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Following the invisible trail: kinematic analysis of mate-tracking in the copepod *Temora longicornis*

Marc J. Weissburg¹, M. H. Doall² and Jeannette Yen²

¹School of Biology, Georgia Institute of Technology, Atlanta, GA 30332-0230, USA

²Marine Sciences Research Center, State University of New York, Stony Brook, NY 11794-5000, USA

We have analysed the fine-scale kinematics of movement of male and female copepods, *Temora longicornis*, to resolve how these small animals find their mates. Location of the trail initially involves rapid random turning and high rates of directional change. Males subsequently increase their rate of movement as they follow the trail, and execute a regular pattern of counter turns in both x,z and y,z planes to stay near or within the central axis of the odour field. Pursuit behaviour of males is strongly associated with female swimming behaviour, suggesting that quantifiable variations in the structure of the odour signal released by females affects male tracking. The behavioural components of mate tracking in *Temora* are very similar to those of other animals that employ chemically mediated orientation in their search for mates and food, and we conclude that male *Temora* find their mates using chemoperception. The kinematic analysis indicates both sequential and simultaneous taxis mechanisms are used by *Temora* to follow the odour signal. This, in turn, indicates that rather than responding to a diffuse plume, males are following a signal more accurately characterized as a chemical trail, and copepods appear to use mechanisms that are similar to those employed by trail-following terrestrial insects such as ants. While *Temora* expresses similar behaviours to those of a variety of chemosensory organisms, the ability to track a three-dimensional odour trail appears unique, and possibly depends on the persistence of fluid-borne odour signals created in low Reynolds number hydrodynamic regimes.

Keywords: chemotaxis; Crustacea; mating behaviour; navigation; orientation; sex-specificity

1. INTRODUCTION

Copepods are small (0.1–10 mm) aquatic crustaceans that live in a vast ocean, often separated by thousands of body lengths. Yet they must find prey and meet mates. To improve their probability of encounter, they can aggregate (Mackas *et al.* 1985; Hamner 1988; Wishner *et al.* 1988). Copepods are in close proximity to each other in these aggregations so that water-borne signals such as fluid mechanical deformations, chemical scents and visual images can reveal their presence. Perception of these signals mediates various interactions, including predatory strikes, prey escapes and the formation of mating pairs. Recently, Doall *et al.* (this volume) observed that copepods in single-species swarms track their mates from many body lengths away.

The observations of Doall *et al.* indicate that males use remote perception to recognize and follow the path of the female, and suggest long-distance cues, either chemical, mechanical or both, are used to guide animals to their mates. Further characterization of the sensory modalities employed by copepods requires a detailed understanding of animal movements during tracking. Kinematic analysis is a powerful tool that has been used to investigate sensory mechanisms in a wide variety of animals (e.g. Schöne 1984), but traditionally has not been used to address questions regarding copepod perceptual abilities. Here we

present a detailed depiction of the movements of male and female *Temora* during mate finding.

Comparative investigations suggest that animals often display locomotory behaviours that represent specific responses to cues of a given modality. For instance, chemosensory guided orientation in crabs and lobsters consists of a number of distinct behavioural elements, such as across stream tacking, that appear part of a generalized strategy for localizing sources of chemical stimuli (Atema 1996; Weissburg 1998). Indeed, the similarity of chemosensory orientation mechanisms may extend across habitats; the behaviour of crustaceans engaged in chemosensory search has strong functional parallels with odour-modulated orientation in terrestrial animals such as moths (Weissburg & Zimmer-Faust 1993, 1994).

An animal may use a particular type of cue in a variety of ways, and the kinematics of locomotory behaviour also furnishes strong evidence as to what particular sensory and behavioural strategies are employed by a given animal. The simplest and most common guidance strategy may be one in which movement is indirectly related to the stimulus source, and is revealed by kinematic analysis as changes in speed or frequency of random turning (kinesis; Schöne 1984; Dusenbery 1992). Kinesis most commonly serves as a mechanism to keep animals within restricted areas of intense stimuli (patches) that may contain objects of interest (prey, mates) (e.g. Buskey 1984;

Weissburg & Zimmer-Faust 1991). Almost all available behavioural observations of small planktonic crustaceans support kinesis as the predominant chemosensory mechanism (Weissburg 1998).

In contrast, kinematic analysis of foraging crabs and lobsters suggests that direct guidance to the odour stimulus (taxis) is used by these animals to find food (Reeder & Ache 1980; Moore *et al.* 1991; Weissburg & Zimmer-Faust 1994; Atema 1996; Weissburg 1998). Taxis mechanisms result in behaviour that is narrowly focused on a specific stimulus source and mediate changes in direction and speed that are always oriented towards (or occasionally away from) the source. Each of these two behavioural strategies has different implications for how animals move through a stimulus field, and each functions best in specific stimulus environments (e.g. Dusenbery 1992; Weissburg & Zimmer-Faust 1993, 1994). Thus, the behavioural strategy is critical in establishing the link between animal distributions and the stimulus space.

In this paper we present arguments that indicate that certain copepods strongly rely on chemical cues to mediate mate-tracking. Kinematic analysis of female swimming patterns in relation to male behaviour during tracking suggests that chemical signals convey the most information and are used by the male *Temora longicornis* to navigate along a scent trail to the female. Differences in male behaviour during tracking arise as a consequence of differences in female swimming, suggesting that predictable properties of diffusive trails constrain male behaviour. Further, the fine-scale kinematic analysis of males during tracking indicates substantial similarities with chemotactic behaviour displayed in a variety of terrestrial and aquatic animals. Although the behavioural mechanisms are similar, *Temora* appear to be unique in that they follow odour trails that are three-dimensional. Our quantification of swimming in *Temora* is the first account in which the paths of both sexes can be determined precisely in three-dimensional space and time, and has led to an understanding of the sensory mode and behavioural strategy involved in mate-tracking.

2. MATERIALS AND METHODS

(a) General methods

Details of copepod collections, videotaping sessions and digitizing methods are described fully in Doall *et al.* (this volume). Briefly, the behaviour of the marine planktonic copepod *Temora longicornis*, collected from Long Island Sound, NY, USA, was videotaped at the Center for Great Lake Studies of the University of Wisconsin, using optical methods described previously (see Strickler & Hwang 1995; Strickler, this volume).

