Estuaries and Coasts

Predator-prey interactions of the polyclad, Euplana gracilis, and the amphipod, Apocorophium lacustre, in the Chesapeake Bay

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To the Editor:

Please find the attached final version of our manuscript. All edits were corrected and contribution numbers added in the Acknowledgements section. We would like to thank you as well as the reviewers for the edits/comments that helped make the manuscript better.

Sincerely,
Dean Janiak

Specific Edits/comments:

-"et al." throughout the text should not be in italics, remove italics

Throughout the text, all italics have been removed.

-where sentences start with E. gracilis (e.g., line 65), please write out the genus name

The full genus was written out for all sentences that started with a species name.

-remove title on Fig. 3

Title for Figure 3 was removed.

-Fig. 3 add per grab on axis labels (e.g., E. gracilis (average no. ind. per grab or similar) and also use the same format on axes of Fig 4 for consistency.

Both Figure 3 and Figure 4 axis labels changed.

-Font size of numbers on y-axes differ for the two species in Fig 3. Also make sure all fonts (numeric and text) font on all figures 3-5 is consistent

Font size on y-axis was corrected. Font size was checked and fixed when needed in Fig 3-5.

-There seems to be an error in the first sentence of the figure legend 3: "from based on the different shoreline types??"

Sentence was corrected, "based on" was deleted.

-incorrect long dash (sorry this was my copy paste error) please use "–" throughout text for long dash and in the citations

The corrected long dash "–" was replaced throughout the text and references.

–

Title:

Predator-prey interactions of the polyclad, *Euplana gracilis*, and the amphipod, *Apocorophium lacustre*, in the Chesapeake Bay

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Abstract

Predation within the marine environment has been well-studied and shown to be of major importance in shaping patterns of biodiversity. Typically larger predators, such as fishes, are examined because of the ease of manipulation and strong detectable results whereas effects of smaller micro-predators are much more difficult to evaluate. Here, we examined the distribution and prey consumption of the polyclad flatworm, *Euplana gracilis*, in the Chesapeake Bay. *Euplana gracilis* is a common, micro-predator but no data exist on its ecological function. Flatworms were found to actively prey upon a single species, the tube-building amphipod *Apocorophium lacustre*, in lab trials when tested against several other commonly encountered species. To examine natural population densities of flatworms, large-scale field sampling was conducted via benthic grabs and *E. gracilis* abundances were found to

be significantly correlated with *A. lacustre* particularly in areas close to the shoreline. Some predator-prey interactions were examined including timed observations of consumption, predator size, and tube protection. Flatworm body size was found to correlate strongly with number of prey consumed over time. Tubes constructed by amphipods were examined as a means of refuge when in the presence of *E. gracilis* but provided very little protection as flatworms could easily penetrate tubes in search of prey. Our results are the first to show predation of an estuarine/marine polyclad flatworm on amphipods as well as provide some insight into the dynamics of this previously unknown predator-prey relationship.

Introduction

Within the marine environment, the role of predation has been well-studied and shown to be an important factor in shaping patterns of biodiversity. Predators can have direct or indirect effects on multiple trophic levels within a community, causing alterations in the interactions among prey and their competitors for resources (Paine, 1980; Duffy, 2002; Bruno and O'Conner, 2005; Clemente et al., 2010; Vieira et al., 2012). Most studies have focused on the effects of larger predators on populations or communities (Lubchenco and Menge, 1978; Myers and Worm, 2003; Thrush et al., 2006; McCauley et al., 2012) whereas few studies have documented the effects of micro-predators on their prey (e.g. Newell et al., 2007). What little work has been done shows the importance of these species as predators in different marine habitats (Ambrose, 1991; Osman and Whitlatch, 1992; Newell et al., 2000; Osman and Whitlatch, 2004; Lavender et al., 2014). One typically overlooked though ubiquitous group of small marine predators are the polyclad flatworms.

The Polycladida are a diverse order within the phylum Platyhelminthes (Class Rhabditophora) consisting of almost entirely marine, non-parasitic forms. They are globally distributed with an extensive dietary breadth, and found in most habitats as well as in close association with a variety of invertebrates (e.g. crustaceans and echinoderms) (Newman and Cannon, 2003). The majority of species within this group primarily consume sessile prey including bivalves, barnacles, corals, and ascidians (see review by Galleni et al., 1980; Newman et al., 2000; Rawlinson and Stella, 2012). Other than feeding selectivity, little is known about the ecological role polyclad flatworms have in the marine environment. What has been identified comes from a group of studies that has focused primarily on members of the Stylochidae (Galleni et al., 1980; Chintala and Kennedy, 1993; Merory and Newman, 2005; Lee et al., 2006). Members of this family are typically recognized as pests on a variety of commercial aquaculture species including clams, mussels, and oysters (Littlewood and Marsbe, 1990; Newman et al., 1993; Jennings and Newman, 1996; O'Connor and Newman, 2003).

