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Wastewater treatment plant (WWTP) effluent is a significant source of anthropogenic N loading to urban streams and has been shown to impact the ability of streams to provide ecosystem services of nutrient retention and denitrification. If a stream is unable to provide these services, then the downstream systems will receive higher N loads potentially causing eutrophication and reduction of biodiversity.

Corbicula fluminae (Asian clam) is an invasive species that has been shown to filter feed at a very high rate. I hypothesized that *Corbicula* functions to remove anthropogenic N at a sufficient rate to impact suspended particulate matter (seston) dynamics in a stream receiving treated urban wastewater. Stable isotope analysis was used as a tool to evaluate trophic relationships between seston and *Corbicula*.

Fieldwork was conducted on North Buffalo Creek, NC, USA. Two laboratory experiments were performed to evaluate *Corbicula* filtering rate both in the presence and absence of stream sediment. Ash free dry mass (AFDM), $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, C/N ratio, and chlorophyll a were measured over the course of 12 h in order to determine *Corbicula* impact on these seston variables over time. Field and experimental results showed that *Corbicula* in North Buffalo Creek was not filtering as has been described previously. My results indicate that *Corbicula* pedal feeds in the sediment.

Therefore, instead of providing an ecosystem service of removing sewage-derived N from the water column, *Corbicula* returns sediment bound nutrients to the water column, thereby contributing further to downstream eutrophication.

IMPACT OF *CORBICULA FLUMINAE* (ASIAN CLAM)
ON PARTICULATE MATTER TRANSPORT
IN AN URBAN STREAM

by
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CHAPTER I

INTRODUCTION

Anthropogenic nitrogen (N) addition to terrestrial systems is increasing worldwide, which increases N loading to freshwater systems (Peterson et al. 2001, Mulholland et al. 2008). Most urban streams receive both point and non-point sources of anthropogenic N. Non-point sources include pet waste, runoff from impervious surfaces, fertilizers, septic tanks and sewage overflows (Lofton et al. 2007). Even though point sources, such as wastewater treatment plants (WWTP) are highly regulated, they continue to have a prominent impact on the ecology of streams (Haggard et al. 2001). WWTP have been shown to contribute 50% to 90% of the annual nutrient inputs into urban streams (Haggard et al. 2005).

Stable isotopes of N have been a useful tool for detecting anthropogenic N in aquatic systems (Ulseth & Hershey 2005). Stable isotope signatures of consumers have been found to be enriched 3-5‰ in ^{15}N and 0.5-1‰ in ^{13}C relative to their food sources. These relationships permit these stable isotopes to be used to trace organic matter through food webs (Peterson & Fry 1987, Udy et al. 2006). WWTP effluent has a distinct isotopic signature that can be distinguished from other potential N sources (Ulseth & Hershey 2005, Nishikawa et al. 2009). Sewage-derived nitrogen (SDN) is readily traced through aquatic food webs because it has a higher concentration of the heavy isotope than most natural N sources (Northington & Hershey 2006). Udy et al. (2006) found that both

plants and sediment had an elevated $\delta^{15}\text{N}$ above the approximate $\delta^{15}\text{N}$ value for treated sewage (10‰), indicating not only an enriched point source of N but also that the N cycle of the stream had been altered in the presence of WWTP effluent.

WWTP effluent released into urban streams has a dramatic and long-lasting impact on these ecosystems by altering their natural ability to process N. WWTP effluent impact is an important environmental concern because increased levels of anthropogenic NO_3^- transport downstream to coastal waters accelerates eutrophication, causes the formation of hypoxic zones and algal blooms, and reduces biodiversity (Rosenzweig et al. 2008, Weijters et al. 2009). Input from a WWTP not only changes the water chemistry for several kilometers downstream of the effluent, but also affects nutrient cycling processes (Haggard et al. 2005). As N input increases, the stream N-processing efficiency decreases, resulting in inorganic N travelling much further downstream before being taken up by the biota (Peterson et al. 2001). If the stream is unable to retain N, then the downstream system will receive higher N loads (Grimm et al. 2005), which is a particularly serious problem in coastal regions of NC, where N loading is occurring from upstream areas. Mulholland et al. (2008) surveyed 72 streams in 8 different regions and determined that the efficiency of both biotic uptake and denitrification decreases as the concentration of WWTP effluent increases, thereby reducing the proportion of in-stream nitrate that is removed from the system. Lofton et al. (2007) found that an urban stream receiving WWTP effluent exhibited a low denitrification rate and therefore exported large quantities of N downstream. Sensitive stream species, such as fish and EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa, are the first to disappear in N-polluted

waters, and are replaced with more tolerant species that are sometimes non-native (Weijters et al. 2009).

Corbicula fluminea (Asian clam) is an invasive species that competes with native fauna for habitat and nutritional resources. *Corbicula* is a self-fertilizing simultaneous hermaphrodite that may reach maturity in as little as 3 months and have a lifespan of 1 to 4 years, with the ability to spawn once or twice a year (Strayer 1999, McMahon & Bogan 2001). *Corbicula* is known to have a high fecundity on average, producing 68,678 juveniles per adult per year (McMahon & Bogan 2001). *Corbicula* is recognized as one of the most notorious invasive species in aquatic ecosystems and has been the focus of several studies in America and Europe. Sousa et al. (2008) found that *Corbicula* has very high secondary production and hypothesized that it may be responsible for changes in benthic assemblages. Delong (2010) reported that *Corbicula* showed an assimilated diet very similar to that of the well-known zebra mussel (*Drissena polymorpha*) when stable isotope signatures were compared between the species within the Mississippi River system. Hakenkamp & Palmer (1999) found that *Corbicula* consumption of oxygen in a shallow streambed was 46%, thus indicating that *Corbicula* was able to consume a significant portion of the dissolved oxygen in the water column.

Corbicula may filter feed, decreasing phytoplankton abundance (Cohen et al. 1984), or pedal feed on particles in the sediment (Strayer 1999). Lauritsen (1986) found that *Corbicula* filtration rate at 20°C was about 905.8 ml/h for 22.4 mm clams, and estimated that clams on the Chowan River could filter the overlying water (mean depth 5.25m) every 1.6 days when chlorophyll concentrations were high. Sousa et al. (2009)

showed that *Corbicula* was able to filter the water column at a sufficient rate to reduce phytoplankton and zooplankton density, thereby increasing water clarity. Hakenkamp & Palmer (1999) estimated *Corbicula* consumption rate of sediment organic matter was 50 mg bivalve⁻¹ day⁻¹ through pedal feeding.

The ability of *Corbicula* to impact the water it inhabits is due to the basic biology of the animal. *Corbicula* is known to have ctenidia with 32-42 cilia/cirrus, which is much higher than many native unionids which have 11-16 cilia/cirrus. Having more cilia/cirrus allows the clam to have a higher clearance rate of water. However, not all particles taken in by the clam are ingested. *Corbicula* is known to create pseudofeces, which are particles that are rejected by the mouth, labial palps, and ctenida, and which are deposited to sediment (Cummings & Graf 2010). A few recent studies have suggested that *Corbicula* is able to use both filter feeding and deposit feeding to meet nutritional needs (Yeager & Cheery 1994, Hakenkamp & Palmer 1999, Hakenkamp et al 2001, Vaughn & Hakenkamp 2001 Cummings & Graf 2010). When pedal feeding, clams use ciliary tracts on the foot to transport sediment particles to the labial palps (Cummings & Graf 2010).

Here, *Corbicula* in North Buffalo Creek was studied to evaluate its role in removing anthropogenic N from the water column of an urban stream. *Corbicula* has been documented in North Buffalo Creek and its stable isotope signature has indicated that WWTP effluent was an important part of its diet (Ulseth & Hershey 2005). I hypothesized that *Corbicula* would be able to remove anthropogenic N at a sufficient rate to substantially impact anthropogenic N in the water column and, therefore, provide an

ecosystem service. This hypothesis was evaluated with the following objectives: (1) to determine abundance of the invasive species, *Corbicula*, in North Buffalo Creek; (2) to evaluate the trophic relationship between seston and *Corbicula* in North Buffalo Creek using stable isotope analysis of seston and *Corbicula*, and ash-free dry mass (AFDM) and C/N ratio of seston; (3) to determine the effect of *Corbicula* filtering activity on quality and quantity of suspended particulate organic matter (seston); and (4) to determine how much wastewater-derived anthropogenic particulate N *Corbicula* removes from the seston of North Buffalo Creek downstream of the wastewater treatment plant. The first three objectives were evaluated through field campaigns and laboratory experiments. The fourth objective was evaluated through integration of field and laboratory studies.

CHAPTER II

METHODS

Site description:

Research was conducted on North Buffalo Creek in Greensboro, North Carolina, USA, a city with a population of 237,316 (Greensboro Convention and Visitors Bureau 2009). The stream originates within the city and is the headwaters of the Cape Fear River Basin. North Buffalo Creek receives both point and non-point pollution throughout the city of Greensboro. The major point pollution source is the North Buffalo WWTP. The North Buffalo Creek WWTP serves the northern half of the city, which is primarily residential. Since 1980, the capacity of the facility has been 60,567 m³/d (City of Greensboro Water Resource Department). North Buffalo Creek has also been identified by the US EPA as impaired due to high levels of fecal coliform bacteria (> 200 colonies L⁻¹; NCDENR 2004). North Buffalo Creek has been studied for a number of years, not only as a part of an EPA mandated Total Maximum Daily Load (TMDL) project to reduce fecal coliform bacteria, but also as a research site for studies to understand urban stream ecology, the incorporation of wastewater-derived N into aquatic food webs (Northington & Hershey 2006, Ulseth & Hershey 2005, Kalconis-Rueppell et al. 2007) and the capacity of the stream to process N (Lofton et al 2007, Hines 2007).

To evaluate objective two, North Buffalo Creek was sampled twice during the summer of 2009 at sites that are easily accessible by road (Fig. 1). Summit Ave. was

the only site upstream of the WWTP that was sampled over the course of this study. Below the WWTP, sample sites used were Rankin Mill Rd., Creekview Rd. and McLeansville Rd., the latter of which is located just above the confluence with South Buffalo Creek (Fig. 1). Discharge data were obtained (on-line) from a USGS gage at Rankin Mill Rd. Below the WWTP, the riparian land use was virtually all forested. Samples were taken at or near base flow conditions as measured by the USGS gage located at Rankin Mill Rd.

