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Effects of the Invasive Asian Shore Crab, Hemigrapsus sanguineus, on New England Trophic Cascade: Diet and Predation

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Abstract

Trophic cascades occur when the community structure is influenced by indirect effects of predation on the lower trophic levels. The trophic cascade can be disrupted when an invasive species is introduced. The Asian shore crab, *Hemigrapsus sanguineus* has invaded ecosystems in New England and we predict this invasion will negatively affect the classic New England trophic cascade. By the predation indirectly affecting lower trophic levels, *H. sanguineus* can influence the community structure and diversity at the lower levels. To understand the trophic cascade, we manipulated the food source and competitors in four different mini-ecosystems. By introducing *H. sanguineus* into an environment with *L. littorea* and two algal species; *Fucus distichus* and *Ulva sp.*, we confirmed that *H. sanguineus* disrupts the trophic cascade. Specifically, the Asian shore crab, consumed the snail's preferred type of algae, which led to the indirect effect on the trophic cascade. The findings of this experiment confirmed that with the presence of the invasive crab, the Asian shore crabs had an indirect effect on the snail. Invasive species can have a negative effect on trophic cascades resulting from competitive exclusion.

Introduction

Trophic cascades consist of indirect effects of predation on lower trophic levels (Singer 2016). Trophic cascades occur when the community structure is influenced by predators and which results in effects multiple trophic levels away from the predator. Nonindigenous species can have detrimental effects on trophic cascades. These species prey on native communities, which lead to the altering of certain food webs, and even the extinction of some of the native species in the trophic cascade (Bourdeau and O'Connor 2003). For example, the predatory zooplankton, *Bythotrephes longimanus*, invaded the Laurentian Great Lakes in the 1980s.

Compared to nearby lakes, it was observed that *B. longimanus* had reached large densities. This invasion led the biomass of the grazer *Daphnia pulicaria* to decrease and caused the water quality to decline. These effects of the invasive species triggered a massive loss of ecosystem services within the trophic cascade (Walsh 2016). Changes in the local species diversity over time have occurred due to either an addition or removal of certain organisms. While some organisms can increase the species diversity in lower trophic levels, others cause certain organisms to become extinct (Lubchenco 1978).

Invasive species can have indirect and direct effects on a trophic cascade and surrounding community. Some species in a community have a greater impact than others, resulting in competitive exclusion. Because the diversity of species in New England is complex, it is hard to prevent competitive exclusion. Many studies observe the introduction or removal of organisms from their native trophic cascades. For example, when *Littorina littorea*, a native snail species, is absent or rare, their preferred algae food choice, *Enteromorpha intestinalis*, outcompetes the other algal species in the algal pools. This reduces the diversity in that area. When the snail species is present, in intermediate densities, the competitive exclusion is prevented and there is a higher species diversity in the pools (Lubchenco 1978).

It is common for invasive species to cause dramatic changes in the community structure and function of the trophic cascade (Singer 2016). The Asian shore crab, *Hemigrapsus sanguineus*, is a crab that is native to Asia, but that has invaded the northwest Atlantic and the Mediterranean Sea (Ledesma 2001). The Asian shore crab has become the dominant species in many of the invaded communities. In previous studies, it has been found that high densities of *H. sanguineus* that occur in the wild completely disrupt the trophic cascade. The large appetite of the Asian shore crab plays an important role in effecting the structure of the prey communities in their newly invaded habitats (Brousseau 2001). While there is still some uncertainty about the long-term ecological impact that this invasive species would have in the community, the rapid dispersal of this invader can be detrimental. Asian shore crabs play an important role in restructuring prey communities in non-native environments, such as the New England trophic cascade (Brousseau 2005). It has been found that, *H. sanguineus* consumes both plant and animal food (McDermott 1999). The gut content analyses of wild-caught crabs suggest algae is its primary food (Lohrer and Whitlatch, 1997; McDermott, 1999; Tyrell and Harris, 2000). None of these studies, however, examine food preference when both plant and animal food items are available. This makes it difficult to assess the importance of both food sources in the overall diet of this invader.

