THE QUALITY OF *PECTEN MAXIMUS* SPERM

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ABSTRACT

Sperm of the scallop *Pecten maximus* (L.) can be collected by various methods for artificial reproduction. Characteristics such as spermiation time, fertilizing capacity, respiration and motility of the spe can be modified by collection method. In the current study, sperm was collected quickly by scarification, but its quality was only average. When thermal shock or serotonin injection was used to stimulate the release of sperm, it was collected and concentrated as "dry" as possible after exit fr the kidney. Its quality was comparable to that of diluted sperm collected after thermal shock, a technique commonly used in bivalve hatcheries. The results also showed that serotonin injection systematically decreased spermiation time and induced the production of high quality sperm and henc is the preferable method to collect sperm for artificial reproduction.

INTRODUCTION

Great variability has been reported in the quality of molluscan bivalve larvae reared in hatcheries (Boucher and Dao 1989; Devauchelle and Mingant 1991). As a result, experiments were undertaken to examine the physiology of gametes and embryos of the scallop, *Pecten maximus*. The goal this study was to control and improve fertilization and embryonic development, which is basic to the success of larval rearing.

This paper presents results obtained on male gametes. Sperm activity was assessed by two criteria: respiration rate and motility. These two criteria are linked to fertilization capacity, which reflects sperm quality. Differences in spermiation time under different methods of stimulation were also compared.

MATERIALS AND METHODS

BIOLOGICAL MATERIAL

Scallops were dredged from the Bay of Brest and stimulated to release spermatozoa by two methods: thermal shock (Cochard and Gerard 1987 and injection of 250 μl serotonin into the male and female parts of the gor The serotonin (5-hydroxytryptamine, creatinine sulfate complex) was prepare in 1 μ filtered seawater, as described by Matsutani and Nomura (1982) and Castagna et al. (1985), but a lower concentration (1 μM) was used. Three t of sperm collection were tested: diluted in a container of seawater (a technique commonly used in hatcheries), or concentrated as "dry" as possibl by collecting the sperm directly from the gonad either by scarification or exit from the anterior part of the kidney. Concentrated sperm was stored a

4°C. In order to test fertilization capacity of the sperm, diluted oocytes were collected in seawater using thermal shock on broodstock.

PERIOD BEFORE SPERM RELEASE

This period was estimated for both stimulation methods: thermal shock and serotonin injection.

SPERM FERTILIZING CAPACITY

Each batch of sperm was used for fertilizing several batches of 50,000 oocytes at a ratio of 50 spermatozoa per oocyte. One aliquot of each batch of unfertilized oocytes was collected without being fertilized in order to estimate self-fertilization. Four hours later, aliquots of previously unfertilized oocytes and aliquots of each batch of fertilized oocytes were fixed in 1 ml GA buffer to which was added 6% formol, as described by Micarelli (1992). GA buffer was composed of 250 μM N-methylglucamine, 250 μM K-gluconate, 50 μM Hepes, and 10 μM EGTA, adjusted to pH 7.4 with acetic acid. Aliquots were resuspended twice in the GA buffer. Oocyte suspensions were then stained with the fluorochrome Hoechst 33258 (Dubey and Raman 1983; Hinckley et al. 1987), stored below 4°C in DMSO (0.5 mg/ml). Just prior to use, this DMSO Hoechst solution was diluted 1000-fold in GA buffer; 1 ml of solution was then added to oocyte suspensions (final concentration: 0.5 μg Hoechst per ml oocyte suspension). After sitting for two hours with the fluorochrome, aliquots were resuspended in GA buffer. The sperm fertilizing capacity was estimated by examining the stained preparations of previously treated aliquots with a microscope equipped with UV epifluorescence.

RESPIRATORY RATE OF THE SPERM

The respiratory rate of spermatozoa was monitored by continuous recording with a Clark oxygen electrode following the procedure of Christen et al. (1982). A 40-fold dilution of dry sperm was made into 2 ml of seawater. The initial rate of sperm respiration was calculated from the line tangent to the slope and expressed as nM O_2 per minute per mg protein (Ohtake 1976). Protein content was estimated by the technique of Lowry et al. (1951) on a sample of sperm taken from the electrode chamber.

