FIELD AND LABORATORY EVALUATION OF AN INDUSTRIAL EFFLUENT CONTAINING ELEVATED LEVELS OF AMMONIA

By

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FIELD AND LABORATORY EVALUATION OF

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CHAPTER I

I. AMMONIA AND EFFLUENT TOXICITY TESTING: AN OVERVIEW

Ammonia is a common component in aquatic systems where it is derived from both natural (metabolism of proteins, product of organic decomposition) and anthropogenic sources including sewage, agricultural run-off and industrial wastes (Goudreau et al., 1993, Wicks et al., 2002, Wicks and Randall, 2002). In aquatic environments, ammonia exists in both ionized (NH_4^+) and un-ionized (NH_3) forms depending on pH and temperature, with NH_4^+ being the dominant species at lower pH (Cherry et al., 2005). Equilibrium between ionized and un-ionized ammonia occurs at a pH of 9.26 with the both species present between a pH of 8.26 and 10.26 (Manahan, 2000). Unionized ammonia is the more toxic form and can accumulate in aquatic systems when there are high temperatures, low water flow and elevated pH (Cooper et al., 2005).

Toxic effects to aquatic organisms from unionized ammonia include reduced survival, growth and higher susceptibility to predation (Hickey and Martin, 1999, Prenter et al., 2004, Neil et al., 2005). For example, Wilkie (2002) determined that increased levels of unionized ammonia caused damage to the gills of fish (*Squalus acanthias*) which can result in decreased gas exchange and death. Hickey et al. (1999) exposed macroinvertebrates to increasing levels of ammonia and found decreased taxonomic

richness and abundance of mayflies, while caddisflies abundance increased. Negative effects to the tiger crab (*Orithyia sinica*) exposed to unionized ammonia included decreased growth and survival with increasing exposure time and concentration. (Koo et al., 2005).

Concentrations of ammonia in freshwater systems can fluctuate diurnally with mid-day concentrations of total ammonia nitrogen at 1.0 mg N/L and mid-night levels of 0.5 mg N/L (Crumpton and Isenhart, 1988). Jofre and Karasov (1999) measured ammonia levels in the Fox River in Green Bay, Wisconsin and found unionized ammonia concentrations of 0.04 mg NH_3/L in ambient water and concentrations greater than 1.0 mg NH_3/L in sediment pore water. In three Illinois Rivers, total ammonia nitrogen concentrations ranged from 0.28 mg N/L to 6.08 mg N/L with the lower levels associated with agriculture runoff and higher values taken near urban areas (Wilkin and Flemal, 1980). In 1999, the United States Environmental Protection Agency established chemical specific limits for ammonia levels with two criteria for total ammonia, the Criterion Maximum Concentration (CMC), which is the acute 1-h average concentration, and a Criterion Continuous Concentration (CCC) or chronic 4-d exposure. Neither of these two limits should be exceeded more than once every 3 years. The criteria for the CMC are based on pH and the presence or absence of salmonid fish species (Table1). The criteria for the CCC are based on temperature, pH, and presence of early life stages of fish (>30 days old) as indicated in Table 2 (USEPA, 1999).

Regulation of wastewater effluent

The Clean Water Act was established in 1972, with its primary objective to restore and maintain the physical, chemical and biological integrity of the Nation's waters (USEPA, 2003). The National Pollutant Discharge Elimination System (NPDES) permit program is part of the Clean Water Act, and the focus of this program is to regulate the types and amounts of wastewater discharges into aquatic systems from industrial, municipal and any other sources released into the Nation's waters (USEPA, 2003). In Oklahoma, the Department of Environmental Quality sets regulations for the state's waters that include chemical specific limits and biological monitoring (Whole Effluent Toxicity Tests) (OKDEQ, 2005).

Field versus laboratory experiments

A field study can verify the results from laboratory bioassays. *In situ* biomonitoring and laboratory bioassays are used to determine if chemicals released into the environment are causing adverse effects. *In situ* biomonitoring evaluates the impacts of chemical inputs by placing organisms in the environment and measuring endpoints such as growth and reproduction. Laboratory bioassays are conducted in a controlled setting to determine the effects of the chemical by measuring acute and chronic endpoints such as survival and reproduction (Smolders et al., 2004). It can be unrealistic to extrapolate bioassay results to responses of organisms in the field. Furthermore, field results can be influenced by variables such as water quality (ex. temperature and pH) and food availability (Anderson et al., 2003). For example, higher temperatures can increase

metabolism, which could increase the amount of contaminants absorbed by organisms (Petts, 2000).

Cauchie et al. (2000) found that environmental conditions were different when comparing laboratory and *in situ* experiments with more variability in the *in situ* tests. Smolders et al. (2003) established that the lipid budget in a common carp (*Cyprinus carpio*) recovered in *in situ* exposures but did not recover in an on line monitoring system. Anderson et al. (2003) discovered that the amphipod (*Eohaustorius estuaries*) survival rate in field experiments was between 30-40% compared to 64-76% in laboratory experiments, which was attributed to the leaching of contaminants from natural sediments in the field. Wang et al. (2004) found that constant light in the laboratory caused an amphipod (*Hyalella azteca*) to burrow, which exposed them to higher levels of contaminants in pore water, which was not a natural behavior observed in field observations.

Laboratory bioassays measure the exposure-response of organisms to chemicals released into the environment (Moore et al., 1997). Laboratory toxicity tests measure the toxicity of contaminants by generating LC50 values. The LC50 is the concentration of a contaminant that causes 50% mortality in test organisms (Rand *et al.*, 1995). Milne et al. (2000) determined that the 6-h LC50 concentration of unionized ammonia to the rainbow trout (*Oncorhynchus mykiss*) was 0.83 mg NH_3/L and the 14-d LC50 was 0.38 mg NH_3/L. Whiteman et al. (1995) calculated LC50 values for an oligochaete *Lumbriculus variegates* and a midge *Chironomus tentans* exposed to ammonia, and compared pore water to water-only LC50 values and found that both values (pore water compared to water only) where similar (9.2mg NH_3/L unionized ammonia). Arthur et al. (1987) found

that pH can fluctuate during laboratory bioassays in their study of the affects of ammonia on fish and macroinvertebrates. Since the toxicity of ammonia is dependent on pH monitoring, these changes during bioassays with ammonia is important (USEPA, 1999).

Benthic macroinvertebrates as biomonitors of environmental stress

Evaluation of macroinvertebrate communities exposed to contaminants in the environment can help verify that results from laboratory bioassays are similar to field results. Benthic macroinvertebrates are frequently used to assess environmental contamination and are a vital component of the aquatic communities in rivers and lakes. Furthermore, macroinvertebrates integrate changes in the environment over time and space, which also make them good indicators for biological monitoring (Basset et al., 2004).

Benthic macroinvertebrates have a number of life history strategies that make them useful for performing water quality assessments. These include: a wide distribution (Rosenberg and Resh, 1993), sedentary nature to facilitate comparisons at different locations (Usseglio-Polatera and Beisel, 2002) and, for at least some groups, established sensitivities to environmental stressors which can help indicate the extent of disturbances. In addition, macroinvertebrate collecting equipment is relatively inexpensive (Klemm et al., 2003).

There are some disadvantages in using benthic macroinvertebrates for biomonitoring. For example, identification can be time consuming and not all disturbances affect macroinvertebrates (Rosenberg and Resh, 1993). In addition, macroinvertebrates can be affected by factors such as available substrate or water

movement (Lancaster, 1999). Principe and Corigliano (2006) found that macroinvertebrates can move into the water column, which can cause them to drift to other areas of rivers they would not normally inhabit. These factors make it important to evaluate multiple sampling sites over time when attempting to characterize macroinvertebrate assemblages.

Many studies have used macroinvertebrates to asses the effects of contaminants, which include ammonia (Arthur et al., 1987, Sarda and Burton, 1995, Boardman et al., 2004), metals (Duzzin et al., 1988, Barata et al., 2005) and pesticides (Ward *et al.*, 1995, Overmyer, et al., 2005). Dickman (2000) examined the impacts of the pesticide Bti on non-target species by comparing treated pools with non-treated pools in the Tai Tan River in the New Territories of Hong Kong. He found that the pesticide decreased chironomid larvae populations in the treated pools during the first year compared to control pools. However, resistant larvae repopulated the treated pools the following year and there was no significant difference in chironomid larvae populating the treated and non treated pools. Malmqvist and Hoffsten (1999) analyzed the influence of old mine deposit drainage into rivers using macroinvertebrates. Although they discovered the drainage had no adverse effects on biomass or abundance, their research found a decrease in taxa richness by 36%. Hickey et al. (1999) found that while a 29 day exposure to un-ionized ammonia reduced the abundance of mayflies *Deleatidium* sp. (Ephemeroptera: Leptophlebiidae) and *Coloburiscus humeralis* (Ephemeroptera: Oligoneuriidae), the abundance of the caddisflies *Beraeoptera roria* (Trichoptera: Conoesucidae) and *Confluens* sp. (Trichoptera: Conoesucidae) increased.

There is a well-defined list of macroinvertebrates that are indicator species for water quality. The US Environmental Protection Agency (2005a) classifies macroinvertebrates as sensitive, moderately tolerant and pollution tolerant (Table 3). It is important to note that non-contaminant factors such as water chemistry and available substrate can also influence where macroinvertebrates occur in aquatic systems (USEPA, 2005a). Sensitivity ratings may also be specific for certain types of contaminants. For example, the index developed by Hilsenoff (1987) emphasized organic contaminants that influence dissolved oxygen levels. Sensitivities of an organism can also vary if the type of contaminant changes over time.

Fish as biomonitors of environmental stress

In addition to macroinvertebrate communities, fish populations can be good indicators of the impacts of environmental disturbances. There are advantages for using fish in biomonitoring that include, the relative ease of fish collection and identification, their well established distribution and life histories, and their long life span, which allows for studies to be done on a seasonal basis (USEPA, 2005b).

Many studies have assessed the impacts of a wide range of contaminants on fish in fresh water systems, including ammonia (Wicks and Randall, 2002, Boardman et al., 2004), pesticides (Parvez and Raisuddin, 2005, Mazet et al., 2005) and metals (Dalman, 2005). In laboratory experiments, fish experience adverse effects including alterations to the central nervous system, ionic imbalances and morphological changes to gill lamellae when exposed to increased levels of ammonia (Cardoso et al., 1996, Vedel et al., 1998). Wang and Walsh (1999) found that fish exposed to ammonia showed signs of stress that

included darkening of the skin and temporary loss of balance. Milne et al. (2000) exposed rainbow trout (*Oncorhynchus mykiss*) to varying levels of ammonia at intervals of 1, 6 and 24 hours and found that at the end of a 7 day recovery period all fish survived exposure to unionized ammonia levels between 0.024-0.2 mg NH_3/L. Only 0.02% of fish died at 0.4-0.43 mg NH_3/L in the 6 and 24-h exposure; however, all fish died within 6-h in the 24-h exposures with unionized ammonia levels between 0.75 mg NH_3/L and 0.82 mg NH_3/L. Hermanutz et al. (1987) examined the effects of ammonia in experimental streams by measuring length and weight of the fathead minnow (*Pimephales promelas*). They found that ammonia influenced the fish length (difference of 4.2 to 5.3mm) and weight (difference of 11.1 to 17.6g) when they compared control streams with unaltered levels of ammonia streams.

Zebra mussels as biomonitors of environmental stress

In addition to macroinvertebrates and fish, zebra mussels (*Dreissena polymorpha*) are valuable tools for biomonitoring. In lotic systems, mobile organisms can avoid disturbances by escaping contaminated areas. Since zebra mussels are sedentary, they can be used *in situ* to determine if chemicals are causing adverse effects in aquatic environments (Lafontaine et al., 1999). Bervoets et al. (2004) describes factors that make zebra mussels good candidates for biomonitoring including, easy collection and handling, availability in large numbers, and the tolerance of contaminants without high mortality rates.

Yu and Culver (1999) exposed zebra mussels to hypoxic conditions to measure survival and growth by putting cages in a lake from 0.05m to 8.0m (0.5m increments).

All of the mussels died in cages more than 5.0m deep, and the survival rate for the other cages ranged from 24% to 76% (increased with depth). Growth from depths of 0.5m to 2.5m was 4.3-5.2mm and 2.5 to 5.0m was 3-4mm. Mersch and Beauvais (1997) exposed zebra mussels *in situ* to different stressors that included effluents from a paper mill plant, nuclear power plant, steel industry, petrochemical industries and PVC manufacturing/metal coating plant. In addition, Mersch and Beauvais (1997) wanted to determine if there was an induction of micronuclei (MN) as a means to determine genetic damage caused by these effluents. Mn induction was observed at all sites when compared with a reference site with the highest induction at the nuclear power plant and PVC manufacturing/metal coating plant locations. The lowest Mn induction was observed at the paper mill effluent with the steel and petrochemical industries falling in the middle. Smolders et al. (2004) examined household wastewater and industrial wastewater effluent to determine if the lipid budget of zebra mussels was affected. The experiment showed that the lipid budget in zebra mussels exposed to the household effluent was not affected but the industrial effluent decreased lipid levels. The industrial effluent *in situ* study was compared to laboratory bioassays and the same results were observed.

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TABLES

Table 1. Chemical specific limits for total ammonia (mg N/L) for acute exposure based on the presence of fish species

pH	Salmonids present	Salmonids absent
7	24.1	36.1
8	5.62	8.40

Table 2. Chemical specific limits for total ammonia (mg N/L) for chronic exposure based on temperature, pH and the presence of early life stages of fish

	Fish present		Fish absent	
Temperature (ºC)	pH 7	pH 8	pH 7	pH 8
0	5.91	2.43	9.60	3.96
10	5.91	2.43	7.91	3.26
20	4.15	1.71	4.15	1.71
30	2.18	0.88	2.18	0.88

Table 3. Summaries of the sensitivities of macroinvertebrates to environmental disturbances (EPA, 2005a).

Sensitive	Moderately Tolerant	Pollution Tolerant
Stoneflies (Plecoptera)	Caddisflies (Trichoptera)	Midgeflies (Diptera)
Mayflies (Ephemeroptera)	Dragonflies (Odonata)	Worms (Oligochaeta)
Dobsonflies (Coleoptera)	Damselflies (Odonata)	Leeches (Hirudinea)
Alderflies (Megaloptera)	Amphiods (Amphipoda)	Pouch Snails (Gastropoda)
Mussels (Pelecypoda)	Blackflies (Diptera)	
Water penny Beetles (Coleoptera)	Craneflies (Diptera)	
Riffle Beetle (Coleoptera)	Crayfish (Decapoda)	
Snipeflies (Diptera)	Isopods (Isopoda)	

CHAPTER II

II. LABORATORY AND FIELD EVALUATION OF AN INDUSTRIAL EFFLUENT CONTAINING ELEVATED LEVELS OF AMMONIA

INTRODUCTION

Ammonia occurs in aquatic systems from both natural (metabolism of proteins, product of organic decomposition) and anthropogenic (sewage, agricultural run-off, and industrial wastes) sources (Goudreau et al., 1993, Wicks et al., 2002, Wicks and Randall, 2002). Based on voluntary reports of chemical releases in the United States (from the United States Environmental Protection Agency's Toxic Release Inventory), approximately 80,486 metric tons of ammonia were released into the environment in 2006 (USEPA, 2008). The majority of this input was derived from agricultural sources, while industry contributed 1-2% from point sources such as food processing plants, fertilizer plants, chemical companies and wastewater treatment plants (USEPA, 2008).

When it does occur in water, "total ammonia" is comprised of both an ionized (NH_4^+) and un-ionized (NH_3) form depending on pH and temperature, with NH_4^+ being the dominant species at lower pH (Cherry et al., 2005). Equilibrium between ionized and un-ionized ammonia occurs at a pH of 9.26 with both species present between a pH of

8.26 and 10.26 (Manahan, 2000). Unionized ammonia is the more bioavailable, and in turn, more toxic form (Thurston et al., 1979, Redner and Stickney, 1979).

Industrial effluents that are released into U.S. surface waters are largely regulated through state regulatory agencies as part of the National Pollutant Discharge Elimination System (NPDES) program that was established through the Clean Water Act (USEPA, 2003). In order to limit the potential for negative impacts on aquatic receiving systems, discharge permits may include "do not exceed" limits for constituent chemicals (chemical criteria), and/or require regular toxicity screening through standardized laboratory bioassays. As for other chemicals that have national regulatory limits, the criteria for total ammonia include a Criterion Maximum Concentration (CMC), which is the acute 1-h average concentration, and a Criterion Continuous Concentration (CCC) or chronic 4-d exposure. Neither of these two limits should be exceeded more than once every 3 years. The criterion for the CMC is based on pH and the presence or absence of salmonid fish species. The CMC for total ammonia nitrogen with salmonids present at pH 7 is 24.1 mg N/L and at pH 8 is 5.62 mg N/L. The CMC with salmonids absent at pH 7.0 is 36.1 followed by 8.4 mg/L total ammonia at pH 8.0. The criteria for the CCC are based on temperature, pH, and presence of early life stages of fish (>30 days old). At pH 7 and a temperature of 24 °C, the CCC for total ammonia nitrogen is 3.21 mg N/L, while at pH 8 and 24 °C it is 1.32 mg N/L (USEPA, 1999). These chemical-specific limits could be made more stringent at the state level based on site characteristics such as flow rate and established total maximum daily loads (TMDL) (USEPA, 1991).

Single chemical criteria are important for regulating wastewater discharges, although they rely on the assumption that the chemical composition of the effluent is

known and quantified (Sarakinos et al., 2000). This approach fails to take into account the potential chemical interactions that could occur in a complex mixture like an industrial effluent, the persistence of the chemicals present, and the potential assimilative capacity of the specific receiving system the effluent is being discharged into (Marcus and McDonald, 1992). In the specific case of ammonia, there is also concern that the chemical criteria are not sufficiently protective of some freshwater species like native mussels even though the ammonia criteria were derived with data from significantly more genera than required under USEPA guidelines (Augspurger et al., 2003).

Some of the potential deficiencies of single-chemical criteria can be overcome by conducting whole effluent toxicity ("WET") tests, which can help characterize unknown toxic effects and assess chemical interactions of effluents (Chapman, 2000). WET tests are an important part of the wastewater regulatory process and are often mandated under the NPDES permit program (USEPA, 1994). These tests are conducted under controlled conditions (light and temperature) to establish effects by measuring acute and chronic endpoints such as survival and reproduction of "standard" test organisms such as the cladocerans, *Ceriodaphnia dubia* and *Daphnia pulex*, and fathead minnows, *Pimephales promelas* (Smolders et al., 2004).

WET tests provide information about effluent quality that the use of single chemical criteria alone may not, although there are potential limitations with this approach as well. For example, the species used in these tests may not always represent organisms present in the receiving system and test results may not effectively indicate the cumulative effects of chemicals in the wastewater discharge (La Point and Waller, 2000). The controlled environment the tests are conducted in may also fail to adequately

represent field conditions where exposure to contaminants may be variable and interactions with predators and/or the availability of food may influence the susceptibility of organisms to the chemical stressor (Anderson et al., 2003, Fleeger et al., 2003).

A third approach used to assess effluent quality is in-field biological assessment. In contrast to chemical-specific criteria and laboratory toxicity tests, field studies evaluate the condition of aquatic systems through collecting/analyzing resident species and species studied *in situ*. Common endpoints in field assessments include measures of community structure such as species diversity of a particular assemblage and/or the presence of key indicator organisms. *In situ* studies may utilize groups of confined organisms that are exposed to the effluent discharge in cages. This approach facilitates evaluation of responses at the individual level (e.g. survival, growth, biochemical endpoints), while also providing a realistic exposure scenario (Chappie and Burton, 1997). While field assessments are not effective for *a priori* assessment of risk (since system impacts would already have occurred if they are being detected in the field assessment), they have the potential to validate the relationship between responses determined in toxicity tests and that occurring in natural systems (Cairns, 1986, Ferraro and Cole, 2002). Taken together, chemical analyses, laboratory toxicity testing, and field assessment can provide a very effective way to evaluate effluent quality and determine if routine methods for effluent monitoring (chemical analyses and laboratory testing) are providing adequate protection for the receiving system.

The focus of my research is on the Verdigris River located east of Tulsa in Verdigris, Oklahoma were a fertilizer manufacturing plant releases its effluent that contains elevated levels of ammonia. The fertilizer plant started production in 1975 and

was purchased by Terra Nitrogen in 1994. Terra Nitrogen's Verdigris plant is the largest producer of Urea Ammonia Nitrate in North America with a total production of 3,200 tons per day and an annual production of 2,050,000 tons. To minimize the affects to the river, the effluent from Terra is passed through biological treatment ponds, two holding ponds and is mixed with unpolluted water in a holding pond before it is released into the river (Terra, 2009).

The Verdigris River originates in Kansas before it enters Oklahoma and has variable flow depending on the amount of rain and water released from Oologah Lake, which is located upriver on the Verdigris. The river has a drainage area of 6,534 square miles and the stream flow from 2001 to 2006 ranged from a low of 0.74 m^3/s in August 2003 to a high of 617.31 m^3/s March of 2004. The river gauge height for this period ranged from 0.99m in September 2006 to 5.38 m in March 2004 (USGS, 2009). The changes indicate the variability in the flow of the river that has the potential to influence the distribution of species by modifying habitats and biotic interactions. For example, high river flow can alter habitats that could influence the distribution of macroinvertebrates and fish, which in turn can influence predator-prey interactions (Thorp and Casper, 2003). Furthermore, elevated stream flows could decrease the effects of chemicals released into the river. The Verdigris River is a dynamic system and many factors can modify the abiotic and biotic composition and these changes can occur both spatially and temporally.

This study used both laboratory and field evaluations to evaluate the aquatic effects of this industrial wastewater effluent from a Terra Nitrogen that contains varying levels of ammonia. Standard acute (48-h) laboratory tests with the fathead minnow (*Pimephales promelas*), were undertaken to determine if constituents in the effluent enhanced or decreased the toxicity of ammonia as compared to bioassays of ammonia in laboratory water alone. Laboratory tests also sought to determine how pH influenced the potential for effluent ammonia loading based on toxicity to the fish. Acute tests with fatheads are a common WET requirement for discharge permits in Oklahoma. For the field component, the objective was to determine the potential effects of the wastewater discharge on a riverine receiving system and determine if the results of the laboratory bioassays were consistent with the condition of the aquatic communities in the vicinity of the effluent outfall. The field assessment included surveys of the macroinvertebrate and fish communities in the vicinity of the outfall in addition to in-situ growth and condition studies with the zebra mussel, *Dreissena polymorpha*, which was a component of the macroinvertebrate community in the effluent receiving system.

RESEARCH QUESTIONS

1. How do elevated levels of pH and ammonia in the effluent affect acute toxicity and how do these compare to the same treatments of pH and ammonia in reconstituted very hard laboratory water?

2. Are laboratory toxicity tests with the effluent providing an accurate representation of potential effects in the field in the vicinity of the effluent outfall when evaluating resident species, which include macroinvertebrates, fish and zebra mussels?

METHODOLOGY

Effluent sample collection

Effluent samples were collected directly from the effluent discharge pipe and the cooling towers of a nitrogen manufacturing plant prior to each test. Each sample was collected in acid-washed 4L polypropylene containers and transported back to the Ecotoxicology and Water Quality Research Laboratory at Oklahoma State University in coolers on ice. The samples were maintained at 4 °C until use in bioassays which usually occurred within 24 h of collection.

