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Trophic Shifts Introduced To The Saco River Estuary By A Central Secondary Consumer, The Invasive European Green Crab (Carcinus Maenas)

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Trophic shifts introduced to the Saco River Estuary by a central secondary consumer, the invasive European green crab (*Carcinus maenas*)

BY

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Introduction

The European green crab (*Carcinus maenus*) is one of the most notorious invasive species found along the East coast of the United States. Green crabs were introduced to the US in the 1800s when they supposedly rode in ballast water across the Atlantic Ocean. Green crabs are native to the Atlantic coasts of Europe and Northern Africa. Once established on the Atlantic coast of North America, they started to migrate mostly north, and will continue to do so with warming waters due to climate change. (Glude 1955). Warmer winters will lead to greater crab egg survival, increasing the green crabs' reproductive success. Green crabs have become one of the main and most dangerous predators of bivalve mollusks on the East coast and have become a major problem for soft shell clam harvesters. Not only are green crabs decimating soft shell clam flats, they also have greatly reduced populations of blue mussels (*Mytulis edulis)*, and recent work in Newfoundland has shown that they are also starting to target shallow water scallop populations (Matheson and Mckenzie, 2014). Larger crabs also tend to choose soft shell clams and mussels over scallops, but crabs of all sizes will eat scallops, if presented to them. Green crabs range from 6 to 10 cm, but some are larger (Perry, 2017). Green crab populations are able to access to a wide variety of types and sizes of prey ranging from bivalves to native crabs, due to their scavenger lifestyle. While many of these consequences of the invasive are regularly observed by fishermen, beachcomber, and researchers, not much is known about the true quantifiable and qualitative results of these crabs. This thesis aims to gain an understanding of the fallouts of the green crab invasion by analyzing the trophic interactions in which the green crab acts as a predator and in which it is the prey.

The first chapter found in this thesis is the paper titled *Stable Isotope Signatures Reflected in Habitat Affinities: Saltwater, Estuarine, and Freshwater Fish in Saco Bay*. Utilizing stable isotope analysis of muscle samples from marine species found in Saco Bay, a biplot was created in which these marine species were characterized by their $\delta^{13}C$ and $\delta^{15}N$ values. Some of the species included in these samples were striped bass (*Morone saxatilis*), sand eels (*Ammodyes americanus*), green crabs, and Atlantic herring (*Clupea harengus*). Studying species like this is incredibly important due to their high recreational and commercial values. The results from this analytical paper allowed for conclusions to be made about the trophic level of organisms and the geographic location of where that organism was caught, simply based on their $\delta^{13}C$ and $\delta^{15}N$ signatures. Where each organism was captured was reflected by the $\delta^{13}C$ value on the graph, supporting that geographic location, even if it is a within a small area, can be determined using carbon stable isotope analysis. Green crabs were the only species on the list of those sampled that are labelled as invasive and based on the biplot, green crabs seemed to have an impact on other species interactions with each other. Discerning the top-down and bottom up effects of the green crab presence on the Atlantic coast of North America is significant in protecting native resources and coastal livelihoods.

The soft shell clam (*Mya arenaria*), has experienced dramatic population shifts with the introduction of green crabs and is currently being studied to determine the true impacts of this invasive predations. The crabs' direct effects on economically important species like the soft shell clam are felt by the clammers who rely on soft shell clams as a portion of their annual income. Reduced juvenile soft shell recruitment and reduced seeded clam survivorship are two of the most observed changes in the clam flat communities in recent times. Clammers have started to use landscape mesh to cover their juvenile clams in order to increase the clams' survivorship and the profit margins for the clammers. With soft shell clams holding 4% of the seafood market value in Maine, it is critical that the true impacts of predation are understood in order to try an reduce the effects and increase both the clammers' yields and also help to maintain this incredibly important ecosystem found within the mudflats. It is estimated that commercial fisheries have experienced upwards of $44 million in losses from green crabs (Perry, 2011). Chapter 2 titled, *The relative impacts of both native predators and the invasive European green crab (Carcinus maenas) on seeded soft shell clams (Mya arenaria) in Southern Maine tidal mudflats,* aimed to quantify the predation of green crabs on soft shell clams by utilizing various predator exclusion methods, one of which was the landscape mesh that clammers already use. Green crabs are not only an efficient and deadly predator on the mudflats, but they also pose another ecological role: as a food source.

Striped bass are well known as having an extremely varied diet throughout each migratory season. Squid, mackerel, lobsters, and herring are just a few of the notable prey items of the striped bass, but their diet changes as they travel south to north and north to south during the spring and fall (Nelson et al, 2003). Green crabs happen to live within the same range that the striped bass do and are regularly found in the mouths and stomachs of striped bass of all sizes. Striped bass constantly move in search of bait, scouring boulder field, sandflats, and river mouths, among other locations along the Atlantic Coast of North America (Walbaum, 1792). Green crabs also habit the same areas that striped bass do, presenting themselves as a potential food source for striped bass. While many of the striped bass' preferred food sources (i.e. baitfish) are constantly travelling to find food and avoid predators, green crabs tend to congregate in the same areas, as they are weak swimmers and do not migrate any more than within the intertidal zone (Green Crab, Washington). Striped bass find themselves in the presence of green crabs on a near day to day basis, due to their high abundance. As striped bass are opportunistic feeders, green crabs pose a potential to be a supplement to the striped bass's diet, especially when other food sources are scarce. Chapter 3, *Trophic shifts in the Saco River Estuary that occur with the arrival and summer residence of the striped bass (Morone saxatilis),* aimed to determine the importance of green crabs in the striped bass diet, and compare its stable isotope signatures with signatures from other prey items of the striped bass.

Invasive species are of utmost concern when it comes to environmental protection as they can dominate important native species and cause major damage to essential ecological processes, like the cycling of nutrients. Green crabs have been established on the Atlantic coast of North America for 200 years now, and the progressive effects of these invaders on native populations is being observed and analyzed in the field and in the lab. This thesis aims to evaluate the top-down and bottom-up effects caused by the green crab in the Saco River Estuary.

Site Maps

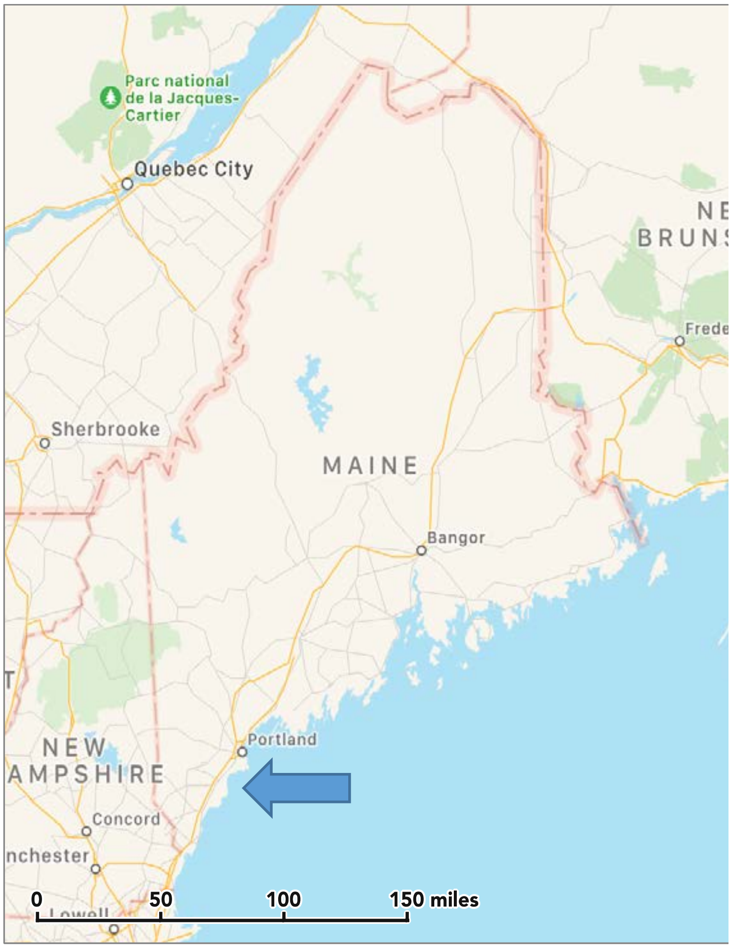

Figure 1: Site location in Maine. Located in Biddeford and Biddeford Pool, 30 minutes south of Portland, Maine.

