Presence of spawn-inducing pheromones in two brittle stars (Echinodermata: Ophiuroidea)

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ABSTRACT: Possible roles of pheromones in inducing spawning of 2 brittle stars, *Ophiocoma dentata* and *Ophiocoma scolopendrina*, were tested with gonad homogenates. In both species, the ovary homogenate induced conspecific, competent males to spawn. The potential pheromone was species-specific and had no effect on conspecific females. The testis homogenate did not have any effect. Of the males 50% could be induced at an estimated concentration of 7.8×10^{-7} g ml^{-1} (wet ovary weight/seawater) with a threshold concentration at 10^{-8} g ml^{-1}. Some males could be induced to spawn at least 3 consecutive times at 1 d intervals. The unidirectional induction, i.e. males induced by female releasate, suggests high selective pressure of sperm limitation in these 2 species.

KEY WORDS: Pheromones · Brittle star · Spawning · Species-specificity

INTRODUCTION

Only in aquatic and marine environments do we find both males and females broadcasting gametes for external fertilization (Strathmann 1990). One potential problem ensuing from this mode of reproduction is the dilution of gametes in the water column, which may prevent or lower rates of successful fertilization (Denny 1989, Yund 2000). Many tactics may be adaptive for this challenge in marine environments (Levitan & Petersen 1995). For example, egg size increase may improve fertilization rate; whereas both sperm speed and duration of activity can enhance the probability of encountering an egg (Levitan 1993, 2000). Since the extent of spatial separation may determine the fertilization rates of benthic organisms (Downing et al. 1993), aggregative behavior of adults is expected in many mobile species during the reproductive season (e.g. Pennington 1985, Shepherd 1986). The most obvious tactic for sessile organisms, however, may be the synchronized release of gametes that occurs in many species (e.g. Harrison et al. 1984).

Synchronization of spawning may involve physical and biological cues from the environment (e.g. Himmelman 1980, Starr et al. 1990, Hastie & Young 2003).

Endogenous mechanisms of biological clocks may also be involved (e.g. Fong 1993). One problem these universal cues present in the environment is that other species may exploit them, too. Thus, mechanisms to avoid out-crossing may be necessary. Moreover, synchronized release of gametes can effectively increase the fertilization rate only if spawning individuals are close by (Downing et al. 1993). If local densities of the population are low and there is no aggregation of organisms before spawning, simple synchronization may not be enough to bring gametes together from distant individuals (Levitan 2004). Inductive mechanisms involving detection of spawns of other individuals, especially those of the opposite sex of the same species, may evolve under such selective pressures (e.g. Beach et al. 1975, Zeeck et al. 1988, Painter 1992, Hardege et al. 1996). Gametes may be released only when those of others are around.

Under the sperm limitation situation, we are more likely to find eggs released first and sperm released later. If sperm were released first, their concentration could be too low to fertilize the eggs when they are released. Under this circumstance, the most practical mechanism to evolve would be for males to be induced to spawn by females.

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An alternative possibility is when sperm competition imposes stronger selection, forcing males to compete with one another to reach unmated females. In this case, the sperm has to compete for unfertilized eggs (see Yund & McCartney 1994, Yund 1998). Late sperm would encounter eggs that have been fertilized. Earlier release of male than female gametes would be expected in this situation; thus, the induction mechanism would more likely be male releasate inducing females to spawn.

Evidence can be obtained from at least 2 kinds of approaches to distinguish whether sperm limitation or sperm competition imposes stronger selection on the life-history evolution of a species. The first is to observe the timing of gamete release between males and females of a species. The second is to determine the direction of induction, if there is one. The advantage of the first approach is that the timing difference between males and females is likely to evolve before induction direction. The advantage of the second approach is that it can be tested in laboratories, resulting in less uncertainty and ambiguity than characteristics observed in the field.

To investigate the above hypotheses, we studied several species of marine invertebrates. It is obvious that not all broadcasting species are suitable for this purpose. For example, the candidate species must be gonochoristic so that any difference in the timing of gamete release can be easily observed and will not be attributed to other constraints. A reasonably long spawning season is required so that multiple observations are possible. Easy handling and culturing in the laboratory are additional requirements for the test organisms. After preliminary tests on small abalones *Haliotis diversicolor*, giant oysters *Crassostrea gigas*, clams *Meretrix lusoria*, and the sea urchin *Tripneustis gratilla*, we found 2 brittle stars (*Ophiocoma dentata* and *Ophiocoma scolopendrina*) with unambiguous patterns.

