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Corbicula fluminea production in three major habitats, including one dominated by the non-native aquatic plant, Hydrilla verticillata, in the tidal freshwater Mattaponi River Estuary

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Corbicula fluminea production in three major habitats, including one dominated by the non-native aquatic plant, *Hydrilla verticillata*, in the tidal freshwater Mattaponi River Estuary

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Abstract

I determined the occurrence and distribution of the Asian clam *Corbicula fluminea* in subtidal benthic habitats of the Mattaponi River estuary in southeastern Virginia. This work was completed in consideration of the effect of vegetation type on *C. fluminea* production. *Hydrilla verticillata*, a non-native species of SAV (submerged aquatic vegetation), has recently become the dominant species of SAV in the tidal freshwater reaches of the Chesapeake Bay. To date, no research has been done to evaluate the ecological value of *H. verticillata* or other tidal freshwater habitats of the Chesapeake Bay for macrobenthic invertebrate secondary production. If exotic *H. verticillata* beds alter macrobenthic production, then food webs and energy flow in the tidal freshwater regions of the estuary may also be affected. For this study, benthic samples were collected in June, July, and September 2009 at sites with *H. verticillata* or mixed vegetation (native and *H. verticillata*), as well as unvegetated sites in the tidal freshwater portion of the Mattaponi River, a sub-tributary of the York River Estuary. Preliminary analyses of data from June 2009 documented the occurrence of *C. fluminea* in benthic samples, and that the clam dominated total macroinvertebrate biomass. In August 2010 *H. verticillata* and unvegetated habitats were again sampled for *C. fluminea*. Daily production (mg AFDM m^{-2} d^{-1}) of *C. fluminea* was compared among the three different habitats sampled in 2009 (unvegetated, *H. verticillata* dominated, and mixed vegetation) and two different habitats for 2010 (*H. verticillata* and unvegetated). Production was estimated for individual clams using an empirical method. Mean total production was computed per 5 mm length class, habitat type and month sampled. In 2009, the mixed and unvegetated samples exhibited greater levels of *C. fluminea* production than the samples

from the site with *H. verticillata*; however, this trend was not statistically significant. In 2010, there were no differences between the sampling sites in *C. fluminea* production. In addition to documenting for the first time the occurrence of *C. fluminea* in the Mattaponi River, the results of this study demonstrate that *C. fluminea* dominated macrobenthic secondary production (up to 300 mg AFDW $m^{-1}y^{-1}$) among three representative shallow subtidal habitats within the freshwater region of the Mattaponi River during 2009 and 2010. Relative to native or unvegetated benthic habitats, however, *C. fluminea* production was not affected by the presence of *H. verticillata*.

Introduction

Submerged aquatic vegetation (SAV), defined as any submerged vascular plant, provides important habitat within the Chesapeake Bay. Important taxa, including many species of fish, blue crabs, clams, shrimp, benthic and epibenthic macrofauna and insects utilize SAV as habitat. The physical complexity of SAV often improves water quality; root rhizome complexes bind sediments to increase sediment stability and deter erosion while complex above-ground leaf and stem structures can trap suspended sediment, lowering the resuspension rate of fine particles. SAV leaves can provide habitat for a large number of plants and animals including epiphytes and many species of invertebrates that are structure dependent. Different species of SAV support faunal communities that differ in species composition (Theel et al. 2007). SAV is also an important component of energy transfer in these communities; leaves produce large quantities of organic material that can be consumed by herbivores or returned to the detrital cycle (Fonseca et al. 1997).

In freshwater regions, these ecological services result in SAV habitats having greater diversity and abundances than comparable unvegetated habitats (Posey et al. 1993).

The Chesapeake Bay historically had much higher densities of native SAV coverage than currently exist (Orth & Moore 1984). During the 1970s, SAV abundance was reduced by approximately 90% in the Chesapeake Bay area as a result of eutrophication caused by agricultural run-off, acid rain and sewage treatment plant effluent (Goldsbourgh 1997). In 1982, the Executive Council of the United States Environmental Protection Agency's Chesapeake Bay Program agreed to use "the distribution of submerged aquatic vegetation (SAV) in the Bay and its tidal tributaries as documented by Baywide and other aerial surveys conducted since 1970, as an initial measure of progress in the restoration of living resources and water quality" (United States Environmental Protection Agency 1982). Since 1982, SAV abundances throughout the Chesapeake have increased. This resurgence is attributed to upgrades in sewage treatment plants, including enhanced phosphorous removal, decreased frequency of algal blooms and increased water clarity. In 2009 there was approximately 86,000 acres of SAV coverage in Chesapeake Bay and its tributaries (Orth et al 2010).

Hydrilla verticillata is an invasive species of aquatic vegetation that first arrived in the Chesapeake Bay during the early1980s, probably introduced with boat drainage (Posey et al. 1993). The proliferation of this exotic SAV has raised many questions within the Chesapeake Bay management community. *H. verticillata* is considered a pest because it may reduce water flow and cause flooding for boaters, landowners, and hydroelectric plants. Furthermore, invasive species are traditionally viewed as a threat to natural communities within an ecosystem. In the case of *H. verticillata*, managers worry

that this exotic plant may outcompete and eliminate native species of SAV and alter the ecosystem functions associated with these beds. However, recent reports suggest that while *H. verticillata* has become the dominant species of SAV in the freshwater regions of the Chesapeake Bay and its watershed during the past 17 years, native populations of SAV have continued to grow throughout the study period as well (Rybicki et al. 2007).

Macrofauna are important components of tidal freshwater benthic communities, including communities associated with *H. verticillata* in the Chesapeake Bay ecosystem (Posey et al. 1993). Secondary production of benthic macrofauna, net heterotrophic production derived from autotrophic primary production, constitutes an important portion of the energy available to the entire estuarine system (Gillett and Schaffner, 2009) and is also an ecological process increasingly used as an indicator of ecosystem health (Dolberth et al 2005). Furthermore, secondary production is a valuable tool for tracking carbon and energy flow through ecosystems and is vital to the understanding of an ecosystem's function. Macrobenthic production in SAV beds provides a link between organic matter sources (e.g. benthic macro -microalgae, detritus, phytoplankton) and economically and ecologically significant fish and crustaceans living within the vegetation. Furthermore, the ecosystem services these benthic communities provide affect estuarine water and sediment quality. For example, filter feeders remove particles from the water column and thereby enhance water quality, which can facilitate the growth of SAV (Diaz and Schaffner 1990). The invertebrate communities associated with SAV beds are an important prey source for higher trophic levels, especially fish and waterfowl.