(b) Kinematic analysis of tracking behaviour

(i) General

Within the loose aggregates of copepods swarming around the centrally positioned blue laser, mating encounters occurred. The digitized paths of male and female *Temora* were used to analyse the fine-scale movements of males during mate-tracking and normal swimming, as described fully in Doall *et al.* (this volume). The kinematics of motion during, and prior to, mate-tracking for males, were quantitatively analysed by calculating swimming

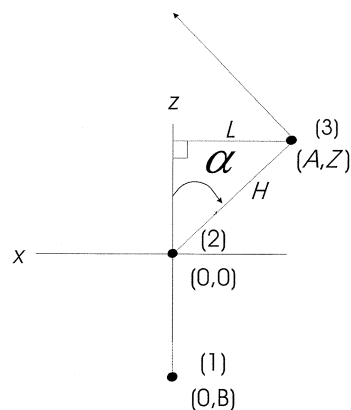


Figure 1. Computation of course angles of copepod paths. Hypothetical track of a section of a path consisting of three points, shown only in the x and z dimensions. They have been translated and rotated so that the first point lies on the x -axis and the second point is at the origin. Dropping a perpendicular line from point 3 to the z -axis forms a right triangle with the length of leg L equal to A . For this two-dimensional plot, the course angle α can then be calculated as arcsine (A/H). This angle represents the change relative to the previous heading. If the animal does not change direction, the calculated angle is 0° , while 180° constitutes complete course reversal. This example shows the two-dimensional case, whereas the rotations on the data are performed in the x,z and y,z planes.

speed, angular bearing, postural angle, net-to-gross displacement ratio (NGDR) and trail age. The x,y,z coordinates of the paths determined using our system had a spatial resolution of $165\ \mu\text{m}$ averaged across the x,y and z planes, and a temporal resolution of 16 ms (one video field at a frame rate of $60\ \text{frames s}^{-1}$).

(ii) Swimming speeds

Speeds for each path represent the average overall framewise speed measurements taken for the female, and for the tracking or non-tracking path segments of the male (Doall *et al.*, this volume).

(iii) Angular bearings and postural angles

Kinematic analysis of trail-following requires quantitative measurements of the turns and directional changes of a tracking male. As there is no relevant stimulus feature of the female path (such as flow or gravity) that remains constant over time, one cannot define a fixed coordinate system that is appropriate for computing the turning behaviour of the male. Examining the angle of the male's body axis with respect to the female path would be acceptable, but is computationally difficult because the paths of the female and her male pursuer are disjunct in time. Such an analysis would require extrapolating the path of the female between sampled points, and presents considerable problems in determining the appropriate reference segment of the female path. Additionally, we have evidence that in certain cases, the location of the trail is offset relative to the female's location. To avoid these difficulties, angular bearing of a given path segment was defined relative to the previous heading of the copepod (figure 1). For every three consecutive points, we determined the angle by remapping the points such that the

second point was located at the origin (0,0,0) and rotating these points so that the coordinates of the first point of the path equal zero on the x - and y -axes. The new coordinates of rotated points were calculated using equations for rotation of axes (Swokowski 1988). The result of these operations is that simple trigonometric relationships can be used to calculate the course angle in the x,z and y,z planes. The accuracy of these measurements is between 7° and 15° , depending on male swimming speed, and the accuracy improves at higher speeds. During the tracking phase males swim most quickly and generally display the smallest course angles (see § 3), so errors in small angles tend to be less than for the larger angles.

Video images also were used to quantify the angle of the body with respect to the animal's translational movement. This measure, referred to as the postural angle, was used to characterize female swimming behaviour in addition to swimming speed and NGDR. In each female path, we randomly selected three frames and measured the body orientation by tracing the path and the animal onto acetate sheets. The resultant postural angle was determined to the nearest degree with a protractor. Repeated measures of the animal were averaged and compared using the Watson–Williams two-sample test for angular data (Batschelet 1981).

(iv) *NGDR*

The net-to-gross displacement ratio (NGDR) provides gross measurements of the degree of path tortuosity, and is used to characterize the tendency of animals to turn during swimming. The NGDR is the ratio of the translational distance relative to total distance travelled and is calculated as the distance between the initial and final points of the path segment divided by the total distance travelled along the path. This value ranges from 0, for a completely circular path, to 1, for a completely straight path. To examine the behaviour of males relative to the female he pursues, we divided the NGDR of males by that of the female whom he tracked to calculate the 'relative NGDR' (rNGDR). We performed this calculation on tracking and non-tracking segments, and because this scalar manipulation does not alter the relative difference between the NGDR in each path segment, the behaviour of males prior to, and during, tracking may still be compared.

(v) *Distance to trail*

Last, we estimated the distance of the male from the female's path for every set of path coordinates of the tracking segment, defined as distance between the male's position and the closest point of the digitized record of the female path. This slightly overestimates the distance of the male relative to the female path, because the shortest distance would be the line segment extending from the male's position that intersects the female path at a 90° angle. At an average initial distance from the path of 2 mm and an average distance of 0.6 mm for consecutive points of the female path, this calculation overestimates the distance by approximately 0.01 mm, or 1% of the distance of the male from the female's path. This is smaller than the resolution of our video system. If the male is 0.3 mm from the path the calculated distance of a male equidistant between two points in a female path

would be about 0.42 mm. Although the relative error increases, it is nonetheless quite small in absolute magnitude and is frequently less than the body length of an individual copepod.

(vi) *Statistical analysis*

Results were analysed using pairwise t -tests to compare the differences in rNGDR and movement speed in tracking versus non-tracking segments. Log-likelihood analysis (Sokal & Rohlf 1981) using partial association χ^2 statistics examined differences in the distribution of angles in both x,z and y,z planes in tracking versus non-tracking path segments. This test determines the factors that significantly determine the frequency of observations in a two- or multiway contingency table. We also examined the relationship between distance to trail and trail age using both linear and non-linear regression. All statistical analysis was accomplished using Statistica (StatSoft, Tulsa, OK).

3. RESULTS

(a) *Kinematics of male trail-following*

(i) *General description*

The analysis shows that males attempting to track female paths perform a variety of manoeuvres that are qualitatively and quantitatively different from normal swimming. One distinct element of the male's behavioural repertoire is the bout of directional changes that immediately precedes the male actually locking on to the female path. This consists of a number (figures 2–4) of large turns as the male alters course in both x,z and y,z planes, and was seen in every tracking event. Thereafter, the male swims rapidly along the invisible trail created by the female, performing alternating turns in both horizontal and vertical planes (figures 2 and 3) to remain within several millimetres of the female's trajectory. When he is within one body length of his target he pauses, then in a quick leap captures her in a mating grasp. Because of the rapid pursuit speed of the male, the tracking phase often lasts less than 1 s, although occasionally events last twice that long.

