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Heterospecific mating and partial prezygotic reproductive isolation in the planktonic marine copepods *Centropages typicus* and *Centropages hamatus*

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Abstract

Using three-dimensional (3D) video observations in laboratory experiments, I describe interspecific and intergeneric mating behaviors and motility patterns of the common planktonic marine copepods *Centropages typicus*, *Centropages hamatus*, and *Temora longicornis*. These observations are then used to estimate heterospecific and conspecific male mate-search volume rates and mate encounter rates in North Sea *Centropages* populations. Behavioral prezygotic reproductive isolation between *Centropages* species is incomplete, since males of each species pursued, contacted, captured, and, in rare cases, placed a spermatophore on the urosome of heterospecific females. *T. longicornis* males also detected the diffusible pheromone trail and pursued *C. typicus* females to the point of mate contact. Male mate-search tracking behavior was equally effective on diffusible pheromone trails of heterospecific and conspecific females, indicating that pheromone and hydromechanical precontact mating cues lack species specificity. Males attempted mating with both heterospecific and conspecific females at high frequencies in the laboratory, and species recognition is inferred to occur through contact chemosensory cues or morphological incompatibility between species. Heterospecific mate encounter rates in the North Sea were maximal in late summer (August), up to ~2,000 encounters m^{-3} d^{-1}, and were comparable in magnitude to conspecific encounter rates during the same period. *C. typicus* females experience the highest incidence of heterospecific mating interactions, since they encounter heterospecific males at rates up to 100^{+} encounters $female^{-1}$ d^{-1}, ca. one order of magnitude higher than encounter rates with conspecifics. Heterospecific mating attempts may be a common feature of the reproductive ecology of planktonic copepods and may incur substantial fitness costs to the individuals involved.

The population dynamics of marine copepods may be constrained by mate encounter and fertilization rates (e.g., Hopkins 1982; Kiørboe and Bagøien 2005; Kiørboe 2006), in addition to suitable food availability, predation, and other extrinsic environmental factors (e.g., Eiane 2002; Li et al. 2006). Population-level mate encounter and fertilization rates are, in turn, controlled by the behaviors of individual zooplankters on small spatial scales. Owing to the broad biogeographic distributions and common co-occurrence of congeneric copepod species (e.g., Frost 1969; Mullin 1969; Goetze 2005), mate-seeking adults often encounter closely related heterospecifics in the water column, and the ability to discriminate heterospecific from conspecific potential mates is an important component of reproductive success for individual copepods. Although we may expect individuals to excel at selective mate-choice behavior, given its importance to fitness, laboratory experiments and field observations on planktonic copepods in freshwater environments often have found significant levels of heterospecific mating and hybridization between species (up to 70% of females in the field) (Chen et. al. 1997; Chow-Fraser and Maly 1988; Maier 1995, and references therein). These studies raise the possibility that heterospecific mating may be an important component to the reproductive ecology, fertilization dynamics, and genetic integrity of co-occurring plankton species. Few comparable studies have been conducted on marine species (but *see* Katona 1973; Jacoby and Youngbluth 1983), and little is known about the frequency and potential importance of heterospecific mating interactions in natural populations. High levels of heterospecific mating could exacerbate conspecific fertilization limitation and influence population dynamics, in addition to causing genetic introgression between species.

How might a mate-seeking adult male copepod distinguish conspecific from heterospecific potential mates? A variety of potential information sources are available to mate-seeking males, which are detectable at encounter, pursuit, capture, and copulation stages of the mating sequence (sensu Buskey 1998). Prior investigations of conspecific mating behavior have demonstrated that both chemical and hydromechanical cues play a role in the remote detection of mates (e.g., Griffiths and Frost 1976; Weissburg et al. 1998; Bagøien and Kiørboe 2005*a*, *b*, and references therein), with the relative importance of each sensory cue dependent on the mating strategy (Yen et al. 1998; Kiørboe and Bagøien 2005). These precontact mating cues, and diffusible pheromone signaling in particular, have been hypothesized to be traits involved in species recognition and reproductive isolation (Jacoby and Youngbluth 1983; Buskey 1998; Lonsdale et al. 1998). However, few observations have been made that bear on the species

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specificity of the diffusible pheromones, and Doall et al. (1998) found males pursuing other males to be evidence that the pheromone signal may lack information regarding the gender of the potential mate.

Upon contact with the female (at capture), males also may use contact chemoreception or morphological cues to determine whether the female is genetically compatible (e.g., Blades 1977; Blades-Eckelbarger 1991; Snell and Carmona 1994, and references therein). The use of contact chemoreception to detect species-specific glycoproteins on the surface of the female exoskeleton has documented importance in mate recognition for mate-guarding marine harpacticoid copepods (Frey et al. 1998; Kelly and Snell 1998) and may also play a role in the reproductive isolation of planktonic species. Morphological shape characters of sexually modified male appendages (e.g., geniculate A1 or P5 limbs) and the female urosome may also interact to serve as "lock and key" mechanisms preventing spermatophore placement in heterospecific mating pairs (Fleminger 1967; Lee 1972). Postzygotic reproductive isolation has been well documented in marine copepods (Lee 2000; Burton et al. 2006, and references therein) but, in the absence of any prezygotic isolating barriers, would not prevent the wasting of gametes in unfertile or nonviable mating crosses and would result in reduced individual fitness for co-occurring congenerics.

This study examines heterospecific mating behavior in the marine planktonic copepods *Centropages typicus*, *Centropages hamatus*, and *Temora longicornis* in order to identify the mating signals that play a role in species recognition, determine the frequency and fate of heterospecific mating attempts, and examine the potential ecological importance of heterospecific mating interactions in natural populations. Laboratory experiments on mating and motility patterns are combined with field data on adult abundances of *Centropages* species in the North Sea. Here I show that adult male copepods appear to have no ability to discriminate heterospecific from conspecific potential mates prior to contact with the female and that males attempt mating with heterospecific females at high frequencies.

Materials and methods

C. typicus and *T. longicornis* were established in continuous culture from animals collected in the North Sea (Dogger Bank) in August 2005. *C. hamatus* cultures were established from Øresund and Baltic Bornholm Basin populations in 2005 and 2006. Animals in culture were fed mixed algal diets including *Rhodomonas baltica*, *Heterocapsa triquetra*, *Thalassiosira weissflogii*, *Oxyrrhis* sp., *Prorocentrum minimum*, and *Gymnodinium sanguinium*. The salinity of the Baltic *C. hamatus* culture was gradually increased to 33, and all cultures were maintained at 14–15°C and a salinity of 33. All experiments were conducted at this temperature and salinity, with saturating food conditions of *R. baltica* and *Oxyrrhis* sp. Newly matured adults, usually obtained from a separately established cohort of animals, were used in mating and motility experiments.

Heterospecific and conspecific mating was examined in no-choice mating experiments, which included 20 adult females and males, each of a single species only. Observations were made in a 1-liter aquarium with three-dimensional (3D) video recording of animal behavior over a 4-h period. Adults of each sex and species were isolated from culture 1–3 d prior to experiments to ensure that animals had not recently mated prior to the experiment. Only females not carrying a spermatophore and coupling device were selected for use in experiments: virgins were used in some cases (isolated at C5 stage). Although direct observations on the frequency of remating in these species have not been reported, females of *Centropages* and *Temora* are often observed with multiple spermatophores on the genital segment in both culture and field populations (Blades 1977; Goetze pers. obs.), suggesting that they do remate. Centropagoid copepods, including these species, also often lack a seminal receptacle for sperm storage and require frequent matings in order to produce fertile eggs (e.g., Katona 1975; Ianora et. al. 1989; Ohtsuka and Huys 2001). All animals used in heterospecific mating experiments were examined for the presence of spermatophores following completion of the experiment. Densities used in mating experiments (40,000 indiv. m^{-3}) were high relative to typical field densities (e.g., up to ~14,000 indiv. m^{-3}, Halsband-Lenk et al. 2004), in order to ensure sufficient numbers of mating observations.

