



Mating behaviours of *Daphnia pulicaria*, a cyclic parthenogen: comparisons with copepods

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The pre- and post-contact mating behaviours of *Daphnia pulicaria* are investigated by direct observations of vertical distributions, swimming behaviours and male–female interactions. Analysis of vertical distributions in a 1 m deep, thermally stratified migration chamber reveals that females were always located in the upper layer of the water column but males exhibited a bimodal depth distribution, in which an individual's depth was a function of body length and water temperature. The observed distributions of males may be the result of several interacting pressures; predation avoidance, life-history optimization, and avoidance of assortative mating. Male swimming behaviour was faster and orthogonal to that of females, which is in agreement with the predictions of encounter-rate maximization models. Video recordings of males and females interacting in a 1-litre vessel showed that males both pursued and contacted other males more often than females. Thus, there was no evidence that *Daphnia* are able to use water-borne chemical signals to locate and identify potential mates. However, the average duration of male–female contacts (13.8 s) was much longer than those between males (1.6 s), suggesting that males can determine the sex of contacted individuals.

Daphnia mating behaviour is significantly more complex than previously acknowledged. In contrast to the conventional view of *Daphnia* males swimming more-or-less randomly and mating with any individual encountered, they exhibit behaviours which increase the potential of mating with females while reducing the risk of predation. Several male behaviours, such as 'scanning' and the performance of area-restricted spirals upon encounter, are similar to those reported for some copepods and may be common to zooplankton that lack sophisticated chemosensory abilities. The possibility that *Daphnia* may also be able to assess such important female attributes as species and reproductive status is discussed.

Keywords: *Daphnia*; mating behaviour; copepod; mechanoreception; copulation

1. INTRODUCTION

Crustacean zooplankton exhibit a variety of reproductive modes, ranging from the obligate sexuality of most copepods to the obligate asexuality of some cladocerans (Gilbert & Williamson 1983). The freshwater cladoceran *Daphnia* typically reproduces by means of cyclic parthenogenesis, in which extended periods of asexual reproduction during favourable environmental conditions are punctuated by bouts of sexual reproduction at the onset of unfavourable conditions (Hebert 1987). Although sexual (mictic) reproduction has important consequences for the long-term persistence of *Daphnia* populations, as it creates genetically diverse egg-banks from which future populations can be established (De Meester 1996; Grebelnyi 1996), investigations focusing specifically on the mating behaviour of *Daphnia* are rare.

The earliest accounts of *Daphnia* mating behaviour were published more than 175 years ago (Jurine 1820; translated in Dodson & Frey 1991), but little progress beyond anecdotal descriptions has occurred since then. Furthermore, most reports of cladoceran mating have focused on post-contact behavioural interactions and descriptions of the process of copulation, with little attention being given to how *Daphnia* find each other in large volumes of water and subsequently choose a mate. For males, the sequence

of events in sexual reproduction—encounter (and recognition), contact and copulation—is fundamentally no different from that of a predator consuming prey (Gerritsen & Strickler 1977). Thus, zooplankton, which are often sparsely distributed in nature (Omori & Hamner 1982), can benefit greatly by maximizing encounter rates with conspecifics, the essential first step in the sequence of events leading to successful mating.

Much of the work on zooplankton mating behaviour has centred on investigating ways in which male copepods increase their probability of encounter with females (Katona 1973; Griffiths & Frost 1976; Blades & Youngbluth 1980; Watras 1983; Uchima 1985; Uchima & Murano 1988; Yen 1988; Van Duren & Videler 1996; Yen & Strickler 1996; Doall *et al.*, this volume; Weissburg *et al.*, this volume). The theoretical model of prey encounter developed by Gerritsen & Strickler (1977) shows that predators of slowly moving prey can maximize encounter rates by increasing their swimming speeds (i.e. acting as cruising predators). Gerritsen (1980) later modified this model to include swimming direction and showed that predators can further increase encounter rates with prey by swimming orthogonal to the predominant swimming direction of their prey. The model predicts both faster and orthogonal swimming behaviour in the 'searcher' and has been supported empirically by observations of cyclopoid

copepod predators (Gerritsen 1980), and in the male–female interactions of calanoid copepods (Watrás 1983; Yen 1988). Similarly, male *Bosmina* have been reported to swim faster and ‘more erratically’ than females (Kerfoot & Peterson 1980).

In addition to a swimming-behaviour component, Gerritsen & Strickler (1977) found that encounter rates increased as the square of encounter radius, defined as the distance at which a male can detect a conspecific female. In this scenario, sex pheromones may enhance mating success by effectively expanding the encounter radius of males for females (Van Leeuwen & Maly 1991; but see Doall *et al.*, this volume). There is strong evidence that some male copepods use water-borne chemical signals to locate females (Katona 1973; Doall *et al.*, this volume; Yen *et al.*, this volume; Weissburg *et al.*, this volume), and cladocerans are known to respond behaviourally to chemical signals from both predators and conspecifics (Larsson & Dodson 1993). However, to date, investigations of *Daphnia* mating have not revealed whether males are able to exploit chemical signals to increase encounter rates with females, or if they must rely on other modes of mate detection such as mechanoreception or vision.

The goal of this paper is to investigate both pre-contact and post-contact mating behaviours of a cyclically parthenogenetic clone of *Daphnia pulex* and to make comparisons with copepod behaviour.

2. METHODS

All experiments were performed using a clone of *D. pulex* from Berry Reservoir, Georgia, USA kept in culture by L. Weider at the Max-Planck Institute for Limnology. *Daphnia* were cultured at 21 °C in 0.45 µm filtered lake water (Plusee, Plön, Germany) at densities around 150 animals l⁻¹ and ambient light conditions. *Daphnia* were fed daily with *Scenedesmus acutus* grown in continuous culture at a concentration of about 0.5 mgC l⁻¹. Under these conditions, this clone produced males readily, at times exceeding 60% of the population in a given culturing vessel.

(a) Vertical distributions

Vertical distributions were determined by using the vertical migration chamber described by Loose *et al.* (1993). The chamber consisted of 12 perspex tubes (1 m length, 5 cm diameter), and a single large-diameter tube (10 cm diameter), placed inside a transparent water-bath. The tubes were illuminated from above by a row of 12 V halogen lamps shining through frosted-glass plates. Experiments were done under a 16 h:8 h light–dark cycle, with both sunrise and sunset simulated by constantly changing the lamp voltage between 0 and 12 V over a period of 1 h. The water bath was thermally stratified from 23 to 9 °C, with the thermocline located between 40 and 50 cm in depth (see figures 1 and 2).

