Escape Behavior in *Temora longicornis* when exposed to *Karenia brevis* and *Alexandrium fundyense*

A Thesis

Presented to

The Academic Faculty

By

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In Partial Fulfillment

Of the Requirements for the Degree Designation

Research Option in Biology

Georgia Institute of Technology

May, 2011

Escape Behavior in *Temora longicornis* when exposed to *Karenia brevis* and *Alexandrium fundyense*

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ACKNOWLEDGMENTS

I would like to thank my thesis adviser, Dr.Yen for her unbelievable support, guidance and encouragement throughout my research experience at Georgia Tech. I could not have asked for a better lab to do research in! She has truly inspired me to think like a scientist and to further explore animal behavior. I would like to thank my PhD. mentor, Rachel Lasley-Rasher for teaching me all about copepods, the schlieren optics system, designing experiments, and introducing me to culturing plankton! This project could not have been possible without her help! Next, I would like to thank Kathy Nagel for teaching me the copepod tracking softwares and for assisting me whenever I needed help. I would like to thank David Murphy for teaching me TechPlot to visually plot the copepod trails. I would also like to thank Kelsey Poulson for helping me run an ELISA on my *Karenia brevis* cultures. I want to especially thank my family and friends in their enthusiasm and willingness to learn about harmful algal blooms and copepods throughout this project.

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ABSTRACT

Recent studies have shown that copepods exhibit complex behaviors. Copepods are ocean-dwelling crustaceans that form the base of the marine food web. With the increase in global temperatures, there has been an increase in naturally occurring harmful algal blooms. The purpose of this project was to determine the effects of harmful algal blooms such as *Karenia brevis* and *Alexandrium fundyense* on the escape behaviors of the small North Atlantic copepod, *Temora longicornis*. The experiments were performed in the schlieren optics system tank. A siphon was used to mimic the fish's mouth. Data were collected via a high speed camera. Detection distance, escape distance, and escape speed were analyzed. *Temora longicornis* escape ability was not affected in terms of escape speed and escape distance after feeding on *A fundyense*. Copepods exposed to *K.brevis*, however, exhibited the furthest escape distance, largest average escape speed, and highest maximum speed of all other treatments. This conspicuous escape behavior increases the probability that they will fall prey to visual predators. Increased predation rates on HAB-affected copepods may facilitate the bioaccumulation of brevetoxins up the marine food chain with possible deleterious effects on humans consuming these fish.

Key words: Harmful Algal Blooms; *Karenia brevis*; *Alexandrium fundyense*; *Temora longicornis*; escape behavior; detection distance; speed; schlieren.

CHAPTER 1- INTRODUCTION

Increases in human population and the continuous increase in global temperatures have triggered an increase in Harmful Algal Blooms (HABs) causing them to occur over a broader range of area and for greater lengths of time (Anderson 2009). HABs occur when a population of single-celled algae reaches such a large size that other species are excluded. Microscopic algae are at the bottom of the marine food web and support most of marine life. There are approximately 4,000 known species of phytoplankton, of which 300 species form high density aggregates commonly referred to as blooms and approximately 40 produce toxins which threaten the marine food web and may lead to deleterious effects to humans (Anderson 2009). Warm temperatures and nutrient rich waters are a few of the factors that are responsible for promoting bloom formation in HABs and many other phytoplankton species. Exposure to HABs, either through inhalation, skin contact, or more commonly, ingesting contaminated seafood, can cause amnesia, stomach cramps, nausea, paralysis and eventually death (Wang 2008, Plakas 2010).

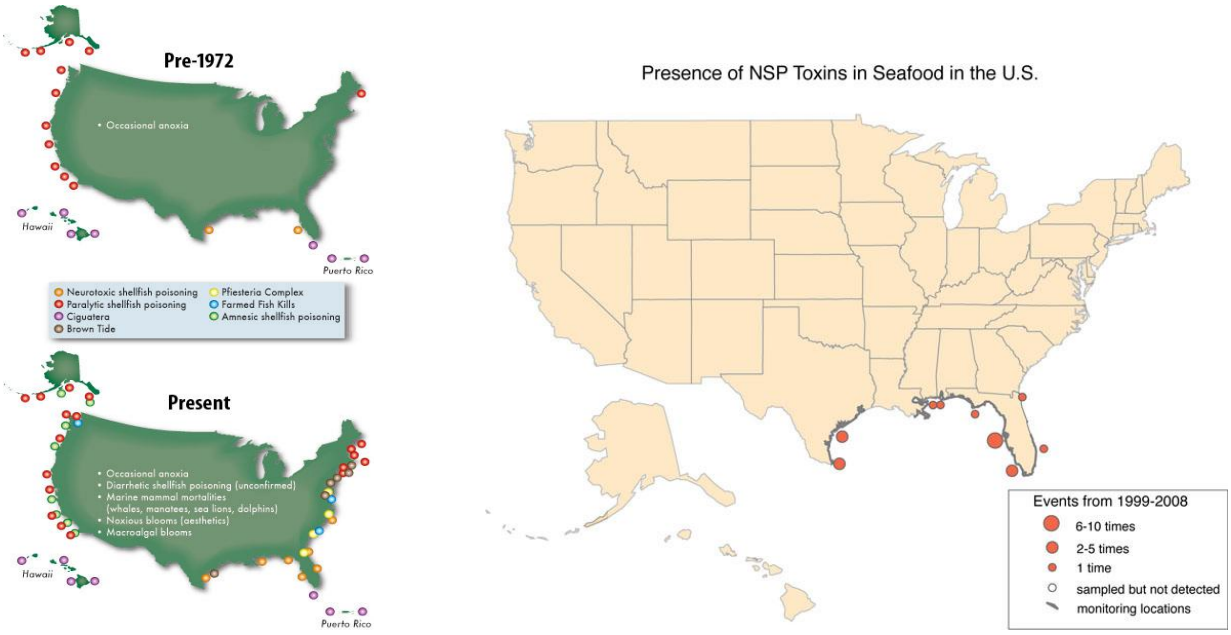

Figure 1: The gradual spread of Harmful Algal Blooms (HABs) in the United States: (Left) Top map is pre-1972, bottom map is post-1972. This shows an increase in HABs over a period of 40 years. An increase is especially noticed along the coast, where overfishing typically occurs. Map on the right shows the presence of Neurotoxic Shellfish Poisoning (NSP) in the seafood in the United States from 1999-2008 (reprinted from (Woods Hole Oceanographic Institution, 2011)

Current research has shown that HABs affect marine life, the environment, and humans. However, little work has been done on the immediate response of plankton to HABs. Here, we chose to study the escape behavior, a critical behavior ending in either life (a successful escape) or death if escape responses are impaired. In this study, the Calanoid copepod, *T.longicornis* were exposed to toxic strains of *Alexandrium fundyense* and *Karenia brevis* recording the fast escape behavior to see how the copepods respond to the suction stimulus. Our hypothesis is that copepods exposed to the treatments with HABs will deviate from their normal swimming

behavior, resulting in an impaired escape behavior due to physiological incapacitation or nutritional deficiencies.