The *Centropages* mating experiments were paired, with heterospecific and conspecific (control) experiments conducted on the same day, in order to control for differences in the state of the copepod cultures. Comparison of the frequencies of mating behaviors was based on four replicate paired experiments for two species combinations: (1) *C. typicus* females and *C. hamatus* males, heterospecific; *C. hamatus* females and males, conspecific, and (2) *C. hamatus* females and *C. typicus* males, heterospecific; *C. typicus* females and males, conspecific. Statistical significance of differences in the frequency of behaviors between heterospecific and conspecific experiments was tested using a Student's *t*-test or Mann–Whitney *U*-test for each mating behavior (track, contact, capture). Heterospecific mating interactions between *T. longicornis* males and *C. typicus* females were recorded using the same experimental approach, but in the absence of a conspecific control experiment. In this case, only qualitative behaviors are reported.

The experimental setup for video recording of animal behavior was as described in Bagøien and Kiørboe (2005*a*). Briefly, two charge-coupled device (CCD) cameras view the aquarium from orthogonal directions, with a date-time generator and video recorder for each camera. Collimated infrared lighting is from behind, with copepods visualized as shadows in the video recording. The positions of males and females in recordings from each camera were integrated to generate a 3D reconstruction of animal movement. All mating captures visible by both cameras (in 3D) were digitized, followed by mating contacts visible in 3D in sequential order up to a total of 24 (heterospecific) or 27 (conspecific) events. Mating sequences selected for analysis were digitized using ImageJ software, with animal

Table 1. Characteristics of 27 digitized conspecific mating events of *C. hamatus*, ordered in increasing along-track distance to the female at the time of detection (along-track distance). Encounter distance is the 3D straight-line distance between the male and female at the time of detection: M and F trail length are calculated from detection to mate capture or contact. The experiment (Exp) and event (Ev) number are arbitrary identifiers of a particular behavioral event. TR = normal tracking behavior observed?

Exp No.	Ev No.	Fate	TR	Encounter distance (mm)	Along-track distance (mm)	M trail length (mm)	F trail length (mm)	Trail age (s)	Duration chase (s)	Male velocity (mm s^{-1})	Initial direction	Trail lost?
40C	2	Contact	Y	6.9	4.8	8.4	17.4	0.2	0.4	19.1	Correct	N
42C	2	Capture	Y	4.1	7.1	325.8	57.9	1.6	9.6	33.8	Correct	Y
39C	2	Capture	Y	6.0	9.7	7.0	11.7	2.4	0.5	13.4	Correct	N
39C	8	Contact	Y	6.3	10.0	8.5	16.0	2.0	0.3	23.7	Correct	N
41C	4	Contact	Y	10.3	12.6	11.5	18.0	3.1	0.4	23.9	Correct	N
39C	11	Contact	Y	4.9	12.9	17.0	20.7	3.2	0.6	25.0	Correct	N
39C	17	Contact	Y	6.6	14.1	35.3	30.0	2.4	1.6	21.5	Incorrect	N
39C	7	Contact	Y	9.5	14.1	11.9	21.1	3.4	0.6	19.9	Correct	N
39C	6	Contact	Y	6.5	14.6	9.6	18.6	4.9	0.4	23.9	Correct	N
39C	5	Contact	Y	8.0	16.9	9.6	20.4	4.7	0.5	18.6	Correct	N
39C	9	Contact	Y	11.0	17.3	20.5	24.3	4.5	0.9	22.2	Incorrect	N
39C	15	Contact	Y	7.2	18.9	26.8	28.3	3.0	1.0	25.8	Incorrect	N
41C	3	Capture	Y	7.4	19.6	12.7	24.1	4.9	0.6	21.2	Correct	N
42C	3	Contact	Y	16.6	24.0	19.5	29.5	6.0	0.7	27.1	Correct	N
39C	12	Contact	Y	11.0	26.2	51.4	41.4	4.9	2.0	25.2	Incorrect	N
39C	16	Contact	Y	7.1	26.9	52.2	50.7	4.2	2.3	22.5	Incorrect	Y
39C	10	Contact	Y	10.0	33.0	44.6	47.5	6.4	2.0	22.3	Incorrect	N
41C	5	Contact	Y	16.2	33.2	41.8	51.7	4.6	1.9	21.3	Incorrect	N
39C	14	Contact	Y	5.4	39.9	33.3	57.3	4.0	1.5	21.9	Correct	N
42C	4	Contact	Y	29.7	41.4	38.1	53.9	7.6	1.4	25.7	Correct	N
41C	1	Capture	N	1.7								
41C	2	Capture	N	2.0								
42C	1	Capture	N	2.1								
40C	1	Capture	N	2.4								
39C	1	Capture	N	2.4								
39C	4	Capture	N	2.6								
39C	3	Capture	N	2.6								

positions recorded at each video frame (0.04 s) before, during, and after each mating event. All calculations of mating behavior (Tables 1, 2) were made from digitized mating sequences. Twenty-seven conspecific *C. hamatus* mating events from control experiments were digitized (114 events observed, total; Table 1) because little prior information is available in the literature regarding normal mating in this species. One hundred and seventy-nine heterospecific mating interactions were observed between *C. typicus* females and *C. hamatus* males (45 captures, 104 contacts, 30 tracking events), 24 of which were digitized for further analysis (Table 2). Sixty-seven heterospecific mating interactions were observed between *C. hamatus* females and *C. typicus* males (5 captures, 57 contacts, 5 tracking events), a few of which were digitized. The Mann–Whitney *U*-test was used to test for differences in male tracking behavior between heterospecific and conspecific mating events (Table 3).

Motility experiments were conducted with adult males and females of *C. hamatus* and *C. typicus*, with swimming behavior recorded in the absence of the other sex and species (20 adults L^{-1}). The same experimental setup for video recording of animal behavior was used as in mating experiments, but with recording only in two dimensions (2D). Copepods were allowed 1-h of acclimation time in the aquarium prior to video recording. Positions of animals in 4 to 6-min video clips were digitized using LabTrack software (BioRas, Kvistgård), and a total of 132–187 swimming tracks were analyzed for each sex and species. Analysis was restricted to swimming paths present in the field of view for longer than 10 s. First antennule lengths of adult male *C. hamatus* from culture populations averaged 1.34 mm (n = 43, SD = 0.065). This length was used in encounter rate calculations below.

Abundance of adult males and females of *C. typicus* and *C. hamatus* in field populations was provided by the GLOBEC Germany project, which sampled and enumerated mesozooplankton in the southeastern sector of the North Sea during spring, summer, and fall of 2004. Copepods were sampled with a 150-μm mesh bongo net (diameter 0.3 m) towed obliquely from depths 5 m above the bottom to the sea surface.