MATERIALS AND METHODS

Two congeneric species of broadcast-spawning brittle stars, *Ophiocoma scolopendrina* (Lamarck) and *O. dentata* (Müller and Troshel), are abundant on the reef flats of southern Taiwan. *O. scolopendrina* inhabits crevices in the intertidal zone. It extends its arms to the water surface at low tide. *O. dentata* is more cryptic, but is common under rocks at 1 to 5 m depth (Applegate 1984). Both species have intermediate disc sizes for brittle stars. They tend to stay quietly in plastic cups. The genders of mature individuals can be assessed by checking the slits between arms; males have white spermaries, whereas females have red

ovaries. These species have a reproductive period from April to July in southern Taiwan (Chang 1999).

Intraspecific induction. Male and female individuals of both species were collected from May to July in 1997 and 1998 during the spawning period at the reef flats of southern Taiwan (22° 00′ N, 120° 42′ E). Each individual was kept in a 300 ml cup with 100 ml of seawater. Ovaries and testes were dissected from 5 individuals of each sex and homogenized separately after tearing off the mesenteries and stirring in seawater. The stock solution had a concentration of 1% (1 g wet weight of gonad in 100 ml of seawater). Three treatments were used: ovary homogenate, testis homogenate, and seawater as control. At least 10 individuals of each sex of each species with fully developed gonads were used in each of the 3 treatments above. A positive response usually occurred within 5 s of the addition of the test solution. Clouds of gametes were ejected from the genital slits following strong pumping action of the discs. If no immediate response was observed, the specimens were kept in the individual beakers and the murkiness of seawater was checked regularly for signs of gamete release. In 1998, after this primary test, individuals without response to the testis treatment and those in the control group were treated with ovary homogenate.

Interspecific induction. Mature male *Ophiocoma dentata* were kept in individual containers with seawater. At least 10 individuals were used in each treatment, with each receiving 1 ml ovary stock solution of either *O. dentata* or *O. scolopendrina*. This experiment was repeated with *O. scolopendrina* as the subject.

Induction by different body parts. Five female *Ophiocoma dentata* were dissected into ovaries, arms, and discs. Homogenates of each body compartment (1 g wet wt 100 ml^{-1} seawater) were prepared. Ten *O. dentata* were used in each treatment following the same procedures as described previously. This experiment was repeated using *O. scolopendrina*.

Repeated spawning of male *Ophiocoma dentata*. Seventy male *O. dentata*, collected during the reproductive season and kept separated in individual containers with seawater, were treated with 1 ml ovary homogenate. Those that spawned were kept in aquaria for 24 h before repeating the assays. The process was repeated for the third time after another 24 h. This test was only done with *O. dentata* since it was more abundant.

Threshold dosage in *Ophiocoma dentata*. Forty competent male *O. dentata* were selected by testing for spawning with a 1 ml ovary homogenate the previous day. Seven concentrations of ovary homogenates (from 10^{-4} to 10^{-10} g ml^{-1}) were prepared by diluting the stock solution with filtered seawater. Five individuals were tested in each concentration with blank seawater as an extra control. This test was only done with *O. dentata*.

RESULTS

Intraspecific induction

In this investigation, 12 of the 33 male *Ophiocoma dentata* (Table 1) and 10 of the 35 male *O. scolopendrina* (Table 2) were induced to spawn by the ovary homogenate. None of the males spawned in the seawater control (n = 30) or under the testis homogenate (n = 30). Of those treated with testis homogenate and the seawater control, 16 of the 40 males of *O. dentata* and 12 of the 40 males of *O. scolopendrina*

Table 1. *Ophiocoma dentata.* Number of males responding to gonad homogenates; only 1 individual released eggs in similar tests with females in the same periods (total n = 90). Numbers in parentheses indicate individuals responding to ovary homogenate after the primary test

Test time	Response (released sperm)	Seawater (control)	Testis homogenate	Ovary homogenate	p (χ^2-test)
July 1997	No	10	11	9	0.02
	Yes	0	0	4	
May 1998	No	10 (5)	10 (7)	4	<0.01
	Yes	0	0	6	
June 1998	No	10 (1)	10 (3)	8	0.12
	Yes	0	0	2	