Laboratory bioassays

Acute laboratory toxicity tests followed methods outlined in USEPA (2002) using the fathead minnow (*Pimephales promelas*), and were conducted under Oklahoma State University Animal Care and Use Protocol AS50110. Bioassays were performed with unadjusted whole effluent, whole effluent that had the pH manipulated to either 8.5 or 9.0, and effluent that had ammonia added to bring the initial total ammonia nitrogen concentration to 10, 20 or 30 mg N/L with pH adjusted as indicated above. Table 2 outlines the diluents used in each series of bioassays with the corresponding pH and ammonia treatments. Effluent pH was adjusted by adding 1N NaOH, drop wise until the desired pH was reached, while ammonia was adjusted by adding ammonium chloride (NH_4Cl) to reach the target concentration. The amount of ammonium chloride added was

calculated by taking the difference between the target concentration of ammonia and that measured in the unadjusted effluent and dividing this by 0.34 to account for the fractional composition of ammonium in ammonium chloride. To initiate a bioassay, the 100% effluent samples (both unadjusted and adjusted) were serially diluted (75%, 56%, 42%, 32%) with very hard (154-280 mg/L as $CaCO_3$) reconstituted laboratory water (USEPA, 2002). This water hardness was selected because it matched that of the receiving system the nitrogen manufacturing plant discharges into. Lower dilutions of effluent were used if mortality of the fish was greater than 50% at the lowest effluent dilution (32%) of the initial bioassay.

An additional series of bioassays was also conducted using total ammonia nitrogen solutions at concentrations of 10, 20 and 30 mg N/L as “100% effluent”. These solutions were prepared by adding ammonium chloride to very hard reconstituted laboratory water. Serial dilution with very hard reconstituted laboratory water was then undertaken to prepare the actual treatment levels as described for the whole effluent. Reconstituted laboratory water was also used as the control treatment in all tests.

All exposures were conducted in covered 250-mL glass bowls (to reduce pH fluctuations), containing 200 mL of test solution, 10 fathead minnows per bowl, and two replicate bowls per test concentration. Test chambers were inspected every 6 h to determine number of live and dead fish with dead fish identified by discoloration and lack of response to gentle prodding. Test solutions were renewed every 24 h by replacing 80% of the water volume with freshly prepared effluent or ammonia solutions. Test temperature was maintained in a temperature controlled room at 25 °C +/- 1 °C with a 16/8 h light/dark cycle.

Laboratory water chemistry

Temperature, dissolved oxygen (DO), pH, total ammonia, conductivity, alkalinity, and hardness were measured in each test solution at the start of each bioassay and at the beginning and end of each solution renewal cycle. Mortality and pH were measured every six hours throughout tests. Ammonia was measured using an Accument® AR25 Ammonia Meter (Fisher Scientific, New Jersey, USA), with unionized ammonia concentrations estimated from the measured total values based on temperature and pH (Thurston et al., 1979). Dissolved oxygen was measured using a YSI® model 550A Dissolved Oxygen meter (YSI Incorporated, Ohio, USA) and pH was measured using a Accument® portable AP62 pH/mV meter (Fisher Scientific, Pittsburg, Pennsylvania). Conductivity was measured with a Hach® conductivity/TDS meter (Hach, Loveland, Colorado) and alkalinity and hardness were measured by titration (APHA 1998). Prior to use, all water quality meters were calibrated according to the manufacturer instructions.

Field study site

The study site was located on the Verdigris River in Rogers County, OK, 1.1 km upriver from the entrance to the Port of Catoosa. All sampling was conducted along an approximately 500 m reach of river that included the discharge zone of the effluent from the plant. The river width in this area is approximately 69 m wide and is dominated by muddy and rocky substrate. To facilitate sampling, 15 stations were established on either side of the river above, within, and downstream of the effluent discharge (Figure 1, Table 1).

Field water chemistry parameters

Water chemistry parameters measured at the field sites included temperature, pH, conductivity, dissolved oxygen (DO), ammonia, alkalinity and hardness. Temperature, DO, pH, and conductivity were determined with a Quanta® Hydrolab multimeter (Hydrolab, Austin, Texas, USA). Water samples for the other parameters were collected at the water surface and the bottom of the river. Bottom samples were taken with a Van Dorn sampler. These samples were placed on ice and transported to Oklahoma State University where they were held at 4° C until the analyses were performed (within 24 h). Analyses for ammonia, alkalinity and hardness followed procedures described for the laboratory bioassays. Air temperatures and wind speeds were also taken at site 1 with a pocket thermo wind meter (Kestrel® 2000 (Kestrel, Santa Cruz, California, USA)).

Macroinvertebrate collection

Invertebrates were collected using Hester-Dendy samplers (Ohio EPA, 1989) from eleven locations (1, 3, 4, 7, 9, 10, 12, 13, and 15 (refer to Figure 1 and Table 1)). To deploy, four samplers were connected to concrete blocks via a steel cable attached to rebar that was pounded into the ground on the bank. At station 7 (outflow) 3 blocks were placed, one upriver (~ 4.6 m) just out of the influence of the effluent, one directly in the effluent, and one directly down (~ 4.6 m) from the effluent. In 2005, invertebrates were sampled three times (June, July and August) and in 2006 two times (July and August). All samplers remained in the system for six-weeks. Samplers were retrieved by cutting them from the concrete blocks and placing them in individual plastic containers which

were then dosed with 70% ethanol and brought back to the laboratory for sorting and identification. For sorting, the Hester-Dendy samplers were placed on a 500 µm mesh sieve, and then disassembled and washed with dechlorinated water while being scraped clean with a dissecting probe and a soft bristle brush. The contents were then rinsed with 70% ethanol and placed onto petri plates for picking and identification. All picking and identification was performed using an Olympus SZX12 (Olympus America Inc., Center Valley, Pennsylvania. USA) dissecting scope between 7 and 90x magnification. All macroinvertebrates were identified to genus except for chironomidae, which were identified to family. Identification was accomplished using a macroinvertebrate key by Merritt and Cummins (1996).

Fish collection

Fish collections were undertaken with a boat-mounted electroshocker (Ohio EPA, 1989). Electroshocking was performed along a reach of river bank that extended 25 m up and down each side of stations 1, 3, 7, 9, 13 and 15. In 2005, sampling was done in October, while in 2006 it was done in June and October. Stunned fish were removed from the water with a dip net and placed in plastic buckets filled with river water prior to identification. The time of shocking (seconds) was recorded for each collecting event. Fish were identified to species (Miller and Robinson, 2004) on site and then released. Fish which could not be identified on site were placed in four liter containers containing 10% buffered-formalin solution and taken back to the laboratory for identification using Miller and Robinson (2004).

Zebra mussel collection

Zebra mussels were known to occur in this section of the Verdigris River since at least 2003 as a result of their presence in Oologah Lake (Rogers County, OK), an impoundment located upstream from the study site. Growth studies with the mussels were performed for six week periods during 2005, 2006 and 2007. In order to avoid any confounding effects of acclimation to the effluent, zebra mussels used in the 2006 study were collected from Oologah Lake. At the time of collection, water temperature range at Oologah Lake was 20-22 °C, and at the time of deployment in the Verdigris River, the water temperature was 22.4-24 °C. The mussels were collected by gently scraping them from the solid surfaces to which they were attached with a metal paint scraper. Mussels were then placed in coolers containing moist paper towels and transported back to the laboratory where they were carefully separated using a scalpel, and measured along their longest axis to the nearest 0.01 mm with digital calipers. Individuals were then placed into growth chambers that consisted of polyethylene tackle boxes (10 x 20 cm) which had internal compartments to accommodate individual fishing lures, one mussel per compartment. The tackle boxes had solid plastic hinged lids on top and bottom and these lids were modified by cutting out most of the plastic panel and replacing it with rigid plastic mesh (2 x 2 mm grids) to allow water exchange when the chambers were deployed in the river. The growth chambers containing the zebra mussels were placed in aerated 38 L tanks containing dechlorinated municipal water before being moved to the Verdigris River to initiate the growth study (within 48 h). During this time, mussels were held at 22 °C and were inspected for attachment to the walls of the growth chambers. Those that did not attach were removed and replaced with ones that attached. For

deployment, two replicate growth chambers were attached to concrete blocks (2005 and 2006 sites 1,3,4,7,9,10,12,13,15; 2007 sites 1,7,10,13) utilized for the Hester-Dendy samplers. After six weeks, the growth chambers were collected, transported back to the laboratory in a cooler containing moist paper towels, and surviving mussels were removed by cutting the byssal threads with a scalpel and measured for growth.

For the zebra mussel growth study in 2007, mussels were collected from Sooner Lake in Pawnee County, OK, because availability of healthy mussels from Oologah Lake was limited. Collection, transport and handling of mussels followed that described above. Growth chambers were placed on the effluent side of the river (see Figure 1) at one site upstream (site 1), one at the outfall (site 7) and two downstream sites (site 10 and 13). After six weeks, the chambers were again collected and transported back to the laboratory where the surviving mussels were removed. In this study, the wet and dry mass of the mussel soft tissue was determined in addition to growth. Once length measurements were made, the byssal thread was cut off at the shell and the soft tissue was removed and weighed to the nearest 0.01 g, dried for 48 h at 60 °C and reweighed. The wet:dry weight ratio and the wet weight and dry weight change were then calculated (Smolders et al., 2004) to compare source populations to river-deployed mussels and also to compare groups of mussels between the different sites on the river.

Statistical analysis

Median lethal concentrations (48-h LC50 values) and associated 95% confidence intervals for the fathead minnow bioassays were generated with the trimmed Spearman-Karber method using the Comprehensive Environmental Toxicity Information System

software (CETIS, Tidepool Scientific Software. Mckinleyville, California. USA). Differences between LC50 values were determined based on overlap of 95% confidence intervals.

For the macroinvertebrate data, total taxa, % Ephemeroptera, Plecoptera and Trichoptera (% EPT,) abundance and diversity (Shannon-Weiner Diversity Index) were generated using Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA). Shannon-Weiner Diversity (*H)* was calculated as described by Stephenson and Mackie (1986). The macroinvertebrate and zebra mussel growth and condition data were tested for normality and homogeneity of variance using Sigma Stat (Systat Software, Inc., San Jose, California, USA). All percentage data were transformed (arcsine square-root) prior to testing for normality. For all tests, analysis of variance (ANOVA) with Holm-Sidak (normal data) or Kruskal-Wallis followed by Dunn's method (non-normal, ranked data) was used to compare results between the field sites. Statistical significance was determined at α=0.05.

Jaccard's similarity index (Ivchenko and Honov, 1998) was also calculated to compare the macroinvertebrate and fish communities between the combined upriver, effluent and downriver sites. Index values closer to one indicate greater similarity between locations while values closer to zero indicate less similarity (Real and Vargas, 1996).

RESULTS

Laboratory toxicity tests

Water chemistry

Water quality parameters for the 100% effluent, effluent with ammonia added, ammonia solution and cooling tower blowdown are summarized in Tables 3 and 4. Total ammonia nitrogen in the base effluent averaged 7.3 mg N/L and ranged from 6.3 to 9.4 mg N/L. Total ammonia nitrogen concentrations for the spiked effluent and ammonia solutions were within 1% of target levels (10, 20, 30 mg N/L), and the manipulated pH values (8.5 and 9.0) were within 0.1 unit of target values. The average total ammonia nitrogen concentration in the cooling tower blowdown samples was 24 mg N/L and ranged between 13.6-58.1 mg N/L. The pH range for the unadjusted base effluent was 7.3-7.8 with an average of 7.6. The average pH for the effluent with ammonia added was 7.7 with a range of 7.5-7.8. The average pH of the unadjusted cooling tower blowdown was 7.2 with a range of 6.7-8.1. The adjusted pH values for the cooling tower blowdown samples were within 0.1 of the target values. Temperature and dissolved oxygen ranges across all toxicity tests were 18.5-24.1 °C and 5.2-15.0 mg/L, respectively. Conductivity ranges for the base effluent, effluent with ammonia added, ammonia solution and cooling tower blowdown were 755-2192, 845-3417, 625-1207 and 840-2210 µS/cm, respectively. Alkalinity (mg/L as $CaCO_3$) ranges for the toxicity tests in the same order were 68-130,

80-140, 126-230 and 6-30, respectively, while the hardness (mg/L as $CaCO_3$) ranges were 230-430, 350-548, 154-192 and 522-2160, respectively.

Bioassays

A total of 43 acute toxicity tests with the fathead minnow were performed with unmanipulated and manipulated effluent and the ammonia solutions. Due to the lack of effects in some of the bioassays, LC50 values could not be generated for all tests. A summary of the number of LC50s generated out of the total number of tests conducted is presented in Table 5.

The interactive effects of ammonia concentration and test solution pH were clearly apparent in the results from the laboratory bioassays. In tests with effluent that had no pH adjustment, mortality of the fish was insufficient to generate an LC50 value with base effluent (no ammonia added) or with samples that had total ammonia nitrogen levels increased to 10 and 20 mg N/L (Figure 2a). An average 48-h LC50 of 65.2% effluent was obtained from bioassays on effluent that had total ammonia nitrogen levels increased to 30 mg N/L without any pH changes. At pH 8.5, insufficient mortality of the fish also resulted in no LC50 value being generated for effluent with no ammonia added and for effluent with total ammonia nitrogen concentrations raised to 10 mg N/L. However, the pH 8.5 effluent samples with total ammonia nitrogen levels of 20 and 30 mg N/L were acutely toxic to the fish and, based on comparison of the 95% confidence intervals for the median lethal concentrations, a significant dose-dependant increase in toxicity was apparent. The 48-h LC50 for the pH 8.5- 20 mg N/L effluent was 58.5% and that for the pH 8.5-30 mg N/L sample was 28.0%. When the effluent pH was increased to

9.0, the base effluent and all effluent samples with ammonia added were acutely toxic to fathead minnows. A clear concentration-dependant increase in toxicity was apparent here as well, with 48-h LC50 values ranging from 81.2% for the base effluent to 16.2% for the effluent with ammonia concentrations increased to 30 mg/L.

The effect of pH on ammonia toxicity is further illustrated by comparing toxicity test results from effluent samples with the same ammonia levels but with different pH. For example, as previously stated, due to insufficient mortality, no LC50 values were generated from bioassays with base effluent at normal effluent pH (7.5-8.3) or pH 8.5, but at pH 9.0 an average LC50 of 81% effluent was obtained (Figure 2a). This same pattern held for the effluent that had total ammonia nitrogen levels increased to 10 mg N/L, with no LC50 at unadjusted pH or pH 8.5, but an LC50 of 66.4% at pH 9.0. Effluent with total ammonia nitrogen levels increased to 20 mg N/L was acutely toxic to the fish at both pH 8.5 and 9.0, but not at the unadjusted pH. In these tests, the effluent at pH 9.0 was significantly more toxic (48-h LC50 = 23.1%) than the samples at pH 8.5 (48-h LC50 = 58.5%). At 30 mg N/L, the effluent was acutely toxic at all pH values tested and toxicity was progressively greater as pH increased.

There was sufficient mortality to generate 48-hr LC50 values at all pH and ammonia levels in the acute tests with the ammonia solutions (Figure 2b). As for the effluent tests, there was a concentration-dependent increase in toxicity with increases in ammonia levels in the ammonia solutions. The unadjusted 10 (48-h LC50 = 93.0%) and 20 (48-h LC50 = 82.0%) mg total ammonia nitrogen/L were the only ammonia solutions that did not exhibit a significant difference in toxicity with increasing ammonia levels based on 95% confidence intervals. A significant increase in toxicity was observed in the

unadjusted pH tests as the total ammonia concentration increased from 20 to 30 mg N/L. The 48-hr LC50 values from the tests with these solutions averaged 82.0% and 57.3%, respectively. The increase in toxicity due to ammonia concentration was also observed at pH 8.5 with 10 (48-h LC50 = 82.0%), 20 (48-h LC50 = 50.0%) and 30 (48-h LC50 = 28.0%) mg N/L solutions all having significantly different 48-h LC50 values. The same pattern occurred at pH 9.0 and the average 48-hr LC50 values from the tests with these solutions were 49.4%, 25.0% and 13.0%, for the 10, 20 and 30 mg N/L solutions, respectively.

There were also obvious pH effects on toxicity of the ammonia solutions to fathead minnows. At l0 mg total ammonia nitrogen/L there was not a significant difference between the median lethal concentrations in tests with unadjusted pH (48-h LC50 = 93.0%) and pH 8.5 (48-h LC50 = 82.0%, Figure 2b) based on comparison of 95% confidence intervals. A significant difference was observed between the average 48-hr LC50 values at 10 mg total ammonia nitrogen/L for pH 8.5 (48-h LC50 = 82.0%) and pH 9.0 (48-h LC50 =49.4%). At 20 mg total ammonia nitrogen/L, toxicity of the ammonia solution significantly increased across all of the pH ranges tested. The average 48-hr LC50 value was 82.0% for unadjusted pH, 50.0% for pH 8.5, and 25.0% for pH 9.0. The same situation was observed for the ammonia solution with 30 mg total ammonia nitrogen/L, with an average 48-h LC50 value of 57.3% for samples with no pH adjustment, 28.0% for pH 8.5 and 13.0% for and pH 9.0.

The ammonia solutions were generally more toxic to the fathead minnows than the effluent samples with similar ammonia concentrations, indicating the effluent matrix ameliorated ammonia toxicity to some degree. For example, at unadjusted pH, LC50

values were generated for the ammonia solution at all ammonia concentrations, while acute toxicity in the effluent was only observed in samples spiked with total ammonia nitrogen of 30 mg N/L. In most cases, when LC50 values could be generated from tests with the spiked effluent samples they were significantly greater (lower toxicity) than values generated for the ammonia solution at comparable pH and ammonia concentration. The exceptions to this were the pH 9.0 samples with 20 mg N/L. In this case, the differences in average 48-h LC50 values for the effluent with ammonia added and ammonia solution were not significant.

The average total ammonia nitrogen levels at the LC50 were usually higher in the effluent with ammonia added as compared to the comparable ammonia solutions (Table 6). For example, the effluent with ammonia added at 10 mg N/L was not acutely toxic until a pH of 9.0. Total ammonia nitrogen at the LC50 generated at this pH was 6.6 mg N/L. The comparable ammonia solution was acutely toxic to the fish at all pH levels. Total ammonia nitrogen levels at these LC50 values were 9.3 mg N/L at unadjusted pH, 8.2 mg N/L at pH 8.5, and 4.9 mg N/L at pH 9.0. Similarly, no LC50 was generated for the effluent with ammonia added at 20 mg N/L unadjusted pH, while the comparable ammonia solution was acutely toxic with a total ammonia nitrogen level at the LC50 of 16.4 mg N/L.

The average 48-h LC50 values generated for the unadjusted pH and pH 9.0 cooling tower effluent samples (n=6) were 86.0% and 55.0%, respectively. There was insufficient mortality to calculate LC50 values for the cooling tower water at pH 8.5 (Figure 3, Table 7). Chlorine levels in the cooling tower water ranged from 0.1 to 1.1 mg/L with an average of 0.7 across all tests (Table 8).

Field study

Water quality

Water quality parameters were monitored at field sites along the Verdigris River from November 2004 to October 2007. Ranges of values for these parameters are presented in Table 9, while data for sites 4, 7 and 10 (those directly associated with the outfall) are graphed in Figures 4-7. Individual data points for each date are included in Appendix 1a-g.

Temperature at sites 4, 7 and 10, ranged from a low of 4.0 °C in January 2005 to a high of 34.8 °C in July 2006 (Figure 4a). Dissolved oxygen for these sites ranged from 4.0 mg/L in April 2006 to 14.4 mg/L in February 2005 (Figure 4b), the range for alkalinity (mg/L as $CaCO_3$) was from 64 in April 2006 to 126 in May 2005 (Figure 5a), while hardness (mg/L as CaCO3) ranged from 118 in May 2006 to 418 in April 2006 (Figure 5b). The conductivity range for sites 4, 7 and 10 was from 0.1µS/cm in October 2006 to1.7 µS/cm in October 2006 (Figure 6). pH at site 4 ranged from 6.4 in January 2005 to 8.3 in July 2006, while site 7 was 6.4 in January 2005 to 8.1 in July 2006, followed by site 10 with a pH ranged from 6.4 in January and February 2005 to 8.6 in July 2006 (Figure 7a). Total ammonia nitrogen (mg N/L) at site 4 ranged from 0.0006 in June 2005 to 4.5 in August 2006, site 7 0.05 in May 2006 to 11.2 in September 2007, and for site 10 0.003 in June 2005 to 5.1 in September 2007 (Figure 7b). Ammonia, conductivity and hardness were consistently higher at site 7 (discharge site) compared with the other locations; however by site 10, concentrations returned to levels similar to those at the upriver site for each of the three parameters.

Macroinvertebrates

I found a total of 36 different macroinvertebrate taxa belonging to 11 orders. Taxa richness ranged from 0 at site 7d in June 2005 to a high of 15 at site 13 in August 2006 (Appendix 2a-e). There was no significant difference in taxa richness between sites in either 2005 ($p=0.81$) or 2006 ($p=0.57$) (Figure 8), although in both years, richness right at the effluent outfall (7 in) was lower than at other sites. In 2006, richness was also lower at most of the upstream sites as compared to downstream stations.

The average Shannon-Weiner diversity index ranged from a low of 0 at site 7d in June 2005 to a high of 1.46 at site 1 in June 2005 (Figures 9-10). There was no significant difference in diversity between sites during any of the sampling periods, although as for taxa richness, diversity was sometimes reduced right in the vicinity of the outfall.

The percent abundance of the four most common macroinvertebrate taxa for each sampling period in 2005 and 2006 are presented in Figures 9 and 10. Chironomid midges were the most common macroinvertebrates at the effluent sites (7u, 7i, 7d) in the sampling periods that ended in June and August of 2005 (Figure 11). In the November 2005 samples, chironomids were the dominant taxon at all sites. Caddisflies from the genus *Hydropsyche* were the most abundant macroinvertebrates in the upriver sites (1, 3, 4) in June 2005 and mayflies from the genus *Caenis* were most common downriver (9, 10, 12, 13, 15). In August 2005, Caenid mayflies were the most abundant taxon up and downriver. Chironomid midges were the most abundant taxa upriver, at the effluent, and downriver for both collections in 2006 (Figure 12).

The percent Ephemeroptera, Plecoptera and Trichoptera (EPT) was significantly lower in macroinvertebrate samples from the effluent zone as compared to both upriver and downriver sites in June ($p<0.001$), August 2005 ($p=0.013$) and July 2006 ($p=0.046$) (Figure13). The percent EPT from the effluent outfall samples were also significantly lower than that from the upriver sites in August 2006 ($p=0.009$). There were no significant differences in % EPT between sites in November 2005. There was a reduction in the % EPT across all sites starting with the November 2005 collection and continuing through the 2006 samples.

Jaccard's similarity index was calculated to compare the macroinvertebrate community between the upriver, effluent and downriver sites. The lowest index value of 0.31, indicating lower similarity in community composition between locations, was calculated from the effluent and downriver sites from the July 2006 samples (Figure 14). The highest index value of 0.71 was obtained for the upriver and down river stations in the June 2005 samples. The average Jaccard values (across all sampling dates) indicated the effluent/upriver stations were the most similar with an index score of 0.60, followed by effluent/downriver with 0.51, and finally upriver/downriver at 0.53 (Table 10). The overall similarity across all sites decreased from 0.68 for the July and August 2005 samples to 0.46 for the November 2005, July 2006 and August 2006 samples.

Fish assemblage

A total of seventeen fish species were collected during electroshocking on the Verdigris River, with the greatest number of species collected in October of 2005 (14 species). Species richness near the effluent outflow (left bank) was highest on each

collection date when compared to all other locations (Figure 15). There was also a trend toward higher species richness on the effluent side of the river with the exception of the upriver sites in June 2006 and downriver sites in November 2006 with the same number of species collected between sites both collection dates (Figure 15).

Total abundance of fish across all sites was lower in June 2006 (18) compared with both October and November sampling dates (2005 – 306, 2006 – 1053). With respect to site comparisons, total abundance was higher around the effluent in October 2005 and June 2006 (Table 11a and b). As indicated above, fish abundance was low at all sites in June 2006. The highest abundance at any site occurred at the upper left bank in November 2006 (Table 11c). Gizzard shad (*Dorosoma cepedianum)* accounted for the majority of fish collected during each sampling event.

