Evaluating chemoreception in the ctenophore *Bolinopsis microptera* through changes in predatory behavior

E. P. Charles

ABSTRACT

Lobate ctenophores such as the Salish Sea species *Bolinopsis microptera* serve as top-down regulators of plankton ecosystems. These generalists have been linked to declines in zooplankton and forage fish populations due to their complex predatory behavior. Prior research demonstrates that lobate ctenophores rely on mechanical cues for prey detection, but also exhibit anticipatory responses to prey prior to contact, suggesting a reliance on additional sensory cues. To determine the role of chemoreception in predatory behavior of *Bolinopsis microptera*, individuals were exposed to a crustacean olfactory cue. We did not observe any significant effect of crustacean olfactory cues on ctenophore predation behavior, however behavioral trends consistent with response to olfactory cues were observed.

INTRODUCTION

Planktonic organisms serve as the foundation for pelagic ecosystems around the world. Within these food webs, large gelatinous zooplankton such as jellyfish and ctenophores are considered vital predators for nutrient cycling and carbon export (Potter et al. 2023). As in other ecosystems, these predators serve as top-down regulators of other planktonic organisms, dictating zooplankton and phytoplankton population dynamics (Potter et al. 2023).

The ecological importance of gelatinous predators is demonstrated through the effects of the invasive lobate ctenophore *Mnemiopsis leidyi* in the Caspian Sea. Following *M. leidyi* introduction, planktonic populations were significantly disrupted, resulting in a decrease in zooplankton (Hamer et al. 2011; Khoshnood et al. 2013). Most notably, this decline in zooplankton resulted in the collapse of forage fish populations from a combination of prey competition and predation on fish larvae by *M. leidyi* (Hamer et al. 2011; Khoshnood et al. 2013). In addition to their ecological importance, these forage fish populations supported local fisheries (Khoshnood et al. 2013). Given the ecological and resulting economic impacts of predation, it is important to better understand the feeding behavior of lobate ctenophores.

While both medusae and ctenophores occupy similar ecological niches, lobate ctenophores have been found to be more efficient predators than medusae (Colin et al. 2014). Lobate ctenophores engage in active predation through generated feeding currents, resulting in higher predation success than passive predators like medusae (Colin et al. 2014). These feeding currents allow them to make direct contact with their prey, allowing them to locate it through tactile cues (Colin et al. 2014). In an experiment designed to understand *M. leidyi* feeding efficiency, researchers found that capture efficiency increased with more instances of contact with their copepod prey (Costello et al. 1999). This study indicates that lobate ctenophores such as *M. leidyi* are tactile feeders, benefitting primarily from tactile sensory cues for prey capture success (Costello et al. 1999, Moss et al. 2004). An ecologically similar species of lobate ctenophore, *Bolinopsis infundibulum*, has also been found to be a tactile predator (Sørnes & Aksnes 2004). Additionally, Sørnes and Aksnes found that the predation success of

B. infundibulum did not vary significantly under different lighting conditions, indicating that they do not rely on visual cues for predation. Locally in the Salish Sea, *Bolinopsis microptera* are present, a species only recently distinguished from *Bolinopsis infundibulum* through genetic analysis (Johnson et al. 2022).

Although current research demonstrates the importance of tactile cues in lobate ctenophore predation, chemoreception of prey has not been widely investigated. The ctenophore *Beroë cucumis*, which preys exclusively on *B. infundibulum*, has shown an increase in activity following exposure to homogenized *B. infundibulum* (Falkenhaug & Stabell 1996). Ecologically, *B. cucumis* exhibit high prey specificity, while *B. infundibulum* are known to be generalists with a high proportion of their prey consisting of copepods and crustaceans (Falkenhaug & Stabell 1996; Granhag & Hosia 2015). Similarly, *M. leidyi* are also considered to be generalists, occupying similar niches to the North Atlantic *B. infundibulum* and the Eastern Pacific species *B. microptera*, one of the driving factors of their significant impact as an invasive species (Granhag & Hosia 2015). Although chemoreception in predation has not yet been investigated in generalists, *M. leidyi* have been shown to rely on chemosensory cues for timing of spawning (Sasson et al. 2018). These studies provide evidence that lobate ctenophores have chemosensory mechanisms, but the role of chemoreception in predation by generalists such as our study species, *B. microptera*, has yet to be explored (Granhag & Hosia 2015).

