Gamete dispersion and fertilisation success of the sea cucumber *Cucumaria frondosa*

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Abstract

During spawning in the field, the buoyant oocytes of Cucumaria frondosa progressed to the surface, and fertilisation occurred as they made their way through a dense layer of spermatozoa, spread over the spawning site. Consequently, the proportion of fertilised eggs increased with distance from the bottom, passing from 27% near the spawning population to about 85% at the surface. Asynchronous gamete release from both sexes combined with oocyte buoyancy and prolonged sperm potency therefore appear as determinant factors leading to fertilisation success in this species.

Introduction

In recent years, a number of studies used field observations, laboratory experiments, or both, to determine the fertilisation success of echinoderms under various current conditions and population densities (Pennington, 1985; Sewell & Levitan 1992; Levitan et al., 1992; Young et al. 1992; Levitan, 1993; Benzie & Dixon, 1994; Babcock et al., 1994; Benzie et al., 1994; Hamel & Mercier, 1995a).

Some even predicted the fertilisation success according to different physical parameters (Denny & Shibata, 1989; Levitan et al., 1991; Denny et al., 1992; Young et al., 1992) or to the in situ gamete abundance and fertilisation rate (Benzie & Dixon, 1994; Babcock et al., 1994; Benzie et al., 1994).

Pennington (1985) even conducted a field experiment in order to estimate the natural fertilisation rate in *Strongylocentrotus droebachiensis*. Most of those studies used KCl to induce spawning in laboratory or directly in the field. Not using this artificial technique, Sewell & Levitan (1992) described the in situ fertilisation success during a natural spawning of the sea cucumber *Cucumaria miniata*.

Other exceptions comprise the field measurement of sperm dispersal and fertilisation in the colonial hydroid *Hydractinia echinata* (Yund, 1990) and the work of Benzie & Dixon (1994), Babcock et al. (1994), Benzie et al. (1994) on the crown-of-thorn *Acanthaster planci*.

However, no continuous observation of the fertilisation success was ever made during a natural spawning, considering the number and position of individuals on the substrate and the influence of environmental parameters.

Our study was not designed to build a predictive model of fertilisation success in *Cucumaria frondosa*, but rather focused on evaluating the sequence of events that takes place upon the release of gametes in the water column. To achieve that, we kept a continuous record of sperm and oocyte concentrations and fertilisation rates in the field over several hours, during a natural spawning event.

We also concurently monitored various environmental factors. This experiment brings new light to the understanding of gamete behavior in *C. frondosa* and perhaps other broadcast spawners.

Materials and Methods

Monitoring of the spawning event

The experiment was conducted at Les Escoumins, Lower St Lawrence Estuary, eastern Canada, in summer 1992. Using SCUBA, we monitored gamete release, dispersion and fertilisation during a natural spawning of males and females *Cucumaria frondosa*. Beginning with the first signs of spawning, divers took turns underwater by groups of up to 10 divers for 50 hrs, until the end of spawning. The divers were positioned at different depths using an electronic depth meter. Each team dived for a maximum of 35 mins at intervals of 3–4 hrs or longer. Most of the measures were taken above 10 m (only a few divers needed to descend at 15 m). The monitoring of environmetal parameters and the spawning sequence are described in a previous paper (Hamel & Mercier, 1995b).

Gamete behaviour (single individual)

These data were collected during the first isolated spawning events, without artificial stimulation. We studied only spawning individuals at least 20 m

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away from the closest spawning neighbour. The distinct peristaltic movement of the body, from the anus to the mouth, and the well-extended gonopore were the major signs visible for divers. After locating a single spawning individual, the divers (without disturbing the animal) placed a transect line (25m long) parallel to the direction of current, maintained by a buoy at the surface, and attached to the nearest boulders.

This operation was performed in less than 15 mins. All individuals that initiated gamete release before the final installation of the transect line were rejected. Water samples were collected with Niskin bottles (2.8 l) at various depths over a spawning individual, from the beginning of spawning (time 0), and at regular interval until 150 mins. The number of spermatozoa was determined in 5 subsamples of 2 ml under a microspcope with an hemacytometer.