The trophic structure within the Chesapeake Bay is fairly well-characterized (Baird and Ulanowicz, 1989; Krause et al., 2003) and an important and well-studied relationship includes the predator-prey interaction between the eastern oyster, *Crassostrea virginica* (Gmelin, 1791) and the polyclad flatworm, *Stylochus ellipictus* (Girard, 1850) (Landers and Rhodes, 1970; White and Wilson, 1996; Newell et al., 2000). Oysters provide economic and ecological services vital to the bay and therefore, have received much attention over the years (Newell, 1988; Ulanowicz and Tuttle, 1992; Rodney and Paynter, 2006). Within the bay, a second flatworm, *Euplana gracilis* Girard, 1853, is found in high densities **(**personal observation) though little is known of its ecological role. *Euplana gracilis* is a common inhabitant in the Chesapeake Bay as well as most of the eastern Atlantic coastline from Maine to the Gulf of Mexico (Hyman, 1940). Despite the fairly large distributional range of *E. gracilis*, there exists little knowledge on the species. The aim of the current study was to examine the local distribution and predatory impacts of *E. gracilis* in the upper Chesapeake Bay. To do this, field collections were made and a series of laboratory experiments were conducted to examine the predatory role of *E. gracilis*. Experiments were designed to test prey selectivity as well as examine specific interactions between *E. gracilis* and its prey. Specifically we asked: 1) what is the local distribution of *E. gracilis* within a representative area of the upper Chesapeake Bay, 2) what is the prey selectivity for *E. gracilis* among commonly encountered benthic species, and 3) what are some specific predator-prey interactions between *E. gracilis* and its prey.

Materials and Methods

Study site and field collections

All sampling and species collections were done in the Rhode River (38° 53.03' N, 76° 32.4' W), a subestuary in the northwestern portion of the Chesapeake Bay in Maryland, USA (Figure 1). The river covers an area of approximately 4 km^2, is shallow (2 – 4 m depth), and mesohaline having salinity ranges from 0 to 20 with highest salinities occurring during the drier parts of the year. The mean tidal amplitude is roughly 35 cm though can be influenced by local wind patterns. For laboratory studies, all species were collected from wooden pilings and docks at the Smithsonian Environmental Research Center located on the western shore of the Rhode River. Structures were scraped with a paint scrapper and all mobile and sessile animals were brought back to the lab for identification and sorting. Animals were retained in the lab with fresh river water changed often under ambient temperature. Artificial habitats were used to collect live specimens due to practicality and a diverse suite of species found throughout the area. Collections of flatworms and potential prey were made as needed throughout the duration of the project. Once an animal was used in a trial or spent > 3 days in the lab, it was returned to the field and new collections were made. Field collections detailed below were done in natural habitats throughout the entirety of the river.

Predation on local species

To examine prey selectivity of *Euplana gracilis*, experiments were conducted with potential prey species commonly encountered and found in close proximity to flatworms in the Rhode River. The prey species used in each of the trials were 1) the tube-building amphipod *Apocorophium lacustre* (Vanhöffen, 1911), 2) the free-swimming amphipod *Gammarus mucronatus* Say, 1818, 3) the barnacle *Amphibalanus improvisus* (Darwin, 1854), 4) the tube-building spionid polychaete *Polydora cornuta* Bosc, 1802, 5) the nudibranch *Cratena pilata* (Gould, 1870), 6) the ctenostome bryozoan *Victorella pavida* Saville-Kent, 1870, 7) the nereid polychaete *Alitta succinea* (Leuckart, 1847), and 8) *Tanypus* sp. larvae (Insecta: Chironomidae). All experiments were run as paired trials, 10 replicates with and 10 replicates without a single individual exposed to a flatworm. For the colonial species *V. pavida*, clumps consisting of 6 actively feeding zooids were used for each replicate. All experiments were done in square 250 mL containers with newly-collected river water and allowed to run for 24 h. All species were collected within 48 h of the start and held in separate containers without food. All trials were monitored to note any particular interactions that occurred. After the allotted time, all prey species were counted as either dead or alive. A Fisher's exact test was used to compare the survivorship (nominal variables "dead" or "alive") of potential prey with the null hypothesis that the proportion of prey alive is the same when exposed or not exposed to a potential predator.

