J. Mar. Biol. Ass. U.K. (1999), **79**, 121–129
Printed in the United Kingdom

Mucus as a mediator of gametogenic synchrony in the sea cucumber *Cucumaria frondosa* (Holothuroidea: Echinodermata)

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The data presented here demonstrate that the sea cucumber *Cucumaria frondosa* secretes a biologically active mucus that helps maintain gametogenic synchrony among conspecifics. Either a whole mature individual or a sample of its freshly collected mucus was able to initiate gametogenesis in conspecifics that were in the gametogenic recovery stage when other environmental conditions, including daylength, were maintained constant. Similar results were obtained when the mucus was kept in seawater for less than 3 h prior to its use, whereas after 6 h in seawater, the mucus had lost its inducing properties. Laboratory experiments showed that the mucus was produced in lesser amount during late summer and autumn; the production rapidly increased in early January to reach a peak a few months before the June spawning, in 1992 and 1993. The increment of mucus production was concurrent with the initiation of gametogenesis. Synthesis of mucus was maximal in individuals having attained gametogenic maturity and minimum in individuals with less developed gonads. At first relatively stable in seawater, the mucus gradually lost its integrity within a period of 3–5 h, suggesting that the mucus may be carried over long distances by currents, thus allowing a transfer of information before its complete degradation. This phenomenon was observed in the field where streams of mucus could be followed by SCUBA divers as far as 20–30 m away from the secreting animal. The data presented here are the first evidence of the important role played by mucus secretion during the gametogenic processes of an echinoderm.

INTRODUCTION

A number of interactions between organisms involve chemical agents rather than physical contact, and the study of these interactions has been termed chemical ecology. Interspecific chemoreception is thought to prevail mainly in food and predator detection. Nevertheless, pheromones are presumed to play a role during conspecific recognition, search of suitable colonizing area, spawning, courtship, moulting, aggregation and communication of potential threat among invertebrates (see reviews by Leroy, 1987; Agosta, 1992; Hay, 1996).

Although reports remain scarce and often speculative, the study of chemical communication in echinoderms has received some degree of attention in recent years. Run et al. (1988) proposed that sex-specific contact chemoreception could partially explain sex recognition in the starfish *Archaster typicus*. Ormond et al. (1973) and Beach et al. (1975) showed that an active component released by the gonad of *Acanthaster planci* induced reproductive behaviour and spawning in nearby mature adults. Chaet (1966) and Kanatani & Shirai (1968) suggested that a chemical substance released into the seawater by starfish could stimulate the spawning in neighbouring individuals. More recently, Hamel & Mercier (1995a) indicated that chemical communication seemed to play a role in the synchronization of gametogenesis during aggregative behaviour in the starfish *Leptasterias polaris*, mostly during the last stages of gamete maturation, before spawning. The paired mating of the sea star *Neosmilater georgianus* was also presumed to be regulated by chemical mediators (Slattery & Bosch, 1993). Miller (1989) studied the periodic presence of a long-lived bioactive sex-specific substance in natural seawater during the reproductive season of starfish. He suggested that benthic invertebrates may communicate their sexual identity and readiness to spawn by means of waterborne pheromones. To our knowledge, no description of the exact mode of action or identification of the chemical compounds involved have been provided for echinoderms (see review by Giese et al., 1991).

In a recent study, we have demonstrated that photoperiod was not the only factor controlling the onset and evolution of gametogenesis in *Cucumaria frondosa* (Hamel & Mercier, 1996a). Laboratory experiments showed that gametogenic processes were significantly asynchronous in individuals maintained separately under natural environmental conditions for more than a year. Conversely, animals kept in groups showed gametogenic synchrony. Gametogenic activity was induced in less mature individuals by exposure to more developed ones, even without the photoperiod cue. These results clearly demonstrated that chemical communication was playing a major role during gametogenesis, in synergism with the increment of daylength, temperature and food supply, allowing an optimal synthesis of gametes to ensure the success of spawning (Hamel & Mercier, 1996a).

The goal of the present work was to determine the origin of the chemical mediator and its annual seasonal cycle in *C. frondosa*. We also aimed at determining its ecological significance in benthic ecosystems, especially among dense populations of sea cucumbers found at all depths from the lower water spring tide to more than 300 m, on the east coast of Canada.

