Chemical Mate-tracking in Copepods: A Comparison of *Hesperodiaptomus shoshone* and *Temora longicornis*

Megan Heaphy
Advisor: Jeannette Yen

ABSTRACT

Males of the marine copepod species *Temora longicornis* have been seen to track the chemical trails of females in order to locate the female for mating. The males of one freshwater copepod, *Hesperodiaptomus shoshone,* have recently been observed to track the chemical trails of females. The actual following behaviors with respect to the trail were previously unknown, and have now been documented. Methods have been developed to allow for the analysis of the orientation with respect to a chemical trail. *H. shoshone* spends more time on the outside of the chemical trail when tracking than *T. longicornis,* which spins in a helical fashion on the inside of the trail. With a chemical trail of radius 0.5 mm, *H. shoshone* has an average distance from the trail of 0.90 mm ± 0.11, whereas *T. longicornis* has an average distance from the trail of 0.49 mm ± 0.39. Future studies will further elucidate the mechanisms used by these copepods and others to accurately locate mates, in an attempt to create models of mechanisms to be used in biologically-inspired chemical detection devices.

Key words: *Hesperodiaptomus shoshone*; *Temora longicornis*; mate-tracking; marine copepods; freshwater copepods; tracking mechanisms

1. INTRODUCTION

Animals need to identify materials by their scents and discern whether they are worth pursuing or need to be avoided. In many cases, the animals are trying to determine the presence of mates, and if it will be worthwhile to actively search for these mates, possibly through tracking. One animal, the copepod, spends its time balancing predator

avoidance, food location, and mate detection. Many copepod species must rely on chemical signals of mates in order to find one another, given the low probability of random encounter. For example, *Temora longicornis*, a marine copepod, and *Hesperodiaptomus shoshone,* a freshwater copepod, have been observed to locate mates by tracking a chemical exuded by the female. There have been some studies on copepods and many studies on other chemical-tracking organisms that provide the basis for characterizing the chemical tracking behaviors of these known trail-tracking copepods. It is still not known how exactly these organisms utilize the chemical scents of their mates and how they orient themselves toward and within the chemical signals. The purpose of this study is to elucidate the orientation of copepods within the trail in order to better characterize how the copepods are taking in the scent and which mechanisms they are using to track.

(a) Chemical tracking abilities

Aside from copepods, many animals perform tracking using chemical signals to locate materials. It is important to study the tracking behaviors of other species in order to determine any patterns in chemical tracking that may arise due to the environment in which the animal lives. These patterns can be applied to make more accurate hypotheses about the behaviors of unstudied species.

The phenomenon of chemical tracking has been observed in blue crabs (Weissburg 2000), shrimp (Hamner 1977), moths (Schofield et al. 2003), and Procellariiform seabirds (Nevitt 2000), among others. Blue crabs have been observed to

track food scents superimposed against a flow field. The crabs will always track upstream in order to locate the source of the odor (Weissburg 2000). Shrimp have been seen to track the chemical exudates from falling food particles, and it is most likely that they were following the concentrations of several amino acids found in the food (Hamner 1977). Catfish, another aquatic animal, have been observed to detect the chemical and hydrodynamic components of the wake left by a prey item, which can be up to 10 seconds old (Pohlmann et al. 2001). Procellariiform seabirds, a group which includes albatrosses, are also known for their tracking abilities. It is postulated that they use their large olfactory glands to detect odors above the ocean, and that they use these odors to guide their decisions on where to forage (Nevitt 2000).

Location of mates using pheromones has been observed in several of these scent-tracking animals. A male gypsy moth is known to search the air by flying back and forth in an area once he senses a conspecific female's scent in order to find the plume, and will home in on the female by flying upwind in the plume until he reaches her location (Bradbury 1998). Contact pheromones are also used in rotifers, where the female secretes pheromones and the male has a corresponding receptor. The male will touch other rotifers to confirm their gender, and will engage in mating with females when detected (Snell et al. 1995).

(b) Copepods and signals

Copepods inhabit large bodies of water, while remaining within relatively small areas. While maneuvering in the water, many have the capability for motion in three dimensions. Due to the constraints on how to characterize 3D movement that have only

recently been solved (Crenshaw et al. 2000), many questions remain unanswered about copepod mobility.

Copepods rely on sensory cues from their environment to guide them while searching for food and mates as well as attempting to avoid predators. One cue from the environment is that of light. Many copepod species are positively phototactic and use sunlight as a cue to migrate away from the surface through the process of diel vertical migration. Other cues from the environment are hydrodynamic signals, or wakes created by other organisms. Copepods can detect the hydrodynamic signals of other copepods, or those of predators, and the components of the signal determine whether the copepod pursues the source or escapes (cite?).

The most important cues in the context of this study are chemical signals. Copepods can respond differently to a material based on its chemical components, and much like with hydrodynamic cues, the different chemicals represent predators, food or mates. The detection of mates by copepods can be broken down into several groups. Bagøien and Kiørboe argue that mates can be detected based on hydrodynamic cues, chemically diffuse plumes or chemical trails, or can involve a combination of the two chemical detection methods. They summarize the methods used for several species (Bagøien 2005). Females of some species, including *T. longicornis*, can make themselves more detectable through the use of "hops," which are small hydrodynamic disturbances around the females (Van Duren 1996).

It was first suggested in 1973 that male copepods could be relying on chemicals exuded by females in order to locate the female for mating (Katona 1973). Katona's work focused on the species *Eurytemora affinis,* the females of which most likely emit diffuse

plumes of chemical in their surroundings. The males of this species exhibit a searching behavior when they first encounter this chemical, and spiral around the female, coming closer and closer to eventually grab her. These chemical compounds, called pheromones (Dusenbery 1995), are used to track females by males of many species. Pheromones often function to give males a remote signal from the female, thereby increasing the probability that the male will encounter the female.