A total of three separate experiments were done to investigate factors influencing the vertical distribution of females and males. In the first two, vertical distributions were determined in the presence of the chemical exudates (kairomones) of two different predators: fish and *Chaoborus*, and in the third, to the elimination of thermal stratification. In all three experiments, five adult females

and five males were placed in each of the 12 perspex tubes in a medium containing a non-limiting concentration of *S. acutus* (2 mgC l⁻¹) which was re-established twice a day by adding concentrated algae to the top of each tube. In the first experiment, half of the tubes were filled with filtered lake (control) water and the other half were filled with water containing fish kairomone. Fish kairomone water was created by placing two small fish (*Leucaspius delineatus*) that had been pre-fed *Daphnia* in 10 l of 0.45 µm filtered lake water for 5 h and re-filtering the water immediately before filling the tubes. In the second experiment, half the tubes contained control water and the other half filtered water from a 20-l culture that held about 50 third and fourth instar larvae of *Chaoborus flavicans* (phantom midge) which were fed *Daphnia*. Experiments 1 and 2 were started between 17.00 and 19.00 of day 1 and the depth of each of the ten animals per tube was noted 3 h before and after the mid-point of the light cycle (10.00 and 16.00) for the next 2.5 d, after which any kairomone was likely to have been degraded by bacteria (Loose *et al.* 1993). The tubes were thermally stratified for the entire experiment. Because each tube contained five individuals of each sex, it was not possible to correlate individual depths among observations. Thus, to avoid pseudo-replication, statistical comparisons of male and female mean population depths are based on only the last reading (day 3, 10.00), though distribution patterns were consistent throughout the experiments. In the third experiment, all 12 tubes contained control water. Experiments were started between 17.00 and 19.00 on the first day, during which the tubes were thermally stratified, and the depth of each animal was noted at 10.00 and 16.00 for the next 6 d. At 19.00 of day 2, the thermal stratification was eliminated by warming the bottom layer to 21 °C over a period of 6 h. After 2 d of isothermy, the thermocline was re-established over a period of 6 h.

(b) Swimming behaviour

To characterize swimming behaviour, individual swimming speeds and turning angles were calculated from video tapes of animals swimming in the large, central tube of the migration chamber. At the beginning of each of the three vertical distribution experiments, a mixed population of about 250 males and females was placed in the large tube in control water. At about 10.00 of day 2 of each experiment, video recordings were made with a Panasonic BP5000 video camera with an LA408C3 auto-iris lens (4.5 mm, 1:0.75) and a Panasonic AG7350 VCR recording at 25 Hz using the migration chamber’s overhead lighting. Based on the observed distribution of the animals, recordings were made for 30 min in each of three zones; the top 10 cm, the thermocline (40–50 cm depth), and the bottom 10 cm.

The necessity of recording through the migration chamber’s water bath created two limitations; first, only two-dimensional (2D) recordings could be made, and second, the camera could be placed no closer than 10 cm from the tube, which prevented resolution of small-scale swimming behaviours. Because of the relatively low resolution of swimming behaviour, both swimming speed and turning angle were estimated based on tracks in which an animal swam 5 cm horizontally. Vertical lines were drawn every 1 cm (at the magnified scale) on a video monitor. The

