Effects of Parafluvial Flows on Interstitial Ammonia and Freshwater Mussels in the Buffalo National River

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EFFECTS OF PARAFLUVIAL FLOWS ON INTERSTITIAL AMMONIA AND FRESHWATER MUSSELS IN THE BUFFALO NATIONAL RIVER

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David A. Johnson

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ABSTRACT

Freshwater mussel populations and distributions have declined over the past several decades in the Buffalo River, Arkansas. Among many possible causes, this study tested the hypothesis that ammonia concentrations in parafluvial flows through gravel bars might be high enough to impair the survival and growth of juvenile mussels. Ammonia, pH, temperature, and dissolved oxygen were measured in interstitial and water column samples from gravel bars to test for possible longitudinal gradients. Samples were taken along 3 large bars at locations within the Buffalo National River, at approximate bi-weekly intervals between June and September of 2010. Water sample ammonia concentrations were compared with revised US Environmental Protection Agency water quality criteria for the protection of aquatic life. In addition, juvenile mussels were caged immediately upstream and downstream at each of the monitoring sites to test for site and location differences in growth and survival. Interstitial DO, temperature, and pH decreased, and ammonia concentrations increased, as water traveled downstream through the gravel bars. During drought periods when the river was at its lowest flow, ammonia concentrations at some sites exceeded levels that have negative effects on mussels in laboratory tests. However, growth of caged mussels was generally strong at both upstream and downstream locations. Growth rate rose as the river warmed during May and June, peaked at 26-27°C in July, and was lower when temperatures exceeded 28-30°C in both May and August-September. Results indicate that while ammonia concentrations increase in parafluvial flows along bars, these concentrations are diluted rapidly in surface waters and may not have significant impacts on local mussel populations.

KEYWORDS: parafluvial flow, ammonia, freshwater mussel, Buffalo National River, Unionidae

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OVERVIEW

Freshwater mussels (Order Unionida) can be found on every continent in the world except for Antarctica. However, the greatest abundance and diversity of mussels, about 300 recognized taxa, is present in North America north of Mexico, most notably the Southeast United States (Williams et al. 1993). Mussels are important members of lotic and lentic freshwater communities and they play crucial roles in ecological functioning as abundant, large-bodied, benthic suspension feeders (Vaughn et al. 2008). Mussel distribution tends to be clumped and many are found in large aggregations (>100 individuals/ m^2) that can exceed 10,000 m^2 (Christian 1995). Due to their dense aggregates, mussel ecological impact is high in these areas (Christian 1995). Mussels feed primarily on bacteria, algae, and fine particulate detritus that they capture by filtering water through their gills. This feeding strategy means mussels can have a significant impact on the plankton community (Nalepa et al. 1991, Arnott and Vanni 1996, Davis et al. 2000). Mussels transfer food energy to the benthic community through their biodeposits (feces and pseudofeces), and their shells create habitat for benthic invertebrates. Mussels are also important as food for a variety of mammals, turtles, and fish (Howard and Cuffey, 2006).

Although often still locally abundant, many mussel species and populations in North America have declined throughout the last century, so that the group has one of the highest proportions of species at risk of extinction of any major taxon in North America (Master 1990, Williams et al. 1993, Ricciardi et al. 1998, Ricciardi and Rasmussen 1999). Of the 297 species found in North America, 91 are classified as Threatened or Endangered (USFWS 2018). There are several reasons for this severe decline, and these reasons vary among species and

populations. However, frequently cited explanations include historical overharvest by the shell button industry, introductions of non-indigenous species such as the zebra mussel (*Dreissena polymorpha*) and Asiatic clam (*Corbicula fluminea*), habitat degradation as a result of impoundments, channelization, sedimentation, and pollution (Williams et al. 1993, Watters 1996, Ricciardi et al. 1998, Box and Mossa 1999, Vaughn and Taylor 1999, Anthony and Downing 2001, Strayer et al. 2004, Bogan 1993). Mussels are highly vulnerable to dissolved, suspended, and deposited pollutants because they are imbedded in the sediment, relatively immobile, and constantly filter water for respiration and feeding. Both laboratory and field studies indicate that mussels are among the most sensitive organisms to certain types of pollutants including copper and ammonia (Augspurger et al. 2007, Angelo et al. 2007).

Study Area

The Buffalo National River is one of the premier free-flowing rivers in the mid-western United States and is well known for its recreational activities that include canoeing, hiking, fishing, hunting, and camping. The Buffalo River flows approximately 150 miles across the north central portion of Arkansas originating in the Boston Mountains and flowing eastward through the Ozarks. The river begins in Newton County and stretches across Searcy, Marion, and Baxter counties where it flows into the White River (Figure 1). The Buffalo National River is a relatively young national park having been established in March 1, 1972. Since then, the National Park Service (NPS) has worked to preserve the biological resources of the river and its riparian zone. About 40% of the 3,427 km^2 watershed is public land. The NPS controls 132 miles of the river corridor, which encompasses roughly 11% of the watershed. The rest of the public land is divided between National Forest Service (26%) and Arkansas Game and Fish

Commission (3%). About 60% of the watershed is privately owned, and primarily used for agriculture, logging and livestock production. (National Park Service 2004).

The Buffalo National River, as with all national parks, is managed in part to preserve natural and cultural resources, including wildlife. The freshwater mussel fauna of the Buffalo River was originally documented by Meek and Clark (1912). Their survey covered roughly two-thirds of the river from the present-day Pruitt access downstream, but they did not survey the upper section of the river (Meek and Clark 1912). From the Pruitt area to the confluence with the White River, Meek and Clark located 26 mussel beds, which were comprised of 22 different species of mussels. Later, after the river became a national park, John Harris of the Arkansas Highway and Transportation Department surveyed mussels in the mid-1990s. Harris found 26 species but was unable to survey approximately one third of the river (Harris 1996). In 2004 and 2005, Matthews (2007) conducted a qualitative and quantitative survey along roughly 146 miles of the Buffalo River. This survey totaled 33 sites surveyed qualitatively and 23 quantitative sites but located only 23 of the 26 species identified by John Harris, despite the increase in area sampled.

Of the mussel species that occur in the Buffalo River (n=26), 78% are species of concern in Arkansas (Matthews 2007). One of these, the Rabbitsfoot (*Quadrula cylindrica*), was recently listed as federally threatened (USFWS 2018). This high proportion of species that are ranked as species of concern indicate that the Buffalo River is of crucial importance for preservation of many freshwater mussel species in Arkansas.

Both the Matthews survey and quantitative surveys of long-term mussel monitoring sites along the Buffalo River by NPS document a severe decline in mussel populations over the last several decades. As with freshwater bivalves elsewhere, it is likely that multiple factors have

contributed to this decline. Potential factors adversely affecting native mussels in the Buffalo River prior to establishment of the park included logging and mining operations within the watershed. Prior to 1972, large areas were cleared within the floodplain, creating unstable banks that introduced large amounts of sediment into the river. Large tracts of upland forest were cleared by the lumber industry and those lands were then converted to pasture. The resulting pastures were stocked with cattle that utilized the river and its tributaries, resulting in soil erosion and transport of additional sediment and nutrients into the Buffalo River. This watershed disturbance is thought to have directly affected mussel habitats. Most of these issues were reduced or minimized once the river was established as the nation's first protected waterway in 1972. However, other factors may presently contribute to the decline of mussels. Releases of hypolimnetic water from Bull Shoals reservoir into the White River, approximately 30 miles upstream of the mouth of the Buffalo River, affects movements and populations of fish hosts of some species (Faron Usery, NPS, personal communication). Other possible impacts on mussels in the Buffalo include inputs of ammonia, herbicides and pesticides and other pollutants from agricultural and urban sources (Wang et al. 2007, Cope et al. 2008).

Ammonia

Dissolved nitrogen occurs in several forms including nitrite (NO_2), nitrate (NO_3), ammonia (NH_3), and ammonium (NH_4+) (Triska and Duff 2000). These four compounds of nitrogen plus molecular dissolved nitrogen (N_2) and organic nitrogen form the basis of the nitrogen cycle in fluvial ecosystems. The four previously mentioned are sometimes referred to as 'reactive nitrogen' because, unlike N_2, they are available to be incorporated into organic material by metabolic processes. Reactive nitrogen in aquatic systems comes from various

sources such as atmospheric precipitation, surface runoff, groundwater exchange, and fixation by bacteria, cyanobacteria, and vegetation (Triska and Duff 2000). During decay processes, certain bacteria convert organic nitrogen back to ammonium, a process known as ammonification. This process is heavily dependent on pH, temperature, and dissolved oxygen (Triska and Duff 2000).

From the 1960 to 1990, the loading rate of reactive nitrogen in Earth's ecosystems has approximately doubled (Vitousek et al. 1997, Carpenter et al. 1998, Galloway et al. 2008). Most reactive nitrogen enters the stream dissolved in groundwater (Triska et al. 1984) with a smaller contribution from decomposition of leaf litter in the riparian environment (Triska and Duff 2000). Other sources, which would be defined as pollution, include industrial waste, municipal wastewater facilities, and most notably agricultural runoff.

Because of nitrogen's dual role as a nutrient and a potential toxicant, nitrogen input and retention in streams are among the leading factors controlling biological communities (Triska and Duff 2000). Ammonia is a contaminant of special concern because freshwater mussels, particularly juveniles, are more sensitive to ammonia toxicity than any other freshwater species that have been tested (Wang et al. 2007, 2011, Augspurger et al. 2003, USEPA 2009). Ammonia concentrations of 1.75 mg/L to 2.5 mg/L at pH 8 have acute effects and chronic concentrations of 0.3 mg/L to 1.0 mg/L can be fatal (Augspurger et al. 2003, Wang et al. 2011). Laboratory studies have shown that previous water quality criteria for ammonia were not effectively protecting freshwater mussels (Wang et al., 2007, 2011). These studies led to an update to the Ambient Water Quality Criterion in 2013 that reduced the allowed levels of chronic and acute ammonia exposure as compared to the 1999 Update (USEPA 1999, USEPA 2013).

Although water quality surveys in the Buffalo River indicated that water column ammonia was below the minimum reporting limit of 0.041 mg/L as N (Galloway and Green,

2004), it is possible that ammonia levels may reach toxic levels in sediments and the hyporheos. The hyporheos is the habitat found below the water column and includes the substrate and the water flowing within the interstices of the substrate. The hyporheos is a very important part of the river as it provides a habitat for many groups of benthic animals, including freshwater mussels. Ammonia concentrations in interstitial water can be higher than in the overlying water column because of metabolic processes that release ammonia from the breakdown of organic material found within sediment along with lower light availability (Triska et al. 1984). The input of nitrogen in fluvial ecosystems is well documented for surface flows (Triska and Cromack 1980, Vincent and Downes 1980, Naiman 1982, Richey et al. 1985, Grimm 1987, Triska et al. 1989, 1994). However, less is known about how nitrogen flows through the hyporheos. Gravel-bottomed rivers, such as the Buffalo, have substantial hyporheic flows because of the porosity of the substrate (Claret et al. 1997). Parafluvial zones occur along emergent gravel bars with horizontal inflow and outflow. Within parafluvial zones, interstitial water may be altered in solute concentrations as a result of biological and chemical processes (Larned and Datry 2013). The longitudinal distribution of nitrogen in parafluvial zones through gravel bars could be related to the recent decline in mussel abundance on the Buffalo National River. Pore-water ammonia concentrations tend to be greater than the concentration of the overlying water, which places the freshwater mussel fauna at greater risk (Augspurger et al. 2003).

Thesis Objectives

The first objective was to determine concentrations of ammonia that occur in gravel bars within the Buffalo River. These concentrations were then compared to USEPA ammonia criteria regarding toxicity to juvenile and adult mussels. This information can be used to examine the

effectiveness of current management and water quality standards in the watershed. The second objective was to test whether mussel growth varied in relation to parafluvial flows and ammonia or other factors. These data could help to evaluate the feasibility of reintroducing mussel species into the Buffalo National River and select prime locations based on available water quality data.

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CHAPTER 1 – LONGITUDINAL PROFILE OF AMMONIA CONCENTRATIONS IN PARAFLUVIAL FLOW IN THE BUFFALO RIVER

Abstract

Freshwater mussels in the Buffalo River have declined in distribution and abundance in recent history. One suspected cause for this decline is elevated ammonia, to which freshwater mussels are particularly sensitive. This study examined gravel bars to determine if gravel bars could be a source for local increases of interstitial ammonia. Three gravel bars were chosen in the Buffalo National River, to represent the upper, middle and lower reaches of the river within the NPS controlled corridor. Each of these gravel bars were then subdivided into four sections and parafluvial interstitial water was sampled several times throughout the summer. A sample was collected from each of these gravel bar sections as well as a surface flow sample collected directly downstream of the gravel bar. Total ammonia nitrogen, pH, and temperature were used to calculate the concentration of unionized ammonia in the sample. Seasonal variation and slight trends of increasing downstream ammonia were observed at all three sites. However, there was only a single event of toxic concentrations of unionized ammonia in the surface flows of the river. In summary, gravel bars appear to produce low levels of un-ionized ammonia (UIA), which is rapidly diluted into surface waters downstream. While these concentrations appear too low to have effects, at least one recent study suggests that mussel recruitment can be impacted by much lower levels that those recommended in the current Water Quality Criterion (Strayer and Malcom 2012).

Introduction

Ammonia is one of the most important pollutants in freshwater, mainly originating from nonpoint sources including agriculture, fertilizers, and human and animal waste (Wang et al. 2008). The toxicity of ammonia depends on factors including pH and temperature. Ammonia exists in a chemical equilibrium between the toxic unionized form, known as ammonia (NH_3), hydrogen ion (H^+), and the less toxic ammonium ion (NH_4^+) (USEPA 2009). The gaseous unionized ammonia molecule readily diffuses across epithelia making it toxic to organisms (USEPA 1999). This equilibrium between ammonia and ammonium is largely dependent on pH (USEPA 1999, Wang et al. 2008). In general, for every unit of rise in pH level (more basic), the ratio of ammonia to ammonium in an aqueous solution increases approximately 10-fold (Erickson 1985, USEPA 2009). To a lesser degree, the ratio is also controlled by temperature. The ratio of ammonia to ammonium increases approximately two-fold for every 10°C increase in temperature (Erickson 1985, USEPA 2009). Another important controlling factor is dissolved oxygen (DO). When DO is abundant, ammonia is readily oxidized into non-toxic nitrate. Therefore, ammonia is more likely to be elevated in areas of depleted DO, such as in areas of organically rich stream sediments (Boulton et al. 1998, Allan and Castillo 2007).

The Buffalo River, being largely a gravel-bottom river, can have substantial subsurface flows because of the porosity of the substrate, and subsurface flows potentially play an important role in nutrient cycling. The first category of subsurface flow is hyporheic flow. Hyporheic flows are located under the surface of the river channel and are characterized by vertical exchange between the substrate and the overlying water column (Datry and Larned 2008). The second category of subsurface flows is parafluvial flow. Parafluvial flows are located adjacent to the river channel within emergent features including gravel bars, terraces, and periodically flooded

side channels. These areas are hydrologically connected to the river along the margins of gravel bars where the subsurface flow paths meet surface flows (Claret 1997, Larned and Datry 2013). The third and final form of subsurface flow is riparian. Riparian flows lie distal to parafluvial zones within the watershed and have no direct exchange with the river channel (Datry and Larned 2008, Larned and Datry 2013). The focus of this paper is on parafluvial zones, or the zones within and adjacent to gravel bars, and their interaction with the river channels.

Parafluvial flow in gravel bars is an important link between surface waters and groundwater (Grimm and Fisher 1984, Triska et al. 1989, Gibert 1990, Valett et al. 1994). While gravel bars appear dry, below the surface in the interstitial areas, the surface water from the river flows through the gravel bar and interacts with the shallow subsurface water through upwelling and downwelling areas in a process known as hyporheic exchange (Tonina and Buffington 2009). Gravel bars can act as a source or sink for nitrates in lotic systems depending on bank conditions (Claret et al. 1997). Some studies have shown progressive increases in nitrate concentrations in these parafluvial zones (Triska et al. 1989, 1993, Naiman and Ford 1989, Valett et al. 1990, Valett 1993, Holmes et al. 1994). A progressive decrease in dissolved oxygen concentrations in water flowing through parafluvial zones has also been reported (Triska et al. 1989, White and Hendricks 1991, Valett 1993, Triska et al. 1993).