Until now, mucus secretion in echinoderms was mainly associated with protection against external abrasions of the body wall and defensive behaviour (Nance & Braithwaite, 1979; Lawrence, 1987). Its role as a buffer during salinity fluctuations was also examined (Binyon, 1976) and some papers described the nature of mucus in marine invertebrates (Fontaine, 1955; Fieser & Fieser, 1956; Drollet et al., 1993). A number of researchers identified saponins as important constituents of mucus in echinoderms, especially in starfish and sea cucumbers (Nigrelli et al., 1955; Fange, 1963; Rio et al., 1963, 1965; Burns et al., 1977). This research is the first to propose that mucus, one of its constituents or a chemical emitted with it, plays a determinant role in the fine tuning of gametogenesis among entire populations of echinoderms.

MATERIALS AND METHODS

Collection and holding of individuals

Sea cucumbers *Cucumaria frondosa* were collected by SCUBA divers in November 1992 at 10 m depth at Sainte-Anne-des-Monts along the south shore of the Lower St Lawrence Estuary, eastern Canada (48°21′N 68°47′W). They were maintained individually in 50-l tanks under stable conditions and left to acclimatize for two weeks. The conditions referred to as stable correspond to a temperature of 6°C, a salinity of 28, a pH of 8 and a daylength of 10 h with an irradiance of 35 μE m^2 s^{-1}. These conditions are naturally found in the field for a large part of the year at 10 m depth. Other sea cucumbers were collected in June 1992 and kept +2, 4 and 6 months out-of-phase of photoperiod (Pearse & Eernisse, 1982; Pearse & Walker, 1986; Pearse et al., 1986a,b; Hamel & Mercier, 1996a). This method yielded individuals at different stages of gonad maturity for the duration of the experiment.

General localization of the chemical mediator and its effect on gametogenesis

The aim of this procedure was to locate the tissue responsible for the synthesis of the chemical mediator which was proposed to play a role in the interindividual fine-tuning and synchronization of gametogenesis in *C. frondosa* (Hamel & Mercier, 1996a). Individuals with gonads in the recovery stage, maintained under stable environmental conditions, were exposed either to: (1) a whole mature animal, in order to verify whether the conditions and the results obtained were comparable to those of our previous research; (2) coelomic fluid collected through the body wall with a syringe, filtered on 100-μm mesh; and finally; (3) mucus freshly collected from the body surface of a mature sea cucumber (male or female). The seawater from which the mucus was separated was not tested because absolute partition could not be achieved. Knowing that the communication was sex-specific, only the following combinations for which the best results were previously obtained were considered (Hamel & Mercier, 1996a): male (recovery) exposed to male (maturity) and female (recovery) exposed to female (maturity). The sex of the individuals was easily visible due to the sexual dimorphism of the gonopore. Each experimental combination was replicated 15 times simultaneously in different tanks.

To conduct the experiment with mucus as the stimulator, the mature individual selected was wiped to remove seawater and mucus from its surface and was placed in a 4-l beaker filled with 2-l of artificial seawater. Within 5 min, about 300 ml of mucus (mixed with seawater) was collected directly on the body wall, using a pipette. The mucus was subsequently filtered on 100-μm Nitex mesh to remove residual impurities and the solution was filtered again at 20-μm to retain the mucus and dispose of excess seawater. A mix of mucus from five different individuals was used (the mucus solution contained a non-negligible fraction of seawater).

About 20 g wet weight of mucus was introduced into the experimental tanks, within 15 min of collection, twice a day for eight weeks. Complementary experiments monitored the influence of filtrated mucus resuspended in seawater at 4°C for 3 or 6 h before its use, following the previously described methods. Controls for all the experiments consisted of individuals maintained under the same condition, without any stimulation.

Gametogenesis was monitored in control and stimulated individuals every week by periodic examination of the gonads. At each collecting date, a biopsy (0.5 cm long) was performed with a scalpel near the middle of the animal, on the anal side, to collect 1 cm gonad samples close to the apical end. To facilitate the regeneration of body wall, one stitch was placed to close the aperture, according to the technique used by Bouland & Jangoux (1988) with *Asterias rubens* and by Hamel & Mercier (1996a) with *C. frondosa*. These previous studies demonstrated that the surgery had no significant influence on gametogenesis.

