

**FINDING FOOD IN AN AQUATIC DESERT:  
HOW CRUISING COPEPODS DETECT THEIR NEXT  
PHYTOPLANKTON MEAL**

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**FINDING FOOD IN AN AQUATIC DESERT:  
HOW CRUISING COPEPODS DETECT THEIR NEXT  
PHYTOPLANKTON MEAL**

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## LIST OF SYMBOLS AND ABBREVIATIONS

NGDR	Net-to-Gross Distance Ratio
“Encounters”	where copepod disturbs trail flow without a reorientation of the body or a follow due to lack of interest
“Follows”	which are encounters with a subsequent follow where the copepod is provoked to reorient its body and disturbs the trail for a length leaving a distinct residue
“Spirals”	where the copepod does not enter the trail but instead rotates around the borders of it due to possible interest
“Mistakes”	where the copepod orients its body toward the trail and follows it, exits the trail, then reorients to follow again

## SUMMARY

Copepods exist in an aquatic food desert where finding food is difficult given the constraints of their environment. They live in a three dimensional world and must filter  $10^6$  times their own body volume to cover their nutritional needs (Kiorboe, 2011). Copepods sense chemical, hydromechanical or both cues for prey and mate detection. Hydromechanoreception is the dominant sensory mechanism used to locate nearby prey (Legier-Visser, et al. 1986, Goncalves et al. 2014)). However, it is relatively unknown how copepods detect their prey from a distance. This study focused on the sensory mechanism and behavior of an abundant pelagic copepod, *Temora longicornis*, tracking a co-occurring phytoplankton prey, *Tetraselmis suecica*. The wake of a mobile prey was mimicked in the form of a trail-like cue and scented with either the smell of *T. suecica*, female *T. longicornis*, or remained scentless. Males were exposed to all three treatments, whereas females only to *T. suecica* and a scentless trail. Preliminary results indicate that male and female *T. longicornis* copepods may rely on chemical and hydromechanical cues for prey detection. Why it is important to determine the mechanism copepods use for remote detection of prey? The ocean contains numerous tiny trails scented with the smell of phytoplankton which leak from the source prey patch. It has been suggested that trail-sensing copepods will be more successful at finding mates and food than those who do not follow trails (Yen et al., 2010). Results from this study may open a new avenue of research for the success of trail-sensing organisms.

# CHAPTER 1

## INTRODUCTION

Copepods are an important member of all aquatic food webs. They serve as a key basal organism since most aquatic life is dependent on their survival, directly or indirectly. Their abundance is key to the survival of marine and freshwater animals. A large portion of the world's ocean protein supply would dwindle due to the interdependency of the oceanic food web on copepod abundance. Despite their ease and abundance as a food source for other aquatic animals, the ocean is an aquatic food desert for copepods. Finding food is difficult given the constraints of their environment, such that they live in a three-dimensional world where encountering prey is rare due to their miniscule body size to habitat ratio. They must filter  $10^6$  times their own body volume to cover their nutritional needs (Kiorboe, 2011). To combat these constraints, copepods have evolved sensory mechanisms to detect prey remotely and nearby. Hydromechanoreceptive cues aid in the recognition of water movement, which helps to capture nearby prey. Using this sensory mechanism copepods will either: (1) generate a feeding current and capture prey that enter the current, (2) wait for prey to pass within their field of detection and then ambush capture them, or (3) swim smoothly through the water and capture prey as they continuously swim (Kiorboe, 2011). Chemoreceptive cues are the detection of diffusible chemical compounds, which help to detect and track footprints from remote prey. Much information is available on hydromechanical detection of phytoplankton prey by copepods (Yen and Strickler, 1996; Kiørboe and Visser 1999; Visser, 2001; Goncalves and Kioboe, 2015), yet chemical cues are less often concluded to be responsible for this proximal detection (Poulet and Gill, 1988; Steinke et

al, 2006). Furthermore, even with feeding experiments such as Schultz and Kiorboe's showing copepods ability to remotely distinguish between different types of prey (2009), the sensory mechanism and tracking behavior for remote prey detection is not well understood.

One research focus of the laboratory of Dr. Jeannette Yen is on understanding the mating behavior of copepods. Males of some marine species detect conspecific female wakes in the form of a trail-like cue laced with female chemical exudates and follow these pheromone trails to find and capture female mates (Yen, et. al. 1998). A successful follow is described as when a copepod detects a chemical trail, follows the trail to its source while disturbing the trail in its wake, as seen in *Figure 1*. These copepods are remarkably able to detect a conspecific mate even though they are usually thirty body lengths away (Yen et al. 1998; Kiorboe, 2005). This remote detection ability is possibly due to keen sensory mechanisms that may allow these copepods to sense other remote organisms, such as prey. To determine if and how *Temora longicornis* remotely detect and track their phytoplankton prey, we conducted a simple trail mimic experiment [Yen et al. 2004]. Male and female *T. longicornis* were exposed to two trail mimics, one scented with the chemical exudates of a co-occurring phytoplankton prey species, *Tetraselmis suecica*, and the other trail remained scentless. Both trails were flowing at the same rate. The mimic imitates the wake left behind from a swimming prey in the form of a trail [Yen et al. 2004]. From this simple experiment, we asked the following two questions:

1. Do males and females rely on a hydromechanical or chemical cue for prey detection?

2. Does the tracking behavior of males and females differ?

In a following experiment we exposed only *Temora longicornis* males to three different trails that included the scentless trail, a trail scented with female *T. longicornis* scent, and a third trail scented with *T. suecica* scent. From this experiment we asked the question:

