A DIET STUDY OF EUROPEAN GREEN CRABS (*CARCINUS MAENAS)* IN THE PLUM ISLAND ESTUARY

by

EMILY JESSEPH

ABSTRACT

European green crabs (*Carcinus maenas*) are an invasive species of crab native to the Atlantic coasts of Europe and North Africa. However, this species has been introduced to 6 out of the 7 continents and is known to disturb native ecosystems and food webs. *C. maenas* arrived to the Northeastern United States in the early 1800's, but the last diet study of *C. maenas* in this region was done in the 1950's. To determine the spatial variability of *C. maenas* diets across habitat types within the Plum Island Estuary, I used stomach content analysis as well as stable isotope analysis to determine assimilation of prey and trophic relationships. I found that the primary producers, such as *Spartina* and microalgae, had the highest presence in their stomach contents, with $\delta^{13}C$/ $\delta^{34}S$ values indicating assimilation of nutrients from small benthic invertebrates. A calculated trophic range of 1.5-2.5 is indicative of an omnivorous diet of *C. maenas*, as shown in stomach contents and stable isotope values.

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INTERNSHIP

Experience

This Master's research internship took place at the Plum Island Estuary LTER site from June 2025 to July 2025, with research continuing from Athens, GA, from January 2026 to March 2026 funded by Plum Island LTER under NSF under award #1238212. This project focused on studying the diets of European green crabs (*Carcinus maenas*) using stomach content analysis and stable isotope analysis. The main goal of stomach content analysis was to establish spatial variability and overall composition of diets. I participated in fieldwork from June 2025 to July 2025 in the Plum Island Estuary, where I collected green crabs from three habitat types: primary creek, main stem, and sound. At falling tide, green crabs were caught in these three habitats and taken back to the lab for stomach content analysis. I learned how to conduct stomach dissections on *C. maenas* and, to the best of my ability, identify the food fragments to the lowest taxonomic level possible.

A major caveat in using stomach content analysis is that it does not tell you what it assimilated in the tissues, but only what food items are present in their stomachs. The second half of my project involved using stable isotope analysis to gain a better understanding of which consumed items were actually assimilated into *C. maenas* tissues. I prepared samples that were collected in 2021 for $\delta^{13}C$/ $\delta^{15}N$ and $\delta^{34}S$ analysis. To analyze the isotope data, I used Bayesian mixing models in R version 4.5.1 to determine the source of material to green crab tissue.

This project provided me with the opportunity to gain more hands-on fieldwork experience, data management, and quantitative analysis skills. This research contributes to the PIE-LTER by investigating how *C. maenas* impacts local food webs and whether the newly introduced species, *M. pugnax,* is being preyed upon by *C. maenas*. Specifically, *M. pugnax* surveys have found that

the population is growing exponentially. My work contributes to understanding whether predation is a factor that might be limiting the growth of fiddler crab populations at the PIE-LTER (Plum Island Ecosystems Long Term Ecological Research). Additionally, recent work has focused on the decline of clam fisheries in Plum Island, and my results are able to inform on whether *C. maenas* predation has been contributing significantly to the observed decline.

Introduction

In 1817, the European green crab (*Carcinus maenas*) arrived at the Northeastern United States on ships traveling from Europe and North Africa (Yamada 2001). *C. maenas* is listed as one of the top 100 most harmful invasive species (Nielsen et al. 2025) and is present on every continent except Antarctica. They owe their success to their phenotypic plasticity (Young and Elliot 2020) and their euryhaline and eurythermal characteristics (Perkins et al. 1969), allowing them to tolerate a large range of salinities and temperatures. With a maximum carapace width of 9-10cm in adults and typical lifespans of 4-7 years (Young and Elliot 2020), *C. maenas* can establish a significant presence in local ecosystems. The presence of an invasive species, such as *C. maenas*, establishes competition with and among native species, allowing for alterations of food webs and energy flow in local systems. (Yamada 2001).

