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Mate-finding behaviour in *Calanus marshallae* Frost

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Mate-finding behaviour by *Calanus marshallae* Frost, 1974, was observed and video recorded in a 1 m diameter kreisel. Newly moulted females signal to males by depositing vertical pheromone trails many tens of centimetres long. Males search for trails along primarily horizontal trajectories. The orthogonality of signal trace and search trail trajectory maximizes the chance of intersection. Males often initiate a dance of rapid, tight turns upon encountering a pheromone trail, then waggle down it (chase swimming) to the signalling female. She jumps away after initial contact, and the male follows. Many successive approach, bump and jump sequences follow, with mating eventually ensuing. The actual copulatory clasp and spermatophore transfer were not observed, although a few instances of brief attachment and tandem swimming were seen. Male dances occur at times when chase swimming does not follow, and the function of dances is not yet known.

Keywords: copepods; *Calanus*; pheromone; mating; kreisel

1. INTRODUCTION

Reproduction of planktonic animals with separate sexes requires females and males to find each other in a huge, three-dimensional and relatively featureless space. Each must identify the other as a suitable mate, then connect for an interval during sperm transfer. Details of how various plankters, particularly copepods, accomplish these tasks are slowly being revealed.

Katona (1973) described swimming patterns of male *Eurytemora affinis* that he associated with mate finding: 'a very quick and complex swimming pattern, involving somersaults and tight-radius turns and loops. The male traces a complex path through several millimetres of water in a localized region . . . . This type of swimming is definitely associated with finding a mate.' Griffiths & Frost (1976) observed similar patterns of swimming for males of *Calanus pacificus* and *Pseudocalanus* sp., and observations of males swimming with tight turns have been reported for a number of other pelagic copepods: Parker (1902) for *Labidocera aestiva*; Jacobs (1961) for *Pseudodiaptomus coronatus*; Roff (1972) for *Limnocalanus macrurus*; Watras (1983) for *Diaptomus leptopus*; Uchima & Murano (1988) for *Oithona davisae*.

Katona (1973) sugggested that the stimulus initiating the mate-finding behaviour of males is probably a pheromonal signal from females. He observed that males exhibit mate-seeking behaviour in the vicinity of a capillary tube filled with females and capped with a dialysis membrane, but did not do so near empty tubes. Similarly, Griffiths & Frost (1976) showed that isolated males of *Calanus pacificus* exhibit patterns of rapid swimming with many tight turns ('zig-zag' and 'figure-eight') almost exclusively when placed in water conditioned by the presence of newly moulted, adult females. They interpreted this behaviour as mate-seeking. The chemical nature of the signal stimulating these dances was supported by exposing males to water collected from around newly moulted females that had been reared on ^{14}C-labelled food, then examining the aesthetascs on the male antennule by autoradiography. There were significant deposits of radiocarbon of female origin on the antennular aesthetascs. There is no doubt that these aesthetascs are olfactory sensors, given both the large number of ciliary dendrites they contain (Barrientos-Chacon 1980; Gill 1986; Kurbjeweit & Buchholz 1991; Bundy & Paffenhöfer 1993) and the olfactory role of similar aesthetascs throughout the crustacea (Derby & Atema 1987; Hallberg *et al.* 1992).

Behavioural events after male–female contact is established have been described in some detail for several heterarthrandrid calanoids (Katona 1975, *Eurytemora*; Blades 1977, *Centropages*; Blades & Youngbluth 1979, *Labidocera*; Jacoby & Youngbluth 1983, *Pseudodiaptomus*). The male latches onto the female with his geniculate right antennule, usually grasping one of her antennules. He then transfers her so that her caudal rami can be grasped by the strong chela of the right fifth swimming leg. After some stroking of sensory areas on the female urosome, the male's left fifth leg collects a spermatophore from his genital aperture and deposits its coupling plate over the female's genital aperture. Similar detail is available for post-capture behaviour in a few pelagic cyclopoids (for example, Gophen 1979, *Mesocyclops leuckarti*), an harpacticoid (Haq 1972, *Euterpina acutifrons*), and an oithonid (Uchima & Murano 1988, *Oithona davisae*).

Much less is known about copulatory mechanics in amphascandrid families. In the Calanidae (for example,

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Calanus, *Neocalanus*, *Nannocalanus*) modification of male limbs for copulation is much less pronounced than in heterarthrandrids, simpler also than in some of the more elaborated amphascandrids like Euchaeta. The antennules have no grasping capability obvious from their anatomy, and the right fifth leg is essentially an unmodified swimming appendage closely resembling the more anterior swimming legs. The exopod of the left fifth leg is relatively longer than that of the right, because of elongation of the first and second segments. The third exopodal segment is flame-shaped and bears a low brush of short hairs on the distal medial surface and two terminal spines. Exactly how this and other limbs are used to transfer spermatophores is unknown. So far as we know, nobody has yet managed to observe the actual mating procedure in *Calanus* or related genera. Mating does not occur readily or often in dishes small enough for convenient observation under a microscope, and it appears that volumes larger than the usual culture containers of a litre or so are needed for successful mating in the laboratory. Mullin & Brooks (1967), for example, were able to obtain multiple generations of *Rhincalanus nasutus* (Eucalanidae) in 19-l carboys, but not in 2- or 4-l containers. Mullin & Brooks interpreted this effect of container size as likely to be caused by interference with mating behaviour from collisions with container walls. Many workers have struggled with apparent mating failures in small containers, and only recently have multiple generations of *Calanus* (P. Blades-Eckelbarger, personal communication) and *Calanoides* (W. Peterson, personal communication) been achieved in the laboratory using containers approaching 1 m^3.

