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Behavior and Ecosystem Effects of the Invasive Asian Clam (Corbicula fluminea) In Urban Streams

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BEHAVIOR AND ECOSYSTEM EFFECTS OF THE INVASIVE ASIAN CLAM (*CORBICULA FLUMINEA*) IN URBAN STREAMS

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ABSTRACT

Invasive species can be detrimental to freshwater ecosystems. By completing laboratory and field studies to observe processes and behaviors of the invasive Asian Clam (*Corbicula fluminea*), I documented pathways whereby this invasive species impacts aquatic ecosystems under conditions typical of urbanized streams. The predominant pathways by which clams impacted nitrogen (N) cycling were through excretion, thus increasing ammonium (NH_4^+) flux out of sediment, and through bioturbation, which increased nitrate (NO_3^-) diffusion to the sediment and dinitrogen gas (N_2) production (i.e., denitrification). The effect was greater under urban conditions, where *C. fluminea* population density and water column NO_3^- were higher than in the rural stream. Urban environmental conditions also negatively impacted the clams' physiology and mortality. The decline in clam condition and high mortality rates, particularly under high nutrient conditions, suggest that it may not be the tolerance of the individuals that allows for the persistence of successful populations, but the life history strategies of the species. Conducting laboratory and field studies on clams' ecosystem effects inspired questions about what factors control clams' burial behavior. In laboratory experiments on clam behavior, I found that larger substrates impeded burrowing ability. Despite ease of movement in smaller substrates, clams did not preferentially choose one substrate over another or move laterally once buried. I also found that presence of predators did not affect burial speed or number of clams that buried unless the predator

was frequently manipulating the clams. Learning how invasive species behave and how they affect the ecosystem is crucial to the management and prevention of initial invasion, and I hope that my research will be of help in those efforts.

CHAPTER I

INTRODUCTION

The threat of non-native species invasions

Biological systems are in a delicate balance in which every component is interconnected. Biological invasion by non-native species are a major threat to ecosystem balance, especially in freshwater environments (Carpenter et al., 2011). A major concern regarding invasions is their impact on native organisms and ecosystem processes such as nutrient cycling and primary production. Invaders form new species assemblages that change ecosystem function or reduce native species abundance through competition, predation, or indirect effects (Sax et al., 2007). There are not only ecological changes associated with invasive species, but economic impacts as well. The economic cost of damages or control measures from invasive species is estimated at $120 billion per year in the United States (Pimentel et al., 2005). Despite these obvious detriments, if there is a silver lining to be found, it may be that invasive species provide new experiments that allow for research on factors associated with global change such as extinctions, speciation and ecosystem functions (Sax et al., 2007).

Research on invasive species in freshwaters is critical because these ecosystems are imperiled, and provide numerous important services (Dudgeon et al., 2006). While intentional introduction of non-native species into aquatic environments has decreased, freshwater ecosystems remain vulnerable to the introduction of new invasive

species through shipping, recreation or accidental release (Ricciardi, 2006). These methods of translocation are exacerbated by human population growth and movement, and anthropogenic stressors such as urbanization (Pimentel et al., 2005).

Some of the most well-known and successful invasive species in freshwater ecosystems are bivalves (Sousa et al., 2009). The most common are the zebra mussel (*Dreissena polymorpha*), quagga mussel (*Dreissena bugensis*), golden mussel (*Limnoperna fortunei*), Chinese pond mussel (*Anodonta woodiana*), and the Asian clam (*Corbicula fluminea*) (Strayer, 2009; Darrigran and Damborenea, 2005; Paunovic et al., 2006; Sousa et al., 2008a). These bivalve taxa often occur at very high densities, becoming the dominant invertebrates in terms of biomass. Their rapid growth and high fecundity allow them to sustain high populations and rapidly recolonize after population crashes (Sousa et al., 2009; Sousa et al., 2008b). Each of these bivalves acquires nutrients through the filtration of phytoplankton, bacteria, and organic material from the water column, and can influence nutrient dynamics through their excretion (Vaughn and Hakenkamp, 2001). In fact, bivalve populations have been shown to have equal or even greater filtration rates than all other filter-feeders in their respective ecosystems (Strayer et al., 1999). Zebra mussels, quagga mussels, and golden mussels attach to hard substrates, or to one another, using byssal threads, and can lead to major biofouling problems. The pond mussel and Asian clam are burrowing bivalves. Their burrowing behavior can disturb the sediment-water interface, increasing sediment oxygen reduction and nutrient diffusion through bioturbation (Vaughn and Hakenkamp, 2001).

Corbicula fluminea as an invasive species and ecosystem engineer

The Asian clam, *Corbicula fluminea*, was first described in 1774 by O. F. Muller as one of three species in the genus *Tellina*, and was later described by Megerle von Mühlfeld in the genus *Corbicula* (Araujo et al.,1993). Originally found in Southeast Asia, the Pacific Islands, and some parts of Europe and Africa, *C. fluminea* was first documented in the United States in Washington State in the 1930's (McMahon, 1983). Exhibiting such characteristics as early maturation, short life-span, and high fecundity, *C. fluminea* has become widespread throughout the United States (Sousa et al., 2008a). Population size and biomass are highly variable depending upon location and time of year, but they are typically in high densities (1-269,000 individuals m^{-2}; Schmidlin and Baur, 2007; Cherry et al., 2005; Sousa et al., 2008b). This species is a burrowing bivalve found in sandy substrata and is also a filter feeder (Araujo et al., 1993). *C. fluminea* resources overlap with native bivalves in the family Unionidae (freshwater mussels) and Sphaeriidae (fingernail clams), and represent a potential competitor to those species (Atkinson et al., 2011).

A unique adaptation of *C. fluminea* is its capacity to supplement its filter feeding with pedal feeding, or ingesting organic material directly from the sediment. This method of obtaining nutrients has an effect on sediment characteristics, organic matter cycling, and other benthic organisms (Hakenkamp et al., 2001; Sousa et al., 2008a). Bioturbation, the mixing of sediments by an organism through behaviors such as burrowing, can increase sediment oxygen (O_2) penetration, exchange of nutrients between the water column and sediment pore spaces, reduce organic matter through consumption, and

dislodge other benthic macroinvertebrates (Vaughn and Hakenkamp, 2001). This is an important adaptation for *C. fluminea* because filter-feeding alone may not provide enough nutrients to fully support the clams' metabolism (Boltovskoy et al., 1995).

Another adaptation that allows *C. fluminea* to contend with low food availability is its valve closure behavior. *C. fluminea* can regularly partake in extended periods of valve closure (10-12 hours), remaining aerobic inside the valve for the first few hours and then becoming anaerobic. Several other bivalve species including zebra mussels (*Dreissena polymorpha*) and pisiid clams (*Sphaerium corneum* and *Pisidium amnicum*) have been documented to exhibit valve closure of several hours. This behavior allows for reduced metabolic costs during periods of food resource limitation or other stressful environmental conditions such as predation or water contamination (Ortmann and Grieshaber, 2003).

Despite its capacity to withstand brief periods of duress through valve closure, *C. fluminea* is subject to large die-offs caused by factors such as siltation, extreme high or low temperatures, and low dissolved oxygen (DO), especially in the winter (Cherry et al., 2005; French and Schloesser, 1996). Consequently, there is a release and accumulation of high concentrations of ammonia (NH_3^+), which can reduce water quality to the detriment of other benthic organisms (Cherry et al., 2005; Wittmann et al., 2012). While the soft clam tissue quickly decomposes or provides food for other organisms, the valves (i.e., shells) remain on the benthos for long periods of time (Sousa et al., 2008b). As a result, one impact of *C. fluminea* invasion is that it can provide a new, hard substrate in otherwise soft-bottomed streams. Empty *C. fluminea* shells left behind after die-offs

reach high densities. For example Werner and Rothhaupt (2007) reported an average density of 2,000 shells m^{-2} in Lake Constance in Central Europe. Hard shells provide habitat for organisms such as epiphytic and epizoic organisms and increase population densities of mayflies and leeches (Vaughn and Hakenkamp, 2001; Werner and Rothhaupt, 2007), but the presence of shells of live clams has negative effects on the abundance of bacteria and flagellates, possibly due to bioturbation or consumption (Hakenkamp et al., 2001).

Along with altering the physical composition of the stream benthos, *C. fluminea* can impact biogeochemical processes (Sousa et al., 2008a) through elevating nutrient concentrations via excretion and mineralization of their biodeposits (i.e, feces; Vaughn and Hakenkamp, 2001) and by enhancing diffusion of water and nutrients across the sediment-water interface through burrowing and pedal feeding (Zhang et al., 2011). *C. fluminea* can increase the inorganic nitrogen (N) concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) in porewaters, which serve as chemical substrates for important N transformations (Chen et al., 2005; Zhang et al., 2011). Because *C. fluminea* can increase concentrations of N species needed for nitrification and denitrification, and occur at high densities, *C. fluminea* has the potential to have a significant effect on nitrification and denitrification at the stream reach scale. However, its influence on sediment N transformation rates have not been previously documented.

Urban Streams

Historically, urban areas have been centered near river ecosystems, which is why many rivers and streams have suffered degradation from urbanization effects (Francis,

2012). Urbanization and the expansion of impervious surfaces can cause higher runoff and flood events, which alters channel morphology (Wang et al., 2001). Higher nutrient and contaminant concentrations associated with effects of urbanization (i.e., effluent from industry, wastewater treatment, and road runoff) decrease species richness by selecting taxa most 'tolerant' of urbanized conditions (Walsh et al., 2005). *C. fluminea* is considered one of these tolerant taxa because it can thrive in poor quality water and habitats due to its short generation time, high fecundity, flexible feeding mechanisms, phenotypic plasticity, and preference for sandy, loose substrates typical of urban streams (Sousa et al., 2008a). Because *C. fluminea* has high population densities in urban streams with high N loads, understanding its influence on N fluxes in urban conditions will be critical for management of water and habitat quality in these ecosystems.

Economic repercussions of *Corbicula fluminea* colonization

Corbicula fluminea invasion has potential economic impacts. Accumulation of empty shells may create new habitats, but they also impact recreation and fishing by becoming trapped in nets (Sousa et al., 2008b). Shells are also associated with biofouling, or the blockage of pipes and water lines, particularly near power plants and industrial water systems (Robinson and Wellborn, 1988; Darrigran, 2002). In the United States, *C. fluminea* is estimated to cost approximately $1 billion per year in damages and control measures (Pimentel et al., 2005). There have been some effective treatments for *C. fluminea*, such as screens and filters, physical removal, or chemical treatments, but most treatment approaches are tailored for power plant intake pipes, not open water, and costs

of *C. fluminea* mitigation remain a prominent conservation concern (Sousa et al., 2008b; Wittmann et al., 2012).

Experimental Design: *C. fluminea* ecosystem effects and behavior

For a comprehensive analysis of how *C. fluminea* affects ecosystem processes, and environmental factors that influence its behavior, I completed two studies. First, a combined laboratory and field study to test the clams' ecosystem effects in urban stream conditions, and second a behavioral study in the lab to observe bottom-up and top-down drivers of *C. fluminea* burrowing behaviors.

My first objective, discussed in Chapter II, was to design a laboratory study to examine the effects of urban stream conditions on *C. fluminea*. We developed a controlled experiment in Loyola University Chicago's artificial stream facility to mimic urbanized stream characteristics. We set up 8 streams consisting of 3 fully-crossed treatments: added nutrients, added sediment organic matter, and clams. We then measured clam condition, N transformations, and ecosystem metabolism over 9 weeks. Results from this laboratory study positioned me well to select factors to test in the subsequent field study.

The second component of Chapter II was to carry out a complementary field study to test the influence of *C. fluminea* on stream biogeochemistry in an urban stream relative to a rural, forested stream. This was done using two streams of similar geomorphology and with persistent populations of *C. fluminea*. For the urban site, we chose a reach in the North Branch of the Chicago River at Harms Woods in Cook County, Illinois. For the rural site, we selected Eagle Creek, part of the Kalamazoo River

Watershed near Augusta, Michigan. The Chicago River reach exhibited characteristics typical of urban streams such as elevated nutrient concentrations and eroded banks. The substrate composition was highly variable, although the majority was sand, gravel, and empty *C. fluminea* shells. The substrate composition in Eagle Creek was predominantly sand and gravel and nutrient concentrations were low. We deployed an experiment where sediment was incubated in plastic trays with and without clams at each site. After six weeks incubation in the streams, the trays were collected and we measured the clams' effects on sediment organic matter and N transformations, as well as clam condition and excretion rates in each stream.

A conceptual diagram for the relationships among *C. fluminea*, N transformations, and gross primary production (GPP) is shown in Figure 1. In this study, I hoped to demonstrate the effects of *C. fluminea* populations on ecosystem processes, in an urban and rural stream. I expected that C. *fluminea* would increase the rate of nitrification due to the high levels of porewater NH_4^+ released through excretion and mortality, and increased oxygenation of sediment through their burrowing. Denitrification should also be increased, as nitrification can increase NO_3^- availability (i.e., indirect denitrification or coupled nitrification-denitrification). In addition, clam burrowing can increase diffusion of water column NO_3^- into sediment where it can be denitrified (i.e., direct denitrification). I also expected to see a decrease in primary production and respiration as clams consume water column and sediment microbes and primary producers. I presumed that *C. fluminea* would exhibit better condition and survivorship in streams with higher nutrients and sediment organic matter due to the increased food resources. Finally, due to

the high concentrations of C and N in urban streams, I expected the clams' effect on N cycling would be masked in the Chicago River, and that the clams in the rural stream, Eagle Creek, would have more of an impact on nitrogen cycling.

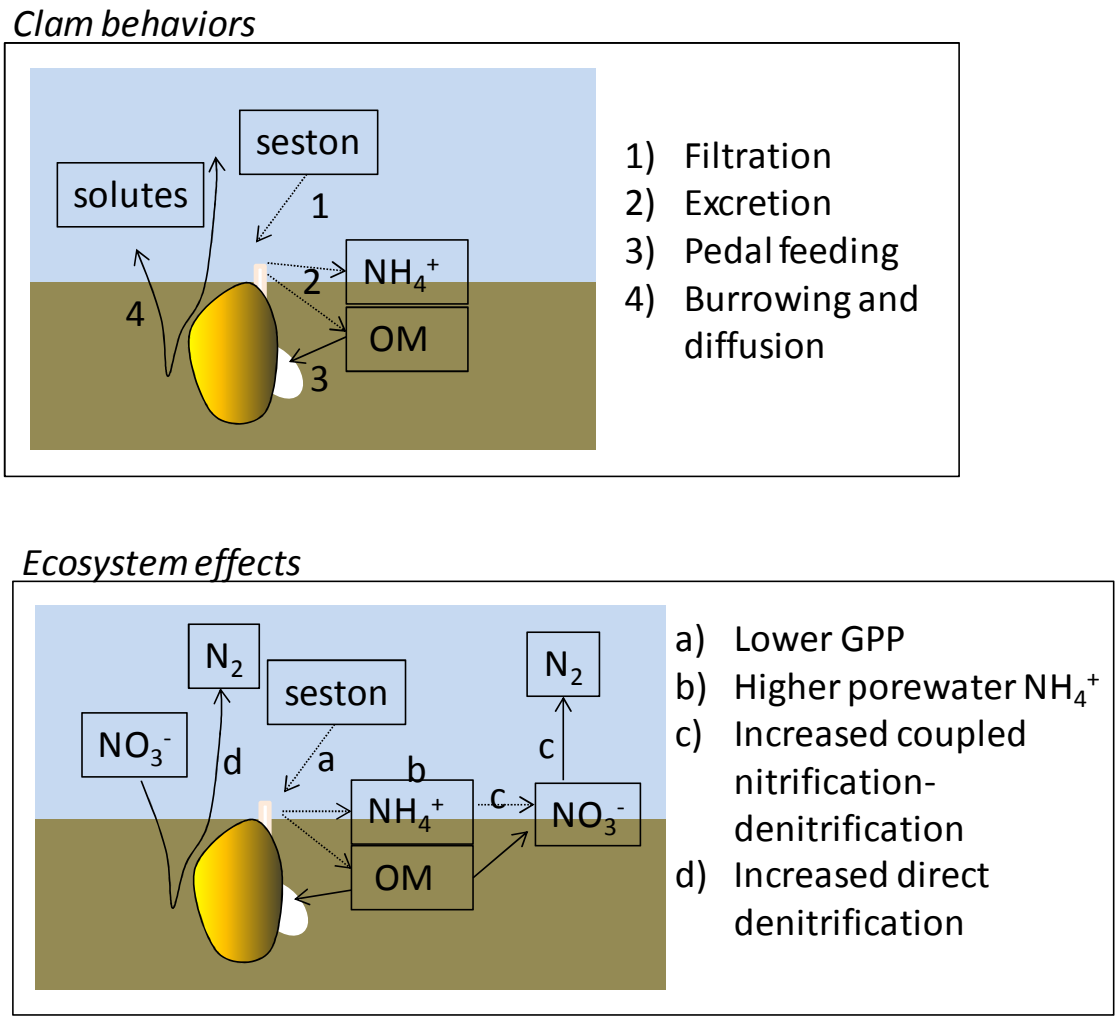

Figure 1. Conceptual diagram of hypothesized ecosystem effects of *Corbicula fluminea*

My second objective, discussed in Chapter III, was to measure external (i.e., substrate composition and crayfish predation) drivers of *C. fluminea* burrowing behavior.