Jaccard's similarity index was calculated to compare the fish community between upriver, effluent and downriver sites. The highest index value of 0.44, indicating higher similarity in community composition between locations, was calculated between upriver and downriver sites in October 2005 followed by the lowest of 0.17 between effluent and upriver in November 2006 (Table 12). Jaccard's values (across all sampling dates) indicate effluent/downriver sites were the most similar with an average index value of 0.33, followed by the upriver/downriver with 0.31. The least similar were the effluent/upriver sites with a similarity index value of 0.23 (Table 11).

In-situ zebra mussel study

There was a significant difference in zebra mussel growth between sites in 2005 ($p<0.001$), 2006 ($p=0.004$) and 2007 ($p=0.022$, Figure 16), although no consistent differences between the effluent and other sites. In 2005, zebra mussels held at site 4

grew significantly less than those held at sites 7u, 9, 10 and 12. In 2006, mussels held at site 7d had significantly higher growth than mussels at site 15. In 2007, site 1 mussels grew significantly more than those held at site 7. Mussels across all sites grew an average of 0.07mm/day in 2005, 0.06mm/day in 2006, and 0.11mm/day in 2007.

In 2007, the ratio of mussel soft tissue wet weight and dry weight was determined as an additional measure of condition. Wet:dry ratios were lower in source populations with a range from 2.1 to 7.2 (Figure 17). The range of wet:dry weight ratios of mussels placed in the Verdigris River was 4.4 to 12.8. There were no significant differences in this ratio between groups of mussels placed in the Verdigris River, however all river-deployed mussels had wet:dry ratios that were significantly higher ($p<0.009$) than mussels from the source population. The change in the wet and dry weights of the zebra mussels over the course of the 2007 growth study are presented in Figure 18 a and b. There was a significantly greater increase in the wet weight of mussels deployed at site 13 compared to site 7i ($P=0.009$). The dry weight increase of mussels deployed at site 13 was significantly greater than that at all other sites ($P<0.001$).

DISCUSSION

Laboratory toxicity tests

Based on ammonia concentrations, the range of 48-h LC50 values for fathead minnows across all test conditions (effluent, effluent with ammonia added, ammonia solutions and pH ranges- 7.6-9.0) was 0.3-1.7 mg NH_3/L for un-ionized ammonia and 3.9-19.6 mg N/L for total ammonia. Comparison of effects levels between studies can be difficult due to differences in test conditions, although this range of LC50 values is similar to those generated under comparable test conditions in previous studies. Thurston et al. (1983) assessed the acute effects of ammonia in a series of 96-h flow-through tests with fathead minnows over a range of test temperatures and fish sizes. pH values for these tests ranged from 7.6 to 8.2. They reported an LC50 range of 0.8 to 3.4 mg NH_3-N/L un-ionized ammonia and 34 to 108 mg N/L total ammonia. While size or source of the fish did not influence toxicity, the effect of ammonia decreased as temperature increased from 12 to 22 °C. Arthur et al. (1987) also conducted acute toxicity tests with fathead minnows exposed to ammonia at different temperatures due to season (3.4-26.1 °C) and a pH range of 7.9-8.1. Their reported range of 96-h LC50 values was 1.8-2.6 mg NH_3-N/L as un-ionized ammonia with no significant effect of temperature on toxicity. Finally, Mayes et al. (1986) report a 96-h LC50 of 1.5 mg NH_3-N/L for fathead minnows exposed to unionized ammonia at a pH of around 8 (7.89-8.39).

Two key differences between the previous studies reviewed above and the bioassays with fatheads from this study are that a number of the previous studies were conducted using flow-through systems while our studies were conducted using a static-renewal exposure. The studies cited were also mostly 96-h tests while those here were 48-h bioassays. Flow-through exposures may lead to lower LC50 values because toxicant levels in the water would be maintained more consistently. The exposure time used here was consistent with Oklahoma Department of Environmental Quality effluent permit testing requirements which specify a 48-h exposure for acute tests and 7-d exposure for chronic tests (OKDEQ 2008). Hasan and Macintosh (1986) exposed common carp (*Cyprinus carpio*) fry to ammonia and found no significant difference between the 48-h and 96-h LC50 values (1.76 vs 1.74 mg NH_3-N/L, respectively). Similarly, Soderberg and Meade (1992) found no difference between 48 and 96-h LC50 values for unionized ammonia in bioassays with both Atlantic salmon (*Salmo salar*) and lake trout (*Salvelinus namaycush*).

pH effects

In the present study, toxicity of the test solutions was evaluated by comparing median lethal effects concentrations based on percent effluent/solution, total ammonia, and unionized ammonia levels. When assessing toxicity based on percent effluent/solution, increased toxicity was observed with increasing pH throughout all tests. For example, at 30 mg N/L, the 48-h LC50 values for the effluent spiked with ammonia were 65.2% for the unadjusted pH (8.0), 38.1% for pH 8.5 and 16.2% for pH 9.0. The total ammonia nitrogen levels at the LC50 followed the same trend as the percent effluent

at 30 mg N/L with values of 19.6 mg N/L for the unadjusted pH, 11.4 mg N/L for pH 8.5 and 4.9 mg N/L for pH 9.0. In contrast, the 48-h LC50 values based on the un-ionized ammonia concentration increased with increasing pH. For instance, the LC50 values for the 30 mg N/L ammonia-spiked effluent based on un-ionized ammonia were 0.3 mg NH_3-N/L at the unadjusted pH, 1.1 NH_3-N/L at pH 8.5, and 1.3 mg NH_3-N/L at pH 9.0. This same general pattern regarding pH effects on the LC50 values held for the tests with the ammonia solution as well.

It is well established that pH influences the speciation of ammonia, with increasing pH leading to increased levels of the un-ionized (NH_3) form that is more toxic to aquatic organisms. In 96-h acute bioassays with the fresh water mussel (*Lampsilis siliquoidea*), Wang et al. (2008) reported EC50 values for survival in exposure to total ammonia of 88 mg N/L at pH 6.6 and 1.0 mg N/L at pH 9.0. In bioassays with fathead minnows, Thurston et al. (1981) reported a 96-h LC50 for fathead minnows with total ammonia nitrogen of 254 mg N/L at pH 7.0 and 18.4 mg N/L at pH 8.5. They also observed an increase in the LC50 value based on unionized ammonia as pH increased. At pH 7.0, the LC50 reported for unionized ammonia was 0.4 mg NH_3-N/L, while at pH 8.5 it was 1.4 mg NH_3-N/L. Similarly, Fairchild et al. (2000) evaluated the effect of pH on ammonia toxicity to the Colorado pikeminnow (*Ptychocheilus lucius*) in a series of 48-h toxicity tests. They report a 48-h LC50 value for total ammonia nitrogen of 11.0 mg N/L at pH 8.5 and 6.3 mg N/L at pH 9.0. The LC50 values based on un-ionized ammonia concentrations at these two pH values were 0.9 and 1.6 mg NH_3-N/L, respectively. Possible explanations for the slightly lower LC50 values based on unionized ammonia

with lower pH include enhancement of NH_3 toxicity by hydrogen ions at lower pH and/or that the ammonium ion is exerting a toxic effect (Thurston et al., 1981, USEPA, 1985).

The average pH values measured in the base effluent (with no pH or ammonia manipulations only) was 7.7 (pH range of 7.4 to 7.9) and the average total ammonia nitrogen level was 7.2 mg N/L. I was unable to generate a 48-h LC50 effluent until pH was manipulated to 9.0 or the total ammonia levels were increased 30 mg N/L in the effluent with no pH adjustment. Over the course of this study, pH values in river water ranged between 6.0-8.6 and total ammonia nitrogen levels in the effluent mixing zone ranged from 0.5-11.2 mg N/L. It is therefore unlikely that any acute toxic effects of effluent ammonia are being realized in the receiving system.

Diluent effects

In this study, effluent with ammonia added was compared to laboratory water spiked with ammonia. In most cases, ammonia in the effluent was either less toxic than that in laboratory water or not significantly different in toxicity. For example, no LC50 values were generated for the effluent with total ammonia at 10 mg N/L for the unadjusted pH (7.7) and pH 8.5, while the ammonia solution at this same pH and ammonia concentration was acutely toxic with LC50 values of 93.0% solution for the unadjusted pH sample and 82.0% solution at pH 8.5. When the effluent was made acutely toxic by either adding ammonia and/or adjusting pH, higher LC50 values were obtained from bioassays with effluent as the diluent in all tests except pH 9.0 at 20 mg total ammonia nitrogen/L and unadjusted pH at 30 mg total ammonia nitrogen/L (no significant difference).

A number of studies have investigated how other water quality parameters other than pH can influence ammonia toxicity. Soderberg and Meade (1992) found decreased toxicity of ammonia to lake trout (*Oncorhynchus mykiss*) with increased ionic strength of the diluent, and Wicks et al. (2002) found increased calcium levels reduced ammonia toxicity to rainbow trout (*Oncorhynchus mykiss*). Ankley et al. (1995) report that ammonia toxicity to the freshwater amphipod, *Hyalella azteca*, decreased with increasing water hardness. In this study, the formulated laboratory water used to prepare the ammonia solutions was in the "very hard" range (154-192 mg/L as $CaCO_3$, USEPA, 2002), while the effluent hardness ranged between 350-548 mg/L as $CaCO_3$. It is therefore possible that water hardness was an important ameliorating factor for ammonia toxicity in the effluent. Interestingly, the ameliorative effects of whatever factor was reducing ammonia toxicity in the effluent appears to have been surpassed at pH 9.0 with total ammonia levels of 20 mg N/L and all three pH levels at 30 mg N/L, since in these treatments, the acute toxicity of the spiked effluent and ammonia solutions was comparable. In a review of the influence of water hardness on ammonia toxicity, Parametrix and Chadwick Ecological Consultants (2006) state that while changes in the ion composition of water does decrease ammonia toxicity for some species, this effect may not be directly related to hardness alone but rather to other ions (e.g. sodium) and their dynamics at the gill surface.

The potential for receiving system water to reduce the toxicity of chemicals has implications for the derivation of site-specific water quality criteria. The U.S Environmental Protection Agency (USEPA, 1999) outlines an approach to develop site specific criteria for ammonia in the form of Water-Effects Ratios (WERs). WERs are

determined by calculating the ratio of the toxicity of ammonia in the site water to the toxicity of ammonia in laboratory water. If the differences are small, the WERs for ammonia are expected to be close to 1 (USEPA, 1999). The actual process of calculating WERs for use in deriving site-specific criteria requires seasonal toxicity data to account for receiving system variation during high and low water flows. The national ambient water quality criterion can then be multiplied by this final WER to develop a site specific water quality criterion (Welsh et al., 2000).

The receiving system for the effluent investigated in the present study is the Verdigris River. River water was not used in any of the bioassays conducted, but rather the whole effluent was used as the diluent for ammonia. The water that makes up the bulk of the effluent matrix is actually derived from Spavinaw Lake, OK via a water line that supplies the City of Tulsa, OK. As such, the calculation of a WER for the receiving system is not possible, although expressing ammonia toxicity in the effluent versus laboratory water as a ratio facilitates comparison with WERs generated for ammonia in other studies.

Based on tests in which the effluent was acutely toxic, WERs could be calculated for the pH 9.0 10 mg N/L solutions (WER= 1.4) and the 20 mg N/L for pH 8.5 (WER =1.1) and pH 9.0 (WER=0.9). Additionally, WERs were calculated at 30 mg N/L for the unadjusted pH (0.5), pH 8.5 (1.1) and pH 9.0 (1.25). The range for the six WERs in this study was thus 0.5 to 1.4 which was similar to values of 0.8 to 1.3 found by Nimmo et al. (1989) when calculating WERs for fathead minnows and johnny darters (*Etheostoma nigrum*) exposed to ammonia in solutions of river water and well water. In the same study, Nimmo et al. (1989) calculated four WERs with a range of 0.5 to 1.5 when they

compared wastewater to the well water (pH 7.8-8.2). Diamond et al. (1993) calculated a WER of 1.1 for fathead minnows when they compared well water to pH-adjusted laboratory water (pH 8.0), and Monda et al. (1995) obtained a WER range from bioassays with the chironomid (*Chironomus riparius*) of 0.6 and 0.8 when comparing sewage effluent (pH=7.86 to 7.94) to well water (pH=8.15 to 8.17).

Cooling tower

The cooling tower water was acutely toxic to fathead minnows at a pH of 7.2 and 8.9, but not at pH 8.4. Chlorine levels in the four samples used to conduct the bioassays with cooling tower water were 0.11, 0.29, 0.71, 0.80 mg/L, with total ammonia nitrogen levels in the range of 13.6 to 55.2 mg N/L. Chlorine and ammonia in water form chloramines and these reactions are primarily dependent on pH and the chlorine and ammonia ratio (Vikesland et al., 2001). Monochloramine is the main species present between pH 6.5-8.5 (Qiang and Adams, 2004). In the present study, no LC50 values were generated for the acute toxicity tests at pH 8.5 and this is approximately the same pH Qiang and Adams (2004) found monochloramine levels to be highest. Previous studies have demonstrated that monochloramine has less-than-additive effects compared to chlorine or ammonia alone (e.g. Cairns et al., 1990; Farrell et al., 2001). These findings support the results that cooling tower water was not acutely toxic to fathead minnows at a pH 8.4.

Macroinvertebrate community structure

Macroinvertebrates were collected on five different occasions over the course of the 2005 and 2006 field seasons. The total ammonia nitrogen levels for the effluent

outfall and stations in the vicinity (sites 4 and 10) ranged from 0.0006 to 11.2 mg N/L and from 0.0004 to 2.6 mg N/L all other stations. Effects on the macroinvertebrate community were not major, but some subtle trends were indicated. Although there were no significant differences, taxa richness was lower around the effluent for both years, and in 2006 the richness was lower at upstream sites compared to the downstream sites. Dyer et al. (2003) investigated the influence of untreated wastewater to aquatic communities at six sites on a 17.7 km reach of a river in the Philippines. Total ammonia nitrogen levels throughout the study area were 1.04 to 2.79 mg N/L. The macroinvertebrate richness was lower at sites that received inputs from storm runoff, domestic waste water and agricultural runoff than at sites in lower populated areas or that received no commercial or domestic wastes. Fries and Bowles (2002) examined macroinvertebrate community structure near the outfall of a sportfish hatchery with total ammonia nitrogen levels ranging from 0.15 to 0.29 mg N/L. There were no significant differences in richness from sites upriver, at the outfall, and downriver during the duration of the 2-year study.

Shannon-Weiner diversity in the present study was also not significantly different between sites, but was again consistently lower around the effluent. The Shannon-Weiner diversity index provides a measure of the richness and evenness of abundance of organisms and normally falls between 1.5 and 3.5 (Sterling and Wilsey, 2001). In their study of the hatchery effluent, Fries and Bowles (2002) found no significant differences in diversity between the outfall and reference sites with a Shannon-Weiner diversity range of 1.28 to 2.88. Kirkagac et al. (2004) examined macroinvertebrate communities exposed to five trout farm effluents in a Turkish brook with total ammonia nitrogen levels of ~0.6 to 1.0 mg N/L. Shannon-Weiner diversity index values in this study ranged from

0 to 1.55, with the higher value from a site upstream from the effluents. Beketov (2004) evaluated macroinvertebrate communities from 10 field sites and found that reductions in mayfly diversity corresponded to increasing levels of total ammonia. The total ammonia nitrogen levels they found were from 0.01 to 0.45 mg N/L with Shannon-Weiner diversity index values from 0 to 2.6.

The percent Ephemeroptera, Plecoptera and Trichoptera (EPT) calculated for the first two collections (June and August) in 2005 were significantly lower at the effluent compared to upriver and downriver sites, while there were no significant differences for the November 2005 collection. The percent EPT at the upriver was significant lower then downriver in July 2006, while effluent was also significantly lower than upriver sites in August 2006. Hickey et al. (1999) exposed macroinvertebrates to varying levels of total nitrogen ammonia (control=0.14, treatments=0.95, 2.32, 6.25 mg N/L) in a simulated stream. The abundance of EPT was significantly lower (59% and 60%) in the mesocosms with the highest ammonia levels when compared to the control. Henriques-de-Oliveira et al. (2007) compared the effects of a sewage effluent (total nitrogen ammonia=11.90-26.19 mg N/L) with a control (total nitrogen ammonia=0.31-2.38 mg N/L) on macroinvertebrate communities. The percent EPT was approximately 23% at the control site while no EPT taxa was found at the effluent site. Dyer et al. (2003) evaluated the influence of untreated wastewater to aquatic communities at six sites on a 17.7 km reach of a river in the Philippines, with total ammonia nitrogen levels of 1.04 to 2.79 mg N/L. EPT species were only found at sites 1 (0.03%), 4 (0.0001%) and 6 (0.003%), which did not always correspond to highest ammonia levels.

Based on Jaccard's index, the similarity in the macroinvertebrate assemblage between sites decreased between the first two collections in 2005, and the November 2005 and 2006 collections. As discussed above, Fries and Bowles (2002) evaluated macroinvertebrate community structure around the outfall of a sportfish hatchery. The Jaccard's similarity values they calculated were generally around 0.4, which they concluded showed moderate similarity between all locations.

Chironomids were consistently the most abundant macroinvertebrate group around the effluent for all sampling periods. Caddisflies were the most abundant group upriver in June 2005 and Caenid mayflies were most abundant downriver in June 2005 and also most abundant both upriver and downriver for the August 2006 sampling. Chironomid midges dominated upriver, effluent and downriver sites in November 2005 and both collections in 2006.

While these data indicate some shifts in community structure around the effluent outfall, temporal and spatial factors not related to contaminants can also have a major influence on the type of macroinvertebrates found in aquatic systems. Punti et al. (2007) found that river size, temperature, substrate and flow influenced the composition of the chironomid assemblage in streams with the flow regime having the greatest influence on their distribution. Chatzinikolaou et al. (2008) established that habitat modification decreased macroinvertebrate diversity, with other influences on community composition coming from river flow, available substrate, and water chemistry. Chatzinikolaou et al. (2008) concluded that seasonal changes and river habitats were the major factors influencing the structure of macroinvertebrate assemblages in Mediterranean rivers. In the present study, some habitat differences existed between the sampling sites. For

example, the depth on the effluent side of the river was approximately 1.5 m lower at sites around the effluent (4 and 10) than at up and down river sites (1 and 13). Furthermore, the substrate on the effluent side in the vicinity of the outfall was dominated by rocks and cobbles, while the upriver, downriver, and opposite side of the river had a muddy substrate. The effluent side of the river also had more trees, which provided more shade.

Fish assemblages

In the present study, electroshocking for fish was performed three times during 2005 and 2006. On each collection date, species richness was higher near the effluent outfall when compared to all other locations. There was also a trend toward higher species richness on the effluent side of the river except for upriver in June 2006 and November 2006 during which the same number of species were collected between sites. Fish abundance was higher around the effluent for the October 2005 and June 2006 sampling dates and the upper left bank had the highest abundance in November 2006. The majority of the fish collected for each sampling was Gizzard shad (*Dorosoma cepedianum*). Jaccard's similarity index value for all of the sampling dates combined was 0.28, which would indicate low similarity between sites.

Many studies have evaluated the effects of ammonia on warm and cold water fish, with effect levels much higher than maximum total ammonia levels observed near the effluent outfall during the present study (11.2 mg N/L). For example, Wicks et al. (2002) calculated a 96-h LC50 value of 174.0 mg N/L for resting rainbow trout (*Oncorhynchus mykiss*). Broderius et al. (1985) exposed the smallmouth bass (*Micropterus dolomieui*) to

total ammonia nitrogen in a series of 96-h toxicity tests and calculated LC50 values of 39.5 at pH 7.7 and 117.0 N/L at pH 7.2.

Other studies that have evaluated effluent effects on fish assemblages mostly report trends toward lower taxa richness and abundance around the inputs with both richness and abundance increasing as the effects of the effluents decrease (Dauba et al., 1997, Ganasan and Hughes, 1998, Northington and Hershey, 2006). The lack of any reduction in fish assemblage metrics in the present study may indicate a lack of any particularly toxic constituents in the effluent. This could be the result of treatment- the effluent passes through biological treatment ponds, two holding ponds and is mixed with unpolluted water in a holding pond before it is released into the river. In addition, the effluent does not comprise more than 4.5% of the river flow at extremely low flow conditions. In contrast, for some of the studies mentioned above, the effluent accounted for 99-100% of the river flow for a majority of the year (Dauba et al., 1997, Ganasan and Hughes, 1998). The apparent attraction of fish to the effluent plume could be regarded as a response to the effluent. Gafny et al. (2000) investigated the effects of domestic effluent in a Mediterranean stream with total effluent ammonia nitrogen levels ranging from 0.2 to 12.1 mg N/L. They found higher species richness at sites considered slightly enriched compared to unpolluted or polluted sites. Furthermore, the highest abundance was seen at a site ~17.5 km downriver from the effluent with total ammonia nitrogen levels of 11.3 mg N/L. In some cases, attraction of fish to industrial effluents with temperatures significantly different from that of the receiving system can lead to negative effects. For example, the warmer water in thermal discharges from power plants may attract fish in winter and lead to a fish kill if the plant shuts down and the temperature changes abruptly

in the mixing zone (Cooke et al., 2004). However, water temperatures of the effluent mixing zone in the present study did not significantly differ from that of the main river, so a similar threat of mortality would probably not exist for fish near the outfall in this study.

In-situ zebra mussel study

There was a significant difference in zebra mussel growth rates between sites for each of the three years the mussels were deployed, although no consistent differences between the effluent and other sites were apparent. In 2005, zebra mussels held at site 4 grew significantly less than those held at sites 7u, 9, 10 and 12. In 2006, site 15 had significantly higher growth than site 7. In 2007, mussels at site 1 grew significantly more than mussels at site 7. Mussels across all sites grew an average of 0.07 mm/day in 2005, 0.06 mm/day in 2006, and 0.11 mm/day in 2007.

In 2007, the ratio of mussel soft tissue wet weight and dry weight was determined as an additional measure of condition. Wet:dry ratios were lower in source populations (Sooner Lake, Noble County, OK) with a range from 2.1 to 7.2 compared to 4.4 to 12.8 for mussels placed in the Verdigris River. There were no significant differences in this ratio between groups of mussels placed in the Verdigris River, however all river-deployed mussels had wet:dry ratios that were significantly higher than mussels from the source population. There was a significantly greater increase in the wet weight of mussels deployed at site 13 compared to site 7i. The dry weight increase of mussels deployed at site 13 was significantly greater than that at all other sites.

Other studies have calculated zebra mussel growth rates that are similar to the values calculated in the present study. For example, growth rates in two Oklahoma lakes were 0.1 mm/day (Sooner Lake, Noble County, OK) and 0.07 mm/day (Oologah Lake, upriver from the present research site, C. Boeckman, personal communication). Allen et al. (1999) calculated zebra mussel growth rates of 0.7 mm/day in the lower Mississippi River, while Dorgelo (1993) examined growth rates of zebra mussels exposed to water from lakes with differing trophic states and found growth rates of 0.08 mm/day in eutrophic conditions with 0.05 mm/day in more oligotrophic systems.

Zebra mussel growth has also been used in previous biomonitoring studies of wastewater discharges. Smolders et al. (2002) exposed zebra mussels to an effluent-dominated stream and found decreased growth at sites directly downstream from the effluent outfall with total ammonia levels were 0.5 to 1.0 mg N/L. In another study, Spada et al. (2002) investigated the effects of an effluent discharge on zebra mussel growth rates in a lake and its outlet, with total ammonia nitrogen levels of approximately 1.5 mg N/L. The zebra mussel growth in the vicinity of the effluent discharge was 0.06 mm/day, which was significantly lower than the outlet site with growth rates of 0.31 mm/day.