To better understand chemoreception in *Bolinopsis microptera* predation we exposed individuals to olfactory cues from planktonic crustaceans while submerged in seawater with a range of background cues. This experiment is designed to assess if *B. microptera* are able to detect prey cues in complex olfactory environments to better understand the role of chemosensory perception in this ctenophore's predation behavior.

MATERIALS & METHODS

Collection and Husbandry

Twenty-eight *Bolinopsis microptera* were collected from the breakwater at Friday Harbor Laboratories (48.545100, -123.012177) from May 21st through May 27th, 2026. Collection was performed using a 500ml cup attached to the end of a 1.5m pole. Once collected, individuals were sorted by size into three categories (0.8-1.2cm, 1.3-3.0cm, 3.0-8.7cm) and housed in glass jars filled with natural seawater and placed in a sea table to maintain temperature. Water was changed once daily to maintain oxygenation and limit nitrate and nitrite accumulation.

Experimental Design

To test the role of olfaction in *Bolinopsis microptera* prey detection, we exposed 28 individuals to one of two treatments in filtered seawater on May 26th or May 27th, 2026. Homogenized planktonic crustaceans were added to 20ml of filtered seawater alongside 8 drops of fluorescein dye to create a prey olfactory cue. The cue was filtered with a coffee filter to remove any particulate. The crustacean plankton was collected from the Friday Harbor Laboratories dock (48.545100, -123.012177) using a 150μm plankton tow net with a plankton tow to 4 meters of depth. Crustaceans were isolated from other planktonic species using a pipette, and placed in filtered seawater. The concentration of crustacean plankton per milliliter in the olfactory cue was just over twice the concentration of the plankton tow sample (Table 1). Crustacean types were counted and recorded for reference (Table 1). The control treatment was 20ml of filtered seawater combined with 8 drops of fluorescein dye.

Prior to introducing the individual to the treatment tank, they were moved through two filtered seawater rinses, each in a one gallon tank. During these rinses, individuals were measured (Figure 1). At the beginning of each trial, a ctenophore was randomly selected and transferred into the experimental tank filled with 6 liters of water (16.5cm x 30.5cm x 21.6cm) and centered in a transparent plastic tube (8cm in diameter) for acclimation (Figure 2). The acclimation tube also served

to keep the individual centered in the tank prior to administering the treatment cue. Ctenophores were acclimated in this tube for 10 minutes. At the end of the acclimation period, the acclimation tube was slowly lifted to prevent damage to the ctenophore. Simultaneously, 1ml of treatment cue was added to the left side of the tank with a 3ml pipette. Behavior was recorded for 5 minutes following release from the acclimation tube. The time spent on the half of the tank with the treatment cue was recorded using a stopwatch. To monitor predation behavior, we tallied the number of times the ctenophore contacted the treatment cue, as well as the number of times it closed its feeding lobes. At the end of each trial, the ctenophore was assigned a number, and returned to an individual housing jar. A total of 14 individuals were exposed to the control cue, and 14 individuals were exposed to the crustacean olfactory cue. Cues were administered in an alternating order to standardize randomization.

Statistical Analysis

To evaluate if there was a statistical difference between the number of lobe closures and cue contacts across my control and crustacean olfactory cue, we performed a Mann-Whitney U test. The amount of time spent on either side of the trial tank was also analyzed using a Mann-Whitney U test. This data was also converted to percentage divergence from expected time on each side of the tank assuming no cue impact using the equation ((time spent in seconds) - 150 seconds)/150 seconds. Statistical tests were performed in R, and data was stored and organized in Excel. All figures were created in R.

