Aversive Conditioning and Scent Discrimination in the Green Crab *(Carcinus maenas)*

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Abstract

In the Northwest Atlantic, green crabs have decimated shellfish and eelgrass populations since the 1800s. This resulted in a decline in the fishing industry. Green crabs use their acute sense of smell to track or locate their next meal. They also have chemoreceptors on their antennae that enable them to identify chemicals in the water and sense their environment. Green crabs can be conditioned to go against their natural behavior by a reward, or penalty. The purpose of our project was to see if we could aversively condition green crabs to avoid a scent, and still recognize the conditioned scent once it was presented with other scents. During the preconditioning, each crab was introduced to four scents five times in a random order (bunker, shrimp, clam and bluefish). The crabs then transitioned to a conditioning phase where they were only presented with one scent, followed by the introduction of a pseudo-predator, which was rapidly moved around the crab to disturb it. The final phase was post-conditioning, where the crabs were introduced each of the four scents again, in random order to see if they reacted aversively to the conditioned scent while having non-aversive behaviors towards the other scents. The results showed that the crabs conditioned to the clam had the best retention rate (90% aversive reaction) while those conditioned to shrimp had the worst retention rate (70% aversive reaction). The crabs were able to reverse their innate behavior because they avoided the shellfish. Green crabs can aversively condition to and discriminate between scents. Through the use of conditioning, green crabs are able to get trained to avoid shellfish.

Introduction

Green crabs originally came to the Americas in the 1800s through ballast water exchanges during the larval stage of their life cycle. Green crabs pose a large risk to the well-being of marine ecosystems in the northeast waters since they change the ecological equilibrium of organisms. This species is known to destroy eelgrass beds and fish habitats. Because of its large population, aggressive behavior, and enormous appetite, green crabs threaten mollusks and shellfish. Unless the green crab population is controlled, it will lead to the endangerment of these species. Green crabs fight for food and shelter with native crabs and other crustaceans, decreasing the number of fish, shellfish, and bivalve species. The aquaculture industry is on the decline because green crabs have decimated clam and other shellfish populations. The green crab population must decline in order to prevent further damage to the ecosystem and the aquaculture industry.

When making important decisions regarding how to act in certain situations, animals might give up obtaining resources and finding a mate to eliminate the risk of predation. Animals use their senses to analyze the area around them and obtain information that may decrease their chance of survival. Chemosensory cues from the surroundings of an animal can provide details about the presence of predators or prey (Kats, 1998).

Aquatic invertebrates detect molecules from other species and use these molecules to interpret animals as prey. The molecules have been described as chemicals released from animals that scatter throughout the sea and reach the chemoreceptors of predators (Kamio, 2017). Chemoreceptors on crabs can be found on their antennae. Antennae are long, segmented appendages that are found close to the crab's eyes that sense chemicals but also help the crab identify its environment. Antennules are smaller structures, but very similar to antennae that serve the same purpose. The lobular giant neurons in the brains of crabs allow them to memorize stimuli. This is a key aspect of their survival. When fishing for crabs, the odor from the bait, releases from the trap into the ocean where crabs can detect it from meters away. Crabs can eat plants, shellfish, fish, and sometimes other crabs. When a crab is eating, they use their claws to manipulate the food and place it into their mouth.

Classical conditioning is a learning procedure where a neutral stimulus is paired with a negative stimulus. Various conditioning techniques based on classical training paradigms have been investigated on green crabs, such as positive reinforcement (Orlosk, 2011). The crabs were conditioned to move into a light environment for food even though Green Crabs stay in dark, or dimly lit environments to avoid predation.

The objective of our project was to see if Green Crabs were able to discriminate scents after being aversively conditioned to a scent. In the preconditioning phase of the experiment, four groups of five green crabs were given the scents of clam, shrimp, bunker, and bluefish in no particular order and their reactions were recorded. Then, each group of crabs were given only one scent and aversively conditioned with a pseudo predator. Finally, in the post-conditioning phase, each group was given four scents and normal and aversive reactions were recorded. It was hypothesized that if the green crabs are being given a negative stimulus while exposed to a scent, then they will actively avoid the scent after conditioning because they will associate the scent with the negative stimulus rather than the other scents. It was shown in spiny lobster that they were able to recognize and distinguish between different scents (Fine-Levy, 1989). This experiment will be used to determine if odor discrimination is present in other decapod crustaceans.

