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Locating a mate in 3D: the case of *Temora longicornis*

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Using laser optics to illuminate high-resolution video-recordings, we revealed behavioural mechanisms through which males of the calanoid copepod species *Temora longicornis* locate females. Males of *T. longicornis* swam at significantly faster speeds than females along more sinuous routes, possibly reflecting adaptations to increase encounter with females. Upon approaching within 2 mm (i.e. two body lengths) of a female's swimming path, males accelerated to significantly higher pursuit speeds. Pursuit trajectories closely traced the trajectories of females, suggesting that males were following detectable trails created by swimming females. Males of *T. longicornis* detected female trails up to at least 10-s old, and tracked trails for distances exceeding 13 cm, or 130 body lengths. Females were positioned up to 34.2 mm away from males (i.e. reactive distance) when males initiated 'mate-tracking'. It was always the males of *T. longicornis* that detected and pursued mates. In rare events, males pursued other males. Behavioural flexibility was exhibited by males during mate-tracking. Males generally tracked the trails of 'cruising' (i.e. fast-swimming) females with high accuracy, while the pursuits of 'hovering' (i.e. slow-swimming) females often included 'casting' behaviour, in which males performed sharp turns in zigzag patterns within localized volumes. This casting by males suggested that hovering females create more dispersed trails than cruising females. Casting behaviour also was initiated by males near locations where females had hopped, suggesting that rapid movements by females disrupt the continuity of their trails. Males were inefficient at choosing initial tracking directions, following trails in the incorrect direction in 27 of the 67 (40%) mating pursuits observed. Males usually attempted to correct misguided pursuits by 'back-tracking' along trails in the correct direction. Males were observed to detect and track their own previous trajectories without females present, suggesting the possibility that males follow their own trails during back-tracking. Observations of males tracking their own trails and the trails of other males bring into question the specificity of trails as a mechanism promoting reproductive isolation among co-occurring planktonic copepods.

Keywords: calanoid copepods; mate location; *Temora longicornis*

1. INTRODUCTION

The calanoid copepod *Temora longicornis* is commonly found in surface plankton communities of the temperate northern hemisphere. This species dominates the copepod biomass in Long Island Sound from January to July, removing up to 49% of the daily primary production (Peterson 1985; Dam & Peterson 1993). Hence these planktonic copepods provide important links in marine food webs, transferring large amounts of carbon to higher trophic levels. For their populations to continue, individuals must meet mates, but little is known about their mating behaviour. Individuals of *T. longicornis* are small (1 mm), and often are separated by relatively large volumes of water. Unlike terrestrial animals they must search for mates within a three-dimensional environment in which motion along all planes is unrestricted.

The mechanisms by which planktonic copepods locate mates are not well understood, due in part to technological barriers preventing detailed observations of these small animals in their three-dimensional fluid environment. Previous observations of mate-searching behaviours have been made in small volumes of water (i.e. 1–50 ml) with

only a two-dimensional frame of reference, sometimes using tethered copepods (Katona 1973; Jacoby & Youngbluth 1983; Uchima & Murano 1988). These past studies suggest that sex pheromones signal males of the presence of females, but exact mechanisms through which male copepods follow chemical signals through three-dimensional space are difficult to discern.

Using a sophisticated optical system designed by Strickler (Strickler & Hwang 1998; Strickler, this volume) we were able to record and quantify the three-dimensional swimming paths of males and females of the calanoid copepod *T. longicornis* during mating interactions. Observations were made in relatively large volumes of water (1.5 l), thereby limiting wall effects and constraints on the animals' sensory range and swimming behaviour. To organize our findings, mating interactions are dissected into a series of sequential steps, similar to the sequence of events described by Gerritsen & Strickler (1977) for predatory interactions in the plankton, as well as Holling's (1959, 1966) classic 'components of predation'. The mating sequence progresses as: encounter, pursuit, capture and spermatophore transfer. Our quantitative observations focus on the steps leading up to mate capture, and

provide a means to evaluate the mechanisms which copepods use to improve their chances of locating mates within a vast ocean. Mechanisms of capture and spermatophore transfer have been described elsewhere for other copepod species (Blades & Youngbluth 1980; Uchima & Murano 1988).

2. METHODS

(a) *Animal collection and maintenance*

Mating behaviour of *T. longicornis* was observed during Summer 1994, and Spring 1996. A 100 µm plankton net pulled alongside a dock was used to collect copepods from Stony Brook Harbor, a small Long Island Sound embayment in Stony Brook, NY. Immediately following collection individuals of *T. longicornis* were sorted under a dissecting microscope and placed into screw-cap containers filled with 4 l of filtered seawater. The cultures were transported by car in thermally insulated containers to the Center for Great Lakes Studies (University of Wisconsin-Milwaukee) for experimental observation. Cultures were maintained at constant temperatures and light cycles in an incubator, and were fed roughly equal portions of the phytoplankton *Thalassiosira weissflogii* and *Isochrysis galbana* every other day. Colourful guts indicated that the copepods were feeding. The filtered seawater in the 4-l cultures was changed on a weekly basis. The emergence of nauplii in cultures indicated that adults of *T. longicornis* were reproducing and were healthy. Adults of *T. longicornis* were sorted from cultures and placed into 2-l containers of filtered seawater several hours before the start of an experiment.

(b) *Experimental observation*

Mating interactions between adults of *T. longicornis* were recorded on videotape using a system of laser photography developed by Strickler (Strickler & Hwang 1998; Strickler, this volume). Two orthogonal views, representing the $x-z$ and $y-z$ plane, were superimposed on to one video camera, allowing for analysis of the three-dimensional movements of individual copepods. To distinguish the two views, the common z -axis was slightly misaligned. The planar views encompassed the entire 1.5-l experimental vessel, which measured 10 × 10 × 15 cm (length × width × height). Copepods appeared as white silhouettes against a black background.