RESULTS

The *B. microptera* collected ranged from 0.8cm to 8.7cm in length, with an average of 2.2cm and a standard deviation of 1.8cm (Figure 1). The mean number of lobe closures per trial when ctenophores were provided a control cue was 2.21 with a standard deviation of 2.55 lobe closures (Figure 3). The mean number of lobe closures in the treatment group provided a crustacean olfactory cue was 2.56 with a standard deviation of 2.30 lobe closures (Figure 3). There was no statistical difference between mean number of lobe closures across treatments (Mann-Whitney U test, W = 89.5, N = 28, p-value = 0.7072) (Figure 3).

The mean number of cue contacts per trial in the control group was 0.50 with a standard deviation of 1.09 contacts. The mean number of cue contacts per trial in the crustacean olfactory cue group was 0.93 with a standard deviation of 1.27 contacts. The means were not statistically different across treatments (Mann-Whitney U test, W = 72.5, N = 28, p-value = 0.1783) (Figure 4).

The mean time spent on the cue side of the tank when a control cue was provided was 122.9 seconds with a standard deviation of 133.1 seconds. When a crustacean olfactory cue was provided, the mean time spent on the cue side of the tank was 201.8 seconds with a standard deviation of 114.9 seconds. The means of time spent on the cue side was not statistically different across trials (Mann-Whitney U test, W = 64, N=28, p-value = 0.1214) (Figure 5).

DISCUSSION

The observed trends in predation behavior and locomotion across control and crustacean cue trials were not statistically significant. However, as hypothesized, all three behaviors we observed increased in the presence of the crustacean olfactory cue (Figure 3, Figure 4, Figure 5). Lobe closures are characteristic of prey capture behavior in lobate ctenophores (Costello et al. 1999). The increase in lobe closures across crustacean trials suggests an increase in attempted prey captures, even in the absence of mechanical cues from prey species (Figure 3). Similarly, the increase in cue contacts suggests that the *B. microptera* may be chemotactic towards prey cues, as expected if chemoreception is present (Figure 4). Additionally, the time spent on the olfactory cue side compared to the empty side of the tank suggests a slight preference towards the crustacean olfactory cue (Figure 5). Despite statistical insignificance, these results provide cause for additional research into the predation behaviors of generalist lobate ctenophores.

A different species of ctenophore, *Mnemiopsis leidyi*, exhibits anticipatory responses, positioning their feeding lobes prior to prey contact (Costello et al. 1999). This behavior is thought to be partially induced via detection of small turbations created by copepod prey, but this mechanical cue alone is not always sufficient to induce anticipatory behavior (Costello et al. 1999). Further research on the predatory behavior of *M. leidyi* suggests that passive prey detection occurs prior to contact with copepods, suggesting that these ctenophores have chemoreception (Colin et al. 2015). Additionally, there is evidence of combined chemoreceptory and mechanoreceptory organs in lobate ctenophores, supporting the finding that a combination of multiple sensory cues enables predation success (Kass-Simon & Hufnagel 1992). In concurrence with prior research, my results suggest that my study system *B. microptera* may rely jointly on olfactory and mechanical cues for prey capture, and as a result shows only slight chemotaxis in the absence of mechanical prey cues.

Ecologically, this conclusion is also supported by ctenophores' role as a predator of mobile species rather than marine snow or phytoplankton (Hamter et al. 2014; Khoshnood et al. 2013; Granhag & Hosia 2015). Ctenophores serve as top-down regulators of planktonic food chains with

wide ranging impacts from increasing carbon export to collapsing forage fish populations (Hamer et al. 2011; Khoshnood et al. 2013). As a result, research in *B. microptera* chemoreception is vital to understanding their sensory ecology and impacts on planktonic communities. To confirm these results, additional trials of this pilot study should be performed to increase statistical power. Increasing the contrast between the treatment cue and surrounding water by using UV-sterilized seawater as in Sasson et al. 2018 may also advance our understanding of *B. microptera* chemoreception specificity. Similar to prior research on other species, future research should also focus on determining the presence of other chemoreception-mediated processes in *B. microptera* such as locating conspecifics, timing reproduction, and predator avoidance (Falkenhaug & Stabell 1996; Sasson et al. 2018).