The objective of this study is to determine the longitudinal profile of ammonia concentrations in parafluvial flows in the Buffalo River. Long gravel bars may act as a trap for detritus, which can release ammonia during decomposition. Ammonia concentrations in parafluvial areas have been shown to be 6 to 30 times more concentrated compared to the overlying water column in the upper Mississippi River (Frazier et al. 1996). Thus, the interstitial water that returns to the water column in parafluvial flow may be sufficiently high in ammonia

that it could impair mussel growth or survival. This concept may be beneficial in understanding the decline of the freshwater mussel populations of the Buffalo River.

Methods

Study Area. The study area included three large gravel bars in the Buffalo River. These bars were selected based on location on the river, ease of access, and location adjacent to known mussel beds. The most upstream gravel bar was located roughly one-quarter mile below the landing at Kyle's Landing located on AR Hwy 74 west of Jasper, AR. Moving downstream, the second site was located at the Saunders Field area, which is towards the lower section of the middle portion of the river. This area is accessible via a dirt road off AR Hwy 14 and is located upstream of the North Maumee access area. The most downstream site, known as Cedar Creek, is located approximately one mile downstream of Rush Landing off AR Hwy 14 (Figure 1).

Longitudinal Profile of Gravel Bar. To determine a rough linear profile of ammonia concentrations along the gravel bar, surface and subsurface water samples were drawn from locations along its length. Five locations were sampled at each gravel bar during each sampling period, except at Kyle's Landing, where only four locations were sampled. The exact positions of the sample locations varied depending on water level, because lower levels expose a greater proportion of the bar thereby increasing the emergent length. Sample locations were selected based on set criteria upon arrival on the bar during each sampling day. The gravel bar was divided into equal quarters and a sample was collected in each of these segments. Each sample was collected approximately five feet from the river's edge to limit direct mixing from the river during sampling. The sample locations were located in relation to a natural marker located on the adjacent bank to ensure the samples were consistently sampled from a similar longitudinal

position on the gravel bar. This method for sample selection was used because the river level would fluctuate and potentially leave specific locations under water or too far from the river, resulting in difficulty of sampling due to lack of water.

Collecting Water Samples. Hyporheic samples were obtained using a sampling probe driven into the gravel. The main body of the sampling probe was constructed of 1-inch diameter PVC pipe with approximately 10 quarter-inch holes drilled on two sides (Figure 2). The lower end was fitted with a solid, pointed cap to allow penetration into the gravel bar. On the upper end a PVC pipe union allowed a modified slide hammer to be attached to aid in driving the device into the gravel bar. The device was always driven up to the bottom lip of the union, approximately 45 cm. Once the probe was buried up to the union, the union could be opened exposing an inner three-quarter inch PVC pipe. This inner pipe was also perforated and wrapped in 1-mm screen mesh. Once driven into the gravel bar, interstitial waters entered the probe, filtered by the screen. The water that passed though the mesh flowed into the secondary inner pipe where it could be sampled via a syringe.

Ammonia can readily diffuse into air, so care was taken to avoid contact between water samples and the atmosphere. Sixty-milliliter polyethylene syringes were used to collect, store, and process samples. Each syringe was fitted with a three-way valve and a piece of vinyl hose that connected it to a pipette. Water was then collected by inserting the pipette into the inner pipe and drawing the water up the line into the syringe. The syringe was flushed three times using the three-way valve, so that the final sample had no contact with air. The final sample was stored in the syringe. A surface water sample was collected from the surface water of the river on the downstream side of the gravel bar. The surface water sample was collected by submersing the collecting syringe into the water and placing the nozzle near the substrate.

Measuring Water Samples. Water samples were stored in the syringes and were processed on site within 30 minutes of collection. Prior to the onsite analysis, all sample syringes were stored in the dark inside an insulated bag. Ammonia concentrations were measured using a Cole-Parmer ammonia electrode (Model No. 27512-00) connected to an Oakton ion meter (Oakton Ion 5 Acorn Series). The ammonia electrode was used for its increased range, accuracy, and sensitivity compared to colorimetric tests for ammonia. The ammonia electrode can measure a concentration range of 0.01 to 17,000 ppm NH_3 with ±2% reproducibility (Cole Parmer 2004). In addition, electrode measurements are not affected by the color or turbidity of the sample. Electrode membrane integrity was tested before each use. The electrode and meter were calibrated using a fresh commercial ammonia standard (100 mg/L) and dilutions of 0.1 mg/L, 1 mg/L and 10 mg/L prior to each sample date. Standards and samples were treated with 1 ml ISA/100 ml sample per the manufacturer's instructions (Cole Palmer 2004).

Each sample was measured inside of the 60 mL syringe that was used to draw the sample from the gravel bar. A four-inch diameter petri plate was modified by gluing a threaded plug onto the middle of the plate (Figure 3). This allowed the syringe to be screwed onto the plate to hold it upright while running the test and plugged the end to prevent losing the sample once the plunger was removed. Before measurement, a small portion of the sample was expelled along with any sediment that may have been collected from the gravel bar. The syringe was then firmly attached to the plate and the plunger was removed. A magnetic stir bar and 0.5 mL of ISA (ionic strength adjuster) were added to the sample. The petri plate and syringe were placed on top of a magnetic stir plate, which was used to mix the sample and ISA for one minute. While air contact occurred at the top of the sample during the mixing process, lab experiments using prepared standards indicated little influence on the sample. The tip of the ammonia electrode was then

lowered into the sample with care to prevent an air bubble from forming on the tip and skewing the sample. Once the reading stabilized, the value was recorded. This process was repeated for each of the samples with the ammonia electrode and the threaded plug on the petri dish being rinsed in distilled water between each sample.

The equilibrium constant for ammonia ionization was estimated using the factors provided by Emerson et al. (1975):

$$pK = 0.09018 + \frac{2729.2}{273.2 + T}$$

where pK is the negative logarithm of the equilibrium constant K and T is degrees Celsius.

The fraction (f_{NH3}) of total ammonia nitrogen (TAN) present as unionized ammonia (UIA) was estimated using the following equations:

$$f_{NH_3} = \frac{1}{1 + 10^{pk-pH}}$$

$$\text{UIA} = f_{NH_3} * \text{TAN}$$

Temperature (°C), pH, and dissolved oxygen (DO) were measured in the field by removing the ammonia sampling tube and placing the probes of a portable Hach HQ40d meter into the sampling well. The two probes used for this study were the pH probe (Model No. PHC101) and the DO probe (Model No. LDO). Both probes were calibrated prior to every sampling trip per the manufacturers' instructions.

Criterion Continuous Concentration and Criterion Maximum Concentration Calculation. The measured ammonia concentrations were compared to Criterion Maximum Concentration (CMC) and the Criterion Continuous Concentration (CCC) for ammonia. CMC

and CCC were calculated for the measured temperatures and pH of each sample site, according to standard methods (USEPA 2013). The equation for calculating CMC is as follows:

$$CMC = MIN\left(\frac{0.275}{1 + 10^{7.204-pH}} + \frac{39.0}{1 + 10^{pH-7.204}}\right), \left(0.7249 * \left(\left(\frac{0.0114}{1 + 10^{(7.204-pH)}}\right) + \left(\frac{1.6181}{1 + 10^{pH-7.204}}\right)\right) * \left(23.12 * 10^{0.036*(20-T)}\right)\right)$$

where T is the measured temperature of the sample in degrees Celsius and MIN means the smaller of the two numbers in the parentheses is used.

The equation for CCC is

$$CCC = 0.8876 * \left(\frac{0.0278}{1 + 10^{7.688-pH}} + \frac{1.1994}{1 + 10^{pH-7.688}}\right) * (2.126 * 10^{0.028*(20-MAX(T,7))})$$

where T is temperature in degrees Celsius and MAX calls for the larger of the two values in parentheses to be used.

Site Sampling. Data were collected throughout the summer of 2010 at the three sampling sites located at Kyle's Landing, Saunders Field, and Cedar Creek (Figure 1). Each site was sampled on four to seven dates throughout the summer with Kyle's Landing and Cedar Creek being sampled less frequently at five and four times, respectively. Deviations from schedule were due to difficulty reaching the sampling location during periods of high or low water conditions. On each sampling date, surface and subsurface samples were measured for pH, temperature, and total ammonia as nitrogen (TAN). Dissolved oxygen was also sampled after August 18, 2010. The subsurface samples were numbered sequentially where the lowest value represented the highest upstream location and the highest value represented the farthest downstream sampling location within each site. Results averaged over all dates for each sampling location are presented in Table 1. Individual data are presented in Table 2.

Results

Several trends in the data were evident among all three of the sample sites. DO, temperature, and pH all generally decreased from upstream to downstream along the parafluvial zone, except that the most downstream subsurface sample at each site showed an increase in pH as compared to the adjacent upstream samples. The surface sample, taken at the lower end of the bar, was the highest pH value at each site (Table 1).

Subsurface TAN values increased downstream with the exception of the most downstream sample, which fell slightly. Surface TAN at the downstream end was similar to the most upstream subsurface sample (Table 1). At Saunders Field, UIA also increased in subsurface samples from upstream to downstream until the most downstream subsurface sample, which decreased (Table 1). However, this trend was not as evident for UIA at the Kyle's Landing or Cedar Creek sites (Table 1).

TAN was also related to seasonal flows at each of the sampling locations. The river received little rainfall from approximately July 20 until September 9, 2010. It was during this period that the highest TAN measurement occurred at all three sites. The highest TAN measurement occurred at the third subsurface sample from the most upstream location of the gravel bars at all three sites (Figure 4, 5, 6).

Discussion

Ammonia in Gravel Bars. Gravel bars are important components of rivers as they serve as a link between sub-surface water and in-channel surface flows (Grimm and Fisher 1984, Triska et al. 1989, Gibert 1990, Valett et al. 1994). Holmes et al. (1994) demonstrated that the parafluvial flow through gravel bars can be an important source for nitrogen in nitrogen-limited

streams. A goal of the present study was to determine if ammonia increased along gravel bars on the Buffalo River and might reach levels toxic to freshwater mussels. Ammonia increased in parafluvial water as it flowed downstream through gravel bars at all three sites (Figure 4, 5, 6). However, the two longer gravel bars, Saunders Field and Cedar Creek, showed a decrease in subsurface ammonia at the most downstream sampling sites. This sharp decrease was not seen in the previous study by Holmes et al. (1994), possibly because in that study no sample was taken at the end of the gravel bar close to the water's edge. It appears that in the present study the lower-most sample was taken in the "gaining reach" of the bar (Larned and Datry 2013) where surface waters are mixing with the parafluvial flow. It appears that the mixing zone for both gravel bars occurred between the third and fourth sampling locations.

Elevated levels of TAN do not necessarily imply a toxic environment to aquatic organisms. The toxicity of ammonia in water is largely dependent on both temperature and pH. As mentioned previously, at higher pH, a greater proportion of the nitrogen is unionized NH_3 and at lower pH, more is in the form of NH_4^+. The pH of water is the primary driving factor behind this proportion as a single unit of change in pH can change the amount of unionized ammonia by ten-fold. Moving downstream through the gravel bar, as TAN increases, the pH decreases (Figure 7, 8, 9). This pattern was seen at all three sites. Therefore, although TAN tended to increase downstream in subsurface samples, UIA generally did not increase (Figure 10, 11, 12). This indicates a possible offsetting effect of pH on the toxicity of ammonia in the parafluvial zones.

Ammonia Concentrations Related to Water Quality Criteria. The EPA sets allowable acute and chronic maximum concentrations of ammonia in waterways in the United States with the Water Quality Criteria (WQC). Research over the past two decades has shown that

freshwater mollusks are among the most sensitive aquatic organisms (Keller and Zam 1991, Augspurger et al. 2003, Wang et al. 2007, 2011). In 2013, the Environmental Protection Agency significantly lowered both the Criterion Maximum Concentration (CMC) and the Criterion Continuous Concentration (CCC). As previously mentioned, both CCC and CMC are calculated using equations that incorporate pH and temperature. Data from this study suggest that the parafluvial concentration of ammonia exceeded CCC at some sites for at least a two-week period during the summer, which indicates a possible impact on the mussels.

At Saunders Field, subsurface samples at positions #2 and #3 as well as the surface sample exceeded the CCC value on August 18, and #2 and #3 exceeded CCC again on September 1 (Table 2). Likewise, at Cedar Creek, all subsurface samples except #2 exceeded the CCC value on August 26, and #3 and #4 exceeded CCC values on September 1 (Table 2). These data are for interstitial water, and only one sample from surface waters slightly exceeded CCC (Table 2). Ammonia produced in parafluvial water dilutes by mixing with in-channel flows. However, without using dye tracing to determine the exact mixing areas along the gravel bar, it is not possible to say that there are not isolated areas of increased ammonia concentrations that exceed recommended values.

Recommended Follow-up Studies. Data from this study showed an increasing trend of total ammonia in parafluvial flows on the Buffalo National River. These concentrations did not reach levels that would be expected to impact the local mussel populations, based on EPA criteria. However, a recent study indicates that recruitment failure in freshwater mussels in small streams in southeastern New York was strongly associated with concentrations of un-ionized ammonia well below current threshold limits established by laboratory studies (Strayer and Malcom 2012).

The paths by which the parafluvial and surface flows mix is also of interest. Future studies could take this study a step farther and look at the actual flow paths associated with each of these gravel bars using a dye and trace method. Once a flow path is located, samples can be collected at the mixing zone to get a better understanding of the ammonia contributions in the river from the parafluvial flows. In addition, it would be beneficial to understand ammonia concentrations in other subsurface flows. While this study and previous studies suggest that parafluvial flows are important, other hyporheic waters may be equally or more important to freshwater mussels. Juvenile mussels may bury six centimeters or more in interstitial areas (Layzer and Madison 1995). Juveniles located deep into these interstitial flows may have little contact with surface flows and may be living completely in sub-surface flows. Multiple studies indicate that sedimentation and alteration of interstitial water quality impair juvenile survival and recruitment of the European Pearl Mussel (*Margaritifera margaritifera*), which lives in relatively oligotrophic streams (Geist 2007, Quinlan et al. 2014). A future study to assess water quality in interstitial waters would be beneficial to understanding impacts on freshwater mussels on the Buffalo River.

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CHAPTER 2 – ASSESSMENT OF IMPACT OF PARAFLUVIAL AMMONIA CONCENTRATIONS ON FRESHWATER MUSSELS CAGED IN THE BUFFALO NATIONAL RIVER

Abstract

Freshwater mussels of the Buffalo National River are in decline. One possible explanation for this decline is poor water quality due to increased agricultural input creating elevated concentrations of interstitial ammonia in the river. Survival and growth of caged juvenile mussels was used as a bioassay, in conjunction with a study (Chapter 1) of parafluvial ammonia in the Buffalo River, Arkansas. Four mussel silos (benthic cages) were placed at each of the three study sites, two upstream of the gravel bar and two downstream. Each silo contained five juvenile Black Sandshell (*Ligumia recta)* mussels. The silos were monitored periodically between May 25 and September 29, 2010 and the mussels photographed for length measurements. Among the three sites, growth was not consistently higher or lower comparing upstream and downstream except in late summer, when growth rate was substantially lower downstream of the gravel bars. Over the study period, growth rate increased in May and June to a peak in mid-July, then fell. Interstitial ammonia concentrations did not seem to correlate with growth rate or survival of caged mussels indicating minimal influence. Temperature or seasonal effects appeared to be most important factor influencing growth rate. Growth rate was highest in a temperature range of approximately 26-28°C and was lower by more than half at higher temperatures in May and in August.

