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Mating behaviour and reproductive cycle of *Archaster typicus* (Echinodermata: Asteroidea)

J.-Q. Run [1]*, C.-P. Chen [2], K.-H. Chang [1,2] and F.-S. Chia [1]**

[1] Institute of Marine Biology, National Sun Yat-sen University, Kaohsiung, 80424 Taiwan, Republic of China
[2] Institute of Zoology, Academia Sinica, Nankang, Taipei, 11529 Taiwan, Republic of China

Abstract

This study examines the reproductive cycle, the mechanism of male-on-female pairing behavior and the spawning behavior of *Archaster typicus* Müller et Troschel. Field studies were conducted in the intertidal zone of the sand beaches at Penghu, Taiwan (23 32'N; 119 33'E) at ebb-tide in 1984 and 1985. The pairing behavior of *A. typicus* is a reproductive behavior which leads to simultaneous spawning, increasing the probability of fertilization. As the breeding season approaches, sea stars, especially males, display increased mobility. Because only males tend to mount another individual, and because males can detect the sex of another individual by contact with the side of their arms, a male-on-female pair is formed when a male encounters a female. Eighty-five percent of the sea stars observed were paired during the pairing season. Spawning by a paired female is closely followed by spawning of its paired male; male spawning, however, does not induce spawning in its paired female. During spawning, the male turns slightly so that its arms overlap the arched arms of the female. The gonad volume of males is much less than that of females. This may result from the high efficiency of fertilization in this species, which does not require a large amount of gametes to be released, or from the higher energetic demands made on the males.

Introduction

Successful fertilization is a critical step in the life-history of all free-spawning marine invertebrates (Thorson 1946, Chia 1974). The percentage of fertilization of echinoid eggs is very low in the field when spawning males and females are 10 cm or more apart (Pennington 1985). Thus, many free-living organisms have developed aggregating behavior during breeding seasons and/or synchronized spawning to increase the probability of fertilization.

Some sea stars show some degree of reproductive aggregation and spawning synchrony (Tyler et al. 1982), but only two species, *Archaster typicus* (Boschma 1924, Mortensen 1931, Ohshima and Ikeda 1934a, b, Clemente and Anicete 1949, Komatsu 1983) and *A. angulatus* (Mortensen 1931), undergo a male-on-female pairing behavior during the breeding season. The present study was undertaken to examine the reproductive cycle, the mechanism of pairing behavior and the spawning behavior of *A. typicus* Müller et Troschel.

Materials and methods

Field studies were conducted during 1984 and 1985 in the intertidal zone of the sand beaches at Penghu, Taiwan (23 32'N; 119 33'E) when *Archaster typicus* Müller et Troschel were covered by shallow water (below 30 cm) at ebb-tide. The top and bottom individuals of the pairs were designated "M" (male) and "F" (female), respectively. Those individuals observed during the non-pairing season were designated "S" (single), since sex could not be determined in the field by external features.

Reproductive cycle

Twenty sea stars (20 singles, or 10 pairs) were collected monthly. The sea stars were damp-dried and weighed; the gonads were removed and their volume was determined by water displacement in a graduated cylinder. Gonad indices (GI) were calculated as the ratio of gonad volume to body wet weight according to the methods of Farmanfarmaian et al. (1958).

* Present address: Institute of Zoology, Academia Sinica, Nankang, Taipei, 11529 Taiwan, Republic of China
** Present address: Department of Zoology, University of Alberta, Edmonton, Alberta T6G 2J9, Canada