The abundance of oocytes was assessed in 2 subsamples of 1 l, using a binocular. The laboratory work involving microscopy was carried out within an hour in nearby installations. We also noted the progression of oocytes in the field along a 3 m long graduated tube, using 5 replicates, during both slack and flood tides.

Gamete behaviour (massive spawning)

Additional underwater data were taken during the massive spawning (when more than 65% of the observed individuals were spawning), to evaluate the gross male and female gamete concentrations at various depths until the end of this event. Again using Niskin bottles, 5 samples of water were taken at regular intervals (between 30 and 50 mins) for more than 6 hrs. Gamete abundance was evaluated as previously described. The first male spawning observed in the study site marked time 0 of collection.

Fertilisation success during spawning

We recorded the percentage of fertilised oocytes present at different depths and at the surface of the water, at regular intervals (about 50 mins), during the entire female spawning. Using Niskin bottles (2.8 l), a sample of water (generally containing oocytes and spermatozoa) was collected at each depth (0, 5, 10, 15 m) and was injected within 5 mins with 25 ml of 37% formaldehyde to stop further development or fertilisation of the eggs.

Fertilisation was verified by staining the sample of gametes with the DNA-specific dye Hoechst 33258 and observing it under fluorescence microscopy (Leitz Diaplan fluorescence microscope) to determine the presence of male pronuclei in the eggs. The fertilization success was also estimated under a light microscope from evidence of fertilization membrane elevation, presence of polar body or cellular division.

Results

Spawning of a single individual

Because of the low variability in gamete densities between individuals (≤15%), the data from all single individuals of the same sex were combined (7 males and 9 females). The results are expressed as an average for all individuals observed over the 150-min period.

Male

Within the first 5 min of gamete release, a rapid increase of the spermatozoa concentration within 3 m of the spawning individual occurred (figure 1). This concentration fluctuated around 1 x 10^5 spermatozoa.ml^{-1} at time 0 near the spawning individual, and increased rapidly to reach a maximum of 10 x 10^5 spermatozoa.ml^{-1} after 10 mins, within 0.5 m of the spawning individual. At first detected only within 0.5 m of the spawning male, the spermatozoa slowly dispersed and could be seen at 3 m after 30 s, at 3.5 m after 5 mins, and at 4.5 m after 10 mins.

At that time, white clouds of sperm were dispersing laterally and vertically in the water column more than 3 m away from the spawning individual. After 15–30 mins, the maximum density of spermatozoa was observed, combining the observations at all depths studied. The spermatozoa concentration reached about 1.8 x 10^5 spermatozoa.ml^{-1} at 7 m of distance and remained very high near the spawning individual where it never exceeded about 10 x 10^5 spermatozoa.ml^{-1} (see figure 1). After 100 mins, the spermatozoa densities decreased rapidly near the spawning individuals (<0.1 x 10^5 spermatozoa.ml^{-1}), which at that time had ceased to release their gametes. However, the concentration remained high away from the individuals, attaining the highest concentration after 150 mins at 7 m, with 4.5 x 10^5 spermatozoa.ml^{-1} (see figure 1). The spermatozoa densities remained considerable in the plume of gametes throughout the spawning, especially near the water surface.

Female

At the time female spawning was initiated, spermatozoa densities were around 1.5 x 10^6.ml^{-1} due to massive male spawning. As soon as a female began