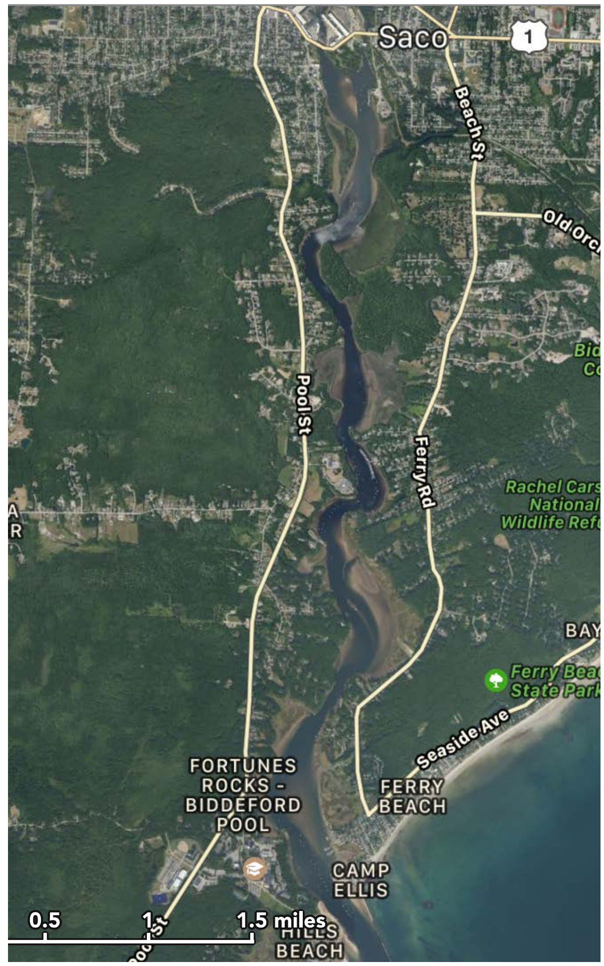

Figure 2: Location of focus #1, the stretch of river between the first dam on the Saco River (Cataract) and the mouth, leading into Saco Bay.

Figure 3: Map of Biddeford Pool. Biddeford Pool is just south of the mouth of the Saco River.

Chapter 1:
Stable Isotope Signatures Reflected in Habitat Affinities: Saltwater, Estuarine, and Freshwater Fish in Saco Bay

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Abstract

Understanding how trophic interactions occur within an ecosystem and being able to map a food web, assists ecologists in understanding the effects humans have on important ecosystems. This benefits our ability to harvest resources from these ecosystems sustainably by giving us an insight into the interconnectedness of these ecosystems. Stable isotope analysis (SIA) is a method used to map trophic interactions by plotting the δ15 Nitrogen (representative of trophic level) by δ^{13} Carbon (representative of primary producer type) on a biplot. SIA was used on samples taken from the Saco River Estuary in order to determine whether or not the primary producer (δ^{13} Carbon) type changed between organisms that were categorized as either freshwater, estuarine, or saltwater. I hypothesize that there will be significant differences between the signatures, indicating that environmental preferences of certain organisms can be determined by analyzing the δ^{13}C signatures of that organism and identifying that there are significant differences between the carbon values of organisms found in freshwater, estuarine, and saltwater ecosystems. δ^{13}C signatures in the consumer reflect the actual δ^{13}C value of the primary producer they are eating or their prey has eaten. ANOVA and Tukey Post Hoc analysis supported my hypotheses by indicating that there were significant differences between the δ^{13}C values of these three groups.

Introduction

Saco Bay is a highly active area with some clamming, commercial fishing, and recreational fishing occurring, among many other human activities. The Saco River is a popular area for recreational and commercial fisherman. Many species migrate to the Saco River during the summer, like striped bass (*Morone saxatilis*), river herring (*Alosa pseudoharengus)*, and sand eels (*Ammodytes americanus*), among many other important species (Furey, 2011). Federally protected species like Atlantic and Shortnose Sturgeon (*Acipenser oxyrinchus* and *Acipenser brevirostrum*) congregate in the estuary as well, most likely to feed on the dense populations of sand eels (Novak et al., 2017) found in the river. The river concentrates these species as it contains the necessary nutrients to support such a high concentration of large predators. The area this project focused on stretches from the first dam in the Saco River to the outside of the mouth of the river, about a mile away from the dam. The interactions between recreational fished species like striped bass, federally protected species like sturgeon, and the many baitfish species in the Gulf of Maine are incredibly important in maintaining a healthy ecosystem. These interactions help to maintain the population sizes of each species at a sustainable level (via interspecific competition) and help to transfer nutrients up and back down the food webs, among other important processes. It is very important for humans to understand these interactions in order to help protect the ecosystem and use the resources located in the Saco River Estuary responsibly and sustainably. One method that is used to gain an understanding of trophic interactions is stable isotope analysis.

Stable isotope analysis (SIA) is a method used to determine various ecological factors, mostly trophic level of an organism and the primary production source of a food chain. Stable isotopes are cycled through the food web via the natural processes of the ecosystem (i.e. consumption, decompositions, etc). The two most commonly analyzed stable isotopes used in food web ecology are carbon and nitrogen, as they are good indicators of the source of primary production in a food web (carbon) and the trophic level of the organisms being analyzed (nitrogen). There are heavy and light isotopes of both nitrogen and carbon circulating through an ecosystem. The ratios of these heavy and light isotopes in organismal tissue are analyzed using a mass spectrometer and elemental analyzer to determine the trophic level (δ15 Nitrogen) and carbon source (δ^{13} Carbon) (Fry, 2006). A higher trophic level organism will have a larger δ15N value than one with a lower trophic level. δ15N accumulates in higher trophic levels, signaling whether or not an organism is a primary producer, consumer, etc. Nitrogen accumulates in higher trophic levels because each single organism in a higher trophic level contains proportionally more nitrogen than a single organism in the level below it. δ^{13}C is used to track the carbon source (base of the food chain) in an ecosystem and can even be used to determine if it is a marine or terrestrial derived or a C3/C4 plant. This is done by comparing your values to previously known value ranges for these types of plants. Analyzing carbon values is extremely important in an estuarine setting because the results can be used to determine which types of primary producers are being consumed in the "in between state" that an estuarine system is, in terms of being between a saltwater and a freshwater ecosystem. Stable isotope ecology plays an essential role in helping us understand the trophic interactions occurring in ecosystems across the globe.

The Saco River estuary is a location that is very significant to many species like the striped bass, sturgeon, and herring. Utilizing SIA, I set out to help characterize the trophic interactions occurring within the Saco River Estuary and to determine whether or not δ^{13}C signatures differed between freshwater, estuarine, and saltwater species within a one mile stretch of the Saco River. I hypothesize that there will be significant differences between the signatures, indicating that environmental preferences of certain organisms can be determined by analyzing the δ^{13}C signatures of that organism and identifying that there are significant differences between the carbon values of organisms found in freshwater, estuarine, and saltwater ecosystems.

Methods

Study Site: The area that was chosen to study was the stretch of the Saco River from the mouth to the Cataract Dam (about a mile up river from the mouth). The mouth of the river is composed of a sandy bottom and, depending on the tide, can have strong currents rushing out. During the summers, this stretch of river houses many species of anadromous fish, including Atlantic and Shortnose sturgeon and striped bass, that congregate here to feed on baitfish like sand eels and river herring.

Methods: Samples were attained using gill nets and were then frozen until further processing. Labelled bags of frozen samples were thawed out and the wet weights and lengths of each individual fish were recorded. Using the fishbase.org, each organism was listed as freshwater, estuarine, or saltwater, based on the very detailed life histories found on the database. Each individual fish was gutted and then a muscle sample was taken from the fillet on either side of each organism. These muscle samples were placed right into a drying oven.