Observations were made in the dark, with illumination for the black and white video camera provided by an infrared laser. A blue laser beam, directed through the centre of the vessel from above, was used to attract the positively phototactic copepods, thereby concentrating the copepods in the centre while limiting interference from the boundaries of the vessel. The filming vessel was submerged in a large water jacket to maintain constant temperature levels during video-recording. Room temperature also was controlled for this purpose.

Mating interactions first were observed unexpectedly during a series of experiments conducted from 11 June–27 June 1994, designed to investigate the swarm kinematics of *T. longicornis*, both in the presence and absence of the predatory calanoid *Euchaeta rimana*. The blue laser served to congregate the phototactic animals, creating the swarms. Mixtures of adult males and females of *T. longicornis*,

totalling from 15 to 35 individuals, were gently spooned into the filming vessel containing filtered seawater, and their interactions were recorded for up to 2 h. Mating interactions recorded during these experiments are analysed here.

In Spring 1996, a series of experiments was conducted to (i) demonstrate the reproducibility of mating behaviour in *T. longicornis* and (ii) examine interactions between members of the same sex. The same observation techniques were employed as in 1994, including the use of the blue laser to congregate copepods, but controlled numbers of males and females were added separately to the filming vessel. At the start of each experiment 12 adults of the same sex were placed in the vessel, and their interactions were recorded for 30 min. After 30 min, 12 adults of the opposite sex were added. The sex-specificity of mating behaviours was addressed by comparing the occurrence of behaviours between the same sex and male/female treatments. On several occasions, coupled pairs (i.e. following capture of one copepod by another) were pipetted out of male/female treatments for sexual identification under a dissecting microscope.

(c) *Video review*

Recordings of eight different swarms of *T. longicornis* were reviewed, providing 6.5 h of observation of male/female mixtures, 1 h of male only and 1 h of female only observations. Sixty-seven mating interactions were identified, defined here as events in which adult males of *T. longicornis* detect and pursue adult females. Pursuits were identified through characteristic behaviours displayed by males, which included tight 'spinning' motions and rapid speeds. The sex of individuals could not be discerned from the videotapes. However, observations presented here, as in previous studies (Katona 1973; Griffiths & Frost 1976; Uchima & Murano 1988), indicated that: (i) only males pursue conspecifics and (ii) males pursue females much more frequently than males pursue males. Therefore males were identified as those copepods that pursue conspecifics, and females were identified as those copepods being pursued by males. However, it is possible that in some of the analysed events the copepod being pursued was another male.

Each mating interaction was qualitatively analysed by marking the successive positions of the male and female, both before and during pursuit, on to the monitor. It was evident from these initial analyses that males were following the trajectories of females during pursuit. The mating interactions were categorized based on the males' success/failure in capturing females. In unsuccessful mating interactions the female was identified as the copepod whose trajectory was being followed. Unsuccessful pursuits in which the female could not be identified were not included in analyses. Mating interactions also were categorized based on the male's initial pursuit direction along the female's trajectory, which was either correct (in the direction the female was going) or incorrect (in the direction that the female had come from).

(d) *Digital tracking of swimming trajectories*

Mating interactions were digitally recorded from videotape on to an IBM compatible computer equipped with a

video-capture card and a 4-Gb hard drive dedicated to video storage. The digital video was controlled from this computer, and individual frames were captured on a second PC and interfaced with video-analysis software (Optimus) on to a separate monitor. This software placed captured video frames within a Cartesian coordinate system, and returned the coordinates for specified points. A calibration measure from the video was used to convert the coordinate system from pixels into mm. The vertical axis (i.e. with respect to gravity) was designated as z , and the x - and y -axes formed the horizontal plane. One planar view provided x and z coordinates, the other provided y and z coordinates. The z coordinate was averaged from the two planar views. The three-dimensional trajectories of copepods were visualized by plotting their sequential coordinate positions. The temporal resolution between video frames was 33.3 ms.

Digital tracking of copepod coordinates started 1–9 s before the initiation of pursuit, in order to visualize the full length of female trails. Digital tracking ended when males and females became coupled or after males stopped pursuing females. The anterior tip of the copepod, the rostrum, was the specific point tracked for each trajectory. Before pursuing females, male coordinates were obtained every three video frames (i.e. 100 ms). During pursuit, males travelled at greater speeds with more frequent and sudden turns, and a temporal resolution of 33.3 ms was required to accurately track their trajectories during pursuit. Female swimming speeds and behaviours did not change when males initiated pursuit, and females were tracked at constant intervals of 100 ms throughout mating events. These temporal resolutions provided detailed information on swimming speeds and trajectories.

(e) *Quantitative analyses*

(i) *Swimming trajectories*

Swimming trajectories were digitally tracked in 23 mating interactions, with males successfully capturing females in 19 of these events. Swimming behaviour before mating interactions was referred to as 'normal'. Measures of normal swimming were obtained from swimming paths immediately preceding pursuit. Normal swimming trajectories ranged from 1.17 to 12.50 s in duration. Pursuit trajectories ranged from 0.40 to 3.67 s in duration.

The distance d between two points in three-dimensional space was computed from the x , y , z coordinates as:

$$d = ((x_1 - x_2)^2 + (y_1 - y_2)^2 + (z_1 - z_2)^2)^{1/2}. \quad (1)$$

Swimming speed was computed as the distance between copepod positions divided by the time interval between those positions. Swimming speeds over consecutive tracking intervals (i.e. 100 or 33.3 ms) were averaged for males and females during normal swimming and pursuit. At the end of pursuit, males made rapid lunges at females, of less than 67 ms in duration. These lunges were not included in average pursuit speeds. Lunge speeds were computed separately as the distance travelled over 33.3 ms (i.e. one video frame). If lunges occurred over parts of two frames, the maximum lunge velocity was taken.