Distribution and abundance

As part of a separate monitoring project to examine the distribution and abundance of infaunal communities, 151 benthic grab samples were collected throughout the entirety of the Rhode River (Figure 1) in June 2014. Approximately half of the samples were collected at nearshore sites (0 – 3 m from shoreline) and the other half collected at offshore sites (> 3 m from shoreline). Environmental data collected for each site included depth, temperature, dissolved oxygen, salinity, and sediment type (visually assessed as fine sand, coarse sand, mud, and mix). Furthermore, samples collected at nearshore sites were classified by their shoreline type (forest, marsh, beach, bulkhead, riprap, and offshore). Samples were collected using a Petite Ponar benthic grab (WILDCO®). This particular grab can sample a variety of benthic substrate types and samples approximately an area of 15.2 cm^2 (2.4 L of sediment). Samples were sieved at 500 µm, fixed in 10 % formalin for one week, and transferred to 70 % ethanol for sorting and enumeration. For the purposes of this study, only *E. gracilis* and *A. lacustre* abundances were used in the data analysis. Data were analyzed using a distance-based analysis of a linear model (DistLM; *PRIMER v7*) (Clarke and Gorley, 2015) to examine species abundance in relation to environmental factors. Abundance data were square-root transformed and a resemblance matrix was constructed using Bray-Curtis similarities. Environmental data was normalized and a resemblance matrix was constructed using Euclidean distances. Selection of environmental factors was step-wise and AICc was used as the selection criterion to choose the best-fit environmental parameters explaining species distribution. A Two-Way ANOVA was run on abundances of both *A. lacustre* and *E. gracilis* using the fixed, categorical factors shoreline type and sediment type. The interaction between the two factors was not included because sediment type within and between shoreline type strongly varied. In both ANOVAs, Student-Newman-Keuls pairwise comparisons test within factors was used. Abundances of flatworms and amphipods were also compared using linear regression to test for any pattern in natural abundances between the two species.

Timed Observations

A series of trials were run to examine the length of time it took for *E. gracilis* to attack and consume *A. lacustre* as well as if that same individual would consume a second amphipod after 24 h. Twenty-seven adult flatworms of a similar size were randomly chosen and placed in separate containers (250 mL) along with a single adult amphipod in each. All flatworms and amphipods were collected within 48 h and starved during that time. Amphipods that were chosen were all of a similar size. The time it took for an initial attack was recorded as well as how long it took for amphipods to become immobilized and fully consumed. After the first amphipod was consumed, a second was added and the amount of time it took the same flatworm to prey upon the second amphipod was recorded.

Size versus consumption

A series of trials were run to examine if the size of individual *E. gracilis* was a significant factor in the number of amphipods consumed over a given time period. Flatworms (n = 45) of various sizes were randomly chosen and each was placed in a drop of water on top of a ruler and photographed at least 3 times when fully extended. The area (mm^2) of each flatworm was measured using ImageJ (Abramoff et al., 2004), and the average area from the separate photographs was used to indicate each individual's size. This was necessary as flatworms were quite active and a single measurement could be misleading. Sizes of flatworms used were found to range from 0.8 mm^2 to 9 mm^2. After photographs were taken, each flatworm was put into a separate square 500 mL container with new river water. In each container, 4 randomly chosen, adult amphipods of a similar size were added. The prey density was kept constant at 4 and dead amphipods were noted every few hours and replaced with lives ones over the duration of the experiment. The experiment was allowed to run for 120 h (5 days) and at the end of each day, water in each container was carefully siphoned out and exchanged with new river water. After the allotted time, all amphipods left were marked as either dead or alive. A control with 45 amphipods in a separate container without flatworms was run simultaneously to monitor the health of amphipods over the duration of the experiment. The number of amphipods consumed was compared against the size of each individual *E. gracilis* (mm^2) via linear regression.

Amphipod tubes and protection

Three separate trials were run in which amphipods were allowed to build tubes prior to predator exposure. Both trials 1 and 2 contained 5 replicates and trial 3 contained 12 replicates. Trial 3 had increased replication to ensure results were consistent. Each pair of replicates consisted of a single adult amphipod all of a similar size, either with a tube or without a tube exposed to a single flatworm. In each trial, half of the amphipods were placed in separate square 250 mL containers with defaunated sediment and new river water and allowed to build a tube whereas the other half were placed in containers without sediment. After amphipods in all replicates had built tubes (approximately 24 h), a single flatworm was randomly picked and added to each of the containers and the experiment was allowed to run for 120 min. Once time had expired, all amphipods were counted as dead or alive. Fisher's exact test was used to compare the survivorship of amphipods either with or without tubes using the nominal variables "dead" or "alive".