This experience, much of it anecdotal, suggested using a very large aquarium for observation of mating in *Calanus*. It was apparent that it should be tall and wide, but narrow from the viewing side to back. Thus, the swimming patterns involved in mating would have large scope, yet be close enough to the viewing window for observers outside the tank to keep individuals continually in sight. We chose a 1 m plankton kreisel of a design by Hamner (1990), which had been constructed for an earlier study of growth in jellyfish. Prolonged observations of male and female *Calanus marshallae* Frost, 1974, in this large tank allowed us repeatedly to observe the sequence of behaviours by which females attract males and males find females. We were neither successful in fully observing the final stages of a copulatory embrace, if there is one, nor in seeing actual spermatophore transfer. Nevertheless, the sequence of events leading to a close encounter between a *Calanus* female receptive to mating and a male was clearly and repeatedly observed.

2. MATERIALS AND METHODS

Copepods were collected gently by drifting a 70 cm net (253 μm mesh) at a station 4 miles offshore from Newport, Oregon, during summer months. The samples were diluted with surface water in 4-l bottles and transferred to a shore laboratory. Intact adult males and fifth copepodids of *Calanus marshallae* were sorted under a dissecting microscope. The copepods were kept in 2-l beakers at a density of 20–30 individuals per beaker in a cold room and fed *Artemia nauplii*. Every day or two, all the copepods were examined and newly moulted individuals were removed. These laboratory-moulted females and males and field-collected males were used for observation of mating behaviour. We confirmed that mating did not occur in the beaker by observing the empty seminal receptacles of the new females.

The sorted males and females were introduced into a 250-l plankton kreisel (Hamner 1990), 1.01 m in diameter and 0.3 m from front to back. A standpipe across the top of the tank and just off centre fed water through a series of jets aimed parallel to the circumference of the tank, causing the interior mass of water in the tank to rotate. Flow was most intense at the perimeter, while water in the centre was very still. The perimeter flow carried sinking particles, such as copepods, up to the top, then they sank across the centre of the tank. The very quiet central zone appeared to elicit semi-natural behaviour from the copepods. Water was continuously removed by a pump from above a screen mounted in the kreisel top next to the standpipe, filtered, then pumped to the top of the standpipe. The kreisel was illuminated from the top with a bank of small incandescent lamps controlled by a rheostat. The light was filtered through a single, 3 mm sheet of blue acrylic plastic. We also tried a red light, but no difference of behaviour was recognized between the two light sources. At times light beams, flood lights and other lighting were also tried. Mate-seeking behaviour was observed with all of these types of illumination.

Many repetitions of male search-swimming, dances, chases and contacts with females were observed only when large numbers of individuals were placed in the tanks. Our most productive day of work involved 45 females (35 introduced that day) and 22 males. During three timed hours of observation we saw 25 contacts.

The behaviour of the copepods was observed directly and with a dissecting microscope mounted horizontally on a multi-joint camera arm (SAC-F). This arm covered about 80% of the area of the kreisel and a copepod located within 8 cm of the front wall could be brought into focus. A CCD video camera (Fujix FH80) equipped with a close-up lens and mounted on the camera arm was used for recordings. A target copepod was followed manually with support from the camera arm. This video system gives a clear image of the 3.8 mm copepods. Images at the highest magnification are 8 cm in body length on a 53 cm monitor.

Detailed analysis of the behaviour was done with the video records. A total of 3.3 h of recordings were made during 11 days of observation. Representative behaviours were traced from a 53 cm monitor onto a transparent sheet using small particles in the water column or dots on the aquarium wall as fixed points. Lengths of the trajectories were measured with an image processor (Hamamatsu, ARGUS-10). Distance (or speed of organisms) was expressed as multiples of body length. Also, we roughly estimated the real distance and speed, supposing the prosome and total lengths of the adult copepods were 3.0 and 3.78 mm, respectively. Image sequences with less fluctuation of the copepod location along the front-to-back axis were selected for swimming speed measurements, except for the dance of males.

Table 1. *Patterns of mating-related swimming behaviour of* Calanus marshallae

(Numbers in parentheses indicate range, s.d., and number of data.)

swim duration pattern	sex rhythm	swimming speed		approximate	
		body lengths s^{-1}	mm^{-1}	(s)	(Hz)
hop and sink	female	0.71 (0.19–1.18, 0.32, 14)	2.69	—	0.27 (0.07–0.68, 0.19, 21)
	male	0.65 (−0.11–1.14, 0.38, 8)	2.45	—	1.13 (0.65–2.18, 0.46, 20)
search	male	14.8 (9.15–20.3, 10.2, 22)	56.1	180–1440	—
chase	male	5.71 (4.18–−7.78, 1.48, 7)	21.6	0.4–30	4.55 (3.18–5.45, 0.84, 7)
escape	female	67.3 (16.0–129.0, 26.8, 41)	254.4	0.03–0.3	—
dance	male	19.8 (11.9–28.0, 5.77, 9)	74.7	5–20	—

3. RESULTS

In preliminary experiments, we observed that the seminal receptacles of almost all unmated females placed with males in the kreisel were filled with sperm during a 2-d interval. This confirmed that our experimental conditions were suitable for mating of *Calanus marshallae*.

