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SHORT COMMUNICATION

Ocean-bottom krill sex

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For the first time the entire sequence of the mating behaviour of Antarctic krill (*Euphausia superba*) in the wild is captured on underwater video. This footage also provides evidence that mating can take place near the seafloor at depths of 400–700 m. This observation challenges the generally accepted concept of the pelagic lifestyle of krill. The mating behaviour observed most closely resembles the mating behaviour reported for a decapod shrimp (*Penaeus*). The implications of the new observation are also discussed.

KEYWORDS: Antarctic krill; mating behaviour; underwater camera; Southern Ocean; animation

The euphausiid crustacean, Antarctic krill (*Euphausia superba* Dana), is reputed to have the largest biomass of any single metazoan species on the planet, playing a key role in the structure and function of the Southern Ocean ecosystem. Antarctic krill serve as both important grazers and critical prey for whales, seals and seabirds (Everson, 2000). Krill are one of the best-studied species of pelagic animals, yet there are still considerable uncertainties about key elements of their biology with few published accounts of their *in situ* behaviour (Nicol, 2006). Reproductive behaviour, in particular, is poorly described. There are very limited descriptions of mating behaviour for this, or any of the 85 species of euphausiid, either in the field or the laboratory (Ross and Quetin, 2000). The only reported observation of reproductive behaviour made in the wild is by Naito *et al*. (Naito *et al*., 1986), who photographed mating behaviour of the surface swarms of Antarctic krill from the deck of a research vessel. Part of reproductive behaviour has also been reported in captive krill with observations of male krill chasing gravid female krill and making brief contact (Ross *et al*., 1987).

Here, for the first time, we report the entire sequence of mating behaviour of Antarctic krill in the wild captured on underwater video and then traced and interpreted using digital animation. This imagery at the same time provides evidence that mating behaviour can take place near the seafloor at depths of 400–700 m.

The traditional view of krill reproduction is that they mate and lay eggs in the surface layer (0–200 m). The embryos subsequently sink, then hatch at depths of 700–1000 m (Ross and Quetin, 1984) and the

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developing larvae actively swim upwards, reaching the surface in autumn (the "developmental ascent", Marr, 1962). There is, however, growing evidence of krill inhabiting much deeper water (Kawaguchi *et al.*, 1986; Gutt and Seigel, 1994; Clarke and Tyler, 2008), and our current observations reinforce the importance of the ocean bottom as a habitat for krill.

In this paper, we first describe the entire process of krill mating behaviour, and second, discuss the implication of our observation of this process occurring at the ocean floor.

Observations were conducted by using an autonomous submersible video camera [Benthic Impacts Camera System (BICS); Kilpatrick *et al.*, 2011] by lowering the system vertically to the seafloor. For details on the sampling gear, see Supplementary Material I.

Deployments of the underwater camera were conducted at 16 stations off East Antarctica from the *RV Aurora Australis* (between 6 and 8 January 2010; Table I).

Video footage of krill mating behaviour was digitally traced frame by frame, by using Flash animation software. Tracings of live krill were combined with animated drawings traced from illustrations of krill anatomy (Kirkwood, 1982).

The presence of Antarctic krill near the seafloor was confirmed for all the 16 stations where the camera gear was deployed, and at 14 of these krill occurred in high densities. In most of the cases, very high densities of krill surrounded the light source within 2 min after the camera reached the bottom.

Adult Antarctic krill can be recognized easily from their size and shape: mature male krill have an elongate shape and prominent antennae; gravid females are distinguished by their markedly swollen thorax (Clarke and Tyler, 2008). At all sites where krill were encountered at high densities, they were moving rapidly and many gravid females were observed. We frequently observed male krill chasing gravid females, which indicated a population of krill in an active reproductive state.

Conspicuous mating behaviour is apparent in video sequences that were captured, which show mating behaviour lasting for ~12 s (Supplementary Material II). The initial behaviour consists of "chase", "probe", "embrace" and "flex" (Fig. 1), which resembles the mating behaviour of decapod shrimp (*Penaeus*; Misamore and Browdy, 1996). The latter half of the mating behaviour observed here we refer to as "push" and this gesture seems to be specific to krill (Fig. 1, Supplementary Material III). Our observations also clearly show that two males can be involved in pursuing a single female at the same time.

