Pheromone trail following in three dimensions by the freshwater copepod *Hesperodiaptomus shoshone*

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Finding mates can pose a particular problem for obligately sexual planktonic organisms, resulting in a variety of adaptations to ensure sufficient mating. Several types of mate-finding behavior have been observed in marine copepods, but the one most effective at low population density, following a pheromone trail, has not been observed in freshwater copepods. Using three-dimensional (3D) videography, we show that males of the large-bodied alpine species *Hesperodiaptomus shoshone* follow pheromones in the female’s trail. Using a trail mimic comprised of female-conditioned water, we found that males followed female scent without the presence of the female. This behavior was reduced when the female scent was diluted, suggesting that the male’s behavior can be modified by the intensity of the chemical signal. Analyses of the 3D trajectories of copepods that formed mating pairs indicate that the male does not make a direct approach to the female, as might be expected if he relied purely on hydrodynamic or visual cues. Instead, males that are >0.5 cm from females react to crossing female trails by making an abrupt turn and spending more than 2 s following the female’s trail. Furthermore, flow field analysis showed that at this distance it was unlikely that copepods could distinguish the hydrodynamic signal from the background flow. This is the first demonstration of chemical trail following in a freshwater copepod and has important implications for encounter rates and viable population densities in similar species.

KEYWORDS: calanoid copepods; mate tracking; *Hesperodiaptomus shoshone*; pheromone

INTRODUCTION

A persistent population of sexually reproducing organisms depends on sufficiently high encounter rates between females and males. Planktonic copepods at low density in the open waters of lakes or oceans are often separated by 100’s of body lengths, making direct random encounters infrequent (Gerritsen, 1980; doi:10.1093/plankt/fbq164, available online at www.plankt.oxfordjournals.org. Advance Access publication January 11, 2011 # The Author 2011. Published by Oxford University Press. All rights reserved. For permissions, please email: journals.permissions@oup.com

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Buskey, 1998). As a result, active searching combined with the ability to detect potential mates from a distance may be required to produce a reasonable chance of encounters between sexes (Gerritsen and Strickler, 1977; Haury and Yamazaki, 1995; Kiorboe, 2006). Some marine copepods increase perceptual distance beyond physical contact via hydrodynamic cues (Strickler, 1998), whereas males of other species follow chemical trails produced by females (Doall et al., 1998; Tsuda and Miller, 1998; Kiorboe and Bagøien, 2005). Because hydrodynamic signals rapidly decay (Yen et al., 1998), pheromone trails that can be followed over many body lengths should lead to larger improvements in encounter rates between potential mates (Gerritsen and Strickler, 1977; Kiorboe and Bagøien, 2005).

Although freshwater copepods experience the same challenges in locating mates as marine species, their ability to increase the encounter rate with trails and chemical signals is not well understood. Watras (Watras, 1983) suggested that in the calanoid copepod Diaptomus leptopus, male preference for gravid females was based on the detection of a chemical signal, but only observed males pursuing females over a distance of one or two body lengths. Males in this species increased swimming speed in the presence of gravid females (van Leeuwen and Maly, 1991) which could lead to higher encounter rates of males with females (Gerritsen, 1980; Kiorboe, 2006) but also increases the risk of encountering predators (Kiorboe, 2008). More recent research on another freshwater copepod, Leptodiaptomus ashlandi, also found evidence for increased swimming speed in response to a chemical signal, but not for directional tracking of females (Nihongi et al., 2004). Because marine species with the greatest ability to locate mates from a distance use trail following (Kiorboe and Bagøien, 2005), it is important to determine if this mechanism is present in freshwater species. If not, freshwater species must maintain higher population densities or employ mate finding behavior distinct from their marine relatives.