Once dried, each sample was crushed in a mortar and pestle and then placed in a labelled Eppendorf tube. These Eppendorf tubes were sent off to an external lab (Colorado Plateau Stable Isotope Facility) to be encapsulated and analyzed for δ^{13}C and δ^{15}N. The samples were packaged in 1 microgram tin balls and run through a mass spectrometer and an elemental analyzer. An ANOVA and Tukey Post Hoc HSD tests were then used to determine significant differences between the three groups established (freshwater, estuarine, and saltwater).

Results

Using, ANOVA and Tukey Post Hoc HSD tests, I was able to determine that there are in fact significant differences in the carbon sources of freshwater, estuarine, and saltwater organisms in the Saco River (*Fig 1*). The $\delta^{13}C$ range for saltwater organisms was the narrowest (*Fig 1*), while freshwater and estuarine organisms had very wide ranges of $\delta^{13}C$ value (*Fig 2 and 3*). Overall, the $\delta^{15}N$ values had a narrow range and were mostly high values across all groups. Striped bass (*Morone saxatilis*) had the highest nitrogen value out of all the samples collected ($\delta^{15}N$=14.34). Some of the lowest observed $\delta^{15}N$ signatures in this project came from the various baitfish that the striped bass feeds on (i.e. *Clupea harengus* and *Alosa aestivalis*) ($\delta^{15}N$=9.69 and 9.5 respectively). Using ANOVA and Tukey Post-Hoc analysis, significant differences were confirmed between the $\delta^{13}C$ signatures of organisms classed as freshwater, estuarine, or saltwater, which helped to support my original hypothesis that $\delta^{13}C$ signatures would be significantly different between freshwater, estuarine, and saltwater organisms.

Discussion

Changes in $\delta^{13}C$ signatures represent a different primary producer at the base of the food chain (Zanden et al, 1999). The significant differences in $\delta^{13}C$ signatures between the groups helps to express the gradient of primary producers that make up the base of the food web in the Saco River Estuary. $\delta^{13}C$ signatures are reflected in different ratios depending on the photosynthetic reactions being used. CAM, C3, C4, and marine photosynthetic organisms usually fall within certain ranges on the biplot; C3 plants ranging from -24 to -34 ‰ and C4 plants ranging from -6 to -19 ‰ (Kendall, 2018). CAM plants tend to fall between these two ranges. All the freshwater, estuarine, and saltwater organisms collected for this project were caught in the same 1 mile stretch of river. There is some evidence that the environmental salinity also influences the $\delta^{13}C$ signature of organisms over time as the salinity varies (Groenigen J-WV, 2002). This helps to explain these significant differences between the three distinct environmental groups. Considering that some of the species collected travel hundreds of miles every year to feed and spawn, it was rather interesting to see that even in a short section of river, there are significant differences in the carbon signatures of those three groups. Niche partitioning within this small section of an ecosystem is also reflected in the data; each group (freshwater, estuarine, and saltwater) is utilizing different organic matter in a small area.

The narrow $\delta^{13}C$ range for the saltwater organisms was most likely due to the very large number of organisms sampled compared to freshwater and estuarine. The large number of samples helped to reduce the standard errors of $\delta^{13}C$ associated with a small sample number. The limited number of estuarine and freshwater organisms sampled contributed to the wide standard errors of $\delta^{13}C$ values for some of the points within those two groups. Wide $\delta^{13}C$ values could mean either/both that the range of resources used by those organisms contains many different food items, or there were not enough samples to limit the standard error (Rader et al, 2017).

The narrow range and high values of $\delta^{15}N$ is a reflection of the fact that most, if not all the organisms sampled were primary and secondary consumers in the Saco River. There were no primary producers sampled for this experiment. As was explained before, nitrogen signatures become stronger in higher trophic levels, so the consumers, like striped bass, expressing higher nitrogen values was expected. The highest of these values came from the striped bass muscle tissue. Striped bass are piscivorous and eat a wide variety of bait, making them an intermediate consumer, somewhere between primary and secondary consumer (Walbaum, 1792).

This project would benefit from more samples of freshwater species located at the base of the dam to further represent the food web the striped bass is incorporated in. These results help to characterize the isotopic niches of the freshwater, estuarine, and saltwater organisms living in the Saco River Estuary. Utilizing the outcomes from this project and ecosystem modelling, biomass shifts, and trophic shifts can be analyzed and understood in order to minimize the negative impacts. It will also assist in determining sustainable harvest limits in order to maintain the integrity of this very important ecosystem in Southern Maine. One of the speculations that can be made are that there are overlapping food webs within this small section of river. Further sampling for this project and different predator-prey interaction analytics will be applied in the future to answer more specific questions about these interactions.

Citations

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Tables and Figures

source	sum of squares SS	degrees of freedom νν	mean square MS	F statistic	p-value
treatment	504.54	2	252.27	45.19	1.11E-16
error	982.48	176	5.58		
total	1,487.02	178			

Table 1: This table shows the ANOVA output that was used when performing the Post Hoc analysis. The P-value indicated that there were significant differences within the data set, but the Post Hoc was used to determine where those differences were.

treatments Pair ($\delta^{13}C$)	Scheffé TT-statistic	Scheffé p-value	Scheffé inferfence
SW vs EST	5.3312	1.90E-06	** p<0.01
SW vs FW	8.5532	5.20E-14	** p<0.01
EST vs FW	5.4203	1.26E-06	** p<0.01

Table 2: This table expresses the results from the Scheffe Post Hoc tests. The interference values between each group tells us that there were significant differences between the $\delta^{13}C$ values of all three groups

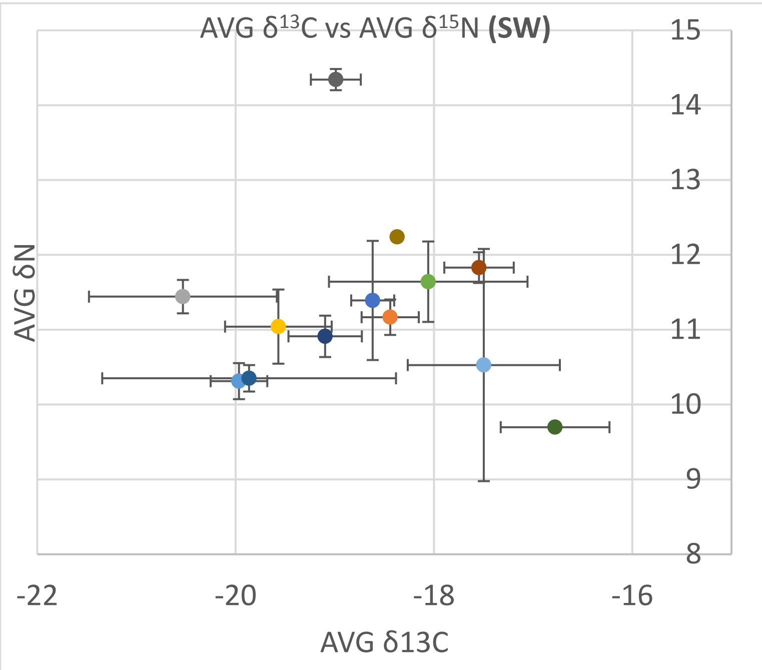

Fig 1: This is a plot comparing the $\delta^{15}N$ values to the $\delta^{13}C$ values of saltwater organisms in Saco Bay Estuary. Error bars represent standard error.