Table 2. *Ophiocoma scolopendrina.* Number of males responding to gonad homogenates; none of 90 individuals released eggs in similar tests with females in the same period. Numbers in parentheses indicate individuals responding to ovary homogenate after the primary test

Test time	Response (released sperm)	Seawater (control)	Testis homogenate	Ovary homogenate	p (χ^2-test)
July 1997	No	10	15	13	0.17
	Yes	0	0	2	
May 1998	No	10 (1)	10 (6)	7	00.04
	Yes	0	0	3	
June 1998	No	10 (4)	10 (1)	5	<0.01
	Yes	0	0	5	

Table 3. *Ophiocoma scolopendrina, O. dentata.* Number of males responding to ovary homogenate of different species. Numbers in parentheses indicate individuals responding to ovary homogenate of its own species after the primary test

Species	Response (released sperm)	Ovary homogenate of *O. scolopendrina*	*O. dentata*	p (χ^2-test)
Male *O. scolopendrina*	No	7	10 (4)	0.06
	Yes	3	0	
Male *O. dentata*	No	10 (5)	4	<0.01
	Yes	0	6	

that did not spawn were later induced to spawn by ovary homogenate (Tables 1 & 2). This indicated that the frequency of competent male brittle stars in each treatment was not different (p > 0.05, chi-square tests) and that ovary homogenate contained the effective substance to induce male spawning. In both species, responding males always spawned within 10 s after the addition of ovary homogenate. The response was immediate if the brittle stars were close to the surface.

Only 1 of the 30 female *Ophiocoma dentata* and none of the 30 female *O. scolopendrina* spawned when treated with testis homogenate. None of the females spawned in the ovary homogenate treatment or the seawater control.

Interspecific induction of spawning

A total of 6 of the 10 male *Ophiocoma dentata* and 3 of the 10 male *O. scolopendrina* spawned when treated with intraspecific ovary homogenate. None spawned when treated with interspecific ovary homogenate (Table 3). After the primary test, 5 of the *O. dentata* and 4 of the *O. scolopendrina* that did not spawn in the primary tests using interspecific homogenate spawned after adding intraspecific ovary homogenate (Table 3).

Induction by different body parts

A total of 5 of the 10 male *Ophiocoma scolopendrina* and 2 of the 10 male *O. dentata* spawned when treated with ovary homogenate. None spawned when treated with arm or disc homogenates. The difference in *O. scolopendrina* was statistically significant (p < 0.01, chi-square test), but not in *O. dentate* (p = 0.12, chi-square test). After the primary test, 1 to 3 of the individuals that did not respond to disc or arm spawned after adding ovary homogenates (Table 4).

Repeated spawning by male *Ophiocoma dentata*

Of the 70 male *Ophiocoma dentata*, 44 (63%) spawned after the first exposure to ovary homogenate in the labora-

Table 4. *Ophiocoma scolopendrina*, *O. dentata*. Number of males responding to homogenates of different body parts of conspecific females, in June 1998. Numbers in parentheses indicate individuals responding to ovary homogenate after the primary test

Species	Response (released sperm)	Homogenate of			p (χ^2-test)
		Ovary	Disk	Arm	
O. scolopendrina	No	5	10 (1)	10 (1)	<0.01
	Yes	5	0	0	
O. dentata	N	8	10 (1)	10 (3)	0.12
	Yes	2	0	0	

tory. Of these, 40 (91%) spawned when exposed a second time to ovary homogenate, 24 h later, and 16 (40%) spawned when exposed to the ovary homogenate a third time, 48 h after the first exposure.

Threshold dosage in *Ophiocoma dentata*

Induction of spawning was dosage-related. All individuals spawned at concentrations of ovary homogenates $\geq 10^{-5}$ g ml^{-1}. No males responded at concentration $< 10^{-8}$ g ml^{-1} (Fig. 1). By intrapolation, a dosage of ca. 7.8×10^{-7} g ml^{-1} can induce 50% males to spawn.