Preliminary sampling in June 2009 for the present study revealed that a non-native macrobenthic species, the Asian clam, *Corbicula fluminea* (also known as *C.*

manilensis), was the biomass dominant in shallow subtidal habitats of the tidal freshwater reaches of the Mattaponi River, a sub-tributary of the York River Estuary, Chesapeake Bay. The Mattaponi River is an important component of the tidal freshwater habitat of the Chesapeake Bay ecosystem (Wooden 1999). *C. fluminea* was first reported on the Atlantic Coast in the 1970s (Sousa et al. 2008) and was first found in the freshwater Potomac in 1975 (Dresler and Cory 1980). When this study began, there were no previous documented records of *C. fluminea* in the Mattaponi (Schaffner, personal communication). This clam is a particularly successful "invasive" species due to its high fecundity, capacity to utilize a variety of substrates, and the ability to rapidly form dense populations. The introduction of *C. fluminea* into freshwater habitats has been correlated with the decrease of native bivalve abundances and diversity in North America and Europe (Sousa et al 2008). Unlike the native American unionid freshwater mollusks, which have a parasitic glochidial larval phase, *C. fluminea* larvae lack a planktonic phase; larvae are brooded and released as shelled larvae (Phelps 1994).

C. fluminea acts as an ecosystem engineer by altering both the biological and physical components of the tidal freshwater ecosystem (Sousa 2009). For example, *C. fluminea* can modify the species composition of an ecosystem. Studies on *C. fluminea* in the River Minho estuary in Portugal, a comparable system to the Chesapeake Bay estuary, indicate that patches with higher clam densities had higher abundances and biomass of several invertebrate species including oligochaetes, freshwater sponges and amphipods, relative to low density clam patches (Sousa 2008). The shells of living and dead *C. fluminea* provide substrate for attachment of sessile organisms (Gray 2002). Furthermore, remnant *C. fluminea* shells accumulated at the bottom of lake Constance

(Central Europe) were found to increase may fly (*Caenis* spp.) density (Werner and Rothhaupt 2007).

C. fluminea may also act as an ecosystem engineer by decreasing water turbidity via filtration; individual *C. fluminea* have been estimated to filter as much as 100 mL an hour (Lauritsen 1986). Other freshwater feeding bivalves native to the Chesapeake Bay (Unionidae and Pisidiiae) have relatively low filtration rates (Mattice 1979) and are not as abundant as *C. fluminea*. Thus, these other bivalves are expected to have a less significant effect on water turbidity. As filter feeders, *C. fluminea* have been shown to exert top-down control on phytoplankton; areas downstream of dense *C. fluminea* populations in the Potomac River estuary showed 40%-60% abundance "sags" in phytoplankton (Cohen et al. 1984). The filtering feeding of *C. fluminea* has been suggested to be responsible for the resurgence of submerged aquatic macrophytes (including *H. verticillata*) in the tidal freshwater Potomac (Phelps 1994).

C. fluminea is an important component of tidal freshwater food webs and is involved in benthic-pelagic coupling. When they filter feed, *C. fluminea* concentrate nutrients from the water column. The production of mucoid feces and pseudofeces keeps substantial quantities of this nutritional material in the surface layer of the sediment (Gray 2002). Finally, *C. fluminea* is an important prey item for fish, waterfowl and otter in the Chesapeake Bay (Perry, 1981; Robinson & Wellborn 1988; Posey et al. 1993).

As *H. verticillata* and *C. fluminea* continue to infiltrate the tidal freshwater of the Chesapeake Bay it is essential to identify the relationships, if any, between these species. Along the Susquehanna Flats of mainstream Chesapeake Bay, *H. verticillata* is associated with higher levels of faunal densities than unvegetated sites (Posey et al. 1993). Further,

Posey et al. (1993) found that transplanted *C. fluminea* showed significantly greater survivorship when transplanted in *H. verticillata* compared to being transplanted into unvegetated sediment and suggests that SAV may provide clams with protection from predation. However, no other studies have been done to determine how *H. verticillata* beds compare to native SAV habitats or unvegetated areas in terms of production in the Chesapeake Bay, or the relative suitability of *H. verticillata* beds as habitat for *C. fluminea*.

The objective of the present study is to compare *C. fluminea* production among three habitat types: *H. verticillata*, mixed vegetation (native and *H. verticillata*) and naturally unvegetated habitats. Specifically, this study estimated *C. fluminea* abundance (density m^{-2}), biomass (mg AFDW m^{-2}) and production (mg AFDW m^{-2} d^{-1}) at four different sites representing the three major habitat types of the Mattaponi sub-tributary of the York River, a major estuary of the lower Chesapeake Bay. Changes in abundance, biomass or production among vegetation habitat type would indicate a change in macrobenthic community structure or function. If exotic *H. verticillata* beds alter macrobenthic production relative to native vegetation and unvegetated habitat, energy flow will also be affected. This could have important implications for production at higher trophic levels and other aspects of tidal freshwater ecosystem structure and function.

Methods

Sites Selection

Sampling was conducted in the Mattaponi River on 16 June, 13, 15 July, 21, 22 September 2009, and 5 August 2010. Three representative sites within the Mattaponi, two vegetated and one unvegetated, were chosen for sampling so that beds of vegetation and unvegetated areas had comparable dimensions, depth and substrate type (when possible). The first site, located at 37.72188 N, 77.02840 W, was mostly unvegetated with a few patches of *H. verticillata* along the fringe of the sampling area. The sample area was adjacent to a very steep clay cliff, which had undergone visible erosion. The second sampling site, located at 37.73287 N, 77.04396 W, was characterized by mixed vegetation consisting of *H. verticillata* and an unidentified species of native SAV. The vegetation at this site was young and still somewhat sparse, with considerably more of the unidentified species than *H. verticillata*. The final sampling site, located at 37.72359 N, 77.02547 W, was characterized by a dense *H. verticillata* bed.

When we returned to the Mattaponi Unvegetated site in August 2010 we found that *H. verticillata* and an unidentified species of SAV had colonized the area and the site was no longer unvegetated. *H. verticillata* and the unidentified species were present both in the intertidal and sub-tidal zones. Furthermore, in 2010 the *H. verticillata* sampling site was moved about 100 m north within the same bed because the original sampling location had become inaccessible.

