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**CHEMOSENSATION IN BLUE CRABS AND THEIR REACTION  
TO ATTRACTIVE AND AVERSIVE CUES**

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**CHEMOSENSATION IN BLUE CRABS AND THEIR REACTION  
TO ATTRACTIVE AND AVERSIVE CUES**

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## **CHAPTER 1**

### **INTRODUCTION**

Animals in nature commonly use their olfactory systems for a variety of critical processes such as finding food or detecting predators (Johnson and Atema 2005; Steullet et al. 2001). In these situations animals must simultaneously discriminate among different types of odors, such as predators and food, and make the appropriate reaction to hide from predators or locate food; a wrong detection could result in the animal's demise. Although various animals' olfactory systems, including humans', have been studied for decades, not much was understood about the specific olfactory pathways (Christensen and Hildebrandt 2002; Horner et al. 2004). Currently, what is known is that many animals, such as insects and crustaceans, have differentiated olfactory systems; this means that they have more than one independent sensory population in separate regions of the body. These different sensory populations are used to independently and simultaneously detect different odors. This type of olfactory system, known as chemosensation, is very different to the drastically more complicated mammalian model that has been typically studied in the past (Spehr et al. 2006; Steullet et al. 2001).

It is important for scientists to understand multiple types of olfactory systems because of the need for basic research in animal biology or ecology for future application. How any animal, in this case blue crabs, detects and reacts to their environment has great implications on the ecosystem as a whole, for example predator-prey relationships. How blue crabs sense their prey and detect a predator's presence significantly impacts not only the predator and prey specifically but every organism in the ecological setting through the

food chain or competition for resources. Understanding these processes can help scientists set up sustainable predator-prey relationships and prevent species extinction. Understanding how blue crabs' behaviors are modified by their olfactory system not only affects blue crabs. How they receive and perceive different odors (food, predator, etc.) affects their prey, their predators, and in effect, the entire food chain and ecosystem. For example, Blundon and Kennedy's research investigates the effects of blue crab food preferences and eating behaviors of different bivalves on the separate bivalve species; their preferences change the population dynamics of the bivalve species in the Chesapeake Bay (1982a; 1982b; Eggleston et al. 1992; Jackson et al. 2007). This research can aid in restoring the slowly declining bivalve species and resurrecting the balanced predator-prey dynamics that use to be in place in the Chesapeake Bay. In addition, this research has applications in such areas as robotics or the detection of the presence or level of an odor or molecule using any sort of device. Specific examples include the use of an olfactory-sensing robot based on a variety of non-human animal models to detect hazardous chemicals in various industrial sites and a metal oxide gas detection system based on both bacteria and silkworms (Distante et al. 2009; Marques et al. 2002).

The human model is not always sufficient or optimal for creating a mechanistic model for many reasons. Often, human olfactory models are too complex for a simpler tracking device (Herz 2009); in humans the olfactory system is not the primary source of sensory input, whereas it is in such animals as blue crabs (Marques et al. 2002). In addition, Martinez et al. discovered that two differentiated sensory populations on separate organs, such as in blue crabs, allows for more precise tracking methods than a single sensory organ (2006). Therefore scientists can and do find this mechanical

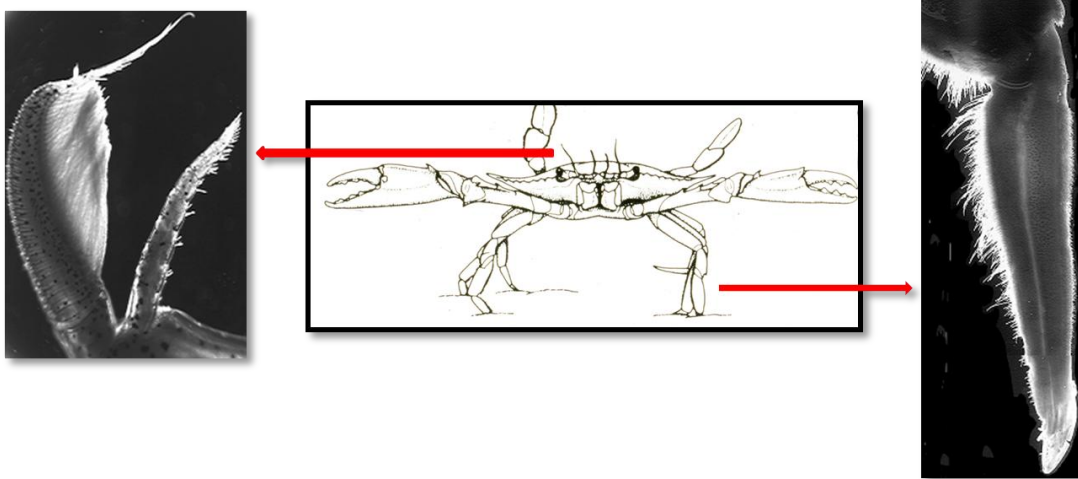
inspiration from other more efficient or simple animal models to understand the best way to create a robot or detection device in a given situation (Marques et al. 2002; Webb et al. 2004; Martinez et al. 2006; Distante et al. 2009).