(ii) *Trail-following as a function of female swimming behaviour*

Initial observations suggested that male trail-following falls into two distinct modes that appear to be associated with differences in the swimming behaviour of the females being tracked. Scatter plots of female swimming kinematics show that females form relatively distinct clusters (figure 5) that yield quantifiable differences in swimming behaviour (table 1). 'Cruising' females tend to swim rapidly and follow straight courses, while 'hovering' females move more slowly and in a more circuitous manner. Swimming speed in cruisers is significantly different from that in hoverers ($t=2.86$, $p<0.025$), and there is a strong trend for cruisers to display increased NGDR relative to hoverers ($p=1.58$, $p<0.15$). In addition, hovering females tend to orient so that their body axis is oriented obliquely to their direction of travel, and they display angles that are significantly different from cruising females, which tend to swim with their body parallel to their forward motion ($F_{1,10}=130.1$, $p<0.001$). Even though the behaviour of these animals may be continuous, the data

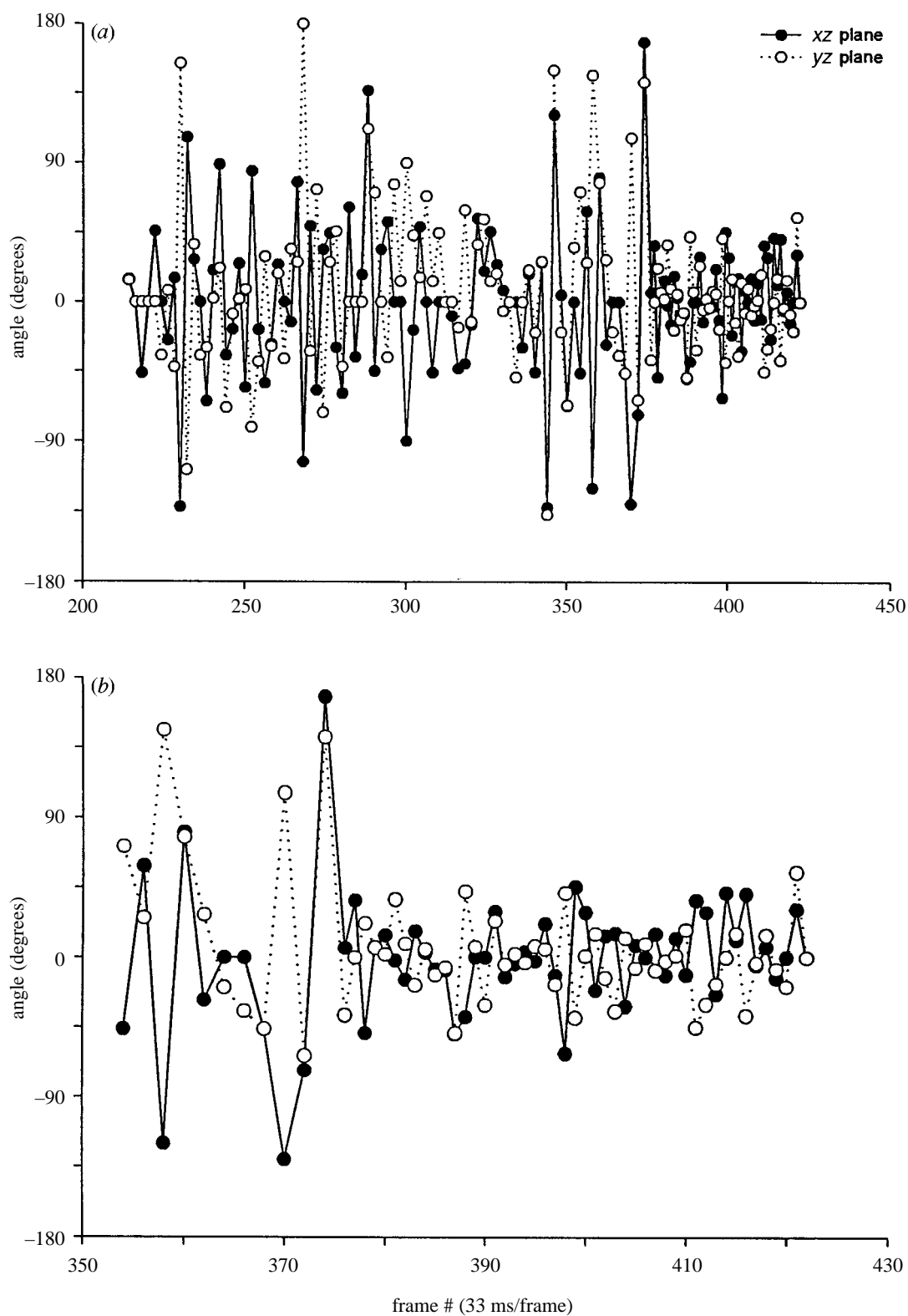


Figure 2. Course angles of copepod during swimming for a male tracking a cruising female. Male begins spinning at approximately frame number 350, then tracks female. (a) Whole path. (b) Close-up of tracking period. Closed symbols indicate course angles in the x,z plane; open symbols are course angles in the y,z plane.

characterize commonly observed patterns that represent the ends of a spectrum of female swimming behaviour. These two modes of locomotion are likely to have considerable effects on the nature of the stimulus directing males to females. Hence, we have separately analysed the males who track hovering versus cruising females.

Males that track cruising females swim very differently during the tracking phase than during time prior to tracking (table 1). Analysis revealed a significantly greater rNGDR in tracking versus non-tracking males ($t=3.4$, $p<0.025$). Males travelled a more direct route during tracking than during the preceding period.