DISCUSSION

Some unidentified substances in ovaries of the 2 brittle stars *Ophiocoma dentata* and *O. scolopendrina* were shown to be able to induce males of their own species to spawn in this investigation. Presumably, the substances were released in water along with eggs
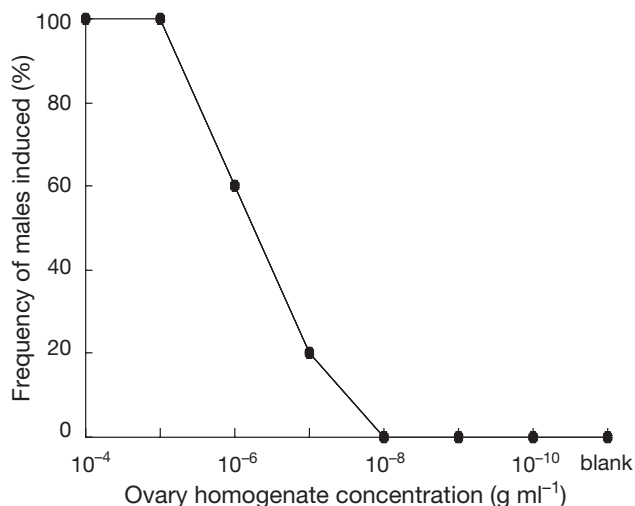

Fig. 1. *Ophiocoma dentata*. Percentage of males induced to spawn by conspecific ovary homogenates at different concentrations (n = 5 in each concentration)

when spawning, and they act as pheromones to 'notify' males of arriving eggs. The unidirectional induction, i.e. ovary homogenate inducing males to spawn, but not the other way around, of the 2 brittle stars was the most noticeable result of this study. In organisms with relatively simple behavioral patterns, exact timing of spawning may be based on relatively little information from conspecifics. The sex to release gametes first may be cued by environmental factors, not information from the other sex. In the 2 species of brittle stars, we observed that males and females were close together during the spawning season. *O. scolopendrina* often had high local densities, but *O. dentatum* aggregates in patches of 2 to 3 individuals. During the relatively long periods of association between individuals, there may have been many opportunities to investigate the reproductive status of their neighbors. Cryptic behavior, especially that of *O. dentatum*, hindered detailed observation of their spawning behavior in nature. The lack of chemical cues to females in the spawns of males, as indicated in this study, did not exclude the possibility of other signals, such as contact. In polychaetes, which have complicated pre-spawning behavior patterns, both males and females were found to cue the other sex at different stages of the courtship during their brief encounter in the water column (see Watson et al. 2003). Either sex could be fine-tuned by the presence of mature individuals of the other gender in the final spawning of gametes.

Besides the sperm-limitation hypothesis, there are other hypotheses to explain how only males evolved the ability to sense the other sex or only females to produce pheromones. Anisogamy, i.e. differentiation of gamete sizes, may be driven by selection for motility of small gametes and selection for pheromone production of large gametes (see Dusenbery 2000). Thus, females may be expected to produce pheromones rather than males. The chemotaxis of sperm in response to egg products works at a much smaller spatial scale to bring eggs and sperm together (e.g. Miller 1985, Yoshida et al. 2002) than synchronization of spawning. The former is a response at the cellular level, whereas the latter involves the whole organism. These 2 mechanisms have the same function of increasing fertilization rate. Since both testes and ovaries are about the same size in these brittle stars (authors' pers. obs.), we suggest that the size-constraint hypothesis of which gender produces pheromones does not apply here.

The discovery of species specificity of spawning induction in brittle stars (Table 3) raises the possibility that prevention of out-crossing may be the principal

selective force behind the evolution of the induction mechanisms. We suggest, however, that the evolution of induction appears to come before the evolution of specificity. Although these 2 species of brittle stars are sympatric, their habitats are different. As the intertidal *Ophiocoma scolopendrina* may have little opportunity to encounter a subtidal and cryptic *O. dentatum*, any selective pressure against outbreeding may be low. In other words, the induction mechanisms here require an explanation other than species recognition.

The order of gamete release between males and females, selected either for sperm limitation or sperm competition, may set the stage for the evolution of induction. The evolution of the inductive mechanism should be obvious once females are selected to spawn ahead of males. Any males able to detect the presence of eggs in the surrounding water will enjoy higher fertilization rates than males who spawn without such cues. The reason females spawn ahead of males is because the selective pressure from sperm dilution is much harsher than that from sperm competition.

