STUDIES ON THE REPRODUCTIVE BIOLOGY OF THE ATLANTIC SEA CUCUMBER CUCUMARIA FRONDOSA

Gonad morphology and gametogenesis of the sea cucumber *Cucumaria frondosa*

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Abstract

The occurrence of a particular gonad morphology, with distinctive large tubules and smaller, less-mature ones, was investigated in sea cucumber Cucumaria frondosa *throughout the north-east American coast. Observed in individuals from high latitudes, this characteristic was not present in populations south of New Brunswick. Monitoring of the reproductive cycle in* C. frondosa *from the Lower St Lawrence Estuary, eastern Canada, showed that gametogenesis was initiated in early winter, after the first increase of day length, and coincided with a transfer of energy from the body wall to the gonad. The subsequent increase of gamete synthesis, in early spring, related to the abundance of food and the rising temperature. The early summer spawning was characterised by a significant decrease in the gonadal tubule size, index and calorific value.*

Introduction

Knowledge of the gonad morphology of sea cucumbers is crucial to the understanding of their reproductive cycle. It was recently realised that the often confusing information given in early studies of the sea cucumber *Cucumaria frondosa* (Jordan, 1972; Coady, 1973) stemmed from an imprecision regarding size disparity among the gonadal tubules. It therefore remains a question weather or not it could have made a difference in the proposed description of the reproductive cycle.

Now we know that while some holothurians possess a single type of gonadal tubules which all undergo an entire gametogenetic cycle in one year (Tanaka, 1958; Costelloe, 1985, 1988; Sewell & Bergquist, 1990), others demonstrate a more complex gonadal development, occurring in tubules of variable size and degrees of maturity (Smiley & Cloney, 1985; Smiley, 1988; Smiley et al., 1991; Tuwo & Conand, 1992; Hamel et al., 1993). In those cases, only a fraction of the gonad becomes mature during the spawning season.

Further, it appears that the presence of different sizes of tubules in the gonad is not always a constant feature in a given species. Gonad morphology can vary according to geographical location, as demonstrated by Sewell (1992) for *Stichopus mollis*. For that reason an extensive sampling over a wide range of latitudes was conducted during our study in order to demonstrate this phenomenon in *Cucumaria frondosa*. Focusing on the St Lawrence population, we then thoroughly monitored the gametogenetic cycle in small and large tubules, in relation to environmental factors.

Material and methods

Gonad morphology under different latitudes

This protocol was designed to verify the occurrence of two classes of gonadal tubules in different populations of *Cucumaria frondosa*. Individuals (about 40 males and 40 females) were collected during spring (before spawning) between 1987 and 1993.

Collections were made in 16 different locations scattered along the east coast of Canada and the United States. The sea cucumbers were collected by dredging or Scuba in the following stations (figure 1):

- *Station 1*: Hopedale at about 20 m depth;
- *Station 2*: St. Anthony at 15 m depth;
- *Station 3*: Grand Bank at 12 m depth;
- *Station 4*: Havre-Saint-Pierre at 3, 13, 25 and 52m depths;
- *Station 5*: Les Escoumins at 5, 10, 20, 40 and 60m depths;
- *Station 6*: Rimouski at 110 m depth;

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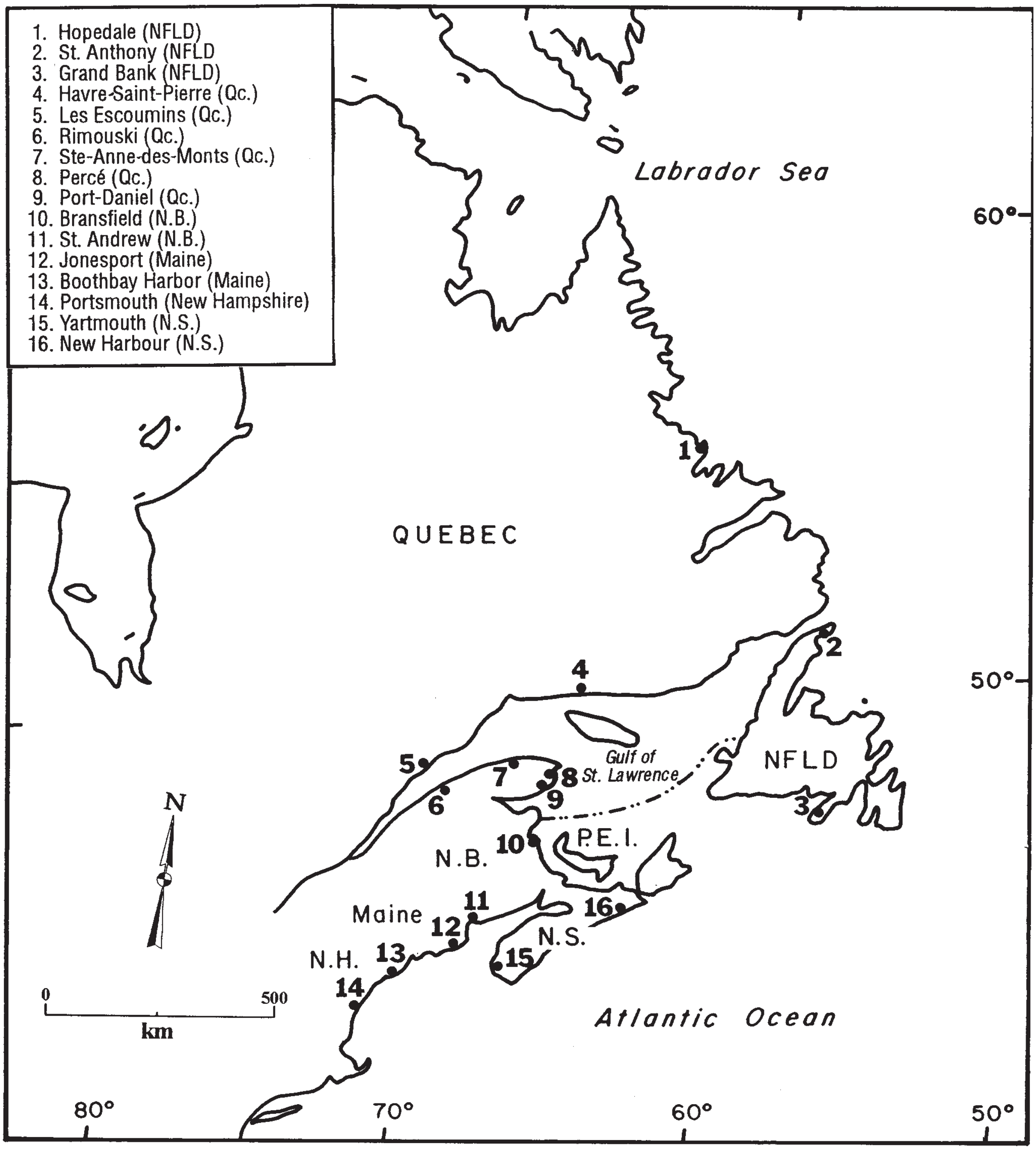

Figure 1: Map of the north-east American coast showing the 16 sampling sites where we studied the gonad morphology. The interrupted line located between stations 2 and 3 indicates the limit between *Cucumaria frondosa* with 2 size classes of gonadal tubules (upper limit) and those with one class of gonadal tubules (lower limit).