3. Does male tracking behavior differ between finding a mate versus a prey?

It was hypothesized that male *T. longicornis* will rely on chemoreception and track a prey-scented trail in a similar manner as they do when exposed to female pheromone trails. Males should successfully interact with a food trail at a similar proportion and employ a similar trail-tracking behavior as a female pheromone trail mimic. Female copepods are expected to follow trails using similar trail-tracking behavior as males. Regardless of sex, energy must be efficiently allocated to maintain the survival of all animals, which is only possible with highly specialized mechanisms. Chemical cues are extremely important in marine ecosystems where background noise of so many other elements such as pheromones, kairomones, and other natural chemical reactions are competing for acknowledgement; yet these animals have to utilize other sensory mechanisms to distinguish these signals due to lack of sight or auditory capabilities (Hay, 2004). The capability of the *T. longicornis* copepods to distinguish between these chemical cues to interact with their mates and prey will allow scientists to inspect their fitness advantages that are responsible for the success of the aquatic food web.

## CHAPTER 2

### MATERIALS AND METHODS

#### **Animal Collection and Care**

Copepods (*Temora longicornis*) were collected from the Darling Marine Center in Walpole Maine USA during the months of May, June and July of 2015. Copepods were collected by hand-retrieving a plankton net. After on-site collection, the copepods were transferred to 1-L sampling bottles and shipped overnight to the Georgia Institute of Technology in Atlanta, Georgia. After shipment arrival, the copepods were cared for by the Yen laboratory animal husbandry team where they were diluted to a density of approximately 300 animals per five gallons of artificial seawater and maintained in a temperature-controlled room at their native temperature of 12°C. The experimental species, *Temora longicornis*, co-occur with other marine species in the collection site so they were separated from the others upon arrival. They were fed a mixed phytoplankton diet of *Tetraselmis suecica* and *Isochrysis galbana*. The phytoplankton species were cared for by the lab of Dr. Terry Snell, School of Biology at the Georgia Institute of Technology, where they were cultured to a density of approximately 2500 cells per microliter.

#### **Trail Mimic Experiment**

To assess the relative importance of hydromechanical and chemical cues for prey detection, the hypothesis that males and females are sensitive to prey diffusible chemical exudates was tested. Male copepods were exposed to three different scent treatments:

female *T. longicornis* scented water, *T. suecica* scented water, and scentless water that the copepods were maintained in. These treatments allowed for the determination if male tracking behavior differed between mates versus prey. Female copepods were exposed to only the *T. suecica* scented water and the scentless water, but not the female *T. longicornis* scented water because females do not react to this cue in nature. These treatments allowed for the determination if females track prey differently from males.

### **Scent Collection**

The scent of *T. suecica* was collected for use in a trail treatment prior to each experimental replicate (protocol determined by Kathryn Nagel, 2012). The *T. suecica* scented trail was made by centrifuging 40-mL of the cell culture at 4000 rpm for five minutes and collecting the cell-free filtrate for immediate trail use. The scent of *T. longicornis* females was collected by creating a copepod “tea.” The tea was created by feeding females a phytoplankton meal and then immersing them in nanopure filtered artificial seawater for 8-12 hours or overnight to perfume the water. Females were removed from the water and the remaining female-scented water was collected into 20-mL scintillation vials and stored at -80C until use in a trail mimic replicate [methods further explained in Pender-Healy and Yen, 2014].). Female scent was used as a positive control because males exhibit a known response to this scent treatment to which they follow in a predicted behavioral manner [Yen et al. 1998]. Artificial seawater at 32ppt was filtered with 22- $\mu$ m bacterial filter and used as a negative control because males exhibit a known negative response to this scentless treatment to which they rarely follow [Yen et al. 1998].

### **Trail mimic bioassay**

Yen et al. (2004) developed a mimic to simulate the wake left behind a swimming copepod in a mating interaction. This mimic can be varied in terms of its chemical content as well as its hydrodynamics. Here, the trails were scented with the perfume of female *T. longicornis*, *T. suecica*, or scentless artificial seawater. The speed of the trail mimic was modified to match a positive tracking response by *T. longicornis* copepods (Doall et al., 1998). Dextran, a high molecular weight sugar, was added to each trail treatment to increase the difference in the refractive index of the water and the trail mimic. This enabled visualization of the trails and the copepod trackers in the Schlieren optical viewing system [Hecht date]. At the start of each experimental trial, thirty males or females were added to the experimental tank, which contained 600-mL of 32 parts per thousand (ppt) artificial seawater (12°C), to acclimate for ten minutes; these conditions match those found in their natural habitat in the Gulf of Maine. After the acclimation period, the trails were placed in the water and programmed to flow at a constant rate of 0.01 mL/minute. The order of the trails was randomized for each replicate to avoid spatial bias. The copepods were allowed to interact with the trails for the duration of the fifty minute experiment. Ten successful replicates were conducted, six male and four female replicates.

### **Visualization and quantification of behavior**

Experiments were conducted in complete darkness, but copepods were visualized using a Schlieren optical viewing system [Schlieren theory and methods further explained in Doall, et al. (1998)]. Their swimming behavior was recorded onto DVD format for

behavioral analysis. These videos were analyzed by tracking their movement on the trails using the tracking software Hedricks for MatLab (Hedrick, 2008) to gather corresponding 'x-y' coordinates to transform into useful locomotory kinematics. To quantify copepod behavior between trail treatments, two types of data were collected. The proportion of trail behaviors for each trail treatment was examined. Trail behavior was quantified by analyzing three locomotory kinematics: the average following speed [cm/sec], the gross distance tracked on a trail [cm], and time spent following a trail [seconds].