Net-gross displacement ratios (NGDRs) were computed during normal swimming according to Buskey (1984):

$$\text{NGDR} = \frac{\text{net displacement of copepod}}{\text{gross displacement of copepod}}. \quad (2)$$

The NGDR provided a measure of the relative linearity of copepod swimming paths, with lower NGDRs implying more curved trajectories than higher NGDRs. NGDRs are fractal, and therefore depend on the temporal scale used. We computed NGDRs over 5-s intervals for eight males and 12 females.

(ii) *Encounter*

The mating sequence commences with encounter, which occurs when males detect the trails of females. Three measures were computed at the moment of encounter, which was taken to occur in the video frame prior to the initiation of pursuit. Straight-line distances between males and females at the moment males react to females, or reactive distances (Holling 1966), were computed using equation (1). The minimum distance between the male's position and the female's trajectory at the moment of encounter, or the initial tracking distance, also was computed using equation (1). The age of the female's trail on detection by the male was computed as the temporal difference between the moment the male reacted to the female trail and the moment that the female was positioned closest to where the male reacted.

(iii) *Tracking accuracy*

A quantity referred to as male-female displacement ratio (MFDR) was developed to describe the accuracy with which males followed female trajectories. Distances between consecutive copepod positions were summed to provide trajectory lengths, and the MFDR in each mating event was computed as:

$$\text{MFDR} = \frac{\text{length of male pursuit trajectory}}{\text{length of female trajectory}}. \quad (3)$$

The female trajectory length used in computing MFDRs started at the point closest to where the male detected her trail, and ended at the point where the male captured the female. Male pursuit trajectories started at the point where males initiated pursuit and ended on capture of the female. MFDRs quantified the level of symmetry between male pursuit trajectories and female trails. MFDRs equal to 1 represented the most accurate tracking, with turns in the male's trajectory perfectly coinciding with turns in the female's trajectory. Values greater than unity indicated that male pursuit covered longer distances than the female trail, as occurred when males swam with more frequent turns than females, or when males swam in incorrect directions along female trails. MFDRs less than unity indicated that males had 'cut corners' along female trajectories.

A second measure, the average tracking distance, also was used to describe the accuracy with which males tracked female trajectories. The average tracking distance was defined as the average minimum distance of the male to the female's trajectory during pursuit. This measure quantified the spatial separation between male pursuit trajectories and female trajectories. For each male position,

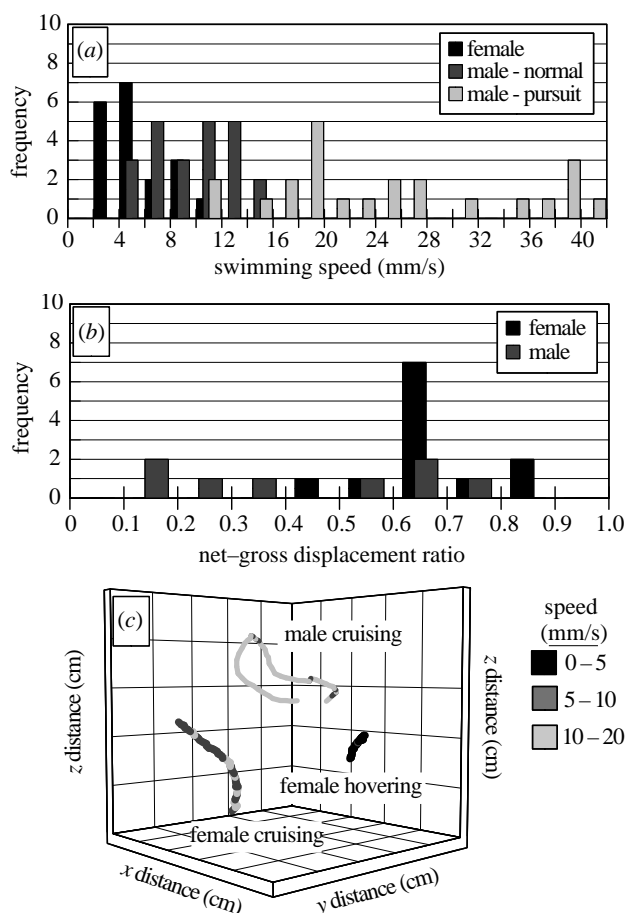


Figure 1. The swimming behaviour of males and females of *Temora longicornis*. (a) Swimming speeds. Normal swimming speeds were measured prior to 23 mating encounters ($n=23$ males and 23 females). Pursuit speeds of males were measured during 23 mating pursuits. Bin limits are labelled along the horizontal axis. (b) Net-gross displacement ratios. Computed for 8 males and 12 females over 5-s swimming intervals during normal swimming prior to mating encounters. Bin limits are labelled along the horizontal axis. (c) Normal swimming patterns. The trajectories, of 5-s duration, are representative of male and female normal swimming patterns.

the distances to every digitized position along the female's trajectory were computed and the minimum was taken as the instantaneous tracking distance. Instantaneous tracking distances were averaged over the course of pursuit to obtain average tracking distances.

3. RESULTS

(a) Normal swimming

(i) Swimming speeds

The normal swimming speeds of adult females of *T. longicornis* ranged from 2.84–10.10 mm s^{-1} (figure 1a), averaging (mean \pm s.d.) $5.91 \pm 2.28 \text{ mm s}^{-1}$ ($n=23$). Males travelled at significantly greater speeds than females (two-tailed t -test, $t=4.68$, $p<0.001$), ranging from 4.18–15.13 mm s^{-1} (figure 1a), averaging $9.73 \pm 3.18 \text{ mm s}^{-1}$ ($n=23$).