- *Station 7*: Sainte-Anne-des-Monts at 15, 30 and 120 m depths;
- *Station 8*: Percé at 15 m depth;
- *Station 9*: Port-Daniel at 20 m depth;
- *Station 10*: Bransfield at 20 m depth;
- *Station 11*: St Andrews at 3 and 90 m depths;
- *Station 12*: Jonesport at 15 m depth;
- *Station 13*: Boothbay Harbor at 10 m depth;
- *Station 14*: the Islands of Shoal in front of Portsmouth at 20 m depth;
- *Station 15*: Yarmouth at 20 m depth; and
- *Station 16*: New Harbour at 15 m depth.

All individuals were dissected and visually inspected for the presence of two classes of gonadal tubules.

Reproductive cycle of **Cucumaria frondosa** *from the St Lawrence Estuary*

Collection of individuals and histological procedures

Male and female *Cucumaria frondosa* were periodically collected at Les Escoumins on the north shore of the Lower St Lawrence Estuary, eastern Canada (48° 21' N : 68° 47' W) from April 1992 to November 1993. They were dredged from a depth of about 20m, usually every month and exceptionally bimonthly when the spawning period was anticipated. The dissections and subsequent mass records all involved fresh animals.

During histological procedures, freshly collected gonads were transferred to Bouin's fixative for four weeks and then processed according to standard embedding and coloration techniques (Hamel et al., 1993). Five longitudinal cuts were made across the tubule and stained with Periodic acid-Schiff (PAS). When applicable, separate examinations were made on small (<2.2 mm in diameter) and large (>2.2 mm) gonadal tubules. Those two classes of tubules were established using the parameters in Hamel et al. (1993).

Gametogenesis

The stage of gonadal development associated with the population at each sampling date was determined in 15 males and 15 females, using the previously described histological techniques. The five gonadal stages (post-spawning, recovery, growth, advanced growth and maturity) were defined in the recent study of *Psolus fabricii* (Hamel et al. 1993). This sea cucumber shows a close anatomical resemblance to *Cucumaria frondosa*, and also presents a very similar reproductive cycle. We also measured the tubule diameters under a microscope.

Determination of body component indices

The wet mass of the body wall, without the aquapharyngeal bulb and the muscle bands, was chosen as a denominator to establish the different tissue indices. The intestine (together with its contents) was removed from the posterior end of the stomach to the beginning of the cloaca, the gonad from its point of attachment to the gonoduct, and the respiratory tree from its point of attachment to the cloaca. The muscle bands of the body-wall and those of the aquapharyngeal bulb and cloaca were removed from the body wall last. All indices were calculated as the ratio of dry organ mass to wet body-wall mass. For each collection date, the various indices were measured in 15 males and 15 females, ranging from 270 to 320 mm distance mouth to anus, previously determined to show minimum inter-individual variations. We established the ratio between mature males and females collected at each sampling date.

Energy content in the tissues

The seasonal fluctuations of energy content in all major organs were measured to give a more complete portrait of the reproductive cycle. We determined the calorific value of the gonad, the intestine including its content, the respiratory tree, the muscle bands and the body wall in 15 males and 15 females, at each sampling date. In the case of gonads, small and large tubules were considered separately. Routinely, the organ samples were dried to constant mass at 55°C in an oven. The material was then grounded to a fine powder and mixed carefully. The caloric content was evaluated by calorimetry (Parr macro oxygen bomb calorimeter), in three samples (≈1g dry mass) of the same organ. These calorimetric measurements provided a quantitative estimate of the total amount of energy present per unit of material combusted. These data in calory.g^{-1} ash free dry mass were transformed into kJ.g^{-1} dry mass.

Seasonal variation of the environmental factors

The chlorophyll a concentration was recorded weekly at the study site by collecting three water samples (8 l) at 15 m depth, during high tide (Hamel & Mercier, 1995). The temperature at 15m was recorded by three Peabody Ryan thermographs. The data of day lengths were obtained from the weather station at the Quebec Airport (Environment Canada, Atmospheric Environment Service). Data of fresh-water run-off, combining four rivers—Montmorency, Batiscan, Sainte-Anne and Chaudière—were provided by Environment Canada (Climatologic Services).

Size at sexual maturity

To establish the growth pattern of the gonads, and the size at sexual maturity, 567 individuals of all sizes, from 1 to 350 mm (distance mouth–anus), were collected in April 1992, a few weeks before the anticipated spawning period. The gonads of individuals over 100 g were inspected under a binocular, while histological procedures were used to determine the degree of maturity in smaller individuals. Sea cucumbers were classified as adults when baring mature gametes, as immature when baring gametes in the early stage of development, and as non-differentiated when no gametes were present. The gonadal index was measured according to the previously described method. We also evaluated the total amount of mature oocytes (fecundity), as well as the number of gonadal tubules and their length in all-sized individuals.

Results

Gonad morphology

The gonads of sea cucumbers collected from the highest latitudes (Labrador coast, station 1) down to the Chaleur Bay (Quebec, station 9), including station 2 on the north shore of Newfoundland, were divided in two classes of gonadal tubules (see figure 1). The sea cucumbers collected from the east coast of New Brunswick (station 10) down south to the Shoals Islands (New Hampshire), including station 3 in Newfoundland, did not present the distinctive classes of tubules observed in the north. All their gonadal tubules were in the same stage of development, roughly had the same diameter and attained maturity after a single year.

In St Andrews (station 11), Saint-Anne-des-Monts (station 6), Les Escoumins (station 5) and Havre-Saint-Pierre (station 4) sea cucumbers were collected at different depths. There was no difference in the gonad morphology at each of those depths. When two classes of gonadal tubules were observed in shallow water (<20 m), they were also present at greater depths.

The St Lawrence Estuary population

The gonad of adult *Cucumaria frondosa* from Les Escoumins was divided into numerous gonadal tubules (120 to 140 tubules.ind^{-1}), each measuring about 160±10 mm in length. Advanced gametic stages (advanced growth and maturity) predominated in the large tubules, and earlier stages (post-spawning, recovery and growth) in the small tubules (see figure 2).

The small tubules (<2.2 mm in diameter) were those undergoing their first year of growth, and the large tubules (>2.2 mm in diameter) those attaining their maturity and involved in the spawning of the current year. During this study, all individuals observed had an approximately equal number of small and large tubules at any time.

Gamete development occurred uniformly all along the germinal epithelium, except in the first centimetre, near the gonoduct, which was empty of gametes, very constricted, and showed a smaller diameter and an atrophied germinal epithelium.