Chemosensation has been studied in animal models such as lobsters, insects, and mice, and, therefore, certain hypotheses about blue crabs can be adapted from previous research (Christensen and Hildebrandt 2002; Horner et al. 2004; Spehr et al. 2006; Horner et al. 2008). Caribbean spiny lobsters, *Panulirus argus*, are known to have separate pathways with different sensory organs and different neuronal pathways. It was found that they have two separate sensilla populations; sensilla are hair-like sensors that are located on antennae between the animal's eyes and on the appendages. The first group is known as the thoracic sensilla. These are responsible for urine detection and aid in sheltering behaviors as well as food detection (Horner et al. 2008). The second group, known as the cephalic sensilla, are also responsible for food detection as well as the implementation of grooming behaviors (Schmidt and Derby 2005). This suggests that various pathways within an animal overlap in certain detection processes such as food detection. The idea of overlapping receptors in different sensory organs provides benefit to the animal. In the case of harm to a single population, the second sensilla can aid or act as a backup for the injured sensory population. Also, two sensilla populations increase sensitivity compared to one; this is because two separate groups increases the probability an animal will detect an odor at any given moment versus having only one (Horner et al. 2008; Steullet et al. 2001; Derby et al. 2001; Horner et al. 2004). This scenario of dual organ sensilla is seen in many male insects, and it has been shown that these different sensory and neuronal pathways act as independent olfactory pathways, detecting both

plant odors and female sex pheromones separately (Christensen and Hildebrandt 2002). It is known that many mammals, reptiles, amphibians, and other crustaceans have multiple sensory pathways, but, the specific detection process and which specific stimuli are detected is less understood (Spehr et al. 2006; Ptacyk and Graves 2002; Miller and Gutzke 1999; Horner et al. 2008).

Although much is known about Caribbean spiny lobsters, little is still known about blue crabs, *Callinectes sapidus*. For scientists to have the most well-rounded view of chemosensation in various animal models, it is important to understand whether animals from the suborder Pleocyemata, which includes all crabs, lobsters, shrimp, and crayfish, have the same olfactory system, or whether it is an evolutionary aspect more specific to the Caribbean spiny lobster family, Palinuridae, which encompasses only lobsters (Williams et al. 1989). If blue crabs have a unique olfactory system from Caribbean spiny lobsters, this could give professionals in the field a completely different understanding of olfaction. This research could then serve to be a different or better animal model in certain robotics scenarios (Marques et al. 2002; Keller et al. 2003; Webb et al. 2004; Martinez et al. 2006).

Similar to lobsters, as mentioned above, blue crabs have two distinctly different hair-like chemosensation organs and pathways. The pathways are the same as lobsters in that they originate in the cephalic appendages, which are on antennules located between the eyes as seen on the left side of *Figure 1*, and thoracic appendages, which are on the legs as seen on the right side of *Figure 1* (Keller et al. 2003; Moir and Weissburg 2008).



*Figure 1.* Diagram showing the cephalic and thoracic sensilla of blue crabs.

Blue crabs are a cannibalistic species, like praying mantises and komodo dragons, which means when starvation occurs they will turn to each other as a form of food (Prete and Wolfe 1992; Wagner and Wise 1996; Moir and Weissburg 2008). However, if one animal detects the scent of an injured crab, it could perceive it as an opportunity for food or as the presence of a predator. Typically crabs react to an injured crab scent as the presence of a predator and will not track, or follow the food odor to its origin (Ferner et al. 2005; Moir and Weissburg 2008).

It is currently known that in blue crabs the cephalic sensilla are responsible for detecting female sex pheromones, detecting food, and encouraging upstream movement and motivation while detecting food (Gleeson 1982; Gleeson et al. 1984; Keller et al. 2003). As mentioned above, in lobsters these sensilla are responsible for food detection and implementation of grooming behaviors (Schmit and Derby 2005; Horner et al. 2008). This shows some overlap between the two species because both cephalic sensilla aid in food detection and some type of pheromone detection. In blue crabs, the thoracic sensilla also aid in food detection, as well as body and food location, and the ability to effectively

detect and find the food odor, also known as localization (Keller et al. 2003); in lobsters, as previously mentioned, the thoracic sensilla are responsible for urine detection, sheltering behaviors, as well as food detection (Horner et al. 2008). Overlap between these species includes food detection as well as body placement, whether it is the act of finding food or shelter. It has been shown that crabs can detect a food scent and a predator scent simultaneously. A predator scent comes from an injured crab that has been hurt, theoretically by a predator. The blue crab's separate sensor populations preferentially detect these different odors similar to the well-studied insects and lobsters (Horner et al. 2008; Christensen and Hildebrandt 2002). Moir and Weissburg showed that crabs, in the presence of an attractive odor, or food, will typically track, or follow, the odor; crabs in the presence of an aversive odor, or a simulated predator, typically will not track. When given a combination of the two scents, crabs can still detect the predator and will not track (2008; Weissburg et al. 2003). However, it is currently unknown which sensilla population is responsible for predator detection.

The purpose of these experiments is to discover which sensilla population is responsible for predator detection. Deafferentation is a key element in testing the hypothesis. Removal of a sensory population, also known as deafferentation, abolishes the ability to respond to the chemical cues that the region typically detects; in short, it deactivates the specific senses (Keller et al. 2003; Horner et al. 2008). The two sensilla will be deafferented separately to test the animal's reaction to different odors, which will be discussed more in the *Methods* section. The ability to separately deafferent the sensory populations is key in testing which sensilla population is responsible. It will show how the animal's reaction changes in comparison with the control animals.

To test whether the cephalic sensilla, thoracic sensilla, or both are responsible for predator detection, a combination of deafferentations and odor combinations must occur. It is expected, based from previous research, that a non-deafferented crab will track an attractive odor and will not track a conflicting odor (Moir and Weissburg 2008). It is also expected that deafferentation of the cephalic sensilla will cause the crabs to have reduced tracking to a food odor due to the fact that both sensilla aid in food detection (Keller et al. 2003). The experiment will test how a cephalic and thoracic deafferented crab will react to a conflicting odor of attractive and aversive cues.