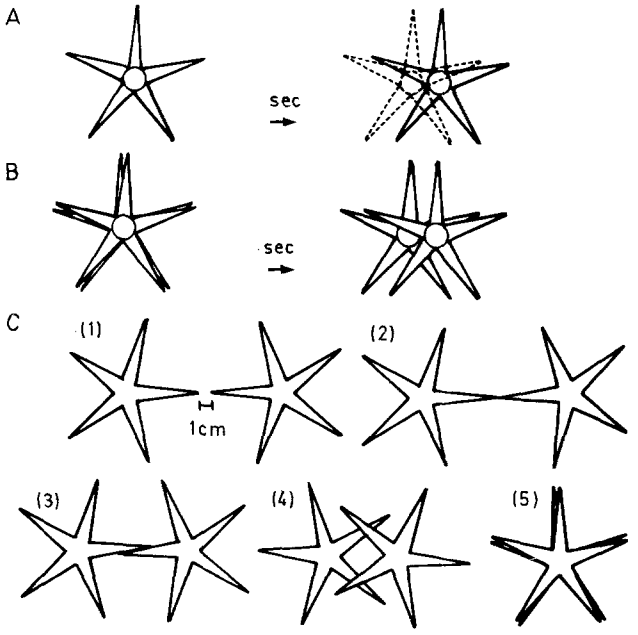

Fig. 1. *Archaster typicus*. Positioning of sea stars in experiments on tendency of individuals to move (A), pair (B), and recognize sex (C). (A) Tendency to move: male or female of pair is placed on sand; time that sea star stays buried until its disc has completely moved from center of burial site is measured. (B) Tendency to pair: two sea stars are placed one on top of the other, with arms overlapping; time that disc of top individual remains until it moves away from disc of bottom individual is measured. (C) Sex recognition: two individuals (M/M or M/F) are placed together in one of five ways: (1) separate, (2) arms tip to tip, (3) arms side by side, (4) arms on top of arms, (5) disc on top of disc; the number of pair-assemblages in each treatment is recorded

Pairing season

The number of sea stars within a 100 m^2 area was counted monthly during the low-tide period of spring tides. The pair index was calculated as the percentage of paired individuals to total number.

Locomotion rate before pairing season

The locomotion rates of moving sea stars were monitored in March, April and May of 1985. The distance that an individual travelled over an interval of 2 min (in April) or 1 min (in May) was measured by using the disc center as the reference point. Because sea stars tend to decrease their locomotion rate before stopping, the data of those sea stars that stopped within the measured interval or the next interval were deleted.

Tendency to move

During the pairing season, when individuals of a pair are separated and placed on the sand, each displays the following behavioral sequence: partial burial in the sand, a period of quiescence, emergence, and then movement away from the burial site. Those sea stars which display a higher tendency to move stay quiescent in the sand for a shorter period. Therefore, tendency to move was determined as that time in which they stayed quiescent after being placed on the sand until their discs moved away from the centers of the burial sites (Fig. 1 A). Because experiments were conducted during the low-tide period, time available was limited and our determinations were limited to 5 min per individual; preliminary observations had indicated 5 min to be a sufficient period to follow this behavioral sequence. The length of the period of quiescence of the male and its paired female was compared by the Wilcoxon signed-ranks test (Sokal and Rohlf 1981).

Tendency to pair

Male sea stars mount females during the pairing season, but females were never observed to mount other individuals. In order to detect the tendency to pair of male (M) and female (F) sea stars in the pairing season and of single sea stars (S) in the non-pairing season, individuals were placed together in the field (Fig. 1 B) in the following five combinations: (1) M/M; (2) M/F; (3) F/M; (4) F/F; (5) S/S (M/F indicates that M is on top of F, etc.). The time for the disc of the top individual to completely move away from that of the bottom individual was measured. Each measurement was conducted over a period of 2 min because of the limited amount of time available.

Sex recognition

Either two males (M/M) or a male and a female (M/F) were placed on the sand in the field in one of the following five ways: (1) separate, (2) arms tip to tip, (3) arms side by side, (4) arms on top of arms and (5) disc on top of disc (Fig. 1 C). The number of pair assemblages was recorded in each treatment. A pair assemblage is defined as the mounting of one individual on the other with their arms interlocked. In the treatment of "disc on top of disc", the pair had to remain in position for at least 2 min. The number of pair assemblages between M/M and M/F in each treatment was compared by the χ^2 test of independence (Sokal and Rohlf 1981). In addition, in the treatment of "arms side by side", the time that male sea stars stayed quiescent in the sand from the beginning of being placed on the sand until their discs moved away from the centers of the burial sites was recorded.

Spawning behavior and percentage fertilization

In the laboratory, 30 μg of 1-methyladenine (1-MeAde) dissolved in 2.5 ml filtered sea water was injected into the coelomic cavity (0.5 ml per arm) of each paired individual to induce spawning (Kanatani 1969). Four treatments were designed: (1) paired individuals were separated and placed in

separate aquaria and then injected; (2) paired individuals were not separated and both male and female were injected; (3) paired individuals were not separated but either the male or the female was injected; (4) paired individuals were separated, the male was removed and the female was injected; the female was removed after it had spawned, and then the original paired male was returned to the aquarium where the female had spawned. For each treatment, more than three pairs were examined. The sequential behaviors of the sea stars were recorded after injection. The spawning behaviors of the sea stars were also observed in the field.