The distinction has been made that copepods do not track odor trails in a manner similar to animals in higher Reynolds number environments, such as crabs and moths (Yen 1998). While crabs and moths rely on a flow direction cue as well as a pheromone, copepods do not respond to flow in one direction. Copepods live in lower Reynolds number environments, and are so small that they are carried where the water takes them. Chemicals in the water around them move mainly by diffusion. Therefore, trails left in the water behind females can be detected and are a fairly reliable source about the proximity of the female. The males of many copepod species, including *Centropages typicus, Centropages hamatus, Calanus marshallae,* and *Temora longicornis* are documented as using pheromone trails as a method to find mates (Bagøien 2005). Males of the species *Temora longicornis* combine female hydrodynamic "hops" with chemical cues in order to locate mates. *Temora longicornis* is known to track female trails for up to 13 centimeters, and sometimes even follows the female trail away from the female, and detects he is traveling in the wrong direction, then doubles back on the trail to follow it to the female (Doall 1998).

While mate tracking in *T. longicornis* has been documented, not much is known about the mechanisms underlying it (Yen 1998). Professor Yen et al. noted that *T.*

longicornis males have sensors on 10% of proximal sectors of the antennae, over a span of approximately 100 micrometers (Fleminger 1967; Griffiths 1976). It was postulated by Yen that the locations of these sensors cause the copepod to dive in and out of the trail in order to test for the edges of the trail (Yen 1998). The actual mechanism that these copepods are using to detect the trail and orient within it to follow it to the female remains unknown and is the focus of this study. Therefore, this study will focus on the hypothesis that *T. longicornis* dives in and out of the trail.

Mate searching has been found in marine copepods as well as freshwater copepods. The freshwater copepod *Leptodiaptomus ashlandi* has been found to search for mates based on a chemical component (Nihongi et al. 2004), which is the basis for the present work with the freshwater copepod *Hesperodiaptomus shoshone.* Additionally, current work in the Yen lab has shown that *H. shoshone* relies on a chemical cue from the female, which has led us to question how this copepod behaves compared to *T. longicornis.* These copepods have struggled to retain populations after the predation from stocked fish in their mountain lakes created dwindling numbers (Sarnelle & Knapp 2004). Therefore, it is extremely important that we determine how they locate mates so that population models can be created and their population growth can be facilitated. Not much is known about this copepod species, and therefore we can only speculate as to the location of chemical receptors on their bodies. It is postulated from prior observations that these copepods have receptors in the mouth regions, rather than on their antennae, due to the manner in which they skim the outside of the trail mimics created in previous experiments. Therefore, they would not dive in and out of the trail like *T. longicornis,* but would rather stay immediately on the outside.

Harpacticoid copepods, which are benthic (bottom-dwellling) plankton, are more likely to rely on contact pheromones (Frey et al. 1998). These copepods have extremely short antennae, and probably have not evolved longer antennae due to the lack of a need for spatial resolution in a three-dimensional environment. The fact that they do not track trails contributes to the hypothesis that the antennae length of the copepod determines the copepod's tracking abilities.

Overall, detection and location of mates is not limited to a pheromone-tracking modality in chemical-tracking copepods. Tracking allows the males to get closer to the females, but there are often contact pheromones or mechanical steps (Blades & Youngbluth 1980) that are involved to assist the male in determining if a particular female is one of his species. While other factors come into play, pheromones are extremely important to the animals that use them. The pheromones are species-specific, and guide the male in deciding whether he should pursue the source of the odor, as well as serving to increase the encounter radius of females. Therefore, it is important to study the benefits of pheromone use for each species, so that we may tell what each species' requirements are for survival.

(c) Motivation

While the study of copepods and other tracking organisms is important in order to demonstrate how they are able to thrive as a species, studying the chemical tracking behaviors can also provide a basis for many models to apply to engineering problems. With the increasing popularity of the study of biomimetics (Benyus 1997), which uses models from the natural world as inspiration for solutions to design problems, animal

tracking strategies have become more widely used in robotics to have robots perform specific tracking tasks. The moth plume-following strategies have recently been implemented for trail following mechanisms in autonomous vehicles (Li 2001). By broadening the knowledge of copepod chemical-tracking behaviors, we will be providing biologically-inspired designers with another animal model that tracks trails on an extremely small scale, which would be optimal for any nano-scale robots that were developed.

The study of chemical trail-tracking copepods will give a better understanding of the mechanisms behind tracking. It will also allow for distinctions between the various environments, which can show how the various species evolved to use such tactics, as well as providing comparative models for biologically inspired design projects. Several components of the tracking behavior of the copepods *H. shoshone*, and *T. longicornis,* including speed, distance from the trail, and angle with respect to the trail, will be analyzed in order to determine any similarities or differences between the tracking strategies. It is hypothesized that there will be a difference in the speed of the copepods before and during tracking, that *T. longicornis* will spend more time within the trail than *H. shoshone,* and that the angles with respect to the trail will be higher for *T. longicornis* than for *H. shoshone.*

2. METHODS

(a) Copepod Care

Copepods were kept in 5-gallon buckets at the correct temperature for their environment, which is 13° C for *T. longicornis* (1.3 mm) and 12° C for *H. shoshone* (2.1

mm). *T. longicornis* was fed *Tetraselmis spp.,* whereas *H. shoshone* was fed *Artemia spp.* nauplii.

