gonzales, Cuero, and Victoria; (Table 1), each contributing non-point source and point source pollution. In addition, agriculture and rural communities contribute non-point source pollution and more than 80 permitted wastewater outfalls serve as sources of point source pollution along the Guadalupe River (Figure 1). As the river flows downstream, there can be an additive effect from the point and non-point source pollution from the surrounding area around the main river channel as well as inputs from tributaries like San Marcos River which flows through San Marcos and Luling. This effect creates a presumed pollution gradient with the most polluted water further downstream and the least polluted water further upstream. This pollution gradient allows for an ideal area for a toxicological study because sites further downstream are presumably exposed to higher concentrations of contaminants. The Guadalupe River Basin historically had a total of 20 mussel species (Randklev at el. 2020); therefore, multiple species can be experiencing the effects of reduced water quality due to anthropogenic activities. In addition, a field study has already investigated the impact of trace elements and nutrients on mussels in the Guadalupe River (Britton 2018), allowing for a direct comparison to this caged transplant experiment.

Study species

The Threeridge (*Amblema plicata*) was used for this study because it is a common and relatively large (max shell length = 178mm; Cummings and Mayer 1992) species and therefore contains enough gill tissue for trace element and biomarker analysis. *A. plicata* is endemic to North America and is widely distributed across the United States and Canada. It is a generalist, which allows it to occupy diverse habitats and a variety of climates (Mulvey et al. 1997). The IUCN Red List categorizes *A. plicata* as a species of least concern and it is abundant throughout Texas (Cordeiro & Bogan 2012). This is important when choosing a species for toxicology studies that require a large number of mussels to be sacrificed. In addition, *A. plicata* can act as a surrogate for other unionid mussel species in Texas rivers that have much smaller population sizes [e.g., Pimpleback (*Cyclonaias pustulosa*) and Guadalupe orb (*Cyclonaias necki*)].

A. plicata has been used in previous studies allowing for a comparison to this study (Naimo et al. 1992; Augspurger et al. 2003; Fritts et al. 2013; Nobles & Zhang 2015; Britton 2018). It is found throughout the Guadalupe River and was collected from already established collection sites. Britton (2018) found that *A. plicata* was exposed to a variety of trace elements and nutrients at different locations in the Guadalupe River basin, but the study could not explain how quickly mussels can accumulate contaminants and physiologically respond to the stress. This thesis will address this knowledge gap.

Contaminants to be investigated

Within rivers, mussels are exposed to a mixture of environmental contaminants.

In this study, the contaminants to be investigated include essential trace elements [cobalt (Co), Cu, iron (Fe), manganese (Mn), nickel (Ni), selenium (Se), and Zn), non-essential trace elements [Ag, arsenic (As), Bi, Cd, Cr, Hg, Pb, and uranium (U)], and nutrients [total nitrogen (TN), nitrate (NO_3), NH_4, total phosphorous (TP), and soluble reactive phosphate (SRP)], TSS and non-volatile suspended solids (NVSS). These contaminants are added to the Guadalupe River basin through wastewater treatment plants, industrial activities, urban runoff, agricultural runoff, and livestock waste. Understanding how exposure to these contaminants changes in mussels over time at several different sites within the river basin and the resulting physiological response is critical to aide in the development of conservation and recovery plans for freshwater mussels in Texas.

Objectives of the thesis

This study investigated the change in trace element concentrations in gill tissue and the resulting physiological response using biomarkers to a mixture of contaminants in *A. plicata* over 12 weeks at six sites within the Guadalupe River basin using a caged transplant experiment.

This study can be broken down into three objectives:

1. Investigate the change in trace element concentrations in gill tissue over a 12-week period and how that varied between time points (week 0, 3, and 12) and among six sites along a presumed contamination gradient within the Guadalupe River basin, with the prediction that trace element concentrations will be greatest at week 12 and at downstream sites.

2. Examine trace element concentrations in gill tissue relative to concentrations in sediment at all time points and six sites, with the prediction that concentrations will be higher in gill tissue than sediment at all sites and at all time points.
3. Use biomarkers in gill tissue to examine the physiological response of exposure to trace elements and nutrients over a 12-week period and how that varied between time points and among sites, with the prediction that there will be a greater response in mussels exposed for a longer period of time and at downstream sites.

II. METHODS

Study area

To conduct this study, cages containing mussels were placed at six sites in the Guadalupe River basin: Lake Wood (LW), Palmetto State Park (PSP), Plum Creek (PC), Sandies Creek (SC), and Victoria (VA and VB) (Figure 2, Table 2). PSP is located on the San Marcos River, a major tributary of the Guadalupe River which begins 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 flows through two large urban centers (San Marcos and Luling; Table 1) before joining the Guadalupe River which contribute a variety of non-point and point source pollutants. As the San Marcos River flows between these two cities, intermittent rural communities contribute contaminants such as agricultural and urban runoff and point source pollutants such as wastewater effluent. PC is located in Plum Creek, a small tributary of the San Marcos River which joins the San Marcos River just upstream of Palmetto State Park. Plum Creek is located near Luling and is surrounded by rural areas which contribute wastewater effluent and non-point source pollutants such as agricultural runoff. LW is the uppermost site on the Guadalupe River and is located just upstream of Gonzales. The Guadalupe River flows through two large cities just prior to LW (New Braunfels and Seguin; Table 1) and another three large cities downstream of LW before reaching San Antonio Bay (Gonzales, Cuero, and Victoria; Table 1). Sandies Creek, a tributary of the Guadalupe River, contributes contaminants from rural communities and from industrial activities such as oil and gas development. SC is located on the Guadalupe River just below the

confluence of Sandies Creek and the Guadalupe River located just upstream of Cuero. VA and VB are located downstream of Victoria with VA placed just below the outfall for a wastewater treatment plant and VB approximately one mile downstream of the wastewater outfall. Both VA and VB receive contaminants from the wastewater effluent along with urban and industrial runoff from Victoria.

Study design and sample collection

This experiment required the collection of 260 *A. plicata* from a control site (Lake Wood) where mussels had the lowest gill trace element concentrations, as determined by Britton (2018). Once collected, 20 mussels were transported directly from the control site to Texas State University for processing so that baseline trace element concentrations and biomarker values in the gills could be determined at the start of the experiment (week 0). Of the remaining 240 mussels, 40 were placed into cages and left at LW as a control and the rest were placed into buckets with wet towels and transported to each site (Figure 2). At each site, 40 mussels were placed into wire crawdad traps (12.7 x 8.7 x 6.7 inches; hereafter referred to as cage) that had a rubber coating over the wire (10 mussels per cages; 4 cages per site) and attached to 30 lb cinder blocks that were wrapped in plastic sheeting. Two cages were collected from each site after three weeks (23 days) and the remaining two cages collected after 12 weeks (86 days).

Environmental parameters including water temperature, dissolved oxygen, conductivity, pH, turbidity, and current velocity where cages were set was recorded at each site and time point using a flow meter and Eureka Mana +30 probe (Eureka Water Probes, Austin, Texas) (Table 3). At each time point, two water samples were collected

from each site in brown 1 L Nalgene bottles to analyze the concentration of nutrients (TN, NO_3, NH_4, TP, SRP), ions, dissolved organic carbon (DOC), chlorophyll a (Chl A), TSS, and NVSS. Sediment was collected at each site at week 12 using a plastic shovel and stored in a 1 gallon Ziplock bag for trace elements analysis, grain size analysis, and to determine the organic carbon content.

Mussel processing

Once collected, mussels were transported to Texas State University, flash frozen at -80°C for 20 minutes and rinsed with deionized water to remove all sediment in preparation for dissection. Mussel length, width, and height were recorded using calipers and whole wet weight was recorded after mussels were opened and drained of water (Table 4). Condition factor (CF) was calculated to evaluate overall health using the following equation described by Crosby and Gale (1990):

CF = whole mussel mass (g)/ shell length (mm)

Before dissection, gills were inspected for the presence of glochidia and removed if present. Gill tissue was removed, rinsed with deionized water, and blot dried using Kimwipes to remove excess moisture. Approximately 0.2 g of gill tissue was subsampled and held at -80°C for biomarker analysis and the remainder was frozen at -80°C and freeze-dried at -54°C for 48 hours, homogenized into a fine powder, and stored for trace element analysis. Wet weight and dry weight were recorded prior to and after freeze drying, respectively, to determine the percent moisture content in gill tissue [mean ±

standard deviation (SD) = 84.1 ± 1.4 %; minimum and maximum percentage = 79.9 and 87.5%].

Trace element analysis

Approximately 0.25 g of gill tissue was digested in 5 ml of 9:1 nitric acid (HNO_3): hydrochloric acid (HCl) using a high pressure, high-temperature microwave (Ethos-UP; Milestone Inc., Shelton, CT) for 75 minutes. After the samples had cooled, they were diluted with 25 ml of Milli-Q water (Millipore, Burlington, MA) to obtain a total volume of 30 ml (dilution factor ~ 120). 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 using an Agilent 7900 and 8900 ICP-MS (Agilent Technologies, Santa Clara, CA) to determine the concentration of 15 trace elements (Ag, As, Bi, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, U, and Zn) following EPA Method 6020A (U.S. EPA, 1998).

To confirm the validity of the data, quality control included blanks, standard reference material (SRM; NIST 1566b oyster tissue, National Institute of Standards and Technology) spiked samples, and duplicate samples. All blanks (n = 13) were below the detection limit (BDL) for each element. The mean percentage recovery for all elements was between 86 and 102% for NIST 1566b (n = 13) and 90 and 100% for the spiked samples (n = 13). The mean relative percentage difference between duplicate samples was < 5% for all elements. One set of quality control was included with every 20 samples analyzed.

Biomarker analysis

This study aimed to measure the concentration of metallothionein, lipid peroxidation, antioxidant capacity against peroxyl radicals, total protein and total lipid in gill tissue collected from all mussels. Approximately, 0.2 g wet weight of gill tissue from each mussel was stored at -80°C, packed with dry ice, and shipped overnight to the University of Lethbridge (Lethbridge, Canada) for biomarker analysis. Due to a three day delay, the samples thawed while awaiting customs clearance in Canada. As a result, samples were only analyzed for total protein which was determined following the method described in Gillis et al. (2014b).

Sediment analysis

Sediment from each site was subsampled and dried at 60°C for 48 hours. Dried sediment was then broken up using a pestle and mortar and further subsampled for the following analyses: 1) trace element analysis, 2) grain size analysis, 3) organic carbon content.

To determine the concentration of trace elements in sediment at each site (except Hg), approximately 10 g of sediment was sent to the Trace Element Analysis Core Lab at Dartmouth College for acid digestion and ICP-MS analysis. Quality control (n =1) included a blank sample, SRM (NIST 2709a San Joaquin soil) and duplicate sample. For all elements, the blank was BDL, the percent recovery of the SRM was between 79 and 112%, and the relative percent difference between duplicates samples was ≤ 8%. Sediment Hg concentrations were determined using a direct mercury analyzer (DMA-80; Milestone, Shelton, CT) at Texas State University. Quality control included blanks

[empty quartz boat, 0.0001 μg/g Hg (n = 2)], certified reference material [MESS-4 marine sediment, National Research Council Canada, 98.2% recovery (n = 2)], and a duplicate sample [6.4% relative percentage difference (n = 1)].

Grain size analysis, to calculate the percent coarse sediment (> 63 μm; n = 3 per site), was determined by rehydrating approximately 30 g of sediment and passing it through a 63 μm sieve; sediment greater than 63 μm was dried at 60°C for 24 hours and the percent coarse sediment determined. Organic carbon content was determined by combusting approximately 4g of sediment in a muffle furnace at 450°C for 6 hours (n = 3 per site); the percent organic carbon was calculated as the percent difference in sample weight before and after combustion.

Water quality analysis

All water quality analysis followed the methods described in the American Public Health Association (APHA) Standard Methods for the Examination of Water and Wastewater (2005). The following methods were used to determine all water quality parameters: TN (APHA method 4500-NO_3 C), NO_3 (APHA method 4500-NO_3 B-2000), NH_4 (APHA method 4500-NH_3 F), TP (APHA method 4500-P E), SRP (APHA, method 4500-P E), ions (APHA method 300.0), DOC (APHA method 5310 B2000), chlorophyll a (APHA method 10200 H 3), TSS (APHA method 2540 D), and NVSS (APHA method 2540 E).

Selenium:mercury molar ratio and biota sediment accumulation factor calculations

Gill tissue Se:Hg molar ratios were calculated for each mussel to determine

whether Se has a protective effect against Hg toxicity in freshwater mussels. Ratios were calculated by first dividing the Se and Hg concentrations (μg/g dry weight) by their corresponding atomic mass (Se = 78.96; Hg = 200.59). The μmol/g Se value was then divided by μmol/g Hg value to calculate the ratio. If Se was in molar excess (i.e., the Se:Hg molar ratio was > 1:1) then Se may have a protective effect against Hg toxicity, whereas if Hg was in molar excess (i.e., the Se:Hg molar ratio was < 1:1) then Se does not have a protective effect.

To compare the concentration of each trace element in mussels to the environmental concentration, the biota sediment accumulation factor (BSAF) was calculated for each mussel at each time point and site. Sediment trace element concentrations are more representative of environmental concentrations than water trace element concentrations because, firstly, mussels are in contact with the sediment, secondly, trace element concentrations are much lower in water and more difficult to measure, and thirdly, water trace element concentrations can vary on an hourly-basis and therefore only represent a snapshot in time, whereas sediment concentrations more accurately reflect what mussels are exposed to long-term. The BSAF was calculated using the following equation:

BSAF = element concentration in gill (μg/g)/element concentration in sediment (μg/g)

The BSAF was used to determine whether mussels or sediment is more enriched in an individual trace element at each site. A BSAF > 1 means mussels are more enriched in a trace element, whereas a BSAF < 1 means the sediment is more enriched.

Statistical analysis

All statistical analysis was performed using R version 3.5.0 and SigmaPlot 13 and 14 (Systat Software Inc., San Jose, CA, USA). Significance was determined at the $p < 0.05$ confidence level. Following the methods of Hopkins et al. (2006) and Glass and Gray (2001), sites and time points in which > 50% of samples were BDL for an element were eliminated from the analysis. Data were natural log transformed as appropriate to meet parametric assumptions of normality and homoscedasticity.

Overall differences in trace element concentrations in gill tissue and BSAF values were first compared using a permutational multivariate analysis of variance (PERMANOVA) with the factors time (week 0, week 3, and week 12), sites (6 sites), and mussel length. Mussel length was found to explain only 5% of the variation in trace element concentrations in gill tissue (PERMANOVA, $F_{1,255} = 13.4$, $R^2 = 0.049$ $p = 0.001$). However, while significant, length was excluded from the analysis because it did not explain a large amount of variation in trace element concentrations and the average length from each site and time point ranged from 96.3mm – 102.9mm. This difference is minor and not biologically significant. A PERMANOVA was chosen over a multivariate analysis of variance (MANOVA) because data were not normally distributed. An one-way analysis of variance (ANOVA) was used to compare individual trace elements, BSAF values, Se:Hg molar ratios, condition factor and total protein concentration between time points (week 0, 3, and 12) within a site and among sites for week 3 and 12. If a significant difference was found ($p < 0.05$), a Tukey post-hoc test was used to determine individual differences between time points and among sites. When data did not meet parametric assumptions, a non-parametric Kruskal Wallis test followed by a Dunn's

pairwise comparison, if needed, was used.

A Spearman's rank order correlation was used to determine the correlation between Se:Hg molar ratios and mussel length and Se:Hg molar ratio and Hg concentration in gill tissue for all sites and time points. A Spearman's rank order correlation was also used to determine the correlation between general health measurements (total protein and condition factor) and trace element concentrations in gill tissue, and nutrients, ions, TSS, and NVSS in the water. Lastly, redundancy analysis (RDA) biplots were used to examine patterns in trace element concentrations in gill tissue in relation to site for week 3 and week 12 collected mussels.

III. RESULTS

Trace elements in mussel gill tissue

The mean (± SD) essential and non-essential trace element concentrations in gill tissue at each site and time point are reported in Table 5 and 6. Overall, the concentration of essential trace elements in gill tissue were greater than non-essential trace element concentrations with the exception of As which had a greater mean concentration than Cu, Se, Ni, and Co after combining all sites and time points. The ranked mean concentration for essential trace elements with all sites and week 3 and 12 time points combined was Mn > Fe > Zn > Cu > Se > Ni > Co and As > Cr > Pb > Cd > U > Hg > Ag for non-essential elements. Bismuth was the only investigated trace element which was BDL at all sites and time points (< 0.0280 µg/g). Week 12 trace element concentrations are the main focus of the results because those mussels we exposed *in situ* for the longest period of time.