Data Collection

At each of the summer 2009 sampling sites, five 13.2 cm diameter cores ("Faunal Samples") were taken to a depth of 15 cm, including associated vegetation (if present), at a series of pre-determined random points in an 8 meter by 7.5 meter sampling grid. Sediment from each core was washed through a 500 um bucket sieve (water entered through the screen at the bottom, and the residue retained on the screen was then collected into a cloth bag which was kept on ice while in the field. Additional samples were collected for chlorophyll and sediment grain size. Benthic chlorophyll samples were taken to a depth of 2 cm using a 10 mL syringe with the bottom cut off. The grain size sample was taken to a depth of 5 cm using a 30 mL syringe. These samples were kept cool in the field and frozen in the lab until analyzed. The sediment grain size was analyzed using standard protocol (Plumb 1981) and sediment chlorophyll was analyzed using a Shimadzu UV-1650 Spectrophotometer according to protocol (Lorenzin 1967). We used a YSI probe in the field to obtain general water chemistry data including temperature (˚C), dissolved oxygen (mg/ L), pH, and salinity (ppt). We used excess sediment collected for grain size analysis in June 2009 to determine LOI (% sample organic). In 2010 we collected 3 replicates of 3 L water samples to determine water column chlorophyll a content (mg/ L) (Shoal & Loum, 1976).

Faunal samples were fixed with 20% buffered formalin with rose bengal dye for a minimum of 48 hours. These samples were then stored in jars with 10% buffered formalin and rose bengal. In the lab, samples were rinsed in a 500 um screen and then sorted using a dissecting microscope. Macrofauna removed from the samples were stored

in 2% buffered formalin. The length (dorsal/ ventral), width (anterior /posterior), and height of all *C. fluminea* were recorded. Dry mass (DM) and ash free dry mass (AFDM) were determined by drying clams (after removing the shells) at 65 ˚ C for ~24 hours and subsequently combusting the dried animals at 550 ˚ C for 4 hours. Organisms were massed using a balance sensitive to 0.1mg.

Field sampling techniques were modified in 2010 in order to capture a more representative sample of *C. fluminea*, which was patchily distributed in the 2009 samples. Six random ring-core samples ("Clam Samples") were collected at each site and processed in the field for clams only. These cores had a greater surface area (26 cm diameter) than the previous cores and were taken to a depth of 15 cm. Subsequent processing was as described as above except that only *C. fluminea* were retained. Clams were held in whirl packs and kept cool while in the field. Clams were then stored at -80˚F for three months prior to production calculations.

Secondary Production Estimation

Mean daily production of *Corbicula fluminea* was estimated using the empirical model of Edgar (1990) (Eq. 2). Edgar (1990) found that body size and temperature accounted for approximately 95% of the total variance in production. This model was derived from meta-analysis of production estimates for benthic faunal productions and has been used to estimate macrobenthic production for Chesapeake Bay (Hagy 2002).

The Edgar model (1990) relates daily production (**P**, $\mu g d^{-1}$) for a single macrobenthic animal to its biomass (**B**, µg AFDW) and water temperature (**T**, ºC) with $r^2=0.94$. Although Edgar (1990) formulated different models for different taxonomic groups (e.g. bivalves, crustaceans, polychaetes), he found them to be indistinguishable from the general model.

Eq. 1 : $$\mathbf{P} = 0.0049\mathbf{B}^{0.89}\mathbf{T}^{0.89}$$

The general Edgar equation was applied to each individual clam in order to provide a better estimate of size-specific production. Mean total production (mg AFDW $m^{-2}d^{-1}$) was then calculated per 5 mm weight class, habitat type and month sampled

Statistical Analyses

Due to the unbalanced sampling design and slight changes in sampling methods from 2009 to 2010, clam abundance, biomass and production data from the two years were analyzed separately.

For 2009 clams, 1-way ANOVAs were run for each month individually with R statistical software version 2.11.1 using site as the treatment variable. Normality and variance were visualized using Q-Q and residual plots. Natural logarithm transformations were used to normalize the abundance and production data, while a square root transformation was used to transform the biomass data. A Bartlett's Test was used to determine that the homogeneity of variance assumption was met before running

ANOVAs. Effects of seasonality were tested for by running Kruskall - Wallis tests for each sampling site using month as the treatment variable on Minitab 16 statistical software. Nonparametric tests were necessary because, due to the uneven sample sizes in June vs. July and September, my data did not meet the assumptions of normality or variance required for parametric statistics.

Because there were only two sampling locations in 2010, clam abundance, biomass, and production were analyzed using a 2-sided Welsh t-test. Equal variance was tested and met using an F-test for abundance and production data. Equal variance was not met for biomass data and an appropriately pooled variance (across treatment groups) was used for this response. Physical data were also separated by year and analyzed for the effects of sampling site and month using 2-way ANOVAs. Water column and benthic chlorophyll concentrations for 2010 were analyzed for site differences with 1-way ANOVAs.

Results

Environmental Parameters

Mean physical parameters, including salinity (ppt), DO (mg/ L), temperature (˚C), pH, and grain size (% sand) for 2009 sampling sites were comparable by month and location and are summarized in Table 1. Mean physical parameters, including salinity, DO, temperature and grain size for the 2010 sampling sites are summarized in Table 2.

Mean values and standard errors for all biological parameters including water column chlorophyll a (mg/ L), benthic chlorophyll a (um/ cm^2), and LOI (% organic sample) are listed in Table 3. Water was only collected for analysis during the 2010 sampling and LOI was only determined for June 2009 sampling. Surface fractions (0-1 cm) were used to determine benthic chlorophyll a content and were collected at every sampling occasion. There were no significant differences among sampling sites in any of the biological parameters and there was no significant difference among months in the benthic chlorophyll. There was a significant interaction between month and sampling site for benthic chlorophyll (df = 2, F = 4.34, p = 0.02), which means that patterns of change within sites among months differed.

Corbicula fluminea *abundance, biomass and production*

To facilitate production calculations, projected shell area (mm^2) was regressed to biomass (g AFDW) with $y = 5E\text{-}08x^2 + 0.0001x - 0.005$, $R^2 = 0.9506$ (Fig 1). This regression was used in the present study to estimate clam biomass for 2010 production estimates and can be used in future projects to analyze *C. fluminea* production in the Mattaponi without going through the lengthy process of determining AFDW.

C. fluminea is patchily distributed and abundance up to 1700 individuals m^{-2} was observed at the unvegetated and mixed sites and up to 450 individuals m^{-2} at the *H. verticillata* site. Mean *C. fluminea* abundances are shown on Figure 2. *C. fluminea* abundance at the *H. verticillata* sampling site differed significantly by month (df = 2, p = 0.044) with greater abundance in June than either July or September 2009. None of the

other sampling sites differed significantly in abundance by month. In 2009, there were no significant differences in *C. fluminea* abundances among the three sampling sites. However, in July 2009, there were marginal differences in *C. fluminea* abundance between the unvegetated sampling site and the *H. verticillata* sampling site ($df = 2$, $p = 0.073$). In 2010, abundances approximately ranged between 20 – 130 individuals m^{-2} at the unvegetated site and approximately between 40 – 400 individuals m^{-2} at the *H. verticillata* site. There was no significant difference between *C. fluminea* abundance in the *H. verticillata* and unvegetated sampling site in 2010.

