

## Effects of Municipal Wastewater Effluent on Freshwater Mussel Growth and Survival

SR-13-02, November 21, 2012

Alex Duncan<sup>1</sup> and Trey Nobles<sup>2</sup>

<sup>1</sup>City of Austin, Watershed Protection Department, Environmental Resource Management Division, <sup>2</sup>Texas State University, Department of Biology

### Abstract

There is growing concern over the impacts of Waste Water Treatment Plant (WWTP) effluent to overall stream health and function. Freshwater mussels are routinely utilized as biological indicators of water quality due to their sessile lifestyle and filter feeding abilities. In order to better understand how freshwater mussels may be affected by WWTP effluent, a caged experiment was performed upstream and downstream of a WWTP with tertiary treatment discharging to Wilbarger Creek in Manor, TX. Both native *Amblema plicata* and non-native *Corbicula fluminea* received a 72 day in-stream exposure period. Native *A. plicata* showed significantly lower mass and condition indices and exhibited lower oxygen consumption rates below the discharge compared to the upstream reference site. Juvenile *C. fluminea* exhibited much lower growth and survival rates below the discharge, whereas all mussels above the discharge survived and grew substantially. Results suggest that freshwater mussels' survival, growth, and reproduction may be negatively impaired after exposure to high quality WWTP effluent. Elevated constituents of copper, potassium, magnesium, and zinc in the water column directly downstream of the discharge may be related to observed detrimental impacts. 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 ecosystem impacts on biodiversity and conservation must be taken into account when planning their location and operation.

### Introduction

---

There are many ways in which growing urban populations negatively impact stream ecosystems. One of these ways is the discharge of wastewater treatment plant (WWTP) effluent into streams and rivers (Paul and Meyer, 2001). Depending on the level of treatment performed on the wastewater, WWTP effluent can contain high levels of nutrients, bacteria, metals, pesticides, polycyclic aromatic hydrocarbons, endocrine disruptors and other pharmaceutical chemicals (Ciccotelli and Colombo, 1998; Kolpin *et al.*, 2002). With many WWTPs discharging into small streams, their effluent can comprise a significant portion of stream baseflow. In many arid and semi-arid environments, WWTP effluent can supply 90% or more of stream flow during droughts or dry periods (Brooks *et al.* 2006), with minimal fresh water instream flow to dilute the effluent. The impact of this effluent on stream ecosystems can vary depending on the quality and amount of effluent and the physical and biological characteristics of the receiving stream.

Generally, WWTP discharge can result in eutrophication, increased community respiration rates and biological oxygen demand, increased organic matter decomposition rates, reduced nutrient uptake, feminization of fish and invertebrates, reduced fitness in biological organisms, and reduced overall biological diversity (Gucker *et al.* 2006).

Freshwater mussels are among the most threatened groups of aquatic organisms. Five central Texas species found in the Colorado River basin are currently being assessed for federal protection under the Endangered Species Act. As sessile filter feeders, they absorb many of the toxins present in the water and cannot move to areas with lower concentrations of pollutants. Most mussel species are relatively intolerant of elevated nutrient and toxin concentrations, especially during their larval and juvenile life stages, and have been shown to be harmed by WWTP effluent (Ciccotelli and Colombo, 1998). Because of these characteristics, freshwater mussels have been effectively used as biomonitors to indicate overall water quality (Hellou and Law, 2003; Martel *et al.*, 2003; Gangloff *et al.*, 2009). The goal of this study was to determine if effluent from a WWTP with tertiary treatment has a negative impact on native freshwater mussels and non-native clams in a Central Texas intermittent stream. Growth, condition indices, respiration, and excretion of the native threeridge mussel (*Amblema plicata*) and growth and survivorship of the non-native Asian clam (*Corbicula fluminea*) were measured after 72 days of exposure to WWTP effluent. Additionally,

## Methods

---

### *Site Description*

The study was conducted on 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<sup>2</sup>. 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. Wilbarger Creek is a seasonally intermittent stream; however, many sections have become perennial due to supplemental effluent additions. Under drought conditions these sections may become completely dominated by undiluted wastewater effluent. Discharge at the Elgin gauge ranged from 1 ft<sup>3</sup>/s to 8,950 ft<sup>3</sup>/s during the study period of February 24 through May 22, 2012. There are eight active municipal WWTPs that cumulatively discharge an estimated 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 additional effluent, more than doubling the current allowed discharge.

In order to investigate the effects of municipal wastewater effluent on freshwater mussels of Wilbarger Creek, we chose four sites near the Wilbarger Creek Wastewater Treatment Facility (TPDES Permit No. WQ0012900001) located in and operated by the city of Manor, TX (Figure 1). Current discharge is up to 0.5 mgd, with future permitted discharge up to 2 mgd (see Table 1 for effluent constituent limitations). 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 2). Based on the preliminary WQ results and the literature, it was determined that a distance of greater than 3.7 km downstream of the discharge should be a sufficient to minimize effluent impacts on water quality of Wilbarger Creek (Goudreau *et al.* 1993). 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). 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 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.

### *Stream Monitoring*

Habitat surveys were performed 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. Water quality parameters were collected at each site on three occasions between early March and mid-June. All surface water quality monitoring was done in accordance with the City of Austin, Water Resource Evaluation Standard Operating Procedures Manual (COA 2010). Water samples were collected from the top one-third of the water column, being careful not to get surface debris or bottom sediments into the container. All samples were iced to at least 4°C during transport to the lab and kept out of sunlight, making sure to meet holding times for each requested analysis. All routine water chemistry analysis was performed by the Lower Colorado River Authority (LCRA) a National Environmental Laboratory Accreditation Program (NELAP) accredited laboratory located at 3505 Montopolis Drive in Austin, TX. Analyzed parameters are listed in Table 4. In addition, dissolved oxygen, water temperature, conductivity, and pH were sampled during every field visit using a Hydro Tech Hydrolab MiniSonde 4a v2.06. All City of Austin sondes receive routine maintenance and are pre/post calibrated after every sampling event (COA 2010).