The bioenergetics of pheromone production and its diffusion rate in water implies that larger animals are more likely to employ pheromones in mate location (Dusenberry and Snell, 1995). Large, freshwater diaptomid copepods are common in alpine lakes in the Sierra Nevada and Rocky Mountains where fish predators are not naturally present (Anderson, 1980). More recently, fish stocking has led to the local extinction of some large calanoid copepod species, including Hesperodiaptomus shoshone and H. arcticus (Knapp et al., 2001; Parker et al., 2001; Sarnelle and Knapp, 2004). These copepod populations sometimes fail to recover following fish removal despite the presence of diapausing egg banks, presumably because of an Allee effect stemming from mate limitation (Sarnelle and Knapp, 2004; Kramer et al., in press). The ability of these species to recolonize will depend on the critical density for recovery which will depend on the encounter rate of males and females (Kramer et al., in press). Here, we describe the mate tracking behavior of H. shoshone in the freshwater environment and provide evidence that this copepod uses chemical trail tracking in mate location, increasing the probability of mate encounter for these obligately sexual organisms living a relatively vast three-dimensional (3D) habitat.

METHOD

Animal collection and maintenance

Hesperodiaptomus shoshone individuals were collected with a plankton net from Dissertation Lake (lake 52121, 3615 m altitude, 37°16′2″N, 118°4′33″W). Adults of this species are available for ∼1 month/year, from the time the lake thaws in July until the lake freezes in September. Immediately after collection, males and females were sorted into separate containers prefilled with lake water. The male can be identified with a hand lens based on its geniculated antennule, which is used to grasp the female prior to mating. Copepods were shipped in insulated containers on ice overnight to Georgia Institute of Technology in Atlanta, GA, USA. Cultures were maintained at 12°C in an incubation room and were fed concentrated Tetraselmis spp. and Rhodomonas lens phytoplankton along with rotifers and low-salinity (5 ppt) Artemia nauplii. Males and females were maintained in separate containers. Average prosome lengths of adults in the collection were 2.46 ± 0.13 mm (males) and 2.63 ± 0.13 mm (females). Experiments were initiated within 4 days of collection.

Experimental observation

Mating behaviors of adult male and female H. shoshone were observed using 3D Schlieren laser videography as developed by Strickler (Strickler and Hwang, 1998) and further described by Doall et al. (Doall et al., 1998). Briefly, two video cameras were mounted to give orthogonal views (x–z and y–z) of the experimental tank. Using the common z-axis, the two orthogonal views were superimposed and slightly offset to facilitate the measurement of the 3D position of each animal. The vertical axis, common to both superimposed orthogonal views, was designated the z-axis. Visualization using the Schlieren optics relies on differences in the refractive indices between the water and
the copepods. Copepods were visible as white silhouettes illuminated by an infrared laser against a black background. Copepods did not respond to this wavelength of light.

The size of the experimental tank ranged from 0.7 to 3.0 L. Filtered, fresh water from Lake Lanier, located just outside of Atlanta, was maintained at 12°C in the tank by circulating distilled water in a large water jacket through a refrigeration unit. The pH was adjusted to match that of the source lake. Animal numbers ranged from 5 to 15 males and 5 to 15 females per observation period.

At the beginning of each observation period, *H. shoshone* males were spooned into an experimental vessel. Male behavior in the absence of females was observed for 20 min at the beginning of each experiment. After 20 min, one female for each male was added to the observation container. Mating interactions were recorded for 2–4 h.