In other marine invertebrates, the direction of spawning induction is not as straightforward as in our brittle stars. For example, in the starfish *Asterias rubens* induction is bidirectional, gametes of either sex can induce spawning in the other sex (Gemmill 1914). In polychaetes, the subjects of most studies concerning marine spawning and pheromones, the pheromones may be released by males and/or females, depending on species (Bartels-Hardege et al. 1996, Muller et al. 1999, Ram et al. 1999, Rohl et al. 1999). In the oyster *Crassostrea virginica*, an intrinsic membrane protein in sperm-triggered spawning is found in both sexes (Rice et al. 2002). Conspecific eggs of this oyster, along with a wide range of chemicals and eggs of other species, can induce males to spawn (Galtsoff 1940).

The roles of pheromones are apparently related to other behavioral characteristics (Watson et al. 2003). Pairing of a male and a female, for example, may reduce the chance of male–male competition after the association is formed, and one is more likely to find females releasing gametes first. On the other hand, aggregation of large numbers of individuals during spawning (see Hagman & Vize 2003) may increase the pressure of male–male competition, and at the same time alleviate the risk of sperm dilution for females. Males are expected to spawn earlier than females under this circumstance. In comparison, an interesting aspect of our study lies in the absence of sex pheromones in the semen of male brittle stars. We suspect this may be due to the small size of the aggregation of brittle stars during spawning. Two-way induction is beneficial when reproductive aggregations are large.

The minimum concentration of the pheromone required for 100% induction of brittle stars of our study

was about 10^{-5} g of ovary ml^{-1} of seawater in the laboratory (Fig. 1). Since it only took a few seconds for males to respond, an average mature female (gonad weight ≈ 0.3 g in *Ophiocoma dentatum*) could induce a male response even after dilution in 30 l of seawater. A female should have little problem inducing neighboring males, since they usually hide in the same crevices, with limited water exchange.

For species inhabiting a more open space, e.g. reef corals, dilution of sperm, as well as possible pheromones, may be a more severe problem. Large colony or aggregation sizes may be a solution for these immobile benthic organisms (Soong 1993), which may also evolve inductive spawning mechanisms (see Twan et al. 2003). It remains to be tested whether individuals in large aggregations enjoy a disproportionately high fitness.

One phenomenon observed in our experiments was the relatively low frequencies of males responding to ovary homogenate. One possible explanation is that some individuals may have spawned before being captured for the experiment. Although some tested males were able to spawn for 3 consecutive evenings in the laboratory, the frequencies did become progressively lower. It is also possible that some pre-spawning priming, e.g. a certain period of association with a female, is necessary before males become inducible. Since any pheromone released with eggs is likely to exist and be detectable regardless of whether these eggs have been fertilized or not, a male may be selected to set a requirement before being inducible. On the female side, it remains to be investigated how they make sure that their neighboring males are in a responsive status. It would be a great loss for a female to spawn in the presence of only non-inducible males.

If sperm limitation is indeed the mechanism for the directional induction of spawning in the brittle stars of this study, instead of dividing sperm among consecutive opportunities (see 'Results'), why do males not release all their sperm upon induction? There are 2 explanations. First, it may be a bet-hedging strategy. The chemical cue does not tell the males about the amount of eggs available, since the pheromone concentration is determined by both ovary size and distance from the males. More importantly, as discussed previously, the arriving eggs may have been fertilized. Second, the inductive mechanism may be sufficient to fertilize eggs effectively, making release of all sperm unnecessary.

Spawning of echinoderms in nature has been the focus of many investigations, although the timing difference between males and females was not necessarily reported (Ormond et al. 1973, Pennington 1985, Giese & Kanatani 1987, Minchin 1987, Pearse et al. 1988, Hamel & Mercier 1995). Both sexes have been observed to release gametes first, although more spe-

cies were reported to show male-first spawning in the phylum (echinoids: Fox 1924, ophiuroids: Thorson 1950, holothurians: McEuen 1988, Hendler 1991, crinoids: Holland 1991, asteroids: Hamel & Mercier 1995). Does the prevalence of male-first cases indicate the relative importance of sexual selection in echinoderms? We suggest that observation bias may also have played a role here. According to the sperm-limitation hypothesis, spawning time difference should be small, i.e. males should follow females closely. Thus, difference in spawning time between sexes may be more difficult to distinguish when sperm limitation is at work. According to the sperm-competition hypothesis, on the other hand, the competition to spawn earlier than other males may inadvertently result in a wider gap in spawning time between the sexes. Thus, male-first release may be easier to observe. Behavioral differences between the sexes during spawning (see Hagman & Vize 2003) may be an additional factor that renders one of the sexes more conspicuous than the other.