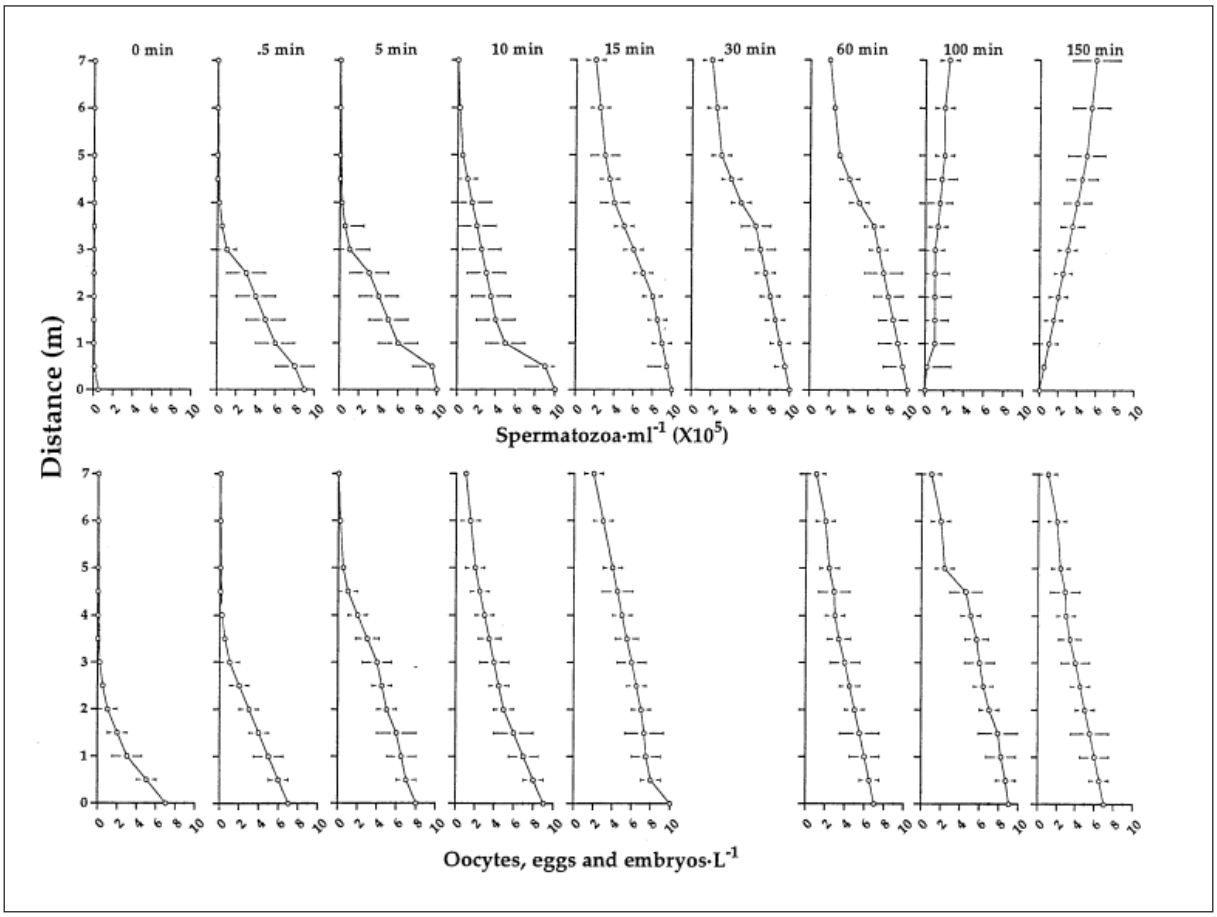
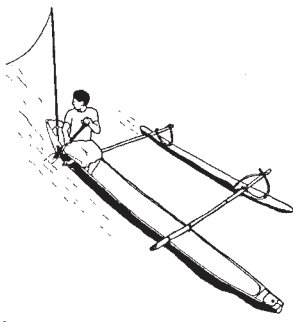


Figure 1: *Cucumaria frondosa*. Density of male or female gametes, released by a single sea cucumber, from the beginning (time 0 min) to the end of spawning (time 150 mins). The horizontal lines represent the 95% confidence interval.

to release gametes, the density of oocytes around it increased rapidly, attaining 7 oocytes.l^{-1} in less than 30 s near the individual, and 2 oocytes.l^{-1} within 3 m.

After 10 mins, the oocyte density attained 9 oocytes.l^{-1} within 0.5 m and gametes were detectable up to 7 m away from the spawning individual with about 2 oocytes.l^{-1}. After 15 mins, the maximum density of oocytes was observed in the water column, both near and away from the spawning individual, attaining about 10 oocytes.l^{-1} and 3 oocytes.L^{-1}, respectively (see figure 1). Following that, no significant change in oocyte density was observed, whatever the depth, until the end of the experiment.