In addition to the routine parameters listed above additional water quality monitoring was performed, following completion of the field sampling portion of the study, in order to better explain the treatment effects observed. These additional water quality parameters were measured once on June 12, 2012 by the LCRA laboratory and are listed in table 5. A portion of the samples were filtered in the field using a Geotech Geopump 2 with Masterflex platinum coated silicon tubing and a Geotech 0.45 micron high capacity disposable filter. In-situ chlorine was also measured in the field using a Hach DR/2000 Spectrometer in accordance with USEPA method 330.5.



**Figure 1.** Map of four selected sampling sites in addition to the WWTP Outfall location.

**Table 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 2.** Preliminary water quality test results used to determine site locations.

Distance from outfall (km)	Cond ( $\mu$ S/cm)	pH	DO (mg/L)	Temp ( $^{\circ}$ C)	TSS (mg/L)	Total phosphorus (mg/L)	E. coli (mpn/100ml)	Ammonia (mg/L)	Nitrate (mg/L)
0	1215	8.02	9.15	18.02	< 1	0.192	582	0.011	13.2
0.61	842	8.04	10.17	10.74	20.3	0.051	344	0.026	3.68
5.79	851.5	8.11	9.35	11.43	25	0.074	226	0.035	3.43
10.21	808	8.15	10.4	10.66	26.5	0.072	192	0.037	3.32
14.33	760	8.12	9.6	10.85	20.3	0.181	323	0.056	2.93

**Table 3.** Physical measurements and description of sites used in study.

Site	Distance downstream of discharge (km)	Habitat type	Substrate	Mean depth (m) <sup>a</sup>	Mean wetted width (m) <sup>a</sup>	Mean bankfull width (m) <sup>a</sup>	Mean canopy cover (%) <sup>a</sup>
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 <sup>b</sup>	3.65	Run	silt/grvl/c obl	0.57 ± 0.19	5.4 ± 0.48	13.0 ± 2.01	77.6 ± 9.81

<sup>a</sup> Value ± SE

<sup>b</sup> Only run habitat data from Site 4 are included

**Table 4.** Routine core laboratory parameters for water samples. Analysis methods and analytical costs.

Lab Parameter	Analytical Method	Cost per sample
Ammonia-Nitrogen (mg/L)	EPA 350.1	\$10.00
Nitrate+Nitrite-Nitrogen (mg/L)	EPA 353.1	\$12.00
Total Orthophosphorus (mg/L)	EPA 300.0	\$12.00
Total Suspended Solids (mg/L)	EAP 160.2	\$8.00
<i>Escherichia coli</i> (cfu/100mL)	SM 9223 B	\$25.00

**Table 5.** List of sampled constituents measured for detailed Water Quality analysis

Volume/Type	Analytes	Preservative
125 mL, plastic	E. coli	Na2SO4
1 L amber glass	Caffeine	none
1 L	TSS, VSS	none
500 mL	Alk, Br, Cl, F, OP, SO4	none (total)
250 mL	NH3, NO3, TKN, TOC, TP	H2SO4 (total)
250 mL	Al, Ba, Ca, Cu, Fe, Pb, Zn	HNO3 (filtered in field)
250 mL	As, B, Mg, Na, Sr, K	HNO3 (total)

#### *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 (Table 8; Table 9; Figure 3) of native *Amblema plicata* and non-native *Corbicula fluminea* both before and after in-situ exposure to Wilbarger Creek water at our four sites. *Amblema 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  $84.3 \pm 3.53$  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 (Vaughn *et al.* 2007). 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.

Mussels were allowed to recover from handling stress for 24 hours before beginning initial physiological status measurements. Initial physiological status (Table 8) of the *A. plicata* was measured 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  $\mu$ m 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 was downloaded at the end of each day, 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, water was filtered from the chamber through a 1  $\mu$ m glass fiber filter and frozen (125 mL sample) for later ammonia concentration analysis using a Cary 50 spectrophotometer (Varian Inc., Palo Alto, CA, U.S.A.) set at 630 nm. Results were calibrated using blanks as zero and known standards 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.

Juvenile *C. fluminea* were used 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  $\pm$  SE  $8.72 \pm 0.05$  mm, mean mass  $0.15 \pm 0.004$  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 the 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 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.

Experimental cages were constructed 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. Two mm plastic canvas mesh was attached 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. Mussels were transported 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. Four cages were placed in the middle of the channel at each site approximately 2 m apart in a checkerboard pattern and each cage was anchored 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 *A. plicata* halfway in the gravel substrate of each cage in their natural

infaunal orientation. Cages were checked 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, cages were collected and brought back to the lab in the 89 L cooler filled with stream water (25° C) from above the discharge. The total instream exposure time was 72 days. Lengths and masses of each *C. fluminea* were measured upon returning to our lab following the same methods as at the beginning of the study. All *A. plicata* were acclimated and re-measured using the same parameters and procedures as at the beginning of the study. In addition to re-measuring the initial parameters, soft tissue of each *A. plicata* was also dried 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). We calculated BCI-dry using dry soft tissue weight (g) X 1000/internal shell cavity volume (ml), and are assumed to be the most accurate measure of assessing the nutritive and stress status of bivalves (Crosby and Gale, 1995).