In addition to solely looking at tracking patterns of the crabs as evidence for the hypothesis, there are other mechanistic functions that can aid in the analysis. It is known from Keller et al. that when crabs have either their cephalic or appendage sensilla removed they can and will typically track an attractive odor using only one set of sensilla; however, their tracking speeds are reduced compared to crabs with all of their sensilla intact (2003). This is true because the crab uses both cephalic and appendage sensilla independently to track food. The deafferentation will eliminate half of the animal's food tracking sensilla, and, therefore, will reduce the speed (Derby et al. 2001; Steullet et al. 2001; Horner et al. 2004).

However, it is unknown how the deafferented and non-deafferented crabs' tracking behavior and mechanisms will be affected by the aversive cue and the conflicting cue. From previous research it is believed that a non-deafferented, or normal, crab will typically not track in these conflicting cues; but if this does occur, the speed will be dramatically reduced due to the presence of the aversive cue, which acts as an inhibitor to the animal (Moir and Weissburg 2008; Weissburg et al. 2003). When looking

at tracking mechanisms, one must look at path linearity, side bias, and the time it takes for crab to initiate tracking in addition to total velocity. It is thought an intact crab will have better path linearity than a deafferented crab tracking the same substance due to the inhibition of a sensory population. It is also thought that an intact crab will have better ability to find and stay on the side of the attractive odor versus a deafferented animal.

Exit time is the time it takes for the crab to leave the starting arena; continuing with previous reasoning an intact crab should be able to detect the attractive odor more quickly and leave the arena faster than a deafferented animal. This data will aid the analysis and give insight to how the deafferentation of the cephalic or thoracic sensilla affects the animal's response.

## CHAPTER 2

### EXPERIMENTAL METHODS

#### Animal Collection and Maintenance

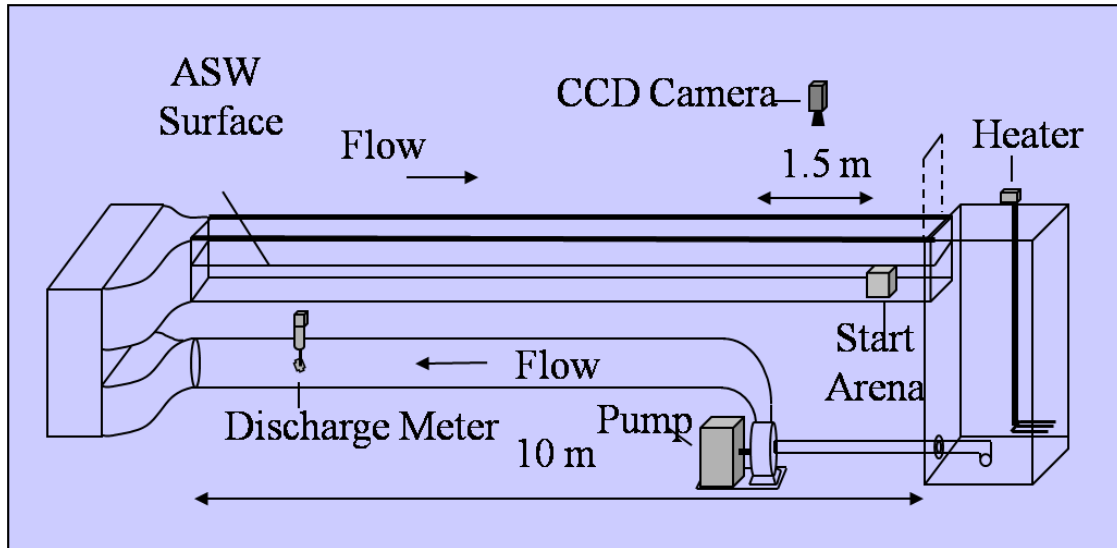
The blue crabs were caught off the coast of Skidaway Island, GA. They were brought to Atlanta, tagged for identification, and housed in holding tanks. The holding tanks are 50 gal flow-through tanks, which are filtered with protein skimmers and carbon particle and UV filters. Salinity, nitrate, phosphate, and ammonia levels were checked weekly to ensure a salinity of 27-30 ppt. and to keep the chemical levels at a healthy level. Water was changed as necessary with fluctuations in the previously mentioned levels and kept at a temperature of  $20\pm 1^{\circ}\text{C}$  (Moir and Weissburg 2008).

#### Solution Preparation

The attractive cue was created by soaking 7 g/L of shelled shrimp wrapped in mesh in saltwater for one hour. The aversive injured crab scent was created by soaking an injured crab in 3.5 L of saltwater for 3 hours (Moir and Weissburg 2008). These scents were delivered into a flume simultaneously through small nozzles using air pressure; the specifics of the process will be discussed more in the next section, *Flume and Apparatus Preparation*.

### Flume and Apparatus Preparation

Experiments were held in a 12m x 0.75m into a re-circulating seawater flume as seen in *Figure 2*. The water velocity was held constant at a  $5 \text{ cm} \cdot \text{s}^{-1}$ , which is a speed



*Figure 2*. Flume set-up diagram

that is environmentally realistic for blue crabs in the wild (Moir and Weissburg 2008).

The water depth was 0.2 m. Prepared solutions were expelled through two pressure-regulated nozzle apparatus each placed 4 in. apart from each other and 2.5 cm. from the bottom of the flume. The diameter of the nozzles was 4.7 mm. These specifications were found to be optimal for odor delivery to a medium to large sized crab in similar flumes from previous research due to the size of the crab and the water velocity (Jackson et. al. 2003; Smee and Weissburg 2006; Moir and Weissburg 2008) and have been adapted to this current research.