Overall, the prediction that trace element concentrations would be greatest at downstream sties was not met. Differences in trace element concentrations among sites were not significant (PERMANOVA, $F_{5,255} = 0.96$, $R^2 = 0.02$, $p = 0.45$); however, differences in the mean concentrations among sites for individual elements were found. For example, mean (± SD) concentrations during week 12 for essential trace elements were greatest at LW for Se (3.67 ± 0.321 µg/g dry weight), PC for Cu (9.02 ± 1.29 µg/g dry weight), SC for Fe (960 ± 419 µg/g dry weight), Mn (4874 ± 797 µg/g dry weight), and Zn (462 ± 77.9 µg/g dry weight), and VA for Co (1.48 ± 0.358 µg/g dry weight) and Ni (2.18 ± 0.396 µg/g dry weight) (Table 5). For non-essential elements, mean (± SD)

concentrations during week 12 were greatest at PC for Hg (0.0823 ± 0.0081 μg/g dry weight), PSP for Cr (0.710 ± 0.162 μg/g dry weight), SC for Ag (0.0631 ± 0.0172 μg/g dry weight), VA for As (9.26 ± 2.17 μg/g dry weight), Cd (0.271 ± 0.104 μg/g dry weight), and U (0.114 ± 0.0314 μg/g dry weight), and VB for Pb (0.409 ± 0.0526 μg/g dry weight) (Table 6). Though these sites had the greatest mean concentration of an individual element, there was no individual site that was significantly greater in multiple trace elements than all other sites during week 12 (one-way ANOVA, $p > 0.05$) and differences were generally seen between individual sites. Furthermore, significant differences ($p < 0.05$) were seen between individual sites, however, the differences in trace element concentrations were not large when comparing the absolute mean trace element concentration. For example, PC had the greatest mean concentration of Hg during week 12 and was significantly greater than the mean concentration at LW (one-way ANOVA, $p = 0.007$, Figure 4) but the difference between these absolute numbers was not large and might have not been biologically relevant. Additionally, there were no statistically significant differences among sites for As, Cd, Cr, Fe, Pb, U, and Zn during week 3 and week 12 (Figure 3 and 4). Sites further downstream (SC, VA, VB) did not consistently have greater trace element concentrations than sites further upstream (LW, PC, PSP) and trace element concentrations varied depending on individual trace elements and time points.

The prediction that trace element concentrations would be greatest at week 12 was not met. Differences in trace element concentrations over time were not significant (PERMANOVA, $F_{2,255} = 2.24$, $R^2 = 0.02$, $p = 0.09$) and mean trace element concentrations were dependent on individual elements and sites. For essential trace

elements, the only elements that showed a consistent change in accumulation over time at all sites were Se and Cu; Se concentrations decreased over time at each site and Cu concentrations were greatest at week 12 at each site (Figure 3). There were no significant differences between week 0 and 3 for Cu concentrations but week 12 was significantly greater than both time points at all sites. For non-essential elements, mean As and Cd concentrations decreased over time and Pb concentrations decreased over time at PSP and VA, whereas Hg concentrations increased over time at PC, (Figure 4). Additionally, the one-way ANOVA only reported a significant decrease for Pb concentrations at VA ($p = 0.006$; Figure 4). Overall, there was no consistent pattern in trace element accumulation over time for each site except for Se and Cu. Week 12 trace element concentrations were not consistently greater than trace elements concentrations found during week 0 or week 3, however, patterns were found for individual trace elements and at individual sites.

Some differences between sites were associated with differences in trace elements. For example, at week 3, mussels transplanted to PC were characterized by high concentrations of Ni, and high concentrations of Cu and Hg occurred at SC. At week 12, mussels transplanted to PC were characterized by high Cu and Hg concentrations, mussels at LW high concentrations of Se, and mussels at VA high concentrations of Ni (Figure 6A, 6B).

The Se:Hg molar ratios in gill tissue at each site and time point are reported in Table 7. The mean Se:Hg molar ratios were > 1:1 at all sites, with the greatest ratio at LW during week 3 (143) and the lowest ratio at PC during week 12 (105) (Table 7). At the end of the caged transplant experiment, mussels at LW had significantly greater Se:Hg molar ratios than all other sites ($p = 0.0002$; Figure 7). Over time, the Se:Hg molar

ratios significantly decreased between week 0 and week 12 at all sites except LW ($p = 0.397$) (Figure 7, Table 7). For all sites and time points combined there was a significant negative relationship between Se:Hg molar ratios and shell length ($R_s = -0.183$, $p = 0.003$) and Se:Hg molar ratios and Hg concentration ($R_s = -0.739$, $p < 0.001$; Figure 8A, 8B).

Sediment trace element concentrations and BSAFs

The organic carbon content and grain size distribution at each site are reported in Table 8 and the mean sediment trace element concentrations are reported in Table 9. Mean (± SD) organic carbon content ranged from 1.8 ± 0.07% at SC to 6.5 ± 0.1% at VA (Table 8). Overall, LW contained the greatest amount of fine sediment with a mean (± SD) percentage of course sediment (> 63μm) of 38.3 ± 0.8% and SC contained the greatest amount of course sediment with a mean (± SD) percentage of course sediment of 79.6 ± 2.5% (Table 8).

The mean concentration of essential trace elements in sediment were greater than the mean concentration of non-essential elements after combing all sites, except for Cr, Pb, As, and U (Table 9). The ranked mean concentration for essential trace elements in sediment with all sites combined is Fe > Mn > Zn > Ni > Cu > Co > Se and Cr > Pb > As > U > Cd > Hg > Ag for non-essential trace elements. VA had the greatest mean As, Cd, Cu, Fe, Mn, Pb, and Zn concentrations, LW contained the greatest concentration of Ag, Se, and U, PSP contained the greatest concentrations of Co, Cr, and Ni, and VB contained the greatest concentration of Hg (Table 9).

The mean (± SD) calculated essential and non-essential trace element BSAFs in

gill tissue at each site and time point are reported in Table 10 and 11. Overall, the prediction that trace element concentrations will be higher in gill tissue than sediment at all sites and at all time points was not met. The trace elements that had a mean BSAF > 1 for all time points and sites combined, and are therefore more enriched in gill tissue than sediment, are Ag, As, Cd, Cu, Hg, Mn, Se, and Zn and the trace elements that had a mean BSAF < 1 for all time points and sites combined, and therefore more enriched in sediment than gill tissue, are Co, Cr, Fe, Pb, Ni, and U (Table 10 and 11). Overall, calculated BSAFs for essential trace elements had the greatest mean values, however, they were not consistently greater than mean BSAFs calculated for non-essential trace elements. For example, the mean Mn, Se, and Zn values were greater than all non-essential trace element values, however, Hg, Ag, As, and Cd had greater mean values than the rest of the essential trace elements. The ranked mean essential trace element BSAFs with all sites and week 3 and week 12 time points combined was Mn > Zn > Se > Cu > Ni > Fe > Cr and Hg > Ag > As > Cd > Co> U > Pb for non-essential trace element BSAFs.

After combining the BSAFs calculated for all trace elements; there were significant differences in mean BSAFs among sites (PERMANOVA, $F_{5,255} = 183.415$, $R^2 = 0.76$, $p < 0.01$) and over time (PERMANOVA, $F_{2,255} = 20.489$, $R^2 = 0.03$, $p < 0.01$). Among sites, mean BSAF values were greatest at SC for all essential and non-essential elements during week 3 (except Mn and U) and week 12 (Table 10 and 11). During week 12, SC had the greatest mean BSAF for all trace elements, however, the difference was only statistically significant at all sites for Co, Cu, Fe, Ni, and Zn for essential elements (one-way ANOVA, $p < 0.05$; Figure 9, Table 10) and As, Cr, and Pb for non-essential

elements (one-way ANOVA, $p < 0.05$; Figure 10, Table 11).

Over time, mean BSAFs calculated for essential trace elements varied based on individual elements and sites (Figure 9). For example, SC increased over time for Co, Mn, Zn, Cu and Fe, PC increased over time for Mn and decreased for Co, PSP increased for Mn, and VB increased over time for Ni and decreased over time for Co and Mn. Overall, for these sites and trace elements, a one-way ANOVA showed that differences between week 3 and week 12 were not statistically significant ($p > 0.05$); however, week 0 was statistically different ($p < 0.05$) from week 3 and week 12. For non-essential trace elements, mean BSAFs calculated for week 0 were significantly different than week 3 and week 12 at all sites (one-way ANOVA, $p < 0.05$) and differences between week 3 and 12 were not significant (one-way ANOVA, $p > 0.05$) (Figure 10). For example, SC increased after week 0 for all non-essential elements except As while other sites showed different trends in BSAF values depending on the trace element. For example, PC and PSP decreased after week 0 for As, Cr, Hg and Pb while PC increased for Cd and U and PSP increased for Ag and U. Overall, As, Hg, and Pb decreased over time at each site except at SC and Ag and U increased over time at all sites except at LW (Figure 10).

Nutrients and other analytes

Nutrients, DOC, chlorophyll a, TSS, and NVSS concentrations collected from each site and time point are reported in Table 12 and ion concentrations are reported in Table 13. Overall, PC had the greatest concentration of mean TN (6.02 mg/L), NO_3 (5.07 mg/L) and SRP (1.02 mg/L) during week 12 and NH_4 was more variable across sites (Figure 11). Overall, sites further downstream (SC, VA, VB) did not have greater nutrient

concentrations than upstream sites (LW, PC, PSP) and over time, TN and NO_3 increased at LW, PSP, SC, and VB, while NH_4 and SRP decreased throughout the study period at VA and VB (Figure 11). Total suspended solids (387 mg/L) and NVSS (321 mg/L) were high at SC during week 3 while there was no clear pattern among sites for DOC and chlorophyll a. TSS and NVSS concentrations varied over time, while DOC increased over time at each site except at PC and SC, and chlorophyll a decreased over time at each site except at PC (Table 12).

Overall, cation concentrations were greater than anion concentrations, however Cl^- and SO_4^{2-} had greater mean concentrations than Na^+, Mg^{2+}, and K^+ after combining all sites and time points. The ranked mean concentration of cations after combing all sites and time points is $Ca^{2+} > Na^+ > Mg^{2+} > K^+$ and $Cl^- > SO_4^{2-} > F^- > Br^-$ for anions (Table 13). Among sites, PC had the greatest concentrations of anions and cations at each time point except for Mg^{2+}. For example, Cl^- concentrations ranged from 15.4 mg/L at SC during week 3 to 268 mg/L at PC during week 0. Over time, concentrations varied based on individual ions. For example, Cl^-, K^+, and Na^+ decreased over time at all sites and Ca^{2+} increased over time at all sites (Table 13).

General health indicators and correlation with contaminant concentrations

Mean condition factor and total protein concentrations are reported in Figure 12 and Spearman's rank order correlation results between general health indicators (condition factor and total protein) and trace elements are reported in Table 14. Overall, the mean (± SD) condition factor calculated in mussels ranged from 1.62 ± 0.211 at SC during week 3 to 1.93 ± 0.249 at PSP during week 12. Mean condition factors were not

greatest at downstream sites or during week 12. Among sites, PSP and VA had the greatest mean condition factor however, there were no sites that were continuously greater than all other sites throughout the study (Figure 12A). Over time, condition factor increased from between week 3 and week 12 at PSP, SC and VA, however, these differences were not large when comparing the absolute number (Figure 12A). During week 3, calculated condition factors had a weak positive correlation with Cr, Co, and U (Table 14) and during week 12 mean condition factors had a weak positive correlation with Cr, Mn, Co and Ni, and negatively correlated with Cu, Pb, and U (Table 14). Mean total protein concentrations ranged from 2.80 ± 0.435 μg/μL at PC during week 12 to 5.87 ± 0.727 μg/μL at PSP during week 3 (Figure 12B). Overall, total protein concentrations varied across sites and over time and were not greatest at downstream sites or during week 12. During week 3, total protein concentration had a weak negative correlation with As concentration in mussels (Table 14).

IV. DISCUSSION

Trace element concentrations in mussel gill tissue

Essential trace elements are required by mussels and are regulated under homeostatic control. Elements such as Cu, Zn and Fe interact with proteins as cofactors, are important in cell metabolism, and are important in other various metabolic and signaling functions (White & Rainbow 1985; Viarengo and Nott 1993; Ahearn et al. 2004; Schimitt et al. 2015). However, essential elements can be toxic at high concentrations in mussels (Loayza-Muro & Elieas-Letts 2007; Oliveria et al. 2016; Jorge et al. 2018). In comparison, non-essential trace elements are not required by mussels and are harmful when excretion rates or detoxification mechanisms do not significantly offset the uptake rate (Wang et al. 2010, 2018; Rocha & Souza 2012). In the present study, essential trace element concentrations were greater in gill tissue than non-essential trace element concentrations except for As, which was greater in gill tissue than Co, Cu, Ni and Se. The same trend was seen in Britton (2018) where mussels were collected from multiple sites in the Guadalupe River basin and analyzed for trace elements. Natural (e.g., erosion of rock and soil) and anthropogenic sources (e.g., fossil fuel combustion, smelter operations, and sewage effluents) of As can be attributing to the As concentrations found in this study (Smedley & Kinniburgh 2002). However, Gong et al. (2014), found low As concentrations (0.95μg/L) in groundwater throughout the Edwards Aquifer, suggesting that As could be entering the study area through surface exposure such as road runoff, wastewater effluents and atmospheric deposition. Mussels can take up As through diet, sediment, and from the surrounding water. A study by Gailer et al. (1995) estimated the

bioconcentration factor (BCF), which calculates the ratio of a contaminant accumulated by an organism divided by the concentration in the water, in blue mussel (*Mytilus edulis)* to range from 454 to 1390. Additionally, another study (Giusti and Zhang 2002) estimated the bioaccumulation factor (BAF), which calculates the ratio of a contaminant in an organism to the concentration in the surrounding environment (through water, diet, and sediment) in Mediterranean mussel (*Mytilus galloprovincialis)* to range from 681 to 1263. This suggests that As can bioconcentrate in mussels and that they can bioaccumulate As from other sources such as phytoplankton and sediment (Henry 2003; Rahman et al. 2012; Zhang and Wang 2018).

Only one other study (Britton 2018) has investigated trace element concentrations in gill tissue from mussels collected in Texas and overall trace element concentrations were comparable to levels in the current study. Trace elements in mussels collected at week 12 from LW, PSP, SC and VB could be compared between the two studies with As, Co, Cu, Mn, and Zn having greater trace element concentrations (1.01 - 1.4-times greater) at each site in the current study and Ag, Fe, Hg, and Pb having lower concentrations (1.02 - 2.5times lower) at each site than the same sites in Britton (2018). Other studies not conducted in Texas that investigated gill tissue in wild unionid mussels show varying trace element concentrations compared to the present study. The mean trace element concentrations during week 12 after combing all sites in the current study for Cr and Pb was 4.2-times lower and 19-times lower, respectively, than Cr and Pb concentrations in mussels exposed to wastewater in Ontario, Canada (Gillis et al. 2014a). Moreover, As, Cd, Cu, Cr, Mn, Ni, and Pb concentrations were lower in the present study after combing trace element concentrations from each site during week 12, compared to mussels

collected from Ranco Bay located in Northern Italy (Rivera et al. 2003). Lead concentrations were 55-times lower; Cd concentrations were 52-times lower and Cr concentrations were 11-times lower in the current study than in the gill tissue from mussels in Ranco Bay. These comparisons suggest that the Guadalupe River basin is lower in trace elements or comparable to the concentrations found in other low to moderately impacted freshwater systems.

The present study focused on trace element concentrations in gill tissue only. Gill tissue is the primary site for the uptake of contaminants because it filters particles from the surrounding water column. Therefore, gill tissue is useful for biomonitoring studies interested in contaminants in the surrounding environment. Other tissues such as foot, mantle and digestive gland accumulate different concentrations of trace elements and this can reflect how trace elements are metabolized and remobilized within mussels (Wang et al. 1996; Giguere et al. 2003; Rivera et al. 2003; Britton 2018; Oliveria et al. 2018). For example, Britton (2018) found greater and more variable concentrations of trace elements in gill tissue compared to foot tissue, suggesting that trace elements are remobilized after uptake and that further regulation such as metallothionein proteins binding to a trace element or the absorption of trace elements by lysosomes can occur after uptake (Viarengo & Nott 1993; Ahearn et al. 2004). Furthermore, elements such as Fe, Mn, and Zn can be redistributed around the body after uptake. These trace elements can interact with proteins as cofactors that affect hormone signaling, enzyme catalysis and gas transport and can be used in the formation of structural connective tissue within mussel gill, mantle, and kidney tissue (Hinzmann et al. 2015; Schmitt et al. 2015). This can result in trace element concentrations changing within individual tissues and should be

considered when using gill tissue as a representation of environmental conditions.

In contrast to my prediction, downstream sites were not consistently greater in trace element concentrations compared to upstream sites and sites such as SC (Ag, Fe, Mn, Zn) and VA (As, Cd, Co, Ni), located upstream of VB, had the most trace elements with the greatest concentration at week 12 (Figure 3 and 4). Additionally, significant differences between sites in the mean concentration of Ag, Co, Cu, Mn, Ni, Se, Hg and Pb were seen during week 12 (Figure 3 and 4). These differences may be statistically different but may not be large enough to cause significant different physiological effects on mussels. Other studies such as Gillis et al. (2014a) found that mussels with higher trace element concentrations had greater concentrations of metallothionein's and showed more evidence of oxidative stress than mussels with lower trace element concentrations. However, the difference in trace element concentrations in mussels located at different sites were greater than the differences seen in the present study.