The wet:dry weight ratio provides an indication of osmotic imbalance in an organism which in turn could indicate degraded physiological condition. Increasing wet weight indicates an increase in tissue water content. As such, in freshwater systems an increase in this ratio would be associated with an osmotic or ionic disturbance. Smolders et al. (2004) placed zebra mussels in the effluent stream from both a municipal and an industrial wastewater treatment plant and determined wet:dry weight ratios after 28 days

of exposure. The wet:dry weight ratios for the mussels exposed to 100% of the industrial effluent significantly increased while those exposed to the municipal effluent were not affected. Furthermore, they concluded that observed changes in concentrations of specific ions in the mussel tissue was due to the disruption of osmoregulatory homeostasis in mussels exposed to effluent waste, which caused mussels to lose physiological integrity. Studies with other invertebrates have also indicated increases in the wet:dry weight ratio that was associated with contaminant exposure (Depledge and Lundebye, 1996; Soto et al., 2000)

Overall, no clear indications of negative effects on the mussels due to exposure to the effluent in the present study were apparent and, as indicated above, the most significant differences in the parameters measured occurred between the organisms in the source population and those deployed in the Verdigris River. These observed differences could be related to factors such as water depth, water flow, water chemistry and substrate (Young et al., 1996, Hincks and Mackie, 1997, Yu and Culver, 1999, Karatayev et al., 2006).

CONCLUSION

There were two primary objectives to this study. The first was to conduct a series of laboratory bioassays with effluent samples from a nitrogen manufacturing plant to determine how the effluent matrix influenced acute ammonia toxicity and also to determine pH and ammonia levels that would lead to acute toxicity of the effluent. The second major objective evaluated the condition of the receiving system in the vicinity of the effluent outfall to determine if laboratory toxicity tests with the effluent were providing an accurate representation of potential effects in the field.

For the laboratory portion, increased toxicity was observed with increasing pH and ammonia throughout all tests and the ammonia solutions were generally more toxic to the fathead minnows than the effluent samples with similar ammonia concentrations. Water hardness is a potentially important ameliorating factor for ammonia toxicity in the effluent, however, other uncharacterized ions and their affect at the gill surface may have also played role in the different response to ammonia observed with the two test diluents. Further studies with equivalent ion composition in the effluent and laboratory water could further support these findings or determine if other constituents in the effluent are responsible for these ameliorating effects.

For the field component of the study, in-stream biomonitoring of macroinvertebrate communities and fish assemblages was undertaken in addition to an *in situ* zebra mussel growth study. For the macroinvertebrates, there were no significant

differences in taxa richness and abundance between sites, although they both were somewhat reduced around the outfall. This could be due to the water chemistry around the outflow or sedimentation effects. For example, on some occasions, the samplers used to collect macroinvertebrates at the outfall were buried in mud which would have contributed to reductions in density and diversity of macroinvertebrates on the samplers. The most abundant macroinvertebrate species around the outfall was the chironomid midge, while in collections from 2005, caddisflies from the genus *Hydropsyche* and mayflies from the genus *Caenis* were the most abundant macroinvertebrates at the upriver and downriver stations. For the August 2005 and 2006 collections, chironomid midges were the most abundant taxon at all sites. Shifts in the distribution of macroinvertebrate taxa could be related to changes in river flow levels which were somewhat reduced in 2005 and 2006 due to low precipitation levels. The river flow could have also influenced the similarity between sites since the Jaccard's similarity index for the first two collections was 0.68 and then the similarity decreased to 0.53 for the November 2005 and both 2006 collections. The macroinvertebrate data demonstrate that ammonia can be assimilated into larger river and not disturb macroinvertebrates richness, diversity and similarity index, although it can influence percent EPT.

Three fish collections were performed throughout the study, with the highest species richness for all collections found around the effluent outfall. The lack of any reduction in fish assemblage metrics around the effluent outfall may indicate a lack of any particularly toxic constituents in the effluent or that the effluent had an enrichment effect, which has been shown in previous studies to attract fish. There was also a trend toward higher richness on the effluent side of the river as a whole which could indicate

habitat differences that were influencing the fish assemblage. For example, the effluent side of the river was generally shallower than the opposite side.

There were some growth differences in zebra mussels that were deployed between sampling sites, although no consistent differences existed between the effluent mixing zone and the other sampling locations. There were also no consistent differences in condition indices of the zebra mussels between sites. This indicates, for the present study, the effluent did not cause adverse effects in growth or wet::dry weight ratios for the zebra mussels.

Based on the laboratory study, total ammonia nitrogen levels in the plant effluent could be as high as 20 mg N/L at a pH of 8.5 without causing adverse acute effects. Total ammonia nitrogen levels in the effluent mixing zone during the course of the study were 0.5 to 11.2 mg N/L with a pH 6.0 to 8.6. These factors, combined with the water hardness in the mixing zone and that during extremely low flows the effluent matrix only constitute 4.5% of the river flow, suggest that the effluent from the plant is not posing any acute risk to the receiving system. The results of this study and the fact the fertilizer plant has been located at this location since 1975 support this conclusion and further indicate that no long-term effects on the receiving system are being realized.

FUTURE RECOMENDTIONS

Future recommendations for the present study could consist of evaluating spatial differences at the tissue or biochemical level in the test organisms collected in the field. To accomplish these objectives the plasma or white mussel ammonia levels in fish could be analyzed, glycogen or lipid content in mussels and body size or mass of macroinveretebrates could establish if there are differences between sites (Wicks et al., 2002, Basset et al., 2004, Smolders et al., 2004). Furthermore, a series of WET tests with water from the river could determine the potential for the receiving system water to reduce the toxicity of chemicals and has implications for a derivation of site-specific water quality criteria (USEPA, 1999). Finally, adjustments to water hardness in bioassays that match hardness found in the effluent could identify if hardness is causing the ameliorating effects found in this study (Parametrix and Chadwick Ecological Consultants, 2006).

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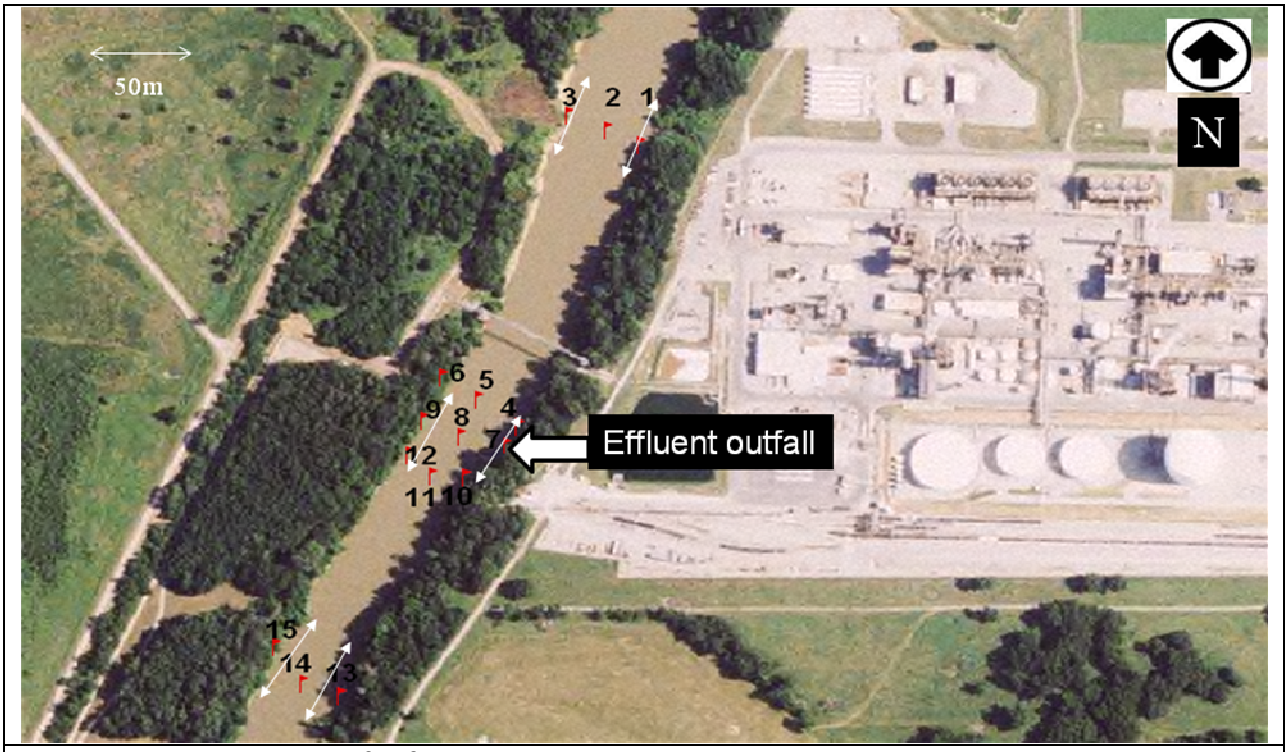


Figure 1. Station location for field data collection (2003 NAIP Air Photo Images Countywide Mosaic Images in UTM Zonal Projections). Numbers represent station locations and white arrows represent areas in which electroshocking was performed.

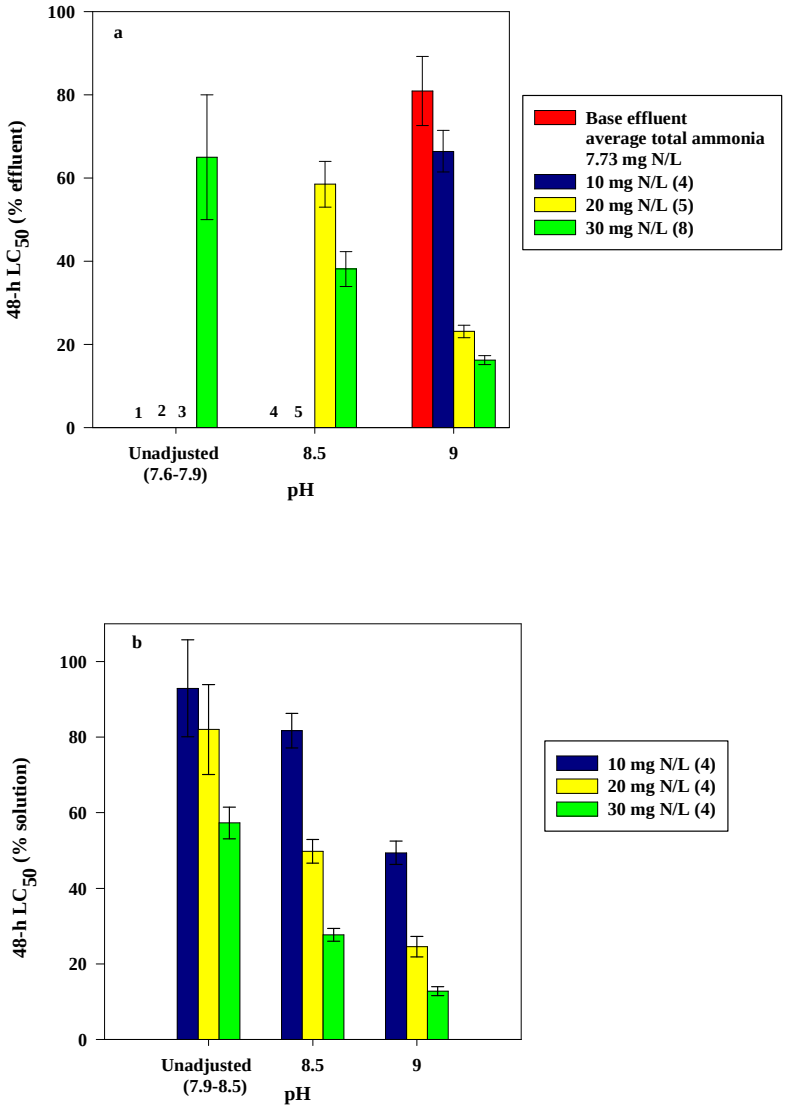


Figure 2. Average 48-h LC50 values (% effluent/solution) from bioassays with effluent both with and without added ammonia (a) and ammonia solution (NH_4Cl) (b) at unadjusted pH and pH 8.5 and 9.0. Numbers in parenthesis on legend indicate number of tests. Numbers in parenthesis under unadjusted are the range of pH values. Error bars are 95% confidence intervals.

[1]Base effluent no LC50 for unadjusted pH (pH average 7.8)

[2]Effluent + 10 mg total ammonia nitrogen/L N: no LC50 for unadjusted pH (pH average 7.7)

[3]Effluent + 20 mg total ammonia nitrogen /L N: no LC50 for unadjusted pH (pH average of 7.7)

[4]Base effluent: no LC50 at pH 8.5

[5]Effluent + 10 mg total ammonia nitrogen/L N: no LC50 at pH 8.5

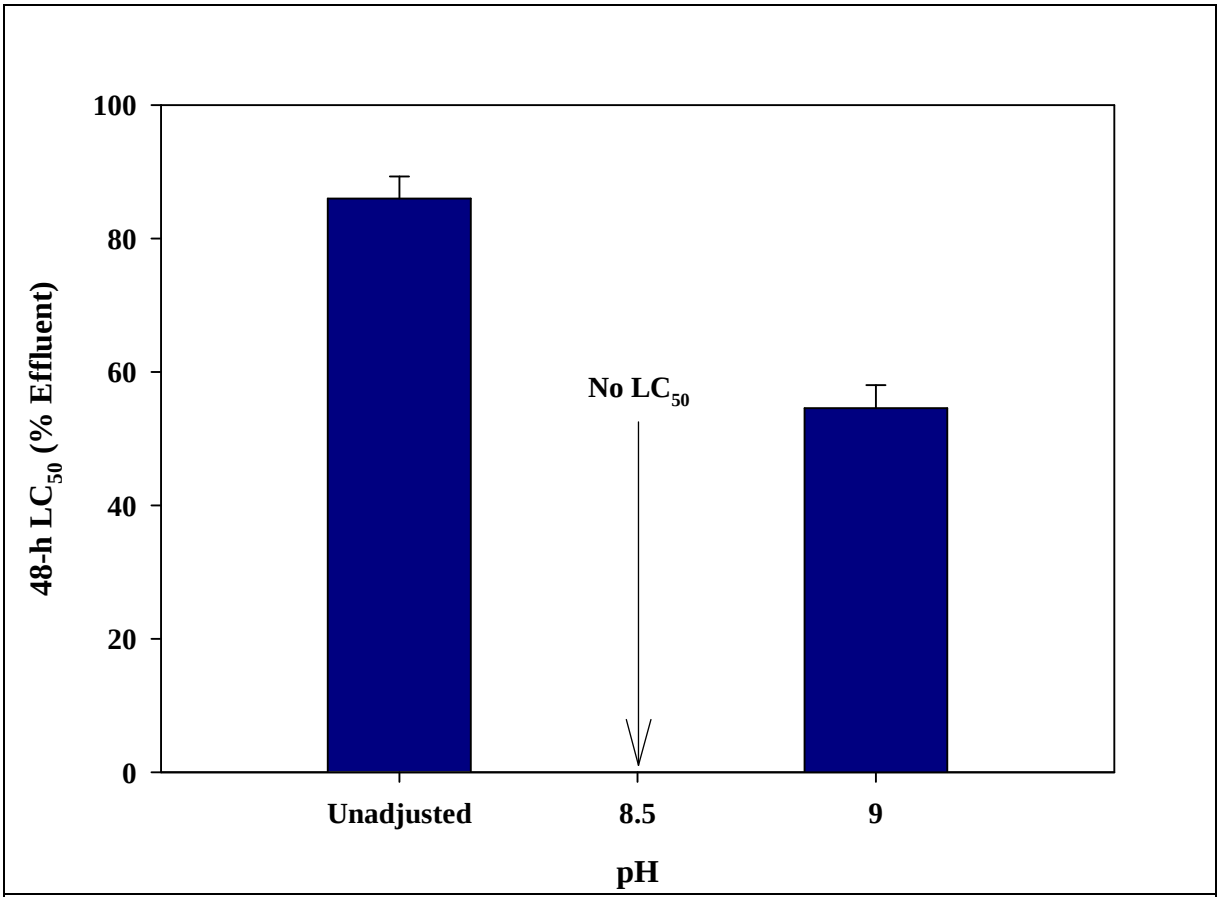


Figure 3. Average (n=6) 48-h LC50 (% effluent) values for bioassays with cooling tower blowdown at unadjusted pH and pH 8.5 and 9.0. Error bars are 95% confidence intervals.

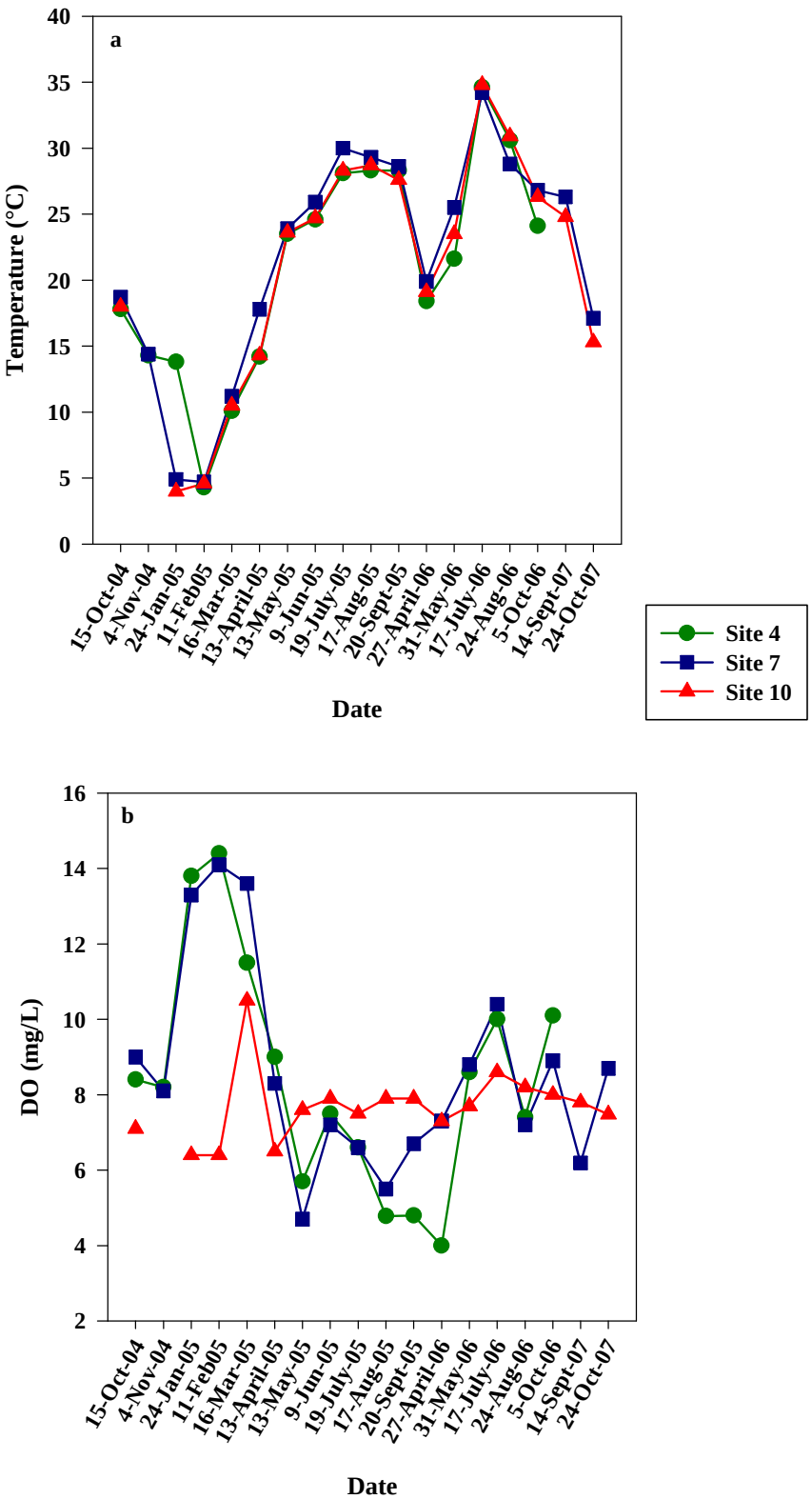
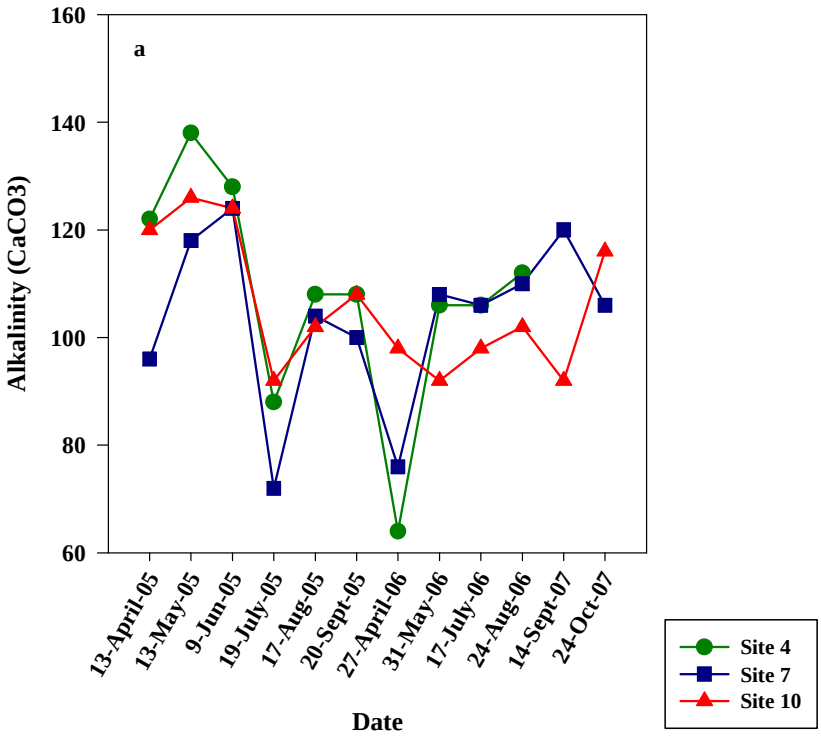


Figure 4. Temperature (a) and dissolved oxygen (b) for sites upriver ~20m (4), at discharge (7) and downriver ~20m (10) on the Verdigris River from 2004 to 2007.



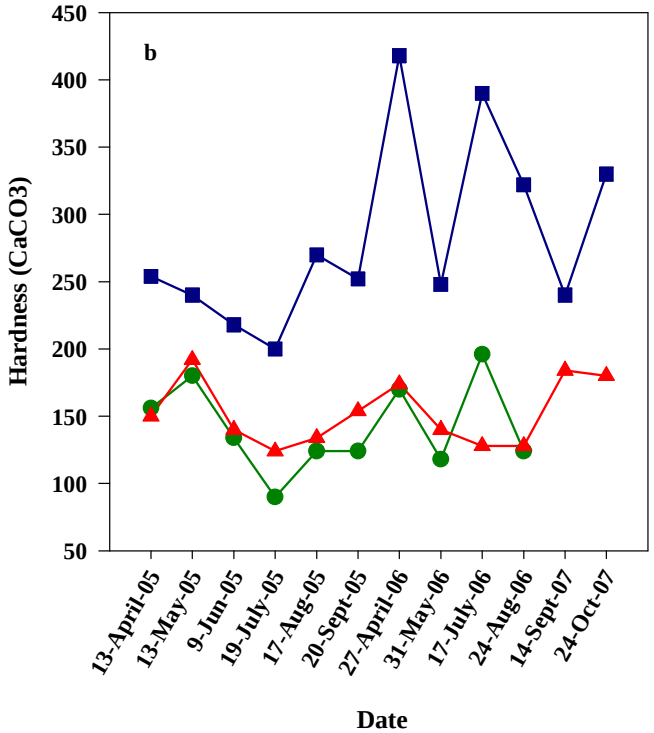


Figure 5. Alkalinity (a) and hardness (b) for sites upriver ~20m (4), at discharge (7) and downriver ~20m (10) on the Verdigris River from 2004 to 2007.