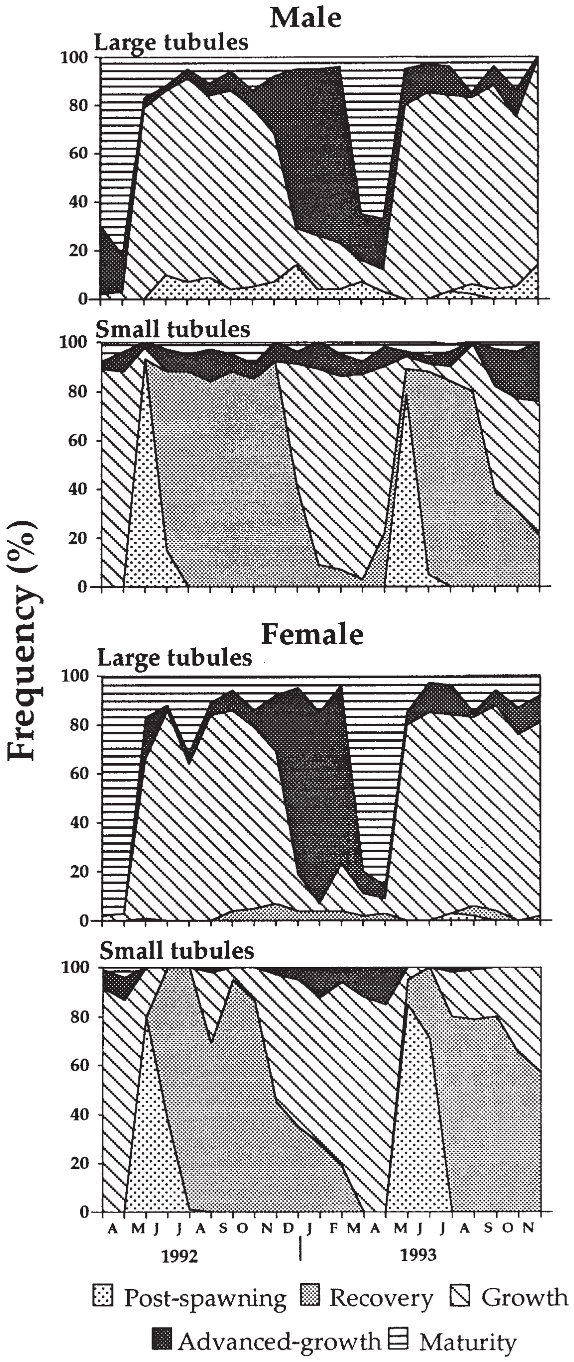

Figure 2: *Cucumaria frondosa*. Relative frequency of different gametogenetic stages in small and large tubules of males and females from April 1992 to November 1993.

All gonadal tubules were joined to a single gonoduct leading to a gonopore located between the tentacles.

No significant departure from a sex ratio of 1 : 1 was observed in any of the samples, and the ratio of the pooled samples was 512 males for 542 females ($X^2_{0.05}$, 14, p>0.05).

Size at sexual maturity

Gonadal tubules were present in all individuals with a body-wall mass superior to ≈1 g. Histological preparations revealed that only undifferentiated precursor cells were present along the germinal epithelium of individuals with a mass under 40 to 42 g, making it impossible to recognise males from females.

The non-differentiated gonads had a uniformly cream colour. The individuals only became sexually distinct (still immature) when reaching 42 to 50 g (see figure 3), at which time histological investigations allowed us to observe many precursors of mature sexual cells, especially along the germinal epithelium. Numerous nests of oogonia, or long and thin layers of spermatogonia, were then observable.

The testis and ovary indices remained comparable up to a body wall mass of 45 to 60 g (see figure 3). Upon reaching that size, males and females attained their sexual maturity and their gonads developed a clear sexual dimorphism; the male gonad becoming light pink and that of the female presented a dark-red colour.

Maturity was confirmed by observation of the first oocytes with PAS-positive yolky reserve and the first few spermatozoa. The relative value of the gonad indices increased sharply as the body-wall mass rose from 47 to 60 g, especially for males (see figure 3). At that time, there were not many mature gametes in the gonad, as they represented less than 5 per cent of the oocytes and less than 8 per cent of the sperm cells.

From that size on, the gonadal index of males was always higher than that of the females, with a minimum of overlapping. Individuals with a body-wall mass between 120 and 180 g showed the smallest variations due to body size (see figure 3) and were therefore used for monitoring the gonadal index and other tissue indices in all experiments.