Methods

Crab Collection

16 green crabs were collected at 40°54'19.5"N 73°13'53.4"W (Kings Park, NY) during the end of incoming tide. They were caught using dip nets and crab pots filled with dead menhaden. The crabs were then separated into four groups of four and each group stored in a 29-gallon tank with 1.02 ppt saltwater. In the last step of the collection phase, the spines on the carapace were clipped so that there was a noticeable difference and individuals could be identified. This was used to correlate the crabs to a name and group.

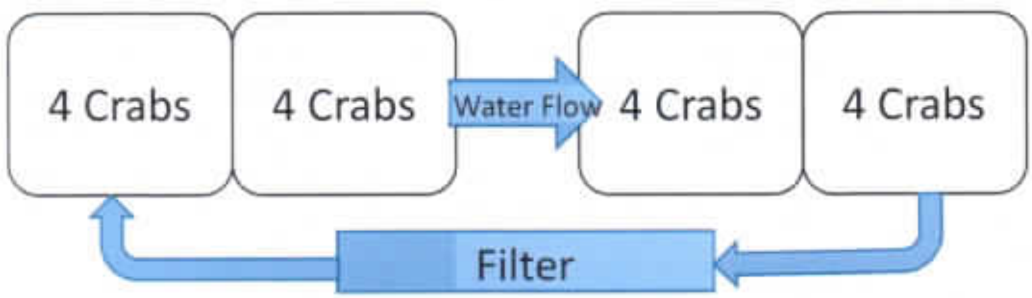

Figure 1. The crabs' storage tank set up for when they are not being tested

Scent Creation

The scents will be derived from clam, shrimp, menhaden and bluefish. 20 grams of clam were added into a blender with 300ml of saltwater (1.02ppt) The liquid was then strained into an empty beaker using filter paper and a funnel. The remaining meat of the fish was disposed appropriately. The mixture was preserved in a refrigerator until experimentation. This was repeated with individually for shrimp, menhaden and bluefish.

Preconditioning

A crab was taken out from any tank and placed in a separate tank by itself. The crab was put in a separate 3-gallon tank with 1.02 ppt saltwater. While the clam acclimated to the new tank, the order of the scents was randomly chosen. Each scent (clam, shrimp, menhaden and bluefish) was written down on a piece of loose leaf five times. The paper was ripped and all the scent names on little pieces of paper were put in a bucket. The scent names were pulled out of the bucket in a random order and written down in the logbook. The crab was given 5 µl of each scent in the order that it was written down in. The crab's reaction was recorded upon consensus. The researchers wrote down the crabs' reaction based on the definitions (refer to Table 1). If there was no consensus, there was a discussion as to why the reaction was written down and the researchers will agree on the most likely behavior of the crab. This was repeated with all 16 crabs. After the testing of each crab, the water in the separate container was poured into the sink, and it was filled with new 1.02 ppt saltwater.

Table 1. Crab reactions to stimulus.

Mandibular Movement	The mandibles (an exterior portion of the mouth) will move or be groomed
Grabbing	The crab will make a direct movement for the pipette or scent cloud
Searching	The crab will begin sifting through the rocks/ moving its claws while its mandibles are moving
False Food	The crab will pick up rocks/sediment and attempt to eat it
No reaction	The crab displays no movement
Active Avoidance	The crab will begin to run away, or attempt to push itself away with its claws
Burrowing	The crab will use its legs to push sediment and rocks aside and burrow itself
Retracting	The crab will bring its legs and claws tight to its body and demonstrate eye retraction

Aversive Conditioning

The 4 different groups of crabs were assigned a scent to be aversively conditioned (Group 1 was clam, group 2 was shrimp, group 3 was bunker, and group 4 was Bluefish). Each crab was aversively conditioned by itself in the separate tank. When given 5 μl of the scent, the pseudo predator was introduced and waved around in the rocks to disturb the crab. The PVC pipe which acted as the pseudo predator was 30 cm in length and 2 cm wide. The pseudo predator did not touch or hit the crabs. The crab's reaction was recorded upon consensus. The researchers wrote down the crabs' reaction based on the definitions (refer to Table 1). If there was no consensus, there was a discussion as to why the reaction was written down and the researchers will agree on the most likely behavior of the crab. Each crab was given its scent that it was assigned 20 times. After the testing of each crab, the water in the separate container was poured into the sink, and it was filled with new 1.02 ppt saltwater. Each crab was conditioned to their appropriate scent.

