Defne Aktuna

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Research Plan

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

A. Rationale

In a natural environment, knowing the difference between a predator and prey can mean the difference between life and death. Many oceanic species will use their acute sense of smell to track or locate their next meal (Kats, 1998). Crabs have chemoreceptors on their antennae that enable them to sense their environment and identify chemicals produced by their prey in the water. Besides antennae, they also have shorter features called antennules which help them feel their surroundings. Crabs have been known to have complex brains and the ability to associate a stimulus with a behavior (Sandeman, 1992). Green crabs have also shown to be responsive to conditioning. They can be conditioned to go against their natural behavior by a reward, or penalty (Orlosk, 2011). It is advantageous for green crabs to be able to distinguish between different olfactory cues. These cues can be from a potential mate, a predator, or prey. Ignoring certain olfactory cues may result in death, failure of reproduction, or starvation (Tran, 2013). By studying a green crab's ability to distinguish scents, we can improve our understanding about green crab behavior and potentially find ways to use this knowledge to help eradicate this invasive species.

B. Hypothesis

Can Green Crabs (*Carcinus maenas*) accurately discriminate between scents after being aversively conditioned?

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. 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 this type of odor discrimination is present in an invasive decapod crustacean to use as a means to eradicate the species.

C. Procedure

Crab Collection

1. 16 green crabs will be collected at 40°54'19.5"N 73°13'53.4"W (Kings Park, NY) during the end of incoming tide. They will be caught using dip nets and crab pots filled with dead porgy.
2. The crabs will be separated into 4 groups of 4 crabs and stored in 4 tanks with saltwater (29 gallons) (1.02 ppt).
3. The crabs will have their spines on the carapace clipped so that there is a noticeable difference and individuals can be identified. This will correlate to a name and group.

Scent Creation: Clam, Shrimp, Menhaden and Bluefish

1. 20 grams of clam will be added into a blender with 300ml of saltwater (1.02ppm).
2. The liquid will then be strained into an empty beaker using filter paper and a funnel.
3. The liquid will be preserved in a refrigerator until experimentation.
4. The meat of the fish will be disposed appropriately.
5. Steps 1-4 will be repeated individually for the shrimp, menhaden and bluefish.

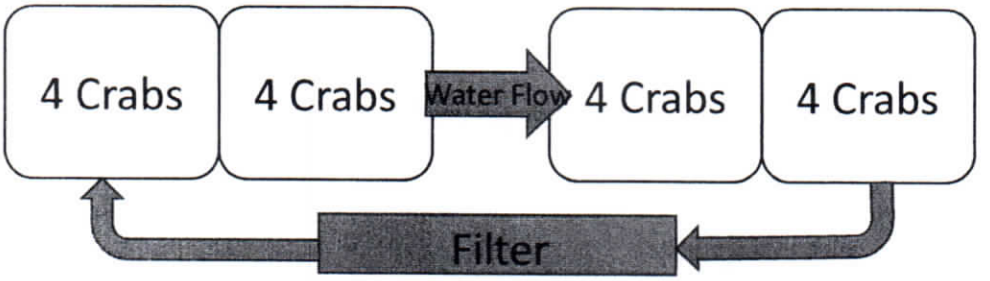

Figure 1. Simulated set up of the experimental crab tanks.

Preconditioning

1. A crab will be taken out from any tank and placed in a separate 3-gallon, 1.02 ppm saltwater tank by itself.
2. Each scent (clam, shrimp, menhaden and bluefish) will be written down on a piece of loose leaf five times. The paper will be ripped and all the scent names on little pieces of paper will be put in a bucket. The scent names will be pulled out of the bucket in a random order and written down in the logbook.
3. The crab will be given 500 µl of each scent in the order that it was written down in. The crab's immediate reaction will be recorded upon consensus. The researchers will write down the crabs' reaction based on the definitions (refer to Table 1). If there is no consensus, there will be a discussion as to why the reaction was written down and the researchers will agree on the most likely behavior of the crab.
4. This will be repeated with all 16 crabs.
5. After the testing of each crab, it will be placed in a separate bin and the water in the testing container will be poured into the sink. It will be filled with new 1.02 ppm 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

Conditioning

1. The 4 different groups will be assigned a scent to be aversively conditioned (Group 1 is Clam, 2 is Shrimp, 3 is Menhaden and 4 is Bluefish).
2. 500 µl of the conditioned scent will be added using a pipette near the mandible of the crab.
3. After 10 seconds, a pseudo predator will be introduced and move in a rapid and random motion. A PVC pipe will act as the pseudo predator and will be 30 cm in length and 2 cm wide.
4. The behavior of the crab will be recorded based off of the guidelines in Table 1. The crab's immediate reaction will be recorded upon consensus. The researchers will write down the crabs' reaction based on the definitions (refer to Table 1). If there is no

consensus, there will be a discussion as to why the reaction was written down and the researchers will agree on the most likely behavior of the crab.

5. After the testing of each crab, it will be placed in a separate bin and the water in the testing container will be poured into the sink. It will be filled with new 1.02 ppm saltwater.
6. This process will be repeated for all 16 crabs and each crab will be conditioned to its appropriate scent.

Post Conditioning

1. The process used in the pre-conditioning stage was repeated with the 16 crabs. No pseudo predator was used. All reactions were recorded and analyzed.

Risk and Safety

Green crabs have strong dactyls and can cause injury when handled improperly. Safety gloves and nets will be used when the crabs need to be moved.

Data Analysis

The crab's immediate reaction will be recorded and imputed into Microsoft Excel. We will then determine the percentage of the crab's reaction and compare the preconditioning, conditioning and post-conditioning to determine if there was a aversive reaction to the conditioned scent.

D. References

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NO ADDENDUMS EXIST