EFFECTS OF ANTHROPOGENIC DISTURBANCE ON FRESHWATER

MUSSEL POPULATIONS

THESIS

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EFFECTS OF ANTHROPOGENIC DISTURBANCE ON FRESHWATER MUSSEL

POPULATIONS

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TABLE OF CONTENTS

Page

CHAPTER

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

EFFECTS OF MUNICIPAL WASTEWATER EFFLUENT ON FRESHWATER
MUSSEL GROWTH AND SURVIVAL

<u>INTRODUCTION</u>

Surface freshwater ecosystems provide many services to human populations around the world, including the dilution and disposal of waste products (Postel and Carpenter, 1997). In the United States, municipal wastewater treatment plants (WWTPs) are ubiquitous in urban and suburban areas, serving over 173 million people (USEPA, 2012). Although modern wastewater treatment technology has greatly reduced the amount of organic pollution, pathogens, and solids discharged into America's streams, they still remain significant contributors of inorganic nutrients, metals, pesticides, industrial chemicals, and pharmaceutical and personal care products (PPCPs) (Halling-Sorrensen et al., 1998; Tchobanolgous et al., 2003; Clara et al., 2012). The release of these substances in surface freshwaters can result in eutrophication and altered stream metabolism (Chambers et al., 1997; Smith et al. 1999; Wassenaar et al., 2010), altered community structure (Newman et al., 1987; Northington and Hershey, 2006; Slye et al., 2011), and reproductive and physiological effects to aquatic organisms (Cicotelli et al., 1998; Gagne et al., 2001; Akaishi et al., 2007; McGee et al., 2012; Tetreault et al., 2012).

Whole effluent testing (WET) is one approach often used when testing the toxicity of wastewater effluent on aquatic organisms. The approach of WET measures the toxicity of all known and unknown compounds in the effluent as well as any synergistic effects that may occur from combining multiple chemicals (Sarakinos et al., 2000; Smolders et al., 2003). While most WET studies are conducted in laboratory settings (USEPA, 2002), in-situ field trials often provide a more complete and relevant analysis of effluent toxicity in real-world settings (Smolders et al., 2003), especially considering that the form, toxicity, and bioavailability of many toxins is dependent on water and sediment chemistry (Naimo, 1995). Active biomonitoring is one method of in-situ WET testing that involves collecting organisms from an unpolluted site and transplanting them to a test site to quantify their physical and biochemical responses to water quality (Smolders et al., 2005). Marine bivalves have been successfully used as active biomonitors for aquatic pollution for over 30 years (Goldberg, 1975), and more recently an increasing number of researchers have begun using freshwater mussels in biomonitoring programs (ASTM, 2001).

Freshwater mussels offer several advantages in in-situ WET testing (Widdows and Donkin, 1992; Forbes, 1993; ASTM, 2001). As benthic filter feeders, they are constantly exposed to dissolved and suspended materials in the water and sediment and ingest particulate matter through their filtering activity. Roditi et al. (2000) found that 77% of Ag, 78% of Cd, and 65% of Hg bioaccumulated in zebra mussels (*Dreissena polymorpha*) was obtained through food, and many metals are found absorbed onto suspended particles (Salomons et al., 1987; Tessier and Campbell, 1987) that would be filtered and processed by mussels. They are also more tolerant of handling stress than

many other commonly used aquatic organisms, and can be placed in smaller enclosures due to their limited mobility. Mussels also have a very high bioaccumulation rate and very low biotransformation potential for both organic and inorganic compounds, making them useful as long-term sentinels (Smolders et al., 2003).

Ecologically, freshwater mussels are among the most threatened groups of aquatic organisms with 67% of North American species considered threatened (Williams et al., 1993), making studies looking at anthropogenic impacts on this group particularly important. Most species are relatively intolerant of elevated nutrient and toxin concentrations, especially during their larval and juvenile life stages (Bogan, 1993; Valenti et al., 2006; Cope et al., 2008), and as sessile organisms cannot move to areas with lower concentrations of pollutants. Despite these facts, few studies have investigated how freshwater mussel populations are influenced by WWTPs. Those that have studied the influence of WWTPs on freshwater mussels have typically found reduced abundance and richness downstream of discharges and increased mortality and reduced growth in cage or laboratory trials (Horne and McIntosh, 1979; Goudreau et al., 1993; Gangloff et al., 2009). None of these studies, however, have investigated the effects of tertiary-treated wastewater in semi-arid streams that may become completely dominated by effluent during periods of drought.

In the present study, we investigated the effects of tertiary-treated municipal wastewater effluent on transplanted native and non-native freshwater mussels placed in enclosures in a small stream in central Texas for 72 days. We measured growth, condition indices, respiration, and excretion of native threeridge mussel *Amblema plicata* (Say 1817), and growth and survivorship in the non-native Asian clam *Corbicula fluminea*

(Muller 1774) both before and after exposure. Based on the results of previously

published studies, and on preliminary water quality testing of the effluent plume at our

study site, we predicted that mussels would have impaired survivorship, growth, and

physiological status immediately below the effluent discharge compared to an upstream

reference site, and that the response in these variables would increase with increasing

distance downstream from the discharge.

MATERIALS AND METHODS

Site description

We conducted our study in Wilbarger Creek, a third order tributary of the

Colorado River located in eastern Travis County, Texas (30°20'47.23"N, 97°32'56.74"W)

that has a watershed of approximately 470 km^2. Soils within the watershed are

predominately dense clay, and land use is mainly pasture and cultivated agriculture,

although the watershed also drains the rapidly growing towns of Pflugerville, Manor, and

Elgin with a combined population 60,108 (http://quickfacts.census.gov). Wilbarger Creek

has a maximum-recorded discharge of 20,000 cubic feet per second (cfs) and is naturally

a seasonally intermittent stream with zero discharge reported 29% of the time although

many sections have become perennial due to supplemental effluent additions (LCRA,

2011). Under drought conditions these sections may become completely dominated by

undiluted wastewater effluent. Discharge at the Elgin gauge ranged from 1 cfs to 8950 cfs

during the study period of February 24 through May 22, 2012. There are eight active

municipal WWTPs that cumulatively discharge 1.95 million gallons of effluent per day

(mgd) into Wilbarger Creek, but are permitted to discharge up to 12.4 mgd. There are two

additional WWTPs permitted, but not yet built, that will add up to 15.9 mgd of effluent, more than doubling the current allowed discharge.

In order to investigate the effects of municipal wastewater effluent on the Wilbarger Creek ecosystem, we chose four sites near the Wilbarger Creek Wastewater Treatment Facility (TPDES Permit No. WQ0012900000l) located in and operated by the city of Manor, TX. Current discharge is up to 0.5 mgd, with future permitted discharge up to 2 mgd (see Table 1.1 for effluent constituent limitations). We had initially planned on conducting a downstream gradient impact study with three 100 m long sites below the WWTP discharge (with the site farthest downstream as reference) as the area upstream of the discharge had been dry for the previous six months due to extreme drought in 2011. Heavy rains in January and February of 2012, however, restored flow upstream of the discharge and another reference site was added above the discharge. In January 2012, we conducted an initial water quality analysis at the discharge and at four sites up to 14.3 km downstream to map the effluent plume, and we used this data to determine our site locations (Table 1.2). Site 1 was located approximately 160 meters upstream of the discharge, Site 2 approximately 50 meters below the discharge, Site 3 approximately 0.61 km downstream of the discharge, and Site 4 approximately 3.85 km downstream of the discharge (Figure 1.1). Sites 1, 2, and 3 were similar to each other and dominated by run habitat, whereas Site 4 was characterized by run and riffle habitat types (see Table 1.3 for a full site description). In order to minimize the influence of different habitat types on the results of the study, we separated Site 4 into run and riffle habitats and only used run habitat data to compare results between sites. We performed stream habitat surveys by taking four transects at each site at the end of February approximately two weeks prior to

beginning the instream impact studies. Flow data were collected using a Flow-Mate Model 2000 Water Current and Flow Meter (Flow-Tronic, Welkenraedt, Belgium), depth using a standard USGS wading staff, wetted and bankfull width using a 50-meter tape, and canopy cover using a convex forest densiometer. We collected water quality parameter data at each site on three occasions between early March and mid June. Dissolved oxygen, water temperature, conductivity, and pH were measured using a Hydro Tech Hydrolab MiniSonde 4a v2.0. The Lower Colorado River Authority (LCRA) National Environmental Laboratory Accreditation Conference (NELAC) certified laboratory located at 3505 Montopolis Drive in Austin, TX, performed all other analyses. Following the completion of the study, we conducted a detailed water quality analysis of 37 common nutrients and pollutants, analyzed by the LCRA lab, in order to gain insights into possible drivers of the differences we found between sites.

Mussel community surveys

We collected freshwater mussels at each site by pulling mussel rakes (Eagle Claw Fishing Tackle Co., Denver, CO, U.S.A.) through the substrate to a depth of approximately 10 cm through the entire 100m reach. We identified mussels to species level using Howells et al. (1996), measured total anterior to posterior shell length, and preserved them in 95% ethanol for conclusive identification in the lab. Out of all four sites, we collected only two native pondmussels (*Ligumia subrostrata*), both from Site 2.

Experiment on mussel growth and physiological status

To study the effects of the wastewater effluent on freshwater mussels, we measured several physical and physiological parameters of native *Amblema plicata* (Say 1817) and non-native *Corbicula fluminea* (Muller 1774) both before and after in-situ exposure to the water at our four sites. *A. plicata* is a common and widespread mussel found throughout the eastern two-thirds of Texas. As previous mussel surveys in the study area showed a very low density of native mussels, we collected the *A. plicata* used in our study from a location on the Guadalupe River near Victoria, TX known to have a high density of mussels. Fifty six *A. plicata* of similar size (mean shell length 84.3 ± 3.53 mm) were collected by hand searching at the end of February 2012, placed into a large (89 L) aerated cooler filled with river water (20° C), and transported back to our lab (approximate drive time 2 hours). The mussels were maintained in aerated river water and allowed to acclimate at room temperature (21° C) overnight. The following day, we removed approximately 15 L of river water from the cooler every hour for four hours and replaced it with artesian spring water, warmed to room temperature, from the Edward's Aquifer formation that is piped into our lab.

After four hours, we removed each mussel from the cooler and gently scrubbed its shell with a soft plastic-bristle brush to remove any periphyton and/or algae. Each mussel was then patted dry with a paper towel, allowed for the shell to air dry, and marked with an individually numbered tag (The Bee Works, Orillia, ON, Canada) affixed to the left valve with cyanoacrylate gel glue. We then measured length, width, and thickness to two decimal places using digital Vernier calipers (ThermoFisher Scientific, Waltham, MA, U.S.A.) and measured total wet mass to one decimal place using an Ohaus ScoutPro

digital balance (Ohaus Corporation, Pine Brook, NJ, U.S.A.). We calculated an initial

live mussel body condition index as the whole wet mass of the mussel divided by shell

length (BCI-wet). This ratio is commonly used to measure growth and nutritive status in

live bivalves (Crosby and Gale, 1990). When all measurements were complete, each

mussel was placed into a second insulated cooler filled with 100% spring water held at

room temperature and circulated and aerated with a power head and air stones. Total air

exposure time for each mussel was approximately 15 minutes. For the remainder of the

time the mussels remained in the lab (8 days), they were maintained in the cooler with the

lid propped slightly open, with half of the water changed out daily.

We allowed the mussels to recover from handling stress for 24 hours before

beginning initial physiological status measurements. We measured initial physiological

status of the *A. plicata* by placing each individual in a 730 ml clear acrylic closed-cell

respiration chamber containing a magnetic stir bar, filled with spring water filtered

through a 1 μm glass fiber filter, and placed on a magnetic stirrer. Oxygen concentration

inside the chamber was measured every 20 seconds by a model 1302 oxygen sensor

(Strathkelvin Instruments, North Lanarkshire, Scotland) inserted through the top of the

chamber and connected to a Strathkelvin model 782 interface unit. The data were

downloaded at the end of each day to a Dell Optiplex 760 computer, analyzed using the

Strathkelvin software, corrected for water volume, temperature, and readings from

control chambers, and normalized by total mussel wet biomass including the shell.

After removing each mussel, we filtered the water from the chamber through a 1

μm glass fiber filter and froze a 125 ml sample for ammonia concentration analysis. We

later thawed the samples and analyzed ammonia concentration colorimetrically using a

Cary 50 spectrophotometer (Varian Inc., Palo Alto, CA, U.S.A.) set at 630 nm. Results

were corrected for blanks and control samples and normalized for total live mussel

biomass. O:N ratios of the mussels were determined using the simultaneous rate of

oxygen consumption and ammonia excretion during each 1-hour trial in the chambers.