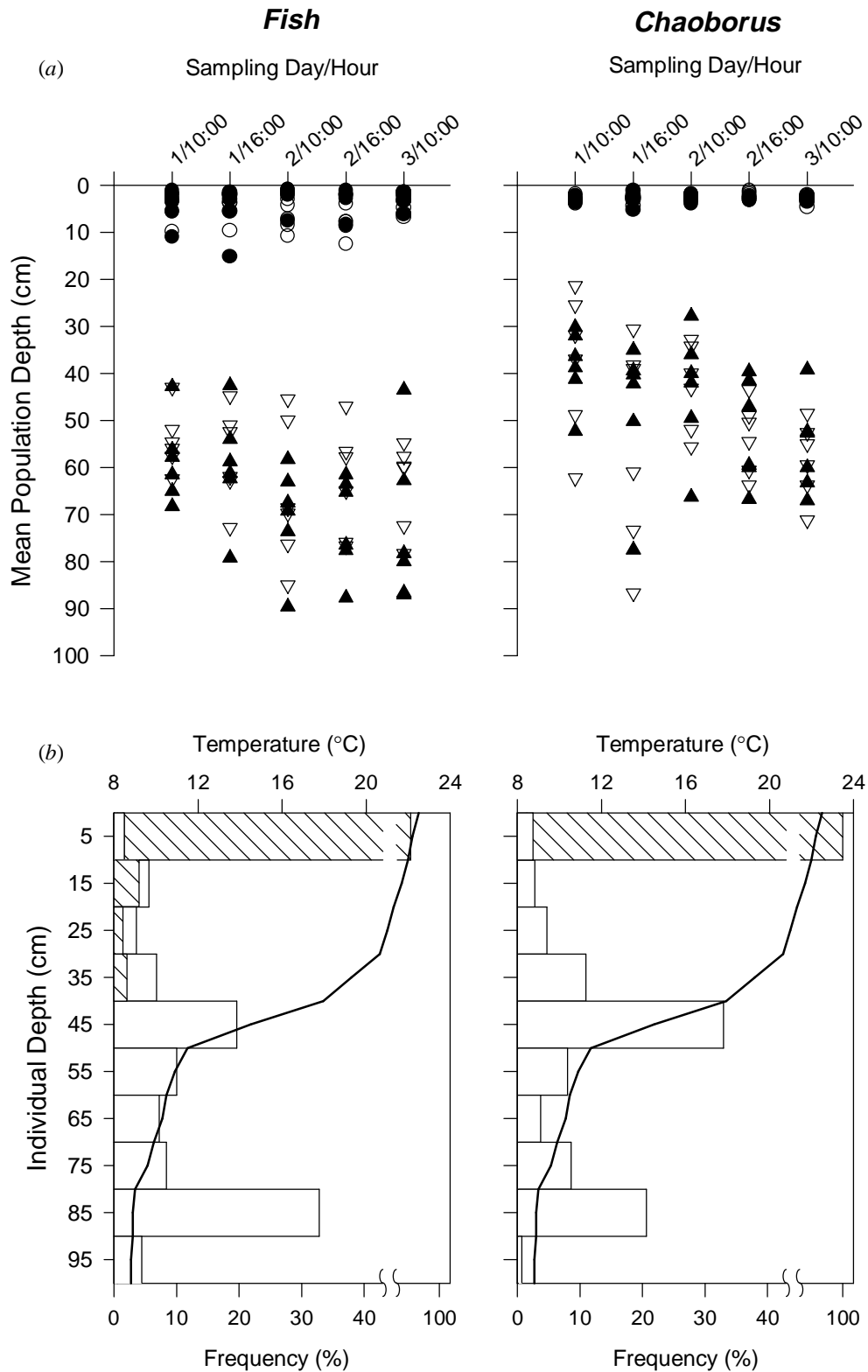


Figure 1. Results of vertical distribution experiments 1 and 2. (a) Mean population depths throughout the experiment. Circles represent females, triangles represent males, open symbols represent controls, filled symbols represent kairomone treatments. (b) Depth-frequency distributions of individual males (open bars) and females (striped bars), plotted with a temperature profile of the migration chamber. Note the break in frequency scale.

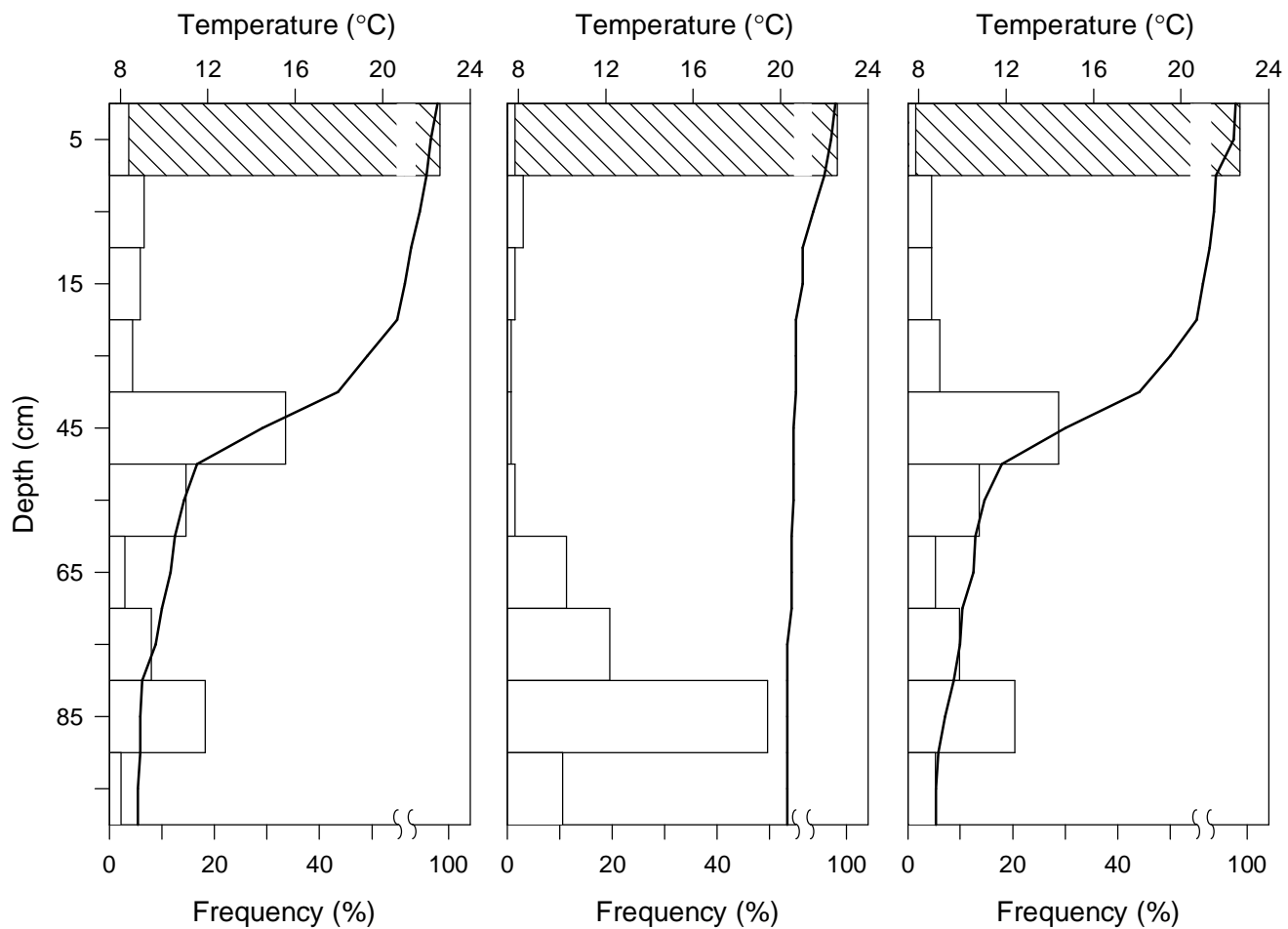


Figure 2. Depth-frequency distributions of individual males (open bars) and females (striped bars) in response to the elimination and re-establishment of a thermocline in the migration chamber. Also shown are temperature profiles. Note the break in frequency scale. (a) Days 1–2: thermocline; (b) days 3–4: isothermal; (c) days 5–6: thermocline.

video tape was paused and an individual that was in focus was haphazardly chosen from the population. The tape was advanced until the animal crossed one of the vertical lines, and that point was marked on the screen (start point). The tape was again advanced until the animal crossed five horizontal lines, and the point on the fifth line was marked on the screen (end point). Swimming speed was calculated by dividing the straight-line displacement of the animal between the start and end points by the time necessary to swim that distance. Swimming angle was determined by measuring the angle (from horizontal) between the start and end points. Paths were rejected if an animal either reversed horizontal direction or swam out of focus (focal depth *ca.* 1 cm) before swimming 5 cm horizontally. This procedure was repeated until 30 different animals had been measured in each of the three recording zones in each replicate. As the animals were always in view, it could be verified that individuals were only measured once.

After filming, 30 animals were haphazardly siphoned out of the central tube from each of the three recording zones and their body lengths measured to the nearest 0.1 mm from the top of the eye to the base of the tail spine.