To investigate fertilization success of the pairs, the female of a pair was injected with 1-MeAde in the field. Fifteen minutes after spawning, eggs were collected close to the gonopores (within 1 cm) and fixed in 5% formalin. The eggs with elevated fertilization envelopes were counted for assaying the percentage of fertilization.

Results

Reproductive cycle

The mean GIs of female and male *Archaster typicus* over the reproductive cycle varied from 0.2 to 10.1 and from 0.1 to 2.1, respectively (Fig. 2A). The GIs of both males and females dropped sharply between June and July, remained very low from August through February of the following year, increased from March, reaching a peak in June. This indicates that the spawning season of *A. typicus* is in late June and July.

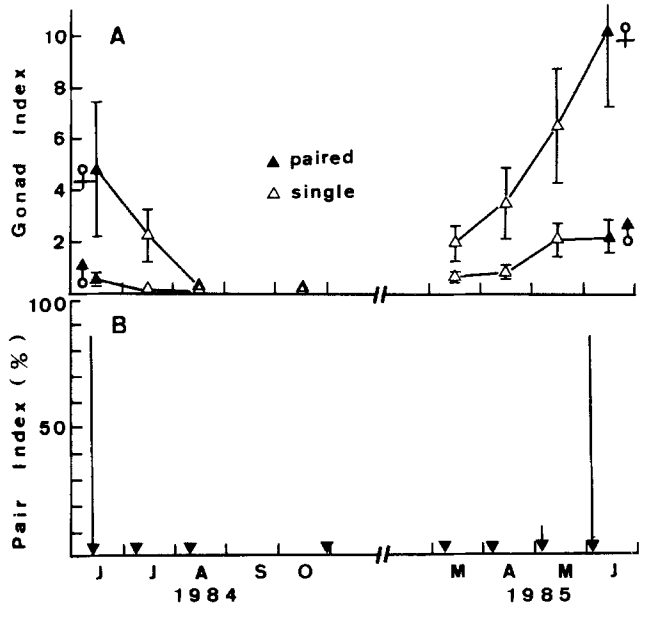

Fig. 2. *Archaster typicus*. Reproductive cycle and pairing season. (A) Monthly mean gonad index; vertical bars show 95% confidence limits around mean. (B) Monthly pairing index (% paired individuals of total number)

Pairing season

Pairing activity commenced in early May, reached and maintained a peak from May to June with a pair index about 85%, decreased in July, and ended in August (Fig. 2B). Pairing of *Archaster typicus* commences after the size of testes has increased to a maximum, about 2 mo before spawning.

Locomotion rate before pairing season

In March 1985, many impressions of sea stars were found on the sand surface, but no moving sea stars were observed, suggesting that they were not very active at that time. More sea stars were seen in the field in April and May. The mean locomotion rate was 15.1 (SD = 5.0, $n = 17$) cm min^{-1} in April and 24.9 (SD = 7.8, $n = 12$) cm min^{-1} in May. The pairing season commenced subsequently. Sea stars increase locomotory activities from March to May just before the pairing season.

Tendency to move

The average time that a sea star stayed buried after being separated from its partner was about 132.3 s for males and about 196.1 s for females (Table 1). The period of quiescence was significantly less ($p < 0.05$) for males than for females, suggesting that males have a higher tendency toward movement.

Tendency to pair

During the pairing season, a male placed on top of another individual of either sex with arms overlapping remained for 110 s (SD = 28, $n = 14$: M/M) and 117 s on average (SD = 10,

Table 1. *Archaster typicus*. Time that seastars remained in their burial sites after being separated from partners and placed on sand during pairing season. Plus sign indicates that individual was still buried at end of 300 s observation period

Pair No.	Burial time (s) of:		Pair No.	Burial time (s) of:	
	♂	♀		♂	♀
1	50	91	11	118	300+
2	54	48	12	127	143
3	55	300+	13	138	163
4	60	60	14	165	98
5	60	300+	15	168	120
6	75	105	16	230	300+
7	79	84	17	260	300+
8	86	239	18	278	300+
9	101	300+	19	300+	300+
10	110	175			
Mean				>132.3	>196.1