The averages of each behavior over all replicates were compared per behavior using a 95% confidence interval to determine significant differences between the trail treatments. These significant differences relayed if the copepods tendency to distinguish between trails. To declare if the tracking behavior between males and females differed when detecting prey, the averages of each of the kinematics described above when the copepods were either in a scentless control or a prey scented trail were compared with a MANOVA test for significant differences that may suggest the patterns observed when in proximity to prey scent. The means of the kinematics when males followed either a pheromone or a prey scented trail were compared using ANOVA test to determine if males varied in tracking behavior when introduced to these treatments. Significant differences for both of these tests were declared if a p-value of 0.05 or less was found.

## **CHAPTER 3**

### **RESULTS**

#### **Relative reliance on chemical or hydromechanical cues for prey detection**

Males and females encountered, followed, and spiraled around the scentless and food-scented trail mimics (Figure 2). The proportion of the total number of trail encounters, follows and spirals between the trail treatments were compared with 95% confidence limits. Results indicate that males rarely followed the scentless trail treatment compared to the food-scented trail treatment (Figure 2). On the other hand, females followed the scentless control just as much as the food-scented trail treatment (Figure 2). Also, the proportion of total trail follows on the food-scented trail treatment may be greater for males (Figure 2). The occurrence of spiraling was only observed in the scented trails. These spirals were quantified based on the radius of the circle, or the furthest point away from the center of the trail on each side. This spiral ring radii did not significantly vary between trail treatments, yet the male copepods were observed to spiral at a larger range than the female copepods (Table 1 and 2).

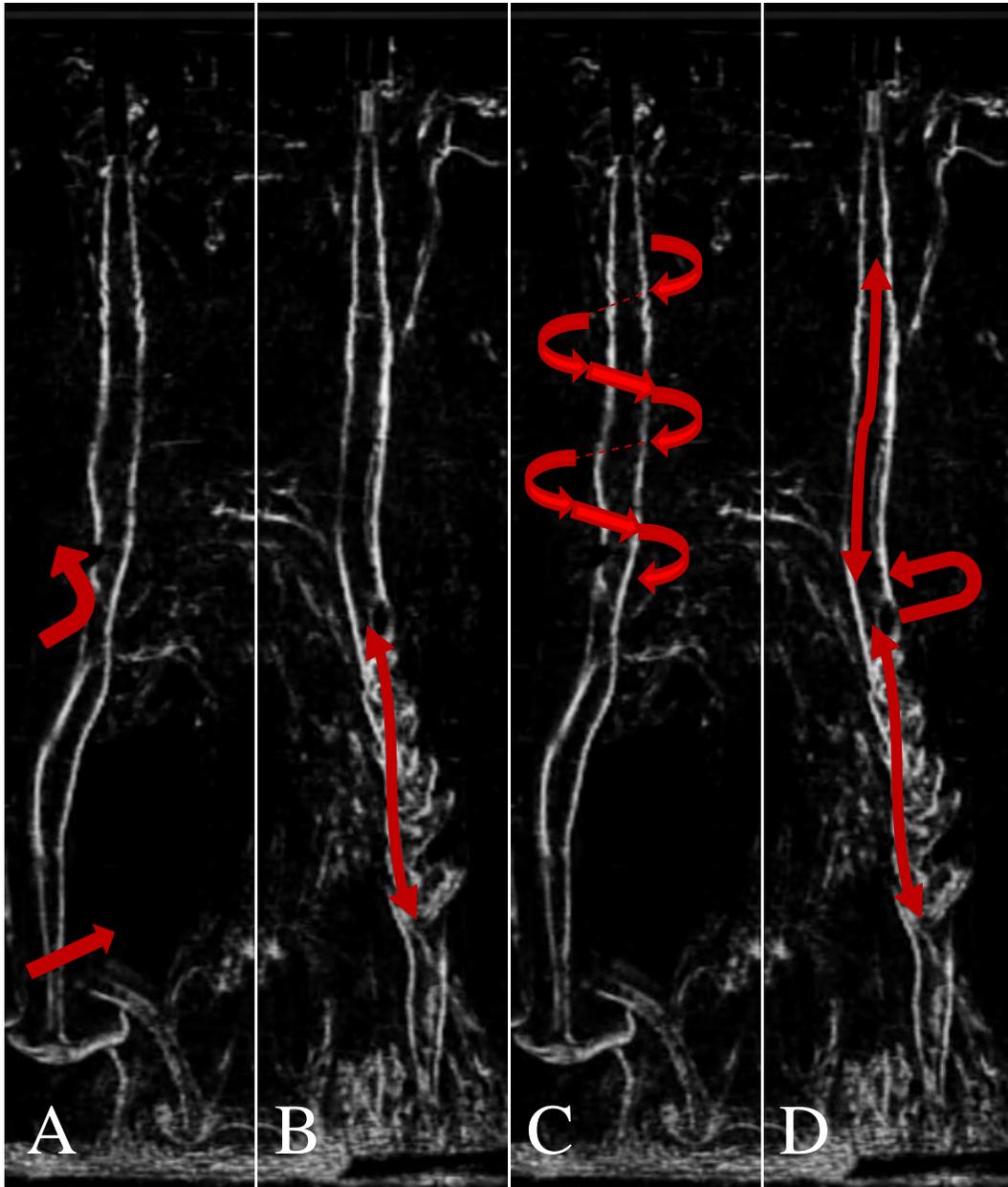
#### **Prey-tracking behavior of males and females**

Four types of trail behaviors were observed (Figure 1). After intersecting a trail, copepods would either not follow a trail mimic (“trail encounter”), follow the trail mimic by disturbing the trail (“trail follow”), perform a “trail follow” but not disturb the trail and instead spiral around the trail (“spiral”), or perform a “trail follow” with continual entrance and exit of the trail during a single follow by the same copepod (“mistake”).