Introduction

There are numerous reasons for the recent declines in freshwater mussel populations in the United States and for the increase in species listed as endangered, threatened, or of special concern (Williams et al. 1993). Of all of the possible threats facing freshwater bivalves, pollutants are believed to be among the biggest threats, with ammonia being of particular concern (Bogan 1993, Richter et al. 1997). In 1985, the United States Environmental Protection Agency (USEPA) set out guidelines for certain pollutants, including ammonia. These guidelines are collectively known as the Water Quality Criteria (WQC) and were created in response to the Federal Water Pollution Control Amendment and Clean Water Act amendments of 1972 and 1977, respectively. The WQC established acute (CMC) and chronic (CCC) allowable limits for certain important pollutants. CMC stands for criterion maximum concentration and is the maximum allowable concentration over a short period, roughly three days to a week. CCC stands for criterion continuous concentration and is the maximum allowed concentration over a longer period on the order of weeks or months.

Ammonia is of special concern because freshwater mussels are more sensitive to ammonia toxicity than any other freshwater species that have been tested (Wang et al. 2007a, 2007b, Augspurger et al. 2003). In response to accumulating data, EPA substantially lowered the acute and chronic levels of ammonia in streams with mussels present (USEPA 2009, 2013). While ammonia has a significant impact on freshwater mussels, certain life stages are more susceptible than others. Freshwater mussels have at least five distinct life stages: gametes, developing embryos, glochidia, juveniles, and adults. Of these five life stages, the early juvenile life stage, i.e. individuals in their first year, is most often tested for sensitivity to pollutants. Juvenile mussels are very small and probably remain completely buried in the substrate at depths

of 1-6 cm for most of their first growth season (Yeager et al. 1994, Layzer and Madison 1995). Contaminant concentrations may be higher in interstitial water (Salomons et al. 1987) including ammonia (Chapter 1). In addition, juvenile mussels have relatively high metabolic rates and feeding rates remain more continuously active compared to adults (Wang et al. 2007a, 2007b).

The objective of this portion of the thesis was to test whether the growth and survival of caged juvenile mussels might be affected by elevated ammonia or other factors relating to parafluvial flow along gravel bars in the Buffalo River. Adult mussels tend to be found in areas with stable substrate while juveniles are more typically found in depositional areas, such as behind rocks or along the margins of rivers (Neves and Widlak 1987). Juveniles in these areas along the margins of rivers may be at a greater risk of exposure of critical ammonia concentrations from parafluvial flows. Therefore, if parafluvial flows are sources of increased ammonia concentrations, juvenile mussels would be at greater risk due to their lower tolerance to ammonia and the connection between parafluvial flows and interstitial flows.

Methods

Study Area. The reaches used for this portion of the study were the same as those identified in Chapter 1 (Figure 1). These three gravel bars were selected based on location, ease of access, and locations adjacent to known mussel aggregates in the Buffalo River. The most upstream gravel bar selected was located roughly one-quarter mile below the river access at Kyle's Landing located on AR Hwy 74 west of Jasper, Arkansas. Moving downstream, the second site was located at the Saunders Field area, which is towards the lower end of the middle third of the river. This area is accessible via a dirt road off AR Hwy 14 and is located upstream

of the North Maumee access area. The most downstream site, known as Cedar Creek, is located approximately one mile downstream of Rush Landing off AR Hwy 14.

Test Organisms. The organism used for this portion of the study was *Ligumia recta*, more commonly known as Black Sandshell. The Black Sandshell is a widely distributed mussel, but it is somewhat uncommon throughout its range. This species was chosen for two reasons. First, Black Sandshell can be propagated in captivity and was available at the time of the study from a cohort reared at Missouri State University. In addition to availability, Black Sandshell is a species of interest for reintroduction at Buffalo National River. Black Sandshell was historically present on the Buffalo River as reported by Gordon et al. (1979). However, extensive sampling in 1996 and 2006 failed to locate the species, suggesting that it may have been extirpated from the Buffalo River (Matthews 2007).

Caging. Mussels placed into the river were housed in benthic cages called mussel silos (Barnhart et al. 2007). Mussel silos are concrete hemispheres approximately 12 inches in diameter and roughly six inches in height. The silo has a hole through the center into which is inserted a cylindrical chamber made of two-inch diameter PVC pipe. The chamber is capped at both upper and lower ends with two-millimeter mesh window screen. The bottom surface of the silo is placed on gravel substrate so that interstitial water can be drawn upward into the silo. The silo is designed to take advantage of the Bernoulli Principle. Water flowing over the top of the silo moves faster, creating an area of low pressure. This low pressure at the top of the silo pulls water up from the bottom of the silo and through the inner chamber. This design, therefore, creates adequate flow for the mussels to provide food and remove waste. Experiments with dye indicate that, on open gravel substrate, the silo can entrain interstitial water (M.C. Barnhart, personal communication).

Design. The main goal of this experiment was to determine whether potential increased ammonia concentrations or other factors in parafluvial flow through the gravel bar might impact freshwater bivalves associated with the habitat adjacent to the gravel bars. To test these effects, four mussel silos containing five Black Sandshell each were placed at each of the gravel bars selected for this study. As indicated in Chapter 1, interstitial ammonia concentrations associated with these gravel bars never reached critical levels, but they did reach the criteria for chronic effects. Therefore, assessing the chronic effects was more valuable than assessing for only mortality. Growth rate, measured as changes in total length per day, was compared among measurement periods and locations.

To determine differences in growth and survival between the upstream side of a gravel bar and the downstream side, two silos, each containing five mussels, were placed at the upstream side of the gravel bar and two were placed at the downstream side of the gravel bar (Figure 13). Locations at the upstream and downstream sides were selected to minimize differences in flow, substrate, and water depth. The silos were placed in at least three feet of water, to deter tampering by making the silos more difficult to be spotted by recreational users of the river and to allow for changing water levels. A temperature logger was secured in the substrate at each of the sites to provide data on the temperatures throughout the summer.

Measuring. Mussels were measured before deployment, and again at the end of the experiment using digital calipers (Mitutoyo). In addition, field measurements were made using photos and image analysis. Mussels initially ranged in length from five to seven millimeters. All the mussels used were pooled and then randomly assigned among silos. After deployment, mussels were photographed biweekly when possible. Silos were pulled to the edge of the water but kept submerged. The chambers were removed and gently rinsed by agitating them in the river

to clear the screens of any debris or sediment. The chamber was then opened to expose the mussels, and magnetic stir bar (7-mm) was placed among the mussels for scale. The mussels were arranged to show each specimen laterally, then photographed along with a tag to help positively identify the picture the laboratory. Measurements from photos were made using ImageJ software (Rasband 2016). In each photograph, length was calibrated with the 7-mm scale bar and the longest anterior-posterior distance of each mussel was determined.

Data Analysis. The rate of growth for each individual and measurement period was calculated by dividing the change in length by the number of days in the river. Measurement periods varied somewhat among sites due to accessibility issues. In some cases, cages were lost to vandalism, so that sample sizes were also variable. Given these limitations, individual mussel growth rates were averaged for each measurement period, upstream and downstream at each site, and 95% confidence intervals were calculated for each mean to allow comparison of differences in growth rate.

Results

Within the sites, sample size was greater at the upstream locations because of problems with access and vandalism at downstream locations. The silos on the downstream side at each site tended to be more visible due to decreased water velocity and depth and were therefore more inviting to vandalism. One silo was lost for all or part of the experiment at each of the three downstream locations. Temperature recorders were recovered at only two of the three sites but both recovered devices showed a similar pattern of fluctuation over time. Therefore, temperature was averaged between the two recovered recorders and used as the temperature for all three sites.

Individual length measurements (Tables 3, 4, 5) were plotted as growth curves (Figures 14, 15, 16). Individuals within groups generally grew at similar rates, so that mussels that were larger at the beginning of the observations were also largest at the end.

Mean growth rates (mm/day) were calculated for each measurement interval and location (Tables 3, 4, 5; Figure 17). At each site, growth rate increased over time during May and June, peaked in late June to mid-July, and then decreased in August and September. Upstream and downstream growth rates were usually not significantly different except in the late summer intervals, when downstream growth was lower than upstream at all three sites (Figure 17). Overall, mussel length increased roughly three-fold during the four-month observation period. The only deaths occurred in vandalized cages.

Discussion

Growth rates of caged mussels varied strongly over time and among locations (Figure 17). Factors affecting growth could be very local, depending on current speed for example, or on differences in parafluvial and hyporheic flow over short distances (Larned and Datry 2013). Therefore, more than one or two cages per location might be needed to reveal larger scale patterns as opposed to differences among individual cage locations. However, the much lower growth rates in late summer at the downstream locations along the gravel bars are suggestive. The sharp decrease in growth for the downstream individuals probably indicates that there was a factor affecting growth. Ammonia should not be ruled out, but other factors appear to be driving growth overall.

The apparent effect of temperature on growth (Figure 17) is interesting because growth rate was highest at intermediate temperature and lower at both lower and higher temperatures.

Effects of temperature on freshwater mussels are not well known. Water temperature in the Buffalo River frequently exceeded 30°C and sometimes 35°Cduring this experiment, while fluctuating 3-4°C degrees daily. The critical thermal maxima of several species of juvenile mussels were tested by Martin (2016) using a ramped temperature exposure that mimicked the daily rise and fall of temperature, with the peak temperature held for two hours. The peak temperature fatal to half of the mussels (LT50) ranged from 33-41°C in summer-acclimated mussels, depending on species and age. These limits are surprisingly high, and although they can be tolerated, the present data indicate that juvenile Black Sandshell may suffer reduced growth at temperatures above 28°C. The margin between peak summer temperatures in this study and lethal limits is not very large, so that climate warming is cause for concern.

The fall of growth rates in late July followed a peak flow event that occurred on July 21, 2010 (Figure 18, 19, 20). After this peak flow event, the river experienced approximately a month and a half without significant precipitation and falling river levels. During this low water period, the highest parafluvial ammonia concentrations were recorded at the Saunders and Cedar Creek sites while Kyle's Landing varied less throughout the summer (Figure 21, 22, 23). Freshwater mussels, particularly juveniles, are among the most sensitive organisms to ammonia (Keller and Zam 1991, Augspurger et al. 2003, Wang 2007a, Cope et al. 2008). Data from Chapter 1 indicate that ammonia concentrations in parafluvial flows are likely to approach and potentially exceed chronic water quality criteria (USEPA 2009).

Future caging studies might be facilitated by developing cages that are less conspicuous or more difficult to be tampered with. Likewise, tags could also be created and placed on each of the silos explaining the purpose and significance of the study so that even if someone examines a silo they would hopefully return it back to the water. On several of the occasions when I found a

silo that had been moved or tampered with, I felt it was probably out of curiosity as nothing was taken or damaged.

Elevated ammonia concentrations could have adverse effects on survival and growth of freshwater mussels, particularly on juveniles. A study of population recruitment should also be taken into consideration as ammonia has been linked to decreases of recruitment to the point of creating relic populations, populations that are comprised of mainly non-reproductive adults (Hanson and Locke 2001, Geist and Auerswald 2007, Osterling et al. 2008, 2010, Strayer and Malcom 2012). Furthermore, this study could be taken a step farther by combining results from a chemical dye and trace study to find parafluvial pathways with extensive population mapping data. If an entire mussel bed was sampled and each quadrant was mapped with the population density in that area, it would be possible to see effects of parafluvial pathway outlets from the gravel bar on population distribution within an area. If the area near an outlet from the gravel bar is significantly less populated than surrounding areas that could indicate a possible effect related to the water quality from the gravel bar.

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Table 1. Water quality results for subsurface and surface water samples for Kyle's Landing (A), Saunders Field (B), and Cedar Creek (C) sites. Position number indicates sample position from the gravel bar with 1 being the most upstream and 4 being the most downstream. Data are means (± standard error) collected on N number of dates during the summer of 2010.

A.

Kyle's Landing						
Position	N	D.O. (mg/L)	Temp ºC	pH	TAN (mg/L)	UIA (mg/L)
1	5	7.50	31.3	7.53	0.036 (±0.005)	0.002 (±0.001)
2	5	4.87	29.1	7.38	0.073 (±0.005)	0.002 (±0.001)
3	5	1.30	28.6	7.41	0.124 (±0.016)	0.003 (±0.001)
Surface	5	6.90	28.8	7.94	0.013 (±0.004)	0.002 (±0.00006)

B.

Saunders Field						
Position	N	D.O. (mg/L)	Temp ºC	pH	TAN (mg/L)	UIA (mg/L)
1	7	6.50	30.3	7.65	0.173 (±0.052)	0.007 (±0.002)
2	7	2.28	29.6	7.54	0.484 (±0.289)	0.014 (±0.008)
3	7	0.93	29.2	7.50	0.752 (±0.355)	0.018 (±0.007)
4	7	1.58	28.8	7.54	0.294 (±0.075)	0.009 (±0.004)
Surface	7	7.81	30.2	7.88	0.127 (±0.028)	0.002 (±0.001)

C.

Cedar Creek						
Position	N	D.O. (mg/L)	Temp ºC	pH	TAN (mg/L)	UIA (mg/L)
1	4	10.47	31.3	8.09	0.183 (±0.038)	0.029 (±0.018)
2	4	4.05	30.6	7.61	0.313 (±0.075)	0.011 (±0.003)
3	4	1.40	31.2	7.41	0.853 (±0.348)	0.019 (±0.008)
4	4	0.72	30.0	7.61	0.637 (±0.223)	0.020 (±0.006)
Surface	4	6.05	29.5	7.99	0.218 (±0.050)	0.003 (±0.001)

D.O. = Dissolved Oxygen

TAN = Total Ammonia Nitrogen

UIA = Unionized Ammonia

Table 2. Temperature, dissolved oxygen, pH, and TAN data collected throughout the summer of 2010 at Kyle's Landing (a), Saunders Field (b), and Cedar Creek (c) sites on the Buffalo National River. Criterion Maximum Concentration (CMC) and Criterion Continuous Concentration (CCC) are calculations based on pH and temperature measured for each sample position, subsurface and surface, on the specified date using algorithms for EPA ammonia criteria (USEPA 2009).

A.

Kyle's Landing							
Date	Position	pH	Temp (°C)	D.O. (mg/L)	TAN (mg/L)	CMC	CCC
25-Jun	1	6.99	33.5	-	0.02	5.52	0.77
	2	6.86	31.6	-	0.09	7.16	0.91
	3	6.95	29.4	-	0.16	8.02	1.03
	Surface	7.69	31.7	-	0.01	2.59	0.53
13-Jul	1	7.09	29.6	-	0.04	6.95	0.96
	2	7.01	28.4	-	0.07	8.28	1.07
	3	7.37	27.9	-	0.11	5.77	0.91
	Surface	7.99	27.2	-	0.03	2.19	0.49
28-Jul	1	7.60	33.8	-	0.04	2.52	0.51
	2	7.40	30.1	-	0.07	4.62	0.77
	3	7.54	31.4	-	0.17	3.38	0.63
	Surface	8.10	30.7	-	0.01	1.33	0.33
18-Aug	1	7.96	30.3	7.97	0.05	1.79	0.41
	2	7.72	29.4	4.82	0.07	2.97	0.60
	3	7.67	27.5	1.06	0.09	3.79	0.72
	Surface	7.90	28.7	6.42	0.01	2.29	0.50
23-Sep	1	8.03	29.4	7.03	0.03	1.69	0.40
	2	7.90	26.1	4.91	0.07	2.84	0.59
	3	7.54	26.7	1.53	0.09	4.99	0.86
	Surface	8.01	26.0	7.37	0.02	2.33	0.51

D.O. = Dissolved Oxygen
TAN = Total Ammonia Nitrogen
CMC = Criterion Maximum Concentration
CCC = Criterion Continuous Concentration

B.