Abundance of *Corbicula* in North Buffalo Creek:

To evaluate objective one, *Corbicula* abundance was measured at four sampling sites on North Buffalo Creek (Fig. 1) using a surber sampler (0.093m^2). *Corbicula* density (no./m^2) was measured three times in pool, riffle and run habitats at each sampling site during the summer of 2009 to determine if *Corbicula* abundance was related to habitat. To evaluate objective one with respect to season, pool habitats also were sampled in December 2009. A canoe transect was conducted on 15 December 2009 from Rankin Mill Rd. (distance = 0 m) to McLeansville Rd. (distance = 7000 m), with samples taken approximately every 500 m. A GPS was used to measure stream distance. At each sampling site, three surber samples (0.093m^2) were taken to assess *Corbicula* density (no./m^2). Stream width was also measured (m). The abundance of *Corbicula* in the reach was estimated using the surber counts and measurements of stream width and transect length.

Samples of *Corbicula* were collected to calculate a length to weight regression. Each *Corbicula* was measured to the nearest mm with a vernier calliper, then weighed (mg) and heated until the shell opened. Tissue was removed and dried at 60°C for ≥ 48 hr. *Corbicula* was then reweighed in order to determine dry weight.

Trophic relationships between seston and *Corbicula* in North Buffalo Creek:

To evaluate objective two, seston and *Corbicula* samples collected during both summer and winter sampling events were prepared for stable isotope analyses. Dried clam tissue was ground into a powder and placed in 4X6 mm pressed tin capsules then sent to UC-Davis Stable Isotope Laboratory for determination of $\delta^{15}\text{N}(\text{‰})$ and $\delta^{13}\text{C}(\text{‰})$. Water for seston analyses was collected in 4-L cubitainers, returned to the lab and 400 ml was vacuum filtered onto six pre-ashed glass fiber filters (0.7 μm) per site. All filters were dried at 60°C for ≥ 48 h. Three filters per site were prepared for stable isotope analyses. One fourth of each of the three filters was placed in a tin capsule and sent to UC-Davis Stable Isotope Laboratory for $\delta^{15}\text{N}$ (‰) and $\delta^{13}\text{C}$ (‰) analyses. C/N ratio was calculated for seston samples at each sampling site using the data received from UC-Davis Stable Isotope Laboratory. The three remaining filters had been weighed prior to use and were reweighed after being dried at 60°C for ≥ 48 h. These filters were then ashed in a muffle furnace at 500 °C and reweighed to determine ash-free dry mass (AFDM).

Laboratory experiments, an estimation of the effect of *Corbicula* filtering on seston quality and quantity:

To evaluate objective three, two laboratory experiments were conducted to evaluate qualitative and quantitative effects of clam filtering activity on seston. The first experiment (hereafter exp. #1) was conducted on 23 September 2009. An experimental treatment (3 aquaria) contained four *Corbicula* (mean length = 21 mm) with sediment (1.5 cm depth) and unfiltered water (34,100 ml). The *Corbicula*, sediment and water were collected from the Rankin Mill Rd. site. This clam density was selected because Lauritsen (1986) estimated *Corbicula* (mean length = 22.4 mm) filtering rate to be 905.8 ± 43.6 ml/h at 20°C. Based on Lauritsen's value, the filtering rate (rate of change in chlorophyll a concentration or AFDM over time) of the four clams in each tank should have been sufficient to filter the entire tank of water over the course of the 12 h experiment. A control treatment (3 aquaria) consisted of sediment (1.5 cm depth) and unfiltered Rankin Mill Rd. water (34,100 ml) but no *Corbicula*. The effect of *Corbicula* on seston quality was assessed by measuring change in seston $\delta^{15}\text{N}$ (‰), $\delta^{13}\text{C}$ (‰) and C/N ratio over time. The experiment was run for 12 h, with samples taken initially (t=0) and at 2 h intervals.

Each aquarium was set up 24 h prior to t=0 in an environmentally controlled room. The room was set to 23.4°C, the ambient temperature of North Buffalo Creek at the time of water, sediment and clam collection. A 1 L solution was made with one gram of 99% ^{13}C sodium bicarbonate and DI water. Each aquarium received 100 ml of that solution. The isotope was added to enrich the seston in ^{13}C (via algal uptake of

bicarbonate) to increase the likelihood of seeing a change in $\delta^{13}\text{C}$ during the 12 h experiment due to clam removal of algal components of seston. The aquaria were allowed to acclimate with full access to light for 24 h in order for the algae to incorporate the ^{13}C label. Each aquarium was equipped with an aquarium pump and the same length of tubing, and aerated to minimize settling of seston and prevent the tanks from becoming anoxic. The tubing was secured to the side of the aquaria half way up the sidewall. 1.2 L of water was removed at each sampling time from each tank starting at $t=0$ and was not replaced. Lights were turned off at $t=0$ and the experiment was allowed to proceed in the dark.

Water collected at each time interval was filtered onto three pre-ashed glass-fiber filters. One filter was used to calculate filtering rate based on AFDM. The second filter was prepared and sent to UC-Davis Stable Isotope Laboratory for stable isotope analyses. These analyses also provided data on sample C/N ratio. The third filter was prepared for chlorophyll a analysis; however, chlorophyll data were not obtained due to equipment failure of the spectrophotometer. Seston quality was determined by comparing the $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and C/N ratio change over time.

The second experiment (hereafter exp. # 2), conducted on 10 March 2010, consisted of three treatments: clams, sediment + water, clams + water and water only. The clams, sediment + water treatment consisted of five *Corbicula* (mean length = 21 mm) placed in three aquaria (38 L) with sediment (1.5 cm) and unfiltered water (32,100 ml) collected from the Rankin Mill Rd. site. The clams + water treatment consisted of three aquaria containing five *Corbicula* (mean length = 21 mm) and unfiltered Rankin

Mill Rd. water (32,100 ml), but no sediment. Five *Corbicula* (mean length = 21 mm) per tank were used in exp. #2 to increase the likelihood of observing a change in AFDM, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, C/N and chlorophyll a of seston. A control treatment (n=3) consisted of unfiltered Rankin Mill Rd. water (32,100 ml). Each aquarium was set up 24 h prior to t=0 in an environmentally controlled room. The room was set to 23.4°C, the same temperature as exp. #1. Effect of *Corbicula* on seston quantity and quality was assessed by measuring change in seston $\delta^{15}\text{N}$ (‰), $\delta^{13}\text{C}$ (‰), C/N ratio and chlorophyll a over 12 h. The experiment was conducted and sampled as described above for exp. #1 except for the following. A 900 mL solution was made containing 900 mg of 99% ^{13}C sodium bicarbonate and DI water. Each aquarium received 100 ml of that solution. For the chlorophyll analysis, 400 ml of water collected from each tank at each time interval was filtered onto a third filter and frozen in a light sealed container. These filters were then processed using 10 ml of 90 % HPLC grade acetone to extract the chlorophyll a (Clesceri et al. 1998). The extract was analyzed using a fluorometer to measure chlorophyll a concentration.

Evaluation of wastewater-derived anthropogenic particulate N removal from seston by *Corbicula*:

Objective four was evaluated by using all data obtained from the first three objectives. I expected to find that *Corbicula* was abundant in North Buffalo Creek. I expected that seston $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ would decrease downstream of the WWTP as seston was removed by *Corbicula*. I expected to confirm that *Corbicula* is filtering suspended

organic matter as a sufficient rate to have a measureable impact on seston. Therefore, I expected that *Corbicula* provides an ecosystem service to North Buffalo Creek by removing a sufficient amount of sewage-derived particulate nitrogen from the water column.

Data Analysis:

All statistical analyses were conducted using SPSS 16.0 and all data sets were examined for normality using Kolmogorov-Smirnov (KS) tests. Clam abundance data and transect AFDM were natural log transformed to correct for non-normality. One-way ANOVAs were used to evaluate the difference in *Corbicula* abundance between different habitats (summer only) and between seasons. Independent-sample t-tests were used to compare $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of *Corbicula* and seston at each sampling site.

Independent-sample t-tests were also used to compare the mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of clams used in exp. # 2.

Linear regression with ANOVA was used to determine if AFDM, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ changed with distance along the transect. Repeated measures ANOVA was used to determine if AFDM, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of seston changed significantly through time in both exp. #1 and exp. #2. Time, treatment and the interaction of time x treatment were all tested to determine if they were significant predictors of AFDM, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, C/N ratio and chlorophyll a (exp. # 2 only). Initial AFDM, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and C/N values between treatments were compared using an ANOVA for exp. 2 and t-test for exp. #1. Final values AFDM, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and C/N between treatments were also compared using an

ANOVA for exp. #2 and t-test for exp. #1. P-values < 0.05 were considered significant with $p < 0.01$ to be convincing of statistical differences. Whereas, marginal or suggestive conclusions could be inferred from $0.05 < p < 0.1$ (Ramsey & Shafter 2002).

CHAPTER III

RESULTS

Corbicula abundance during the summer of 2009 in North Buffalo Creek was 136 ± 52 (mean \pm SE) clams/m², 32 ± 20 clams/m² and 46 ± 25 clams/m² in pool, riffle, and run habitats, respectively (Table 1). The pattern was suggestive that there were more clams in the pools compared to riffles or runs ($F=2.569$, $df=2,33$, $p<0.086$), but clam abundance was not significantly different between habitats.

In December 2009, I estimated that there were 12,168,000 *Corbicula*, along the 7000 m transect of North Buffalo Creek from Rankin Mill Rd. to McLeansville Rd. The average width of North Buffalo Creek was 13.0 ± 0.7 m. Clam abundance in pools was significantly lower in winter (41 ± 8.1) than in summer (136 ± 52 clams m⁻²) ($t=3.030$, $df=45$, $p<0.004$).