Post-Conditioning

The process used in the preconditioning stage was repeated with the 16 crabs. All reactions were recorded and analyzed.

Results

Preconditioning

During the preconditioning stage, all the crabs displayed the various natural reactions of grabbing, searching, false food, mandibular movement, or had no reaction. For the crabs that were conditioned to the clam and bunker, on average, grabbing is the most common preconditioning reaction for the four scents. Relatively, searching was the most common preconditioning reaction in the group that was conditioned to shrimp. The crabs that were conditioned to bluefish showed no reaction a majority of the time, but mandibular movement was the second highest in the preconditioning stage.

Figure 2. The preconditioning reactions performed by the crabs that were conditioned to clam.

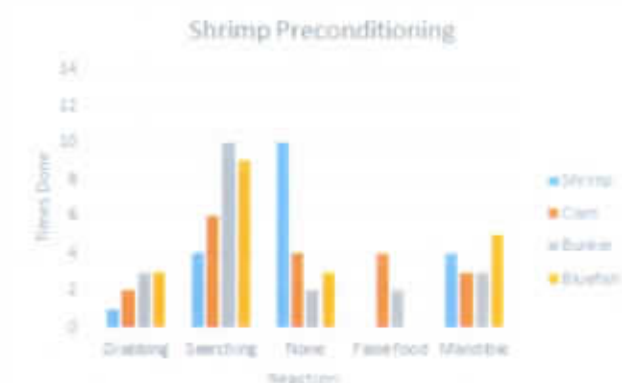

Figure 3. The preconditioning reactions performed by the crabs that were conditioned to shrimp.

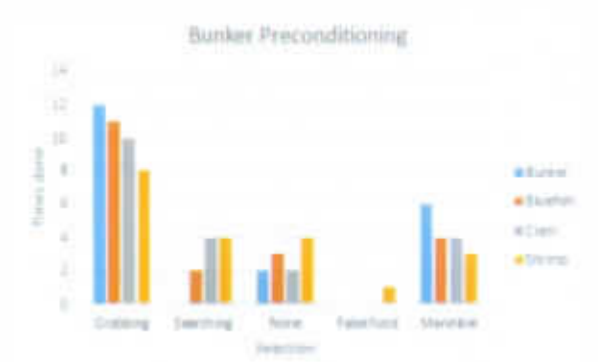

Figure 4. The preconditioning reactions performed by the crabs that were conditioned to bunker.

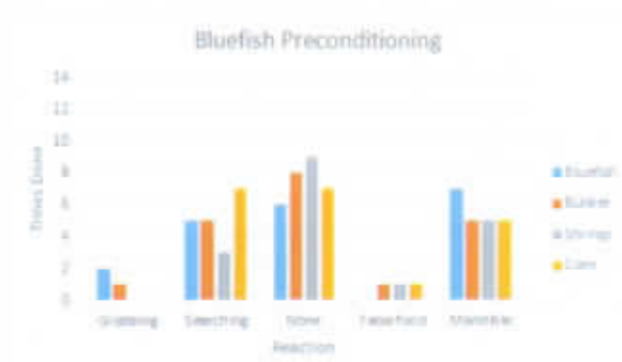

Figure 5. The preconditioning reactions performed by the crabs that were conditioned to bluefish.

The reactions of the conditioning stage showed active avoidance as the highest out of the aversive reactions during the conditioning stage of clam, shrimp, bunker, and the bluefish groups. Only aversive reactions of active avoidance, retracting, burrowing, or no reaction were displayed by the crabs.