## **Deafferentation**

Deafferentation is the removal of a sensory population. In this experiment, the cephalic or thoracic sensilla are being removed. The crab's reaction to specific odors in combination with deafferentation will aid in determining the hypothesis, which sensory population is responsible for predator scent detection. Cephalic deafferentation is conducted by removing the cephalic sensilla from the crab's antennules (as seen in *Figure 1* in the Introduction section) with a scalpel. During this time the gills are moistened with a damp seawater cloth, which is located on the ventral side of the crab near the mouth. The crab was exposed to cold temperatures of approximately 0°F for about 30 minutes to slow down its movement, and a microscope was used to aid in seeing the small sensilla. Control crabs that are not deafferented are referred to as shams. These animals go through the same process as a deafferented animal. They are cooled, placed under a microscope, and have a metal object rubbed against its sensilla, but the actual sensilla are left intact. This was done to ensure that the animal's reaction was due to the cues and not due to the stress of the procedure. Typically a cephalic deafferented crab was tested within three days, which is consistent with other studies using this technique (Horner et al. 2003 and Keller et al. 2008).

Thoracic deafferentation is a chemically mediated process versus a mechanical process like in the cephalic deafferentation. Again, the crabs were exposed to low temperatures in order to slow their movements and make them easier to work with.

Syringe tubes were secured around each of the crab's walking legs and claws, as seen in *Figure 3*, and covered the tips of the legs where the thoracic sensilla are. Deionized



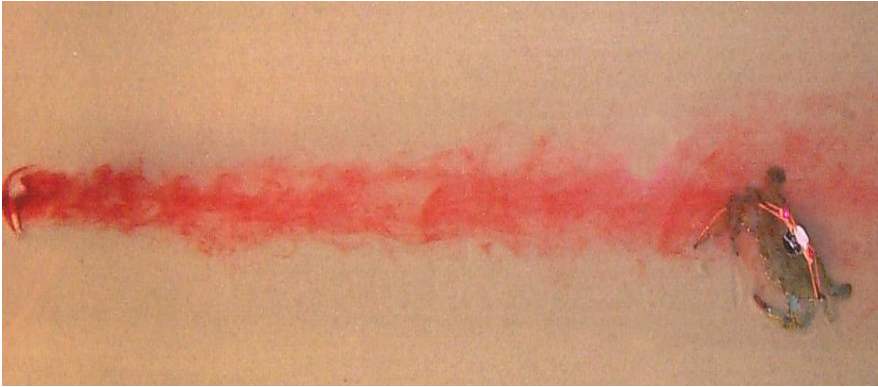
*Figure 3.* Thoracic deafferentation process

water was then poured into each test tube for approximately 15 minutes; this disrupts the osmotic balance of the legs and eliminates sensory abilities for approximately 12 hours (Derby and Atema 1982; Gleeson et al. 1997). This method was different than the cephalic deafferentation because it is impossible to physically remove the sensilla without disrupting other mechanical-sensory sensilla (Gleeson et al. 1997). These process are well outlined and supported in various previous research, and more detailed outlines can be found there (Derby and Atema 1982; Gleeson et al. 1997). A control, or sham, was

also necessary for the thoracic sensilla. Shams were placed in the same scenario with test tubes surrounding each leg, but instead of deionized water, regular salt water was used as to not affect the osmotic gradient and keep the sensory abilities intact. These experiments were typically executed 1-3 hours after deafferentation (Horner et al. 2003; Keller et al. 2007).

### **Experimental Design**

To begin my experiments, I recorded the sex, size, and tag marker of each crab as well as place a glow stick on its carapace for more efficient monitoring, which will be described more in the next section, *Flow/Video Analysis*. The tag markers are used to tell the crabs apart because the same crab is not used for more than one experiment. The crab was then placed in a small holding box 1.5 m downstream from the sources to ensure the appropriate height and width for crab scent detection. The lights were turned off in order to keep any outside light or movements from interfering with the crab's detection process. Crabs stayed in the holding box for 20 minutes to allow time for adjustment to the environment. After 20 minutes, the cue-dispensing apparatus was turned on to allow the crab to sense the downstream odor. After 10 minutes of having the odor present, the gate was removed and the crab had 15 minutes to react by tracking the attractive cue or



*Figure 4.* Crab perceiving stimulus from downstream nozzle

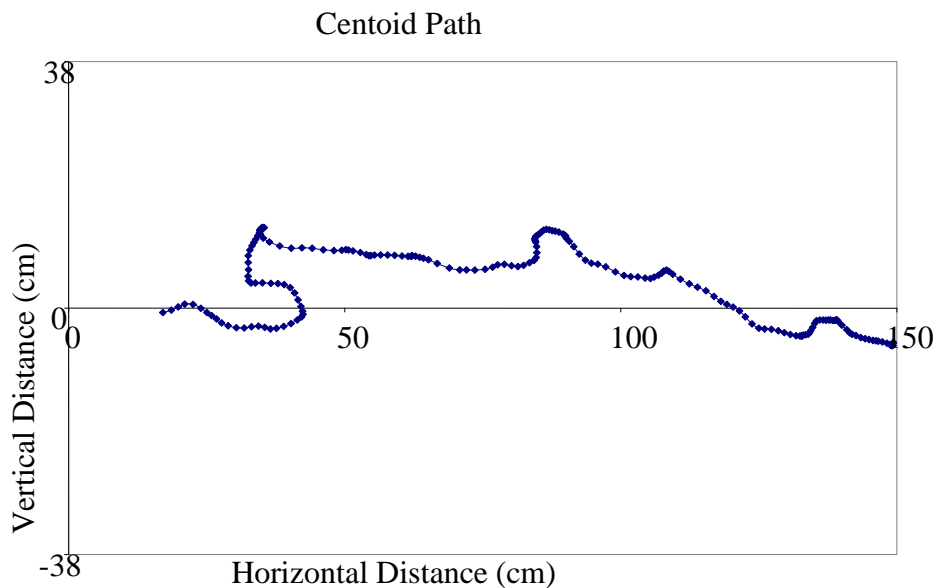
not tracking the attractive cue. *Figure 4* shows what the stimulus dispersion looks like in the flume. All observations were recorded at this time. The experiments will be separated into four separate groups to test the various crabs' reaction to the attractive and conflicting odors.