One explanation why a contamination gradient, and large differences in trace element accumulation was not seen, could be that mussels were placed in a river system that was large and complex. Sites within the study area were spread out over 100 miles of the Guadalupe River (Figure 1) and discharge ranged from 1.10 cfs to 7272 cfs (Figure 13). In contrast, Gillis et al. (2014a), found a contamination gradient moving downstream in the Grand River located in Ontario, Canada. However, the study area only stretched over approximately three miles and discharge levels in the Grand River can range from 200 to 1200 cfs (Gillis et al. 2014b). Since that system was smaller and had lower discharge levels, trace element concentrations were less likely to get diluted. In the current study, multiple sources could be influencing trace element uptake because the

study area was large and discharge levels fluctuated throughout the experiment. For example, PC is highly impacted by wastewater effluent and agricultural runoff while other sites located in the lower Guadalupe River (SC) could be influenced by oil and gas activity form the Eagle-Ford shale (GBRA 2018). Mussels placed at these sites could be influenced by different contaminant sources and suggest that gradients might only stretch over small distances.

Additionally, accumulation patterns between sites could have been influenced by flooding events that occurred throughout the study. Flooding events could influence trace element accumulation through runoff (urban or agricultural), diluting existing trace elements or causing mussels to close their shells for the duration of the disturbance, thus decreasing filtration rates. Mussels in the current study were placed in cages with a cinder block attached to the bottom, which prevented them from burrowing into sediment. This could have caused mussels to close their shells for a significant portion of the experiment and could explain why trace element accumulation did not differ between sites. Furthermore, dissolved trace elements were not measured in the water, so it is unknown if dissolved trace elements were washed into the system or diluted. Angelo et al. (2007), found that trace metal accumulation in mussels correlated with flow rates. Mussels collected after a period of moderate runoff had the greatest amount of Cd, Pb, and Zn concentrations and mussels collected after a period of major discharge events had the lowest levels of Cd, Pb, and Zn (Angelo et al. 2007). Additionally, mussel trace element concentrations did not correlate with dissolved trace element concentrations in the water, however, they did correlate with trace element concentrations in the sediment. This was not seen in the current study and could have occurred because mussels were unable to

burrow into the sediment since they were caged and placed on top of a cinder block. This prevented mussels from burrowing into the sediment and could have prevented them from sediment trace element exposure.

Mean trace element concentrations were compared over time between week 0, week 3 and week 12 with the prediction that trace element concentrations would increase over time. This prediction was not met because concentrations varied based on individual elements and the differences were not large when comparing the absolute concentration in mussels. For example, Cu concentrations increased over time at all sites while Se concentrations decreased over time. Other elements such as Co, Hg, and Ni increased at an individual site but decreased at others. Additionally, significant differences over time, for at least one site, were seen for all trace elements except for Cd, Fe, and Zn (Figure 3 and 4). However, these differences were not large when comparing the absolute number. For example, the largest difference between time points for Cu was 2.66 μg/g between week zero and week 12 at PC and the largest change over time for Se was 0.85 μg/g between week zero and week 12 at VA (Figure 3 and 4).

One reason why concentrations did not increase over time could be that multiple flood events occurred throughout the duration of the experiment. For example, discharge levels between week 0 and week 3 at LW ranged from 238 – 1105 cfs and 496 - 3572 cfs between week 3 and week 12 (Figure 13). This would impact the ability for mussels to accumulate trace elements because dissolved trace elements in the water are highly influenced by dilution from groundwater and precipitation events (Angelo et al. 2007). This could also explain why sites with smaller mean discharge did not accumulate greater trace element concentrations than sites with larger mean discharge. For example, normal

discharge in the San Marcos River ranges between 150-300 cfs and discharge levels were greater than 300 cfs for 70% of the entire study period and reached as high as 1331 cfs (USGS 2018). Furthermore, these river conditions could have resulted in greater stress on mussels because mussels were unable to bury into the sediment during floods. This could have resulted in mussels closing their shells for the duration of the experiment and therefore decreasing their ability to accumulate trace elements.

Mercury is well known for causing negative health effects in freshwater organisms (Wester 1991; Monteir et al. 2010; Flanders et al. 2019). For example, oxidative stress and DNA damage was found in mussels exposed to 40 μg/L of Hg for five days and accumulated mean concentrations of methylmercury (196 ± 21.2 μg/g) and inorganic mercury (42.7 ± 0.66 μg/g) in whole tissue samples (Faria et al. 2009). Additionally, the U.S. Environmental Protection Agency (U.S. EPA) acute water quality criteria for Hg is 1.4 μg/L and Valenti et al. (2005), found LC_{50} values that ranged from 99 – 162 μg/L in juvenile mussels. In the current study, mussels accumulated a mean Hg concentration that ranged from 0.0678 μg/g at VB during week 3 to 0.0823 μg/g at PC during week 12 (Table 6) suggesting that mussels were not exposed to levels of Hg that would cause deleterious effects. Selenium has a known antagonistic relationship with Hg and has been found to offer protection from Hg toxicity in aquatic organisms (Tran et al. 2007; Ralston et al. 2007, 2008). Se:Hg molar ratios are an effective tool in analyzing this relationship and in the present study Se:Hg molar ratios were greater than 1:1 in all mussels (Figure 7) suggesting that Se has a protective role against Hg. Furthermore, a comparison between Se:Hg molar ratios and Hg concentrations showed a significant negative relationship suggesting that ratios were most influenced by Hg concentrations in

gill tissue (Figure 8b). Se:Hg molar ratios were also compared against mussel shell length where a weak correlation was found (Figure 8a). This could be due to the large variation in Se:Hg molar ratios and the small range in shell lengths. For example, 93% of mussels collected in the present study had a shell length that ranged between 90-110mm. Recent studies have found a significant relationship between shell length and Hg concentrations and suggest that Hg concentrations depend on the shell length of mussels (Cossa & Tabard 2020). However, a larger range in shell length is needed to more properly analyze the relationship between shell length and Se:Hg molar ratios.

Sediment trace elements and BSAFs

Sediment is a good representation of long-term trace element exposure in aquatic systems (Grapentine et al. 2008; Maranho et al. 2015; Taylor et al. 2017). In contrast, water measurements only represent the concentration of trace elements at the time of collection and rarely correlate with concentrations found in mussel tissues (Rivera et al. 2003; Angelo et al. 2007; Taylor et al. 2017). Therefore, comparing trace element concentrations in mussels to sediment concentrations gives a more accurate representation of the surrounding environmental conditions and how they are affecting mussels (Rivera et al. 2003; Angelo et al. 2007). Calculating the BSAF is a useful tool for analyzing trace element concentrations in mussels in comparison to sediment concentrations and gives a better understanding of the bioavailability of elements (García-Luque & DelValls 2003). In the current study the BSAF for Ag, As, Cd, Cu, Hg, Mn, Se, and Zn were > 1 at all sites and Co, Cr, Fe, Pb, Ni, and U were < 1 at all sites (Table 10 and 11). Essential elements were expected to be greater in mussels because

they are necessary for biological function. However, Fe was greater in sediment due to the naturally high levels within sedimentary rock (Algül & Beyhan 2020). Non-essential elements were expected to be greater in sediment. For example, Cr was higher in sediment because it is particle reactive and will bind to dissolved organic matter in the water column before binding to gill tissue (Dutton & Fisher 2012) and Pb could be greater in sediment because of its low bioavailability in mussels (Mejdoub et al. 2019).

Mean BSAFs were most influenced by trace element concentrations in sediment because concentration in sediment varied more across sites than concentrations in mussels. For example, SC had significantly larger BSAFs at all time points for all trace elements. This is because concentrations in sediment were significantly lower at SC than all other sites. Additionally, SC had the largest percentage of course sediment (79.6%) and the lowest percentage of organic carbon (1.8%) compared to all of the other sites (Table 8). Increased trace element concentrations for Ag, Cu, Mn, Hg, Zn and Pb have been found to correlate with increased sediment organic carbon content (Wang & Fisher 1996; Algul & Beyhan 2020). These trace elements can bind with organic carbon and ingested by mussels (Roditi et al. 2000). Additionally, dissolved trace elements can bind to organic carbon and then accumulate in the sediment; thus, increasing trace element concentrations within river sediment (Algul & Beyhan 2020). Sediment grain size can corelate with trace elements found in sediment with finer sediment having a greater affinity to trace elements than coarser particles because finer sediment has greater surface area to volume ratio. (Horowitz & Elrick 1987; Angelo et al. 2007; Maranho et al. 2015). Sites such as VA had the lowest BSAF for Cd, Fe, Mn, Pb, and Zn and had one of the lowest percentages of course sediment (38.9 ± 0.3 %) and largest percentages of organic

carbon (6.5% ± 0.1) suggesting that the low BSAF values calculated for this site could be due to higher concentrations of trace elements in the sediment (Table 8).

Sediment was only collected from sites at week 12 and trace element concentrations found in sediment during week 12 was used to calculate BSAFs across all time points. Therefore, BSAFs should reflect trace element concentrations found in mussels. However, it should be noted that BSAFs calculated for week 0 used trace element concentrations in mussels collected from LW and the sediment trace element concentrations from week 12. If sediment was collected during each time point, we could hypothesize that concentrations would vary over time because of the large flood events throughout the experiment. These flood events could have resulted in the disturbance of river sediment composition by transporting sediment downstream and therefore affecting the trace element concentrations in the sediment at each location.

Nutrients and other analytes

Overall, nutrient concentrations collected from PC were greater at all time points than all other sites. Mean concentrations of TN (6.02 mg/L), NO_3 (5.07 mg/L), were consistent with total nitrogen, and nitrate levels recorded throughout Plum creek in 2016 (GBRA, 2018). Plum Creek was listed on the Texas 303 (d) list of impaired water bodies because of high *E. coli* levels and nitrogen levels exceeding the screening criteria of 1.95 mg/L (TCEQ 2008, GBRA 2018). High nutrient concentrations can be attributed to wastewater effluent from five major discharge outputs along the length of the creek (GBRA 2018). Additionally, failing septic systems, fertilizer from agricultural operations and cattle operations could be adding to nitrogen and phosphorous levels.

Ammonium levels varied between sites and no site was significantly larger than other sites. Concentrations may have not followed trends seen by other nutrients because NH_4 is easily influenced by environmental factors such as water temperature and pH (Jones and Hood 1980). Mean concentrations ranged from 0.263 at PSP during week 3 to 1.14 mg/L at SC during week 3 meaning that all mean concentrations were lower than the 2013 EPA criteria continuous concentration (CCC) of 1.24 mg/L.

Ion concentrations followed similar trends to nutrients with the largest levels recorded from PC. Chloride levels ranged from 96.7 – 268 mg/L at PC and levels from all other sites were significantly lower ranging from 15.4 – 54.5 mg/L (Table 13). Overall, concentrations were lower than EPA ambient water quality criteria for juvenile clams (EC_{50} = 472 mg/L), however, EC_{50}s reported by Gillis (2011) for glochidia and juvenile mussels ranged from 113 – 1430 mg/L and Salerno et al. (2020) reported EC_{50}s that ranged from 435.5 – 525.5 mg/L. Chloride can enter aquatic systems via runoff from a variety of anthropogenic sources such as fertilizers, industrial and wastewater effluents, and roads. TCEQ is currently developing state specific surface water quality criteria. However, maximum concentrations continue to be limited for nutrients and other analytes.

General health indicators and correlation with contaminant concentrations

Condition Factor (CF) and biomarkers (metallothionein, lipid peroxidation, antioxidant capacity against peroxyl radicals, total protein, and total lipid) were predicted to have a greater response over time and at sites located further downstream. However, condition factor and total protein concentrations were the only measurements used

because biomarker samples thawed while waiting in customs in Canada and total protein is the only biomarker that was stable after being thawed. Therefore, only overall general health of mussels could be investigated in the study.

Mean condition factor and total protein did not consistently increase or decrease overtime or between sites. Mean condition factor ranged from 1.62 at SC during week 3 to 1.93 at PSP during week 12 and statistical differences between sites were seen during week 3 and week 12 (Figure 12). Condition factor gives information about long term condition status in mussels and can indicate overall general health of mussels. No clear pattern was seen across sites, however mean condition factor significantly increased between week 3 and week 12 for PSP, SC, and VA but the differences between week 3 and week 12 ranged from 0.18 – 0.25. These differences may have been significantly different but the change in the absolute number is small and may not indicate significant physiological differences and more time might be needed to see large differences in condition factor over time. Recent studies have found decreased condition factor from mussels exposed to wastewater effluent (Gillis 2012) and positive correlations between condition factor and trace elements measured in clams collected in the St. Lawrence Estuary in Quebec, Canada were seen (Gagne et al. 2006). Furthermore, positive correlations between metal seeking proteins such as metallothionein and condition factor were seen in clams collected in the Saguenay Fjord in Quebec, Canada were seen (Blaise et al. 2002). The current study found week positive correlations between condition factor and Cr, Co U, Mn, and Ni and weak negative correlations with Cu and Pb (Table 14) indicating that trace elements could influence mussel condition however more research is needed to have a clear understanding between trace elements and overall condition in

mussels.

Mean total protein concentrations ranged from 2.82 at PC during week 12 to 4.24 at PSP during week 3 and statistical differences between sites were seen during week 3 and week 12. Over time, significant decrease in total protein was seen at PC, PSP, and VB between week 3 and week 12. This suggests that mussels experienced stress throughout the study at these sites. Protein synthesis is known to be affected by a variety of environmental contaminants such as pesticides and trace elements and environmental disturbances such as floods (Gillis et al. 2014a; Ali et al. 2019). In the current study, mussels were exposed to varying flooding events and were unable to bury into the sediment to avoid stressful conditions and could have played a role in total protein concentrations. However, we cannot narrow down the source of stress on mussels because we were unable to test a suite of biomarkers.

Conclusions and future research

There was no clear pattern in trace element concentrations over time and across sites and the lack of biomarker analysis made it difficult to understand how mussels physiologically responded to exposure to contaminants over time. Sediment concentrations allowed for the comparison between trace element levels in mussels and sediment. However, we were unable to compare sediment over time because sediment was only collected during week 12 because of high flows during week 3. Overall, these results were the consequence of a large river system and a flashy regime. The study was planned during the dry season in Texas; however, large rain events still occurred during the study. The Guadalupe River is a large, complex river system making it a difficult

location for caged transplant studies. Large rain events can increase dissolved trace elements in the water column from increased agricultural and urban runoff, however, this increase can be affected by the amount of water diluting the dissolved trace elements in the water column, producing varying results. Additionally, contaminant plumes can be completely missed during low flows if cages are not placed in the correct location within the river. The San Marcos River and Plum Creek are more manageable systems because they are smaller. However, flow levels can still increase quickly within these systems. Therefore, future caged transplant studies should focus on smaller river systems and should be conducted during the dry season. Other studies (Gillis et al. 2014b) have proven that caged transplant studies can be successful however environmental conditions must be constant.

Additionally, flood events could have resulted in abnormal mussel behavior because they were unable to burrow into sediment. However, this could not be concluded because of a lack of biomarkers. Therefore, future studies should focus on placing mussels in a location where they can burrow naturally while keeping them contained and a suite of biomarkers is needed to fully understand the physiological response of mussels to contaminant exposure.

In conclusion, caged transplant studies are important for understanding how fast mussels respond to environmental contamination and are useful because mussels can be transferred to areas where they would not be normally present. Previous caged transplant experiments have been successful in identifying accumulation patterns in mussels placed in small riverine systems; however, data collected in this study identified the difficulties associated with conducting the same experiment in a large river system with a flashy

regime. The results of this study will provide useful data for Texas Parks and Wildlife and the U.S. Fish and Wildlife Service, along with other environmental organizations, for future conservation and recovery plans for Texas freshwater mussels. For example, trace element concentrations in mussels can be compared to other mussels collected in Texas and the accumulation patterns over time and across sites can be used to evaluate how effective mussels are at reflecting water quality in Texas Rivers.

Table 1. Population estimates of Texas cities located on the San Marcos River and Guadalupe River. (U.S. Census Bureau, 2019)

City	River	Population
San Marcos	San Marcos	63,220
Luling	San Marcos	5,830
Kerrville	Guadalupe	23,370
New Braunfels	Guadalupe	79,438
Seguin	Guadalupe	28,894
Gonzales	Guadalupe	9,740
Cuero	Guadalupe	8,297
Victoria	Guadalupe	75,900

Table 2. GPS coordinates of all study sites.

Study site	Latitude	Longitude
Lake Wood (LW)	29.468413	-97.491116
Plum Creek (PC)	28.657383	-97.601922
Palmetto State Park (PSP)	28.588411	-97.585961
Sandies Creek (SC)	29.100599	-97.332584
Victoria WWTP (VA)	28.753284	-97.006460
Victoria 1-mile (VB)	28.749665	-97.000103

Table 3. Environmental parameters reported for each time point at each site. LW = Lake Wood, PC = Plum Creek, PSP = Palmetto State Park, SC = Sandies Creek, VA = Victoria wastewater treatment plant (WWTP), VB = Victoria 1 mile downstream of the WWTP. ND = not determined.