(ii) NGDRs

The NGDRs of females ranged from 0.45 to 0.82, averaging 0.68 ± 0.10 (figure 1b). The NGDRs of males were

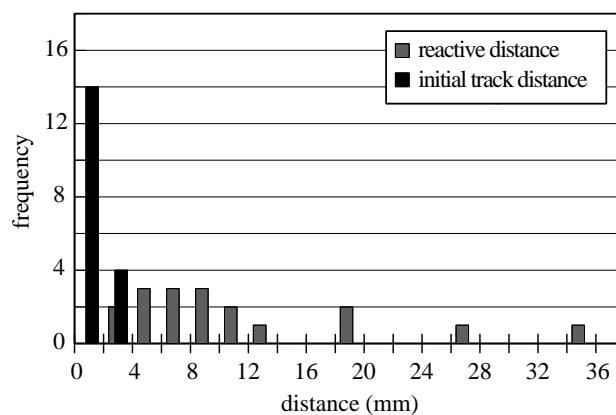


Figure 2. Comparison of reactive distances and initial track distances in mating encounters. Bin limits are shown on the horizontal axis.

lower than those of females (figure 1b; two-tailed Mann-Whitney U -test, $U_s=73$, $0.1 > p > 0.05$), reflecting sharper and more frequent curves and loops in male swimming trajectories (figure 1c).

(iii) Swimming styles

Males typically displayed a 'cruising' style of swimming, in which their rostro-caudal body axes were aligned in the direction of motion, whether they were swimming up, down or horizontally. Sinuous trajectories and relatively fast speeds were associated with this swimming style of males (figure 1c). The swimming style of females varied between the ends of their velocity range. At slow speeds, the motion of females is described as 'hovering'. The alignment of the rostro-caudal body axis maintained a relatively vertical orientation during hovering, as females slowly travelled upward through the water, often with horizontal components to motion (figure 1c). Hovering females travelled in relatively linear directions, but frequent small-scale oscillations reduced their NGDRs. At higher speeds, female swimming resembled the cruising mode (figure 1c). A combination of hovering and cruising characteristics was displayed by females at intermediate speeds.

(b) Encounter

Females were located from 2.4 to 34.2 mm away from males (straight-line distance) when males initiated pursuit (figure 2; table 1). Unlike this wide range of reactive distances, encounter always occurred when males were near the females' previous trajectories, usually within 2 mm (figure 2; table 1). These consistently low distances to female trajectories, or initial track distances, suggested that males were detecting signals in the path of the female rather than detecting females directly. Males detected female trails up to 10.3-s old (table 1).

(c) Pursuit

(i) Mate-tracking

On detecting the trails of females, males accelerated to significantly greater speeds (figure 1a; paired t -test, $t=-8.07$, $p<0.001$), averaging $24.96 \pm 9.39 \text{ mm s}^{-1}$ ($n=23$). Males did not swim directly at females during pursuit, but travelled along the trajectories of females

Table 1. *Mate-tracking variables for 18 successful mating pursuits*

(Successful mating pursuits are ones in which males capture females. The mating events are ordered by female speed, from lowest (hovering) to highest (cruising).)

event	reactive distance (mm)	initial track distance (mm)	trail age (s)	female trail speed (mm s^{-1})	initial pursuit direction	pursuit distance (mm)	trail length (mm)	MFDR	average track distance (mm)
1	8.22	3.17	10.3	2.41+0.88	incorrect	42.10	28.84	1.46	—
2	2.43	0.78	1.3	2.66+0.86	incorrect	10.29	4.85	2.12	1.31+0.50
3	18.59	1.41	8.3	3.03+0.72	correct	22.04	26.82	0.82	1.12+0.62
4	5.15	0.25	2.1	3.29+1.07	incorrect	37.00	13.27	2.79	2.06+0.95
5	4.43	1.56	1.9	3.46+1.28	correct	6.42	8.52	0.75	0.90+0.37
6	9.44	1.90	3.6	3.73+0.90	correct	13.48	16.78	0.80	1.22+0.53
7	10.33	1.71	2	4.74+1.24	correct	14.82	13.95	1.06	1.59+0.56
8	2.59	1.75	0.5	4.81+0.98	correct	4.33	4.06	1.07	0.93+0.39
9	19.85	1.88	5.5	4.89+1.31	correct	26.44	32.61	0.81	1.98+0.46
10	6.70	0.74	2.1	5.39+2.3	incorrect	34.99	21.44	1.63	0.86+0.51
11	11.05	3.33	2.6	5.4+1.21	correct	26.98	22.94	1.18	1.53+1.2
12	13.01	1.84	2.2	5.77+1.53	incorrect	137.81	33.85	4.07	1.73+1.15
13	27.60	3.04	5.2	5.71+1.02	correct	36.19	37.32	0.97	1.38+0.84
14	9.91	1.56	1.7	6.56+1.5	correct	14.98	16.55	0.91	0.91+0.40
15	4.11	0.95	0.6	7.01+0.79	correct	6.63	6.75	0.98	0.43+0.24
16	7.76	2.17	2.4	8.58+1.54	correct	30.21	28.19	1.07	0.89+0.35
17	6.93	0.67	1	9.07+1.7	correct	22.57	19.83	1.14	0.97+0.27
18	34.20	1.22	5.47	9.86+2.36	correct	55.11	65.53	0.84	1.02+0.34

(figure 3). Males typically maintained an average tracking distance of less than 2 mm (table 1), tracing female swimming paths with all their turns. This pursuit behaviour, termed 'mate-tracking', suggested that males were following detectable trails along the swimming paths of females. The swimming speed of females did not change significantly when males initiated pursuit (paired *t*-test, $t=0.45$, n.s.). Males were easily able to overtake females, swimming at average pursuit speeds five times greater than female swimming speeds.