Saunders' Field							
Date	Position	pH	Temp (°C)	D.O. (mg/L)	TAN (mg/L)	CMC	CCC
25-Jun	1	6.96	33.8	-	0.07	5.52	0.76
	2	7.01	32.5	-	0.08	5.89	0.82
	3	6.96	30.9	-	0.29	7.03	0.92
	4	6.93	30.1	-	0.15	7.69	0.99
	Surface	7.20	33.4	-	0.15	4.52	0.70
30-Jun	1	7.58	31.9	-	0.06	3.04	0.58
	2	7.53	30.6	-	0.08	3.67	0.67
	3	7.43	30.1	-	0.11	4.43	0.75
	4	7.39	28.9	-	0.11	5.17	0.84
	Surface	7.60	30.5	-	0.02	3.31	0.63
19-Jul	1	7.60	30.1	-	0.11	3.42	0.65
	2	7.53	29.6	-	0.13	3.98	0.71
	3	7.61	28.4	-	0.15	3.88	0.72
	4	7.56	28.7	-	0.31	4.10	0.74
	Surface	7.79	28.6	-	0.06	2.81	0.58
28-Jul	1	8.01	31.1	-	0.09	1.53	0.36
	2	7.62	29.9	-	0.12	3.37	0.64
	3	7.90	32.7	-	0.31	1.64	0.38
	4	7.65	30.9	-	0.20	2.95	0.58
	Surface	8.01	31.6	-	0.02	1.46	0.35
18-Aug	1	7.74	29.4	7.53	0.42	2.87	0.58
	2	7.52	30.1	2.24	2.15	3.88	0.70
	3	7.38	29.2	0.75	2.40	5.11	0.83
	4	7.65	29.2	1.90	0.56	3.40	0.65
	Surface	8.58	31.5	10.40	0.17	0.49	0.14
1-Sep	1	7.77	30.6	2.84	0.31	2.47	0.52
	2	7.80	28.4	1.89	0.68	2.81	0.58
	3	7.56	27.8	0.74	1.80	4.41	0.78
	4	7.87	28.4	1.30	0.53	2.48	0.53
	Surface	7.98	30.3	5.40	0.18	1.73	0.40
23-Sep	1	7.92	25.5	9.13	0.15	2.87	0.60
	2	7.80	26.1	2.70	0.15	3.40	0.68
	3	7.63	25.3	1.30	0.20	4.86	0.86
	4	7.72	25.5	1.53	0.07	4.11	0.77
	Surface	7.98	25.8	7.63	0.03	2.51	0.54

D.O. = Dissolved Oxygen
TAN = Total Ammonia Nitrogen
CMC = Criterion Maximum Concentration
CCC = Criterion Continuous Concentration

C.

Cedar Creek							
Date	Position	pH	Temp (°C)	D.O. (mg/L)	TAN (mg/L)	CMC	CCC
30-Jun	1	7.90	31.2	-	0.20	1.86	0.42
	2	7.51	31.1	-	0.37	3.63	0.66
	3	7.47	32.6	-	0.60	3.40	0.62
	4	7.50	30.9	-	0.52	3.74	0.67
	Surface	7.89	30.7	-	0.21	1.97	0.44
29-Jul	1	7.64	34.4	-	0.07	2.25	0.47
	2	7.42	32.8	-	0.10	3.59	0.63
	3	7.26	34.5	-	0.15	3.84	0.63
	4	7.63	32.2	-	0.17	2.74	0.55
	Surface	8.01	34.2	-	0.14	1.18	0.30
26-Aug	1	8.84	30.0	11.84	0.23	0.35	0.10
	2	7.73	29.5	4.30	0.33	2.90	0.59
	3	7.47	29.4	1.48	1.80	4.43	0.76
	4	7.74	29.0	0.66	0.76	2.97	0.60
	Surface	8.05	27.0	6.50	0.36	1.99	0.45
1-Sep	1	7.99	29.7	9.10	0.23	1.78	0.41
	2	7.76	28.8	3.80	0.45	2.92	0.59
	3	7.43	28.2	1.32	0.86	5.18	0.85
	4	7.56	27.9	0.78	1.23	4.38	0.78
	Surface	7.99	26.1	5.60	0.16	2.40	0.52

D.O. = Dissolved Oxygen
TAN = Total Ammonia Nitrogen
CMC = Criterion Maximum Concentration
CCC = Criterion Continuous Concentration

Table 3. Lengths (mm) of each individual grouped based on sample position at Kyle's Landing.

Site	Ind	5/25	6/10	6/17	6/25	7/1	7/13	7/27	8/18	9/29
Upper 1	1	4.61	5.07	-	6.23	7.16	10.37	12.36	-	17.71
	2	6.35	6.80	-	8.30	9.33	13.26	13.75	-	19.79
	3	6.40	7.21	-	9.29	9.78	13.74	15.01	-	20.83
	4	7.27	Mort	8.90*	10.04	11.51	14.27	16.81	-	22.87
	5	10.07	Mort	10.42*	11.58	12.31	15.74	17.46	-	23.95
Upper 2	1	4.81	4.88	-	5.25	6.33	9.03	11.36	-	15.61
	2	6.41	6.60	-	8.06	9.44	12.69	14.14	-	21.84
	3	7.14	7.27	-	8.23	9.52	13.54	15.06	-	21.84
	4	8.00	8.48	-	8.85	9.84	13.66	15.75	-	22.89
	5	8.55	8.65	-	9.97	11.03	15.88	16.23	-	23.94
Lower 1	1	6.50	Mort	4.70*	6.88	7.58	10.34	-	-	15.59
	2	8.09	Mort	7.58*	7.58	7.75	10.99	-	-	15.61
	3	8.57	Mort	7.87*	9.36	10.07	13.60	-	-	17.66
	4	9.54	Mort	9.28*	9.55	10.37	14.07	-	-	18.73
	5	10.19	Mort	11.13*	11.13	11.93	16.42	-	-	21.83
Lower 2	1	8.51	Mort	5.04*	5.04	-	7.02	-	9.34	Mort
	2	9.09	Mort	6.69*	6.69	-	8.53	-	11.44	Mort
	3	9.23	Mort	6.76*	7.80	-	10.66	-	15.59	Mort
	4	9.71	Mort	7.26*	7.88	-	12.05	-	15.58	Mort
	5	9.95	Mort	9.22*	9.26	-	12.63	-	15.60	Mort

"-" Indicates a measurement was not taken on that sampling date

*Indicates individual placed in silo to replace a mortality

"Ind" = Individual

Table 4. Lengths (mm) of each individual grouped based on sample position at Saunders Field.

Site	Ind	25-May	10-Jun	25-Jun	1-Jul	27-Jul	29-Sep
Upper 1	1	7.29	8.62	13.49	14.97	18.56	27.06
	2	8.47	9.52	13.74	15.07	19.09	27.07
	3	8.96	10.18	14.00	15.53	20.01	28.11
	4	9.19	10.61	14.83	16.92	20.20	28.18
	5	10.47	11.90	15.27	17.04	21.04	29.16
Upper 2	1	5.50	5.50	8.57	8.98	15.16	25.00
	2	5.98	6.33	8.94	11.25	17.89	26.04
	3	6.33	6.33	9.91	11.59	18.23	29.14
	4	10.25	11.11	15.00	16.55	24.38	34.33
	5	10.35	11.32	15.27	17.48	25.51	37.46
Lower 1	1	4.73	5.29	**			
	2	5.00	6.08	**			
	3	6.75	7.95	**			
	4	7.63	8.56	**			
	5	9.61	11.18	**			
Lower 2	1	6.48	8.06	11.20	12.89	19.71	25.09
	2	7.96	9.78	12.74	14.43	21.62	27.06
	3	8.57	10.07	12.90	14.84	22.32	27.07
	4	9.16	10.20	13.30	15.08	22.35	27.09
	5	9.96	11.14	14.56	16.13	23.72	28.11

"-" Indicates a measurement was not taken on that sampling day

*Indicates individual placed in silo to replace a mortality

**Indicates silo was lost and never recovered

"Ind" = Individual

Table 5. Lengths (mm) of each individual grouped based on sample position at Cedar Creek.

Site	Ind	10-Jun	1-Jul	27-Jul	25-Aug	29-Sep
Upper 1	1	7.84	10.48	14.19	21.28	25.84
	2	8.34	12.22	22.11	24.44	26.89
	3	10.69	15.79	22.70	26.41	30.36
	4	11.56	16.62	26.01	26.79	32.85
	5	12.29	17.22	26.79	32.80	33.15
Upper 2	1	5.01	7.46	14.33	18.14	19.97
	2	8.69	12.38	22.13	26.19	27.64
	3	8.82	13.06	22.94	27.82	28.67
	4	10.77	15.38	26.48	31.77	32.68
	5	11.91	15.85	26.94	32.66	33.78
Lower 1	1	6.31	11.40	20.84	21.79	**
	2	6.62	12.66	23.79	25.45	**
	3	6.74	13.15	24.75	25.15	**
	4	10.96	17.58	28.49	28.98	**
	5	12.15	19.26	31.14	31.35	**

"-" Indicates a measurement was not taken on that sampling day

*Indicates individual placed in silo to replace a mortality

**Indicates silo was lost and never recovered

***Removed Lower 2 due to incomplete data

“Ind” = Individual

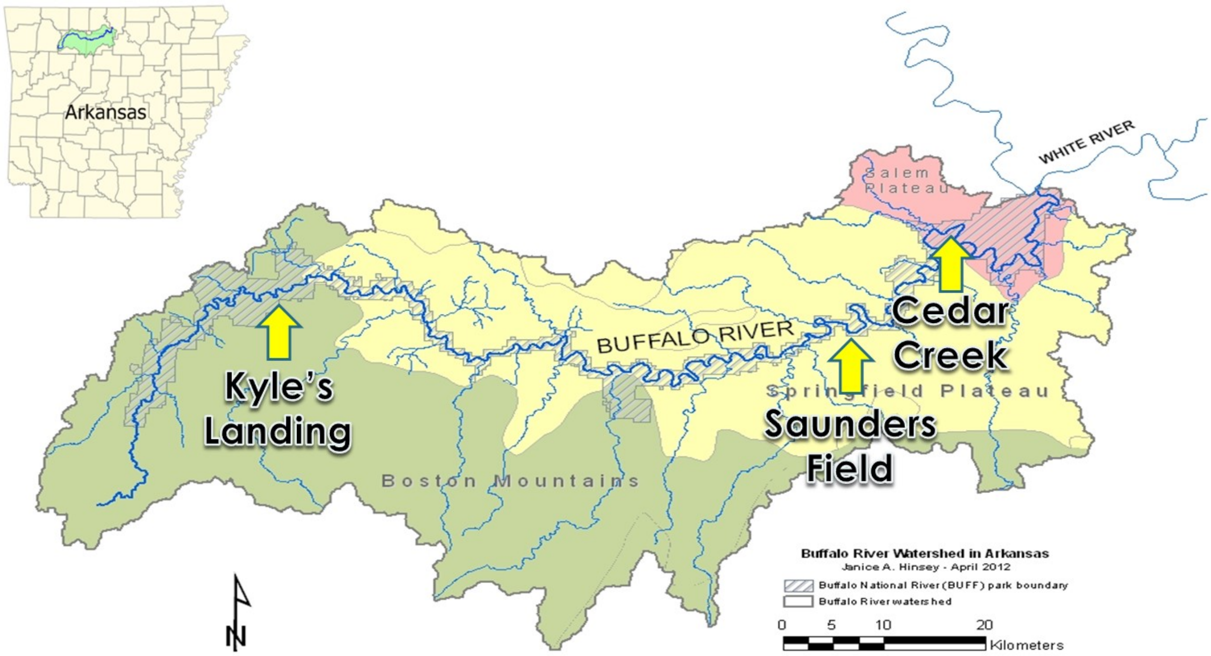

Figure 1. Map of Buffalo National River and study sites.

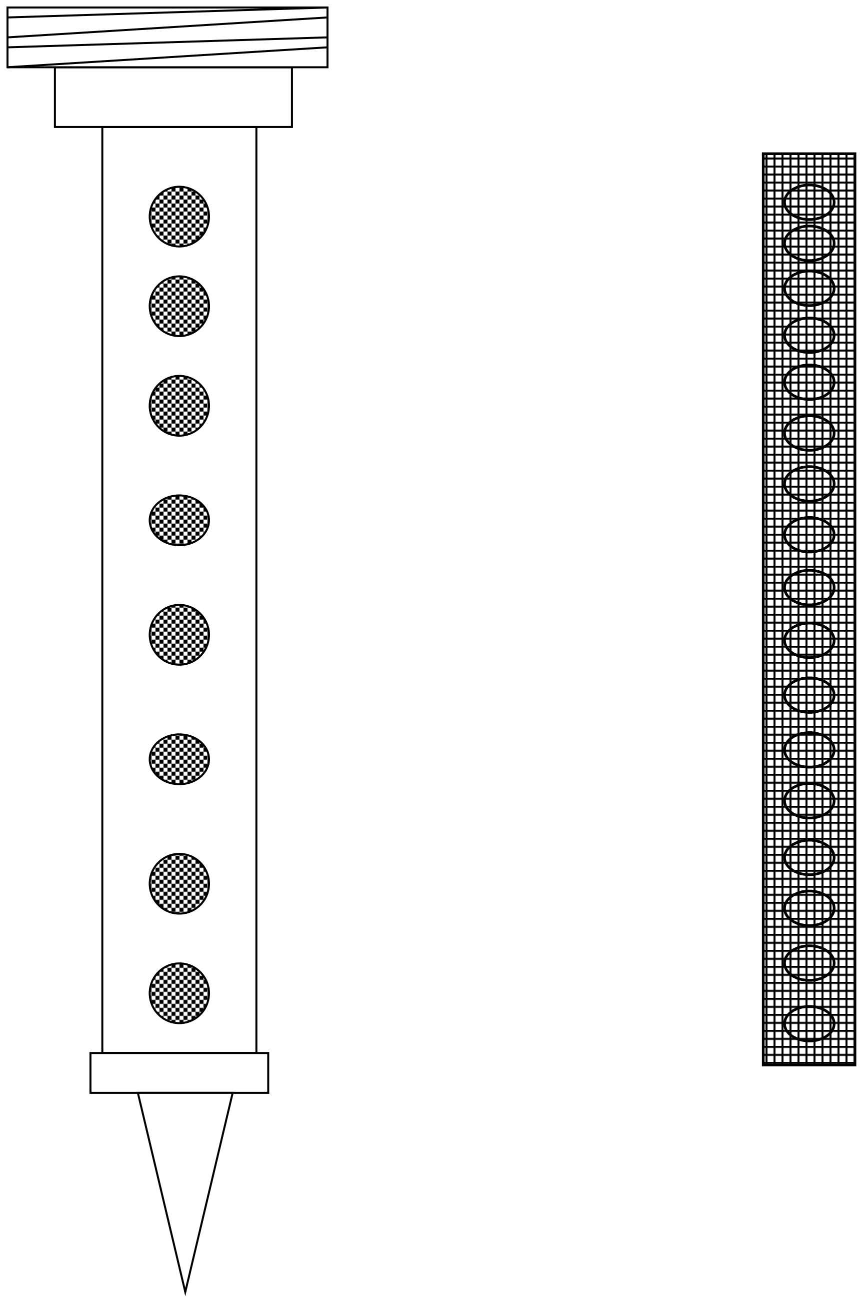

Figure 2. Diagram showing the sampling device (not to scale) used to collect parafluvial water samples from gravel bars. The outer housing (left) is constructed of 1-inch PVC pipe and is 18 inches in length with ¼-inch holes drilled in the side. The inner housing (right) is constructed of ¾-inch PVC piping also with ¼-inch holes and is wrapped in 1-mm screen before being placed into the outer housing.