In this study, the Asian shore crab was introduced into a classic New England trophic cascade involving *L. littorea* and two types of algae; *Fucus distichus* and *Ulva sp*. This experiment was proposed to determine whether the Asian shore crab would have a direct or indirect effect on the classic New England trophic cascade. We hypothesize the introduction of the Asian shore crab will have a negative impact on classic New England trophic cascade. This is because of its indirect effects. With the presence of the crab, there will be a decreased number of algae due to the Asian shore crab's direct effect on the snail's food.

Materials and Methods

Study species

All the organisms were collected from Stratford Point Beach in Stratford, CT. The sex of the Asian shore crabs was determined by observing the carapace. The carapace of the male crab has a thin triangular abdominal flap, while the carapace of the female crab has a rounded shorter abdominal flip. The legs were counted to see if they had the correct total of 10 legs, without any deformities. The width of the carapace was measured in mm. *L. littorea* were measured in mm from the bottom of the body to the highest point of the shell to determine its height.

The dry weights of the two algae, *F. distichus* and *Ulva sp.* were determined in grams. A piece of *F. distichus* was obtained. A single blade was used to cut one piece per tank at a length of 7 cm. The holdfast (bottom) or the receptacles (top swollen portion) were not included in these pieces. The *F. distichus* were blotted gently with a paper towel. Each piece of *F. distichus* was weighed to the nearest 0.01 grams using a weigh boat and a balance. A paper clip was carefully attached to one end of the blade of the piece of algae. Each piece was placed into one tank. The same was done for *Ulva sp.*

Experimental setup

Four treatment tanks with 12 replicates of each were set up (48 tanks total). The four treatment tanks were set up in 0.5-gallon aquaria filled with sea water: 1) Both algae species alone (A), 2) Both algae and 2 snails (AS), 3) Both algae species and 2 crabs (AC), 4) Both algae species, 2 snails, and 2 crabs (ASC).

Data Collection

After one week, the tanks were taken down and, snail and crab survival and conditions were assessed. The snails were examined for missing pieces of their shell or other damages. The crabs were observed for damages, such as missing legs or pieces of legs. For each tank, signs of predation were noted. After assessing the organisms, the crabs and snails were placed in the freezer for 45 minutes.

The study organisms were removed from the freezer to analyze their gut content. For the snails, a hammer was used to crack open the shell of the snail. The snail's body was exposed by removing the pieces of the cracked shell. The entire intestinal track of the snail was removed. A dissecting microscope was used to determine whether the snails consumed algal or animal tissues. For the crabs, the carapace was removed by cutting around the edge of it with scissors. Without disturbing the hepatopancreas and the stomach, the carapace was lifted to expose the epidermis. The dissecting scope was used and allowed for a stage to gently remove the epidermis with forceps. The hepatopancreas, stomach, muscle tissue, and hindgut were then able to be seen. The forceps were then used to remove the hepatopancreas, allowing access to the other organs, such as the reproductive organs. Then, the stomach and digestive tract were removed and carefully sliced open to view the crab's last meal and used the dissecting microscope to see if any algae or snails were consumed.

The final algal wet masses were taken. The algal appearance was qualitatively assessed, and the pieces of algae were dabbed to remove excess water and their weights were taken and recorded. A tissue penetrometer was used to measure the volume of water needed to pierce through the algal tissue. The amount of water it took to allow the pushpin to pierce the algae, was recorded in mL.

Data analysis

In order to determine if the force required to pierce *F. distichus* and *Ulva sp.* differs, an independent sample t-test was conducted. Using SPSS Statistics, the means were analyzed and compared using an independent samples t-test. The mean sizes, standard deviations, and T-test statistics (T, df, and p) were calculated and recorded.