We observed several types of swimming behaviour which presumably relate to mating. Video recordings (VHS or SVHS) of these swimming patterns can be borrowed from either author for viewing and copying.

(a) *Hop and sink (female)*

Females spend most of the time slowly sinking, intermittently punctuating that with brief upward hops, much like the 'hop and sink' pattern described by Bainbridge (1952) and considered theoretically by Haury & Weihs (1976). The upward hops of females in the kreisel were mainly driven by the movement of the oral appendages, particularly the second antennae. Hops were not fast, not like escape movements driven by the swimming legs. The hop pattern, with frequency of 0.07 to 0.66 Hz (table 1), was smooth and the upward phase was 1–2 body lengths. Between hops, individuals slowly sank in the water column at 0.19–1.18 body length s^{-1} (table 1). They occasionally turned loops with a diameter of 1.5–2 body lengths.

(b) *Hop and sink (male)*

Males had various swimming patterns, but they also spent much of the time in hop and sink swimming, especially in the absence of newly moulted females. Hop motion was more frequent (0.65–2.18 Hz) than in females, and the upward phase was shorter (0.5–1.1 body length) than in the female (table 1). Hop motion seemed to be mainly generated by flapping of the urosome. Although some individuals showed net upward movement, the average sinking speed during this hop and sink was not different from that of the female. Hop and sink behaviour was sometimes combined with a small loop or incomplete loop which produced horizontal displacement.

(c) *Search swimming (male)*

This movement pattern (figure 1) consisted of horizontal swimming and loops of various diameters (more than 80 body lengths). The range of speeds was 9.15–20.3 body length s^{-1} (table 1). The ventral side of the copepod always faced upward during the horizontal swim, and the antennules were held rigidly at right angles to the body. The swimming legs were kept tightly folded anteriorly. The trajectories of horizontal swims often showed smooth waves with an amplitude of 1–5 body lengths. The propulsion was most likely generated by movements of the mouthpart appendages and occasional flaps of the urosome. Timed instances of search swimming by individual males lasted 3 to 24 min ($n=8$). We observed this search swimming only in the presence of newly moulted females.

(d) *Dance (male)*

The dance is also a gender-specific swimming pattern observed in males. They move through very complicated trajectories at speeds faster than search swimming (table 1, figure 2). The dance was a combination of loops and turns (quick changes of body direction with little movement of the head position). Males changed direction five to ten times within a second. Dances last a few to 20 s. The maximum dimension of the overall space filled by dances ranged from 8–40 cm, usually within 20 cm. In other swimming patterns the antennules were almost always kept at right angles to the body, but in the dance they were often folded back. Because the swimming legs remain folded anteriorly, the swimming seems to be maintained by antennal movements and beats of the urosome. The dance was rarely observed in the absence of newly moulted females, and the frequency of dances notably increased with their presence. The dance sometimes started from hop and sink, and the male then returned to hop and sink. However, dances most commonly started during search swimming and preceded a chase swim.

(e) *Chase swimming (male)*

The chase is a downward swimming pattern observed in males approaching females located below them. The overall

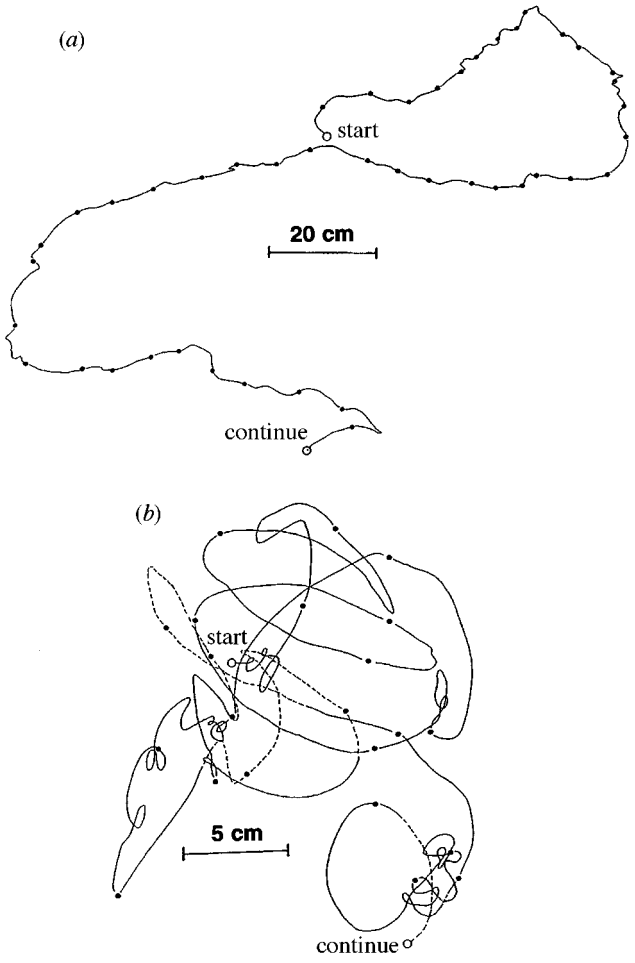

Figure 1. *Calanus marshallae*. Typical search swimming trajectories of the male traced from video records. The narrow front–back dimension of the kreisel forced most of the trajectory into the left–right, up–down plane in which the motion is characterized here. Dots along the track are 1 s apart. (*a*) 45 s open trajectory, scale bar 20 cm. (*b*) 24 s trajectory with tight turns, scale bar 5 cm.