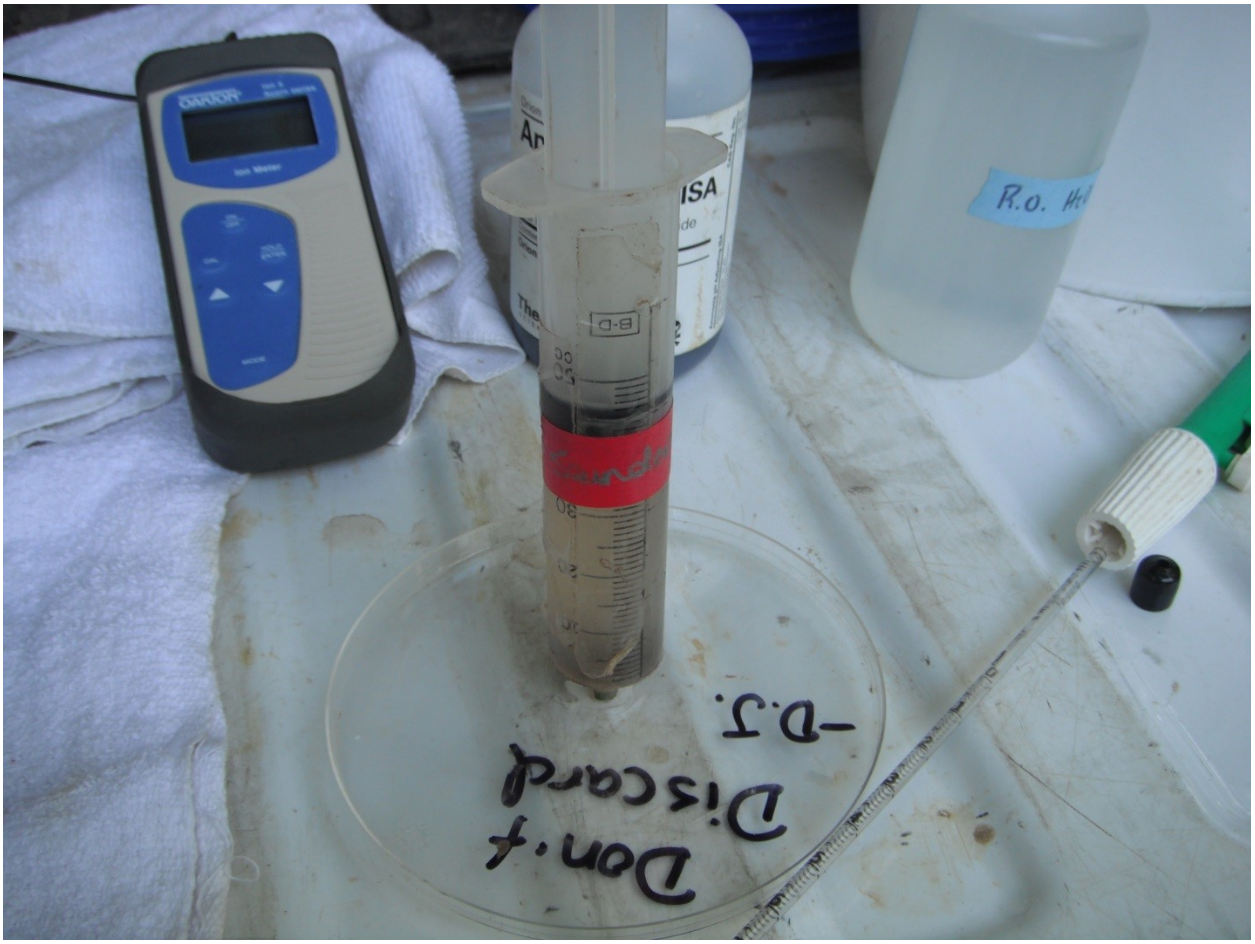

Figure 3. Modified petri plate designed to contain sample and hold sample tube upright during measurement.

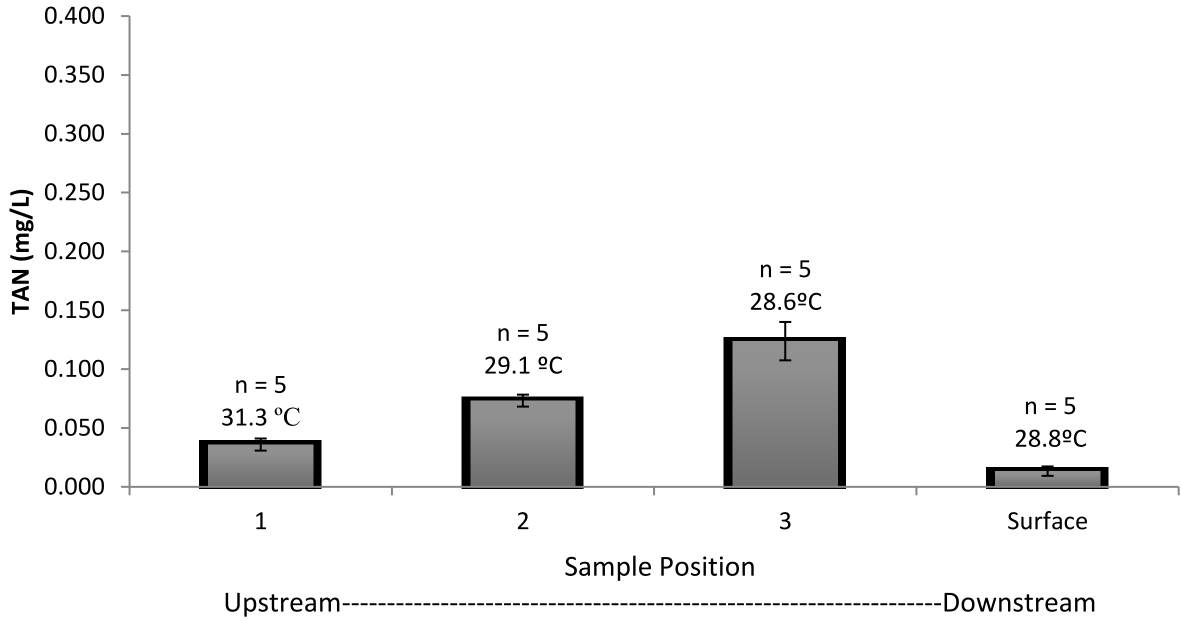

Figure 4. Comparison of mean Total Ammonia Nitrogen (TAN) (mg/L) between sampling positions at Kyle's Landing with standard error.

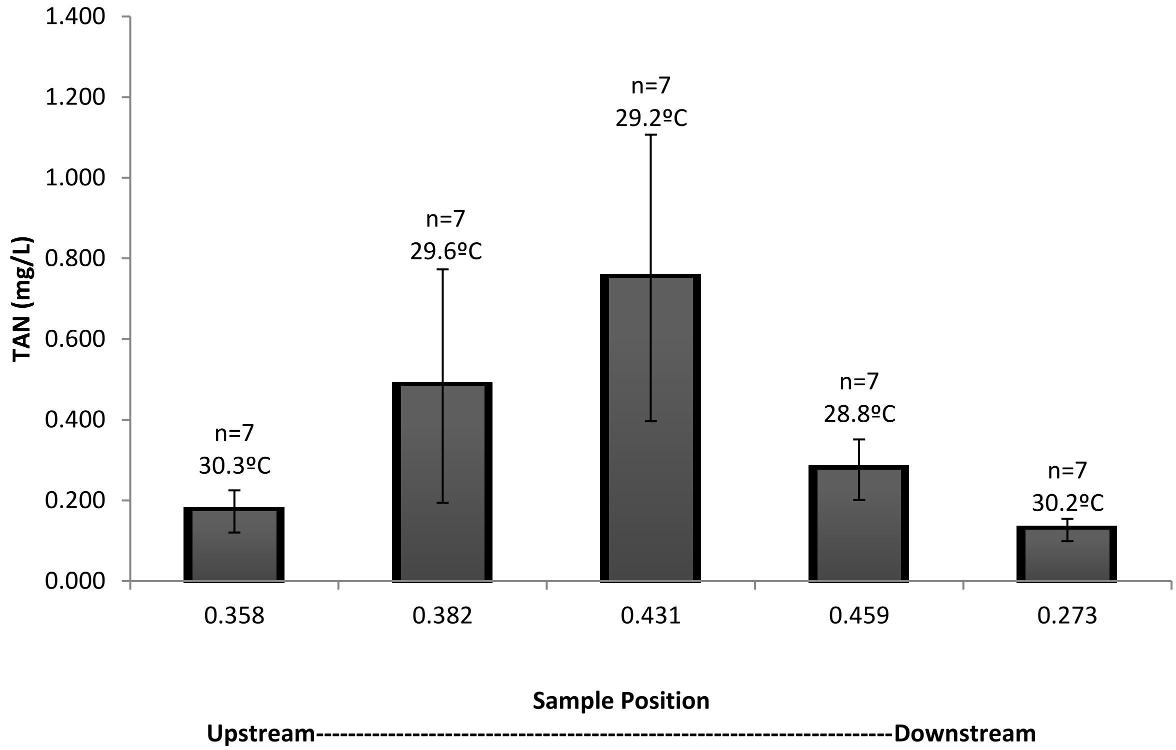

Figure 5. Comparison of mean Total Ammonia Nitrogen (TAN) (mg/L) between sampling positions at Saunders Field with standard error.

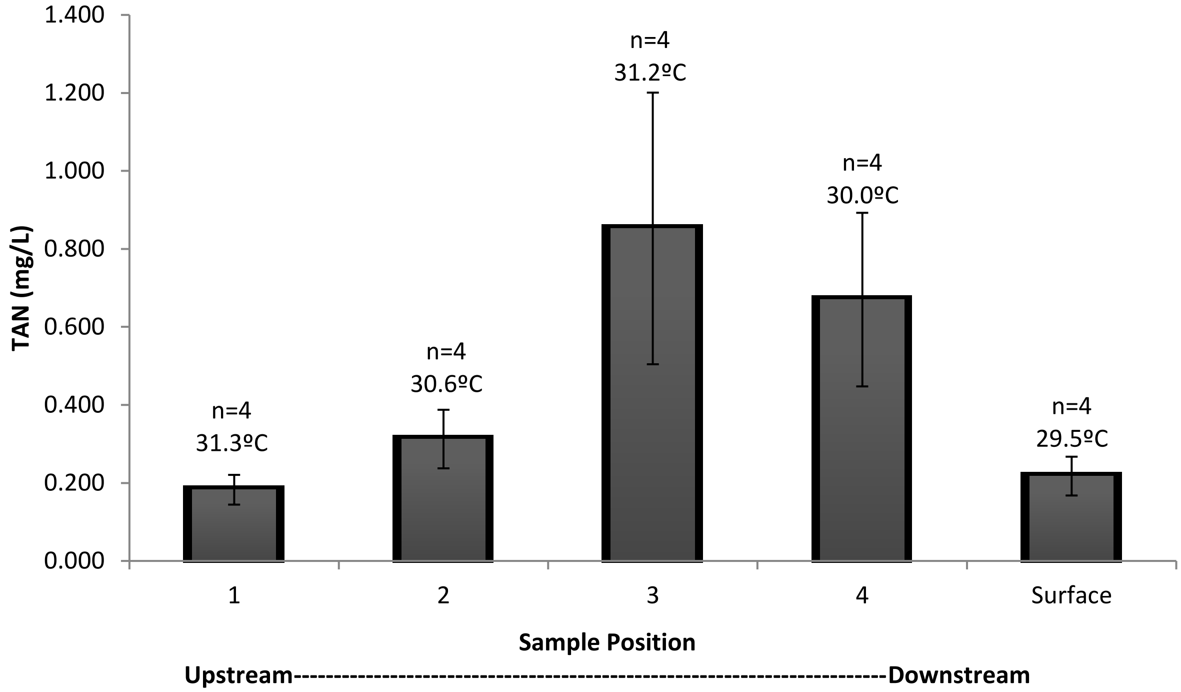

Figure 6. Comparison of mean Total Ammonia Nitrogen (TAN) (mg/L) between sampling positions at Cedar Creek with standard error.

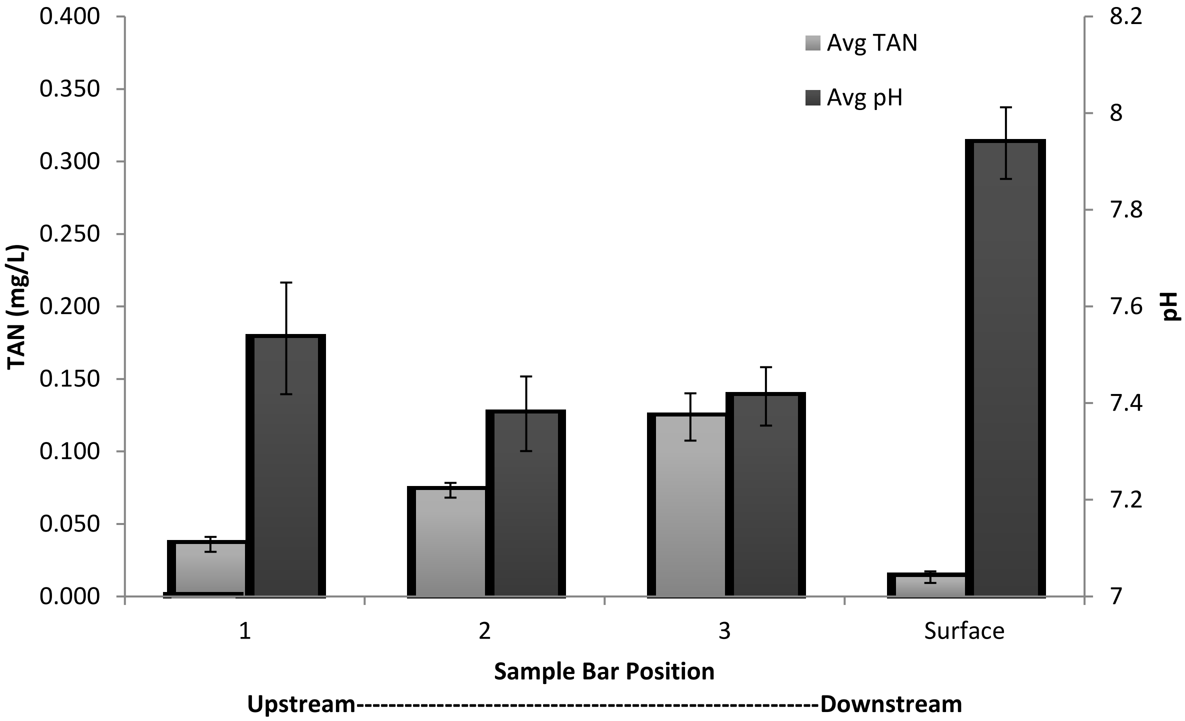

Figure 7. Comparison of mean Total Ammonia Nitrogen (TAN) (on the left y-axis) to mean pH (on the right y-axis) at all sampling positions in the gravel bar at Kyle's Landing.

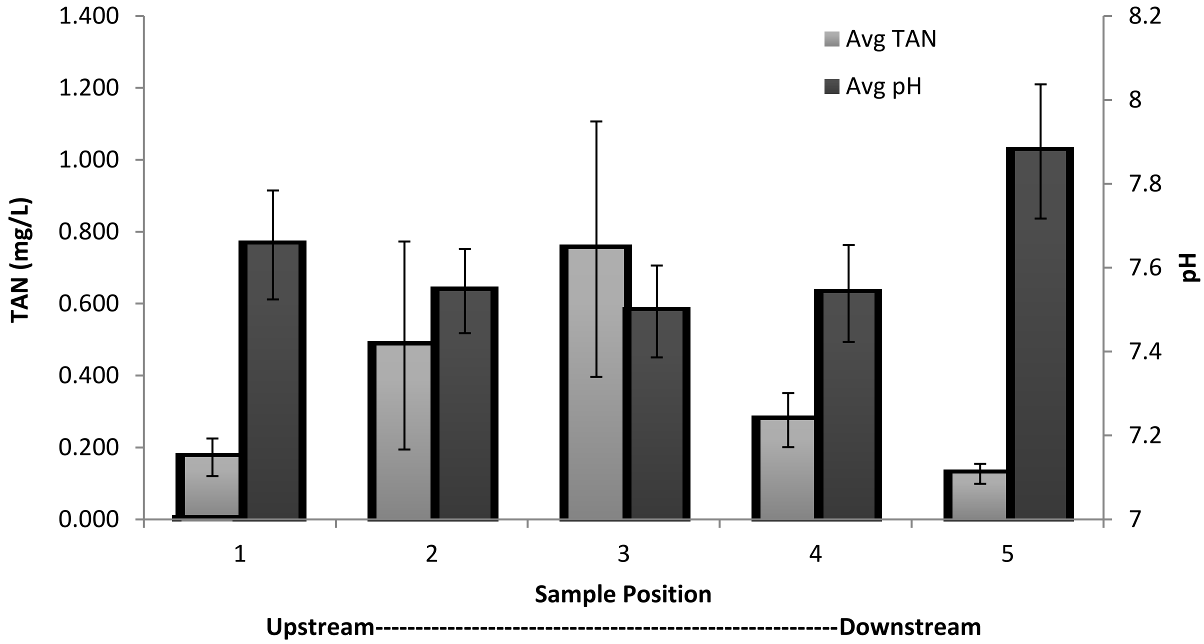

Figure 8. Comparison of mean Total Ammonia Nitrogen (TAN) (on the left y-axis) to mean pH (on the right y-axis) at all sampling positions in the gravel bar at Saunders Field.