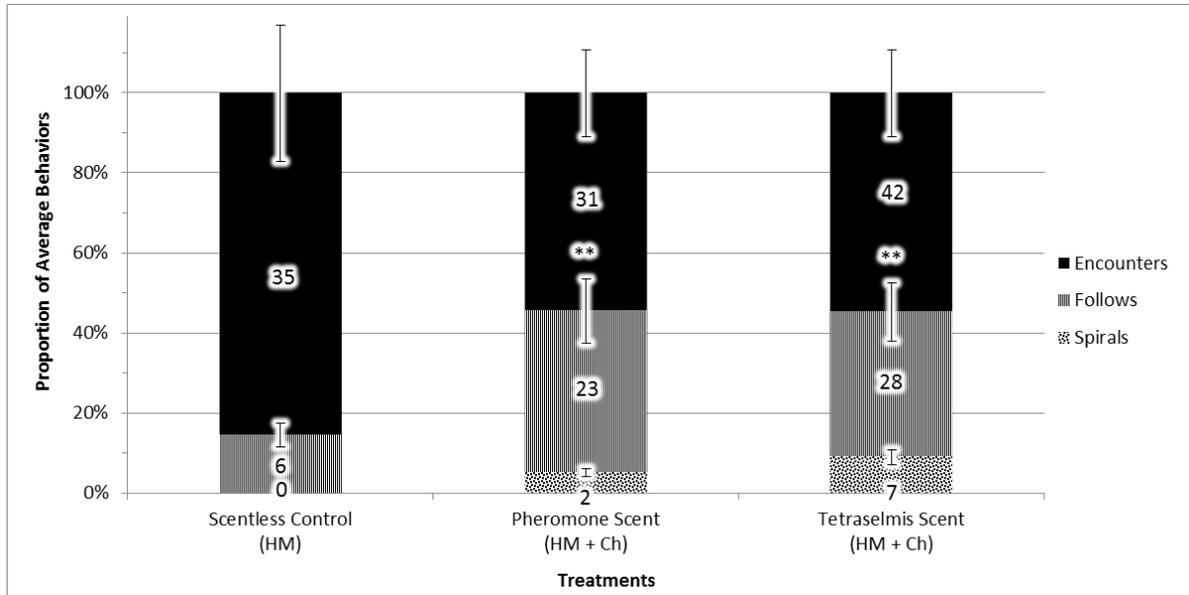
Males and females exhibited all four trail behaviors (Figure 2). Females followed both the scentless control and the food-scented treatment at a significantly shorter distance and time duration than the males (Table 1 and 2). The females rarely made mistakes in comparison to the number of mistakes and direction changes of male copepods during following (Table 1 and 2). Other aspects such as average return distance of mistakes did not significantly vary between sex or trail treatment (Table 1 and 2).

### **Male tracking behavior of a mate versus a prey**

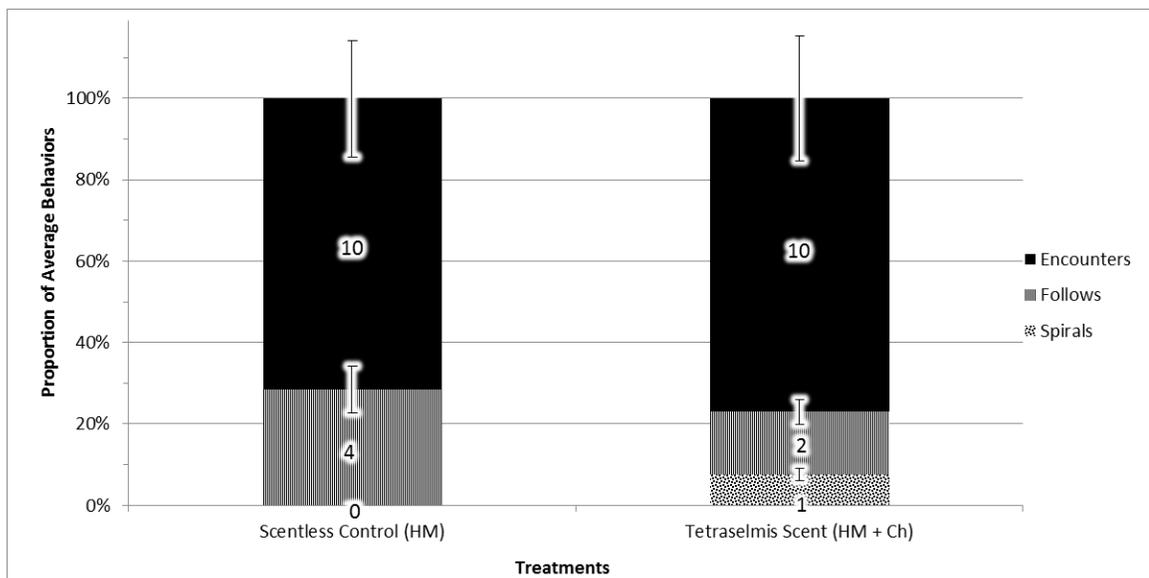
Males encountered, followed, and spiraled around the female-scented and food-scented trail mimics (Figure 2). Males followed a food-scented trail for a shorter net distance compared to the female-scented trail, which males typically followed the entire length of the trail mimic (Table 3). The gross distance, speed, number of mistakes, and ring radii of spirals was similar for males and females. There was not significant difference in the proportion of trail encounters, follows and spirals between trail treatments. There was not a significant difference in the locomotory kinematics of male tracking between trail treatments (Figure 2).