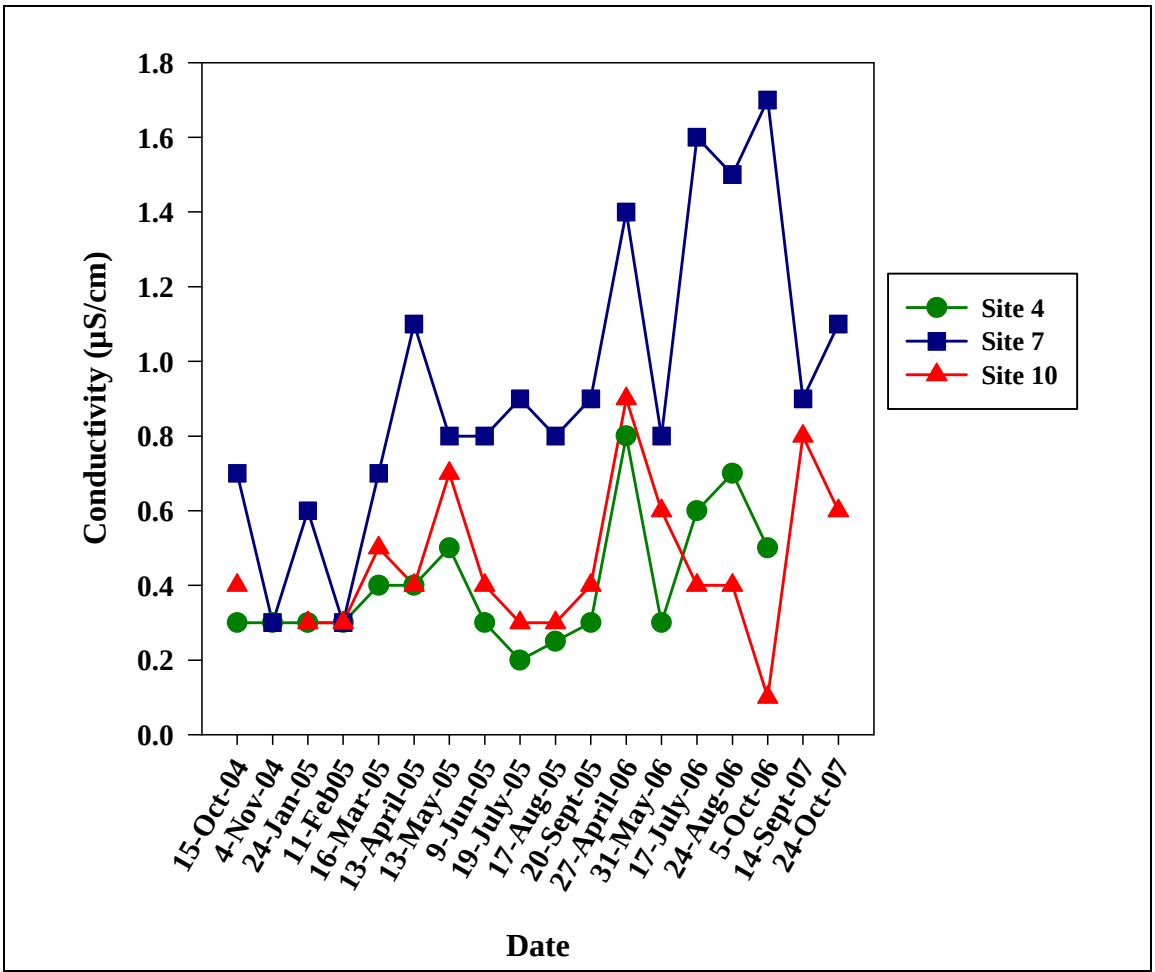


Figure 6. Conductivity for sites upriver ~20m (4), at discharge (7) and downriver ~20m (10) on the Verdigris River from 2004 to 2007.

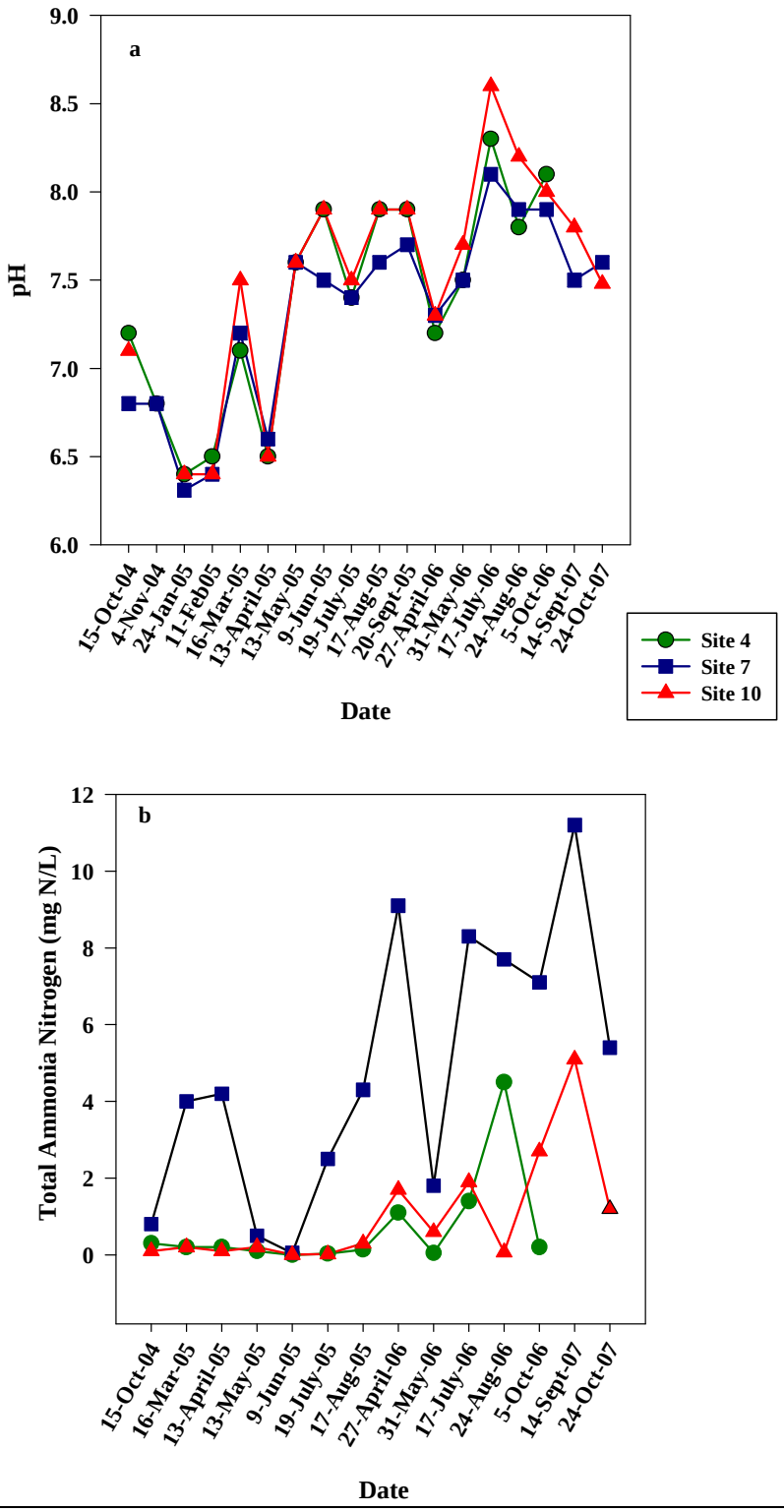
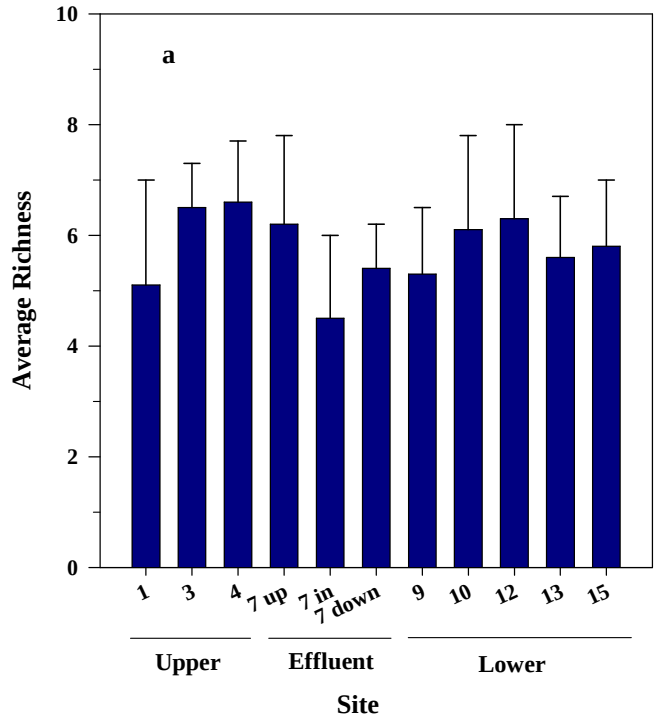


Figure 7. pH (a) and total ammonia (b) for sites upriver ~20m (4), at discharge (7) and downriver ~20m (10) on the Verdigris River from 2004 to 2007.



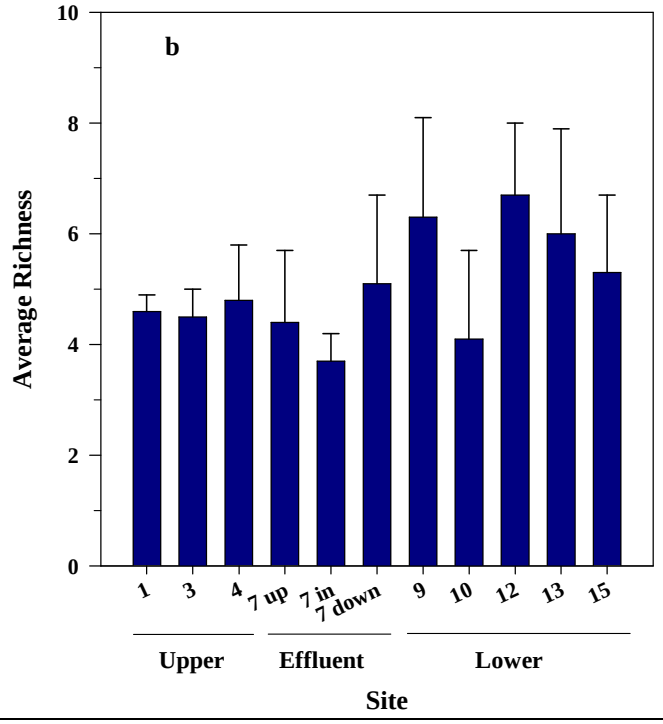


Figure 8. Average macroinvertebrate taxa richness (n=4) by site for 2005 (a) and 2006 (b) samples from the Verdigris River. Upper: sites upstream from effluent outfall, Effluent: sites within effluent outfall, Lower: sites across and downstream from effluent outfall. Error bars are ±1 standard deviation.

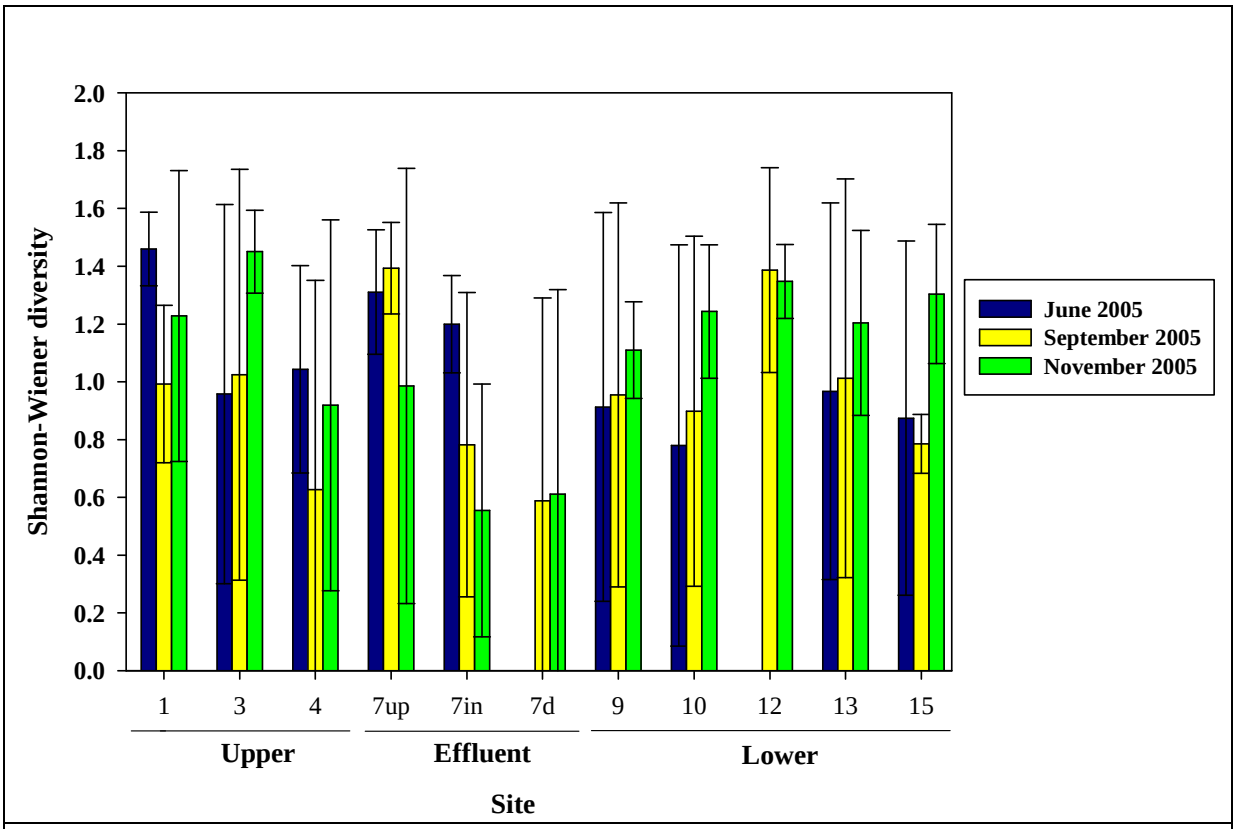


Figure 9. Average Shannon-Weiner diversity values (n=4) for macroinvertebrates collected at the different sampling stations on the Verdigris River in 2005. Upper: sites upstream from effluent outfall, Effluent: sites within effluent outfall, Lower: sites across and downstream from effluent outfall. In June 2005, no macroinvertebrates were found in sample 7d and at site 12 the sampler was missing. Error bars are ±1 standard deviation.

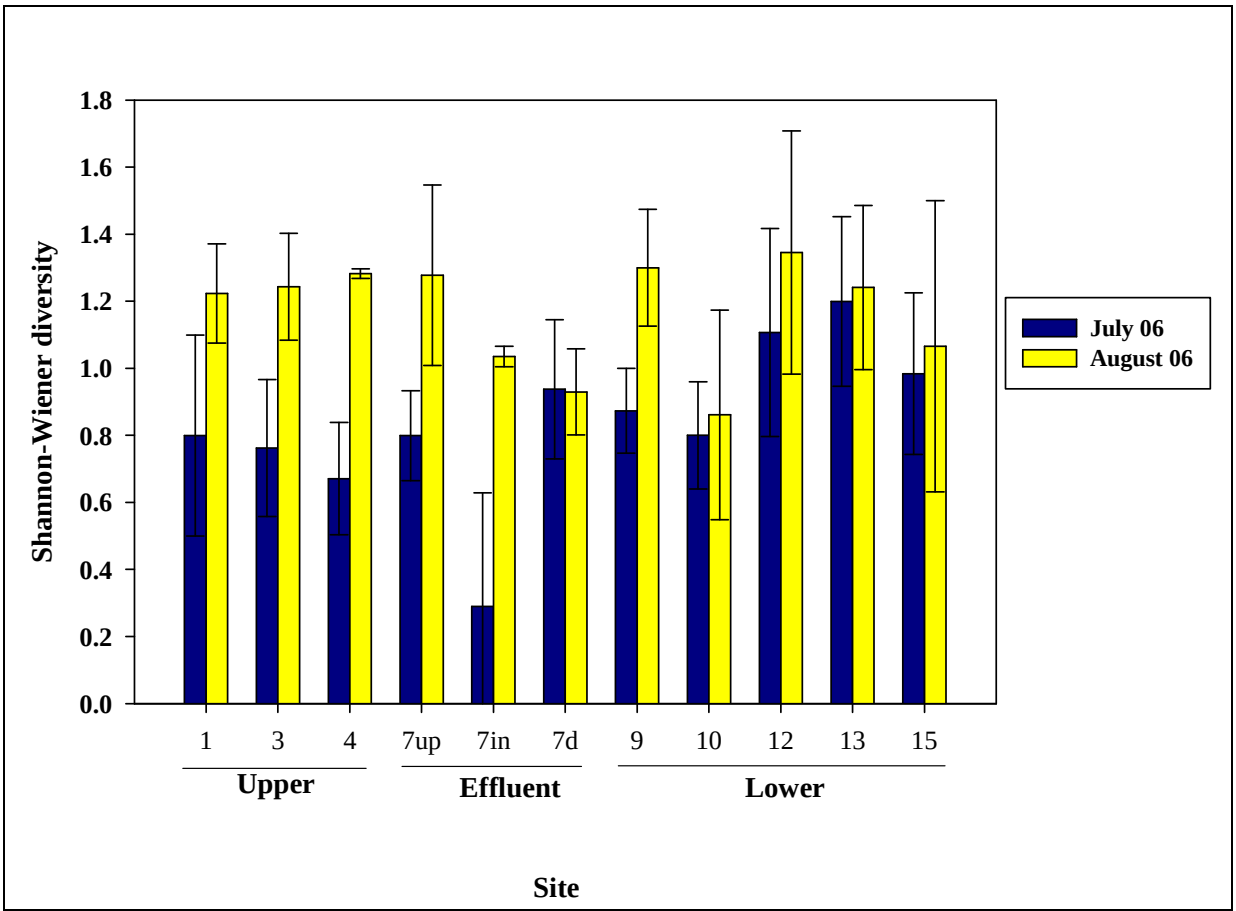


Figure 10. Average Shannon-Weiner diversity values (n=4) for macroinvertebrates collected at the different sampling stations on the Verdigris River in 2006. Upper: sites upstream from effluent outfall, Effluent: sites within effluent outfall, Lower: sites across and downstream from effluent outfall. Error bars are ±1 standard deviation.

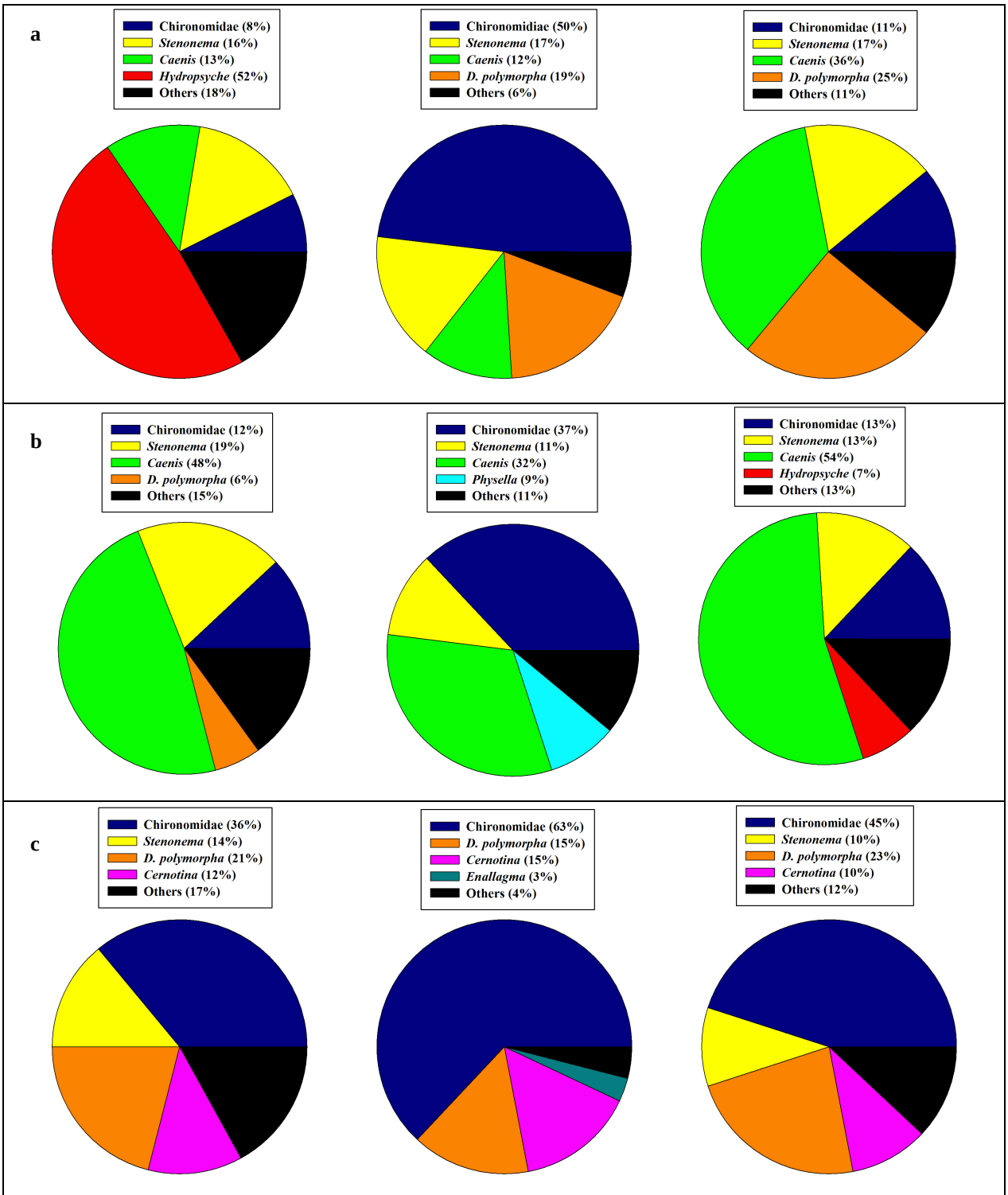


Figure 11. Percent abundance of the top four macroinvertebrate taxa from upriver, effluent and downriver sites for the six week sample periods ending in June (a), August (b), and November (c) in 2005. Numbers in parenthesis are the actual % abundance values. Upriver: sites upstream from effluent outfall, Effluent: sites within effluent outfall, Downriver: sites across and downstream from effluent outfall.