### *Statistical Analysis*

In order to determine the overall effect of site on the physical and physiological status of *A. plicata*, a Multiple Analysis of Covariance (MANCOVA) test was conducted with our seven measured parameters as dependent variables, site as factor variable, and pre-exposure whole wet mass as covariate (Vaughn *et al.* 2007). 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 during the ANCOVA test, Fisher's LSD test was used to identify differences between the sites. A paired T-tests was also conducted 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 was tested for normality and homogeneity of variance using Kolmogorov-Smirnoff and Levene's tests, respectively.

## **Results**

### *Water Quality*

Other than conductivity, there were no significant spatial or temporal differences in water quality parameters measured throughout the study period (Table 6). Although nitrogen and phosphorous concentrations were elevated at the effluent discharge, there were not enough data collection points to make statistical comparisons between the effluent and other sites. The effluent discharge had significantly higher conductivity levels than all other measured sites (Table 6); however, this difference is considered negligible because its contributions to overall stream conductivity levels were minimal. Of the 28 parameters measured after the completion of the study (Table 5) only copper, magnesium, potassium, and zinc had any detectable elevated differences between the upstream reference, effluent discharge, and farthest downstream comparison site (Table 7).

**Table 6.** Water quality parameters as measured throughout the study period.

Site (distance downstream from discharge)	Conductivity ( $\mu\text{S/cm}$ )	pH	Dissolved Oxygen (mg/L)	Temperature ( $^{\circ}\text{C}$ )	Ammonia As N (mg/L)	Nitrate/Nitrite As N (mg/L)
Site 1 (-0.16 km)						
Maximum	1190	8.03	7.65	27.35	0.049	4.29
Minimum	877	7.75	5.15	19.51	0.008	0.024
Mean ( $\pm$ SE)	993 $\pm$ 71	7.9 $\pm$ 0.06	6.3 $\pm$ 0.6	23.4 $\pm$ 1.6	0.03 $\pm$ 0.008	1.8 $\pm$ 1.0
Effluent (0 km)						
Maximum	1677	8.02	9.15	27.42	0.626	19.9
Minimum	1215	7.75	7.54	18.02	0.011	13.2
Mean ( $\pm$ SE)	1475 $\pm$ 86	7.8 $\pm$ 0.05	7.9 $\pm$ 0.3	24.1 $\pm$ 1.7	0.3 $\pm$ 0.3	16.5 $\pm$ 3.4
Site 2 (0.05 km)						
Maximum	1093	8.00	7.83	24.55	0.084	4.05
Minimum	967	7.86	6.37	19.84	0.008	1.57
Mean ( $\pm$ SE)	1049 $\pm$ 41	7.9 $\pm$ 0.04	6.9 $\pm$ 0.4	22.7 $\pm$ 1.5	0.04 $\pm$ 0.02	2.6 $\pm$ 0.7
Site 3 (0.6 km)						
Maximum	1063	8.04	10.17	25.33	0.111	3.68
Minimum	842	7.85	5.52	10.74	0.026	2
Mean ( $\pm$ SE)	988 $\pm$ 48	7.9 $\pm$ 0.04	6.1 $\pm$ 1.1	22.7 $\pm$ 3.2	0.06 $\pm$ 0.02	2.8 $\pm$ 0.4
Site 4 (3.85 km)						
Maximum	1320	8.05	7.45	26.49	0.045	6.87
Minimum	978	7.63	4.14	19.24	0.026	2.79
Mean ( $\pm$ SE)	1108 $\pm$ 74	7.9 $\pm$ 0.09	5.8 $\pm$ 0.7	23.5 $\pm$ 1.6	0.04 $\pm$ 0.004	4.1 $\pm$ 0.4
Site (distance downstream from discharge)	Total Phosphorus As P (mg/L)	Orthophosphorus As P (mg/L)	Total Suspended Solids (mg/L)	Chlorine (mg/L)	<i>E. coli</i> Bacteria (MPN/100 mL)	
Site 1 (-0.16 km)						
Maximum	0.139	0.053	75.7	0.36	727	
Minimum	0.083	0.004	31.4	0.1	131	
Mean ( $\pm$ SE)	0.1 $\pm$ 0.01	0.02 $\pm$ 0.01	48.3 $\pm$ 9.8	0.2 $\pm$ 0.07	381.0 $\pm$ 129.3	
Effluent (0 km)						
Maximum	1.41	1.18	1	0.66	582	
Minimum	0.192	1.18	< 1	0.66	23	
Mean ( $\pm$ SE)	0.8 $\pm$ 0.6	1.2 $\pm$ 0	1.0 $\pm$ 0	0.7 $\pm$ 0	302.5 $\pm$ 279.5	
Site 2 (0.05 km)						
Maximum	0.261	0.148	56.5	0.1	345	
Minimum	0.058	0.004	24.3	0.1	42	
Mean ( $\pm$ SE)	0.2 $\pm$ 0.07	0.1 $\pm$ 0.05	40.9 $\pm$ 9.3	0.1 $\pm$ 0	169.7 $\pm$ 90.7	
Site 3 (0.6 km)						
Maximum	0.179	0.071	69	0.1	344	
Minimum	0.051	0.028	20.3	0.1	36	
Mean ( $\pm$ SE)	0.1 $\pm$ 0.03	0.06 $\pm$ 0.01	45.8 $\pm$ 13.4	0.1 $\pm$ 0	179.0 $\pm$ 63.5	
Site 4 (3.85 km)						
Maximum	0.341	0.68	62.5	0.11	651	
Minimum	0.062	0.028	11	0.1	30	
Mean ( $\pm$ SE)	0.2 $\pm$ 0.06	0.3 $\pm$ 0.1	33.9 $\pm$ 11.4	0.1 $\pm$ 0.003	304.3 $\pm$ 130.6	