**Figure 1.** *Trail Behaviors.* The paired white vertical lines define the trail location and width where this image is visualized using Schlieren optics [Hecht date]. Since the copepod also is a phase object, this optical technique enables visualization of both the deformation of the signal and the trajectory of the copepod. Four types of trail interactions were observed between among male and female copepods. A) “Encounters”, where copepod passes through a trail without a subsequent follow, B) “Follows”, which are encounters with a subsequent reorientation of the body and disturbance of the trail, C) “Spirals”, where the copepod does not enter the trail but instead rotates around the borders of it, and D) “Mistakes”, where the copepod orients its body toward the trail and follows it, exits the trail, then reorients to follow again.



**Figure 2. Male Behavior Proportions.** Proportion of male copepod trail behaviors for each trail treatment compared with a 95% confidence interval as the error bars showed that scented trails were followed more than unscented trails. The resulting proportions are shown in the “scentless” hydromechanical (HM) negative control, positive control of “female pheromone scent” with hydromechanical and chemical cues (HM + Ch) for males only, and “prey scent” of hydromechanical and chemical cues (HM + Ch).



**Figure 3. Female Behavior Proportions.** Proportion of female copepod trail behaviors between trail treatments compared with a 95% confidence interval as the error bars shows what???. The resulting proportions are shown in the “scentless” hydromechanical negative control (HM) and “prey scent” of hydromechanical and chemical cues (HM + Ch).

**Table 1. Locomotory Kinematics of females. Kinematics analyses quantitatively show that what???** The resulting means of each behavior  $\pm$  standard deviation for female copepods' follows and spirals in the scentless negative control and *T. suecica* scented trails.

		CONTROL	TETRASELMIS
FOLLOWS	1) Distance traveled		
	Gross (mm)	0.84 $\pm$ 0.48	0.98 $\pm$ 0.56
	Net (mm)	0.32 $\pm$ 0.12	0.34 $\pm$ 0.30
	NGDR	0.52 $\pm$ 0.26	0.52 $\pm$ 0.60
	2) Time spent tracking a trail (s)	1.78 $\pm$ 1.66	1.30 $\pm$ 1.65
	3) Swimming speed during a track (mm/s)	0.59 $\pm$ 0.19	2.46 $\pm$ 2.70
	4) Average number of direction changes	0.00 $\pm$ 0.00	0.50 $\pm$ 0.71
5) Average number of mistakes	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	
6) Average return distance of mistakes (mm)	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	
SPIRALS	1) Distance traveled		
	Gross (mm)	0.00 $\pm$ 0.00	4.69 $\pm$ 2.10
	Net (mm)	0.00 $\pm$ 0.00	1.97 $\pm$ 0.88
	Ratio	0.00 $\pm$ 0.00	0.42 $\pm$ 0.19
	2) Time spent tracking a trail (s)	0.00 $\pm$ 0.00	9.37 $\pm$ 4.19
	3) Swimming speed during a track (mm/s)	0.00 $\pm$ 0.00	0.50 $\pm$ 0.22
	4) Ring radii (mm)	0.00 $\pm$ 0.00	0.31 $\pm$ 0.14

**Table 2. Locomotory Kinematics of males: Control and Tetra. Kinematics analyses quantitatively show that what???** The resulting means of each behavior  $\pm$  standard deviation for male copepods' follows and spirals in the scentless negative control and *T. suecica* scented trails.

		CONTROL	TETRASELMIS
FOLLOWS	1) Distance traveled		
	Gross (mm)	3.87 $\pm$ 1.10	10.08 $\pm$ 5.00
	Net (mm)	2.42 $\pm$ 0.01	6.41 $\pm$ 3.10
	NGDR	0.69 $\pm$ 0.09	0.67 $\pm$ 0.07
	2) Time spent tracking a trail (s)	3.84 $\pm$ 0.61	8.56 $\pm$ 3.09
	3) Swimming speed during a track (mm/s)	1.03 $\pm$ 0.02	1.14 $\pm$ 0.16
	4) Average number of direction changes	2.00 $\pm$ 2.83	1.75 $\pm$ 1.71
5) Average number of mistakes	1.00 $\pm$ 1.41	1.38 $\pm$ 0.95	
6) Average return distance of mistakes (mm)	0.12 $\pm$ 0.17	0.16 $\pm$ 0.12	
SPIRALS	1) Distance traveled		
	Gross (mm)	0.00 $\pm$ 0.00	17.55 $\pm$ 6.06
	Net (mm)	0.00 $\pm$ 0.00	1.29 $\pm$ 0.92
	Ratio	0.00 $\pm$ 0.00	0.07 $\pm$ 0.02
	2) Time spent tracking a trail (s)	0.00 $\pm$ 0.00	7.35 $\pm$ 3.24
	3) Swimming speed during a track (mm/s)	0.00 $\pm$ 0.00	2.67 $\pm$ 1.02
	4) Ring radii (mm)	0.00 $\pm$ 0.00	0.24 $\pm$ 0.16

**Table 3.** *Locomotory Kinematics of males: Pheromone and Tetra. Kinematics analyses quantitatively show that what???* The resulting means of each behavior  $\pm$  standard deviation for male copepods' follows and spirals in the positive control pheromone and *T. suecica* scented trails.