LITERATURE CITED

Applegate AL (1984) Echinoderms of southern Taiwan. Bull Inst Zool Acad Sin (Taipei) 23:93–118

Bartels-Hardege HD, Hardege JD, Zeeck E, Muller C, Wu BL, Zhu MY (1996) Sex pheromones in marine polychaetes. 5. A biologically active volatile compound from the coelomic fluid of female *Nereis* (*Neanthes*) *japonica* (Annelida Polychaeta). J Exp Mar Biol Ecol 201:275–284

Beach DH, Hanscomb NJ, Ormond RFG (1975) Spawning pheromone in crown-of-thorns starfish. Nature 254: 135–136

Chang D (1999) Spawning induction of two brittle stars, *Ophiocoma dentata* (Muller and Troschel) and *Ophiocoma scolopendrina* (Lamarck) (Echinodermata: Ophiuroidea). MS thesis, National Sun Yat-sen University, Kaohsiung, Taiwan

Denny MW (1989) Consequence of surf-zone turbulence for settlement and external fertilization. Am Nat 134:859–889

Downing JA, Rochon Y, Perusse M, Harvey H (1993) Spatial aggregation, body-size, and reproductive success in the fresh-water mussel *Elliptio complanata*. J North Am Benthol Soc 12:148–156

Dusenbery DB (2000) Selection for high gamete encounter rates explains the success of male and female mating types. J Theor Biol 202:1–10

Fong PP (1993) Lunar control of epitokal swarming in the polychaete *Platynereis bicanaliculata* (Baird) from central California. Bull Mar Sci 52:911–924

Fox HM (1924) The spawning of echinoids. Proc Camb Phil Soc Biol Sci 1:71–74

Galtsoff PS (1940) Physiology of reproduction of *Ostrea virginica*. III. Stimulation of spawning in the male oyster. Biol Bull (Woods Hole) 78:117–135

Gemmill J (1914) The development and certain points in the adult structure of *Asterias rubens*. Phil Trans R Soc Lond B Biol Sci 205:213–294

Giese AC, Kanatani H (1987) Maturation and spawning. Reprod Mar Invertebr 9:251–329

Hagman DK, Vize PD (2003) Mass spawning by two brittle star species, *Ophioderma rubicundum* and *O. squamosissimum* (Echinodermata: Ophiuroidea), at the Flower Garden Banks, Gulf of Mexico. Bull Mar Sci 72:871–876

Hamel JF, Mercier A (1995) Prespawning behavior, spawning, and development of the brooding starfish *Leptasterias polaris*. Biol Bull (Woods Hole) 188:32–45

Hardege JD, Bentley MG, Beckmann M, Muller CT (1996) Sex pheromones in marine polychaetes: volatile organic substance (VOS) isolated from *Arenicola marina*. Mar Ecol Prog Ser 139:157–166

Harrison PL, Babcock RC, Bull GD, Oliver JK, Wallace CC, Willis BL (1984) Mass spawning in tropical reef corals. Science 223:1186–1189

Hastie LC, Young MR (2003) Timing of spawning and glochidial release in Scottish freshwater pearl mussel (*Margaritifera margaritifera*) populations. Freshw Biol 48: 2107–2117

Hendler G (1991) Echinodermata: Ophiuroidea. In: Giese AC, Pearse JS (eds) Reproduction of marine invertebrates, Vol VI. Echinoderms and lophophorates. Boxwood Press, Pacific Grove, CA, p 355–511

Himmelman JH (1980) Synchronization of spawning in marine invertebrates by phytoplankton. In: Clark WH, Adams TS (eds) Advances in invertebrate reproduction, Vol II. Elsevier, New York, p 3–19

Holland ND (1991) Echinodermata: Crinoidea. In: Giese AC, Pearse JS (eds) Reproduction of marine invertebrates, Vol VI. Echinoderms and lophophorates. Boxwood Press, Pacific Grove, CA, p 247–299

Levitan DR (1993) The importance of sperm limitation to the evolution of egg size in marine invertebrates. Am Nat 141: 517–536

Levitan DR (2000) Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin *Lytechinus variegatus*. Proc R Soc Lond B Biol Sci 267:531–534