**Table 7.** Contaminants detected in elevated concentrations downstream of the Manor WWTP effluent 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

*Physical and Physiological Response by A. Plicata*

Results of MANCOVA testing of physical fitness of *A. Plicata* showed a significant overall difference between sites (Table 8). There were significant differences between sites for all four physical parameters: percent change in whole wet mass, percent change in BCI-wet, BCI-dry, and dry tissue mass (Table 8). Mussels at Site 1 above the discharge consistently showed the greatest increase in physical and physiological status compared to those downstream of the discharge (Table 9). In contrast to the physical parameters, there were no significant ANCOVA results among oxygen consumption, ammonia excretion, and O:N ratio (Table 8; Figure 2E-G). This is likely due to high variability in the responses of individual mussels at all sites (Table 9).

**Table 8.** 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.

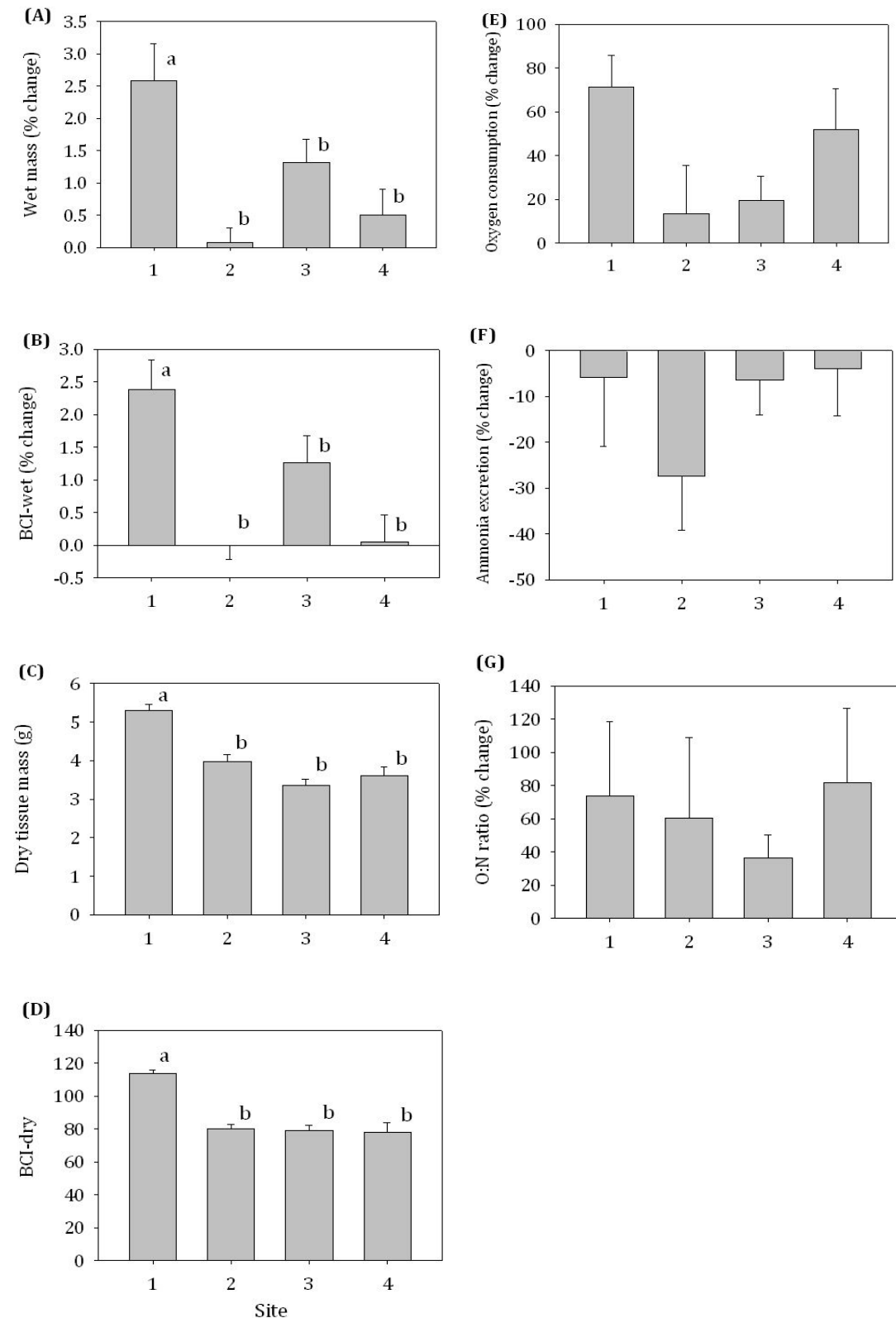
	df (num, den)	F	P Value
MANCOVA (all 7 parameters)	21, 6.3	3.858	0.046
Whole Wet Mass (% change)	3, 11	8.706	0.003
BCI-wet (% change)	3, 11	9.88	0.002
Oxygen consumption (% change)	3, 10	2.278	0.142
Ammonia excretion (% change)	3, 11	0.577	0.642
O:N ratio (% change)	3, 8	0.362	0.782
BCI-dry	3, 11	18.666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

**Table 9.** Mean *A. plicata* pre- and post-exposure measurements and percent change ( $\pm$  SE) for the physical and physiological parameters measured in our study for each site.