**Trail-mimic bioassay**

To determine if *H. shoshone* would follow a chemical trail in the absence of the females, males were exposed to chemical trails created from female-conditioned water. To create chemical trails of *H. shoshone*, females were placed in beakers containing filtered spring water at a density of 1 animal per 10 mL. Females were allowed to condition the water overnight (between 8 and 10 h). After incubation was completed, female-conditioned water was transferred to sterile plastic vials and frozen. Prior to chemical trial bioassays, female-conditioned water was thawed and was doubly-labeled with high-molecular weight dextran to increase the difference in the refractive index, enabling imaging of the trail using the Schlieren optics. To test the effect of cue intensity, the conditioned water was first diluted with non-conditioned spring water (Crystal Spring) to 50 and 10% the original strength. Control trails contained filtered spring water and dextran. Trail-mimic bioassays were conducted with 20 reproductively mature *H. shoshone* (California) males in a 3-L tank according to the methods described by Yen [*et al.*](Yen et al., 2004). Trails were fed into the tank by an electronic syringe pump via capillary tubes at 0.01 mL min$^{-1}$, resulting in a trail velocity of 0.2 mm s$^{-1}$. The speed of flow of mimic is much less than the speed of the female (1.4 cm s$^{-1}$) and unlikely to be a source of a hydrodynamic signal. Once the trails were fully developed (after a few minutes), males were added into the tank. Trail following behavior was visualized using the Schlieren optical system (see above). Trials were conducted for 2 h. Upon trial termination, animals were returned to their stock cultures and untested copepods were used in replicates. The experimental tank was rinsed with distilled water and all syringes and capillary tubes were rinsed with acetone and distilled water and allowed to dry between trials to remove any chemical scent.

**Digital tracking of swimming trajectories**

Mating interactions of *H. shoshone* were demonstrated by the pursuit and/or capture of females by males. The sex of animals was not obvious from the recording, so when smoothly swimming pairs remained together for more than one second in the video sequences, the pursuer was assumed to be a male and the pursued a female and analyzed for this study of male-female mating interactions. This assumption was based on the following observations. In female-only observations, no trackings were observed. In male-only observations, couplings were infrequent: with 10 males, one coupling was noted in an hour, whereas when 10 females were added to the experimental vessel with the same 10 males, 5 couplings were noted in half an hour. Another trial with 20 males showed no couplings during 45 min. Pairings also were distinct: male–male pairings were short with rapid movements and antennal flicking whereas male–female pairings remained attached for longer periods with calm swimming.

A total of 16 h of observation were recorded with a charged-coupled device camera in VHS tape format. Mating events that occurred centrally in the tank and providing more than 1 s of tracking were chosen for trajectory analysis (*n* = 11). Analog video clips containing mating interactions were converted to a digital format using a dphVelocity 8 (Leitch, Ackworth, GA, USA) interface between the video recording VCR and an IBM-compatible computer as a sequence of digital image frames at 33.3 ms intervals. Scion Image (Scion Corporation, Frederick, MD, USA) video analysis software was used to track the position of the rostrum of individuals on a Cartesian coordinate system. Position measurements were taken for each frame, resulting in a temporal resolution of 33.3 ms. A 10.0-mm calibration measurement from the video was used to convert pixel measurements into millimeters. Calibrated position measurements were used in subsequent quantitative analyses of swimming trajectories and mating interactions.

**Swimming speed**

The temporal sequence of swimming behaviors was classified into three stages with respect to pursuit and
capture; “before” initiation of mate pursuit, “during” pursuit and “after” mate capture. Swimming speeds of individuals performing these behaviors were calculated as the distance between consecutive 3D positions divided by the time elapsed between those two positions (i.e. 33.3 ms). The distance \( d \) between points was calculated using the \( x, y, z \) coordinates in the following equation:

\[
d = \left( (x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2 \right)^{1/2}.
\]

The average male and female swimming speeds before, during and after pursuit were calculated as the means of consecutive 33.3 ms interval speeds.

**Perceptive distance**

Two perceptive distances were calculated for each mating event; chemical and hydrodynamic. The chemically mediated perceptive distance was calculated as the direct linear distance between the male and the female at the initiation of mate pursuit behavior. The hydrodynamic perceptive distance was calculated as the linear distance between the male and the female when the male lunges to catch the female. At this distance, the hydrodynamic wake created by the female provides the directional stimulus to evoke the high-speed lunge that results in mate capture (Yen et al., 1992, 1998). The increase in perceptive ability conferred by using chemical perception versus hydrodynamic perception was characterized as the ratio of chemical perceptive distance to hydrodynamic perceptive distance for each mating event.

**Event durations**

The age of the trail at the time of detection was calculated as the difference between the time when the female was positioned at the detection point to the time when the male initiated pursuit from this point. The time tracking was taken to be the time elapsed between male initiation of pursuit and attempted capture of the female. The time coupled was recorded as the time elapsed between successful capture of the female and separation of the mating pair or disappearance of the mating pair from the field of view.

