Blind dating—mate finding in planktonic copepods. III. Hydromechanical communication in *Acartia tonsa*

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ABSTRACT: Mate-finding behaviour in the marine copepod *Acartia tonsa* was examined by video analysis. *A. tonsa* appears to depend on hydromechanical signals in the location of mates, detected at distances of up to 5 or 7 mm. Series of up to 7 or 8 synchronised hops in closely situated individuals, interpreted as an expression of hydromechanical communication or assessment of a potential partner, often concluded with mating. We use previously published models of hydrodynamic signalling to explain the observed behaviour and extrapolate the results to other species.

KEY WORDS: Mating behaviour · Hydromechanical signals · Detection · Communication · Model

INTRODUCTION

Most pelagic copepods are capable of remote detection, location, and identification of potential mates. This substantially enhances the rate at which males and females encounter one another and enables population maintenance, even at relatively low population densities. The majority of species for which remote mate detection has been described appear to involve pheromone (i.e. chemical) signals. Pheromones can be deposited as a trail in the wake of a cruising female (Doall et al. 1998, Tsuda & Miller 1998, Bagøien & Kiørboe 2005, this volume) or alternatively be spread out as a diffuse plume by a hovering female (Nihongi et al. 2004, Kiørboe et al. 2005, this volume). Remote detection of mates by hydromechanical signals has been described only for very few species. Strickler (1998) observed that males of the freshwater copepod *Cyclops scutifer* detect and pursue females at close range via hydromechanical signals. Similarly, *Acartia tonsa* (Doall et al. 2001), *Acartia hudsonica* (Colin 1995), *Tortanus* sp. (Colin 1995), and possibly *Cyclops fimbriatus* (Holmes 1903) rely on hydromechanical rather than chemical signals for detection of mates. The aim of this study is to examine the mate finding behaviour of *A. tonsa* and to analyse the features of the signal used for mate detection in this species.

MATERIALS AND METHODS

The methods employed are described in Bagøien & Kiørboe (2005). Mating behaviour was observed in a 1 l aquarium by means of 2 perpendicularly mounted video cameras. The aquarium was illuminated by infrared light only. Light from 2 infrared LEDs was collimated through condenser lenses, with one light source directed towards each camera, thereby providing shadow images of the swimming copepods.

Continuous cultures of *Acartia tonsa* were bred on the phytoplankton *Rhodomonas baltica* at 18°C. Freshly moulted adult males and females were sorted and the sexes separated 1 to 3 d prior to the mate finding video experiments; 20 to 30 preseparated individuals of each sex were brought together in the aquarium and filmed for 4 h while swimming freely. All experiments were made at rearing temperature and with additions of *R. baltica* at roughly 10^7 cells l^{-1}.

A total of 53 events involving an individual capturing another individual, or synchronised hops between individuals, were recorded on video; 11 of these events were digitised as described in Bagøien & Kiørboe (2005). By combining information from the 2 cameras, 3D positions of target individuals were determined with a temporal resolution of 1/25 s, enabling compu-

tation of 3D distances and velocities. It was not possible to distinguish between males and females on the video recordings. In this paper we make the assumption that the individual which first displays a behavioural response to a potential partner is the male, and vice versa.

RESULTS

Males and females of *Acartia tonsa* displayed similar swimming patterns and velocities. Both sexes typically swam at speeds around 4 mm s^{-1}, making frequent shifts in direction and performing small hops at irregular points in time.