The most recent diet study on *C. maenas* in the Plum Island Estuary, Massachusetts, was conducted in 1954 (Ropes 1968), where the most consumed prey items were determined to be invertebrates, bivalves, and crustaceans. However, the Plum Island Estuary system has seen changes in both environmental conditions, such as increasing temperatures salinity, and changes in the species present (Nelson et al., submitted). By determining the preferred prey items of *C. maenas*, we can further understand how energy is transferred across habitats in the Plum Island Estuary and how local food webs are responding to these changes. Stomach content and stable isotope analysis are common methods for diet studies. One method alone may not be sufficient (Cordone et al. 2022) so by pairing these two analyses, we can determine what prey items are being ingested and what nutrients are being assimilated.

Species of Interest

Two species of interest were a focus during the course of this diet study: the Atlantic marsh fiddler crab (*Minuca pugnax*) and the Soft-shell clam (*Mya arenaria*). In 2014, the first Atlantic marsh fiddler crab was observed in the Plum Island Estuary, showing regional expansion northward from previous studies (Johnson 2014). As a range-expanding species, this species' niche changes as temperature increases, and it is introduced to local food webs. It is currently unknown if *M. pugnax* is preyed upon by *C. maenas*, but prior work has found *C. maenas* targets other crustaceans (Ropes 1968), suggesting that *M. pugnax* could be a potential food source.

Since the 1900's, *M. arenaria* populations have been declining and *C. maenas* is known to prey on *M. arenaria* (Tan and Beal 2015). Throughout the course of this study, abundance of *M. arenaria* was monitored to determine if the quantity present is indicative of large-scale predation.

Methods

Sample Collection

Green crabs were collected along the Plum Island Estuary in high, mid-, and low estuarine environments between June 2025 and July 2025. Sites were categorized based on habitat type from three geomorphologically different habitats: primary creek, main stem, and sound sites (Fig. 1). Green crabs would eat on the marsh platform during high tide and then, with potentially full stomachs, follow the falling tide down the center of the creeks where minnow traps were set to collect them. Minnow traps were deployed for approximately 45 minutes and used raw chicken as bait. A total of 271 crabs were sampled and crabs with a carapace width under 4cm and gravid females were excluded, as their frequency of capture was extremely low. A power analysis was performed to determine the minimum sample size required at each site to achieve 80% power.

Stomach Content Analysis

Stomach content analysis was conducted using a dissection scope. Stomach contents were identified to lowest possible taxonomic level, with unidentified species being placed in an unidentified category following the closest identification. Prey items were recorded on a presence/absence (0/1) basis and for each site, presence/absence of food items was averaged for all crabs sampled in that site. Sites were then organized by habitat type, where the average was taken between all sites so that each food item was represented by a single value.

A PERMANOVA was conducted to test overall diet composition differences among habitats using the vegan package in R version 4.5.1.

Stable Isotope Analysis

Green crabs used for stable isotope analysis were sampled in 2021 from three different primary creek sites, with five crabs coming from each: Sweeney, Clubhead, and West. Samples for stable isotopes were taken by grinding whole crabs into a fine powder using a mortar and pestle after being dried for 72h at 60°C. To test for carbonates, a subsample of each crab was rinsed with 5% HCl in a small petri dish and if bubbles formed, the entire sample would be decarbed by rinsing it twice with 5% HCl and drying it on a 47mm GFF filter using a vacuum pump. 0.8mg per sample was wrapped for C/N analysis in a 5 x 9 mm tin capsule and ~3.0-3.5mg per sample wrapped for S analysis in a 4 x 6 mm tin capsule. Sulfur samples were wrapped again in a 5 x 9 tin capsule which included Niobium(V) oxide as a combustion agent. Fifteen total specimens were sent to the Washington State University Stable Isotope Lab for analysis. Trophic discrimination factors used for analysis for producer and consumer sources were 0.5‰ and 1.5‰ for $\delta^{13}C$, 1.5‰ and 3.0‰ for $\delta^{15}N$, and 0.6‰ and 1.2‰ for $\delta^{34}S$.