The gonad of *C. frondosa* develops two classes of tubules; the small ones roughly represent the first year of gamete growth and the large ones contain maturing gametes of the second year. The distinction between the two size-classes of tubules was established by Hamel & Mercier (1995b) for *C. frondosa* and by Hamel et al. (1993) for the sea cucumber *Psolus fabricii*. In order to follow the gametogenic development in the two classes of tubules, small (<2.2 mm in diameter) and large tubules (>2.2 mm) were collected from the gonad. Five histological slides, each holding four to five gonad sections (5–6 μm) per gonad tubule sample, were stained with periodic acid-Schiff reaction (PAS) and examined under a light microscope. The stages of gametogenic development for males and females (post-spawning, recovery, growth, advanced-growth and maturity) were described in Hamel & Mercier (1995b, 1996a,b,c,d) along with the complete histological techniques. These methods were used for all the experiments.

Stages of gametogenic development in individuals from various depths

A third group of adult sea cucumbers *C. frondosa* were collected at Les Escoumins (48°32′N 69°41′W) along the north shore of the St Lawrence Estuary in November 1992 and May 1993. The study site was a small bay of

$\sim 0.4\,\text{km}^2$ (Anse aux Basques) characterized by a substrate of bedrock and boulders, with the presence of caves and a shipwreck. The depth increased rapidly from the shore line with a slope of about 35–60°. There were plateau at various depths where rocks or boulders accumulated and created diverse habitats. One hundred individuals were collected at different depths (5, 10, 20, 40, 60, 100 and 200 m) using SCUBA divers or dredging. The light is significantly reduced at 40 m depth and completely absent at 60 m depth in the St Lawrence Estuary (Babin et al., 1993). Another 100 individuals were collected in caves (in complete darkness) at 20 m depth. A third sample (N=100) was collected inside a shipwreck at 37 m, where the light is also absent. Finally, 100 individuals were collected at 20 m depth, under rocks or boulders, where the light is attenuated but present. The presence or absence of light was evaluated using black & white photographic paper (Kodak Polycontrast III RC) rolled in a water-tight container and placed among the sea cucumbers in the caves and ship wreck for a week. The absence of light was confirmed when the paper was unexposed. The sea cucumbers were all dissected and their level of gonad maturity was established (in small and large tubules) using the previously described histological method.

Seasonal abundance of mucus

The monthly abundance of mucus synthesized by the same 50 males and 50 females *C. frondosa* maintained separately in two large 500-l tanks under natural environmental conditions (including food supply and photoperiod) was monitored for 19 months. Males and females were gently cleaned to remove sediment and other debris attached to them prior to their introduction into the tank. Special care was taken to avoid exposing the animals to air during the manipulation. Each month, the sea cucumbers were carefully transferred individually to a tank filled with 20 l of artificial seawater (salinity 28 psu, temperature 4°C). After 30 min, the sea cucumber was thoroughly rinsed with 10 l of seawater and removed from the tank. Within 1 h of collection, the mucus solution (30 l) was frozen at −80°C and lyophilized (Virtis Uni-Trap 10-100) for 72 h. The total weight obtained always included the seasalts contained in the 30 l of seawater. To evaluate the proportion of organic matter, the sample was dried to ash in a preweighed aluminium dish at 500°C for 24 h and weighed to the nearest 0.001 g.

The gonads of another group of 600 sea cucumbers maintained under the same environmental conditions were monitored to establish the gonad index (wet gonad weight/wet body wall weight), using the techniques described by Hamel & Mercier (1995b, 1996a,b). Fifteen females and 15 males were dissected once a month.

Mucus behaviour in seawater

Qualitative observations were noted to assess the behaviour of mucus in seawater. About 4 l of mucus, mixed with seawater, was collected as previously described from individuals with mature gonads. Three hundred ml of the mucus in solution was placed in test tubes and centrifuged at 1000*g* for 15 s. Mucus samples were tested hourly for 10 h, from the time of collection, to assess the presence or absence of a distinct phase over the seawater after centrifugation. All experiments were conducted at 4°C under constant lighting.