CHAPTER 2- LITERATURE REVIEW

There are many types of harmful blooms that occur in the oceans. The two species we targeted in this study are *Alexandrium fundysense* and *Karenia brevis*. *Alexandrium fundyense* commonly forms blooms in the North Atlantic Ocean, and produces a neurotoxin called saxitoxin, which when ingested by humans, can cause fatal paralytic shellfish poisoning (Lefebvre et al. 2004). Symptoms consist of nausea, vomiting, dizziness, and sometimes death due to respiratory failure (Lefebvre et al. 2004). These toxins threaten public health, marine ecosystems, and the global economy (Lefebvre et al. 2004). Studies show that toxic algal blooms have caused massive deaths in invertebrates, Atlantic herring, finfish, birds, and marine mammals (Lefebvre et al. 2004, Cohen et al. 2007).

Another type of toxin, known as a brevetoxins, is produced by the red-tide causing plankton, *Karenia brevis* (found in the Gulf of Mexico), and produces similar symptoms as the saxitoxin (Lekan 2010). Brevetoxins ranging from PbTx 1-9 can cause severe neurological symptoms in organisms that are exposed to concentrations ranging from picomolar to nanomolar g/L of brevetoxin (Baden 1989). The major brevetoxin produced by *K.brevis* is PbTx-2 (Lekan 2010). Brevetoxins are depolarizing substances that open the sodium ion channels in the cell walls, which lead to an uncontrolled influx of sodium ions into the cells (Baden 1983). The derivatives of PbTx-1, PbTx-2 and PbTx-3 have shown to produce a rapid increase in calcium ions (Baden 1983).

Since copepods are usually exposed to a mixture of phytoplankton in the ocean, it is important to know whether or not the copepods can selectively choose their food. A study was done to see whether copepods have the ability to actively select which phytoplankton they eat

(Schultz and Kiorboe 2009). Two species of copepods, *Temora longicornis* and *Pseudocalanus elongata* were exposed to a mix treatment of toxic *Karenia mikimotoi* and non-toxic *Gyrodinium instriatum* (Schultz and Kiorboe 2009). Both copepods had reduced clearance rates of *K. mikimotoi* relative to *G.instriatum*, which suggests active prey selection (Schultz and Kiorboe 2009). It was suggested that these species may have consumed the algal species without tasting their food (Schultz and Kiorboe 2009). This is because if the copepod used chemical cues to taste its prey before consumption, then the rejection rate for *K.mikimotoi* would have been close to 50% (Schultz and Kiorboe 2009). In the experiment, the rejection rates were less than 20% for both species, which suggests that prey selection was independent of their diet (Schultz and Kiorboe 2009). Due to this fact, it can be hypothesized that copepods will ingest the same number of toxic cells as non-toxic cells.

Another active prey selection study was done with three Maine copepods (*Acartia tonsa, Centropages hamatus,* and *Eurytemora herdmani*) that co-occur with *Alexandrium spp*. were exposed to different concentrations of this bloom based on the varying concentrations of paralytic shellfish poisoning (PSP) (Teegarden 1999). When given the choice between toxic *Alexandrium fundyense*, non-toxic *Alexandrium tamarense,* and non-toxic palatable mixture of phytoplankton, all three copepods chose *A.tamarense* over the other choices (Teegarden 1999). Additionally, when clones with various concentrations of PSP of *Alexandrium spp*. were introduced to the copepods, they were all able to distinguish between the different cell toxicities by their chemoreceptors (Teegarden 1999). This shows a possibility of active prey selection. It was also found that the grazing rates of the toxic cell consumption varied from species to species (Teegarden 1999).

Copepods are zooplankton that inhabit the pelagic environment with minimal protection and are vulnerable to predation. It is important to know how HABs will affect a copepod's swimming behavior, since swimming in erratic motions may make them more conspicuous to predators. A behavioral study on copepods' swimming patterns tested sublethal effects of *K.brevis* on two sympatric copepod species (*Acartia tonsa* and *Temora turbinata*) and one allopatric copepod species, *Centropages typicus* (Cohen et al. 2007). It was found that the sympatric species stopped grazing at high concentrations of *K.brevis* whereas the allopatric species grazed at all the concentrations of *K.brevis*. In terms of the swimming behavior, *C.typicus* showed suppressed swimming behavior of low-mid *K.brevis* treatments with PbTx-2 concentrations ranging from 0.15-1.5µg/L and the cell concentrations ranging from 1×10^5 - 1×10^7 cells/L (Cohen et al. 2007). When *Temora turbinata* was subjected to these concentrations, half of the copepods were observed swimming except at the highest *K.brevis* cell concentration. *Acartia tonsa* showed no difference amongst the different treatments (Cohen et al. 2007).

Understanding how the copepod escapes in the presence of a predator is an important behavior strategy which can maximize their life and allow them to reproduce. The escape reaction is a rapid response to a threatening stimulus such as a hydrodynamic disturbance that in nature would be caused by an approaching predator (Wagget and Buskey 2008; Yen 2000). The mechanoreceptors on a copepod's antennules can detect the gradient in flow, as shown in Figure 2 below (Yen 2000). There is a high speed flow near the proximal end for detecting prey and low speed at the distal end for detecting predators (Yen 2000). By using the coordinated motion by its pereiopods, a copepod can propel itself forward by as much as 200-800 body lengths per

second during an escape reaction (Davis et al. 1999; Buskey et al. 2002; Lenz et al. 2000). An escape attempt by the copepod can lower the predation risk by 50% (Lenz et al. 2000).

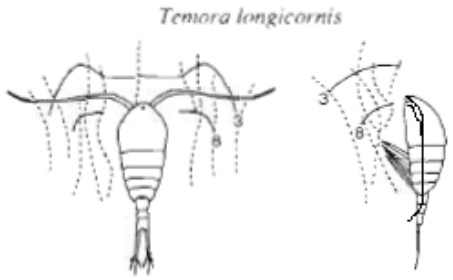

Figure 2: ***Temora longicornis* escape**: Show trajectories of flow velocities (Tiselius and Jonsson, 1990)

The topic of my investigation is to study the escape behavior of copepods when they are exposed to a mid algal bloom concentration. I used quantitative analyses to test the hypothesis that when copepods are exposed to HABs, they change in escape behavior that makes them conspicuous to predators.