O:N ratios have been used to measure stress in bivalves (Aldridge et al., 1987 and 1995;

Naimo et al., 1992), and indicate whether the animal is predominately metabolizing

carbohydrates, lipids, or proteins.

We used juvenile *C. fluminea* to compare growth rates between sites, as they are

known to grow up to 0.95 mm in length per week under warm water conditions (Welch

and Joy, 1984) and adult unionid mussels are unlikely to exhibit measureable growth in

length over the course of a short-term study. Two days prior to beginning the field

portion of the study, we collected 80 juvenile *C. fluminea* (mean length ± SE 8.72 ± 0.05

mm, mean mass 0.15 ± 0.004 g) upstream of the WWTP discharge at Site 1 on Wilbarger

Creek by sifting substrate through a 2 mm mesh sieve. The *C. fluminea* were transported

back to our lab in buckets of stream water (travel time approximately 45 minutes), where

they were randomly assigned to one of sixteen groups and held in individual containers of

aerated stream water. We measured each individual's length to two decimal places using

digital Vernier calipers (ThermoFisher Scientific, Waltham, MA, U.S.A.) and the

combined mass of all five mussels to four decimal places using a Mettler Toledo Classic

Plus digital scale (Mettler Toledo LLC, Columbus, OH, U.S.A.). We used the average

length and mass of the mussels in each group to measure response to the effluent, as they

were too small to mark individually for re-measurement.

We constructed our experimental cages out of $27.3 \times 37.6 \times 42.7$ cm plastic milk crates completely covered in 2.5 cm wire poultry mesh to prevent predation by fish or mammals. We attached 2 mm plastic canvas mesh to the bottom half of the crate's sides using non-toxic hot glue, and filled the crates halfway with pea gravel for substrate. We constructed $8.5 \times 8.5 \times 8.5$ cm cubes out of the 2 mm plastic canvas mesh to hold the *C. fluminea*, which were also filled halfway with pea gravel. We transported the mussels to the field sites in an 89 L cooler filled with spring water, half of which was replaced with stream water (19° C) from above the discharge upon our arrival to acclimate the mussels to ambient stream conditions. We placed four cages in the middle of the channel at each site approximately 2 m apart in a checkerboard pattern and anchored each cage with two 61 cm and one 122 cm long 0.95 cm diameter steel concrete reinforcement rods. We placed one plastic mesh cube containing a group of five *C. fluminea* in each cage, and buried three randomly selected *A. plicata* halfway in the gravel substrate of each cage in their natural infaunal orientation. We checked the cages every two weeks to remove any accumulated debris and to ensure the cages had not been moved or lost by high flows.

Near the end of May, we collected the cages and brought the mussels back to our lab in the 89 l cooler filled with stream water (25° C) from above the discharge. The total instream exposure time was 72 days. We measured the lengths and masses of the *C. fluminea* upon returning to our lab following the same methods as at the beginning of the study. For the *A. plicata*, we acclimated the mussels and re-measured their same parameters using the same procedures as at the beginning of the study. In addition to re-measuring the initial parameters, we also dried the soft tissue of each *A. plicata* at 63° C for 48 hours to use in calculating a more accurate body condition index based on the

proportion of the available internal shell cavity volume to actual soft tissue occupying

that cavity (BCI-dry) (Crosby and Gale, 1990). The equation we used to calculate BCI-

dry was dry soft tissue weight (g) X 1000/internal shell cavity volume (ml), and is

assumed to be the most accurate measure of assessing the nutritive and stress status of

bivalves (Crosby and Gale, 1990).

Statistical analysis

In order to determine the overall effect of site on the physical and physiological

status of *A. plicata*, we conducted a Multiple Analysis of Covariance (MANCOVA) test

with our seven measured parameters as dependent variables, site as factor variable, and

pre-exposure whole wet mass as covariate (Spooner and Vaughn, 2006). For parameters

that were measured both before and after the field study (wet mass, BCI-wet, oxygen

consumption, ammonia excretion, and O:N ratio), we used the percent change in those

parameters from pre-exposure to post-exposure in our analysis, and for parameters only

measured post-exposure (dry tissue mass and BCI-vol), we used the recorded data from

that single time. We followed the MANCOVA with one-way ANCOVA tests on each

individual parameter, again with pre-exposure whole wet mass as covariate. When a

significant difference was found, we conducted a Fisher's LSD test to identify differences

between the sites. We also conducted paired T-tests on the pre- and post-exposure

measurements for each site to determine significant changes over time. Our experimental

units for the statistical analyses of *A. plicata* were the sixteen cages, with measurements

of individual mussels within each cage averaged to obtain an overall value for that cage.

Due to the high mortality of *C. fluminea* at Sites 2 and 3, statistical analyses for those

parameters were not conducted. All analyses were conducted in SPSS with an alpha level of 0.05. Data were tested for normality and homogeneity of variance using Kolmogorov–Smirnoff and Levene's tests, respectively.

<div align="center">RESULTS</div>

Water quality testing

Preliminary water quality testing was conducted in January 2012, during a period of time when there were no upstream flows entering Wilbarger Creek in the immediate area above the WWTP discharge. This resulted in a distinct difference for several of our measured variables between the area just below the discharge and our next testing site 0.61 km downstream (Table 1.2). Conductivity at the discharge measured 1215 µS/cm and ranged between 760 and 851 between 0.61 and 14.33 km downstream. Total phosphorus measured 0.192 mg/L at the discharge, 0.051 – 0.072 mg/L between 0.61 and 10.21 km downstream, and increased back up to 0.181 at our testing site 14.33 km downstream. Nitrate was substantially higher at the discharge with 13.2 mg/L than downstream sites, which ranged from 2.92 – 3.68 mg/L. *E. coli* bacteria counts were highest at the discharge with 582 mpn/100ml, declined from 344 mpn/100ml at 0.61 km downstream to 192 mpn/100ml at 10.21 km downstream, then increased again to 323 mpn/100ml at 14.33 km downstream. Ammonia was lowest at the discharge with 0.011 mg/L, and increased steadily to 0.056 mg/L at 14.33 km downstream. Temperature was 18.0 °C at the discharge, and declined to 10.6 – 11.4 °C downstream. Total suspended solids were below detectable limits (< 1 mg/L) at the discharge, and increased to 20.3 – 26.5 mg/L downstream. pH remained relatively consistent between all sites (8.0 – 8.2)

and showed no obvious pattern. Dissolved oxygen followed a similar pattern to pH, and ranged from 9.2 to 10.4 mg/L.

After late winter rains restored upstream flows and diluted the effluent, the water quality variables that had been elevated in the stream channel below the discharge in January's testing remained elevated only in the undiluted effluent (Table 1.4). Measurements at Site 2 (50 m downstream of the discharge) were similar to those at the other three sites for most variables tested for. The effluent showed substantially higher mean conductivity (1475 ± 86 µS/cm), ammonia (0.3 ± 0.3 mg/L), nitrate/nitrite (16.5 ± 3.4), total phosphorus (0.8 ± 0.6 mg/L), and orthophosphorus (1.2 ± 0 mg/L) that at the four mussel enclosure sites. The means of the measured variables showed relatively little difference between the four enclosure sites, with the exception of nitrate/nitrite and orthophosphorus, which were higher at Site 4 than at the other three sites. *E. coli* was also higher at Sites 1 and 4 than at Sites 2 and 3.

After removing the enclosures from Wilbarger Creek, we conducted a more detailed water quality analysis of the effluent and water from Sites 1 and 4 (Table 1.5). We tested for 37 variables, including nutrients and several common pollutants. Of these, copper, magnesium, potassium, and zinc were found to be in higher concentrations downstream of the discharge than upstream. Concentrations of copper were 1.9 µg/L at Site 1, 5.5 µg/L in the effluent, and 2.1 µg/L at Site 4. Magnesium was 10.7 mg/L at Site 1, 15.9 mg/L in the effluent, and 11.3 mg/L at Site 4. Potassium concentrations were 5.94 mg/L at Site 1, 19.9 mg/L in the effluent, and 11.6 mg/L at Site 4. Zinc was 10.1 µg/L at Site 1, 66.2 µg/L in the effluent, and 23.1 µg/L at Site 4.

A. plicata growth and physiological status experiment

Results of MANCOVA testing showed a significant overall difference between

sites ($F_{21, 6.3}$ = 3.858, p = 0.046) (Table 1.6). There were significant differences between

sites for all four physical parameters: percent change in whole wet mass (ANCOVA: $F_{3, 11}$ = 8.706, p = 0.003), percent change in BCI-wet ($F_{3, 11}$ = 9.88, p = 0.002), BCI-dry ($F_{3, 11}$ = 18.666, p < 0.000), and dry tissue mass ($F_{3, 11}$ = 27.14, p < 0.000) (Table 1.6).

Mussels at Site 1 above the discharge consistently showed the greatest increase in

physical and physiological status compared to those downstream of the discharge.

Increase in whole wet mass was highest at Site 1 with a 2.58 ± 0.58% ($\bar{x}$ ± SE) increase

and lowest at Site 2 with 0.08 ± 0.22% (Fig. 1.2A). Site 1 was significantly higher than

the other three sites, and along with Site 3 exhibited a significant increase in wet mass

over the course of the study (Table 1.7). Sites 3 and 4 showed an increase of 1.32 ±

0.28% and 0.50 ± 0.40% respectively, and were not significantly different from Site 2.

Percent increase in BCI-wet showed a similar pattern as whole wet mass. Site 1 showed

the greatest increase of 2.38 ± 0.46%, and Site 2 the lowest with a slight decrease of -0.01

± 0.42% (Fig. 1.2B). Site 3 exhibited an increase of 1.26 ± 0.42% and Site 4 an increase

of 0.05 ± 0.41%. Site 1 was again significantly higher than the downstream sites, which

were all statistically similar to each other. Only Site 1 showed a significant increase in

BCI-wet over time (Table 1.7). Post-exposure dry tissue mass was highest at Site 1 at

5.29 ± 0.18 g, and lowest at Site 3 at 3.37 ± 0.15 g (Fig. 1.2C). Sites 2 and 4 showed a

final dry mass of 3.98 ± 0.18 g and 3.60 ± 0.23 g respectively. Mean dry mass at Site 1

was significantly higher than at the downstream sites, which were not significantly

different from each other. BCI-dry was highest at Site 1 with 113.96 ± 1.91 and lowest at

Site 4 with 78.11 ± 6.04 (Fig. 1.2D). Site 2 showed a final BCI-dry of 80.39 ± 2.41 and Site 3 was 78.87 ± 3.52. Site 1 was significantly different from Sites 2, 3, and 4, which were all similar to each other.

In contrast to the physical parameters, there were no significant ANCOVA results among the three physiological parameters (oxygen consumption, ammonia excretion, and O:N ratio) we measured due to high variability in the responses of individual mussels at all sites (Table 1.6). Oxygen consumption showed a distinct pattern of the greatest increase in respiration at Site 1 with 71.39 ± 14.52 %, the lowest increase at Site 2 with an increase of only 13.54 ± 22.04 %, and increasing to 19.53 ± 10.95 at Site 3 and 51.89 ± 18.67 at Site 4. Mean ammonia excretion followed a similar pattern, but with all changes being negative. Site 1 showed a decrease in excretion of -5.75 ± 15.14, and Site 2 had the greatest decrease of -27.35 ± 11.89. Sites 3 and 4 exhibited decreases of -6.48 ± 8.23 and -3.93 ± 10.25 respectively. Change in O:N ratios was highly variable and showed no clear pattern, with the greatest change being an increase of 81.92 ± 44.6 % at Site 4, and smallest increase of 36.42 ± 13.73 at Site 3. Site 1 showed an increase of 73.92 ± 44.64 % and Site 2 showed an increase of 60.17 ± 48.17.

C. fluminea growth experiment

Survival and growth of the *C. fluminea* differed greatly between upstream and downstream sites. Survivorship ranged from 100% above the discharge at Site 1 to 0% below the discharge at Sites 2 and 3, with Site 4 showing intermediate survivorship of 37.8% (Fig. 1.3A). Growth in whole wet mass at Site 1 increased from an average of 0.16 ± 0.01g/mussel pre-exposure to 0.41 ± 0.007g/mussel post-exposure. Mussels at Site 4

increased slightly from 0.15 ± 0.008g/mussel to 0.17 ± 0.01g/mussel (Fig. 1.3B). Growth in length at Site 1 increased from 8.85 ± 0.13mm/mussel to 11.94 ± 0.11mm/mussel. At Site 4, mussels increased slightly from 8.59 ± 0.08mm to 9.37 ± 0.14mm (Fig. 1.3C). In addition to the five *C. fluminea* we placed in each cage at Site 1, we found a total of 33 additional juvenile *C. fluminea* in the cages upon retrieval. These new individuals were easily identifiable as recruits due to their smaller size than the five original ones we started with. No additional *C. fluminea* were found at any other site.