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

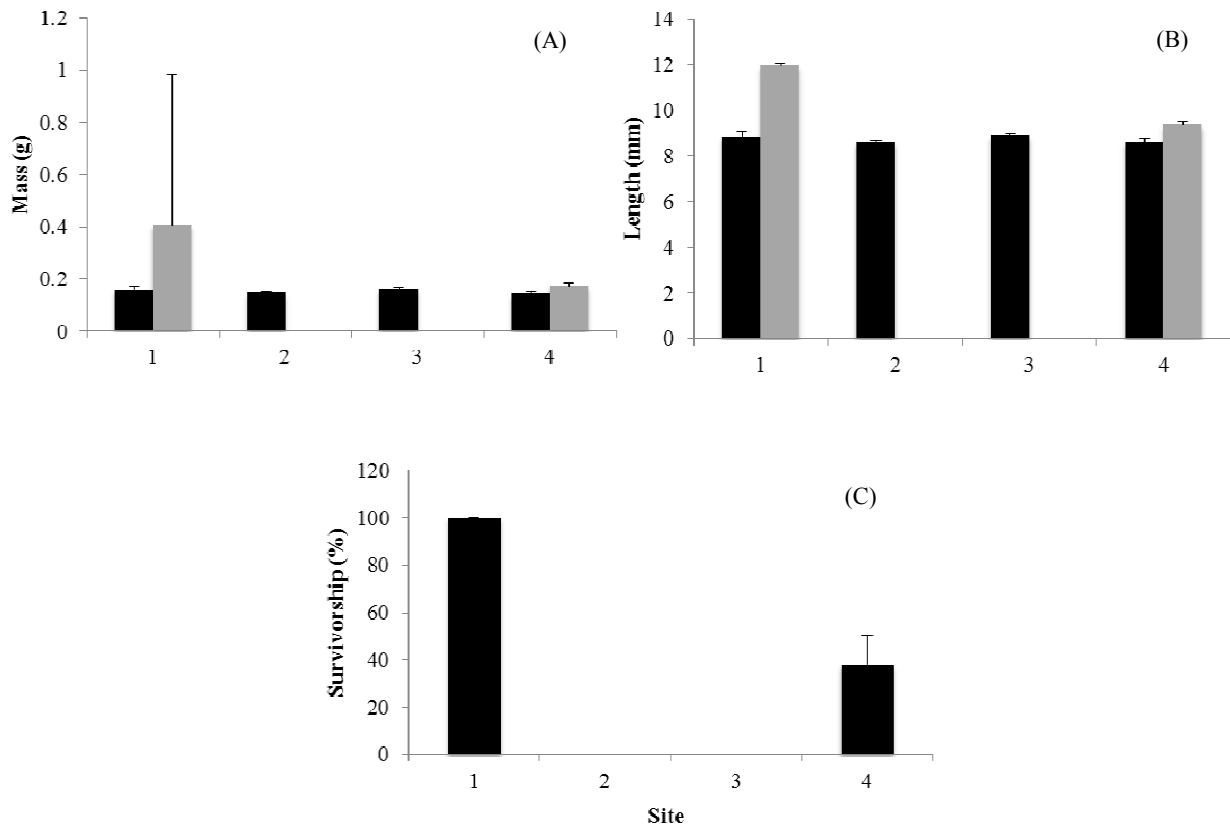
**Figure 2.** Physical (A-D) and physiological (E-G) responses of *A. Plicata* after 72 days exposure to WWTP Effluent at 4 site locations in Wilbarger Creek. Columns with the same letter were not significantly different from each other at the p=0.05 level.



### Survival and Growth of *C. fluminea*

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. 3A). Growth in whole wet mass at Site 1 increased from an average of  $0.16 \pm 0.01$  g/mussel pre-exposure to  $0.41 \pm 0.007$  g/mussel post-exposure. Mussels at Site 4 increased slightly from  $0.15 \pm 0.008$  g/mussel to  $0.17 \pm 0.01$  g/mussel (Fig. 3B). Growth in length at Site 1 increased from  $8.85 \pm 0.13$  mm/mussel to  $11.94 \pm 0.11$  mm/mussel. At Site 4, mussels increased slightly from  $8.59 \pm 0.08$  mm to  $9.37 \pm 0.14$  mm (Fig. 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.

**Figure 3.** *C. fluminea* growth and survival after 72 days of exposure to effluent discharge in Wilbarger Creek at for sampling locations. (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  $\pm 1$  standard error.



## Discussion

---

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 above the discharge at Site 1 survived and grew substantially.

### *Effluent effects on distribution and mortality*

The results of this study 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<sup>2</sup> upstream of discharge from a WWTP with secondary treatment on the Blanco River in Texas to 0.0 mussels/m<sup>2</sup> immediately downstream, and density increased to only 0.2 mussels/m<sup>2</sup> at 2 km downstream. Horne and McIntosh (1979) 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. Horne and McIntosh (1979) 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<sub>50</sub> 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 Burns, 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. The Goudreau *et al.* (1993) 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. Goudreau *et al.* (1993) water quality analyses revealed that total residual chlorine at sites just below the WWTPs regularly exceeded the 24 hour LC<sub>50</sub> of 0.084 mg/L they established through laboratory testing. While instream ammonia levels only exceeded the Goudreau *et al.* (1993) determined LC<sub>50</sub> 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. Gangloff *et al.* (2009) 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 Goudreau *et al.* (2009) LC<sub>50</sub> of 0.084 mg/L. 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 and clams there.