$n=12$: M/F) before its disc moved away from that of the bottom individual. The data were restricted to the observation period of 2 min per individual. However, most males did not move away within 2 min (12 out of 14 individuals for M/M, 11 out of 12 for M/F). The time for a female in the pairing season in an identical set-up was 32 s (F/M, SD = 35, $n=15$; 1 individual did not leave within 2 min) or 32 s (F/F, SD = 25, $n=14$), similar to the time displayed by single individuals of either sex during the non-pairing season (S/S, mean = 38 s, SD = 31, $n=22$; 2 individuals did not leave within 2 min). These results indicate that during the pairing season only males tend to remain paired with another individual, even a male, females do not. During the non-pairing season, individuals of neither sex remain paired.

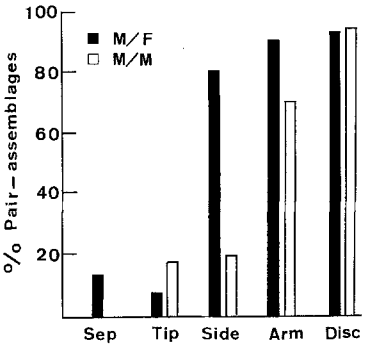

Fig. 3. *Archaster typicus.* Percentage of pair-assemblages occurring when individuals were placed separately in field (Sep), with tips of arms touching (Tip), with side of arms touching (Side), with arms superimposed (Arm), with discs superimposed (Disc)

Sex recognition

The percentage of pair assemblages was directly correlated with the amount of body-surface contact of the sea stars (Fig. 3). When a male did not contact another individual (separate), or only the tip of arm was in contact (tip to tip), the percentage of pair assemblages was low. When a male was placed on top of another sea star (arms on top of arms, disc on top of disc), a much higher percentage of pair-assemblages occurred. The percentages for M/M and M/F were not significantly different, except in the case of "side by side" contact ($p<0.05$). When a male contacted a female with the side of its arms, the male moved away from its own burial site in an average of 77 s (Table 2). In most cases (80%), after leaving their sites males mounted the females. However, when a male contacted another male, both males sank into the sand and remained quiescent for a long period. Generally, one individual emerged first, the other remaining buried for longer than 5 min observation period. These results suggest that *Archaster typicus* can recognize the sex of the encountered individual through contact of the sides of its arms.

Table 2. *Archaster typicus.* Time that males stayed buried after contacting other individuals of either sex with side of arm. 1st and 2nd mover: time (s) taken by each of two males to emerge and move away. Asterisk indicates that male and the contacted individual subsequently paired. Plus sign indicates that male was still buried at end of 300 s observation period

Individual No.	Burial time (s) after contact with:		
	female	male	
		1st mover	2nd mover
1	20*	31	89
2	29*	39	300+
3	40*	59	300+
4	68*	67	300+
5	77*	100	300+
6	95	101*	222*
7	97*	155	300+
8	106*	234*	300+*
9	108	300+	300+
10	133*	300+	300+
Mean	77	>139	>271

Spawning behavior and percentage fertilization

Separated males and females shed gametes independently when injected with 1-MeAde. When both sea stars of a pair were injected with 1-MeAde, the male remained on top of the female with its arms alternating with those of the female. After about 20 min, the female gradually arched its arms and began to spawn through the serial gonopores on the aboral surface of the arms. Meanwhile, the male turned slightly to one side, so that its arms were placed just above those of the female, and also began to spawn. This position was held for about 20 min (Fig. 4).

Identical spawning behavior was observed when only the female of the pair was injected. On the other hand, when the male of the pair was injected alone, the female did not spawn although sperm were released from the male. It is evident that males are induced by female spawn, but not the other way round.

Male sea stars were induced to spawn when placed in a container in which a female had just spawned and then been removed, indicating that the male is stimulated chemically to spawn by the eggs or by other chemicals discharged with the eggs.

The results of each treatment listed above were replicable. In addition, some pairs of *Archaster typicus* were observed spawning in nature. Their spawning behavior was consistent with that in the laboratory. In the field, 95% of eggs collected 15 min after induced spawning of pairs, were fertilized.