(c) Behavioural interactions

Observations of males interacting with females were recorded in three dimensions (3D) in a $10 \times 10 \times 10 \text{ cm}^3$

perspex vessel containing 800 ml of water. The 3D filming set-up was as used by Brewer (1996), in which two orthogonal video cameras and infrared back-lighting produced silhouetted front and side views of the vessel. These views were electronically spliced into a single video signal and recorded on a VHS video cassette recorder. A total of five replicates of 20 adult males ($1.4 \pm 0.1 \text{ mm}$) and 20 non egg-bearing adult females ($1.8 \pm 0.1 \text{ mm}$) were placed in the recording vessel in filtered lake water with 1 mgC l^{-1} of *S. acutus* and recorded for 2 h in dim overhead halogen light (*ca.* $2 \mu\text{E m}^2 \text{ s}^{-1}$). The sex of individuals could be distinguished on the video by the difference in their size. From the video record, the number of interactions in which a male pursued another individual was noted, as was the sex of the individual being chased. Also, the distance at which the male began a pursuit (detection distance), the number of contacts, and the length of time the male held on to the individual (contact duration) were measured for each encounter. The 3D coordinates of the interactions were not available for analysis.

3. RESULTS

(a) Vertical distributions

In vertical distribution experiments 1 and 2, the mean population depth of males was consistently greater than that of females (figure 1a; Mann–Whitney *U*-test,

$p < 0.001$), and neither sex exhibited a significant response (in vertical distribution) to either fish or *Chaoborus* kairomone (figure 1a; Mann–Whitney U -test, $p = 0.11$). As kairomone had no effect on vertical distributions, in further analyses control and kairomone treatments were pooled within sex. Frequency distributions of the depths of individuals in experiments 1 and 2 (figure 1b, $n = 60$ per sex), revealed that females were found exclusively in the top 15 cm of the tubes and males, while present at all depths, had a bimodal distribution with one group concentrated at the thermocline (40–50 cm) and the other near the bottom (80–90 cm).

In vertical distribution experiment 3, which tested for a response of individuals to the elimination of the thermocline, females were distributed as in experiments 1 and 2, always inhabiting the top layer of the water column (figure 2). During the first 2 days in a stratified water column, males exhibited the same bimodal distribution as in experiments 1 and 2. Upon eliminating the thermocline however (days 3–4), most of the males (91%) were located below 60 cm, with a modal depth of 80–90 cm. After re-establishing the thermocline (days 5–6), the males again took on the typical bimodal distribution of ‘thermocline-males’ and ‘deep-males’ (figure 2).

(b) Swimming behaviour

In addition to the differences in their vertical distributions, which were also apparent in the large central tube, females and males exhibited clearly distinct swimming behaviours. There were no differences in mean swimming speed within each of the three groups (females, thermocline-males and bottom-males) between replicates (Model II ANOVA, d.f. 2,88, $p > 0.10$), so the three replicates were pooled for a single ANOVA. Comparisons among the three groups revealed that females ($0.68 \pm 0.21 \text{ mm s}^{-1}$) swam about half as fast as thermocline-males ($1.24 \pm 0.20 \text{ mm s}^{-1}$) and about two-thirds the speed of deep-males ($0.95 \pm 0.15 \text{ mm s}^{-1}$), which in turn swam about 23% slower than the thermocline-males (figure 3). The average swimming speed of each group was significantly different from the other two groups (ANOVA, d.f. 2,357, $p < 0.001$, and Tukey’s multiple comparisons, all $p < 0.001$). Male and female swimming angle distributions were significantly different (Kolmogorov–Smirnov two-sample-test, $p < 0.01$; Sokal & Rohlf 1995). Females exhibited a strong vertical swimming component, ranging from -60° to $+75^\circ$ from the horizontal, whereas male swimming behaviour was primarily linear and horizontally orientated, with swimming angles deviating no more than $\pm 15^\circ$ from the horizontal (figure 3).

In the mixed populations, the mean body length of females siphoned from the top 10 cm ($1.74 \pm 0.25 \text{ mm}$) was significantly greater than that of both thermocline-males ($1.16 \pm 0.15 \text{ mm}$) and deep-males ($1.37 \pm 0.06 \text{ mm}$), and deep-males were significantly larger than thermocline-males (ANOVA, d.f. 2,267, $p < 0.001$, and Tukey’s multiple comparisons, all $p < 0.001$) (see figure 4). Females ranged in size from 0.94 to 2.35 mm, and therefore overlapped in size with males found at all depths. In contrast, there was very little overlap in size between the thermocline-males, which ranged from 0.72 to 1.32 mm and deep-males, which ranged from 1.26 to 1.53 mm in length (figure 4).

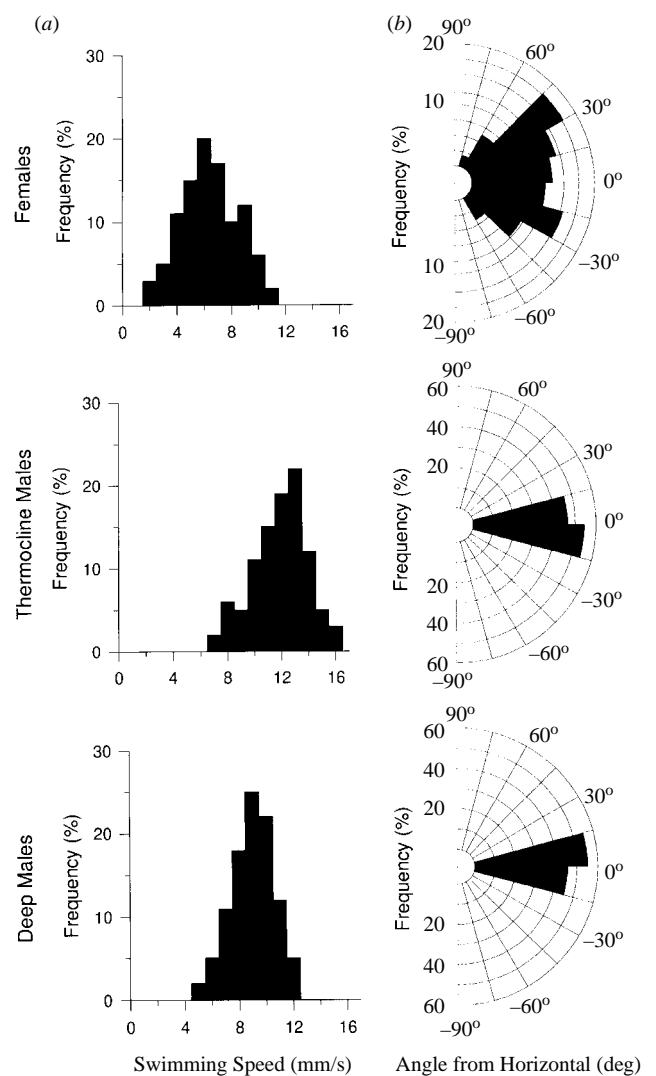


Figure 3. Summary of swimming behaviours of females, thermocline-males and deep-males in the vertical migration chamber. (a) Swimming speeds calculated over a 5 cm horizontal interval. (b) Swimming angle, from horizontal, measured over a 5 cm horizontal interval. Note the difference in frequency scales between females and males. In all graphs, $n = 90$.