In summary, despite the statistical insignificance of the trends in behavior we observed, this pilot study's results align with our initial hypotheses. This study supports further research into the olfactory capabilities of *Bolinopsis microptera* to better understand the sensory ecology of generalist ctenophores.

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FIGURES

Table 1: Crustacean plankton counts included in the olfactory crustacean cue. Collected via plankton tow from the Friday Harbor Laboratories dock (48.545100, -123.012177) using a 150μm plankton tow net from 4 meters of depth. Individuals were identified by life stage. The average number of planktonic crustaceans per milliliter of plankton tow sample was 2.611.

Crustacean Type	**Count**
Copepoda Adult	19
Copepoda and Cirripedia Nauplius	80
Decapoda Zoea	2
Cirripedia Cyprid	1
Decapoda Shrimp	7

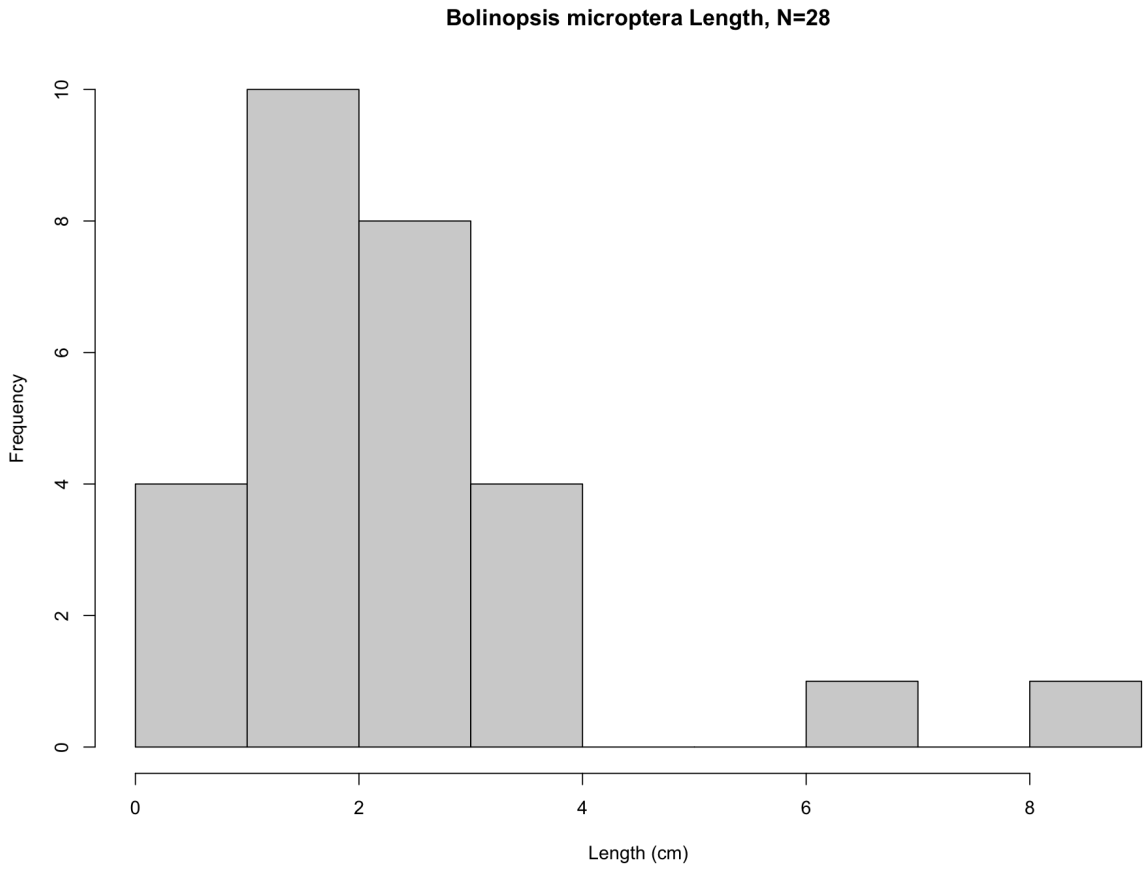

Figure 1: Length distribution of *B. microptera* used in control and crustacean olfactory cue trials. Length was measured from the tip of the feeding lobes to the tip of the aboral region.