trajectories of chase swimming were usually directly oriented toward females macroscopically, whereas side-to-side oscillations were observed microscopically (figure 3). The male follows a wavy trajectory, flexing his body (prosome and urosome) ventro-dorsally and laterally at a frequency of 3.2–5.5 Hz (table 1). This swimming was different from all other swimming patterns in that the orientation of the antennules with respect to the overall trajectory was kept relatively constant, more or less horizontal. The antennules did not rotate relative to the vertical when the body did, which implies they repeatedly rotated with respect to the body. The speed was slower than in search swimming (4.18–7.78 body lengths s^{-1}). Approach to females by males was almost always accompanied by this type of swimming over various distances, although males sometimes swam in this pattern after an interval of search swimming without finally approaching a female. Possibly they were tracing trails abandoned by females. Distance of this type of swim ranged from a few millimetres to over 50 cm.

(f) *Approach and escape (male and female)*

Males approach females by chase swimming, almost always on a downward directed trajectory, then the female escapes from the approaching male. Sometimes this followed the male bumping into the female head first (although this was never video recorded). More usually it occurred as the male passed very nearby, at a separation of 0.77–2.0 body lengths (average 1.48, s.d. 0.45, $n=7$). The escape swim was the fastest recorded motion observed, (16.0–129 body lengths s^{-1}), which lasted 0.03–0.3 s (average 0.13, s.d. 0.08, $n=4$, table 1).

The distance of the escape jump ranged from 1.58–14.5 body lengths (average 7.7, s.d. = 3.76, $n=41$). The propulsion

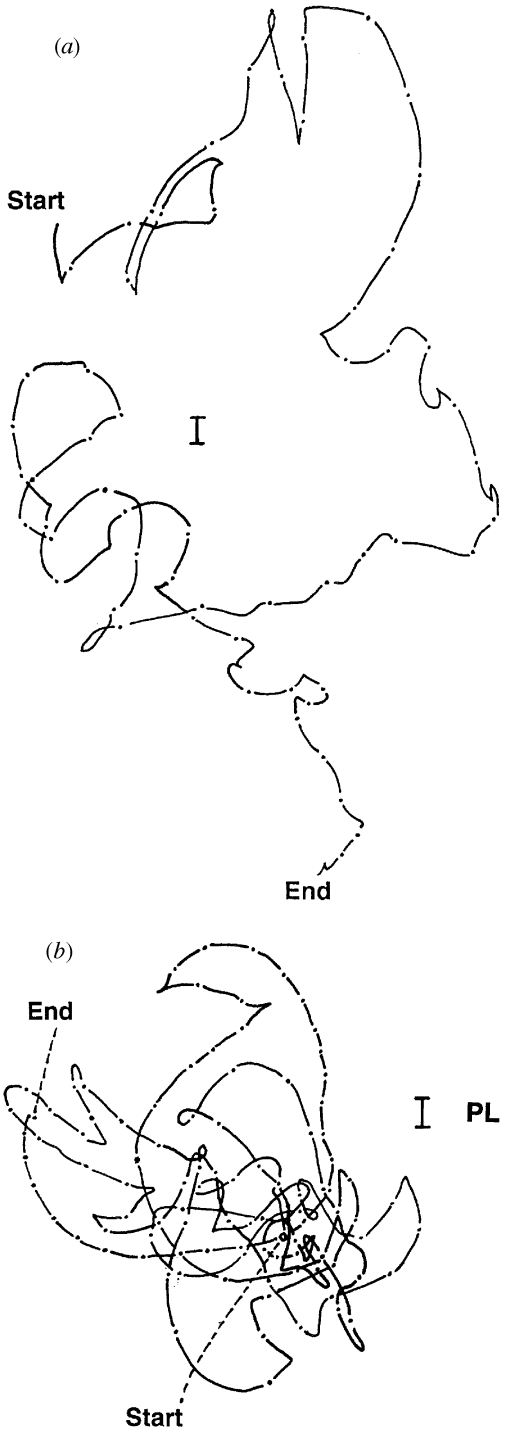

Figure 2. *Calanus marshallae*. Two x–z projections of male dance sequences traced from video recordings. Scale bars show the male prosome length (3 mm) on the same scale as the trajectory. Total times for these trajectories were 4.8 s (*a*) and 8.18 s (*b*).

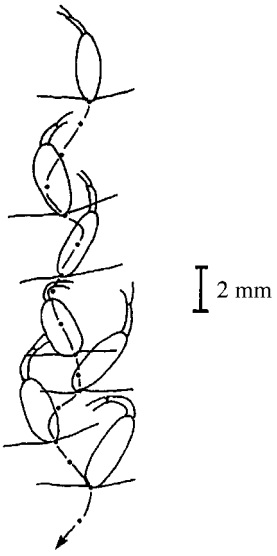

Figure 3. *Calanus marshallae*. Detail of the oscillation pattern of the male during downward chase swimming. The front of the cephalosome moved side-to-side; the antennule remained close to horizontal.