(c) Behavioural interactions

Males displayed the same fast, primarily horizontal ‘scanning’ behaviour in the small vessel as in the large migration tube. Males were the aggressor in the mating process, pursuing individuals upon encounter. In the 10 h of recording, a total of 483 interactions was observed (table 1), of which significantly more were males pursuing males (315) than males pursuing females (168) (G -test, Sokal & Rohlf 1995; d.f. 1, $p < 0.001$). Males exhibited a stereotypical response upon encountering another individual, interrupting their scanning and performing a spiral restricted-area search in the vicinity of initial encounter. In contrast, females almost always performed an escape response upon encounter, swimming rapidly in the direction opposite to contact with the male. Males would generally pursue the female for a distance of several body lengths, displaying some ability to follow the female along a curved path, after which the male either appeared to lose the female’s ‘trail’ or was successful at capturing the female.

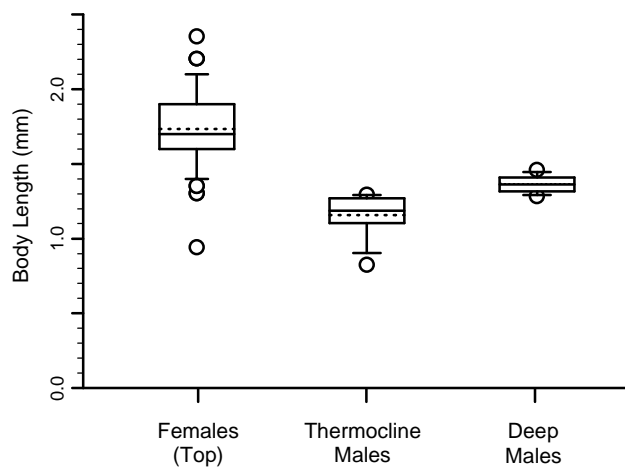


Figure 4. Box plot of body lengths of females, thermocline-males and deep-males in the large migration tube used for recording swimming behaviour. Boxes represent 25th to 75th percentiles, bars represent 10th and 90th percentiles and circles represent data points outside the 10th and 90th percentiles. Dashed lines indicate means. For each group, $n=90$.

In figure 5, diagrammatic representations are presented of the three classes of interactions observed in the recording vessel: (a) a male unsuccessfully pursuing a female; (b) a male successfully grasping a female; and (c) two males pursuing one another. In figure 5a, a 'scanning' male (solid line) encounters a female (dashed line), which responds by rapidly swimming away from the male. Failing to contact the female, the male performs a restricted-area spiral in an apparent attempt to re-encounter the female. The male succeeds and makes contact for several seconds, during which time the female again swims rapidly. The male is dislodged and performs another spiral but does not re-encounter the female and returns to scanning. In figure 5b a scanning male encounters a female, is successful at grasping her and maintains contact for more than 25 s. During contact the female swims in a rapid, sinuous manner, essentially dragging the attached male. In figure 5c, two males encounter and spiral around each other, contact one another for less than 2 s and then separate and swim in opposite directions.

Males initiated pursuits of other individuals at an average distance of 3.85 mm, ranging from 0.3 to 6.4 mm, and the average detection distance for other males was no different than that for females (table 1; ANOVA, d.f. 1,9, $p=0.36$). Males, in addition to chasing males more often, were more successful at capturing other males than females (table 1; ANOVA of arcsine transformed percentages, d.f. 1,9, $p<0.001$). However, the average duration of contact was much greater for females (13.6 ± 2.8 s) than males (1.6 ± 0.2 s; Mann-Whitney U -test, $p<0.001$). Frequency distributions of contact durations show that males rarely held on to other males for longer than 2 s, and that most female contacts were also of short duration (figure 6). However, if male-female contacts lasted for more than a few seconds, they tended to last longer than 15 s. Apparently, most often males were unable to get a solid grasp of the females, in which case contacts were very short. But, if males were able to grasp females well, the contacts were considerably longer, lasting up to 44.5 s.

4. DISCUSSION

(a) Vertical distributions

The differences in vertical distributions found between males and females, and among males may be the result of several interacting pressures: predation avoidance, life-history optimization, and avoidance of assortative mating. Zooplankton diel vertical migration is a well-documented predation avoidance behaviour, in which individuals move down in the water column during the day to avoid the danger of visual predation in the light (Lampert 1993). As visual predation is size-selective, with larger individuals being more vulnerable (Hrbacek 1962; Brooks & Dodson 1965), the bimodal distribution of male depths may be the manifestation of an anti-predation response. The smaller males (less than ca. 1.3 mm) were concentrated at the upper boundary of the thermocline; as deep in the water column as they could be and still remain in warm water (figure 1). Though in the experimental tubes the difference in light intensity between the surface and the thermocline (depth=45 cm) was negligible, in lakes the light intensity at the thermocline is often significantly lower than at the surface (Hutchinson 1957), thereby affording individuals found there some protection against predation. The larger males (more than ca. 1.3 mm), being more vulnerable to visual predation, may move as deep in the water column as possible, which would presumably be darker in lakes, to minimize this risk. However, predation risk alone cannot explain the observed distributions of males because if this were the only consideration, one could expect that all the males would be as deep in the water column as possible at all times. Also, the fact that the larger, and therefore more vulnerable, females did not move down in response to predator kairomones (figure 1), suggests that this clone of *D. pulex* is not specialized at avoiding visual predators.