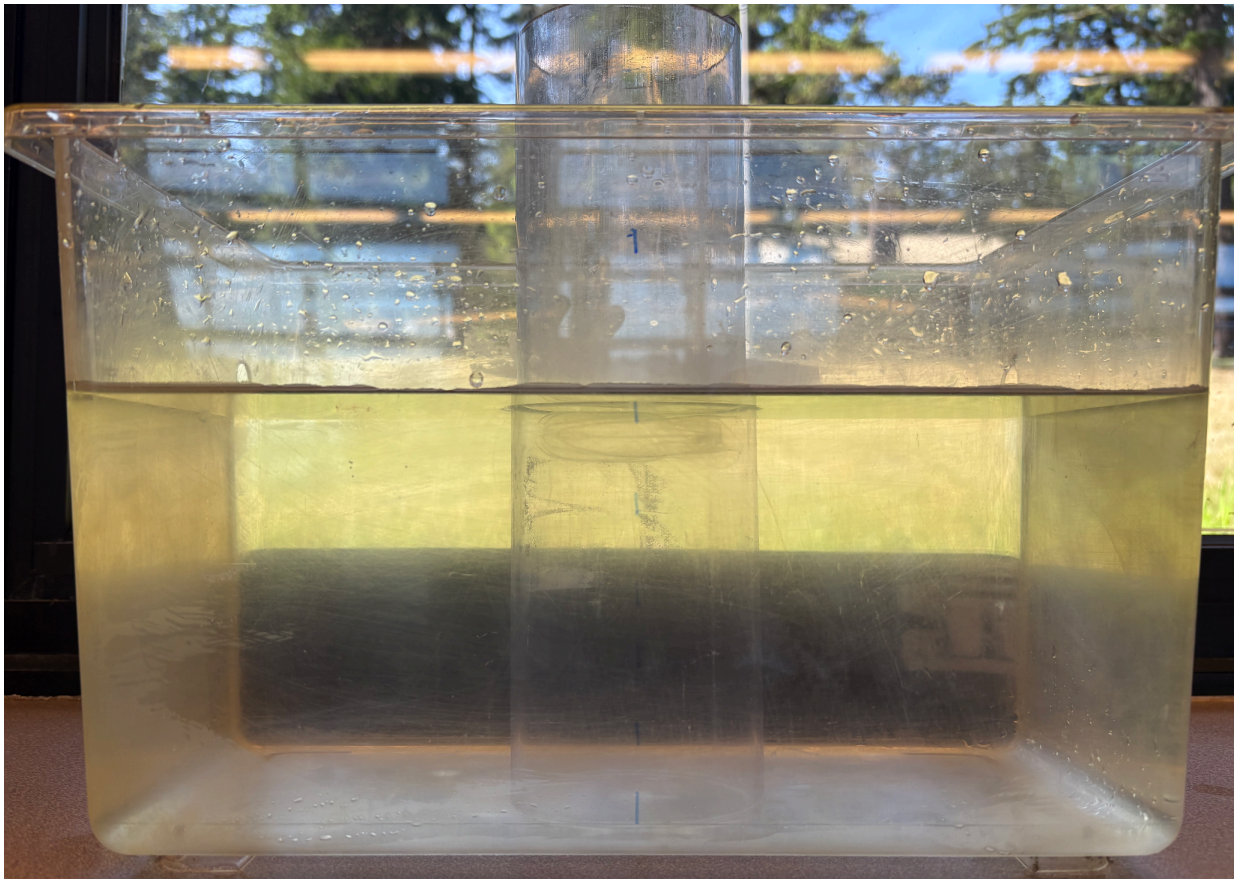

Figure 2: Treatment tank and acclimation tube. The treatment tank was 16.5cm x 30.5cm x 21.6cm. The tube was 27.5cm tall and had an internal diameter of 8cm. The tank was filled with 6L of filtered seawater prior to each trial.