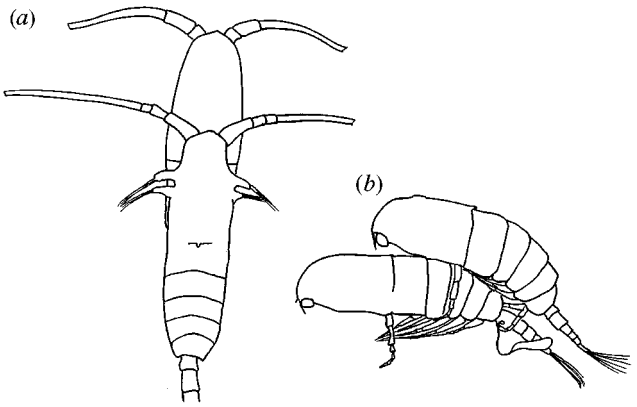

Figure 5. *Calanus marshallae*. Relative positions of male and female during two separate contacts captured by video recording. The sketches were enhanced from knowledge of the anatomy. (*a*) Dorsal view, exact orientation. (*b*) Lateral view, showing speculations that the female is grasped by the male maxilliped and that the left male fifth exopod attaches the spermatophore by reaching around the female genital somite. The pair in (*b*) moved together in the position shown.

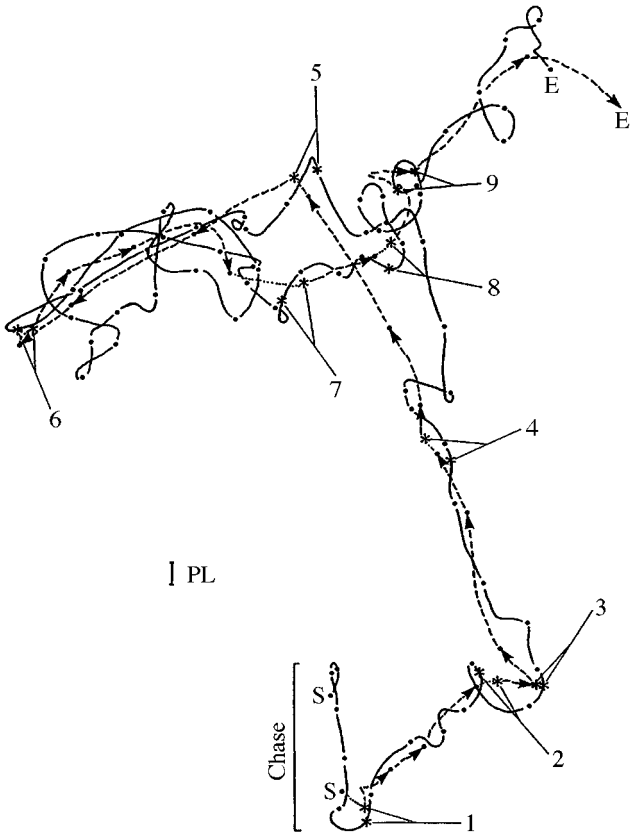

Figure 4. *Calanus marshallae*. Trajectories of both sexes during a search and approach sequence. The male (solid line with head positions as dots each 0.1 s) terminated a search swim and chased downward to the female. She initiated a long sequence of escapes (dashed line), with the male pursuing and repeatedly approaching. Scale bar shows prosome lengths.

of this swim was presumably generated by a stroke of the swimming legs. The male precisely followed the female trajectory, sometimes spiralling around it (see figure 4). Then, successive approaches and escapes were observed to recur from 1–17 times. In a variant sequence observed six times, the male ended the chase at a distance of 2–3 cm from the female, possibly having lost track of her, then danced in a spherical vicinity near her and finally jumped to her. She again jumped away. Most of the sequences ended without copulation, the male losing the female after two to three approaches.

(g) *Contact*

In the present study, we did not observe transfer of spermatophores. However, several contacts were observed between a female and a male. In these instances, the females abandoned the intensive escape jumps of the consecutive approach and escape sequence. The male approached from a postero-dorsal direction, and both swam together, apparently touching (see figure 5) for 0.1 to a few seconds. On one occasion, a male clasped a female by wrapping her with his antennule. On another a male was observed 'attaching' his head near the female prosome–urosome articulation (duration 0.8 s). Our observations are not sufficient to establish exactly how the male and female interact for spermatophore transfer. However, several observations of a posture with the male over the female's dorsum, set back by about one-third of her prosome length, suggest the general transfer process illustrated in figure 5. It seems likely that the greater length of the male maxiliped, compared with that of the female, is an adaptation for clasping females to the male ventrum.

4. SUGGESTED MATE FINDING BEHAVIOUR OF *CALANUS MARSHALLAE*

Newly moulted females swim slowly, mostly in the hop and sink mode, which presumably produces a vertical trail of pheromone in the water column. Males are inactive in the absence of female stimuli. They slowly hop and sink, possibly alert for traces of pheromone. When the males detect faint traces of pheromone, presumably