Our observations indicate that *Acartia tonsa* depends on hydromechanical signals for detecting mates (examples in Figs. 1 & 2). In the typical prelude, a male and female approach each other by what appears to be chance, both making small and temporally irregular hops. When the 2 individuals reach some critical distance from each other, a hop made by the female is detected by the male and immediately triggers a response hop (Figs. 1 & 2). During the subsequent seconds, 1 or more such synchronised hops typically occur. Up to 7 or 8 synchronised precatch hops were
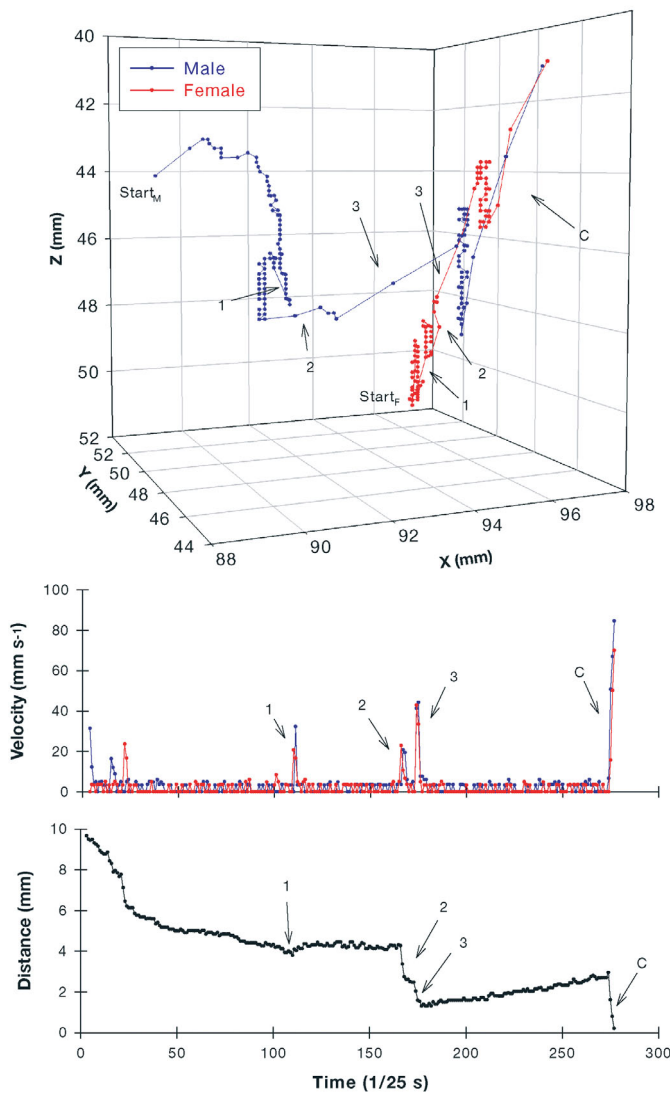

Fig. 1. *Acartia tonsa*. Expt 1. Top: Swimming trajectories of the male (blue) and female (red). Subsequent points are temporally separated by 1/25 s. Corresponding male and female positions at time of synchronous hops are indicated by numbers; C: catch leap. Middle: Swimming velocities of male (blue) and female (red) with time. Bottom: 3D-distance between male and female with time
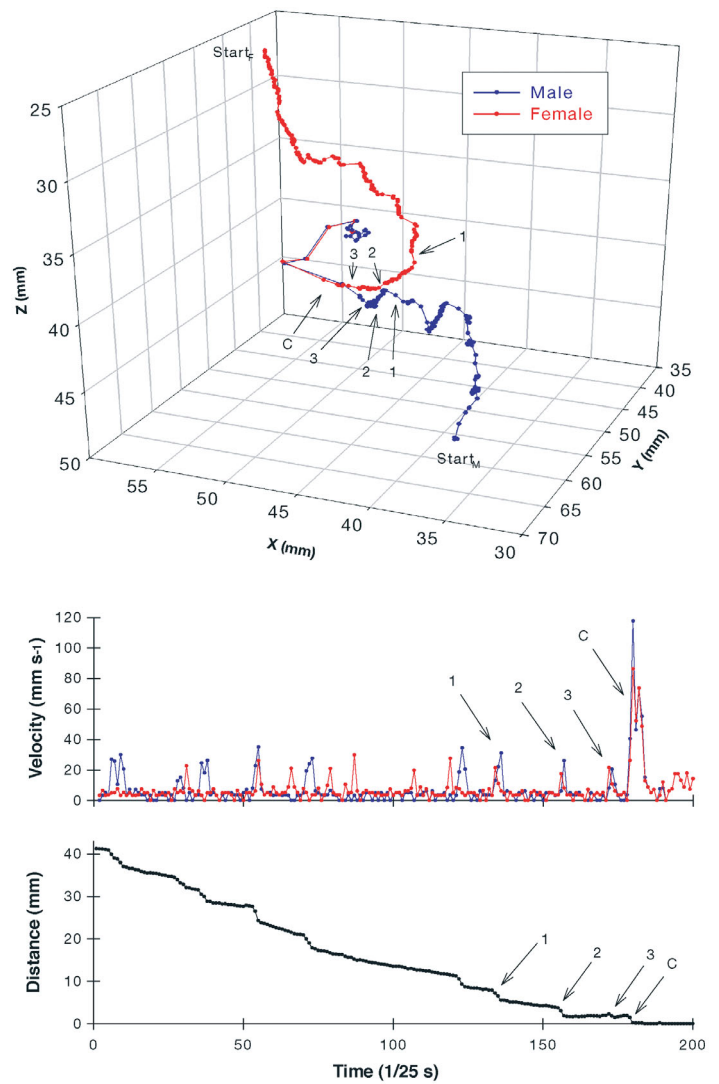
Fig. 2. *Acartia tonsa*. Expt 2. Top: Swimming trajectories of the male (blue) and female (red). Subsequent points are temporally separated by 1/25 s. Corresponding male and female positions at time of synchronous hops are indicated by numbers; C: catch leap. Middle: Swimming velocities of male (blue) and female (red) with time. Bottom: 3D-distance between male and female with time