Biomass (mg AFDW m^{-2}) of *C. fluminea* was variable within sampling sites (Fig. 3). In 2009, there were no significant differences in *C. fluminea* biomass among sampling sites or within sampling sites among month. In 2009 sampling site biomass averages ranged from approximately 1057 – 2117 mg AFDW m^{-2} at the *H. verticillata* site, 1505 – 1747 mg AFDW m^{-2} at the unvegetated site, and approximately 3310 – 6825 mg AFDW m^2 at the mixed vegetation site. In 2010, there was significantly more biomass at the *H. verticillata* sampling site than at the unvegetated site ($df = 6.55$, $t = 1.65$, $p = 0.05$). Biomass ranged from approximately 610 mg AFDW m^2 at the unvegetated site and approximately 703 – 9044 mg AFDW m^2 at the *H. verticillata* sampling site (Fig. 3).

Production (mg AFDW m^{-2} d^{-1}) of *C. fluminea*, like biomass, was variable within sampling sites (Fig 5). In 2009, sampling site production averages ranged from approximately 13 - 30 mg AFDW m^{-2} d^{-1} at the *H. verticillata* site, approximately 38 – 92 mg AFDW m^{-2} d^{-1} at the mixed vegetation site and approximately 34 – 142 mg AFDW m^{-2} d^{-1} at the unvegetated site. In 2009, there were no significant differences in *C. fluminea* production among sampling sites or within sampling sites among month. However, in

July 2009 there was a marginally significant difference in *C. fluminea* production between the *Hydrilla* sampling site and the unvegetated site (df = 2, p = 0.094). In 2010, *C. fluminea* production ranged between approximately 9 – 135 mg AFDW m^{-2} d^{-1} at the unvegetated site and approximately 8 – 105 mg AFDW m^{-2} d^{-1} at the *Hydrilla* sampling site. There was no statistical difference between *C. fluminea* production between sampling sites in 2010 (Fig 4).

Individual *C. fluminea* collected from July and September 2009 and August 2010 were classified into one of four size classes. Size class specific abundance, biomass and production are listed in Tables 4-7. Most notably, the mixed vegetation site was the only sampling site to have individuals in the largest size class, SC4, during 2009 while the unvegetated site was the only sampling site to have individuals in the smallest size class, SC1, during 2009. In 2010, individuals that were representative of all four size classes characterized both the *H. verticillata* site and the unvegetated site.

Discussion

This is the first study to document the occurrence of the Asian clam in the Mattaponi River estuary. All environmental parameters measured confirmed that the three sampling sites within the Mattaponi River were well within the typical environmental range of *C. fluminea*, which can survive in temperatures as high as 37.2 ˚C (Mudkhede & Nagabhushanam 1977) and salinity ranging from 0.03 – 22 ppt (Evans 1979) .

The density values I recorded (up to 1700 individuals m^{-2}) for the Mattaponi sites lie within range of densities reported from other areas where *C. fluminea* has invaded. *C. fluminea* densities have been reported between 230 – 4130 individuals m^{-2} in the River Minho Estuary (Sousa 2005), which is a comparable system to the tidal freshwater of the Chesapeake Bay.

Among the three sites studies in 2009, bivalves (primarily *C. fluminea*) accounted for 90-94% of the total macrobenthic biomass (Fig 5). These results are consistent with studies comparing the total biomass contributions of *C. fluminea* relative to other macroinvertebrates in other regions dominated by the Asian clam. In lower reaches of the Yangtze River, its native habitat, *C. fluminea* has been found to constitute approximately 98% of the total macrobenthic biomass (Wu et al. 1986). Following colonization, *C. fluminea* accounted for 95.4 % of bivalve biomass in the River Minho Estuary in Portugal (Sousa 2005). Therefore, these results confirm that *C. fluminea* is the dominant bivalve in the habitats sampled and that *C. fluminea* is the greatest contributor to macrobenthic production in tidal freshwater of the Mattaponi sub-tributary.

Biomass (mg AFDW m^{-2}) of *C. fluminea* was also variable due to small scale patchiness. The overall range of average values I observed of 610 mg AFDW m^{-2} at the unvegetated site in 2010 to 9044 mg AFDW m^{-2} at the H. verticillata sampling site in 2010 are consistent with *C. fluminea* average biomass records of between approximately 1000 – 50000 mg AFDW m^{-2} in a Georgia (USA) river (Stites 1995).

Production (mg AFDW m^{-2} d^{-1}) of *C. fluminea*, like abundance and biomass, was variable within sampling sites. While *C. fluminea* production is typically described to reach its peak early to mid spring (Gottfried 1982, Stites 2005, Sousa 2008) production

for the present study (2009) was observed to be highest in July rather than June, but, again, this trend was not significant and may be an artifact of the patchy distribution of the bivalve. Even so, it is likely that *C. fluminea* dominates macrobenthic production of the tidal freshwater reaches of the Mattaponi River.

My production results demonstrate that mean daily production of *C. fluminea* should not be estimated by biomass alone. In the 2009, the mixed vegetation site appeared to have greater biomass than the unvegetated site; however, the unvegetated site exhibited greater values for *C. fluminea* production than the mixed site. This is because larger densities of smaller clams characterized the unvegetated site; in fact the unvegetated site was the only location to have clams less than 9 mm in length. Per capita production for clams is greater for smaller, younger individuals than for larger, mature individuals and, this trend is exemplified in my data. This may support the hypothesis that smaller clams contribute more to site-specific mean daily production than do larger clams (Stites 1995, Sousa 2008).

A marginally significant trend occurred in July 2009 where both *C. fluminea* abundance and production were considerably lower at the *H. verticillata* sampling site than at the unvegetated sampling site. This trend seems to suggest that *H. verticillata* may support lower densities of clams and less production compared to the unvegetated habitat. However, in 2010, the *H. verticillata* sampling site has significantly higher *C. fluminea* biomass than the unvegetated sampling site. It is important to note that by August 2010 the unvegetated site had become colonized by both *H. verticillata* and an unidentified species of SAV, so differences between these two sampling sites in 2010 may not be representative of the 2009 habitat types.

The sampling methods used for the 2009 sampling were designed to study the entire benthic community and were not optimal for sampling bivalves. For that reason, clam sampling methods were modified in 2010 when it became apparent that *C. fluminea* was the dominant driver of production at the sampling sites we are monitoring. As is commonly observed (Schaffner, personal communication), the observed bivalve distributions are highly variable due to both the patchy distribution of *C. fluminea* and relatively small sample sizes. Nonetheless, the results of this study are highly useful because they demonstrate that *C. fluminea* production can be very high within the tidal freshwater region of the Chesapeake Bay ecosystem and that *C. fluminea* is able to successfully colonize a variety of habitat types.