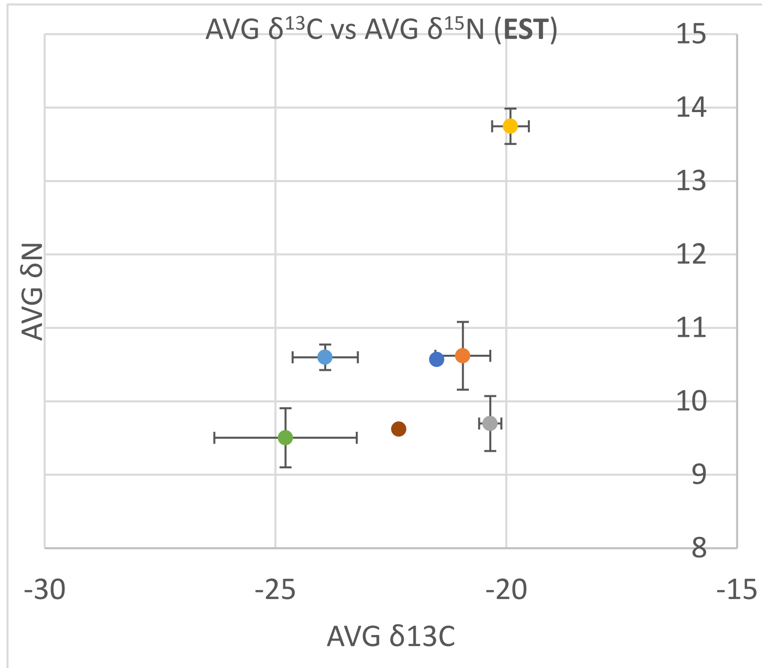

Fig 2: This is a plot comparing the $\delta^{15}N$ values to the $\delta^{13}C$ values of estuarine organisms in Saco Bay Estuary. Error bars represent standard error.

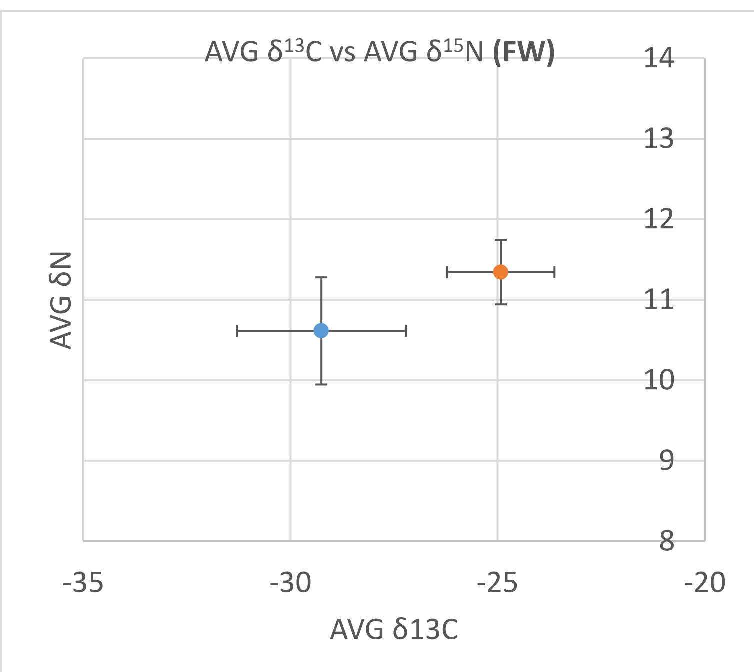

Fig 3: This is a plot comparing the $\delta^{15}N$ values to the $\delta^{13}C$ values of freshwater organisms in Saco Bay Estuary. Error bars represent standard error.

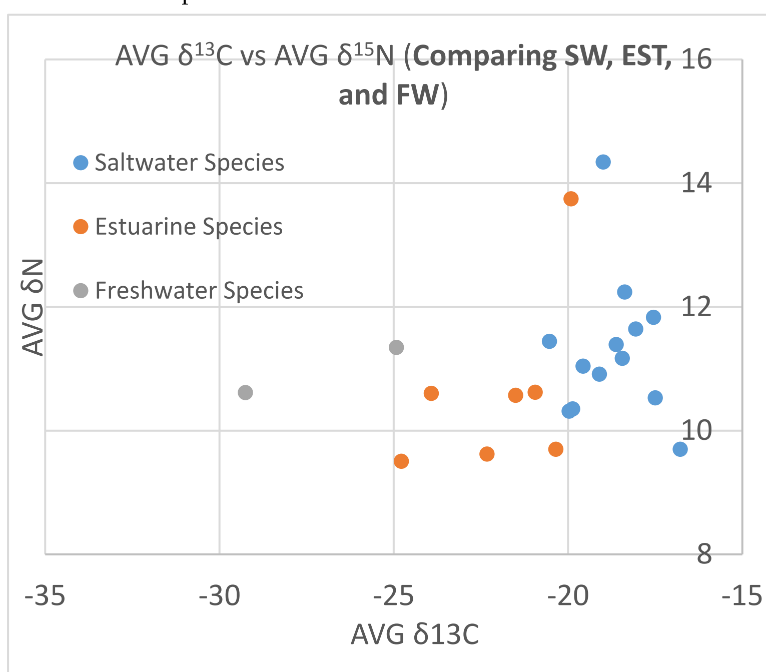

Fig 4: This plot compares the $\delta^{13}C$ and $\delta^{15}N$ of all three groups (saltwater, estuarine, freshwater). Error bars represent standard error.

Chapter 2:

The relative impacts of both native predators and the invasive European green crab (*Carcinus maenas)* on seeded soft shell clams (*Mya arenaria)* in Southern Maine tidal mudflats

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Abstract

Soft shell clams (*Mya arenaria*) hold 4% ($24,660,884.72) of the seafood market value in Maine, or 3% (8,294,244.[15] lbs) of the total poundage of seafood taken annually. In Maine, annual soft shell harvests have declined over 40 years by $30 million. At the same time, populations of invasive green crab (*Carcinus maenas*) have been increasing and are known predators of soft shelled clams, among many other important species. Recent work in eastern Maine demonstrates high predation rates of unprotected clams on mudflats by green crabs. However, little is known about the relative impacts of these invasive species compared to other predators in southern Maine mudflats. A field experiment was conducted to quantify survivorship of clams in the presence and absence of predators using different treatments: netting, flashing, and a control. For each of these three treatments, four plots of five replicate tubes (10 cm PVC) were arranged in Biddeford Pool mudflat, Biddeford, Maine. Netting, a typical method of protecting clams from crabs for commercial harvest, may also deter other predators, such as birds or fish. Aluminum flashing was used to create a 0.5 meter wall around four plots thereby excluding crabs but still allowing access to flying, burrowing, and swimming predators. Results show that netting was more effective in promoting clam survivorship than flashing with 84% clam survivorship compared to 63%, respectively. Interestingly, both exclusion methods (<1%) were equally effective compared to the open control plots (25%) in deterring green crabs, indicated by evidence of crushed shells, suggesting that clam populations are impacted by a diverse suite of predators.

Introduction

The soft shell clam (*Mya arenaria*) is a major aquaculture product produced and harvested in Maine. Biddeford Pool has a population of soft shell clams that is well monitored and harvested by local clammers. A major threat to these clams along the east coast is the invasive European green crab (*Carcinus maenus*). Green crabs are already high in numbers and increasing every day, due to their high rate of reproduction. These crabs are such a problem because they kill and eat many shellfish, including the soft shell clams, especially the juveniles. As the number of green crabs increases on the East coast, the threat to soft shell clams also increases as a result of the green crabs' high predation rates upon the soft shell clams.

Soft shell clams (*Mya arenaria*) are found in mudflats along the northeastern coast of the United States. These clams are very important to the shellfish industry in Maine and support a major portion of commercial clammers' income every year. These clams make up 4% ($24,660,884.72) of the seafood market value in Maine and 3% (8,294,244.15 lbs) of the total pounds of live seafood harvested in Maine (Watts 2016). Soft shell clams bring the state around

$21 million every year, but prices fluctuate as clam population increases and decreases (Alden 2001). Soft shell clams range in an adult size of about 3 to 4 inches and have a white, chalky shell that is "soft", meaning it can be crushed rather easily. The shell does not actually seal the clam all the way as the clam has a large siphon on one end that extends out to reach the top of the sediment so it can filter feed. The clams feed on dinoflagellates and diatoms in this method (Newell 1986). The clams rarely move from their established location, especially once they are an adult. This sessile nature leaves them rather exposed to predation from many organisms, most importantly the European green crab.

The European green crab (*Carcinus maenus*) is an invasive species that was established in the United States several hundred years ago, most likely via ship ballast (ref). Since that moment, the green crabs have flourished and caused many detriments to the invertebrate species it feeds on. One of the many species it preys on is the soft shell clam. Green crab presence has been mentioned as a factor in the fluctuations of clam numbers over the years, and some research has been done on this hypothesis. Dr. Brian Beal at the Downeast Institute of Maine has looked at survivorship rates of juvenile clams and determined that green crabs are in fact an important predator of soft shell clams, but did not quantify the percentage of predation on the clams is in fact done by the crabs (Beal 2006). The green crab has the potential to be a major detriment to the soft shell clam populations on the East coast and research needs to be done in order to determine the actual effect of this predation.