#### *Effluent effects 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. 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.*, 2002), 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.*, 2002). 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; Herwig, 1989; 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 elevated metal concentration measured during this study is correlated 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, 1990). 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. Smolders *et al.* (2002) found that zebrafish showed lower condition indices and increased respiration when exposed to high levels of wastewater effluent in laboratory tests. Smolders *et al.* (2002) 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 geometrically increasing respiration downstream at Sites 3 and 4. Smolders *et al.* (2002) 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, 1990). However respiration rates have also been shown to decline when exposed to high concentrations of toxins (Widdows and Johnson, 1989; 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 significantly increased downstream of the discharge, but overall shell length did not, causing a shell growth abnormality known as "shell-length-to-tissue-weight growth decoupling." Gagne *et al.* (2001) concluded that the estrogen-like compounds in the effluent caused the mussels to redirect energy into Vt production at the expense of somatic growth. Blaise *et al.* (2003) performed a follow-up study at the same sites as Gagne *et al.* (2001) using *E. complanata* and *D. polymorpha* again finding 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. 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). 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 could have resulted from a 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 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). Fewer adult mussels not only reduce the immediate population, but also reduce the number of potential recruits needed to repopulate a particular area. 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 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; Augsburg *et al.*, 2003), and can be killed or immobilized at concentrations below that known to

adversely 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). Effluents can also affect mussel populations through alteration in adult reproduction. 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 fluoxetine, 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, 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.

## Conclusions

---

In this study, we have shown that both native and non-native mussel species can be significantly impaired by 72 days exposure to domestic wastewater effluent for at least up to 3.85 km downstream from the effluent discharge. Native *A. plicata* showed significantly lower mass and condition indices and exhibited lower oxygen consumption rates below the discharge compared to the upstream reference site. Juvenile *C. fluminea* exhibited much lower growth and survival rates below the discharge, whereas all mussels above the discharge survived and grew substantially. 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; specifically the association of elevated copper, magnesium, potassium and zinc on reduced mussel growth and survival. 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 ecosystem impacts on biodiversity and conservation must be taken into account when planning their location and operation.

## Recommendations

---

1. Evaluate the health of extant populations of freshwater mussels in Austin's lakes and streams; specifically monitoring for changes in survival, growth, and reproduction.

2. Repeat the Caged Mussel experiment at Wilbarger and other creeks with WWTP discharges around Austin to see if trends are similar in different watersheds and repeatable over time. Include monitoring of elevated Water Quality constituents identified during this study: copper, magnesium, potassium and zinc.
3. Additional Surveys in our larger watersheds (Onion, Barton, Dry, Gilleland, and Wilbarger) and protected ponds and oxbows of the Colorado are warranted to rule out the presence of rare and endangered mussels.

## References

---

- Akaishi, F. M., St-Jean, S. D., Bishay, F., Clarke, J., Rabitto, I. S., Oliveria Ribe, C.A. 2007.** Immunological responses, histopathological finding and disease resistance of blue mussel (*Mytilus edulis*) exposed to treated and untreated municipal wastewater. *Aquat. Toxicol.*, 82(1):1-14.
- Aldridge, D. W., Payne, B. S., and Miller, A. C. 1987.** The Effects of Intermittent exposure to suspended solids and turbulence on three species of freshwater mussels. *Environ. Poll.*, 45:17-28.
- Aldridge, D. W., Payne, B. S., and Miller, A. C. 1995.** Oxygen consumption, nitrogenous excretion, and filtration rates of *Dreissena polymorpha* at acclimation temperatures between 20 and 32°C. *Can. J. Fish. Aquat. Sci.*, 52:1761-1767.
- Allen, Y., Calow, P., Baird, D.J., 1995.** A mechanistic model of contaminant-induced feeding inhibition in *Daphnia magna*. *Environ. Toxicol. Chem.* 14, 1625–1630.
- Anderson, D. T., White, B. M., and Egan, E. A. 1976.** The larval development and metamorphosis of the ascidians *Pyura praeputialis* (Heller) and *Pyura pachydermatina* (Herdman), (Pleurogona, Family Pyuridae). *Proc. Linn. Soc. New South Wales* 100:205-217.
- Augsburger, T., Keller, A. E., Black, M. C., Cope, W. G., and Dwyer, F. J. 2003.** Water quality guidelines for protection of freshwater mussels (Unionidae) from ammonia exposure. *Environmental Toxicology and Chemistry*, 22: 2569-2575.
- Ausley, L.W., 2000.** Reflections on whole effluent toxicity: The Pellstone workshops. *Environ. Toxicol. Chem.* 19, 1–2.
- Baker, S.M. and Hornbach, D.J. 2000.** Physiological status and biochemical composition of a natural population of Unionid mussels (*Amblema plicata*) infested by zebra mussels (*Dreissena polymorpha*). *American Midland Naturalist* 143: 443-452.
- Bayne, B. L., Moore, M. N., Widdows, J., Livingstone, D. R. and Salkeld, P. 1979.** Measurements of the responses of individuals to environmental stress and pollution: studies with bivalve molluscs. *Phil. Trans. R. Soc. Lond. B* 286, 563-581.
- Beyers, D. W., Rice, J. A., Clements, W. H., and Henry, C. J. 1999.** Estimating physiological cost of chemical exposure: integrating energetics and stress to quantify toxic effects in fish. *Can. J. Fish. Aquat. Sci.* 56, 814– 822
- Billoir, E, Pery, A. R. R., and Charles, S. 2007.** Integrating the lethal and sublethal effects of toxic compounds into the population dynamics of *Daphnia magna*: a combination of the DEBtox and matrix population models. *Ecol Model* 203(3–4):204–214
- Blaise, C., Gagné, F., Salazar, M., Salazar, S., Trottier, S., Hansen, P. D. 2003.** Experimentally induced feminisation of freshwater mussels after long-term exposure to a municipal effluent. *Fresenius Environ Bull*;12:865–70.