Figure 6. Reactions performed by the clam group during the conditioning stage.

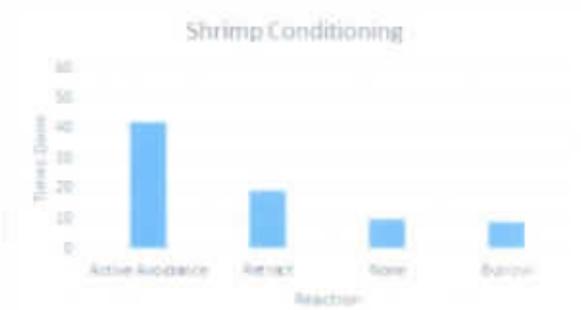

Figure 7. Reactions performed by the shrimp group during the conditioning stage.

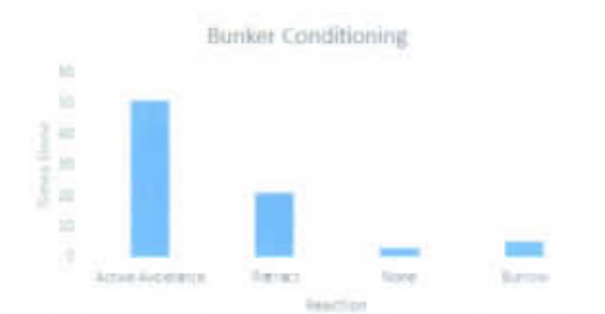

Figure 8. Reactions performed by the bunker group during the conditioning stage.

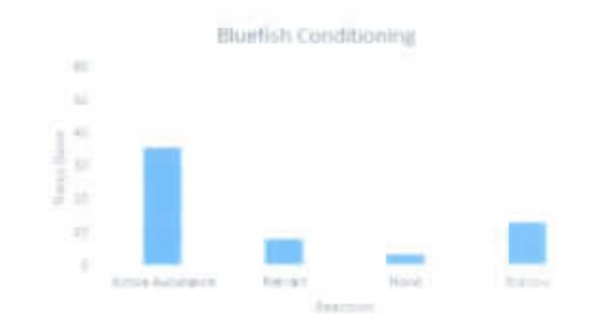

Figure 9. Reactions performed by the bluefish group during the conditioning stage.

The results from the post-conditioning confirm that green crabs are able to aversively condition to different scents. The crabs that were conditioned to the clam showed 90% aversive and 10% showed no reaction during the post-conditioning. This scent showed the best retention rate among the other crabs because they showed the highest aversive reaction. The post-conditioning results of bunker showed 80% aversive, 15% natural and 5% had no reaction. The bluefish post-conditioning reactions were 75% aversive, 23% natural, and 2% no reaction. The shrimp post-conditioning reactions remained 70% aversive, 10% natural, and 20% showed no reaction. Shrimp held the least retention rate because it showed the least amount of aversive reaction. No reaction was considered as its own category as the reactions were calculated since it could be argued both as a natural and an aversive reaction.

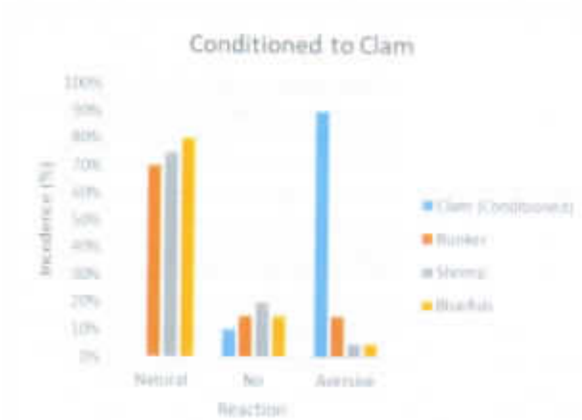

Figure 10. The percentage of natural vs aversive or no reaction for the crabs conditioned to clam.