The model was run with two different source compositions: basal and consumer. Isotope data was gathered from the PIE-LTER yearly isotope survey data (Deegan et al. 2021). Basal sources consisted of *Ulva* (sea lettuce), *S. alterniflora,* and zooplankton. *S. alterniflora* leaves were collected for macrophyte end-members and rinsed with distilled water before drying. Leaves were collected within the PIE system at multiple sites over a 9 yr. sampling period (Nelson et al. 2015). Zooplankton samples consisted of copepods which acted as a proxy for POM since bulk POM contains significant amounts of detritus. Samples were collected using light migration to separate zooplankton from phytoplankton and detritus, where zooplankton was hand-picked, filtered, and dried (Deegan and Garrett 1998). Consumer sources consisted of *M. pugnax, M. arenaria, H. diversicolor,* as well as *S. alterniflora* to reflect common prey items in Plum Island. Consumer samples were prepared by collecting 3-20 tissue samples per species, washing with deionized water, and drying at 50-60°C. Samples were then ground into a fine power with a Wig-L-Bug and processed at the Marine Biological Laboratory (Nelson et al. 2015).

Each source composition was run between two different trophic levels (1 and 2 for consumers, 2 and 3 for basal sources) as well as both $\delta^{13}C/\ \delta^{34}S$ and $\delta^{13}C/\delta^{15}N/\ \delta^{34}S$ to determine which model was the best fit. Bayesian mixing models were run in R using the rjags and MixSIAR packages to plot data on mixing spaces for visualization of source value and consumer values. Statistical output used the "very long" model in rjags to obtain diagnostics.

Trophic level of *C. maenas* was calculated using the following formula from Nelson et al. 2019:

$$TL = \frac{\delta^{15}N_{ind} - \sum(\delta^{15}N_{source} * mean\ \%\ cont_{source})}{\Delta\delta^{15}N} + 1$$

where $\Delta\delta^{15}N$ = 2.9 ± 0.5, $\delta^{15}N_{ind}$ is the nitrogen value of an individual consumer, $\delta^{15}N_{source}$ is the nitrogen value of each particular source of primary production, and % $cont_{source}$ is the proportion contribution of each source to the consumer diet.

Results

Results from the PERMANOVA show no difference among habitat types (p=0.6312). An R^2 value of 0.071 shows that habitat explains 7.1% of variation in site-level stomach-composition data. Pairwise PERMANOVAS also showed no difference between main stem and creek sites (p=0.9513), main stem and sound sites (p=0.8231), and primary creek and sound sites (p=0.5814). The NMDS plot (Fig. 3) shows tight clusters and overlapping ellipses between habitat type, indicating so significant difference between habitat types. Ordination yielded a low stress value of 0.096, suggesting the two-dimensional configuration provides a reliable representation of sample dissimilarities.

The model results using $\delta^{13}C/\delta^{34}S$ data for basal resources (zooplankton, benthic microalgae, *Spartina*) with *C. maenas* show ~32% contribution from *Spartina*, ~45% contribution from *Ulva*, and ~24% from POM (Fig. 3). For consumer resources (*M. pugnax, M. arenaria, H. diversicolor*, as well as *S. alterniflora)*, model results show ~23% contribution from *H. diversicolor*, ~34% contribution from *M. pugnax*, ~19% from *M. arenaria*, and ~24% from *S. alterniflora* (Fig. 4). The Geweke diagnostic showed that none of the three chains fell below the ±1.96 z-score threshold for basal resources, which is indicative of a stable distribution. For consumers, six

chains fell below the z-score threshold. The Gelman-Rubin diagnostic for both basal and consumer sources indicate the chains converged to the same posterior distribution, with all 19 variables <1.05, suggesting that the chains converged successfully, and the length of the model was appropriate. Trophic level range was calculated to be around 1.5 to 2.5, spanning one trophic level. The $\delta^{13}C/\delta^{34}S$ (Fig. 4) mixing space shows a diet similar to those of other benthic invertebrates, with the lower $\delta^{34}S$ indicative of smaller, benthic invertebrate influence. Similarly, $\delta^{15}N$ values extend over multiple trophic levels, with *C. maenas* looking closely like small benthic invertebrates (Fig. 5).