- Bouchard, B., Gagne, F., Fortier, M. and Fournier, M. 2009.** An in-situ study of the impacts of urban wastewater on the immune and reproductive systems of the freshwater mussel *Elliptio complanata*. *Comparative Biochemistry and Physiology, Part C* 150: 132–140.
- Bringolf, R. B., Heltsley, R. M., Newton, T. J., Eads, C. B., Fraley, S. J., Damian, S., and Cope, G. W. 2010.** Environmental occurrence and reproductive effects of the pharmaceutical fluoxetine in native freshwater mussels. *Enviro. Tox, and Chem.* 29(6):1311-1318
- Calow P, Sibly RM. 1990.** A physiological basis of population processes: ecotoxicological implications. *Funct. Ecol.*, 4(3): 283-288.
- Ciccotelli, M., Crippa, S. and Colombo, A. 1998.** Bioindicators for toxicity assessment of effluents from a wastewater treatment plant. *Chemosphere* 37: 2823-2832.
- City of Austin. 2010.** Standard Operating Procedures Chapters 3, 5 & 6.
- Crosby, M. P., AND Gale, L. D. 1990.** A review and evaluation of bivalve condition index methodologies with a suggested standard method. *J. Shellfish Res.* 9: 233-237.
- Curtis, T.M., Williamson, R., and Depledge, M.H. 2000.** Simultaneous, long-term monitoring of valve and cardiac activity in the blue mussel *Mytilus edulis* exposed to copper. *Mar. Biol.* 136, 837–846
- Dietz, T. H., and Byrne, R. A. 1990.** Potassium and rubidium uptake in freshwater mussels. *J. Exp. Biol.* 150: 395-405.
- Doherty, F.G. and Cherry, D.S., 1988.** Tolerance of the Asiatic Clam *Corbicula* sp. to Lethal Levels of Toxic Stressors - A Review. *Envir. Pollution*, 51:236.
- Donkin, P., Widdows, J., Evans, S. V., Staff, F., and Yan, T. 1997.** Effects of neurotoxic pesticides on the feeding rate of marine mussels *Mytilus edulis*. *Pesticide Science.* 49: 196-209.
- Encomio, V., and Chu, F. L. 2000.** The effect of PCBs on glycogen reserves in the eastern oyster *Crassostrea virginica*. *Mar Environ Res* 50: 45–49.
- Farcy, E., Gagne, F., Martel, L., Fortier, M., Trepanier, S., Brousseau, P., and Fouriner, M. 2011.** Short term physiological effects of a xenobiotic mixture on the freshwater mussel *Elliptio complanata* exposed to municipal effluents. *Environmental Research*, 111(8):1096-1106.
- Fisher, S. W., Stromberg, P., Brunner, K. A., and Boulet, L. D. 1991.** Molluscicidal activity of potassium to the zebra mussel, *Dreissena polymorpha*: Toxicity and mode of action. *Aquatic Toxicol.* 20: 2 19-234.
- Gagné, F., Blaise, C., Salazar, M., Salazar, S., Hansen, P.D. 2001.** Evaluation of estrogenic effects of municipal effluents to the freshwater mussel *Elliptio complanata*. *Comp. Biochem. Physiol. C* 128, 213–225.
- Gangloff, M.M., Siefferman, W.S., Seesock, W. and Webber, E.C. 2009.** Influence of urban tributaries on freshwater mussel populations in a biologically diverse piedmont (USA) stream. *Hydrobiologia* 636: 191-201.

- Gnyubkin, V. F. 2009.** An early warning system for aquatic environment state monitoring based on an analysis of mussel valve movements. *Russian Journal of Marine Biology*, 35(5):431-436.
- Goudreau, S. E., Neves, R. J., and Sheehan, R. J. 1993.** Effects of wastewater treatment plant effluents on freshwater mollusks in the upper Clinch River, Virginia, USA. *Hydrobiologia*, 252:211-230.
- Gucker, B., Brauns, M. and Pusch, M.T. 2006.** Effects of wastewater treatment plant discharge on ecosystem structure and function of lowland streams. *Journal of the North American Benthological Society* 25: 313-329.
- Hellou, J. and Law, R.J. 2003.** Stress on stress response of wild mussels, *Mytilus edulis* and *Mytilus trossulus*, as an indicator of ecosystem health. *Environmental Pollution* 126: 407-416.
- Herwig, H.J. 1989.** Effects of cadmium on valve-gape patterns of a freshwater bivalve. In Vernet, J.P. ed. *Proceedings of an international conference: Heavy Metals in the Environment*, pp. 566-9. Edinburgh: CEP Consultants.
- Horne, S., and MacIntosh, F. R. 1979.** Factors influencing distribution of mussels in the Blanco River of central Texas. *Nautilus* 94:119-132.
- Imlay, M. J. 1973.** Effects of potassium on survival and distribution of freshwater mussels. *Malacologia*; 12:97-113.
- Kraak, M. S.H., Wink, Y. A., Stuijzand, S. C., Buckert-de Jong, M. C., de Groot, C. J., Admlraal, W. 1994.** Chronic ecotoxicity of Zn and Pb to the zebra mussel *Dreissena polymorpha*. *Aquat Toxicol* 30:77-89
- Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B. and Buxton, H.T. 2002.** Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. *Environ. Sci. Technol.* 36: 1202-1211.
- Klok, C., and de Roos, A. M. 1996.** Population level consequences of toxicological influences on individual growth and reproduction in *Lumbricus rubellus* (Lumbricidae, Oligochaeta). *Ecotoxicology and Environmental Safety* 33: 118-127.
- Kooijman, S. A. L. M. 2009.** *Dynamic Energy Budget Theory for Metabolic Organization*, 3rd edn. Cambridge University Press, Great Britain.
- Kooijman, S. A. L. M. 2000.** *Dynamic energy and mass budgets in biological systems*, Cambridge University Press, Cambridge, 2nd Edition
- Kooijman, S. A .L. M., and Bedaux, J. J. M. 1996.** The analysis of aquatic toxicity data. *Chemosphere* 57, 745-753.
- Liao, C. M., Jou, L. J., Lin, C. M., Chiang, K. C., Chou, B. Y. H., and Yeh, C. H. 2007.** Predicting acute copper toxicity to valve closure behavior in the freshwater clam *Corbicula fluminea* supports the biotic ligand model. *Environmental Toxicology*, 22, 295–307.
- Maltby L. 1999.** Studying stress: The importance of organism-level responses. *Ecological Applications* 9, 431-440