The length-weight regression model for *Corbicula* was significant ($R^2=0.93$, $F=1742.5$, $df=2,213$, $p<0.000$), using both wet mass ($t=12.296$, $p<0.000$) and length ($t=3.953$, $p<0.000$) as predictors of clam dry weight:

$$\text{Dry wt (mg)} = -23.28 + 2.21(\text{length (mm)}) + 0.027 (\text{wet wt (mg)}) \quad (\text{eq. 1})$$

Fig. 2 shows the mean $\delta^{15}\text{N}$ values of three seston and three *Corbicula* samples from four locations on North Buffalo Creek. Summit Ave., the only site upstream of the WWTP, was the only site where $\delta^{15}\text{N}$ values of *Corbicula* and seston were not significantly different (Table 2). At Rankin Mill Rd., seston $\delta^{15}\text{N}$ ($9.32\text{‰} \pm .25$) was significantly enriched compared to *Corbicula* $\delta^{15}\text{N}$ ($6.80\text{‰} \pm 0.07$). Rankin Mill Rd. was the only site where the seston was more ^{15}N enriched than *Corbicula*. At both Creekview Rd. and McLeansville Rd. sampling sites, the $\delta^{15}\text{N}$ values of seston were significantly lower than those of the *Corbicula* (Table 2). At each site except Rankin Mill Rd., the expected shift of 3-5‰ N from resource to consumer was observed.

Seston $\delta^{13}\text{C}$ was significantly greater than *Corbicula* $\delta^{13}\text{C}$ at each of the four sites (Table 2, Fig. 3). The difference in seston versus *Corbicula* $\delta^{13}\text{C}$ was greatest at Summit Ave. (4.6‰) followed by Creekview Rd. (2.89‰), McLeansville Rd. (2.59‰), and Rankin Mill Rd. (1.49‰). Seston $\delta^{13}\text{C}$ became more progressively more enriched with distance downstream of the WWTP. A $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ biplot (Fig. 4) for mean \pm SE *Corbicula* and seston at four sampling sites illustrates that the isotopic signature did not correspond to the expected pattern for a trophic shift from resource to consumer.

Data to evaluate objective two are shown in Fig. 5. AFDM declined linearly with distance (Table 3, Fig. 5A) from Rankin Mill Rd. to McLeansville Rd. ($R^2 = 0.328$, $F = 19.495$, $df = 1, 41$, $p < 0.000$). If North Buffalo Creek provided the ecosystem services of organic matter processing AFDM, would have decreased with distance downstream from the point source of pollution, which was the observed pattern.

The mean \pm SE C/N ratio for seston at the 15 sampling sites on the North Buffalo Creek transect are shown in Fig. 5B. There was no trend and no significant model could be found ($R^2=0.01$, $F=0.434$, $df=1,42$, $p=0.514$). Therefore, factors other than distance influenced the C/N ratio along the transect.

Seston $\delta^{13}\text{C}$ (Table 3, Fig 5C) decreased significantly ($R^2=0.282$, $F=16.138$, $df = 1,42$, $p<0.000$) with distance downstream ($t=-4.017$, $p<0.000$). However, $\delta^{13}\text{C}$ of *Corbicula* did not show a trend with distance downstream of the WWTP (Fig.6B, $df = 1,42$, $F=0.688$, $p=0.412$). This result indicates that *Corbicula* was not using seston as its primary carbon source.

$\delta^{15}\text{N}$ for both seston and *Corbicula* along the North Buffalo Creek transect are shown in Fig. 5D. Both seston and *Corbicula* $\delta^{15}\text{N}$ increased with distance downstream. The (ln) distance- $\delta^{15}\text{N}$ regression for seston was significant but weak ($R^2=0.14$, $F=6.632$, $df = 1,42$, $p<0.014$, Table 3), indicating that distance is a poor predictor of seston $\delta^{15}\text{N}$ ($t = 2.575$, $p<0.014$). In contrast, distance ($t=4.219$, $p<0.000$) downstream is a stronger predictor of *Corbicula* $\delta^{15}\text{N}$ ($R^2=0.293$, $F=17.804$, $df = 1,44$, $p<0.000$, Table 3). At each site along the transect, *Corbicula* $\delta^{15}\text{N}$ was 4-5‰ greater than seston $\delta^{15}\text{N}$.

AFDM of the seston in exp. #1 (Fig. 6A, Table 4) was not significantly different between sediment + water and clams, sediment + water treatments at the initial ($t = 0$) sampling ($t = -0.405$, $p=0.706$). The final AFDM values also were not significantly different ($t=-1.001$, $p=0.374$). The repeated measures ANOVA did not indicate a significant time, interaction, or treatment effect over the course of the experiment (Table 5).

In exp. #1, $\delta^{13}\text{C}$ of the seston did not differ at time zero between the clams, sediment + water treatment and the sediment + water treatment (Fig 6B, Table 4). The $\text{NaH}^{13}\text{CO}_3$ enriched the seston by approximately 13‰. The trend observed was that seston $\delta^{13}\text{C}$ became more enriched through time in the clams, sediment + water treatment but did not change in the sediment + water treatment. The $\delta^{13}\text{C}$ values at the end of the experiment were marginally different ($t=-2.504$, $p<0.066$), indicating that the treatments behaved differently through time. The repeated measures ANOVA showed a significant time effect ($F=28.212$, $p<0.006$, Table 5), indicating that the $\delta^{13}\text{C}$ changed significantly over time. The repeated measures ANOVA also showed a significant interaction effect ($F=9.711$, $p<0.036$), which confirmed that the $\delta^{13}\text{C}$ of the seston within each treatment changed differently over the course of the experiment. This result indicates that the presence of the clams altered the $\delta^{13}\text{C}$ of the seston.

The $\delta^{15}\text{N}$ for each treatment type in exp. #1 is shown in Fig. 6C (Tables 4-5). The trend observed was that the $\delta^{15}\text{N}$ in the sediment + water treatment increased while that of the clams, sediment + water treatment remained relatively constant over the course of the experiment. However, neither initial nor final $\delta^{15}\text{N}$ were significantly different between treatments ($t=-1.222$, $p<0.287$, and $t=-1.811$, $p<0.166$ respectively). The repeated measures ANOVA suggested that there was an interaction effect ($F=4.373$, $p<0.070$), and a treatment effect ($F=4.595$, $p<0.062$). However, only time was found to be a significant factor ($F=76.52$, $p<0.000$) in exp. #1 with respect to seston $\delta^{15}\text{N}$.

C/N ratio for exp. #1 increased in the sediment + water tanks over the course of

the experiment, which indicates that the food quality deteriorated over the course of 12 h. The initial C/N ratios were not significantly different between treatments ($t=0.443$, $p<0.681$, Table 4). However, the final C/N ratios were significantly different ($t=3767$, $p<0.020$). The repeated measures ANOVA resulted in a significant treatment effect ($F=11.986$, $p<0.026$, Table 5) and time effect ($F=8.756$, $p<0.042$), indicating that when clams were present the C/N remained fairly constant which didn't occur without the clams. The interaction between time and treatment was marginally significant ($F=6.939$, $p<0.058$), consistent with the conclusion that the treatments changed over time in different ways.

AFDM in exp. #2 (Fig. 7A, Tables 6-7) showed no trends indicating a change over the 12 h experiment. ANOVA results indicated that neither initial ($F=2.074$, $p<0.201$) nor final ($F=0.726$, $p<0.522$) AFDM values differed between treatments. The repeated measures ANOVA showed no treatment ($F=1.289$, $p<0.342$), interaction ($F=1.822$, $p<0.241$) or time effect ($F=0.024$, $p<0.883$). These results indicated that clams were not filtering at a sufficient rate to reduce the amount of particulate organic matter in the water to change the AFDM with or without the presence of sediment.

Initial mean $\delta^{13}\text{C}$ values of seston were -12.28‰ , -11.55‰ , -9.51‰ in the water only, clams + water, and clams, sediment + water treatments, respectively (Fig. 7B, Tables 6-7), which were not significantly different ($F=0.734$, $p<0.519$). The final $\delta^{13}\text{C}$ values also were not significantly different ($F=0.138$, $p<0.874$). The repeated measures ANOVA showed that there was a significant increase in $\delta^{13}\text{C}$ through time across all treatments ($F=55.82$, $p<0.000$), but there was not a significant treatment ($F=0.321$,

$p < 0.737$) or interaction effect ($F = 0.014$, $p < 0.986$). These results show that all three treatments changed the same way.

$\delta^{15}\text{N}$ of seston in exp. #2 (Fig. 7C, Tables 6-7) increased over the course of the experiment in all treatments. Initial $\delta^{15}\text{N}$ values were significantly different ($F = 6.770$, $p < 0.029$) and post hoc analysis indicated that the clams + water treatment was significantly different than clams, sediment + water treatment ($F = 6.770$, $p < 0.026$, $df = 2, 8$). The final $\delta^{15}\text{N}$ values were not significantly different ($F = 1.674$, $p < 0.264$). Repeated measures ANOVA resulted in a significant time effect ($F = 76.52$, $p < 0.000$). However, the interaction ($F = 4.373$, $p < 0.07$) and treatment effects ($F = 4.595$, $p < 0.062$) were only suggestive of a differential change in $\delta^{15}\text{N}$ between treatments over time.

C/N ratios of seston in exp. #2 (Fig. 7E, Tables 6-7) declined during the experiment indicating a trend of improved seston quality. Neither initial ($F = 0.283$, $p < 0.763$) nor final ($F = 2.728$, $p < 0.144$) C/N ratios were significantly different between treatments. Clams + water treatment showed the greatest decrease in C/N ratio (2.2) followed by clams, sediment + water (1.8) and water only (1.1). Repeated measures ANOVA showed that treatment ($F = 0.540$, $p < 0.609$) was not significant. However, the interaction ($F = 10.02$, $p < 0.012$) and time effects ($F = 294.3$, $p < 0.000$) were significant, indicating that the presence of the clams increased the quality of seston compared to water only.

Chlorophyll a in exp. #2 (Fig. 7D, Tables 6-7), showed a decrease after time zero. Initial chlorophyll a values were not significantly different ($F = 2.292$, $p < 0.197$).

However, final values were significantly different ($F=8.864$, $p<0.016$). Post hoc analysis found that the clams, sediment + water treatment was significantly different ($p<0.033$) from the water only and the clams + water treatment ($p<0.020$). Repeated measures ANOVA showed neither a significant time ($F=3.283$, $p<0.130$) nor interaction effect ($F=0.729$, $p<0.527$). The treatment effect ($F=5.428$, $p<0.056$) was suggestive that the tanks changed differently over time. Chlorophyll a in the water only treatment seemed to remain fairly consistent throughout the experiment, whereas in the clams, sediment + water treatment it declined initially, and then increased again.