observed on the video recordings. At the same time the distance between the 2 individuals tends to decrease somewhat. In some cases the male makes a small 'additional' hop, not an immediate response to any particular hop of the female, apparently to get in position for catching her. After a variable period of time, when the male may be positioned at any angle relative to the female, the male leaps towards the female and catches her.

Catch events in *Acartia tonsa* where 2 individuals become attached were conspicuous as the couple typically displayed a great burst in swimming velocity while moving together in a cyclic manner. The whole attachment phase tended to last only a few seconds. We assumed that such catch and attachment events represented mating, although spermatophore transfer could not be verified from the recordings due to both the great swimming velocities and small size of the individuals. A close up video clip of the catch event demonstrating 7 or 8 synchronised precatch hops and the cyclic swimming behaviour of the attached couple is available as an Electronic Supplement (www.int-res. com/articles/suppl/blind-dating/). Series of synchronised hops in closely situated individuals did not necessarily lead to a catch or physical encounter. A number of observations were made of 2 copepods performing synchronous hops during a shorter or longer time period, and then calmly parting by swimming separate ways. No indications of chemical mate detection or trail tracking were observed for *A. tonsa*. The male and female trajectories prior to the observed mating events did not concur and the individuals seemed to be unaware of the presence of the potential mate before coming within a range of few millimetres of each other (cf. Figs. 1 & 2).

The reaction distance, i.e. the 3D distance between the individuals when the male initially responded to a female hop, ranged between 2 and 7 mm; 7 out of 9 digitised cases which could be evaluated with a reasonable level of confidence indicated reaction distances of between 3.5 and 5 mm. Even if the phenomenon of synchronised hops was obvious in these cases, it could be difficult to positively identify the initial 'response hop'. There is no objective way of distinguishing between 'response hops' and merely hops occurring more or less simultaneously due to coincidence. The problem of positively identifying the initial response hop rendered determination of the number of synchronous hops during an event, as well as the duration of the communication period (i.e. the time elapsed between the first synchronous hop and the catch or encounter), difficult in some cases. The communication period typically lasted 2 to 3 s, although in one case its duration was as long as 13 s. Here, the 2 copepods initially performed a synchronous hop and thereafter

sank in close proximity for almost 13 s until the catch was made. In some cases mate catches were made directly, with no synchronised hops preceding the attachment. This was observed when swimming females passed more or less stationary males at short range.

DISCUSSION

The mate-finding behaviour for *Acartia tonsa* resembles that of the freshwater copepod *Cyclops scutifer* (Strickler 1998) but is strikingly different from mating behaviours reported for species employing chemical signalling (e.g. Doall et al. 1998, Tsuda & Miller 1998, Weissburg et al. 1998, Yen et al. 1998, Nihongi et al. 2004, Bagøien & Kiørboe 2005, Kiørboe et al. 2005). The distribution of chemical signals may be described as ranging between well-defined trails and diffuse plumes. Different signal distributions tend to be reflected by characteristic pursuit behaviours in the males, which may include increased swimming velocities, zigzag motion, frequent shifts of direction, etc. (see references above). *A. tonsa* males displayed no swimming mode resembling the 'trail-tracking' or 'signal-scanning' behaviours described for 'chemical species'. The male and female trajectories prior to the observed mating events did not concur, and in most cases the 2 individuals approached each other from different directions. Nor were the males observed to increase their swimming speed at any point prior to the mate encounters, except when making hops.