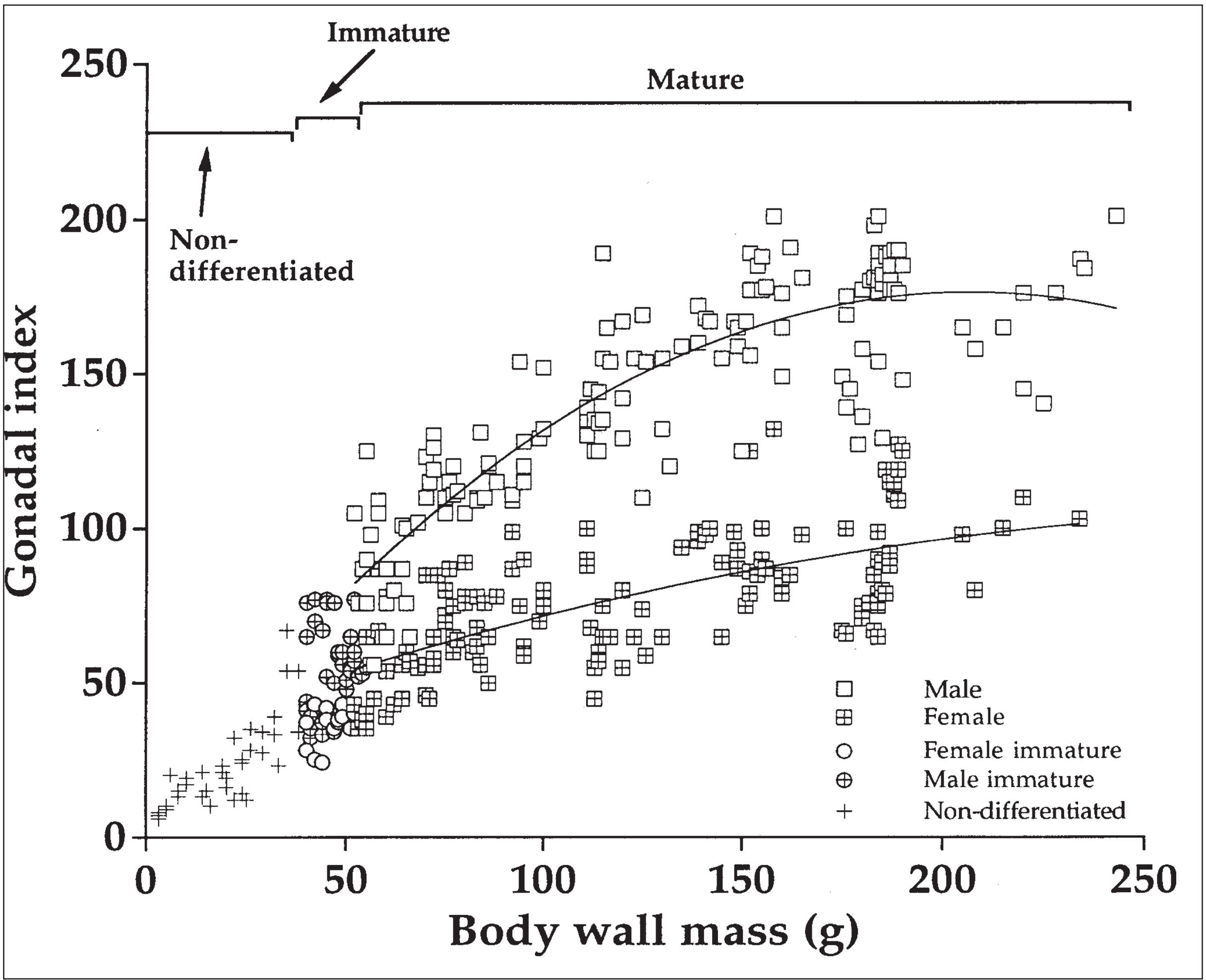

Figure 3: *Cucumaria frondosa*. Relation of the gonadal index to the wet body-wall mass (n=567) in April 1992. The state of maturity of the sea cucumber is indicated for all sampling sizes.

The presence of mature oocytes was monitored in females of increasing body-wall mass. We found that females between 70 to 80 g possessed around 300 mature oocytes (figure 4). After reaching 120 g of body wall mass, the number of mature oocytes increased rapidly to attain the maximum mean density of 8,100±2,300 oocytes for individuals with 180 to 250 g of body-wall mass (see Figure 4).

Male reproductive cycle

After the early summer spawning, the small tubules mainly contained residual gametes and nutritive phagocytes, characteristic of the recovery stage, until December 1992 and January 1993 (figure 2). During that same period, no significant gametogenetic development was recorded in the large tubules (Kruskal-Wallis, $p>0.05$). Spermatogenesis was first initiated in the small tubules in early January 1993.

This process was characterised by the appearance of a thin opaque structure, composed mainly of densely-conglomerated spermatogonia, along the inner surface of the germinal epithelium. Following that, the proportion of individuals with small tubules in the recovery stage decreased to less than 10 per cent between December 1992 and February 1993 (see figure 2). The importance of the growth stage increased abruptly during that same interval, and then more subtly until May 1993, when it was observed in the small tubules of 85 per cent of the individuals, prior to spawning.

Female reproductive cycle

The female cycle closely resembled that of the males. In May and June of 1992 and 1993, advanced stages (advanced growth and maturity) were found in ≥80% of the large tubules, whereas the earlier stages predominated in the small tubules (see figure 2). In the large gonadal tubules, the post-spawning and recovery stages were almost always absent, and the major evidence of both 1992 and 1993 spawnings was the decrease in mature stages.

In contrast, the mature stages in the small tubules remained rare and the major evidence of spawning was a sharp increase of the post-spawning stage. Oogenesis was initiated in early January 1993, after the characteristic resting period that prevailed just after spawning and through the beginning of January. Subsequent oocyte development was characterised by the growth of the nucleus and cytoplasm and the increase in the ratio of cytoplasm to nucleus volume. With continued growth, oocytes often be-

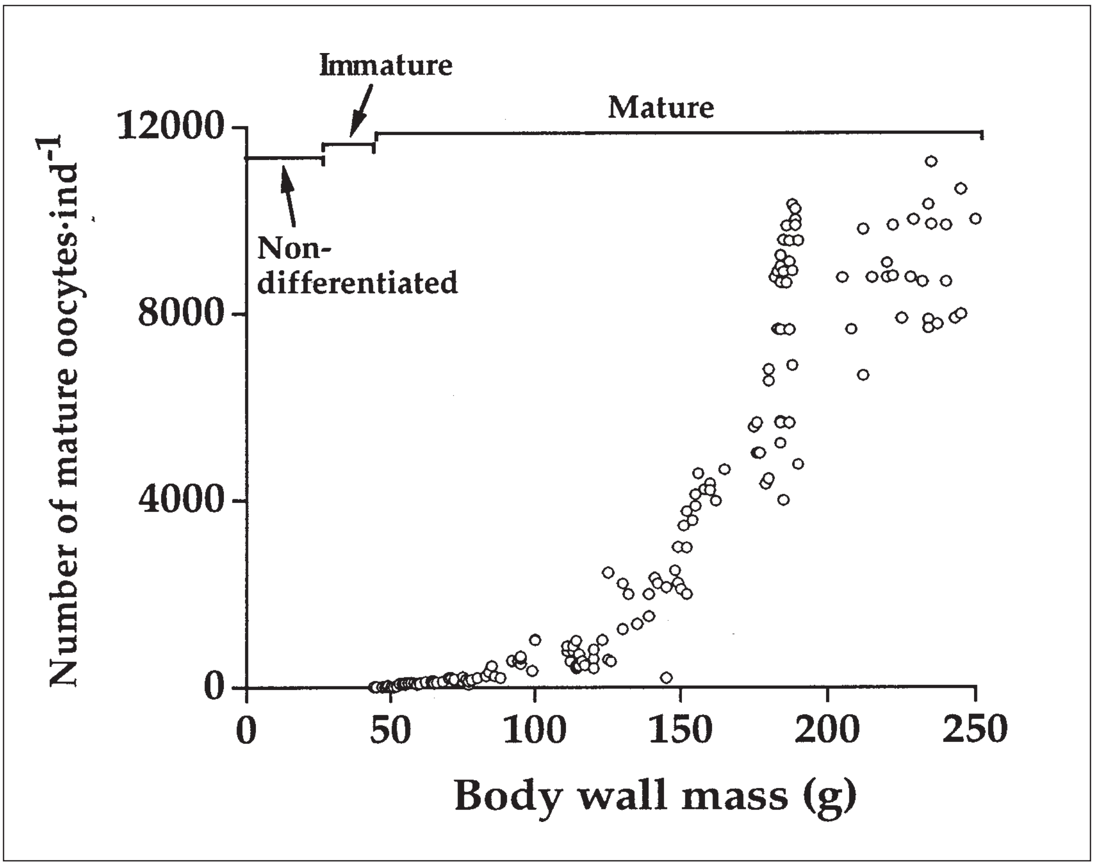

Figure 4: Fecundity and state of maturity of females *Cucumaria frondosa* from less than 1 g to 250 g. These data were collected from individuals dredged in April 1992 before the spawning period.

came irregularly shaped due to spatial constraint within the gonadal tubule.

For males and females, the approach of spawning and the event itself corresponded to an important transition in the gonadal tubule morphology. The small tubules became larger (up to 2.2 mm in diameter), due to the increasing presence of maturing gametes, and progressively entered the lower levels of maturity.