CHAPTER 3- METHODOLOGY

3.1 Copepod, Temora longicornis

3.1a Copepod Collection in Darling Maine Center, Walpole,Maine

Copepods were collected by towing a plankton net (250 micrometers) behind a boat at approximately 10 meters in depth. They were then transferred to 20 liter containers of surface seawater and transported to the lab. Within a day of collection, copepods were carefully sorted and transferred to 1 liter wide-mouthed bottles. They were then sealed and shipped in an insulated box at approximately 12°C.

3.1b Copepod Lab Care

Calanoid copepod, *T. longicornis* (1.3mm) were shipped from Walpole Maine to Georgia Tech in June and July. They were diluted in 5-gallon buckets with artificial seawater at 12 °C. *T.longcornis* were fed a mixed diet of *Tetraselmis spp*. and *Rhodomonas lens*.

3.2 Preparation of Harmful Algal Cultures

3.2a Karenia brevis

Karenia brevis is a dinoflagellate with a cell length ranging from 24-28 µm and cell width from 18-22µm (CCMP 2010). A culture of *K.brevis,* strain #2228, has been kept in lab for a few years. It is maintained in 35 ppt of filtered and autoclaved seawater with f/2 media (Guillard and Ryther, 1962) at 22 °C on a 14:10 light: dark cycle (CCMP, 2010). In order to observe the growth phase, cells were counted using a Sedwick rafter every other day. Cells were cultivated in the late exponential phase because of the high toxicity levels. Since the culture had

been in lab for a few years, a brevetoxin- ELISA analysis was run on *K.brevis* cultures to ensure high toxicity levels (Naar et al 2002). This ELISA detects for Type-2 brevetoxins, which account for 90-95 % of the toxins produced during a bloom (Naar et. al, 2002). The working range for the assay is expected to be between 0.2 – 20 ng/ml. Of the two *K.brevis* samples analyzed, one sample had a concentration of 9.223 ng/ml and the other sample had a concentration of 4.822 ng/ml. After the calculations were done, it was found that the toxin content per cell was between 8-12pg/cell. This is a reasonable toxicity test for a mid-algal bloom concentration.

3.2b Alexandrium fundyense

Alexandrium fundyense , a slow growing dinoflagellate of length, 30-40µm, and width of 30-40µm depending on the availability of nutrients, produces sexually and asexually (Guillard and Ryther, 1962; CCMP, 2010). A strain of *Alexandrium fundyense* (strain # 1978) was ordered from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP, 2010) in late Summer, 2010. The culture was inoculated in 32ppt of L1 media. The media was made by mixing 1L of filtered Maine seawater with 1ml of $NaNO_3$, 1ml of $NaH_2PO_4 \cdot H_20$ and 1ml of $Na_2SiO_3 \cdot 9H_2O$ (Guillard and Ryther, 1962). The media was autoclaved in bottles for 1 hour, and cooled to 10°C. The cultures were inoculated with 20ml of L1 media and 2ml of *A.fundyense* culture in a pre-autoclaved 50ml Erlenmeyer flask. The cultures were stored at 12°C for two weeks at a 24 hour light cycle using cool-white fluorescent bulbs without stirring. No mixing was found crucial in the preliminary study of growing and maintaining cultures in the lab. The reason thought to be is because *A.fundyense* have two delicate flagella when seen from a light microscope. One flagella surrounds the body like a belt and the other runs along the body. If the flagella are damaged, then they cannot swim to another mate and reproduce for sexual

reproduction (Anderson, 2004). The flagella are also needed for asexual reproduction. Thus, stirring should be very limited in the first few weeks. A saxitoxin analysis is scheduled later in May using either the method of LC-MS or ELISA. The toxin analysis will be run when cultures are in their late exponential phase to insure high toxicity levels.

3.2c Rhodomonas lens

Rhodomonas lens is an alga with a length of 8-13 µm and cell width from 5-8µm (CCMP, 2010). A culture of strain #739 is maintained in 35ppt of filtered and autoclaved seawater with f/2 media (Guillard and Ryther, 1962) at 22°C on a 14:10 light: dark cycle (CCMP, 2010). The different treatment of cells was counted using a hemocytometer.

3.3 Preparing the Treatments

Three treatments were tested on the copepods. Treatment 1 consisted of 80% *K.brevis* and 20% *R.lens*, with a concentration of 320:1120 cells/ml of *K.brevis* and *R.lens* respectively (Prince et al 2006). Treatment 2 consisted of 80% *A.fundyense* and 20% *R.lens* with a concentration of 320:1120 cells/ml of *A.fundyense* and *R.lens* respectively (Prince et al 2006). Lastly, treatment 3 consisted of 20% *R.lens* with a concentration of 1120cells/ml (Prince et al 2006). All cells were counted in the late exponential phase and added to 200ml of filtered seawater at the same salinity as the animals. Then, approximately 10 male and 10 female *T.longicornis* were added to the treatment, covered and placed at 12°C for 2 hours so the copepods could get in their "intoxicated" state. Treatment 3 with 20%*R.lens* served as a starvation control with 1120 cells in 200ml. If no escape difference occurred in treatments 1 and 2, then they should be similar to the starvation control of treatment 3.

After 2 hours of exposure time in the treatment, the animals were filtered out using a 250µm mesh net, rinsed with filtered seawater, and stored in a beaker at 12°C until ready for use.

3.4 Setting up the Siphon

A Pasteur pipette was used to construct the siphon. It was attached to a rubber tube that sucked up water between a rate of 0.5 and 0.55ml/second. This flow rate mimicked the natural predators of the copepod. The rubber tube emptied the water into a bucket. In order to keep the water level constant and to maintain the flow, in the 1 liter tank, a rubber tube was in another smaller bucket, which was attached to the peristaltic pump, and at the end was a bubbler, which gently bubbled in the water.

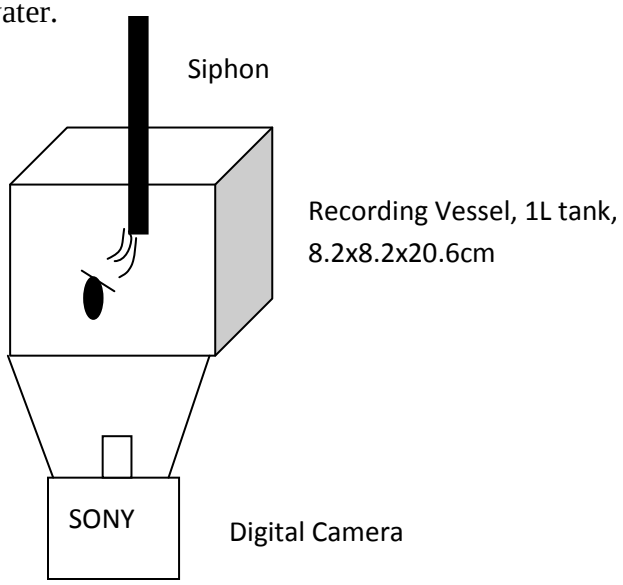

Figure 3: **Schlieren Tank**:Diagram shows a schematic illustration of the experimental setup to investigate the effects of HABs on copepod escape behavior. A mixture of 20 copepods, both male and female were tested for each experiment. Videos were recording via a high-speed camera for 3 hours.