The male and female seemed unaware of the presence of a potential mate until one individual revealed itself by hopping within a few millimetres from the other individual. The observed synchronised hops are interpreted as an expression of hydromechanical communication, or possibly just a means of maintaining an appropriate 'striking distance', allowing for further assessment and catch of a potential partner. Synchronised hops imply that the male and female become aware of each other almost simultaneously (a delay of ~10 ms reported by Doall et al. 2001), as compared to some chemical signallers where the male may track a trail-producing female for several seconds before she notices him (e.g. Doall et al. 1998, Bagøien & Kiørboe 2005). Thus, mate-finding behaviour in hydromechanical signallers may be considered more 'symmetric' than in species which depend on chemical cues, both due to the comparatively short delay between mutual detection in the 2 individuals as well as the more similar pre-detection roles played by the male and female.

The delay between a pair of synchronised hops in *Acartia tonsa* is too short to be assessed using standard video equipment with a temporal resolution of 1/25 s.

However, Doall et al. (2001), who studied mating behaviour in *A. tonsa* using high-speed video, measured the delay between the initialization of the female hop and the initialisation of the male response hop as 9 to 12 ms. Males and females were described as encountering each other during hop-sink swimming; a female hopping within several body lengths of a male would trigger the male to hop towards her and position himself within 3 mm of her. After a period that included 1 to 4 'tandem hops', during which the male would come closer to the female, the male would lunge and capture his mate. Colin (1995) filmed mating behaviour in *Acartia hudsonica* and *Tortanus* sp. and found no indications of chemical trail tracking of mates in these 2 species. Instead of detecting and pursuing the trail of the female, males would respond directly to her present position. In both species the individuals seemed able to detect each other at distances of several millimetres, and the catch would be made up to many seconds later. During the period between detection and capture of the partner, the male would follow the hopping female and maintain a rather constant distance to her.

The lack of indications of chemical mate signalling in our study does not invalidate this hypothesised mechanism in *Acartia tonsa*. However, when also considering the results reported by Doall (2001) and Colin (1995) for *A. hudsonica*, we find it most likely that mate detection in *A. tonsa* is governed by hydromechanical signalling only.

The detection distance of up to 5 or 7 mm observed in the present study is consistent with present knowledge of generation and perception of hydromechanical signals in copepods (e.g. Visser 2001) and with the sensitivity to hydromechanical signals estimated for adult *Acartia tonsa* (Kiørboe et al. 1999). Copepods perceive hydromechanical signals by means of setae on the antennules, and bending of these setae may elicit neurophysiological (Yen et al. 1992) and motor responses (Lenz & Hartline 1999). Setal bending is caused by a velocity difference between the copepod and the ambient water, resulting from, for example, a fluid disturbance generated by a jumping mate. The signal strength can be estimated as this velocity difference, and signals that exceed a critical value, *s*, may elicit a behavioural response. For low Reynolds numbers, the hydromechanical disturbance generated by a hopping copepod attenuates approximately with the square of the distance to the copepod, and the disturbance may be estimated by describing the copepod as a force dipole (Svensen & Kiørboe 2000, Visser 2001). If the jump velocity is *U*, then the jumping copepod may be perceived at a maximum distance *R* by a mate with sensitivity *s* (Visser 2001):

$$R \cong \left(3a^2 b \frac{U}{s}\right)^{1/3} \qquad (1)$$

where *a* is the equivalent spherical radius of the copepod (~0.05 cm; 0.13 cm total length) and *b* the antennal length (0.12 cm). With $U = 5$ cm s^{-1} and $s = 0.02$ cm s^{-1} (Kiørboe et al. 1999), we can estimate the maximum reaction distance as 0.6 cm (measured from the tip of the antennules), similar to what we observed. Thus, our hypothesis of mates of *A. tonsa* perceiving one another by means of hydromechanical signals at distances of up to 5 or 7 mm, as indicated by our observations, is consistent with the model prediction.

Sensitivity to hydromechanical signals varies widely between copepod species but, in general, decreases with size so that *s* increases in direct proportion to the size of the copepod (Fig. 3). Strickler (1998) estimated the hydromechanical mate reaction distance in the freshwater cyclopoid *Cyclops scutifer* to be approximately 2 to 3 mm (in 2 dimensions). This copepod has a cephalothorax length of only 0.1 cm ($a \sim 0.04$ cm), short antennules ($b = 0.06$ cm), and a jump velocity of about 1.5 cm s^{-1} (Strickler 1998). If we assume that it has a hydromechanical sensitivity which is 3/4 that of *Acartia tonsa* (due to its smaller size), then *R* can be estimated as 3 mm, again consistent with the observed mate reaction distance.