The very buoyant oocytes of *Cucumaria frondosa* reached the water surface and spread in a dense layer (2–3 m thick) below the sea surface, where thousands of eggs developed. The majority of oocytes (≈87%) progressed at 0.75+0.2 m.min^{-1} during slack tide, but this rate was increased drastically when measurements were made during the flood tide. Then, the oocytes or eggs moved up at an average of 2.2+0.5 m.min^{-1}, while the current velocity near the bottom was between 2 and 5 cm.s^{-1}. Only immature oocytes, with a poorly developed vitelline reserve, sank to the bottom.

Massive spawning of males and females

Occurring well after the first isolated spawnings in both sexes, the massive spawning was also monitored. Between 65 and 80% of males and females were spawning (Hamel & Mercier, 1995b), and the amount of gametes in the water column was already high as we began to record them (see figures 1 & 2). Sperm densities at the beginning of the observations were uniform between the surface and 15 m, showing an average of 6.5 x 10^6 spermatozoa.ml^{-1}. Those densities remained uniform until

850 mins, despite the continuous supply of new gametes from spawning individuals. The maximum densities recorded were observed after 850 min, with about 15.5 x 10^6 spermatozoa.ml^{-1} near the bottom (15 m). By 950 min, the majority of males (≥93%) had stopped spawning and the sperm densities began to decrease rapidly near the bottom, reaching their lowest value (≈2.5 x 10^6 spermatozoa.ml^{-1}). When female spawning was at its maximum, involving about 83% of individuals (roughly 60 mins after maximum male spawning (see Hamel & Mercier 1995b), the fertilized oocytes undergoing the first cleavage were very abundant just below the surface (about 40 oocytes.l^{-1}).

However, the oocyte densities increased continuously in the water column to reach about 100 oocytes.l^{-1} after 800 min near the bottom, and about 40 oocytes.l^{-1} near the surface. After more than 850 mins, the concentration of oocytes increased rapidly at the surface and reached 180 oocytes.l^{-1} after 950 mins. After 1050 mins, the vast majority of females stopped spawning and the oocyte densities decreased near the bottom, falling to less than 10 oocyte.l^{-1}, while the density remained high (140 oocytes.l^{-1}) near the surface (see figure 2). Spawning within the entire population was over after about 24 hrs. A single individual, male or female, spawned during about 2 or 3 hrs. Spawning of the majority of individuals occurred in a small 3-hrs interval, during which more than 65% of the population was releasing gametes.

Fertilisation success

Immediately after the first release of oocytes in the water column (600 mins), the fertilisation rate remained low close to the gonopore with only 9% of the oocytes fertilized (see figure 3). After 620 mins, about 82% of the oocytes collected near the surface were fertilised compared to 15% near the spawning females. The proportion of fertilization success was never higher than 27% near the spawning female (figure 3).