(b) Trail Preparation

Females of the species being studied were placed in a small volume of filtered water that was free of food particles with a concentration of one copepod per 20 mL, and were left for 2 hours so that their scent could infuse the water around them. The female copepods then were pipetted out of the water and returned to their bucket. The scented water was filtered and dextran, a density agent, was added at a concentration of .01 g/mL water. The water with dextran then was placed into a syringe and added to an electric syringe pump, which released the scented water into the tank at a rate of .01 mL per minute, in order to reduce any hydrodynamic effects from adding the water. The trails represent mimics of the female's pheromone trail as she swims through the water. By releasing them with a steady flow of .21 mm/s, the hydrodynamic effects were minimized and were standardized. A second trail containing only dextran and seawater for *T. longicornis* and filtered lake water for *H. shoshone* was run along with the first trail as a control to ensure that the copepods were following the pheromone and were not simply reacting to the dextran in the water.

(c) Visualization of data

The tank containing the copepods rests within the outer vessel of the Schlieren optics system (Doall 1998). In this setup, illumination is provided by a laser beam, which displays the objects based on the differences in refraction index throughout the tank. The

copepods and trails appear as white objects on a black background. The X-Z and Y-Z views from the splitting of the laser are combined onto one image, and are offset slightly so that it is clear which is the X-Z and which is the Y-Z axis. The camera used in the experiment is oriented on its side so that it may capture the entire view of the tank, and all images are therefore retrieved so that the Z (up-down) axis is from left to right. Additionally, the laser beam is at a wavelength that does not interfere with the behavior of the copepods, which controls for any visual cues that the copepod may be responding to in nature.

The trails placed in the tank were allowed to settle so that they were running straight. Ten to fifteen males of the species being studied then were placed gently in the tank, so as not to disturb the trails. The males first were observed using a low-magnification lens (85 mm), which gave a view of the entire tank to determine whether the males were following the trails. The view then was switched to a high-magnification (200 mm lens), and a calibration was performed using the calibration stick, a cylindrical rod with an indentation that is one centimeter high with a diameter of once centimeter. This calibration stick allowed for the adjustment of data to scale later in the digitization process. The behavior of the males then was recorded onto VHS tapes at 30 frames per second for 2 to 4 hours.

The videos then were reviewed and the trail followings were noted. The sequences that included the trail follows then were digitized into TIFF sequences using dpsVelocity (version 8), an image acquisition program. The digitized clips were analyzed using Scion Image (Scion Corporation, Frederick, MD), and the male's location in the x-z and y-z axes were taken to give the speed of the copepod along the trail. The

distance from the trail was determined by measuring points on the x-z and y-z trail with the same z-coordinates as the copepod. The tail of the copepod was measured, as well as a second point on the trail. These two points, along with the male's location and the initial trail point, were used to calculate the angle between the copepod and the trail.

(d) Calculations

(i) Velocity

The velocity of the copepod was calculated using the change in location between two points divided by the change in time for the points used (Equation 1), as used by Weissburg et al (Weissburg et al. 1998). The velocity of the trail was measured to be .212 mm/s. The trail velocity was added to copepods traveling up the trail, and subtracted from copepods traveling down the trail, due to the fact that the trail hindered the copepod from traveling faster when going up the trail, and caused the copepod to swim faster when going down the trail. The velocity of the copepod on the trail was calculated with and without the trail velocity (Appendix Figures 1 and 2).

$$v = \frac{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2}}{(t_2 - t_1)} \quad (1)$$

(ii) Distance from trail

The points for the distance from the trail were obtained by clicking on a point on the trail that corresponds in z-value to the rostrum of the copepod. The distance from the trail then was calculated by subtracting the x value for the trail from the x value for the copepod, then subtracting the y value for the trail from the y value for the copepod. These

values were then squared, summed, and the square root was taken, giving the Pythagorean distance between the two (Equation 2). The z-value was omitted when calculating the distance from the trail, as the z values for the trail were taken at the z value of the copepod, giving a contribution of zero to the overall distance from trail. The distances from the trail will also be represented at each z-value, and therefore considering this position is not necessary.

$$D_t = \sqrt{(x_t - x_c)^2 + (y_t - y_c)^2} \qquad (2)$$

(iii) Angle with respect to the trail

The x-z and y-z angles were calculated separately. The x and z values of the copepod's rostrum and tail were used to calculate the x-z angle of the copepod with respect to the frame (Equation 3). The same was done for the y-z angle, and for the x-z and y-z angles of the trail. The angle of the trail within the frame at the position of the copepod was also calculated using additional point on the trail upstream from the original point. The angle between the two is taken by subtracting the value for the trail from the value for the copepod (Equation 4). The x-z and y-z angles are left separate, so that they may be plotted against one another to observe whether the angles are out of phase. The angles that are out of phase will represent a helical pattern of the copepod's trajectory as it is traveling around the trail.

$$A_{x,z} = \left[\tan^{-1}\left(\frac{C_{x2} - C_{x1}}{C_{z2} - C_{z1}} \right) \right] * \frac{180}{\pi} \qquad (3)$$

$$A_{c,t} = A_c - A_t \qquad (4)$$

3. RESULTS

(a) Direction of the copepod within the trail

The direction of tracking was determined from the analysis of videos. All events noted involved the following of the experimental trail as opposed to the control trail, indicating a clear preference for the pheromone-scented trails. All but one tracking event for *Hesperodiaptomus shoshone* (n = 20) involved the copepod tracking up the trail, while all *Temora longicornis* (n=5) tracking events showed the copepod tracking downstream. In the *H. shoshone* tracking event that was not tracking upstream, the copepod remained on the trail only briefly, before hopping away quickly.

(b) Velocities of the copepods

The velocities of the copepods were obtained by analysis of the sequences with Scion Image. The average velocity of *T. longicornis* within the trail, adjusted for the speed of the trail, was 0.865 cm/s (n=5, SD .124), while the average velocity of *H. shoshone* was 1.134 cm/s (n=18, SD = .259).