Site	Week	Temperature (˚C)	pH	Dissolved oxygen (mg/L)	Dissolved oxygen (% Sat)	Conductivity (µS/cm)	Turbidity (ntu)
Lake Wood (LW)	0	29.6	8.07	7.78	105.1	489	14.2
	3	26.5	8.09	8.23	105.7	485	99.0
	12	17.3	8.06	9.86	106.1	510	34.5
Plum Creek (PC)	0	28.4	8.17	7.94	105.4	1378	ND
	3	23.9	7.86	7.23	88.9	805	107
	12	13.1	8.08	11.4	112.0	990	38.6
Palmetto State Park (PSP)	0	30.3	8.21	8.09	110.1	594	ND
	3	24.9	8.09	7.66	95.7	562	129
	12	15.4	8.01	9.74	100.7	609	57.9
Sandies Creek (SC)	0	30.0	8.25	7.90	107.6	526	1.57
	3	25.4	7.82	6.47	81.6	313	871
	12	16.7	8.07	8.99	96.2	531	59.9
Victoria WWTP (VA)	0	30.3	8.06	7.67	105.1	588	7.20
	3	24.4	8.10	7.22	89.3	ND	454
	12	17.4	8.02	8.88	95.7	543	55.4
Victoria 1-mile (VB)	0	28.8	8.75	8.31	110.6	ND	180
	3	24.4	8.10	7.22	89.3	ND	454
	12	17.4	7.89	8.62	93.1	540	107

Table 4. Biological measurements (mean ± standard deviation; minimum and maximum in parentheses) of *Amblema plicata* collected at each site and time point (n = 20 per site and time point).

Site	Week	Length (mm)	Width (mm)	Height (mm)	Wet weight (g)
Lake Wood (LW)	0	99.9 ± 5.79	40.5 ± 2.30	71.7 ± 3.66	179 ± 31.6
		(91.0 - 112)	(35.5 - 44.5)	(67.0 - 79.5)	(144 - 248)
	3	101 ± 5.75	40.6 ± 2.59	72.0 ± 2.78	188 ± 31.5
		(92.5 - 114)	(36.5 - 47.0)	(66.5 - 77.5)	(147 - 272)
	12	98.5 ± 4.81	40.7 ± 2.88	71.0 ± 3.20	179 ± 28.4
		(90.5 - 109)	(33.0 - 45.0)	(67.5 - 78.0)	(128 - 248)
Plum Creek (PC)	3	99.0 ± 3.71	40.5 ± 2.26	71.9 ± 3.04	181 ± 25.2
		(93.5 - 106)	(36.0 - 44.0)	(66.0 - 78.5)	(136 - 229)
	12	97.3 ± 5.60	39.0 ± 2.18	70.1 ± 3.17	164 ± 22.4
		(86.5 - 106)	(35.0 - 43.0)	(62.0 - 75.0)	(122 - 200)
Palmetto State Park (PSP)	3	98.7 ± 6.85	38.6 ± 3.05	70.6 ± 4.48	171 ± 37.2
		(89.5 - 111)	(31.5 - 45.0)	(63.0 - 79.0)	(110 - 254)
	12	101 ± 4.45	42.4 ± 2.60	72.6 ± 2.33	196 ± 30.6
		(93.0 - 107)	(38.0 - 47.0)	(69.0 - 78.5)	(141 - 240)
Sandies Creek (SC)	3	96.4 ± 6.93	38.5 ± 2.63	69.6 ± 4.82	158 ± 30.8
		(84.0 - 111)	(34.0 - 43.5)	(61.5 - 78.5)	(108 - 218)
	12	100 ± 5.32	40.2 ± 1.56	71.2 ± 4.16	182 ± 28.8
		(92.0 - 110)	(37.0 - 42.5)	(65.5 - 81.0)	(144 - 236)
Victoria WWTP (VA)	3	98.4 ± 5.25	39.0 ± 2.00	70.3 ± 4.04	167 ± 26.4
		(89.0 - 106)	(35.5 - 41.5)	(60.0 - 78.0)	(114 - 208)
	12	103 ± 5.40	42.4 ± 2.65	73.5 ± 4.28	200 ± 31.4
		(94.5 - 115)	(36.0 - 48.5)	(66.0 - 80.5)	(157 - 280)
Victoria 1-mile (VB)	3	102 ± 5.80	40.3 ± 3.21	72.4 ± 3.59	194 ± 37.3
		(92.0 - 113)	(35.5 - 47.0)	(67.0 - 79.5)	(141 - 271)
	12	97.1 ± 3.89	38.3 ± 2.21	69.8 ± 2.10	161 ± 22.1
		(88.0 - 107)	(36.0 - 43.0)	(66.0 - 74.0)	(128 - 199)

Table 5. Concentration of essential trace elements (μg/g dry weight; mean ± standard deviation (n = 20 per site and time point), minimum and maximum concentrations in parentheses) in gill tissue of *Amblema plicata* at each site and time point.

Site	Week	Co	Cu	Fe	Mn	Ni	Se	Zn
Lake Wood (LW)	0	1.37 ± 0.266 (0.944 - 1.77)	6.36 ± 0.841 (4.45 - 8.46)	784 ± 276 (383 - 1464)	4385 ± 850 (2567 - 5632)	1.79 ± 0.338 (1.33 - 2.85)	4.04 ± 0.414 (3.06 - 4.95)	425 ± 84.8 (283 - 534)
	3	1.42 ± 0.346 (0.892 - 2.15)	6.53 ± 0.981 (5.06 - 8.94)	893 ± 366 (464 - 1732)	4666 ± 711 (3784 - 5754)	1.94 ± 0.307 (1.42 - 2.55)	3.83 ± 0.277 (3.16 - 4.24)	447 ± 72.5 (340 - 588)
	12	1.28 ± 0.234 (0.843 - 1.78)	7.35 ± 1.25 (5.29 - 9.29)	885 ± 372 (316 - 1545)	4602 ± 796 (3440 - 6401)	2.02 ± 0.28 (1.60 - 2.68)	3.67 ± 0.321 (3.13 - 4.32)	433 ± 64.3 (325 - 549)
Plum Creek (PC)	3	1.45 ± 0.276 (0.975 - 1.89)	6.55 ± 1.03 (4.56 - 9.36)	866 ± 449 (364 - 1604)	4680 ± 699 (3202 - 5692)	2.08 ± 0.368 (1.51 - 2.99)	3.43 ± 0.312 (3.07 - 4.29)	413 ± 62.1 (297 - 516)
	12	1.23 ± 0.267 (0.964 - 2.08)	9.02 ± 1.29 (6.55 - 11.6)	823 ± 317 (408 - 1455)	4775 ± 693 (3850 - 5818)	1.75 ± 0.216 (1.26 - 2.18)	3.38 ± 0.267 (3.08 - 4.07)	444 ± 61.2 (346 - 519)
Palmetto State Park (PSP)	3	1.37 ± 0.224 (1.09 - 1.98)	5.86 ± 0.62 (4.85 - 7.40)	650 ± 214 (374 - 1329)	4451 ± 619 (3549 - 5452)	1.78 ± 0.202 (1.44 - 2.20)	3.61 ± 0.34 (2.99 - 4.33)	409 ± 64.8 (298 - 557)
	12	1.44 ± 0.360 (0.938 - 2.15)	7.73 ± 1.37 (6.03 - 11.2)	830 ± 409 (311 - 1709)	4735 ± 772 (3358 - 6346)	1.85 ± 0.386 (1.09 - 2.91)	3.38 ± 0.418 (2.66 - 4.71)	415 ± 67.5 (323 - 524)
Sandies Creek (SC)	3	1.20 ± 0.284 (0.761 - 2.12)	6.86 ± 0.944 (4.70 - 7.97)	729 ± 359 (344 - 1669)	4075 ± 787 (2878 - 6066)	1.93 ± 0.234 (1.64 - 2.58)	3.66 ± 0.305 (2.95 - 4.03)	427 ± 94.1 (280 - 627)
	12	1.30 ± 0.279 (0.879 - 1.91)	7.64 ± 1.18 (5.87 - 10.6)	960 ± 419 (336 - 1759)	4874 ± 797 (3143 - 5884)	1.87 ± 0.307 (1.40 - 2.42)	3.35 ± 0.291 (2.86 - 3.98)	462 ± 77.9 (321 - 578)
Victoria WWTP (VA)	3	1.25 ± 0.248 (0.826 - 1.72)	6.27 ± 0.640 (5.41 - 7.30)	814 ± 416 (346 - 2149)	4590 ± 688 (3464 - 6076)	1.92 ± 0.314 (1.38 - 2.67)	3.35 ± 0.288 (2.91 - 4.03)	426 ± 71.0 (291 - 529)
	12	1.48 ± 0.358 (0.995 - 2.16)	7.94 ± 0.901 (6.38 - 9.62)	852 ± 420 (304 - 1781)	4841 ± 870 (3401 - 6545)	2.18 ± 0.396 (1.63 - 3.05)	3.19 ± 0.337 (2.72 - 4.03)	438 ± 73.6 (342 - 555)
Victoria 1-mile (VB)	3	1.37 ± 0.268 (1.01 - 2.01)	6.06 ± 0.824 (4.73 - 7.75)	886 ± 426 (364 - 1944)	4877 ± 854 (2729 - 6527)	1.93 ± 0.295 (1.52 - 2.52)	3.50 ± 0.389 (2.92 - 4.36)	440 ± 80.5 (309 - 563)
	12	1.26 ± 0.360 (0.952 - 2.20)	7.83 ± 1.64 (5.29 - 12.6)	912 ± 440 (403 - 1991)	4781 ± 838 (3414 - 6202)	2.02 ± 0.32 (1.28 - 2.61)	3.26 ± 0.258 (2.67 - 3.60)	451 ± 67.5 (340 - 583)

Table 6. Concentration of non-essential trace elements (μg/g dry weight; mean ± standard deviation (n = 20 per site and time point), minimum and maximum concentrations in parentheses) in gill tissue of *Amblema plicata* at each site and time point.

Site	Week	Ag	As	Cd	Cr	Hg	Pb	U
Lake Wood (LW)	0	0.0516 ± 0.0124 (0.0305 - 0.068)	9.51 ± 2.34 (6.65 - 35.5)	0.255 ± 0.0733 (0.153 - 0.460)	0.688 ± 0.184 (0.364 - 1.02)	0.0782 ± 0.0161 (0.0487 - 0.112)	0.435 ± 0.0367 (0.369 - 0.484)	0.104 ± 0.0268 (0.0635 - 0.168)
	3	0.0447 ± 0.0172 (0.0199 - 0.0729)	8.38 ± 1.85 (6.40 - 12.6)	0.277 ± 0.124 (0.162 - 0.759)	0.711 ± 0.176 (0.433 - 1.08)	0.0691 ± 0.0092 (0.0527 - 0.0836)	0.438 ± 0.0526 (0.337 - 0.555)	0.113 ± 0.0204 (0.0879 - 0.157)
	12	0.0547 ± 0.0158 (0.0321 - 0.0799)	8.67 ± 1.94 (5.29 - 12.5)	0.237 ± 0.0486 (0.168 - 0.331)	0.696 ± 0.224 (0.335 - 1.25)	0.0705 ± 0.0099 (0.0575 - 0.0978)	0.389 ± 0.0417 (0.314 - 0.460)	0.0981 ± 0.0205 (0.0632 - 0.139)
Plum Creek (PC)	3	0.0406 ± 0.0136 (0.0227 - 0.0672)	9.14 ± 2.64 (5.91 - 15.8)	0.245 ± 0.0615 (0.146 - 0.413)	0.722 ± 0.219 (0.433 - 1.16)	0.0793 ± 0.0113 (0.0595 - 0.100)	0.453 ± 0.0533 (0.337 - 0.543)	0.114 ± 0.027 (0.0835 - 0.180)
	12	0.0541 ± 0.0130 (0.0310 - 0.0713)	8.30 ± 1.57 (5.34 - 12.2)	0.217 ± 0.0563 (0.134 - 0.327)	0.617 ± 0.118 (0.391 - 0.809)	0.0823 ± 0.0081 (0.0700 - 0.104)	0.397 ± 0.0464 (0.293 - 0.484)	0.0985 ± 0.0193 (0.0612 - 0.131)
Palmetto State Park (PSP)	3	0.0466 ± 0.0157 (0.0261 - 0.0763)	7.70 ± 1.65 (5.74 - 13.2)	0.250 ± 0.0386 (0.183 - 0.310)	0.581 ± 0.195 (0.355 - 1.22)	0.0729 ± 0.0084 (0.0565 - 0.0855)	0.421 ± 0.0636 (0.279 - 0.506)	0.112 ± 0.0204 (0.0796 - 0.154)
	12	0.0605 ± 0.0184 (0.0379 - 0.0954)	8.69 ± 1.94 (6.46 - 14.1)	0.254 ± 0.0774 (0.134 - 0.466)	0.710 ± 0.162 (0.404 - 1.04)	0.0754 ± 0.0136 (0.0523 - 0.110)	0.398 ± 0.0652 (0.297 - 0.512)	0.0979 ± 0.0163 (0.0669 - 0.132)
Sandies Creek (SC)	3	0.0503 ± 0.0201 (0.0265 - 0.0890)	8.73 ± 2.23 (5.69 - 13.9)	0.244 ± 0.0536 (0.167 - 0.370)	0.557 ± 0.183 (0.296 - 1.04)	0.0800 ± 0.0097 (0.0612 - 0.0959)	0.435 ± 0.0567 (0.296 - 0.525)	0.0993 ± 0.0253 (0.0579 - 0.147)
	12	0.0631 ± 0.0172 (0.0346 - 0.0990)	8.96 ± 1.77 (5.12 - 11.7)	0.241 ± 0.0601 (0.171 - 0.375)	0.656 ± 0.158 (0.469 - 1.14)	0.0782 ± 0.0110 (0.0626 - 0.116)	0.399 ± 0.0434 (0.328 - 0.480)	0.102 ± 0.0183 (0.0698 - 0.138)
Victoria WWTP (VA)	3	0.0488 ± 0.0175 (0.0264 - 0.0869)	9.44 ± 4.24 (6.46 - 26.3)	0.241 ± 0.0426 (0.191 - 0.353)	0.67 ± 0.215 (0.424 - 1.21)	0.0723 ± 0.0091 (0.0572 - 0.0911)	0.425 ± 0.0529 (0.359 - 0.590)	0.114 ± 0.0185 (0.0851 - 0.165)
	12	0.0478 ± 0.0108 (0.0346 - 0.0712)	9.26 ± 2.17 (6.31 - 13.7)	0.271 ± 0.104 (0.166 - 0.642)	0.675 ± 0.172 (0.364 - 0.942)	0.0734 ± 0.0095 (0.0550 - 0.0864)	0.386 ± 0.0551 (0.279 - 0.479)	0.114 ± 0.0314 (0.0728 - 0.172)
Victoria 1-mile (VB)	3	0.0428 ± 0.0171 (0.0205 - 0.0749)	7.85 ± 1.15 (6.26 - 10.9)	0.235 ± 0.0679 (0.134 - 0.456)	0.685 ± 0.249 (0.345 - 1.32)	0.0678 ± 0.0074 (0.0572 - 0.0932)	0.413 ± 0.0618 (0.308 - 0.546)	0.118 ± 0.0212 (0.0781 - 0.174)
	12	0.0567 ± 0.0191 (0.0329 - 0.0903)	8.56 ± 1.60 (6.84 - 12.0)	0.259 ± 0.0815 (0.155 - 0.438)	0.687 ± 0.179 (0.428 - 1.06)	0.0730 ± 0.0089 (0.0597 - 0.0958)	0.409 ± 0.0526 (0.284 - 0.503)	0.0993 ± 0.0139 (0.0739 - 0.125)

Table 7. Se:Hg molar ratios [mean ± standard deviation (SD), minimum and maximum values] in gill tissue of *Amblema plicata* at each site and time point.

Site	Week	Mean	SD	Minimum	Maximum
Lake Wood (LW)	0	136	25.0	84.2	169
	3	143	24.1	103	175
	12	134	19.5	96.0	159
Plum Creek (PC)	3	111	14.9	83.1	133
	12	105	10.0	87.8	120
Palmetto State Park (PSP)	3	127	20.4	93.9	185
	12	117	25.4	82.9	162
Sandies Creek (SC)	3	118	14.7	91.0	139
	12	110	13.4	83.3	133
Victoria WWTP (VA)	3	119	13.2	100	139
	12	112	19.8	90.7	147
Victoria 1-mile (VB)	3	132	18.2	94.5	170
	12	114	11.6	94.4	133

Table 8. Organic carbon content and grain size distribution as determine by percentage of coarse sediment (> 63μm) for sediment at each site (mean ± standard deviation) at week 12. LW = Lake Wood, PC = Plum Creek, PSP = Palmetto State Park, SC = Sandies Creek, VA = Victoria wastewater treatment plant (WWTP), VB = Victoria 1 mile downstream of the WWTP.

Site	Organic carbon (%)	Coarse sediment (%)
LW	2.6 ± 0.08	38.3 ± 0.8
PC	3.8 ± 0.3	73.4 ± 0.5
PSP	3.6 ± 0.02	52.3 ± 0.8
SC	1.8 ± 0.07	79.6 ± 2.5
VA	6.5 ± 0.1	38.9 ± 0.3
VB	4.0 ± 0.01	47.9 ± 2.8

Table 9. Trace element concentrations (μg/g dry weight) in sediment collected from each site at week 12. LW = Lake Wood, PC = Plum Creek, PSP = Palmetto State Park, SC = Sandies Creek, VA = Victoria wastewater treatment plant (WWTP), VB = Victoria 1 mile downstream of the WWTP.