3.5 Setting up the camera and schlieren

Movie sequences were collected on a high speed camera attached to an 85mm lens. Treated copepods in a 1-liter glass tank with dimensions of 8.2cm x 8.2cm x 20.6cm were filmed. The tank was filled gently with filtered seawater at 12°C, and any bubbles were removed. The tank was placed in the Schlieren optical system. Copper wire was wrapped around the tank and attached to the chiller which was kept at 12°C. The flow rate was checked and calibration was done with a 1cm x 1cm x 1cm stick before adding in the animals. Animals were monitored closely and 6-second clips were recorded of their escape. All the videos were compiled and analyzed in LabTrack or Hedrix Software. A Kruskal-Wallis one-way ANOVA was performed to determine difference in escape kinematics between treatments (GraphPad Prism 2005). A G-test of independance test was performed to determine differences between the proportion of successful escapes from each treatment (GraphPad Prism 2005)

3.6 Calculations

Calibration Stick

$$C_s = \frac{1}{\sqrt{(x_s - x_2)^2} + \sqrt{(z_s - z_2)^2}}$$

Equation 1- **Converting from pixels to centimeters:** In this equation, x and z represent two points taken from the calibration stick. The difference was squared, rooted and divided by 1.

Middle of the siphon

$$M_s=\left(\frac{x_1+x_2}{2},\frac{y_1+y_2}{2}\right)$$

Equation 2- **Midpoint formula**: Two endpoints were taken of the siphon. The midpoint formula seen above was used to find the middle of the siphon.

Conversion from pixels to centimeters

$$M_{cm}=\frac{1}{\sqrt{(x_s-x_2)^2}+\sqrt{(z_s-z_2)^2}}\times\left(\frac{x_1+x_2}{2},\frac{y_1+y_2}{2}\right)$$

Equation 3- **Converting siphon from pixels to centimeters:** Conversion was successful by multiplying equation 1 and 2.

Standardizing the values

$$\text{Value}_{zero}=\left(P_{x,y,z}\times C_s\right)-M_{cm}$$

Equation 4- **Standardizing the values**: Here, $P_{x,y,z}$ is the point of the copepod in pixels, which is then converted into centimeters and then subtracted from the middle of the siphon.

Detection Distance

$$\sqrt{\left(z_i v_x-M_{cm,x}\right)^2}+\sqrt{\left(z_i v_y-M_{cm,y}\right)^2}+\sqrt{\left(z_i v_z-M_{cm,z}\right)^2}$$

Equation 5- **Detecting the distance away from the siphon**: Equation shows the point where the copepod first elicits the escape response, shown by $z_i v_{x,y,z}$ and was subtracted from the middle of the siphon.

Escape Distance

$$\sqrt{(z_f v_x - M_{cm,x})^2} + \sqrt{(z_f v_y - M_{cm,y})^2} + \sqrt{(z_f v_z - M_{cm,z})^2}$$

Equation 6- **Distance escaped from the siphon**: Equation shows the last point of the copepod's escape response, shown by $z_f v_{x,y,z}$ and was subtracted from the middle of the siphon.

Average Escape Speed

Average speed(cm/sec)=Sum (Initial Velocity->Final Velocity)/Total number of frames

Equation 7- **Average speed at which the escape occurred**: Average velocities from the first instance of the escape response to the final instance, divided by all of the occurrences.

The Threshold Deformation Equation

$$\Delta = Q/2\Pi r^3$$

Equation 8- **Deformation rate** is the rate at which fluid around the copepod's sensors is being deformed. This has been shown to be the primary signal that causes copepods to escape (Fields & Yen 1997, Kiorboe et al. 1999). Q is the flowrate of the siphon (measured w/ the graduated cylinder) and r is the radial distance between the copepod and the center of the siphon opening at the point that the animal escapes.

Signal Strength

$$S\Delta = \Delta * L$$

Equation 9- **Signal strength** takes into account the deformation rate of the fluid relative to the size of the copepod (Kiorboe et al. 1999). Where L is the radius of the copepod. For *Temora longicornis* we used 1.3 mm (Dam & Peterson 1998).

RESULTS

T.longicornis that were exposed to *Karenia brevis* and *Alexandrium fundyesnse* detected the predator mimic from significantly shorter distances relative to control treatments(Fig. 1, p = 0.022), Copepods exposed to *K. brevis* exhibited longer escape distances (Fig. 2, p = 0.055), and faster escape speeds (Fig. 3, p = 0.010). Overall, copepods escape success was reduced by ingesting either *A. fundyense* or *K. brevis* (Fig. 4, p = 0.0005)

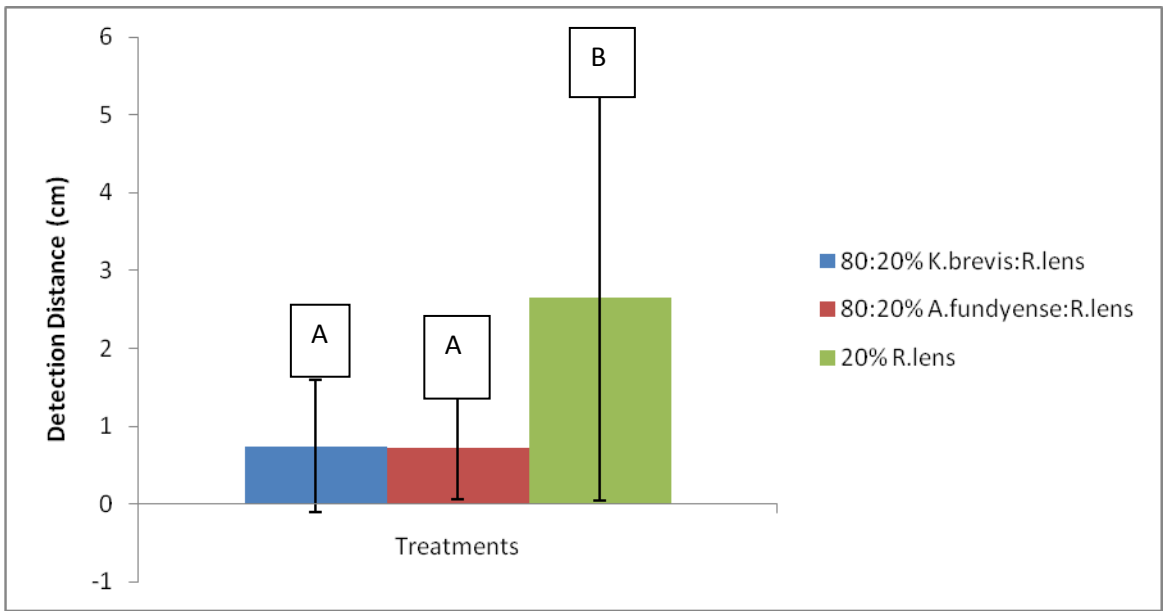

Figure 4- **Average Detection Distance**: *Temora longicornis* executed an escape following exposure to mixed harmful algal bloom treatments: 80:20% *Karenia brevis:Rhodomonas lens(n=4),* 80:20% *Alexandrium fundyense:Rhodomonas lens(n=10)* and 20% *Rhodomonas lens(n=10).* Letters indicate significant differences between the HABs and the control, *R.lens* treatment according to a Kruskall Wallis ANOVA, followed by a Tukey post-hoc analysis.