DISCUSSION

The reduction in growth at sites downstream of the effluent discharge in both *Amblema* and *Corbicula* and the significant mortality of the *Corbicula* generally agree with our initial hypothesis of decreased growth and increased mortality downstream of the effluent discharge, although we did not see a consistent pattern of increased growth with increasing distance from the discharge. Results of this study suggest that the effluent from the Wilbarger WWTP could have a significant negative impact on the ecology of Wilbarger Creek downstream of its discharge for at least 3.85 km. Native *A. plicata* showed significantly lower mass and condition indices below the discharge compared to the upstream reference site after 72 days exposure. *A. plicata* from downstream sites also exhibited lower oxygen consumption rates than those from the reference site, indicating a lower overall metabolism rate. The juvenile *C. fluminea* also exhibited much lower growth and survival rates below the discharge, whereas all mussels at Site 1 survived and grew substantially. Our mussel surveys found only two live mussels downstream of the effluent discharge, whereas we found one live and several dozen recently killed adult

pond mussels in the dewatered streambed upstream of the discharge during our initial site visit in January.

Effluent effects on distribution and mortality

Our results add to the growing body of knowledge suggesting the negative effects of wastewater effluent to bivalves. Horne and McIntosh (1979) found that mussel abundance declined from an average of 7.1 mussels/m^2 above a secondary treated wastewater discharge on the Blanco River in Texas to 0.0 immediately below it, and density increased to only 0.2 mussels/m^2 at 2km downstream. They also found zero survival of three species of native mussels (including *Amblema plicata*) after 28 days of exposure in cages to diluted effluent downstream of the discharge, with *Corbicula* showing 50% survival downstream. They attributed this decline to elevated concentrations of ammonia and potassium in the diluted effluent (6.8 and 7.8 mg/L, respectively). Single sample ammonia concentrations in our study never exceeded 0.11 mg/L at any of our test sites, which is only slightly higher than the lowest reported acute LC$_{50}$ concentration (concentration of the chemical required to kill 50% of the test animals in a given time) for juvenile *C. fluminea* which are more sensitive to ammonia than native unionid mussels (Augsburger et al., 2003; Mummert et al., 2003). Although ammonia toxicity studies using *A. plicata* have not been conducted, the concentrations of ammonia measured in our study are below the 0.3-0.7 mg/L range recommended by Augsburger et al. (2003) as safe for continuous exposure to all life stages of freshwater mussels, including glochidia which are typically more sensitive to contaminants than adults. Freshwater mussels are known to be sensitive to potassium (Imlay, 1973; Horn and

McIntosh, 1979; Dietz and Byrne, 1990), and potassium has been investigated as a possible biocidal compound to control Asian clam and zebra mussel (*Dreissena polymorpha*) infestations (Dietz and Byrne, 1990; Fisher et al., 1991). Imlay (1973) found potassium concentrations of 11 mg/L toxic to 90% of freshwater mussels tested between 36-52 days, and that 7 mg/L was lethal to two species after 8 months exposure. Based on his findings and an analysis of freshwater mussel distribution and potassium concentrations in 49 rivers, he recommended potassium levels should not exceed 4-10 mg/L for mussels. We measured potassium concentrations of 19.9 mg/L in the effluent and 11.6 mg/L 3.85 km downstream at Site 4, whereas concentrations upstream of the discharge at Site 1 were 5.9 mg/L. While these concentrations may explain the differences we found in growth of *A. plicata*, they are much lower than acute concentrations (120 mg/L) reported to induce shell gaping (a stress response) for *C. fluminea* (Anderson et al., 1976).

Goudreau et al. (1993) also found greatly reduced densities of unionid mussel and *C. fluminea* below two WWTPs on the Clinch River in Virginia compared to upstream sites, but no differences in density above and below communities served by on-site septic systems. Their study suggested that mussels had been eliminated below the WWTP discharges and glochidia from above the discharges were prevented from recolonizing downstream areas by some chemical pollutant in the effluent, most likely unionized ammonia and chlorine. Their water quality analyses revealed that total residual chlorine at sites just below the WWTPs regularly exceeded the 24 hour LC_{50} of 0.084 mg/L they established through laboratory testing. While instream ammonia levels only exceeded their determined LC_{50} of 0.284 mg/L on one occasion at one site, they hypothesized that

sublethal concentrations of both chlorine and ammonia could prevent the glochidia's ability to successfully infest host fish and complete their life cycle. Gangloff et al. (2009) found similar differences in mussel abundance above and below a WWTP on Parkerson Mill Creek in Alabama, and also reported increased mortality (78%) and decreased condition of caged mussels downstream of the WWTP. They, too, hypothesized that chlorine and/or other untested compounds were driving these differences (although not measured in their study, the WWTP being investigated had been frequently cited for high levels of chlorine). While ammonia concentrations at our sites only exceeded 0.284 mg/L in the undiluted effluent, total residual chlorine at all sites on all sampling dates was higher than the LC_{50} of 0.084 mg/L of Goudreau et al. (1993). However, we found the highest mean concentration of chlorine (0.165 mg/L) *upstream* of the discharge at Site 1, where growth of both *A. plicata* and *C. fluminea* was highest and where we also noted the presence of many small juvenile *C. fluminea,* suggesting that chlorine from the Wilbarger WWTP is not significantly impacting mussels there.

Effects of effluent on energetic condition

In testing the effects of chronic whole effluent exposure, sublethal endpoints such as growth, condition, and respiration are biologically appropriate because they are sensitive, holistic measures of an organism's well-being that incorporate the effects of toxins on a multitude of processes at several levels of biological organization (Munkittrick and McCarthy, 1995; Ausley, 2000). Energy budgets are often used to quantify the intake and assimilation of energetic resources by an organism and their allocation to various energy sinks such as growth, maintenance, and reproduction (Beyers

et al., 1999; Kooijman, 2000; Muller et al., 2010). Different energy budget models have been developed such as the scope for growth model (SfG) proposed by Warren and Davis (1967) and the dynamic energy budget model (DEB) put forth by Kooijman (2000), but generally they assume that food taken in by an organism is either assimilated or lost to respiration or excretion. Energy not lost is put into reserves and then directed to somatic maintenance, growth, maturity development in juveniles, or maturity maintenance and/or reproduction in adults (Fig 3). It is also generally accepted that somatic maintenance has an absolute priority for energy over other sinks (Muller, 2010; Kooijman, 2009), and that only when energy assimilation exceeds the basic cost of maintenance will resources be directed toward growth or reproduction. Thus, anything that reduces food intake or assimilation rates or increases maintenance costs will reduce the amount of energy available for growth or reproduction (Callow and Silby, 1990; Kooijman et al., 2009; Muller et al., 2010). Exposure to environmental toxins has been shown to both reduce feeding rate and increase maintenance costs in fish (Kooijman and Bedeaux, 1996; Smolders et al., 2002a and 2003), mussels (Widdows et al., 1995; Donkin et al., 1997; Muller et al., 2010), and other aquatic invertebrates (Allen et al., 1995; Billoir et al., 2007).

There are many toxins and other stressors known to affect mussels' energetic balance. Some studies have found strong inverse correlations between scope for growth (the amount of energy available for growth) and pollution concentration gradients for the marine mussel *Mytilus edulis* (Bayne et al., 1979; Widdows et al., 1981), and Encomio and Chu (2000) found that polychlorinated biphenyls (PCBs) reduced glycogen concentration (the main energy storage molecule in bivalves) in oysters. Baker and

Hornbach (2000) found that *A. plicata* infested by *D. polymorpha* showed lower

clearance rates (a measure of food intake ability) and a lower O:N ratio indicative of

starvation. A reduction in clearance rates and condition indices has also been shown in

caged *D. polymorpha* exposed to both municipal and industrial effluents (Smolders et al.,

2002b). A typical response of many mussel species to the presence of toxins is to tightly

shut their valves (Horne and McIntosh, 1979; Doherty et al., 1986; Curtis et al., 2000;

Valenti et al., 2006), which limits the amount of time a mussel can filter water and ingest

food. This valve-closure response has been shown for chlorine (Valenti et al., 2006),

copper (Sloof et al., 1983; Curtis et al., 2000; Liao et al., 2007), cadmium (Sloof et al.,

1983; Tran et al., 2003), zinc (Doherty et al., 1987; Kraak et al., 1994), and other

substances. Because of the sensitivity of valve closure and filtration rates to toxins, these

behaviors have been suggested as screening tools to detect low levels of environmental

contaminants (Mouabad et al., 2001; Gnyubkin, 2009). Both copper and zinc were found

in higher concentrations in the effluent and downstream sites than at our reference site,

although in lower concentrations than previously reported to cause valve closure or

reduced filtration rates. However, the pattern of metal concentration shows correlation to

the pattern of growth we saw in *A. plicata* and growth and survival in *C. fluminea*.

Although not statistically significant due to wide variation between individual mussels,

mean respiration and excretion rates were much lower at Site 2 than at other sites, which

could indicate that mussels at that site kept their shells closed more often. Valve closure

is a common response by mussels to avoid adverse conditions while simultaneously

reducing metabolism by 90% or more of standard metabolic rate (Ortmann and

Grieshaber, 2003). While this behavior allows mussels to remain relatively protected

from unfavorable environmental conditions and reduce metabolic requirements for short periods of time, it also greatly reduces their ability to ingest food needed for growth in the long-term.

In addition to reducing the amount of energy an organism takes in, exposure to pollutants also increases the energy required for somatic maintenance by forcing the organism to allocate resources to maintain homeostasis in the presence of stress (Callow, 1991). Kooijman and Bedeaux (1996) found that an energetic-based model including increased maintenance costs was the best-fit model describing the growth of zebrafish exposed to toxins. Other research (Smolders et al., 2002a) has shown zebrafish showed lower condition indices and increased respiration when exposed to high levels of wastewater effluent in laboratory tests. They attributed the higher respiration rate to increased homeostatic costs because of the effluent, and the lower condition to the higher maintenance costs when food availability is held constant. In our study, we found the highest respiration above the discharge at Site 1, the lowest just below the discharge at Site 2, and increasing geometrically downstream at Sites 3 and 4. Smolders et al. (2002b) found that *D. polymorpha* initially increased their respiration when exposed to both municipal and industrial wastewater effluent, but later became depressed at the industrial effluent site while rates at the municipal site remained elevated above those at the reference site. It is generally assumed that respiration rates should increase with increasing concentrations of pollutants due to increased metabolic requirements for maintenance (Callow, 1989; Callow, 1991), however respiration rates have also been shown to decline when exposed to high concentrations of toxins (Widdows and Johnson, 1988; Widdows and Donkin, 1991). This could explain our pattern of respiration in our

downstream sites, with toxin concentrations near the discharge being high enough to depress respiration but declining enough downstream to allow recovery. We did not, however, observe significant differences in whole wet mass or dry tissue mass between our three downstream sites, which may indicate differences in the amount of time the mussels closed their valves to lower metabolic requirements.

Several studies published in the last decade have examined physiological biomarkers to measure the impact of wastewater effluent on mussels, and while most don't investigate energetic balances specifically, they do report effects that increase energetic costs. Gagne et al. (2001) found significant increases in the egg yolk protein precursor vitellogenin (Vt) in both male and female *Elliptio complanata* placed in cages for two months downstream of a WWTP in the St. Lawrence River in Canada. They also noted that soft tissue weight increased downstream of the discharge increased significantly, but overall shell length did not, causing a shell growth abnormality known as "shell-length-to-tissue-weight growth decoupling." They concluded that the estrogen-like compounds in the effluent caused the mussels to redirect energy into Vt production at the expense of somatic growth. Another study performed at the same sites as Gagne et al. (2001) using *E. complanata* and *D. polymorpha* again found elevated levels of Vt in both test species, along with elevated levels of metallothioneines (MT), a stress-response protein that binds to and protects against metals (Gagnon et al., 2006). They also found higher numbers of heterotrophic bacteria circulating throughout the hemolymph and decreased phagocytosis, indicating an immunosuppressive effect of the effluent, as well as damage to DNA in *D. polymorpha*. Blaise et al. (2003) also found increased Vt levels, shell growth abnormalities, and altered sex ratio in a yearlong study in the St. Lawrence

River. Several other studies using *E. complanata* and *M. edulis* in Canada found that exposure to municipal effluent resulted in depressed immune capabilities (Akaishi et al., 2007; Bouchard et al., 2009; Farcy et al., 2011), decreased resistance to bacterial challenge (Akaishi et al., 2007), activated detoxification mechanisms (Bouchard et al., 2009; Farcy et al., 2011), increased mortality (Bouchard et al., 2009; Farcy et al., 2011) and lower overall condition indices (Farcy et al., 2011), with some responses being detectable after only one week. All of these responses require an organism to redirect resources from growth to maintenance and repair, and although we did not specifically measure immune or reproductive biomarkers in our study it is possible that the differences in growth and condition in the mussels in our study were caused similar physiological responses to the effluent from the Wilbarger WWTP.