Discussion

Stomach content analysis showed the most popular food item to be primary producers, such as *S, alterniflora* and *Ulva* (Fig. 2). Stable isotope analysis revealed from the consumer model that ~24% contribution came from *S. alterniflora*, with the other 76% coming from consumers, showing a mixed diet of primary producers and consumers. Stomach content analysis introduces bias towards taxonomic groups (Cordone et al. 2022), so pairing this analysis with stable isotope analysis provides additional information on what is being assimilated, as not all ingested nutrients are taken up into the body. This is due to many reasons, such as an inability to break down and digest certain food items. Most commonly, plant items such as *Spartina* contain cellulose, which is difficult to digest and take up into tissues. The stable isotope data indicated that green crab tissues look like that of small invertebrates, with *Ulva* contributing the most out of the three basal resources. Since the stable isotope data is sourced from a different year than the stomach content data, it is not fully representative of green crab diets in the summer of 2025 and with whole green crabs being ground during the preparation process, results may incorporate

error associate with differing tissue-specific turnover rates. Future studies would benefit by conducting stomach content analysis and stable isotope analysis within the same year to get better representation of the current population. A calculated trophic level range of 1.5 to 2.5 indicates that *C. maenas* are not strict herbivores, instead they have a mixed diet containing nutrients from primary producers and small primary consumers.

While fragments similar to those of *M. pugnax* were identified in low abundance in each habitat type, as a new species in this system, it would be beneficial to conduct further stomach studies as *M. pugnax* population numbers increase to determine with more certainty if they are being preyed upon by *C. maenas*. *M. arenaria* fragments had site-specific results, with Carolton Creek (primary creek) and Rowley River (main stem) showing the largest presence in *C. maenas* stomach contents. However, with few crabs showing a large abundance in their stomachs, it is not possible to claim that *C. maenas* are the sole contributor to *M. arenaria* population declines in Plum Island.

Conclusion

This study established broad-scale diets of *C. maenas* in the Plum Island Estuary. *C. maenas* diets show little spatial variability between the three different habitat types: primary creek, main stem, and sound. Observations from stomach content analysis revealed the most abundant food items to be algae and plant matter, followed by bivalves and clam worms, which is in line with past literature (Ropes 1968). The stable isotope analysis showed relatively even contribution from our three basal sources, with a calculated trophic level range between 1.5 and 2.5, indicating an omnivorous diet. Future studies should continue to monitor the diets of *C. maenas* to determine if *M. pugnax* shows a larger presence due to their increasing population.

Figures



Fig. 1 Map of sites in the Plum Island Estuary sorted by habitat type: primary creek (green), main stem (red), and sound (blue). Samples were taken using a minnow trap with raw chicken as bait.