Results

Conspecific mating: C. hamatus—Conspecific mating behavior in *C. hamatus* was broadly similar to that reported for *C. typicus* (Fig. 1; Blades 1977; Bagøien and Kiørboe 2005*a*), although males did not appear to detect or track females over comparable distances. The maximum along-track distance to the female at the time of detection in *C. hamatus* was 4.14 cm (Table 1), while in *C. typicus*, males

Table 2. Characteristics of 24 digitized heterospecific mating events involving a *C. typicus* female and a *C. hamatus* male, ordered in increasing along-track distance to the female at the time of detection (along-track distance). Encounter distance, M and F trail length, TR, and Exp and Ev No. as defined in Table 1.

Exp No.	Ev No.	Fate	TR	Encounter distance (mm)	Along-track distance (mm)	M trail length (mm)	F trail length (mm)	Trail age (s)	Duration chase (s)	Male velocity (mm s^{-1})	Initial direction	Trail lost?
39H	7	Contact	Y	3.4	5.6	4.1	9.8	0.4	0.2	20.3	Correct	N
42H	10	Contact	Y	4.6	6.0	5.3	8.7	1.3	0.2	22.3	Correct	N
42H	4	Capture	Y	2.7	9.6	6.3	13.2	2.8	0.2	31.3	Correct	N
19	4	Contact	Y	8.3	11.8	17.2	13.8	2.9	0.6	26.9	Incorrect	N
42H	3	Capture	Y	3.7	12.7	13.9	17.0	2.5	0.5	24.8	Correct	N
39H	5	Capture	Y	5.2	12.8	16.5	19.8	1.6	0.6	27.4	Correct	N
42H	5B	Capture	Y	4.3	12.9	24.8	23.5	1.9	1.8	13.5	Correct	N
19	3	Contact	Y	4.1	13.9	50.5	25.9	2.7	1.9	25.8	Incorrect	N
19	1	Capture	Y	11.7	14.8	42.0	22.9	4.1	1.4	28.4	Incorrect	N
42H	1	Capture	Y	10.8	15.5	27.2	23.7	2.4	1.2	22.7	Correct	N
39H	8	Contact	Y	12.5	16.2	29.2	30.9	3.2	1.2	23.5	Correct	N
42H	6	Contact	Y	8.0	16.3	19.7	22.4	4.6	0.8	23.5	Correct	Y
42H	5A	Contact	Y	4.4	18.2	43.4	36.3	2.6	2.5	17.2	Incorrect	N
41H	1	Capture	Y	5.4	20.3	142.0	54.8	2.9	4.5	31.1	Incorrect	Y
19	2	Capture	Y	14.6	20.4	19.9	26.3	5.7	0.6	31.0	Correct	N
42H	9	Contact	Y	5.9	23.9	24.6	28.8	6.4	1.0	24.6	Correct	N
41H	2	Capture	Y	20.8	24.9	30.1	33.5	5.5	1.2	23.5	Correct	N
42H	2	Capture	Y	10.5	35.9	39.4	41.0	11.8	1.3	29.0	Correct	Y
12	1	Capture	Y	23.6	43.1	174.4	94.3	7.1	8.6	20.2	Incorrect	Y
39H	6	Contact	Y	8.0	60.9	164.7	82.7	10.4	2.8	57.2	Correct	Y
42H	7	Contact	Y	35.6	64.2	54.1	70.5	26.5	1.6	33.8	Incorrect	N
39H	4*	Capture	Y	14.4	97.8	133.1	138.8	12.1	4.6	28.9	Correct	N
39H	1	Capture	N	3.6								
39H	2	Capture	N	4.3								

* Case 1 described in text, illustrated in Fig. 1A,B.

were able to detect females at up to 16.6 cm away along the track line (Bagøien and Kiørboe 2005*a*). There is one outlier event, in which the *C. hamatus* male pursued and contacted the female twice, lost the pheromone trail twice at the female escape hop, before successfully tracking and capturing her (Fig. 1; Table 1, Exp 42C Ev 2). With the exception of this event, males pursued females over a range of along-track distances from 4.8 to 41.4 mm to the female at the time of trail detection (~4–40 body lengths), over pursuit durations of 0.4 to 2.3 s. Males detected pheromone trails up to 7.6-s old. Males also initiated tracking in the incorrect direction ca. 35% of the time and occasionally lost and recovered the pheromone trail through accelerated swimming in what appeared to be a ''signal-scanning'' behavioral mode. During signal scanning, the male accelerated (to ~35–50 mm s^{-1}) above normal tracking speeds and searched a restricted volume in an effort to regain the pheromone trail (Fig. 1B,C), as has been observed in *C. typicus* (Bagøien and Kiørboe 2005*a*). Mate capture often occurred without any prior pheromone trail tracking, and males were able to detect and capture females directly at distances of ~2–3 mm (Table 1).

Heterospecific mating: qualitative description—Behavioral prezygotic reproductive isolation between *C. typicus* and *C. hamatus* is incomplete, since males of each species were observed to successfully detect and follow pheromone trails, make contact, capture, and place spermatophores on

Table 3. Effectiveness of male tracking behavior in heterospecific and conspecific mating events (captures and contacts). Medians and (ranges) listed for tracking parameters. Heterospecific experiments = *C. typicus* female and *C. hamatus* male. Data for conspecific *C. typicus* mating from Bagøien and Kiørboe (2005); dash indicates no data available.

Tracking parameters	Heterospecific *typicus*/*hamatus*	Conspecific *C. hamatus*	Conspecific *C. typicus*
Lost trail	23%	10%	—
Incorrect initial tracking direction	32%	35%	35%
Male velocity during pursuit (mm s^{-1})	25.3 (13.5–57.2)	22.4 (13.4–33.8)	—
Duration of chase (s)	1.2 (0.2–8.6)	0.8 (0.3–9.6)	1.6 (0.1–6.0)
Trail age at detection (s)	3.1 (0.4–26.5)	4.1 (0.2–7.6)	9.6 (1.2–30.8)
Length of pursued trail (mm)	26.1 (8.7–138.8)	26.3 (11.7–57.9)	46.3 (6.1–198.5)
Along-track distance at detection (mm)	16.3 (5.6–97.8)	17.1 (4.8–41.4)	51.1 (11.0–165.6)