Figure 11. The percentage of natural vs aversive or no reaction for the crabs conditioned to shrimp

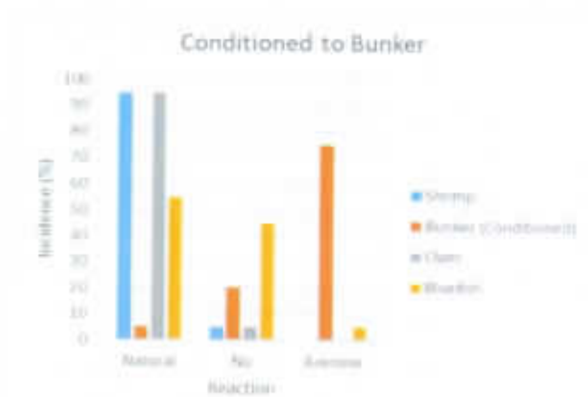

Figure 12. The percentage of natural vs aversive or no reaction for the crabs conditioned to bunker.

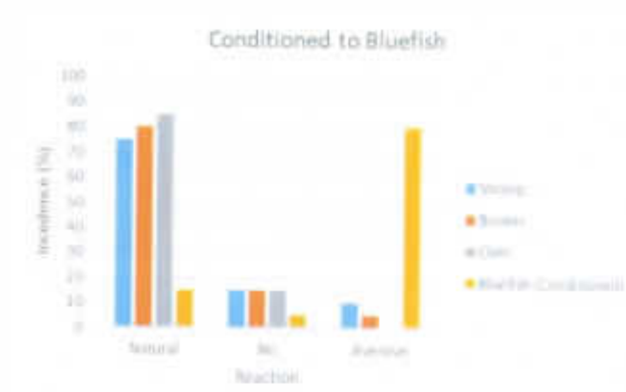

Figure 13. The percentage of natural vs aversive or no reaction for the crabs conditioned to bluefish

Discussion and Conclusion

In the post-conditioning, 70% to 90% of the crabs in each group avoided the scent that was conditioned with a *pseudo-predator*. The results were similar to those conducted in previous research. In research done by Orlosk (2011), 70% of the crabs were conditioned to go into the lit area, without food. Green crabs are a successful invasive species in the North East waters of the United States because the skill of rapidly learning increases their survival chances versus other indigenous species.

In our experiment, green crabs showed the most aversive reaction to the clam scent. Clam must have produced a distinct odor that is easily detected by the chemoreceptors, and the information is stored in the brain. It was found that on the coast of Maine, green crabs' prey on the softshell clam. According to Wilson and Walsh (2019), green crabs have decimated local clam populations in the region. The outcome of our research indicates that green crabs and possibly other crustaceans can deviate from or reverse natural habits when trained to do so.

Similar results from the bunker and bluefish suggest that they have a similar chemical composition in their odor. Their impact on the neurons in the brain of a green crab could be a result of the chemical composition in fish oils'. Within their taxonomy, both have similar orders.

The different behavior of crabs was witnessed in the data. Briffa (2008), found that hermit crabs behavioral plasticity varied between situations. The timid crabs show a lot of burrowing or retracting rather than making large movements such as active avoidance. Some crabs barely moved toward any stimulus whether it was the regular scent or the pseudo predator. Other crabs were more active and bolder. This was seen in the crabs that reacted by grabbing or searching. The different behavior of crabs affected the distribution of the data.

To conclude, green crabs were able to discriminate scents after being aversively conditioned to a scent. The data supported our hypothesis that if the green crabs are being given a negative stimulus while exposed to a scent, then they will actively avoid the scent after conditioning because they will associate the scent with the negative stimulus rather than the other scents. The crabs aversively reacted to the scents 70% to 90% of the time in post-conditioning.

Future Research

There are many possibilities for the expansion of research. First, a main aspect is to have a larger sample size of green crabs with an increased number of trials of preconditioning, conditioning, and post-conditioning. For a conclusion to be sufficiently supported, great amounts of data need to align with the concluding statements. Additionally, different scents such as oysters, flounder, or black seabass could be used to condition the green crabs. Certain scents may affect the area of the brain that that retains memory differently. This may facilitate or make it more difficult for crabs to discriminate between scents. Furthermore, the same experiment can be done on different types of crustaceans such as crayfish, or other types of crabs.