		<b>PHEROMONE</b>	<b>TETRASELMIS</b>
<b>FOLLOWS</b>	1) Distance traveled		
	Gross (mm)	8.20 $\pm$ 3.44	10.08 $\pm$ 5.00
	Net (mm)	6.19 $\pm$ 2.46	6.41 $\pm$ 3.10
	NGDR	0.79 $\pm$ 0.11	0.67 $\pm$ 0.07
	2) Time spent tracking a trail (s)	6.22 $\pm$ 2.46	8.56 $\pm$ 3.09
	3) Swimming speed during a track (mm/s)	1.43 $\pm$ 0.23	1.14 $\pm$ 0.16
<b>SPIRALS</b>	4) Average number of direction changes	2.32 $\pm$ 1.27	1.75 $\pm$ 1.71
	5) Average number of mistakes	2.44 $\pm$ 1.54	1.38 $\pm$ 0.95
	6) Average return distance of mistakes (mm)	0.27 $\pm$ 0.09	0.16 $\pm$ 0.12
	1) Distance traveled		
	Gross (mm)	14.21 $\pm$ 5.82	17.55 $\pm$ 6.06
	Net (mm)	1.97 $\pm$ 1.98	1.29 $\pm$ 0.92
	Ratio	0.12 $\pm$ 0.09	0.07 $\pm$ 0.02
	2) Time spent tracking a trail (s)	7.94 $\pm$ 6.79	7.35 $\pm$ 3.24
	3) Swimming speed during a track(mm/s)	2.49 $\pm$ 1.37	2.67 $\pm$ 1.02
	4) Ring radii (mm)	0.31 $\pm$ 0.07	0.24 $\pm$ 0.16

## CHAPTER 4

### DISCUSSION

#### **Relative reliance on chemical or hydromechanical cues for prey detection**

The results of this experiment indicate that male and female *T. longicornis* copepods may use different sensory mechanisms to detect their prey. Males and females followed each trail treatment, including the scentless negative control, indicating reliance on a hydromechanical cue. Results from the relative proportion of behaviors between the two trail treatments showed females interacted with the scentless control and the food scented trail at similar occurrences indicating that females do not need a chemical cue to detect prey. However they exhibited a different behavior unique to the food trail, spiraling. Females exhibited spiraling, a behavior associated with prey detection, only in the chemically scented food trail and not in the control. The obscure “spiral” behavior, where the copepods were observed to circle around the perimeter of the trail with no disturbance, yet seemingly interested, was interestingly only seen in the chemically scented trails by both sexes. Lombard et al. showed that copepods follow sinking particles of marine snow due to the copepods ability to sense these chemicals at remote distances (2013). The spiraling behavior may allow the copepods to remain interested in the trail while distinguishing the direction and type of scent for more accurate tracking and following behavior. This behavior termed “spiral” seems important to these results although they are proportionately less prevalent than the other behaviors because of its occurrence only with the scented trails. Although this behavior did not seem to differ based on the type of scented trail, it did differ between the sexes as far as ring radii or

swimming speed during the spiral. Female copepods were closer proximity to the prey-scented trail based on the smaller ring radii during a spiral. This is supportive evidence that *T. longicornis* copepods rely primarily on chemical cues to remotely detect their prey from larger distances which may give insight into the gradient or distance necessary for chemical cue detection by the copepods.. However, males followed a food-scented trail at nearly twice the proportion of a scentless trail mimic (Figure 2), indicating a primary reliance of a chemical cue with the addition of a hydromechanical cue to detect prey. A further experiment exploring their hydrodynamic sensitivity will be needed to tease apart the relative influence of hydrodynamics in prey detection. Due to the nature of the bioassay, one cannot remove the hydromechanical cue without making the trail motionless. However, the rapid decay of hydrodynamic signals makes them poorly informative; therefore, chemoreception is believed to be the main contributor to increase encounter rates (Yen et al., 1998). The use of multiple sensory cues allows males to detect changes in their surrounding environment from multiple sources that are proximal and distant to their location (Woodson, 2007). The ability to utilize both chemical and hydromechanical cues may prove to be advantageous to the males when mating or feeding to survive in these huge oceans with so many competing cues. .

### **Prey-tracking behavior of males and females**

When comparing male and female tracking behaviors in the food scented trails, they were found to significantly differ during their interactions. In general, female copepods interacted with the trails at a lower occurrence over all of the replicates for any behavior. The males were observed to follow the *T. suecica* scented trail significantly

more than the females. Males followed the food trail for longer distances and time as well as a higher number of mistakes compared to the females. The number of mistakes gives insight into the detection distance for prey based on the average distance from the trail when the copepod returns from a mistake. These differing results may be due to females less prominent tracking mechanisms since they are not responsible for other tracking events such as mating. This possible deficiency of sensitivity in female detecting mechanisms may also be responsible for their lack of interaction with pheromone trails. Previous studies have shown that female *Temora longicornis* copepods do not change their swimming behavior when exposed to heterospecific pheromone-conditioned water which entails their lack of heterospecific trail following behavior in other experiments (Serount et al, 2014). Females may not track trails since prey are usually present in scented plumes that do not require the following behavior that was recorded in this experiment. The plumes are observed to emit a chemical cue that can be sensed by the copepod (Steinke et al., 2006) but may not be strong enough in this type of bioassay containing thin hydrodynamic cues. The analysis of these locomotory kinematics indicates the relative significant difference in males higher time spent, distance, and mistake occurrence during tracking of prey than females.