Population level consequences

Although ecotoxicological studies typically measure the responses of individual organisms, it is the population scale that is ultimately of concern. The long-term success of populations, however, depends on the success of the individuals that comprise the population. There are several mechanisms by which wastewater effluent can affect mussels at the population level. The first is through direct or indirect mortality on adult mussels. Several studies have shown increased mortality to transplanted adult mussels below WWTP discharges (Horne and McIntosh, 1979; Bouchard et al., 2009; Gangloff et al., 2009; Farcy et al., 2011) or to effluent in laboratory settings (Ciccotelli et al., 1998; Akaishi et al., 2007). Unfortunately, to our knowledge there have not been any studies published that were able to monitor extant mussel populations near newly built WWTPs,

so we are unable to make any definitive conclusions about how population structure

changes when WWTPs begin discharging effluent. Fewer adult mussels not only reduce

the immediate population, but also reduce the number of potential recruits needed to

repopulate a particular area. A second way effluent can affect mussel populations is

through mortality or impairment of larval and juvenile mussels. The presence of

relatively healthy mussel populations above wastewater discharges and lack of mussels

below them, as was the case in our study, indicates that recruitment of larvae is not

occurring in areas of high effluent concentrations (Horne and McIntosh, 1979; Goudreau

et al., 1993; Gangloff et al., 2009). Mussel glochidia are known to be the among the most

sensitive aquatic organisms for many environmental contaminants commonly found in

wastewater effluents (Horne and McIntosh, 1979; Goudreau et al., 1993; Naimo, 1995;

Augsburger et al, 2003), and can be killed or immobilized at concentrations below that

known to affect adults. Glochidia exhibit the same valve-closure response to toxins as

adults do, which can reduce their likelihood of successfully attaching to the gills of a host

fish. Juvenile mussels often spend much of their time completely buried in the top layers

of stream substrate and filter pore water (Yeager et al., 1994), which can contain higher

concentrations of ammonia and other toxins than surface water (Naimo, 1995;

Augsburger et al., 2003). A third way effluents can affect mussel populations is through

alteration in the reproduction of adult mussels. Bringolff et al. (2010) found that female

mussels altered their lure display behavior and released more nonviable glochidia than

those in controls and that males released their spermatozeugmata prematurely in the

presence of fluoxitene, the active ingredient in Prozac that is commonly found in

municipal effluents (Kolpin et al., 2002). Another study found that exposure to effluent

had reduced the size of the seminiferous tubules in male *Dreissena*, reducing the sperm producing areas of the gonads and potentially reducing fecundity (Quinn et al., 2004). Estrogen-like compounds present in wastewater effluent have also been shown to induce feminization and skew sex ratios toward females in caged *E. complanata* (Blaise et al., 2003). Altering sex ratios to proportions not seen naturally could have dramatic long-term population effects for mussels in areas affected by wastewater effluents. Changes in energy budgets can also affect individual fecundity and reproductive success. Decreased food assimilation and/or increased somatic maintenance costs caused by pollutants can reduce energy available for reproduction, reducing fecundity and delaying maturity in juveniles (Maltby, 1999; Kooijman et al., 2009). An energetic-based model predicting the response of the worm *Lumbricus rubellus* to copper accurately predicted severe population declines at high concentrations of copper because juveniles were not able to reach reproductive size and be able to reproduce (Klok and de Roos, 1996). Bayne et al. (1979) also found reduced fecundity and egg viability in *M. edulis* when placed under toxic stress. These population-level impacts of wastewater effluent can have drastic long-term consequences for freshwater mussels, which are essentially sedentary and thus cannot move to more favorable areas.

<div align="center">CONCLUSIONS</div>

In this study, we have shown that both native and non-native mussel species can be significantly impaired by 72 days exposure to municipal wastewater effluent for at least up to 3.85 km downstream from the effluent discharge. Municipal effluents are chemical cocktails comprised of substances known and suspected to be harmful to

aquatic organisms. Because the behavior of these chemicals is ultimately controlled by site-specific environmental conditions and water quality characteristics, more in-situ studies investigating the chronic effects of effluent are needed. Although wastewater treatment facilities are a necessary component of urban communities, and are ultimately beneficial to the environment at the large scale, consideration of their impacts on biodiversity and conservation must be taken into account when planning their location and operation.

Table 1.1. Discharge limitations of effluent from the Wilbarger Wastewater Treatment Facility at 0.5 MGD discharge stage.

Effluent Characteristic	Daily Average (mg/L)	7-Day Avg. (mg/L)	Daily Max (mg/L)	Single Grab (mg/L)
Flow, MGD	Report	N/A	Report	N/A
Carbonaceous Biochemical Oxygen Demand (5-day)	5	10	20	30
Total Suspended Solids	5	10	20	30
Ammonia Nitrogen	2	5	10	15
Total Phosphorus	1	2	4	6
Total Dissolved Solids	Report	N/A	Report	N/A

Table 1.2. Preliminary water quality test results used to determine site locations.

Distance from outfall (km)	Cond (µS/cm)	pH	DO (mg/L)	Temp (°C)	TSS (mg/L)	Total phosphorous (mg/L)	E. coli (mpn/100ml)	Ammonia (mg/L)	Nitrate (mg/L)
0	1215	8.0	9.2	18.0	< 1	0.192	582	0.011	13.2
0.61	842	8.0	10.2	10.7	20.3	0.051	344	0.026	3.68
5.79	851	8.1	9.4	11.4	25	0.074	226	0.035	3.43
10.21	808	8.2	10.4	10.7	26.5	0.072	192	0.037	3.32
14.33	760	8.1	9.6	10.9	20.3	0.181	323	0.056	2.93

Table 1.3. Physical measurements and description of sites used in study.

	Distance downstream of discharge (km)	Habitat type	Substrate	Mean depth (m)[a]	Mean wetted width (m)[a]	Mean bankfull width (m)[a]	Mean canopy cover (%)[a]
1	-0.16	run	silt	0.49 ± 0.09	6.8 ± 0.17	9.0 ± 0.41	64.5 ± 10.33
2	0.06	run	silt	0.46 ± 0.14	6.0 ± 0.23	9.5 ± 0.65	76.5± 15.91
3	0.61	run	silt	0.77 ± 0.19	5.4 ± 0.78	7.0 ± 0.71	0
4[b]	3.65	run	silt/grvl/cobl	0.57 ± 0.19	5.4 ± 0.48	13.0 ± 2.01	77.6 ± 9.81

[a] Value ± SE

[b] Only run habitat data from Site 4 are included

Table 1.4. Water quality parameters as measured throughout the study period.

Site (distance downstream from discharge)	Conductivity (µS/cm)	pH	Dissolved Oxygen (mg/L)	Temperature (°C)	Ammonia As N (mg/L)	Nitrate/Nitrite As N (mg/L)	Total Phosphorus As P (mg/L)
Site 1 (-0.16 km)							
Maximum	1190	8.03	7.65	27.35	0.049	4.29	0.139
Minimum	877	7.75	5.15	19.51	0.008	0.024	0.083
Mean (± SE)	993 ± 71	7.9 ± 0.06	6.3 ± 0.6	23.4 ± 1.6	0.03 ± 0.008	1.8 ± 1.0	0.1 ± 0.01
Effluent (0 km)							
Maximum	1677	8.02	9.15	27.42	0.626	19.9	1.41
Minimum	1215	7.75	7.54	18.02	0.011	13.2	0.192
Mean (± SE)	1475 ± 86	7.8 ± 0.05	7.9 ± 0.3	24.1 ± 1.7	0.3 ± 0.3	16.5 ± 3.4	0.8 ± 0.6
Site 2 (0.05 km)							
Maximum	1093	8.00	7.83	24.55	0.084	4.05	0.261
Minimum	967	7.86	6.37	19.84	0.008	1.57	0.058
Mean (± SE)	1049 ± 41	7.9 ± 0.04	6.9 ± 0.4	22.7 ± 1.5	0.04 ± 0.02	2.6 ± 0.7	0.2 ± 0.07
Site 3 (0.6 km)							
Maximum	1063	8.04	10.17	25.33	0.111	3.68	0.179
Minimum	842	7.85	5.52	10.74	0.026	2	0.051
Mean (± SE)	988 ± 48	7.9 ± 0.04	6.1 ± 1.1	22.7 ± 3.2	0.06 ± 0.02	2.8 ± 0.4	0.1 ± 0.03
Site 4 (3.85 km)							
Maximum	1320	8.05	7.45	26.49	0.045	6.87	0.341
Minimum	978	7.63	4.14	19.24	0.026	2.79	0.062
Mean (± SE)	1108 ± 74	7.9 ± 0.09	5.8 ± 0.7	23.5 ± 1.6	0.04 ± 0.004	4.1 ± 0.4	0.2 ± 0.06

Table 1.4 - Continued. Water quality parameters as measured throughout the study period.

Site (distance downstream from discharge)	Orthophosphorus As P (mg/L)	Total Suspended Solids (mg/L)	Chlorine (mg/L)	E Coli Bacteria (MPN/100 mL)
Site 1 (-0.16 km)				
Maximum	0.053	75.7	0.36	727
Minimum	0.004	31.4	0.1	131
Mean (± SE)	0.02 ± 0.01	48.3 ± 9.8	0.2 ± 0.07	381.0 ± 129.3
Effluent (0 km)				
Maximum	1.18	1	0.66	582
Minimum	1.18	< 1	0.66	23
Mean (± SE)	1.2 ± 0	1.0 ± 0	0.7 ± 0	302.5 ± 279.5
Site 2 (0.05 km)				
Maximum	0.148	56.5	0.1	345
Minimum	0.004	24.3	0.1	42
Mean (± SE)	0.1 ± 0.05	40.9 ± 9.3	0.1 ± 0	169.7 ± 90.7
Site 3 (0.6 km)				
Maximum	0.071	69	0.1	344
Minimum	0.028	20.3	0.1	36
Mean (± SE)	0.06 ± 0.01	45.8 ± 13.4	0.1 ± 0	179.0 ± 63.5
Site 4 (3.85 km)				
Maximum	0.68	62.5	0.11	651
Minimum	0.028	11	0.1	30
Mean (± SE)	0.3 ± 0.1	33.9 ± 11.4	0.1 ± 0.003	304.3 ± 130.6

Table 1.5. Contaminants detected in higher concentrations
downstream of the discharge.

	Copper (µg/L)	Magnesium (mg/L)	Potassium (mg/L)	Zinc (µg/L)
Site 1	1.9	10.7	5.94	10.1
Effluent	5.5	15.9	19.9	66.2
Site 4	2.1	11.3	11.6	23.1

Table 1.6. Results of MANCOVA and ANCOVA tests on *A. plicata* data.
Tests on whole wet mass, BCI-wet, oxygen consumption, ammonia excretion,
and O:N ratio were performed on the percent change from pre- to post-
exposure measurements. Tests on BCI-dry and dry tissue mass were performed
on data collected post-mortem. MANCOVA was analyzed using all seven
measured parameters as dependent variables. Both MANCOVA and
ANCOVA tests were run with the average pre- and post-exposure mussel
whole wet mass as covariate.

|	df (num, den)	F	Sig.
MANCOVA (all 7 parameters)	21, 6.3	3.858	0.046
Whole Wet Mass (% change)	3, 11	8.706	0.003
BCI-wet (% change)	3, 11	9.88	0.002
Oxygen consumption (% change)	3, 10	2.278	0.142
Ammonia excretion (% change)	3, 11	0.577	0.642
O:N ratio (% change)	3, 8	0.362	0.782
BCI-dry	3, 11	18.666	< 0.000
Dry tissue mass (g)	3, 11	27.14	< 0.000

Table 1.7. Mean *A. plicata* pre- and post-exposure measurements and percent change (± SE) for the physical and physiological parameters measured in our study for each site. Mean tissue dry mass and mean BCI-dry were only measured at day 72.