**Biologically generated flow**

The hydrodynamic trail generated by a free-swimming, female \( H. \) shoshone individual was quantified using a Particle Image Velocimetry (PIV) system. The copepods were housed in a \( 6 \times 6 \times 15 \) cm glass container filled with Crystal Geyser spring water and seeded with titanium dioxide. The tank containing the copepods was placed inside a 15-cm cubic glass recirculation tank where deionized water was recirculated from the tank into a Fisher Scientific chiller that maintained a temperature of \( 13^\circ \)C. To visualize the flow fields, a planar laser sheet (width of 0.7 mm) was produced from a pulsed, infrared Oxford laser (model HSI-500) to illuminate the titanium dioxide particles. A high-resolution (1280 \( \times \) 1024 pixel) VDS Vosskühler CMC-1300 digital camera was placed parallel to the laser sheet. High-resolution pictures were taken at a frequency of 50 Hz. Since the laser beam was pulsed with a displacement of 7 ms, the camera captured sequential images with a 7-ms time displacement. A smaller camera that was used to monitor the location of copepods in the laser sheet was located perpendicular to the VDS Vosskühler digital camera. Data were only collected while copepods were swimming within the laser sheet. A more detailed description of this set-up is available in Catton et al. (Catton et al., 2007).

PIV is a non-intrusive method used to quantify velocity fields by measuring the displacement of seeding particles over a time interval (Raffel et al., 1998). The displacement of the particles is found by comparing the location of the particles between image pairs in \( 32 \times 32 \) pixel interrogation windows using a cross-correlation analysis. To calculate the velocity, the displacement of the particles is divided by the time displacement between the two images. The velocity field was validated by comparing a local grid value to the median value of a neighboring \( 3 \times 3 \) grid. Typically, less than 1% of the velocity vectors within the velocity field were identified as bad vectors and replaced with a spatially interpolated value (Westerweel, 1994; Nogueira et al., 1997). A Cartesian coordinate system was used where \( x \) denotes the horizontal direction and \( y \) denotes the vertical direction, and where \( y \) values increase from the bottom of the tank to the top of the tank. The \( u \) and \( v \) velocity components were calculated as:

\[
u = \frac{\partial u}{\partial t} \quad \text{and} \quad v = \frac{\partial v}{\partial t}.
\]

The shear strain rate has been identified as a fluid component that elicits a behavioral response in copepods. Shear strain rate values >0.4 \( \text{s}^{-1} \) cause an escape reaction in certain copepod species (Fields and Yen, 1997; Kiørboe et al., 1999). Thus, a shear strain rate value of 0.4 \( \text{s}^{-1} \) was used to determine the spatial extent of the hydrodynamic trail:

\[
\varepsilon_y = \frac{1}{2} \left( \frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} \right).
\]

\[v\]
The shear strain rate is only one component of the strain rate tensor, although it has been shown to be the largest component in copepod flow fields (Catton et al., 2007). However, the orientation of the copepod in the flow field will determine what components of the strain rate tensor are potentially sensed by the copepod sensor array. The maximum deformation rate is a measure of the maximum strain rate in the fluid that could be sensed by the copepod (Kiørboe and Visser, 1999). The maximum deformation rate is determined by solving for the eigenvalues ($\lambda_1$, $\lambda_2$) of the strain rate tensor using the equation below and taking the maximum of the two eigenvalues. Kiørboe and Visser (Kiørboe and Visser, 1999) found that copepods in both Couette flow and siphon flow respond with escape behaviors to a maximum deformation rate in the fluid of $\sim 0.4$ s$^{-1}$. Therefore, 0.4 s$^{-1}$ has been set as the threshold maximum deformation rate to determine the spatial extent of the hydrodynamic disturbance:

$$\det \begin{vmatrix} \varepsilon_{xx} - \lambda_1 & \varepsilon_{xy} \\ \varepsilon_{yx} & \varepsilon_{yy} - \lambda_1 \end{vmatrix} = 0$$