SPERM MOTILITY

One μl of a 50-fold dilution of "dry" sperm was mixed on a slide with 50 μl of seawater to which was added 2.5 μl Bovine Serum Albumin (1%). The motility was measured with stroboscopic illumination and dark-field optics connected to a video camera, as described by Cosson et al. (1985). The sperm solution was rated for the percentage of motile spermatozoa and the frequency of flagellar movement. Trajectory and displacement speed were estimated using image by image analysis.

CORRELATION BETWEEN FERTILIZING CAPACITY, RESPIRATION RATE, AND MOTILITY, ACCORDING TO STIMULATION METHOD AND SPERM COLLECTING SITE

Correlations between fertilizing capacity, respiration rate, and motility were calculated from average values obtained for each stimulation method and sperm collecting site, which were represented in five categories.

RESULTS

PERIOD BEFORE SPERM RELEASE

Stimulation with serotonin enabled sperm to be collected very quickly (15 ± 5 min). Spermiation time was longer with thermal shock stimulation (57 ± 33 min).

SPERM FERTILIZING CAPACITY

Spermatozoa collected in the gonad were the least fertile, regardless of stimulation method (Fig. 1). Student T-test results are shown Table 1. The fertilizing capacity was almost nil when sperm were collected i the gonad of a non-stimulated animal (Fig. 1 and Table 1). Spermatozoa collected on exit from the kidney were as fertile as those collected in containers of seawater following release under both induction methods (Fig. and Table 1).

RESPIRATORY RATE OF THE SPERM

Respiration rate of several batches of spermatozoa is shown in Figure 2. Respiration rate was different for sperm batches (1) (2) (3) and a large variation was observed between individuals. Seasonal variations were also recorded, as well as differences related to the degree of gonad maturit The total inhibition of oxygen consumption was seen as a slope of zero (1') after adding a final concentration of 1 μM NaCN to the first batch of sperm (1).

SPERM MOTILITY

When spermatozoa were not in contact with seawater, their movements were reduced. However, the period of activation was rapid: motilit was observed within a few seconds of dilution in seawater.

More than 90% of the spermatozoa collected in seawater or on ex: from the kidney were motile and appeared normal. In contrast, motility of spermatozoa collected in the gonads after scarification was low (from 0 to 20%) and the sperm were damaged (flagella without head or isolated heads).

Activation of *P. maximus* spermatozoa continued for several hour: the percentage of motility and flagellar frequency beat began to decrease t hours after dilution in seawater (at room temperature: 20°C), as shown in Figures 3 and 4. Duration of activation was increased to more than four hou by storage at 4°C, but in this case a decrease in the number of motile spermatozoa and flagellar frequency beat appeared more than six hours after dilution.

In natural seawater, the trajectory was circular. The diameter of the circle was on average 63 ± 8 mm (n = 10). The majority of the circling sperm travelled in the same direction, depending on the location of the interface: clockwise for spermatozoa near the surface and counterclockwise for spermatozoa near the bottom of the slide (as observed under the cover slip). Generally, displacement speed was 0.25 ± 0.05 mm per hour (n = 10) under standard conditions at 25°C. The flagellar beat frequencies were similar in different batches of spermatozoa and measured on average 38 ± 3 Hz (n = 20).

CORRELATION BETWEEN FERTILIZING CAPACITY, RESPIRATION RATE, AND MOTILITY, ACCORDING TO STIMULATION METHOD AND SPERM COLLECTING SITE

A positive correlation was found between spermatozoa fertility and respiration rate, according to the stimulation mode of the animal and the sperm collecting site. The coefficient of correlation was 0.996 (n = 5). The fertility of spermatozoa also correlated positively with percentage motility (correlation coefficient 0.996 (n = 5)) for each stimulation method and sperm collecting site.

CONCLUSIONS AND DISCUSSION

The period before sperm was released, which on average ranged from 10 to 20 minutes, was similar to that observed in many other bivalves, e.g. within 15 minutes for ocean quahogs, *Arctica islandica*, hard clams, *Mercenaria mercenaria*, and surf clams, *Spisula solidissima*, from 10 to 15 minutes for bay scallops, *Argopecten irradians*, and after 15 minutes for ribbed mussels *Geukensia demissa* (Gibbons and Castagna 1984; Castagna et al. 1985). *Crassostrea virginica* oysters are an exception, requiring only 2-10 minutes for sperm release (as indicated by previous authors).

As for fertilization capacity of the sperm with respect to collecting site, spermatozoa collected directly from the gonad were the least fertile. This observation suggests that sperm must probably undergo a capacitation phenomenon in the kidney or in the pallial cavity to acquire its fertilizing capacity, as demonstrated by Widowati (1990) and Widowati et al. (In Press) in oocytes.