The clams are most commonly found in soft-bottomed streams (Araujo et al., 1993) and their predators in invaded habitats are predominantly fish and crayfish, but include some birds and mammals as well (Saloom and Duncan., 2005). The research questions for the bottom-up effects included:

(1) Do large substrates inhibit *Corbicula* burrowing behavior?

(2) Does *C. fluminea* move horizontally?

(3) Is choice involved in substrate association?

I expected that clams would burrow more quickly in finer substrates such as sand and organic matter versus larger substrates like gravel due to ease of movement. I also expected to see more lateral movement in finer substrates. Finally, I expected that clams would show a preference for sand + organic matter over gravel due to ease of burrowing and additional food resources present.

To examine predator interactions, I tested the effect of another invasive species, the rusty crayfish (*Orconectes rusticus*) on clam burrowing behavior. Crayfish are natural predators and scavengers of *C. fluminea* (Covich et al., 1981). Our questions were:

(1) Does the predator presence influence clam burrowing behavior?

(2) Does the intensity of the interaction of a predator affect clam burrowing behavior?

We measured clam burrowing behavior as the speed of burial and the proportion of clams buried in a 24 hour period. I expected that clams would burrow more quickly in the presence of a predator that was unrestricted and if the predator was sensed (i.e., caged predator) than in the absence of a predator, because increased burial speed could be an important defense mechanism for the bivalves. I also predicted that more interactions

with the predator would reduce burrowing success due to repeated predator-induced valve closure. By answering these questions, I hoped to better understand the abiotic and biotic controls on *C. fluminea* burrowing behavior, as these could be important considerations for management applications that mitigate current invasions or prevent additional *C. fluminea* range expansion.

CHAPTER II

ECOSYSTEM EFFECTS OF THE INVASIVE ASIAN CLAM (*CORBICULA FLUMINEA*) IN URBAN STREAMS

Introduction

The introduction of invasive species can have multiple negative environmental and ecological effects, and is now considered to be one of the predominant causes of environmental change globally (Vitousek et al., 1990; Mack et al., 2000; Carpenter et al., 2011). A species is typically labeled invasive or non-indigenous if it has been introduced to a novel area through anthropogenic means and has established a subsistent population (Sax et al., 2007). Not all species that are introduced can persist in a new environment, and of those that do, only some will be detrimental to the ecosystem (Williamson and Fitter, 1996; Carpenter et al., 2011). Characteristics typical of successful invaders are: large geographical distribution, genetic variability and phenotypic plasticity, tolerance to abiotic changes, short generation times, rapid sexual maturity, high fecundity, and opportunistic feeding behavior. These characteristics can be advantageous for species colonizing an area with regular disturbances (Sousa et al., 2008b).

Aquatic invasive species affect physicochemical conditions and biodiversity of their invaded habitats (Strayer et al., 1999). Invaders can establish a niche in their invaded range because native organisms have no evolutionary history with the invader. Invasive species can also have multiple effects on ecosystem structure and function, as

invaders can alter pools or fluxes of nutrients including carbon, nitrogen, and oxygen (Sax et al., 2007). Aside from ecological effects, invasive species cause millions of dollars per year in damages or control measures (Pimentel et al., 2004).

Along with other factors such as changes in climate, land use, and pollution (i.e., eutrophication and industrial chemicals), aquatic invasive species are a major reason freshwater ecosystems are among the most highly altered worldwide (Carpenter et al., 2011). In the Great Lakes Basin for example, there are nearly 200 non-indigenous species that have established populations since 1840. While intentional introduction has decreased, the rates of unintentional introduction through activities like shipping have continued to increase (Ricciardi, 2006).

Some of the most well-studied invasive species in freshwaters include the Eurasian round goby (*Neogobius melanostomus*) and dreissenid mussels (i.e. zebra and quagga mussels). The round goby is native to the Black and Caspian Seas but populations have spread rapidly in the United States, particularly in the Great Lakes region (Kipp and Ricciardi, 2012). Dreissenid mussels are widespread in the US and cause dramatic ecological changes such as eutrophication and habitat destruction (Strayer, 2009). Quagga mussels are similar in morphology and function, however they are able to persist in soft-bottomed bodies of water where zebra mussels prefer hard substrates (Patterson et al., 2002).

Aside from dreissenid mussels, one of the most abundant bivalve invaders of freshwater ecosystems is the Asian clam, *Corbicula fluminea* (Muller1774). It first invaded the United States in the 1930’s, and is present throughout much of the

continental US (Araujo et al., 1993). Like other invasive species, *C. fluminea* exhibits typical r-strategy life history including early maturity, rapid growth, and high fecundity (Sousa et al., 2008b). One prominent reason for *C. fluminea*'s success as an invasive species is its non-selective diet. It can filter particles from the water column (i.e., filter-feed) and feed on sediment particles using its foot (i.e., pedal feed), thus allowing for maximum exploitation of available resources (Reid et al., 1992).

Recent studies have demonstrated multiple effects of *C. fluminea* on native taxa. High densities of the clam were negatively correlated with the population density of benthic bacteria and flagellates (Hakenkamp et al., 2001). The large number of shells in invaded habitats provides hard substrates in otherwise soft-bottomed areas, which have been associated with an increase in other species such as mayflies and leeches (Werner and Rothhaupt, 2007). In addition, *C. fluminea* is also subject to mass die-offs caused by low dissolved oxygen and overwintering mortality, which elevates ammonia (NH_3) concentrations. This can accumulate in porewater to levels that are lethal to native mussels (French and Schloesser, 1996; Cherry et al., 2005).

Corbicula fluminea is of particular concern where it occurs in association with native mussels in the family Unionidae, which are the most highly threatened freshwater species in the world (Atkinson et al., 2011). Unlike *C. fluminea*, unionid mussels are long-lived, have highly specialized relationships to fish species that host their parasitic juveniles (i.e., glochidia), and live in dense colonies within select benthic habitats (Neves and Widlak, 1987). In contrast, *C. fluminea* are short-lived, reproduce without fish hosts or long-lived juvenile stages, and may be less selective for benthic substrates. By filling a

broader trophic niche than their native competitors, *C. fluminea* may be able to better utilize the available resources (Atkinson et al., 2010). The decline in native mussels and the increase of *C. fluminea* could cause significant changes in ecosystem processes. While native mussels and *C. fluminea* share some of the same functional roles (i.e., burrowing and filter feeding), there may be differences in rates of feeding, excretion compounds, and sources of food (Vaughn and Hakenkamp, 2001; Atkinson et al., 2011).

Corbicula fluminea, like other burrowing bivalves, affect nutrient cycling in aquatic ecosystems through excretion, biodeposition, and bioturbation (Vaughn and Hakenkamp, 2001). Excretion contains high amounts of inorganic nutrients which are released into the ecosystem (Sousa et al., 2008a), and *C. fluminea*, excretion can release nutrients in excess of benthic nutrient demand (Lauritsen and Mozley, 1983). Their burrowing action indirectly impacts nutrient dynamics by increasing exchange of solutes and oxygen between the water column and sediment (Vaughn and Hakenkamp, 2001). Burrowing can also increase sediment microbial activity, leading to more rapid degradation of organic matter and nutrient mineralization and flux from sediment (Zhang et al., 2011). As *C. fluminea* range expansion continues, it is increasingly important to understand environmental drivers of its effects on different types of aquatic ecosystems.

Corbicula fluminea is a successful invader of aquatic environments with low human impact (i.e., “pristine” ecosystems), as well as streams and lakes influenced by urban land-use. Urban rivers are characterized by multiple environmental stressors, including higher nutrient concentrations, changes in the width and depth of the channel (i.e., increased flooding during storms and decreased flow during dry periods), and

changes in species richness and diversity (i.e., decrease in species richness and increase in tolerant species) (Walsh et al.,2005). Most previous studies of *C. fluminea* have taken place in aquatic ecosystems with low-human influence, so their ecosystem effects in urbanized locations have not been quantified. Our research questions were (1) does *C. fluminea* change rates of nitrogen (N) cycling and ecosystem metabolism via their filtration, burrowing and excretion? and (2) how does the influence of *C. fluminea* differ in an urban relative to a rural stream? We addressed these questions in a laboratory study and a field experiment.

Materials and Methods

Artificial stream study

This 9 week study was conducted in the artificial stream facility at Loyola University Chicago. The purpose of the study was to measure clam survivorship and ecosystem effects under conditions typical of urbanization. Artificial streams were re-circulating chambers with a paddle wheel, channel width = 14.0 cm, and total flowpath length = 2.0m. The streams were filled to 60 L, and water level was marked and maintained throughout the study. Streams were refilled with tap water that had been allowed to dechlorinate for a minimum of 2 d.

In each stream we placed 12, 22.86 cm X 13.97 cm X 8.89 cm plastic trays for the experimental units (Plastic Take-Out Container, Hangzhou Yusheng Plastic Products Co. Ltd., Hangzhou City,China). We tested 3 factors in a fully crossed design: the presence of organic matter, added nutrients, and presence of clams. Trays were filled with either 400 mL all-purpose sand (KolorScape All Purpose Sand, Oldcastle,Inc., Atlanta, GA) or a

mix of 200 mL all-purpose sand and 200 mL potting soil (Miracle-Gro Organic Choice potting soil, The Scotts Company LLC, Marysville, OH) to represent organic matter. Those streams designated to have clams received 14 clams in each tray to correspond with the 200 m^{-2} average density found in literature (Lauritsen and Mozley 1983, Schmidlin and Baur 2007, Brown et al.2007) for a total of 168 clams artificial $stream^{-1}$. Clams were collected from the North Branch of the Chicago River on 21 February 2012. The streams designated to contain nutrients received enrichment with 100 mL 40.64 g L^{-1} $NaNO_3$ and 1.85 g L^{-1} KH_2PO_4 solution once a week (target concentration in stream= ~8 mg N L^{-1} and 0. 6 mg P L^{-1}) consistent with a highly eutrophic stream. All 8 streams were inoculated with 100 mL of sediment-periphyton slurry collected from the Chicago River on the same date as the clams. 2 mL of non-viable marine algae (Shellfish Diet 1800, Reed Mariculture Inc., Campbell, CA) was distributed evenly into each stream every Monday, Wednesday and Friday for the duration of the study.

Ecosystem metabolism

2 data-logging sondes were rotated among the study streams so that each stream had a sonde in it for 24 h each week for the duration of the study. Sondes measured water temperature and dissolved oxygen (DO; as percent saturation and mg L^{-1}) every 15 min for 24 h using a luminescent DO probe (Hach Hydromet, Loveland, CO). Reaeration (kO_2 at 20^oC) was equal to 0.015 min^{-1} and was estimated from velocity-reaeration measurements previously established for these artificial streams (T. Hoellein, unpublished data). Community respiration (CR) was the average reaeration-corrected oxygen (O_2) flux during the dark, and gross primary production (GPP) was the sum of the

instantaneous change in O_2 concentration (reaeration-corrected) during daylight hours (Marzolf et al. 1994, Young and Huryn 1998).

Trays from each stream were sampled at 3 weeks, 6 weeks, and 9 weeks after the start of the experiment. On each date, 3 trays from each stream were removed for sampling. For those streams containing clams, one clam from each of the 3 sampled trays was used to measure excretion rates, and a different clam from each of the 3 trays was used to calculate condition index. From each of the streams, a composite sediment sample was collected from each tray. Sediment was taken from 3 areas within the tray and homogenized with a metal stir bar. From this composite sample, measurements were taken for sediment AFDM, exchangeable NH_4^+, nitrification, and denitrification potential (see below for details on these methods). Once the trays were sampled they were returned to the streams but were marked and were not re-sampled.

Condition index and excretion rates

Condition index was calculated as the volumetric meat-to-shell ratio using the dry weight of meat (g) X 100 divided by shell-cavity volume (mL) (Mann 1978). Live clams were preserved for 24 h in 95% ethyl alcohol. Clam tissue was removed, dried, and weighed. To determine the shell-cavity volume, we filled one clam valve with Kolorscape All-Purpose sand (Kolorscape, Oldcastle,INC, Atlanta, GA) and weighed the sand. A mass to volume regression for playground sand was calculated by weighing sand from known volumes (i.e. 1.25 mL and 5 mL). We doubled the sand mass from 1 valve to account for both halves of each individual's shell and used our standard regression to

calculate shell volume for each clam. Shell length was measured at the widest part of the clam.

To measure excretion rates, we adapted a protocol from Lauritsen and Mozley (1983). 500 mL of site water was filtered through a vacuum into a 1000 mL, acid washed plastic beaker (N=6). One clam from each tray was placed in the filtered water, and one beaker was left with only water to serve as a control. The beakers were then covered with foil perforated with small holes. Only four clams were used from the Chicago River site due to mortality. A 20 mL water sample was taken from each cup at 2, 8, and 24 h, filtered using Whatman 25mm Glass Microfibre Filters (Whatman,Ltd., GE Healthcare, Piscataway, NJ) and frozen until analyzed on a Seal Auto-analyzer 3 for NH_4^+ concentrations (see below). Excretion was calculated as the linear increase in NH_4^+-N relative to the control.

Sediment ash-free dry mass and exchangeable NH_4^+

A 5 ml subsample from the composite sediment sample was collected and used to measure AFDM. The sample was placed in pre-ashed and weighed tins. The samples were dried at a temperature of 60°C for a minimum of 2 d, and then the dry weight was recorded. Next, the samples were ashed at 550°C for 3 h and cooled in desiccators for minimum of 1 h before measuring the ash weight. Our protocol for exchangeable NH_4^+ measurement was adapted from Maynard et al. (1993). 10 mL of the homogenized sediment sample was placed into a 50 mL centrifuge tube. The samples were weighed and an equivalent volume of 2M potassium chloride (KCl) was added (1 ml KCl per 1 g wet sediment). The centrifuge tubes were then placed on a shaker table at 150 rpm for 1

h. We centrifuged the tubes at 6000 rpm for 10 min., and the supernatant liquid was filtered using Whatman 25mm Glass Microfibre Filters (Whatman,Ltd., GE Healthcare, Piscataway, NJ) into 20 mL scintillation vials and frozen until analysis.

Nitrification and denitrification enzyme activity

We measured nitrification via the nitrapyrin inhibition method. Nitrapyrin blocks the conversion of NH_4^+ to NO_3^- (Frye 2005, Strauss and Lamberti 2000). A nitrapyrin solution was made from 0.5 g of nitrapyrin dissolved in 10 mL dimethyl sulfoxide (DMSO). We added 25 mL sample sediment and 50 mL site water to each of two flasks, one with 20 µl of the nitrapyrin + DMSO and the other with 20 µl of DMSO only. All flasks were then covered loosely with foil and placed on a shaker table at 150 rpm for 2 days. Samples were covered to block light which could affect the nitrifying bacteria. After 2 d, 25 mL of 2M KCl was added to each flask and shaking resumed for an additional 2 h. Then, using a modified 20 mL syringe, 30 mL of the slurry from each flask was placed in 50 mL centrifuge tubes, centrifuged for 10 min at 6000 rpm, and the supernatant was filtered and frozen in the same manner as for the exchangeable NH_4^+.

Denitrification via acetylene-block was used to measure denitrification enzyme activity (DEA; Smith and Tiedje 1979). 25 mL sample sediment from each tray in the artificial stream was funneled into 125 mL media bottles along with 45 mL unfiltered site water(N=3 per stream, N=24 total). 5 mL of chloramphenicol solution was then added to the media bottles to prohibit bacteria from producing additional enzymes (final chloramphenicol concentration 0.3mM). The headspace of the media bottles were then purged for 5 min with N_2 and simultaneously vented with a syringe needle. The media

bottles were re-equilibrated to atmospheric pressure. At this point, 15 mL of pure acetylene gas was added to each media bottle and then shaken for several seconds. Triplicate gas samples were collected 15 min after the addition of acetylene and then every hour for a total of 3 sampling times for each media bottle. Our sampling technique was to pull a 5 mL gas sample from the media bottle and inject it into a 3 ml silicone-coated vacutainer (Kendall Monoject Blood Collection Tube, Covidien, Mansfield, MA). The 5 mL was replaced with an 1:9 acetylene:N_2 mixture to maintain constant volume. The samples were sealed with silicone caulking until they could be run on the gas chromatograph (GC 2014, Shimadzu Scientific Instruments, Inc, Columbia, MD) with an autosampler (AOC-5000, Shimadzu Scientific Instruments, Inc). Using the gas chromatograph we could measure the nitrous oxide (N_2O) and calculate the rate of N_2O accumulation as DEA (Murray and Knowles 1999).

Field study

For the field study, we selected an urban stream and a rural stream which each had persistent populations of *Corbicula fluminea*. The urban stream was the North Branch of the Chicago River at Harm's Woods in Cook County, Illinois (42.06^o N latitude and 87.77^o W longitude). Preliminary data showed this stream exhibited characteristics typical of urban streams such as elevated nutrient concentrations and decreased macroinvertebrate species richness. The substrate composition is highly variable, although the majority is sand, gravel, and *C. fluminea* shells. Discharge at this location of the North Branch at the time of our sampling was ~0.43 m^3 s^{-1}. Our rural site was Eagle Lake Outlet (i.e., Eagle Creek), part of the Kalamazoo River watershed in the

Fort Custer Recreation Area near Augusta, Michigan (-42.33^{o} W latitude and 85.32^{o} N longitude). The substrate composition is predominantly sand and gravel and at the time of our sampling had a discharge of ~0.015 m^3s^{-1}. In addition to high *C. fluminea* populations, both streams had full riparian canopy cover during summer, and drained lentic habitats (i.e., a lake or a small impoundment) 500-1000 m upstream of the study sites.