Levitan DR (2004) Density-dependent sexual selection in external fertilizers: variances in male and female fertilization success along the continuum from sperm limitation to sexual conflict in the sea urchin *Strongylocentrotus franciscanus*. Am Nat 164:298–309

Levitan DR, Petersen C (1995) Sperm limitation in the sea. Trends Ecol Evol 10:228–231

McEuen FS (1988) Spawning behaviors of northeast Pacific sea cucumber (Holothuroidae: Echinodermata). Mar Biol 98:565–585

Miller RL (1985) Demonstration of sperm chemotaxis in Echinodermata: Asteroidea, Holothuroidea, Ophiuroidea. J Exp Zool 234:383–414

Minchin D (1987) Sea-water temperature and spawning behavior in the sea star *Marthasterias glacialis*. Mar Biol 95:139–143

Muller CT, Beckmann M, Hardege JD (1999) Sex pheromones in *Nereis succinea*. Invertebr Reprod Dev 36: 183–186

Ormond RFG, Campbell AC, Head SM, Moore RJ, Rainbow PS, Sanders APL (1973) Formation and breakdown of aggregation of *Acanthaster planci* in the Sudanese Red Sea. Nature 246:167–169

Painter SD (1992) Coordination of reproductive activity in

Aplysia: peptide neurohormones, neurotransmitters, and pheromones encoded by the egg-laying hormone family of genes. Biol Bull (Woods Hole) 183:165–172

Pearse JS, McClary DJ, Sewell MA, Austin WC, Perez-Ruzafa A, Byrane M (1988) Simultaneous spawning of sex species of echinoderms in Barkley Sound, British Columbia. Int J Invertebr Reprod 14:279–288

Pennington JT (1985) The ecology of fertilization of echinoid eggs: the consequences of sperm dilution, adult aggregation, and synchronous spawning. Biol Bull (Woods Hole) 169:417–430

Ram JL, Muller CT, Beckmann M, Hardege JD (1999) The spawning pheromone cysteine-glutathione disulfide ('nereithione') arouses a multi-component nuptial behaviour and electrophysiological activity in *Nereis succinea* males. FASEB (Fed Am Soc Exp Biol) J 13:945–952

Rice P, Ray SM, Painter SD, Nagle GT (2002) Intrinsic membrane protein in oyster sperm stimulates spawning behaviors in *Crassostrea virginica*: implications for aquaculture. J Shellfish Res 21:715–718

Rohl I, Schneider B, Schmidt B, Zeeck E (1999) Ovothiol A: the egg release pheromone of the marine polychaete *Platynereis dumerilii*. Z Naturforsch 54:1145–1147

Shepherd SA (1986) Studies on southern Australian abalone (genus *Haliotis*). VII. Aggregative behavior of *H. laevigata* in relation to spawning. Mar Biol 90:231–236

Soong K (1993) Colony size as a species character of reef corals. Coral Reefs 12:77–83

Starr M, Himmelman JH, Therriault JC (1990) Direct coupling of marine invertebrate spawning with phytoplankton blooms. Science 247:1071–1074

Strathmann RR (1990) Why do life histories evolve differently in the sea? Am Zool 30:197–208

Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates. Biol Rev 25:1–45

Twan WH, Hwang JS, Chang CF (2003) Sex steroids in scleractinian coral, *Euphyllia ancora*: implication in mass spawning. Biol Reprod 68:2255–2260

Watson GJ, Bentley MG, Gaudron SM, Hardege JD (2003) The role of chemical signals in the spawning induction of polychaete worms and other marine invertebrates. J Exp Mar Biol Ecol 294:169–187

Yoshida M, Murata M, Inaba K, Morisawa M (2002) A chemoattractant for ascidian spermatozoa is a sulfated steroid. Proc Natl Acad Sci USA 99:14831–14836

Yund PO (1998) The effect of sperm competition on male gain curves in a colonial marine invertebrate. Ecology 79:328–339

Yund PO (2000) How severe is sperm limitation in natural populations of marine free-spawners? Trends Ecol Evol 15:10–13

Yund PO, McCartney MA (1994) Male reproductive success in sessile invertebrate: competition for fertilizations. Ecology 75:2151–2167

Zeeck E, Wesselmann G, Hardege J, Bartels-Hardege H (1988) Sex-pheromone in a marine polychaete-determination of the chemical structure. J Exp Zool 246:285–292