Tight 'spinning' motions were exhibited by males during mate-tracking. Spinning was most pronounced at the moment of encounter, sometimes appearing as a single, narrow spiral in the male's trajectory with a maximum diameter of less than 1 mm (about one body length). Spinning generally occurred throughout pursuit without interruption to forward motion, appearing only as small oscillations in swimming trajectories. It is speculated that spinning behaviour involves rotations around the longitudinal body axis of males, but this could not be discerned at the level of magnification used.

When within approximately one body length (i.e. 1 mm) of females, males lunged at females and attempted to secure them for spermatophore transfer. This final lunge was brief, lasting less than 66.7 ms (i.e. two video frames) before contact with the female. Lunge velocities averaged $54.64 \pm 32.36 \text{ mm s}^{-1}$ ($n=19$), with velocities up to 140 mm s^{-1} being measured. Shorter temporal intervals (i.e. high-speed film) and greater magnifications are required to describe these rapid events precisely. Lunges were often preceded by brief deceleration of the male. On capture of the female, coupled pairs initially swam rapidly, making sharp turns and loops. Coupled pairs then sank slowly, often to the bottom of the vessel. Examination of coupled pairs pipetted off the bottom showed the males clutching the urosomes of females with their geniculate antennules.

Mating pursuits were observed in groups of males only, but infrequently. In two 30-min observation periods of males only, only two mating pursuits were observed, with one resulting in the seizure of a male by another male. When females were added to these male groups, the frequency of mating pursuits increased five-fold over the same interval. Mating pursuits were not observed in groups of females only. When males were added to groups of females, mating pursuits occurred within minutes.

(ii) *Tracking cruising versus hovering females*

Males successfully tracked trails created over the range of female swimming speeds (table 1). However, behavioural differences were observed between males tracking cruising versus hovering females. Males generally tracked cruising females with high accuracy, swimming within narrow corridors that closely traced female trajectories (figure 3*a*). On detecting the trails of hovering females, the initial pursuits of males often were characterized by erratic turns in zigzag patterns (figure 3*b*). These sharp turns did not correspond with turns by females, but occurred within relatively wide volumes below the females' trajectories, both in correct and incorrect directions. This 'casting' behaviour usually led into more directed pursuits that paralleled the females' trajectories from below. Males typically did not display casting behaviour when tracking cruising females.

(iii) *Back-tracking*

Males tracked female trails in either the direction the female was going (correct), or the direction the female had come from (incorrect). Males were inefficient at choosing tracking directions, following trails in the incorrect direction in 27 of the 67 (40%) mating interactions observed. Males generally did not give up if their initial pursuit was incorrect, eventually turning and 'back-tracking' to the

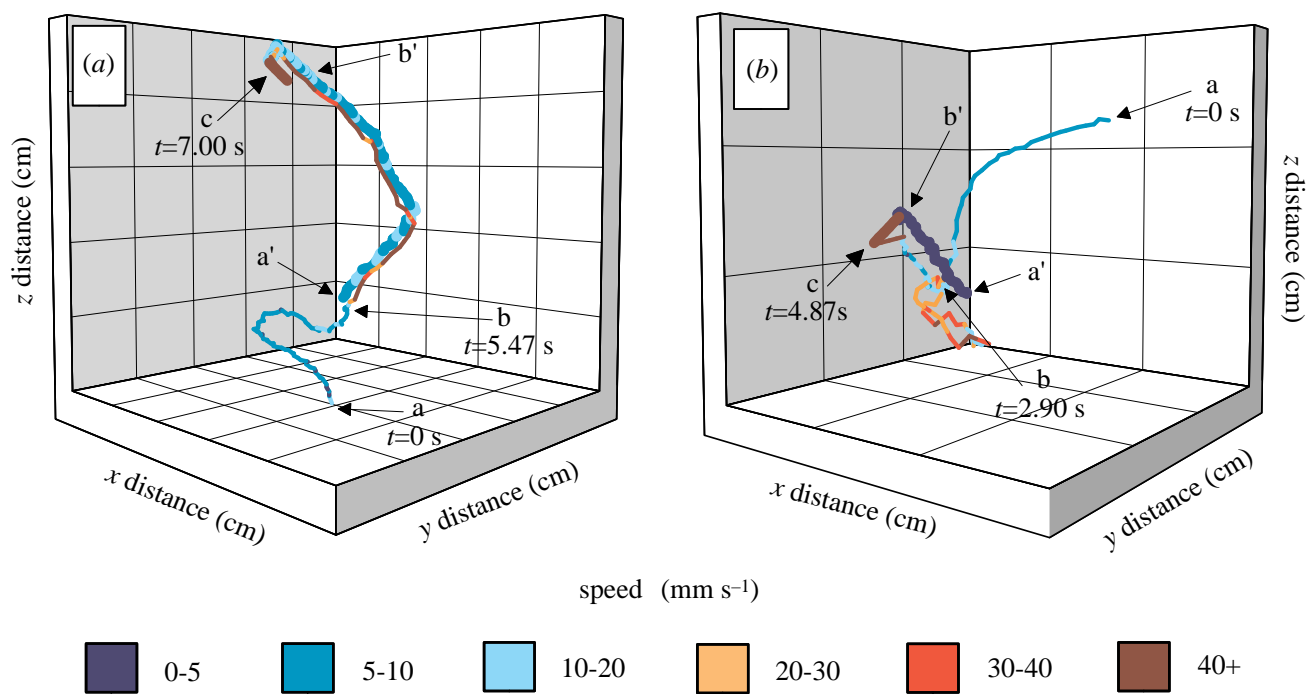


Figure 3. Mate-tracking by males of *Temora longicornis*. Male trajectories are represented by thinner lines than female trajectories. Time points are labelled with letters along male trajectories as follows: a, start of trajectory; b, male detects female's trail; c, male seizes female. The position of females at simultaneous time points are labelled with a' and b'. (a) Tracking a cruising female (event 18 in table 1). The male copepod closely follows the sinuous trajectory of the cruising female, maintaining an average tracking distance of 1.02 mm. (b) Tracking a hovering female (event 4 in table 1). The male initiates casting behaviour on encountering the hovering female's trail.