I did not statistically compare results from 2009 to 2010 for three reasons: First, the sampling method was altered from taking five benthic cores to taking six ring samples exclusively for *C. fluminea* to account for the patchy distribution of *C. fluminea*. Second, the *H. verticillata* sampling site was moved about 100 m north within the same bed. Third, the unvegetated sampling site was colonized by both *H. verticillata* and an unidentified species of SAV by the time it was sampled in 2010. Nonetheless, while statistical comparisons between the two years would be inappropriate, visual comparisons provide some useful insights. In 2010 we found greater densities within the *H. verticillata* habitat type than we did in 2009, although, that may have been a combined result of better sampling techniques and moving the sampling location. Also, in 2010 we found fewer individuals at the same unvegetated sampling site with the new ring sampling method, despite the presence in 2010 of some vegetation. Thus, results were opposite of what I would have predicted.

While I expected that vegetated sites would have greater total mean production relative to unvegetated sites, this was not supported by either the 2009 or 2010 data. Posey et al. (1993) found that *C. fluminea* transplanted into beds of *H. verticillata* had greater survivorship than *C. fluminea* transplanted into similar unvegetated habitats, however, there was no significant difference in biomass in surviving clams. This suggests that while vegetation may serve as protection from some predators (mainly terrestrial predators like otters and waterfowl), vegetation does not directly alter *C. fluminea* production.

To sum, this study documents that the Asian clam *Corbicula fluminea* has successfully invaded the Mattaponi River estuary and appears to be the biomass dominant macrobenthic species. This study shows that the shallow subtidal habitats of the Mattaponi River can support high secondary production of *C. fluminea* relative to other native taxa, while abundance, biomass, and secondary production do not appear to differ among the major habitat types. I found no clear evidence that the presence of *H. verticillata* has a major impact on *C. fluminea* secondary production.

Recommendations for Future Studies

The next step in understanding carbon transfer and energy flow in these habitats is to better define food web relationships using stable isotope tracers, which will help to elucidate carbon sources and consumer trophic shifts for the organisms we have sampled (Appendix). Animals have been collected from all three habitat types and prepared for stable isotope analysis at the Stable Isotope Lab at the University of California at Davis.

Preliminary isotope analysis suggests that large (>20mm) *C. fluminea* feed at a higher trophic level than small (<20mm) *C. fluminea* and that large *C. fluminea* feed at the same trophic level as Unionidae, a mussel genus native to the Mattaponi River. Sediment, SAV, epiphytes, algae, select invertebrates and fish samples have been collected during July and August 2010 for further stable isotope analysis. These samples will continue the time series and add replication to the study and answer questions concerning trophic structure in these habitats. The data collected to date are summarized in the Appendix.

In addition, due to time constraints, production estimates of other taxonomic groups from the 2009 benthic cores were not completed. These data, in conjunction with the production estimates provided by this study and the samples that have not yet been processed could be used to construct a more detailed understanding of how total habitat production may change as a result of the introduction of the non-native species *Hydrilla verticillata* and *Corbicula fluminea.* Tidal freshwater estuaries support a diverse community of nekton and are very productive systems. These estuaries serve as nurseries for many species of crustaceans and fish creating communities that are a mixture of marine and freshwater species with interactions that are not normally considered in either freshwater or marine systems (Wooden 1999). These habitats are important producers of carbon, detritus, and secondary production that serve as important inputs into adjacent systems. While energy and carbon transfer in these systems is very complex and often difficult to trace, it is important to understand how these communities function and how community changes, such as the introduction and proliferation of exotic and invasive species may alter the existing ecosystem functions. *C. fluminea* is a fundamental element in the tidal freshwater of the Chesapeake Bay. The results presented for my study suggest

that this clam sequesters a large portion of the carbon available for benthic production and any change in the abundance, biomass or production of this species would alter the ecosystem functioning of these habitats, potentially making *C. fluminea* an important ecosystem engineer. Understanding how *C. fluminea* responds to the infiltration of *H. verticillata* is one of the first steps in understanding how both species might affect the tidal freshwater regions of the Chesapeake Bay.

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Figures and Tables

Site/ Month	salinity (ppt)	DO (mg/ L)	temperature (˚C)	pH	% sand
Hydrilla					
June	0.03	5.23	25.42	7.73	36.4
July	0.03	7.16	28.19	7.76	
September	0.05	6.84	23.05	7.56	
Mixed					
June	0.03	6.57	26.30	6.96	76.5
July	0.03	5.74	27.64	7.89	
September	0.05	7.02	22.07	7.11	
Unvegetated					
June	0.03	5.27	24.53	7.11	65.9
July	0.03	5.58	28.24	7.62	
September	0.05	6.74	22.29	7.95	

Table 1: Mean values for physical parameters collected in 2009. Grain size was only analyzed for June 2009 sediment cores.

Site	salinity (ppt)	DO (mg/ L)	temperature	% sand
Hydrilla				
	0.10	6.19	30.73	84.7
Mixed				
	0.01	6.06	31.37	/
Unvegetated				
	0.10	5.58	30.86	72.3

Table 2: Mean (standard error) values for physical parameters collected in August 2010. There was no grain size analysis for the mixed sampling site in 2010.

Site/ Mo(yr)	water column Chl a (mg/ L)	benthic chl a (ug/ cm^2)	LOI (% Organic)
Hydrilla			
June (09)		2.48 (0.17)	8.36 (1.04)
July (09)		0.42 (0.31)	
September (09)		7.11 (3.55)	
August (10)	7.69 (0.45)	2.06 (0.15)	
Mixed			
June (09)		2.48 (0.96)	4.05 (1.49)
July (09)		3.43 (0.60)	
September (09)		7.11 (3.06)	
August (10)	10.37 (0.83)	1.54 (0.51)	
Unvegetated			
June (09)		4.37 (0.54)	4.86 (2.13)
July (09)		3.51 (2.36)	
September (09)		1.63 (0.23)	
August (10)	8.31 (0.55)	0.77 (0.39)	

Table 3: Mean (standard error) biological parameters from 2009 and 2010. Water column chlorophyll a content was only collected during 2010. Benthic chlorophyll a represents the chlorophyll the surface (0-1cm fraction) of benthic chlorophyll. Both 2009 and 2001 values for benthic chlorophyll are listed in this table to save space, they were analyzed with different tests. LOI (% organic content) was only calculated for the June 2009 samples. No significant interactions.