The $\delta^{15}\text{N}$ of the *Corbicula* used in exp. #2 is shown in Fig. 8A. The $\delta^{15}\text{N}$ means ($n=3$) of the clams + water tanks were significantly lower than the clams, sediment + water tanks ($t=-2.074$, $df=4$, $p<0.05$) using a one-sided independent t-test. The mean $\delta^{13}\text{C}$ ($n=3$) of the *Corbicula* (Fig 8B) was not significantly different between the two treatments with clams ($t=-1.306$, $df=4$, $p<0.13$).

Clams, sediment + water treatment was the only treatment repeated in both experiments and therefore I was able compare that patterns from both experiments. The AFDM results of both experiments were very similar showing very little change over the course of 12 h. Carbon isotope data indicates a ^{13}C enrichment through time in both experiments. Nitrogen isotope data was the only trend that was different between the two experiments. In exp. #1, the $\delta^{15}\text{N}$ remained fairly consistent over the course of 12 h which was in contrast to exp. #2, where the clams, sediment + water treatment became more enriched in ^{15}N over time. The ^{15}N values at time zero in exp. #2 were significantly lower than in exp. #1 time zero values, which was more typical of a stream receiving

WWTP effluent. The C/N ratios between the two experiments both remained fairly consistent.

CHAPTER IV

DISCUSSION

This study was designed to test the hypothesis that *Corbicula* impacts seston quality and quantity in an urban stream through its filtering activity. It has been suggested that *Corbicula* populations clear the water column of the Chowan River, NC, in 1.04-1.6 days (Lauritsen 1986), the Potomac River, VA, in 3-4 days (Cohen et al. 1984) and Meyers Branch, SC, in 21 h (Leff et al. 1990). However, as discussed below, stable isotope data from North Buffalo Creek and results from laboratory studies of the effect of *Corbicula* on seston indicate that *Corbicula* is not filtering in North Buffalo Creek. In contrast, this study indicates that *Corbicula* impacts water quality of seston in North Buffalo Creek by returning sediment C and N to the seston.

Objective one of this study was designed to determine whether clams were abundant enough in North Buffalo Creek to have a significant impact on seston, similar to that described for other systems. The evaluation of objective one provided evidence that *Corbicula* was abundant in North Buffalo Creek, with a trend toward greater abundance in pools compared to riffle habitats. In the winter, the *Corbicula* population was estimated as 12,168,000 clams along a 7000 m reach of North Buffalo Creek. Assuming the filtering rate of *Corbicula* in North Buffalo Creek was similar to that measured for clams in the Chowan River, I expected that the clams would have been able to filter the stream water three times per day in North Buffalo Creek during the winter,

and, therefore, have a strong impact on seston dynamics. Furthermore, *Corbicula* was approximately three-fold more abundant in summer than in winter, consistent with expected higher abundances during the breeding season (Cummings & Graf 2010). Therefore, an even greater impact of clams on seston would be expected during the summer.

One component of the evaluation of objective two was a comparison of the isotope signatures of both seston and *Corbicula* at different sampling sites on North Buffalo Creek in the summer. As illustrated in the isotope biplot for *Corbicula* and seston (Fig. 4) at four different locations, none of the sites showed the expected trophic enrichment of 3-5‰ for $\delta^{15}\text{N}$ and 0.5-1‰ for $\delta^{13}\text{C}$ (Peterson & Fry 1987) during the summer. At Rankin Mill Rd., the seston was more enriched in both ^{15}N and ^{13}C than *Corbicula*, which indicates two things. First, the seston was reflecting a ^{15}N enrichment due to WWTP point source input. Second, *Corbicula* was not relying primarily on seston N or C at this location. At the other three sites sampled during the summer, clam $\delta^{15}\text{N}$ was enriched compare to seston, suggesting that they could have assimilated seston N, but clam $\delta^{13}\text{C}$ was depleted rather than enriched compared to seston $\delta^{13}\text{C}$, indicating that seston was not the primary C source. Ulselth & Hershey (2005) also indicated that *Corbicula* was assimilating C from different sources at various sampling sites along North Buffalo Creek. These results suggest that seston was not the major food source for *Corbicula*, and that *Corbicula* must have relied on pedal feeding from the sediments.

The evaluation of objective two also involved comparisons of seston and *Corbicula* along the canoe transect of North Buffalo Creek on 15 Dec 2009. Seston

AFDM showed a significant linear decline with distance downstream, indicating that the amount of particulate organic matter in the water declined with distance downstream of the WWTP. However, stable isotope analyses of seston and *Corbicula* along the transect (Fig. 5) do not indicate a clear trophic relationship between *Corbicula* and seston, suggesting that the decline in AFDM was not due to removal of seston by clams. *Corbicula* was 3-5‰ enriched in ^{15}N compared to seston, which was the expected $\delta^{15}\text{N}$ shift from resource to consumer (Peterson & Fry 1986). This trend was consistent with *Corbicula* assimilation of seston N. However, a direct trophic relationship between seston and *Corbicula* cannot be implied from these data alone because alternate sources were not examined and, therefore, cannot be ruled out. If *Corbicula* was relying on seston organic matter as its primary food source, I would also expect a $\delta^{13}\text{C}$ shift of 0.5-1.0‰ from seston to *Corbicula*, which did not occur. In contrast to the $\delta^{15}\text{N}$ data, the pattern of $\delta^{13}\text{C}$ values for *Corbicula* and seston along the 15 Dec transect was not consistent with the conclusion that seston was the primary C source for *Corbicula*. Seston $\delta^{13}\text{C}$ showed a clear trend of decreasing downstream of the WWTP, while *Corbicula* $\delta^{13}\text{C}$ did not change consistently along the transect. Furthermore, as seston $\delta^{13}\text{C}$ progressively decreased, it approached -28‰, the approximate $\delta^{13}\text{C}$ value of terrestrial leaf litter. Although the seston $\delta^{13}\text{C}$ pattern was not related to that of *Corbicula*, it does indicate that processing of sewage-derived C along the stream reach progressed to the point that sewage-derived C was no longer detectable isotopically in the seston, which was also consistent with the observed decline in AFDM. Rather, the stable isotope and seston

AFDM data suggest that that seston dynamics were controlled by processes other than filter feeding by *Corbicula*.

One approach used to evaluate the effect of *Corbicula* filtering activity on seston quantity and quality (objective three) was a laboratory experiment to measure *Corbicula* influence on seston AFDM, stable isotope composition and C/N ratio when seston and sediment were present (Exp. #1, Fig. 6). Based on literature values for filtering rates from studies where *Corbicula* was housed in aquaria with seston as its only resource (Cohen et al. 1984, Lauritsen 1986, Leff et al. 1990), exp. #1 was designed so that the *Corbicula* could have been able to filter all the water in the tank over the course of 12 h. However, in exp. #1, AFDM did not change in either treatment over the course of 12 h. This result suggests that *Corbicula* filtering either did not occur or did not occur at a rate sufficient to impact seston organic matter concentration. This result was unexpected. Seston $\delta^{13}\text{C}$ increased in exp. #1 when clams were present with sediment, but showed no change when clams were absent. This change in seston $\delta^{13}\text{C}$ when *Corbicula* was present suggests that *Corbicula* was impacting seston qualitatively. Three potential explanations for this result were considered.

First, a mechanism for the increase in seston $\delta^{13}\text{C}$ may be that the detrital component of seston differentially settled while the algal component (which was the seston component that was expected to increase in $\delta^{13}\text{C}$ in response to ^{13}C -bicarbonate addition to the tanks), stayed in suspension due to clam activity. That possibility was rejected because AFDM did not change, such that differential settling of detritus could not have been quantitatively significant. Furthermore, if the detrital component had

decreased relative to the algal component, the C/N ratio of seston in the clams + sediment treatment should have decreased, which also did not occur. Thus, differential settling of detritus could not explain the observed increase in seston $\delta^{13}\text{C}$ in the clams, sediment + water treatment.

A second mechanism for the increase in seston $\delta^{13}\text{C}$ is respiration or bioturbation of algal exudates by *Corbicula*, followed by assimilation of the labeled ^{13}C by the microbial component of seston. *Corbicula* respiration has been shown to be higher during combined pedal and filter feeding compared to filter feeding alone (Hakenkamp & Palmer 1999). Sediment algae as well as pelagic algae would have assimilated the ^{13}C -bicarbonate during the pre-experiment incubation period. For clam respiration of the sediment ^{13}C to increase $\delta^{13}\text{C}$ of the seston during the experiment, respired CO_2 would have to have been assimilated by pelagic algae or bacteria in the dark. Significant dark assimilation of CO_2 was not expected. However, previous studies have shown that dark carbon fixation occurs in the microbial community. Granum & Mykkestad (1999) found that when NH_4^+ is present, dark C fixation was stimulated by a marine diatom at a rate of $4.0 \text{ fmol cell}^{-1}\text{h}^{-1}$. Li & Dickie (1991) showed that the role of bacteria in ^{14}C fixation is important when conditions limit photosynthesis by phytoplankton. Coveney (1982) reported that $^{14}\text{CO}_2$ assimilation was 32-95% of estimated heterotrophic bacterial production. Thus, these studies suggest that dark $^{13}\text{CO}_2$ uptake by both algal and bacterial components of seston likely occurred in the tanks, and could have caused the 4.9‰ (or 0.49%) shift in seston $\delta^{13}\text{C}$ that was observed in the clams, sediment + water treatment. Since the shift did not occur in the control treatment, respiration or

bioturbation by *Corbicula* is the most likely mechanism for providing labeled ^{13}C to the water column for microbial uptake.

A third mechanism for the increase in seston $\delta^{13}\text{C}$ in the clams, sediment + water treatment is that *Corbicula* egestion returned labeled POM to the water column that was derived from sediment algae. The latter two mechanisms are not mutually exclusive and cannot be distinguished with existing data. However, previous studies indicate that *Corbicula* egestion increases deposition to the sediments through feces and pseudofeces and releases dissolved nutrients to the water column from the sediment via bioturbation (Vaughn & Hakenkamp 2001, Hakenkamp & Palmer 1999). Therefore, this third mechanism is less likely than the dark uptake mechanism discussed above.