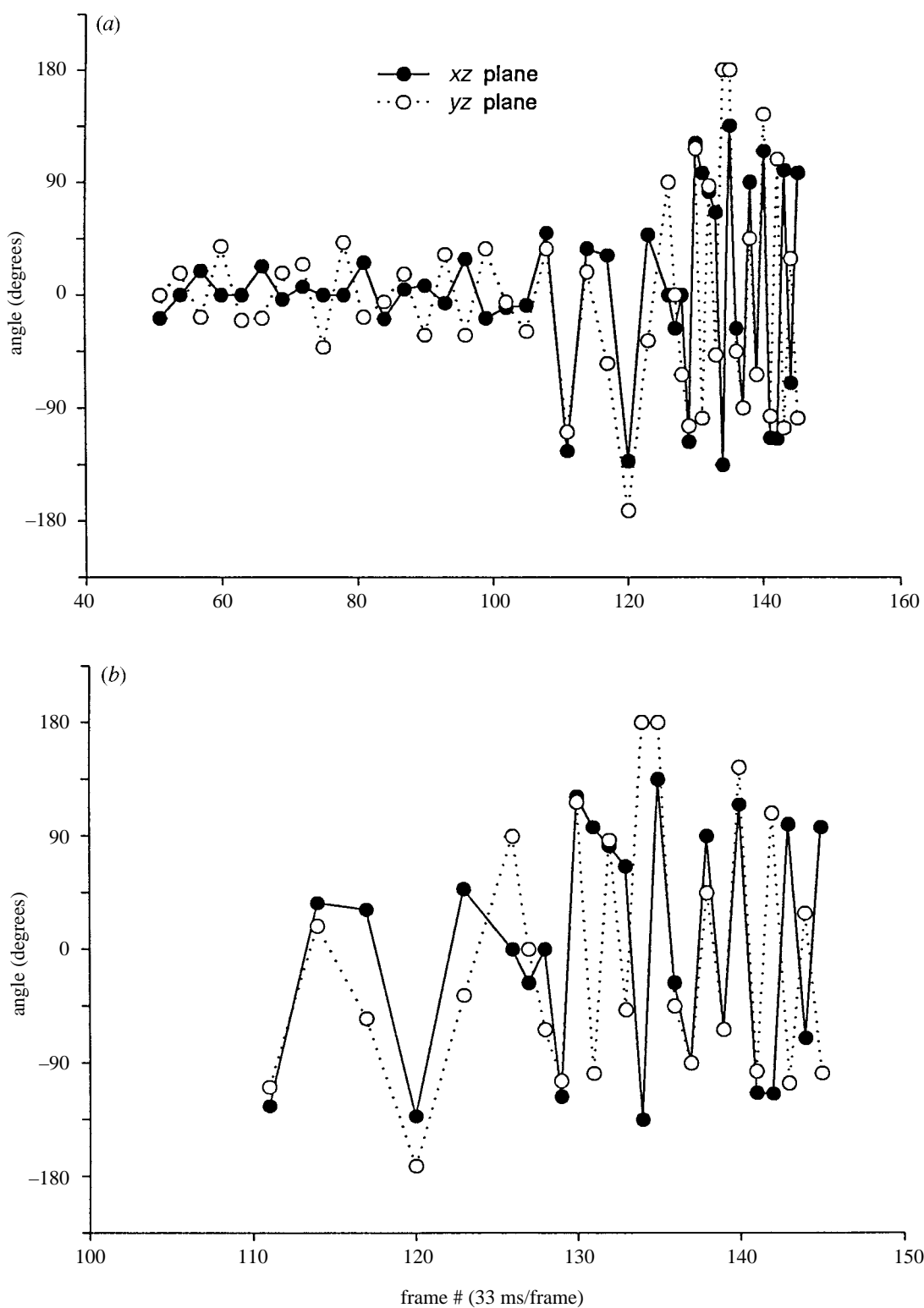


Figure 3. Course angles of copepod during swimming for a male tracking a hovering female. Male begins spinning at approximately frame number 110, then tracks female. (a) Whole path. (b) Close-up of tracking period. Closed symbols indicate course angles in the x,z plane; open symbols are course angles in the y,z plane.

The straighter trajectory of the male during tracking reflects a tendency for the male to constrain his range of turning angles; the distribution of angles is non-uniform for both groups of males (table 2, figure 6). Overall, the distribution of track angles is narrower in males following a female path, than prior to tracking (the

angle \times behaviour interaction). In general, the frequency of angles between 0 and $\pm 45^\circ$ increases, whereas the frequency of angles greater than $\pm 45^\circ$ decreases, in males following females. There is a striking absence of turns through angles greater than 90° in tracking males, whereas such directional changes are seen roughly 6% of

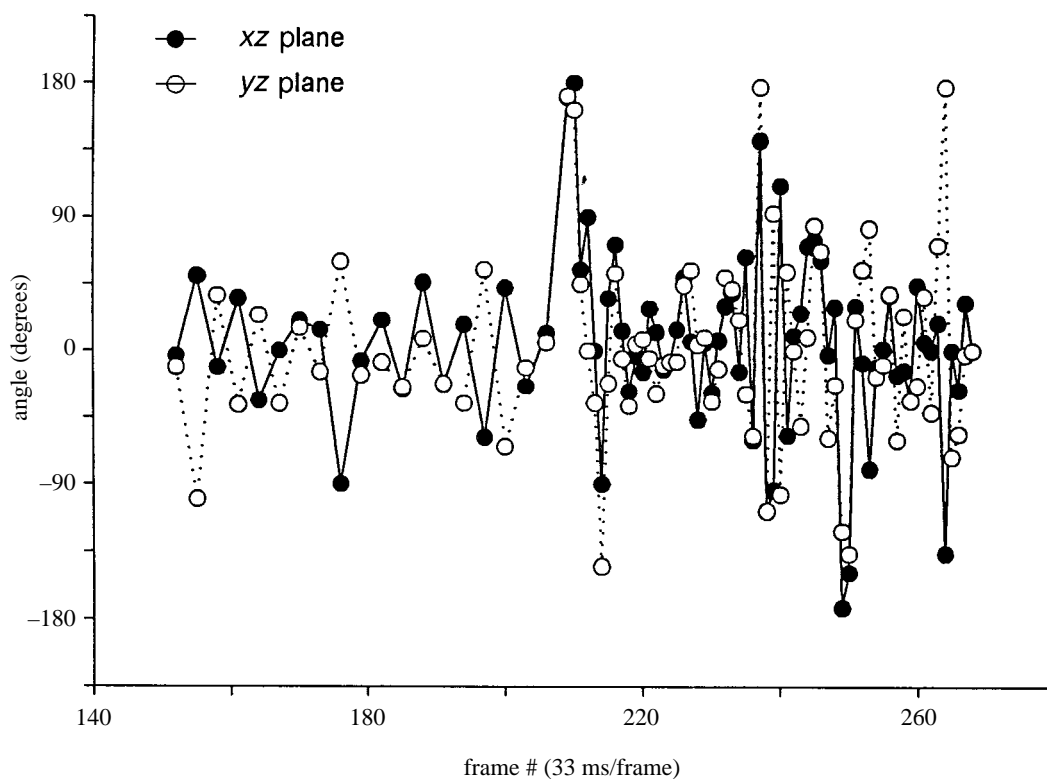


Figure 4. Course angles of copepod during swimming for a male who backtracked, then reversed course towards the female and subsequently lost the trail. Male begins spinning at approximately frame number 210, proceeds along the trail the wrong way until frame number 220 when he reverses course, finally losing the trail and spinning again at frame 240. Closed symbols indicate course angles in the x,z plane; open symbols are course angles in the y,z plane.