We began the field experiment in the rural and urban streams on 15 June 2012, and 20 June, 2012, respectively. We divided a 38.1 cm X 27.94 cm X 10.16 cm plastic tray in half vertically with rubber landscape edging. Each side was filled to the top with a mixture of playground sand and pea gravel. One side was left as a control (i.e., no clams) and 15 individual clams were put on the opposite side, corresponding to approximately 280 clams m^{-2}, within the range we expected from literature values (Lauritsen and Mozley 1983, Schmidlin and Baur 2007, Brown et al.2007). The clams were collected just downstream from where the trays were placed. A lid with 1.7 cm^2 aperture plastic mesh was then placed on top of the tray and secured with zip-ties. Trays were submerged in the stream and held in place with metal rebar that was hammered into the benthos. We deployed 5 trays at each site, and trays were left for 6 weeks.

Prior to tray placement, we collected several measurements to represent the physicochemical characteristics and macroinvertebrate communities at each site. We marked a 100 m reach just upstream (Eagle Creek) or downstream (Chicago River) of the tray placement site. We collected 5 benthic macroinvertebrate samples at random locations in the reach using a modified Hess sampler approach (Hess Stream Bottom

Sampler, WILDCO, Yulee, FL). The Hess sampler (area=0.088 m^2) was inserted ~10 cm into the stream benthos, the sediment surface was vigorously stirred by hand, and the dislodged benthic macroinvertebrates were collected in the Hess sampler net. We collected the sediment and benthic material from ~10 cm depth by scooping it directly into the Hess sampler net (mesh size=250 mm) in a modified approach that allowed for collection of burrowing bivalves. All material from the Hess sampler net was preserved with 80% ethanol. We quantified stream discharge by measuring the depth and water velocity at every 1 m subsection across a width transect. In addition, we collected 3 water samples by filtering stream water using a 60 mL plastic syringe fitted with Whatman 25mm Glass Microfibre Filters (Whatman,Ltd., GE Healthcare, Piscataway, NJ) into 20 mL plastic scintillation vials.

Trays were removed from the stream on August 1(urban) and August 8 (rural), 2012, and immediately brought back to the laboratory. We removed all clams from the trays, and one clam from each tray was used to measure condition index and excretion rates using methods described above. We then collected a composite sediment sample from the control and +clam sides of each tray. A 28.27 cm^2 core was inserted ~3 cm into the sediment, a flat plastic tool was slid underneath, and the sediment was placed into 2, 160 ml sediment containers. This was repeated at 4 locations in both the control side and +clam side of each tray. The sediment was homogenized using a metal stir bar and the sample was then used for all sediment measurements. We measured ash-free dry mass (AFDM), exchangeable NH_4^+, and nitrification on sediment from the control and +clam sides of each tray using methods identical to those described above.

Nutrient and gas fluxes

Fluxes of NH_4^+, NO_3^-, N_2, and O_2 were measured using a flow-through method, an approach modified from Gardner and McCarthy (2009). 150 ml of homogenized sediment from the control side of the replicate trays was placed in each of 3 acrylic cores (30cm X 7.62 cm), and same amount of homogenized sediment from the +clam side of the sediment trays was placed in another 6 cores. We filled each of the cores with site water to a height of ~5 cm (251 cm^3). We added 4 individual clams directly to 3 of the cores that contained sediment from the +clam side of the tray. As a result, we had 3 replicate cores of sediment from the control side of the tray, 3 replicate cores of sediment that was exposed to clams in the stream but did not have clams in the core (ex clams), and 3 replicate cores that had sediment that was exposed to clams in the stream and had live clams in the cores (+clams). A plunger with a rubber o-ring was fit snugly into each core to create a seal. The plunger lid was plumbed with an inlet and outlet tube made of polytetrafluoroethylene (PET). We placed an aerator in 3 separate carboys which contained 20 L of site water. Un-amended site water (i.e., no NO_3^- enrichment or isotope tracers were added) was pumped from the carboys into the cores, then out into plastic beakers at a rate of 1 ml min^{-1}.

Water was passed over sediment for 3 d. After 24 h, 60 mL from the in-flow carboy and each of the outflows was collected and filtered into 3, 20 mL scintillation vials for later measurement NH_4^+ and NO_3^- flux. In addition, water from the inflows and outflows was collected into triplicate 12 mL glass exetainers for measuring dissolved gasses. For this process, we filled each exetainer slowly from the bottom and allowed

them to overflow for several seconds. We added 200 µL zinc chloride (ZnCl), capped the vials ensuring no air bubbles in the headspace, and stored them underwater at room temperature or below until they were run on the Membrane Inlet Mass Spectrometer (MIMS, Bay Instruments, Easton, MD). The water sampling procedure was repeated at 24h, 48h, and 72h (Gardner and McCarthy 2009).

On the MIMS, a peristaltic pump sampled the water from the glass exetainers and dissolved gasses were extracted from the sample across a membrane under vacuum. The mass spectrometer measured abundance of $^{28}N_2$,$^{32}O_2$, and ^{40}Ar. Standards consisted of purified water (18 mohms resistance; E-Pure, Barnstead International, Dubuque, IA) was maintained at constant temperature (24.5^{o}C; Circulating Bath, VWR International, Radnor, PA), equilibrated to atmospheric gasses by stirring at low speed (Lab Egg RW11 Basic, IKA Works, Inc., Wilmington, NC). Samples were corrected for instrument drift with standard water throughout the run.

Fluxes for each core were first calculated for each of the 3 dates of the flow-through measurement, and then averaged across the 3 dates (Gardner and McCarthy 2009). Flux was equal to the difference between concentration in the outflow minus concentration in the inflow, and corrected for surface area of the core and pump flow rate (flux units = mass element m^{-2} h^{-1}). A negative value indicates net retention (i.e., net uptake) and a positive value net production or flux out of the sediment.

Water chemistry

Samples for water column NH_4^+, excretion, exchangeable NH_4^+, nitrification, and NH_4^+ fluxes were run on an Autoanalyzer III (Seal Analytical, Inc., Mequon, WI) using

the phenol hypochlorite technique (Solorzano 1969). For exchangeable NH_4^+, and nitrification, standard matrices were adjusted to account for KCl concentrations in the samples. Water column NO_3^- and soluble reactive phosphorus (SRP) were also run on an Autoanalyzer III using cadmium reduction and antimonyl tartrate techniques, respectively (APHA 1988, Murphy and Riley 1962).

Data analysis: Artificial stream study

To measure effects of the 3 fully-crossed treatments on clam physiology, ecosystem metabolism, and sediment biogeochemistry in the artificial stream study, we used a 3 X 2 factorial ANOVA (Tank and Dodds 2003) for the presence or absence of the three treatment factors. Due to available resources and time constraints, each treatment was not replicated in separate artificial streams. Instead, replicates consisted of separate trays deployed within each treatment stream. We note this reduces the independence of replicate treatments for each date. However, this allowed us to test a wider breadth of factors. All statistical analyses were run using SYSTAT 13 (Systat Software, Cranes Software International Ltd., Chicago, IL).

Data analysis: Field study

For the field study, we used a two-way Analysis of Variance (ANOVA) by site and clam treatment to quantify effects of site and clams on AFDM, nutrient fluxes, nitrification rates, denitrification potential, and porewater NH_4^+ concentrations. We ran an ANOVA based on site on clam condition index and excretion rates. The data for condition index in the artificial stream study and the field study were exponentially transformed (X^2 and X^3, respectively) and the O_2 flux measurements in the artificial

stream study were reciprocally transformed to meet the assumptions of normality and equal variance.

Results

Artificial stream study

In general, organic matter addition had the strongest effect on stream ecosystem function and clam condition and excretion (Table 1). The trays with organic matter increased nitrification rates ($p<0.001$), while clams and nutrient addition had no effect on nitrification. As expected, organic matter addition to the tray increased organic matter concentration relative to the trays with no organic matter (Table 1). However, when clams were present in trays with organic matter, there was a decline in organic matter content relative to those trays with organic matter and no clams (Table 1 and Figure 2). For DEA, significant interaction effects among all three factors precluded simple interpretation of each factor's impact (Figure 3). We had a large die-off of clams in week 6 in the stream that contained trays with no organic matter and added nutrients. This was likely reflected in the very high rate of DEA in that site at week 3, and contributed to the significant interaction terms (Figure 3). Clams decreased GPP ($p=0.027$), especially in the sediment with organic matter added (Figure 4), but there were no significant effects on CR among treatments. Clam condition decreased over time in all treatments; the clams in trays with organic matter present were in better condition than in trays with no organic matter ($p=0.021$). The stream with the lowest clam mortality rate was that with organic matter present but no added nutrients. Finally, there was no consistent pattern of treatment effect on excretion rates (Figure 5).

Table 1. P-values for 3 x 2 Factorial ANOVA for sediment characteristic and clam physiology in the artificial stream study across 3 sampling dates and 8 treatments. Organic matter (O), Nutrient addition (N), Clams (C). Significant p-values are in bold.

Process	**O**	**N**	**C**	**OXN**	**OXC**	**NXC**	**OXNXC**
Nitrification (ug N m^{-2} h^{-1})							
Date 1:	**<0.001**	0.321	0.922	0.166	0.747	0.554	0.975
Date 2:	**<0.001**	0.339	0.334	0.863	0.824	0.719	0.451
Date 3:	**<0.001**	0.339	0.334	0.863	0.824	0.719	0.451
Denitrification enzyme activity (DEA; ug N m^{-2} h^{-1})							
Date 1:	**0.001**	0.380	0.283	0.465	0.299	0.570	0.593
Date 2:	**<0.001**	**0.002**	**<0.001**	**0.043**	**<0.001**	**0.001**	**0.012**
Date 3:	0.085	**0.004**	0.066	**0.033**	**0.003**	**0.038**	**0.007**
Ash-free dry mass (g)							
Date 1:	**<0.001**	0.944	**0.017**	0.335	**0.001**	0.813	0.152
Date 2:	**<0.001**	0.200	**0.009**	0.279	**0.016**	0.798	0.462
Date 3:	**0.001**	0.332	0.092	0.120	**0.020**	0.743	0.598
Porewater NH_4^+ (ug L^{-1})							
Date 1:	**<0.001**	**<0.001**	0.310	**0.008**	**0.001**	0.994	0.054
Date 2:	**<0.001**	**0.020**	**<0.001**	**<0.001**	**<0.001**	0.555	**<0.001**
Date 3:	0.854	0.311	**0.022**	0.304	**0.017**	**0.040**	**0.037**
Gross Primary Production (g O_2 m^{-2} d^{-1})							
	0.717	0.097	**0.027**	0.946	0.416	0.208	0.726
Community Respiration (g O_2 m^{-2} d^{-1})							
	0.213	0.074	0.403	0.695	0.251	0.497	0.668
Excretion (ug NH_4^+ h^{-1})							
Date 1:	0.844	0.304		0.920			
Date 2:	**0.008**	0.310		**0.004**			
Date 3:	0.280	0.430		0.255			
Clam condition index (g mL^{-1})							
Date 1:	0.318	0.321		0.522			
Date 2:	**0.021**	0.171		0.164			
Date 3:	**0.024**	0.236		0.772			

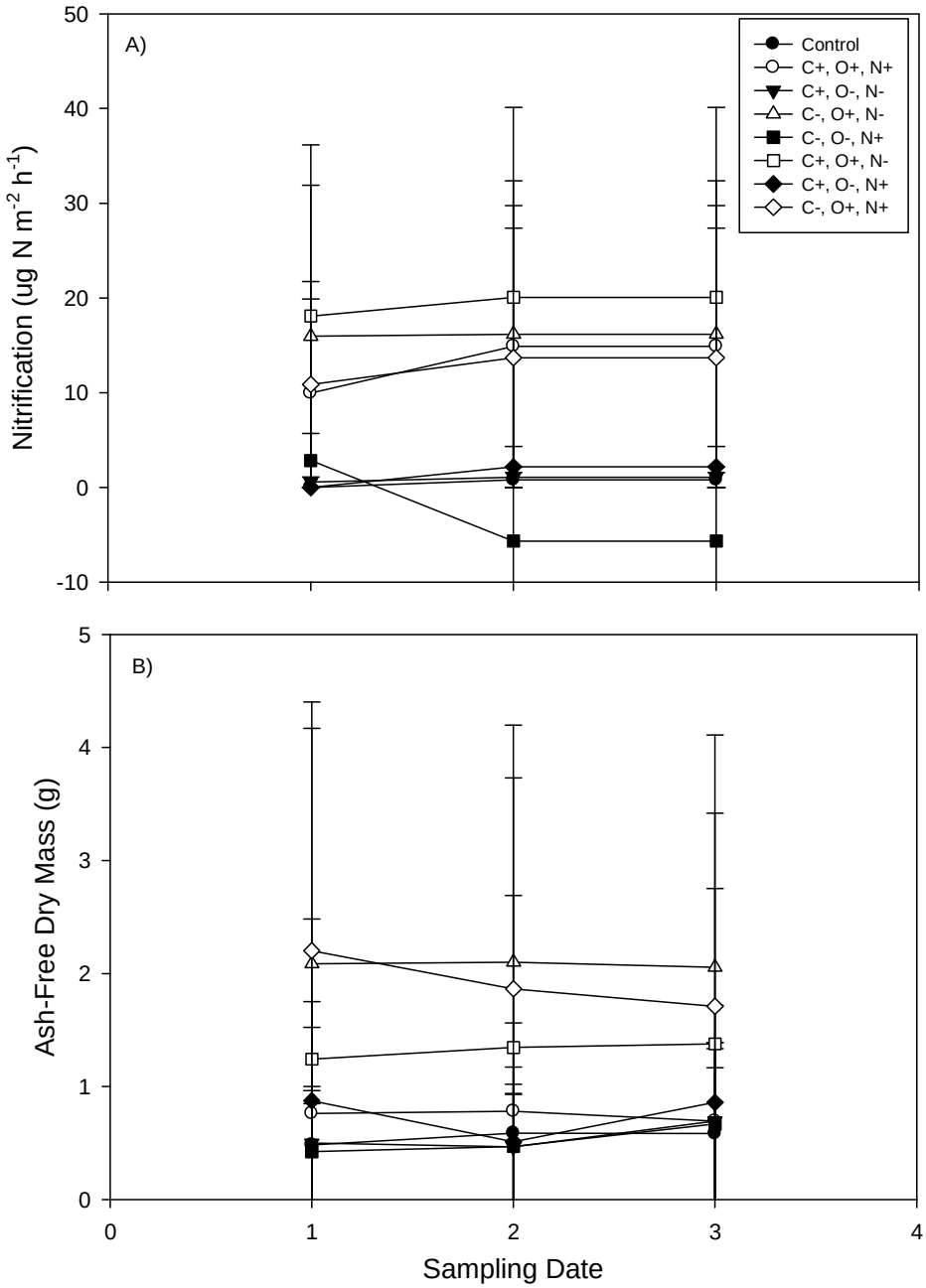

Figure 2. Average nitrification rates (A) and Ash-Free Dry Mass (B) for each of the eight experimental streams across three sampling dates. Unfilled symbols represent treatments with added organic matter and filled symbols represent treatments without organic matter. Error bars represent standard error.

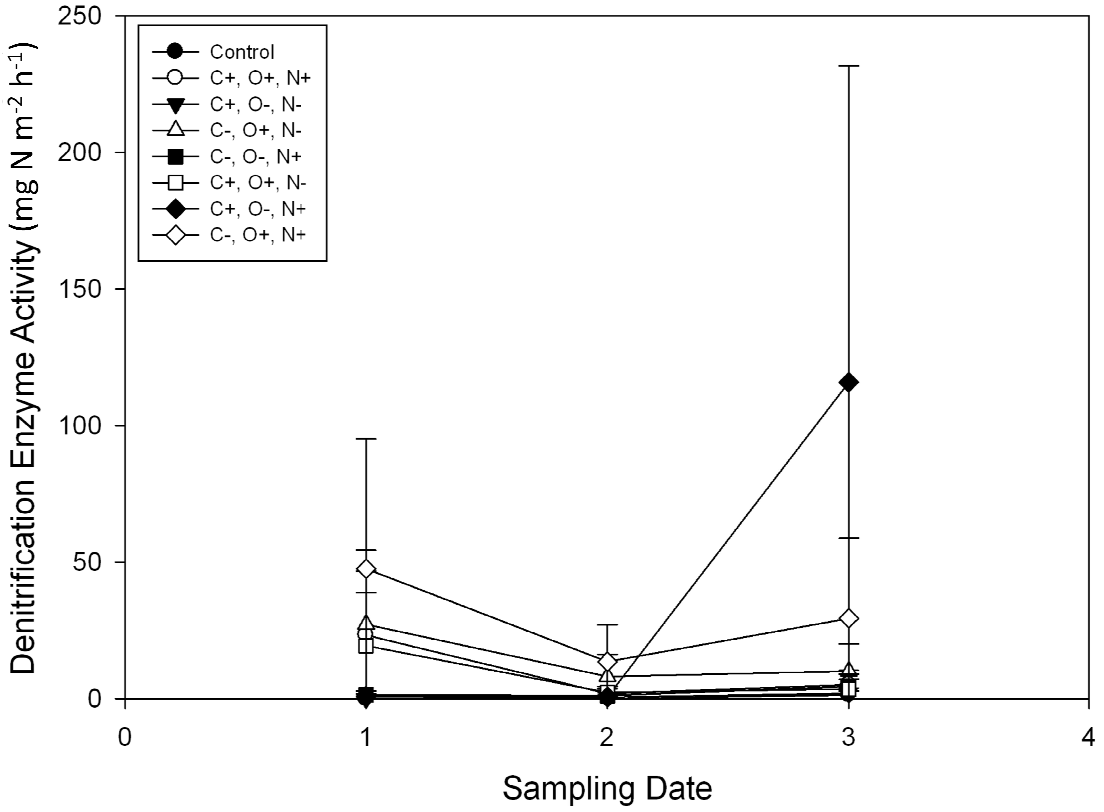

Figure 3. Denitrification enzyme activity in all eight treatment streams across three sampling dates. Unfilled symbols represent treatments with organic matter added, filled symbols represent treatments without organic matter. Error bars represent standard error.