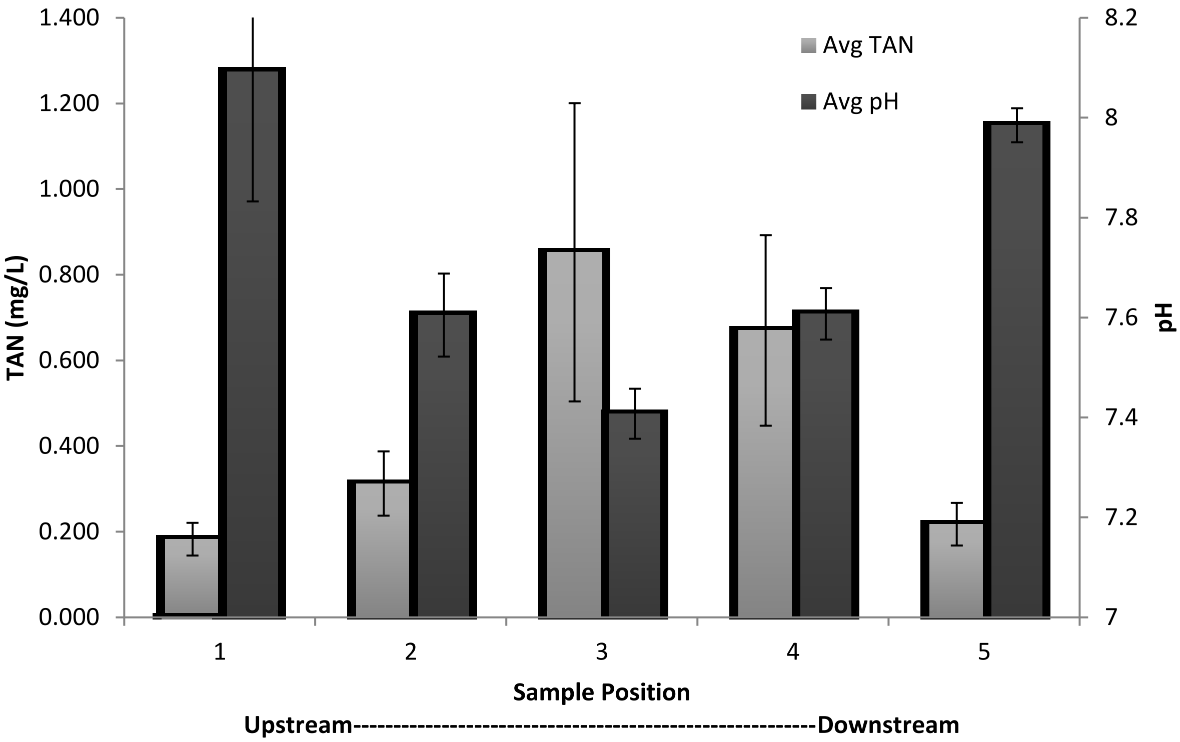

Figure 9. Comparison of mean Total Ammonia Nitrogen (TAN) (on the left y-axis) to mean pH (on the right y-axis) at all sampling positions in the gravel bar at Cedar Creek.

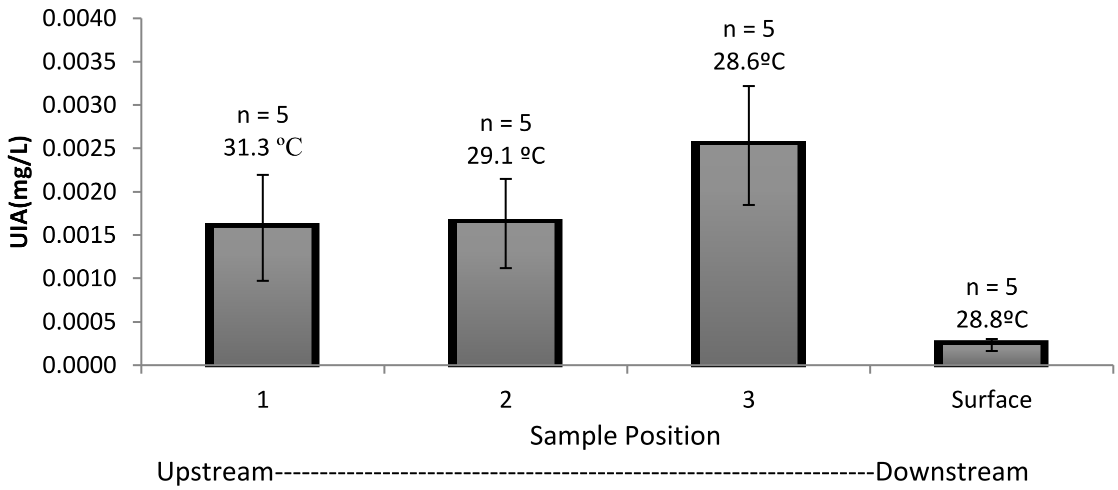

Figure 10. Mean unionized ammonia (UIA) concentrations across the sampling positions at Kyle's Landing calculated with pH and temperature of sample.

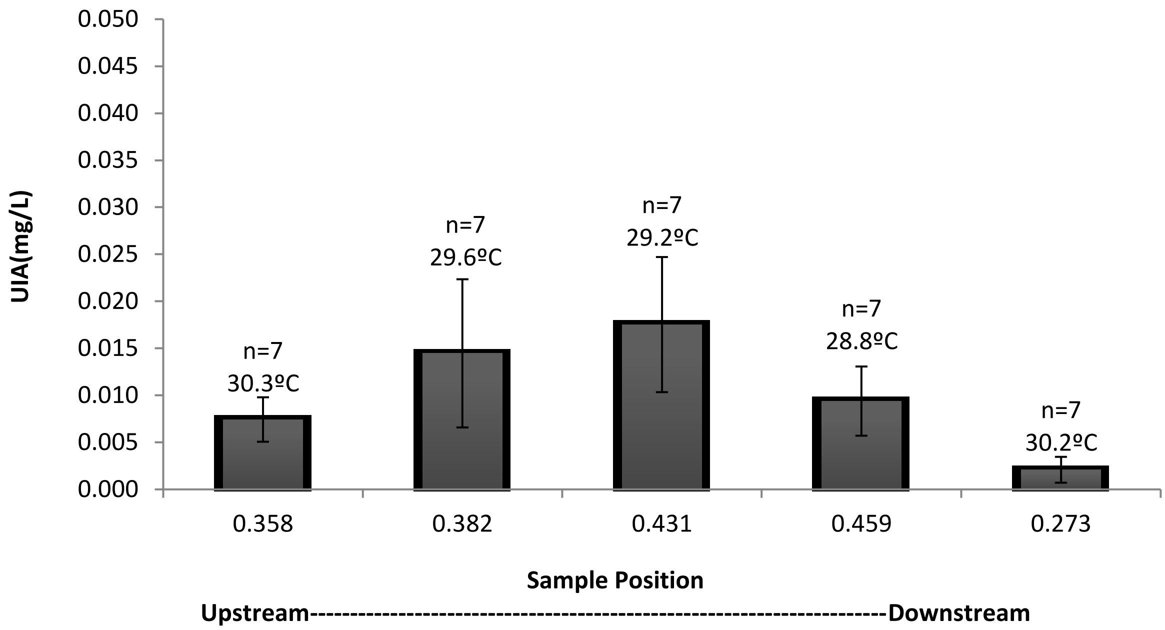

Figure 11. Mean unionized ammonia (UIA) concentrations across the sampling positions at Saunders Field calculated with pH and temperature of sample.

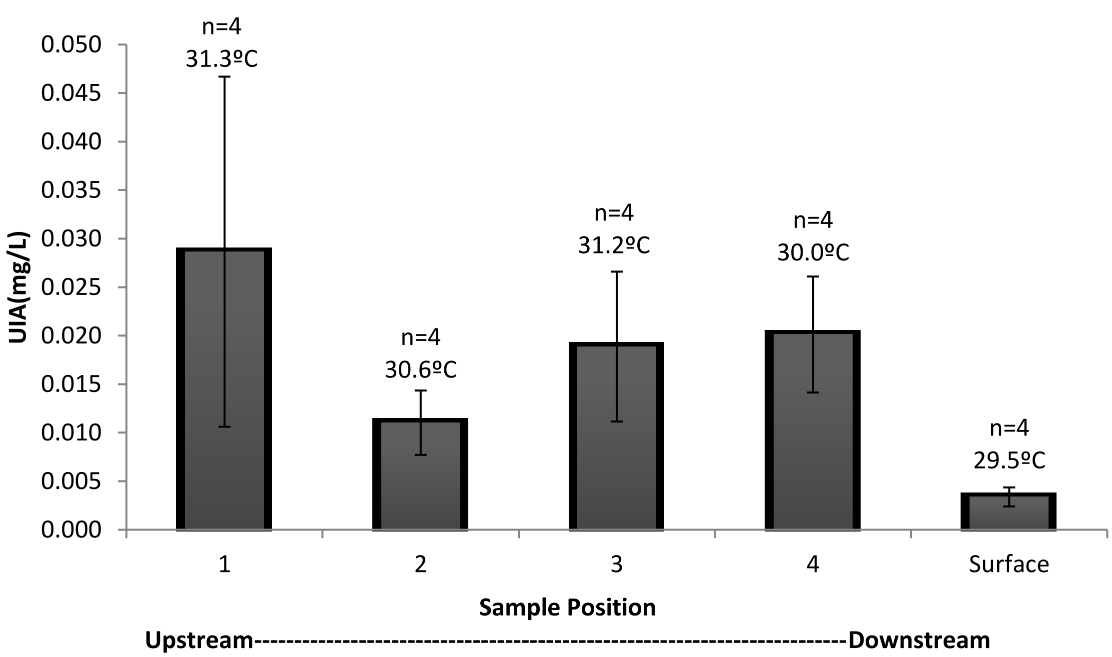

Figure 12. Mean unionized ammonia (UIA) concentrations across the sampling positions at Cedar Creek calculated with pH and temperature of sample.

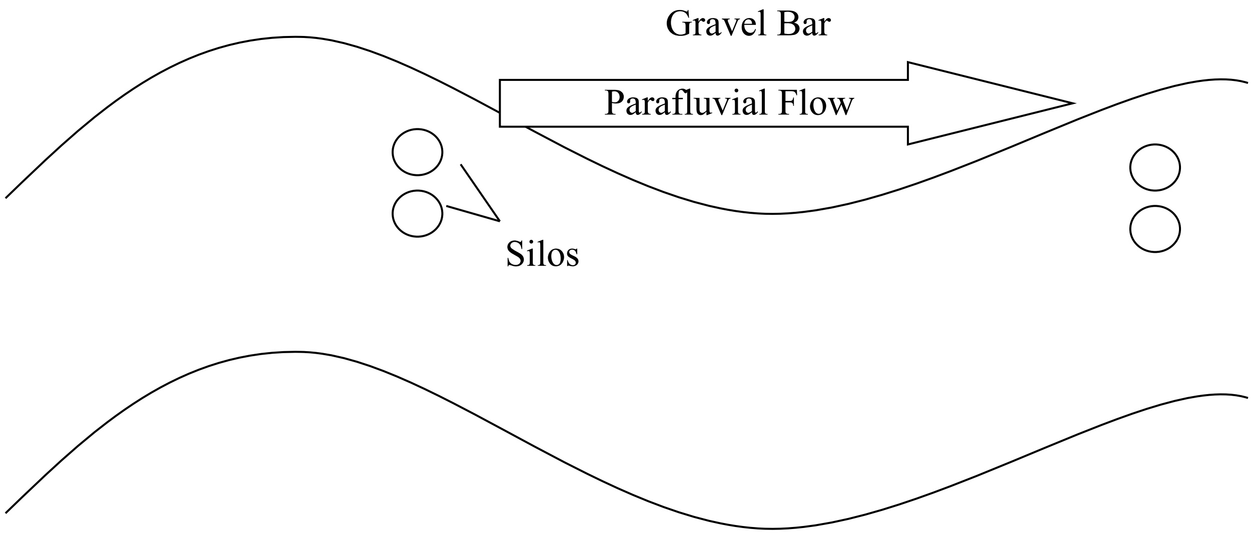

Figure 13. Diagram showing the basic sample design for the caged mussel experiment where two silos are placed upstream of the gravel bar and two are placed downstream.

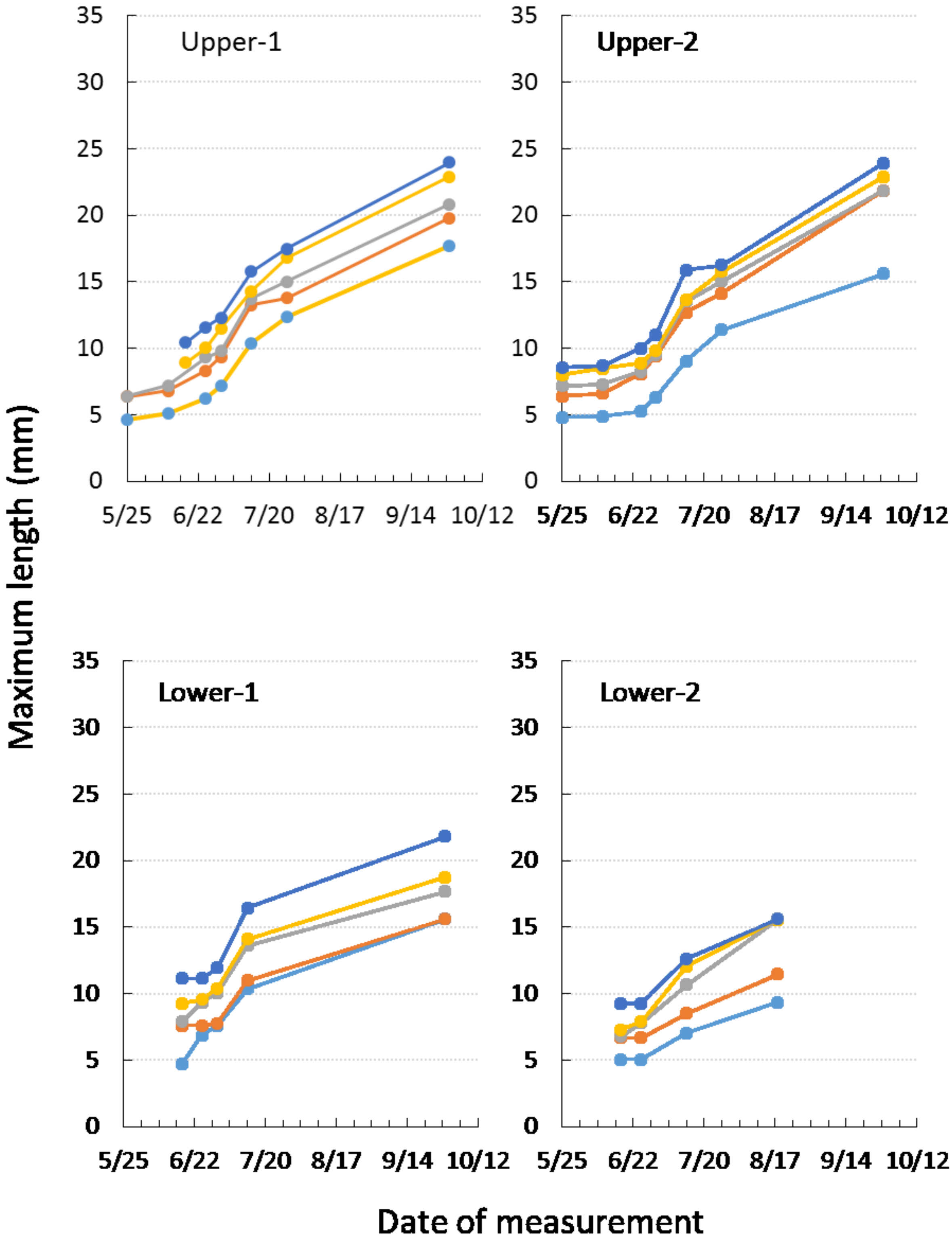

Figure 14. Growth curves (length vs date) of individual mussels in the 4 cages at Kyle's Landing. Upper and lower are upstream and downstream sites.