	LW	**PC**	**PSP**	**SC**	**VA**	**VB**
Essential						
Co	2.79	3.22	3.79	1.79	3.60	3.28
Cu	3.07	3.53	4.14	1.66	4.17	3.68
Fe	8726	8324	9630	5140	10479	9402
Mn	136	106	92.3	88.8	205	178
Ni	6.74	5.50	7.58	3.24	6.90	5.96
Se	0.411	0.192	0.234	0.143	0.241	0.355
Zn	16.1	21.1	22.5	10.4	24.9	22.9
Non-essential						
Ag	0.0195	0.0170	0.0122	0.0040	0.0103	0.0058
As	1.89	2.32	2.20	1.58	2.76	2.60
Cd	0.0829	0.0647	0.0787	0.0622	0.110	0.100
Cr	12.5	15.0	18.7	6.81	14.4	12.9
Hg	0.0081	0.0114	0.0132	0.0051	0.0111	0.0249
Pb	4.88	5.85	5.92	3.52	6.77	5.88
U	0.663	0.446	0.471	0.453	0.584	0.497

Table 10. Essential trace element biota sediment accumulation factors (BSAF) (mean ± standard deviation, minimum and maximum values in parentheses) in gill tissue of *Amblema plicata* at all sites and time points.

Site	Week	Co	Cu	Fe	Mn	Ni	Se	Zn
Lake Wood (LW)	0	0.490 ± 0.0954 (0.339 - 0.635)	2.07 ± 0.274 (1.45 - 2.75)	0.0899 ± 0.0317 (0.0439 - 0.168)	32.2 ± 6.25 (18.9 - 41.4)	0.265 ± 0.0501 (0.197 - 0.423)	9.82 ± 1.01 (7.45 - 12.0)	26.4 ± 5.25 (17.5 - 33.1)
	3	0.508 ± 0.124 (0.320 - 0.771)	2.13 ± 0.319 (1.65 - 2.91)	0.102 ± 0.0420 (0.0532 - 0.198)	34.3 ± 5.22 (27.8 - 42.3)	0.287 ± 0.0455 (0.211 - 0.378)	9.31 ± 0.675 (7.69 - 10.3)	27.7 ± 4.50 (21.1 - 36.4)
	12	0.460 ± 0.0841 (0.303 - 0.640)	2.39 ± 0.408 (1.72 - 3.02)	0.101 ± 0.0427 (0.0362 - 0.177)	33.8 ± 5.85 (25.3 - 47.1)	0.299 ± 0.0415 (0.238 - 0.398)	8.92 ± 0.782 (7.62 - 10.5)	26.8 ± 3.99 (20.1 - 34.0)
Plum Creek (PC)	3	0.452 ± 0.0857 (0.303 - 0.586)	1.85 ± 0.291 (1.29 - 2.65)	0.104 ± 0.0539 (0.0438 - 0.193)	44.0 ± 6.58 (30.1 - 53.6)	0.378 ± 0.0671 (0.274 - 0.545)	17.8 ± 1.62 (15.9 - 22.3)	19.5 ± 2.94 (14.0 - 24.4)
	12	0.383 ± 0.0830 (0.299 - 0.647)	2.55 ± 0.366 (1.85 - 3.28)	0.0988 ± 0.0381 (0.0490 - 0.175)	44.9 ± 6.52 (36.2 - 54.8)	0.318 ± 0.0393 (0.230 - 0.396)	17.6 ± 1.39 (16.0 - 21.1)	21.0 ± 2.90 (16.4 - 24.6)
Palmetto State Park (PSP)	3	0.361 ± 0.0592 (0.289 - 0.524)	1.42 ± 0.150 (1.17 - 1.79)	0.0675 ± 0.0222 (0.0388 - 0.138)	48.2 ± 6.70 (38.5 - 59.1)	0.236 ± 0.0266 (0.190 - 0.290)	15.4 ± 1.45 (12.8 - 18.5)	18.2 ± 2.87 (13.2 - 24.7)
	12	0.381 ± 0.0950 (0.248 - 0.568)	1.87 ± 0.332 (1.46 - 2.71)	0.0862 ± 0.0425 (0.0323 - 0.177)	51.3 ± 8.37 (36.4 - 68.8)	0.244 ± 0.0510 (0.144 - 0.385)	14.5 ± 1.79 (11.4 - 20.1)	18.4 ± 2.99 (14.3 - 23.2)
Sandies Creek (SC)	3	0.669 ± 0.159 (0.425 - 1.18)	4.13 ± 0.568 (2.83 - 4.80)	0.142 ± 0.0698 (0.0669 - 0.325)	45.9 ± 8.86 (32.4 - 68.3)	0.595 ± 0.0723 (0.505 - 0.797)	25.6 ± 2.14 (20.6 - 28.2)	41.0 ± 9.04 (26.9 - 60.3)
	12	0.726 ± 0.156 (0.491 - 1.07)	4.60 ± 0.711 (3.53 - 6.41)	0.187 ± 0.0815 (0.0653 - 0.342)	54.9 ± 8.98 (35.4 - 66.3)	0.577 ± 0.0947 (0.432 - 0.748)	23.5 ± 2.04 (20.0 - 27.9)	44.4 ± 7.49 (30.8 - 55.5)
Victoria WWTP (VA)	3	0.348 ± 0.0688 (0.229 - 0.477)	1.50 ± 0.154 (1.30 - 1.75)	0.0777 ± 0.0397 (0.0331 - 0.205)	22.3 ± 3.35 (16.9 - 29.6)	0.278 ± 0.0455 (0.200 - 0.387)	13.9 ± 1.20 (12.1 - 16.7)	17.1 ± 2.85 (11.7 - 21.2)
	12	0.410 ± 0.0994 (0.276 - 0.600)	1.90 ± 0.216 (1.53 - 2.31)	0.0813 ± 0.0401 (0.0290 - 0.170)	23.6 ± 4.23 (16.6 - 31.9)	0.316 ± 0.0574 (0.236 - 0.441)	13.2 ± 1.40 (11.3 - 16.7)	17.5 ± 2.95 (13.7 - 22.3)
Victoria 1-mile (VB)	3	0.418 ± 0.0818 (0.308 - 0.613)	1.65 ± 0.224 (1.29 - 2.11)	0.0942 ± 0.0453 (0.0388 - 0.207)	27.4 ± 4.81 (15.4 - 36.7)	0.325 ± 0.0495 (0.255 - 0.423)	9.86 ± 1.10 (8.23 - 12.3)	19.2 ± 3.52 (13.5 - 24.6)
	12	0.383 ± 0.110 (0.290 - 0.670)	2.13 ± 0.446 (1.44 - 3.43)	0.097 ± 0.0468 (0.0428 - 0.212)	26.9 ± 4.71 (19.2 - 34.9)	0.338 ± 0.0536 (0.215 - 0.438)	9.19 ± 0.729 (7.53 - 10.1)	19.7 ± 2.95 (14.9 - 25.5)

Table 11. Non-essential trace element biota sediment accumulation factors (BSAF) (mean ± standard deviation, minimum and maximum values in parentheses) in gill tissue of *Amblema plicata* at all sites and time points.

Site	Week	Ag	As	Cd	Cr	Hg	Pb	U
Lake Wood (LW)	0	2.65 ± 0.636 (1.57 - 3.49)	5.03 ± 1.24 (3.52 - 18.8)	3.07 ± 0.884 (1.85 - 5.54)	0.0549 ± 0.0146 (0.0290 - 0.0812)	9.65 ± 1.99 (6.02 - 13.8)	0.0892 ± 0.0075 (0.0756 - 0.0993)	0.158 ± 0.0404 (0.0959 - 0.253)
	3	2.29 ± 0.884 (1.02 - 3.74)	4.43 ± 0.979 (3.39 - 6.68)	3.34 ± 1.49 (1.96 - 9.16)	0.0567 ± 0.0140 (0.0345 - 0.0864)	8.53 ± 1.13 (6.51 - 10.3)	0.0899 ± 0.0108 (0.0691 - 0.114)	0.171 ± 0.0308 (0.133 - 0.237)
	12	2.81 ± 0.813 (1.65 - 4.10)	4.58 ± 1.03 (2.80 - 6.58)	2.86 ± 0.587 (2.02 - 4.00)	0.0555 ± 0.0179 (0.0267 - 0.0994)	8.70 ± 1.23 (7.10 - 12.1)	0.0797 ± 0.0085 (0.0644 - 0.0943)	0.148 ± 0.0310 (0.0953 - 0.210)
Plum Creek (PC)	3	2.39 ± 0.801 (1.34 - 3.95)	3.94 ± 1.14 (2.54 - 6.83)	3.79 ± 0.951 (2.26 - 6.38)	0.0483 ± 0.0147 (0.0290 - 0.0778)	1.23 ± 0.175 (0.920 - 1.55)	0.0776 ± 0.0091 (0.0576 - 0.0930)	0.256 ± 0.0605 (0.187 - 0.404)
	12	3.19 ± 0.768 (1.82 - 4.20)	3.58 ± 0.677 (2.30 - 5.24)	3.36 ± 0.870 (2.07 - 5.05)	0.0412 ± 0.0079 (0.0261 - 0.0541)	1.27 ± 0.125 (1.08 ± 1.61)	0.0680 ± 0.0079 (0.0502 - 0.0829)	0.221 ± 0.0432 (0.137 - 0.293)
Palmetto State Park (PSP)	3	3.82 ± 1.28 (2.14 - 6.24)	3.50 ± 0.749 (2.61 - 6.00)	3.18 ± 0.491 (2.33 - 3.93)	0.0311 ± 0.0104 (0.0190 - 0.0651)	5.52 ± 0.64 (4.28 - 6.48)	0.0712 ± 0.0108 (0.0472 - 0.0854)	0.239 ± 0.0433 (0.169 - 0.328)
	12	4.95 ± 1.50 (3.10 - 7.81)	3.95 ± 0.885 (2.94 - 6.41)	3.23 ± 0.983 (1.70 - 5.92)	0.0380 ± 0.0087 (0.0216 - 0.0557)	5.72 ± 1.03 (3.97 - 8.32)	0.0672 ± 0.0110 (0.0502 - 0.0865)	0.208 ± 0.0346 (0.142 - 0.280)
Sandies Creek (SC)	3	12.7 ± 5.07 (6.69 - 22.5)	5.52 ± 1.41 (3.60 - 8.81)	3.93 ± 0.862 (2.68 - 5.95)	0.0818 ± 0.0269 (0.0435 - 0.153)	15.7 ± 1.89 (12.0 - 18.8)	0.124 ± 0.0161 (0.0839 - 0.149)	0.219 ± 0.0558 (0.128 - 0.325)
	12	15.9 ± 4.33 (8.73 - 25.0)	5.67 ± 1.12 (3.24 - 7.43)	3.87 ± 0.967 (2.74 - 6.03)	0.0963 ± 0.0232 (0.0688 - 0.167)	15.3 ± 2.16 (12.3 - 22.7)	0.113 ± 0.0123 (0.0932 - 0.136)	0.226 ± 0.0403 (0.154 - 0.303)
Victoria WWTP (VA)	3	4.73 ± 1.70 (2.56 - 8.42)	3.42 ± 1.54 (2.34 - 9.54)	2.19 ± 0.387 (1.74 - 3.21)	0.0466 ± 0.0149 (0.0295 - 0.0840)	6.52 ± 0.822 (5.15 - 8.21)	0.0628 ± 0.0078 (0.0531 - 0.0871)	0.196 ± 0.0317 (0.146 - 0.282)
	12	4.63 ± 1.05 (3.36 - 6.90)	3.36 ± 0.786 (2.29 - 4.96)	2.46 ± 0.947 (1.51 - 5.84)	0.0469 ± 0.0120 (0.0253 - 0.0654)	6.62 ± 0.853 (4.96 - 7.78)	0.0570 ± 0.0081 (0.0412 - 0.0707)	0.195 ± 0.0537 (0.125 - 0.295)
Victoria 1-mile (VB)	3	7.32 ± 2.92 (3.51 - 12.8)	3.02 ± 0.442 (2.40 - 4.20)	2.34 ± 0.678 (1.34 - 4.55)	0.0532 ± 0.0193 (0.0268 - 0.102)	2.72 ± 0.299 (2.30 - 3.74)	0.0702 ± 0.0105 (0.0524 - 0.0929)	0.237 ± 0.0425 (0.157 - 0.350)
	12	9.70 ± 3.26 (5.62 - 15.4)	3.29 ± 0.613 (2.63 - 4.61)	2.58 ± 0.814 (1.55 - 4.38)	0.0534 ± 0.0139 (0.0333 - 0.0820)	2.93 ± 0.358 (2.40 - 3.85)	0.0695 ± 0.0089 (0.0482 - 0.0855)	0.200 ± 0.0279 (0.149 - 0.251)

Table 12. Concentration of nutrients dissolved organic carbon (DOC), chlorophyll a, total suspended solids (TSS), and non-volatile suspended solids (NVSS) in water collected at each site and time point.

Site	Week	Total nitrogen (mg/L)	Nitrate (mg/L)	Ammonium (mg/L)	Soluble reactive phosphorus (mg/L)	DOC (mg/L)	Chlorophyll a (µg/L)	TSS (mg/L)	NVSS (mg/L)
Lake Wood (LW)	0	0.552	0.340	0.377	0.0176	42.0	3.50	21.4	16.1
	3	2.05	1.97	0.421	0.0656	47.2	2.01	15.5	11.0
	12	1.80	1.97	0.448	0.0314	52.3	0.905	12.8	9.35
Plum Creek (PC)	0	4.81	4.59	0.566	1.02	74.2	3.86	60.6	51.3
	3	3.52	3.18	0.455	0.469	54.0	1.48	23.5	18.2
	12	6.02	5.07	0.783	0.483	64.5	3.17	1.40	0.377
Palmetto State Park (PSP)	0	1.24	1.04	0.440	0.0923	47.1	2.76	28.9	22.3
	3	1.76	1.83	0.263	0.0526	50.8	1.30	34.4	26.8
	12	1.76	1.71	0.409	0.0400	57.4	0.930	15.2	11.6
Sandies Creek (SC)	0	0.469	0.189	0.295	0.0229	43.7	6.63	33.5	26.2
	3	2.12	1.58	1.14	0.191	31.4	3.43	387	321
	12	1.73	1.58	0.276	0.0485	53.7	1.22	42.7	35.2
Victoria WWTP (VA)	0	2.26	0.911	1.02	0.261	45.2	9.42	25.2	16.7
	3	2.08	1.90	0.729	0.161	48.7	3.37	81.0	68.3
	12	2.07	1.73	0.432	0.107	54.7	1.84	33.5	27.1
Victoria 1-mile (VB)	0	1.20	0.750	0.451	0.119	45.1	6.46	33.9	26.7
	3	1.90	1.62	0.636	0.122	47.2	3.43	80.0	67.1
	12	12.0	1.65	0.475	0.0784	57.7	1.86	58.9	50.1

Table 13. Concentration of anions and cations (mg/L) in water collected at each site and time point.

		Anion				Cation			
Site	**Week**	**Bromide**	**Chloride**	**Fluoride**	**Sulphate**	**Calcium**	**Magnesium**	**Potassium**	**Sodium**
Lake Wood (LW)	0	0.416	31.3	1.71	36.5	59.0	18.5	3.54	24.5
	3	0	18.8	1.57	29.5	72.9	14.8	3.27	15.5
	12	0	21.8	1.35	24.8	81.1	16.7	1.81	14.3
Plum Creek (PC)	0	4.21	268	6.75	118	142	19.0	18.0	211
	3	1.04	96.7	3.10	73.8	88.6	10.6	9.56	73.2
	12	1.02	123	2.83	96.6	118	12.9	8.74	89.8
Palmetto State Park (PSP	0	0.895	54.5	1.74	37.8	67.3	18.1	4.21	41.1
	3	0.840	27.7	1.74	34.9	82.3	16.7	3.42	21.5
	12	0.707	32.7	1.49	35.9	90.5	18.6	2.14	23.1
Sandies Creek (SC)	0	0.858	38.7	1.76	38.4	58.8	18.9	3.94	32.9
	3	0	15.4	1.66	19.9	44.0	7.04	5.96	16.9
	12	0.684	23.6	1.41	27.9	80.9	16.9	2.09	17.6
Victoria WWTP (VA)	0	0.886	54.2	1.88	41.1	59.7	17.4	4.90	44.1
	3	0	38.7	1.77	32.6	70.6	15.1	4.47	26.1
	12	0.679	26.0	1.44	27.4	80.9	16.4	2.42	20.0
Victoria 1-mile (VB)	0	0.883	47.2	1.80	36.0	60.5	17.6	4.38	38.4
	3	0.415	41.8	1.74	32.2	71.1	15.2	4.40	24.7
	12	0.341	25.0	1.43	27.2	81.4	16.5	2.35	19.4

Table 14. Spearman's rank order correlation results between general health indicators (condition factor and total protein) and trace elements. Only significant relationships are presented. No significant relationships were found between general health indicators and nutrients, ions, total suspended solids, and non-volatile suspended solids.