Previous anatomical observations of krill indicate that the transfer of spermatophores is carried out by the use of special hooks developed in the front two pairs of male pleopods (petasma). The hooks fix the spermatophores in the female's genital area (the thelycum) while lying abdomen-to-abdomen "embracing" (e.g. Bargmann, 1937). However, the actual position adopted by krill during spermatophore transfer has not yet been subjected to detailed observation. Ross *et al.* (Ross *et al.*, 1987) wrote: "the contact point was the ventral surface of the female just behind the thelycum and the head of the male near the base of the antennae". Our observations lead us to speculate that this position is the prologue to mating. In decapod

Table I: Details of sites observed

Region	Sample code	Start date/time	Bottom time (h:mm)	Start latitude	Start longitude	End latitude	End longitude	Start bottom depth (m)	End Bottom depth (m)
SHELF BREAK	LC01	6 January 2010/16:34	0:13	−65.86	88.87	−65.86	88.87	452	462
CANYON	LC02	6 January 2010/17:57	0:14	−65.85	88.87	−65.85	88.87	535	562
	LC03	6 January 2010/19:29	0:06	−65.84	88.85	−65.84	88.85	837	869
	LC04	6 January 2010/20:28	0:06	−65.85	88.84	−65.85	88.84	578	576
	LC05	6 January 2010/22:00	0:46	−65.87	88.87	−65.86	88.85	400	417
	LC06	7 January 2010/1:00	0:23	−65.90	89.21	−65.90	89.19	393	416
	LL28	7 January 2010/9:57	8:22	−65.89	89.14	−65.88	89.06	493	422
	LC07	7 January 2010/20:56	0:17	−65.88	89.08	−65.88	89.08	507	481
	LC08	7 January 2010/22:58	0:18	−65.87	89.07	−65.88	89.07	588	571
	BTC29	8 January 2010/4:42	0:03	−65.87	89.03	−65.87	89.04	561	588
	LC09	8 January 2010/4:42	0:05	−65.85	89.32	−65.85	89.32	779	781
	LC10	8 January 2010/5:59	0:06	−65.86	89.34	−65.86	89.34	534	535
	LC11	8 January 2010/7:49	0:07	−65.84	89.42	−65.83	89.42	601	598
	LC12	8 January 2010/10:31	0:06	−65.84	89.53	−65.84	89.52	576	578
	LC13	8 January 2010/14:37	0:08	−65.73	89.97	−65.73	89.97	467	460
	LC14	8 January 2010/15:55	0:08	−65.72	89.97	−65.72	89.97	636	630
	LC15	8 January 2010/17:03	0:08	−65.72	89.97	−65.72	89.97	664	659
	LC16	8 January 2010/20:08	0:07	−65.82	89.53	−65.82	89.53	654	668
	BTC30	8 January 2010/22:45	0:05	−65.84	89.54	−65.83	89.54	547	502