### **Male tracking behavior of a mate versus a prey**

In further statistical analysis of the following behavior, it was found that males did follow both the food-scented trail and the pheromone-scented trail using similar behavior with similar proportions of behavior occurrences, as seen in *Figure 2*. The male copepods did not show any significant differences between the pheromone and *T. suecica*

scented trail treatments during the following behavior. As seen in *Table 3*, their speed and NGDR averages were similar in both trail types over a similar time spent during the tracking event. The even made mistakes at similar amounts which is very important in understanding the ability of the copepod to determine the source of the chemical cue. It has been suggested that copepods tend to swim toward the source of the pheromone cue to eventually engage in copulation with the emitting mate (Serount, 2014), while they are seen to fall with food particles that are leaving a scent to track (Lombard et al, 2013). These mistakes seem to imply that the copepods were not clearly able to immediately and consistently determine the source of the scent. This may be due to the characteristic of constantly flowing trail or a disturbance in the trail could possibly be detouring the tracking ability. Copepods swimming speed in the spiral behavior were found to vary widely but were averaged to be significantly faster than the swimming speed during following. This is an interesting result because it contradicts the previous results of Lombard where the copepods were found to follow at slower speeds in proximity to the marine snow compared to pheromone trails (2014). They were thought to do this because, but as seen in the current results, the faster speed during spirals may be necessary to capture prey in fast moving water where the copepod must travel further distances while remotely tracking the scent of the prey. These results allow for us to see the possible competitive advantage that may occur between these male *T. longicornis*. If there is a variance in the ability of males to track prey or mates with less mistakes during a follow as suggested in other studies (Byers et al, 2010; Titelman et al, 2007), these males will be more efficient in finding these necessary resources by getting to the source sooner than the others. This is also important for the spiral speed where the faster copepod can

capture the mate or prey before another copepod can to collect more food or copulate with more females for a higher fitness and survival. The characteristics of these copepods tracking behavior gives much insight into their competition for the scarce and dispersed resources in the huge, vast aquatic desert that inhabit.

## **Significance**

What is so special about copepods that can follow food-scented trails? The ocean is a vast aquatic food desert for copepods, in which small patches of phytoplankton exist. These food patches leak small trails of concentrated prey smell. Copepods that are able to detect these food-scented trails will be more successful at finding the larger food patches by following the cue to the source of prey. Hence, copepods that are trail followers will be more successful at finding food thus having a competitive advantage for survival against other phytoplankton consumers who do not follow trails [who said this? I don't think I've ever read this in the literature just in my proposal. Please send me the paper that states this], such as fish larvae and shrimp (Texas A&M University, 2009). This ability may also help in the fitness advantage allowing male copepods to find females mates from farther distances compared to those males that cannot successfully detect these cues remotely. Think about it... there is a vast ocean with lots of conversational noise cues. If one has a means of detecting a specific cue that persists for a relatively long period of time and it can lead you right to the source, then you should be more successful than other copepods that cannot detect these trail-like cues. This is specific to chemical cues which last longer compared to the hydromechanical cues that diffuse quicker among the constantly moving water that these copepods live in (Serount, 2014). These results are

important to understand the competitive advantage necessary to maintain high fitness in copepods for the maintenance of the food web's order. Results from this study may open a new avenue of research for the success of other trail-sensing organisms such as insects like ants or mammals like dogs (Goldstein, 2008). Stay tuned.

## CHAPTER 5

### FUTURE WORK

In this current bioassay, the hydrodynamic signal could not be removed without rendering the trail motionless. Therefore, in a future experiment the relative influence of hydromechanical cues can be tested by varying the flow rate of the chemical trail. If the change in flow rate increases the proportion of follows, then you can conclude that copepods rely on hydromechanical cues for detection, not chemical cues. This result will help to understand the copepods' detection ability of these cues.

Also, it would be interesting to determine if copepods have a preference for food type. With this experiment, it is now known that they will follow a food trail. A future experiment would be to vary the scent of each food trail by incorporating different types of prey that co-occur are consumed by *T. longicornis* copepods. For instance, copepods are known to eat *Isochrysis galbana* which is a phytoplankton that is high in lipids. These lipids are important in a copepods diet because it increases the copepods buoyancy level (Schründer, 2014) which allows them to use less energy when swimming for better allocation of resources (Hiltunen, 2014). Also, females are known to increase their egg production when on high-fat diets. It is possible that the short length follows observed in this experiment can be extended when the copepods are enticed by a more beneficial food source in comparison to the less efficient choice.

There is also a need to compare the tracking behavior of other copepod species and their ability to remotely detect food trails. Due to the different body structures as well as environments, not all copepods rely on the same environmental cues when interacting with the objects around them (Tsuda, 1998). For instance, the *Hesperodiaptomus shoshone* copepod is a much larger copepod than the *T. longicornis* that resides in freshwater (Yen, 2011). It is possible that copepods in freshwater have a lower need for

detection sensitivity in their less “noisy” environment (Hay, 2004). On the other hand, they may prove to be more capable of detecting certain cues at larger detection ranges because of the ability for cues to persist longer over time due to the smaller amount of competitive cues. The copepods residing in different environments energy clearly must allocate their energy differently due to environmental constraints, such as growth in body size or detection mechanisms, so it may in turn cause unique behavior in the tracking behavior of these animals that should be explored in further experiments.

It is ultimately very important to figure out what sensory structures are responsible for the detection of prey and mate cues. For instance, the antennae are a likely source of useful sensors that may be sensitive to changes in the environment, whether chemical or hydrodynamic. They are equipped with small sensory setae (Lenz, 1993) that are currently under investigation in the Yen lab for physiological purpose in trail sensing. Therefore, it would be beneficial to determine the different structures that assist in the copepods ability to remotely detect environmental cues and whether it differs between trail types.

### **Broader Impact**

Results from this study will provide insight into copepod swimming behavior and sensory perception of prey cues. Other fields are likely to benefit from this study as well. For instance, cruising copepods may follow trails for both mate-seeking and prey-detection. Therefore trail-following behavior may be an important evolutionary advantage for cruising copepods that needs to be further investigated. Additionally, copepod trail-following behavior has important applications in bio-inspired design as it relates to the sensors as well as propulsion. For example, these natural mechanisms may be used to build underwater sensors on vehicles for the U.S. Navy or develop self-propelled noninvasive medical devices to repair the site of a heart attack.