RESULTS

Qualitative description

A mating event is identified by the observation of two copepods attached to each other via their antennule or thoracic appendages. Copepods attached by their mouthpart appendages were considered to be engaged in a predation event and therefore were not included as a mating event. Analyses of the events preceding the observed pair formation provided the following sequence of events. (i) The male crosses the path of the female and then adjusts his bearing to follow the female's path (the female copepod can be over 10 body lengths ahead of the male [DF]). After the male detects the female, he turns, finds the female's chemical trail and begins tracking (TM). Female position at initial time of male tracking is denoted “TT”. As the male tracks the female’s trail, he speeds up until he catches her and copulation begins (C). [Supplemental materials provide 3D animations of visualized trajectories of the mate tracking events presented in Fig. 1A and B. Male and female trajectories are shown in red and blue, respectively.]

Fig. 1. Graphical representations of 3D mating trajectories of *H. shoshone*. (A) Typical mating trajectory. (B) Trajectory where male leaves the trail but is able to relocate the trail and continue tracking. Male and female trajectories are shown in grey and black, respectively. Start positions are labeled as “Sm” (male start position) and “Sf” (female start position). A male exhibits normal cruising behavior until he detects a female’s chemical signal (Dm). At the point of signal detection, the female is $\sim$5 mm ahead of the male (DF). After the male detects the female, he turns, finds the female’s chemical trail and begins tracking (TM). Female position at initial time of male tracking is denoted “TT”. As the male tracks the female’s trail, he speeds up until he catches her and copulation begins (C). [Supplemental materials provide 3D animations of visualized trajectories of the mate tracking events presented in Fig. 1A and B. Male and female trajectories are shown in red and blue, respectively.]

Analyses of 3D trajectories of the male and female copepods involved in pair formation showed that males follow the path of the female. Instead of swimming directly toward potential mates, *H. shoshone* males closely follow the previous swimming trajectory of females (Fig. 1). Note the overlap in the male trajectory on the female trajectory. This is considered the tracking event. In Fig. 1A, the male is able to stay on track, whereas in Fig. 1B, the male loses track of the female but is able to re-intersect her path to continue following it for his subsequent successful capture of the female copepod.

Trail mimics

*Hesperodiaptomus shoshone* males are able to follow a trail mimic that contains the scent of female conspecifics (Fig. 2). In these experiments, males encountering trails containing female-conditioned water followed them nearly half the time, and never followed control trails.
containing only filtered water and dextran (Table I). As the female-conditioned water was diluted, the tendency to follow trails decreased (Table I). Once males intersect the trail they tend to swim up, following it to the source (95% of 20 documented trail follows).

The average distance the trail mimic was followed (3.16 ± 1.8 cm) was similar to that in the live trials (3.84 ± 3.08 cm, Kolmogorov–Smirnoff (K–S) test for non-normal data, \( D = 0.2222, P = 0.803; \) Zar, 2009). Males spent the same amount of time following a trail mimic (2.8 ± 1.3 s) as they did the trails in live trials (2.4 ± 2.5 s, K–S test, \( D = 0.4495, P = 0.1267 \)). These observations indicate that males follow trail mimics in a manner similar to how they follow female copepods.

**Kinematic analyses**

**Swimming speed**

Before locating the female trail, male *H. shoshone* swimming speed (1.25 ± 0.14 cm s\(^{-1}\)) was similar to female swimming speed (1.36 ± 0.25 cm s\(^{-1}\), K–S test, \( D = 0.3636, P = 0.4792, n = 11 \)). After detecting the female’s trail, male swim speed increased significantly (1.67 ± 0.21 cm s\(^{-1}\), K–S test, \( D = 0.8182, P = 0.0006549, n = 11 \)). Males accelerated further to 6.8 ± 2.8 cm s\(^{-1}\) (\( n = 11 \)) in the final lunge that resulted in coupling with the female (at point C in Fig. 1). Once coupled, mating pairs of *H. shoshone* swam an average of 1.58 ± 0.39 cm s\(^{-1}\) (\( n = 10 \)). The female appears to lead the pair because the male must use his appendages to capture the female and transfer the spermatophore.