Simultaneously, the large tubules attained full maturity (>3.5 mm in diameter) and were emptied of mature gametes during spawning, causing a decrease in their diameter down to less than 2.2mm. After spawning, the large tubules had consequently transformed into small ones, with two years of growth ahead, while newly produced large tubules were at the beginning of their last year of growth.

Changes in the body component parameters in relation to environmental conditions

The major event that occurred in 1992 and 1993 was the drastic drop of the gonadal index in May to June of both years, for both sexes (see figure 5). It coincided with a net decrease in the mean tubule diameter and with a significant decrease in the calorific value of the gonad. This value dropped by 45 per cent in females and by 41 per cent in males (Kruskal-Wallis, $p<0.01$) (see figures 5 & 6), strongly suggesting a spawning event.

Following this drop was a period of low gonadal index until December 1992, coinciding with the simultaneous and progressive decrease in the energetic content and index of the intestine (content included). The gonadal tubule diameter, the gonadal index and the energy stored in the gonad also remained at their minimum of the year in the June to December interval (see figures 5 & 6).

However, from early January 1993, we observed a slight increase in the gonadal index coinciding with the first sign of tubule diameter increment and growing energy content in the gonad. In March, the energy content increased from 21 $kJ.g^{-1}$ to 27 $kJ.g^{-1}$ in the female gonads and 16 $kJ.g^{-1}$ to 20 $kJ.g^{-1}$ in the male gonads. The gonadal index significantly increased from March 1993 to attain a peak in May 1993 (Kruskal-Wallis, $p<0.01$). An increment was also observed in the tubule diameter and in the calorific value of the gonad, both of which attained their maximum levels for the year (figures 5 & 6).

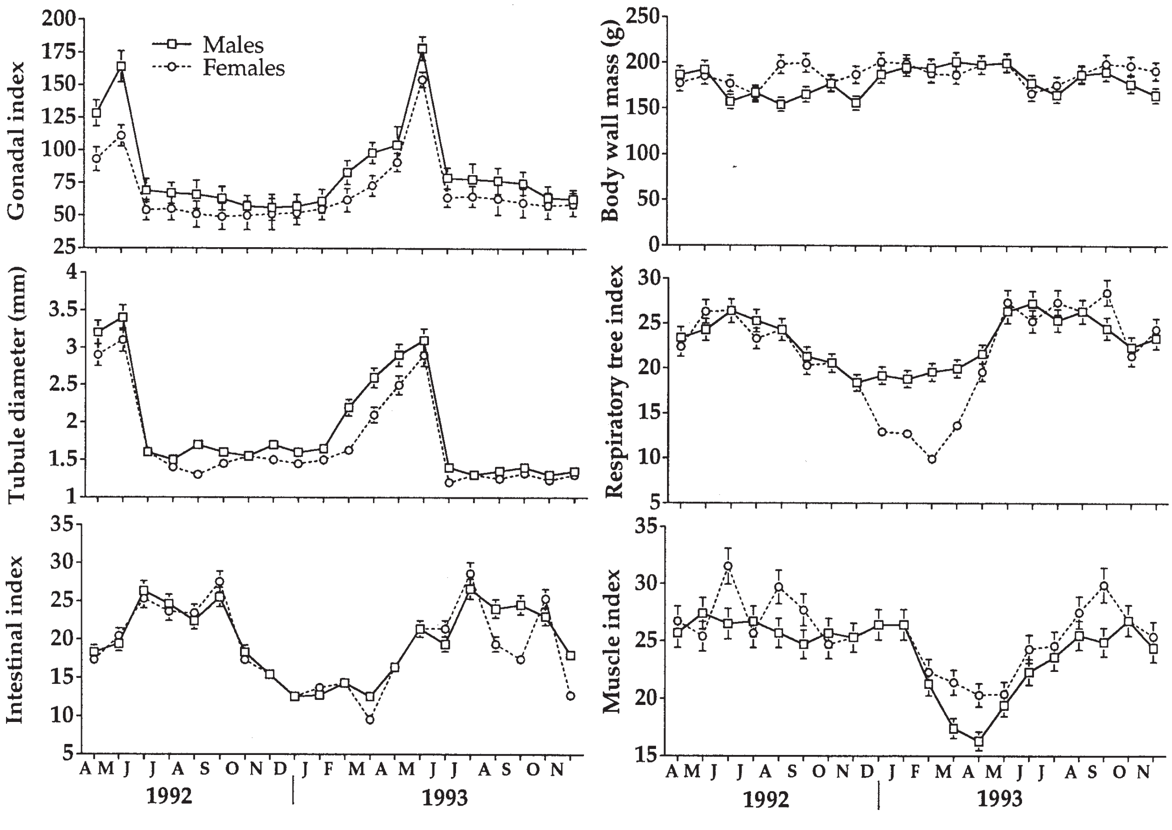

Figure 5: *Cucumaria frondosa*. Seasonal variation of the different body component indices, the mean body-wall mass and the tubule diameter, for males and females, from April 1992 to November 1993. The vertical lines represent the confidence interval (95%).

After the spawning of June 1992 and until February 1993, the index of the respiratory tree decreased progressively and attained its minimum value (figure 5).

The progressive decrease of the index and calorific value of the muscle bands coincided with the initiation of gametogenesis (January – February 1993). It attained a minimum value in April – May, just before spawning in 1993. In the case of male body wall and respiratory tree, indices and calorific value did not show any significant variation over the two years of experiment (Kruskal-Wallis, $p<0.01$).

Although the female body-wall mass remained constant, its energy content dropped significantly (Kruskal-Wallis, $p<0.01$) when the food supply decreased in January 1993.

The energy content increased rapidly again the next spring, in correlation with rising food supply in the field (see figure 6). The respiratory-tree index also marked a significant decrease in females (Kruskal-Wallis, $p<0.01$) (see figure 5).

Total energy content prior to the 1992 spawning was ≈114 $kJ.g^{-1}$ for females and 98 $kJ.g^{-1}$ for males. In both sexes, the gonad accounted for ≈ 35 per cent of this value, the intestine (including its content) for ≈17 per cent, the body wall for ≈19 per cent, the respiratory tree for ≈12 per cent and the muscle bands for ≈16 per cent.

Just after spawning, the total amount of energy in the animals dropped to ≈86$kJ.g^{-1}$ in both sexes. This decrease was correlated to a clearly less important contribution from the gonad, representing only ≈19 per cent of the total amount of energy in a single individual.

Relation of gametogenesis with environmental factors

Temperature

Spring conditions prevailed at the beginning of the experiment, with a water temperature fluctuating between 3 and 5°C and rising rapidly to attain 3 to 9°C in early spring, until the end of August 1992. The final maturation of oocytes in large tubules and the appearance of the growth stage in small tubules coincided with the rapid increase of water temperature in early spring.

The spawning event, occurring between mid-May and mid-June of both years, coincided with the peak of this warming period. Beginning in early September 1992, the water temperature dropped abruptly to reach its minimum annual value in October, with fluctuations between -1 and 1.5°C. This minimum threshold remained until early March 1993, when the warming cycle began again.