Total inhibition of oxygen consumption after adding NaCN confirmed that the oxygen consumption measured was the mitochondrial respiration of the spermatozoa. Scallop spermatozoa were shown to become active within a short period and remain active for a long duration, similar to sea urchin spermatozoa (Christen et al. 1982). On the other hand, some fish have a very short duration of activation, e.g. several minutes for turbot (Suquet et al. 1991), 30 seconds for trout (Cosson et al. 1985; Christen et al. 1987) and one to two minutes for carp (De et al. 1984; Redondo-Müller et al. 1991). Since the duration of activation is correlated with fertility in numerous animal species, it would be interesting to optimize this activation duration in order to increase the fertilizing capacity of the spermatozoa. In the case of the summer whiting, activation duration was increased by adding Mg^{2+} or Ca^{2+} or by varying the ratio K^+:Na^+ in the medium (Goodall et al. 1989). The circular trajectory observed for the scallop spermatozoa has also been previously described for sea urchin spermatozoa (Gibbons and Gibbons 1972; Gatti 1987), but the speed of scallop spermatozoa (0.25 mm per second at 25°C) was close to the average speed of sea urchin spermatozoa (0.160 mm per second at the same temperature (Gibbons and Gibbons 1972)). The flagellar beat frequency of scallop sperm (33 Hz) was lower than that of sea urchin sperm (50 Hz) (Gibbons et Gibbons 1972) and frequencies obtained for some fish, e.g. 50 to 60 Hz for trout (Cosson et al. 1985; Cosson and Gagnon 1988), 60 to 70 Hz for carp (Redondo-Müller et al. 1991) and 100 Hz for conger eel (Gibbons et al. 1985).

Release mechanisms are now being studied in our laboratory, particularly the relationship between motility and ionic concentration of the sperm dilution media. The results will soon be published (Faure et al., In Prep.). Preliminary data shows that scallop sperm responds to variable levels of pH and Na^+. Media with acidic pH or with low sodium concentration inhibit both respiration rate and motility in scallop sperm, as previously demonstrated in sea urchin sperm (Christen et al. 1982; Christen et al. 1986). In addition, external calcium was not necessary for both species (Gatti 1987). However, a medium with a high potassium concentration (200 mM) did not affect respiration rate and motility in scallop sperm, contrary to results with sea urchin sperm (Christen et al. 1982). The aim of this research is to develop blocking and then reactivating media for scallop sperm.

In conclusion, fertilization using concentrated sperm obtained on exit from the kidney after serotonin injection is as effective, if not more so, than fertilization carried out with diluted sperm preparations obtained after thermal shock. In the near future, the criteria of activity will be used to distinguish between effective and non-effective sperm. Indeed, fertilizing capacity, respiration rate and motility are three closely related factors which allow evaluation of concentrated sperm quality.