Results

Predation on local species

Out of all potential prey species tested, only *Apocorphium lacustre* was significantly consumed by *Euplana gracilis* (Table 1). Predation on *A. lacustre* was rapid and in all trials happened within 30 min. There was a 90 %

and 80 % survival rate of *Gammarus mucronatus* and *Amphibalanus improvisus* and based on personal observations, mortality resulted from damage during collection rather than predation. One *Polydora cornuta* was found dead and after the experiment ended, a flatworm was found on the worm but it was unclear whether or not the worm was being preyed upon by the flatworm. The last 4 species, *Cratena pilata*, *Victorella pavida*, *Alitta succinea*, and *Tanypus* sp., were all alive by the end of the trials. In all trials with flatworms absent, all species were accounted for and alive.

Distribution and abundance

A total of 151 benthic ponar grabs were taken throughout the majority of the Rhode River (Figure 1). *Apocorophium lacustre* was found in 93 samples (62 % of total) and *E. gracilis* was found in 51 samples (34 % of total). Based on the DistLM analysis, the environmental parameters temperature (p = 0.001), depth (p = 0.008), and salinity (p = 0.085) were the best predictors of abundances for the two species (Figure 2) though only explained 20 % of the variation in the data ($r^2 = 0.202$). Both *A. lacustre* and *E. gracilis* were found to be more abundant at nearshore sites (collectively for all shoreline types, 78.36 ± 18.41 S.E.) as compared to offshore sites (10.04 ± 3.78 S.E.). In separate Two-Way ANOVAs, there was a significant effect of shoreline type found for both *E. gracilis* (p = 0.028) and *A. lacustre* ($p < 0.001$) however no effect was found for substrate type. In both cases, the shoreline type forest had the highest abundances (Figure 3). There was a strong positive relationship ($r^2 = 0.59$, $p < 0.001$) between the abundance of *A. lacustre* and the abundance of *E. gracilis* (Figure 4). Out of all samples containing *A. lacustre*, 55 % had flatworms present and there was roughly a 10:1 ratio of amphipods to flatworms. Of those without flatworms, amphipod densities were quite low (1 – 10 per benthic grab) and no samples were collected that contained only flatworms with no amphipods.

Timed Observations

In all trials, predation occurred at a rapid rate and all initial *A. lacustre* were consumed by *E. gracilis*. The initial attack took on average 14 min (± 18 S.D.) from when the two species were added together. When an amphipod was added, the flatworm would increase its speed of movement in search of the prey and once encountered, would swiftly attack the ventral portion between two pereopods, injecting its pharynx into the tissue. The flatworm then moved to the dorsal side of the amphipod and in many cases with the pharynx removed, while the amphipod was still mobile. After approximately 3 min (± 1 S.D.), the amphipod was fully immobilized and the flatworm moved back to the ventral side and began to actively digest the internal tissues. Flatworms fed on average

65 min (± 28 S.D.) prior to abandoning the carcass and after, went into a short "resting" phase whereby movement was limited. In 85 % of trials, flatworms had already consumed the second amphipod within 24 h.

Size versus consumption

There was a significant positive linear relationship between the size of *E. gracilis* and the number of amphipods consumed in 120 h ($r^2 = 0.49$, $p < 0.001$, Figure 5). Flatworms in the smaller size classes exhibited increased handling time and some variation in prey consumption but still consumed at least 3 amphipods in the allotted amount of time. Observations suggest that all amphipod mortality was due to predation by flatworms. In the control container, with no flatworms added, all amphipods were accounted for and alive.

Amphipod tubes and protection

In all 3 trials, there was no significant difference between amphipods with tubes present and those without tubes (Fishers exact test, $p > 0.05$). In the first and second trial, 4 of 5 amphipods having tubes were consumed whereas all 5 were consumed without tubes and in the third trial, 10 of 12 amphipods with tubes were consumed whereas all 12 were consumed without tubes. During the trials with tubes present, prey attack and consumption occurred both in and out of tubes. Flatworms were attracted to tubes that contained amphipods and either entered the tube or attacked the amphipod from the outside.