General health indicator	Trace element	Week	Correlation coefficient (rho)	p-value
Condition factor	Cr	3	0.31	< 0.01
	Co	3	0.41	< 0.01
	U	3	0.18	0.056
	Cr	12	0.32	0.004
	Mn	12	0.21	0.023
	Co	12	0.62	< 0.01
	Ni	12	0.29	0.0012
	Cu	12	-0.33	< 0.01
	Pb	12	-0.19	0.038
	U	12	0.37	< 0.01
Total protein	As	3	-0.33	0.02

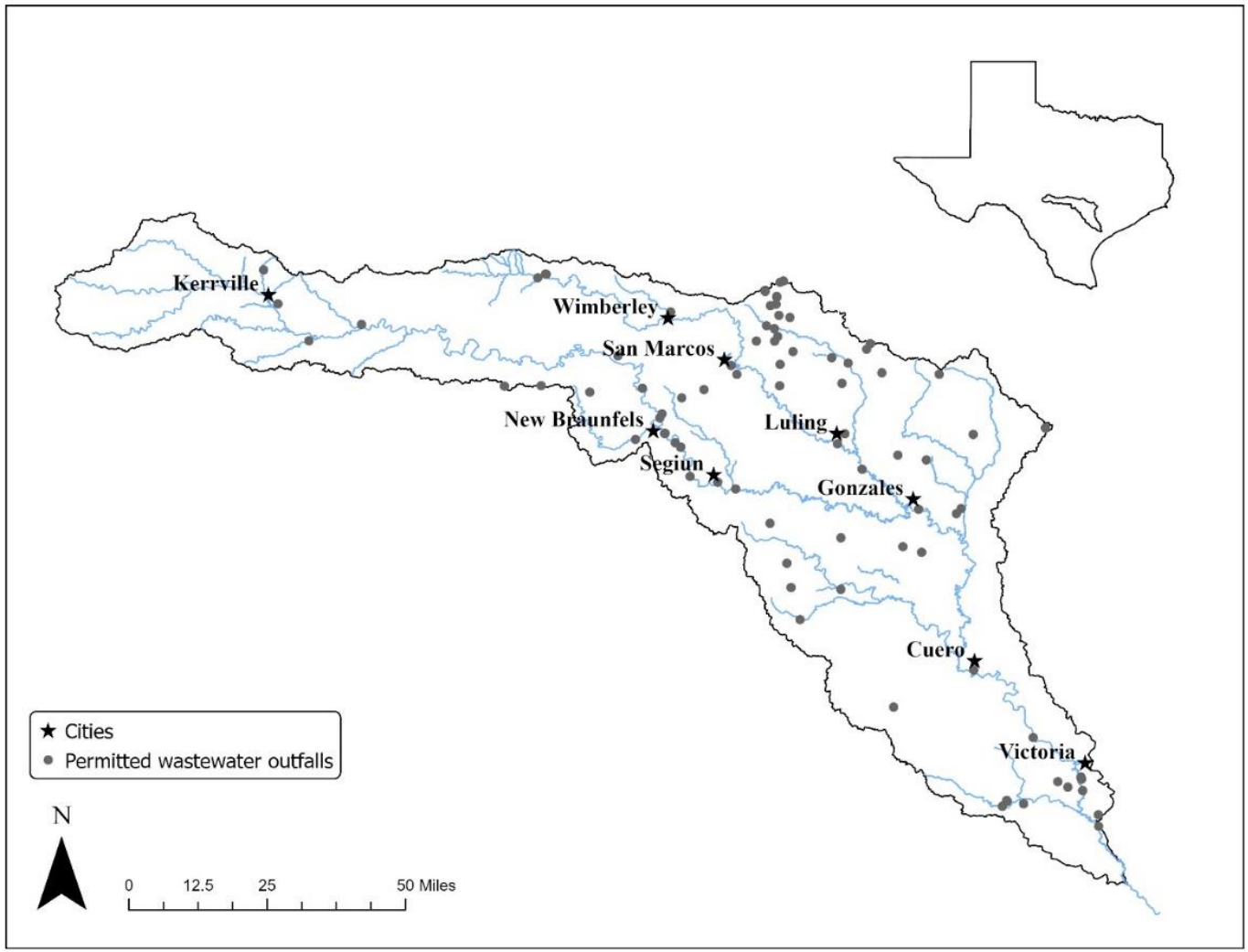

Figure 1. Location of permitted wastewater outfalls within the Guadalupe River basin. (TCEQ, 2014)

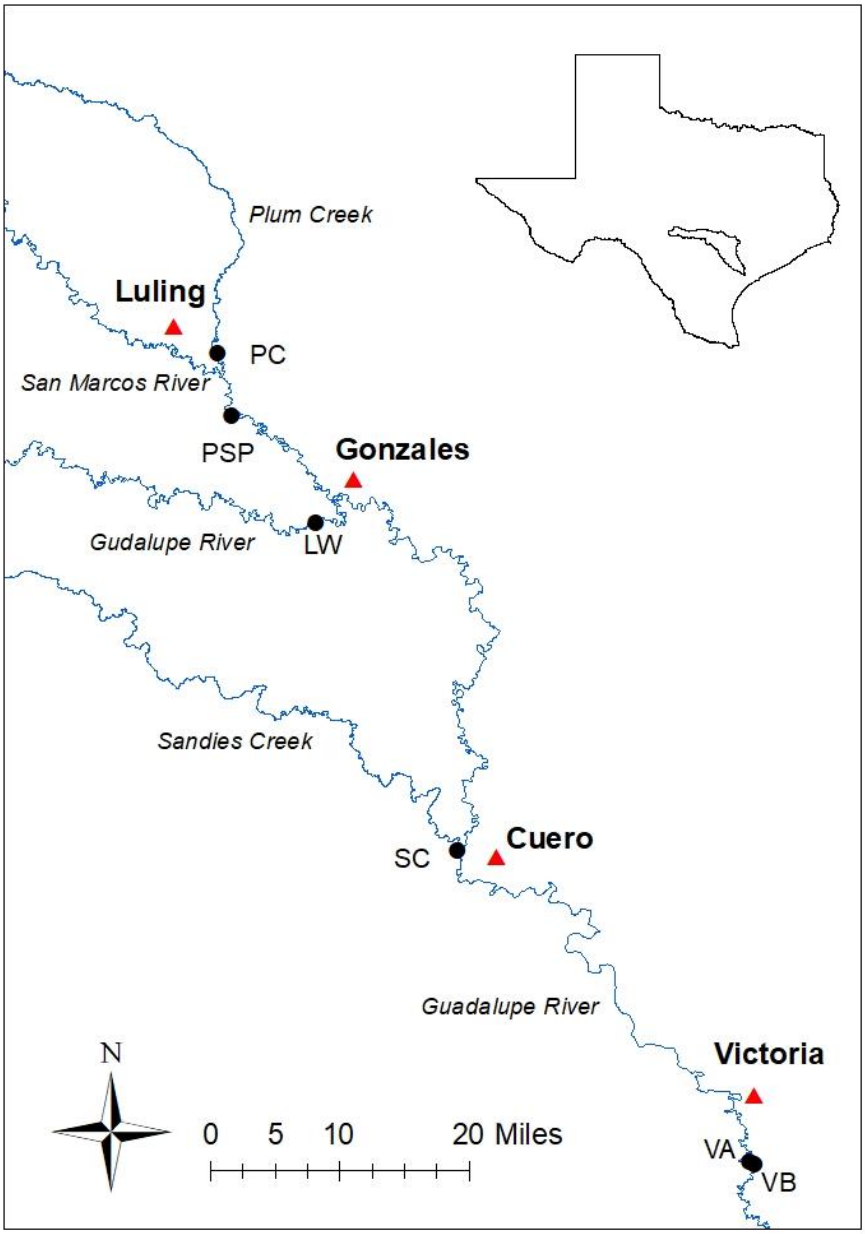

Figure 2. Location of study sites within the Guadalupe River basin. LW = Lake Wood, PC = Plum Creek, PSP = Palmetto State Park, SC = Sandies Creek, VA = Victoria wastewater treatment plant (WWTP), VB = Victoria 1 mile downstream of the WWTP

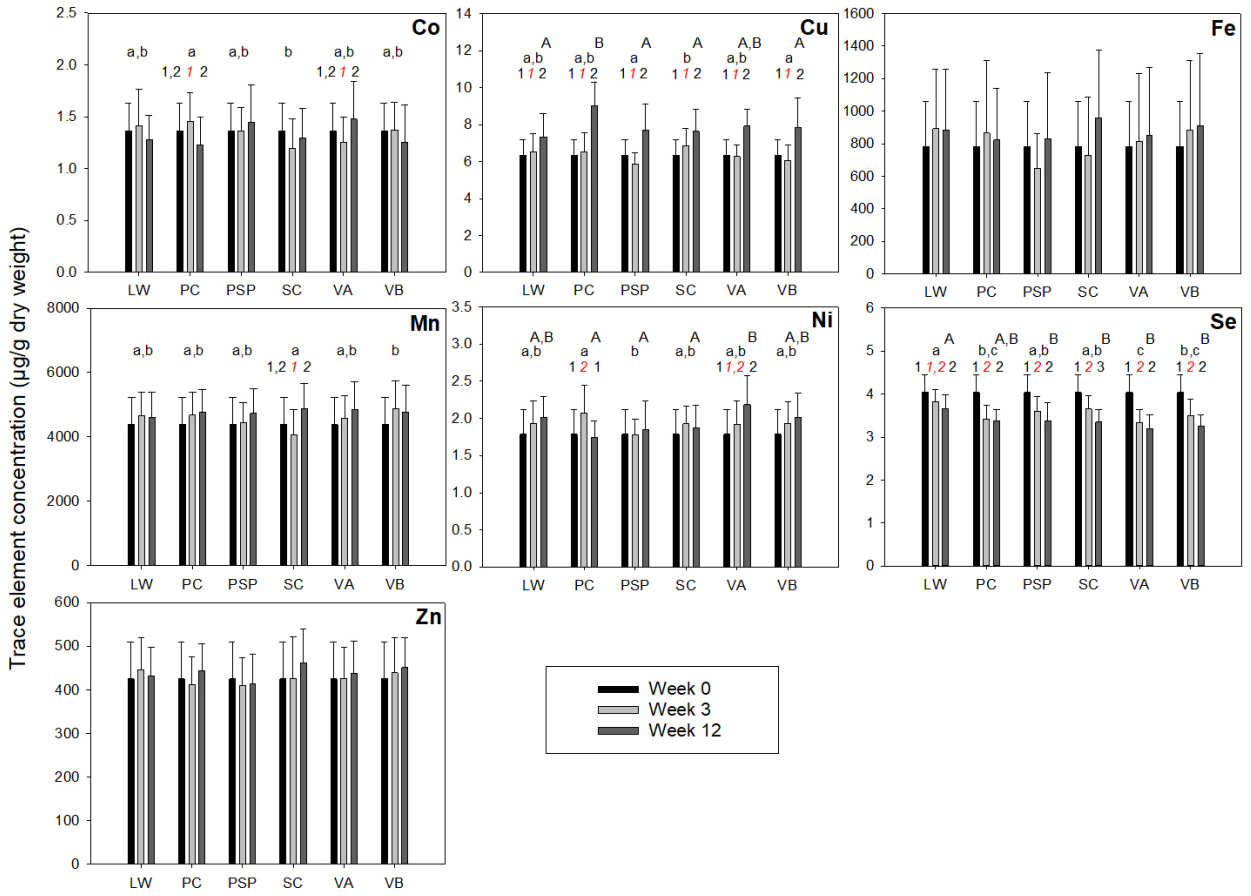

Figure 3. Concentration of essential trace elements (mean ± standard deviation) in gill tissue of *Amblema plicata* at each site at week 0 (LW control), 3, and 12 of the caged transplant experiment. Different numbers, lowercase letters, and uppercase letters represent significant differences ($p < 0.05$, one-way ANOVA) among timepoints within sites (for clarity, red italicized numbers represent week 3 differences), among sites for week 3, and among sites for week 12, respectively. LW = Lake Wood, PC = Plum Creek, PSP = Palmetto State Park, SC = Sandies Creek, VA = Victoria wastewater treatment plant (WWTP), VB = Victoria 1 mile downstream of the WWTP.

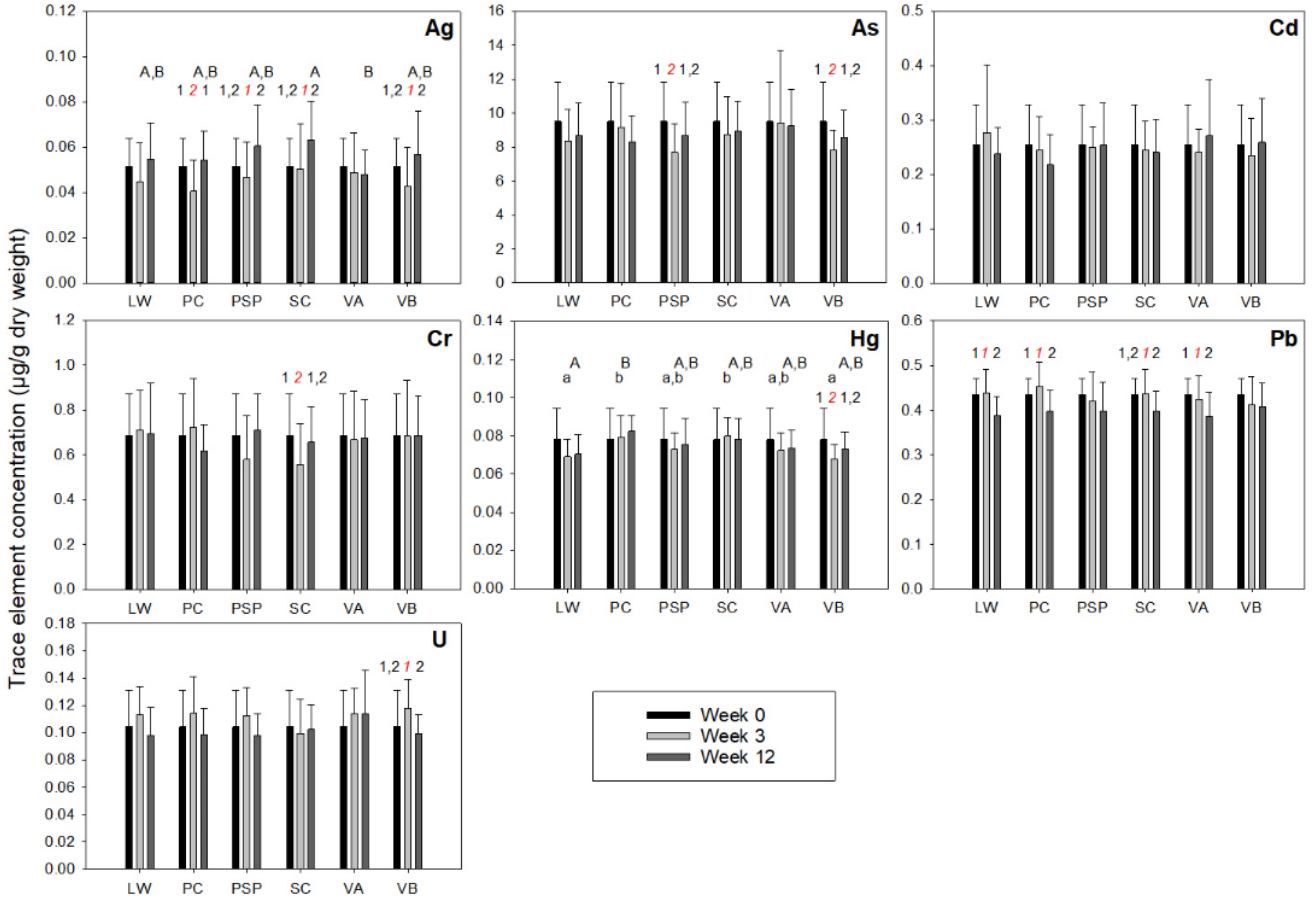

Figure 4. Concentration of non-essential trace elements (mean ± standard deviation) in gill tissue of *Amblema plicata* at each site at week 0 (LW control), 3, and 12 of the caged transplant experiment. Numbers, lowercase letters, and uppercase letters represent significant differences ($p < 0.05$, one-way ANOVA) among timepoints within sites (for clarity, red italicized numbers represent week 3 differences), among sites for week 3, and among sites for week 12, respectively. LW = Lake Wood, PC = Plum Creek, PSP = Palmetto State Park, SC = Sandies Creek, VA = Victoria wastewater treatment plant (WWTP), VB = Victoria 1 mile downstream of the WWTP.