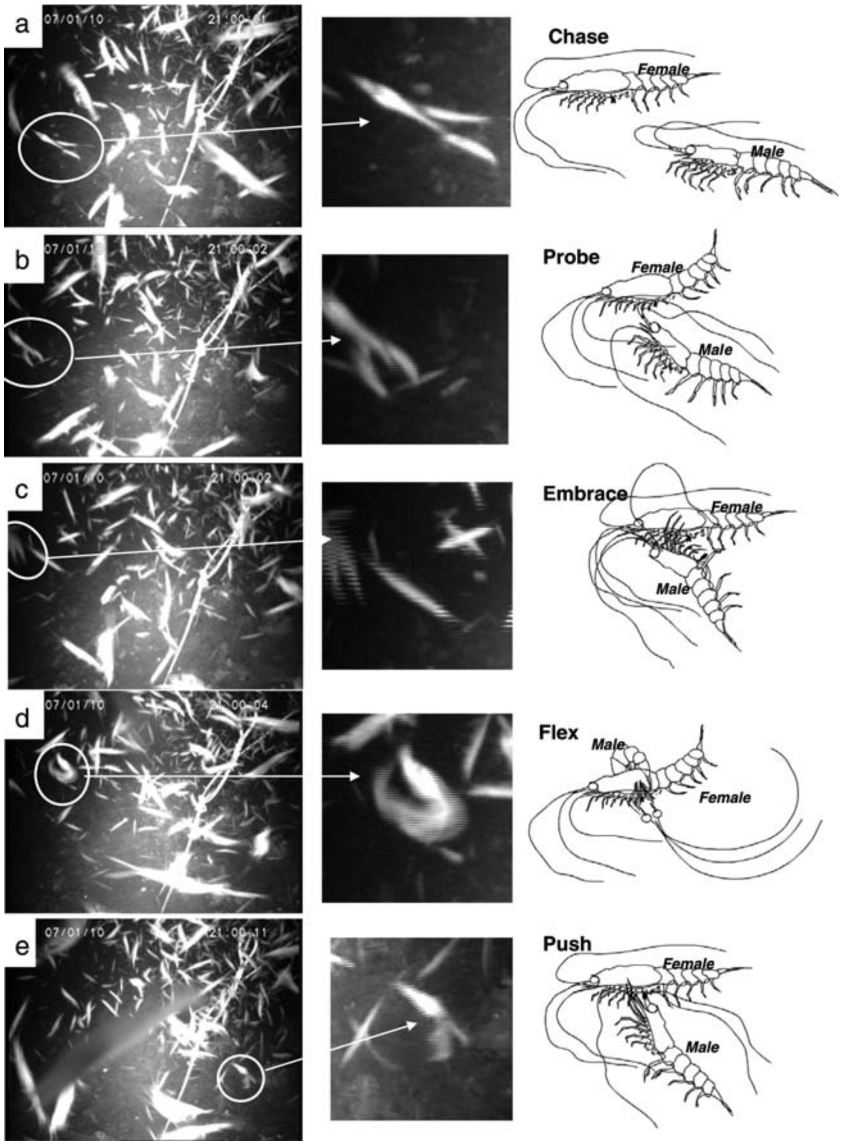

Fig. 1. Sequence of Antarctic krill mating behaviour. Left panels, frames from the video with mating krill circled; centre panels, close ups of mating krill and right panels, line drawings of each of the mating phase. (**a**) Chase, (**b**) probe, (**c**) embrace, (**d**) flex, and (**e**) push.

mating, the involvement of the antennae is thought to indicate the role of a sex pheromone (Misamore and Browdy, 1996).

In order to mate, male krill must first prepare the spermatophores on their first pair of petasmas (on their pleopods). This process is unlikely to take place until the male finishes the "chase", because preparing spermatophores on the pleopods (swimming appendages) for mating would significantly reduce his capacity to swim. However, while in the "embrace" position, the male's pleopods can be free to transfer spermatophore in the manner described in Bargmann (Bargmann, 1937): he can withdraw spermatophores from his genital pores, using the 2nd petasma, and then pass them to his 1st petasma for transferring to the female's thelycum.

Spermatophore transfer seems to take place towards the end of embracing position, or when the male wraps his abdomen around the female's abdomen ("flex" position; Fig. 1). On one occasion in the video footage (Supplementary Material II), two males appear to be involved in mating one female at the same time. The "flex" and "wrapping" gestures with rapid spinning, lasted for ~5 s, and there was only a limited opportunity within these 5 s for sexual organs to make contact in order for spermatophore transfer to occur.

After this act, both males appear to continue "pushing" with their rostrum/antennae against the female's ventral surface and to swim in larger circles for a further 6 s. During this period, one of the males can be seen to detach and swim away. This pushing behaviour is similar to the observation in Naito *et al*. (Naito *et al*., 1986); "a male krill chased a female and mated in the form of letter T, and the couple, keeping the same posture, swam in a circle".

In the field, we mostly see mated females with empty spermatophores, which suggest that spermatozoa are immediately emptied out of the spermatophores (Bargmann, 1937). The surfaces of spermatophores are covered with circular chitin plates which overlap; this type of surface structure allows the swelling and contraction processes of the spermatophore (Thomas and Nash, 1987). The post-mating pushing that we observed in krill, coupled with the flexible chitinous spermatophore surface, may work together as forces that assist the sperm mass in being efficiently extruded from the spermatophore. The exact role of this "pushing" behaviour, and the mechanism of how the sperm intrude into the thelycum, warrant further studies.

Contrary to the traditional view that post-larval krill are typically confined to the top 150 m of the water column with reproduction occurring in surface waters, this study shows the existence of a population of krill at 400−720 m depth and that mating can take place near the seafloor, although it is possible that mating was induced by the camera light. Recent developments in underwater equipment allow for wider deployment of underwater imaging systems, which has resulted in an accumulation of extensive evidence for the existence of populations of krill near the seafloor (Clarke and Tyler, 2008; K. Schmidt *et al*., submitted for publication). Observations reveal the existence of significant numbers of Antarctic krill feeding at abyssal depths and the presence of fully gravid females (Clarke and Tyler, 2008).

Antarctic krill are broadcast spawners and gravid females are thought to spawn shortly after mating. If krill are mating at depth, do they spawn their sinking eggs near the seafloor, or do they need to swim to the surface to release the eggs? Or do they spawn in mid-depth layers just to ensure eggs do not sink to the seafloor? Larvae need to start feeding 3 weeks after hatching, when they develop their feeding appendages and have consumed the energy inherited from their mother (Ross and Quetin, 1989). If females do spawn at depth, then they would need to choose areas with currents or upwelling that would help their larvae to reach the surface. Alternatively, krill larvae might be able to find suitable food at depth and may not need to undergo the developmental ascent (Marr, 1962).

The current conceptual understanding of the life history of krill has largely been based on research activities that focus on the pelagic zone. The upper reaches of the ocean (<200 m) are accessible for sampling by nets and acoustics (Kawaguchi and Nicol, 2007) and the general assumption is that an insignificant portion of the krill population lives below 200 m (Atkinson *et al*., 2009). Recent observations, including ours, are challenging this assumption and this may have considerable implications for understanding the Antarctic marine ecosystem and for management of the krill fishery.

Our study for the first time described the entire sequence of Antarctic krill's mating behaviour and reveals that the process is similar to the general mating behaviour reported in decapod shrimp (*Penaeus*). At the same time, our observation raises important questions about the life history of krill as well as their population structure.

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

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