An independent samples t-test was then conducted to determine if there were differences in the size of the snails and crabs between the treatments. Two t- tests were conducted including one on the size of the snails which was determined by shell height, and a second one on crab size which was determined by carapace width. The mean sizes, the standard deviations, and the T-test statistics (T, df, p) were recorded.

Two one-way analyses of variance (ANOVAs) were conducted on the change in weight for *F. distichus* and the change in weight for *Ulva sp*. The ANOVA test was used to determine if there were differences in the *Ulva sp.* and *F. distichus* among the treatments. If the p-value was less than 0.05, a post-hoc Tukey test was conducted to determine which treatments differ from each other.

Results

Based on the results of the penetrometer tissue tests, significantly more water was needed to pierce though the tissue of *F. distichus*, (mean ± standard deviation mL of water; 66.25 ± 40.93 mL; N=72) compared to *Ulva sp.* (14.37 ± 7.11 mL; N=72) (**Fig. 1**; T= 10.60, df = 75.28, $p < 0.001$). There was a significant change in the amount of *Ulva sp.* consumed between treatment groups (**Fig. 2**; F = 7.31, df = 3, $p < 0.01$). The *Ulva sp.* mean and standard deviation in the algae tanks (A; 0.24 ± 0.26 g; N = 12), algae and crab (AC; -0.21 ± 0.33 g; N = 12), algae and snail (AS; 0.07 ± 0.42 g; N = 12), and algae, snail, and crab (ASC; -0.26 ± 0.13 g; N = 12) was significantly different. Based on the Tukey post-hoc test, A was significantly different from AC or ASC, while AS did not differ from any group. The mean change and standard deviation of *F. distichus* weight was calculated for treatments: A (0.03 ± 0.24 g; N = 12), AC (-0.37 ± 0.37 g; N = 12), AS (-0.04 ± 0.32 g; N = 12), ASC (-0.44 ± 0.39 g; N = 12). The treatment groups were significantly different (**Fig. 3**; F = 5.82, df = 3, p = 0.002). Based on the Tukey post-hoc tests, A was significantly different from AC and ASC, while AS was significantly different from ASC.

The average heights of the *L. littorea* shell (mm) in treatment tank AS (mean ± standard deviation shell height (mm); 10.49 ± 3.92 mm; N = 24) was not significantly different from snails in the treatment tank ASC (11.92 ± 2.16 mm; N = 24) (**Table 1**; T = -1.56, df = 35.77, p = 0.13). The mean *H. sanguineus* carapace widths per treatment tank were not significantly different because tank AC (mean + standard deviation carapace width (mm); 17.68 ± 4.84 mm; N = 24) and tank ASC (17.34 ± 7.21 mm; N = 24) (**Table 2**; T = 0.19, df = 40.24, p = 0.85).

The conditions of the algae differed between the two species. *Ulva sp.* was either smaller in size and weight or completely eaten. The *F. distichus* species was either left in good condition or smaller in size and weight. Overall, the final condition of *Ulva sp.* was worse than that of *F. distichus*. Regarding the crab and snails final condition, most of the organisms had similar conditions that they started with. Most of them were still in relatively good condition, while some crabs and snails died. However, almost all of the organisms were present at the end of the experiment. The gut contents of these organisms were different based on the food preferences. The majority of the gut contents for these organisms was *Ulva sp.*, while a few organisms consumed both algae and other organisms. In a few of the crabs' gut content analysis, other crab body parts were found, along with the algae.