	Speeds before tracking (J. Sehn unpublished data)		Speeds while tracking the trail mimic (mm/s), adjusted for trail speed of .212 mm/s	
	T. longicornis	*H. shoshone*	*T. longicornis*	*H. shoshone*
Mean	9.32	12.52	8.44	11.52
SD	1.82	1.38	1.24	2.62
N	6	11	5	18
95% CI upper	7.41	11.59	6.72	10.71
95% CI lower	11.24	13.45	10.16	12.34

Table 1. Speeds of *T. longicornis* and *H. shoshone* before and during tracking.

The mean velocities during tracking of the trail were obtained for *T. longicornis* and *H. shoshone* (8.44 ±1.24 mm/s and 11.52± 2.62 mm/s, respectively). The confidence intervals were calculated using Formula 5, as seen in Table 1 with the confidence interval for *T. longicornis* being from 6.72 mm/s to 10.16 mm/s and the confidence interval for *H. shoshone* being from 10.71 mm/s to 12.34 mm/s. There is no significant difference between the speeds of the copepods within the trail and outside of the trail, although both species seemed to be moving slower while on the trail.

$$CI_{95\%} = \left(\bar{x} + \frac{t_{\alpha/2,v} \times s}{\sqrt{n}}, \bar{x} - \frac{t_{\alpha/2,v} \times s}{\sqrt{n}} \right) \qquad (5)$$

(c) Distance Away from Trail

(i) Temora longicornis

The counts of each distance away from the trail for *T. longicornis* were plotted as a histogram (Figure 1a). The average value of the distance away from the trail was 0.49 mm ± 0.39. As shown in the figure, the majority of the points fall within 0.5 mm from the center of the trail, demonstrating that *T. longicornis* spends most of its time within the trail, with 0.5 mm representing the average radius of the trail. The percent of time spent within a certain distance from the trail was plotted as well (Figure 1b). Given that the first bar represents all points within the trail, and the second bar represents all values up to one trail radius away from the trail, this figure demonstrates that *T. longicornis* spends significantly more time within the trail than immediately outside of the trail.

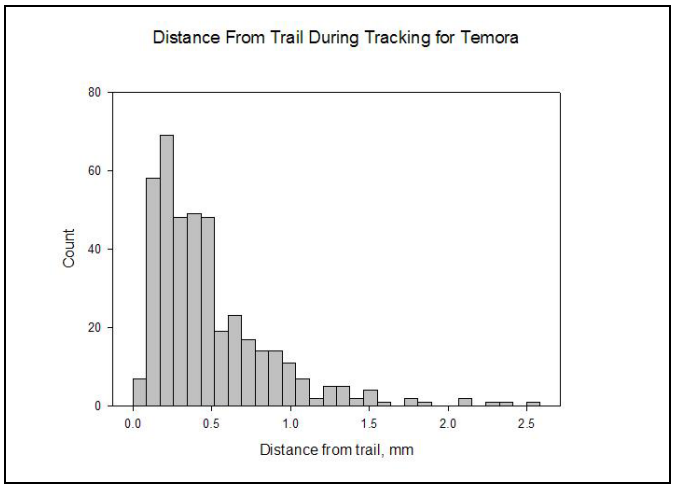

Figure 1a. Distance from the trail during tracking for *T. longicornis*. All values of the 3D distance away from the trail for all tracking events (N=5) were plotted on the same histogram.

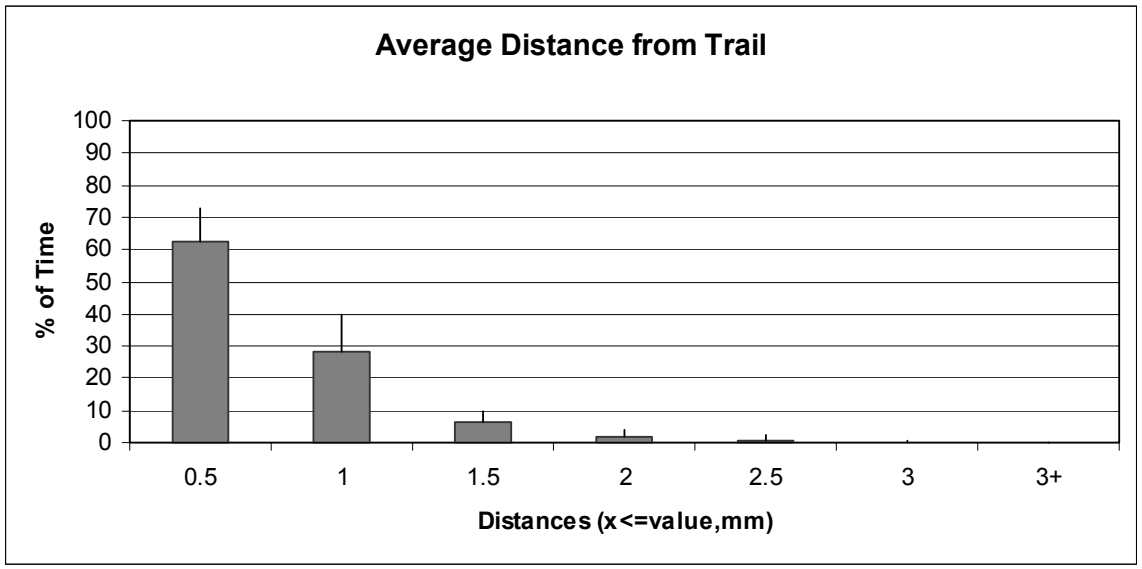

Figure 1b. Distance away from the trail for *T. longicornis*. The percent of time at each distance away from the trail for all tracking events (N=5) is grouped in increments of 0.5 mm, so that the first bar represents all values within the trail.