Green crab populations on the east coast are constantly expanding because they have ample resources to reproduce, eat, and live. With this increase comes the potential for intraspecific competition and extreme amounts of predation on local species that are very important to the ecosystem and the economy. Considering that green crabs do in fact prey on the soft shell clams in Maine, this is a potentially large problem because of the clams' importance to Maine's shellfish economy and the clammers' income. This project will address and quantify how much of an impact the green crabs have on the populations of soft shell clams in a closed mudflat in Biddeford Pool, Maine. Biddeford Pool has a population of soft shell clams that is well monitored and harvested by local clammers. The recent enforcement of a conditional closure to clamming in the Pool adds to the importance to this study in determining the impact of invasive species on a commercially important shellfish. Based off of the results from the experimental design of this project, I hypothesized that (1) soft shell clams will have higher mortality in the presence of predators and (2) predation on adult soft shell clams by green crabs will be higher than predation by any other predators in the Pool.

This project will attempt to measure the impact of predators on clams by using an experimental field-based design that excludes predators to determining the rate that green crabs consume the soft shell clams. The results of this project can be compared to the results of Dr. Beal's (2006) work in northern Maine thereby allowing for generalizations regarding the spatial heterogeneity of green crab impact on soft shell clams across clam flats in Maine.

Methods

Study site: The site being used for the experiment is Biddeford Pool in Biddeford, Maine. There is a large intertidal mudflat located in the actual pool. The pool has one inlet/outlet by which it fills with the incoming tide and empties with the outgoing. The Pool drains completely when it is low tide, exposing a large clam flat that is utilized by people and other organisms. An area was chosen away from the inlet as to avoid any current. This avoidance of the inlet is a result of a study that suggests that crabs will not look for bait in a high flow area; the inlet of the

Pool has high flow during incoming and outgoing tides, which would result in little to no crab predation (Robinson, 2011). All of the field work was performed during low tide when the mudflat was exposed so there was easy access to the clams.

Design: 60 one foot long, four inch diameter PVC tubes were sunk into the mud, in groups of five, so they were flush with the surface (Beal 2006). Four of the groupings were left completely exposed on both ends (the control), four groups were covered with landscape mesh on either end (total predator exclusion), and four of the groups were surrounded by a 24 inch tall sheet metal ring (selective predator exclusion). The main goal of the metal ring was to act as a crab deterrent as it was too slippery for the crabs to climb up and it was too tall for them to swim up and over, excluding an unexpected algae raft with crabs attached. These different groupings were randomly placed throughout the study site. Two juvenile soft shell clams (10-16 mm) were placed in each tube. Each of the plots were checked every two weeks to look for signs of predation (damage to the shell, dead clams, missing clams, etc.). Crushed clam shells were counted as predation by crabs (Tan, 2015). Living, empty, crushed, and missing clams were recorded in order to determine the green crab predation rate. Observing the plots every two weeks reduced human disturbance of the clams and sediment and also gave a considerable time window for any predation to occur. The survivorship rates of the clams in each treatment were calculated by taking the number of dead clams for each plot in each treatment, averaging them together, and dividing it by the total number of clams in each plot (10). The mortality rates for each type of plot (exclusion of all predators, exclusion of crab predators, no exclusion) were compared to determine if the crabs are the major predator, fish are a major predator, or if all the clam's predators have a relatively equal impact on the clams ANOVA testing was performed along with Tukey Post-Hoc analysis to determine the differences between the mortalities of the closed, open, and flashing plots and to discern whether or not these differences are significant..

Results

When comparing the differences between treatments, it was found that there were significant differences in the mortality rates of clams in tubes with no predator exclusion, all predator exclusion, and crab-only exclusion. All the p-values between the treatments were less than 0.01, which translates to significant differences between the mortality rates in each treatment (Table 1). This supports the hypothesis that predator exclusion would have an effect on mortality rates and that the three treatments would have different mortality rates. The same tests were done between sampling dates to test if the progression of the summer had any effect on the mortality rates of the clams. This set of results came out inconclusive, with all p-values for mortality rates between trial dates being greater than 0.01. There was no relation between clam mortality and progression of the summer in this project.

The results of this project supported both of the original hypotheses. The preliminary hypotheses were that the clams with no predator exclusion placed around them would have the highest mortality rate in comparison to clams with predator exclusion placed around them. The second hypothesis was that green crab predation would be responsible for the most clam death. In the presence of all predators (open plots), there was a 17.5% survivorship rate. To compare, the closed plots had a survivorship rate of 87.5% and the plots surrounded by the metal flashing had a survivorship rate of 62.5%. It was also found that, in comparison to other causes of death (worms, birds, etc.), crabs caused the most deaths, with 45% of all deaths recorded in the duration of this project were perceived to be the result of green crab predation (Fig. 1).

Discussion

My project aimed to determine which predators had the most effect on the survival of these hatchery sized clams. I used Dr. Brian Beal's conclusion that predation was the number one cause of clam mortality as one of the inspirations for this project. His project looked at spatial variations as the variable being tested. He found that it was in fact predators that were the main cause of mortality in soft shell clams, not the spatial variations in which they live (Beal 2006). I wanted to determine which of these predators was the most detrimental to the populations of clams. It was easiest to divide the predator categories in crabs, worms, and an "other causes" category. With predation being the number one cause of death for soft shells according to Dr. Beal, my project showed that 45% of all death was caused by crabs. This high percentage of soft shell clams being lost to green crabs is extremely detrimental to the commercial clamming industry.

The results of this project are extremely important to the commercial clamming industry. With Biddeford Pool theoretically representing all clam flats in Maine, we can make an estimate of how much money is lost each year through clam mortality as a result of crab predation. According to the results of this project, 45% of all clam population is lost to crab predation over a course of just 8 weeks. $24 million is brought in each year from softshell clams alone (Watts 2016). If it is assumed that 45% of the total population was taken by crabs, that is over $11 million lost every year to crabs. If crabs were not an issue, the total net worth of the clams harvested in Maine alone would be around $35 million dollars. This allows an inference to be made that any predator exclusion is better than no exclusion when it comes to seeding and farming soft shell clams in Maine. This project had two types of predator exclusion on different plots: a metal flashing ring and landscape netting. Both of these predator exclusion methods had higher clam survival rates than the unprotected clam plots. Some of the covered tubes also had quite a few recruited wild clams in them. They were protected by the tubes and the mesh, much like the planted clams, and were able to settle and grow considerable amounts between trial days.

Throughout the duration of the project, there were many clams recorded as missing. This was determined to be the result of human error (not digging deep enough) or bird predation (there were empty shells around the tubes and bird footprints). Fish predation was also considered, but literature review revealed that mummichogs seem to be the only fish that predate on soft shell clams. Much of this predation on soft shell clams is on larval clams by mummichogs larger than 55 mm, so I did not expect to see many of the size class clams used in this experiment to be eaten by finfish (Kelso, 1979). During the last two trials, the tubes were checked top to bottom for clams and many clams from the first trial were still in the tubes, but buried much deeper than we had dug previously. These clams were also much larger than the other ones that had been counted earlier. One inference that can be made about this is that the larger clams had somehow buried deeper than others on the placement day and were able to avoid most predation and were able to double and even triple in size in some cases. It is known that soft shell clams dig proportionally deeper as they grow larger (Zwarts and Wanink 1991), meaning that as they grew larger in the tube they also dug deeper. It has also been observed that soft shell clams will bury deeper in response to green crab presence, so this is another possible reason why these clams were so much bigger and deeper than the other (Whitlow 2010).