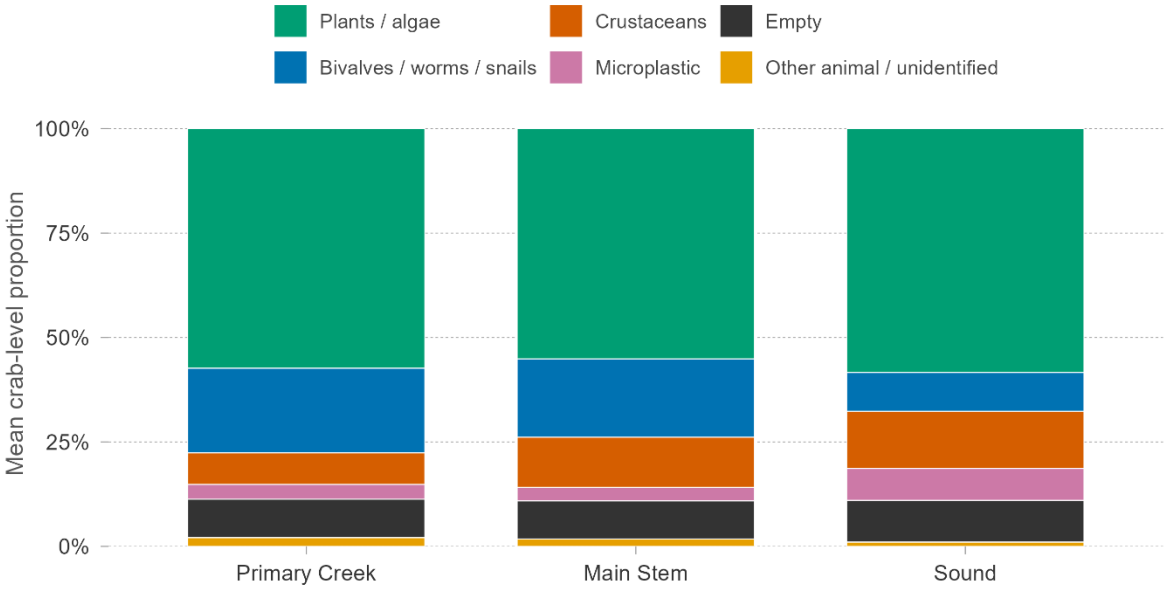


Fig. 2 Mean stomach composition of primary creek, main stem, and sound sites. Bars reoresent mean crab-level proportion of food items and values are normalized to sum to 1 in each habitat type.

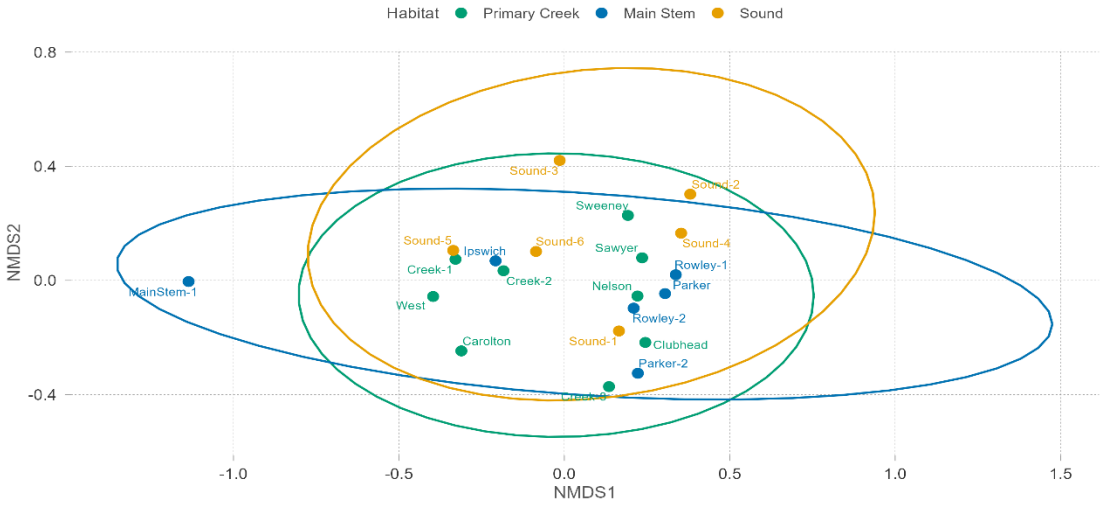


Fig. 3 NMDS for primary creek (green), main stem (blue), and sound (yellow) sites. Each dot represents a site. Tight clusters of sites and overlapping ellipses indicate no variability in diet between the three habitat types.