(ii) Hesperodiaptomus shoshone

The distance from the trail during tracking is illustrated for *Hesperodiaptomus shoshone* in the figures below (Figures 2a-c). The trail radius of 0.5 mm is represented by the first bar of Figure 2a. This demonstrates a nearly equal distribution between points in

the trail and directly outside of the trail, also illustrated in the third figure. The average value of the distance away from the trail was 0.90 mm ± 0.11. There was a much larger variation in distance away from the trail for *H. shoshone* than for *T. longicornis,* causing the data bins to be much smaller on a comparable graph to *T. longicornis.* The region of *H. shoshone* data up to 2.5 mm away was plotted in a separate histogram (Figure 2b), to illustrate the differences immediately around the trail, in the area of *T. longicornis* data. The average distance from the trail for this graph was 0.695 mm ± .489. Figure 2b illustrates the fact that there is a peak in the data immediately on the outside of the trail range, with the average value being .2 mm away from the trail edge, supporting the hypothesis that *H. shoshone* remains on the outside of the trail.

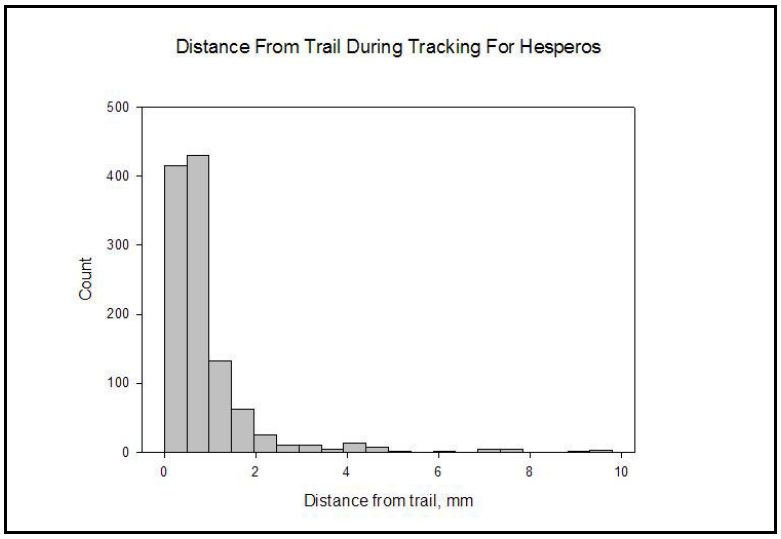

Figure 2a. Distance from the trail for *H. shoshone.* As with *T. longicornis,* all values of the 3D distance away from the trail for all tracking events (N=18) were plotted on the same histogram. The first bar represents the values within the radius of the trail.

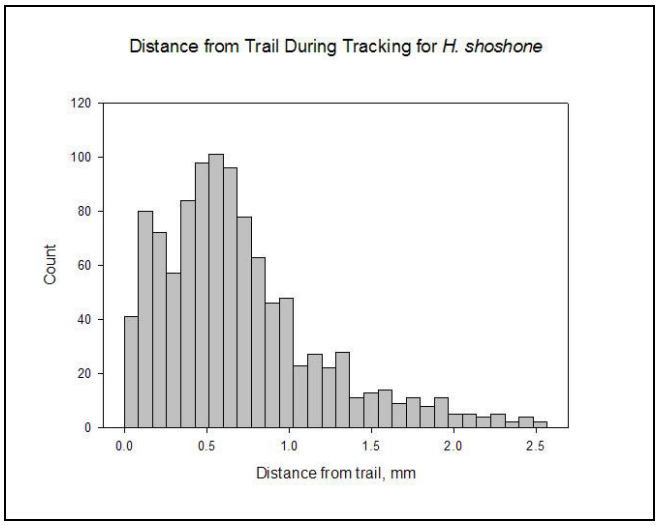

Figure 2b. Magnified view of distances from the trail for *H. shoshone*. The data was cut off at the maximum value found in *T. longicornis* tracking events, so that the values closer to the trail could be compared for the two sets of data.

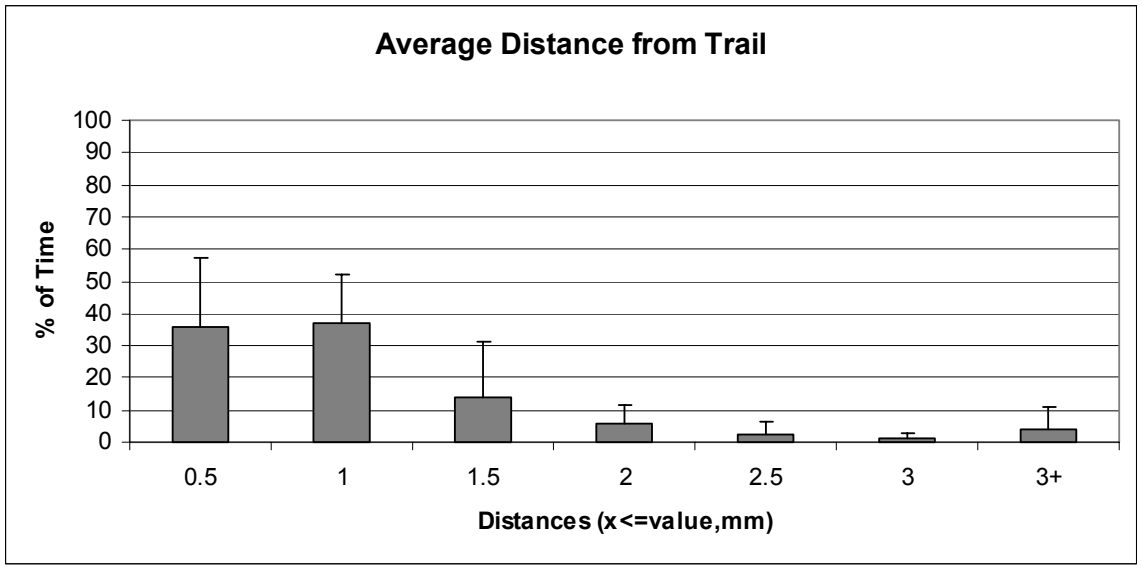

Figure 2c. Distance away from the trail for *H. shoshone*. The percent of time at each distance away from the trail for all tracking events (N=18) is grouped in increments of 0.5 mm, so that the first bar represents all values within the trail.