Conclusion

This predator exclusion project brings to attention the understanding that green crabs are one of the soft shell clam's major predators, with 45% of recorded clam deaths being caused by green crabs. Clam diggers who seed their flats need to work hard to protect their harvest from the green crabs' high predatory rate. I hope that this project will produce more effort in research into creating a more sustainable and manageable soft shell production method. If an efficient and simple way of spawning and growing soft shell clams in an aquaculture setting is developed, the wild populations would be spared our portion of wild soft shell removal. Ideally, I hope this project can help us develop overall better aquaculture for soft shell clams, and a better understanding of the species itself, so we can better protect it and utilize it in the future.

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Figures and Statistics

SUMMARY

Groups	Count	Sum	Average	Variance
Column 1	4	7	1.75	0.25
Column 2	4	35	8.75	0.916667
Column 3	4	25	6.25	0.916667

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	100.6667	2	50.33333	72.48	2.82E-06	4.256495
Within Groups	6.25	9	0.694444			
Total	106.9167	11				

Table 1: ANOVA results from the dataset. The p-value of the entire data set is p<.01, representing significant differences within the data set.

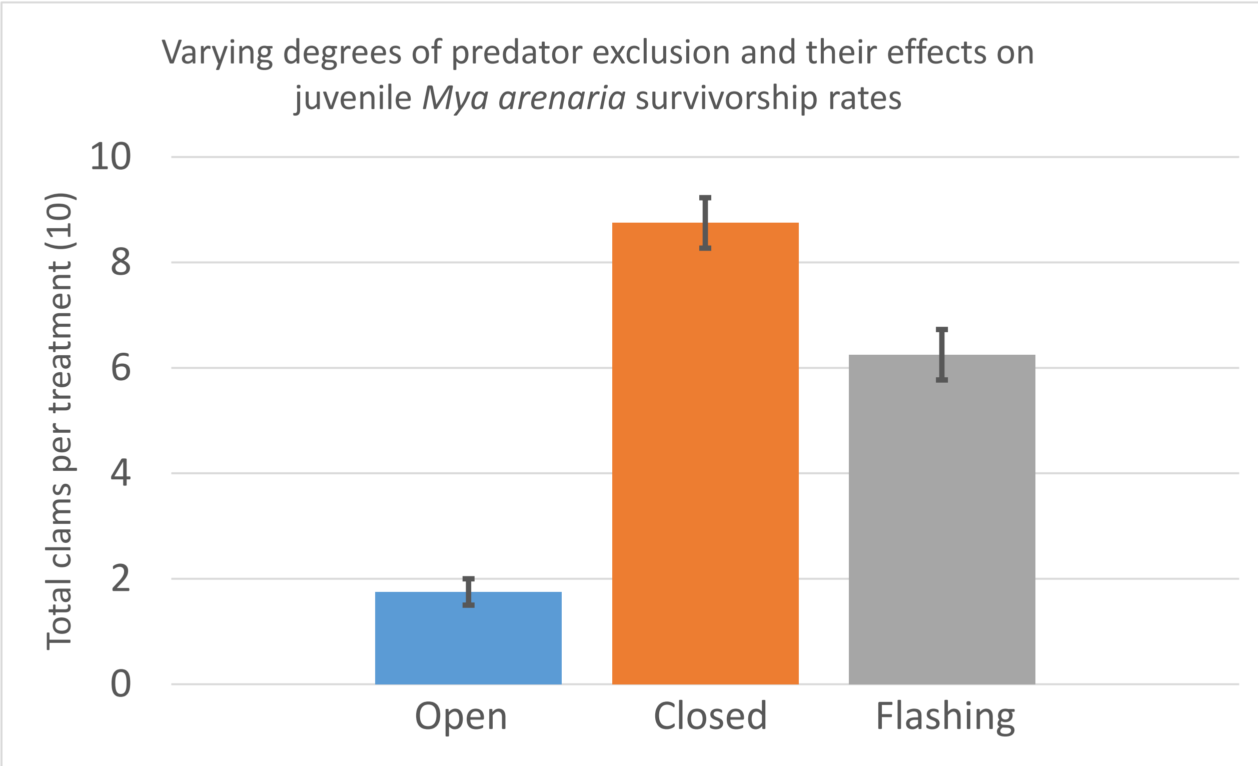

Fig 1: A graph representing the survivorship rates of juvenile soft shell clams with varying degrees of predator exclusion. Error bars represent standard error of the population.

Table 2: The results of the Tukey Post Hoc Analysis, checking for significant differences in survivorship between the 3 predator exclusion treatments. Each treatment had an n of 4, with each sampliong day occurring every 2 weeks for the entire summer (May through August) each tube contained 2 clams that were checked on and replaced every two weeks, if needed. Green boxes represent significant differences between the three treatments.

treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
Open vs Closed	10.2616	0.001	** p<0.01
Open vs Flashing	6.8411	0.001	** p<0.01
Closed vs Flashing	3.4205	0.045	* p<0.05

Chapter 3:
Trophic shifts in the Saco River Estuary that occur with the arrival and summer residence of the striped bass (*Morone saxatilis*)

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Abstract

As an important recreational fish, striped bass (*Morone saxatilis*) help create revenue for southern Maine bait shops, hotels, etc, by attracting anglers to the area. They rely on a host of migratory baitfish to fuel their yearly migrations north and south. Throughout the summer in New England, baitfish of all kinds can be found in the waters, alongside of some resident invasive species, like the European green crab (*Carcius maenas*). This leaves a large variety of food for the striped bass to feed off during the summer months, while they are preparing to travel south to spawn. Utilizing stable isotope analysis and stomach content analysis of striped bass, green crabs, and other bait species in the Saco River Estuary, I intend to track the trophic shifts occurring in the estuary when the striped bass arrive and encounter green crabs as a potential food source. Stable isotopes are still being processed, and the results are expected to arrive in mid to late September. It is expected that due to their high availability, green crabs will play a major part in the striped bass' diet throughout the summer, and this will be expressed through both stomach contents and stable isotope signatures. Understanding the effects of these invasive species is very important to maintaining a healthy population of striped bass in order to sustain the recreational, economic, and most importantly ecological benefits of these fish.

Introduction

With the arrival of May, the Saco River Estuary becomes an important feeding ground for the migratory striped bass (*Morone saxatilis*) in Southern Maine. Two locations where striped bass concentrate in the estuary are Biddeford Pool and the immediate area, and the mouth of the Saco River. These fish are an important part of the local economy, bringing money into the bait shops, boat sales/maintenance companies, restaurants, etc, located in Southern Maine. Striped bass are known to have a wide variety of prey ranging from baitfish to lobsters, to invasive species like the very well-known European green crab (*Carcius maenas*). The presence of these green crabs potentially provides the striped bass another food source to fuel their yearly migration. Striped bass constantly move in search of bait and can be found in many locations including boulder fields, flats, deep water, and coastal rivers, like the Saco (Walbaum, 1792). Their diet changes as they move further north and encounter different ecosystems, but it centers predominately around migratory baitfish such as sand eels (*Ammodytes americanus*), menhaden (*Brevoortia tyrannus*) and Atlantic mackerel (*Scomber scombrus*) (Nelson et al, 2003). **The plethora of bait presented and consumed by the striped bass in the Gulf of Maine leaves a major question to be answered: what role do green crabs play in the striped bass' diet as they migrate up and down the east coast of the United States??** Striped Bass are a pivotal predator in the summer waters of the Saco River Estuary.

With such a wide variety of bait, striped bass can find food just about anywhere, although, baitfish can be very scarce during certain times of the year, particularly when human harvesting of mackerel (January-May) and menhaden (summer) reduce the numbers of these baitfish (Atlantic mackerel, 2017). However, the underutilized European green crab can be found everywhere the striped bass swims, and in high numbers. This provides a potentially reliable food source for the striped bass throughout the year, especially if baitfish are scarce. Green crabs are an invasive species originating from northern Europe. Since their introduction to the east coast over 200 years ago, the green crabs have made significant changes to ecosystems, causing trophic shifts and likely opportunistic diet changes for many other secondary consumer organisms, like the striped bass

(*Carcinus maenas*, 2011). Green crabs live in a range of locations but frequent shallow water in jetties, clam flats, and sandy bottom flats. They are opportunistic feeders and will eat virtually anything as long as it is easily accessible. Juvenile shellfish are a favorite prey item of the green crab, especially soft shell clams (*Mya arenaria*) (Tan, 2015). My previous research looked to quantify green crab predation rates on juvenile soft shell clams, and the results showed that green crabs accounted for 45% of all juvenile clam mortalities in Biddeford Pool. This research from the summer of 2016 focused on the analysis of the top-down pressure on soft shell clams caused by the green crabs, while my proposed 2017 summer research analyzed the bottom-up pressure on striped bass caused by the high abundance of the green crab.