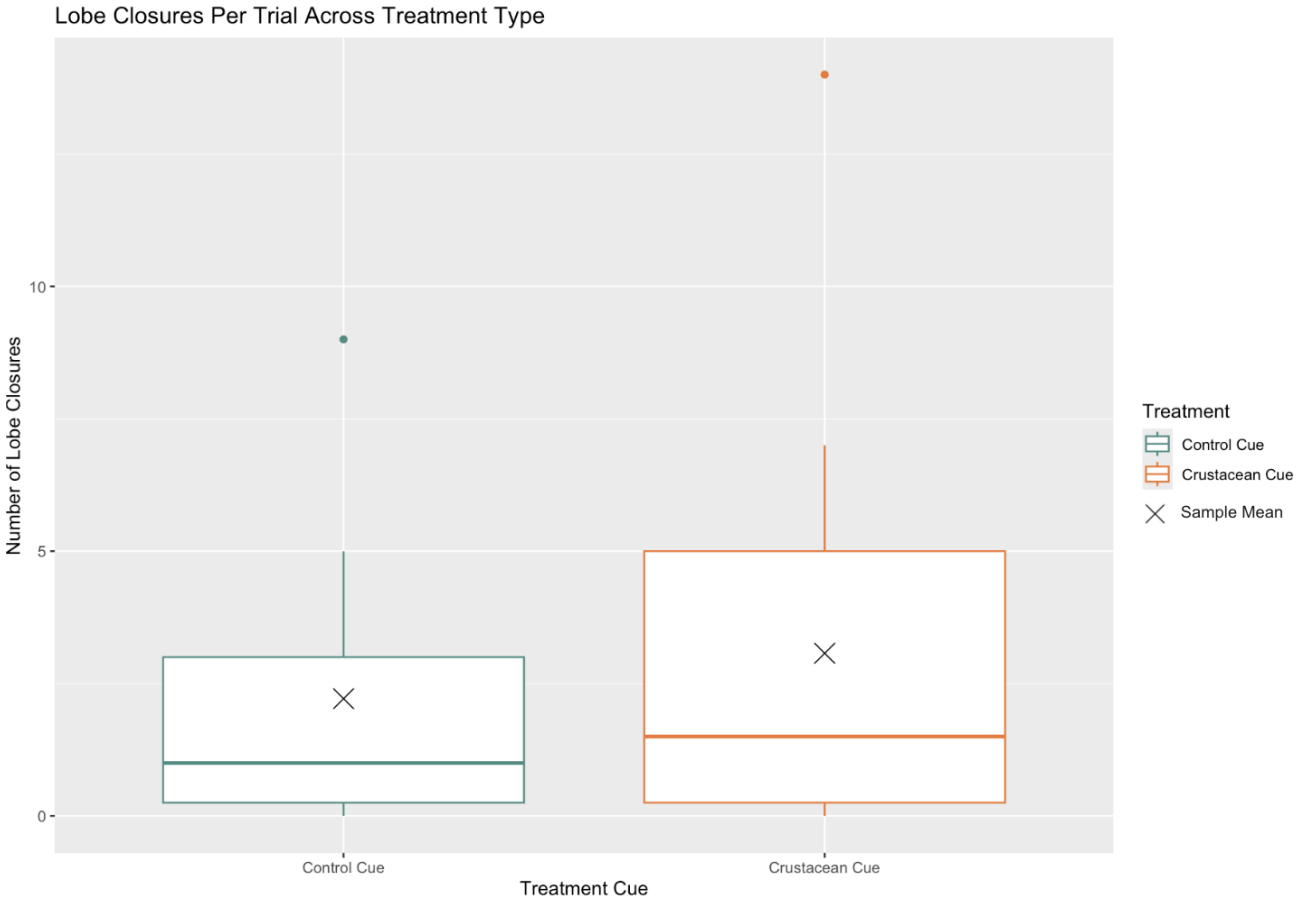

Figure 3: The number of feeding lobe closures per 5 minute trial across control and crustacean olfactory cue treatments. The mean number of feeding lobe closures across groups are not statistically different (Mann-Whitney U test, W = 89.5, N = 28, p-value = 0.7072)