The two polar plots illustrate the distribution of distances from the trail as would be seen if looking down the trail. Coordinates were taken using the x distance from trail and the y distance from the trail, giving a value falling on a Cartesian coordinate system.

All points are on the same plot, illustrating how the points appear when values at all z coordinates are condensed. The polar plot of the *H. shoshone* data is much more spread across the graph, due in part to the sample size.

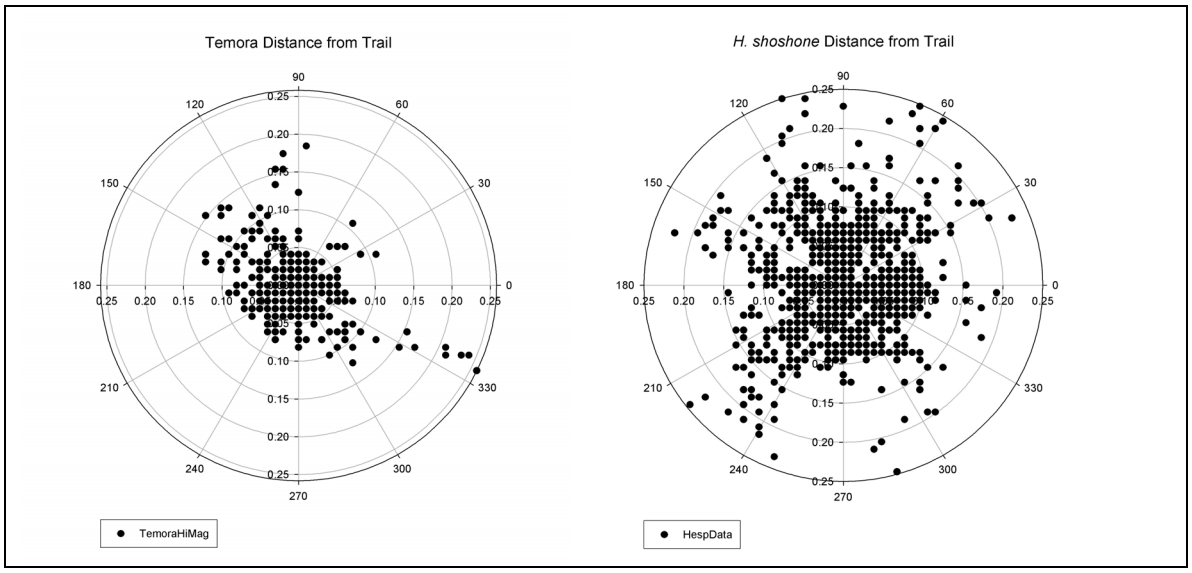

Figure 3a and b. Polar plot of distance from trail for a.) *T. longicornis* and b.) *H. shoshone.* The x and y distances from the trail for all tracking events were plotted together on the same graph, illustrating the distribution with respect to the trail for each species.

(d) Angle with respect to the trail

The X-Z and Y-Z angles were plotted on the same graph as a function of time for *T. longicornis* (Figure 5) and for *H. shoshone* (Figure 6). The maximum angles for *T. longicornis* are 68.3 and -80.5 (X-Z and Y-Z) while the maximum angles for *H. shoshone* are 29.6 and -46.9.