Discussion

This study has shown that the male-on-female pairing behavior of *Archaster typicus* is a mating behavior which leads to simultaneous spawning with complicated spawning be-

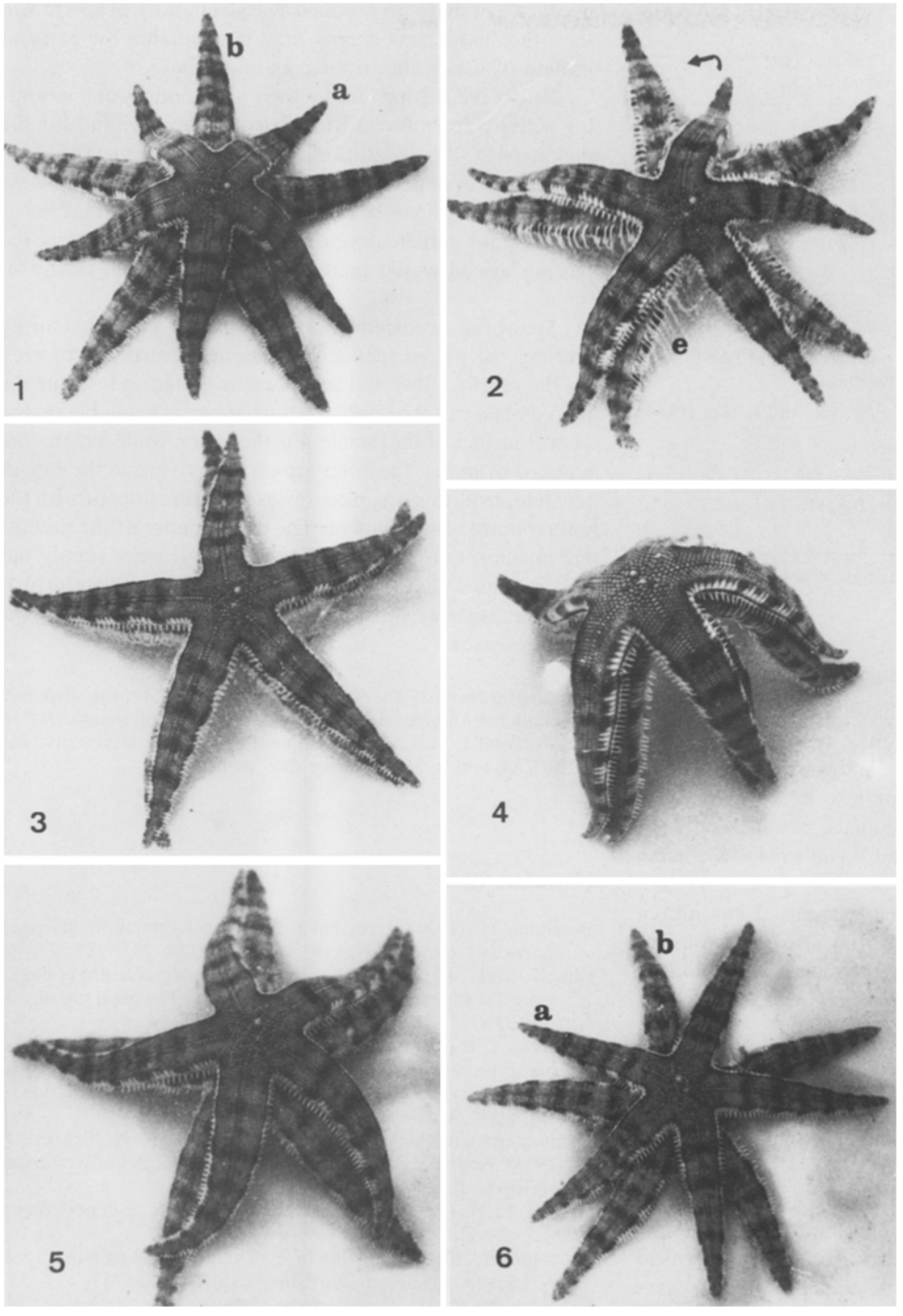

Fig. 4. *Archaster typicus*. Male-on-female pairing and spawning behavior. 1: Male on top of female (M/F) with their arms positioned alternately; a and b indicate male arm and female arm, respectively. 2: 20 min after being injected with 1-MeAde, female gradually arches its arms and begins to release eggs (e); meanwhile, male turns slightly to one side, as indicated by arrow. 3, 4: Male has placed arms just above those of female and begins to spawn. 5: After spawning, male turns again and female flattens its arch. 6: Arms are again positioned alternately (a, b as in Fig. 4:1)