initial location of trail detection (figure 4a). Casting motions sometimes preceded and/or occurred during back-tracking by males. Back-tracking paid off for males in 9 of 22 attempts, allowing them to relocate the female's trail and correctly track it to the female. During back-tracking, it was unclear if males were following either (i) their own trail, (ii) the female's trail, or (iii) a combination of both. Males were observed to detect and track their own previous trajectories without females present (figure 4b), suggesting the possibility that males follow their own trails during back-tracking.

When initial pursuit was in the correct direction, MFDRs typically deviated less than 0.2 from the perfect value of 1 (table 1). MFDRs were significantly higher when initial pursuits were in the incorrect direction (two-tailed Mann-Whitney U , $U_s=65$, $p<0.002$), due to the extra distance travelled by the male.

(iv) *Unsuccessful tracking*

Mate-tracking by males was not always successful, with males capturing females in only 46% of the 67 mating pursuits analysed. The computed tracking efficiency of males would have been even lower if unsuccessful pursuits in which the female could not be identified were included in the total. In 12 of the 36 unsuccessful mating pursuits reviewed, males veered off course near locations where females had made rapid hops or turns (figure 5). This suggested that rapid accelerations by females leave interruptions in their trails. These interruptions evoke behavioural responses from males. In the event illustrated in figure 5a the male initiated casting behaviour approximately 2.9 mm

away from the location where the female had hopped. In figure 5b the male initiated back-tracking in the wrong direction at a location 2.2 mm away from where the female had hopped.

In nine unsuccessful pursuits, the females escaped from the males during the males' final lunge. It was unclear whether the females had sensed the approaching males and initiated escape, or if they were momentarily captured and then rejected by the males, or escaped from the males' grasp.

4. DISCUSSION

Mating interactions between copepods can be dissected into a series of sequential events: encounter, pursuit, capture and spermatophore transfer. Success of the male at each step permits continuation of the mating sequence, resulting in deposition of a spermatophore. The rate of successful mating interactions is an important variable underlying the growth of populations. Our observations reveal behaviours in *T. longicornis* that increase the rate of encounter and the probability of successful pursuit, thereby promoting mating success in this species.

(a) *Encounter rate*

Mating interactions between adults of *T. longicornis* begin when males encounter the trails of females. Encounter rates will vary as a function of: (i) the densities of males and females; (ii) the swimming speeds of males and females; and (iii) the encounter radius of the male (Gerritsen & Strickler 1977).

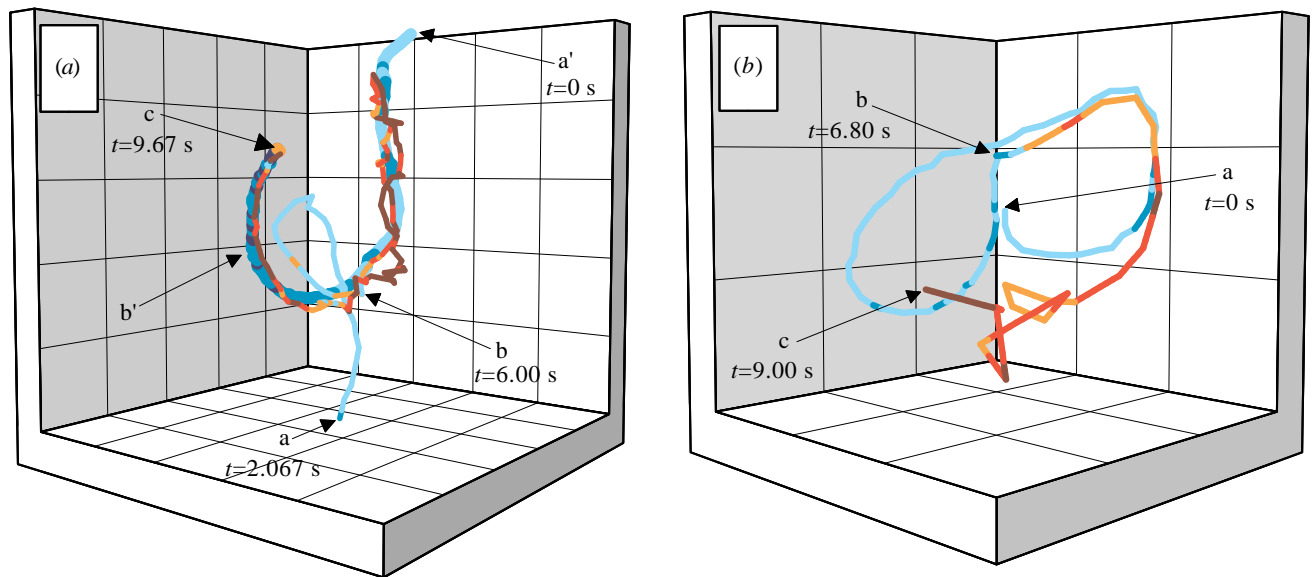


Figure 4. Back-tracking behaviour. Axis labels and trajectory colour codes are the same as in figure 3. Divisions along axes are in cm. (a) Incorrect pursuit and back-tracking (event 12 in table 1). The male corrects his incorrect pursuit by back-tracking, travelling for a total distance of 137.8 mm until capturing the female. Time points are labelled as in figure 3. Note that the male trajectory starts 2.067 s after female trajectory. (b) Male tracking his own trail. The male loops around, intersects his own trail and tracks it. He initiates casting behaviour on losing his trail. Time points are labelled with letters along the male trajectory as follows: a, start of trajectory; b, male detects his own trail; c, end of trajectory.