Discussion

The flatworm, *Euplana gracilis*, was found to consume a single species when tested against several common species in the Rhode River. Flatworms readily consumed the corophioid, *Apocorophium lacustre,* but prior to our study, consumption of amphipods in an estuarine or marine setting has only been reported once as anecdotal observations (Jennings, 1957). Our study is the first to present and quantify any ecological data for *E. gracilis*, including its natural distribution and predator-prey interactions. Polyclad flatworms are generally highly selective in their prey choice (Galleni et al., 1980 and references within) and it is therefore not surprising that *E. gracilis* was found to consume one species throughout the study. Both *E. gracilis* and *A. lacustre* are found throughout the eastern Atlantic coastline though *A. lacustre* is restricted to brackish waters (Bousfield, 1973) whereas *E. gracilis* has been found in salinities ranging from 0 – 37 (personal observation). We included in our laboratory trials a free-swimming amphipod (*Gammarus mucronatus)* though this species was found to easily avoid any encounters when flatworms were in search of food. It is possible that flatworms could be more general in prey choice though the escape behavior of non-tube building amphipods could separate them as prey. In contrast, *A. lacustre* tended to be a very poor swimmer and was easily captured. It is feasible that *E. gracilis* can consume other species, possibly other tube-building amphipods, within its range though this needs further study.

An extensive survey of the benthic habitat within the Rhode River showed a significant relationship between *E. gracilis* and *A. lacustre*. Flatworms were only found in samples that contained *A. lacustre* and increases in flatworms were concomitant with prey density. Both flatworm and amphipod densities were highest in nearshore habitats as compared to offshore though it is unknown why *A. lacustre* was more abundant along the coast as the majority of the river is soft, unsorted mud. Krause et al. (2003) developed an empirical food web model for the Chesapeake Bay which included several fish species (i.e. spot, catfish, and hogchoker) as the main predators of *A. lacustre* so increased predation may occur away from the shoreline. Sites with a forested shoreline also had the highest abundances though this type of shoreline made up the majority of the sampling sites and therefore could be an artifact of site selection.

Our study investigated some specific predator-prey interactions between *E. gracilis* and *A. lacustre*. Timed observations of feeding were relatively consistent with predation occurring within 15 min of adding prey and took roughly 65 min for consumption. Predator size was also a significant factor in consumption rates and as size increased, there was a steady positive increase in the number of amphipods a single individual could consume. Interestingly, the smallest flatworms used (0.8 – 2 mm^2) were found to easily capture and consume an average-sized amphipod, though with increased handling time.

Corophioid amphipods are a common tube-building group found throughout much of the world. Tubes are thought to have several uses including feeding, acting as a storage deposit for food, and facilitating mating efficiency by limiting search time (Shillaker and Moore, 1987; Borowsky, 1991; Dixon and Moore, 1997). The majority of studies have examined tubes in relation to feeding and it is generally thought that *Corophium* spp. are primarily filter feeders (Foster-Smith and Shillaker, 1977; Gerdol and Hughes, 1994), and use their tubes to filter water through to capture particles (Dixon and Moore, 1997). The tubes themselves have never been tested as a means of refuge from predators. Our results suggest that tubes provided very little protection from flatworms. Collectively in all 3 trials, approximately 80 % of amphipods were consumed with tubes as compared to 100 % without tubes. Although with or without tubes was not found to be statistically different, some amphipods did survive and therefore tubes could be somewhat useful. However, increasing the allotted time during the experiment could have increased the consumption rate to 100 %. Flatworms were attracted to occupied tubes and would actively examine the tube either attempting to enter or attacking the amphipod from the outside. In rare cases, amphipods would leave tubes in an attempt to crawl away though with little success. Throughout the trials, *E. gracilis* was also found to deposit eggs on the inside surface of tubes. The successful development and release of larvae was not followed but other species of polyclad flatworms are known to deposit eggs within shells of their prey after consumption (Hurley, 1976; Galleni et al., 1980; Lee et al., 2006).

Studies on polyclad flatworms have generally shown that they can have a strong negative effect on populations, particularly those with long generation times such as corals (Rawlinson et al., 2011; Rawlinson and Stella, 2012), barnacles (Hurley, 1975; Branscomb, 1976) and bivalves (Pearse and Wharton, 1938; Loosanoff, 1956; Littlewood and Marshe, 1990). Corophioids have short generation times (1 – 4 cohorts per year), with direct development leading to high local abundances (Fish and Mills 1979; Moore 1981; Peer et al., 1986; Cunha et al., 2000; Pérez et al., 2007). Several studies have examined the population dynamics of *Corophium volutator* (Pallas, 1766) and have shown negative effects of larger predators including shorebirds (Hicklin and Smith, 1984) and fish (McCurdy et al., 2005). Our results demonstrate that *E. gracilis* is a predator on *A. lacustre*, but it is unclear whether or not there is any top-down control on populations given their high recruitment throughout the year. Data from field collections did show that flatworms were positively correlated with amphipod abundances and only found in samples that contained amphipods, however, this correlation does suggest that flatworms do not limit populations of amphipods.