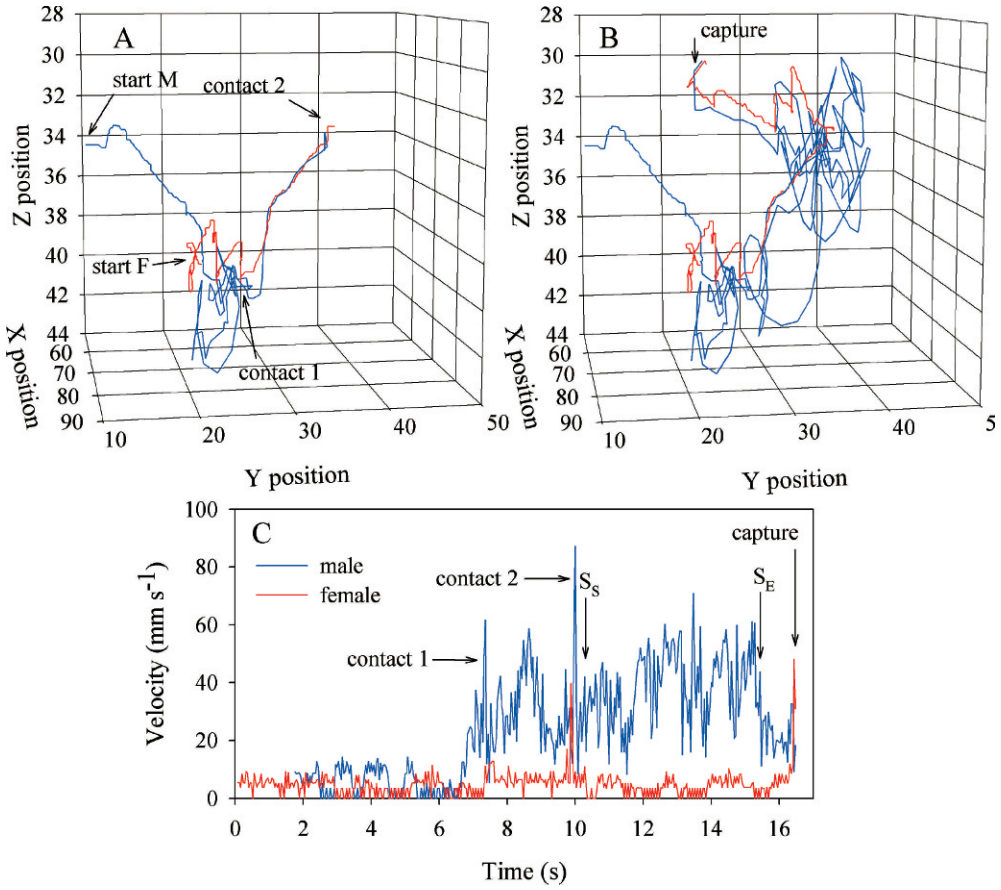

Fig. 1. Example of a conspecific mating event of *C. hamatus*. (A, B) The swimming trajectories of male (blue) and female (red) prior to and during the mate capture. The male tracks the pheromone trail and contacts the female twice prior to successful capture. (C) The swimming velocities of male (blue) and female (red) during the mating event. S_S and S_E mark the beginning and end of "signal-scanning" behavior during which the male is searching for the pheromone trail at accelerated speeds (38.2 ± 13.02 mm s^{-1}) relative to normal tracking speeds (23.3 ± 10.5 mm s^{-1}).

the urosomes of females of the other species (Fig. 2A–D, Table 2). Furthermore, females of *C. typicus* were sexually pursued by *T. longicornis* males, who detected the pheromone trail and made contact with females in at attempt to capture them (Fig. 2E,F).

Centropages heterospecific mating attempts closely resembled conspecific mating in these species, as the same characteristic behaviors were observed. Males appeared to detect a chemical pheromone trail laid down by the heterospecific female and initiated tracking behavior by accelerating from background swimming speeds of ~5–10 mm s^{-1} to a tracking speed of 20–30 mm s^{-1} (Table 2) when they were within 1–2 mm of the female track line (about one body length). Males pursued females with a tight zigzag swimming movement along the track and attempted capture of the female when they reached within 1–2 mm. Males initiated a "signal-scanning" behavior upon losing the pheromone trail in heterospecific mating events of *C. typicus* females and *C. hamatus* males, but this behavior was not observed in reverse mating crosses (*C. hamatus* females, *C. typicus* males). In many cases, males were able to recover a lost pheromone trail and successfully follow it to contact or capture with a heterospecific female (Table 2).

In heterospecific mate captures, males usually caught the female in the correct position, by catching the caudal setae of the female with his geniculate right antennule (as observed for *C. typicus* by Blades 1977). In most cases, however, captured females were released without spermatophore placement. Of 179 heterospecific mating interactions observed (*C. typicus* female, *C. hamatus* male), two spermatophore transfers were found to have occurred at the end of the experiment. Similarly, in the reverse mating cross (*C. hamatus* female, *C. typicus* male) a total of five spermatophores were transferred to a heterospecific female in 67 mating interactions observed. Although comparable data on spermatophore transfers in conspecific crosses are not available from these paired experiments, in additional conspecific mating experiments the number of captures observed (2D or 3D) closely matched the number of spermatophores found to have been transferred at the end of the experiment (n = 3 experiments). Following these observations, we would expect ca. 20 spermatophore