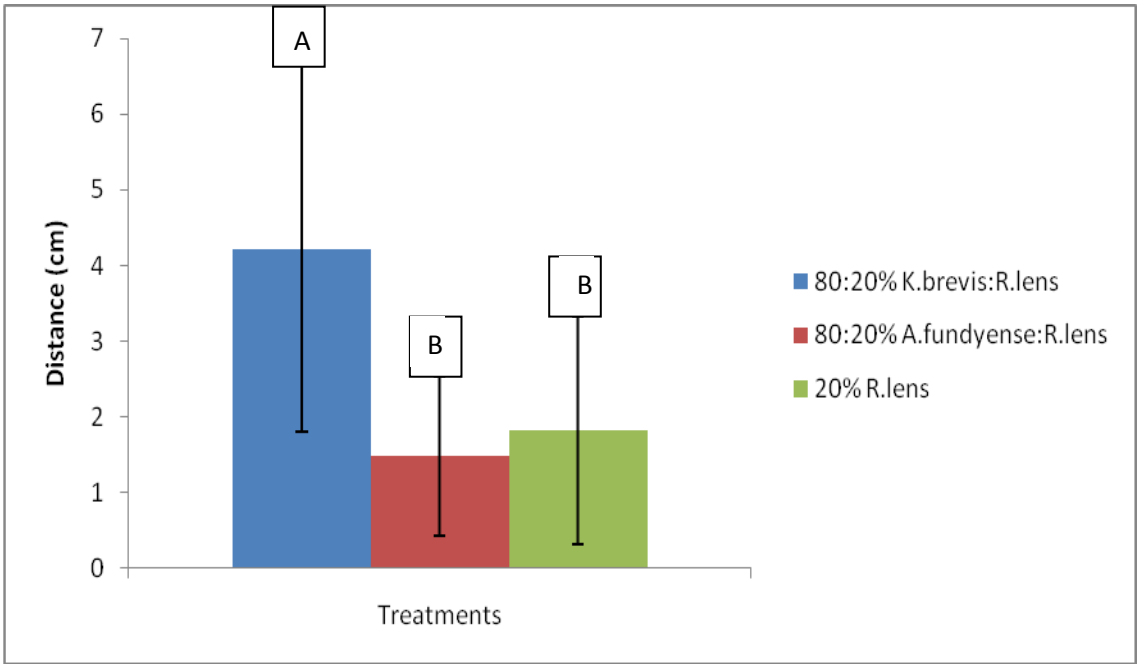

Figure 5- **Escape Distance from the Siphon**: Copepods in the 80:20%*K.brevis:R.lens(n=4)* treatment exhibited the furthest escape distance at 4.1 cm, followed by 20%*R.lens(n=10)*, at 2.1 cm and lastly, 80:20%*A.fundyense:R.lens(n=10)* at 1.7cm. Letters indicate significant differences between the *K.brevis*, *A.fundyense* and *R.lens* treatments according to a Kruskall Wallis ANOVA, followed by a Tukey post-hoc analysis

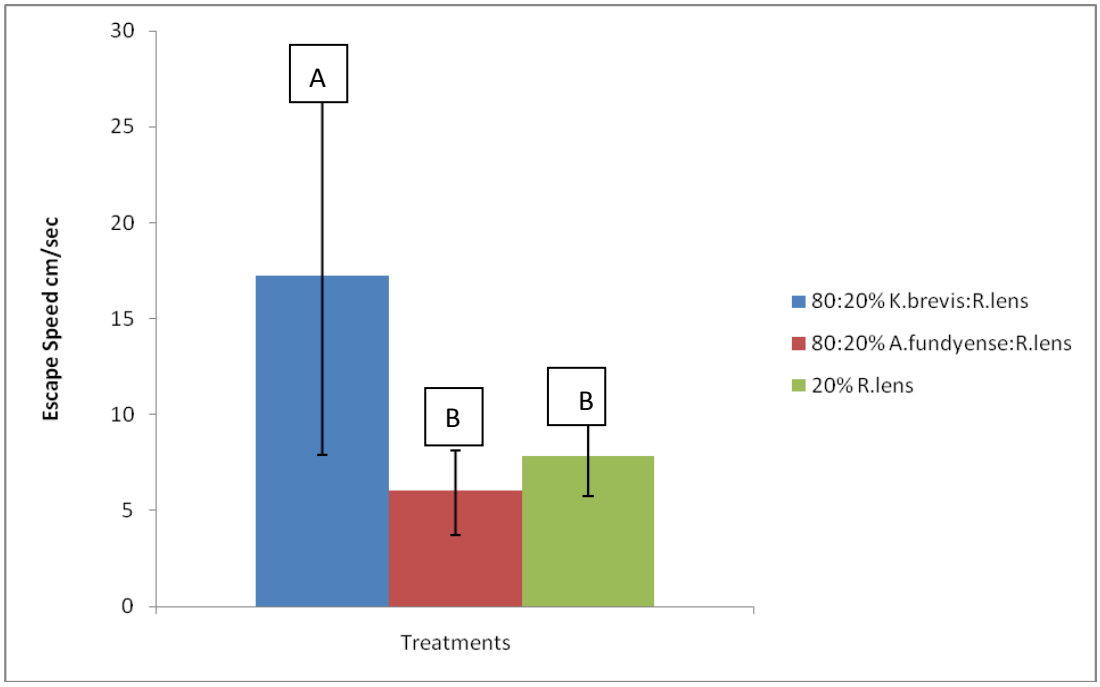

Figure 6- **Average Escape Speed**: Copepods in the 80:20%*K.brevis:R.lens (n=4)* treatment exhibited the highest escape speed, followed by 20%*R.lens (n=10)*, and lastly, 80:20%*A.fundyense:R.lens (n=10).* Letters indicate significant differences between the *K.brevis*, *A.fundyense* and *R.lens* treatments according to a Kruskall Wallis ANOVA, followed by a Tukey post-hoc analysis.