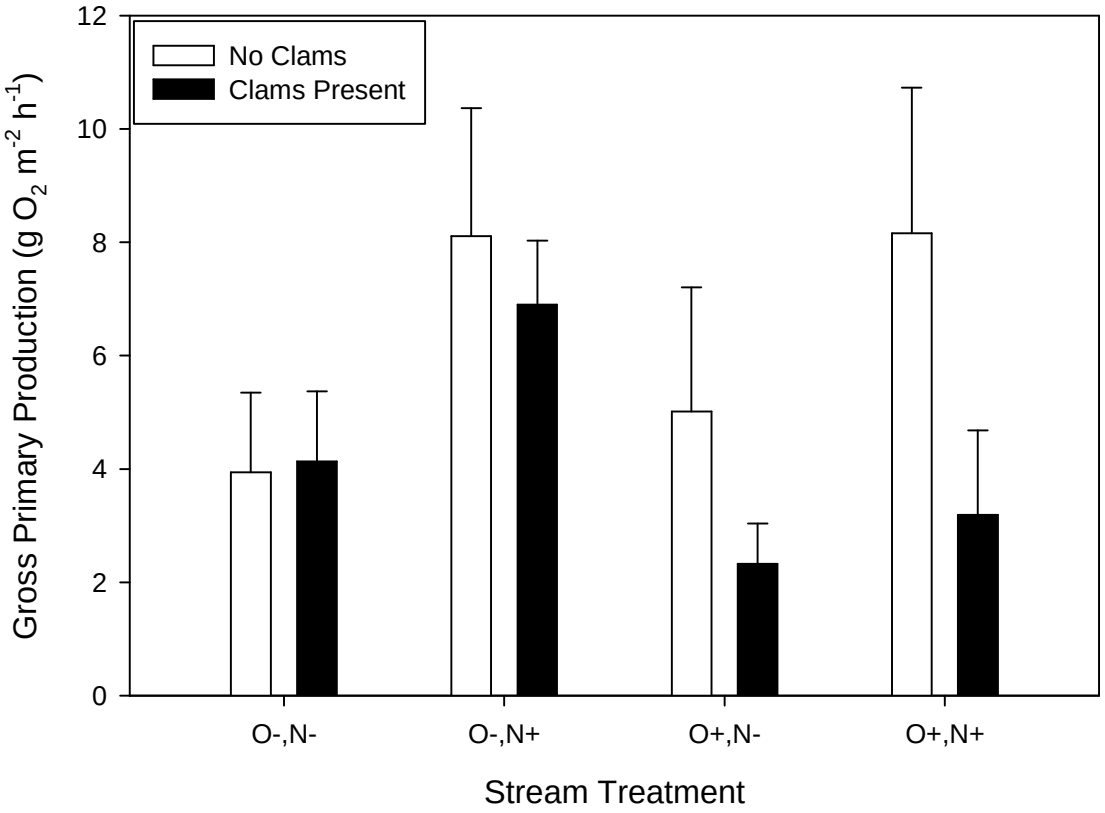

Figure 4. Average gross primary production from all eight treatment streams over nine weeks. White bars indicate treatments with no clams and black bars indicate treatments with clams present. + or – indicates presence or absence of treatment (O=organic matter, N=nutrient solution). Overall, clams decreased primary production (ANOVA p=0.027). Error bars represent standard error.

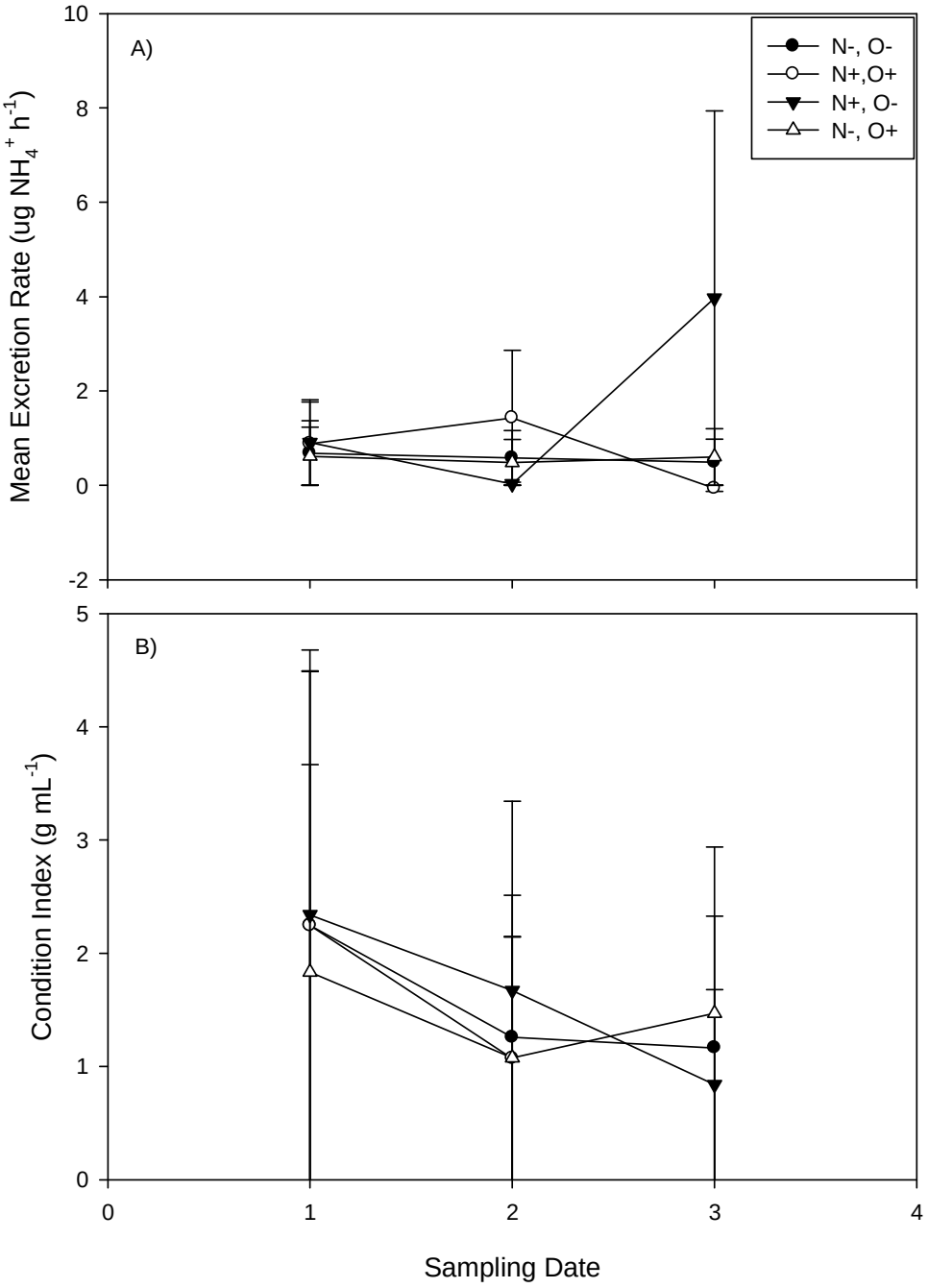

Figure 5. Mean excretion rate (A) and average condition index (B) of clams across three sampling dates. Unfilled symbols represent treatments where organic matter was added and filled symbols represent treatments without organic matter. Error bars represent standard error.

Field study

The Chicago River exhibited higher concentrations of NO_3^- and NH_4^+, higher discharge, and greater benthic density of clams (Table 2). With over 1,500 individuals m^{-2}, the Chicago River is at the high end of the range of *C. fluminea* reported in locations from the northern limit of its distribution, and the density in Eagle Creek (174 m^{-2}) was closer to literature values (French and Schloesser 1996, Ortmann and Grieshaber 2003, Lauritsen and Mozley 1983). The substrate in Eagle Creek was more than 60% sand and gravel, however, in the Chicago River it was less than 50% sand and gravel with a higher proportion of shells, silt, and woody debris.

Overall, *C. fluminea* drove similar changes to N biogeochemistry at both sites (Table 3), but the magnitude of change differed between locations. Like in the artificial stream study, clams did not affect nitrification rates, and there were no differences in nitrification between sites. The amount of organic matter was lower in the Chicago River than in Eagle Creek ($p=0.045$), but the presence of clams did not affect sediment organic matter, which is contrary to results from the artificial stream study (Figure 6). N_2 flux out of the sediment (i.e., denitrification) was greater when live clams were present (2-way ANOVA, $p=0.011$) relative to control sediment and sediment exposed to clams, and N_2 flux was higher in the Chicago River (2-way ANOVA $p=0.006$) than Eagle Creek (Figure 7). There was more O_2 uptake when live clams were present (2-way ANOVA $p<0.001$), but the two streams were not significantly different (2-way ANOVA $p=0.197$; Figure 7). In both sites, the presence of live clams increased NH_4^+ flux out of the sediment (2-way ANOVA $p=0.020$; Figure 8). There was more NH_4^+ and NO_3^- uptake in the Chicago

River than in Eagle Creek (2-way ANOVA, p=0.003, and p=0.045, respectively; Figure 8), and Eagle Creek showed net release of both solutes across control and clam treatments. There was no significant difference in individual clam excretion rates between sites, although the Chicago River clams showed a trend of higher excretion (Figure 9). Finally, while there was clam greater mortality in sediment trays in the Chicago River, the condition of those in the Chicago River was better than that at Eagle Creek (p=0.045).

We used benthic density of clams in each stream to scale up their effects on N fluxes to the level of 1 m^2 of streambed (Figure 10). Results showed an increase in NH_4^+ and N_2 flux out of the sediment when exposed to clams relative to control sediment, and fluxes were even higher when live clams were present. However, the difference between rates in the control sediment and in sediment with live clams was different at each site. For example, the difference in NH_4^+ flux between control and live clams was 466. μg N m^{-2} h^{-1} at Eagle Creek, and 3,623 μg N m^{-2} h^{-1} in the Chicago River. The difference in N_2 flux in control and live clam sediment was also smaller in Eagle Creek (280 μg N m^{-2} h^{-1}) relative to the Chicago River (6,069 μg N m^{-2} h^{-1}). Also, the fluxes in the Chicago River were much more variable than in Eagle Creek as indicated by the error bars.

Table 2. Comparison between North Branch of the Chicago River (urban) and Eagle Creek (rural) for physicochemistry, benthic substrate composition, and benthic macroinvertebrates community.

Measurement	**Chicago River**	**Eagle Creek**
Water Column NO_3 (ug L^{-1})	2,490.8	42.2
Water Column NH_4^+ (ug L^{-1})	126.1	17.9
Porewater NH_4^+ (ug L^{-1})	228.5	270.4
Discharge (m^3 s^{-1})	0.43	0.01
Number of clams m^{-2} (ind m^{-2})	1,516.5	174.7
Benthic Composition		
Sand and Gravel	41%	65%
Silt	6%	5%
Boulder and Cobble	1%	15%
Shells	27%	1%
Other	25%	14%
Benthic macroinvertebrate biomass g m^{-2} (%)		
Corbiculidae	228.67 (99.18%)	8.06 (78.21%)
Unionidae	0.79 (0.34%)	2.19 (21.22%)
Dreissenidae	0.48 (0.21%)	-
Chironomidae	0.05 (0.02%)	0.01 (0.10%)
Oligochaeta	0.01 (0.01%)	-
Hirudinae	0.25 (0.11%)	0.04 (0.36%)
Other	0.30 (0.13%)	0.01 (0.11%)

Table 3. P-values for a 2-way ANOVA by site (rural and urban stream) and clam treatment for sediment physicochemistry and clam physiology measurements from the field study. Bold values are significant at $p<0.05$.

Process	**Site**	**Treatment**	**Interaction**
Nitrification (ug N m^{-2} h^{-1})	0.320	0.390	0.589
N_2 flux (ug N m^{-2} h^{-1})	**0.006**	**0.011**	0.342
O_2 flux (ug m^{-2} h^{-1})	0.197	**<0.001**	0.325
Sediment organic matter (g)	**0.045**	0.418	0.356
NH_4^+ flux (ug N m^{-2} h^{-1})	**0.003**	**0.020**	0.993
NO_3^- flux (ug N m^{-2} h^{-1})	**0.045**	0.993	0.989
Porewater NH_4^+ (ug N L^{-1})	0.466	0.369	0.409
Excretion rate (ug NH_4^+-NgAFDM^{-1} h^{-1})	0.257		
Clam condition index (g mL^{-1})	0.045		

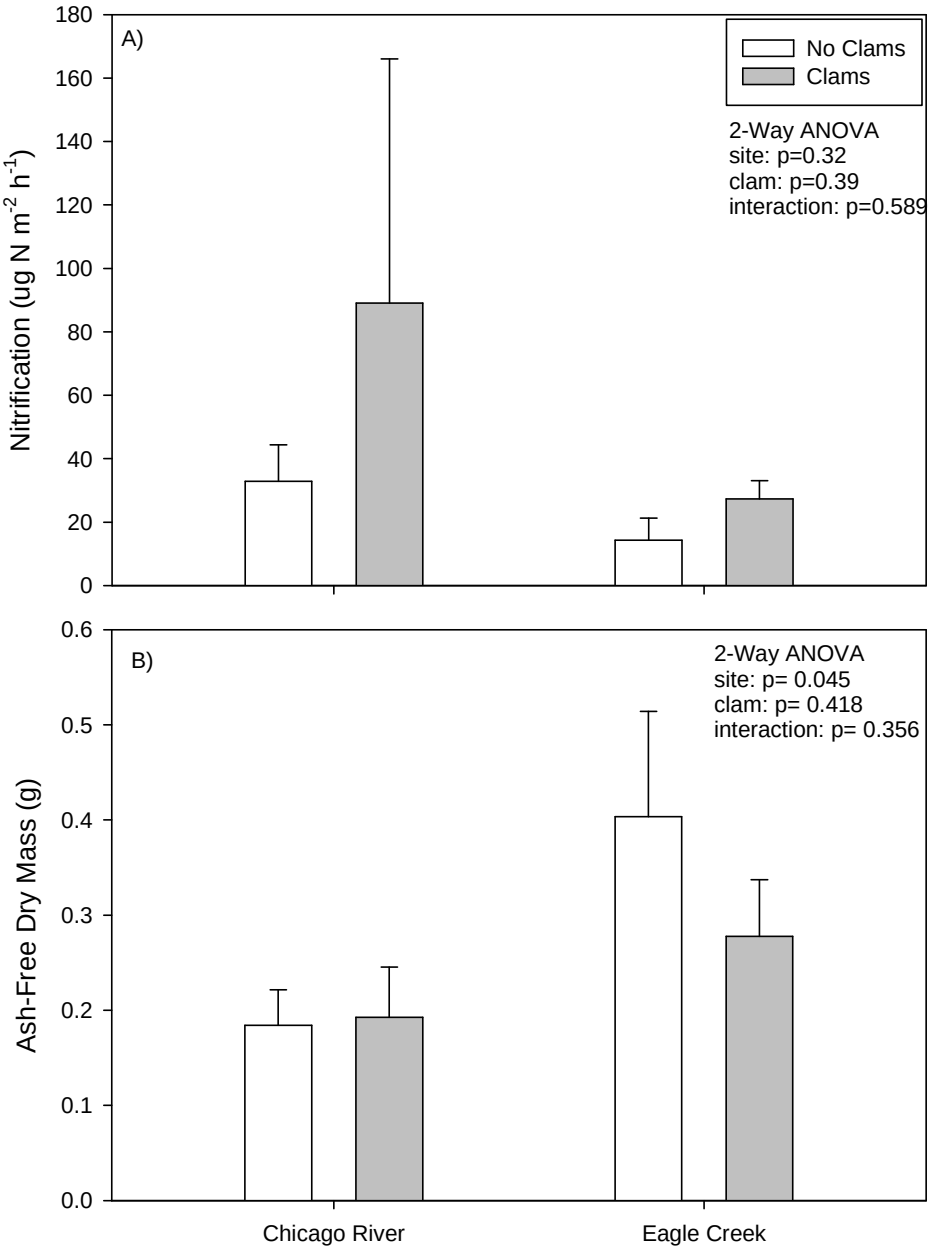

Figure 6. Average nitrification rates (A) and sediment ash-free dry mass (B) at the two study sites Chicago River and Eagle Creek. White bars indicate no clams and grey bars indicate clams present. Although there was no clam effect, Eagle Creek had higher organic matter (2-Way ANOVA p=0.045).