Additional observations in the field were conducted at 15 m depth in May 1993 in Les Escoumins to evaluate the dispersion of mucus after its release from undisturbed individuals. The formation of filaments and the distance covered by the mucus were noted using SCUBA divers.

RESULTS

General localization of the chemical mediator and its effect on gametogenesis

The results obtained for males and females are similar (Figure 1). When exposed to the coelomic fluid, individuals from both sexes showed unmodified gametogenesis: development was indistinguishable from controls over the eight week experiment. However, when exposed to individuals possessing mature gonads or to the freshly collected mucus from mature individuals of their own sex, males and females initially in the recovery stage showed a significant increment in the rate of gamete synthesis (Kruskal–Wallis $P<0.001$, for males and females). The tubules of females entered the growth stage within five weeks, and the advanced-growth stage after seven weeks, without any significant difference between the experiments using whole females with mature gonads or only their mucus as stimulators (Kruskal–Wallis $P=0.087$). The results obtained with males were similar (Figure 1). The first signs of gonad growth were noted after four weeks when males with mature gonads were used as stimulators and after five weeks in the presence of mucus only. The stimulated males reached the advanced-growth stage within six or seven weeks after the two types of stimulation, respectively. Complementary experiments were conducted with 3-h old mucus. When added to the tank that contained an individual with a gonad in the recovery stage, this slightly aged mucus had the same ability to induce maturation than freshly collected mucus for all the combinations tested (Kruskal–Wallis $P<0.05$). In contrast, 6-h old mucus produced no visible gametogenic stimulation; the results were not significantly different from controls (Kruskal–Wallis $P=0.087$).

Mucus behaviour in seawater

The mucus of the sea cucumber *Cucumaria frondosa* is clear, sometimes slightly amber, showing the presence of brown body wall pigmentation, and synthesized roughly throughout the surface of the body wall. Mucus secreted by males and females was very easy to observe in the field by SCUBA divers. In some cases, the mucus remained attached to the secreting individuals, forming slimy streams which sometimes extended to more than 20 cm, especially when the current was weak. The particles that were rapidly trapped into the mucus facilitated its observation underwater. After its release, part of the mucus could cover total distances between 20 and 30 m away from the secreting individual. However, the bulk of mucus remained close to the animal, depositing on the nearest substrate (algae, rocks, etc.) or directly on