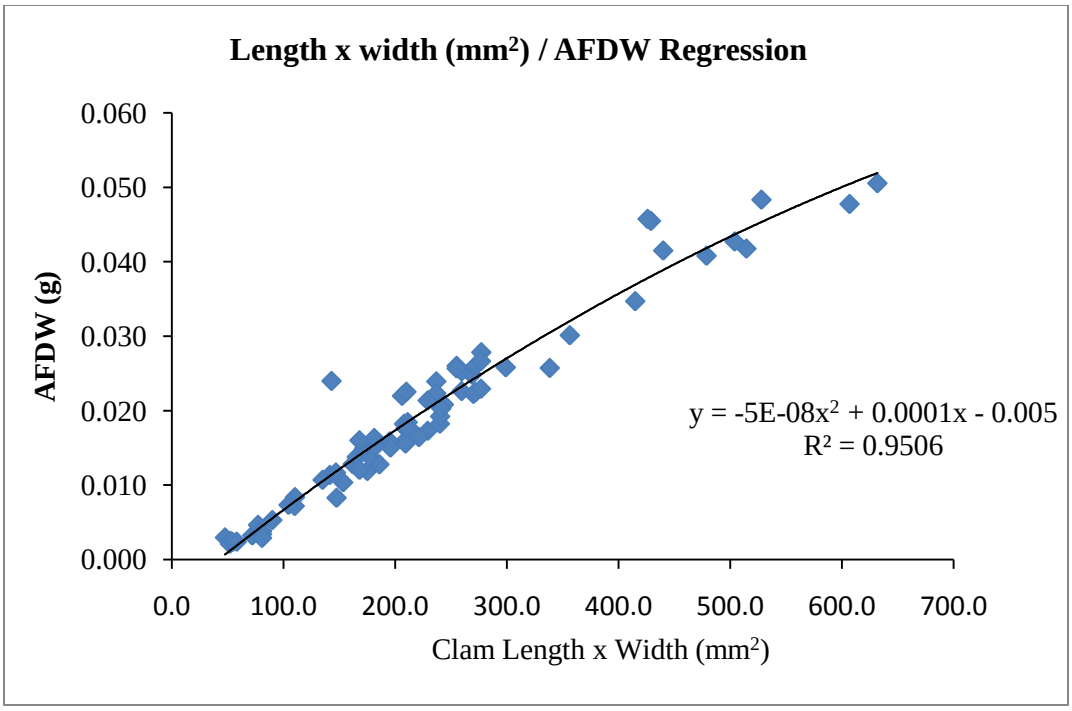

Fig 1. Projected shell area (mm^2) / Biomass (g AFDW) Exponential Regression

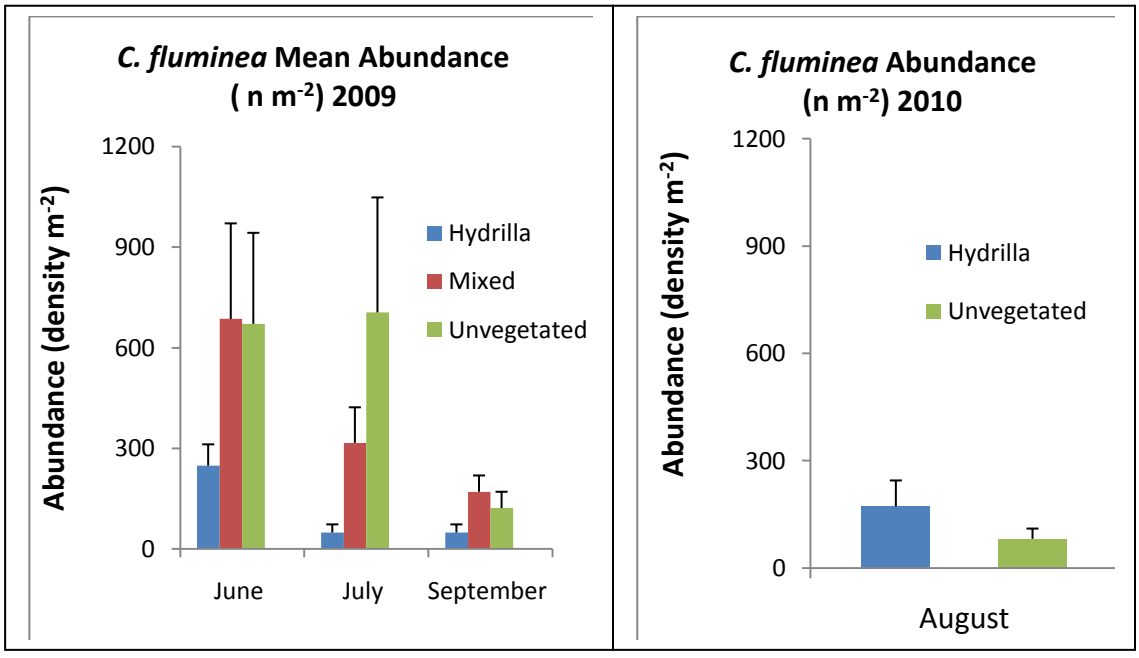

Fig 2: ***C. fluminea*** abundances (density m^{-2}) for 2009 and 2010. Error bars are SE.

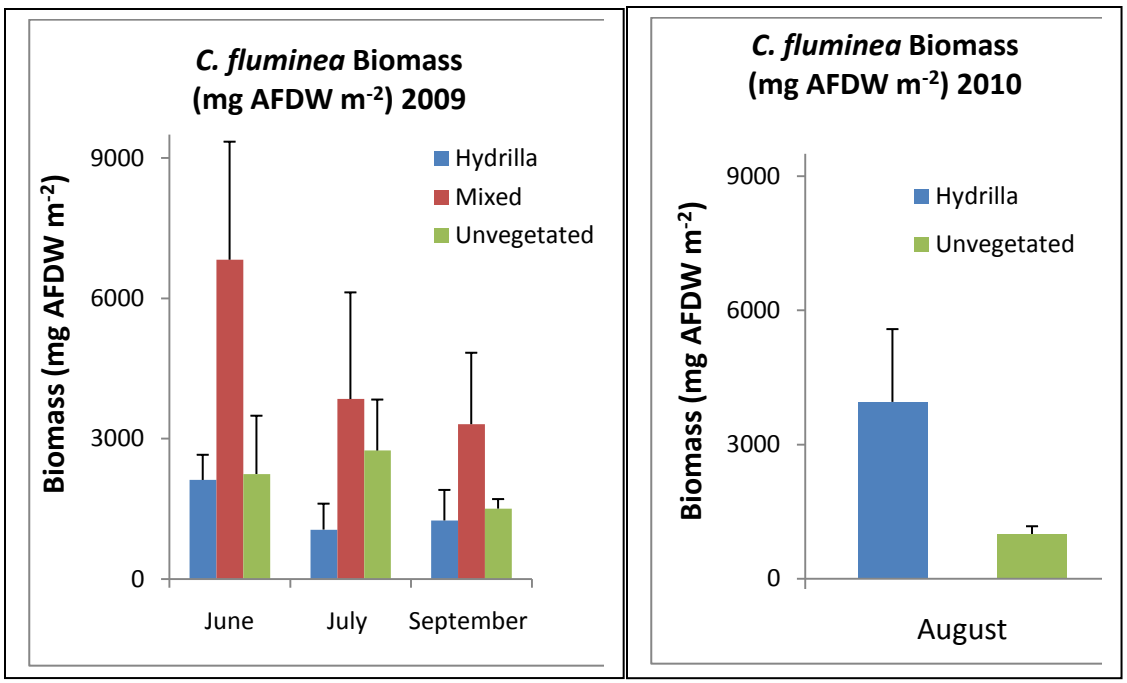

Fig 3: *C. fluminea* biomass (mg AFDW m^{-2}) for 2009 and 2010. Error bars are SE.