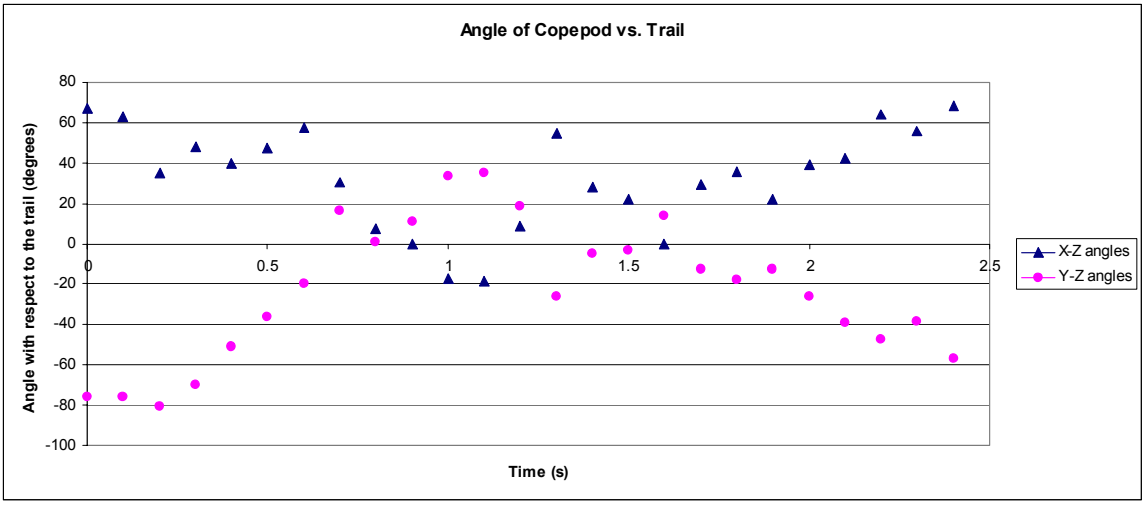

Figure 5. *T. longicornis* angles with respect to the trail

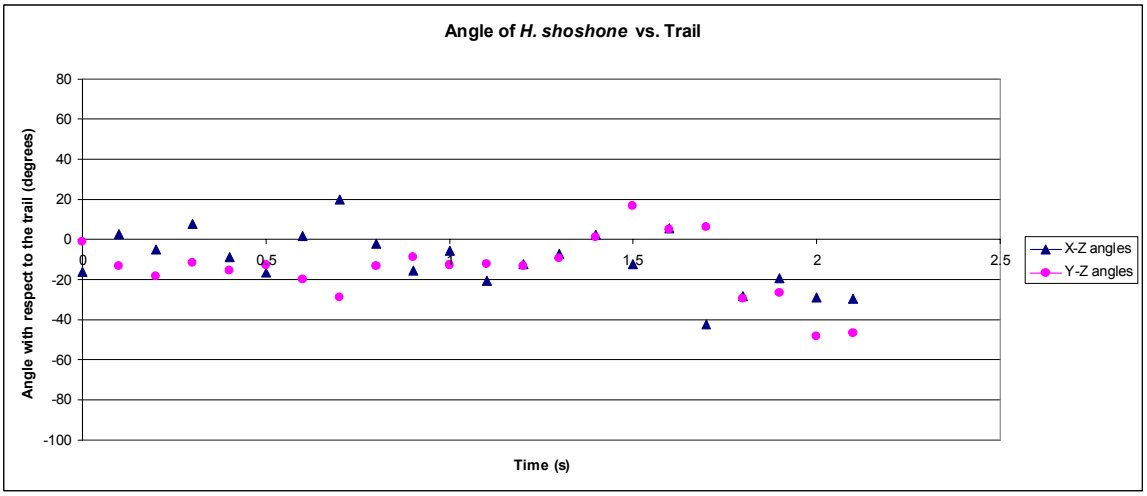

Figure 6. *H. shoshone* angles with respect to the trail

4. DISCUSSION

The results of the data analysis illustrated several differences between *T. longicornis* and *H. shoshone* that may be due to their different sensor locations as well as their differing environments.

(a) Directions

The direction of the trail follow was different in *T. longicornis* (downstream) when compared to *H. shoshone* (upstream). In the one tracking event where *H. shoshone* traveled down the trail, the copepod possibly detected that it was traveling in the wrong direction, and suddenly hopped away from the trail. This would account for its significantly lower time in the trail (Table x, event 6, probably include data in appendix). It seems somewhat strange that all *T. longicornis* events involved the copepod tracking down the trail, when one would expect the copepod to track toward the source. This could be because of the characteristics of the scent that is interacting with the environment. The tracking behavior has evolved in the copepods to best suit their environment, and therefore the different tracking may be evidence of the evolution of the copepod to respond to any chemical that they are tracking (Arnold & Houck 1982). This may also be due to the hydrodynamic cues of the trail. While they were standardized across all experiments, *T. longicornis* and *H. shoshone* could be responding to them differently based on what occurs in their respective environments.

(b) Speeds

The speeds of *T. longicornis* and *H. shoshone* within the trail were significantly different, and were comparable to speeds in prior experiments (Doall 1998, unpublished Yen et al data). The ratio of speeds between *T. longicornis* and *H. shoshone* also correspond with differences that would be expected if body size is correlated with speed. *Hesperodiaptomus shoshone* (2.1 mm) is approximately 1.6 times larger than *Temora longicornis* (1.3 mm, Gerber 1999), therefore if speed is correlated with body size we

would expect the speeds for *H. shoshone* to be 1.6 times faster than *T. longicornis*, which is the case.

There was not a significant difference in the speeds before tracking and during tracking, although the speeds decreased slightly. This could be because both species have reduced their speed to "take in" the pheromones at a slower rate. Additionally, males of *T. longicornis* have often been seen to slow down as they approach the female, possibly responding to hydrodynamic cues from the female and attempting to reduce their own wake (Doall 1998). The slightly lower speed on the trail may have been due to the increased hydrodynamic cues of the highly viscous dextran trail. However, it is also possible that the trails did not contain a sufficient concentration of pheromone to elicit an acceleration reaction from the males.

(c) Distances from trails

The average distance away from the trail was higher for *H. shoshone* than for *T. longicornis*, as expected. It is likely, therefore, that the histograms of the 3-dimensional distances (Figures 1 and 2) serve to illustrate the sensory array of each copepod. It was hypothesized by Dr. Yen (Yen 1998) that the sensors of *T. longicornis* located on the first .1 mm of each antennule contribute to their behavior on trails, leading them to dive in and out of the trail. If the copepod were keeping part of these sensing regions within the trail region, this would indicate that *T. longicornis* could possibly be sensing the chemical component of the trail up to 0.6 mm away from the trail. This appears feasible from the data, as most data fall within the 0.6 mm region. *T. longicornis's* sensor length is 1.35 mm (Gerber 1999), indicating that any contact with the trail could occur up to 1.85 mm

away from the trail center (1.35 mm away from the approximate trail edge). However, we see that the majority of the time is spent within 0.5 mm of the center, and a significant portion is spent up to 1 mm away from the trail center. Therefore, *T. longicornis* males are not utilizing their entire antennae to keep in contact with the trail. For *H. shoshone*, however, the antennae length is approximately 1.6 mm based on measurements of preserved species from the experiments. This is smaller than the expected length of the sensor (2 mm) based on the ratio of body sizes between the two copepods. While it is not known where the region of pheromone receptors is located on the copepod, from personal observation of high magnification trail following events in preliminary experiments, I believe that *H. shoshone's* receptors may be found in their mouth region, which could be supported by their nearly equal distribution inside and directly outside of the trail. The magnified portion of the histogram for *H. shoshone* (Figure 2c) also illustrates a peak around 0.5 mm, indicating a preference for the outside of the trail.