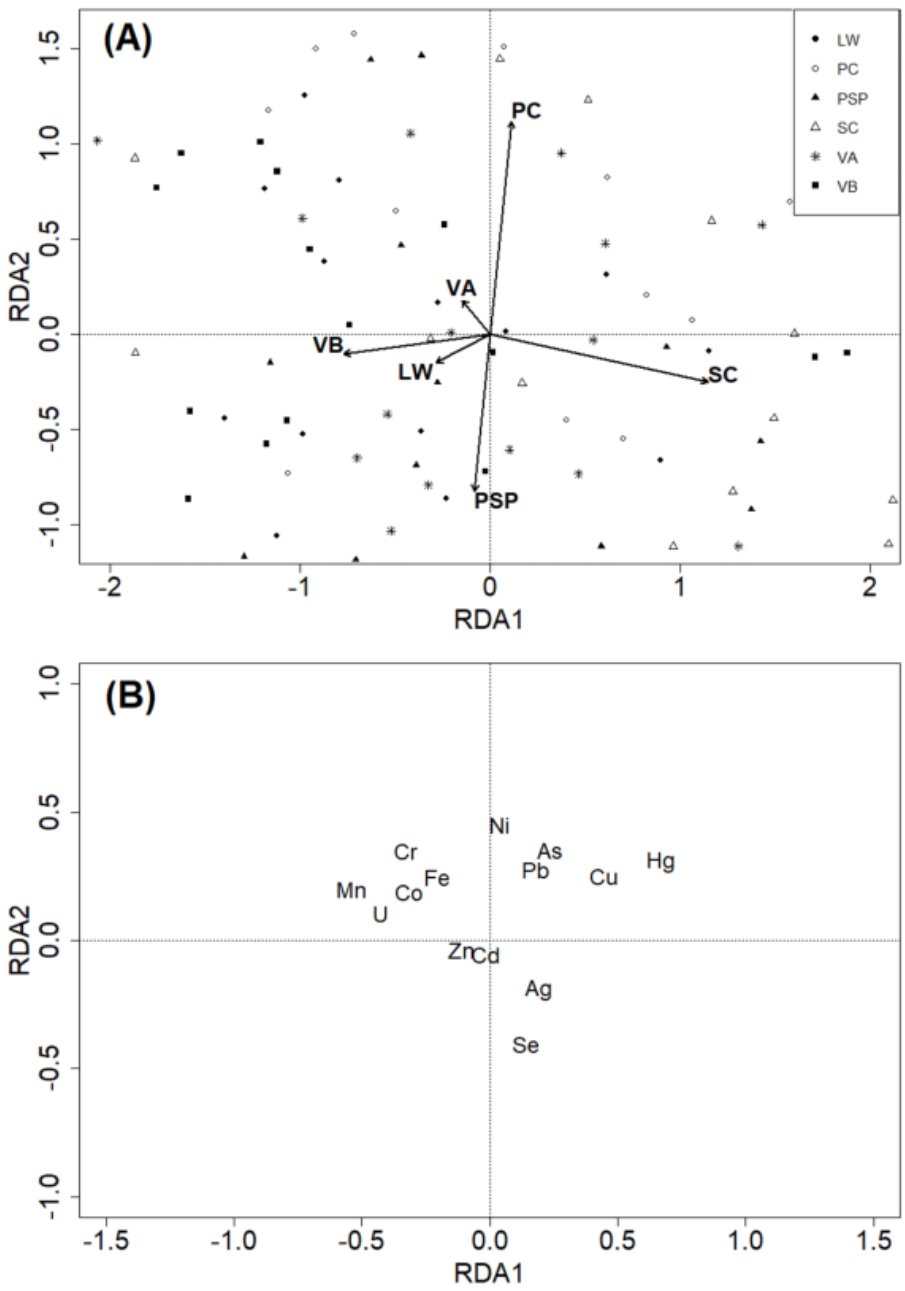

Figure 5. Redundancy analysis (RDA) biplots of trace element concentrations in gill tissue of *Amblema plicata* at all sites at week 3. Site data presented as (A) symbols representing individual mussels at different sites and (B) trace element distribution patterns. LW = Lake Wood, PC = Plum Creek, PSP = Palmetto State Park, SC = Sandies Creek, VA = Victoria wastewater treatment plant (WWTP), VB = Victoria 1 mile downstream of the WWTP. Note: scale difference between (A) and (B).

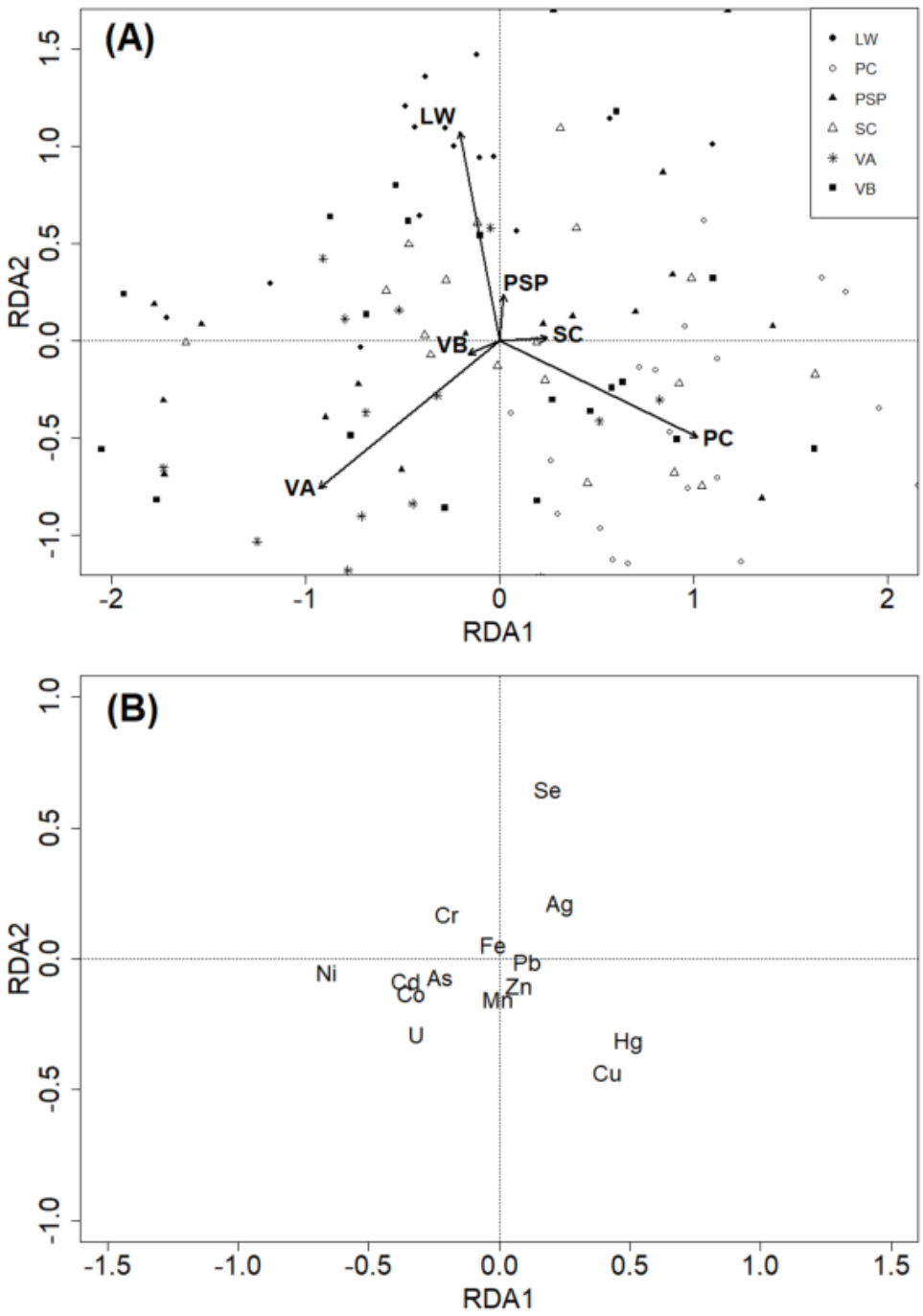

Figure 6. Redundancy analysis (RDA) biplots of trace element concentrations in gill tissue of *Amblema plicata* at all sites at week 12. Site data presented as (A) symbols representing individual mussels at different sites and (B) trace element distribution patterns. LW = Lake Wood, PC = Plum Creek, PSP = Palmetto State Park, SC = Sandies Creek, VA = Victoria wastewater treatment plant (WWTP), VB = Victoria 1 mile downstream of the WWTP. Note: Scale difference between (A) and (B).

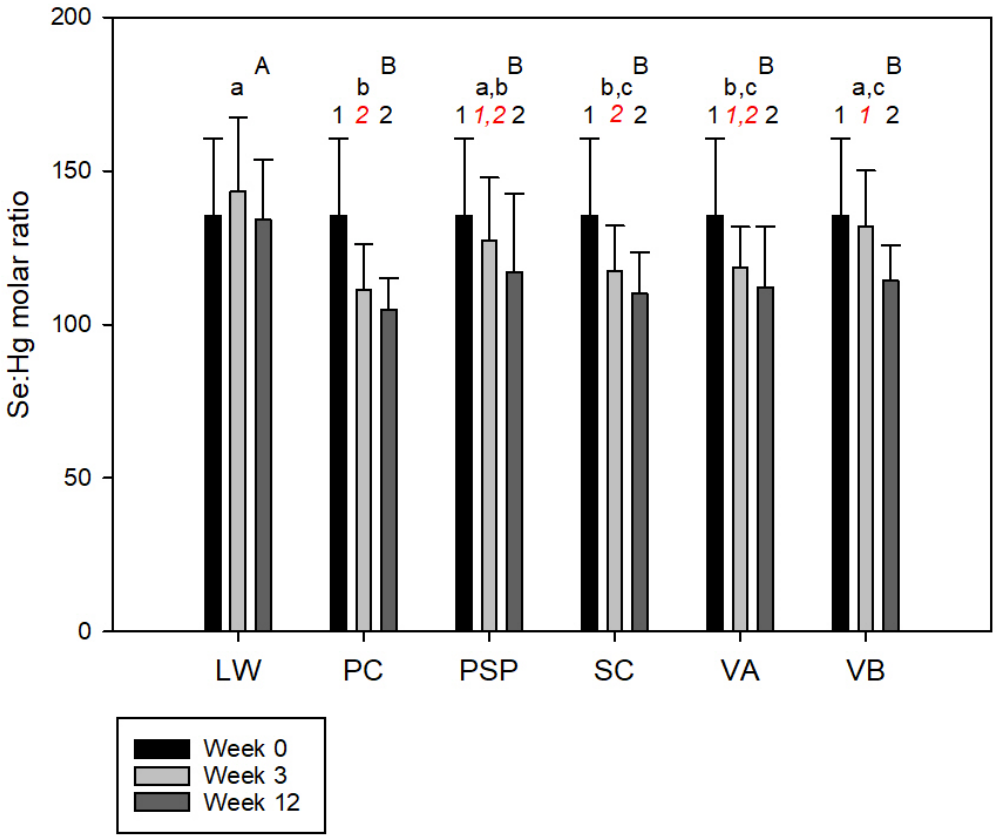

Figure 7. Se:Hg molar ratios (mean ± standard deviation) in gill tissue of *Amblema plicata* at each site at week 0 (LW control), 3, and 12 of the caged transplant experiment. Numbers, lowercase letters, and uppercase letters represent significant differences ($p < 0.05$, one-way ANOVA) among timepoints within sites (for clarity, red italicized numbers represent week 3 differences), among sites for week 3, and among sites for week 12, respectively. LW = Lake Wood, PC = Plum Creek, PSP = Palmetto State Park, SC = Sandies Creek, VA = Victoria wastewater treatment plant (WWTP), VB = Victoria 1 mile downstream of the WWTP.

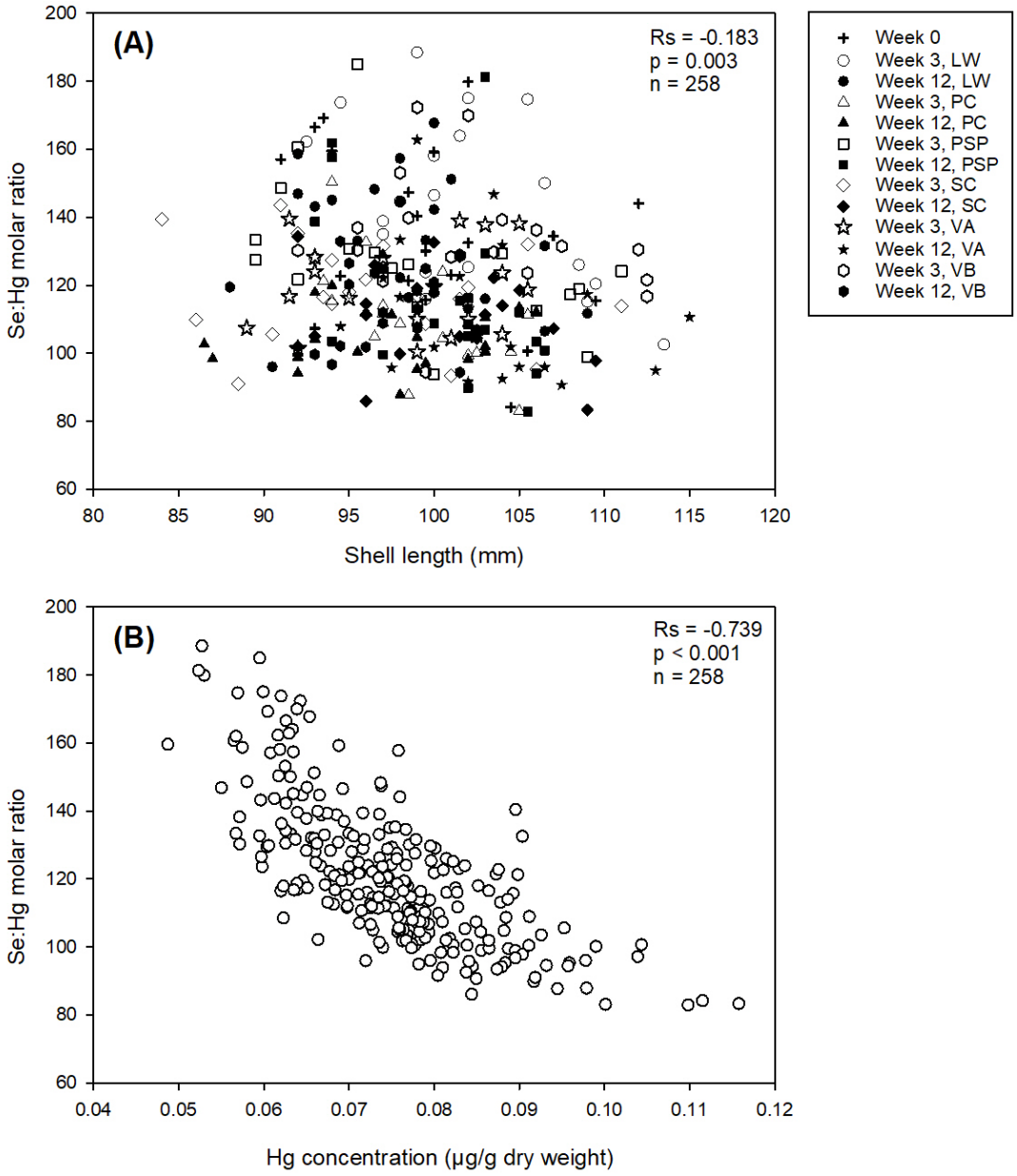

Figure 8. Relationship between Se:Hg molar ratios and shell length (A) and Se:Hg molar ratios and Hg concentration (B) in gill tissue of *Amblema plicata* at all sites and time points. LW = Lake Wood, PC = Plum Creek, PSP = Palmetto State Park, SC = Sandies Creek, VA = Victoria wastewater treatment plant (WWTP), VB = Victoria 1 mile downstream of the WWTP.

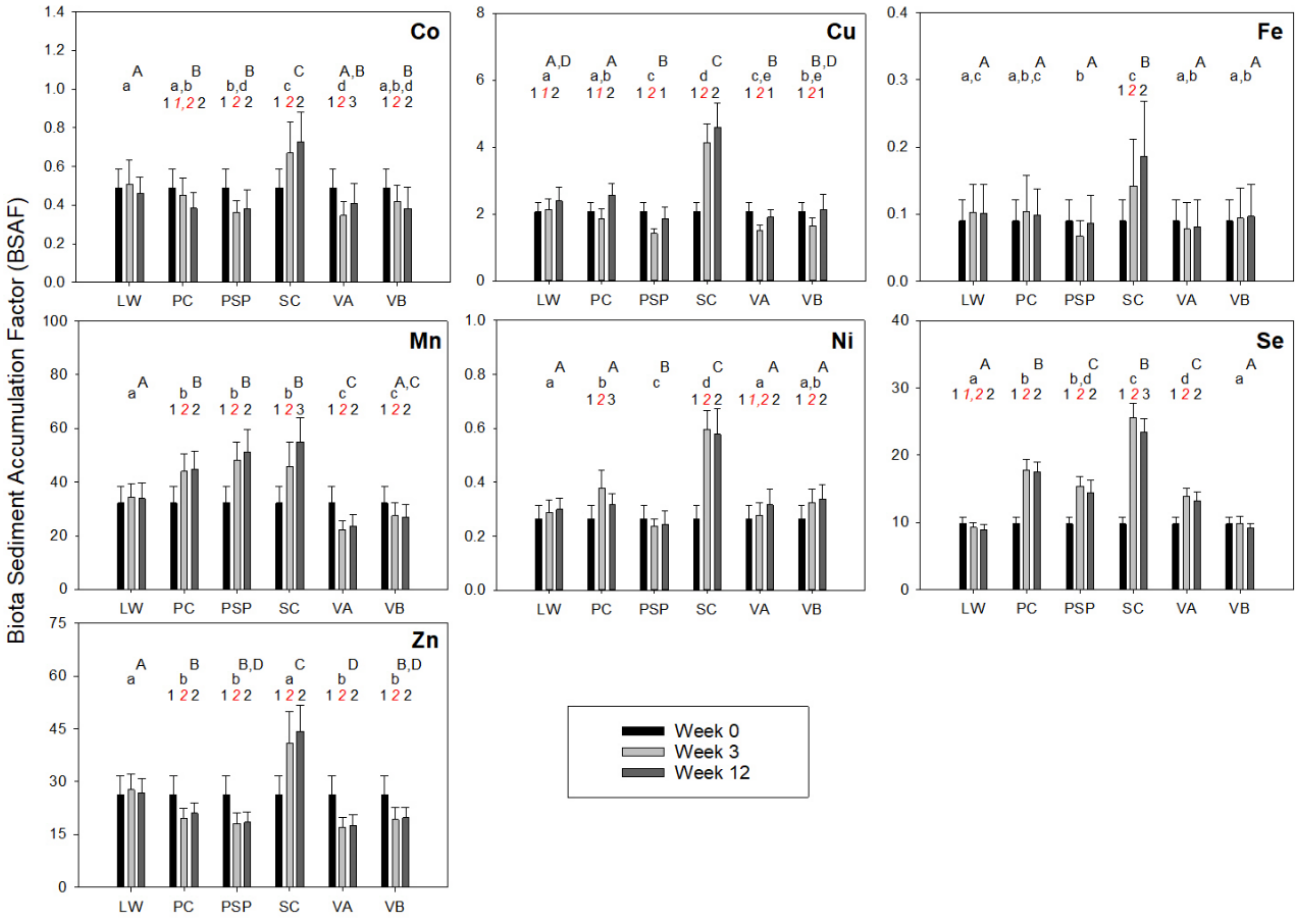

Figure 9. Essential trace element biota sediment accumulation factors (BSAF) (mean ± standard deviation) in gill tissue at each site at week 0 (LW control), 3, and 12 of the caged transplant experiment. Numbers, lowercase letters, and uppercase letters represent significant differences ($p < 0.05$, one-way ANOVA) among timepoints within sites (for clarity, red italicized numbers represent week 3 differences), among sites for week 3, and among sites for week 12, respectively. LW = Lake Wood, PC = Plum Creek, PSP = Palmetto State Park, SC = Sandies Creek, VA = Victoria wastewater treatment plant (WWTP), VB = Victoria 1 mile downstream of the WWTP.