Over the course of exp. #1 the control tanks showed a marginally significant increase in $\delta^{15}\text{N}$ ($p < 0.056$), while $\delta^{15}\text{N}$ in the clams, sediment + water tanks remained fairly constant. The $\delta^{15}\text{N}$ data from Dec 2009 transect was consistent with seston as an N source for *Corbicula*. However, the same result could also have occurred if seston $\delta^{15}\text{N}$ along the transect was controlled by *Corbicula* excretion to the seston of isotopically light N. Animal excretion favors the light isotope, which is the primary mechanism for the approximate 3-5‰ trophic enrichment that is typically observed for ^{15}N (Peterson & Fry 1987). Lauritsen & Mozley (1989) measured the excretion rate of *Corbicula* as $2.06 \mu\text{mol bivalve}^{-1} \text{h}^{-1} \text{NH}_4^+$ at 23°C . Excretion by freshwater bivalves has been shown to be readily taken up by phytoplankton (Vaughn & Hakenkamp 2001). Thus, *Corbicula* could be altering seston quality through nutrient translocation from the sediment to water column (Vanni 2002). Furthermore, the significant increase in C/N ratio in the sediment

only treatment of exp. #1 indicates that seston became more dominated by the detrital component in the absence of *Corbicula*, which did not occur in the clams, sediment + water treatment. Meanwhile, the C/N ratio stayed the same when the clams were present, indicating no change in the algal component of the seston during the experiment. This result suggests that clam excretion of sediment nutrients maintained the seston N concentration.

Exp. #2 (Fig. 7) permitted the evaluation of *Corbicula* influence on seston AFDM, Chlorophyll a, C/N ratio and stable isotope composition with and without the presence of sediment. In exp. #2, AFDM did not change in any treatment over the course of 12 h. This unexpected result indicates that filter feeding by *Corbicula* did not occur or was not sufficient to reduce the seston organic matter concentration even when pedal feeding was not an option.

The $\delta^{13}\text{C}$ of the seston in exp. #2 (Fig. 7B) increased over the course of 12 h in all three treatments. This pattern can be explained by dark uptake of C by the microbial community as discussed in exp. #1. The sediment + water only treatment in exp. #1 was the only one of the 4 treatments from exp. #1 and #2 combined in which $\delta^{13}\text{C}$ did not increase. It is possible that the sediment outcompeted the water column for the labeled ^{13}C . When the clams were present with sediment in both experiments, the seston became enriched in ^{13}C through time due to the clam activity of returning labeled ^{13}C into the water column as respired CO_2 , or through bioturbation of algal exudates followed by subsequent assimilation by the seston microbial community.

The $\delta^{15}\text{N}$ of seston in exp. #2 (Fig. 7C) showed a trend of seston enrichment over the course of 12 h in all three treatments. The initial values were significantly different ($F=6.770$, $p<0.029$), and showed a pattern that indicated that the sediments had removed light N from the water column in the 32 h period when the tanks were set up, but before clams were introduced and $t=0$ samples were taken. Although all three treatments increased in $\delta^{15}\text{N}$ through time, the clams, sediment + water treatment changed the least, such that there was no longer a significant difference in $\delta^{15}\text{N}$ between treatments by the end of the experiment. Seston $\delta^{15}\text{N}$ changed less when clams were present, even though clams were not filtering, consistent with the conclusion that clam excretion was important for maintaining seston $\delta^{15}\text{N}$. The initial $\delta^{15}\text{N}$ in exp. #2 was considerably lower than the initial values of exp. #1. These differences reflect conditions in the stream water used for the experiments due to differences in discharge and/or seasonality. But since seston $\delta^{15}\text{N}$ in the two experiments was not compared directly, these $\delta^{15}\text{N}$ differences between the two experiments are not important to interpreting the results.

The C/N ratio in exp. #2 (Fig. 7D) showed a slight decreasing trend through time in all treatments, but the interaction term indicates that not all of the treatments changed in the same way. In this case, C/N of the control treatment did not decrease as much as the two treatments with clams present. Thus, the presence of clams appears to have partially maintained the seston C/N ratio through excretion of isotopically light N to the water column as discussed for exp. #1.

Chlorophyll a in exp. #2 (Fig. 7E) gave further evidence that clams maintained the algal community when sediment was present. The initial chlorophyll a values were

not significantly different between treatments. However, by the end of the experiment, chlorophyll a was significantly higher in the treatment with clams + sediment than when sediments were absent. This result is consistent with the C/N ratio pattern discussed above and also suggests that clams were maintaining the algal community by pedal feeding and returning nutrients to the water column through excretion. Higher chlorophyll a in the clams + sediment treatment when compared to other treatments also confirms that filter feeding was not an important process in this experiment.

In the presence of sediment, *Corbicula* was significantly more enriched in ^{15}N than the *Corbicula* with water only. This result provides further evidence that that *Corbicula* was using the sediment as a source of N. Deposit feeding has been shown as an important method of feeding for juveniles bivalves (Cummings & Graf 2010). Hakenkamp & Palmer (1999) suggested that *Corbicula* impacted carbon dynamics in Goose Creek through both pedal feeding on buried organic material and filter-feeding from the water column. It has been suggested that *Corbicula* combine both suspension and deposit feeding (Yeager & Cheery 1994, Hakenkamp & Palmer 1999, Hakenkamp et al 2001, Vaughn & Hakenkamp 2001 Cummings & Graf 2010). Although the present study was not designed to partition the contribution of sediment versus water column sources, the experimental results show no evidence that filter feeding was of consequence.

Objective four was designed to synthesize the field and laboratory experiments to determine how much N *Corbicula* were removing from North Buffalo Creek. However, in contrast to previous studies (Cohen et al. 1984, Lauritsen 1986), this study did not find

that *Corbicula* was filtering seston at all. Instead, this study provides further evidence that deposit feeding by *Corbicula* results in translocation of both N and C to the water column. Although a decline in AFDM was observed along a 7 km transect in North Buffalo Creek, consistent with clam removal of seston derived from the WWTP, the $\delta^{13}\text{C}$ data from North Buffalo Creek did not support seston as a C source for *Corbicula*. In contrast, $\delta^{15}\text{N}$ from three of four sites sampled in the summer, and along a 7 km transect sample in winter were consistent with seston as an N source for *Corbicula*. The laboratory experiments resolved these apparently conflicting results. Clam excretion of isotopically light N resulted in a seston $\delta^{15}\text{N}$ signature that was isotopically light compared to that of *Corbicula*. This same shift in $\delta^{15}\text{N}$ would occur if the clams were eating seston. In the absence of another source of N, microbial processing in the water column would result in progressive enrichment of seston. Thus, the laboratory experiments, which demonstrated that filtering did not occur but that clam presence decreased the seston $\delta^{15}\text{N}$ value compared to when clams were absent, resolved the apparent discrepancy between the isotopic signatures of seston and clams observed in North Buffalo Creek. Clams maintain the relatively low seston $\delta^{15}\text{N}$ through excretion and use sediment organic matter as their food source.

I hypothesized that *Corbicula* would be able to remove anthropogenic N at a sufficient rate to substantially impact anthropogenic N in the water column, and thereby provide an ecosystem service. However, it appears that *Corbicula* feeds on sediment-bound organic matter and translocates sediment-bound nutrients back to the water column. In the absence of this translocation activity, denitrification occurs in sediments,

partially mitigating downstream delivery of N (e.g., Lofton et al. 2007). Therefore it appears that rather than providing an ecosystem service, *Corbicula* activity increases N loading to the water column, increasing downstream eutrophication.

CHAPTER V

CONCLUSIONS

In conclusion, this study strongly suggests that *Corbicula* in North Buffalo Creek used deposit feeding primarily over filter feeding as its source for nutrients. Further investigation is needed to evaluate why *Corbicula* does not filter feed in North Buffalo Creek. However, by deposit feeding, *Corbicula* appears to have an impact on seston quality of streams not by reducing AFDM, but by removing nutrients from the sediment and returning carbon dioxide or algal exudates and ammonium back to the water column for assimilation by the water column microbial community. The original justification for this study was that the invasive *Corbicula* might be performing an ecosystem service by removing sewage-derived N from the water column to the sediments, where it can be denitrified. Instead, they return sediment-bound nutrients to the water, thereby decreasing water quality and exacerbating downstream eutrophication.

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APPENDIX A: TABLES

Table 1: Mean clam abundance data in three habitats and two seasons on North Buffalo Creek. Clam abundance was counted at four different habitats using a surber sampler on North Buffalo Creek.

	Pool	Riffle	Run
	Mean ± SE (n)	Mean ± SE (n)	Mean ± SE (n)
June	136 ± 52 (11)	32 ± 20 (12)	46 ± 25 (12)
Dec	41 ± 8 (36)	-	-

Table 2: Stable isotope comparisons between three seston and three *Corbicula* samples from four different sampling sites on North Buffalo Creek. The mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C} \pm \text{SE}$ of seston and *Corbicula* sampled in June of 2009 are graphed on Fig. 2 and 3 respectively.

Date	Site	Isotope ratio	T stat	p-value	DF
26 June	Summit Ave.	$\delta^{15}\text{N}$	-1.640	p=0.176	4
26 June	Summit Ave.	$\delta^{13}\text{C}$	11.313	p<0.005	4
23 June	Rankin Mill Rd.	$\delta^{15}\text{N}$	9.802	p<0.001	4
23 June	Rankin Mill Rd.	$\delta^{13}\text{C}$	6.663	p<0.003	4
26 June	Creekview Rd.	$\delta^{15}\text{N}$	-2.767	p<0.050	4
26 June	Creekview Rd.	$\delta^{13}\text{C}$	8.382	p<0.001	4
26 June	McLeansville Rd.	$\delta^{15}\text{N}$	-3.537	p<0.024	4
26 June	McLeansville Rd.	$\delta^{13}\text{C}$	6.766	p<0.002	4

Table 3: Regression models for transect data of North Buffalo Creek (15 Dec 2009). A canoe transect was made from Rankin Mill Rd. (distance = 0 m) to McLeansville Rd. (distance = 7000 m), with samples taken approximately every 500 m. Three seston samples taken per sampling site were measured for AFDM, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. The mean AFDM, $\delta^{15}\text{N}$ and $\delta^{13}\text{C} \pm \text{SE}$ are shown in Fig.5. *Corbicula* samples were analyzed for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. The mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C} \pm \text{SE}$ are shown in Fig. 5.