**Perceptive distance**

Males can respond to females by following trails or by reacting to hydrodynamic wakes (Table II). The average distance that a male is from the female when he begins tracking is 0.5 cm (range = 0.31–0.96 cm), whereas the average distance he is when executing the mate capture

**Table I: The frequency that an encountered trail mimic (Yên et al., 2004) is followed by the male planktonic copepod *H. shoshone***

<table>
<thead>
<tr>
<th>Medium</th>
<th># encounters</th>
<th># trail follows</th>
<th>% follows</th>
<th>Duration of trial (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female-scented lake water</td>
<td>13</td>
<td>6</td>
<td>46.2</td>
<td>2</td>
</tr>
<tr>
<td>50% dilution</td>
<td>78</td>
<td>6</td>
<td>7.7</td>
<td>6</td>
</tr>
<tr>
<td>90% dilution</td>
<td>63</td>
<td>1</td>
<td>1.6</td>
<td>6</td>
</tr>
<tr>
<td>Control*</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

*Control contained spring water (Crystal Spring) and dextran.

**Table II: Detection distances and trail following behavior in *H. shoshone***

<table>
<thead>
<tr>
<th></th>
<th>Chemical</th>
<th>Hydro-dynamic</th>
<th>Ratio</th>
<th>Trail age (s)</th>
<th>Pursuit distance (cm)</th>
<th>Time tracking (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.529</td>
<td>0.250</td>
<td>2.337</td>
<td>0.57</td>
<td>3.838</td>
<td>2.40</td>
</tr>
<tr>
<td>SD</td>
<td>0.224</td>
<td>0.070</td>
<td>1.390</td>
<td>0.39</td>
<td>3.062</td>
<td>2.28</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Min–max</td>
<td>0.31–0.96</td>
<td>0.14–0.37</td>
<td>0.97–5.7</td>
<td>0.13–1.3</td>
<td>1.59–11.5</td>
<td>0.87–8.63</td>
</tr>
</tbody>
</table>

*Perceptive distance is the straight line distance between male and female at the initiation of trial following (chemical) or coupling behavior (hydrodynamic).
is 0.25 cm (range = 0.14–0.37 cm) from the female. Hence, males of *H. shoshone* more than doubled the distance through which they were able to perceive potential mates by using chemically mediated detection.

**Event durations**

Trail age at the time of detection varied in a range from 0.13 to 1.3 s (Table II). Pursuit distance (1.59–11.5 cm) and time spent tracking (0.87–8.63 s) was variable (Table II) since it depended on trail age and on male and female swimming speeds. Once mating was initiated, pairs could remain coupled for long periods (105 ± 235 s, n = 10) with one pair remaining together longer than 12 min.