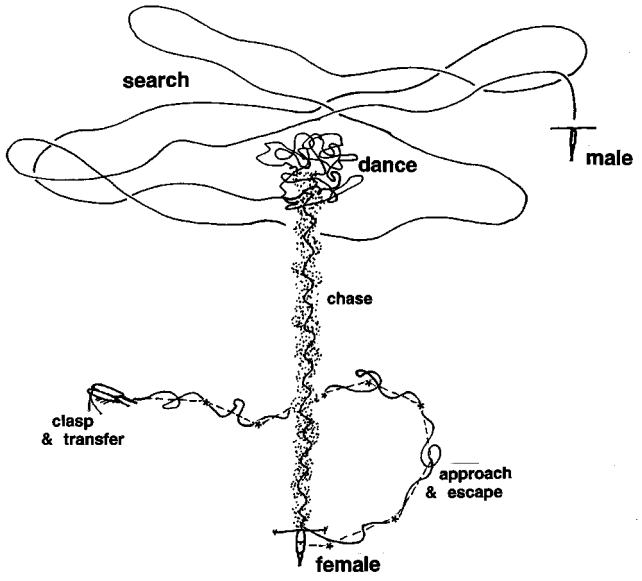

Figure 6. *Calanus marshallae*. A conceptual interpretation of mate-attraction–mate-search behaviour. The sequence of events is: (i) a female generates a vertical pheromone trail; (ii) a male alerted by pheromone to females in the general vicinity swims in smooth loops of mostly horizontal orientation; (iii) on crossing a pheromone trail the male performs (or sometimes doesn't) a dance; (iv) the male chases down the pheromone trail to the female; (v) the female jumps away repeatedly with the male pursuing, sometimes bumping her; and (vi) a mating clasp is established and a spermatophore is transferred from the male to the female.

diffusing from old trails, mate-seeking (figure 6) is induced and search swimming begins. Initially, search swimming consists mainly of horizontal trajectories. Increasing stimulus leads them to perform frequent loops, which might help them to locate pheromone trails at relatively short distances. When the male detects concentrated pheromone, he sometimes performs a dance. The function of this intense activity is unclear, but it often terminates with a brief rest followed by downward tracing of the female's trajectory by chase swimming. The dance may also be left out, the male turning downward sharply from the search trajectory and chase swimming to the female. At the end of the chase, he collides with the female or passes her at close range, in which case he turns toward her from just below. Then the female escapes from the male and the male follows the female through a series of short jumps (figure 4). A sequence of approach and escape moves is eventually terminated by clasping, possibly with the maxillipeds, of the female by the male situated dorsally and about one-third prosome length posteriorly to her. Spermatophore transfer could readily be accomplised in this position. The coiled position of the left fifth leg often seen in preserved specimens would be appropriate for reaching around the female's first urosomal somite from above (figure 5).

5. DISCUSSION

Patterns of swimming exhibited by *Calanus marshallae* females in signalling to males and by males in searching for receptive females show clear signs of optimization for efficient locating in a large and featureless volume. Specifically, the orthogonality of vertical female pheromone trails to the predominantly horizontal search swimming of males maximizes the probability of male encounter with a trail. Once a trail is encountered, it also provides directional information independent of any gradient in pheromone concentration; the female will be below the point of trail encounter. Long directional pheromone trails enhance the likelihood of male encounter with female signal, overcoming some of the size limitations to pheromone communication by diffusion in water theoretically examined by Dusenberry & Snell (1995). In our observations, females increased their 'effective size' in the sense of Dusenberry & Snell by the ratio of trail length to body length: 50 cm: 3.8 mm, about 130-fold. That is by no means the limit of such increase for the field situation.

Vertical pheromone trails as long as 1 m should persist and remain close to vertical for periods of at least minutes in suitably stable parts of the water column. This has been documented (Woods 1968, 1971) for vertical dye tracks established within laminar flow layers in a seasonal thermocline. Woods (1971) observed that a sinking 3 mm pellet of fluorescein dye leaves a wake (likely to approximate the initial dimensions of a *Calanus* pheromone trail) which remained visible within these 1–2 m, low shear layers of the thermocline for 'about 5 minutes'. He also observed that tilting of trails within layers to *ca*. 45° from vertical required times in the order of 2 min. Visibility of a fluorescein trail is not necessarily a good model for detectability of a pheromone trail by male *Calanus*, but it sets a reasonable lower limit on trail longevity. All successful signalling by females in our tank attracted males within about 5 min, or the female was carried away in the lateral flow near the tank bottom. Thus, while our experiments don't address the ultimate durability of pheromone trails in the ocean, or the limits of their useful vertical extent, timescales of multiple minutes and vertical scales of 1 m or more are likely.

Given the enormous volume of oceanic habitats (effectively infinite horizontally and up to hundreds of metres deep for *Calanus*), we suspect that female and male *Calanus* probably gather to signal and search in rather restricted layers of the water column. One candidate case has been seen by M. Heath (personal communication), who finds that male *Calanus finmarchicus* emerging in spring from diapause in the Shetland–Faeroe Channel area rise vertically from the resting level and stop at a sharp thermohaline stratification just below 400 m. We suspect that many more candidate locations for vertically defined mating leks will be evident with suitably targeted sampling. The best locales for painting pheromone tracks of great longevity are likely to resemble those studied by Woods (1968): 1–2 m layers within pycnoclines, stabilized by strong density stratification. Such layers are separated by thin sheets of strong, internal wave driven shear (Woods 1968). Possibly such shear would allow copepods to identify layer boundaries (Mackas *et al.* 1993). Other likely sites will be at subthermocline depths, where mixing energy is consistently small (Gargett 1989), allowing trail persistence even if density stratification is weak.

The male dance is certainly the aspect of mate searching studied by Griffiths & Frost (1976). Dances are clearly part of searching for females ready to mate,

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because the frequency of dances strongly increased with introduction of virgin females to our aquarium. More explicit confirmation of this connection was developed in the experiment of Griffiths & Frost (1976). We usually observed a burst of dance to intervene between mostly horizontal searching and the chase. In fact, dances became cues to us to look for a female directly below the dance site. Probably its function has something to do with the moment of finding a concentrated pheromone trail. However, dances were not always in a sequence with search swimming and the chase. Often the chase would not follow a dance, and dances sometimes erupted during slow hop and sink swimming, the male subsequently returning to hop and sink. A few dances were performed at the end of a chase sequence before actually touching the female.