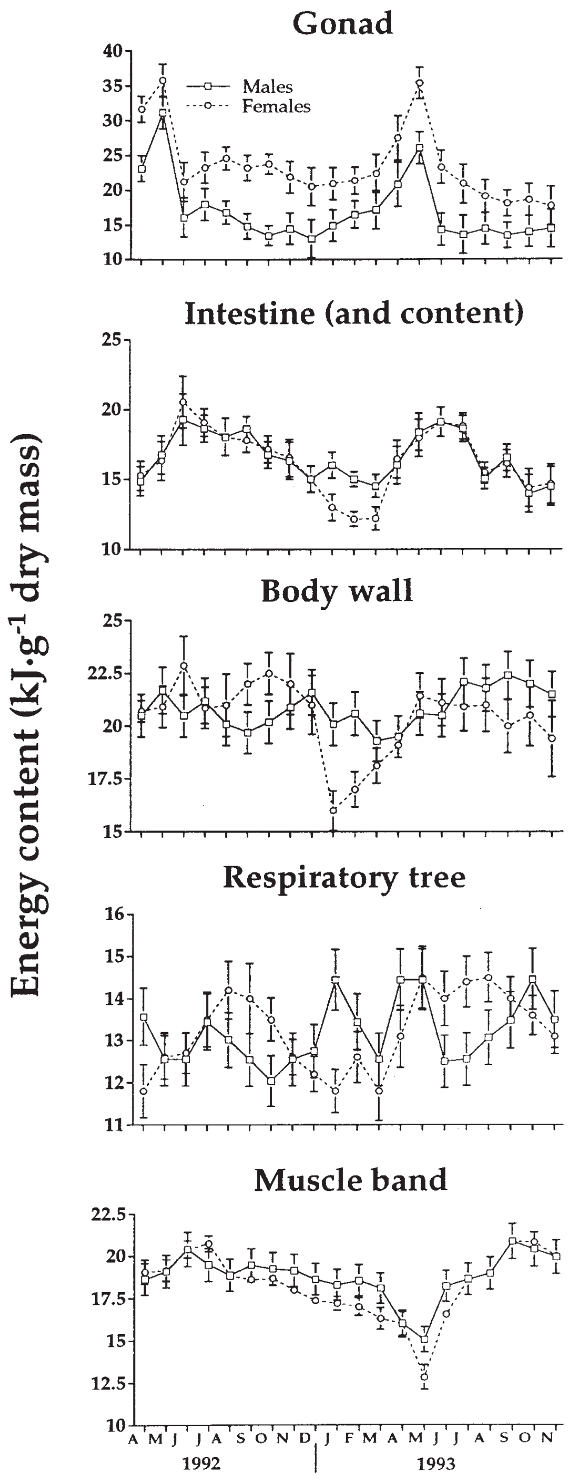

Figure 6: *Cucumaria frondosa*. Seasonal variation of the energy content in 1 g dry mass of gonad, intestine, body wall, respiratory tree and muscle band from April 1992 to November 1993. The vertical lines represent the confidence interval (95%).

Freshwater run-off and chlorophyll a

Chlorophyll a concentrations at the beginning of the experiment (May 1992) fluctuated around 0.5 and 1 $mg.m^{-3}$. A strong increase in chlorophyll a occurred in mid-June 1992 with a peak at 6 $mg.m^{-3}$, indicating an important phytoplanktonic biomass. Spawning occurred at that time. This high production persisted until August, although the pigment concentrations were not constant and fluctuated sometimes from the highest values (≈7 $mg.m^{-3}$) to less than 1 to 2.2 $mg.m^{-3}$.

During late fall and winter, the chlorophyll a concentrations were minimal and fluctuated around 0.2$mg.m^{-3}$, until next spring. The same cycle roughly repeated istelf in 1993. The fresh-water run-off reached a maximum in March and April (1992 and 1993), attaining 3,000 to 8,000 $m^3.s^{-1}$. The abrupt decrease of freshwater run-off coincided with the increased concentration of chlorophyll a in the water column, in June of both years. The minimum value of about 500 to 700 $m^3.s^{-1}$ was maintained until the next spring.

Photoperiod

The minimum day-length was observed during the third week of December 1992 (8.2 $h.d^{-1}$). From this time, the day-length increased progressively and attained its maximum during the third week of June (15.5 $h.d^{-1}$). After reaching this maximum, the day-length began to decrease progressively to return to its minimum value, in December 1993.

Discussion

Gonad morphology versus geographical location

The gonad morphology and reproductive cycle of *Cucumaria frondosa* was not uniform over the scope of sampling sites, along the north-east American coast. The gonad of *C. frondosa* was divided into two distinct classes of gonadal tubules in northern latitudes, while uniformity among the tubules was evident south of mid-New Brunswick. Very different local environmental conditions may regulate this morphological disparity.

Satellite images and in situ data indicate a mean annual difference of 8°C, between the northern and the southern surface of the Gulf of St Lawrence (J. Chassé & D. De Lisle, personal communications). This temperature transition, occurring along the coast of New Brunswick, is probably a consequence of depth variations. Specific gonad morphology was also correlated with the length of the vegetative season in those areas.

Favourable conditions for primary production spread from April to October in the southern regions (Roman & Tenore, 1978; Fournier et al., 1979; Bowman et al., 1981), whereas they occur only from June to September–October around the St Lawrence Estuary (Levasseur et al., 1984; Therriault & Levasseur, 1985, 1986).

As a result, sea cucumbers south of New Brunswick benefit from a longer feeding period. This, combined to a higher mean annual temperature, may favour the development of their entire gonad over a single year, and increase the number of gametes that reach maturity in that interval.

From their work on *Stichopus californicus*, Smiley & Cloney (1985) and Smiley (1988) inferred that the presence of different classes of gonadal tubules, and their successive maturation, was as a constant trade among many species of sea cucumbers.

However, Sewell (1992) demonstrated that the reproductive cycle and gonad morphology varied within samples of *S. mollis*, collected at different latitudes. Like our data on *Cucumaria frondosa*, those results tend to promote the occurrence of a certain plasticity in the gonad morphology of sea cucumbers, when members of the same species are submitted to different environmental conditions.

Cucumaria frondosa *from the St. Lawrence Estuary*

Size at sexual maturity and fecundity

It is well known that a certain minimal body size is necessary before an individual becomes reproductive (Lawrence, 1987). Young *Cucumaria frondosa* reached sexual maturity at about 55g (wet body-wall mass), a very small size compared to the maximum 350 g encountered in the field. This may suggest that, from that moment on, gonadic and somatic growths have to share the available energy, with the probable consequence of reducing the rate of somatic development soon in the life of the sea cucumber.

However, Hamel & Mercier (in press) observed that the growth rate of *C. frondosa* increased significantly well before and after reaching the sexual maturity, in laboratory and in the field. The growth rate did not seem affected by the level of development of the gonad. This may be related in part to the fact that food availability, in terms of planktonic material, is not limiting in the St. Lawrence Estuary, exceeding by far the need of the young sea cucumber and allowing the concurrent growth of somatic and reproductive tissues.