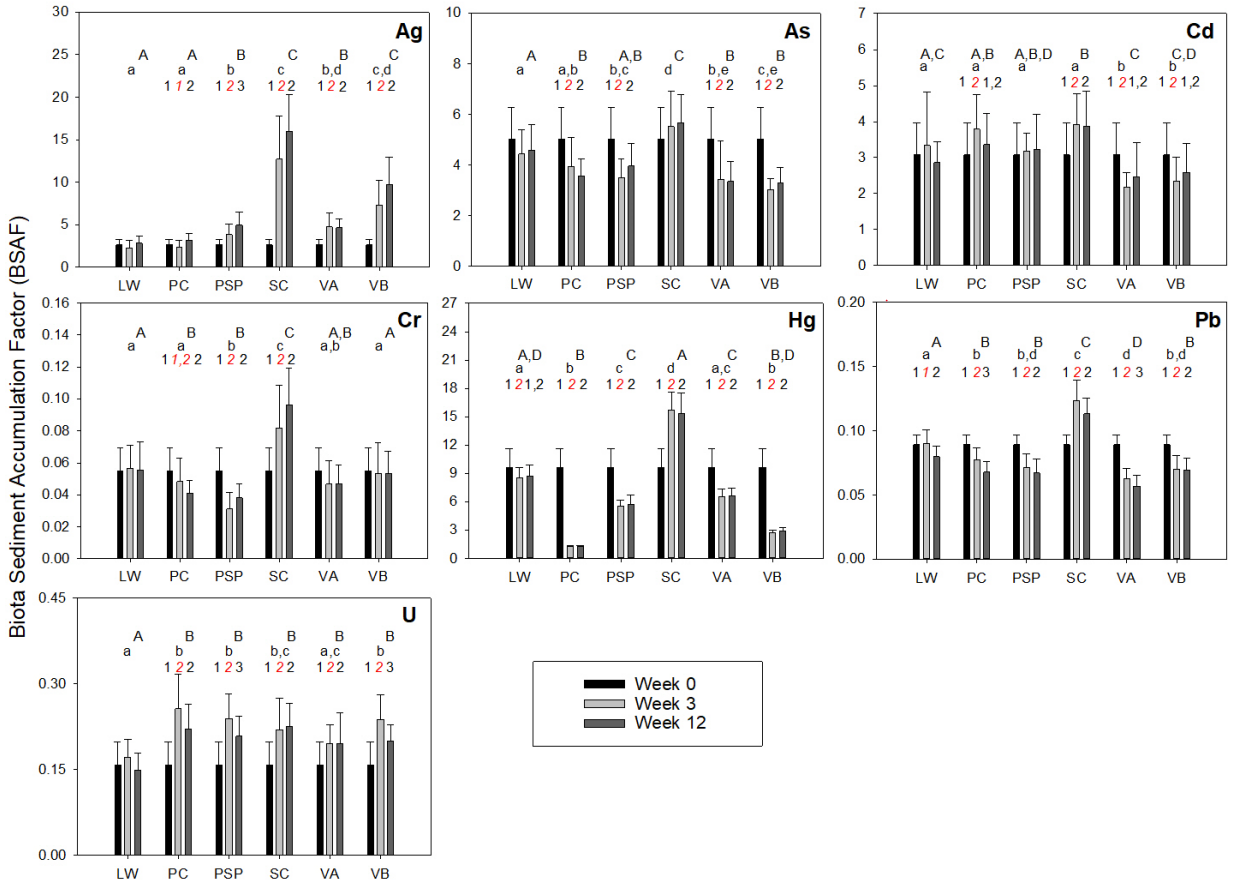

Figure 10. Non-essential trace element biota sediment accumulation factors (BSAF) (mean ± standard deviation) in gill tissue at each site at week 0 (LW control), 3, and 12 of the caged transplant experiment. Numbers, lowercase letters, and uppercase letters represent significant differences ($p < 0.05$, one-way ANOVA) among timepoints within sites (for clarity, red italicized numbers represent week 3 differences), among sites for week 3, and among sites for week 12, respectively. LW = Lake Wood, PC = Plum Creek, PSP = Palmetto State Park, SC = Sandies Creek, VA = Victoria wastewater treatment plant (WWTP), VB = Victoria 1 mile downstream of the WWTP

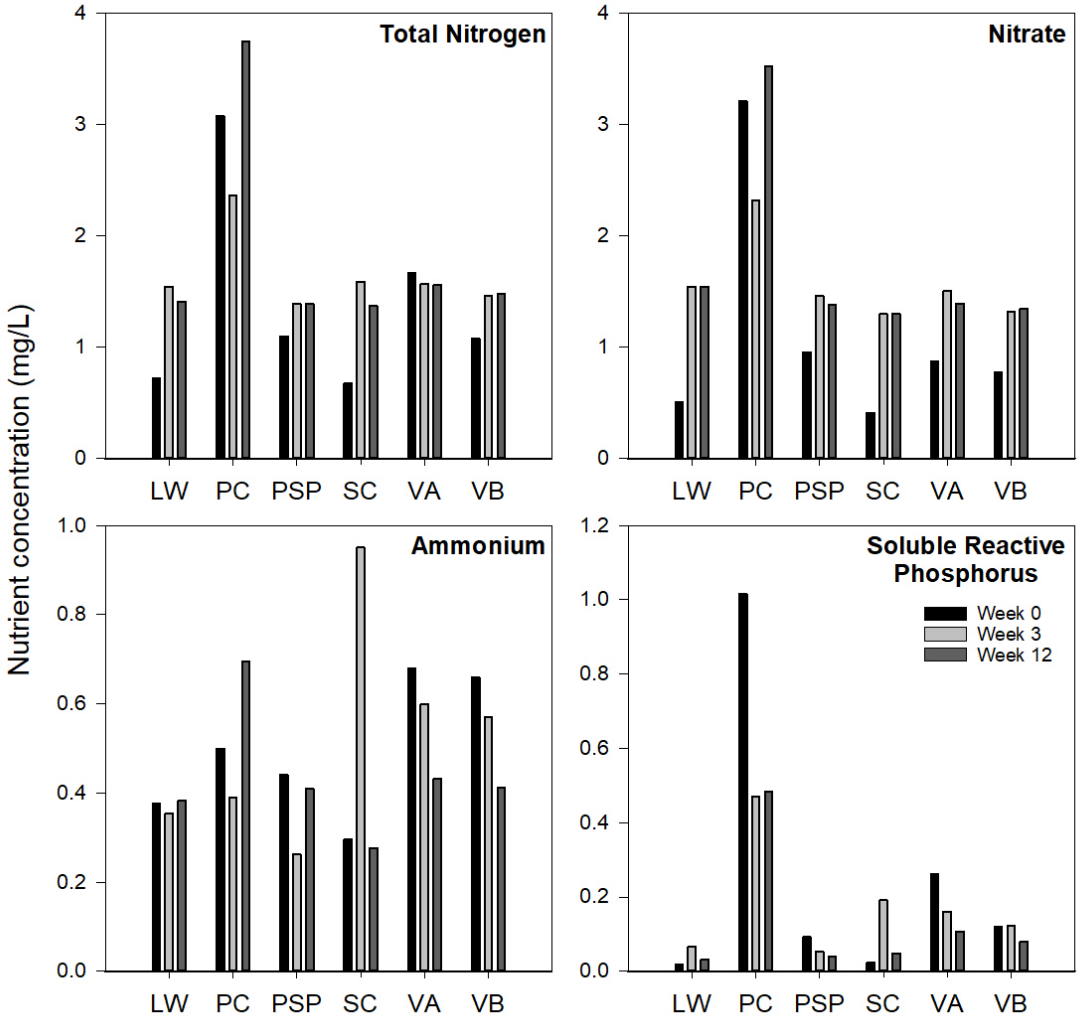

Figure 11. Nutrient concentrations in water collected at each site at week 0, 3, and 12 of the caged transplant experiment. LW = Lake Wood, PC = Plum Creek, PSP = Palmetto State Park, SC = Sandies Creek, VA = Victoria wastewater treatment plant (WWTP), VB = Victoria 1 mile downstream of the WWTP.

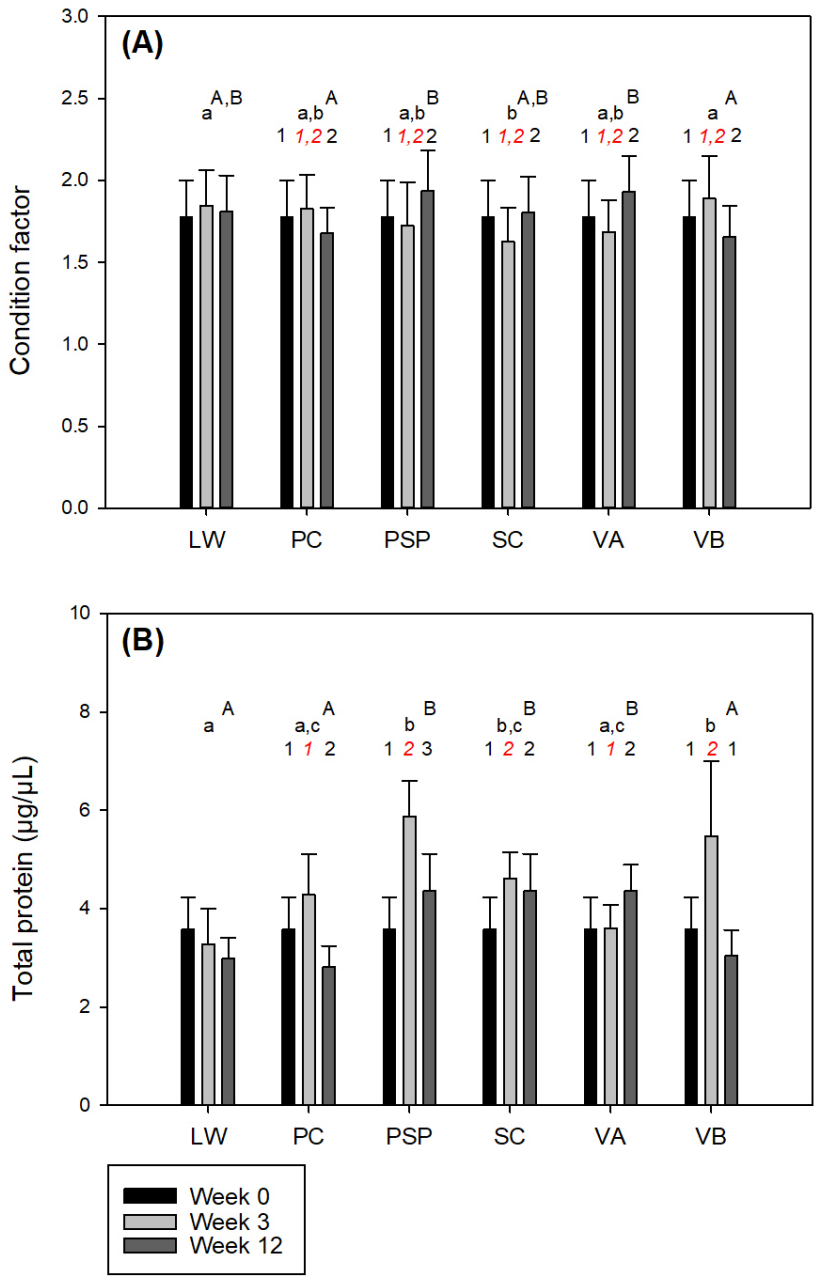

Figure 12. Condition factor (A) and total protein (B) (mean ± standard deviation) in gill tissue at each site at week 0 (LW control), 3, and 12 of the caged transplant experiment. Numbers, lowercase letters, and uppercase letters represent significant differences ($p < 0.05$, one-way ANOVA) among timepoints within sites (for clarity, red italicized numbers represent week 3 differences), among sites for week 3, and among sites for week 12, respectively. LW = Lake Wood, PC = Plum Creek, PSP = Palmetto State Park, SC = Sandies Creek, VA = Victoria wastewater treatment plant (WWTP), VB = Victoria 1 mile downstream of the WWTP

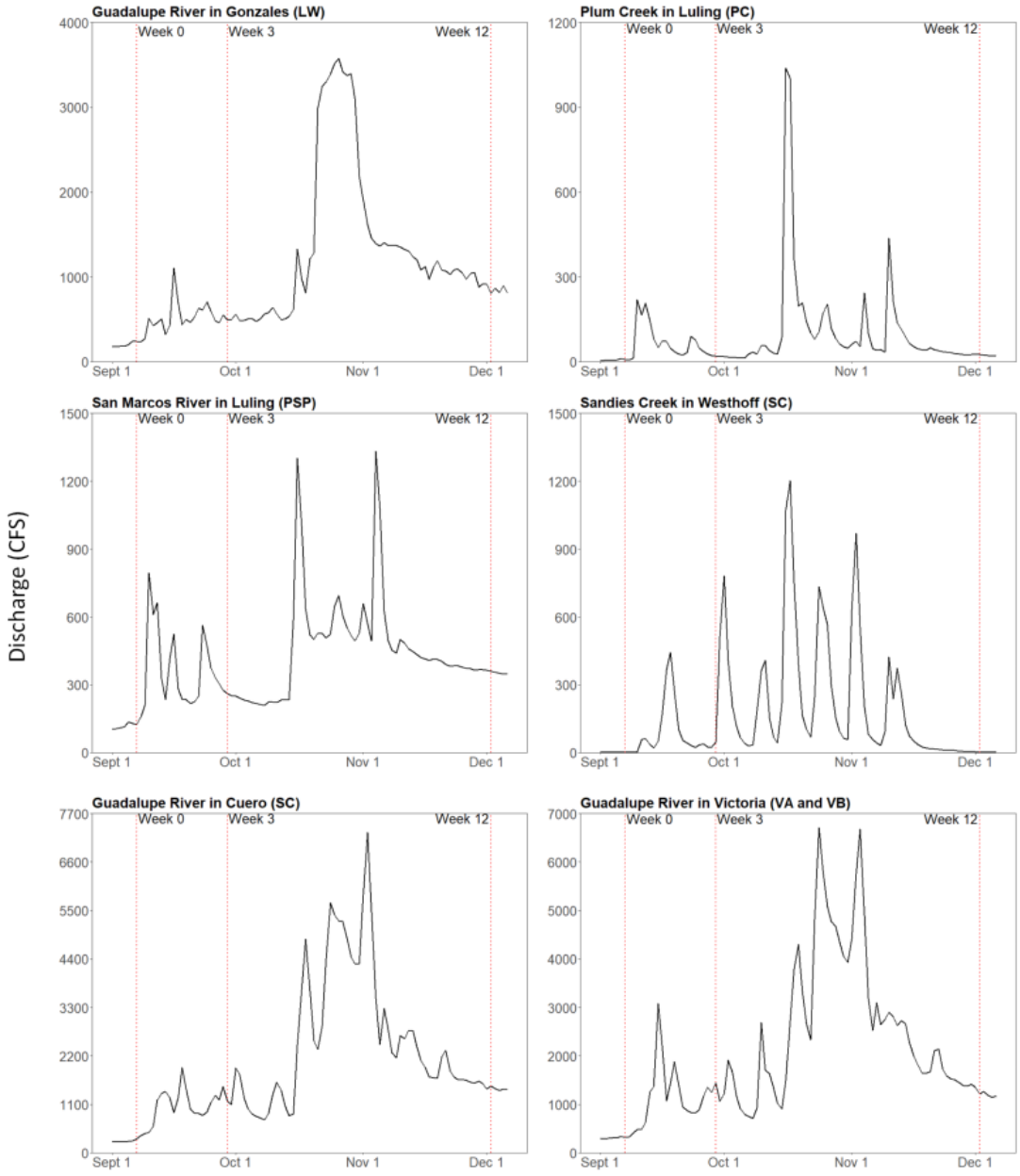

Figure 13. Hydrographs showing river discharge at each site throughout the 12 week caged transplant experiment (USGS, 2020)

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