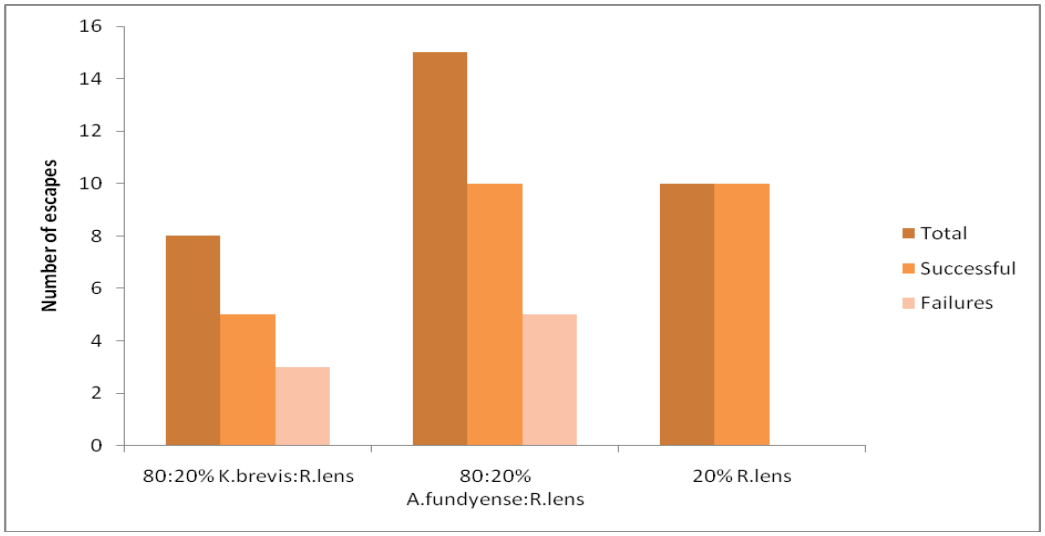

Figure 7-**Quantifying Escape Behavior:** Total number of escapes along with the number of successful and unsuccessful escapes. The non-HAB treatment, 20% *R.lens* shows 100% escape. (Chi-square analysis, p = 0.0005)

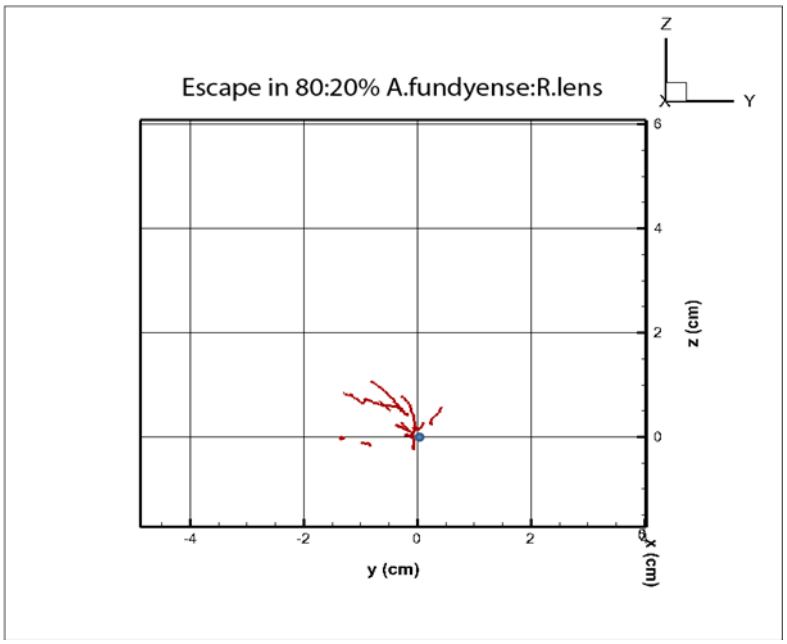

Figure 8-**Escape in 80:20% *A.fundyense:R.lens*:** Shows the trajectories (n=10) in the Y-Z polar view. The siphon is depicted by the blue circle.

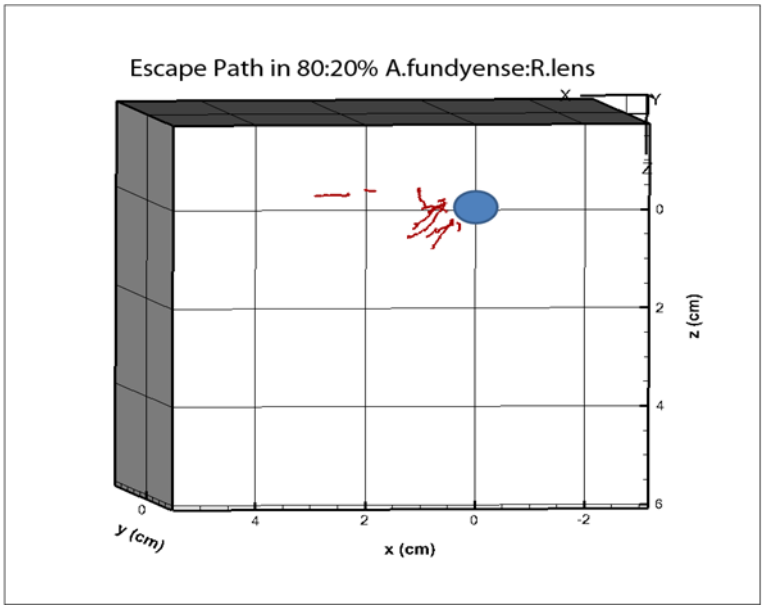

Figure 9-**Escape in 80:20% *A.fundyense:R.lens*:** Shows the trajectories (n=10) in the X-Z 3-D Cartesian view. The siphon is depicted by the blue circle.

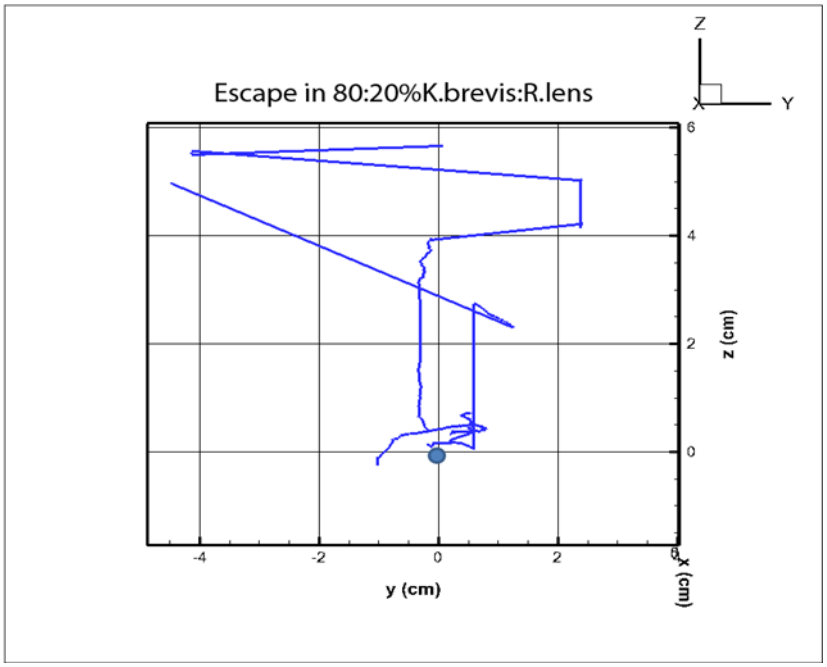

Figure 10-**Escape in 80:20% *K.brevis:R.lens*:** Shows the trajectories (n=4) in the Y-Z polar view. The siphon is depicted by the blue circle.