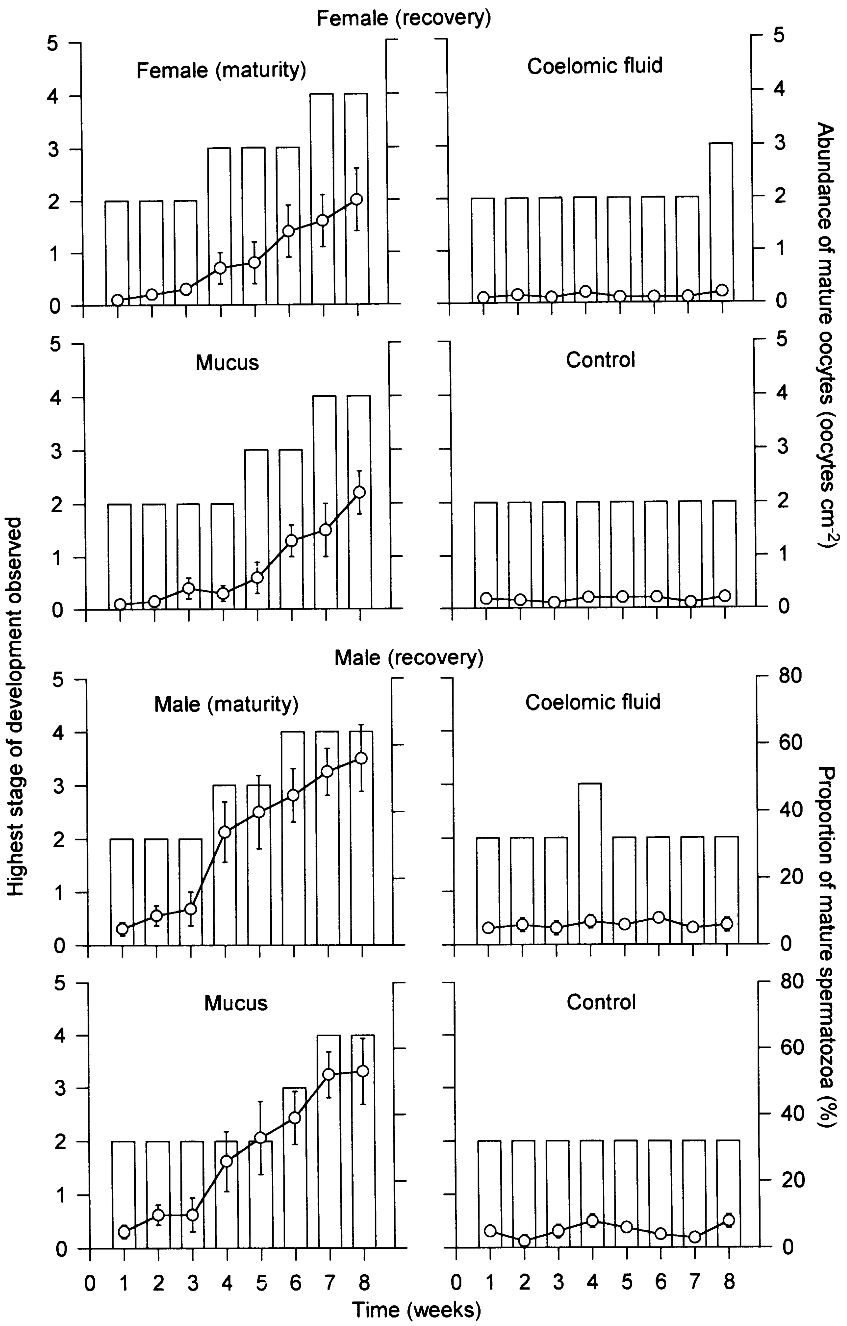

Figure 1. *Cucumaria frondosa*. Gametogenic development in the gonadal tubules of individuals initially in the recovery stage and submitted to three different sex-specific stimulations: a whole mature individual, mucus and coelomic fluid from a mature individual. The highest stages of development observed are presented as histograms and the number of mature oocytes or the proportion of mature spermatozoa as lines. The gametogenic stages observed in both sexes were divided into 1, post-spawning; 2, recovery; 3, growth; 4, advanced-growth; and 5, maturity. Control experiments were performed using individuals without the stimulation. The vertical lines represent the confidence interval (95%).

conspecifics. The movements of mucus streams in the water column were gathered from nine different observations. The mucus was never observed to naturally float at more than 1 m over the sea-floor. However, in the majority of cases, the mucus did not conglomerate sufficiently to become detectable underwater.

Centrifugation experiments showed that mucus maintained a lower density than seawater for ~4 h, spreading as a distinct upper layer in the test tube. This distinct phase was not observed 5 h or more after collection. Instead, the mucus solution, which was still translucent and distinguishable from the seawater, formed a slightly yellow precipitate on the bottom of the centrifuge tubes.

Seasonal abundance of mucus

The quantity of mucus synthesized by males and females throughout the 19 months of study did not differ significantly (Kruskal–Wallis $P=0.091$) (Figure 2).

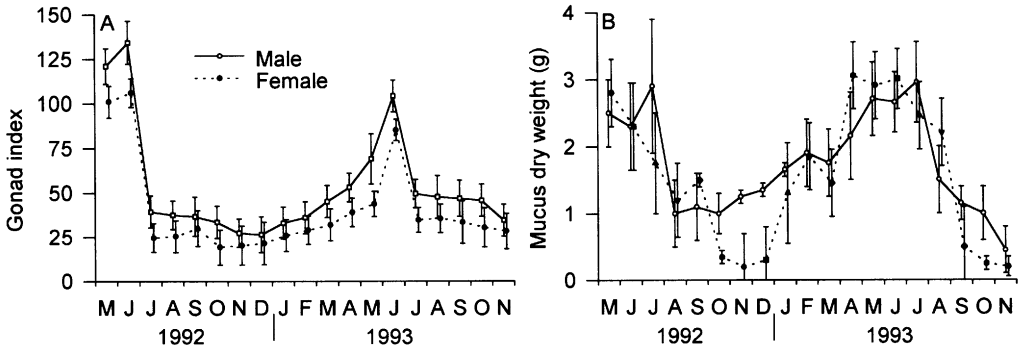

Figure 2. *Cucumaria frondosa*. Seasonal cycle of (A) gonad index of 50 sea cucumbers collected monthly from May 1992 to November 1993 correlated with (B) the abundance of mucus synthesized by another group of individuals submitted to similar environmental conditions of daylength and temperature. The vertical lines indicate the confidence interval (95%).