Discussion

For both *L. littorea* and *H. sanguineus*, gut content analysis showed that both algal species were consumed, while *Ulva sp.* was consumed more by both species. The final conditions of the *Ulva sp.* described that the pieces of algae were either torn up or entirely gone. There were few organisms that had a brown color of gut contents along with the green, which confirmed that they consumed both *F. distichus* and *Ulva sp*. The change in wet mass for *Ulva sp.* and *F. distichus* were significantly different between the treatment groups, confirming that the type of algae was an important factor in this experiment (**Figs. 2** and **3**). In previous studies, it has been found that the green algae was consumed more than the red or brown species (Bourdeau and O'Connor 2003). The tissue penetrometer results supported the difference in algal consumption. *F. distichus* had tougher algal tissue than *Ulva sp.* (**Fig. 1**). This conclusion implies that *F. distichus* is a stronger algal tissue and could be harder for organisms to eat and digest, therefore leading to the preferred consumption of *Ulva sp.*

There are many different foraging patterns of *H. sanguineus,* and although we created their environment for this experiment, some factors may still play a role in the diet choice of the crabs. Some factors include the presence of seasonal diet shifts, changes in diet with size, and individual specialization in diet (Griffen 2012). In our experiment, the results differed between the treatment groups, specifically AS and ASC. In the AS treatment group, it was evident that the snails ate most of the algae that was present. In the ASC treatment group, the crabs ate most of the algae, but did not touch the snails (**Figs. 2** and **3**). The gut content analyses did not show snail remains in the crabs, concluding that *H. sanguineus* does not have a strong impact on the populations of *L. littorea* through direct predation. The results of past studies suggested that the snails are protected by the morphology or thickness of their shells. In the presence of *L. littorea* and algae, *H. sanguineus* consumed green macroalgae more than *F. distichus* (Bourdeau and O'Connor 2003).

Based on the results, *H. sanguineus* could change and effect the structure of tide pool communities. With it being an invasive species, there are possible negative effects. These effects could include competitive exclusion which could lead to eventually the extinction of certain organisms. Certain effects like these would be likely to happen if the experiment was over a longer period of time, where it could be observed that predatory behavior of *H. sanguineus* would have a detrimental effect on the structure of tide pool communities. The structure of tide pool communities is very complex and can be easily impacted by many factors. As seen in various other studies, it is common for one species to be the dominate competitor for space. For example, the green crab, *Carcinus maenas,* is the native member of the classic New England trophic cascade. *C. maenas* predominantly feeds on *L. littorea* (Lubchenco 1978). Since the green crab feeds on the snail, the introduction of the Asian shore crab would have a negative impact on the trophic cascade because it eats the algae which is the snail's food. This can result in the extinction of the snails and possibly the green crab. With the Asian shore crab replacing the green crab in our experiment and disrupting the trophic cascade, it can be concluded that the Asian shore crab has detrimental effects. In our experiment, the results differed between the treatment groups, AS and ASC. The introduction of the crab into the tank did not have a direct effect on the *L. littorea* (**Figs. 2** and **3**).

The experimental removal of *L. littorea* resulted in an abundance of their preferred algae, *E. intestinalis*, and a decrease in abundance of the less preferred algae, *Chondrus crispus*. The additions of the snail resulted in the exclusion of *E. intestinalis* and the dominance by *C. crispus*. This suggests that *E. intestinalis* is the dominant competitor for space and outcompetes the other algal species present. The abundance of this dominant competitor decreases when the snail is introduced, while *C. crispus* dominates because it is not eaten (Lubchenco 1978). As *E. intestinalis* was the dominant competitor for space, so was *H. sanguineus* in the presence of *L.*

littorea and the two algal species. *H. sanguineus* caused a trophic cascade by indirectly affecting the algal species, which were the food source of the snails. The results suggest that invasive species can have a large impact on community structure. The effect of *H. sanguineus* as a predator, can cause a trophic cascade and disrupt the community structure. *H. sanguineus* are fierce predators of both algal and animal species that, once they are introduced, have the ability to restructure prey communities (Brousseau 2001).