The overall undergraduate research goal that I am working towards is to quantitatively and qualitatively catalogue the trophic interactions occurring within the Saco River Estuary. I worked with previously gathered stable isotope data, and used it to create a "food web" plot that presented a good representation of the trophic levels within the estuary and also provided the evidence needed to confidently state that strictly saltwater organisms had a significantly different carbon source from "estuarine" fish, and both saltwater and estuarine fish had significantly different carbon sources from freshwater fish that were caught a mile up river. This project gave me a basic understanding of the trophic interactions in the estuary, and also provided the necessary background in order to pursue more organism specific projects, like last summer's soft shell clam project, and hopefully, this summer's project looking at striped bass trophic interactions. By utilizing my first overarching project as a baseline, I hope to be able to gain an in depth understanding of the interactions occurring within the Saco River Estuary, which will open the doors to many other research projects that aim to conserve this amazing ecosystem. **The specific purpose of this project is to determine the importance of the green crabs in the diet of the striped bass, during the spring to fall migration, and residence in Maine, striped bass experience every year**. This migration is driven by migratory baitfish, like menhaden, but the striped bass come across many other types of prey, including the green crabs. The introduction of these invasive green crabs presents pressure on the organisms they consume, and it also puts different pressure on organisms that consume the green crabs. Using stomach content analysis and stable isotope analysis, it is hopeful that there will be observable shifts in the diet of the striped bass as the summer continues on from the time the striped bass arrive and they leave. Variability in striped bass stable isotope signatures and stomach contents will also be compared between size classes and between study sites. My research proposes to analyze if green crabs are a truly utilized food source for striped bass in comparison to two other well-known striped bass food sources in the Saco River Estuary, sand eels and mackerel. **I hypothesize that green crabs will make up a major percentage of the striped bass diet, especially smaller striped bass that are not able to eat larger bait like mackerel and menhaden.**

Methods

Study Site: The sites that were studied in this project were Biddeford Pool and the immediate area, and the mouth of the Saco River and the immediate area outside of the jetties. Biddeford Pool is a large pool with one inlet/outlet that fills and empties with the incoming and outgoing tide. Green crab populations are known to be high in the area, as explained to me by the local clammers. Striped bass also frequent the pool during the summers, chasing bait in and out. The Saco River empties into the Atlantic Ocean in Biddeford, ME, right on the University of New England campus. The mouth of the Saco River is a hotspot for striped bass fishing, and also hosts many mackerel, sand eels and green crabs, that the striped bass follow and eat.

Sample Collection: Rod and reel were used to collect all of the striped bass menhaden, and mackerel. Rods and reels were used in order to minimize our impact and allow us to control the number of fish that were sacrificed for the project. Seine nets were used to collect sand eels, as they are too small and fast to try to catch any other way. Green crabs were collected while searching under rocks and macroalgae, and also by combing the beach for them. All other organisms were caught in the seine nets. Sampling occurred every week rather than bi-weekly, due to the shifting tides and availability of the actual organisms being sampled. Sampling occurred during 12 weeks in the summer when striped bass are known to be at their highest abundance in the Gulf of Maine (Walbaum, 1792).

Analysis: Samples were collected, processed, and encapsulated, and then sent off site to UC Davis Stable Isotope Facility. Stable isotope analysis (SIA) is performed using an elemental analyzer and a mass spectrometer. The values obtained are paired with two laboratory standards and measured in reference to these standards in order to correct the obtained values. The values sent back to us are conveyed relative to international standards (Carbon, 2017). Carbon-14 stable isotope signatures are tied to certain carbon sources (primary producers) and are expressed up and down the food chain i.e. the δ^{13}C signature of algae eaten by a crab is reflected in the δ13C of the crab, and the δ^{13}C of the crab is reflected in the δ^{13}C of the fish that eats that crab. Alongside stomach content analysis of the striped bass, SIA provides a window into the natural changes in the striped bass' diet as the summer proceeds (via δ^{12}C- δ^{13}C signature shifts) and will also help to support or refute the hypothesis that green crabs are being utilized by striped bass as a major food source and make up a significant portion of their overall diet in Biddeford Pool and the Saco River estuary.

Results

Striped bass (*Morone saxatilis*) had the highest average δ15N value out of the eight species collected for this project, at a value of 16.17. All other species fell under the striped bass' δ15N value by at least 3.5. Mackerel (*Scomber scombrus*) and menhaden (*Brevoortia tyrannius*) occupied very similar δ15N values, as did 9-spine stickle backs (*Pungitius pungitius*) and winter flounder (*Pseudopleuronectes americanus*), and sand eels (*Ammodytes Americana*) and sand shrimp (*Crangon spetimspinosa*). δ^{13}C values varied widely, with some overlaps occurring between organisms. Striped bass showed a strong overlap with winter flounder and sand shrimp, with sand shrimp being the most common stomach content among striped bass during this project. Error bars on each point represent standard deviation of both δ15N and δ^{13}C. There was some overlap in the δ^{13}C error bars of the striped bass and the green crabs as well, but not as strong as striped bass, sand shrimp, and winter flounder.

Discussion

Green crabs were not found to make up a major portion of the striped bass' diet in the Saco River Estuary. The δ^{13}C values of the green crab were not strongly reflected in the δ^{13}C values of the striped bass, and crabs were found in limited amounts in the bass' stomachs. The striped bass had the highest δ15N value out of all the organisms sampled, which was expected. This was anticipated because it was the consumer of focus and none of the species sampled in this project were secondary consumers or even tertiary consumers that would eat a striped bass. δ15N levels increase within an organism's flesh as it consumes and assimilates the energy and nutrients from other organisms (Fry 2006). δ15N therefore accumulates in higher concentrations per organism in higher trophic levels, explaining the high δ15N value for striped bass in this project.

δ^{13}C values and stomach content analysis were much more useful in analyzing whether to support or refute the hypothesis that greens crabs make up a major portion of the striped bass' diet during the migration than δ15N. δ^{13}C values reflect the carbon source a primary producer is using to form energy (Fry, 1991). Overlap in δ^{13}C indicates that there is upstream nutrient cycling occurring between two organisms. Once a consumer eats another organism, the prey's δ^{13}C signature is incorporated into the predator's δ^{13}C signature and changes it to more closely reflect the prey's δ^{13}C signature. The δ^{13}C values of the striped bass and green crabs did have some overlap

(Figure 1), but it was minimal. The wide spread of $\delta^{13}C$ values (Figure 1) express the wide variety of bait the striped bass consume. In reference to Chapter 1 of this thesis, the wide array of $\delta^{13}C$ values also expresses the differences in the carbon sources of estuarine and saltwater organisms. The striped bass' access and ability to handle to a range of salinities and habitats and have access to wide variety of bait suggests that the green crabs are presented to the striped bass, but there is so much other bait that it is not energetically favorable to be eating one single crab at a time.

The one definite green crab appearance in a striped bass' stomach, was later in the summer (July), and in a bass in the 40+ cm range (Table 1). For many of the fish, much of the stomach contents were digested, but evidence of crab carapaces was found in some the fish. Green crabs were prevalent at both sites the fish were being caught, presenting themselves as a viable food source for the striped bass. However, many more striped bass were found with sand eels and sand shrimp in their stomachs than green crabs (*Table 1*). These organisms congregate in large schools, making them easier to prey on in larger quantities for the bass. Another factor considered was the size classes of the fish that were sampled. The majority of the fish (15 of 24) were under 40 cm, and these fish had stomach contents consisting mostly of sand shrimp and some small sand eels. Fish above 40 cm were found with larger organisms in their stomachs, like larger bait fish and green crabs. In the coastal waters of Massachusetts, the average size of green crabs eaten by striped bass increases linearly with the length of the striped bass eating them, with green crabs being most abundant in striped bass that were less than or equal to 42 centimeters (Nelson, 2003). Crabs in general are also very common food for striped bass, with green crabs making regular appearances in the stomach of striped bass in the coastal waters of Massachusetts (Nelson, 2003). These findings from Massachusetts help to explain why green crabs were not found in high numbers in the stomach of striped bass in the Saco River Estuary. The striped bass that were caught for this project were much smaller than the fish caught in Nelson et al., suggesting that the striped bass in the Saco River Estuary are only beginning to consumer green crabs at larger sizes (>40 cm).