Table 1. Cucumaria frondosa. *Relative abundance of sea cucumbers (%) in the different gametogenic stages. Individuals were collected from various depths in November 1992 and May 1993. Each sampling site is characterised by its exposure to sun light: lighted area (5, 10, 20 m); area receiving reduced lighting or shade (20*, 40 m); total darkness (20**, 37***, 60, 100, 200 m). One hundred individuals were analysed at each depth.*

		Gametogenic stages									
		Male					Female				
Depth		Recovery	Growth		Adv.-Growth	Maturity	Recovery	Growth		Adv.-Growth	Maturity
(m)	Nov 1992	S	S	L	L	L	S	S	L	L	L
5		82	18	60	5	35	72	28	70	9	21
10		85	15	55	8	37	79	21	65	13	22
20		77	23	53	7	40	80	20	81	1	18
20*		89	11	50	9	41	80	20	83	5	12
40		90	10	52	9	39	80	20	69	12	19
20**		91	9	49	10	41	82	18	72	6	22
37***		84	16	44	9	47	84	16	77	3	20
60		84	16	51	8	41	89	11	71	12	17
100		81	19	54	8	38	82	18	74	10	16
200		78	22	47	7	46	80	20	76	4	20
	May 1993										
5		21	79	27	5	68	13	87	22	1	77
10		14	86	22	7	71	12	88	15	4	81
20		6	94	16	4	80	10	90	13	3	84
20*		19	81	19	3	78	18	82	18	4	79
40		20	80	25	6	69	16	84	13	7	80
20**		18	82	21	9	70	13	87	13	5	82
37***		18	82	27	3	70	20	80	10	6	84
60		21	79	22	4	74	16	84	15	6	79
100		14	86	23	5	72	21	79	15	4	81
200		11	89	18	3	79	18	82	9	5	86

*, individuals collected under rocks and boulders; **, individuals collected inside caves; ***, individuals collected inside a ship wreck; S, small gonadal tubules; L, large gonadal tubules.

However, a clear seasonal variation was observed in both sexes. From August to December 1992, mucus synthesis was at its minimum and increased significantly, but slowly, from the beginning of the year (Kruskal–Wallis $P<0.01$) and rose in March (Kruskal–Wallis $P<0.001$) to attain a maximum plateau in May–July 1993. Subsequently, the amount of mucus synthesized decreased rapidly until the end of the experiment. A similar pattern seemed to have occurred during the previous year, as shown by the short interval from May to August 1992 (Figure 2).

Corresponding gonad indices are also presented in Figure 2. The major event occurring in 1992 and 1993 was the drastic drop in the gonad index in May–June of both years, in both sexes. Following this drop, there was a period of low gonad index until December 1992. However, from early January 1993, a slight increase of the gonad index was measured. This index significantly increased from March 1993 to attain a peak in May 1993 (Kruskal–Wallis $P < 0.01$).

Stages of gametogenic development in individuals from various depths

The results in Table 1 clearly show that the proportion of individuals in the different stages of gametogenic development were similar for males and females at the different depths studied considering the two dates of collection (November 1992 and May 1993). Gametogenic synchrony was apparent in almost all sea cucumbers, including those sampled below the rocks and inside the shipwreck where the light was either reduced or absent (Table 1).

DISCUSSION

There are important advantages for marine invertebrate populations to synchronize the maturation of their gametes. The most obvious is probably the resulting simultaneous spawning within each population, especially for species that broadcast their gametes into the water (see review Giese et al., 1991). Broadcasters need to maximize the rate of fertilization and minimize the wastage of gametes due to undesirable cross-fertilization (Hamel & Mercier, 1994) in order to maintain sufficient recruitment.