	Site 1 0.16 km above	Site 2 0.05 km below	Site 3 0.61 km below	Site 4 3.65 km below
Total mean wet mass, g (± SE)				
0 days	124.56 ± 4.45	131.67 ± 5.53	112.08 ± 3.02	122.76 ± 2.06
72 days	127.57 ± 4.14	131.73 ± 5.41	113.53 ± 2.77	123.39 ± 1.90
% Change	2.58 ± 0.58 *	0.08 ± 0.22	1.32 ± 0.28 *	0.50 ± 0.40
Mean BCI-wet (± SE)				
0 days	1.48 ± 0.04	1.52 ± 0.05	1.35 ± 0.04	1.46 ± 0.02
72 days	1.52 ± 0.04	1.52 ± 0.05	1.36 ± 0.04	1.46 ± 0.01
% Change	2.38 ± 0.46 **	-0.01 ± 0.42	1.26 ± 0.42	0.05 ± 0.41
Mean oxygen consumption rate, µg/h/g whole wet mass (± SE)				
0 days	7.50 ± 0.35	7.74 ± 0.86	7.53 ± 0.84	7.28 ± 0.45
72 days	12.01 ± 0.64	8.20 ± 1.34	9.64 ± 0.73	9.74 ± 0.94
% Change	71.39 ± 14.52 *	13.54 ± 22.04	19.53 ± 10.95	51.89 ± 18.67
Mean ammonia excretion rate, µg/h/g whole wet mass (± SE)				
0 days	2.51 ± 0.38	2.27 ± 0.15	2.06 ± 0.34	2.08 ± 0.13
72 days	2.07 ± 0.19	1.55 ± 0.16	1.78 ± 0.17	1.97 ± 0.23
% Change	'-5.75 ± 15.14	-27.35 ± 11.89	-6.48 ± 8.23	-3.93 ± 10.25
Mean O:N ratio (± SE)				
0 days	3.41 ± 0.49	3.43 ± 0.36	4.25 ± 0.77	3.46 ± 0.37
72 days	5.36 ± 0.60	5.10 ± 1.18	5.55 ± 0.86	5.35 ± 0.81
% Change	73.92 ± 44.64	60.17 ± 48.71	36.42 ± 13.73 *	81.92 ± 44.60
Mean tissue dry mass, g (± SE)				
72 days	5.29 ± 0.18	3.98 ± 0.18	3.37 ± 0.15	3.60 ± 0.23
Mean BCI-dry (± SE)				
72 days	113.96 ± 1.91	80.39 ± 2.41	78.87 ± 3.52	78.11 ± 6.04

Significant results of t-tests comparing pre- and post-exposure data indicated by asterisks
(* Indicates p-value of < 0.05, ** indicates p-value of < 0.01)
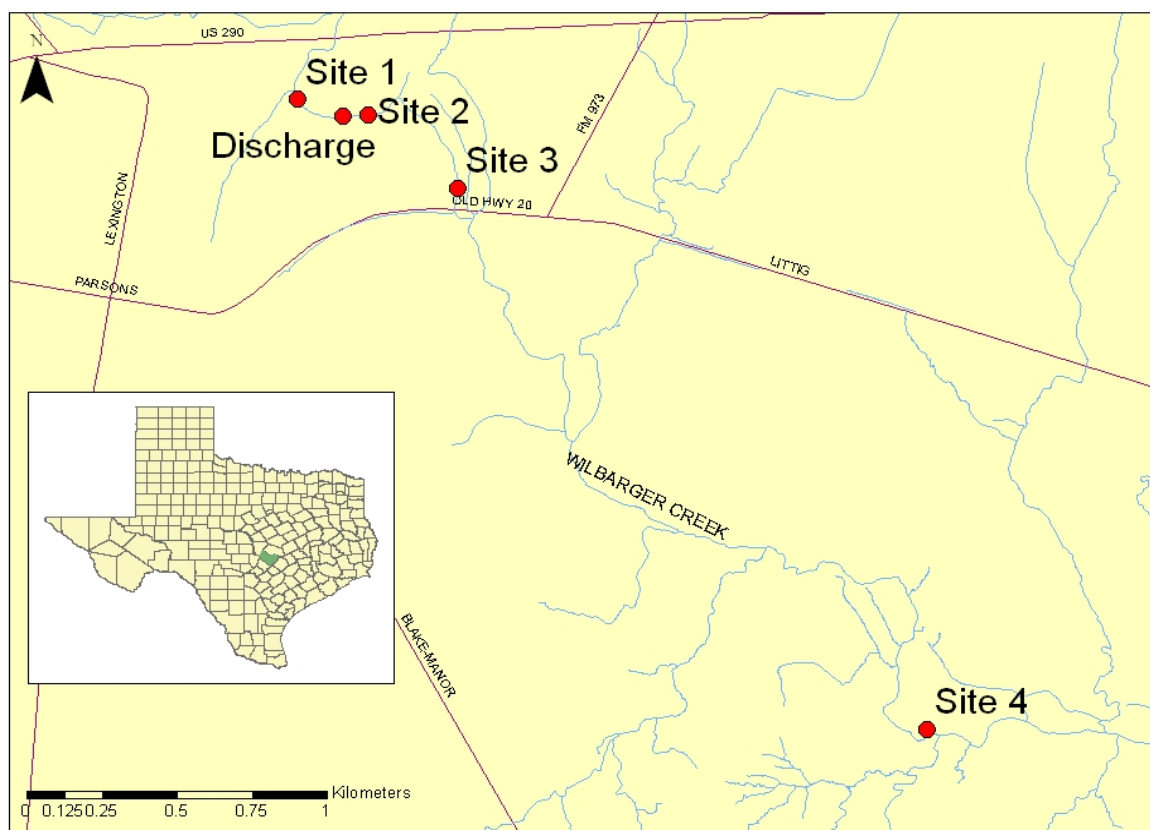
Figure 1.1. Map showing location of the four study sites in relation to the Wilbarger WWTP discharge.
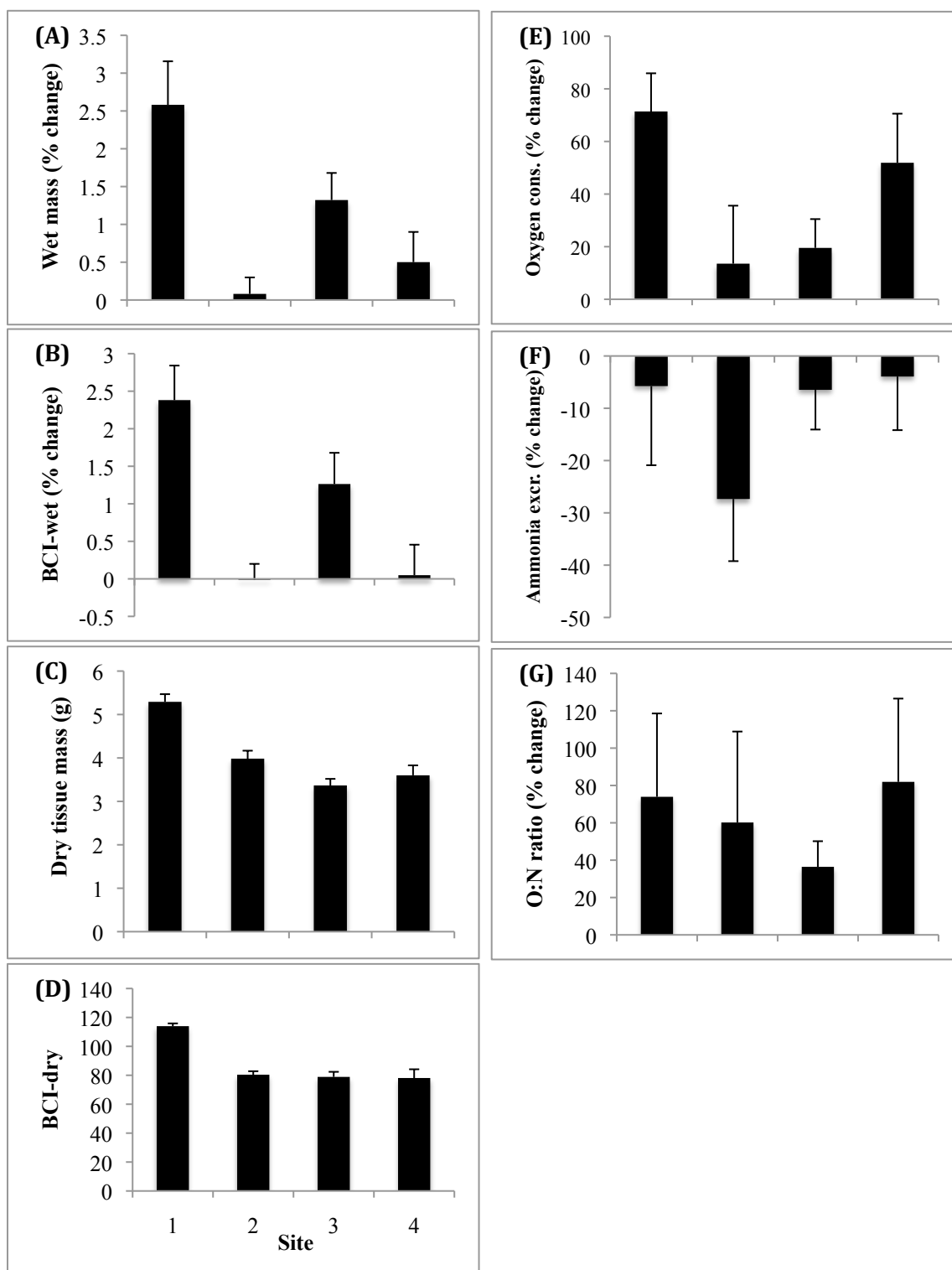
Figure 1.2. Physical (A-D) and physiological (E-G) responses of *A. plicata* to 72 days of exposure to effluent at four sites in Wilbarger Creek. Error bars represent ± 1 standard error.
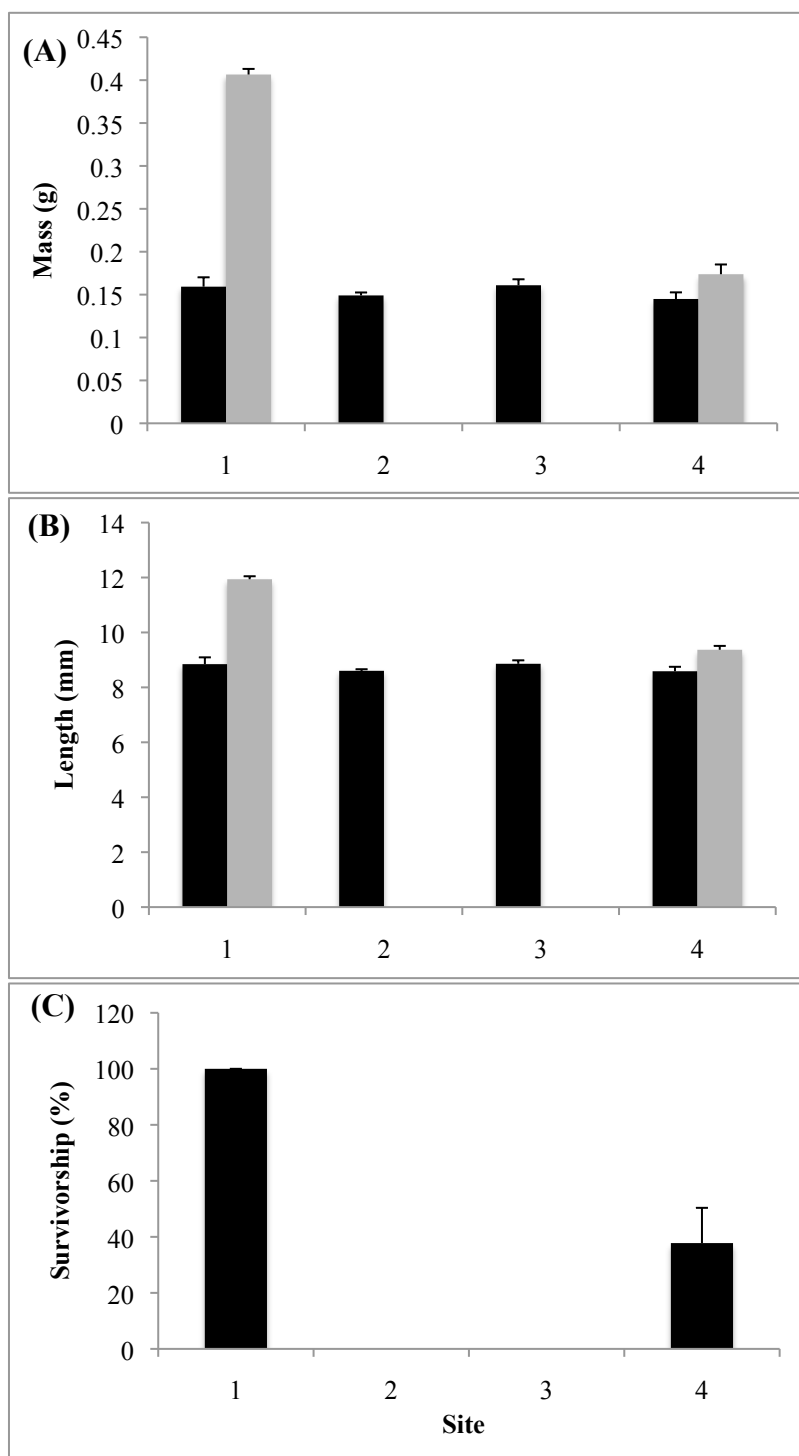
Figure 1.3. *C. fluminea* growth and survival after 72 days of exposure to
effluent at four sites in Wilbarger Creek. (A) Mean whole wet mass in
grams at beginning (black bars) and end (gray bars) of the study; (B) mean
total length in millimeters at beginning (black bars) and end (gray bars) of
the study; (C) mean percent survivorship at end of study. Error bars
represent ± 1 standard error.