(d) Angles with respect to the trail

The angles of *T. longicornis* with respect to the trail seem to support the hypothesis that they are diving through the trail. In the plot of the angles over time, the X-Z and Y-Z angles appear to be out of phase, alternating about the trail, indicating a possible spiraling behavior in the trail. If the copepod were spinning perfectly, the graph would appear as two cosine waves that are 180 degrees out of phase. *Temora longicornis* has been seen to spin wildly when a mate is detected (Doall 1998), and the analysis of angles with respect to the trail demonstrate that this is indeed a spinning behavior. The

angles of *H. shoshone* are much lower and do not seem to be out of phase, also indicating that they are staying closer to parallel with the trail.

(e) Scent trail and video tracking issues

There were several possible sources of unwanted variability in this experiment, the first of which is the preparation of the scent trails. The *T. longicornis* and *H. shoshone* scents were prepared at different times by different students in the lab. This could have caused different preparation methods to be used. The concentrations of pheromone could have varied due to differences in the amount of time that the females were left in the water or the amount of water that was used per copepod, which could have increased or decreased the intensity of the chemical within the trail, and may have affected the copepod's willingness to track the trail. Another difference in the scents could have arisen from the pheromone production rate of the females. While these rates are not known for *T. longicornis* or *H. shoshone* due to the difficulties in isolating the pheromones, they could have contributed to any potential difference between the two behaviors.

A possible source of error for this experiment is human error during analysis of the digitized clips. It was often hard to determine the exact location of the copepods due to their small size on the screen, and the copepod's location was often determined by playing back a video of the tracking event to anticipate the next position. Another contributing factor for the error is the low resolution of the images. Even using a 200 mm lens, the resolution was low and the copepod's body spanned only a few pixels. The trail mimics also spanned only a few pixels on the screen, therefore plotting the minute

differences was not possible. This difference is evident on the polar plots for the two species (Figures 3a and b). The gaps in between the points on the graph represent the distance between two pixels that could be chosen as points on Scion Image. In order to obtain more accurate measurements for the distance to the trail, it would be necessary to use a device with a much higher resolution.

5. CONCLUSION

While it is known that many factors contribute to the location of females by male copepods, chemical tracking is not well understood due to the lack of knowledge about the specific chemicals. This study has begun to elucidate the differences in tracking mechanisms between the marine copepod *T. longicornis* and the freshwater copepod *H. shoshone*. The study has also been useful in developing methods that can be used to illustrate tracking patterns in a standardized form. Future work on the trail-tracking abilities of copepods will include more replicates of the performed experiments in order to increase the statistical power of the results. Another area of future work will be the study of other species so that their tracking methods can be compared using the same analysis methods in this study. Other methods of analysis such as those described by Crenshaw (Crenshaw et al. 2000) will also be used to more fully characterize the kinematics of these copepods while in a trail mimic. Additionally, even higher-resolution footage of mimic-tracking events will elucidate the differences between the tracking abilities of the copepod. The study of trail-tracking by copepods is important for biologically-inspired tracking models. The study of various copepods will provide tracking models for the environments and size range in which they live. By learning how

these animals track in their specific environments, we can apply knowledge to robots or sensors that will be designed on the same scale.

ACKNOWLEDGEMENTS

I would like to thank Dr. Yen for her support, assistance and advice throughout this project, as well as Dr. Gable and Dr. Weissburg. I would also like to thank Rachel Lasley for her assistance and advice, and my friends and family for being so willing to learn about copepods while I complete this project. This research was supported, in part, by contract N0014-03-0366 from the Office of Naval Research.

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APPENDIX

Table 1. Summary of all trail-tracking events for *T. longicornis.*

Event	Distance of follow (cm)	Direction of follow	time in trail (s)	speed in trail (mm/s)	speed in trail (mm/s), adjusted for trail speed (.212 mm/s)	Standard Deviation
7_21a	3.040	up	3.533333	8.522634	8.310633935	3.757988
7_21b	1.526958	up	2.167	6.837126	6.625126313	2.915772
7_21c	2.875122	up	3.2	8.984757	8.772757362	3.573927
7_21d	2.108795	up	2.533333	8.216083	8.004082655	3.554965
7_21e	0.953852	up	0.933333	10.68117	10.46916986	4.880602
Average	2.101		2.473333	8.648354	8.436354024	
Std Deviation	0.7907		0.907842	1.243279	1.243279318	

Table 2. Summary of all trail-tracking events for *H. shoshone.*

Event	Distance of follow (cm)	Follow direction	Time in Trail (s)	Speed in Trail (mm/s)	Speed in Trail (mm/s), Adjusted for Trail Speed (.212 mm/s)	Standard Deviation
1	7.294	up	5.233	13.850	14.062	0.727
2	3.896	up	3.200	12.051	12.263	0.583
3	3.131	up	2.833	10.767	10.979	0.521
4	2.609	up	2.300	10.870	11.082	0.625
5	2.353	up	1.800	13.070	13.282	0.720
6	0.623	down	0.700	8.496	8.284	0.311
7	3.178	up	3.267	9.632	9.844	0.538
8	3.531	up	3.500	9.965	10.177	0.440
9	1.155	up	0.833	13.328	13.540	0.583
10	1.569	up	1.600	9.607	9.819	0.599
11	4.344	up	3.700	11.637	11.849	0.581
12	1.919	up	2.033	9.283	9.495	0.445
13	2.187	up	2.633	8.203	8.415	0.402
14	6.154	up	5.200	11.705	11.917	0.774
15	3.263	up	3.133	10.305	10.517	0.532
16		up				
17		up				
18	5.855	up	4.633	12.547	12.759	0.465
19	2.426	up	2.500	9.451	9.663	0.477
20	1.443	up	1.567	19.286	19.498	0.411
Mean	3.163		2.815	11.336	11.525	
Standard Deviation	1.804049494		1.328925	2.58645	2.615604	
N	18.000		18.000	18.000	18.000	