This small size at sexual maturity was also observed in the sea cucumber *Psolus fabricii* (Hamel et al., 1993) and in the sea urchin *Strongylocentrotus droebachiensis* (Munk, 1992).

The fecundity of females *Cucumaria frondosa* increased rapidly with size. It attained about 9,000 mature oocytes.year^{-1} in individuals of 170 g. The annual fecundity of *C. frondosa* is higher than that of other dendrochirotida, such as *C. pseudocurata* (340 oocytes) and *Psolidium bullatum* (3,000 oocytes), but roughly similar to the fecundity of *C. fallax* (8,800 oocytes) and much lower than that of *C. piperata* (1.1 x 10^4 oocytes), *Psolus chitonoides* (3.5 x 10^4 oocytes), *Pentamera populifera* (3.5 x 10^4 oocytes) and *C. miniata* (1.32 x 10^5 oocytes) (see review McEuen 1987). In regards to the size of the organism, the number of gametes synthesised by *C. frondosa* seems quite low, compared for instance with *P. chitonoides*, a smaller species that produces larger amounts of gametes.

However, the great abundance of *C. frondosa* in the study site (5–15 ind.m^{-2}), their spawning behavior (Hamel & Mercier, 1995), and the large size of their oocytes may ensure a high fertilisation success despite the low fecundity of the species. The same conclusion was formulated by Levitan (1993) for the sea urchin *Strongylocentrotus*, which also synthesises small amounts of large oocytes.

Gametogenesis and reproductive cycle

The annual reproductive cycle of *Cucumaria frondosa* from the St Lawrence Estuary culminated in an early summer spawning. Gametes were released from large gonadal tubules that had matured over the previous year, while small gonadal tubules contained only gametes that would need another year of growth to become mature. Two years of growth seem necessary for each tubule to reach maturity.

Our data of gonad morphology, gametogenesis initiation and development, as well as spawning period, are similar to those obtained with *Psolus fabricii* (Hamel et al., 1993), another sea cucumber found in the Lower St Lawrence Estuary.

However, our results differ from those obtained by Smiley & Cloney (1985) and Smiley (1988) with *Stichopus californicus*. The location of the different classes of gonadal tubules was correlated with their degree of maturity in *S. californicus*, but was totally random in *Cucumaria frondosa.*

Also, the tubules that were implicated in the spawning of *S. californicus* resorbed afterward, whereas they did not in *C. frondosa*. In fact, precursor cells became visible along the germinal epithelium of those gonadal tubules the next January, while growth continued for a subsequent year.

Gametogenesis in *Cucumaria frondosa* was initiated in early January, after a long period of recovery. Intense gamete synthesis and reserve storage began in March and continued until the spawning event, in mid-June. Such results are quite different from those obtained for the same species by Jordan (1972) on the east coast of Maine and by Coady (1973) in Newfoundland.

Coady (1973) indicated that the spermatogenesis in *C. frondosa* was initiated in early June, following the spawning period, and continued until late November. He observed that females did not mature in synchrony with males.

Moreover, Coady (1973) mentioned that the majority of males reached maturity several months before spawning, while the final maturation of oocytes occurred just before their release. As for Jordan (1972), he stated that oogenesis was initiated in early summer and maturity was attained in early winter, while spermatozoa were constantly produced.

Those two studies came up with different results which are also opposed to our own. The sea cucumbers collected close to the study sites of Jordan (1972) and Coady (1973), on the southern coast of Newfoundland (station 3) and along the coast of New England (stations 12, 13, 14), present a single class of gonadal tubules. This may explain in part the difference observed between the gametogenetic cycles of *C. frondosa* from the St Lawrence Estuary and those living southerly.

The similarity between male and female gametic development strongly suggests the importance of environmental factors in its regulation. The only environmental factor that can be correlated to the initiation of gametogenesis in early January appears to be photoperiod. The first appearance of oogonia and spermatogonia nests along the germinal epithelium of the small gonadal tubules coincided with the winter solstice, which marks the increase of daylength. At that time, salinity and water temperature in the study site were roughly constant, around 29 and 0°C, respectively, while chlorophyll a concentration was virtually nil and the intestine was mainly filled with non-living material, indicating that food was scarce.

Those factors could not have had much influence on the initiation of gametogenesis. Photoperiodic regulation of gametogenesis was proposed for various species of echinoderms such as the sea urchin *Strongylocentrotus purpuratus*, the sea stars *Pisaster ochraceus* (Pearse & Eernisse, 1982) and *Asterias vulgaris* (Pearse & Walker, 1986) as well as other species of sea stars from the west coast of the United States (Pearse et al., 1986a, b). Photoperiodic control was also suggested for the sea cucumber *Psolus fabricii* (Hamel et al. 1993). Later on, in early spring, the analysis of intestinal content and chlorophyll a concentration in the field showed that food availability was increasing, along with rising temperature. This period coincided with a net increase of gamete synthesis in *Cucumaria frondosa*.

According to Lawrence (1987), rarely are the testes larger than the ovaries. However, the gonadal index of male *Cucumaria frondosa* was higher than that of female by as much as 20 per cent, before the spawning event. This was correlated with the proportionally larger and heavier gonad of male, also observed in the sea cucumber *Psolus fabricii* (Hamel et al., 1993).

Inversely, Tuwo & Conand (1992) indicated that female *Holothuria forskali* had higher gonadal indices than male. Other sea cucumbers, like *Aslia lefevrei* (Costelloe, 1985), *Parastichopus californicus* (Cameron & Fankboner, 1986) and *Stichopus mollis* (Sewell & Bergquist, 1990) present no disparity between male and female gonadal indices.

The calorific value of 1 g dry mass of gonad was about 10 to 18% higher in female than in male *Cucumaria frondosa*, prior to the spawning period. Thus, male gonads were probably bigger, mainly because less energy is required to synthesise an equivalent amount of gametes. Especially since no difference can be observed in the maximum body size attained by males and females. Lawrence & Lane (1982) indicated that ovaries are richer than testis even if they are of the same size and more sperm may be produced per unit of gonad volume. It therefore appears that the reproductive effort of *C. frondosa*, in terms of the energy invested in gamete synthesis, is equivalent in males and females.

During the period of low food availability, the energy content in the female body wall seemed to decrease, even though its mass did not vary significantly. This suggests that a metabolic activity requiring energy was in progress, as also proposed by Ong Che (1990) for the sea cucumber *Holothuria leucospilota*. A translocation of energy to the gonad, during the early phase of gametogenesis, possibly occurred at that time. Female gamete synthesis probably required more energy, at first, than that of male, in which the translocation process is less clearly defined.

References

Bowman, M. J., W. E. Esaias & M. B. Schnitzer. (1981). Tidal stirring and the distribution of phytoplankton in Long Island and Block Island Sound. J. Mar. Res. 39: 587–603.