Conclusion

The invasive *H. sanguineus* has the potential to disrupt the community structure and can result in the diversity of the lower trophic levels changing over time. The green crab is a predator of *L. littorea*, while the Asian shore crab competes with *L. littorea* for its food. The Asian shore crab replacing the green crab in the classic New England trophic cascade, would negatively impact the structure and diversity of that ecosystem. Based on the results of this experiment, the Asian shore crab had an indirect effect on *L. littorea* because it preyed on its main food source, *Ulva sp.* If furthered, this could have eventually led to the extinction of the snail species, due to the competitive exclusion by this invasive, non-native crab. The findings of this experiment confirmed the hypothesis that with the presence of the invasive crab, the New England trophic cascade was disrupted. There was a decreased amount of the snail's preferred algae, *Ulva sp.*, because *H. sanguineus* preyed on it, as shown by the gut content analyses. The findings of this experiment confirm that invasive species can negatively impact trophic cascades.

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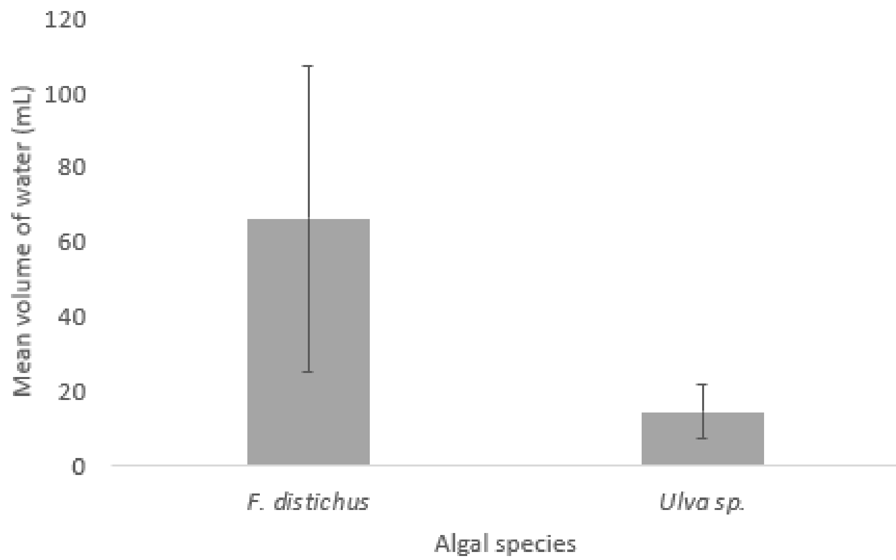

Fig. 1. Mean ± standard deviation of amount of water (mL) required to pierce *F. distichus* and *Ulva sp.* The difference of the mean ± standard deviations between the two species were significantly different ($p < 0.001$).