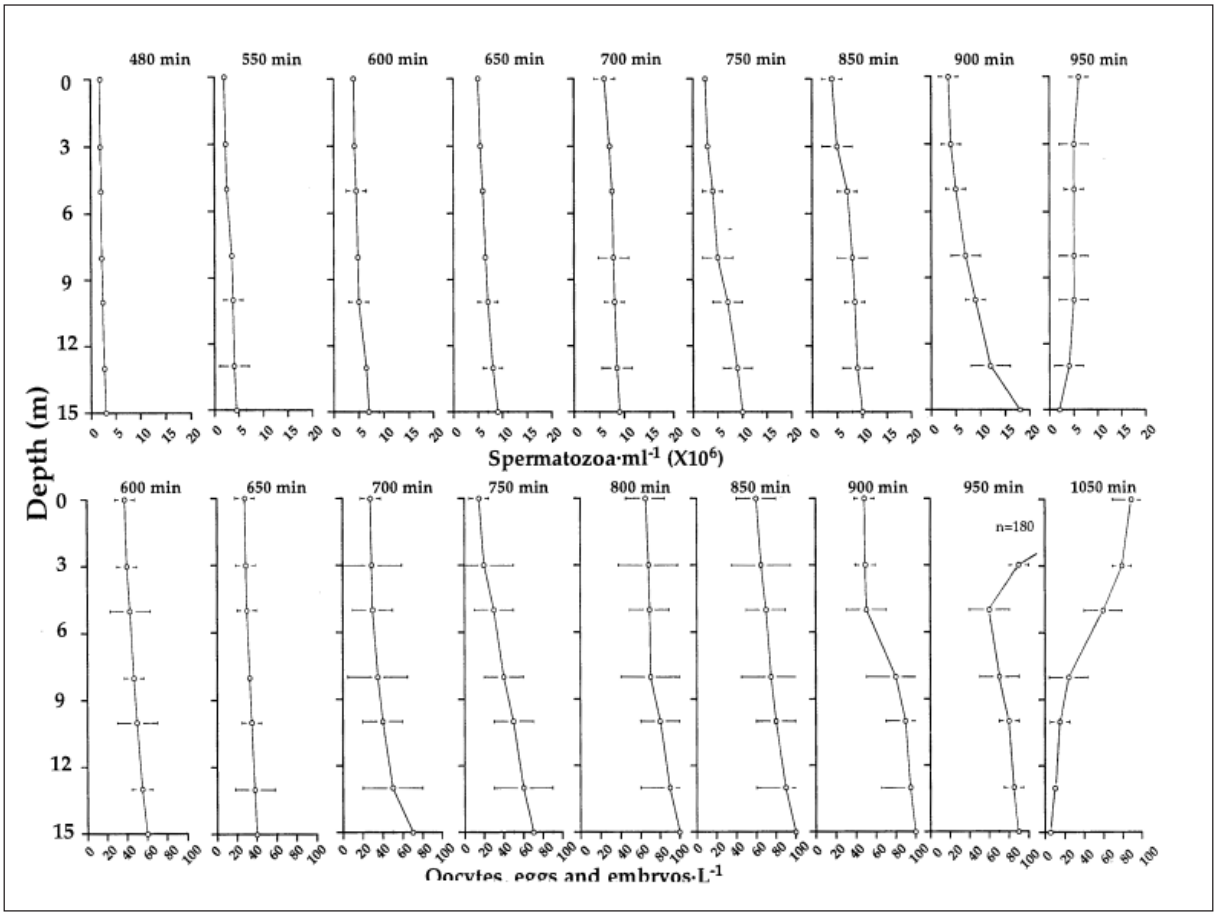


Figure 2: *Cucumaria frondosa*. Integrated value of gamete densities in the water column (from 15 m to the surface) for males and females, throughout their respective massive spawning, in June 1992. The time above the graphics corresponds to the time elapsed since the first record of male spawning. The horizontal lines represent the confidence interval (95%).