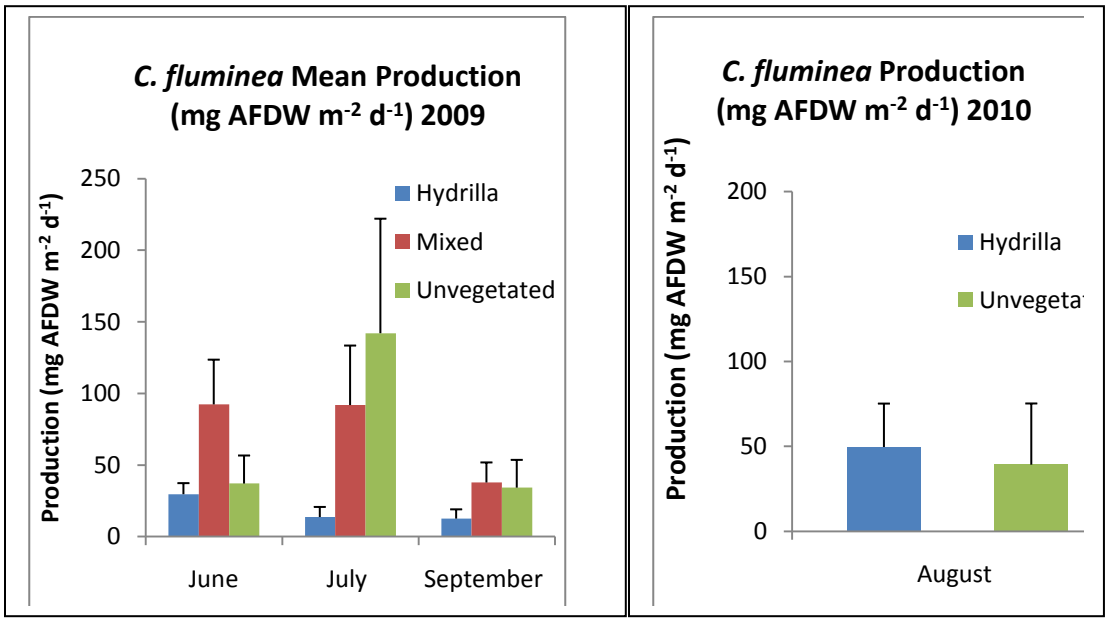

Fig. 4: Corbicula fluminea Production (mg AFDW m^{-2} d^{-1}) in 2009 and 2010. Error bars are standard error.

Sample date / Site	Abundance				
	CS1	**CS2**	**CS3**	**CS4**	**N**
July 2009					
Hydrilla	0.00	50.00	50.00	0.00	48.66
Mixed	0.00	46.15	38.46	15.38	316.30
Unvegetated	10.36	68.97	20.69	0.00	705.60
September 2009					
Hydrilla	0.00	0.00	100.00	0.00	48.66
Mixed	0.00	57.14	28.57	14.28	170.32
Unvegetated	0.00	40.00	60.00	0.00	121.65
August 2010					
Hydrilla	32.28	5.45	49.01	12.73	172.33
Unvegetated	61.53	26.92	7.69	3.85	81.47

Table 4: Sample abundances (density m^{-2}) are given as percentages of predetermined size classes. All values listed are given in percent (%) of sample total (N, density m^{-2}). Total site/ date specific densities are listed in the most right column. Class sizes were determined by measuring the height (D/V length) of every individual *C. fluminea* within a sample. CS1 (5- 9 mm), CS2 (10- 14 mm), CS3 (15- 19 mm), CS4 (20- 24 mm)

Sample date / Site	Biomass				
	CS1	**CS2**	**CS3**	**CS4**	**N**
July 2009					
Hydrilla	0.00	40.94	59.06	0.00	1057.2
Mixed	0.00	28.06	43.94	28.00	3848.3
Unvegetated	31.34	36.80	31.86	0.00	2445.6
September 2009					
Hydrilla	0.00	0.00	100.00	0.00	1252.3
Mixed	0.00	34.13	34.48	31.40	3310.7
Unvegetated	0.00	60.85	39.15	0.00	1505.8
August 2010					
Hydrilla	7.24	3.72	64.29	24.75	3951.2
Unvegetated	31.12	37.26	18.42	13.20	999.48

Table 5: Sample biomass (mg AFDW m^{-2}) is given as percentages of predetermined size classes. All values listed are given in percent (%) of sample total (N, mg AFDW m^{-2}). Total site/ date specific biomass is listed in the most right column. Class sizes were determined by measuring the height (D/V length) of every individual *C. fluminea* within a sample. CS1 (5- 9 mm), CS2 (10- 14 mm), CS3 (15- 19 mm), CS4 (20- 24 mm)

Sample date / Site	Production				
	CS1	**CS2**	**CS3**	**CS4**	**N**
July 2009					
Hydrilla	0.00	42.80	57.20	0.00	13.71
Mixed	0.00	34.19	39.88	25.94	91.90
Unvegetated	3.38	67.01	29.53	0.00	142.06
September 2009					
Hydrilla	0.00	0.00	100.00	0.00	12.60
Mixed	0.00	43.96	30.98	25.06	37.83
Unvegetated	0.00	28.96	71.04	0.00	34.21
August 2010					
Hydrilla	9.73	55.52	31.42	3.33	45.61
Unvegetated	8.35	4.09	64.26	23.30	32.31

Table 6: Sample biomass (mg AFDW m^{-2} d^{-1}) is given as percentages of predetermined size classes. All values listed are given in percent (%) of sample total (N, mg AFDW m^{-2} y^{-1}). Total site/ date specific biomass is listed in the most right column. Class sizes were determined by measuring the height (D/V length) of every individual *C. fluminea* within a sample. CS1 (5- 9 mm), CS2 (10- 14 mm), CS3 (15- 19 mm), CS4 (20- 24 mm)