Model Description	P-value	Model	R²	DF	F-value
AFDM-(ln) distance Transect	p<0.000	AFDM(mg/ml) = 4.566 - 0.286 (ln) distance(m)	0.33	1,41	19.495
$\delta^{13}\text{C}$ (‰)of seston -(ln) distance Transect	p<0.000	$\delta^{13}\text{C}$ of seston (‰) = -26.98 - 0.124 (ln) distance(m)	0.28	1,42	16.138
$\delta^{15}\text{N}$ (‰)of seston -(ln) distance Transect	p<0.014	$\delta^{15}\text{N}$ of seston (‰) = 3.271 + 0.13(ln) distance (m)	0.14	1,42	6.632
$\delta^{15}\text{N}$ <i>C. fluminea</i> (‰)- (ln) distance Transect	p<0.000	$\delta^{15}\text{N}$ <i>C. fluminea</i> (‰) = 7.379 + 0.216(ln) distance (m)	0.29	1,44	17.804

Table 4: Statistical comparisons between initial and final variables of seston evaluated in exp. #1. An independent t-test was performed comparing the seston variables between clams, sediment + water and sediment +water treatment at both the initial and final time points. Each variable was measured three times per treatment. Mean \pm SE values are graphed in Fig. 6.

Variable	T0 p-value	T0 t-stat	DF	T12 p-value	T12 t-stat	DF
AFDM	p=0.706	-0.405	4	p=0.374	-1.001	4
$\delta^{13}\text{C}$	p=0.819	-0.244	4	p<0.066	-2.504	4
$\delta^{15}\text{N}$	p=0.287	-1.222	4	p=0.166	1.692	4
C/N	p=0.681	0.443	4	p<0.020	3.767	4

Table 5: Repeated measures ANOVA results for exp. #1. A repeated measures ANOVA was performed comparing the seston variables between clams, sediment + water and sediment + water treatment comparing the initial and final time points. Repeated measures ANOVA was used to determine if the changes were significant through time, the interaction of time and treatment or by treatment only.

Variable	Time			Interaction			Treatment		
	p-value	F	DF	p-value	F	DF	p-value	F	DF
AFDM	p=0.667	0.20	1,4	p=0.613	0.3	1,4	p=0.255	1.77	1,4
$\delta^{13}\text{C}$	p<0.006	28.2	1,4	p<0.036	9.71	1,4	p=0.166	2.87	1,4
$\delta^{15}\text{N}$	p=0.117	4.18	1,4	p<0.083	5.28	1,4	p=0.693	0.69	1,4
C/N	p<0.042	8.76	1,4	p=0.058	6.94	1,4	p=0.026	11.99	1,4

Table 6: Statistical comparison between initial and final variables of seston evaluated in exp. #2. An ANOVA was performed comparing the seston variables between water only, clams + water and clams, sediment +water treatments at both the initial and final time points. Each variable was measured three times per treatment. The mean values \pm SE are graphed in Figure 7.

Variable	T0 p-value	T0 F-stat	DF	T12 p-value	T12 F-stat	DF
AFDM	p=0.201	2.074	2,8	p=0.522	0.726	2,8
$\delta^{13}\text{C}$	p=0.519	0.734	2,8	p=0.874	0.138	2,8
$\delta^{15}\text{N}$	p<0.029	6.770	2,8	p=0.264	1.674	2,8
C/N	p=0.763	0.283	2,8	p=0.144	2.728	2,8
Chloro a	p=0.197	2.292	2,7	p<0.016	8.864	2,8

Table 7: Repeated measures ANOVA results for exp. #2. A repeated measures ANOVA was performed comparing the seston variables between water only, clams + water and clams, sediment +water treatments comparing the initial and final time points. Repeated measures ANOVA was used to determine if the changes were significant through time, the interaction of time and treatment or by treatment only.

Variable	Time			Interaction			Treatment		
	p-value	F	DF	p-value	F	DF	p-value	F	DF
AFDM	p=0.883	0.02	1,6	p=0.241	1.82	2,6	p=0.342	1.29	2,6
$\delta^{13}\text{C}$	p<0.000	55.8	1,6	p=0.986	0.01	2,6	p=0.737	0.32	2,6
$\delta^{15}\text{N}$	p<0.000	76.5	1,6	p=0.070	4.37	2,6	p=0.062	4.60	2,6
C/N	p<0.000	294.3	1,6	p<0.012	10.0	2,6	p=0.609	0.54	2,6
Chloro a	p=0.130	3.28	1,6	P=0.527	0.72	2,6	p<0.056	5.43	2,6

APPENDIX B: FIGURES

Figure 1. Map of study sites on North Buffalo Creek, NC, USA. The location of study sites on North Buffalo Creek within Guilford County is shown in the inset of Guilford County. The stream flow is eastward. The study sites (circles) and the WWTP (square) are shown as well as major highways.

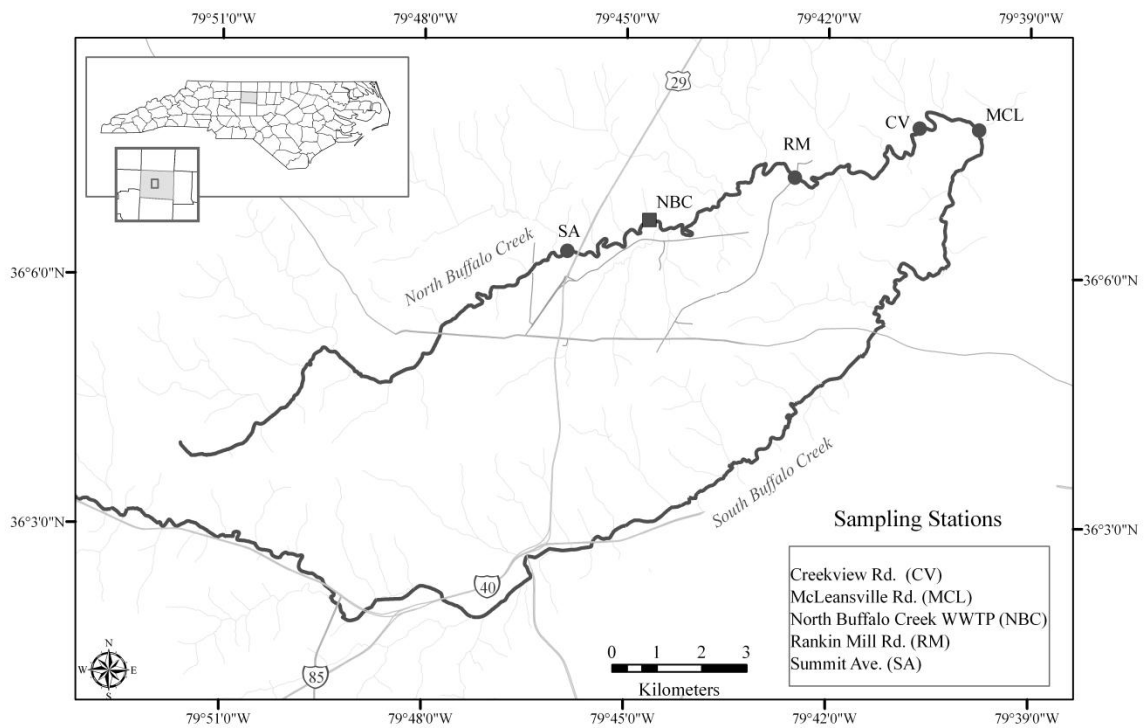


Figure 2. $\delta^{15}\text{N}$ (‰) (mean \pm SE) of seston and *C. fluminea* at Summit Ave. (A), Rankin Mill Rd. (B), Creekview Rd. (C), and McLeansville Rd. (D) on North Buffalo Creek late June 2009. Three clams and three seston samples were taken at each of the four sampling sites and processed for $\delta^{15}\text{N}$. Means that were significantly different ($p < 0.05$) are indicated with non-matching lower case letters.

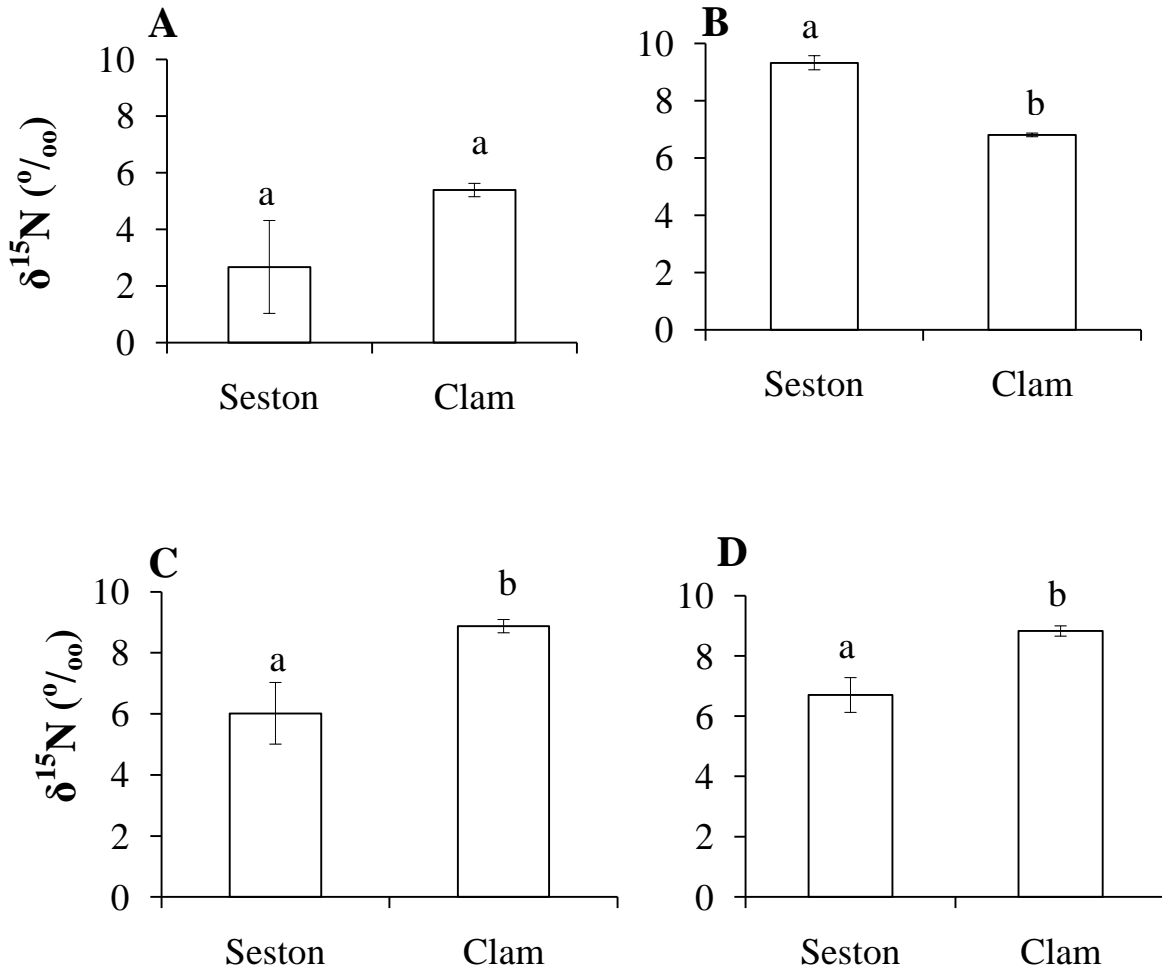


Figure 3. $\delta^{13}\text{C}$ (‰) (mean \pm SE) of seston and *C. fluminea* at Summit Ave. (A), Rankin Mill Rd. (B), Creekview Rd. (C), and McLeansville Rd. (D) on North Buffalo Creek late June 2009. Three clams and three seston samples were taken at each of the four sampling sites and processed for $\delta^{13}\text{C}$. Means that were significantly different ($p < 0.05$) are indicated with non-matching lower case lettering.