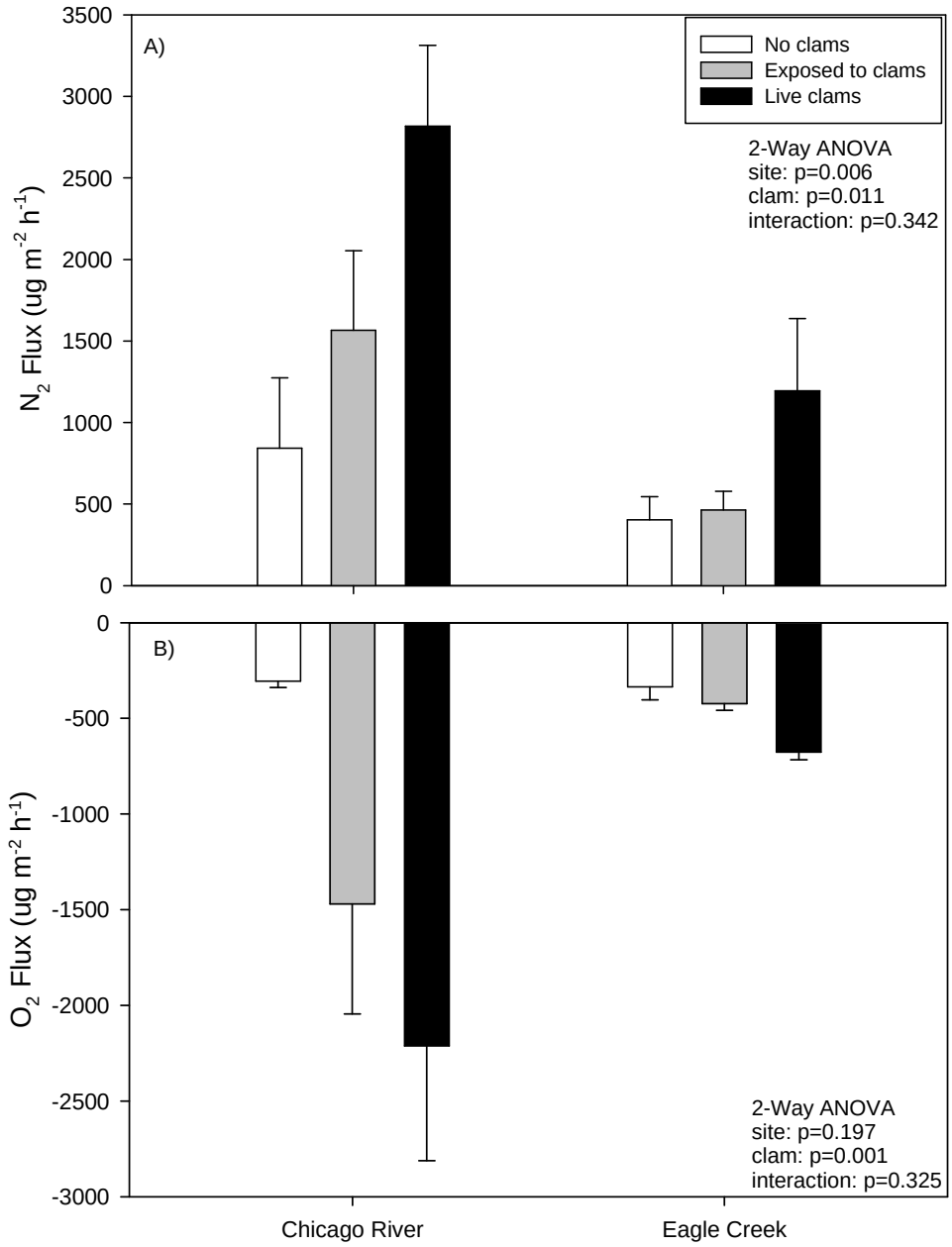

Figure 7. N_2 flux (A) and O_2 flux (B) in sediment without clams, exposed to clams, and with live clams present in the Chicago River and Eagle Creek. N_2 flux (i.e., denitrification) was higher in the Chicago River and when live clams were present (2-Way ANOVA p=0.006, p=0.011). There was more O_2 uptake (i.e., respiration) when live clams were present (2-Way ANOVA p=0.001).

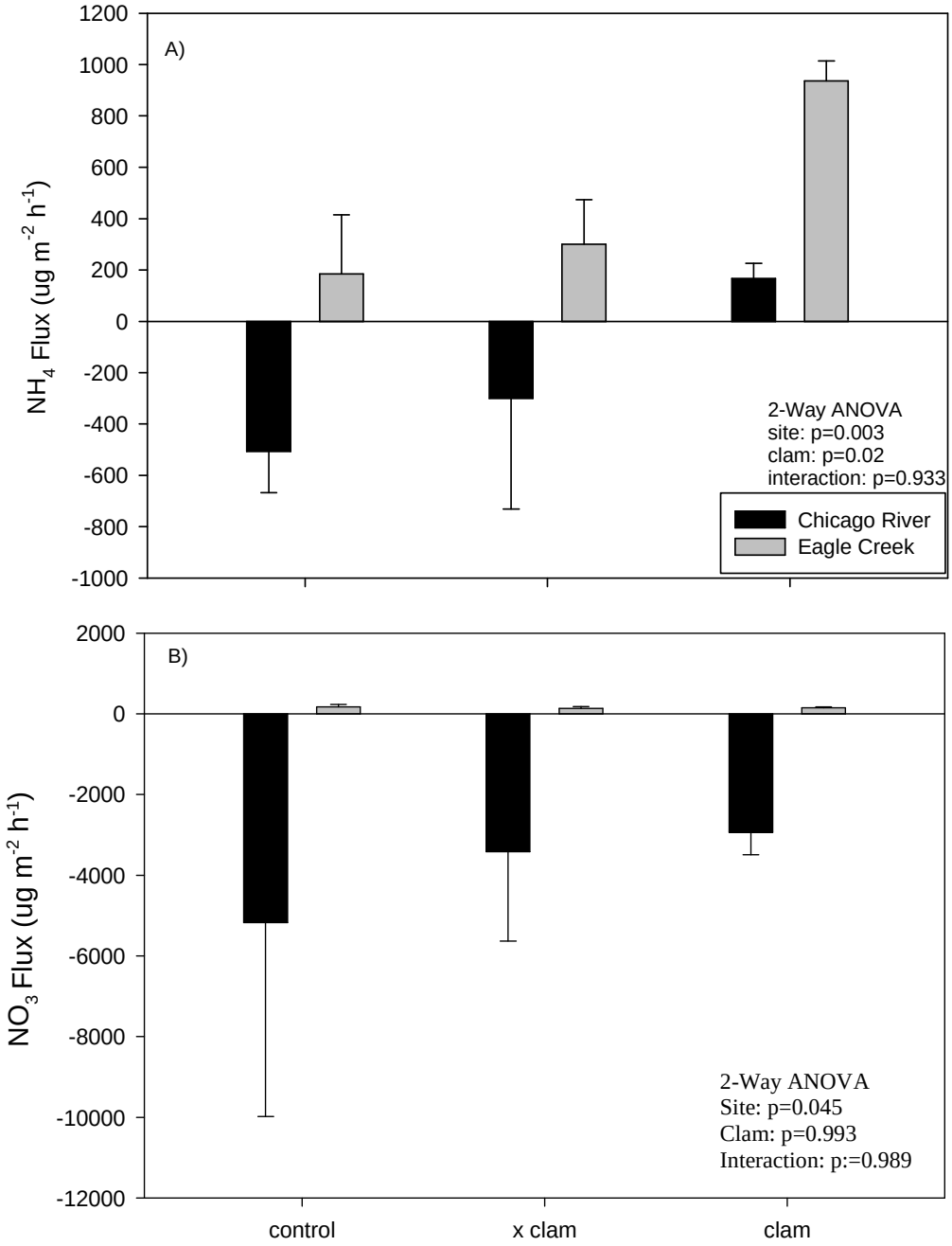

Figure 8. NH_4^+ flux (A) and NO_3^- flux (B) in sediment with no clams (control), exposed to clams (x clam) and with live clams present (clam). Black bars represent measurements from the Chicago River and grey bars represent measurements from Eagle Creek.

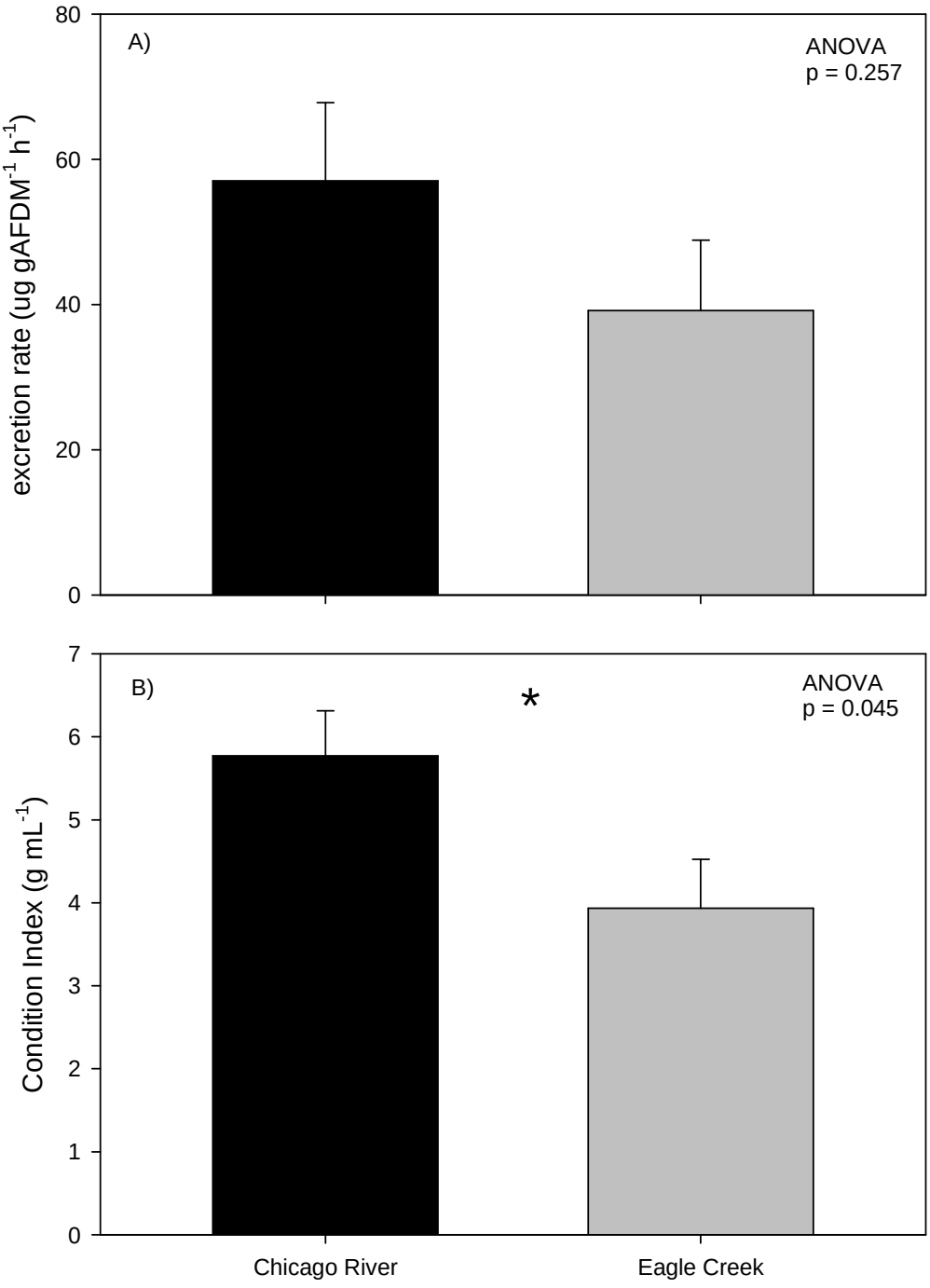

Figure 9. Average excretion rate (A) and condition index (B) in clams from the Chicago River and Eagle Creek. Clams from the Chicago River had a higher tissue:shell cavity volume ratio than those in Eagle Creek (t-test p=0.045). Error bars represent standard error.

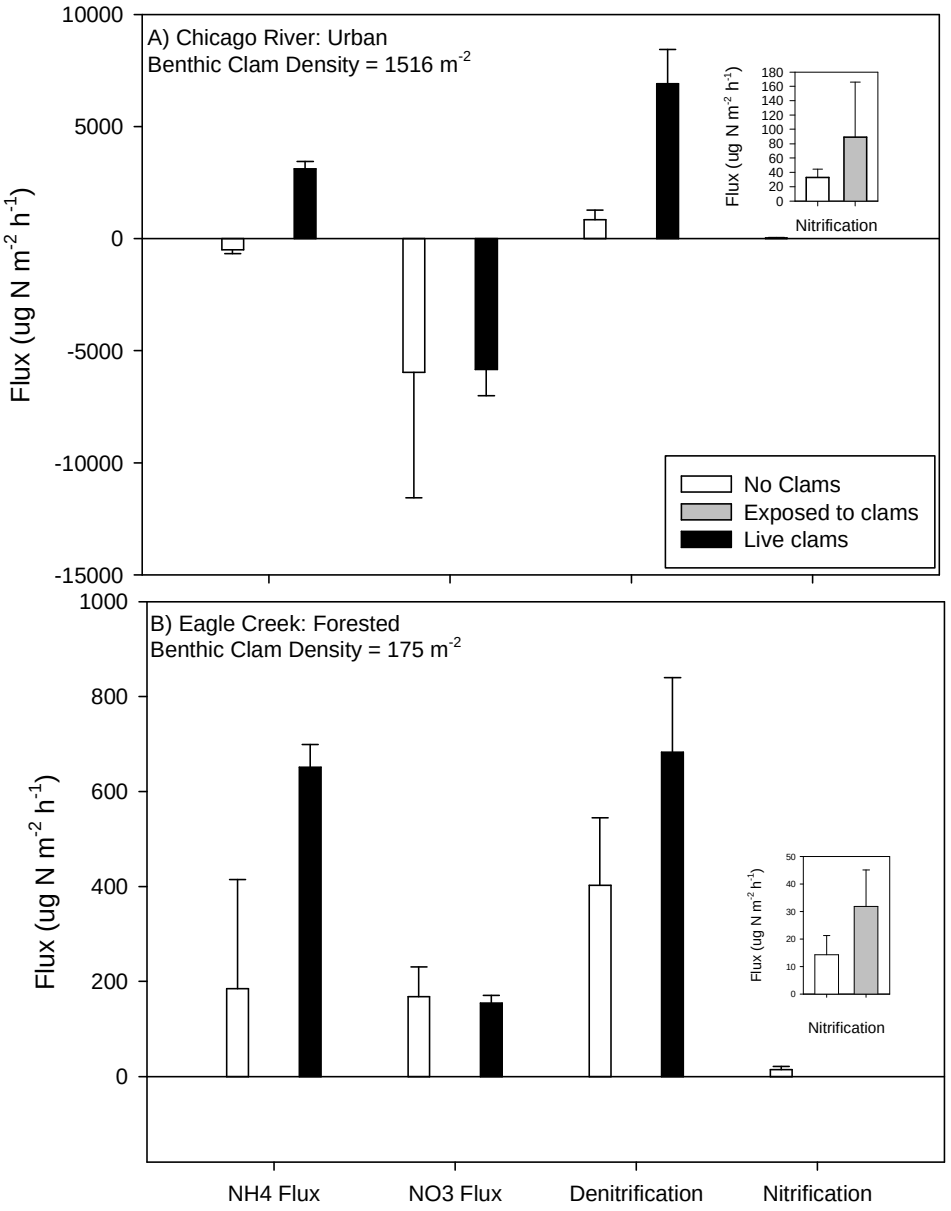

Figure 10. Comparison of biogeochemical processes between the Chicago River (A) and Eagle Creek (B), adjusted for benthic clam density. White bars represent sediment without clams, grey bars represent sediment exposed to clams, and black bars indicate sediment with live clams present. Error bars represent standard errors.

Discussion

Overall, our results showed that *C. fluminea* affected key aspects of N biogeochemistry, but the magnitude of their effect varied according to environmental conditions, both in laboratory streams and *in situ*. In addition to the environmental effects, clam condition and mortality was also affected by the stream environment. In general, it appears clams interacted with stream N and C dynamics most through excretion, which increased NH_4^+ flux, and through burrowing activity, which increased water column diffusion and thereby higher rates of N_2 flux and respiration, as well as changes in sediment AFDM.