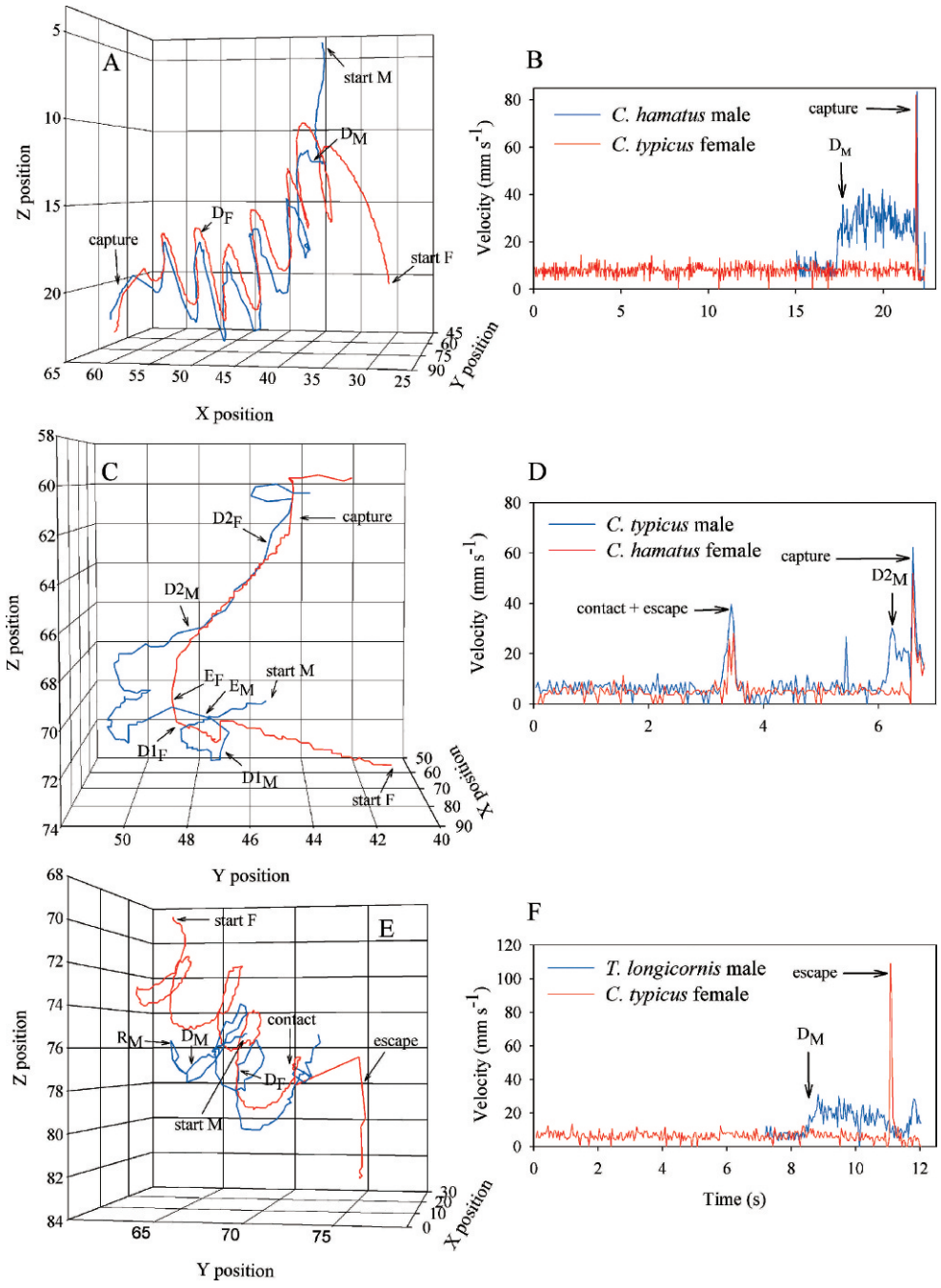

Fig. 2. Examples of heterospecific mating attempts between *C. typicus*, *C. hamatus*, and *T. longicornis* species pairs. (A, B) The mate capture of a *C. typicus* female by a *C. hamatus* male. (C, D) The mate capture of a *C. hamatus* female by a *C. typicus* male. (E, F) A mate encounter between a *C. typicus* female and *T. longicornis* male. (A) Swimming trajectories of a *C. hamatus* male (blue) chasing and capturing a *C. typicus* female (red). D_F and D_M = location of female and male when the pheromone trail was detected. (B) Swimming velocities of male (blue) and female (red) before and during the mating event. (C) Swimming trajectories of a *C. typicus* male (blue) chasing, encountering, losing, and then chasing and capturing a *C. hamatus* female (red). $D1_F$, $D1_M$ and $D2_F$, $D2_M$ mark female and male locations at trail detection during the mate encounter and mate capture events, respectively. E_F and E_M indicate positions at the female escape. (D) Swimming velocities of male (blue) and female (red) before and during the mate contact and capture events. (E) Swimming trajectories of a *T. longicornis* male (blue) chasing and encountering a *C. typicus* female (red). D_F and D_M as defined above; the male reverses tracking direction at R_M. (F) Swimming velocities of male (blue) and female (red) before, during, and after the mate encounter. *See text* for further description of each mating event.

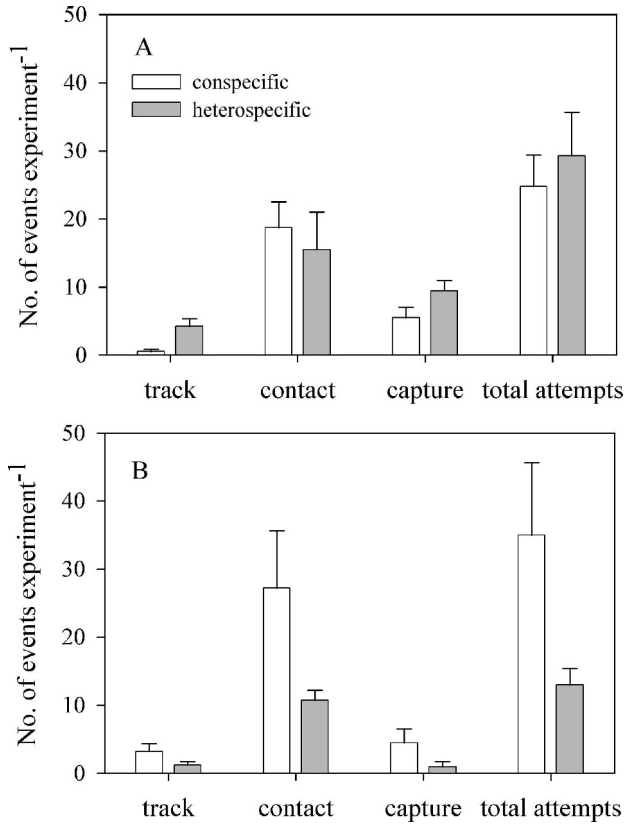

Fig. 3. Frequency of heterospecific and conspecific mating behaviors in four replicate paired experiments for the species combinations: (A) *C. typicus* females and *C. hamatus* males, heterospecific; *C. hamatus* females and males, conspecific (B) *C. hamatus* females and *C. typicus* males, heterospecific; *C. typicus* females and males, conspecific. Mean and standard error (SE) are shown.

transfers to have occurred in conspecific mating experiments reported in both Fig. 3A and 3B. In some cases heterospecific females were released immediately after capture, in others, the male retained grasp of the female for up to a maximum of 409 s (6 min 49 s).

I describe a few case examples below to illustrate typical mating interactions between adults of different species and genera.

Case 1 (Fig. 2A,B; Table 2)—A *C. typicus* female swimming in helical loops at a speed of ~5–10 mm s^{-1} is the male target. The *C. hamatus* male encounters the female pheromone trail when it is 12.1-s old, and the female is 97.8 mm away along the track line. The straight-line, or encounter, distance to the female is only 14.4 mm at the time of trail detection. The male, initially swimming at ~5–10 mm s^{-1} (average = 8.7 mm s^{-1}), accelerates to an average speed of 28.9 mm s^{-1} for pursuit of the female. The male initiates tracking in the correct direction and does not lose the trail during pursuit of the female. He maintains an average track distance of 1 mm during pursuit of the female (±0.4 mm, $n = 113$ positions) and carefully follows the 3D helical structure of the track line. The male tracks the female pheromone trail for a total of 4.6 s and 133.1 mm before capturing the female, and he succeeds in capturing her in the correct position. This behavioral sequence is indistinguishable from that of a successful conspecific mating event of *C. typicus*.

Case 2 (Fig. 2C,D)—In this case, a *C. typicus* male pursues and captures a *C. hamatus* female. The male, swimming at a background speed of 5–10 mm s^{-1}, encounters the 0.7-s old female pheromone trail and accelerates to 27.4 mm s^{-1} for 0.2 s. The female detects his approach and executes a successful escape hop (E_F). The male, in contrast to typical conspecific mating behavior, does not accelerate to seek and recover the lost pheromone trail, but rather decreases his speed to background levels (~5–10 mm s^{-1}). The female continues swimming upward, and the male reencounters the female trail after 2.6 s ($D2_M$). The female pheromone trail is 2.3-s old at the time of reencounter. The male again accelerates (to an average speed of 23.9 mm s^{-1}) and pursues the female for 0.44 s over 11.5 mm until mate capture.

Case 3 (Fig. 2E,F)—This is an intergeneric mating interaction between a *C. typicus* female and *T. longicornis* male. The *Centropages* female swims in a helical looping pattern at speeds of 5–10 mm s^{-1}. The *Temora* male detects the female pheromone trail when it is 4.7-s old and the female is 29.7 mm away along the track line. The encounter distance to the female is 5.5 mm at the time of trail detection. The male initiates tracking in the incorrect direction and reverses his direction after 0.2 s (R_M). The male pursues the female for a total of 2.5 s and 47.5 mm before the female detects his approach and executes a successful escape hop. The male continues searching for the pheromone trail after losing the female (signal scanning) but does not succeed in recovering her position.

In examining a larger number of digitized heterospecific mating events (Table 2), it is apparent that Case 1 above is quite typical for mating interactions between *C. typicus* females and *C. hamatus* males. Males were able to detect heterospecific pheromone trails up to 26.5-s old and pursued females who were up to 97.8 mm away along the track line (~100 male body lengths). The duration of male pursuit ranged up to 8.6 s, and, as was observed in this event, was often longer in cases where the male lost the trail or initiated tracking in the incorrect direction. Male velocities during the mate chase ranged from 13.5 to 33.8 mm s^{-1}, and males pursued females over a total distance of 4.1 to 174.4 mm (M trail length) until contact or capture. Two capture events were also digitized in which tracking behavior was not observed: in these cases, the male lunged and captured the female directly upon detection, which occurred at distances of ~4 mm.