Female *Daphnia* have generally evolved life-history strategies that maximize population growth rate, which is strongly affected by ambient water temperature through its effects on metabolism (Peters 1987), and feeding and assimilation rates (Lampert 1987). For example, female *D. magna* that migrate from warm surface water down into colder, deep water on a daily basis have one-third the growth rate of non-migrating females which stay in the warm water (Dawidowicz & Loose 1992). The effect of low temperature on growth rate is manifest throughout the entire lifespan of females: in juveniles it delays the onset of first reproduction and in adults it results in decreased fecundity (Vijverberg 1980). Therefore, in the presence of adequate food, as in this study, females may remain in the epilimnion to maximize fecundity. Males on the other hand, are more likely to be adversely affected by low temperatures only as juveniles, through the delaying of maturity, assuming that once they mature they do not have the same pressure as females to maximize reproductive rate through continuous gamete production. This assumption however, remains untested.

In this study, the *D. pulex* males matured in the fourth instar, as indicated by the presence of spermatozoa in their testes and spermiducts, at which time their average body length was 1.15 ± 0.03 mm (M. C. Brewer, unpublished data). The minimum size at which males were found at the bottom of the migration chamber was

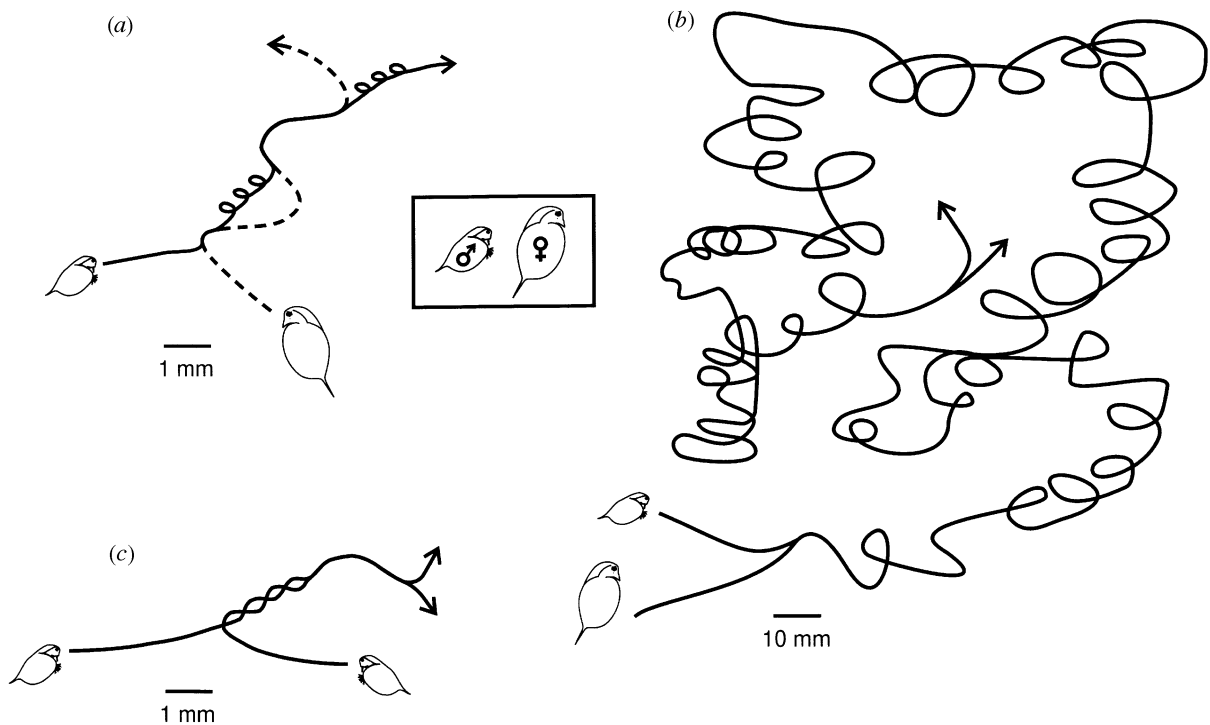


Figure 5. Two-dimensional representations of the three classes of behavioural interactions observed among *Daphnia* in the 1-l recording vessel. (a) Male unsuccessfully pursuing female; (b) male successfully grasping female; (c) two males pursuing one another. See text for further description.

Table 1. Summary of behavioural interactions among 20 females and 20 males recorded for 2 h in a 1-l vessel

(Detection distances, contact durations and row averages are mean \pm s.d. Detection distances are the distances at which males initiated pursuits. Percentages of males and females contacted are the proportions of each sex contacted, of those that were pursued.)

	replicate					average
	1	2	3	4	5	
total number of pursuits	91	107	66	133	86	96.6 \pm 25.1
% males pursued	63.7	69.2	59.1	70.7	58.1	64.2 \pm 5.69
male detection distance (mm)	4.1 \pm 1.3	3.8 \pm 1.4	3.2 \pm 1.1	4.2 \pm 1.1	3.6 \pm 0.9	3.8 \pm 0.4
female detection distance (mm)	3.9 \pm 1.2	4.1 \pm 1.0	3.9 \pm 1.4	3.7 \pm 1.5	4.1 \pm 1.6	3.9 \pm 0.2
total % contacted	40.7	38.3	30.3	42.1	26.7	35.6 \pm 6.74
% of males contacted	43.1	45.9	38.5	47.8	38.0	42.8 \pm 14.6
% of females contacted	27.3	30.3	22.2	33.3	16.7	26.0 \pm 6.6
male contact duration (s)	1.7 \pm 0.3	1.3 \pm 0.3	1.2 \pm 0.3	1.4 \pm 0.2	1.6 \pm 0.4	1.6 \pm 0.2
female contact duration (s)	14.4 \pm 11.2	15.7 \pm 13.4	16.8 \pm 18.3	12.2 \pm 10.1	9.9 \pm 8.7	13.8 \pm 2.8

about 1.3 mm, which corresponds to the sixth instar in this clone. As males require almost three times as long to reach the fourth instar at 10 °C (17 days) than at 20 °C (6 days) (M. C. Brewer, unpublished data), juveniles could benefit considerably by remaining in the warm water to mature as fast as possible. After maturing, movement down into cold water would increase their longevity (MacArthur & Baillie 1929), which could in turn increase their lifetime encounter rate with females. The importance to the small males of remaining in warm water, and its potential trade-off with predation risk, is illustrated by the distributions in experiment 3. Shortly after elimination of the thermocline, and therefore the metabolic cost, all of the smaller males

were found with the larger males, as far down the water column as possible (figure 2). As soon as the bottom was re-cooled, the metabolic costs returned, and the smaller males were again found just above the thermocline. The reason for males waiting for two instars (around 3 days) after reaching maturity to move down is not clear, though it is possible that the mere presence of spermatozoa in the testes is not a reliable indication of sexual competence. Fourth instar males may be physically unable, or less able, to copulate with females and therefore remain in the warm water until reaching functional maturity.