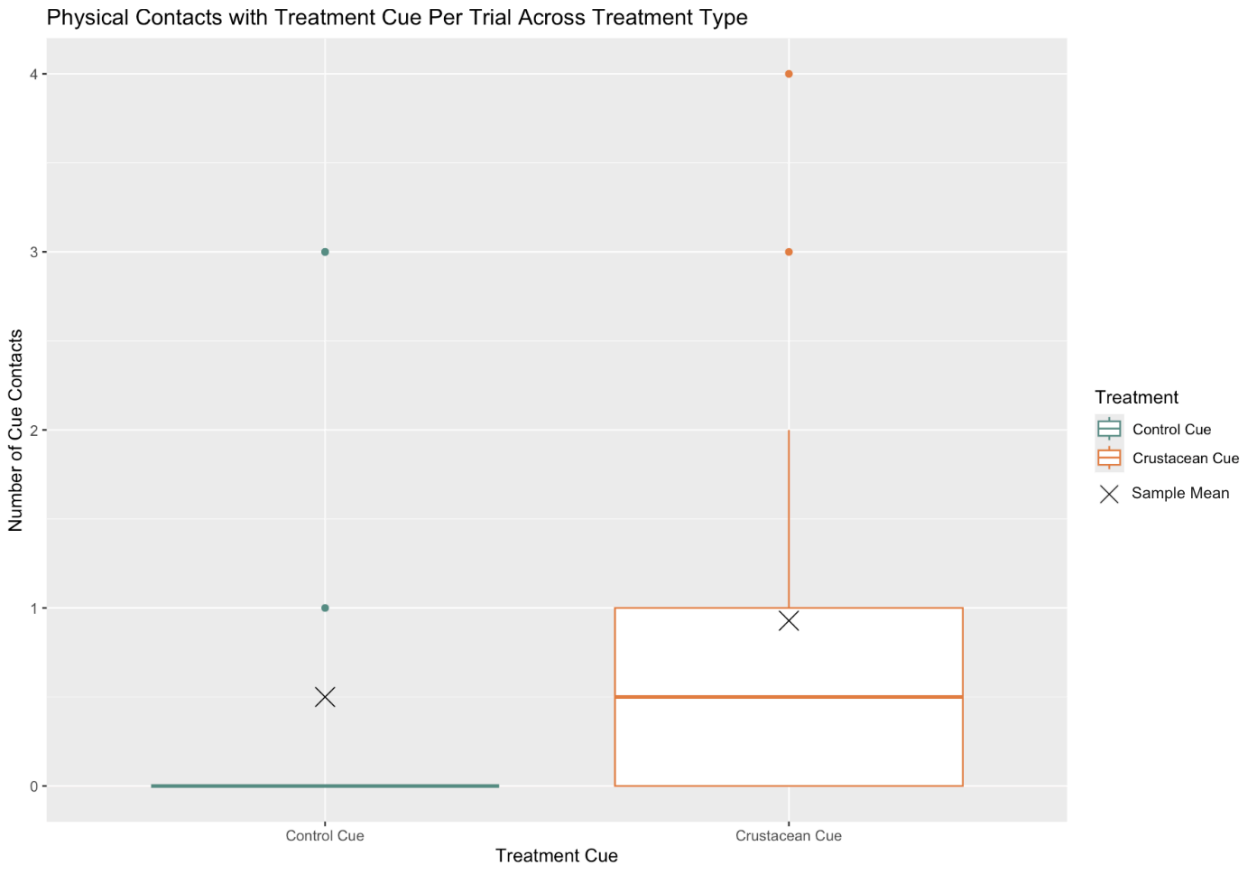

Figure 4: The number of cue contacts per 5 minute trial across control and crustacean olfactory cue treatments. The mean number of cue contacts across groups are not statistically different (Mann-Whitney U test, W = 72.5, N = 28, p-value = 0.1783)

Figure 5: The percent deviation from expected time spent on the cue side per five minute treatment. Above values were calculated based on the assumption that in the absence of preference, we would observe a 50:50 split of time spent on each side of the tank.