The ecological role of micro-predators within the marine environment is poorly understood particularly because of the challenges in constructing manipulative experiments. Despite this, these predators are typically thought of as abundant and important components within the habitats that they are found. Traditionally, micro-predators have been associated with newly settled or juvenile prey altering the composition of communities over succession (e.g. Osman et al., 1992). *Euplana gracilis* preys upon adult corophioids and is an example of a micro-predator that could potentially have a large effect on amphipod populations given their rapid rates of consumption as well as observed field densities found throughout the study site. Despite the fact that this species is fairly ubiquitous throughout the eastern Atlantic coastline, this is the first study to acknowledge its ecological importance and therefore, this is significant for future considerations of the trophic structure within the Chesapeake Bay as well as within the distributional range of *E. gracilis*.

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Figure 1. Map of the Rhode River. Star indicates the location of the Smithsonian Environmental Research Center where live collections were made for experimental trials. Grey shading indicates water and the river empties to the south-east into the mainstem of the Chesapeake Bay. Black circles indicate field sampling sites used for benthic ponar grabs (*n* = 151).

Figure 2. Distance-based linear model (DistLM) plot based on step-wise selection of environmental parameters fitted to abundances for *E. gracilis and A. lacustre* taken from benthic grabs. Vectors indicate the direction of effect for environmental parameters in the plot. Split-plot bubbles represent the number of individuals of each of the two species found at each site.

Figure 3. Average *A. lacustre* and *E. gracilis* abundances (± S.E.) per benthic grab (approximate area = 15.2 cm^2) from the different shoreline types. Offshore indicates all samples that were taken roughly 3m or more from the shoreline. Note the difference in scale on the left and right *y*-axis.

Figure 4. Linear regression for the relationship between amphipod (*x*-axis) and flatworm (*y*-axis) abundances per benthic grab (*n* = 151) collected during field sampling.

Figure 5. Linear regression for the relationship of *E. gracilis* (*n* = 45) size to the amount of *A. lacustre* consumed over 120 h.

Figure 1

Fig. 1

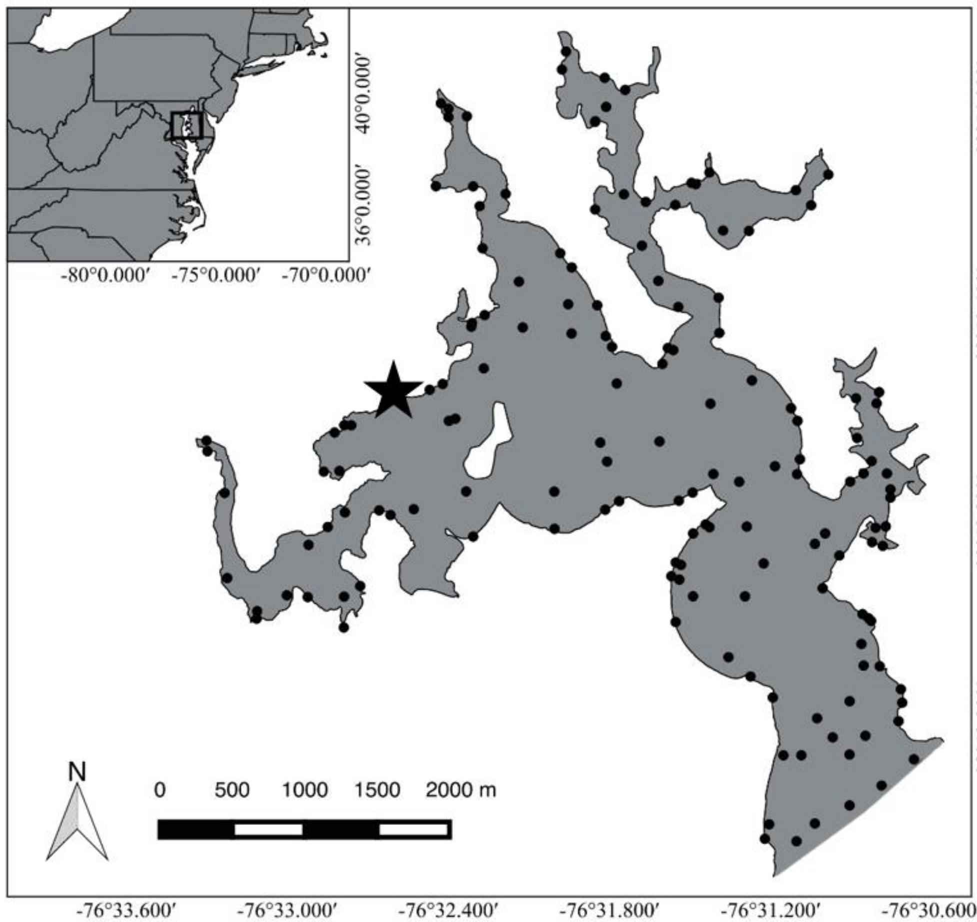

Figure 2

Fig 2.