The absence of a metabolic disadvantage for adult males moving down into colder water does not in itself

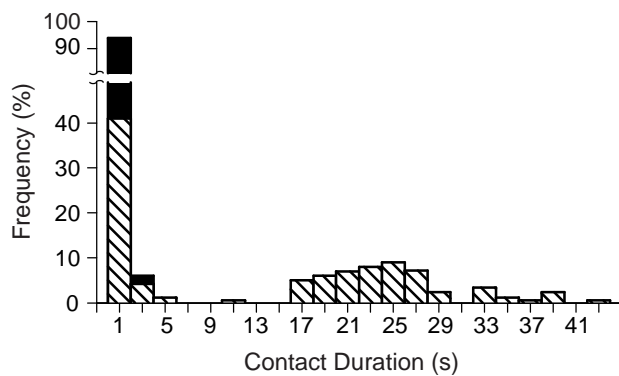


Figure 6. Frequency distributions of contact durations for males grasping males (black bars) and females (striped bars). Note the break in frequency scale.

explain why they would do so, especially given that the larger, more vulnerable females did not vertically migrate. If the adult males are not moving down solely to avoid predation, the explanation may involve a form of habitat partitioning that promotes random mating among clones. De Meester (1992) found that in some clones of *D. magna*, males and females of the same clone differed in phototactic behaviour. He suggested that these differences may increase the chance that males will mate with females of other clones, thereby avoiding the possible deleterious effects of assortative mating such as inbreeding depression. Though the females in this study were always located near the surface, they can be found deeper depending on variables such as light intensity and food concentration (M. C. Brewer, unpublished data) and any habitat partitioning suggested by the current distributions is not as complete as it appears. There is evidence for sex-related habitat partitioning in natural populations also. Haney & Hall (1975) found that male *D. pulex* in Three Lakes occurred at 'intermediate depths', with only 4% of males ever being found in the epilimnion, as opposed to more than 50% of females at night. In comparison, such differences in male and female depths have not been reported for copepods, which do exhibit vertical habitat partitioning among developmental stages (Ambler & Miller 1987). Copepods however, reproduce sexually and therefore do not face the same problem of potentially mating with genetically identical conspecifics.

To summarize the proposed explanations for the observed vertical distributions in this clone; females, which did not respond to predator kairomones, were always in the warm, food rich, shallow water, maximizing their fecundity. Juvenile males remain as deep as possible in the warm water to mature quickly while reducing predation risk. With maturity comes larger size and increased predation risk. As adult males, presumably, are not constrained like females to maximize fecundity, they move deeper into the cold water which may result in them: (i) reducing predation risk; (ii) avoiding mating with the genetically identical females of their own clone; and (iii) extending their longevity, thereby increasing life-time encounter rate with females.

(b) *Swimming behaviour*

The fast, primarily horizontal swimming behaviour of both the thermocline and deep males (see figure 3) is

clearly suggestive of the type of optimal searching behaviour predicted and found by Gerritsen (1980) in the predatory copepod *Cyclops scutifer*. Similarly, Watras (1983) described the swimming behaviour of male *Diaptomus leptopus* as 'in a horizontal plane at a relatively fast speed' in comparison to females which had 'a greater vertical component and a slower speed'. In another example, Yen (1988) found that in *Euchaeta rimana*, a marine calanoid copepod, females swam horizontally whereas adult males swam principally in a vertical direction. As in the copepods, the faster, orthogonal swimming orientation of the male *D. pulicaria* would result in an increased encounter rate with females, which swam more slowly and vertically. The actual degree to which contact rate would be enhanced for *D. pulicaria* males will depend on clonal and environmental effects on the swimming behaviour of females. The fact that this scanning behaviour has been documented in both marine and freshwater copepods and now *Daphnia* suggests that this may be a widespread and effective method by which zooplankton increase their encounter rate with mates. This is further supported by general searching models, which show that simply moving in a linear fashion, with few sharp turns, increases the probability of encountering randomly distributed resources (Dusenbury 1992).

(c) *Behavioural interactions*

The present observations of male-female interactions provide no evidence that male *Daphnia* are able to use chemical signals to increase their probability of encountering females. If males were able to reliably determine the sex of individuals at a distance they would probably have chased and contacted other males less often than females, rather than more often, as was observed (table 1). Furthermore, the fact that detection distances were almost identical for both sexes (table 1) suggests that males have only limited information about what they are pursuing. The apparent preference for males though, is probably artefactual. Based on swimming speeds alone, the Gerritsen & Strickler (1977) encounter model predicts that about 60% of encounters in the recording chamber should have been with other males, which is close to the observed mean of 64.2%. The higher rate of male-male contacts probably stems from the fact that upon encounter, both males perform a restricted-area spiral and try to contact each other, rather than escape like females (see figure 5). The female escape responses upon encounter with males appear to be typical of both cladocerans (Jurine 1820; Weismann 1880; Forró 1997), and copepods (Watras 1983; Yen 1988; Doall *et al.*, this volume). One possible explanation for this response is that females cannot differentiate among different hydrodynamic signals and therefore it is safer to simply escape from all disturbances. A more intriguing, though untested, hypothesis is that both the initial escape and the prolonged fast swimming after contact may be forms of sexual selection by females (Forró 1997).