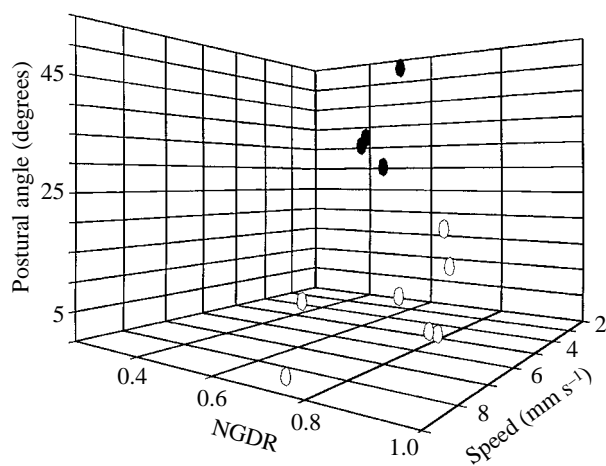


Figure 5. Three-dimensional scatter plot of swimming behaviour of females, showing differences between animals that hover (filled circles) versus those that cruise (open circles). These two clusters were used to define the two groups of females into respective groups for the statistical analysis in table 1.

the time in non-tracking males. Many of these large course angles occur immediately preceding tracking, suggesting that during this phase males are ranging widely to localize the signal produced by the female. Males also make large directional changes when they are nowhere near the path of the female, in an apparently random fashion. The movement of males during tracking is three-dimensional. Angular excursions in both planes

Table 1. Summary of gross movement patterns in 'cruising' versus 'hovering' females and the males that follow the paths laid down by these two types of females

(Note that for males, the NGDR is actually the rNGDR. Sample sizes are 8 and 4 for the two groups, respectively. Standard deviations are given in parentheses, except for postural angles, where the quantity given is r , the measure of variance in circular statistics.)

	speed (mm s^{-1})	NGDR	body posture (degrees)
females (cruising)	6.56 (1.77)	0.71 (0.17)	9.25 (0.99)
males (pre-tracking)	9.76 (1.27)	0.66 (0.34)	—
males (tracking)	27.55 (7.77)	1.02 (0.18)	—
females (hovering)	3.73 (1.01)	0.58 (0.07)	37.29 (0.98)
males (pre-tracking)	9.11 (3.78)	1.23 (0.21)	—
males (tracking)	16.69 (4.16)	0.81 (0.31)	—

are equally variable and there are no differences in the angular distributions of males in x,z versus y,z planes (the plane main effect and the plane \times angle interaction).

Males tracking paths of hovering, rather than cruising, females showed a very different pattern. They still exhibited the burst of directional changes that precedes

Table 2. Summary statistics for log-likelihood analysis of angular distributions in non-tracking versus tracking path segments

(a) Analysis of males that track cruising females. The analysis indicates that the distribution of angles is non-uniform, and that the angular distributions differ in tracking versus non-tracking path segments, whereas the angular distributions of a given behaviour are similar in x,z and y,z planes. Number of observations (frames) is 732 and 426 for non-tracking and tracking paths, respectively. (b) Males that track hovering females. The analysis indicates that angular distributions are non-uniform, and behaviour, but not plane, has a significant impact on the distributions. Number of observations is 200 and 172 for non-tracking and tracking paths, respectively.)

effect	d.f.	χ^2	p
(a)			
angular class	8	1179.6	0.001
plane	1	0.01	0.91
behaviour	1	1522	0.001
angle \times plane	8	5.34	0.72
angle \times behaviour	8	24.1	0.01
behaviour \times plane	1	0.01	0.93
(b)			
angular class	8	293.1	0.001
plane	1	0.25	0.62
behaviour	1	0.81	0.37
angle \times plane	8	5.98	0.64
angle \times behaviour	8	52.82	0.001
behaviour \times plane	1	0.11	0.60

tracking, but had a lower rNGDR while tracking females than during normal swimming (table 1). Every male displayed this pattern although the pairwise t -test was marginally significant ($t=3.25$, $p<0.10$). This strongly suggests that males swam more twisted paths while pursuing the female than in the period preceding tracking. More significantly, the distribution of turning angles broadened (rather than narrowed) in males that were tracking hovering females (figure 7, table 2), indicating that the more convoluted paths result from a higher incidence of large changes in course angle. The angular distributions of the two groups of males are no different in the non-tracking segments. Thus the reversal of the pattern seen in males tracking cruising females cannot be accounted for by general changes in male behaviour. The more broad angular distribution displayed by males tracking hovering females, relative to previous turning behaviour, solely reflects the kinematics of following the somewhat meandering paths created by slowly swimming females.

All males significantly increased their swimming speed when pursuing a female (table 1; $t=6.35$, $p<0.001$; $t=3.92$, $p<0.05$, for males following cruisers and hoverers, respectively), although the change in velocity was greater for males that tracked the paths of cruising females ($t=2.62$, $p<0.05$). Within 2–5 frames (66–150 ms) after detecting the path of a cruising female, a male typically accelerated to an average velocity of 27.55 mm s^{-1} , nearly three times his previous (non-tracking) speed of 9.76 mm s^{-1} . Initial accelerations were quite dramatic, in the order of several hundred mm s^{-2} and there was no tendency for males to

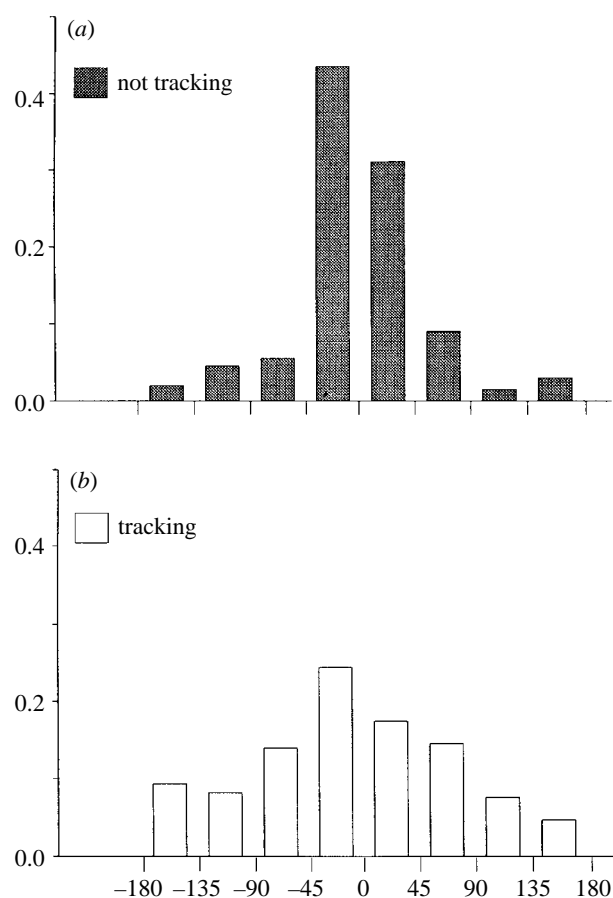


Figure 6. Angular distribution in non-tracking and tracking path segments for males that track cruising females. The x,z and y,z planes have been pooled. Figure shows proportion of observations in each angular class. (a) Non-tracking path segments. (b) Tracking path segments. The main effects 'behaviour' and 'plane' examine whether the frequency of observations differs in tracking versus non-tracking paths, and in different planes, respectively. The effect 'angle' examines if the distribution of angles is non-uniform across angular classes.

accelerate consistently along the path to the female. Immediately prior to the male catching up to the female, males often decreased velocity to levels approaching that of their pre-track speed, and subsequently lunged at a very high velocity directly at his prospective mate. Speeds during this lunge ranged from 35 to 100 mm s^{-1} , with accelerations equal to, or exceeding, those observed when the male initially detected the female's trail. Males that tracked hovering females behaved in a qualitatively similar way, although the pursuit velocity and corresponding accelerations were not as dramatic.