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CHAPTER 2

THE EFFECT OF EXTREME FLOOD EVENTS ON ENDANGERED FRESHWATER MUSSEL POPULATIONS IN THE BIG BEND REGION OF THE RIO GRANDE RIVER

INTRODUCTION

Over the last 100 years, freshwater mussels of the order Unioniformes have declined in abundance and diversity in almost all parts of the world. In North America, where unionid mussels show their greatest diversity, 67% of the over 300 species found there are considered threatened (Williams et al. 1993), making them America's most imperiled group of freshwater animals (Strayer et al. 2004). Unionids are especially sensitive to changes in the hydraulic conditions of their habitat (Watters 1999, Strayer et al. 2004). As essentially sessile, benthic animals that spend their lives buried in the substrate, mussels cannot effectively move to more favorable areas during periods of extreme high or low flows. Thus, their effective habitat is restricted to areas that are submerged during low flow drought periods, but that also provide refuges during flood conditions. These specific habitat requirements result in mussels being found in characteristic "mussel beds", or patchy areas of relatively high densities of individuals.

Recent research has shown that complex hydraulic parameters such as Froude and Reynolds numbers, shear stress and shear velocity are more accurate predictors of mussel distribution (Strayer 1999, Johnson and Brown 2000, Hardison and Layzer 2001). Several

studies have also found mussel abundances to be lower in areas with high shear stress at high flows (Howard and Cuffey 2003, Gangloff and Feminella 2007, Allan and Vaughn 2010). At high current velocities, shear stress can mobilize the sediment particles, and can result in the dislodging, redistribution, and burying of the mussels themselves. High shear stress can also prevent the settling of juvenile mussels into the substrate and thus limit recruitment (Hardison and Layzer 2001, Morales et al. 2006). Taken together, these studies suggest that areas providing low shear stress and stable substrate at high flow provide crucial refuges during spates and allow the formation of mussel beds, provided that other resource requirements are met. This hypothesis has been tested and supported by Strayer (1999) who found that mussel abundances were higher in areas where marked stones moved the least during a spate (i.e. flow refuges).

This dependence on suitable flows puts unionids at risk from extreme flow events. Studies focusing on changes in unionid mussel abundance before and after flood events are rare, as large infrequent floods are difficult or impossible to predict both spatially and temporally and having recent survey data on streams affected by major floods is typically coincidental. Those studies that have looked at changes in abundance before and after major floods have found decreases in abundance (Hastie et al. 2001), no change in abundance (Miller and Payne 1998), or both increases and decreases in abundance in different streams and with different mussel species (Fraley and Simmons 2006). This paucity of data represents a blind spot in our knowledge of how a highly threatened group of aquatic animals responds to a regularly occurring natural phenomenon that has the potential to significantly impact their populations. This is especially concerning given

that human activities and global climate change is altering the frequency and severity of flood events (Poff et al., 1997; Gibson et al., 2005; Knutson et al., 2010)

Here, we present the effects of major floods on unionid mussel populations in the middle section of the Rio Grande River based on pre- and post-flood surveys at five sites between Dryden and Laredo, Texas.

MATERIALS AND METHODS

Site description

The Rio Grande is the fifth longest river in North America, and in Texas forms the international border between Mexico and the United States (Figure 2.1). Its flow is highly dependent on inputs from the Rio Conchos River system in northern Mexico, which depending on rainfall contributes between 69 and 86 percent of the Rio Grande's discharge below its confluence near the town of Presidio, TX approximately 300 km west of Dryden (Dean and Schmidt, 2011). The Rio Conchos is also highly regulated by seven major reservoirs with 3,942 Mm^3 of total storage capacity (Kelly 2001). The Rio Grande at Dryden is a predominately confined river flowing through sandstone canyons, with a median daily discharge of 22 cubic meters per second at the USGS gaging station located at Foster Ranch, approximately 70 km downstream of the Dryden survey site near the town of Langtry, TX. Our other four survey sites were located downstream of Amistad Reservoir, which was constructed in 1969 near the confluence of the Rio Grande and Pecos Rivers just upstream of the town of Del Rio, TX. Amistad has a surface area of 26,300 hectares and a storage capacity of 3,886 Mm^3 at conservation pool, and was constructed primarily for flood control and water storage (IBWC, 2011). The

construction of dams both on the Rio Conchos and the Rio Grande have significantly altered the timing, duration, magnitude, and frequency of flooding in the Rio Grande River (Dean and Schmidt, 2010; Sandoval-Solis et al., 2010).

The Rio Grande drainage in Texas, New Mexico, and Mexico has historically supported at least 16 species of unionid mussels, including 3 endemic species (Howells et al. 1996). Surveys have shown that unionid populations have declined precipitously in the Rio Grande. One species, the Rio Grande Monkeyface (*Quadrula couchiana*), is thought to be extinct, and another, the Texas Hornshell (*Popenaias popei*) is currently being petitioned for federal listing under the Endangered Species Act. The Salina Mucket (*Potamilus metnecktayi*) and Mexican Fawnsfoot (*Truncilla cognata*) were until recently thought to be extinct, but several live specimens of each have been found in recent surveys. Howells (2003) suggested this decline is most probably due to pollution, flow regulation, and other human disturbance, but could also be caused by an increased frequency of extreme storm events. The study area lies in an arid region of west Texas, receiving an average of 370 mm of precipitation annually, and nearly all major flood events are the result of hurricanes or tropical storms.

Magnitude of flooding at Dryden

The two flood events discussed here, caused by Tropical Depression Lowell in 2008 and Hurricane Alex in 2010, differ in both magnitude and duration, but both events represent catastrophic floods having significant impacts on the Rio Grande ecosystem. TD Lowell crossed northern Mexico from the Pacific, bringing massive rainfall to the Conchos watershed. The six major reservoirs on the Rio Conchos, already at or near

conservation level, absorbed the rainfall until they were at capacity and then began

releasing water to prevent overflow. The La Boquillas dam, the largest in the Rio

Conchos system, released water at a rate of 1,318 cms (Conagua, 2008). The reservoirs

continued to release water at a steady rate for weeks afterward, resulting in the flood of

record at Presidio and a protracted period of high flows in the Rio Grande downstream.

At Dryden, discharge reached the fifth highest on record (1,540 cms), and remained

above 1,000 cms for 13 days and over 500 cms for 30 days. Whereas the major impact of

TD Lowell was the unnaturally extended duration caused by reservoir release in Mexico,

Hurricane Alex was characterized by a brief but intense spike in discharge that receded

within a few days. Rainfall totals exceeded 530 mm over the Rio Conchos basin and 380

mm at Dryden, which was 800% above normal for that period. Discharge reached the

highest recorded at the Dryden gaging station (2440 cms), but only remained above 1,000

cms for one day and over 500 cms for four days (Figure 2.2), a typical pattern seen in

pre-dam construction hydrographs (Sandoval-Solis et al., 2010).

Magnitude of flooding at Laredo

Unlike the Rio Grande above Amistad Reservoir, the area downstream was not

significantly affected by the 2008 flood caused by Tropical Depression Lowell. The

reservoir, at 70% storage capacity before the influx of floodwaters from upstream, was

able to provide a buffer to downstream areas. Discharge at Laredo only rose to 605 cms,

although it remained above 500 cms for 24 days (Figure 2.3). Just prior to Hurricane

Alex, however, Amistad was already at 99% capacity, and was unable to buffer the

floodwaters as it did in 2008. Discharge at Laredo peaked at 3170 cms and remained

above 500 cms for 19 days (Figure 2.2). The floodwaters reached 12.9 m, the third

highest ever recorded and the highest since Amistad Dam was constructed in 1969.

Similarly to the hydrograph above Amistad, the river at Laredo only stayed above major

flood stage for two days, again in a pattern more typically seen prior to dam construction

(Figure 2.3).

Field surveys

A preliminary mussel survey was performed at seven locations on the Rio Grande

River between Dryden and Laredo in March 2008, six months before the flood event

caused by Tropical Depression Lowell. A follow-up survey was performed at five of the

same locations in 2011 and 2012 (a fence constructed in 2009 by the Department of

Homeland Security prevented us from accessing two sites in Del Rio, TX). Surveys were

conducted by semi-quantitative timed tactile searches of the substrate surface and up to

25 cm below the substrate in water depths less than 1.5 m, along with quantitative 0.25

m^2 random quadrat surveys conducted at one location in Laredo where we found

relatively high abundance during the timed searches. All mussels found were identified,

measured for length, and returned to the substrate except for a few voucher specimens,

which were preserved in 95% ethanol. Survey effort was calculated by:

T (total search time) × N (number of surveyors) = E (total effort in person-hours).

Total and species catch per unit effort (CPUE) was calculated by dividing the total

number of mussels and the number of each species of mussel found by the total effort in

person-hours. Quantitative results were calculated by dividing the number of unionid

mussels found by the total area sampled. Latitude and longitude coordinates, as measured

by hand-held GPS unit, were recorded for each sampling location, along with general habitat parameters including depth, substrate type. General observations on the effects of the flood events were also recorded during the 2011 survey. All collecting was performed under appropriate state and federal permits.

<div align="center">RESULTS</div>

General post-flood observations

While the study sites were not revisited between the 2008 and 2010 floods, local river expert, Keith Bowden, reported that massive riparian and geomorphic changes occurred to the river at the study site in Dryden, and there were thousands of dead mussel shells on the banks, more than he had seen in over 30 years of rafting the Rio Grande (personal communication, 2008). The National Park Service (NOAA, 2009) reported breached levees on both the American and Mexican portions of the river, damaged building structures, major bed scouring in the river channel, loss of riverbanks, and the removal of riparian vegetation in Big Bend National Park upstream of Dryden. Although the peak discharge of the 2010 flood was nearly 800 cms higher than that of the 2008 flood, the duration was much shorter and the effects were mainly felt below Amistad Reservoir near Laredo. During the follow-up survey of 2011 at the Dryden site, however, no sign of the massive piles of mussel shells reported by Keith Bowden in 2008 were found, and were presumably washed downstream during the flood. Only 7 valves were found in nearly 8 km of searching in 2011. A horizontal band of large cobble to small boulder-sized river rock was also found on a perched bench approximately 10 m above the riverbed that we presume was deposited there by the recent floods. Near Laredo, the

impacts of the 2010 flood could be seen by the flattened riparian vegetation to the top of the channel (approximately 10 m).

Pre- and post-flood mussel data

A total of seven mussel species were found during the 2008 survey: Tampico pearlymussel (*Cyrtonaias tampicoensis*), Texas hornshell (*Popenaias popei*), Salina mucket (*Potamilus metnecktayi*), Mexican fawnsfoot (*Truncilla cognata*), yellow sandshell (*Lampsilis teres*), washboard (*Megalonaias nervosa*), and southern mapleleaf (*Quadrula apiculata*). A total of four species were found in 2011-2012 surveys: *P. popei*, *P. metnecktayi*, *L. teres*, and *Q. apiculata*. At three survey sites (Vega Verde Rd., Normandy, and Eagle Pass), no mussels were found during the course of either survey. At Dryden, the 2008 survey found 24 individuals of three species (*C. tampicoensis, P. metnecktayi, and P. popei*) during 3.5 person hours at one site, for a total CPUE of 6.86 (Table 2.1). During the 2011 survey, we only found one *P. metnecktayi* in 30 person hours, resulting in a much lower total CPUE of 0.03. At Laredo just downstream of the International Bridge, we found 17 individuals of five species in 8 person hours during the 2008 timed search: *T. cognata, P. popei, L. teres, Q. apiculata,* and *M. nervosa*. The total CPUE for 2008 was 2.13 (Table 2.1). In 2012, we only found three species: *P. popei, L. teres*, and *Q. apiculata*. However, we collected 31 mussels in 3 person hours, resulting in a much higher total CPUE of 6.86 (Table 2.1). For the quantitative quadrat survey of the Laredo site, we collected a total of 15 individuals of three species in 2008: *T. cognata, P. popei*, and *Q. apiculata*. We sampled a total of 9.75 m^2 for an overall density of 1.54 mussels/m^2 (Table 2.2). In 2012, we found fewer species and fewer individuals. We

collected one *P. popei* and one *L. teres* in 15.5 m^2, resulting in an overall density of 0.13 mussels/m^2.