The mate detection abilities of male *D. pulicaria* appear considerably less well-developed than those observed for some calanoid copepods. For example, Katona (1973) found male detection distances for females of up to 20 mm for *Eurytemora* sp. and Doall *et al.* (this volume), report that male *Temora longicornis* initiated pursuits of



Figure 7. Photograph of hydromechanical trail ($20\times$) created by a female *Daphnia* swimming through water. Trail is visualized by using a Schlieren optical path and a smooth density gradient as in Strickler (1977).

females at distances of up to 34 mm (34 body lengths), and more than 10 s after the female had swum past. Furthermore, male *T. longicornis* were able to follow relatively complex female swimming paths that were more than 65 mm long. These interactions occur at scales too large to be based on mechanoreception of fluid disturbances, which dissipate quickly, and thus it is likely that these copepods use chemoreception to follow chemical trails left by females as they swim through the water (Yen *et al.*, this volume). In contrast, male *D. pulicaria* had a maximum detection distance of only 6.4 mm, or about four body lengths, and though males showed a limited ability to follow the paths of females, they were generally unable to

follow females that got more than a few body lengths away or performed a sharp turn during escape. The relatively meager detection and tracking ability of *Daphnia* males suggests that they orientate via mechanoreception of fluid disturbances and not by detection of chemical signals. This is certainly feasible, as swimming *Daphnia* create fluid disturbances that persist for at least several body lengths (see figure 7; Kerfoot *et al.* 1980) and female *Daphnia* are able to detect and escape from fluid disturbances (Drenner *et al.* 1978). The males' indiscriminate pursuit of both males and females and their relatively poor tracking ability make it seem unlikely that *Daphnia* have a mechanosensory system sufficiently sophisticated to identify specific groups of organisms (i.e. predators versus potential mates) based on the hydromechanical signal they generate, an ability which has been inferred for some copepods (Wong 1996; Yen & Strickler 1996).

The mating behaviours of *Daphnia* reported here are similar to those described for several diaptomid copepod species (Watras 1983). For example, male *Diaptomus leptopus*, in addition to their swimming with the same scanning behaviour described above, had detection distances of only one or two body lengths and pursued both males and females, though they would usually only contact gravid females. Furthermore, Watras (1983) reported that male *D. leptopus* 'circled or swivelled briefly' when out-distanced by their pursued target, a behaviour resembling the spiral swimming of male *Daphnia* in the present study. Such spiral restricted-area searches are generally considered an effective means of locating a nearby resource *in lieu* of precise information about its location (Bell 1991; Dusenbery 1992), and have also been reported in both marine cyclopoid and calanoid copepods (Uchima & Murano 1988; Doall *et al.*, this volume). Like the orthogonal scanning behaviour, spiral swimming seems to be a common and effective means by which zooplankton encounter mates at close range, even when endowed with relatively sophisticated chemoreceptive abilities.

The fluid disturbances created by swimming *Daphnia* create a potential dilemma for the males which concentrate at the thermocline. The structure imparted to the water by thermal stratification will cause the fluid disturbances to persist longer than in the water of the epi- and hypolimnion. Therefore, the males at the thermocline should be able to detect females at a greater distance and perhaps follow their trails more accurately, increasing copulation success. However, predators such as calanoid copepods should also be able to detect the males at the thermocline more easily, increasing their risk of predation. This may be especially important given that the thermocline males are smaller and thus as a rule more vulnerable to invertebrate predation. Further experimentation is necessary to determine the relative importance of the opposing components of this potential trade-off. At the least, the observed male distributions lend support to the idea that the thermocline is a dynamic location in terms of behavioural interactions.

Observations such as males contacting males and multiple males clinging to single females have led to the widespread view that male *Daphnia*, and cladocerans in general, do not discriminate in their choice of mates (Jurine 1820; Scharfenberg 1910; Shan 1969). However, the view of male *Daphnia* as being wholly indiscriminate

in their mate selection is not accurate. Though males both pursued and contacted other males more often than females, they were never in contact with males for longer than 2.6 s. In contrast, males were in contact with females for 13.8 s on average, and when not dislodged by the fast swimming of the females, for much longer (figures 5c and 6). The marked difference in contact durations indicates that male *Daphnia* can indeed distinguish between males and females upon contact. How this is accomplished is not clear. One possibility is that males use surface structural characteristics in a 'lock and key' fashion to distinguish among species and sexes (for examples, see Goulden 1966; Kerfoot & Peterson 1980). Alternatively, Carmona & Snell (1995) hypothesized that males use glycoproteins on the surface of females' ovaries (i.e. contact pheromones) to determine sex. Supporting this view is recent evidence that, like copepods (Watras 1983; Uchima 1985), male cladocerans may be able to discriminate not only between males and females, but among females of different reproductive states.

Forró (1997) observed the mating of the cladoceran *Moina brachiata* in small drops of water and found that males contacted females more than twice as often as males and never actually copulated with males. Furthermore, males preferentially copulated with receptive females over parthenogenetic females and females already carrying resting eggs. That males did not contact other males led Forró (1997) to conclude that they may be able to determine the sex of the intended mate at a distance, a conclusion not supported for *Daphnia* by the present study. This disparity may be a real difference between *Moina* and *Daphnia*, or may be caused by differences in experimental set-up, i.e. observations in a drop of water versus a 1-l vessel. Nevertheless, upon contact, males did preferentially copulate with receptive females, implying that *Moina* can determine reproductive status on contact. Whether *Daphnia* also have this ability is unknown.

The apparent inability of male *Daphnia* to determine what exactly they are pursuing may be an important element of their ecology. First, the males may be more vulnerable to invertebrate predation because they do not try to escape from hydromechanical disturbances like females do, and instead turn into such disturbances. However, it is possible that males can distinguish among signals of different magnitude or frequency and adjust their response accordingly, as do some copepods (Wong 1996). Second, the fact that males cannot determine what they are capturing until contact is made could play a role in interspecific hybridization, an aspect of *Daphnia* ecology that is increasingly recognized as a significant factor in their evolution (Schwenk 1997). The prevalence of interspecific hybrids within the genus *Daphnia* (Hebert 1985) suggests that if males do use contact pheromones to identify the sex of females it must be a chemical of low specificity, common to at least a given species complex, and possibly the entire genus. Differences in contact pheromones among *Daphnia* species or species complexes may play a role in their isolation, as in some copepods (Frey *et al.*, this volume).

This study indicates that mating behaviour in *Daphnia pulicaria* is not as simple as previously believed, and in some ways it is similar to that described for copepods.

Though some light has been shed on a poorly understood area of *Daphnia* ecology, many questions remain. For example, the frequency distribution of male–female contact durations suggests that a coupling of about 15 s may be the minimum necessary for successful copulation in *D. pulicaria*. However, it was not possible to determine from the video records if a contact, even an extended one, resulted in successful transfer of sperm. Unfortunately, questions pertaining to mate choice and sexual selection require assessment of copulation success to be answered. This is difficult to determine in *Daphnia* under relatively natural conditions (i.e. free-swimming in large volumes of water), because they do not have spermatophores like copepods. Continued progress in this area would be facilitated by the development of methods to assess male copulation success.

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