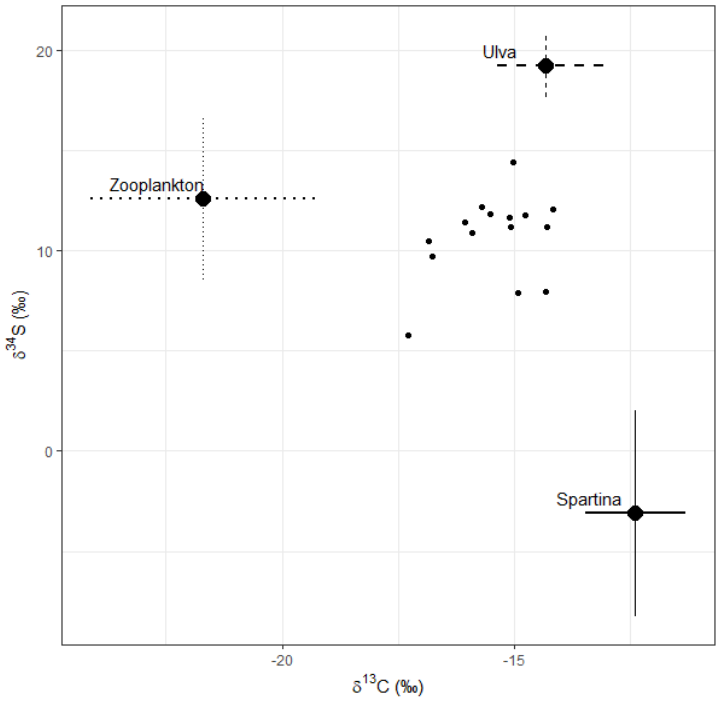


Fig. 3 $\delta^{13}C/\delta^{34}S$ mixing space of three basal resources: *Spartina*, *Ulva*, and Zooplankton as a proxy for POM. The black dots represent isotope values of *C. maenas*.

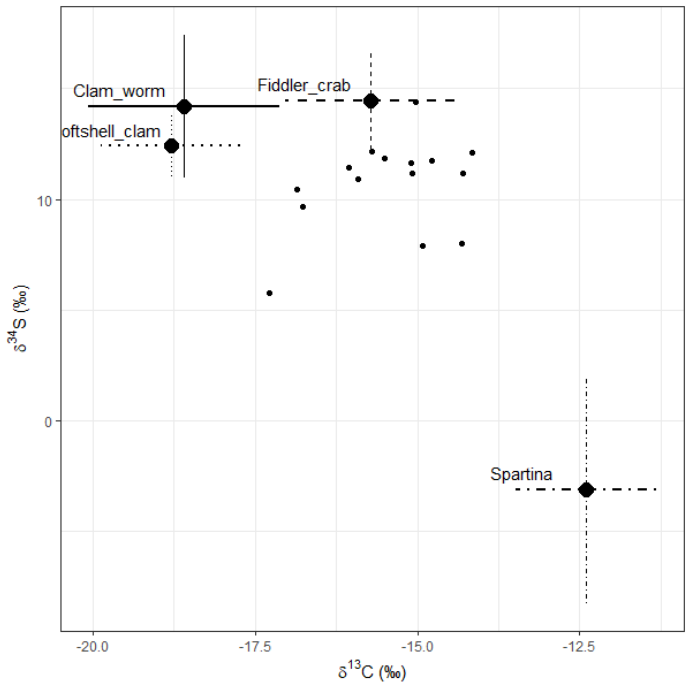


Fig. 4 $\delta^{13}C/\delta^{34}S$ mixing space of three consumer sources: *M. pugnax*, *M. arenaria*, *H. diversicolor*, as well as *S. alterniflora.* The block dots represent isotope values of *C. maenas.*

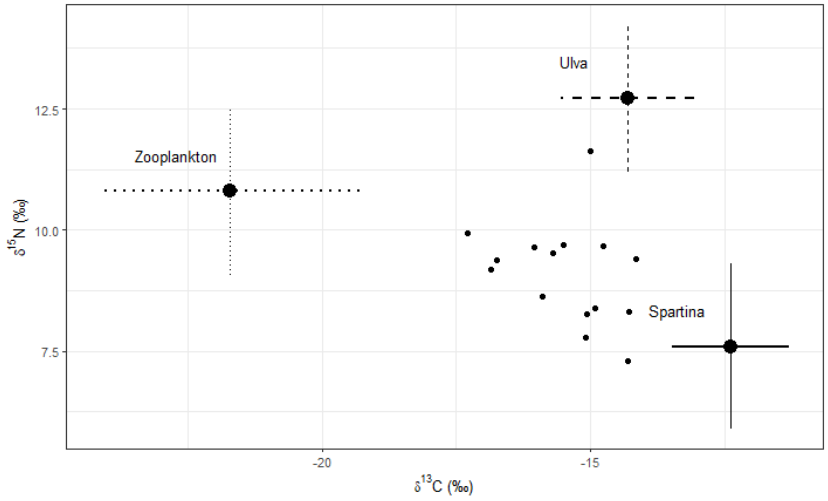


Fig. 5 $\delta^{13}C/\delta^{15}N$ mixing space of three basal sources: *Spartina*, *Ulva*, and Zooplankton as a proxy for POM and the black dots represent isotope values of *C. maenas*. Spread of green crab values along the y-axis represent the spread across a trophic level.

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