### **Crab Tracking/Video Analysis**

During this process each animal's movements were recorded with a mounted camera elevated approximately 2 meters above the flume looking directly down on the flume. The camera was able to capture the animal's movements in the dark due to the fluorescent glow stick, which is 2.5 cm long and 4 mm in diameter, attached to the animal's back. Motion analysis was conducted on all of the animals that successfully tracked or found the origin of the food scent using Motion Analysis VP110 equipment (Keller et al. 2003; Moir and Weissburg 2008). This equipment digitized the light emitted by the glow stick at 30 Hz to allow the path to be seen on a computer screen. The final kinematics were expressed at a rate of 5 Hz. Different kinematics that were analyzed were total velocity, NGDR or net-to-gross displacement ratio, and side bias, the side of

the flume the animal tended to stay on. In NGDR or linearity of the path; 1 represents a completely straight path from origin to destination and in side bias 1 represents an animal that spent all of its time on the side of the attractive odor (Moir and Weissburg 2008).

*Figure 5* is an example of the documented path the Motion Analysis VP110 will give. It shows the path the animal took to track an attractive cue. The Motion Analysis VP110



*Figure 5.* An example of a crab's tracking patterns.

as gives a compilation of velocity, NGDR, and side bias that helps us interpret the animal's path. This information is not given in *Figure 5*.

### Statistical Analysis

Chi-square analysis was completed to investigate whether the crab tracked or did not track in each of the four experimental groups. The groups are *sham attractive* (sham crabs in the presence of only an attractive cue), *sham conflicting* (sham crabs in the

presence of both an attractive and aversive cue), *deaff attractive* (cephalic deafferented crabs in the presence of only an attractive cue), and *deaf conflicting* (cephalic deafferented crabs in the presence of an attractive and aversive cue). Statistical analysis was also performed on the kinematics of the animal's tracking paths. Using the Motion Analysis VP110 data, as mentioned in the *Flow/Video Analysis* in the section above, a one-way ANOVA was performed to look at velocity, NGDR, and side-bias. An arcsine transformation of the data had to be taken for the NGDR and side bias groups because of the proportions are not normally distributed and to remove the variance/mean codependency. The Mann-Whitney Test was performed on the exit time data. This type of statistics is non-parametric which does not assume a probability distribution, or a bell-shaped curve scenario, which is necessary in this situation due to the limit.

## CHAPTER 3

### RESULTS

Because of unforeseen circumstances, the animals quit tracking completely which prevented beginning the thoracic deafferentation experiments. In addition, cold temperatures prevented animal collection and caused these experiments to never be able to be completed. Therefore, only the cephalic deafferentation experiments were performed.

#### **Tracking vs. Non-Tracking**

The tracking data supports previous research as well as indicating that the cephalic sensilla are responsible for detecting the predator scent. As seen in *Figure 6*, 6/6 or 100% of the *sham attractive* tracked while only 1/14 or 7% of the *sham conflicting* tracked; a statistical significance was found ( $X^2=15.918$ ,  $df =3$ ,  $p=0$ ). This shows that a crab's tracking decisions (whether to track or not track) are due the specific odor present; an intact crab typically tracks in the presence of an attractive odor, and will not track in

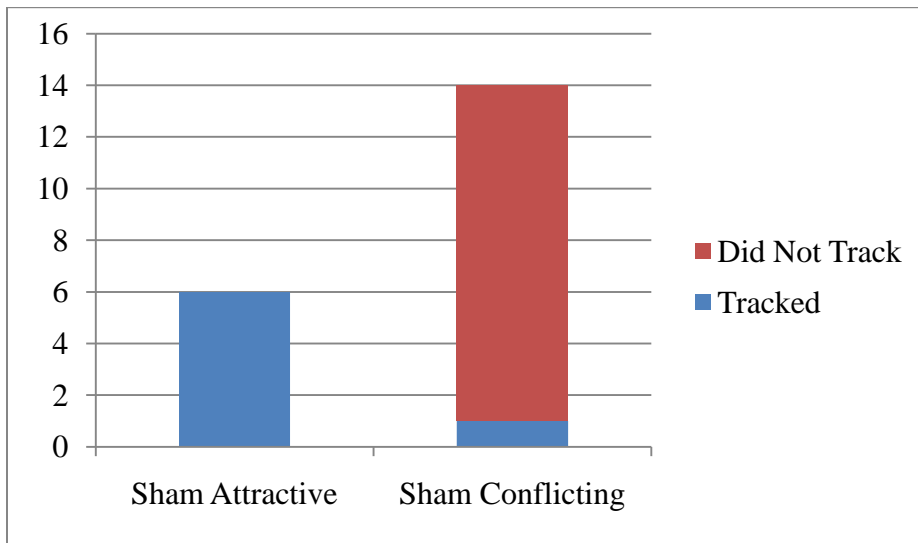


Figure 6. Number of crabs that tracked vs. did not track in sham groups.

the presence of an aversive odor, even if the attractive odor is still present. Figure 7 shows that the *deaff attractive* tracked 6/10 or 60% of the time while the *deaff conflicting* tracked 9/19 or 47% of the time. There is no statistical significance in this situation