The exact function of the dance could not be specified from our observations. It seems most likely that it usually is to provide the precise location of the pheromone trail by a thorough search of the vicinity of the first contact. Possibly dances during search swimming, but not followed by chases, fail to show a clear path location. When chases are not preceded by dances, the location of the pheromone trail may already be sufficiently defined at its intersection with the male search path. Instances of dance after the chase and before actual contact especially, seem to indicate a function in defining the small-scale pheromone pattern. Alternately, dances may provide precopulatory exercise to prepare muscles for encounter with a female or to ready the reproductive tract for spermatophore ejection. The high and similar male speeds in the dance and in the approach and escape sequence suggest that a warm-up might be needed to keep pace with the female. She uses the more powerful swimming leg-propulsion, whereas the male appears to continue relying on the antennae alone. Possibly dances during hop and sink swimming, or even some of those during searching, are displacement activity. Uchima & Murano (1988) suggest that rapid movement of male *Oithona* around and along the trajectory of a female being approached could serve to disperse her pheromones, leaving no trail for other males to follow. This seems unlikely for the dances of *Calanus*, because so little of the trail is usually included in the volume filled by the dance.

Progress of the male to the female in the downward chase is remarkably direct. The male may be partly directed by gravity, much as benthic crabs move upstream in the presence of prey odour (Zimmer-Faust *et al.* 1995). However, also like prey-seeking by crabs, chemotaxis appears to be important. The value of the sinuous trajectory is probably to keep the movement centred on the pheromone trace by checking alternately for reduced concentration to either side. Maintaining the antennules almost exactly horizontal, at right angles to the trace, perhaps facilitates this tracking. The linear array of olfactory aesthetascs would move smoothly across the central core of the trace. Shifting of maximum stimulation from one sensor to the next could provide maximum information about trace direction and possibly reduce olfactory accommodation. The proposed mechanism is similar to that suggested for crabs tracing food odour plumes by Zimmer-Faust *et al.* (1995): 'Because the plume edges were very sharp, when crabs partially exited the plume, some pereiopods (legs or claws) were outside the plume while others remained inside. A comparison of simultaneous chemosensory inputs from the appendages inside and outside would presumably allow the crabs to determine the correct direction and return to the plume.'

We are not sure why the female jumps away from the male approach. However, alternation of male approach and female escape is observed in the mating rituals of a very wide range of animals (see, for example, Lorenz 1963). Consecutive male approach and female escape could serve somehow in mate selection by females, and in copepods that may incorporate tactile olfaction of surface chemical recognition signals (Snell & Morris 1993; Snell & Cremona 1994; Cremona & Snell 1995; Lonsdale *et al.* 1996; Kelly & Snell 1996) between male and female. We could not observe the transfer of spermatophores, but we sometimes observed that a male approached a female from a postero-dorsal direction, followed by swimming together. Therefore, this could be the final position during transfer. Again, mechanical stimuli and possibly tactile chemoreception may help with orientation, because it seems impossible that the male could orient correctly to the female for actual targeting of the spermatophore coupler just from information provided by a diffusing trail of pheromone.

Much remains to be done. The trail pheromone is probably responsible at low concentrations for switching on general searching behaviour in males and for providing a directed trail at higher concentrations. It can be characterized as to molecular weight by size-dependent dialysis of water conditioned by newly moulted females, as was done by Lazzaretto *et al.* (1994) for male-attracting pheromones of *Tigriopus*. A molecule in the 100–1000 Da range they found is likely, given the size and character of fluid dispersed pheromones in general. The trail pheromone eventually can be identified by testing of components of glandular secretions of newly moulted females for their capacity to elicit dances. Sighting and characterization of the final clasping and spermatophore transfer phases of copulation remain to be done. Frequency of completed matings is lower than those of search, dance and chase behaviours, so many hours of observing time will be required.

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REFERENCES

Bainbridge, R. 1952 Underwater observations on the swimming of marine zooplankton. *J. Mar. Biol. Assoc. UK* **31**, 107–112.

Blades, P. I. 1977 Mating behavior of *Centropages typicus* (Copepoda: Calanoida). *Mar. Biol.* **40**, 57–64.

Blades, P. I. & Youngbluth, M. J. 1979 Mating behavior of *Labidocera aestiva* (Copepoda: Calanoida). *Mar. Biol.* **51**, 339–355.

Barrientos-Chacon, Y. 1980 Ultrastructure of sensory units on the first antennae of calanoid copepods. MS thesis, University of Ottawa, Canada.

Bundy, M. H. & Paffenhöfer, G.-A. 1993 Innervation of copepod antennules investigated using laser scanning confocal microscopy. *Mar. Ecol. Prog. Ser.* **102**, 1–14.

Cremona, M. J. & Snell, T. W. 1995 Glycoproteins in daphnids: potential signals for mating. *Arch. Hydrobiol.* **134**, 273–279.

Derby, C. E. & Atema, J. 1987 Chemoreceptor cells in aquatic invertebrates: peripheral mechanisms of chemical signal processing in decapod crustaceans. In *Sensory biology of aquatic animals* (ed. J. Atema, R. R. Fay, A. N. Popper & W. N. Tavolga). New York: Springer.

Dusenberry, D. B. & Snell, T. W. 1995 A critical body size for use of pheromone signals for mating. *J. Chem. Ecol.* **21**, 427–438.