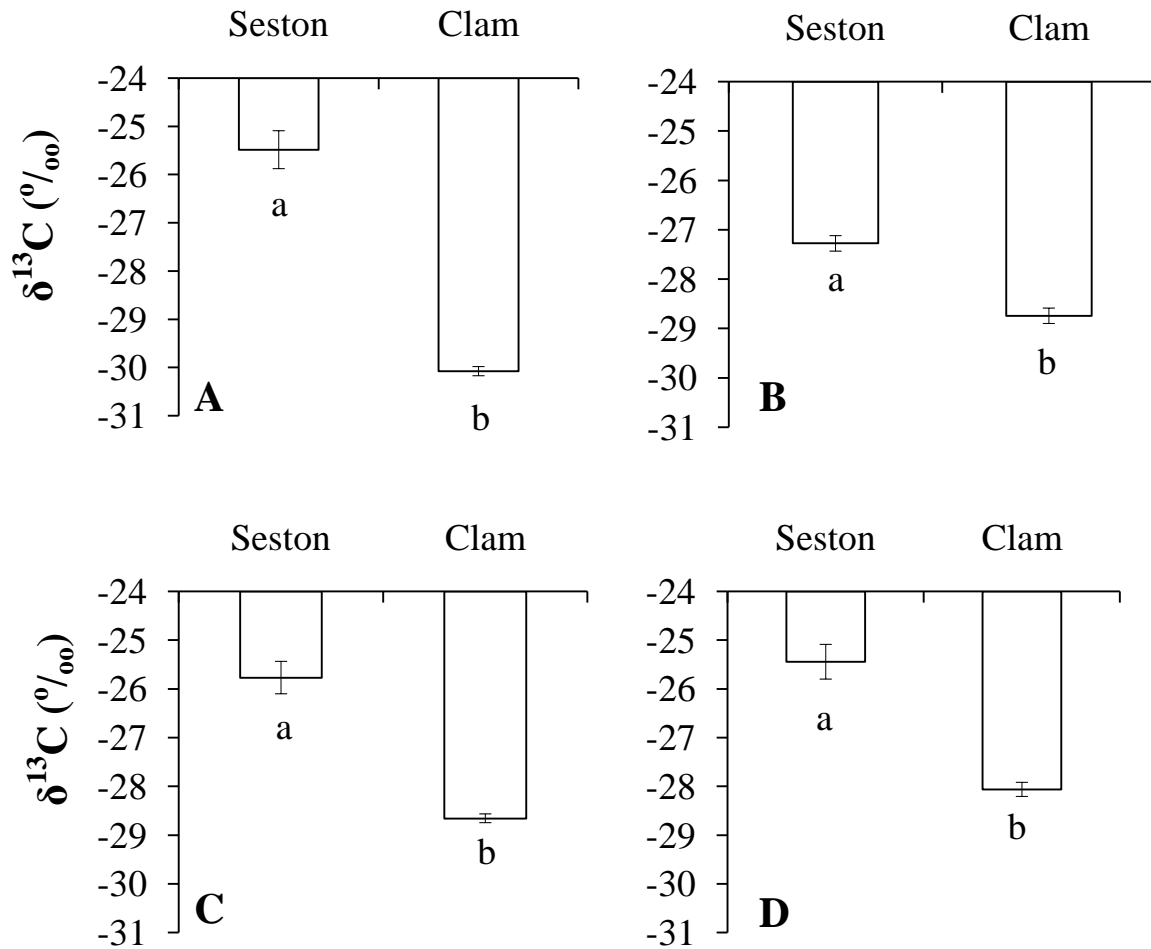


Figure 4. $\delta^{13}\text{C}$ (‰) and $\delta^{15}\text{N}$ (‰) biplot (mean \pm SE) of seston and *C. fluminea* for Summit Ave., Rankin Mill Rd., Creekview Rd., and McLeansville Rd. Three clams and three seston samples were taken at each of the four sampling sites and processed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Arrow shows expected shift between resource and consumer at a given site.

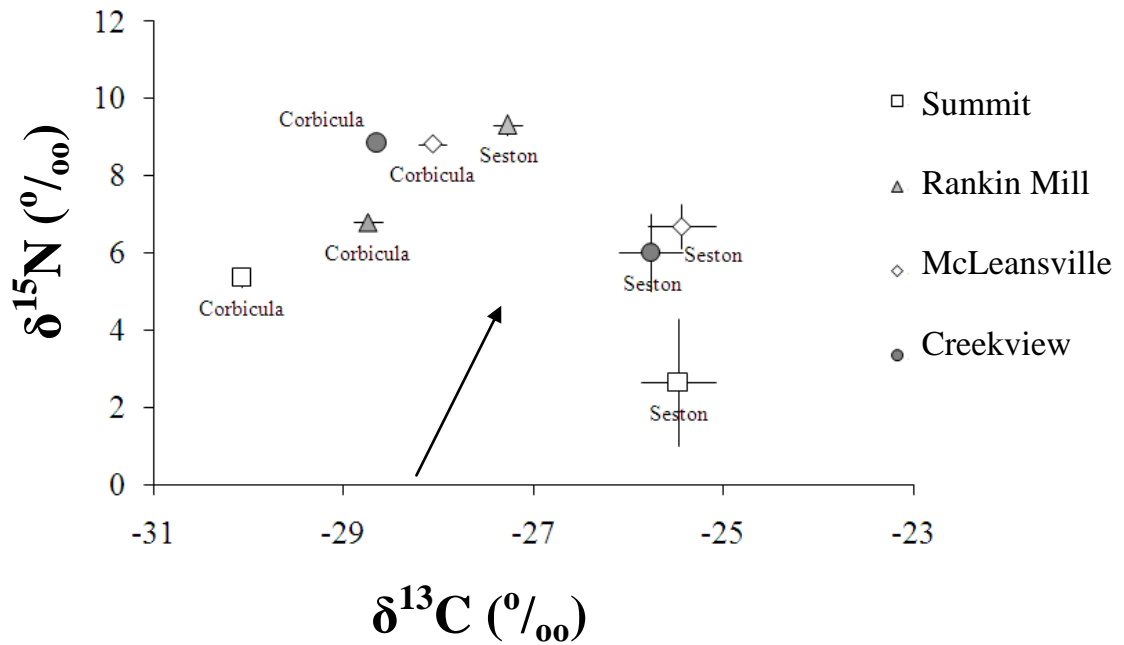


Figure 5. AFDM (A) and C/N (B) (mean \pm SE) of seston along transect of North Buffalo Creek and $\delta^{13}\text{C}$ (‰) (C) and $\delta^{15}\text{N}$ (‰) (D) (mean \pm SE) of seston (■) and *Corbicula* (◇) on North Buffalo Creek 15 December 2009. A canoe transect was made where m=0 was Rankin Mill Road and m=7000 McLeansville Rd. 15 sites were sampled for water and *Corbicula*. Each site was sampled three times for water which was processed for AFDM, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and C/N ratio. For each site, three clams were processed for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$.