**Biologically generated flow**

The velocity field created by a female *H. shoshone* is shown in Fig. 3. To generate motion, fluid was entrained from above the copepod by the swimming appendages on the ventral side of the organism and expelled downward along the body of the copepod. Females produced a velocity disturbance of ~1 cm in length and 0.9 cm in diameter. The trail decay time, or time period between maximum velocity to background velocity (0.025 cm s\(^{-1}\)), was estimated to be 0.7 s. Re-plotting the velocity vectors (in cm s\(^{-1}\)) coupled with a contour plot of shear strain rates (equal to or greater than ±0.05 s\(^{-1}\) in colored contours; Fig. 4A) defines the hydrodynamic structure that can be perceived. Contours were included at shear strain rates of ±0.05 s\(^{-1}\) and ±0.4 s\(^{-1}\) as these are two strain rate thresholds that are related to changes in behavior in turning and escaping responses (Fields and Yen, 1997; Kiorboe et al., 1999; Woodson et al., 2005). Coincidentally, the average background shear strain rate prior to the entry of the copepod was ±0.05 s\(^{-1}\) \((n = 4)\) and, as shown in Fig. 4A, this threshold level did not adequately define the spatial extent of the hydrodynamic disturbance. At distances >0.4 cm (Table III, Fig. 4A), the strain rate \(\bar{x}y\) is below the threshold response level and close to the background levels. The plot of the maximum deformation rate was similar to the plot of the shear strain rate, but the boundaries of the fluid disturbance were more clearly demarcated (Fig. 4B). Due to the addition of the linear strain rate, the maximum trail length was larger when considering the maximum deformation rate but still under a value of 0.6 cm (Table III). The profiles of the maximum deformation rate along the path projections denoted by the black lines in Fig. 3 and Fig. 4 show that in two of the three positions, the hydrodynamically perceived trail length was <0.2 cm, so in most cases hydrodynamic perception would have to occur at distances <0.6 cm. Thus, the male copepod would need to be closer than 0.6 cm from the female to be able to discern her wake by hydrodynamic sensing.
DISCUSSION

Remote mate detection: chemical or hydrodynamic cues?

We found that males of the freshwater copepod species *H. shoshone* followed trail mimics containing female scent, demonstrating that a chemical signal can guide the male copepod to the female. Trail following behavior was reduced when the female scent was diluted, indicating that the male’s behavior was sensitive to the intensity of the chemical signal. Using 3D videography, we confirmed that the freshwater copepod *H. shoshone* followed the female along her path. In darkness, males of *H. shoshone* would track conspecific female copepods often and form persistent mating pairs. Analyses of the 3D trajectories of male and female copepods that formed mating pairs showed that the male copepod did not make a direct approach to the female as might be expected if the copepod relied on hydrodynamic or visual cues. Instead, when the male crossed the female trail, he made an abrupt turn and spent several seconds following the female’s trail prior to capture. On average, the male crossed the trail at distances greater than that at which the hydrodynamic signal matches that of the background flow (>0.5 cm).

These multiple lines of evidence confirm male *H. shoshone* can use a chemical trail to detect females, but the male’s behavior just prior to capture suggests that hydromechanical cues also are used during mate tracking. Previous studies have shown that copepods behaviorally respond to strain rates or the spatial gradient of velocity, rather than the velocity magnitude. Typically, a strain rate value of 0.5 s\(^{-1}\) is used as the threshold for copepod escape behavior (Fields and Weissburg, 2005). However, recent research (Woodson et al., 2005) shows that the threshold for copepod turning behavior may be an order of magnitude lower in intensity (0.05 s\(^{-1}\)). Here, we have often found that *H. shoshone* can begin trail following when >1.5 cm from the female on trails older than 1 s. The flow field analysis shows that, at this distance, the strain rate is not different from the background and this supports the conclusion that males are responding to a chemical cue. However, once engaged in pursuit, the male can experience hydrodynamic strain rates >0.5 s\(^{-1}\) in close proximity to the female, which may guide his final lunge before capture. We conclude that *H. shoshone* probably relies on both chemical and hydromechanical cues to guide the male to a female.

The mating behavior of *H. shoshone* contrasts with that of *Leptodiaptomus ashlandii* (Nihongi et al., 2004), another member of the family Diaptomidae. The Diaptomidae is a wholly fresh water family derived from calanoids originating in the marine environment (Boxshall and Jaume, 2000). Female diaptomids do not store sperm (like other members of this superfamily Diaptomidea) and must mate again for each batch of eggs, which intensifies the need to find suitable mates to maximize reproductive success. *Leptodiaptomus ashlandii* males do not swim directly to mates nor along the female’s path. Instead, they may advect scent into their feeding current flow field from a female swimming nearby, thus detecting a diffuse chemical signal that evokes an increase in swimming speed. *Leptodiaptomus ashlandii* also does not respond to fluid mechanical cues by tandem hopping, as noted for another freshwater copepod, *Cyclops scutifer* (Strickler, 1998). *Hesperodiaptomus shoshone* inhabits environments having exceedingly low productivity (alpine lakes) and so occurs at densities that are low relative to most other copepods. These factors seem likely to select for effective strategies to ensure mating.