(iii) Turning behaviour during trail-following

We further investigated the turning behaviour of males by examining the regularity of his directional changes. Broadly speaking, turns in the horizontal plane can be characterized as either to the left or the right, and those in vertical plane can be either up or down. A runs test was used to analyse the turning sequence in each plane, as this test examines whether two (or more) conditions in an ordered list change more, or less, often than predicted

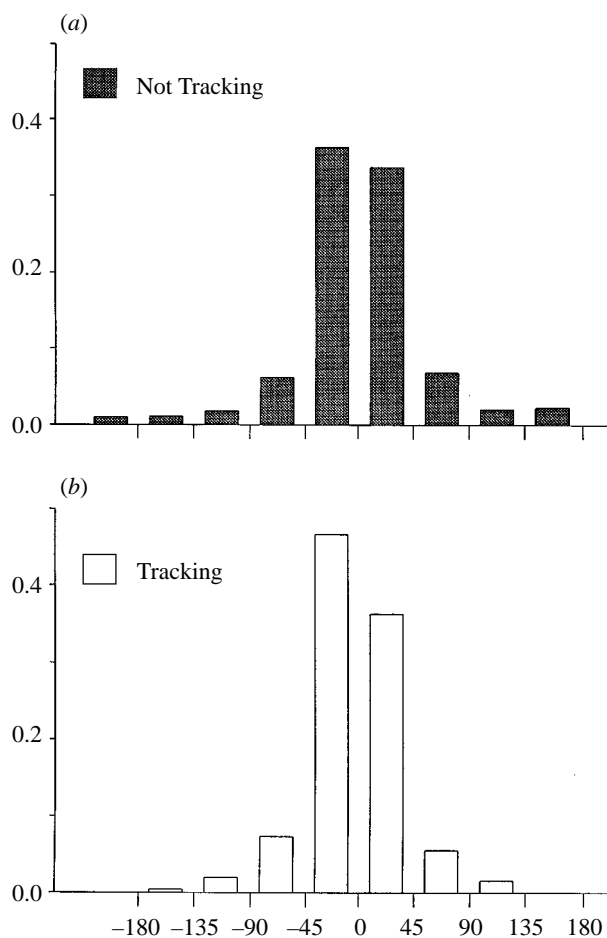


Figure 7. Angular distribution in non-tracking and tracking path segments for males that track hovering females. The x,z and y,z planes have been pooled. Figure shows proportion of observations in each angular class. (a) Non-tracking path segments. (b) Tracking path segments.

by chance. Directional changes that occur more often than expected indicate a regular pattern of alternating turns in a given plane. As the paths of males tracking hovering females were quite short, this analysis was restricted to males that followed cruising females.

When not tracking, males almost never displayed a sequence in which turn frequency deviated from random expectations (table 3). Of the 16 separate analyses (eight paths and two planes per path), only three males exhibited a greater than expected number of runs in a single plane. In contrast, three males made regularly alternating turns in both planes, and seven of eight males displayed a pattern of alternating turns during tracking in at least one plane (table 3). Such alternating turns suggest that during tracking, males traverse across the female path moving up/down and left/right in a semiregular pattern, and continually turn towards the centre of the trail once they have reached its opposite edge. The existence of alternating turns in both planes implies that the spatial distribution of odours corresponds more closely to a corridor, rather than to a two-dimensional trail laid down on a flat surface.

(iv) *Distance to trail as a function of trail age*

The relationship of trail age to distance from the trail reveals several phenomena. First, the upper age limit for

Table 3. Analysis of runs in non-tracking and tracking males, giving the number of left/right and up/down turns in x,z and y,z planes, respectively, and the corresponding number of runs, for non-tracking and tracking males

event	non-tracking		tracking		x,z	y,z	x,z	y,z
	no. of events	no. of runs	no. of events	no. of runs				
path 1	26/23	25/24	21	24	14/15	12/17	21**	21*
path 2	45/36	53/28	47	42	12/21	15/22	31***	28**
path 3	21/9	11/19	14	19 ^a	13/10	12/11	14	19*
path 4	31/25	32/24	28	27	10/9	11/8	13 ^a	16**
path 5	30/28	44/24	43*	39	20/18	12/26	26*	23*
path 6	26/35	32/29	38*	38*	16/17	12/21	18	18
path 7	17/15	14/18	16	15	11/9	11/9	16*	16*
path 8	23/47	24/16	25	30*	10/13	13/10	15 ^a	17*

^a $0.05 < p < 0.10$; * $p < 0.05$; ** $p < 0.025$; *** $p < 0.001$.

detection by most males appeared to be in the order of 5 s, although several males detected trails as old as 10 s. Second, males appeared to decrease their distance from the female's trail as they proceeded along this path en route to the female (figure 8). Third, the decrease in distance from the female trail does not appear linear, but is more accurately modelled by an exponential saturating function. The linear model failed on several criteria. First, it does not accurately predict the steep decrease in distance to the trail that occurs when the trail is very young, and in fact, the y -intercept is significantly different from 0 (intercept = 0.64, $t = 9.10$, $p < 0.001$, $n = 426$). The linear model also shows a poorer fit to the data ($r^2 = 0.29$ for the linear versus $r^2 = 0.35$ for the non-linear model) and, last, the distribution of residuals is non-random, showing a concentration of negative values near the origin. The non-linear regression is more robust and accurately predicts both the drastic decline of distance to trail that occurs for very young trail ages, as well as the relatively constant distance to trail that characterizes the behaviour of males responding to trails older than 1–2 s. The analysis shows that, regardless of the age of the trail when first encountered, males seem to maintain a distance of approximately 1.5–2 mm from the path until the trail is approximately 2 s old. At this point the male is hot on the trail of the female, and rapidly decreases his distance from the trail as he follows it to his mate.