Clam effects on sediment AFDM

We did not expect that the clams would have a significant effect on sediment organic matter. While we expected some consumption by pedal-feeding, we provided a high quality algae mix food to clams in the lab, and clams in the field were downstream of lentic habitats. Our field and lab studies produced mixed results. In the artificial stream study, the presence of clams decreased sediment organic matter, but there was no effect of clams on AFDM in the field study. Most likely, there was no effect of clams on sediment organic matter in the field study because there is a constant import and export of organic matter in natural streams. However, the decline in sediment AFDM in trays that contained clams + organic matter in the lab study could be due to 1) pedal feeding, 2) displacement via burrowing and locomotion, or 3) erosion in the early stages of the experiment.

Evidence from a concurrent study on clam behavior (see Chapter 3 of this thesis) suggests that the displacement explanation is unlikely because we found very little clam horizontal movement. Also, AFDM declined even though not all the clams were buried in the trays. In a combination laboratory and field study, Hakenkamp and Palmer (1999) showed that pedal-feeding reduced sediment organic matter when conditions favored pedal-feeding. However, we think pedal feeding reduction was at most only part of the cause in organic matter reduction in our study, since those clams in sediment with organic matter showed a decline in condition index similar to the other treatments, and our clam behavior study suggested there was no preference for substrates containing organic matter relative to those without. We observed that some of the displaced organic matter in the artificial streams seemed to float in the water column or line the bottom of the stream (i.e., was not consumed).

The reduction in organic matter in the trays with clams happened within the 1st 3 weeks of the experiment, and stayed uniform thereafter (Figure 2), suggesting erosion as a possible explanation for the clams effect. The organic matter seemed to be displaced from the physical presence of the clams, not through their burrowing or feeding. Allen and Vaughn (2011) also found that bivalve-induced erosion disturbs sediment organic matter within high density assemblages of unionid mussels in artificial streams. This is important because the abundance of sediment organic matter was a driving factor in several biogeochemical rates, including nitrification and DEA. In addition, the presence of organic matter in the artificial stream study sustained higher clam condition indices by the end of the 9 week study. The magnitude of AFDM in the +organic matter treatment

was much higher in the lab study relative to AFDM in the field study (5 g and 0.6g, respectively). Overall, while the laboratory experiment clearly demonstrated the effect of organic rich sediment on N dynamics, the high AFDM in sediment trays limits the extension of these results to exploring clam effects on N dynamics under oligotrophic and eutrophic environments.

Clam mortality and condition index is influenced by environmental conditions

C. fluminea populations are subject to large-scale die-offs that have multiple ecosystem effects (Cherry et al. 2005). Our results suggest that high nutrient conditions, combined with low organic matter abundance, can generate a population crash of *C. fluminea*. The results we observed in one of our artificial streams were consistent with those findings. The sudden clam die-off in the stream with no organic matter and high nutrients caused unusually high numbers in our DEA measurements in week 9 (Figure 3). Overall, the clams with the best survival had organic matter present but no added nutrients. In a similar fashion, clam mortality in the Chicago River field experiment was higher than in Eagle Creek, and Eagle Creek had more organic matter (Figure 6) and lower nutrients (Table 2). Wittmann et al (2012) also observed that high NH_4^+ concentrations combined with low DO, increased clam mortality and subsequent algal growth. Previous studies have suggested that low food quantity and quality, associated with higher water temperatures create metabolic expenses that trigger clam mortality events (McMahon 2002, Ilarri et al 2010).

Condition indices are often used in bivalve aquaculture and there are several variations in the measurements (Lucas and Beninger 1985). We used a volumetric meat-

to-shell ratio (Mann 1978) to determine environmental effects on the clams' condition in both lab conditions and in the field. Measuring the condition index is most often done in oyster studies (Lawrence and Scott 1982, Mason and Nell 1995) but has been used previously with *C. fluminea* (Cataldo et. al 2001). A low condition index would be indicative of poor environmental conditions or some other kind of stress on the clam (Lucas and Beninger 1985). Under laboratory conditions, we found that there was a general decline in the condition index of the clams across the course of the study. The results of condition index in the field suggest that clams in the Chicago River have a higher body tissue to shell cavity volume ratio than those in Eagle Creek. What confounds these results, however, is that we found 73% mortality in the clams from the Chicago River while there was only 13% mortality from Eagle Creek. An assessment of the condition indices by Mann (1978), found that results can sometimes be misleading because they only account for fluctuations in water content, not any other potential factors. Therefore, this index may not have been very helpful in assessing the physiological condition of *C. fluminea*. Lucas and Beninger (1985) proposed another index known as Net Growth Efficiency, which calculates the amount of energy allocated toward tissue growth. While it may be more informative, it is also more complicated. This index requires calorimetry and lipid extraction as well as multiple measurements throughout bivalve development.

Corbicula fluminea *excretion rates, porewater* NH_4^+*, and* NH_4^+ *flux*

Our combined field and lab data suggest that NH_4^+ produced by *C. fluminea* via excretion was one of its major impacts on stream N dynamics. Excretion rates were

similar across lab and field studies, and NH_4^+ in cores with live clams were higher than in sediment with no exposure to clams in both the Chicago River and Eagle Creek. Excretion rates show high variation among taxa, organism size, age, and environmental conditions (Vaughn and Hakenkamp 2001), so it was difficult to compare the rates we found to other studies. However, James et al. (2000) found relatively similar rates in zebra mussels in the upper Mississippi River (0-200 μg N $gAFDM^{-1}$ h^{-1}).

Clams significantly increased sediment NH_4^+ flux relative to control sediment at both sites, which was likely due to clam excretion. NH_4^+ flux at Eagle Creek increased by a factor of 3, and in the Chicago River, flux shifted from net uptake to net NH_4^+ release that was nearly 5 times greater than the flux in Eagle Creek. While this difference in NH_4^+ flux cannot be accounted for solely by excretion rates, the excretion rates were higher in the Chicago River than in Eagle Creek. We predicted that NH_4^+ flux in the sediment with clams may increase NH_4^+ in sediment porespaces, however we have no evidence for this in the Chicago River, Eagle Creek, or laboratory study. Overall, the data suggest clams increase NH_4^+ in the water column only, which may be available for biofilm growth or nitrifiers downstream of clam excretion sites.

Clam effects on nitrification and N_2 flux

Clams could increase nitrification through two pathways: increased porewater NH_4^+, and increased oxygenation of sediments. Bivalves elsewhere have been shown to increase sediment nitrification through bioturbation, accelerating the degradation of organic matter and releasing ammonium (Henriksen et al. 1983, Chen et al 2005). We found no evidence for an increase in nitrification rates, but there were some patterns in

the data that suggest an influence of clams on nitrification may be possible. Nitrification was slightly higher at the Chicago River relative to Eagle Creek (although not significantly so), which could be attributed to higher water column NH_4^+. In addition, there was a trend of higher nitrification in the sediment exposed to clams (Figure 6). It may be that if we had incubated clams at higher densities in the trays we would see the trend continue to increase, since inorganic N and P fluxes out of sediments tend to increase directly with clam density (Zhang et al. 2011, Figure 10). Finally, because of the toxicity of DMSO and nitrapyrin, we were unable to run nitrification assays in sediment with living clams (as we did for N_2 and O_2 flux). We may have measured higher nitrification rates in cores with living clams, as this also increased denitrification rates relative to sediment which was simply exposed to clams in the field. However, our results for NH_4^+ flux, porewater NH_4^+ and nitrification suggest that while clams contribute to the NH_4^+ pool in the water column, any influence they have on nitrification likely occurs downstream, where NH_4^+ may again contact the sediment-water interface.

Live *C. fluminea* clearly and significantly increased N_2 flux from sediments in the field study. To our knowledge, this is the first study to examine the effects of live *C. fluminea* on N_2 production. This was captured during our flow-through analysis in which live clams were left in the cores for 3 d during the assay. Since there was a constant flow of water, the clams survived and were actively burrowing, excreting, and feeding within the cores. This flow-through technique is commonly used in lakes (Zhang et al. 2011) and coastal sediments (Gardner and McCarthy 2009), but less frequently used in analysis of nutrient fluxes in stream sediments (but see Juckers et al 2013). We acknowledge the

water velocity in the flow-through cores is lower than stream water velocity. However, the technique has advantages over static water incubations (e.g., acetylene block for denitrification or light/dark bottle methods for respiration) as it maintains constant replacement of water, allows organisms to be relatively unperturbed during the assay, and there is no manipulation of ambient light, dissolved gas, or water chemistry.

Denitrification is driven by the availability of NO_3^-, organic carbon, and anoxic conditions (Newell et al. 2002). Denitrification was higher in the Chicago River than in Eagle Creek, as was NO_3^- concentration. There was less organic matter in the Chicago River compared to Eagle Creek, suggesting NO_3^- availability, rather than C, was driving differences in N_2 flux. Nitrate can be supplied to denitrifying microbes directly from the water column or sediment porespaces (i.e., direct denitrification), or may be provided by nitrifiers in coupled nitrification-denitrification reactions (i.e., indirect denitrification). Results from the field study strongly suggest that clams increased direct denitrification. N_2 flux and water column NO_3^- were 2 and 59 times higher, respectively, in the Chicago River than in Eagle Creek. In addition, there were no effects of clams on nitrification or porewater NH_4^+ concentrations, also supporting the conclusion that the mechanism of clams' influence was direct denitrification. Finally, there was an increase in sediment N_2 flux when live clams were present in the cores relative to sediment that was exposed to clams, suggesting that the presence of live, burrowing clams was necessary for increasing N_2 flux. This burrowing activity could increase the diffusion of NO_3^- in the water column to sediment microbes, while NH_4^+ in clam waste expelled through their siphons into the water column. Zhang et al. (2011) used microelectrode profiles to show that burrowing *C.*

fluminea increase sediment oxygenation and organic matter mineralization, and proposed that increased water column diffusion may increase nitrification and denitrification.

Denitrification rates are typically higher in rivers than other aquatic ecosystems, but can vary greatly due to temperature, season, and other spatial and temporal factors (McCutchan and Lewis 2008). Denitrification rates measured via N_2 flux in this study were in the same range as studies that have used ^{15}N tracers and acetylene-block to measure denitrification. Using isotopic tracers, the range for denitrification in 24 urban streams throughout North America was ~500-4000 μg N m^{-2} h^{-1} and in 24 rural streams was ~90-800 μg N m^{-2} h^{-1} (Mulholland et al. 2008). The N_2 flux we measured in the Chicago River and from the non-urban stream were also similar (~400-700 μg N m^{-2} h^{-1}). Bruesewitz et al. (2006) measured effects of zebra mussels on denitrification using acetylene-inhibition. Their results showed DEA to be N limited and highly variable, but their measurements were well above our measurements at ~500-250 mg N m^{-2} h^{-1} (Bruesewitz et al. 2006), relative to ~0-115 mg N m^{-2} h^{-1} (this study).

The lab study suggested that organic matter, rather than clams or nutrients, were the primary driver of DEA. However, the field study did not show organic matter to drive N dynamics or that clams affected organic matter. This discrepancy could be due to (1) differences in organic matter abundance in the field vs. lab, or (2) differences in the technique for measuring N_2 production in the 2 studies. Organic matter content was approximately 6 times higher in the field study than in the lab. At these levels, it did not appear to be limiting to N_2 production. In the lab study, there were no live clams present in the DEA assay, while in the field, we had live clams in the flow through incubations.

This is because the conditions in the DEA bottles do not allow for living clams (i.e., anoxia, acetylene, and chloramphenicol). We did not use flow through cores for the lab study simply because we did not have the resources or equipment available at the time. Overall, our results suggest that the nutrients and sediment organic matter were more important to DEA than the clams in the lab study, while in the field the ambient concentrations were high enough that organic matter was not limiting to N transformations.

Overall effect of Corbicula fluminea *on N dynamics at study sites*

C. fluminea increased rates of biologically active NH_4^+ in stream water via excretion, but they also increased the amount of inert N_2 flux from sediments most likely produced through burrowing and diffusion of water column NO_3^- (rather than through coupled nitrification-denitrification). We compared these fluxes of inorganic N by scaling up the rates from the cores using benthic *C. fluminea* density at each site to generate conclusions regarding their net effect on inorganic N dynamics at the scale of 1 m^2 of streambed.

In Eagle Creek, the additional NH_4^+ flux out of the sediment with live clams (relative to control sediment) was higher than the increase in N_2 flux (466 μg N m^{-2} h^{-1} NH_4^+ flux and 280 μg N m^{-2} h^{-1} N_2 flux). This suggests that while clams increased the amount of N in the N_2 pool, they increased the amount N in the NH_4^+ pool even more, so their net effect was to increase biologically active inorganic N in the stream. In the Chicago River, however, the additional NH_4^+ flux out of the sediment from the live clams was less than their increase in N_2 flux (3,623 μg N m^{-2} h^{-1} NH_4^+ flux and 7,121 μg N m^{-2}

h^{-1} N_2 flux). This increase may represent an ecosystem service of *C. fluminea.* However, we note that a considerable amount of N is stored in clam tissues given their high density (Table 2), and their death and decomposition, especially during cold weather months, likely increases bioavailable N at those times. A full accounting of *C. fluminea*'s influence on inorganic and organic N pools is a prerequisite to claiming the ecosystem service of denitrification enhancement.

Our understanding of the clams' net impact on inorganic N fluxes is complicated by the NO_3^- flux patterns, which showed high NO_3^- uptake in the Chicago River and release of NO_3^- in Eagle Creek, regardless of the presence of clams (Figure 10). This NO_3^- release in Eagle Creek could be partially attributable to nitrification, however, the rate of nitrification in sediment exposed to clams was lower (27 μg N m^{-2} h^{-1} in Eagle Creek) relative to NO_3^- flux out of these sediments (169 μg N m^{-2} h^{-1} in Eagle Creek). Zhang et al. (2011) found NO_3^- release from sediments in cores with live *C. fluminea* attributed to increased nitrification and diffusion. It is unclear where the source of the NO_3^- flux from sediments in Eagle Creek originates, but it could at least partially represent the nitrification effect we were unable to measure in the cores with live clams.

Macroinvertebrate community and relative clam density

Our survey of benthic macroinvertebrates showed that *C. fluminea* dominates benthic biomass in both the Chicago River and Eagle Creek, but particularly in the urban stream. We have no data on past *C. fluminea* densities at either site, but our evidence for the 'snapshot' of macroinvertebrate communities in June 2012 suggest *C. fluminea* can thrive under urban and rural stream conditions, while other bivalves are less successful.

However, there were more native mussels present in Eagle Creek, which represented about 25% of benthic macroinvertebrate biomass. Our data cannot address whether or not *C. fluminea* outcompetes native bivalves in the urban or rural stream, or if their relative composition is determined by other environmental factors (i.e., urbanization, substrate composition, and reproduction). Vaughn and Spooner (2006) found no significant relationship between *C. fluminea* and native mussels. Karatayev et al. (2003) found no correlation between the biomass of *C. fluminea* and that of other invertebrates in a eutrophic Texas lake. Our expectation is that the external factors affecting water and habitat quality drive relative community composition of lotic bivalves, but it is unclear how bivalve interactions change in urbanized streams relative to more pristine habitats.

Conclusion

Corbicula fluminea is a conservation concern because of its invasibility, biofouling potential, and potential for competition with native species. The rapid growth and high fecundity this species exhibits allows them to invade a variety of freshwater ecosystems and sustain high population densities (Sousa et al. 2008b). The high abundance and unique feeding capabilities allows this species to alter food webs and stream ecosystem function, particularly if populations continue to increase while native species decline (Atkinson et al 2010). Due to its wide distribution and high density this species merits increased attention and monitoring to document its population and ecosystem effects. Our results indicate that *C. fluminea* can be the major driver of pools and fluxes N in the stream benthos. More studies are needed to determine the fate of N taken up in *C. fluminea* biomass over longer time scales, their effect on N transformations

relative to native mussels, and to document seasonality in *C. fluminea*-mediated fluxes of NH_4^+ and N_2.

C. fluminea can contribute to economic problems through biofouling and subsequent clean-up procedures (Darrigran 2002). It is estimated that this species alone accounts for approximately $1 billion annually in control measures and damages just in the United States (Pimentel et al. 2005). While some treatments such as filters, physical removal, and chemical controls have been employed, they are not often appropriate for open water systems. Even those that have been developed for use in lakes or streams may have short-term success, but the long-term status is unknown (Wittmann et al. 2012).

CHAPTER III

EXTERNAL EFFECTS ON BURROWING BEHAVIOR OF THE ASIAN CLAM (*CORBICULA FLUMINEA*)

Introduction

Studying locomotion can provide insight into animal life history strategies. Bivalves are generally considered to be sedentary organisms, but they engage in different types of locomotion across their life stages (Kondo 1997). Larvae can be planktonic, parasitic, or have their own basic swimming capacity (Neves and Widlak 1987). As adults, bivalve behavior includes feeding, filtration, mating, excretion, burrowing, and lateral movement (Amyot and Downing 1997, Vaughn and Hakenkamp 2001). Some bivalves also experience seasonal migration (Watters et al. 2001). In addition, external factors such as sediment contamination, the type of substrate, and presence of predators can affect bivalve locomotion (McCloskey and Newman 1995, Schmidlin and Baur 2007, Saloom and Duncan 2005).