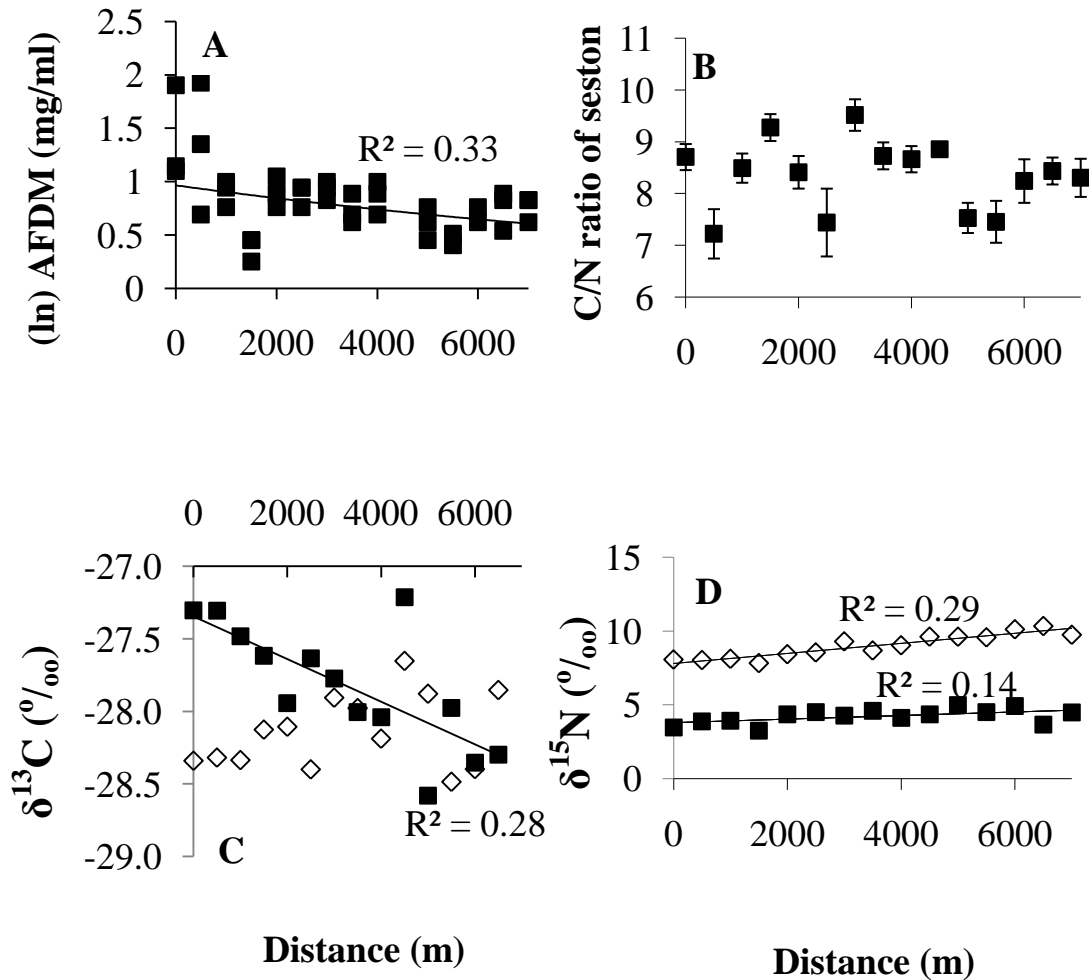


Figure 6. AFDM (A), $\delta^{13}\text{C}$ (‰) (B), $\delta^{15}\text{N}$ (‰) (C), and C/N (D) (mean \pm SE) of seston in exp. #1. Seston samples were taken every 2 hours for 12 hours during exp. #1. The seston samples were assessed for AFDM, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and C/N ratio. The control treatment was sediment + water (■). The experimental treatment was clams, sediment + water (□).

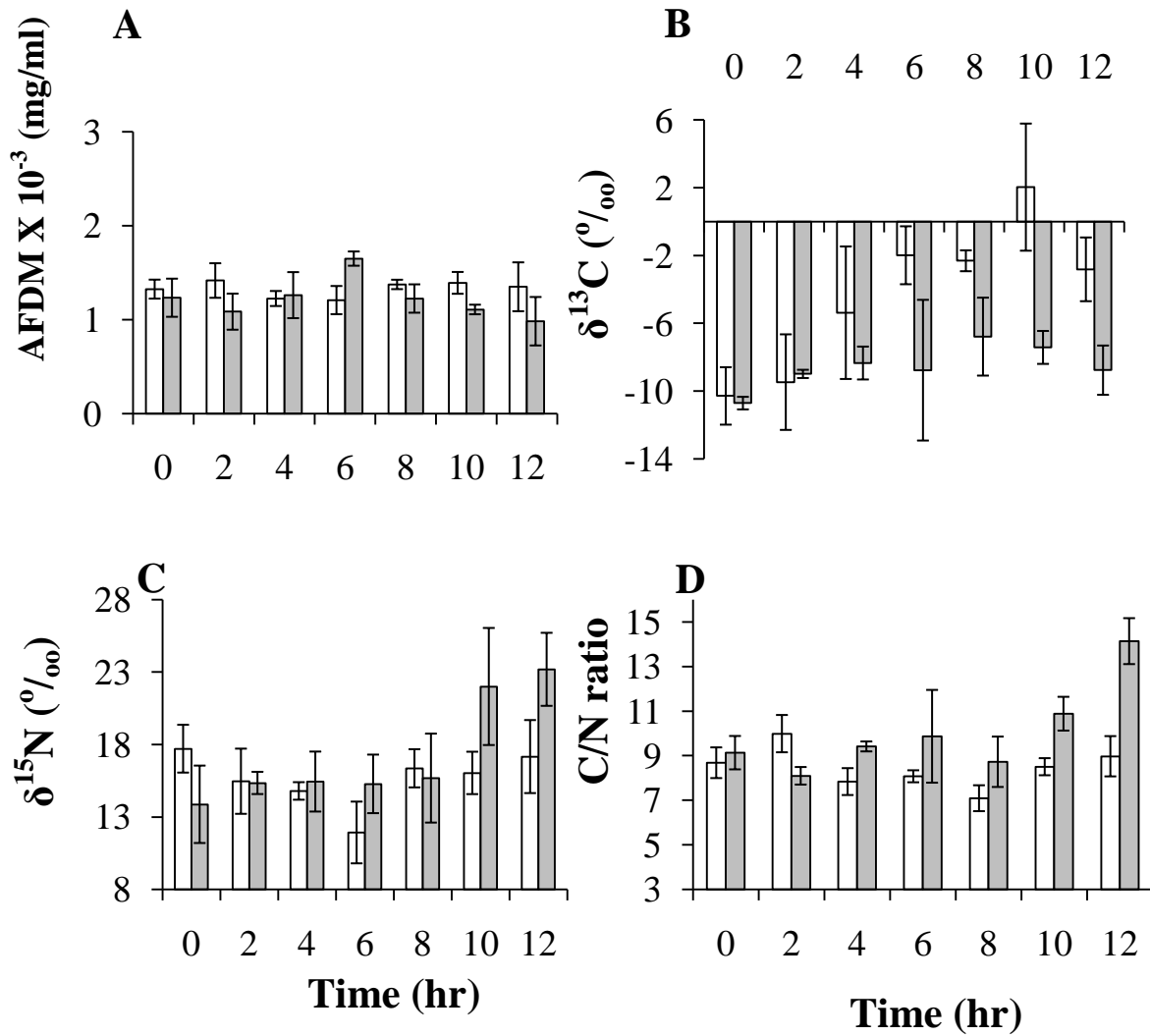


Figure 7. AFDM (A), $\delta^{13}\text{C}$ (‰) (B), $\delta^{15}\text{N}$ (‰) (C), C/N (D), and chlorophyll a (E) (mean \pm SE) of seston in exp #2. Seston samples were taken every 2 hours for 12 hours during exp. #2. The seston samples were assessed for AFDM, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and C/N ratio. The control treatment was water only (□). The experimental treatments were clams, sediment + water (▤) and clams + water (■).

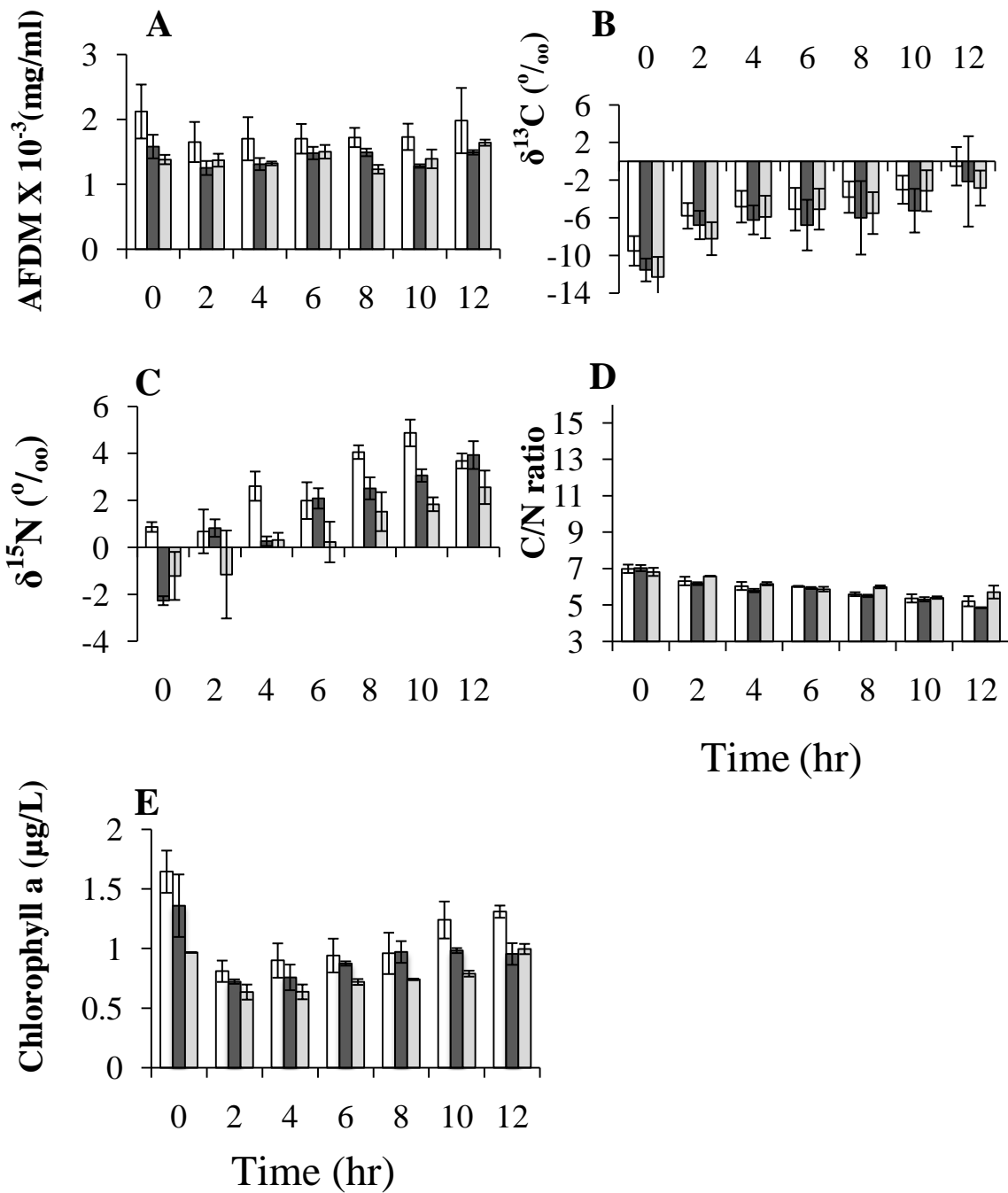


Figure 8. $\delta^{15}\text{N}$ (‰) (A) and $\delta^{13}\text{C}$ (‰) (B) (mean \pm SE) of *C. fluminea* in exp. #2. Three clams from each treatment aquaria (n=3) of clams only and clams, sediment + water were sent for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Means that were significantly different ($p < 0.05$) are indicated with non-matching letters.