4. DISCUSSION

(a) *Kinematics of trail-following and sensory mechanisms*

Almost all motile animals have a strategy for locating and recognizing mates. Our analysis shows that, like many other organisms, copepods have sophisticated sensory and behavioural strategies for finding their mates (figure 9). Male copepods are able to track prospective mates through a three-dimensional realm in a precise and directed manner, and swim differently during tracking than during other periods. Further, the behaviour of males during tracking is affected by the swimming

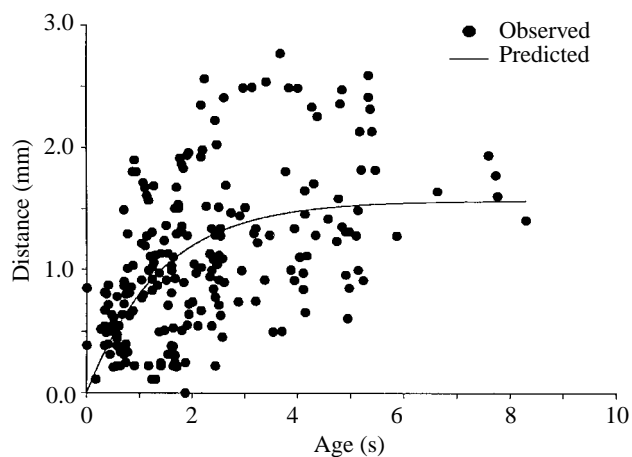


Figure 8. Distance to trail versus trail age. Data were fitted to an exponential saturating function $y = \beta(1 - e^{-rx})$, where y is distance, x is trail age, β is the asymptote and r is the rate constant. The calculated model parameters are $\beta = 1.57 \pm 0.09$ (standard error), and $r = 0.72 \pm 0.01$. $n = 426$.

behaviour of females that create the trail. For males tracking cruising females there is an increase in swimming speed and path straightness on encountering and following a female trail. Males following hovering females also increase their speed, although by less than for males following cruisers, and increase the frequency of wide-angle turns relative to the non-tracking period. During the tracking period, males perform alternate left–right and up–down turns with very narrow angular excursions relative to their behaviour prior to tracking, and they decrease their distance from the path until they ultimately encounter the female and attempt to mate. Using this repertoire of behaviours males can follow trails as old as 10 s, over distances approaching 10 cm.

A recent review of copepod mating biology suggests that many copepods rely on chemical cues to mediate processes critical to reproduction, including mate recognition and spermatophore placement (Lonsdale *et al.* 1998). Other studies have shown changes in swimming patterns, presumably elicited by chemical cues, as part of mate pursuit behaviour. Such differences generally consist of changes in swimming speed and rate of turning when males are exposed to water through which females have swum (Uchima & Murano 1988; van Leeuwen & Maly 1991). Uchima & Murano (1988) also observed *Oithona* males changing their swimming pattern from paddling to a spiralling when they pass through water immediately vacated by the female. Spirals were greater than 2 mm in diameter and gradually decrease over time. *Eurytemora affinis* also exhibited looping behaviour in response to water occupied by females (Katona 1973). Tsuda & Miller (this volume) describe rapid and frequent directional changes when male *Calanus* move to locations near or through which females have passed. Such increases in the rate of undirected turning have been interpreted as promoting efficient area-restricted search (Buskey 1984; Weissburg & Zimmer-Faust 1991). These behaviours are consistent with kinesis mechanisms, where changes in velocity or rates of random turning result in indirect localization of a stimulus source (Schöne 1984; Dusenbery 1992).

The behaviours executed by male *Temora* are indicative of taxis, that is, directed orientation to a source (Schöne

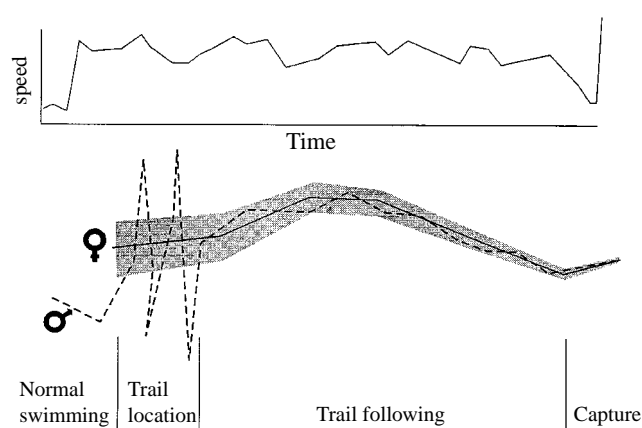


Figure 9. Summary of trail-following in a *Temora*, showing the paths of a male and female, the different phases of tracking and orientation, and speeds during these phases. The path of the pursuing male is the dashed line, the path of the (cruising) female is the dotted line, and the shaded region represents trail borders (not to scale). Note that this is a two-dimensional view, and a similar sequence of turns also occurs in a plane perpendicular to the one shown here.

1984; Dusenbery 1992) rather than kinesis. Movement patterns by tracking male *Temora* are strikingly similar to those of other chemotactic animals, and most of the elements of a tracking male *Temora* have analogues in the behaviour of other animals that directly orient to odour plumes or trails. The brief period of rapid directional changes preceding trail-following in *Temora* is mirrored by the crosswind casting behaviour of moths exhibited during detection of pheromone plumes (Baker & Vogt 1988; Willis & Arbas 1991; Mafra-Neto & Cardé 1994). Such casting behaviour in moths is used to rapidly sample corridors of air to locate a plume, and it appears that male *Temora* perform a three-dimensional analogue of this manoeuvre to initially locate the trail. Additionally, the similar, but more vigorous casting-like behaviours when a male loses a female track (figure 4) appear to be part of a generalized strategy to relocate the boundaries of an odour plume or trail. Upon loss of an odour plume moths also cast widely to re-establish contact and resume odour-guided navigation (Baker & Vogt 1988; Willis & Arbas 1991; Mafra-Neto & Cardé 1994). Analyses of the directionality show that, unlike moths that cast perpendicular to the wind, a copepod casts with equal frequency in all directions, suggesting that flow is not important during mate location. Rather, because of the three-dimensional quality of the trail, copepods cast in both x, z and y, z planes to maximize the probability of encountering the trail.

The subsequent pattern of alternating turns displayed by male *Temora* during trail-following occurs in a variety of aquatic animals that orient to odour plumes (Weissburg 1998) and may result from simultaneous sampling by paired chemosensory organs (tropotaxis). This mechanism may be of considerable utility in locating trail or plume borders, since it functions to detect stimulus asymmetry. Differences in stimulus strength, such as when the animal has one sensor within and the other outside of the trail, is presumed to mediate the degree and direction of turning towards the trail centre. Animals as diverse as ants (Calenbuhr & Deneubourg 1992) and crabs and lobsters

(Weissburg & Zimmer-Faust 1994; Atema 1996) appear to use tropotaxis as a mechanism to locate odour signals, and evince tacking behaviour similar to that displayed by *Temora*. We conclude that, at least in this one case, copepods also are capable of tropotactic orientation in response to a chemosensory trail.