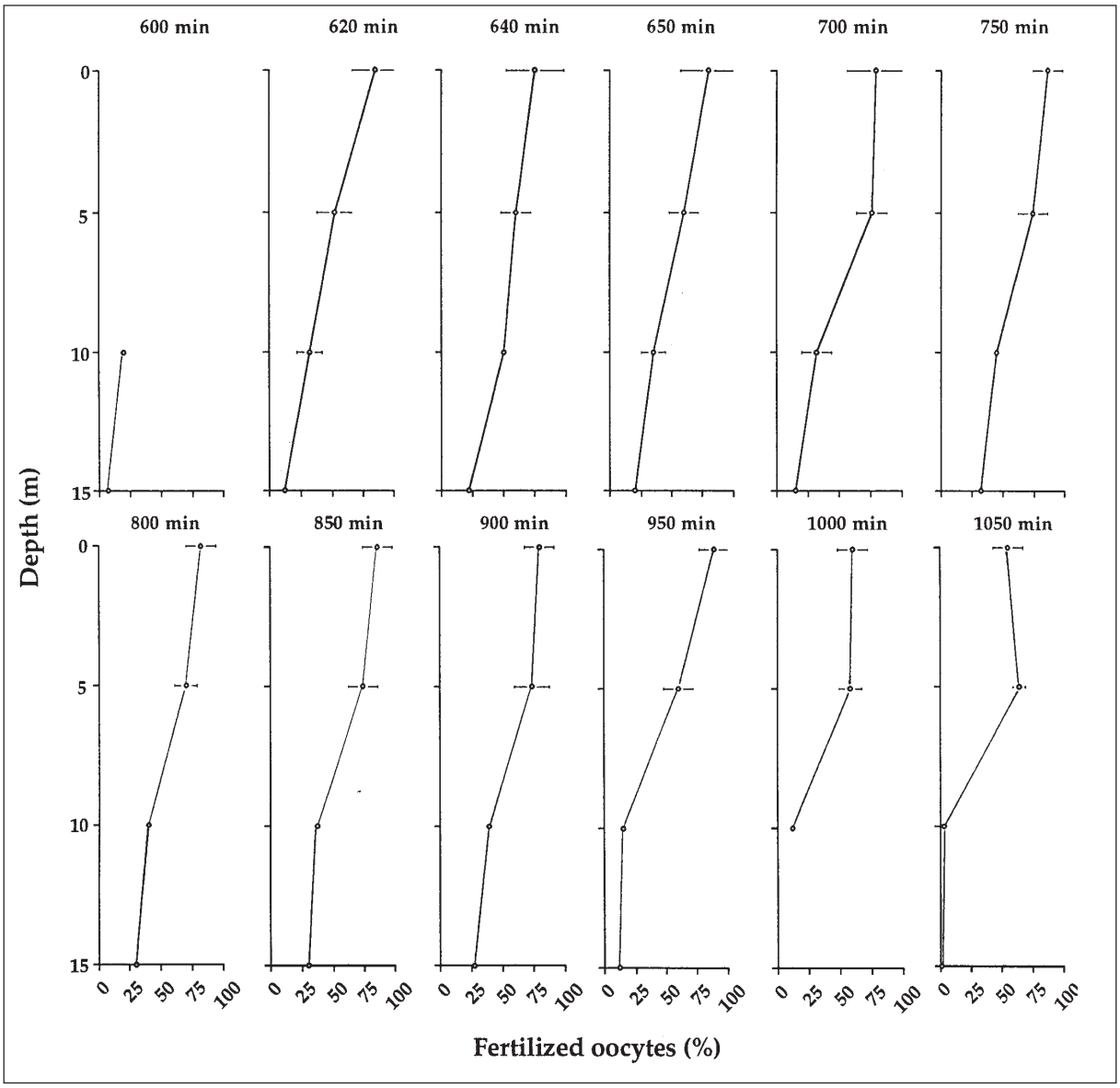


Figure 3: *Cucumaria frondosa*. Percentage of fertilised oocytes at 15, 10, 5 m and at the surface of the water, throughout the entire female spawning. The time above the graphics corresponds to time elapsed since the first record of male spawning. The horizontal bars represent the confidence interval (95%).

The overall proportion of fertilised eggs in the entire water column increased to attain a maximum after 640 mins. Following that time, the proportion of fertilised oocytes remained stable until 950 mins. At that moment, the majority of males had stopped spawning.

Nonetheless, the fertilisation rate remained high near the water surface, where it never decreased below 75 per cent, until the end of the observations. However, from 1000 to 1050 mins the fertilisation success decreased rapidly near the spawning females, becoming virtually nil, but was still high near the surface where it reached about 45% at the end of the experiment.

Discussion

The data of gamete dispersion and fertilisation success measured in the field during the spawning of *Cucumaria frondosa* differed somewhat from almost every model prediction. They also differed from empirical data collected in nature during the spawning of other marine invertebrates. The only closely resembling results are those presented by Sewell & Levitan (1992) for a congeneric species, *C. miniata* from the west coast of Canada.

Despite the fact that most models took various factors such as current conditions, substrate morphology, density of individuals, distance between individuals and gamete abundance into acount, the differences we observed may result from numer-

ous other factors that are difficult to consider with enough accuracy during modelisation. Spawning synchrony between the sexes, complex aggregative behaviours, chemical communication, gamete viability and behaviour, along with environmental conditions prevailing during natural spawning events, are among the most important variables rarely taken into account. A 1-hr delay between the peak of male and female spawnings of *Cucumaria frondosa* (Hamel & Mercier, 1995b) could appear negligible, but it allowed a maximum concentration of sperm to be attained in the water column (3 to 18 x 10^6 spermatozoa.ml^{-1}) prior to female spawning (figure 2).