Gametogenesis in the sea cucumber *Cucumaria frondosa* occurs simultaneously with the first increase of daylength and is influenced by several physical and chemical factors, acting either in synergism, successively or independently of one another (Hamel & Mercier, 1995b, 1996a). Environmental factors, and mostly photoperiod, are usually described in the literature as the major triggers of gametogenesis in marine invertebrates (Pearse & Eernisse, 1982; Pearse et al., 1986a,b; McClintock & Watts, 1990). However, during this study, most individuals sampled in the photic zone inside caves and a shipwreck presented similar gametogenic development and matured along with the others, despite the fact that they were not exposed to light (Table 1).

It has previously been found that an interindividual communication plays a determinant role, in synergism with the increment of daylength, in the generalized initiation of gametogenesis and, further, in its harmonious progression among entire populations of sea cucumbers (Hamel & Mercier, 1996a). The data presented here support and refine our previous results by showing that the seasonal production of mucus, secreted by the body wall of the sea cucumber *C. frondosa*, plays the role of chemical mediator in this interindividual sexual exchange (Figure 2). Synchronous gamete synthesis in the study site (Table 1) is apparently related to mucus synthesis which enables the transfer of sexual information between conspecifics. Either a component of the mucus itself or a chemical secreted simultaneously and carried with it acts as the messenger. To our knowledge, mucus has never before been described to have a role during gametogenesis or spawning in any species of marine invertebrate. The most closely related mention was made by Caspers (1984) who suggested that mass coral reproduction in Papua New Guinea, Fiji and along the Great Barrier Reef may be responsible for the characteristic mucus discharge found on Samoan coral reefs two days before the annual swarming of the palolo worm.

Our data indicate that mucus synthesis follows the gonad cycle (Figure 2), hence it is proportional to the level of maturity of the gonadal tubules (Hamel & Mercier, 1996b), enabling mature individuals to stimulate conspecifics with more efficiency. This suggests that the chemical nature or the quantity of mucus synthesized by an individual changes over time and modulates the intensity of stimulation applied on conspecifics over the seasonal cycle. In fact, the seasonal synthesis of mucus seems to be correlated with the initiation of gametogenesis, occurring in January, and with the progressive increase of the gonad index. The very stable temperature, salinity and the virtual absence of food at that time of the year in the study site (Hamel & Mercier, 1995b, 1996b) suggest that these factors are not linked with the increasing mucus synthesis.

Until now, mucus secretion by various species of echinoderms was mainly associated with a response to threatening conditions (Binyon, 1976; Nance & Braithwaite, 1979, 1981). Nance & Braithwaite (1979, 1981) indicated that the starfish *Pteraster tesselatus* secreted copious amount of mucus when disturbed or in close contact with its predator. Contrary to these observations, the secretion of mucus by *C. frondosa* did not seem to be related to threats. When *C. frondosa* was confronted with its major predator, the starfish *Solaster endeca*, it did not show any increase in mucus secretion, and no mention of such behaviour was noted by Legault & Himmelman (1993) who studied the escape strategy of this sea cucumber.

The only reports of the chemical nature of mucus in marine invertebrates were those of Fontaine (1955), Nigrelli et al. (1955), Fieser & Fieser (1956), Fange (1963), Rio et al. (1963, 1965), Feder (1972), Burns et al. (1977), Nance & Braithwaite (1979) and Drollet et al. (1993). The majority of these studies detected saponin in the mucus. Girard et al. (1990) isolated saponin (Frondoside A) from the external epithelium of the body wall and from the tube feet of *C. frondosa*. These tissues are common sites of mucus-secreting cells in other echinoderms (Nichols, 1960; Nance & Braithwaite, 1981). This suggests that the mucus of *C. frondosa* may also contain saponins, as observed by others in different echinoderms (Nance & Braithwaite, 1979; Drollet et al., 1993). Voogt & Huiskamp (1979) showed that saponins in the starfish *Asterias rubens* were found exclusively in oocytes and that the accumulation of these chemicals was directly related to the gonad index throughout the year, with a maximum occurring when the oocytes reached maturity. Seasonal studies of starfish (Yasumoto et al., 1966; Mackie et al., 1977) described a close relation between saponin levels in the tissues and spawning activity in females. Moreover, the structure of saponins found in the starfish *Leptasterias polaris* by Garneau et al. (1989) varied seasonally, suggesting a possible change in their role or effect over the year.