havior, increasing the probability of successful fertilization. As the breeding season approaches, sea stars increase their locomotory activity, with males displaying a higher tendency for movement. At the study site, the population density of *A. typicus* was about three individuals per square meter; thus, the possibility of male/female encounters should be high for these large (length from anus to arm tip, $R_1 = 7$ cm) sea stars. Because the males recognize the sex of individuals encountered through contact with the sides of their arms, and because only males mount another individual, male-on-female pairing occurs when a male encounters a female. When two males meet, each buries itself in the sand for some

Table 3. Sea stars. Ratio of maximal gonad index (GI) of females to males calculated from the literature

GI ratio	Species	Source
4.8	*Archaster typicus*	This study
2.1	*Asterias vulgaris*	Lawrence and Lane (1982)[a]
1.9	*Oreaster reticulatus*	
1.8	*Pisaster giganteus*	
1.8–1.2	*Asterias rubens*	
1.5–1.3	*Pisaster ochraceus*	
1.3	*Pisaster brevispinus*	
1.2	*Asterias amurensis*	
1.2	*Bathybiaster vexillifer*	Tyler et al. (1982)
1.1	*Leptasterias pusilla*	Lawrence and Lane (1982)[a]
1.0	*Astropecten latespinosus*	
1.0	*Astrostole scabra*	
1.0	*Marthasterias glacialis*	Nichols and Barker (1984)
0.9	*Oreaster hedemanni*	Lawrence and Lane (1982)[a]
0.6	*Solaster stimpsoni*	
0.5	*Echinaster echinophorous*	
0.4	*Echinaster* sp.	
0.4	*Leptasterias hexactis*	

[a] GI ratios calculated from data on maximum gonad indices cited in this review

period of time. However, males have such a high urge to mount that occasionally male-on-male pairing does occur, whereas female-on-female, or female-on-male pairing does not. This male mounting behavior also explains observations of piles of three to five individuals, of which only the bottom one is female (Ohshima and Ikeda 1934a, Komatsu 1983).

Contact chemoreception is well recognized among sea stars (Hyman 1955), and this ability may also be responsible for intraspecific behaviors of aggregation and avoidance (Sloan 1984). Therefore, we believe that *Archaster typicus* recognizes the sex of another individual by contact-mediated chemoreception. Mayo and Mackie (1976) have reported that the tissue extract of the marginal and furrow spines of *Crossaster papposus* can induce avoidance behavior in *Asterias rubens*. In *Archaster typicus*, the marginal and furrow spines are located at the sides of the arms, and it is therefore reasonable to assume that the areas of contact chemoreception enabling sex recognition are located there.

The pairing and spawning behavior of *Archaster typicus* enables the synchronous release of gametes from both sexes in close proximity. Moreover, the serial arrangement of gonopores in *A. typicus* enables a large number of gametes to be released during a short time-period (Tyler and Pain 1982). The eggs tend to aggregate at the spawning site as they are heavier than water. These facts ensure that the gametes are well mixed and increase the probability of fertilization.

The GI of *Archaster typicus* is lower for males than for females. The maximum female:male GI ratio is 4.8, which is much higher than that recorded for other species of sea stars (Table 3). This great difference in male and female GIs in *A. typicus* may be due to the high efficiency of fertilization resulting from their pairing behavior, which reduces the necessity for males to produce a large quantity of sperm. On the other hand, less energy may be available for gonadal growth of males due to their mating behavior:

Before the pairing season, their high motility and mounting activity constitute an increased energy demand for the males. Also, in preparation for the subsequent energy requirements in maintenance of the mounting position, some energy ingested may be dispatched to storage so that less is available for gamete development. The male GI before the pairing season would therefore be less than that of the female.

Spawning occurred about 2 mo after pairing. During pairing, the paired female continuously everts its stomach on the sand to feed and increases its gonad index, but the paired male can feed only from whatever is available on the aboral surface of the female. Furthermore, while females are covered by males, the latter are exposed to air, in the sun, at low tide, requiring again energy expenditure to cope with the unfavourable environment. The maintenance of the mounting position not only depletes the males, energy supply but also increases their energy deficit. The foregoing are probably the reasons why the male GI does not increase during the pairing season.

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