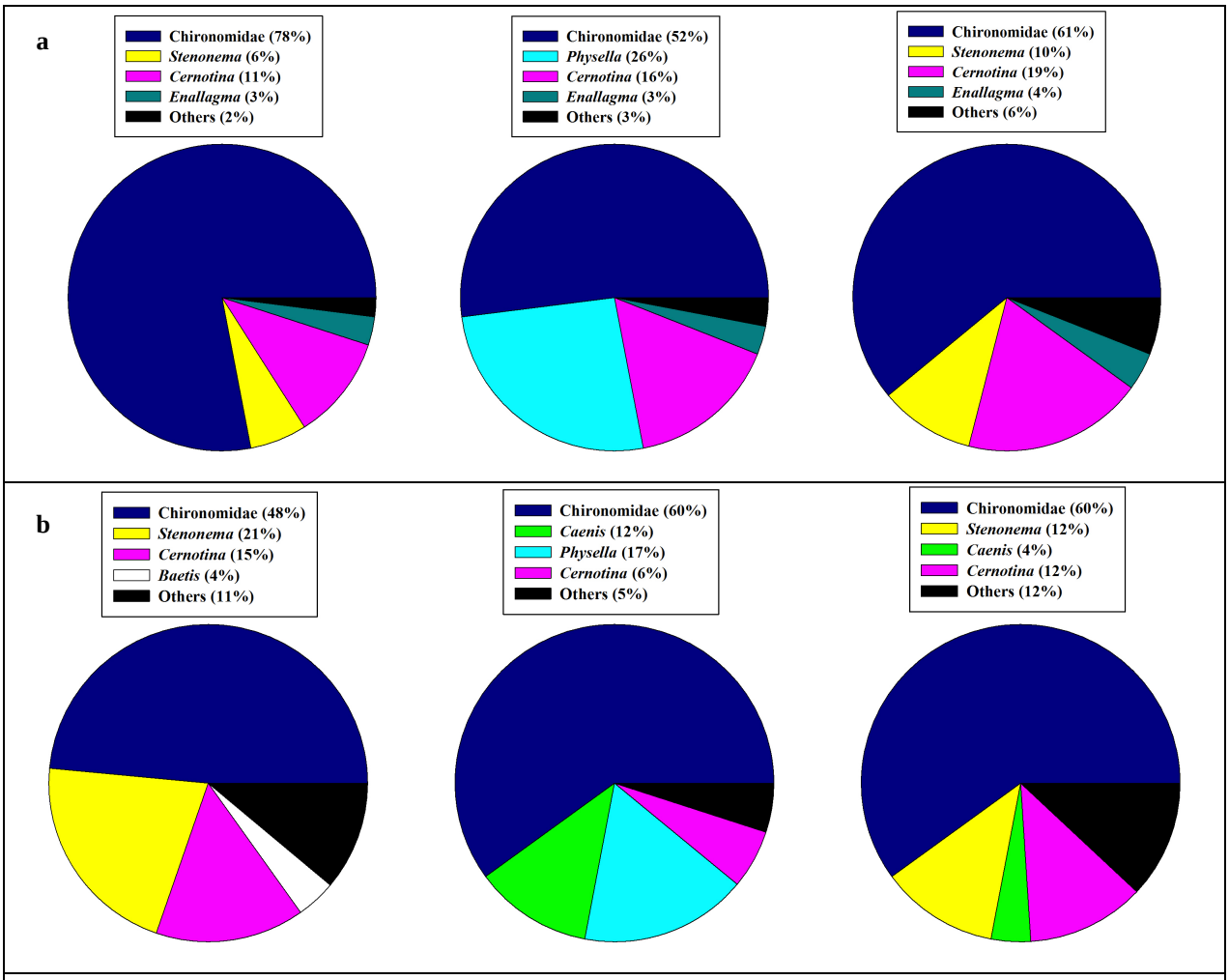


Figure 12. Percent abundance of the top four macroinvertebrate taxa from upriver, effluent and downriver sites for the six week sample periods ending in July (a) and August (b) in 2006. Numbers in parenthesis are the actual % abundance values. Upriver: sites upstream from effluent outfall, Effluent: sites within effluent outfall, Downriver: sites across and downstream from effluent outfall.

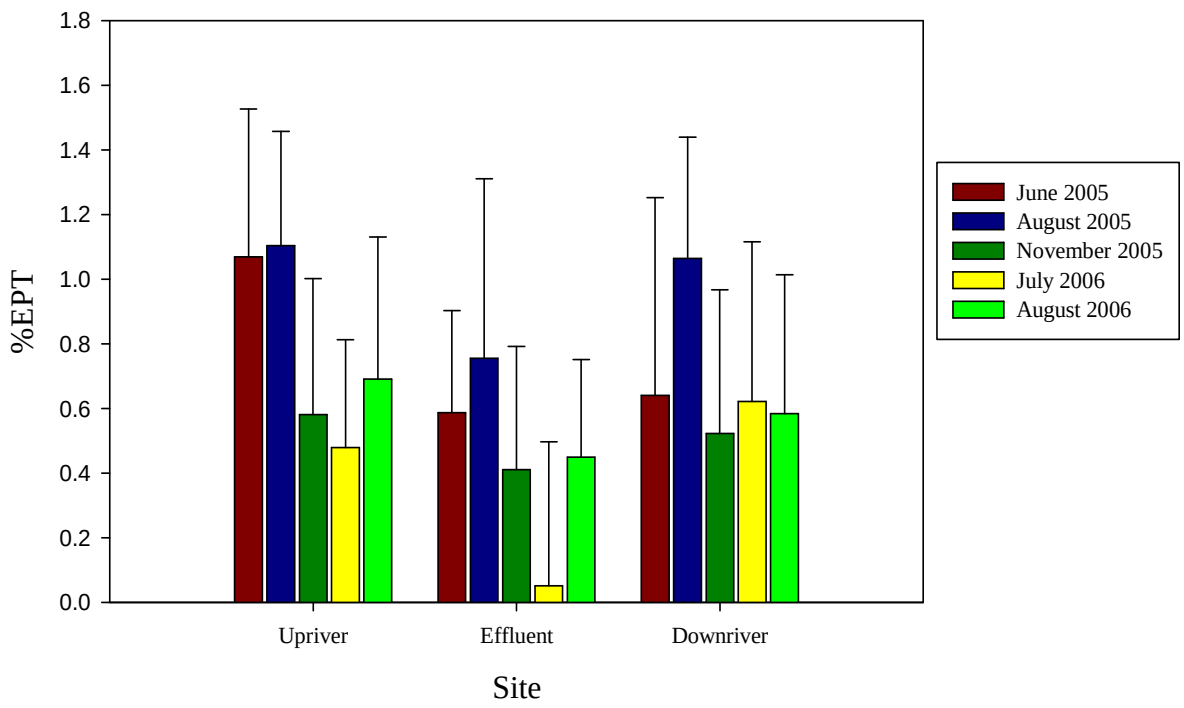


Figure 13. Percent Ephemeroptera, Plecoptera and Trichoptera (EPT) in samples taken from sites upstream from the effluent ("Upriver"- 1, 3, 4), around the effluent ("Effluent"- 7u, 7i, 7d) and downstream from the effluent ("Downriver"- 9, 10, 12, 13, 15) for each of the six week sample periods in 2005 and 2006. Error bars are ±1 standard deviation.

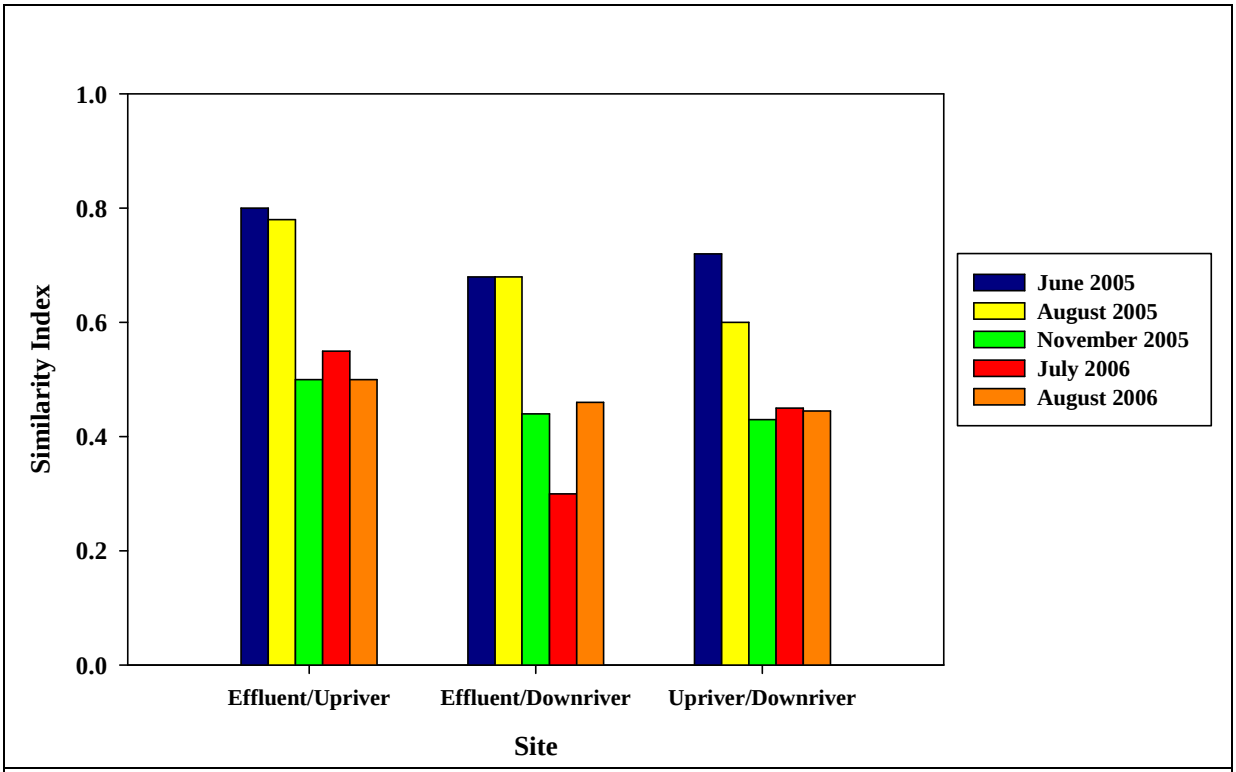


Figure 14. Values for Jaccard's Similarity Index calculated for the macroinvertebrate data from the Verdigris sampling sites for 2005 and 2006. Upriver: sites upstream from effluent outfall, Effluent: sites within effluent outfall, Downriver: sites across and downstream from effluent outfall.

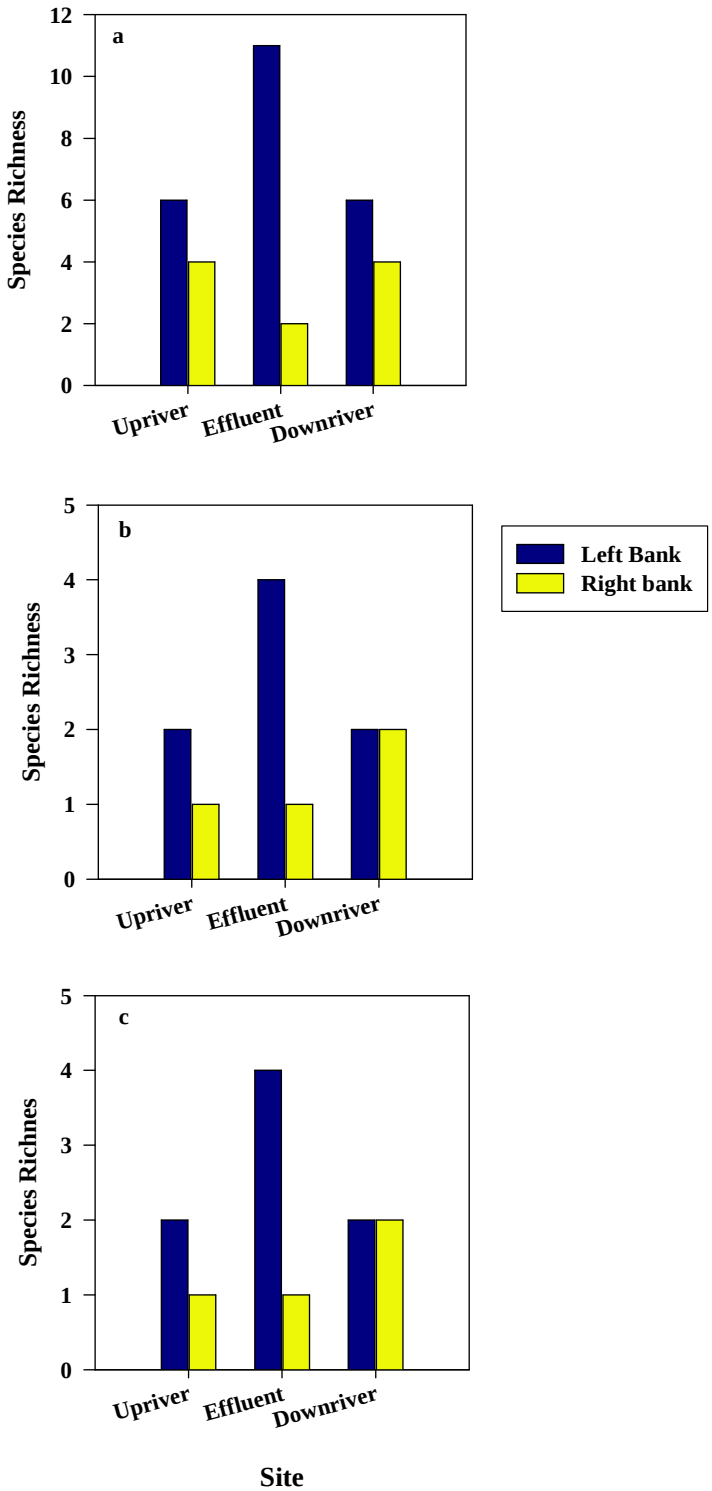


Figure 15. Fish species richness by site for October 2005 (a), June 2006 (b) and November 2006 (c) sampling periods. Collection occurred at left and right bank, upriver at effluent and downriver. Left bank represents effluent side. Note the different Y-axis scales.

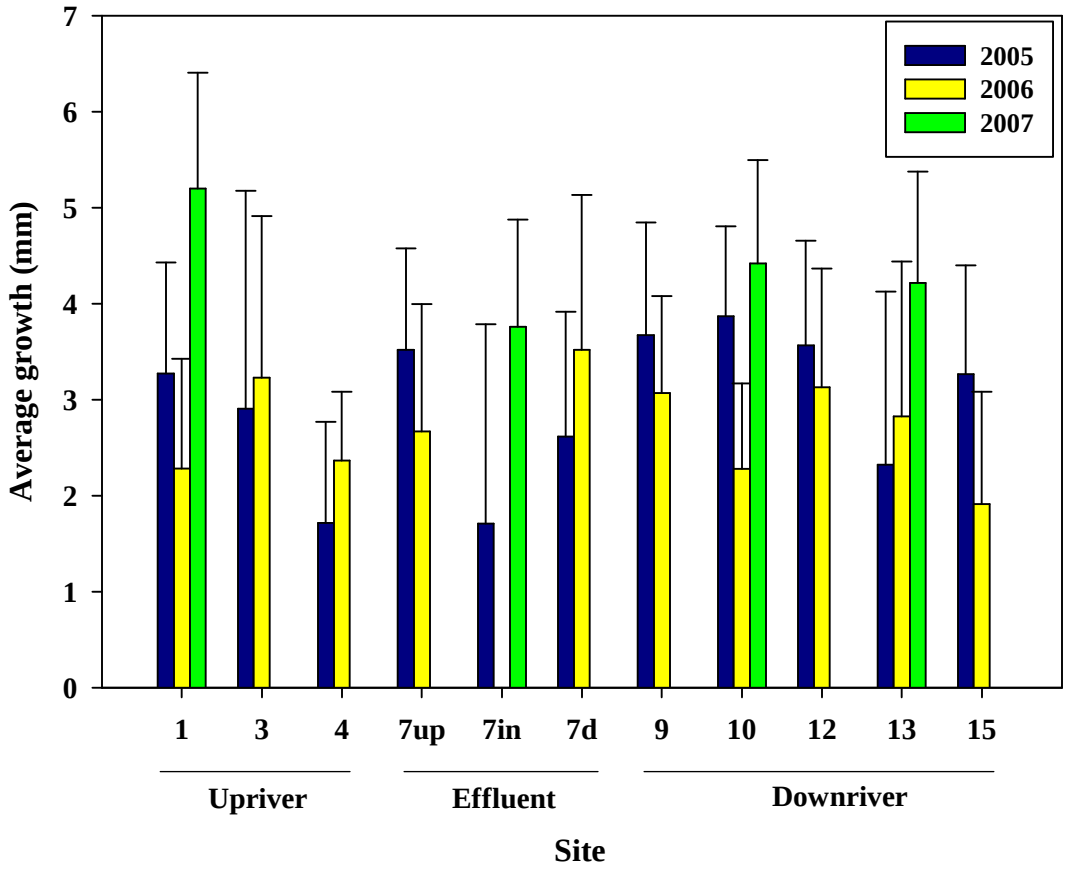


Figure 16. Average zebra mussel growth (mm) by site for 2005, 2006 and 2007. Upper sites: sites upstream from effluent outfall (1,3, 4), Effluent: sites within effluent outfall (7u, 7i, 7d) Lower: sites across and downstream from effluent outfall (9, 10, 12, 13, 15). In 2006 sample 7in was missing and in 2007 four sites were utilized. Error bars are ±1 standard deviation.

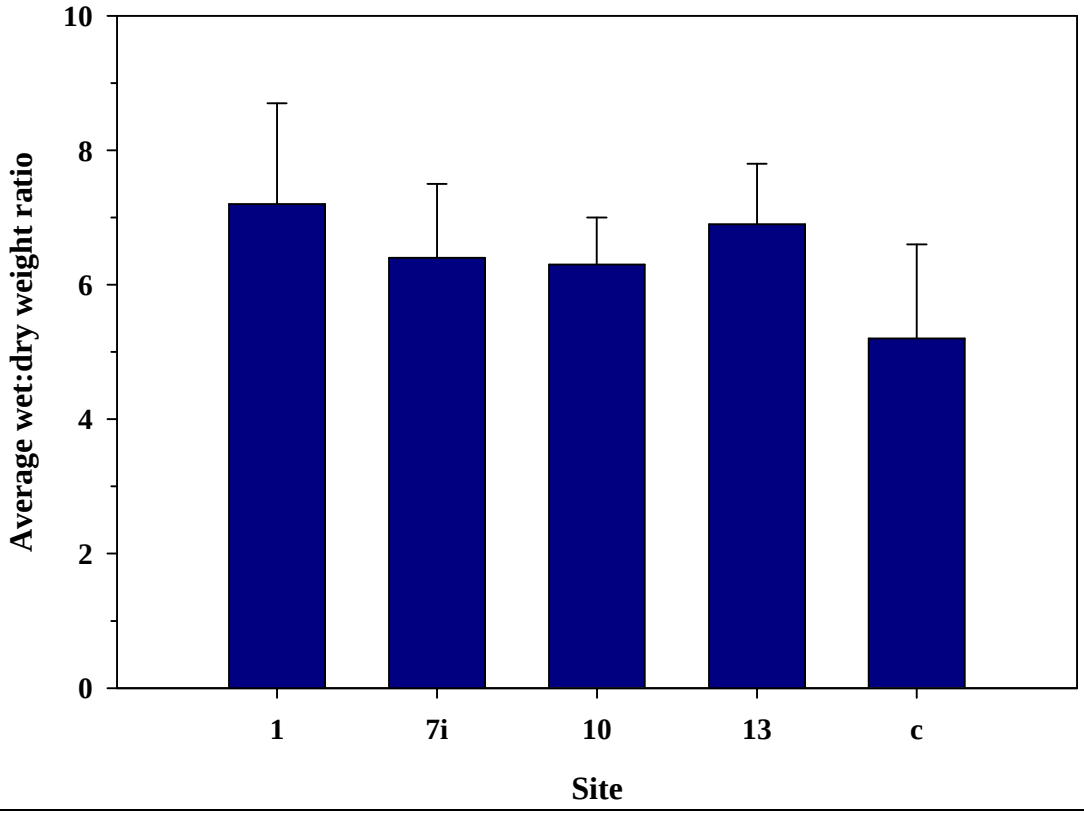


Figure 17. Average zebra mussel wet: dry weight ratios by site for the six week sampling period in 2007. Error bars are ± 1 standard deviation.

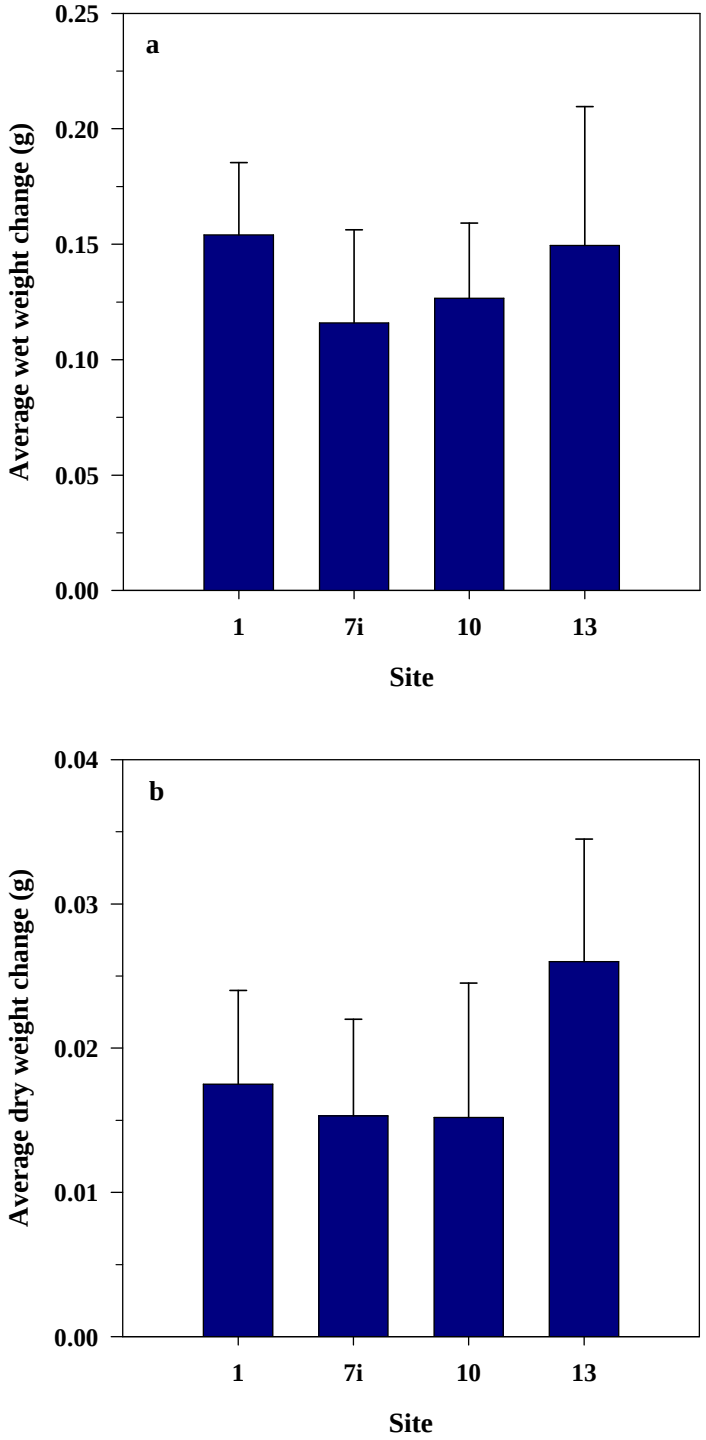


Figure 18. Average zebra mussel wet weight change (a) and dry weight change (b) by site for the six week sampling period in 2007. Error bars are ± 1 standard deviation.

TABLES

Table 1. Locations on the Verdigris River utilized to evaluate water chemistry, macroinvertebrate communities, fish communities and zebra mussel in-situ research.

Station	Location compared to effluent discharge	Universal Transverse Mercator (UTM) Coordinates (Zone 15)	
		Y	X
1	~250m upriver effluent side	4013457.95	255318.37
2	~250m upriver middle river	4013464.98	255280.44
3	~250m upriver across river	4013481.19	255254.84
4	~25m upriver effluent side	4013219.45	255220.46
5	~25m upriver middle river	4013232.33	255186.17
6	~25m upriver across river	4013249.53	255167.73
7	Effluent outfall	4013185.34	255205.12
8	Middle river at effluent	4013191.18	255166.57
9	Across river from effluent	4013207.81	255147.26
10	~25m downriver effluent side	4013177.18	255177.28
11	~25m down and middle river	4013177.62	255161.84
12	~25m down and across river	4013189.66	255136.13
13	~250m downriver effluent side	4012965.90	255085.92
14	~250m down and middle river	4012976.21	255058.21
15	~250m down and across river	4012997.77	255032.76

Table 2. Diluents used in 48-h bioassays with the corresponding pH and ammonia treatments.

Diluents	**pH Treatment**			**Ammonia Treatment (mg N/L)**			
Base Effluent	unadjusted	8.5	9.0	unadjusted	+	+	+
Effluent with ammonia added	unadjusted	8.5	9.0	*	10	20	30
Ammonia solution	unadjusted	8.5	9.0	*	10	20	30
Cooling Tower	unadjusted	8.5	9.0	unadjusted	+	+	+

* Only manipulated ammonia used in bioassays

+No manipulations to ammonia in bioassays

Table 3. Initial average pH and total ammonia nitrogen levels at 0 and 24-h for 48-h toxicity tests with fathead minnows (*Pimephales promelas*) exposed to base effluent, effluent with ammonia added, ammonia solution and cooling tower blowdown at the highest percent exposed. Numbers in parenthesis are the pH ranges.