Conclusion

Striped bass are a pivotal part of both the ecosystem and economy in New England. Recreational fishing, limited commercial fishing, and the culture behind striped bass bring in money from across the country. This importance of the bass stresses how crucial they are to both us and the ecosystem. Their wide diet range and nondiscrimination appetite makes them a key secondary consumer in the coastal waters of New England. Understanding the ecology behind these predators is key in preserving the resource and ecosystem surrounding them. In Maine, the story is no different. This project presented several key findings and speculations about the striped bass in the Saco River Estuary. Striped bass below 40 cm in the Saco River Estuary were not found to have any green crabs in their stomachs. Above 40 cm, the bass were found to have larger baitfish and green crabs in their stomachs, indicating a diet shift with growth in striped bass. SIA signatures reflected the stomach contents of the striped bass, which helped to build the picture of the striped bass' diet in the Saco River Estuary.

Figures

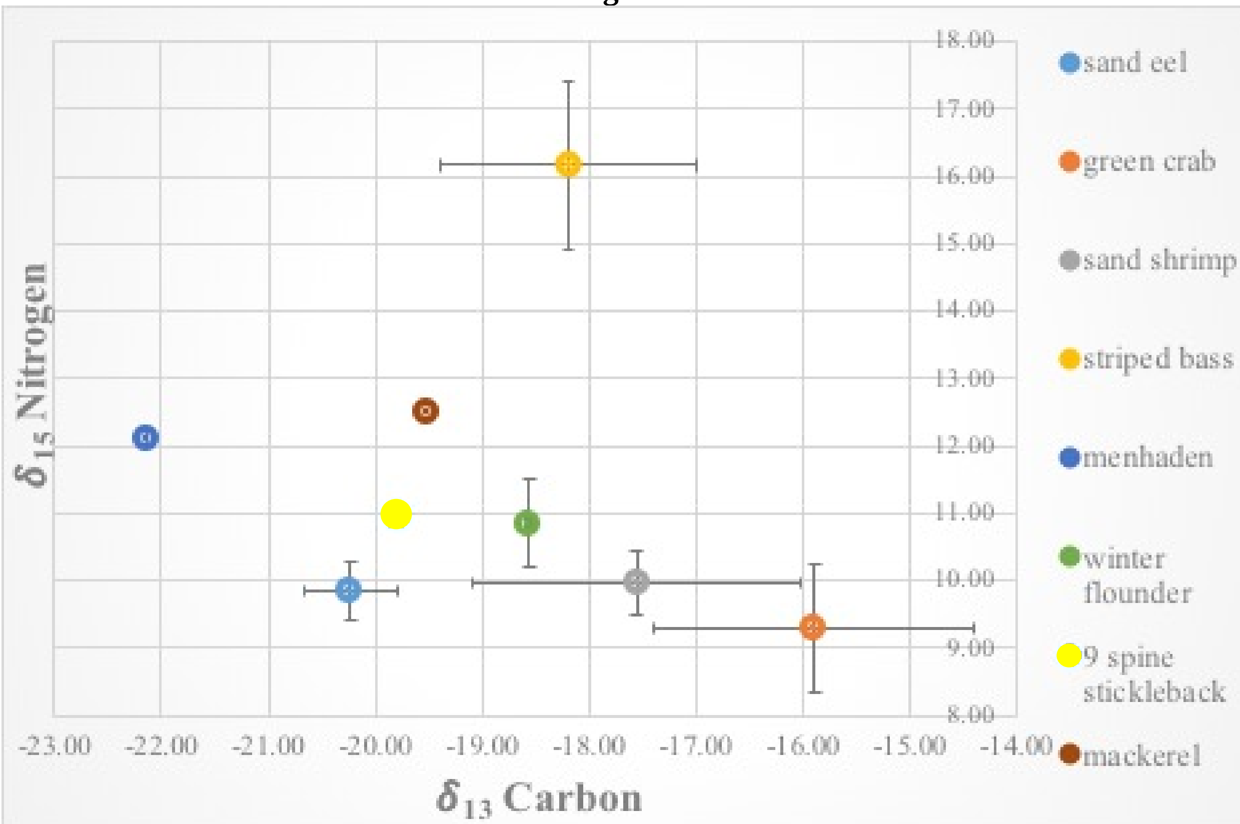

Fig. 1: A biplot comparing the δ^{13}C and δ^{15}N of the species collected in Saco Bay for this project.

Table 1: Striped bass stomach contents from the Saco River Estuary (Summer 2017)

Individual Label	Length of bass (cm)	Date Caught	Species (w/in)	Length (cm)
MS1	34	5/30/17	unidentifiable	
MS2	37	5/30/17	unidentifiable	
MS3	31	5/30/17	scud	
MS4	39	5/30/17	unidentifiable	
MS5	33	5/30/17	unidentifiable	
MS6	37.5	5/30/17	sand eel	4.5
			sand eel	4
MS7	30.5	5/30/17	empty	
MS8	32	5/30/17	unidentifiable	
MS9	47	6/12/17	none	
MS10	35.5	6/13/17	sand shrimp	2
			sand eel	3
MS11	33	6/13/17	unidentifiable	
MS12	76.2	6/26/17	none	
MS13	31	6/26/17	bloodworms	digested
MS14	33	6/26/17	sand shrimp/bloodworms	SS: 3.5
MS15	32	6/26/17	sand shrimp/fish spine	SS: 4
MS16	40.5	6/28/17	scud	
MS17	41	6/28/17	unidentifiable	
MS18	33	7/6/17	sand shrimp	
		7/6/17	bloodworms	
MS19	69	7/6/17	empty	
MS20	75	7/6/17	large baitfish skeleton	
MS21	75	7/6/17	empty	
MS22	46	7/18/17	green crab/sand shrimp	
MS23	70	7/20/17	empty	
MS24	65	7/20/17	mackerel (half digested)	27

Thesis Conclusion

My overall hypothesis that **green crabs play an intermediary role in the food web between soft shell clams and striped bass** was supported through both results from my research at UNE, but also through literature review of studies analyzing similar factors surrounding green crabs, striped bass, and soft shell clams. This thesis expresses the importance of understanding the impacts of the European green crab by analyzing the impacts they have on a commercially important species, soft shell clams, and on a recreationally important species, the striped bass. Stable isotope analysis of the samples taken from the Saco River provided several important outcomes. Significant differences between the $\delta^{13}C$ signatures of freshwater, estuarine, and saltwater organisms were found within a one mile stretch of river, from the mouth of the river up to the first dam. This represents the gradient of primary producers and detritus being utilized by the various food webs that converge in this short section of river. The niche partitioning between these three classes of organisms that is represented by the significant differences in $\delta^{13}C$ values highlights that there are some overlaps between the freshwater, estuarine, and saltwater food webs being analyzed, but there are distinct lines that can be drawn between the types of organic matter being utilized at the base of each food web. Out of all the species sampled in the river, the striped bass had the highest $\delta^{15}N$ value, representing that it was the top predator sampled. Their wide range of food sources is represented by the fact that the striped bass' $\delta^{13}C$ value is leaning towards the estuarine group. Predator-prey interactions between green crabs and soft shell clams also suggested significant upstream nutrient cycling, with 45% of all soft shell clam deaths used in the project being attributed to green crabs. This was based on physical characteristics of green crab predation expressed in the dead clams. This, with evidence of predation on green crabs by striped bass in both this project and in the literature, aids in the understanding of the importance of these interactions introduced by this established invasive species. The nutrient flow between soft shell clams and striped bass is being facilitated by these green crabs and their high predation rates upon juvenile soft shell clams.

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