The differences in the angular distributions in males that track hovering versus cruising females further supports the existence of tropotactic orientation. Schlieren visualizations of the wake of hovering females show a wide trail with vague borders, and the more upright body posture tends to promote sinking and diffusion of the odorants in the vertical plane. In contrast, a cruising female produces a very distinct and narrow hydrodynamic signature consisting of a discrete and clearly definable trail. Thus, males tracking a hovering female are confronted with a relatively diffuse chemical signal, which is reflected by the frequent directional changes they display. The direct and precise tracking behaviour of a male pursuing a cruising female is a consequence of the well-defined nature of her trail type. Hydrodynamic effects on signal structure often are correlated with the behaviour of animals that follow turbulent odour plumes (Weissburg & Zimmer-Faust 1993, 1994; Weissburg 1998). The behavioural differences displayed by male *Temora* who track females with disparate swimming modes confirm the existence of hydrodynamic constraints on behaviour in diffusive, low Reynolds number regimes as well. Here, it is the particular mode of locomotion that mediates male behaviour, through its effects on the structure of the odour trail left in the wake of a swimming female.

The relationship of trail age to distance from the trail is consistent with a tropotactic orientation mechanism. As the trail ages, diffusion (which is the only transport mechanism redistributing odorants in low *Re* flows) will widen the trail. Thus, the male will range more widely before contacting a trail border as trail age increases.

In addition to defining the borders of an odour field, tropotactic comparisons are known to play a role determining the appropriate (up-gradient) direction. Tropotaxis is used in this way by terrestrial animals that follow trails (ants, Calenbuhr & Deneubourg 1992; snakes, Ford 1986), and perhaps also by *Temora* as well. If the male approaches the trail at angles near 90°, then the difference in signal strength across the antennular reach will be maximized and tropotactic comparisons may allow for orientation in the up-gradient direction (i.e. towards the female). However, as the angle becomes less perpendicular, the copepod will be sampling an increasingly thin cross-section of the trail where odour intensities are more nearly equal. Additionally, as the trail ages, diffusion will smear the concentration gradient, possibly making differences in odour concentration across the antennular span difficult to distinguish even if the approach angle is optimal. Other mechanisms, such as klinotaxis (sequential sampling), also are used by animals, such as ants, to navigate along an odour trail by allowing them to determine if they are moving in the direction of increasing stimulus strength.

Interestingly, males do not always choose the correct direction on their initial approach (figure 3 and Doall *et al.*, this volume), but sometimes proceed away from the

female, turn around (without exhibiting casting behaviour) and then move up-gradient towards the female. Our analysis indicates that males never move inappropriately down-gradient unless the trail is older than 2 s. We interpret this behavioural sequence as resulting from the failure of tropotaxis to correctly code for the correct (up-gradient) direction, followed by down-gradient movement over a distance sufficient for the decrease in concentration to be registered by the sensory system. Once the male has ascertained he has headed away from the female, he reverses course. The alternative explanation is that the decision to turn may be mediated by a perception of stimulus absence, implying a simple klinotactic binary coding of stimulus intensity, rather than true gradient detection. In any case, it seems that there are at least two mechanisms mediating trail following by male *Temora*. As copepods occupy a diffusion-dominated fluid regime, chemical stimulus concentrations may be accurately modelled and it becomes possible to determine the range of chemical stimulus concentrations necessary to cause course reversal. A full quantitative analysis of stimulus concentrations in the odour field is presented in a companion paper (Yen *et al.*, this volume).

Other sensory mechanisms may come into play during the course of mate-tracking. The decrease in velocity and subsequent rapid pounce of the male at the culmination of his journey to the female is suggestive of an open loop behaviour similar to that which controls predatory lunges in a variety of animals. Katona (1973) notes that *Eurytemora*, like *Temora*, pauses its search in close proximity to the female, then pounces on her. The pounce may be elicited by the mechanical disturbance, as at this stage the male is close enough to the female to possibly detect her wake. Yen & Fields (1994) and Van Duren *et al.* (this volume) show that the mechanosensory information found in signals shed by swimming copepods includes signal strength (speed of water movement) and signal direction. Mechanosensory abilities in copepods, including directionally specific responses of antennular setae necessary to allow such precise guidance, have been documented by numerous investigators (reviewed in Weissburg 1998).

5. CONCLUSIONS

Investigation of mate-tracking behaviour indicates that trail-following in *Temora* is the result of chemically mediated guidance using both tropo- and klinotactic mechanisms. The former essentially represents spatial comparisons of odourant strength, whereas the latter is the temporal comparison of stimulus intensity.

Large marine crustaceans (such as lobsters and crabs) also use tropotaxis to locate boundaries of turbulent odour plumes. This strategy is most effective in hydrodynamically smooth-turbulent flows where plume borders are relatively distinct (Weissburg & Zimmer-Faust 1993, 1994). In these situations the accuracy of tracking resembles that which we have documented for *Temora*. However, klinotactic mechanisms depend on a perceptible and stable gradient in odourant strength. Because plumes in naturally turbulent waters are highly variable and intermittent (Zimmer-Faust *et al.* 1988; Moore *et al.* 1994), strict klinotactic mechanisms of gradient detection probably cannot

be part of the behavioural repertoire of these large animals. The very precise tropotaxis and, more significantly, the klinotaxis revealed by our kinematic analysis indicates that *Temora* is following a coherent chemical stimulus field that more properly resembles an odour trail rather than a turbulent odour plume.

Chemotactic trail-following in *Temora*, although similar in mechanism to chemosensory navigation by animals that traverse a surface, occurs in a three-dimensional realm, and we know of no other example of directed chemosensory guidance occurring in three dimensions. Other animals either localize odours in two dimensions, use kinesis rather than taxis or depend on other cues in addition to chemical stimuli. As discussed, terrestrial animals follow surface-bound trails, and even benthic aquatic crustaceans cannot be said to exhibit true three-dimensional localization because they, if not the odour signal, are substrate-bound. Bacteria may orient to gradients in three dimensions, but use indirect guidance mechanisms (Berg & Brown 1972). Swimming or flying animals commonly use either gravity (e.g. shrimp; Hamner & Hamner 1977) or information about the prevailing wind direction (e.g. moths; Arbas *et al.* 1993) to determine polarity of the odour signal.

Terrestrial odour trails are navigable over a long time-span, since the slow diffusion of the chemical out of the trail creates a stable gradient that remains for several hours. As the direction in which the trail leads is so reliable, terrestrial organisms such as ants can use chemotaxis to follow the chemical concentration gradient to locate its source (Hölldobler & Wilson 1990). Similarly, the mechanisms used by *Temora*, and perhaps other zooplankton, are possible because of their peculiar hydrodynamic environment, which promotes persistence of chemical trails and preserves a chemical gradient (Yen *et al.*, this volume). In the low *Re* viscous fluid regime experienced by a copepod, fluid physical calculations predict that odorants released by a moving animal will remain as a coherent trail for times in the order of seconds. Such persistence promotes the evolution of sensory mechanisms similar to those that are effective for following more conventional odour trails created in terrestrial realms, so comparisons of chemosensory behaviour of zooplankton and insects may help outline general conditions for environments where chemotaxis is an advantageous sensory strategy.

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