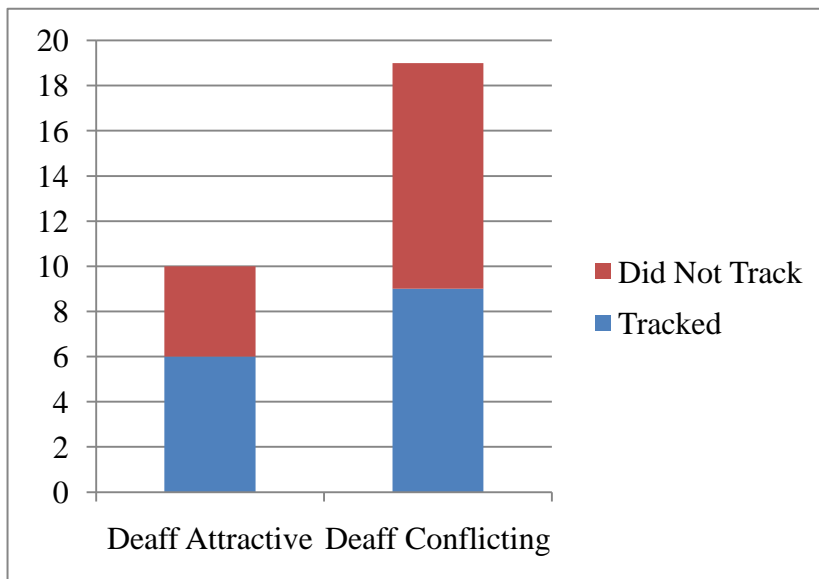
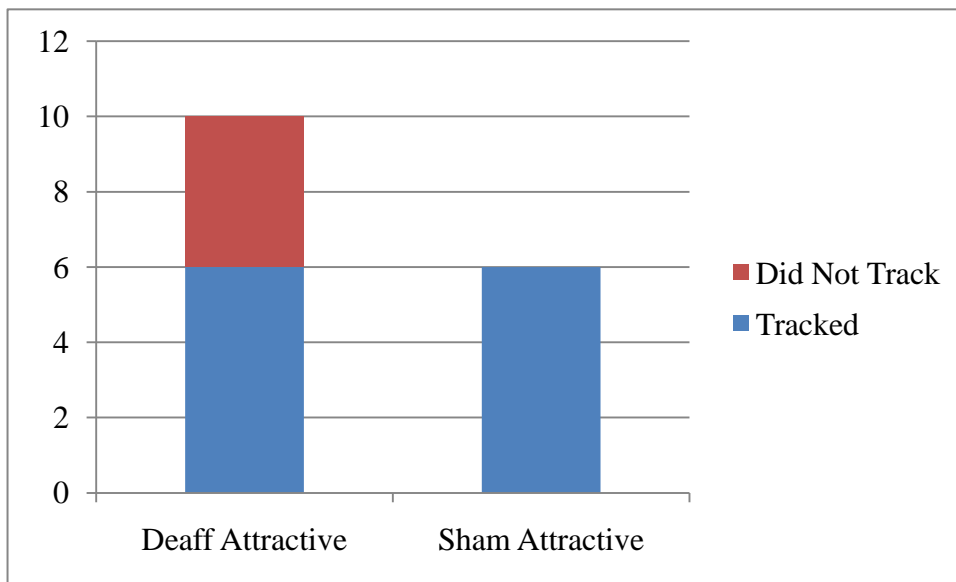


Figure 7. Number of crabs that tracked vs. did not track in a deaf group.

( $X^2=0.418$ ,  $df=3$ ,  $p=0.518$ ). *Figure 8* shows that the *sham attractive* tracked 6/10 or 60% and *deaff attractive* tracked 6/6 or 100% of the time. *Figure 8* has marginally significant results ( $X^2=3.2$ ,  $df=3$ ,  $p=0.074$ ). This shows that cephalic deafferentation affects the crab's decision of whether to track or not track, changing its typical decision of tracking



*Figure 8.* Number of crabs that tracked vs. did not track in an attractive odor.

(100%) to only tracking 60% of the time. *Figure 9* shows that the *sham conflicting* tracks 9/19 or 47% of the time and *deaff conflicting* tracks 1/14 or 7% of the time.

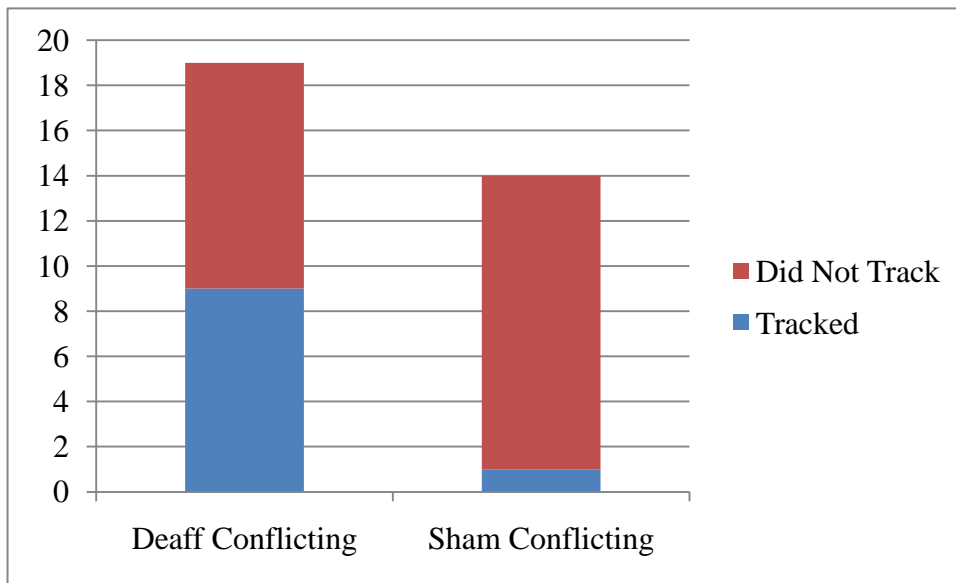


Figure 9. Number of crabs that tracked vs. did not track in a conflicting odor

This shows that deafferentation of the cephalic sensilla may be the cause of the crabs' decision to track or not track in the presence of a conflicting cue and therefore may be responsible for the detection of the aversive/predator scent ( $X^2=6.17$ ,  $df=3$ ,  $p=0.013$ ).