<div align="center">DISCUSSION</div>

Flood effects on mussel populations

In their study of the effects of a major flood on endangered mussel populations in the River Kerry in Scotland, Hastie et al. (2001) identified four main causes of mortality to the mussels at their site: desiccation due to stranding after flood waters receded, damage by mobile bedload, crushing by newly deposited sediment, and washing out to sea. The abundance of shells high on the bank following the 2008 flood at Dryden indicates high mortality due to stranding. It would be impossible at this point to determine number killed by crushing or burying, and although our site is far away from the Gulf of Mexico, it is approximately 90 km upstream of Amistad Reservoir, and it is possible that many of the mussels dislodged by the flood events were washed into it. It is also possible that some unionids were deposited live in downstream portions of the river or in the lake itself. Hastie et al. (2001) found several previously unmapped mussel beds after the flood in their study, although Strayer (1999) asserts that unionids dislodged by high flows are unlikely to settle in suitable locations.

Comparison of pre- and post-flood surveys

We only have thorough post-flood survey data for the section of river between the confluence with the Rio Conchos and Lake Amistad. Our 2008 trip was a preliminary survey of a large portion of the Rio Grande conducted to direct future efforts, and as

access to this part of the river is extremely limited this was the only site above Lake Amistad that was sampled. However, the change from the relatively high abundance of mussels discovered during the 2008 survey to only one individual found in 30 person-hours of searching the same portion of river in 2011 indicates that densities there were impacted severely by the extreme flood events and have declined significantly. To our knowledge, there have been no significant changes in land use or water quality to this part of the Rio Grande between the 2008 and 2011 surveys. This suggests that the decline in abundance and species richness of the mussels at Dryden was likely due to the two major flood events in 2008 and 2010. Because we did not conduct a survey between the 2008 and 2010 floods, we cannot determine which event impacted populations more. We assume that the mussel populations could be severely impacted by a combination of two floods of this magnitude occurring within two years. The reported presence of large numbers of mussels killed after the 2008 event suggests (anecdotally at least) that this flood had a strong impact on mussel populations. Similarly, the 7 shells found in 2011 suggests that fewer mussels were killed during the 2010 flood, although we could not determine whether this was due to the 2010 flood being less destructive or to the fact that fewer mussels were present in 2010 due to mortality from the 2008 flood.

Mussel abundance at the Laredo site did not seem to be greatly affected by the 2010 flood there. Although density based on quadrat surveys declined significantly in 2012, quantitative sampling is not highly effective in populations found in low densities (Strayer et al., 1997; Vaughn et al., 1997) as they are in much of the Rio Grande. Our timed searches at Laredo indicate abundance has increased following the 2010 event. Species richness declined from five species found in 2008 to three in 2012, although the

two species missing in 2012 were already found in low abundance in 2008 and may have been missed in the 2012 survey. The almost total absence of unionids found at Dryden and seemingly stability of the population at Laredo begs the question as to what caused the differences between these two sites. We propose that the highly lengthened duration of the 2008 flood in Dryden caused by month-long emergency dam releases upstream affected this population significantly more than the more natural high-magnitude-but-short-duration flood of 2010 in Laredo that more closely resembles the flood pattern the mussels have adapted to over the millennia.

Conservation and management concerns

Flooding is of course not a new phenomenon to the Rio Grande system. Tropical storms have and will continue to bring large amounts of precipitation to the region in short periods of time. What has changed in the last 100 years though is the human regulation of the river through the use of dams and levees. The 2008 flood wreaked such havoc on the Rio Grande ecosystem because of its duration, which was unnaturally prolonged due to the steady release of waters from the Rio Conchos reservoirs in Mexico. Since the 1940s, the magnitude and duration of floods in the Rio Grande has declined, in some parts by more than 50% (Schmidt et al. 2003). The reduction in flood intensity has created a positive feedback loop, altering the morphology of the river by narrowing the channel width and aggrading the streambed, changing the Rio Grande from a wide, meandering braided stream to a narrow, single-channel one (Dean and Schmidt 2010). These morphological changes were also exacerbated by the presence of dense stands of non-native vegetation (*Tamarix spp*. and *Arundo donax*), which stabilizes banks and

floodplains and encourages sediment deposition. As a result of this narrower, shallower

channel, over-bank flood events have paradoxically become more common and occur at

lower peak discharges since major dam construction (Dean and Schmidt 2010). It is

believed that the simplification of the stream channel has also reduced the amount of

ecologically important in-stream habitat, including low-flow refuge areas (Dean 2009).

These geomorphic changes could be halted or reversed, however, through riparian

vegetation management and by regulating dam releases to provide major sediment-

flushing flows along with more frequent smaller, but still overbank, floods that could

inhibit non-native vegetation establishment and growth (Dean 2009).

Mussel population recovery after the floods

To our knowledge, there have been no studies looking at the long-term recovery

of unionid mussel populations following major flood events. In a study on the recovery of

non-mussel macroinvertebrate communities after two major floods on the Colorado

River, Rader et al. (2005) found that both abundance and richness rebounded to pre-flood

levels within 10 years after the floods. A similar study on the Staunton River in Virginia

found a full post-flood recovery of non-mussel invertebrates compared to reference sites

after only 3 years (Snyder and Johnson 2006). The rapid recolonization of non-mussel

invertebrates observed in disturbed areas has been attributed to downstream drift,

upstream within-water movement, vertical migration within the substrate, and aerial

movement by adults (Williams and Hynes 1976). As previously mentioned, it is possible

for adult unionids to be dislodged during floods and colonize downstream habitat, and

while this is unlikely to be a significant source of mussel population recovery (Strayer

1999), the downstream drift of larvae and juveniles is common (Strayer 2004). Likewise, upstream movement of adults is unlikely as unionids are only capable of lateral movement at the scale of a few meters. Vertical migration, while important to small invertebrates living in interstitial spaces, is possibly a recovery mechanism for mussels during less intense flooding, as juveniles often bury themselves beneath the surface of the substrate and are therefore not directly exposed to high shear stress which can dislodge larger adults (Hastie et al. 2001). During extreme flood events that mobilize large areas of substrate, however, it is likely that most or all mussels in the mobilized sediment will be dislodged, as was seen in the River Kerry study by Hastie et al. (2001). While unionids do not have aerial adult stages, they do have a parasitic larval stage that utilizes fish as hosts. This is considered the dominant method of juvenile dispersal in unionids, as the infected hosts can move considerable distances both up- and downstream before the larvae detach and drift to the substrate (Strayer et al. 2004).

The lone *P. metnecktayi* found during the 2011 Dryden survey was 20 mm in length, and while growth rates are unavailable for this species, it is likely that it was less than one year of age. A small, 18 mm *P. popei* was also found when draining a cooler of river water used to transport live captured fish after returning from the survey, and was also likely to be less than one year of age. We did not report this individual in the results section, as it was not found in the course of the formal survey. The presence of these two juvenile unionids implies that reproduction and recruitment is taking place in the study site, most likely through movement of fish hosts or downstream drift of larvae. As no adult mussels were found in the 8 km of river surveyed in 2011, we hypothesize that a source population of breeding adults exists somewhere upstream of the Dryden site.

Vannote and Minshall (1982) suggested that stable boulder-dominated refugia are critical in the recovery of unionids after floods in the Salmon River, Idaho. In an earlier survey of the Rio Grande near Laredo, TX, large numbers of *P. popei* were found in flow refuges under large, flat stones (unpublished data). It is possible that adult populations have persisted in such flow refuges upstream of Dryden, and that recruitment of juveniles is taking place there as well.

<div align="center">CONCLUSIONS</div>

Flooding is a natural and necessary disturbing force in stream and river ecosystems that populations have adapted to over millennia. Unionids, along with other benthic stream organisms, would not have persisted if they had not found ways of surviving and recovering after extreme disturbance events. Human activities and disturbances, however, have changed the frequency, magnitude, and duration of flood events in the Rio Grande River. When coupled with the loss of instream habitats and the impacts of other stressors such as pollution, unionid mussels are facing grave threats on many fronts. The results of surveys suggest that mussel populations in the Dryden area significantly declined following the extreme events of floods of 2008 and 2010, whereas populations in Laredo remained reletively stable. Population information is still lacking for most parts of this river, but especially for the area between Big Bend and Amistad. This basic data is crucial for determining the status of these already threatened mussels, and must be collected if long-term population trends are to be identified.

Table 2.1. Total and by-species results of pre-flood (2008) and post-flood (2011-2012) timed surveys conducted at five sites on the Rio Grande River. Results are presented as Catch-Per-Unit-Effort (CPUE). Sites where no mussels were found during either survey have been ommitted.

Site	Year	Species	Total Live	Person hours	CPUE	
Dryden	2008	*Popenaias popei*	1	3.5	0.29	
	2011		0	30	0.00	
	2008	*Cyrtonaias tampicoensis*	8	3.5	2.29	
	2011		0	30	0.00	
	2008	*Potamilus metnecktayi*	15	3.5	4.29	
	2011		1	30	0.03	# Species
	2008	Total	24	3.5	6.86	3
	2011		1	30	0.03	1

Site	Year	Species	Total Live	Person hours	CPUE	
Laredo	2008	*Truncilla cognata*	2	8	0.25	
	2012		0	3	0.00	
	2008	*Popenaias popei*	5	8	0.63	
	2012		15	3	5.00	
	2008	*Megalonaias nervosa*	1	8	0.13	
	2012		0	3	0.00	
	2008	*Lampsilis teres*	2	8	0.25	
	2012		3	3	1.00	
	2008	*Quadrula apiculata*	7	8	0.88	
	2012		13	3	4.33	# Species
	2008	Total	17	8	2.13	5
	2012		31	3	10.33	3

Table 2.2. Total and by-species results of pre-flood (2008) and post-flood (2011-2012) quadrat surveys conducted on the Rio Grande River at Laredo. Results are presented as density in mussels per m^2.