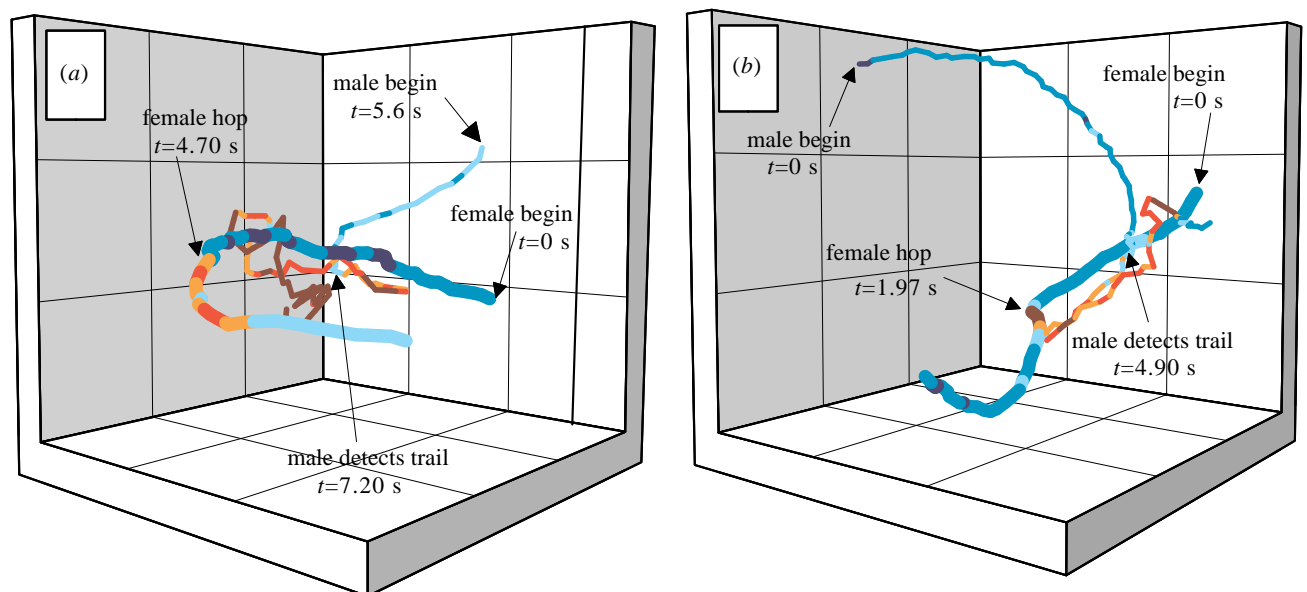


Figure 5. Unsuccessful mating pursuits. Axis labels and trajectory colour codes are the same as in figure 3. Divisions along axes are in cm. Time points are directly labelled on the figure. Note that initial and final time points do not correspond between male and female trajectories. (a) Male initiates casting behaviour. Trail deformation created by a rapid female hop elicits casting behaviour in the male. (b) Male initiates back-tracking in the wrong direction. Trail deformation created by a female hop elicits back-tracking behaviour in the male.

(i) *Density*

A basic variable underlying encounter rates among zooplankton is the density of individuals (Gerritsen & Strickler 1977). To increase the probability of mating encounters, some copepod species may aggregate around a common stimulus. For instance, swarms of phototactic copepods have been observed within the light shafts filtering through the root system of mangrove swamps (Ambler *et al.* 1991). In this study we mimicked this

natural situation by using a blue laser to attract individuals of *T. longicornis* to a common area. When the blue laser was off, most copepods hovered against the walls and at the surface or stayed on the bottom. Incursions into the centre of the vessel were infrequent, and vessel boundaries often blocked a clear view of the copepods. When the blue laser was turned on, copepods immediately migrated to the centre of the vessel, swimming in and out of the shaft of blue light. Encounter rate greatly increased in the

presence of this stimulus. Although swarming activity has not been observed for *T. longicornis* in nature, diel vertical migrations have been documented (Dam & Peterson 1993). These synchronous movements may be a mechanism through which this species and others increase their densities and hence rate of encounter with mates.

(ii) *Swimming speed and behaviour*

In addition to density, encounter rates will depend on the relative swimming speeds of males and females, with encounter rate increasing as swimming speeds increase (Gerritsen & Strickler 1977). Males of *T. longicornis* swim significantly faster than females, along more winding and sinuous paths. These behaviours may have evolved as a mechanism to increase encounter with female trails.

The swimming strategies employed by males do not come without costs or risks. Faster swimming speeds place males at a greater risk of encountering predators. Winding swimming patterns may also make males more attractive to visual predators, such as fish. As flow fields generated by copepods appear to narrow as copepods swim faster (Strickler 1982; Tiselius & Jonnson 1990; Yen & Strickler 1996), males may be less efficient than females at suspension feeding. Lower ingestion rates have been reported for males than for females of *T. longicornis* (Harris & Paffenhöfer 1976). In some copepod species males lose their feeding appendages and become non-feeding on the final moult into adulthood, concentrating their resources on the pursuit of females (Boxshall *et al.* 1997).