Male tracking behavior on diffusible pheromone trails—Male mate tracking behavior was equally effective on heterospecific and conspecific pheromone trails, in a comparison of heterospecific (*C. typicus* female–*C. hamatus* male) and conspecific (*C. hamatus*) contact and capture mating events (Table 3). Males initiated tracking in the incorrect direction and lost the pheromone trail at broadly

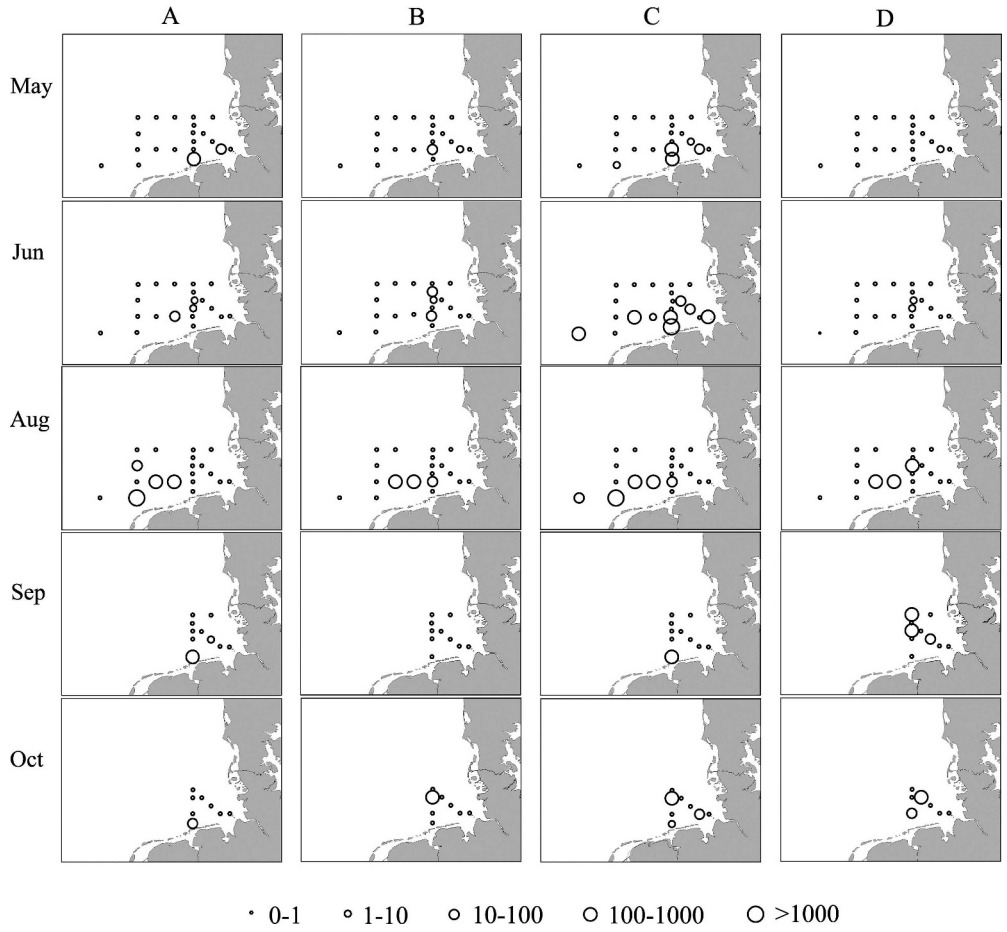

Fig. 4. Encounter rates of heterospecific and conspecific mating pairs of *C. typicus* and *C. hamatus* in the southeastern sector of the North Sea between May and October 2004. Units are encounters m^{-3} d^{-1}. From left to right, panels present encounters between (A) *C. hamatus* males and *C. typicus* females, (B) *C. typicus* males and *C. hamatus* females, (C) *C. hamatus* males and females, and (D) *C. typicus* males and females. Magnitude of encounter rates as indicated in the symbol legend. Heterospecific values range from 0 to 2,000 encounters, conspecific values from 0 to 32,000 encounters (2 cases >1,000). Abundance data courtesy of the GLOBEC Germany project.

comparable rates in both mating types: ~35% and 10–23%, respectively. No significant differences were observed between heterospecific and conspecific mating events in the male velocity during pursuit, duration of the mate chase, trail age at detection, length of the pursuit, or in the along-track distance to the female at the time of trail detection (Mann–Whitney *U*-test, $p >> 0.05$ in all cases; Table 3). Although there were no differences in central tendency for the two mating types, if we consider the range of the data, *C. hamatus* males were able to detect and pursue heterospecific females at longer maximum along-track distances and at older maximum trail ages than conspecific females (Table 3); they also pursued heterospecific females over longer maximum pheromone trails. Published values for conspecific *C. typicus* mating events are included for comparison (Table 3; Bagøien and Kiørboe 2005*a*), although it is unclear whether these higher median values represent actual longer male pursuits (trail age at detection, length of pursued trail, along-track distance at detection) or are due to bias in the selection of mating events for analysis. Larger bodied *C. typicus* females are a priori expected to produce longer pheromone trails (Bagøien and Kiørboe 2005*a*), which would result in longer tracking distances for both heterospecific and conspecific males.

Heterospecific mating: frequency—No significant differences were observed in the frequency of mate contact or capture behaviors between paired heterospecific and conspecific mating experiments in which *C. hamatus* males were the active mate-searching partner (Fig. 3A). Although males contacted heterospecific females at slightly lower mean rates (15.5 vs. 18.8 experiment^{-1}, SE = 5.5, 3.8; or 3.9 vs. 4.7 h^{-1}) and captured them at slightly higher mean rates (9.5 vs. 5.5 experiment^{-1}, SE = 1.5, 1.6; or 2.4 vs. 1.4 h^{-1}) relative to conspecific females, these differences were nonsignificant ($t = 0.491$, -1.852; df = 6, 6; $p > 0.1$ both cases). However, the frequency of male pheromone trail tracking behavior was significantly higher in heterospecific than conspecific experiments ($t = 3.5$, df = 6, $p <$

Table 4. Summary of swimming velocities and time budgets for *C. typicus* and *C. hamatus* males and females, categorized by motility type. C+S = crawl and sink, C/C+S = between crawl and crawl/sink behavior, dash indicates motility type not observed.

	Loop	Cruise	C+S	Sink	C/C+S	Overall average
C. typicus female						
Fraction of time spent	0.79	0.14	0.02	0.05	—	
Velocity, average (mm s^{-1})	5.62	5.49	4.44	4.48	—	5.50
Velocity, SD (mm s^{-1})	1.26	1.40	2.54	0.78	—	3.14
C. typicus male						
Fraction of time spent	0.36	0.28	0.31	0.05	—	
Velocity, average (mm s^{-1})	4.85	4.08	3.16	3.31	—	3.92
Velocity, SD (mm s^{-1})	1.03	1.18	0.50	1.37	—	3.15
C. hamatus female						
Fraction of time spent	0.23	0.20	0.03	—	0.54	
Velocity, average (mm s^{-1})	5.07	5.02	2.54	—	3.16	3.81
Velocity, SD (mm s^{-1})	1.03	1.14	0.80	—	0.85	2.89
C. hamatus male						
Fraction of time spent	—	0.04	0.46	—	0.50	
Velocity, average (mm s^{-1})	—	3.41	2.53	—	2.85	2.48
Velocity, SD (mm s^{-1})	—	1.30	0.77	—	0.89	2.74

0.025). Mate contact, in which the male tracked the pheromone trail to the point of contact but failed to capture the female, was the most common type of mating behavior observed in both heterospecific and conspecific experiments (Fig. 3A). A total of 99 conspecific and 117 heterospecific mating events occurred during the four experiments.