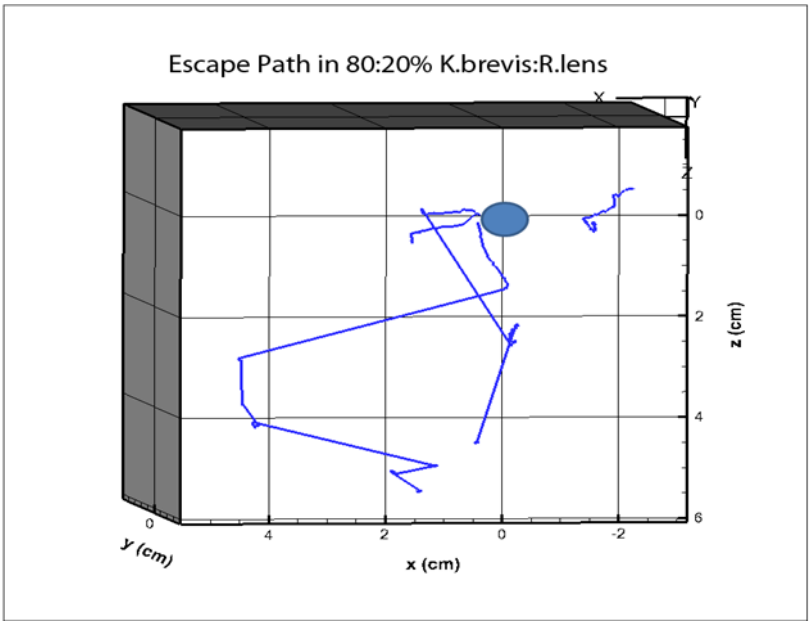

Figure 11-**Escape in 80:20% *K.brevis:R.lens*:** Shows the trajectories (n=4) in the XYZ 3-D Cartesian view. The siphon is depicted by the blue circle.

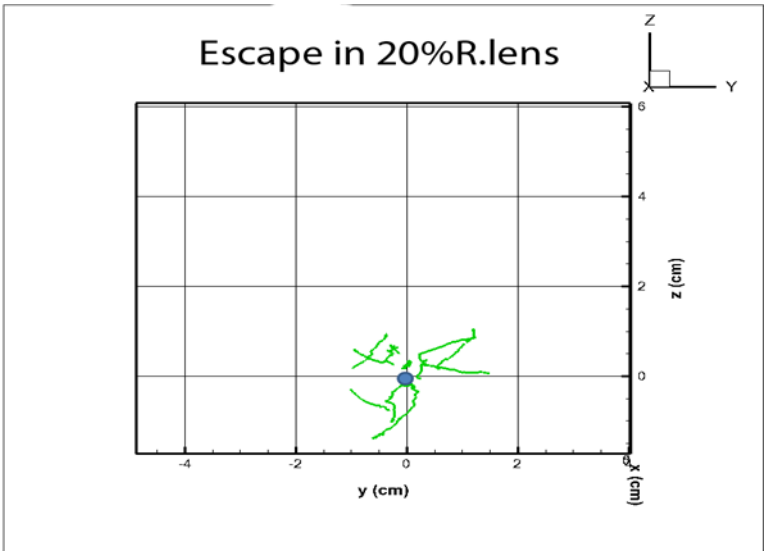

Figure 12-**Escape in 20% *R.lens*:** Shows the trajectories (n=10) in the Y-Z polar view. The siphon is depicted by the blue circle.

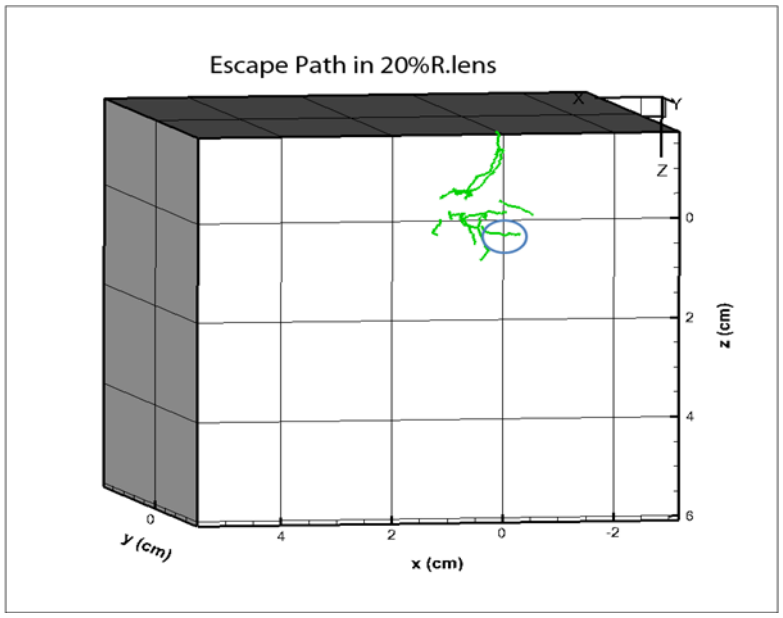

Figure 13-**Escape in 20% *R.lens*:** Shows the trajectories (n=10) in the X-Z 3-D Cartesian view. The siphon is depicted by the blue circle.