Remote detection of females is an efficient means of improving reproductive success by increasing the probability of mating encounters (Kiørboe, 2006). Encounter rates can be increased by other tactics as well, including aggregative behavior (Ambler et al., 1996) and faster swimming speeds (Kiørboe, 2008; Kramer et al., in press). However, whereas aggregative behavior promotes conspecific encounters, it also may increase intraspecific competition. Likewise, faster swimming may increase encounters with predators as well as mates, where fast movements can make copepods more conspicuous to visual predators. Faster swimming also is correlated

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**Table III: The spatial extent of the hydrodynamic disturbances generated in the side view by free-swimming female H. shoshone**

<table>
<thead>
<tr>
<th>Trail length (cm)</th>
<th>n</th>
<th>Velocity extent</th>
<th>Shear strain rate extent</th>
<th>Maximum deformation rate extent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female <em>H. shoshone</em></td>
<td>4</td>
<td>0.96 ± 0.35</td>
<td>0.43 ± 0.24</td>
<td>0.59 ± 0.22</td>
</tr>
</tbody>
</table>

The threshold values that were used to define the length of the hydrodynamic trail for velocity, shear strain rate and maximum deformation rate were 0.025 cm s\(^{-1}\), 0.4 s\(^{-1}\), 0.4 s\(^{-1}\), respectively. The spatial extents are presented as the mean maximum length of the disturbance greater than the threshold value ± SD.
with larger body size (Kiorboe, 2008), a major factor driving a zooplankter’s vulnerability to visually feeding fish (Brooks and Dodson, 1965), the dominant zooplanktivores in most lakes. Neither of these two alternative tactics is specific to improving interactions with mates. As shown here, the use of chemical and hydro-mechanical cues improves encounter rates with mates without these drawbacks, although there are likely metabolic costs for the chemical production-detection system. Additionally, there is a chance, though not documented, that predators might eavesdrop on these cues.

Animals that can increase the probability of mating encounter via remotely detected cues should be more able to establish populations from low-density colonization events. Thus, chemical detection of mates can be seen, at the population level, as a strategy that mitigates the negative effects of low density on population growth (i.e. Allee effects, Courchamp et al., 2008), by reducing the “critical density” for positive population growth (Gerrissen, 1980; Kiorboe, 2006). Using behavioral data on perceptive distances and swimming speed, along with a detailed encounter model, we are able to estimate the critical density for colonization events in this copepod species (Kramer et al., in press).

Conclusions
With this study, we increase the set of calanoid families having members that rely on diffusible chemicals to track mates to three: Temoridae, Centropagidae and now Diaptomidae, all of which are grouped into the superfamly Diaptomoidea. Within this superfamily, the pheromone trail following behavior differs for the freshwater and marine species of copepod. The chemical trails of the freshwater diaptomid H. shoshone are shorter (3.8 cm max) and their trail follows of 2.4 s are briefer than those of the marine species. Temora longicornis tracked trails for distances exceeding 13 cm, with residence times in the trail of up to 5.5 s (Doall et al., 1998). Centropages typicus performed similarly, tracking up to 17 cm for 5.7 s (Bagøien and Kiorboe, 2005). Male H. shoshone follow females closely, usually remaining <1 cm (~3 BL) from the female, whereas males of the marine species T. longicornis are often separated from their females by 3 cm (~25 BL; Doall et al., 1998), suggesting either weaker sensitivity to chemicals by H. shoshone or less stability of the freshwater pheromone. Our results demonstrate, for the first time, that chemically mediated mate tracking behavior occurs in a large freshwater copepod. This behavior may help to resolve the paradox of this species’ widespread distribution in isolated alpine lakes despite the operation of Allee effects limiting population establishment (Kramer et al., 2008).

SUPPLEMENTARY DATA
Supplementary data can be found online at http://plankt.oxfordjournals.org.

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