- Martel, P., Kovacs, T., Voss, R. and Megraw, S. 2003.** Evaluation of caged freshwater mussels as an alternative method for environmental effects monitoring (EEM) studies. *Environmental Pollution* 124: 471-483.
- Mouabad, A., Ait Fdil, M., Maarouf, A., Pihan, J.C. 2001.** Pumping behaviour and filtration rate of the freshwater mussel *Potomida littoralis* as a tool for rapid detection of water contamination. *Aquat Ecol* 35:51-60
- Muller, E. B., Nisbet, R. M., and Berkley, H. A. 2010.** Sublethal toxicant effects with dynamic energy budget theory: model formulation. *Ecotoxicology*, 19:48-60
- Mummert, A. K., Neves, R. J., Newcomb, T. J., and Cherry, D. S. 2003.** Sensitivity of juvenile freshwater mussels (*Lampsilis fasciola*, *Villosa iris*) to total and unionized ammonia. *Environmental Toxicology and Chemistry*, 22: 2545-2553.
- Munkittrick, L.R., McCarthy, L.S., 1995.** An integrated approach to ecosystem health: Top-down, bottom-up or middle out? *J. Aquat. Ecosyst. Health* 4, 77-90.
- Naimo, T. J. 1995.** A review of the effects of heavy metals on freshwater mussels. *Ecotoxicology* 4:341-362.
- Naimo, T. J., Atchison, G. J., and Holland-Bartels, L. E. 1992.** Sublethal effects of cadmium on physiological responses in the pocketbook mussel, *Lampsilis ventricosa*. *Environ. Toxicol. Chem.*, 11: 1013-1021.
- Ortmann, C., and Grieshaber, M.K. 2003.** Energy metabolism and valve closure behavior in the Asian clam *Corbicula fluminea*. *J. Exp. Biol.* 206, 4167-4178.
- Paul, M.J. and Meyer, J.L. 2001.** Streams in the Urban Landscape. *Annu. Rev. Ecol. Syst.* 32: 333-65.
- Quinn, B., Gagné, F., Costello, M., McKenzie, C., Wilson, J., Mothersill, C. 2004.** The endocrine disrupting effect of municipal effluent on the zebra mussel (*Dreissena polymorpha*). *Aquat Toxicol*; 66:279-92
- Sloof, W., de Zwart, D., Marquenie, J.M., 1983.** Detection limits of a biological monitoring system for chemical water pollution based on mussel activity. *Bull. Environ. Contam. Toxicol.* 30, 400-405.
- Smolders, R., Bervoets, L., De Boeck, G., Blust, R. 2002.** Integrated condition indices of whole effluent toxicity testing in zebra fish (*Danio rerio*). *Environ. Toxicol. Chem.* 21 (1): 87-93.
- Tran, D., Ciret, P., Ciutat, A., Durrieu, G. and Massabuau, J. C. (2003).** Estimation of potential and limits of bivalve closure response to detect contaminants: Application to Cadmium. *Environ. Toxicol. Chem.* 22:914 -920.
- Valenti, T.W., Cherry, D.S., Currie, R.J., Neves, R.J., Jones, J.W., Mair, R. and Kane, C.M. 2006.** Chlorine toxicity to early life stages of freshwater mussels (*Bivalvia: Unionidae*). *Environmental Toxicology and Chemistry* 25: 2512-2518.
- Vaughn, C. C., Nichols, S. J., and Spooner, D. E. 2008.** Community and foodweb ecology of freshwater mussels. *J. N. Am. Benthol. Soc.*, 27(2):409-423.

- Warren, C. E., and Davis, G. E. 1967.** Laboratory studies on the feeding bioenergetics and growth of fish. Pages 175-214 in S. D. Gerking, ed. The biological basis of freshwater fish production. Blackwell Scientific, Oxford.
- Widdows, J., Phelps, D. K., and Galloway, W. 1981.** Measurement of physiological condition of mussels transplanted along a pollution gradient in Narragansett Bay. Mar. environ. Res. 4, 181-194.
- Widdows, J., and Donkin, P. (1989).** The application of combined tissue residue chemistry and physiological measurements of mussels (*Mytilus edulis*) for the assessment of environmental pollution. Hydrobiologia 188/189, 455-461.
- Widdows J. and Donkin P. 1991.** Role of physiological energetics in ecotoxicology. Comp. Biochem. Physiol;100:69-75
- Widdows, J, Donkin, P. Brinsley, M. D., Evans, S. V., Salkeld, P. N., Franklin, A., Law, R. J., Waldock, M. J. 1995.** Scope for growth and contaminant levels in North Sea mussels *Mytilus edulis*. Mar Ecol Prog Ser 127:131-148
- Yeager, M. M., Cherry, D. S., and Neves, R. J. 1994.** Feeding and burrowing behaviors of juvenile rainbow mussels, *Villosa iris* (Bivalvia:Unionidae). Journal of the North American Benthological Society, 13:217-222.