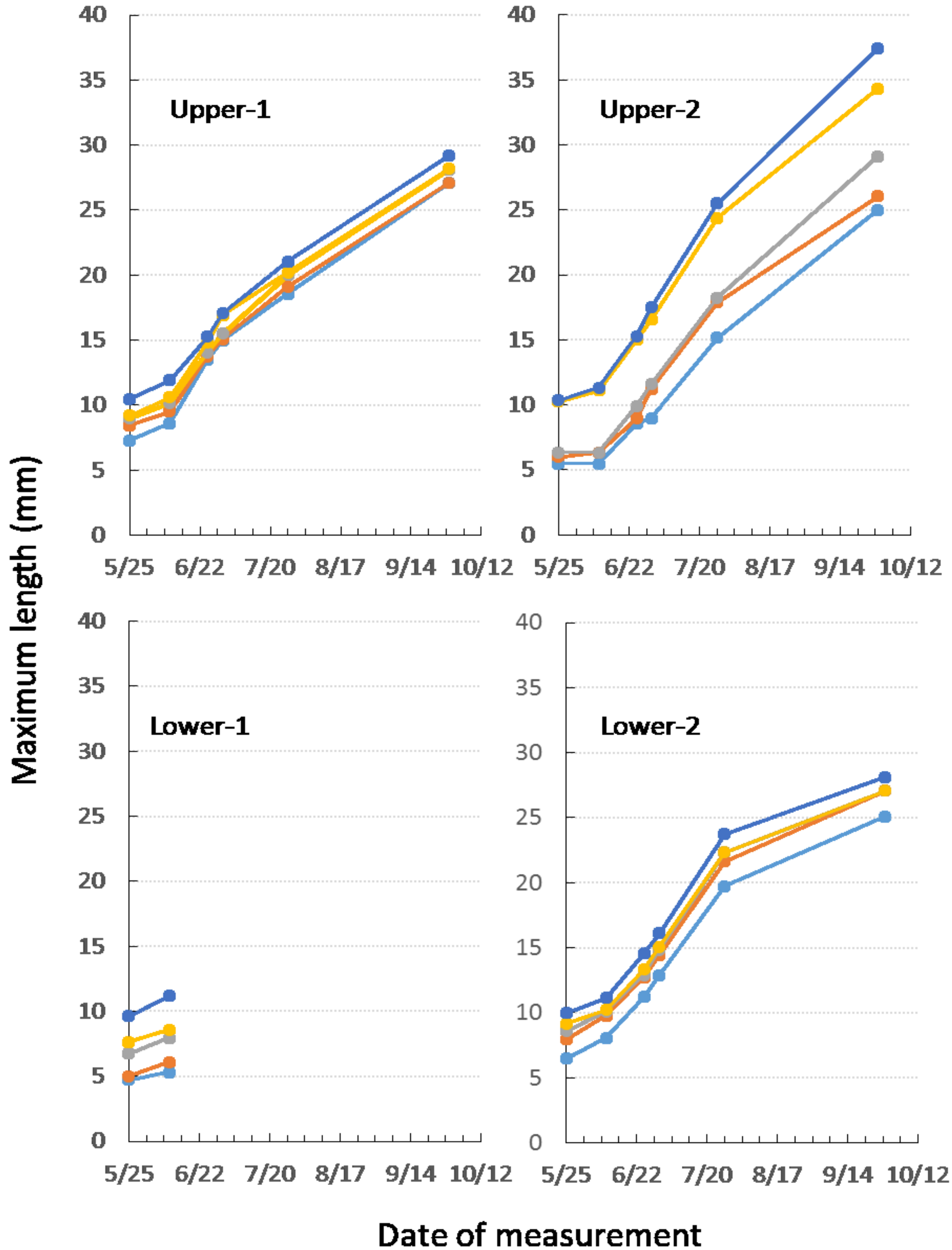

Figure 15. Growth curves (length vs date) of individual mussels in the 4 cages at Saunders Field. Upper and lower are upstream and downstream sites.

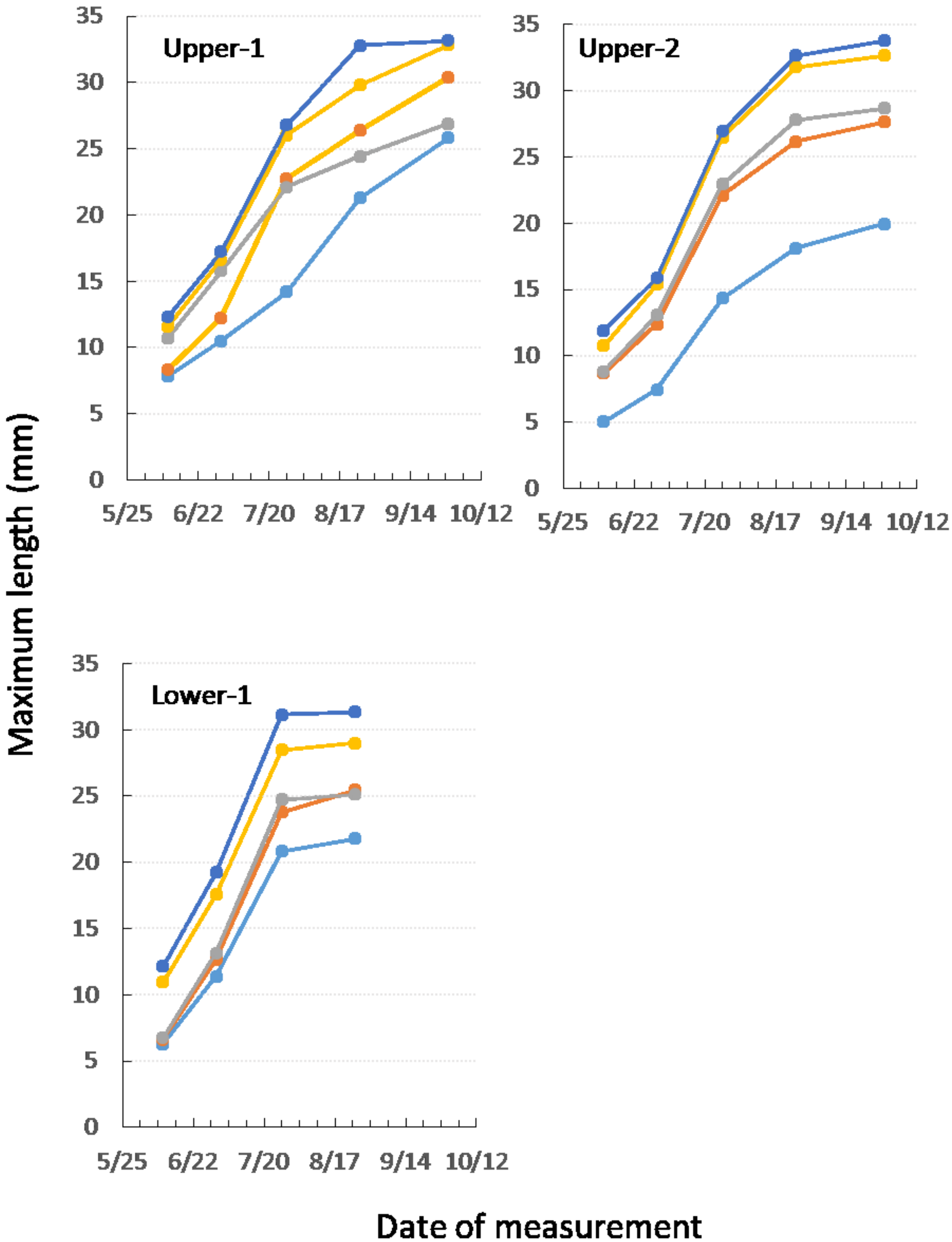

Figure 16. Growth curves (length vs date) of individual mussels in the 3 cages at Cedar Creek. Upper and lower are upstream and downstream sites.

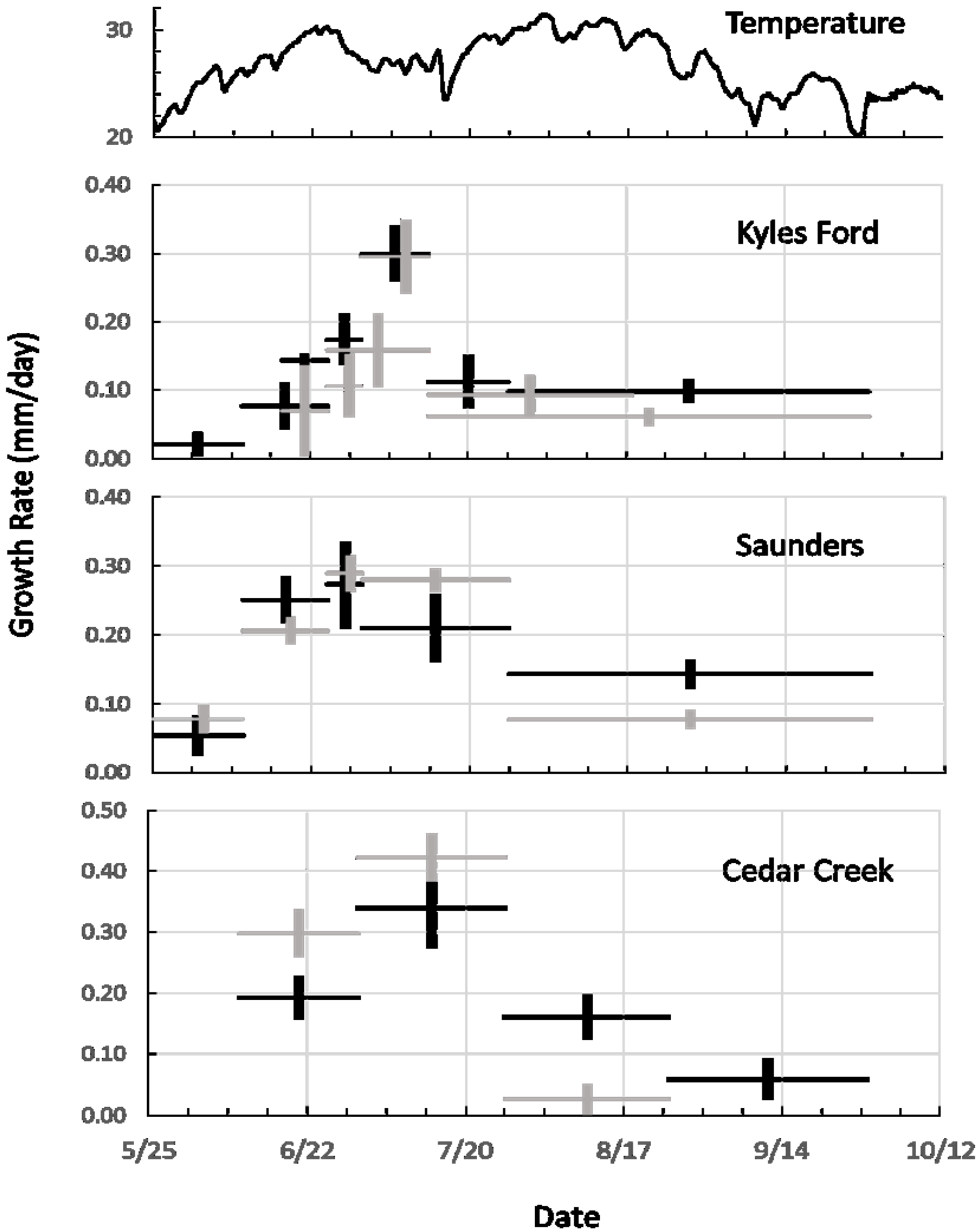

Figure 17. Growth rates (mm/day). Black and gray indicate upstream and downstream sites, respectively. Vertical position of the horizontal lines indicate the mean growth rate (5-10 mussels) and horizontal extent indicates the measurement interval. Vertical bars are 95% confidence intervals for each mean. Temperature line at top is the approximate 24-h running average (data pooled from two of the three sites).

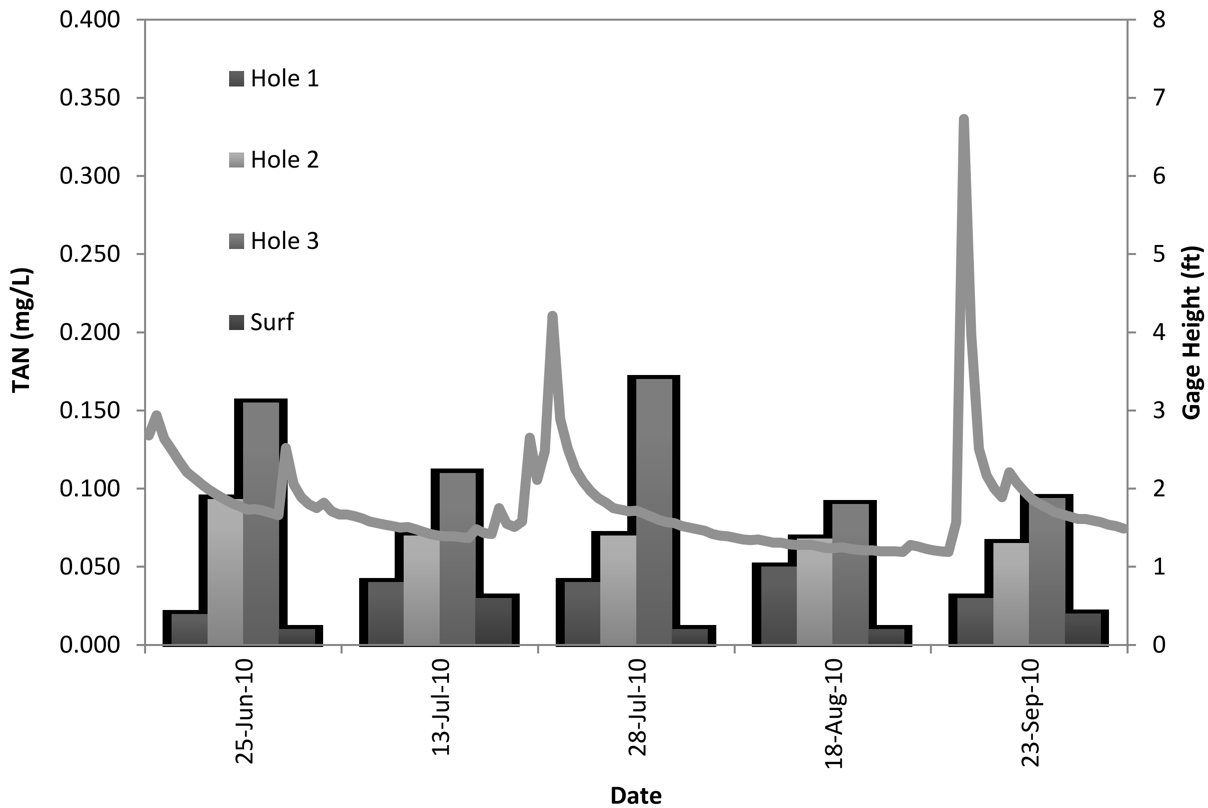

Figure 18. Comparison of mean Total Ammonia Nitrogen (TAN) at each sampling position at Kyle's Landing throughout the summer of 2010 with the hydrograph (represented in gage height).