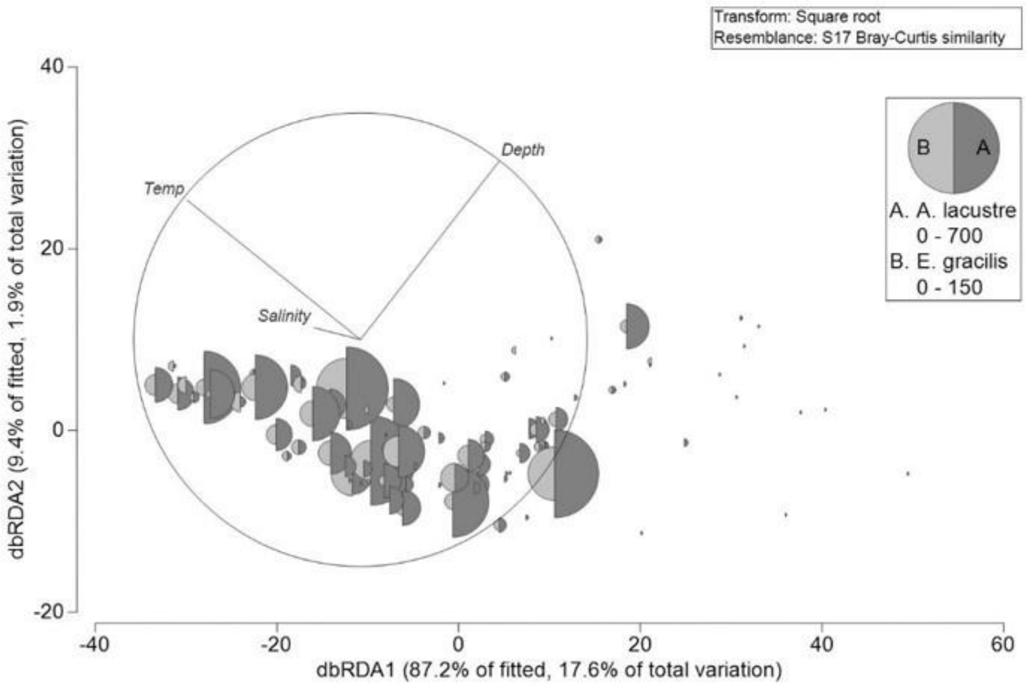

Figure 3

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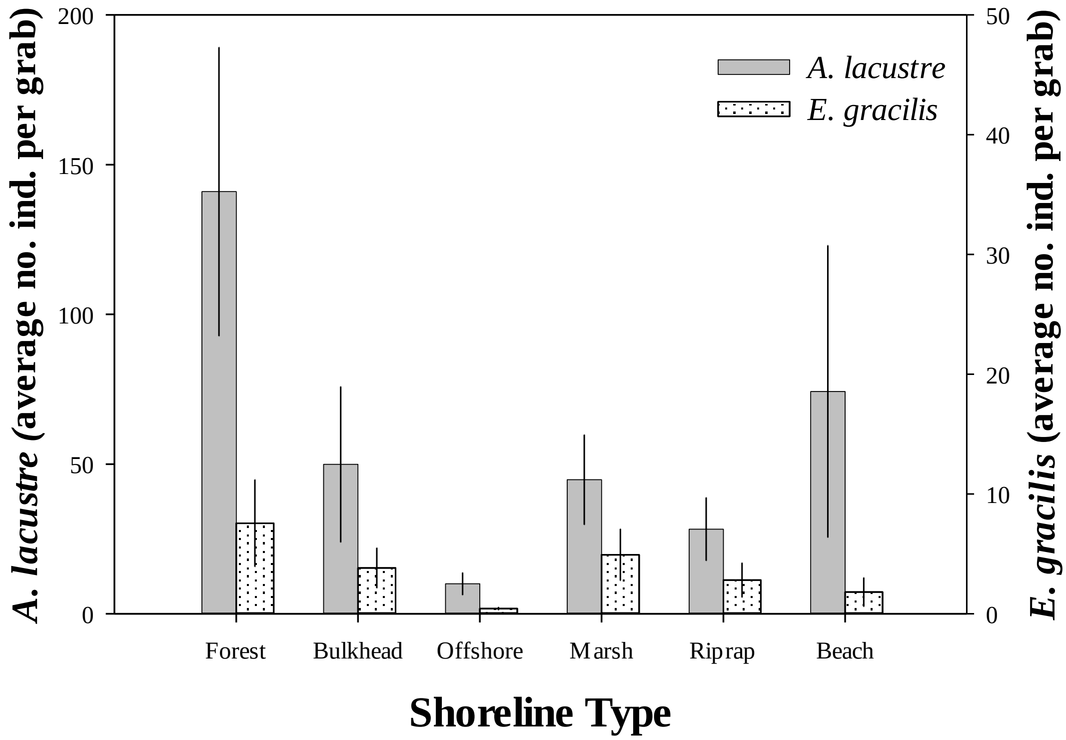