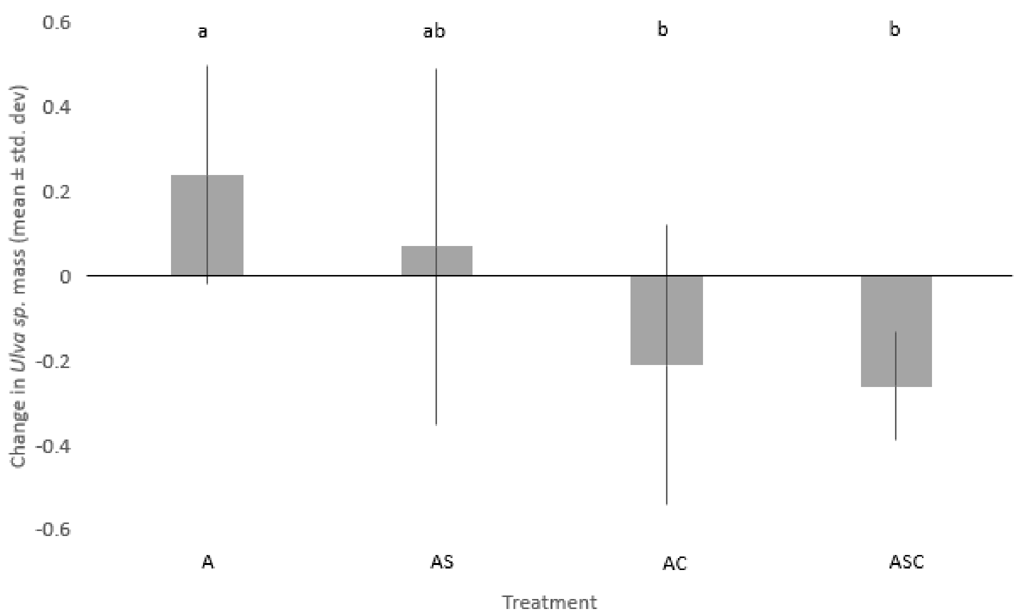

Fig. 2. Mean ± standard deviation of results for change in *Ulva sp.* mass by treatment. The letters below the error bars represent the different treatment groups: algae (A), algae and snails (AS), algae and crabs (AC), and algae, snails, and crabs (ASC). The results of the Tukey tests are

displayed by using different letters over the error bars to indicate which treatments differed from each other. There was a significant difference between treatment groups ($p < 0.001$).

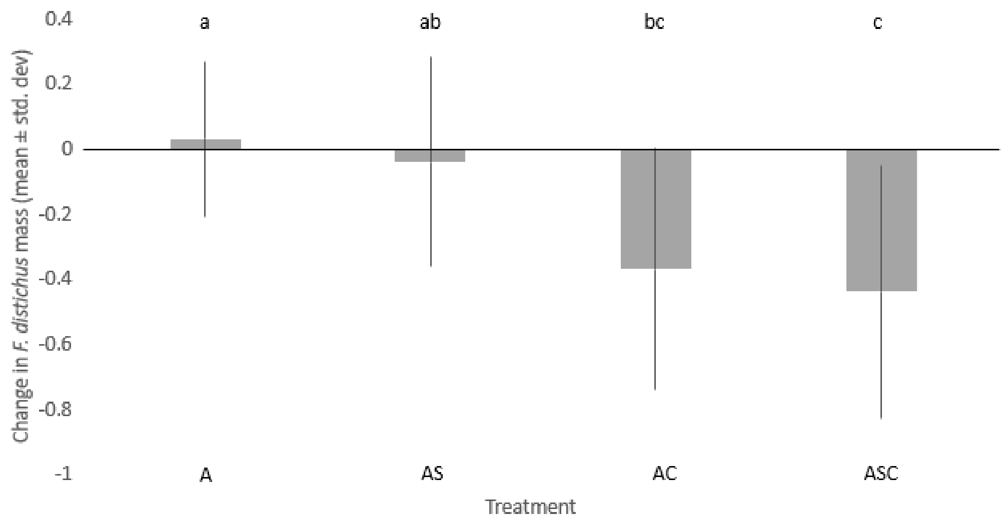

Fig. 3. Mean ± standard deviation of results for change in *F. distichus* mass by treatment. The letters below the error bars represent the different treatment groups: algae (A), algae and snails (AS), algae and crabs (AC), and algae, snails, and crabs (ASC). The results of the Tukey tests performed are displayed by using different letters over the error bars to indicate which treatments differed from each other. There was a significant difference between treatment groups ($p = 0.002$).

Table 1. Mean ± standard deviation of *L. littorea* shell height (mm). The two treatments groups were algae and snails (AS) and algae, snails, and crabs (ASC). The mean shell heights of *L. littorea* between treatment groups were not significantly different ($p = 0.127$).

Treatment	**Mean ± standard deviation of *L. littorea* shell height (mm)**
AS	10.49 ± 3.92
ASC	11.92 ± 2.16

Table 2. Mean ± standard deviation of *H. sanguineus* carapace width (mm). The two treatment groups were algae and crabs (AC) and algae, snails, and crabs (ASC). The mean carapace widths of *H. sanguineus* between treatment groups were not significantly different ($p = 0.85$).

Treatment	**Mean ± standard deviation of *H. sanguineus* carapace width (mm)**
AC	17.68 ± 4.84
ASC	17.34 ± 7.21