Frost, B. W. 1974 *Calanus marshallae*, a new species of calanoid copepod closely allied to the sibling species *C. finmarchicus* and *C. glacialis*. *Mar. Biol.* **26**, 77–99.

Gargett, A. E. 1989 Ocean turbulence. *A. Rev. Fluid Mech.* **21**, 419–451.

Gill, C. W. 1986 Suspected mechano- and chemosensory structures of *Temora longicornis*. *Mar. Biol.* **93**, 449–456.

Gophen, M. 1979 Mating processes in *Mesocyclops leuckarti* (Crustacea: Copepoda). *Israel J. Zool.* **28**, 163–166.

Griffiths, A. M. & Frost, B. W. 1976 Chemical communications in the marine planktonic copepods *Calanus pacificus* and *Pseudocalanus* sp. *Crustaceana* **30**, 1–9.

Hallberg, E., Johansson, K. U. I. & Elofsson, R. 1992 The aesthetasc concept: structural variations of putative olfactory receptor cell complexes in crustacea. *Microsc. Res. Tech.* **22**, 325–335.

Hamner, W. M. 1990 Design developments in the plankton kreisel, a plankton aquarium for ships at sea. *J. Plankton Res.* **12**, 397–402.

Haq, S. M. 1972 Breeding of *Euterpina acutifrons*, a harpacticoid copepod with special references to dimorphic males. *Mar. Biol.* **15**, 221–235.

Haury, L. & Weihs, D. 1976 Energetically efficient swimming behavior of negatively buoyant zooplankton. *Limnol. Oceanogr.* **21**, 797–803.

Jacobs, J. 1961 Laboratory cultivation of the marine copepod *Pseudodiaptomus coronatus* Williams. *Limnol. Oceanogr.* **6**, 443–446.

Jacoby, C. A. & Youngbluth, M. J. 1983 Mating behavior in three species of *Pseudodiaptomus* (Copepoda: Calanoida). *Mar. Biol.* **76**, 77–86.

Katona, S. 1973 Evidence for sex pheromones in planktonic copepods. *Limnol. Oceanogr.* **18**, 574–583.

Katona, S. 1975 Copulation in the copepod *Eurytemora affinis* (Poppe, 1880). *Crustaceana* **28**, 89–95.

Kelly, L. S. & Snell, T. W. 1996 Surface glycoproteins as mating signals in the marine copepod *Tigriopus japonicus*. *EOS, Trans. Am. Geophys. Union* **76**, OS73 (abstract).

Kurbjeweit, F. & Buchholz, C. 1991 Structures and suspected functions of antennular sensilla and pores of three arctic copepods (*Calanus glacialis*, *Metridia longa*, *Paraeuchaeta norvegica*). *Meeresforsch.* **33**, 168–182.

Lazzaretto, I., Franco, F. & Battaglia, B. 1994 Reproductive behavior in the harpacticoid copepod *Tigriopus fulvus*. *Hydrobiologia* **292/293**, 229–234.

Lonsdale, D. J., Snell, T. W. & Frey, M. A. 1996 Lectin binding to surface glycoproteins on *Coullana* spp. (Copepoda: Harpacticoida) can inhibit mate guarding. *Mar. Freshwater Behav. Physiol.* **27**, 259–294.

Lorenz, K. 1963 Das Sogenannte Böse, Zur Naturgeschichte der Aggression. Vienna: Dr C. Borotha-Schoeler (Japanese translation).

Mackas, D. L., Sefton, H., Miller, C. B. & Raiche, A. 1993 Vertical habitat partitioning by large calanoid copepods in the oceanic Pacific during spring. *Prog. Oceanogr.* **32**, 259–294.

Mullin, M. M. & Brooks, E. R. 1967 Laboratory culture, growth rate, and feeding behavior of a planktonic marine copepod. *Limnol. Oceanogr.* **12**, 657–666.

Parker, G. H. 1902 The reaction of copepods to various stimuli and the bearing of this on daily depth migrations. *Bull. US Fish Comm.* **21**, 103–123.

Roff, J. C. 1972 Aspects of the reproductive biology of the planktonic copepod *Limnocalanus macrurus* Sars, 1863. *Crustaceana* **22**, 155–160.

Snell, T. W. & Cremona, M. J. 1994 Surface glycoproteins in copepods: potential signals for mate recognition. *Hydrobiologia* **293/294**, 255–264.

Snell, T. W. & Morris, P. D. 1993 Sexual communication in copepods and rotifers. *Hydrobiologia* **255/256**, 109–116.

Uchima, M. & Murano, M. 1988 Mating behavior of the marine copepod *Oithona davisae*. *Mar. Biol.* **99**, 39–45.

Watras, C. J. 1983 Mate location by diaptomid copepods. *J. Plankton Res.* **5**, 417–425.

Woods, J. D. 1968 Wave-induced shear instability in the summer thermocline. *J. Fluid Mech.* **32**, 791–800.

Woods, J. D. 1971 Micro-oceanography. In *Underwater science, an introduction to experiments by divers* (ed. J. D. Woods & J. N. Lythgoe), pp. 275–317. London: Oxford University Press.

Zimmer-Faust, R. K., Finelli, C. M., Pentcheff, N. D. & Wethey, D. S. 1995 Odor plumes and animal navigation in turbulent water flow: a field study. *Biol. Bull.* **188**, 111–116.

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