Dilution type	Target pH	pH	Total Ammonia (mg N/L)
Base Effluent (001)	Unadjusted	7.6 (7.3-7.8)	7.3
	8.5	8.4 (8.4-8.4)	7.3
	9.0	8.9 (8.8-8.9)	7.3
Effluent + 10 mg N/L	Unadjusted	7.7 (7.2-7.8)	10.0
	8.5	8.4 (8.4-8.5)	10.0
	9.0	8.9 (8.8-9.0)	10.0
Effluent + 20 mg N/L	Unadjusted	7.7 (7.4-7.8)	20.0
	8.5	8.5 (8.4-8.5)	20.0
	9.0	8.9 (8.9-9.0)	20.0
Effluent + 30 mg N/L	Unadjusted	7.6 (7.2-7.8)	30.0
	8.5	8.4 (8.4-8.6)	30.0
	9.0	8.9 (8.9-9.0)	30.0
10 mg N/L solution	Unadjusted	8.2 (7.7-8.4)	10.0
	8.5	8.5 (8.4-8.6)	10.0
	9.0	8.9 (8.9-9.0)	10.0
20 mg N/L solution	Unadjusted	8.0 (7.8-8.1)	20.0
	8.5	8.5 (8.4-8.5)	20.0
	9.0	8.9 (8.9-9.0)	20.0
30 mg N/L solution	Unadjusted	8.0 (7.8-8.0)	30.0
	8.5	8.5 (8.4-8.5)	30.0
	9.0	9.0 (8.9-9.0)	30.0
Cooling tower blowdown	Unadjusted	7.2 (6.7-7.5)	24.0
	8.5	8.4 (8.3-8.5)	24.0
	9.0	8.9 (8.8-9.1)	24.0

Table 4. Ranges of water quality parameters measured in 48-h toxicity tests with fathead minnows (*Pimephales promelas*) exposed to base effluent, effluent with ammonia added, ammonia solution and cooling tower blowdown.

Dilution type	Target pH	Temperature (°C)	DO (mg/L)	Conductivity (µS/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Base Effluent (001)	unadjusted	19.3-22.7	6.9-9.0	781-2174	68-130	230-430
	8.5	19.8-23.2	6.8-8.9	761-2192	68-130	230-430
	9.0	19.9-22.9	6.7-8.9	755-2133	68-130	230-430
Effluent + 10 mg N/L	unadjusted	19.3-23.0	7.0-8.8	863-2731	104-120	478-510
	8.5	19.5-23.0	6.8-8.5	967-2619	104-120	478-510
	9.0	19.6-23.3	6.8-8.9	901-2613	104-120	478-510
Effluent + 20 mg N/L	unadjusted	19.0-22.4	5.4-8.7	988-3417	82-140	362-480
	8.5	19.0-22.5	5.2-8.7	947-3213	82-140	362-480
	9.0	19.1-22.7	6.0-8.7	876-3323	82-140	362-480
Effluent + 30 mg N/L	unadjusted	19.1-23.1	6.3-8.6	1026-2776	80-140	350-548
	8.5	19.4-23.0	6.3-8.7	845-2581	80-140	350-548
	9.0	19.0-23.2	6.4-8.8	876-2551	80-140	350-548
10 mg N/L solution	unadjusted	18.5-23.2	6.7-9.3	625-956	126-186	154-192
	8.5	19.2-23.5	6.4-9.2	630-985	126-186	154-192
	9.0	19.1-23.5	6.6-8.8	639-1073	126-186	154-192
20 mg N/L solution	unadjusted	19.2-23.7	8.2-12.2	782-985	160-230	156-162
	8.5	19.3-23.6	7.9-15.0	775-1002	160-230	156-162
	9.0	19.2-23.5	8.1-12.8	756-909	160-230	156-162
30 mg N/L solution	unadjusted	18.8-24.1	7.6-14.9	702-1207	214-220	154-192
	8.5	18.8-23.7	7.9-14.2	670-1078	214-220	154-192
	9.0	19.0-23.7	7.9-14.5	670-984	214-220	154-192
Cooling tower blowdown	unadjusted	19.2-24.0	7.6-9.8	862-2196	6-30	522-2160
	8.5	19.3-23.5	7.5-9.2	852-2200	6-30	522-2160
	9.0	19.4-23.6	7.3-9.1	840-2210	6-30	522-2160

Table 5. Total number of 48-h LC50 values generated / total number of tests performed on base effluent, effluent with ammonia added and ammonia solution. Average total ammonia nitrogen levels for base effluent were 7.73 mg/L NH_3-N.

pH	Base Effluent	Effluent with total ammonia added (mg N/L)			Ammonia solution (mg N/L)		
	7.73	**10**	**20**	**30**	**10**	**20**	**30**
Unadjusted	0/8	2/4	3/4	4/4	0/4	0/5	4/8
8.5	0/8	3/4	4/4	4/4	0/4	5/5	7/8*
9.0	4/8	4/4	4/4	4/4	4/4	4/5*	8/8

* mortality too great to generate LC50 values

Table 6. Average total and unionized ammonia at LC50 for 48-h toxicity tests with fathead minnows (*Pimephales promelas*) exposed to base effluent, effluent with ammonia added, ammonia solution and cooling tower blowdown. [1]Ammonia levels presented in Table 2.

Dilution type	Target pH	Total Ammonia (mg N/L)	48h LC50 (%)	Total Ammonia at LC50 (mg N/L)	Un-ionized Ammonia at LC50 (mg NH_3-N/L)
Base Effluent (001)	Unadjusted	7.3	-	-[1]	-
	8.5	7.3	-	-[1]	-
	9.0	7.3	81.0	5.9	1.5
Effluent + 10 mg N/L	Unadjusted	10.0	-	-[1]	-
	8.5	10.0	-	-[1]	-
	9.0	10.0	66.4	6.6	1.7
Effluent + 20 mg N/L	Unadjusted	20.0	-	-[1]	-
	8.5	20.0	58.5	11.7	1.4
	9.0	20.0	23.1	4.6	1.2
Effluent + 30 mg N/L	Unadjusted	30.0	65.2	19.6	0.3
	8.5	30.0	38.1	11.4	1.1
	9.0	30.0	16.2	4.9	1.3
10 mg N/L solution	Unadjusted	10.0	93.0	9.3	0.6
	8.5	10.0	82.0	8.2	1.0
	9.0	10.0	49.4	4.9	1.3
20 mg N/L solution	Unadjusted	20.0	82.0	16.4	0.7
	8.5	20.0	50.0	10.0	1.2
	9.0	20.0	25.0	5.0	1.3
30 mg N/L solution	Unadjusted	30.0	57.3	17.2	0.7
	8.5	30.0	28.0	8.4	0.9
	9.0	30.0	13.0	3.9	1.0
Cooling tower blowdown	Unadjusted	24.0	86.0	20.6	0.2
	8.5	24.0	-	-[1]	-
	9.0	24.0	55.0	13.2	3.4

Table 7. Average LC50 % from laboratory toxicity tests fathead minnows (*Pimephales promelas*) exposed to cooling tower water.

Date	Unadjusted pH	pH 8.5	pH 9.0
8/26/05	87.3	0*	82.9
9/11/2005	0*	0*	42.2
10/14/2005	86.5	0*	43.4
11/24/2005	84.1	0*	56.4
12/20/2005	0*	NA	NA
1/5/2006	0*	0*	47.9

* insufficient mortality to generate LC50 values

Table 8. Chlorine, ammonia, alkalinity, hardness and unadjusted pH collected from laboratory toxicity tests with cooling tower water.

Date	Chlorine (mg/L)	Total Ammonia (mg N/L)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Unadjusted pH
8/26/05	1.10	19.5	2160	12	6.9
9/11/2005	0.80	16.8	700	18	7.1
10/14/2005	0.71	19.3	660	12	7.0
11/24/2005	1.10	16.8	662	30	7.4
12/20/2005	0.29	55.2	522	6.0	7.4
1/5/2006	0.11	13.6	840	28	7.6

Table 9. Ranges of water quality parameters measured at 15 sites on the Verdigris River from November 2004 to October 2007.

Station	Temp. (°C)	DO (mg\L)	pH	Conductivity (µS/cm)	Total Ammonia (mg N/L)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
1	3.7-33.0	4.0-14.9	6.0-7.9	0.2-0.8	0.01-2.6	90-134	116-180
2	3.7-33.3	4.0-14.0	6.5-7.8	0.3-0.8	0.0006-1.0	94-180	118-190
3	3.7-32.9	3.3-14.5	6.3-7.8	0.3-0.7	0.0006-1.4	72-108	108-158
4	4.3-34.6	4.0-14.4	6.4-8.3	0.3-1.8	0.0006-4.5	64-112	118-196
5	4.3-34.4	3.8-14.5	6.5-8.4	0.3-0.7	0.0004-1.1	60-106	112-162
6	14.0-33.8	3.4-9.6	6.8-8.1	0.3-0.8	0.0004-1.0	68-114	121-168
7	4.7-34.2	6.2-14.1	6.3-8.1	0.3-1.7	0.05-11.2	76-120	240-418
8	4.5-34.2	3.7-14.5	6.6-8.4	0.3-0.7	0.0004-0.7	90-108	112-170
9	4.4-33.5	3.9-14.1	6.5-8.0	0.3-0.8	0.0005-0.8	94-108	118-160
10	4.0-34.8	4.3-14.1	6.4-8.6	0.1-0.9	0.003-5.1	92-116	128-184
11	4.5-34.5	4.8-14.4	6.5-8.4	0.3-4.1	0.0004-0.8	90-110	114-172
12	4.4-33.5	3.6-13.9	6.4-8.3	0.2-0.8	0.0004-0.7	90-102	116-168
13	3.7-35.0	4.0-14.1	6.0-8.6	0.3-1.9	0.0006-0.8	92-120	112-174
14	4.5-33.0	3.7-14.0	6.5-8.6	0.3-0.8	0.0006-0.8	96-112	118-192
15	4.5-34.0	4.0-14.0	6.5-8.5	0.3-0.9	0.0003-0.7	94-108	110-186

Table 10. Values for Jaccard's Similarity Index calculated for the macroinvertebrate data from the Verdigris sampling sites. Upriver: sites upstream from effluent outfall, Effluent: sites within effluent outfall, Downriver: sites across and downstream from effluent outfall. Numbers in parenthesis are standard deviations. [1]Combined average for June and August 2005. [2]Combined average for June 2005, July and August 2006.

Site/Date	June 2005	August 2005	November 2005	July 2006	August 2006	Average across dates (1 S.D.)
Effluent/Upriver	0.69	0.77	0.50	0.56	0.50	**0.60 (0.12)**
Effluent/Downriver	0.67	0.67	0.44	0.31	0.46	**0.51 (0.16)**
Upriver/Downriver	0.71	0.59	0.42	0.46	0.46	**0.53 (0.12)**
Avg. across sites (1 S.D.)	**0.69 (0.02)**	**0.68 (0.09)**	**0.45 (0.04)**	**0.44 (0.13)**	**0.47 (0.02)**	**0.55 (0.13)**
		[1]0.68 (0.06)		**[2]0.46 (0.07)**		

Table 11a. Total fish species collected on October 2005 from the Verdigris River.

Fish Species	Upper left bank	Upper right bank	Effluent	Across from effluent	Down left bank	Down right bank
Bluegill (*Lepomis macrochirus*)	1	1	2		1	
Bluntnose minnow (*Pimephales notatus)*						
Brook silverside (*Labidesthes sicculus)*			4		7	
Channel catfish (*Ictalurus punctatus*)			1			
Flathead catfish (*Pylodictis olivaris*)						1
Fresh water drum (*Aplodinotus grunniens)*	1					
Gizzard shad (*Dorosoma cepedianum)*	22	7	127	73	2	18
Green sunfish (*Lepomis cyanellus*)			3			
Largemouth bass (*Micropterus salmoides)*	1	2	3			
Longear sunfish (*Lepomis megalotis)*	2	4	3		1	
Redear sunfish (*Lepomis microlophus*)						
River carpsucker (*Carpiodes carpio)*	2			1	5	2
Smallmouth bass (*Micropterus dolomieu*)			1			
Smallmouth buffalo (*Ictiobus bubalus*)						
Striped bass (*Marone saxatilis*)			4			
White bass (*Marone chrysops*)			1			
White crappie (*Pomoxis annularis*)			2			1
Total richness	**6**	**4**	**11**	**2**	**5**	**4**
Total abundance	**29**	**14**	**151**	**74**	**16**	**22**

Table11b. Total fish species collected on June 2006 from the Verdigris River.

Fish Species	Upper left bank	Upper right bank	Effluent	Across from effluent	Down left bank	Down right bank
Bluegill (*Lepomis macrochirus*)						
Bluntnose minnow (*Pimephales notatus)*						
Brook silverside (*Labidesthes sicculus)*						
Channel catfish (*Ictalurus punctatus*)						
Flathead catfish (*Pylodictis olivaris*)			1			
Fresh water drum (*Aplodinotus grunniens)*					1	
Gizzard shad (*Dorosoma cepedianum)*	1	1	2	3	1	1
Green sunfish (*Lepomis cyanellus*)						
Large mouth bass (*Micropterus salmoides)*	1					
Longear sunfish (*Lepomis megalotis)*			4			1
Redear sunfish (*Lepomis microlophus*)			1			
River carpsucker (*Carpiodes carpio)*						
Smallmouth bass (*Micropterus dolomieu*)						
Smallmouth buffalo (*Ictiobus bubalus*)						
Striped bass (*Marone saxatilis*)						
White bass (*Marone chrysops*)						
White crappie (*Pomoxis annularis*)						
Total richness	**2**	**1**	**4**	**1**	**2**	**2**
Total abundance	**2**	**1**	**8**	**3**	**2**	**2**

Table 11c. Total fish species collected on November 2006 from the Verdigris River.

Fish Species	Upper left bank	Upper right bank	Effluent	Across from effluent	Down left bank	Down right bank
Bluegill (*Lepomis macrochirus*)			3			
Bluntnose minnow (*Pimephales notatus)*			3			
Brook silverside (*Labidesthes sicculus)*						
Channel catfish (*Ictalurus punctatus*)						
Flathead catfish (*Pylodictis olivaris*)						
Fresh water drum (*Aplodinotus grunniens)*						
Gizzard shad (*Dorosoma cepedianum)*	437	22	59	263	241	13
Green sunfish (*Lepomis cyanellus*)						
Large mouth bass (*Micropterus salmoides)*			9			
Longear sunfish (*Lepomis megalotis)*						
Redear sunfish (*Lepomis microlophus*)						
River carpsucker (*Carpiodes carpio)*	1					
Smallmouth bass (*Micropterus dolomieu*)		1				
Smallmouth buffalo (*Ictiobus bubalus*)					1	
Striped bass (*Marone saxatilis*)						
White bass (*Marone chrysops*)						
White crappie (*Pomoxis annularis*)						
Total richness	**2**	**2**	**4**	**1**	**2**	**1**
Total abundance	**438**	**23**	**74**	**263**	**242**	**13**

Table 12. Jaccard's Similarity Index for the fish collections made in 2005 and 2006. Upriver: sites upstream from effluent outfall, Effluent: sites within effluent outfall, Downriver: sites across and downstream from effluent outfall.

Site/Date	10/14/05	6/26/06	11/11/06	Average across dates (1 S.D.)
Effluent/Upriver	0.31	0.20	0.17	**0.23 (0.07)**
Effluent/Downriver	0.38	0.40	0.20	**0.33 (0.11)**
Upriver/Downriver	0.44	0.25	0.25	**0.31 (0.11)**
Avg. across sites (1 S.D.)	**0.38 (0.07)**	**0.28 (0.10)**	**0.21 (0.04)**	**0.29 (0.05)**

Appendix 1: Field Study Water Chemistry

Table 1a. Field water quality parameters from each station on the Verdigris River by site and date. For the Secchi depth ctf=current too fast.

	Temp. (°C)	DO (mg\L)	pH	Conductivity (µS/cm)	Ammonia (mg/L)	Secchi depth (m)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Station 1								
4-Nov-04	14.3	11.0	6.0	0.3		8.5		
24-Jan-05	3.7	14.3	6.4	0.3		0.2		
11-Feb-05	4.3	14.9	6.6	0.3		0.2		
16-Mar-05	10.0	11.2	7.0	0.4	0.60	0.9		
13-April-05	14.3	9.4	6.7	0.4	0.10	0.4	118	150
13-May-05	23.3	5.0	7.6	0.5	0.20	0.9	134	180
9-Jun-05	24.6	7.4	7.6	0.3	0.01	0.1	126	136
19-July-05	28.2	7.0	7.3	0.3	0.03	0.5	98	122
17-Aug-05	28.7	5.3	7.8	0.2	0.20	0.6	120	120
20-Sept-05	26.8	7.2	7.7	0.3		0.5	11	122
27-Apr-06	18.2	4.0	7.4	0.8	1.00	0.3	90	180
31-May-06	21.6	9.0	6.3	0.3	0.07	0.5	102	116
17-Jul-06	33.0	7.7	6.3	0.3	0.80	0.3	98	118
24-Aug-06	30.0	7.2	6.1	0.4	0.02	0.4	116	121
5-Oct-06	23.5	8.9	7.5	0.6	0.70	0.3		
14-Sep-07	23.9	6.7	7.9	0.4	0.05		90	116
24-Oct-07	15.4	8.1	7.6	0.4	2.60		96	144
Station 2								
4-Nov-04	14.0	8.6	6.7	0.3	0.30	ctf		
24-Jan-05	3.7	14.0	6.5	0.3		ctf		
11-Feb-05	4.5	13.6	6.7	0.3		ctf		
16-Mar-05	10.1	10.2	7.0	0.4	0.40	>Depth		
13-April-05	14.2	9.5	7.7	0.4	0.10	ctf	114	
13-May-05	23.5	7.0	7.7	0.5	0.10	1.3	136	
9-Jun-05	24.5	7.3	7.9	0.3	.0006	ctf	130	
19-July-05	28.0	7.0	7.4	0.3	.04	ctf	92	
17-Aug-05	28.6	5.6	7.9	0.3	.12	0.8	108	
20-Sept-05	26.8	7.0	7.9	0.3		0.5	108	
27-Apr-06	18.2	4.0	7.4	0.8	1.0	0.3	180	190
31-May-06	21.4	9.1	7.3	0.3	0.10	ctf	108	118
17-Jul-06	33.3	9.0	7.7	0.3	1.00	0.3	94	120
24-Aug-06	30.2	7.3	7.1	0.4	0.01	0.6	110	118
5-Oct-06	23.6	9.0	7.8	0.5	0.20	0.4		

Table 1b. Field water quality parameters from each station on the Verdigris River by site and date. For the Secchi depth ctf=current too fast.

	Temp. (°C)	DO (mg\L)	pH	Conductivity (µS/cm)	Ammonia (mg/L)	Secchi depth (m)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Station 3								
4-Nov-04	14.0	8.5	6.7	0.3		6.3		
24-Jan-05	3.7	14.0	6.3	0.3		0.4		
11-Feb-05	4.3	14.5	6.8	0.3		0.3		
16-Mar-05	10.3	11.2	7.1	0.4	0.20	>Depth		
13-April-05	14.2	9.1	6.7	0.4	0.10	0.6	118	152
13-May-05	23.4	6.9	7.8	0.5	0.10	>Depth	134	178
9-Jun-05	24.5	7.5	7.9	0.3	.0006	0.2	128	140
19-July-05	28	6.9	7.4	0.3	.04	9.4	92	118
17-Aug-05	28.6	5.3	7.9	0.2	.05	0.6	106	124
20-Sept-05	26.8	6.7	7.9	0.3		0.5	110	126
27-Apr-06	17.9	3.3	7.3	0.7	1.4	0.3	72	158
31-May-06	21.4	8.8	7.4	0.4	0.1	1.0	102	134
17-Jul-06	32.9	8.6	7.6	0.3	0.5	0.3	96	108
24-Aug-06	30.1	7.3	7.5	0.4	0.0	0.5	108	124
5-Oct-06	23.6	9.7	7.8	0.5	0.1	0.4		
Station 4								
15-Oct-04	17.8	8.4	7.2	0.3	0.3			
4-Nov-04	14.3	8.2	6.8	0.3	0.1	0.7		
24-Jan-05	13.8	13.8	6.4	0.3		0.4		
11-Feb-05	4.3	14.4	6.5	0.3		0.4		
16-Mar-05	10.1	11.5	7.1	0.4	0.2	>Depth		
13-April-05	14.2	9.0	6.5	0.4	0.2	0.3	122	156
13-May-05	23.5	5.7	7.6	0.5	0.1	>Depth	138	180
9-Jun-05	24.6	7.5	7.9	0.3	.0006	0.1	128	134
19-July-05	28.1	6.6	7.4	0.2	0.03	0.6	88	90
17-Aug-05	28.3	4.78	7.9	0.250	0.14	0.0	108	124
20-Sept-05	28.3	4.8	7.9	0.3	0.1	0.7	108	124
27-Apr-06	18.4	4.0	7.2	0.8	1.1	>Depth	64	170
31-May-06	21.6	8.6	7.5	0.3	0.1	>Depth	106	118
17-Jul-06	34.6	10.0	8.3	0.6	1.4	0.1	106	196
24-Aug-06	30.6	7.4	7.8	0.7	4.5	>Depth	112	124
5-Oct-06	24.1	10.1	8.1	0.5	0.2	>Depth		

Table 1c. Field water quality parameters from each station on the Verdigris River by site and date. For the Secchi depth ctf=current too fast.

	Temp. (°C)	DO (mg\L)	pH	Conductivity (µS/cm)	Ammonia (mg/L)	Secchi depth (m)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Station 5								
4-Nov-04	14.0	8.5	6.8	0.3	0.1	ctf		
11-Feb-05	4.3	14.5	6.5	0.3		ctf		
16-Mar-05	10.0	11.0	7.1	0.4	0.2	1.2		
13-April-05	14.3	9.3	7.3	0.4	0.1	ctf	115	150
13-May-05	23.6	5.8	7.7	0.5	0.1	1.5	134	172
9-Jun-05	24.5	7.4	7.9	0.3	.0004	ctf	130	134
19-July-05	28.1	6.8	7.4	0.3	.03	ctf	88	128
17-Aug-05	28.5	5.2	7.9	0.2	.05	0.8	106	124
20-Sept-05	27.3	7.0	8.0	0.3		0.5	110	124
27-Apr-06	18.4	3.8	7.2	0.7	1.0	0.3	60	162
31-May-06	21.5	9.1	7.5	0.3	0.1	ctf	106	112
17-Jul-06	34.4	11.0	8.4	0.6	1.1		94	120
24-Aug-06	30.5	30.5	8.0	0.5	0.04	0.6	104	118
5-Oct-06	24.1	9.6	8.0	0.6	0.2	0.5		
Station 6								
15-Oct-04								
4-Nov-04	14.0	7.9	6.8	0.3		5.0		
16-Mar-05	10.1	10.9	7.1	0.4		1.0		
13-April-05	14.3	9.2	6.5	0.4	0.1	0.5	120	138
13-May-05	14.3	9.2	6.5	0.4	0.1	0.5	120	138
9-Jun-05	24.5	7.4	7.9	0.3	.0004	ctf	130	134
19-July-05	24.5	7.4	7.9	0.3	.0004	ctf	130	134
17-Aug-05	28.5	4.8	7.9	0.3	0.17	0.8	108	126
20-Sept-05	26.9	6.6	7.9	0.3		0.4	11	128
27-Apr-06	18.0	3.4	7.3	0.8	1.0	0.3	68	168
31-May-06	21.5	8.6	7.4	0.3	0.04	ctf	114	124
17-Jul-06	33.8	9.6	8.1	0.3	0.7	0.3	100	126
24-Aug-06	30.3	7.4	7.9	0.4	0.01	0.5	108	121
5-Oct-06	24.3	8.4	7.9	0.6	0.60	0.4		
17-Jul-06	33.8	9.6	8.1	0.3	0.70	0.3	100	126
24-Aug-06	30.3	7.4	7.9	0.4	0.01	0.5	108	121
5-Oct-06	24.3	8.4	7.9	0.6	0.60	0.4		

Table 1d. Field water quality parameters from each station on the Verdigris River by site and date. For the Secchi depth ctf=current too fast.