These results and the abundance of saponin detected in the body wall of *C. frondosa* by Girard et al. (1990) suggest that this chemical potentially diffuses through the coelomic fluid into the body wall, or directly from the body wall, toward the water column along with the abundant mucus. How and through which channels the message is perceived by conspecifics remain the more intriguing questions. McEuen (1988) suggested that the circumanal pores in holothuroids could serve as a route for pheromones to enter the coelom and stimulate radial nerves or gonad to initiate the sequence of physiological steps leading to gamete release. In fact, if the chemical messengers or pheromones enter the respiratory tree, which is the finest external epithelium in sea cucumbers, this may ensure a rapid transfer of information through the coelomic fluid and eventually into the gonad, during gametogenesis and spawning.

In a previous study on chemical communication, it was found that the message was distinct in males and in females *C. frondosa*. The experiments that tried to stimulate a female with a more developed male, and vice versa, always failed (Hamel & Mercier, 1996a). The transfer of information via a chemical found in or bound to the mucus, as inferred by our present study, makes the sex specificity more understandable, especially since similar observations were reported for other species. For instance, Dunham (1978, 1988) and Salmon (1983) showed that, in various species of crustaceans, and more precisely in the lobster *Homarus americanus* (Cowan, 1991), hormone chemoreception seemed to be sex-specific. However, the sex-specificity we discovered leaves one question open: how can male and female gametogenesis progress harmoniously in *C. frondosa* within a restrictive sex-specific communication? The different levels and types of saponins synthesized over the year in males and females of the same species of echinoderms (Burnell & ApSimon, 1983), may provide part of the answer.

The laboratory experiments showed that the effectiveness of mucus changes after its first contact with seawater. Mucus freshly collected from individuals with mature gonads and maintained for only 3 h in seawater induced a stimulation in individuals with gonadal tubules in the recovery stage. However, 6-h old mucus had lost its efficiency to stimulate sexual activity in conspecifics. That delay roughly corresponds to the time after which the mucus also lost its slight positive buoyancy and emulsification properties, suggesting a partial or total degradation of the product after that time. Thus, the mucus released by an animal seems to have between 3–6 h to carry its message before it becomes inactive. Nonetheless, the transmission of information from individuals found in shallow and lighted areas toward the populations of deeper waters remains probable in *C. frondosa* because the species is generally continuously distributed from low water spring tide to 200 m depth. It should therefore be possible for chemical communication to play a role in the synchronization of gametogenesis and spawning of individuals from all depths. In fact, mucus was observed floating over 20–30 m away from the emitting individual in the field.

The structural organization of *C. frondosa* populations seems to favour communication through mucus secretion. This species does not aggregate during larval settlement, during reproduction, nor at any other time of the year. The populations are distributed in large patches of abundant and uniformly spread individuals. Further, the sex ratio of 1:1 observed in the field (Hamel & Mercier, 1996d) could facilitate the transfer of information between conspecifics, in order to enhance harmonious gametogenesis and synchronous massive spawning. The applicability of mucus-mediated chemical communication to other marine invertebrates remains to be verified. For instance, Ferrand et al. (1988) found a restricted seasonal reproductive cycle over the whole bathymetric range of 60–1000 m in the spantagonid *Brissopsis lyrifera* that shows a close similarity with our results. However, this type of communication seems less convincing for abyssal species that are restricted to great depths without any shallow-water conspecifics. The distance between individuals of the same population is often great and the chances are very small that currents may favour this kind of large-scale chemical exchange. Thus, although chemical interaction cannot be ruled out, the ultimate factors by which deep sea species orchestrate gametogenesis and spawning still remain to be discovered, as mentioned by Young et al. (1992). The only probable exception would be for the populations that form aggregations which may allow chemical exchanges.

We would like to thank Dr Stephen Battaglene and two anonymous referees for their valuable comments. We are indebted to Ms M. Dion (Entreprises Ondine Inc.) for her assistance during the collection of sea cucumbers.

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Submitted 12 May 1997. Accepted 19 February 1998.