Figure 4

Fig. 4

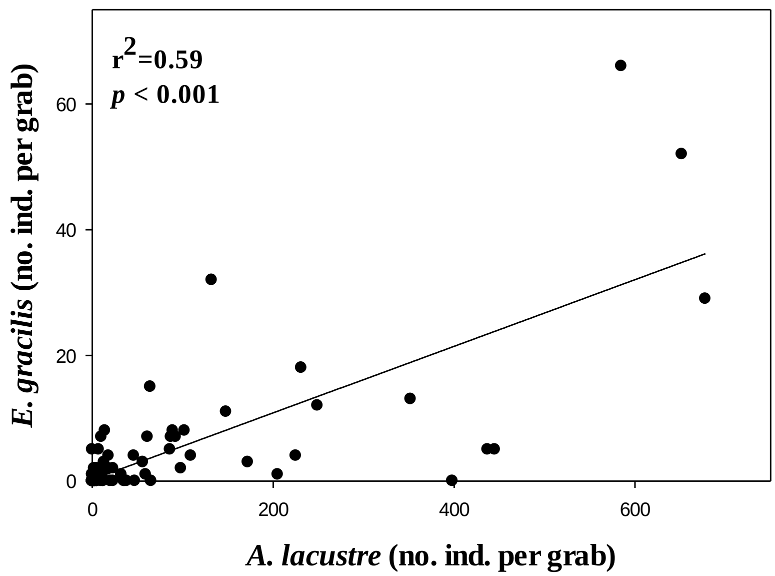

Figure 5

Fig. 5

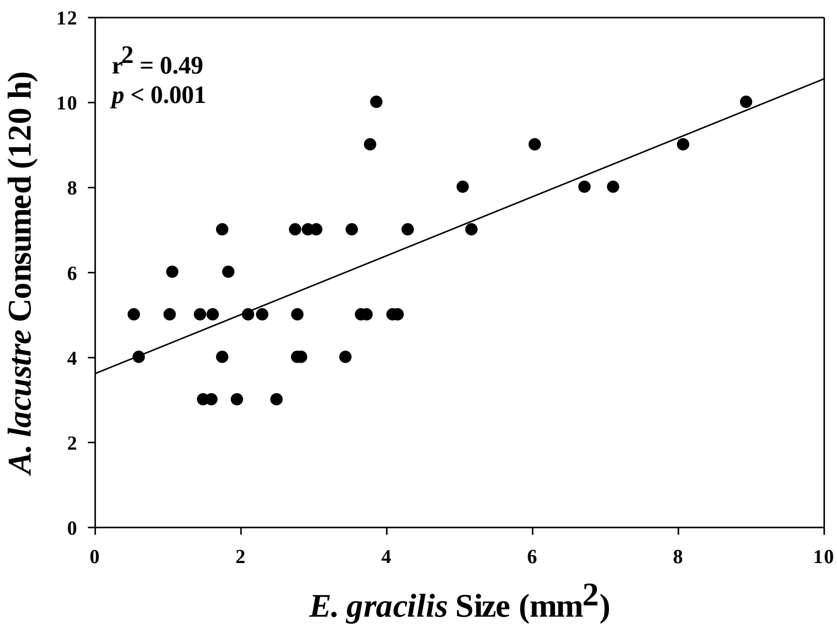

Table 1

Common species tested as potential prey of *E. gracilis.*

	Percent Alive	Fisher's exact test
Apocorophium lacustre	0	$p < 0.001$
Gammarus mucronatus	90	NS
Amphibalanus improvisus	80	NS
Polydora cornuta	90	NS
Cratena pilata	100	NS
Victorella pavida	80	NS
Alitta succinea	100	NS
Tanypus sp. larvae	100	NS

Fisher's exact test on survivorship ("dead" or "alive", $n = 10$). NS = not significant.