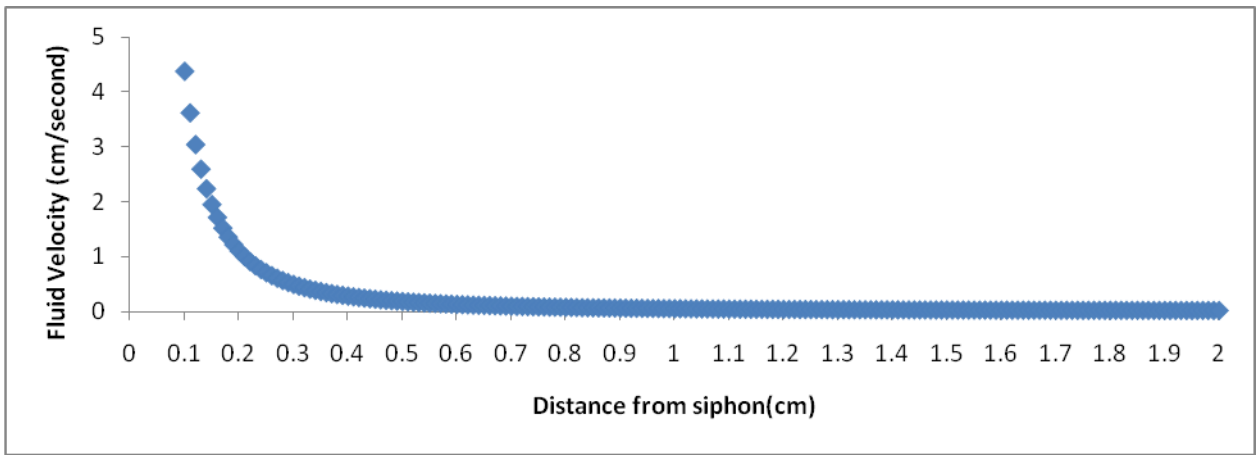

Figure 14- **Signal Strength of the Siphon:** Obtained by tracking particles and plotting data away from the siphon at a constant flow rate.

CHAPTER 5- DISCUSSION AND CONCLUSION

This was a preliminary escape experiment that tested 2-hour HAB exposure on the escape behavior of *T.longicornis*. The escape data are significant because in the HAB treatments, only 67% of the copepods escaped in the treatment with *A.fundyense* and 62% in the *K.brevis* treatment, compared to 100% successful escapes in the 20% *R.lens* treatment (Fig. 7). *T.longicornis* suffered a reduced predator-detection ability, as evident by the 3-fold reduction in detection distance (Fig. 1, $p = 0.022$). The copepods in the 20% *R.lens* treatment could detect the siphon further away, almost 2.5cm when compared to the copepods in the HABs treatments. This could indicate that HABs interfere with the copepod's sensory abilities, and that for them to effectively detect the suction from the siphon, they have to come closer to it. These behavioral alterations are likely due to some sort of physiological incapacitation as opposed to starvation since the copepods had the same concentration of nutritious food (1120 cells/ml of *R.lens*) in both treatments and control. There are two possible consequences to this reduced detection ability. First, if the copepods get too close to an approaching predator, they may be unable to execute a successful escape and will likely be ingested by the predator. Furthermore, if they do execute a successful escape but swim erratically following their escape (as the case for *K. brevis* treatment, Fig. 7) they will create a large fluid disturbance, likely increasing their conspicuousness and hampering their ability to get away from the predator. Alternatively, this erratic swimming behavior could help the copepods get away from a predator because they swim faster and may be able to "out-run" the predator. What actually makes the copepod conspicuous is depicted by the Reynolds number¸ which describes the fluid disturbance created from an animal when swimming through a medium (Batchelor 2001). The Reynolds number varies for different species of copepods, and ranges from 0.1 to over 1000 (Yen & Strickler 1996).

Reynolds number also varies according to the activity exhibited by the copepod. When it is feeding, the Re is less than one (Yen & Strickler 1996). When it is freely swimming, the Re is between 2 and 20 (Yen & Strickler 1996). In both of these instances, the copepod barely leaves a trace in the water (Yen & Strickler 1996). However, when the copepod is initiating an escape response, the Re is around 2000, and it leaves an intense wake opposite to the momentum of the escape, making it more conspicuous to the predator (Yen & Strickler 1996). Also, when a copepod escapes, its legs deliver a force that is 60-400 times more than when it is feeding (Duren & Videler 2002). As argued by (Strickler 1975, 1977) it would be 400 times as much energetically costly for the copepod to elicit an escape response as opposed to it normal cruising or feeding behavior, since an escape can use up to 0.07-0.3% of the copepods' total food energy (Duren & Videler 2002), meaning that in order to survive the longest, the copepod must graze on nutritious food to keep initiating escape responses. Escaping not only refers to the conspicuousness of the copepod, but also to the distance achieved from the escape.

When we measured escape distance travelled, we found that copepods exposed to the *K.brevis* treatment swam at a much faster distance than the *A.fundyense* and *R.lens* treatments (Fig. 6). Even though studies have been done on the effects of brevetoxins on the swimming behavior of *Acartia tonsa*, and have found no correlations, (Prince et al. 2006), there is a probability that brevetoxins could affect *T.longicornis* because it is of a different species of copepod. Brevetoxins are depolarizing substances that open the sodium ion channels in the cell walls, which lead to an uncontrolled influx of sodium ions into the cells (Baden 1983). This erratic behavior possessed by copepods while in the HAB treatment may have caused them to be more susceptible to the predator than a copepod in the *R.lens* treatment. Predator-prey encounter rate models show that when copepods increase their swimming speed, they increase their

conspicuousness to predators and predator-encounter probabilities (Gerritsen & Strickler 1977, Yen & Strickler 1996). Our data supports the hypothesis that copepods escape ability is impaired because there were fewer successful escapes in the either HAB treatment relative to the *R.lens* control treatment (Fig. 7). However, in *K. brevis* treatment, it was surprising to learn that the copepods that did escape, had the highest escape speed and travelled the furthest distance (Fig. 2 and 3). However, it appears that these increases in escape speed and escape distance do not compensate for their lowered predator-detection ability since their overall escape success was reduced. One of the limitations of this project was the use of a siphon instead of a live predator. *T.longicornis* not only has fish predators which use a suction to capture their prey, but they are also predated upon by larger, carnivorous copepods such as *Calanus*. Also, since the siphon is positioned in only one place, it is not very accurate when looking at the after-effects of the behavioral response of the predator to the escape turbulence of *T.longicornis.* The next steps of this project would be to run more experiments with more replicates. Another step would be to test a treatment with 100% *R.lens*, which would show whether or not the escape behavior is caused by starvation. Recent copepod-behavior research indicates that analyzing approximately 50 animal trajectories from 4 different independent replicates are necessary to address the individual variability in copepod's swimming behavior (Seuront et al. 2004). In this preliminary project, only a few clips were analyzed due to the time constraints. It will also be vital to get the initial escape distance from the siphon to see how far the copepod escaped from the siphon. Measuring the first initial jump that the copepod makes will also contribute to learning more about this behavior. Since a study done by Seuront et al. 2004 showed that *T.longicornis* are likely to get a higher number of escapes in turbulent waters, it would be interesting to compare the number of escapes in HAB-laden copepods in turbulent vs. non-turbulent waters.

Furthermore, experiments should be conducted in the presence of actual predators to determine how the affects of HABs on copepods conspicuousness and escape ability combine to alter predator-prey interactions. Determining the impact of HAB ingestion on copepod's vulnerability to predators is important understanding the complex pathway of HABs through marine food webs.

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