Cameron, J. L. & P. V. Fankboner. (1986). Reproductive biology of the commercial sea cucumber *Parastichopus californicus* (Stimpson) (Echinodermata: Holothuroidea). I. Reproductive periodicity and spawning behavior. Can. J. Zool. 64: 168–175.

Coady, L. W. (1973). Aspects of the reproductive biology of *Cucumaria frondosa* (Gunnerus, 1770) and *Psolus fabricii* (Düben et Kören, 1846) (Echinodermata: Holothuroidea) in the shallow waters of the Avalon Peninsula, Newfoundland. M.Sc. thesis, University Memorial, Newfoundland, Canada, 110 p.

Costelloe, J. (1985). The annual reproductive cycle of the holothurian *Aslia lefevrei* (Dendrochirotida: Holothuroidea). Mar. Biol. 88: 155–165.

Costelloe, J. (1988). Reproductive cycle, development, and recruitment of two geographically separated populations of the dendrochirote holothurian *Aslia lefevrei*. Mar. Biol. 99: 535–545.

Fournier, R. O., M. Vandet, J. S. Wilson & N. B. Hargreaves. (1979). Influence of the shelf-break front off Nova Scotia on phytoplankton standing stock in winter. J. Fish. Res. Bd. Can. 36: 1228–1237.

Hamel, J.-F., J. H. Himmelman & L. Dufresne. (1993). Gametogenesis and spawning of the sea cucumber *Psolus fabricii*. Biol. Bull. 184: 125–143.

Hamel, J.-F. & A. Mercier. (1995). Spawning of the sea cucumber *Cucumaria frondosa* in the St. Lawrence Estuary, eastern Canada. SPC Beche-de-mer Information Bulletin 7: 12–18.

Hamel, J.-F. & A. Mercier, A. (in press). Early development, settlement, growth and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). Can. J. Fish. Aquat. Sci.

Jordan, A. J. (1972). On the ecology and behavior of *Cucumaria frondosa* (Echinodermata: Holothuroidea) at Lamoine Beach, Maine. Ph.D. Thesis, University of Maine, Orono, 74 p.

LAWRENCE, J. M. (1987). A functional biology of echinoderms. The Johns Hopkins University Press, Baltimore, USA, 340 p.

LAWRENCE, J. M. & J. E. M. LANE. (1982). The utilization of nutrients by post-metamorphic echinoderms. **In:** Jangoux, M. & J.M. Lawrence (eds) Echinoderm nutrition. A. A. Balkema, Rotterdam, p. 331–371.

LEVASSEUR, M., J. C. THERRIAULT & L. LEGENDRE. (1984). Hierarchical control of phytoplankton succession by physical factors. Mar. Ecol. Prog. Ser. 19: 211–222.

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

MCEUEN, F. S. (1987). Phylum Echinodermata, Class Holothuroidea. **In:** M. F. Strathmann, (ed.). Reproduction and development of marine invertebrates of the northern Pacific coast, data and methods for the study of eggs, embryos, and larvae. University of Washington Press, Seattle, p. 556–596.

MUNK, J. E. (1992). Reproduction and growth of green sea urchins *Strongylocentrotus droebachiensis* (Müller) near Kodiak, Alaska. J. Shellfish Res. 11: 245–254.

ONG CHE, R. G. (1990). Reproductive cycle of *Holothuria leucospilota* Brandt (Echinodermata: Holothuroidea) in Hong Kong and the role of body tissues in reproduction. Asian Mar. Biol. 7: 115–132.

PEARSE, J. S. & C. W. WALKER. (1986). Photoperiodic regulation of gametogenesis in a North Atlantic sea star, *Asterias vulgaris*. Int. J. Invert. Reprod. Develop. 9: 71–77.

PEARSE, J. S. & D. J. EERNISSE. (1982). Photoperiodic regulation of gametogenesis and gonadal growth in the sea star *Pisaster orchraceus*. Mar. Biol. 67: 121–125.

PEARSE, J. S., J. D. EERNISSE, V. B. PEARSE & K. A. BEAUCHAMPS. (1986a). Photoperiodic regulation of gametogenesis in sea stars, with evidence for an annual calendar independent of fixed daylight. Am. Zool. 26: 417–431.

PEARSE, J. S., V. B. PEARSE & K. K. DAVIS. (1986b). Photoperiodic regulation of gametogenesis and growth in the sea urchin *Strongylocentrotus purpuratus*. J. Exp. Zool. 237: 107–118.

PENNINGTON, J. T. (1985). The ecology of fertilization of echinoid eggs: The consequences of sperm dilution, adult aggregation and synchronous spawning. Biol. Bull. 169: 417–430.

ROMAN, M. R. & K. R. TENORE. (1978). Tidal resuspension in Buzzards Bay, Massachusetts. 1. Seasonal changes in the resuspension of organic carbon and chlorophyll a. Estuar. Coast. Mar. Sci. 6: 37–46.

SEWELL, M. A. (1992). Reproduction of the temperate Aspidochirote *Stichopus mollis* (Echinodermata: Holothuroids) in New Zealand. Ophelia 35: 103–121.

SEWELL, M. A. & P. R. BERGQUIST. (1990). Variability in the reproductive cycle of *Stichopus mollis* (Echinodermata: Holothuroidea). Int. J. Invert. Reprod. Develop. 17: 1–7.

SMILEY, S. (1988). The dynamics of oogenesis and annual ovarian cycle of *Stichopus californicus* (Echinodermata: Holothuroidea). Biol. Bull. 175: 79-93.

SMILEY, S. & R. A. CLONEY. (1985). Ovulation and fine structure of the *Stichopus californicus* (Echinodermata: Holothuroidea) fecund ovarian tubules. Biol. Bull. 169: 342–364.

SMILEY, S., F. S. MCEUEN, C. CHAFFEE & S. KRISHNAN. (1991). Echinodermata: Holothuroidea. **In**: Giese, A. C., J. S. Pearse & V. B. Pearse. (eds). Reproduction of marine invertebrates, Echinoderms and Lophophorates. Boxwood Press, California, p. 663–750.

TANAKA, Y. (1958). Seasonal changes occurring in the gonad of *Stichopus japonicus*. Bull. Fac. Fish. Hokkaido Univ. 9: 29–36.

THERRIAULT, J.-C. & M. LEVASSEUR. (1985). Control of phytoplankton production in the lower St. Lawrence Estuary: light and freshwater run-off. Nat. Can. 112: 77–96

THERRIAULT, J.-C. & M. LEVASSEUR. (1986). Freshwater run-off control of spatio-temporal distribution of phytoplankton in the lower St Lawrence Estuary (Canada). **In:** Skresled, S. (ed.) Proceedings of the NATO Freshwater, sea Workshop, Norway. NATO ASI Series, Vol. G7, Springer-Verlag, New York, p. 251–260.

TUWO, A. & C. CONAND. (1992). Reproductive biology of the holothurian *Holothuria forskali* (Echinodermata). J. Mar. Biol. Ass. U. K. 72: 745–758.