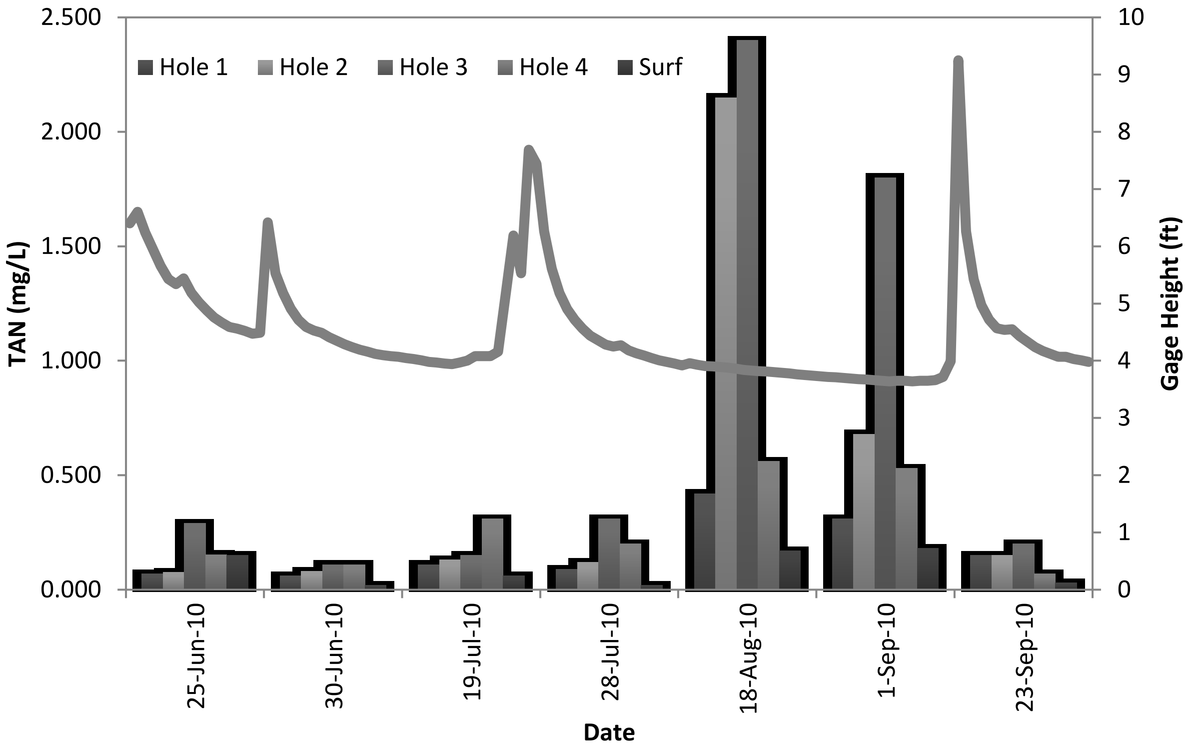

Figure 19. Comparison of mean Total Ammonia Nitrogen (TAN) at each sampling position throughout the summer of 2010 with the hydrograph (represented in gage height) at Saunders Field.

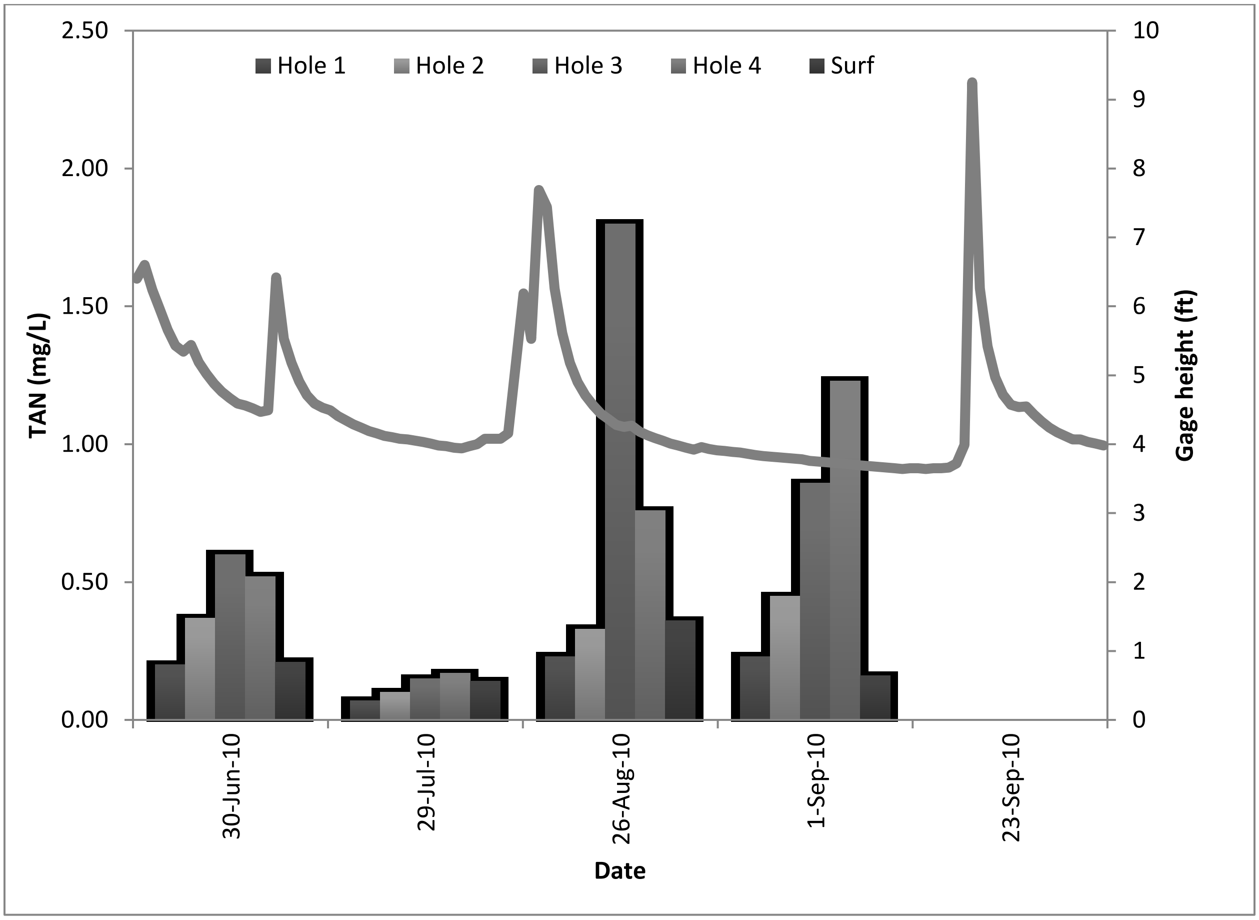

Figure 20. Comparison of mean Total Ammonia Nitrogen (TAN) at each sampling position throughout the summer of 2010 with the hydrograph (represented in gage height) at Cedar Creek.

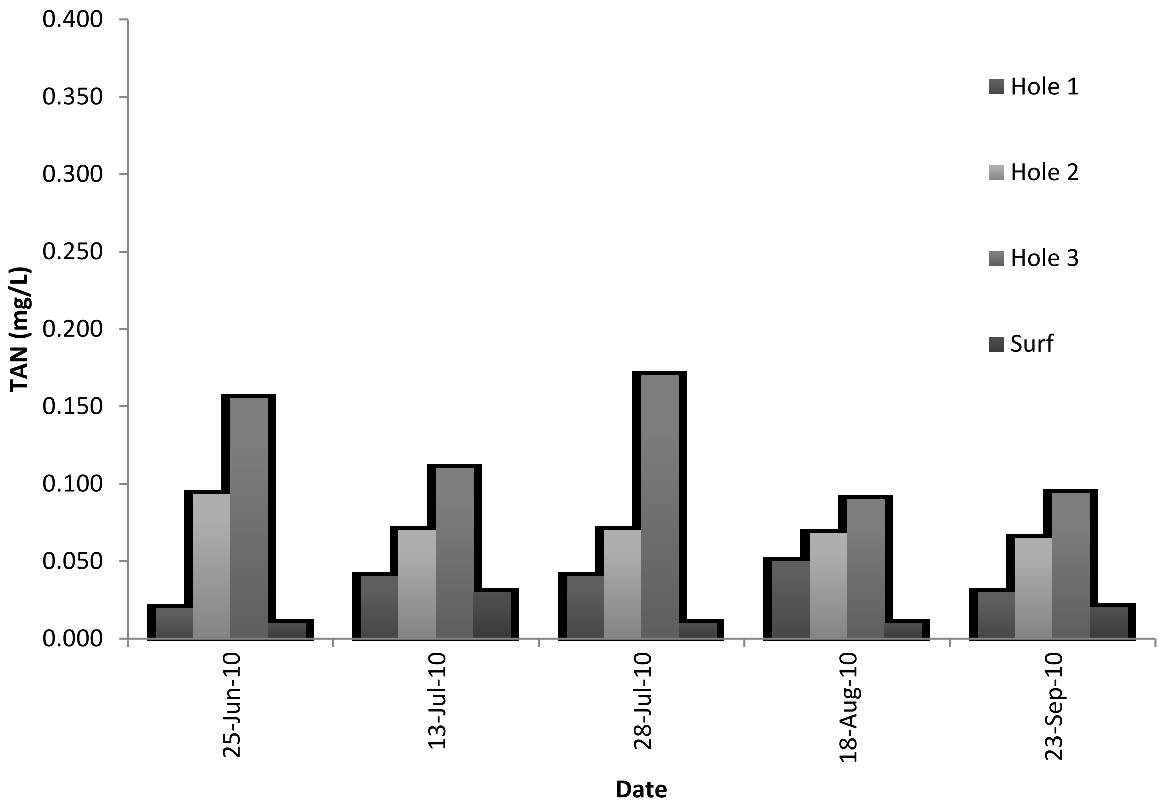

Figure 21. Seasonal effects on mean Total Ammonia Nitrogen (TAN) at Kyle's Landing.

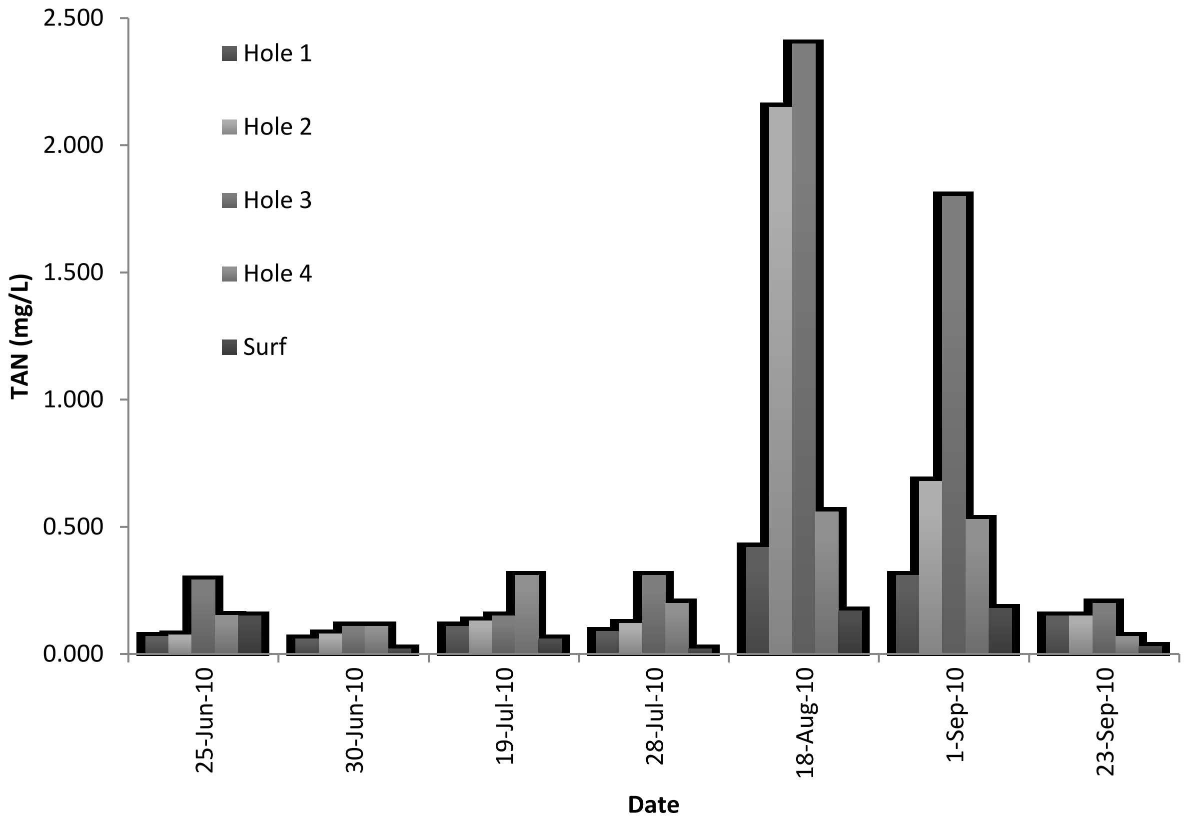

Figure 22. Seasonal effects on mean Total Ammonia Nitrogen (TAN) at Saunders Field.

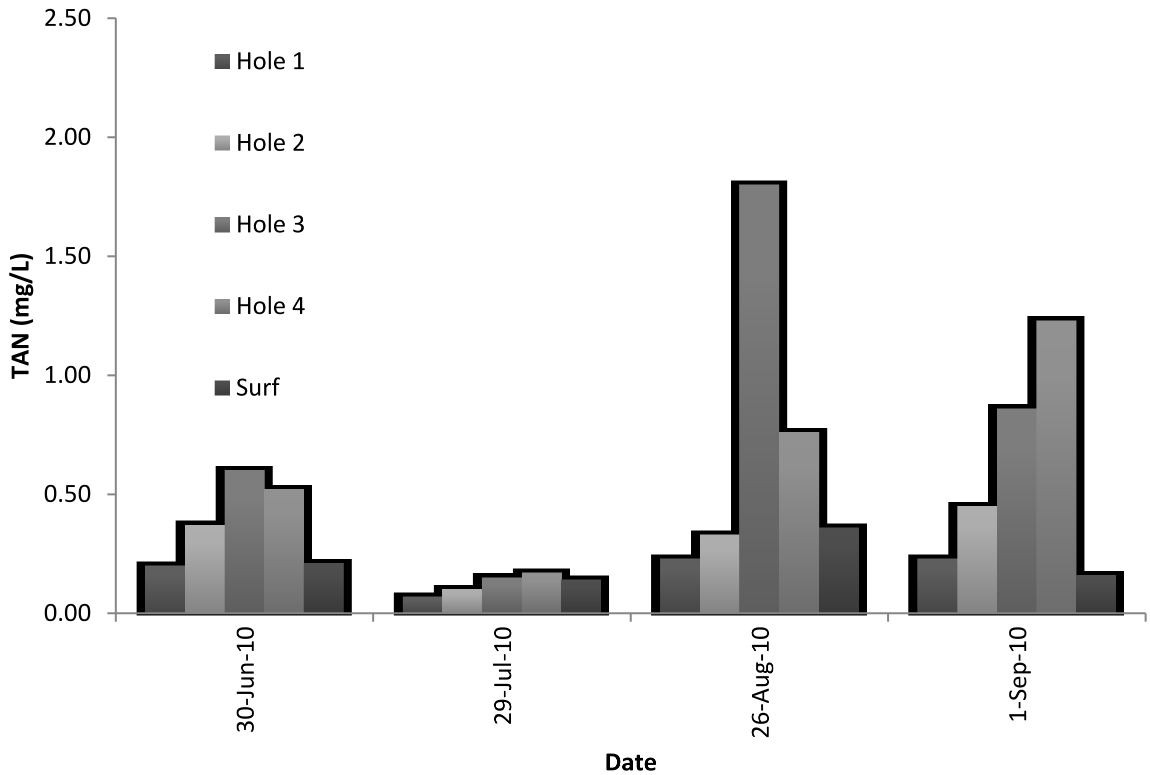

Figure 23. Seasonal effects on mean Total Ammonia Nitrogen (TAN) at Cedar Creek.