In experiments with *C. typicus* males as the active mate-seeking partner, no significant differences were observed in mate tracking, contact, or capture rates between heterospecific and conspecific experiments ($U_{0.05}$ = 22.5, 24, 23; $p > 0.1$ all cases; Fig. 3B). Average rates of pheromone trail tracking, mate contact, and mate capture for heterospecific interactions were 1.3, 10.8, and 1 events $experiment^{-1}$, respectively (SE = 0.5, 1.4, 0.7; or 0.3, 2.7, and 0.3 h^{-1}). Conspecific rates were 3.3 tracking, 27.3 contact, and 4.5 capture events $experiment^{-1}$ (SE = 1.1, 8.4, 2.0; or 0.8, 6.9, and 1.1 h^{-1}). Again, mate contacts were the most frequently observed behavior in both heterospecific and conspecific experiments (Fig. 3B). A total of 140 conspecific and 52 heterospecific mating events were observed during the four replicate paired experiments. Total mating attempts were marginally nonsignificantly different in heterospecific and conspecific mating experiments (t = 2.0, df = 6, $p > 0.09$).

Owing to species differences in the rate at which males can search a given volume of seawater for potential mates (β, *see* calculations in Discussion below), expected encounter rates of heterospecific and conspecific potential mates are not equivalent in these experiments, despite the constant densities (20 males + 20 females L^{-1}). In experiments with *C. hamatus* males, males encounter heterospecific females four times more often than conspecific females: in experiments with *C. typicus* males, males encounter heterospecific females at one quarter the conspecific rate. This difference in search capacity implies that expected encounter rates are higher for the heterospecific case in experiments in Fig. 3A, and higher for the conspecific case in experiments in Fig. 3B.

Motility—In the *Centropages* species, five distinct movement patterns were observed: loop, cruise, crawl and sink (C+S), sink, and between crawl and crawl/sink (C/C+S) swimming behaviors (Table 4; *see* Kiørboe and Bagøien 2005 fig. 1 for illustration of movement types). In *C. hamatus*, females spent the majority of their time looping (23%), cruising (20%), and in C/C+S swimming mode (54%), with only 3% of their time spent in crawl and sink behavior (Table 4). Swimming velocities for each movement type varied between ~3–5 mm s^{-1}, with a total average swimming velocity of 3.81 mm s^{-1}. *C. hamatus* males were not observed looping, and spent nearly all of their time in crawl and sink (46%) and C/C+S behaviors (50%). The total average swimming velocity was 2.48 mm s^{-1}, with averages for individual swimming types ranging between ~2.5 and 3.4 mm s^{-1}. Observations for *C. typicus* were similar to previous reports (Kiørboe and Bagøien 2005) and were reanalyzed here only to ensure comparability to *C. hamatus* measurements. *C. typicus* males spent less time cruising and more time looping than was found in the previous study (Table 4). Total average velocities for females and males were 5.5 and 3.9 mm s^{-1}, respectively.

Discussion

The qualitative and quantitative observations of heterospecific mating behavior reported here demonstrate that behavioral prezygotic reproductive isolation between *C. typicus* and *C. hamatus* is absent. Males of each species are able to detect, pursue, and capture females of the alternate species, and will, in some cases, place a spermatophore on the urosome of a genetically incompatible female. In the discussion below, I first consider the mating cues likely to play a role in species recognition in marine calanoids before

estimating mate-search volume capacities and the frequency of heterospecific and conspecific mate encounters in natural populations.

Species recognition in marine calanoids—Although diffusible pheromones have confirmed importance in the remote detection of copepod mates in the pelagic water column (e.g., Tsuda and Miller 1998; Weissburg et al. 1998; Kiørboe et al. 2005), results reported here indicate that these chemical mating cues contain no information regarding the species identity of the potential mate. Detailed analyses of male tracking behavior on diffusible pheromone trails of heterospecific and conspecific *Centropages* females indicated that males are able to detect and pursue pheromone trails of heterospecific females with comparable efficacy to conspecific female trails (Table 3). Males do not appear to be aware that they are pursuing heterospecific females prior to mate contact or capture. The observation that *T. longicornis* males also detect and follow pheromone trails laid by *C. typicus* females further implies that the pheromone signal is highly nonspecific and may be detectable by males of diverse species. Additional preliminary observations on mating interactions between *T. longicornis* and *Temora stylifera* also support the inference that the diffusible pheromone signal is not species specific (author's unpubl. obs.). Up to nine *Centropages* (Vervoort 1964; Bradford-Grieve and Markhaseva 1999; Continuous Plankton Recorder Survey Team 2004) and two *Temora* (Continuous Plankton Recorder Survey Team 2004) species co-occur in epipelagic waters of the North Atlantic, and this assemblage may be sexually cross-reactive. These results confirm early observations by Katona (1973) and Jacoby and Youngbluth (1983) that mating errors do occur and that males attempt mating with females in intergeneric as well as interspecific crosses. Experimental results on the frequency of heterospecific and conspecific mating behaviors are the first to suggest, in marine populations, that the frequency of "mating errors" may be high (Fig. 3A,B), with males pursuing heterospecific and conspecific females at comparable rates. This absence of a precapture mating barrier between species raises the possibility that males in natural populations spend considerable time or effort in pursuing, capturing, and holding heterospecific females.

Species recognition between *Centropages* potential mates occurs after the encounter and pursuit stages of copepod mating and likely results from species-specific chemical compounds detectable on the surface of the female exoskeleton or from morphological incompatibility between heterospecific mating pairs. Although males of both *Centropages* species were observed to readily attempt mating with heterospecific females, they rarely placed spermatophores on the female urosome, despite holding the females for up to 6+ minutes in the capture position (male A1 grasping caudal setae of female). Males were rarely observed to shift their grasp on the female to the copulatory position (described in Blades 1977), and hybrid crosses are probably deterred at this step in the mating sequence. Further observations on male–female interactions at this mating stage are required. The fate of the rare hybrid crosses observed is currently unknown; there may or may not be a failure of the fertilization tube, gametic incompatibility, or postzygotic isolation between species.

These planktonic copepods are unusual in using a remotely detected sex pheromone that lacks species specificity. Airborne sex pheromones in the Lepidoptera, arguably the most well-studied arthropod chemical communication system, are typically species specific, with distinct chemical compounds or blends used by each species (e.g., Löfstedt 1993; Roelofs and Rooney 2003; Howard and Blomquist 2005, and references therein). Although cross-attraction has been observed in some cases (e.g., Hendrikse 1986, 1988), field manipulations have found that females rarely attract heterospecific males under natural conditions because of differences in both pheromone composition and host plant use (Löfstedt 1993). Furthermore, interspecific mating interactions, or communication interference, has been demonstrated to exert directional selection on females to produce distinct pheromone blends (Groot et al. 2006). Sex and aggregation pheromones used by other insects are also often species specific (e.g., Symonds and Elgar 2004). Many noncopepod marine crustaceans are also known to use remotely detected sex pheromones to attract mates (e.g., Dunham 1978; Dunham 1988), but little information is available regarding their specificity.