### Kinematics

All kinematic analyses (velocity, NDGR, side-bias, and exit time) are analyzed on only the animals that successfully tracked the attractive cue to try to understand the differences behind the searching patterns of deafferented vs. sham animals. Although there are four experimental groups, *deaff conflicting*, *sham attractive*, and *deaff attractive*, only three groups could be analyzed due to the limitations of the specific statistical analyses because the group *sham conflicting* had only one animal that successfully tracked. The ANOVA was used to test velocity, and there was no statistical

significance found between the three tested groups ( $F=1.47$ ,  $df=2$ ,  $p=0.259$ ). The averages, standard deviation, and standard error are given for references in *Table 1*. The

	Deaff Conflicting	Sham Attractive	Deaff Attractive
Average	5.797	8.106	8.229
Standard Deviation	2.231	3.790	4.298
Standard Error	0.673	1.547	2.482
N	11	6	3

*Table 1.* Individual velocity data of crabs that tracked

ANOVA for NGDR, path linearity, was found to be marginally insignificant ( $F=2.62$ ,  $df=2$ ,  $p=0.102$ ). *Table 2* shows the average, standard deviation, and the standard error of

	Deaff Conflicting	Sham Attractive	Deaff Attractive
Average	0.566	0.788	0.640
Standard Deviation	0.193	0.146	0.300
Standard Error	0.058	0.060	0.173
N	11	6	3

*Table 2.* Individual path linearity/NDGR data of crabs that tracked

NDGR for all crabs that successfully tracked. In NDGR analysis, 1 means complete path linearity and 0 means no path linearity; the *sham attractive* group has the highest path linearity with the *deaff conflicting* have the lowest. Side bias was found to have no

notable connection between the groups ( $F=0.32$ ,  $df=2$ ,  $p=0.73$ ). Three Mann-Whitney tests were performed on the exit time data between the three groups to make a comparison to see whether the exit time is affected by deafferentation of the cephalic sensilla. The comparison between the *deaff attractive* and *deaff conflicting* groups was shown to have marginal statistical significance ( $W=11.5$ ,  $significance=0.1017$ ) while the comparisons between the *deaff attractive* and the *sham attractive* ( $W=31.5$ ,  $significance=0.795$ ), and the *deaff conflicting* and the *sham attractive* ( $W=105.5$ ,  $significance=0.546$ ) were not statistically significant. This indicates that the deafferentation process most likely has no effect on exit time. *Figure 10* shows the various averages, distribution, dispersion and outliers of the data for exit times. The bottom on the box indicates the 25<sup>th</sup> percentile, while the top represents the 75<sup>th</sup> percentile of the average number. The middle line is the 50<sup>th</sup> percentile, or the average. Because there was only one animal in the sham conflicting group, no box was able to be created, and simply the only number was plotted. The asterisk is indicative of an outlier that is excluded from the other average sets.