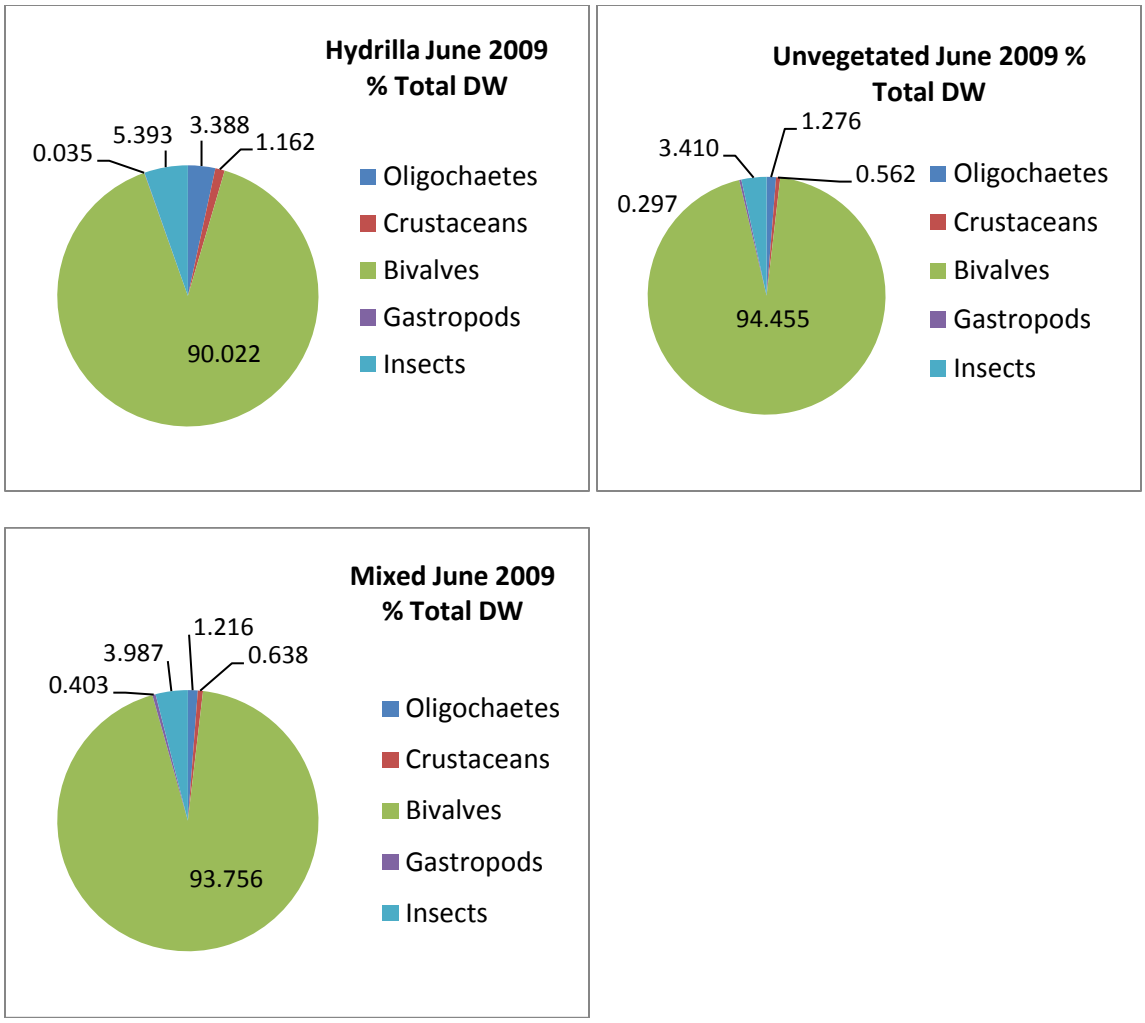

Fig 5: Taxon specific components of Total June 2009 Sample Dry Weights. Values presented are percentages of sample total DWs.

Appendix

Stable Isotope Study

As *H. verticillata* becomes progressively more prevalent throughout the freshwater regions of the Chesapeake ecosystem, it will be important to examine how macrobenthic food webs are structured and how they function within SAV habitats with *H. verticillata* relative to beds of native vegetation and unvegetated habitats. Dual stable carbon and nitrogen isotopic analysis can provide insights into how organic matter is transferred and transformed in complex estuarine habitats. Nitrogen isotopes are highly fractionated during the feeding process, much more so than carbon isotopes, and therefore nitrogen stable isotope data can be used to elucidate trophic shifts within a community of consumers while carbon stable isotope data can be used to determine which primary producers in a system are acting as the foundation of the food web (Minagawa & Wada 1984; Fry 1991).

Although not a major focus of this thesis due to sampling processing time constraints, animals, vegetation and sediments were collected during the course of the study for stable isotope analysis in order to better understand food web relationships in the habitats samples. Standard protocols employed by the Benthic Processes Laboratory at the Virginia Institute of Marine Science (Gillett and Schaffner, unpublished) were followed for collection and processing. Briefly, organisms were collected from all sampling locations by physically separating them from the sediment using a sieve and forceps when necessary. In the lab, samples were dried, ground, (acidified when samples contained excess $CaCO_3$, including sediment and mollusk samples) and encapsulated in

tin capsules. Analysis was by mass spectrometry at the Stable Isotope Facilities at the University of California at Davis for dual stable carbon ($\delta^{13}C$) depletion and nitrogen ($\delta^{15}N$) enrichment.

Isotope analysis from samples collected in June 2009 suggest that large (>20mm) *C. fluminea* feed at a higher trophic level than small (<20mm) *C. fluminea* and that large *C. fluminea* feed at the same trophic level as Unionidae, a mussel genus native to the Mattaponi and Pamunkey rivers (Table 4).

Sample Animal	**$\delta^{15}N$**	**Feeding Type**
Trinectes maculates	10.01 (0.08)	carnivore
Chironomidae	7.73 (1.03)	carnivore
Odonata	7.50 (0.43)	carnivore
Gastropoda	7.10 (1.20)	herbivore, omnivore, carnivore
***Corbicula* (big)**	7.09 (0.30)	planktivore
Unionidae	7.07 (0.29)	planktivore
Oligochaete	6.87 (1.53)	detritivore, omnivore
***Corbicula* (small)**	6.34 (0.54)	planktivore
***Gammarus* spp.**	6.16 (0.83)	herbivore
Ephemeroptera	5.56 (0.77)	herbivore, detritivore

Table 1: Pooled mean nitrogen enrichment ($\delta^{15}N$) and standard error for the most common taxa among all three sampling sites. Tentative feeding classifications are given for each taxonomic grouping (Schaffner, personal communication).