| Year	Species	Total Live	Area surveyed (m^2)	Density	
2008	*Popenais popei*	4	9.5	0.42	
2011		1	15.5	0.06	
2008	*Lampsilis teres*	0	9.5	0.00	
2011		1	15.5	0.06	
2008	*Quadrula apiculata*	8	9.5	0.84	
2011		0	15.5	0.00	
2008	*Truncilla cognata*	3	9.5	0.32	
2011		0	15.5	0.00	# Species
2008	Total	15	9.5	1.58	3
2011		2	15.5	0.13	2
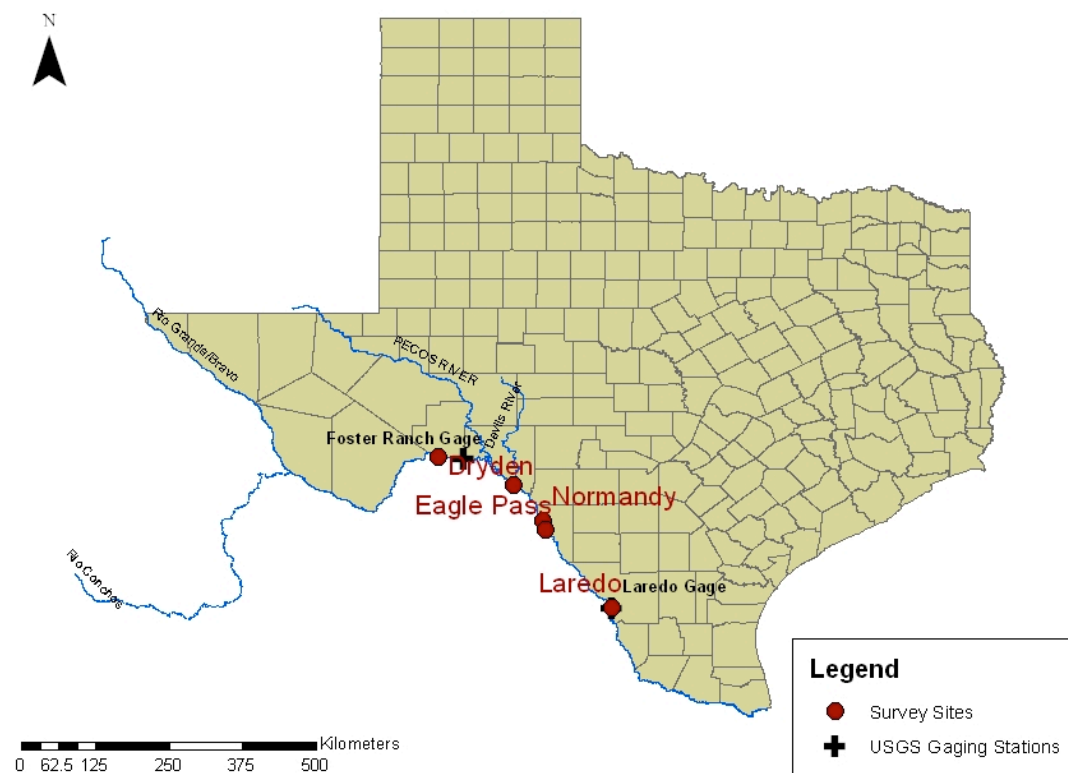
Figure 2.1. Map of the mussel survey sites (circles) and USGS gaging stations (crosses) used in this study.
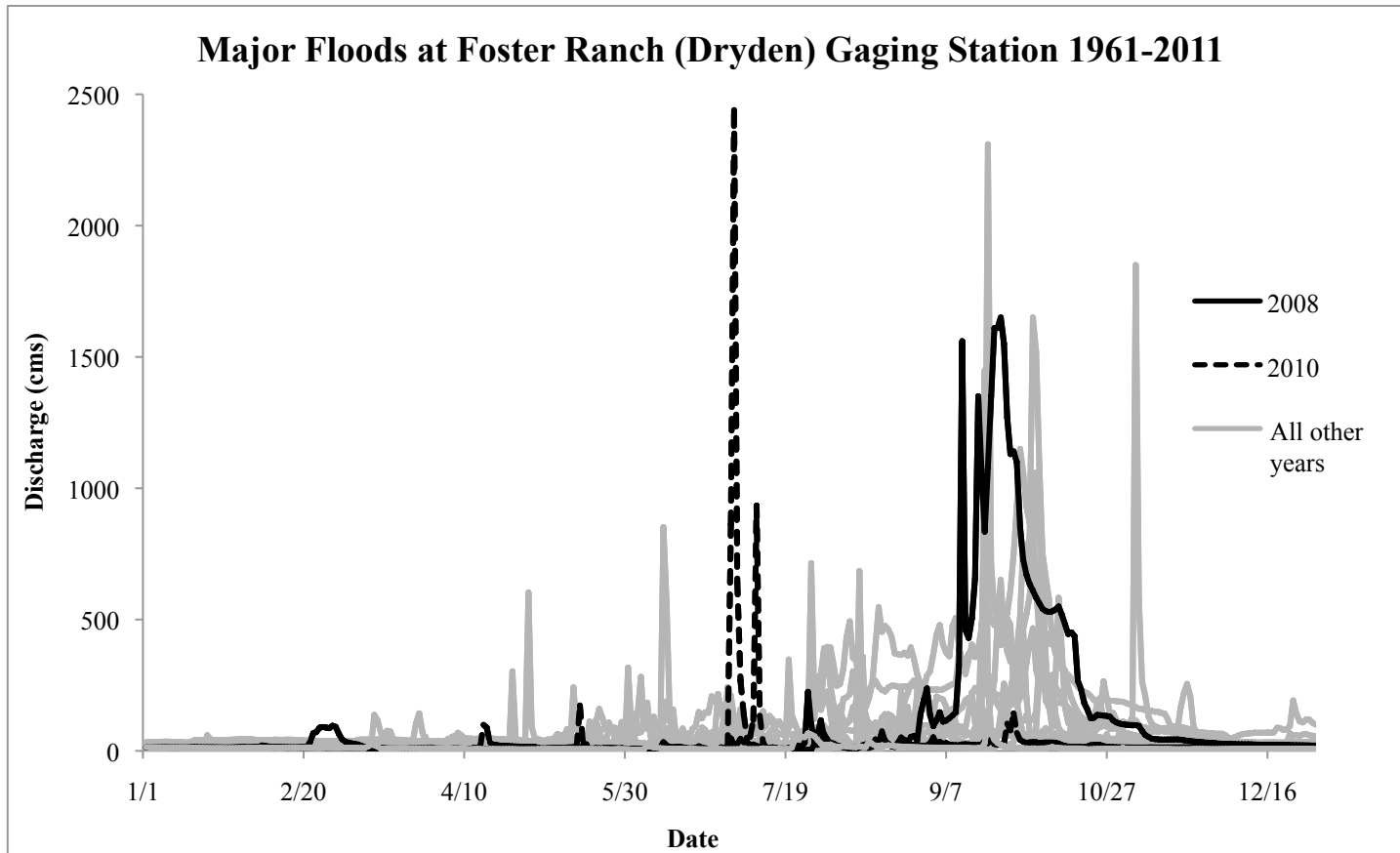
Figure 2.2. Hydrograph of major floods on the Rio Grande River at the Foster's Ranch gaging station for the years 1961-2011. The flood of 2008 caused by Tropical Depression Lowell is shown by the solid black line, and the flood of 2010 caused by Hurricane Alex is shown by the dotted black line. All other years are shown in gray.
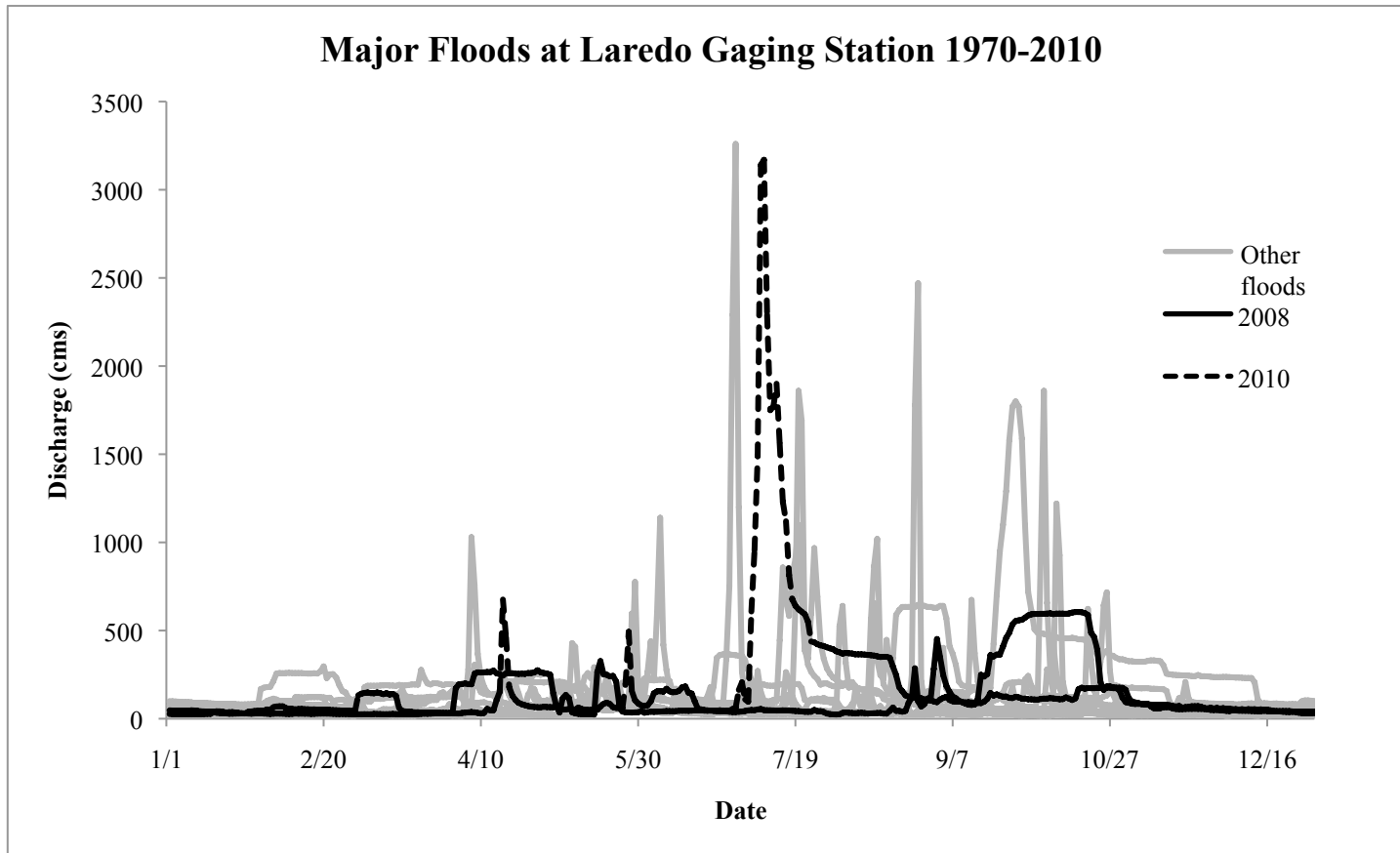
Figure 2.3. Hydrograph of major floods on the Rio Grande River at the Laredo gaging station for the years 1970 -2011 following construction of the Amistad Reservoir dam upstream of Laredo. The flood of 2008 caused by Tropical Depression Lowell is shown by the solid black line, and the flood of 2010 caused by Hurricane Alex is shown by the dotted black line. All other years are shown in grey.
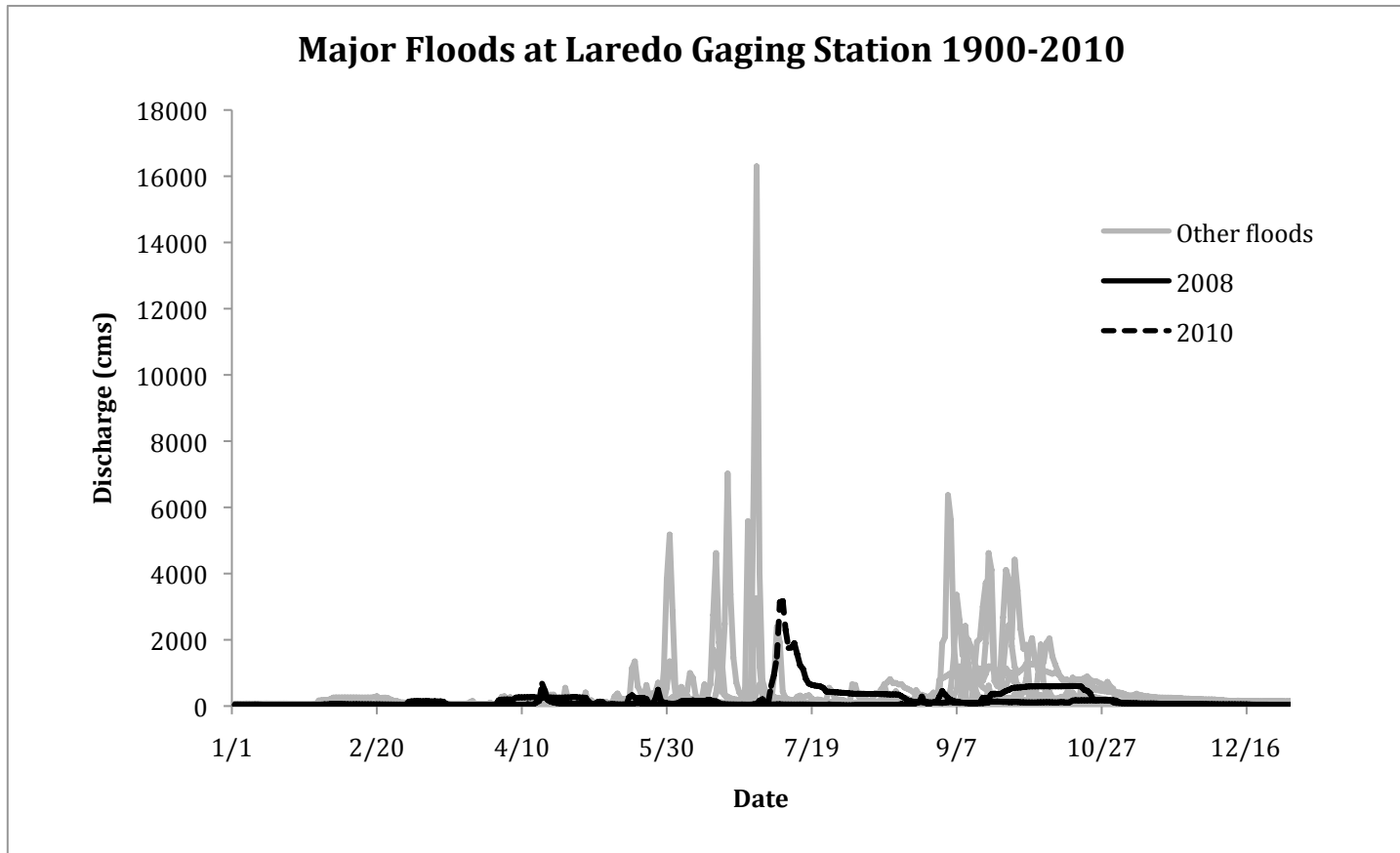
Figure 2.4. Hydrograph of major floods on the Rio Grande River at the Laredo gaging station for the years 1900 -2011. All floods except 2008 and 2010 occurred prior to the construction of the Amistad dam in 1970. The flood of 2008 caused by Tropical Depression Lowell is shown by the solid black line, and the flood of 2010 caused by Hurricane Alex is shown by the dotted black line. All other years are shown in grey.

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