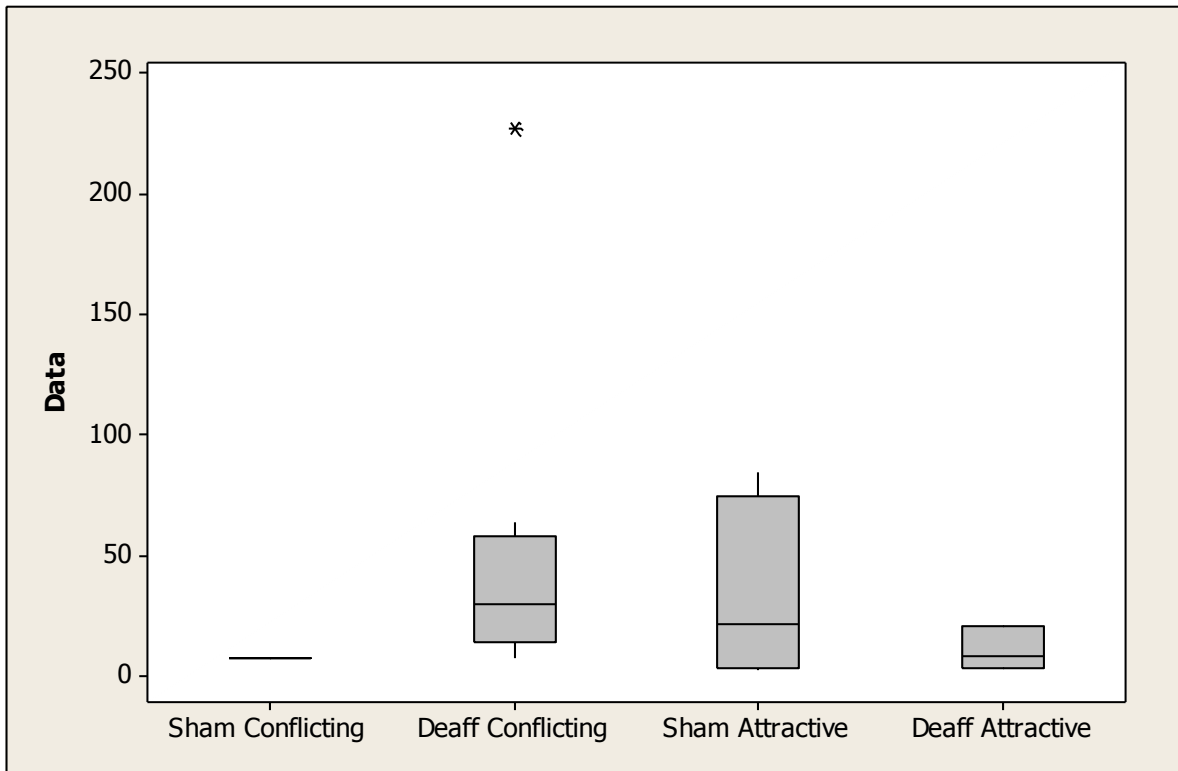


Figure 10. Exit time data of crabs who tracked

## CHAPTER 4

### DISCUSSION

It was known previously that the different sensory populations of blue crabs reacted to different odors (Schmidt and Derby 2005 and Keller et al 2008). It was also known that crabs typically track an attractive food odor, but will not track it in the presence of an aversive odor (Moir and Weissburg 2008). However, it was unknown which sensilla population is responsible for detecting and differentiating the aversive cue, or injured crab, odor. *Figure 7* shows that deafferented crabs track both attractive and conflicting odors at frequencies; 6 out of 10 (60%) in the attractive only, and 9 out of 19 (47%) in the conflicting treatment. This shows that the aversive cue does not affect the cephalic deafferented crab's ability to track in comparison to the *deaff attractive* animals. This is evidence that the cephalic deafferented crab is unable to detect the aversive odor, and therefore tracks in a manner of a deaff crab tracking only an attractive odor. *Figure 8* shows that deafferentation changes how a sham crab would typically track an attractive odor. This gives evidence that removing the cephalic sensilla hinders the ability for a crab to track an attractive odor. This supports previous research that both sensilla (cephalic and thoracic) are responsible for food detection and deafferenting one sensory population will hinder the ability to track the odor (Keller et al. 2004). *Figure 9* shows that deafferentation affects a sham crab's reaction to the conflicting cue. Once the cephalic sensilla are removed the crab begins to track in a similar way a deafferented crab tracks an attractive as seen in *Figure 7*. This indicates that the crab without its cephalic sensilla

cannot detect the aversive odor in the conflicting cue. Therefore, the conclusion can be drawn that the cephalic sensilla are responsible for the detection of an aversive cue or a predator.

Although differences in animal velocity in each treatment are not statistically significant, there is a notable pattern. *Deaff attractive* and *sham attractive* have a similar averages compared to *deaff conflicting*. It makes sense that *sham attractive* would have a high velocity because it still has both sensilla populations intact, and therefore could detect and track the attractive cue better. It also makes sense that *deaff conflicting* would have a low velocity because the deafferentation would remove one set of sensilla making it more difficult to accurately track the cue. It is also possible that another set of sensory populations can detect the predator scent; however, the detection could not have been strong enough to deter the crab from tracking causing the low velocity for the *deaff conflicting* group. However, *deaff attractive*, as shown in previous research, should have a lower velocity due to the deafferentation procedure also (Keller et al. 2003). This may not have been evidence in the data due to a small sample size for this group (N=3).

NGDR data supports the fact that *sham attractive* would have high path linearity because it still has its cephalic sensilla that aids in food detection and upstream motivation (Gleeson 1982; Gleeson et al. 1984; Keller et al. 2003). It would be thought, as in velocity, that both deafferented groups would have equally lower path linearity than their sham counterpart. This again could be attributed to the lower group number for *deaff attractive*.

Side bias was found to have no statistical significance. It is known that odor spreads out in a fan shape from its origination point, as seen in *Figure 4* (Weissburg et al.

2003; Jackson et al. 2007). If a crab is tracking, independent of what side the crab begins its tracking endeavors on, it will not necessarily stay on one side or another as long as the odor is still present. The odor of the attractive cue could originate from the left side, but if the crab is on the right, and can still detect the attractive odor, it has no motivation to switch sides. However, a deafferented crab could have more difficulties determining the odor source than a sham crab and could possibly have less side bias. This theory in combination with the previous idea makes side bias not the most accurate kinematics to test.

Exit times could potentially give information on how long it takes for sham versus deafferented crabs to react to the given odor. This would aid in the understanding of how deafferentation affects the animal's behavior. Although there is little statistical significance, the *deaff conflicting* group has the highest average time it took the animal to leave the box. This supports previous idea, in such topics as speed, that deafferentation will hinder the animal's ability to track an attractive substance. Although it would be expected that the *deaff attractive* group would have a high average as well, the low group number can be attributed to this discrepancy.

In an ideal situation there would be larger group numbers for each group. Also, even though thoracic deafferentation is not absolutely necessary, extra data could have made the previous arguments more grounded. However, even without this extra information many general conclusions and applications can be drawn. More information is now known about the specific reasons on how blue crabs track. This can further aid to understand predator-prey dynamics as well as try to prevent animal extinctions or

endangerments. Also, a singular sensilla population for predator detection can be another option while making dual or multiple organ sensing robots.

In conclusion, the deafferentation of cephalic sensilla makes the crab oblivious to the aversive cue. Therefore, it is likely that the cephalic sensilla are responsible for the detection of predators or aversive cues in an experimental setting. This is supported by the fact that velocity and path linearity are both reduced after cephalic deafferentation.

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