We may consider why these planktonic copepods have not evolved species specificity of the diffusible sex pheromone, given the powerful demonstrations of such evolution in other arthropod (insect) systems. One primary element that will underlie selection toward species specificity is the frequency with which males encounter both conspecific and heterospecific females to which they are sexually attracted. The fitness cost of pursuing heterospecific females in the case of *Centropages* is expected to occur as a result of the time and energy spent in mating pursuit (spermatophore transfers are rare) and the potential increase in predation mortality. These fitness costs will be low if heterospecific females are rarely encountered in natural populations. To address this issue, I estimated encounter rates of heterospecific and conspecific mating pairs in North Sea populations of *Centropages*.

Male mate-search capacities—Given the motility and mating data reported above, I can now estimate mate-search volume rates of males of both species that are searching for heterospecific and conspecific females. I use the encounter model of Kiørboe and Bagøien (2005) for a cruising male pursuing an elongated female pheromone trail,

$$\beta_{\text{trail,cruiser}} = 2Lu_{2D}\left(\sqrt{\frac{D_pL}{v}} + S\right) \quad (1)$$

where L is the length of the pheromone trail, u_{2D} is the 2D swimming velocity of the male, D_p is the diffusion coefficient of the pheromone (taken here to be 10^{-5} cm^2 s^{-1}), v is the 3D swimming velocity of the female, and S is the sensory reach of the male (antennule length). Including average swimming speeds (u_{2D}, v), average male antennule lengths (S), and maximal observed pheromone trail lengths (L), mate-search volume rates (β) were

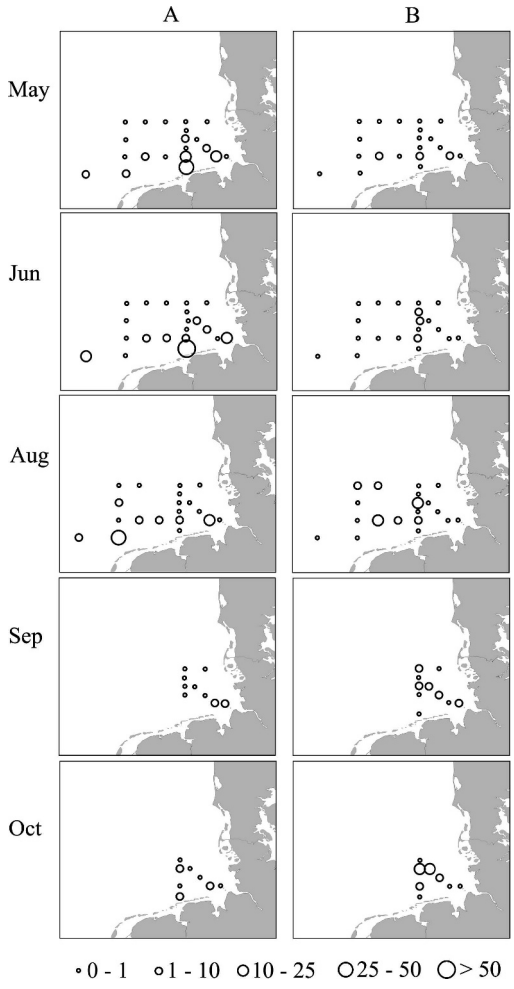

Fig. 5. Specific encounter rates for *C. typicus* females, with (A) heterospecific *C. hamatus* males and (B) conspecific males, in the southeastern sector of the North Sea between May and October 2004. Units are encounters female^{-1} d^{-1}. Magnitude of encounter rates as indicated in symbol legend. Maximum heterospecific and conspecific encounter rates are 104 and 16 encounters d^{-1}, respectively.

estimated to be 168 and 24 L d^{-1} for *C. typicus* and *C. hamatus* males, respectively, searching for conspecific mates, and 43 and 96 L d^{-1} for *C. typicus* and *C. hamatus* males searching for heterospecific potential mates. This general approach assumes that females produce a pheromone trail of constant length, with males encountering the trail at variable positions along the track line (variability in along-track distance, Tables 1, 2). Note that the results suggest that males of *C. hamatus* have a substantially higher search volume capacity for heterospecific than conspecific females because of the longer pheromone trails left by *C. typicus* females. This property makes *C. typicus* females more "chemically conspicuous" to mate-searching males of both species. *C. typicus* males, in contrast, have a heterospecific mate-search volume rate approximately one quarter of their conspecific rate. This difference affects the frequency at which females of each species are pursued by heterospecific males (*see below*).

Heterospecific and conspecific encounter rates in the field—Given the observation that males are approximately equally sexually reactive to females of both *Centropages* species, we can assume that males will pursue females of either species whenever they are encountered in the water column. Encounter rates between potential mates therefore provide a good estimate of heterospecific mating attempts in natural populations.

Encounter rates (E) of heterospecific and conspecific mating pairs can be estimated by combining the β search volume capacities reported above with abundances of adult males (C_M) and females (C_F), according to

$$E = \beta C_M C_F \quad (2)$$

Using adult abundances of both *Centropages* species in the North Sea from the spring, summer, and early fall of 2004, I find that (1) heterospecific encounter rates are up to ~2,000 encounters m^{-3} d^{-1} and are of the same order of magnitude as conspecific encounter rates during the same period (Fig. 4A–D) and (2) the highest rates of heterospecific mate encounter occur in late summer (August), when the species overlap maximally in time and space (Fig. 4A,B). These results imply that heterospecific encounters represent a large fraction of the mating events per day occurring during late summer. The temporal difference in maximum population abundance of the two *Centropages* species was noted previously in the Helgoland Roads and Continuous Plankton Recorder time series for the North Sea (Lindley and Reid 2002; Halsband-Lenk et al. 2004, and references therein) and results in substantial temporal isolation between the congeners. However, *C. typicus* is increasing in abundance in this sector of the North Sea relative to its long-term average (Lindley and Reid 2002; Beaugrand et al. 2007), and heterospecific mate encounters may also be increasing.

If specific encounter rates are considered ($E_F = \beta C_M$), *C. typicus* females experience the highest frequency of heterospecific mating attempts and will often be encountered by *C. hamatus* males at rates higher than conspecific males (Fig. 5A,B). Although the number of heterospecific encounters for individual *C. hamatus* males and females does not exceed 10 d^{-1}, *C. typicus* females will at times experience up to 100^{+} heterospecific encounters d^{-1} (Fig. 5A), while *C. typicus* males experience up to ~50 encounters d^{-1}. For comparison, conspecific encounters for *C. typicus* females and males do not exceed 20 d^{-1} (Fig. 5B), and *C. hamatus* female and male conspecific encounters are on the order of 30 d^{-1}. Thus, the larger bodied (greater pheromone producing) and faster swimming *C. typicus* may, at certain times and locations, experience higher levels of mating activity with heterospecific than conspecific potential mates and may bear the higher fitness cost for heterospecific mating. Selective mating behavior, or the ability to reject unwanted suitors, would therefore be of greater benefit in this species.

In summary, precontact mating cues used by *Centropages* copepods were found to be highly nonspecific, resulting in frequent heterospecific mating attempts between congenerics. Estimates of mating encounters between

heterospecific and conspecifics in North Sea populations show that, despite substantial temporal isolation between species, heterospecific encounters constitute a large fraction of the mating events per day during the late summer. Further analysis of the fitness costs of heterospecific mating attempts is required to fully understand why planktonic copepods have not evolved species specificity of the remotely detected sex pheromones, as have many other arthropods. Heterospecific mating interactions may be a common and important feature of the reproductive ecology of planktonic copepods.

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