The asynchronous spawnings also favoured the formation of a cloud of spermatozoa, through which the oocytes passed on their way to the surface, enhancing fertilisation. That is probably why the maximum proportion of fertilised eggs (≈85%) was observed in the upper water layer. It was also the case in *C. miniata*, studied by Sewell & Levitan (1992). The maximum fertilisation success we measured in the field corresponded to the maximum success obtained in laboratory with *C. frondosa* by Hamel & Mercier (in press). The 10-hrs sperm potency observed in *Cucumaria frondosa* (Hamel & Mercier, in press) probably played a major role in the concentration of active sperm in the water column prior to female spawning.

This potency is much longer that the 20 mins recorded for the sperm of sea urchin *Strongylocentrotus droebachiensis* by Pennington (1985). He and Denny (1988) indicated that the sperm longevity was probably relatively unimportant in high current conditions, because sperm is rapidly diluted by turbulence. The population of *C. frondosa* observed in the St Lawrence Estuary does live in an area of high energy level, where the tidal amplitude and the current enhanced by the wind are strong.

However, the main spawning event occurred during slack current at low tide (Hamel & Mercier 1995b). The sperm and oocyte concentrations remained high over the spawning population during the experiment, especially during the massive spawning (figure 2). Although some gametes may have been exported outside the bay continually, we suggest that a net residual current and the wind that blew toward the coast favoured the retention of a large proportion of gametes inside the bay and reduced their exportation. This allowed the maintenance of a density as high as 18 x 10^6 spermatozoa.ml^{-1} during the massive spawning of the females.

The majority of gametes were spawned during the period of slack current (Hamel & Mercier, 1995b). The low tide and current conditions, along with the massive spawning of males and females, apparently contributed to minimise gamete dispersion prior to fertilisation, as also suggested by McEuen (1988) for some species of dendrochirotes and by Sewell & Levitan (1992) in *C. miniata*. McDowall (1969) and Sewell & Levitan (1992) proposed that low tide reduces the volume of water into which gametes are diluted and increases fertilisation success.

The density of *Cucumaria frondosa* varied between 5 and 18 ind.m^{-2} in the study site, representing a biomass of about 3 to 15 kg.m^{-2}, between 10 and 15 m (Hamel & Mercier, in press). This density, combined with an equal distribution of males and females, may also have favoured a high level of fertilisation by maintaining an important concentration of sperm and limiting gamete dilution. The population density was also previously suggested as a major factor influencing the fertilisation success in other species of echinoderms (Levitan, 1991; Levitan et al., 1992; Sewell & Levitan, 1992). Some models of fertilisation success in marine invertebrates tend to integrate many important and realistic factors. The more abundant those parameters, the more closely related to field data are the predictions.

Despite that, numerous difficulties are inherent to in situ measurements of gamete dispersion and fertilisation success, and we believe that more direct and careful observations of the various conditions prevailing during spawning are an essential prerequisite to increase the real applicability of models. Especially since most marine invertebrates demonstrate important strategies to regulate their gamete release in order to achieve a maximum fertilisation success (see reviews of Chia & Walker 1991; Smiley et al., 1991, Pearse & Cameron, 1991). Furthermore, the natural spawnings often last several hours or even days, over which the fine environmental conditions vary continuously.

Numerous factors seem to optimise fertilisation in marine invertebrates, and more precisely in *Cucumaria frondosa*. Population densities, distance between each individual, male to female ratios are certainly important in all species.

In *C. frondosa*, the delay between male and female spawning, the long sperm potency and maintenance of high sperm concentrations in the water column, despite the variable current conditions in the study site, are also essential factors. Finally, the oocyte buoyancy seems to optimize the fertilisation success by increasing the chances of contact with sperm. This assemblage of factors probably explains in part the high annual recruitment and very dense populations of *C. frondosa* along the North shore of the St Lawrence Estuary.