	Temp. (°C)	DO (mg\L)	pH	Conductivity (µS/cm)	Ammonia (mg/L)	Secchi depth (m)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Station 7								
15-Oct-04	18.7	9.0	6.8	0.7	0.8			
4-Nov-04	14.4	8.1	6.8	0.3				
24-Jan-05	4.9	13.3	6.3	0.6		0.4		
11-Feb-05	4.7	14.1	6.4	0.3		0.3		
16-Mar-05	11.2	13.6	7.2	0.7	4.0	ctf		
13-April-05	17.8	8.3	6.6	1.1	4.2	>Depth	96	254
13-May-05	23.9	4.7	7.6	0.8	0.5	>Depth	118	240
9-Jun-05	25.9	7.2	7.5	0.8	.05	ctf	124	218
19-July-05	30.0	6.6	7.4	0.9	2.5	ctf	72	200
17-Aug-05	29.3	5.5	7.6	.8	4.3	ctf	104	270
20-Sept-05	28.6	6.7	7.7	0.9		ctf	100	252
27-Apr-06	19.9	7.3	7.3	1.4	9.1	ctf	76	418
31-May-06	25.5	8.8	7.5	0.8	1.8	ctf	108	248
17-Jul-06	34.2	10.4	8.1	1.6	8.3	ctf	106	390
24-Aug-06	28.8	7.2	7.9	1.5	7.7	ctf	110	322
5-Oct-06	26.8	8.9	7.9	1.7	7.1	ctf		
14-Sep-07	26.3	6.2	7.5	0.9	11.2	ctf	120	240
24-Oct-07	17.1	8.7	7.6	1.1	5.4	ctf	106	330
Station 8								
15-Oct-04	18.1	8.2	7.0	0.3	0.3			
11-Feb-05	4.5	14.5	6.6	0.3		ctf		
16-Mar-05	10.4	10.6	7.2	0.4	0.2	1.0		
13-April-05	14.3	9.3	7.3	0.4	0.5	ctf	120	148
13-May-05	23.6	5.7	7.7	0.5	0.1	1.3	120	186
9-Jun-05	24.5	7.5	7.9	0.3	.0004	ctf	124	134
19-July-05	28.2	6.7	7.4	0.3	.02	ctf	88	130
17-Aug-05	28.6	4.9	7.9	0.2	0.06	0.9	108	126
20-Sept-05	27.2	7.1	7.9	0.3		0.6	122	124
27-Apr-06	18.4	3.7	7.2	0.7	0.7	0.4	90	170
31-May-06	21.7	8.5	7.5	0.3	0.04	ctf	106	114
17-Jul-06	34.2	11.1	8.4	0.4	0.1	0.3	98	128
24-Aug-06	30.8	8.9	8.2	0.4	0.01	0.6	108	112
5-Oct-06	24.2	9.5	8.0	0.6	0.3	0.4		

Table 1e. Field water quality parameters from each station on the Verdigris River by site and date. For the Secchi depth ctf=current too fast.

	Temp. (°C)	DO (mg\L)	pH	Conductivity (µS/cm)	Ammonia (mg/L)	Secchi depth (m)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Station 9								
11-Feb-05	4.4	14.1	6.5	0.3		0.3		
16-Mar-05	10.4	10.6	7.2	0.4	0.1	>Depth		
13-April-05	14.3	8.9	6.5	0.4	0.2	>Depth	120	146
13-May-05	23.9	5.3	7.6	0.5	0.1	1.2	124	170
9-Jun-05	24.5	7.3	7.9	0.3	.0005	0.1	120	140
19-July-05	28.1	6.6	7.5	0.3	0.02			
17-Aug-05	28.6	5.0	8.0	0.2	0.04	0.8	104	124
20-Sept-05	27.0	6.5	7.9	0.3			112	124
27-Apr-06	18.8	3.9	7.3	0.8	0.8	0.3	96	160
31-May-06	21.5	8.6	7.5	0.3	0.0	ctf	104	118
17-Jul-06	33.5	8.2	8.0	0.3	0.6	0.2	94	122
24-Aug-06	30.2	7.5	7.9	0.4	0.0	0.4	108	120
5-Oct-06	24.2	8.9	7.8	0.6	0.6	0.3		
Station 10								
15-Oct-04	18.0	8.0	7.1	0.4	0.1			
24-Jan-05	4.0	14.1	6.4	0.3				
11-Feb-05	4.6	13.6	6.4	0.3		0.5		
16-Mar-05	10.5	10.5	0.5	7.5	0.2	>Depth		
13-April-05	14.3	8.3	6.5	0.4	0.1	>Depth	120	150
13-May-05	23.6	5.0	7.6	0.7	0.2	>Depth	126	192
9-Jun-05	24.7	7.4	7.9	0.4	0.003	0.2	124	140
19-July-05	28.3	6.3	7.5	0.3	0.02	0.7	92	124
17-Aug-05	28.7	4.9	7.9	0.3	0.3	>depth	102	134
20-Sept-05	27.6	6.9	7.9	0.4			108	154
27-Apr-06	19.1	4.3	7.3	0.9	1.7	0.3	98	174
31-May-06	23.5	8.6	7.7	0.6	0.6	>depth	92	140
17-Jul-06	34.8	13.1	8.6	0.4	1.9	>depth	98	128
24-Aug-06	30.9	9.7	8.2	0.4	0.1	0.4	102	128
5-Oct-06	26.3	9.1	8.0	0.1	2.7	0.3		
14-Sep-07	24.8	5.9	7.8	0.8	5.1		92	184
24-Oct-07	15.3	8.1	7.5	0.6	1.2		116	180

Table 1f. Field water quality parameters from each station on the Verdigris River by site and date. For the Secchi depth ctf=current too fast.

	Temp. (°C)	DO (mg\L)	pH	Conductivity (µS/cm)	Ammonia (mg/L)	Secchi depth (m)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Station 11								
11-Feb-05	4.5	14.4	6.5	0.3		ctf		
16-Mar-05	10.6	10.6	7.2	0.4	0.4	1.1		
13-April-05	14.3	9.0	6.5	0.4	0.2	ctf	122	146
13-May-05	23.7	5.0	7.6	0.6	0.2	1.4	124	184
9-Jun-05	24.5	7.3	7.9	0.3	.0004	ctf	126	140
19-July-05	28.2	6.6	7.5	0.3	0.2	ctf	88	112
17-Aug-05	22.6	5.1	7.8	0.2	0.1	0.8	106	124
20-Sept-05	27.3	7.0	8.0	0.3		0.4	120	124
27-Apr-06	18.4	4.8	7.3	0.7	0.7	0.3	90	172
31-May-06	21.5	8.6	7.5	0.3	0.1	ctf	96	116
17-Jul-06	34.5	9.9	8.4	4.1	0.8	0.3	110	130
24-Aug-06	30.6	9.9	8.2	0.4	0.1	0.5	102	114
5-Oct-06	23.9	8.8	7.9	0.6	0.3	0.4		
Station 12								
11-Feb-05	4.4	13.9	6.4	0.3		0.4		
16-Mar-05	10.5	10.4	7.2	0.4	0.2	>Depth		
13-April-05	14.3	9.0	7.2	0.4	0.2	0.6	122	156
13-May-05	23.9	5.5	7.6	0.5	0.2	1.1	122	176
9-Jun-05	24.4	7.3	7.9	0.3	.0004	ctf	124	140
19-July-05	28.1	6.4	7.4	0.3	0.02	0.6	84	124
17-Aug-05	28.7	4.8	7.9	0.2	0.04	0.8	108	
20-Sept-05	27.0	6.4	7.9	0.4		0.4	110	128
27-Apr-06	17.9	3.6	7.3	0.8	0.7	0.3	90	168
31-May-06	21.5	8.8	7.5	0.3	0.04	ctf	96	136
17-Jul-06	33.5	10.0	8.3	0.3	0.40	0.2	102	122
24-Aug-06	30.3	8.0	8.0	0.4	0.02	0.4	100	116
5-Oct-06	24.2	8.7	7.9	0.7	0.7	0.3		

Table 1g. Field water quality parameters from each station on the Verdigris River by site and date. For the Secchi depth ctf=current too fast.

	Temp. (°C)	DO (mg\L)	pH	Conductivity (µS/cm)	Ammonia (mg/L)	Secchi depth (m)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Station 13								
24-Jan-05	3.7	14.1	6.3	0.3		0.4		
11-Feb-05	4.6	13.9	6.4	0.3		0.3		
16-Mar-05	10.4	10.4	7.2	0.4	0.2	1.3		
13-April-05	14.3	8.9	6.6	0.4	0.1	0.6	122	156
13-May-05	24.5	5.8	7.7	0.6	0.2	>Depth	124	182
9-Jun-05	24.5	7.2	7.9	0.3	.0006	0.1	120	144
19-July-05	28.3	6.4	7.5	0.3	0.02	0.7	86	112
17-Aug-05	28.7	5.0	8.0	0.3	0.07	0.7	108	124
20-Sept-05	27.2	6.5	8.0	0.3			120	128
27-Apr-06	17.8	4.0	7.3	0.9	0.8	0.4	98	174
31-May-06	22.1	9.1	7.6	0.3	0.1	1.1	96	112
17-Jul-06	35.0	13.2	8.6	0.7	0.1	0.3	102	154
24-Aug-06	31.3	7.4	8.1	0.4	0.1	0.5	1	122
5-Oct-06	23.8	8.1	6.0	0.6	0.4	0.4		
14-Sep-07	24.1	5.0	6.3	0.4	0.02		92	128
24-Oct-07	15.5	8.7	6.5	0.4	0.03		120	160
Station 14								
11-Feb-05	4.5	14.0	6.5	0.3		ctf		
16-Mar-05	10.3	10.3	7.3	0.4	0.3	1.1		
13-April-05	14.3	9.2	6.6	0.4	0.1	ctf	122	154
13-May-05	24.1	5.6	7.7	0.6	0.2	1.3	124	180
9-Jun-05	24.4	7.5	7.9	0.3	.0006	ctf	120	138
19-July-05	28.2	6.6	7.5	0.3	0.03	ctf	86	120
17-Aug-05	28.6	4.6	7.9	0.3	0.08	0.8	108	124
20-Sept-05	27.3	7.0	7.8	0.4		0.4	112	130
27-Apr-06	18.1	3.7	7.2	0.8	0.7	0.4	96	192
31-May-06	22.1	9.1	7.6	0.3	0.04	ctf	96	126
17-Jul-06	33.0	12.8	8.6	0.4	0.30	0.3	98	140
24-Aug-06	31.0	9.5	8.3	0.4	0.04	0.4	112	118
5-Oct-06	23.8	8.1	7.0	0.6	0.8	0.6		

Table 1h. Field water quality parameters from each station on the Verdigris River by site and date. For the Secchi depth ctf=current too fast.

	Temp. (°C)	DO (mg\L)	pH	Conductivity (µS/cm)	Ammonia (mg/L)	Secchi depth (m)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Station 15								
11-Feb-05	4.5	14.0	6.5	0.3		0.4		
16-Mar-05	10.2	10.2	7.3	0.4	0.4	1.2		
13-April-05	14.3	9.0	6.6	0.4	0.1	0.5	122	146
13-May-05	24.7	5.5	7.7	0.6	0.1	1.1	122	180
9-Jun-05	24.5	7.4	7.9	0.3	.0003	0.1	126	136
19-July-05	28.2	6.6	7.5	0.3	0.03	0.6	84	128
17-Aug-05	28.6	4.7	7.9	0.3	0.13			
20-Sept-05	27.3	6.5	8.0	0.3		2.3	112	128
27-Apr-06	18.2	4.0	7.3	0.9	0.7	0.4	98	186
31-May-06	21.9	8.5	7.6	0.3	0.03	>Depth	94	114
17-Jul-06	34.0	11.4	8.5	0.4	0.4	0.3	108	136
24-Aug-06	30.5	9.8	8.3	0.4	0.0	0.4	102	110
5-Oct-06	21.4	7.6	7.2	0.5	0.3	0.5		

Appendix 2: Aquatic Macroinvertebrate Taxa Data

Table 2a. Total macroinvertebrates collected 6/9/05 from the Verdigris River.

Taxa	**Site**										
Ephemeroptera	1	3	4	7u	7i	7d	9	10	12	13	15
Baetis											
Caenis	41	66	40	27	25	0	68	3	missing	57	64
Isonychia			17								
Stenonema	68	43	64	57	20		26	14		23	27
Trichoptera											
Cernotina	4	5	34	5	1						
Hydropsyche	3	3	409	5	1			36			
Wormaldia											
Diptera											
Chironomidae	47	25	13	130	93		28			10	20
Culicoides											
Tipulidae											
Odonata											
Argia											
Erythemis											
Coenagrionidae											
Didymops											
Enallagma	5		5	4	1					5	
Gomphus											
Lestes											
Neurocordulia											
Progomphus											
Coleoptera											
Berosus larvae											
Gyretes adult											
Gyretes larvae											
Haliplus adult											
Stenelmis adult	4				1					1	
Stenelmis larvae		4	6	1			7	1		2	1
Veneroida											
D. polymorpha	23	8	8	43	23		17	11		62	43
C. fluminea											
Sphaeridae	1										
Megaloptera											
Corydalus	1	1		1	2						1
Gastropoda											
Ferrissia		1									1
Physella				13							
Others											
Hirudinidae											
Oligochaeta											1
Richness	**10**	**9**	**9**	**10**	**9**	**0**	**5**	**5**	**0**	**7**	**8**
Average Shannon diversity	**1.46**	**0.96**	**1.04**	**1.31**	**1.20**	**0**	**0.91**	**0.78**	**0**	**0.97**	**0.87**

Table 2b. Total macroinvertebrates collected 8/9/05 from the Verdigris River.

Taxa	Site										
Ephemeroptera	**1**	**3**	**4**	**7u**	**7i**	**7d**	**9**	**10**	**12**	**13**	**15**
Baetis											
Caenis	143	153	120	147	29	68	210	351	169	24	199
Isonychia											
Stenonema	52	90	24	77		3	39	40	52	50	43
Trichoptera											
Cernotina	7	38	6	9	3	9	18	51	20	1	15
Hydropsyche		44		1			52	8	48		9
Wormaldia											
Diptera											
Chironomidae	24	40	41	35	53	193	58	51	77	30	20
Culicoides											
Tipulidae							2				
Odonata											
Argia											
Erythemis											
Coenagrionidae											
Didymops											
Dromogomphus					1					2	
Enallagma	9	7	3	9		6	13	2	2	20	6
Gomphus											
Lestes											
Neurocordulia								1			
Progomphus											
Coleoptera											
Berosus larvae											1
Gyretes adult											
Gyretes larvae											
Haliplus adult											
Stenelmis adult				15			3	3	7		8
Stenelmis larvae	1		12	7	6	2	2	2	4		2
Veneroida											
D. polymorpha	1	46	6		1		2	31		9	1
C. fluminea											
Sphaeridae										1	
Megaloptera											
Corydalus				3					1		1
Gastropoda											
Ferrissia				4	1			1			
Physella					4	68		6			
Others											
Hirudinidae											
Oligochaeta					8						
Richness	**7**	**7**	**7**	**10**	**9**	**7**	**10**	**12**	**9**	**8**	**11**
Average Shannon diversity	**0.99**	**1.02**	**0.63**	**1.39**	**0.78**	**0.59**	**0.95**	**0.90**	**1.39**	**1.01**	**0.95**

Table 2c. Total macroinvertebrates collected 11/11/05 from the Verdigris River.

Taxa	Site										
Ephemeroptera	**1**	**3**	**4**	**7u**	**7i**	**7d**	**9**	**10**	**12**	**13**	**15**
Baetis											
Caenis		9	90	3	1		1	116	9		
Isonychia			6	6							
Stenonema	12	21	94				38	36	28	54	25
Trichoptera											
Cernotina	14	55	36	26	35	15	32	58	26	50	22
Hydroptila			6								
Hydropsyche								6			
Wormaldia											
Plecoptera											
Acroneuria								1			
Diptera											
Chironomidae	33	84	210	113	154	52	269	173	83	158	158
Culicoides											
Tipulidae											
Odonata											
Argia											
Erythemis											
Coenagrionidae											
Didymops											
Enallagma	3	6	7	2	6	7	10	6	7	8	10
Gomphus											
Lestes											
Neurocordulia											
Progomphus											
Coleoptera											
Berosus larvae											
Dytiscus larvae	1										
Georyssus larvae			1								
Gyretes adult											
Gyretes larvae											
Haliplus adult											
Stenelmis adult	1						4		1		1
Stenelmis larvae	2	2	1	1				1	2	3	7
Veneroida											
D. polymorpha	33	96	65	27	31	16	114	41	98	78	97
C. fluminea	1										
Sphaeridae							1			2	
Megaloptera											
Corydalus		1	1	2							
Gastropoda											
Ferrissia	1	6	8					9		1	
Physella				2	2	3		6			4
Others											
Hirudinidae											
Oligochaeta											
Richness	**10**	**9**	**12**	**9**	**6**	**5**	**8**	**11**	**8**	**8**	**8**
Average Shannon diversity	**1.23**	**1.45**	**1.39**	**0.99**	**0.55**	**0.61**	**1.11**	**1.24**	**1.35**	**1.20**	**1.30**

Table 2d. Total macroinvertebrates collected 7/17/06 on from the Verdigris River.

Taxa	Site										
Ephemeroptera	**1**	**3**	**4**	**7u**	**7i**	**7d**	**9**	**10**	**12**	**13**	**15**
Baetis											
Caenis	1	9	3			1	1	3	1		
Isonychia										158	
Stenonema	27	16	10				36	10	46		16
Trichoptera											
Cernotina	31	20	46	48			25	59	79	8	15
Hydropsyche								1	35	2	
Wormaldia											
Diptera											
Chironomidae	233	139	299	70	11	76	215	151	228		38
Culicoides							1			3	
Tipulidae											
Odonata											
Argia										78	
Erythemis											
Coenagrionidae										1	
Didymops											
Enallagma	8	2	13	1		9	10	1	12		16
Gomphus											
Lestes											
Neurocordulia									1	1	
Progomphus											
Coleoptera											
Berosus adult	1					8					
Berosus larvae											
Gyretes adult											
Gyretes larvae											
Haliplus adult											
Stenelmis adult			2				2	1	9		
Stenelmis larvae										1	
Veneroida											
D. polymorpha							1			1	
C. fluminea											
Sphaeridae										2	2
Megaloptera											
Corydalus											
Gastropoda											
Ferrissia			2								
Physella				5	19	53					
Others											
Hirudinidae											
Oligochaeta											
Richness	**6**	**5**	**7**	**4**	**2**	**5**	**8**	**7**	**8**	**10**	**5**
Average Shannon diversity	**0.80**	**0.76**	**0.67**	**0.80**	**0.29**	**0.94**	**0.87**	**0.80**	**0.80**	**1.20**	**0.98**

Appendix 2e. Total macroinvertebrates collected 8/24/06 on from the Verdigris River.

Taxa	Site										
Ephemeroptera	**1**	**3**	**4**	**7u**	**7i**	**7d**	**9**	**10**	**12**	**13**	**15**
Baetis							2				1
Caenis	3	3	7	15	23	43	15	6	26	6	8
Isonychia											
Stenonema	36	23	9	1			47	10	68	12	33
Trichoptera											
Cernotina	10	25	13	34	5	5	10	24	69	19	41
Hydropsyche											
Wormaldia	1					1				1	
Diptera											
Chironomidae	52	74	27	105	150	169	55	97	306	127	255
Culicoides		3					1		1	5	2
Tipulidae											
Odonata											
Argia	6	2	1	3	8	3		1	19	6	27
Erythemis										2	
Coenagrionidae				2							
Didymops				1			1	1			
Enallagma						8	1		1	1	
Gomphus							1				
Lestes							3				
Neurocordulia		1									
Progomphus		1									
Coleoptera											
Berosus larvae		1				3					
Gyretes adult		3									
Gyretes larvae	1	1				1			1		
Haliplus adult							3		8	2	2
Macronychus larvae							1				
Stenelmis adult				1		1			37	2	15
Stenelmis larvae								1	4	1	
Veneroida											
D. polymorpha											
C. fluminea											
Sphaeridae		5							1	1	1
Megaloptera											
Corydalus											
Gastropoda											
Ferrissia	4									5	
Physella	5			20	93	8				1	
Others											
Hirudinidae					1						
Oligochaeta		1		3							
Richness	**9**	**13**	**5**	**10**	**6**	**10**	**12**	**7**	**12**	**15**	**10**
Average Shannon diversity	**1.22**	**1.24**	**1.28**	**1.28**	**1.04**	**0.93**	**1.30**	**0.86**	**1.35**	**1.24**	**1.07**

VITA

Phillip Lee Cravatt

Candidate for the Degree of

Master of Science

Thesis: FIELD AND LABORATORY EVALUATION AN INDUSTRIAL EFFLUENT CONTAINING ELEVATED LEVELS OF AMMONIA

Major Field: Environmental Science

Biographical:

Personal Data: Born Tulsa, Oklahoma on November 23rd, 1968.

Education: Received Bachelor of Science Degree in Zoology from Oklahoma State University from Stillwater, Oklahoma in May 2004. Completed the requirements for the Master of Science in Environmental Science at Oklahoma State University, Stillwater, Oklahoma in July, 2009.

Experience: Employed by Oklahoma State University Department of Zoology as a research assistant from September 2003 to December 2007.

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Name: Phillip Lee Cravatt Date of Degree: July, 2009

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: FIELD AND LABORATORY EVALUATION OF AN INDUSTRIAL EFFLUENT CONTAINING ELEVATED LEVELS OF AMMONIA

Pages in Study: 127 Candidate for the Degree of Master of Science

Major Field: Environmental Science

Scope and Method of Study: This study evaluated the effects of an industrial wastewater effluent that contains varying levels of ammonia on the biota in a reach of the Verdigris River in Oklahoma. This investigation was undertaken using both laboratory toxicity tests, as well as field site monitoring including community assessments and *in situ* techniques, water quality monitoring, macroinvertebrate and fish community sampling and an *in situ* zebra mussel (*Dreissena polymorpha*) study. A series of 48-hr laboratory bioassays using the fathead minnow (*Pimephales promelas*) were performed in unadjusted and pH manipulated effluent and laboratory water (pH 8.5 and 9.0). Additionally ammonia levels (total) were adjusted to 10, 20 and 30 mg NH_3-N/L, for another series of toxicity tests with both unadjusted and manipulated test waters. Unadjusted (pH) effluent at all ammonia levels showed no toxicity until total ammonia levels reached 30 mg NH_3-N/L (65.2%) whereas at pH 8.5 LC50 values at 20 and 30 mg NH_3-N/L total ammonia were 58.5% and 38.1%, respectively. The largest effect on toxicity was observed in the effluent at pH 9.0 with LC50 values of 66.4% at 10mg/L, 23.1% at 20 mg/L and 16.2% at 30 mg/L total ammonia. The ammonia solutions were generally more toxic to the fathead minnows than the effluent samples with similar ammonia concentrations, which could be an indication that the effluent matrix ameliorated ammonia toxicity. In the on-site field study, there were no significant differences at the effluent outflow site compared with sites up and down–river on the macroinvertebrate communities, zebra mussel growth and wet: dry weight, although the effluent did attract more fish in the immediate vicinity of the outflow (not significant). Overall, the results from the study indicate the effluent is not having any significant adverse effects on the receiving system.

ADVISER'S APPROVAL: Dr. Joseph R. Bidwell