(iii) *Encounter radius*

The encounter model of Gerritsen & Strickler (1977) is most sensitive to changes in the encounter radius, with encounter rate being proportional to the square of the encounter radius. The encounter radius defines a spherical volume around individual zooplankton in which other animals (i.e. prey, predators, mates) may be detected. Males of *T. longicornis* locate distant females through detectable trails. In essence, these trails serve to increase the encounter radius of males beyond that of physical contact, thereby increasing the rate of mating encounters.

Reactive distances (Holling 1966), or the distance between two animals at the moment one animal reacts to the other, have been used to quantify the encounter volumes of planktivorous fish for prey (Werner & Hall 1974; O'Brien *et al.* 1976; O'Brien 1979). Fish detect prey directly using vision, and reactive distances reflect their visual range. Males of *T. longicornis*, on the other hand, do not directly detect females, but detect signals along the female swimming paths. Reactive distances therefore do not reflect the sensory range of males of *T. longicornis*. Rather, encounter depends on the chance occurrence of males intersecting detectable trails created by females. In addition to the densities and speeds of individuals, as discussed above, the rate at which males intersect female trails will depend on the persistence of these trails over time (see Yen *et al.*, this volume).

(b) *Pursuit behaviours*

It is always the males of *T. longicornis* that pursue mates, as has been noted in other copepod species (Katona 1973; Griffiths & Frost 1976; Jacoby & Youngbluth 1983; Uchima & Murano 1988). Three behaviours that help males track

trails to females are identified: (i) rapid pursuit speeds; (ii) back-tracking; and (iii) casting. Pursuits always are characterized by high velocities which allow males to overtake females. Back-tracking is a behavioural strategy that corrects mistakes made by males in choosing directions of pursuit. Decreasing signal strength during incorrect tracking may be the cue that elicits back-tracking in males (see Yen *et al.*, this volume).

Casting behaviour often is performed by males during pursuit of hovering females. The feeding currents of hovering females may disperse trails, and localized turning may allow males to determine trail boundaries and directions of pursuit (see Weissburg *et al.*, this volume). The feeding currents of *T. longicornis* are posteriorly directed (Yen & Fields 1992). The observation that pursuit trajectories followed below (i.e. with respect to gravity) the trajectories of hovering females supports the idea that feeding currents disperse signals.

Males also initiate casting behaviour near regions where females had hopped. Rapid movements by females may create deformations in trail structure, such as sharp gradients in signal strength, and casting may act as a localized search strategy to relocate structured trails. These searches often are not successful, thereby discontinuing the mating sequence.

The mate-tracking behaviours documented here have some notable differences from male–female interactions for the same species of copepod observed by Van Duren & Videler (1995, 1996) and Van Duren *et al.* (this volume). They never observed mate-tracking by males, which may be due to short observation times (5 min), low animal densities (4 copepods) and/or the absence of a swarming stimulus. To provoke mate-tracking, it also may require certain threshold levels of pheromones or defined structure in chemical gradients (i.e. trails) not present in female-conditioned water that is well mixed. Males did swim faster than females in their experiments, as we observed, but they also found that females hopped more when males were around. Our study documented that males lose trails when females hop, lowering the probability of successful mating. As Van Duren *et al.* (this volume) did not observe mating in these copepods they could not test their hypothesis that hydromechanical signals produced during female hops increase the probability of mating encounters. The relative importance of chemical versus mechanical signals in mating interactions may vary between species and types of copepods, producing differences in copepod responses.

(c) *Reproductive isolation*

Co-occurring copepod species not separated by temporal or spatial barriers require mechanisms to prevent interspecific breeding. In the nearshore marine copepod *Labidocera aestiva*, morphological adaptations provide such a mechanism (Blades & Youngbluth 1980). The spermatophore is attached via a complex plate, or coupler, that corresponds in shape to the external morphology of the female urosome. This 'key and lock' match between conspecifics prevents males of other species from attaching spermatophores to females of *L. aestiva*, thus promoting reproductive isolation. However, other copepod species (i.e. *Calanus finmarchicus*, *Euchaeta* sp.) do not have this post-capture isolating mechanism.

Another mate recognition system, such as specific pheromones, may be needed to prevent hybridization.

To use a pheromone, chemosensory perception is needed. Several behavioural experiments have indicated that chemosensory mechanisms are involved in mating interactions (Katona 1973; Griffiths & Frost 1976; Uchima & Murano 1988). For instance, males of *Eurytemora affinis* cannot locate heat-killed females, perhaps due to degradation of chemical signals, yet they can be 'tricked' into seizing dead females and inanimate objects that are coated in the juices of crushed females (Katona 1973). Males of several species alter their swimming behaviour when placed in female-conditioned seawater without the physical presence of females (Griffiths & Frost 1976; Uchima & Murano 1988). The spatial and temporal scales over which we observed mating pursuits to occur are consistent with a chemosensory mechanism of mate location (see Weissburg *et al.* and Yen *et al.*, this volume).

We observed males tracking conspecific females. We also noted on occasion a male following the trail of another male, bringing into question the specificity in the trail composition. Male copepods have been observed to pursue females of closely related species, although at much lower frequencies than for conspecific females (Katona 1973; Jacoby & Youngbluth 1983). There appears to be some specificity in the contact chemicals used in mate recognition by the harpacticoid copepod *Coullana canadensis* (Frey *et al.*, this volume). Some species, such as *Pseudodiaptomus coronatus*, may also use hydromechanical signals to distinguish mates (Katona 1973). The exact nature of the mating signal, in both its chemistry and hydrodynamic structure, will contribute to its function of attracting suitable mates.

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