More generally, hydromechanical mate reaction distance is expected to scale approximately with copepod size: one can assume that jump velocity, like hydromechanical sensitivity, scales with size, and jump velocity over sensitivity in Eq. (1), *U/s*, is therefore largely size independent. Since *a* and *b* in Eq. (1) both scale with size, it follows that mate reaction distance would be approximately proportional to size. For hydromechanical detectors, mate encounter rate scales with (reaction distance squared, R^2) × (swimming velocity, *v*), where *v* scales approximately with size. These considerations therefore imply that mate encounter rate scales
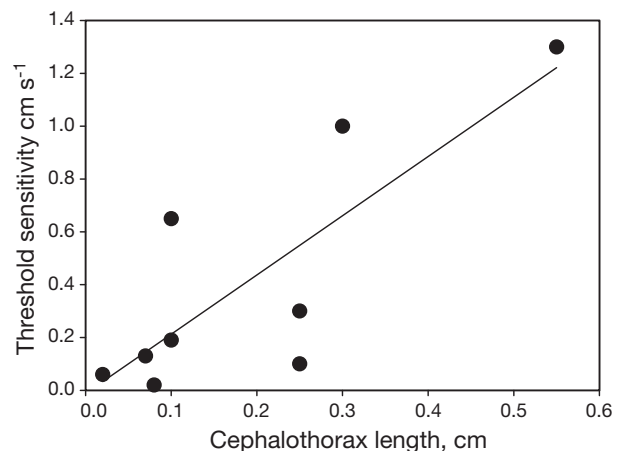

Fig. 3. Estimates of critical signal strength (*s*, cm s^{-1}) as a function of prosome length (*L*, cm) in pelagic copepods. Data from Kiørboe et al. (1999). The regression is $s = 0.0 + 2.24\,L$ ($R^2 = 0.65$)

approximately with (length)^3 of the copepod, or with its mass. Thus, large copepods can search larger volumes of water for mates, which is necessary in order to offset the declining abundance of copepods of increasingly larger size. These considerations apply, however, only at small Reynolds numbers. At Re $\gg$ 1, the fluid disturbance generated by a jumping copepod attenuates faster than at low Re—with (distance)^3 (Visser 2001)—and hydromechanical signalling thus becomes relatively less efficient in large than in small copepods. As a result, mate encounter rate in copepods scales with (length)$^{2.5}$.

Most copepods seem to use chemical signals for remote location of mates. Although hydromechanical signals may be involved during the final phase of the mate chase in 'chemical' species (e.g. *Temora longicornis*, Doall et al. 1998; *Calanus marshallae*, Tsuda & Miller 1998; *Centropages typicus*, Bagøien & Kiørboe 2005), only 4 to 5 cases of pure hydromechanical signalling have been reported so far: *Acartia tonsa*, *Acartia hudsonica*, *Cyclops scutifer*, *Tortanus* sp., and, presumably, *Cyclops fimbriatus*. Why is hydromechanical signalling so rare, when most copepod species are capable of detecting hydromechanical signals? Hydromechanical signals are susceptible to 'noise' generated by turbulence (Visser 2001), and the threshold sensitivity required to elicit a behavioural response is tuned so that the copepod does not overrespond to turbulent noise (Kiørboe et al. 1999). However, chemical signals are also eroded by turbulence (Visser & Jackson 2004), and a scaling analysis based on Visser (2001) and Visser & Jackson (2004) demonstrated that hydromechanical signals should be no more susceptible than chemical signals to the effects of turbulence as a function of copepod size. It is striking, however, that of the ~23 species of pelagic copepods for which remote mate detection to our knowledge has been described (see overview in Bagøien & Kiørboe 2005), 4 of the 5 species apparently depending on hydromechanical signalling are among the smallest. We noted above that hydromechanical communication becomes relatively less efficient with increasing copepod size and suggest that hydromechanical communication is at a disadvantage relative to chemical communication in larger copepods. For copepods producing pheromone trails, for example, mate encounter rate scales with (trail length) $\times$ (copepod size) $\times$ (swimming velocity) (Jackson & Kiørboe 2004, Kiørboe & Bagøien 2005). The length of pheromone trails produced by copepod females increases with copepod length raised to a power >1 (data summarised in Bagøien & Kiørboe 2005). If, as above, we assume that swimming velocity is proportional to size, it follows that mate encounter rate scales with (size)$^{>3}$. Thus, with increasing size it should become increasingly more efficient to use chemical compared to hydromechanical cues to signal presence and position to potential mates.

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