Corbicula fluminea (Muller1774) has been highly invasive to freshwater ecosystems in the United States since 1938 (Araujo et al. 1993). This species is a burrowing clam, which impacts the physical and biogeochemical properties of the ecosystem through bioturbation and sediment mixing (McCall et al 1986, Allen and Vaughn 2009). Burrowing evolved as a mechanism to continue feeding while avoiding predation or harmful environmental factors (Amyot and Downing 1997). *C. fluminea* are

most often found in sandy substrates (Schmidlin and Baur 2007), and have been shown to select sand substrates over gravel. In addition, there is some evidence to suggest *C. fluminea* avoid contaminated sediments, settling instead in uncontaminated sediments (McCloskey and Newman 1995). However, it is unclear if clams "choose" which substrate to burrow in, if they move among substrates, or if the portion of the population which settles in less favorable substrates die off, while those in better habitats thrive. Understanding drivers of *C. fluminea* burrowing behavior is important because they can be present in very high densities and have large effects on stream ecosystem communities and processes.

Little is known about horizontal movement in freshwater mussels or clams. Horizontal movement is theorized to occur in response to stressful environmental conditions such as low food resources or anoxic conditions (Saarinen and Taskinen 2003). Amyot and Downing (1997) examined horizontal movement in a freshwater mussel, *Elliptio complanata*, to document spatial population dynamics. They found that mussels did not move horizontally once they burrowed and travelled relatively short distances annually (i.e., <3 m per year; Balfour & Smock 1995). Schwalb and Pusch (2007) also showed that unionid mussel annual movements are small and appear to be erratic. To our knowledge, there have been no studies on horizontal movement in *C. fluminea*.

Bivalves respond to predators largely by closing their valves, but they could also respond through changes in burrowing rate or horizontal movement. *C. fluminea* have a wide range of predators, predominantly fish and crayfish, but also birds, raccoons, and

muskrats (Robinson and Wellborn 1988, Strayer 1999, Saloom and Duncan 2005). Predation and environmental factors affect clams behavior including increased burial depth and longer valve closure times (Ortmann and Grieshaber 2003). Like other burrowers, *C. fluminea* may face a physiological trade-off during predator evasion. Burying allows for protection from predators, but can inhibit valve opening for feeding or ventilation (Saloom and Duncan 2005).

Crayfish are a natural predator of *C. fluminea* and may potentially benefit from the introduction of *C. fluminea* as a novel food source in invaded ecosystems (Covich et al. 1981). An invasive species in the northern Midwest, the Rusty Crayfish, *Orconectes rusticus*, inhabits some of the same ecosystems as *C. fluminea* (Taylor and Redmer 1996). *O. rusticus* is widespread throughout the United States and was first found in Illinois in 1973. Since then it has become the most abundant crayfish species in most of the sites at which it is found, often to the detriment of native crayfish taxa (Taylor and Redmer 1996). Studies using *Procambarus clarkii* and *Cambarus bartonii* showed the crayfish easily consume small clams, larger clams with damaged shells, and clams that had recently died (Covich et al. 1981). However, clams were less likely to be eaten when they were buried in the substrate (Klocker and Strayer 2004). While clams are clearly at risk to predation from crayfish, no previous studies have documented if *C. fluminea* changes its burrowing behavior when exposed to crayfish predators. Understanding the predator-prey dynamics between these macroinvertebrates will be useful for predicting their ecosystem effects and managing their populations.

Our objectives in this study were to: (1) determine the effect of substrate type on *C. fluminea* burrowing behavior and horizontal movement and (2) determine the effect of a predator, *O. rusticus*, on *C. fluminea* burrowing behavior.

Materials and Methods

The influence of sediment type on clam burrowing and horizontal movement

The objective of the first set of studies was to measure the effect of substrate type on *C. fluminea* burrowing rates and horizontal movement. Clams were collected from the North Branch of the Chicago River on 21 February 2013 and brought to the artificial stream facility at Loyola University Chicago. Artificial streams are re-circulating chambers with a paddle wheel, where channel width = 13.97 cm and total flowpath length = 203.2 cm.

We set up 3 replicate streams, each containing trays with different substrate types: (1) playground sand, (2) a 50/50 mix of sand and potting soil, (3) small gravel (mean diameter = 5.9 mm), (4) large sized gravel (mean diameter = 12.3 mm), and (5) extra large gravel (mean diameter = 19.1 mm). Each stream had 3 trays of each sediment type. Trays were 23 cm X 14 cm X 9 cm plastic take-out containers (Plastic Take-Out Container, Hangzhou Yusheng Plastic Products Co. Ltd., Hangzhou City, China). We marked a grid in units of 1 cm on all sides of the tray and placed 5 evenly spaced clams in each tray. The clams in each tray were painted with a different color nail polish on one valve to identify and track them. Immediately after being placed in the stream, each tray was recorded with a video camera suspended 40 cm above the tray on a ring stand for approximately 1 hour. Researchers watching the video considered clams fully buried if <

1/2 of the shell was visible above the substrate. Using this parameter, clams were considered either buried or not buried after 60 min. The next day, we counted the number of clams buried after 24 h.

To measure horizontal movement, we took a picture of each tray to document the clams' initial positions. One tray was collected each week for each of the substrate types, and we recorded how far each of the 5 clams had moved from their initial position. Some of the clams were visible at the surface, but if they were not, we gently probed with a closed pen to find the position of buried clams. To calculate horizontal movement, we measured a direct line from initial position to final position using the grid marked on the sides of the tray.

Sediment preference experiment

The sediment preference experiment was set up in separate artificial streams. In each stream, we placed 3 trays containing 6 clams. The trays were filled with 2 types of sediment so there was a line of separation running down the center of the tray, parallel to the direction of stream flow. The trays were filled with sediment that contained a 50/50 sand and organic matter mixture on one side of the tray, and gravel (mean diameter 5.9 mm) on the other, with a piece of cardboard in the middle. When the cardboard was removed, this established a clear distinction between substrate types. The clams were marked with nail polish to follow individuals on video. The clams were positioned in the center of the trays so that 3 had their foot facing one substrate and the other 3 had their foot facing the other substrate. Immediately after placing the trays in the stream, each tray was recorded with a video camera to observe the direction and speed of clam burial. We

considered clams buried in a particular substrate if more than half of the body was buried in that substrate. Any clams that buried straight down or did not bury at all were noted and recorded in separate categories. We recorded preference for each clam for 1 h after placing them in the tray, and then again at 24 h.

Effects of predator presence on clam burrowing

Crayfish are natural predators for clams (Covich et al. 1981). We used rusty crayfish (*Orconectes rusticus*) as a model predator to document its effect on *C. fluminea*'s behavior. This study was conducted in aquaria, rather than artificial streams, because monitoring crayfish interactions with clams was much more successful under aquarium conditions. We set up 9, 38 L aquaria as experimental replicates. Three aquaria had an unrestricted crayfish which were able to directly contact the clams. To represent a perceived predator, another three aquaria had crayfish contained in 1 cm^2 wire mesh cages (20 cm x 15 cm x 15 cm). The final three tanks were controls (no crayfish). To start the experiment, 10 clams from crayfish-free artificial streams were added to each aquarium. Each aquarium was recorded with a video camera for 1 h, beginning when all clams were placed into the tank. We recorded clam burial speed, and the number of clams buried after 1 h and after 24 h as described above.

Effects of predator intensity on clam burrowing

The final experiment measured crayfish behavior influenced clam burrowing using a video camera. Crayfish behaviors included walking on the clams, manipulating clams, and picking up/moving clams. We ran 10 new trials using aquaria with clams and unrestricted crayfish. We tallied how many interactions occurred in each trial. We then

categorized the crayfish-clam encounters as “low interaction” when ≤ 14 manipulations occurred during 1 hour, and “high interaction” if ≥15 manipulations occurred during 1 hour.

Statistical analyses

We analyzed burrowing rate across substrate types using a one-way ANOVA followed by Tukey’s multiple comparison test. We used simple linear regression to test the relationship between horizontal movement and substrate particle size. To determine substrate preference, we ran a t-test on burial location, and a t-test on those clams that buried in the direction of their foot relative to those which buried in a different direction. We used an ANOVA to test for the effects of an unrestricted and caged predator on the burrowing speed as well as the proportion of clams that buried. Finally, we used a t-test to analyze if the crayfish interaction intensity affected the proportion of clams that successfully buried. All statistical tests were run in SYSTAT 13 (Systat Software, Cranes Software International Ltd., Chicago, IL).

Results

Substrate size affected both clam burrowing speed and horizontal movement. Larger gravel substrates slowed down the rate of burial, with the largest gravel size (19.1 mm) greatly slowing down the burial process (ANOVA $p<0.01$, Figure 11). With increasing particle size, the horizontal distance moved by the clams decreased (regression, $R^2=0.257$, $p=0.05$; Figure 12). However, the distance measured over the course of the 21 d experiment was very small across treatments. The average distance

was 11 mm per 21 days and the maximum distance moved by any clam was just over 40 mm during the 3 week period.

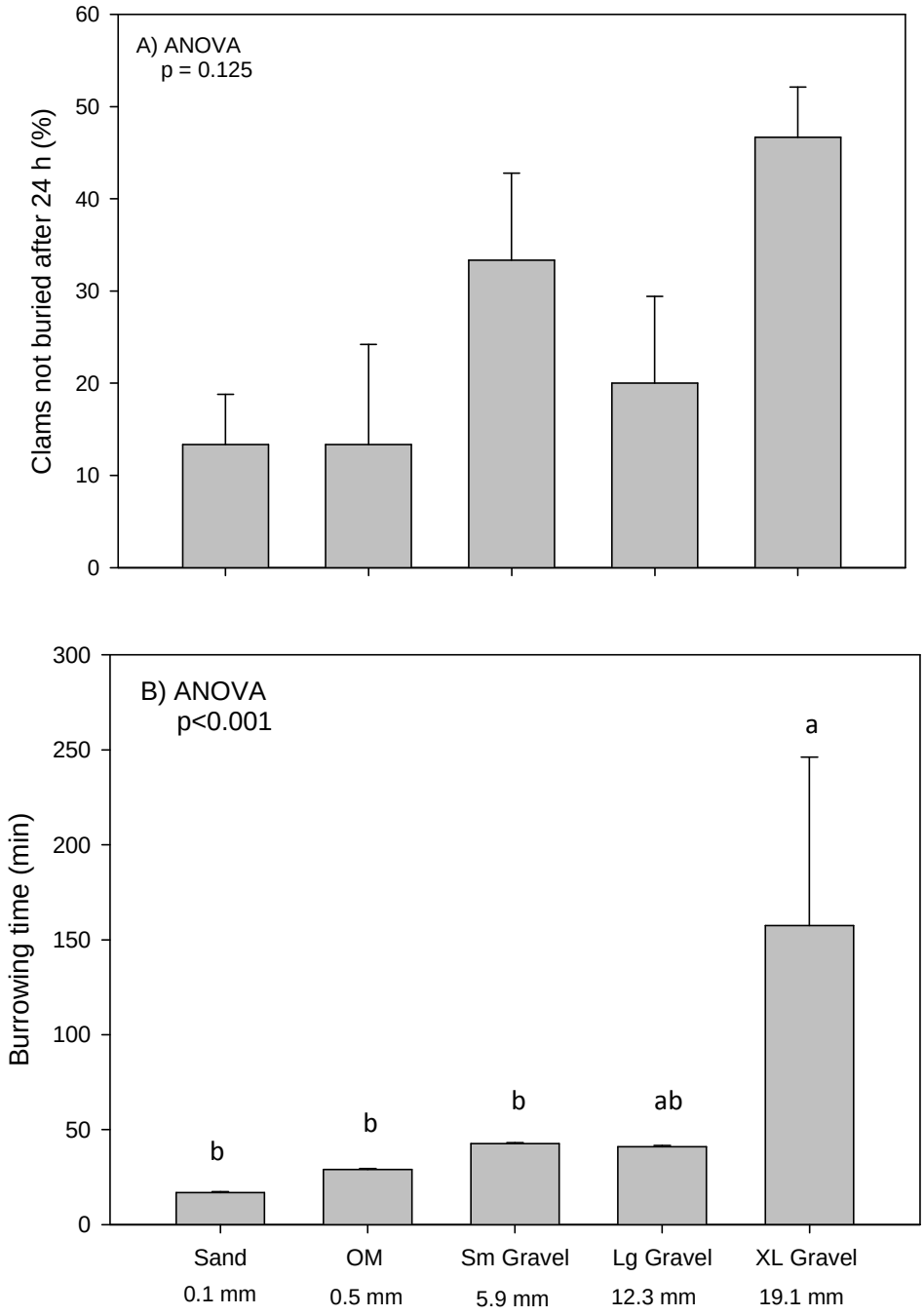

Figure 11. The percentage of clams that did not bury (A) and the average burrowing time of clams in varying substrates ordered from smallest particle size to largest (B). XL Gravel significantly slowed the burial of *C. fluminea* (ANOVA $p<0.001$). Error bars represent standard error.

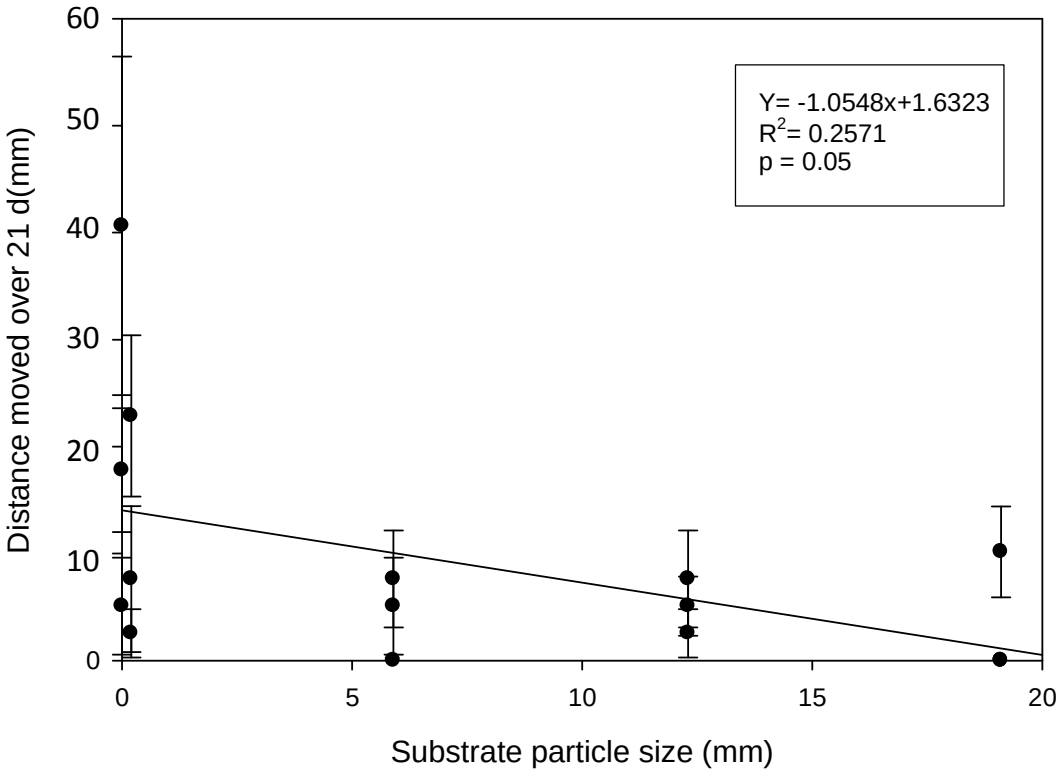

Figure 12. Regression showing the decrease in horizontal distance moved over 21 d as particle size increases.

Despite the differences in burial rate among sediment size classes, clams did not show a preference for one substrate over another (t-test p=0.395). Instead, clams displayed a tendency to burrow in the direction of their foot (t-test p=0.05, Figure 13).