## REFERENCES

- Byers, John, Eileen Hebets, and Jeffrey Podos. "Female mate choice based upon male motor performance." *Animal Behaviour* 79.4 (2010): 771-778. Doall, Michael, et al. "Locating a mate in 3D: the case of *Temora longicornis*." *Philosophical Transactions of the Royal Society B: Biological Sciences* 353.1369 (1998): 681-689.
- Goldstein, E. Bruce. "Tracking odor trails and spatial information present in odors dispersed in fluid." *Encyclopedia of Perception* (2008).
- Gonçalves, Rodrigo J., et al. "Mechanisms of prey size selection in a suspension-feeding copepod, *Temora longicornis*." *Mar. Ecol. Prog. Ser* 517 (2014): 61-74.
- Hay, Mark E. "Marine Chemical Ecology: Chemical Signals and Cues Structure Marine Populations, Communities, and Ecosystems." *Annual Review of Marine Science* 1 (2009): 193–212.
- Hedrick, Tyson. "Software techniques for two-and three-dimensional kinematic measurements of biological and biomimetic systems." *Bioinspiration & Biomimetics* 3.3(2008): 034001.
- Hiltunen, Minna, et al. "Distinctive lipid composition of the copepod *Limnocalanus macrurus* with a high abundance of polyunsaturated fatty acids." *Lipids* 49.9 (2014): 919-932.
- Katona, Steven K. "Evidence for sex pheromones in planktonic copepods." *Limnology and Oceanography* 18.4 (1973): 574-583.
- Kjørboe, Thomas, and Espen Bagøien. "Motility patterns and mate encounter rates in planktonic copepods." *Limnology and Oceanography* 50.6 (2005): 1999-2007.
- Kjørboe, Thomas. "How zooplankton feed: mechanisms, traits and trade-offs." *Biological Reviews* 86.2 (2011): 311-339.
- Legier-Visser, M. F., et al. "Mechanoreception in calanoid copepods." *Marine Biology* 90.4 (1986): 529-535.

- Lenz, Petra, and Jeannette Yen. "Distal setal mechanoreceptors of the first antennae of marine copepods." *Bulletin of Marine Science* 53.1 (1993): 170-179.
- Lombard, Fabien, et al. "Copepods use chemical trails to find sinking marine snow aggregates." *Limnology and Oceanography* 58.1 (2013): 185-192.
- Schründer, Sabine, et al. "Observations of neutral buoyancy in diapausing copepods *Calanoides acutus* during Antarctic winter." *Polar biology* 37.9 (2014): 1369-1371.
- Schultz, Mette, and Thomas Kiørboe. "Active prey selection in two pelagic copepods feeding on potentially toxic and non-toxic dinoflagellates." *Journal of plankton research* 31.5 (2009): 553-561.
- Seuront, Laurent, and H. Eugene Stanley. "Anomalous diffusion and multifractality enhance mating encounters in the ocean." *Proceedings of the National Academy of Sciences* 111.6 (2014): 2206-2211.
- Steinke, Michael, et al. "Dimethyl sulfide (DMS) triggers search behavior in copepods." *Limnology and Oceanography* 51.4 (2006): 1925-1930.
- Texas A&M University. OceanWorld: Marine Food Webs. 2009. Web. 28 Nov 2015. <http://oceanworld.tamu.edu/resources/oceanography-book/marinefoodwebs.htm>
- Titelman, Josefin, et al. "Copepod mating: chance or choice?." *Journal of Plankton Research* 29.12 (2007): 1023-1030.
- Tsuda, Atsushi, and Charles B. Miller. "Mate-finding behaviour in *Calanus marshallae* Frost." *Philosophical Transactions of the Royal Society B: Biological Sciences* 353.1369 (1998): 713-720.
- Woodson, Clifton Brock, et al. "Cue hierarchy and foraging in calanoid copepods: ecological implications of oceanographic structure." *Mar Ecol Prog Ser* 330 (2007): 163-177.
- Yen, J, et al. "The fluid physics of signal perception by mate-tracking copepods." *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 353.1369 (1998): 787-804.

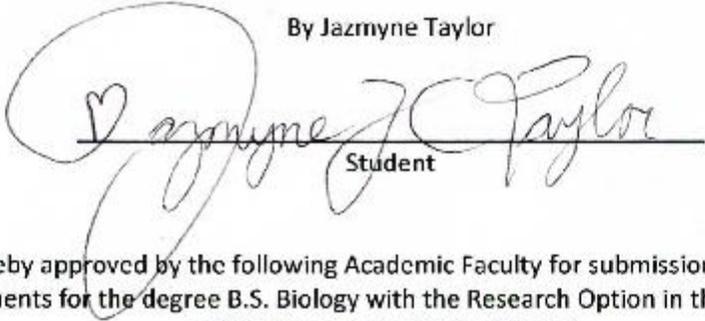
Yen, Jeannette, et al. "Pheromone trail following in three dimensions by the freshwater copepod *Hesperodiptomus shoshone*." *Journal of plankton research* 33.6 (2011): 907-916.

Yen, Jeannette, et al. "Signaling during mating in the pelagic copepod, *Temora longicornis*." In Seuront, L. and Strutton, P. (eds.), *Scales in Aquatic Ecology: Measurements, Analysis, Modelling*. CRC Press, New York, NY, (2004): 149–159.

Yen, J. and Rachel Lasley. "Chemical communication between copepods: finding the mate in a fluid environment." In Thomas Breithaupt & Martin Thiel (eds.), *Chemical Communication in Crustaceans*. Springer, 9 (2010): 177-197.

**Finding Food in an Aquatic Desert:  
How cruising copepods detect their next phytoplankton meal**

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