REFERENCES

BABCOCK, R. C., G. D. BULL, P. L. HARRISON, A. J. HEYWARD, J. K. OLIVER, C. C. WALLACE & B. L. WILLIS. (1986). Synchronous spawnings of 105 scleractinian coral species on the Great Barrier Reef. Mar. Biol. 90: 379–394.

BABCOCK, R. C., C. N. MUNDY & D. WHITEHEAD. (1994). Sperm diffusion models and in situ confirmation of long distance fertilisation in the free-spawning asteroid *Acanthaster planci*. Biol. Bull. 186: 17–28.

BENZIE, J. A. H. & P. DIXON. (1994). The effects of sperm concentration, sperm: egg ratio, and gamete age on fertilisation success in the crown-of-thorns starfish (*Acanthaster planci*) in the laboratory. Biol. Bull. 186: 139–152.

BENZIE, J. A. H., K. P. BLACK, P. J. MORAN & P. DIXON. (1994). Small scale dispersion of eggs and sperm of the crown-of-thorns starfish (*Acanthaster planci*) in a shallow reef habitat. Biol. Bull. 153–167.

CHIA, F.-S. & C. W. WALKER. (1991). Echinodermata: Asteroidea. **In:** Giese, A. C., J. S. Pearse & V. B. Pearse (eds). Reproduction of marine invertebrates, Echinoderms and Lophophorates. Boxwood Press, California, 301–353.

DENNY, M. W. (1988). Biology and the mechanics of the wave-swept environment. Princeton University Press, Princeton, New Jersey. 329 p.

DENNY, M. W. & M. F. SHIBATA. (1989). Consequence of surf-zone turbulence for settlement and external fertilization. Am. Nat. 134: 859–889.

DENNY, M. W., J. DAIRIKI & S. DISTEFANO. (1992). Biological consequences of topography on wave-swept rocky shores: I. Enhancement of external fertilisation. Biol. Bull. 183: 220–232.

HAMEL, J.-F. & A. MERCIER. (1995a). Prespawning behavior, spawning and development of the brooding starfish *Leptasterias polaris*. Biol. Bull. 188: 32–45.

HAMEL, J.-F. & A. MERCIER. (1995b). 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. (in press). Early development, settlement, growth and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). Can. J. Fish. Aquat. Sci.

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

LEVITAN, D. R., M. A. SEWELL & F. S. CHIA (1991). Kinetics of fertilization in the sea urchin *Strongylocentrotus fransciscanus*: Interaction of gamete dilution, age, and contact time. Biol. Bull. 181: 371–378.

LEVITAN, D. R., M. A. SEWELL & F. S. CHIA (1992). How distribution and abundance influence fertilization success in the sea urchin *Strongylocentrotus franciscanus*. Ecology 73: 248–254.

MCDOWALL, R. M. (1969). Lunar rhythms in aquatic animals. A general review. Tuatara 17: 133–144.

MCEUEN, F. S. (1988). Spawning behaviors of the northeast Pacific sea cucumbers (Holothuroidea: Echinodermata). Mar. Biol. 98: 565–585.

PEARSE, J. S. & R. A. CAMERON. (1991). Echinodermata: Echinoidea. **In:** Giese, A. C., J. S. Pearse & V. B. Pearse. (eds). Reproduction of marine invertebrates, Echinoderms and Lophophorates. Boxwood Press, California. 513–662.

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

SEWELL, M. A. & D. R. LEVITAN (1992). Fertilisation success during a natural spawning of the dendrochirote sea cucumber *Cucumaria miniata*. Bull. Mar. Sci. 51: 161–166.

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.

YOUNG, C. M., P. A. TYLER, J. L. CAMERON & S. G. RUMRILL. (1992). Seasonal breeding aggregations in low-density populations of the bathyal echinoid *Stylocidaris lineata*. Mar. Biol. 113: 603–612.

YUND, P. O. (1990). An in situ measurement of sperm dispersal in a colonial marine hydroid. J. Exp. Zool. 253: 102–106.