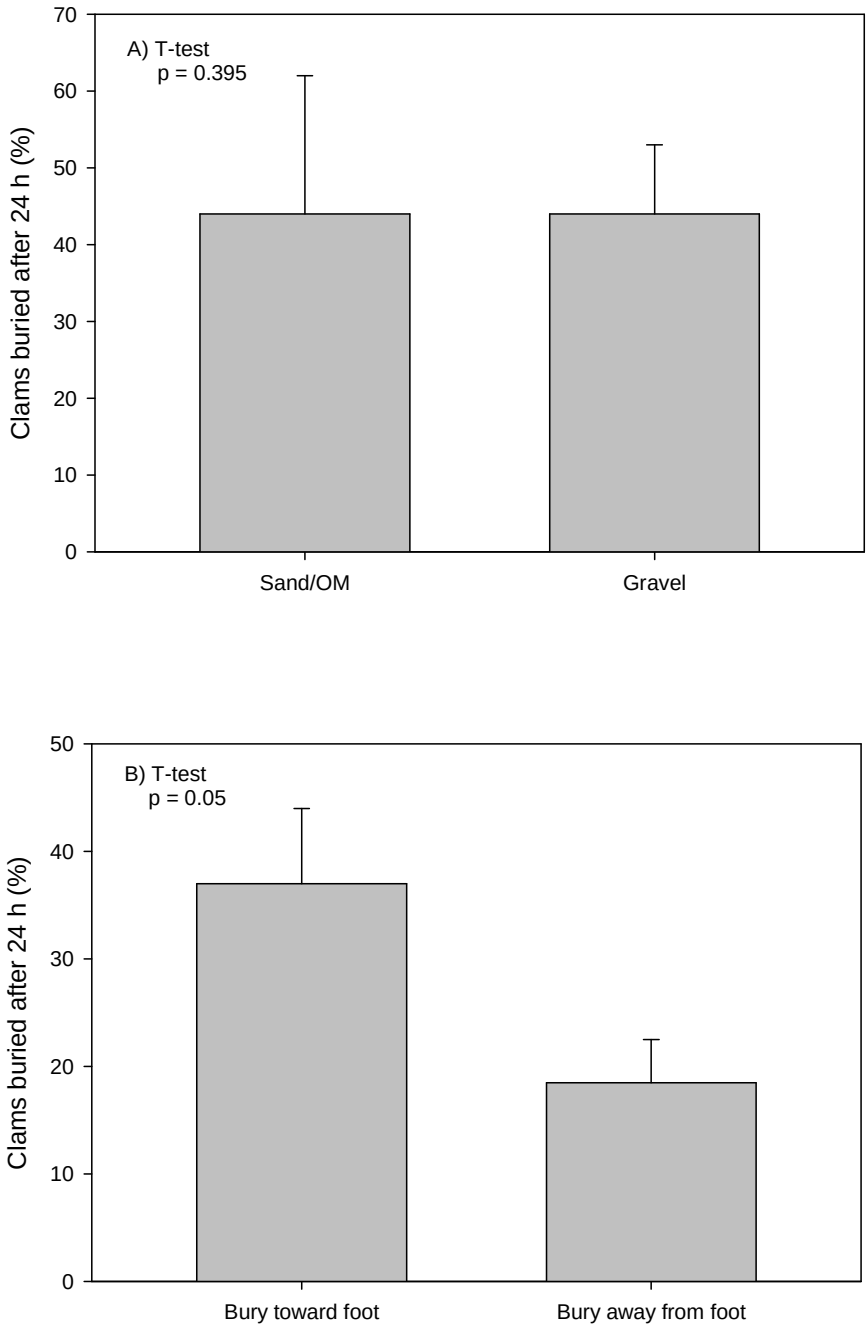

Figure 13. *C. fluminea* showed no preference in substrate between the sand/organic mixture and gravel. Clams burrowed in the direction of their foot more often than away from their foot or straight down (8%). Error bars represent standard error.

To examine the effects of a predator on clam burrowing behavior, we first tested if clams would sense and respond to a predator in the water by altering burial speed and proportion of clams buried. Burial speed and proportion of clams buried were the same among the control, caged predator, and unrestricted predator treatments (Figure 14). However, when the crayfish were able to manipulate the clams, the frequency of their manipulations reduced the proportion of clams that successfully buried. Where predators infrequently touch the clams, their burial proportion was over 30% more than when the crayfish frequently manipulated the clams (t-test $p=0.021$, Figure 15).

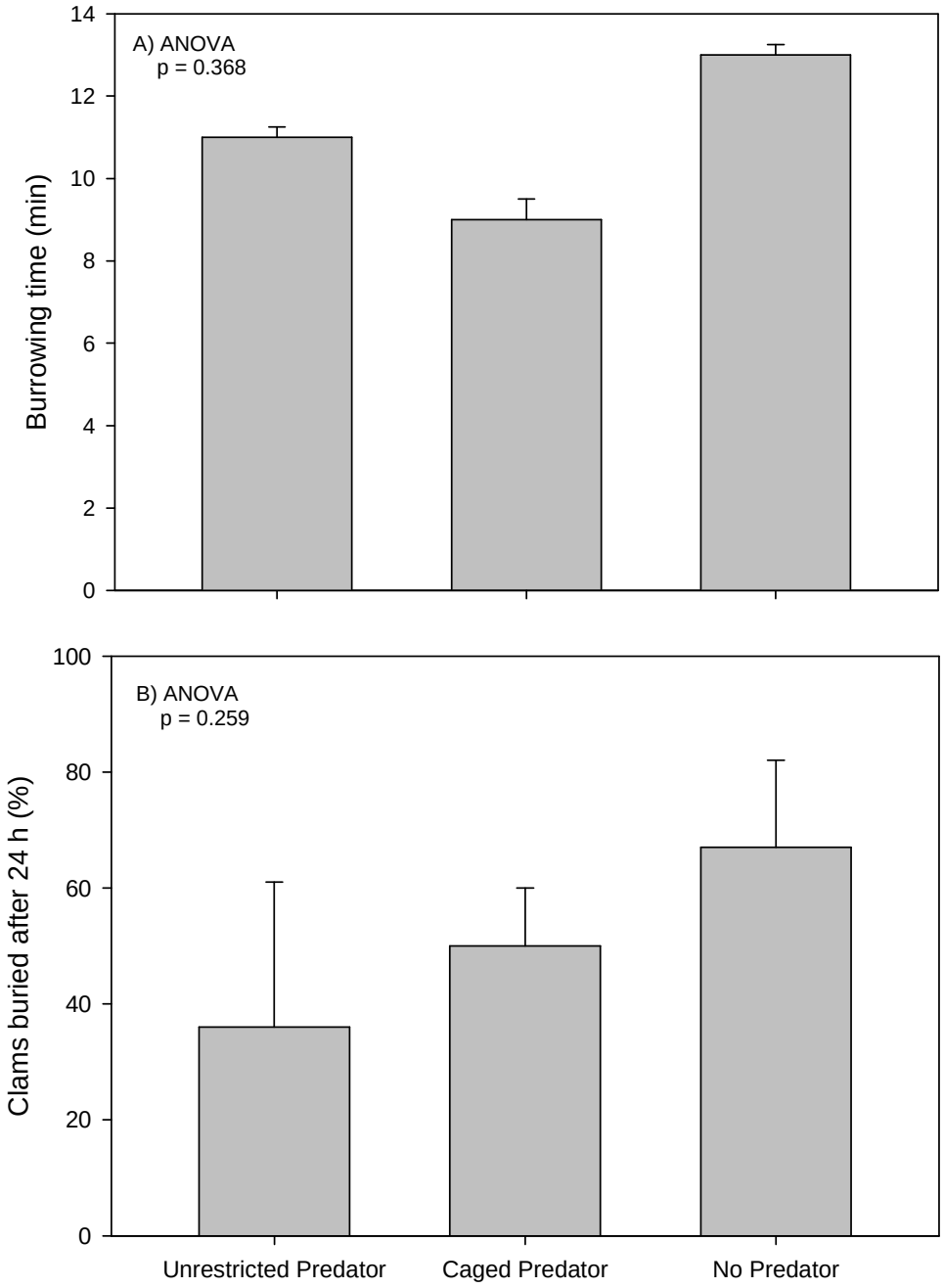

Figure 14. Burrowing time (A) and percentage of clams buried (B) when a predator was able to directly manipulate clams, was present but unable to access clams, and no predator present. Error bars represent standard error.

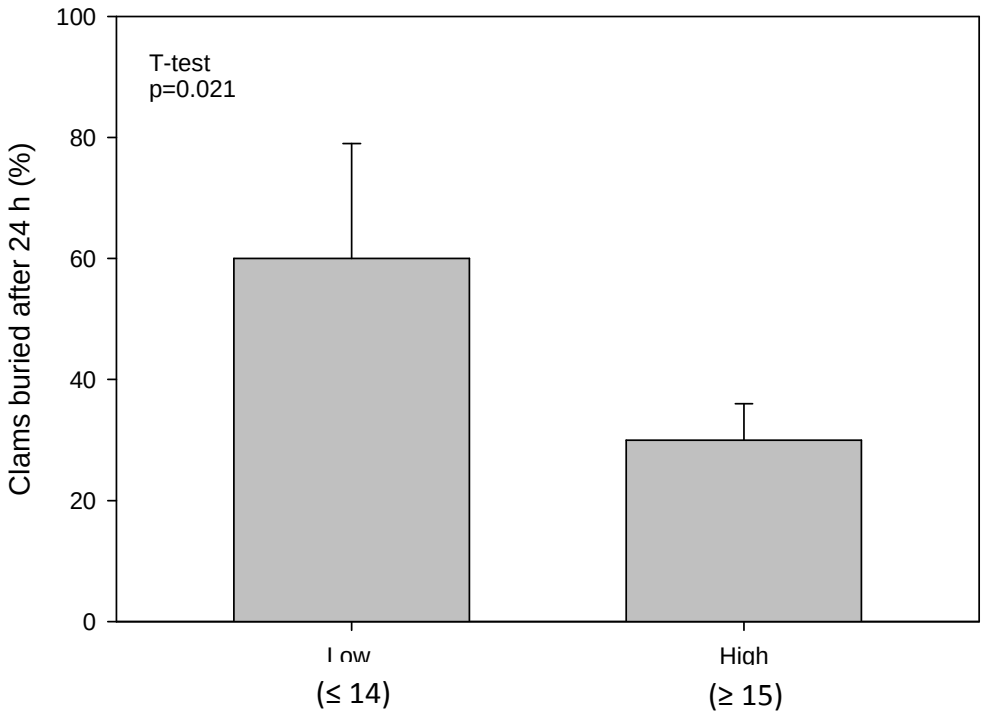

Figure 15. More clams buried in the substrate when there were fewer crayfish interactions within one hour compared to when crayfish frequently manipulated the clams.

Discussion

Documenting environmental controls on burrowing behavior in bivalves is important for understanding the aggregation of their populations in particular substrate types, predator avoidance strategies, and reproductive success (Downing et al. 1993). In addition, as burrowing and horizontal movement come at some energetic cost, documenting abiotic and biotic drivers of these behaviors can help establish conditions that might benefit species conservation (i.e., endangered Unionidae mussels) or present new options for mitigating effects of invasive species such as *C. fluminea*.

As expected, increased substrate particle size, particularly the largest gravel (19.1mm), slowed *C. fluminea* burrowing speed and impaired horizontal movement. It was not surprising to find little lateral movement after burial, however, there were no previous studies of lateral movement in *C. fluminea*. In a similar fashion, freshwater mussels have been known to move only very small distances over the course of a year (Balfour and Smock 1995). Amyot and Downing (1997) found nearly all of the freshwater mussels, *Elliptio complanata*, which buried into the sediment in the fall emerged in the spring at the same location. The only vertical and horizontal movements in *E. complanata* were associated with seasonal variation and spawning periods. A subsequent study by Amyot and Downing (1998) suggested that lateral movement in *E. complanata* could bring males and females closer together, with females moving less because of the energetic cost. *C. fluminea* populations have high numbers of hermaphrodites (Hillis and Patton 1989) and high population densities, so lateral movement for reproductive behavior is likely unnecessary.

We expected *C. fluminea* to prefer finer substrates over larger substrates, but found no evidence for substrate preference. Instead, *C. fluminea* typically buried in the direction their foot was facing. Our results are in contrast to a similar preference study on *C. fluminea*, which reported a selection for finer substrates over gravel (Schmidlin and Baur 2007). However, this study was conducted *in situ* and did not examine the direction of the foot. McCloskey and Newman (1995) showed that *C. fluminea* chose uncontaminated sediment over contaminated sediment when given a choice. However, the authors note external factors affected these results, as both the clams and the snails in the study (*Campeloma decisum*) preferred the left side of the aquarium over the right side of the tank despite sediment contamination.

Previous studies on sediment preference assume *C. fluminea* sense the substrate available, and either change burrowing direction or move horizontally towards a substrate that is more appealing (i.e., smaller grain size or uncontaminated sediments; Schmidlin and Baur 2007). However, previous research has not measured horizontal movement or 'course correction' during burrowing. Our results show no evidence that either mechanism for sediment preference occurs. Instead, it appears the clams largely burrow in the direction in which they were placed (Fig 13), and there was little horizontal movement in any of the substrate types (Fig 12). In general, substrate preference in *C. fluminea* is not well documented, and should be examined further for a better understanding of how clams' sensory capacity (or lack thereof) influences their burrowing behavior.

Another factor that might impact *C. fluminea* burrowing behavior is the presence of predators. Robinson and Wellborn (1988) suggested that the burrowing activity of *C. fluminea* evolved to reduce the risk of predation. This anti-predator behavior is also exhibited by other freshwater bivalves such as unionid mussels (Waller et al. 1999). Klocker and Strayer (2004) showed that fingernail clams (Sphaeriidae) were less likely to be consumed when they were buried in the sediment compared to clams at the surface. Therefore, we expected that more clams would burrow into the substrate, and do so more quickly, in the presence of an unrestricted predator that could actively manipulate them, and when there was a caged predator nearby (sensed via a chemical cue). Our results did not show this pattern. Instead, we found that *C. fluminea* showed neither a burrowing speed response nor a change in the percentage of individuals that buried when an unrestricted or caged predator was present relative to control conditions. We acknowledge that an alternative explanation for this pattern is that this species of crayfish was not perceived as a predator by the clams. Studies using other predators (i.e., fish) or predators from the clams' native habitat may be needed to resolve this question. However, there was little behavioral change in the clams aside from valve closure when touched directly by predators. As with the results for substrate preference and horizontal movement, these data suggest that clams did not sense and then respond to the presence of the predator in their environment by changing burrowing rate.

The strongest effect of predators on clam burrowing behavior was when they were frequently manipulated by the crayfish. More clams buried successfully when they were left untouched or when there were few interactions by the crayfish, and 30% fewer clams

buried when the crayfish frequently manipulated them. The explanation for this pattern is that when the clams are touched, they instinctively close their valves to protect damage to their soft tissue. While this behavior avoids immediate predation, frequent valve closure due to predator manipulation could generate some energetic cost to clams over the long term. If clams must continually close and re-bury themselves, they lose potential feeding time, have restricted respiration while closed, and expend extra energy during repeated burrowing. This predator effect on was shown in a study of blue mussels (*Mytilus edulis*). Robson et al. (2010) found there was a trade-off between maximizing feeding and avoiding predation with respect to valve closure. These energetic costs have not previously been quantified for *C. fluminea*, and represent a potentially overlooked, sub-lethal effect of predators on *C. fluminea* physiology.

To our knowledge, little is known about the physical capacity for *C. fluminea* to sense their environment and respond to stimuli through a change in locomotion. That is, it is unclear if *C. fluminea* can distinguish among substrate types with touch receptors or if they can sense predators in water using olfactory or other chemosensory organs. In addition, it is unknown if clams have the neural capacity to translate those senses into a change in behavior. Except when the crayfish were physically manipulating the clams, it appears chemical cues of predator presence were not received or were not processed into anti-predator behavioral responses. Many superorders of marine bivalves such as *Limoidea* and *Mytiloidea*, along with some freshwater bivalves are known to have photoreceptors (Morton 2008), and zebra mussels can detect certain contaminants through chemoreceptors (Kraak et al. 1992), but published research on *C. fluminea*

sensory capacity is minimal. Our data suggest the ability for either sensory capacity or behavioral response are limited, but more studies are needed that address their physiology and behavior, conducted in the context of meaningful ecological parameters such as predation and substrate variation.

Low sensory capacity for substrate selection or predator avoidance in *C. fluminea* suggests that if individual clams are located in habitats that inhibit their burial, or in locations with predators, they are likely at a higher risk of predation as they will not search for different substrate or increase speed of burial. However, *C. fluminea* is a successful invasive species worldwide. This indicates that even if individuals are not particularly well-equipped to move themselves to avoid predation or non-suitable substrates, the *populations* persist because other life history strategies such as high fecundity and rapid growth facilitate invasion success.

A wide range of studies have focused on various aspects of *C. fluminea* ecology and invasion (Robinson and Wellborn 1988, Hornbach 1992, Sousa et al. 2008a, Ilarri et al. 2011). Several studies have examined valve closure behavior (Ortmann and Grieshaber 2003, Ham and Peterson 1994), but in general, research on other *C. fluminea* behaviors is sparse. However, behavior is a crucial part of what makes species successful as invaders or as ecosystem engineers (Holway and Suarez 1999). This study and subsequent analyses of *C. fluminea* behavior will be helpful in predicting the potential ecosystem effects and possible management options of the Asian clam in established or newly invaded habitats

CONCLUSION

Corbicula fluminea is a widespread invasive species in the United States and throughout the world (Araujo et al 1993). Concurrent with the drastic decline in native mussel populations in North America, the continued spread of this species can cause a multitude of alterations to taxa and ecosystem processes (Sax et al. 2007, Atkinson et al. 2011). My study showed that *C. fluminea* can have an effect on key processes in nitrogen cycling. However, the magnitude of its effects were variable based on density and surrounding environmental conditions. The results of the behavioral study show that instinctive responses of valve closure dominate clams response to environmental stimuli, rather than more sophisticated responses of substrate preference or predator sensing. I conclude that *C. fluminea* is such a successful invader not because it is an aggressive competitor, highly tolerant organism, or capable of avoiding predators, but rather because its life history strategies have evolved to allow populations to thrive, even to the detriment of an individual. Future studies regarding *C. fluminea* should be executed locally for the most accurate observation of the species effects on its ecosystem. Since biomass and abundance can vary so dramatically, the magnitude of the impact on the environment will likely vary as well (Sousa et al 2008a). It is also important to increase research on behavioral aspects of *C. fluminea* as it may be helpful in analyzing the interactions between this invader and native species. Also, maximizing knowledge

regarding the behavior and life history strategies of any invasive species will aid in management and preventative measures.

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