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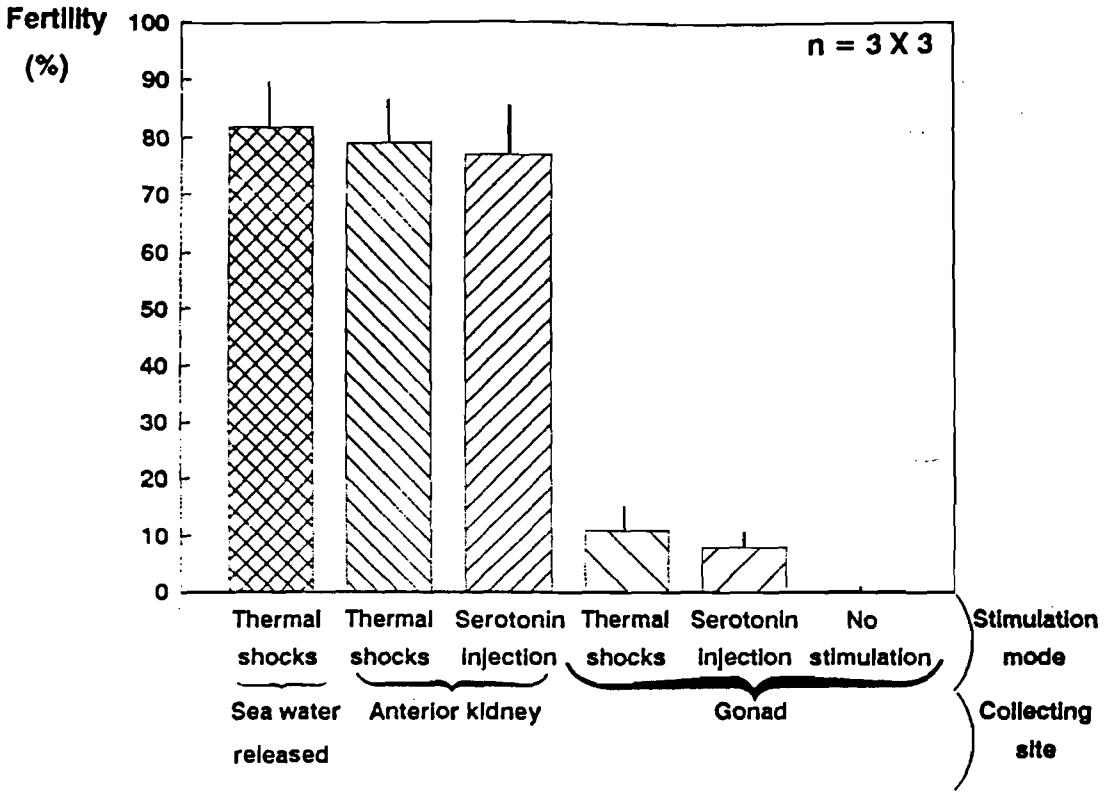

Fig. 1. Fertility of scallop sperm based on the stimulation mode and the sperm collecting site.

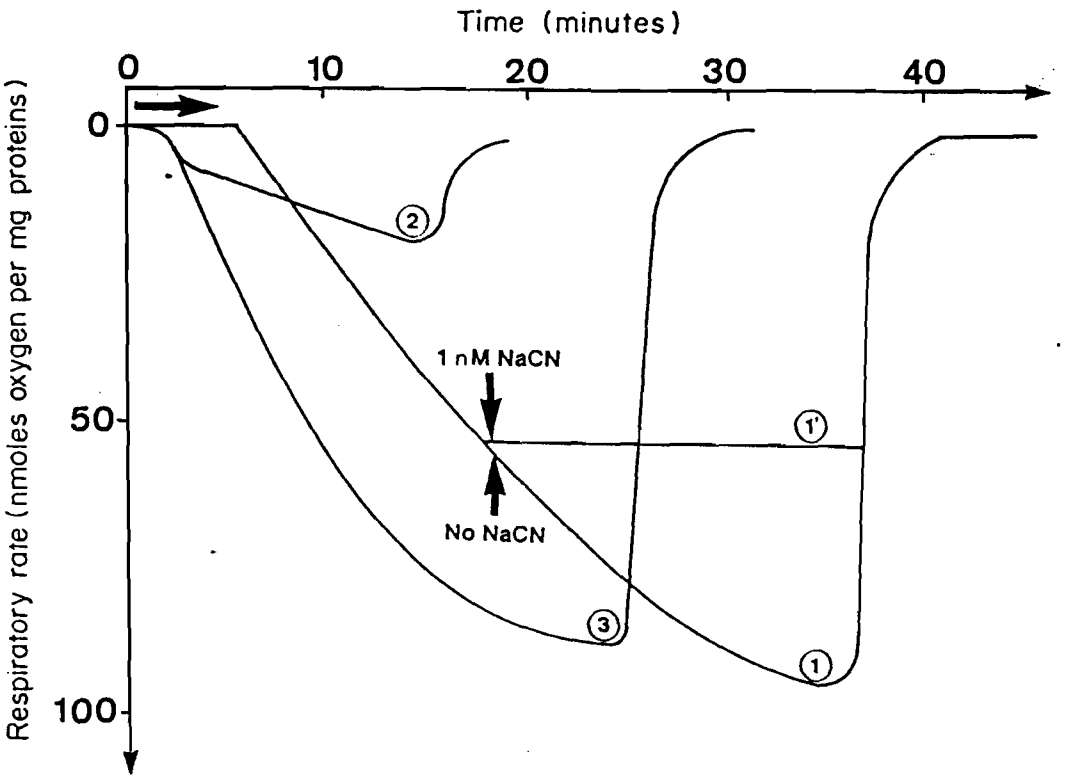

Fig. 2. Respiratory rate of different batches of scallop spermatozoa.

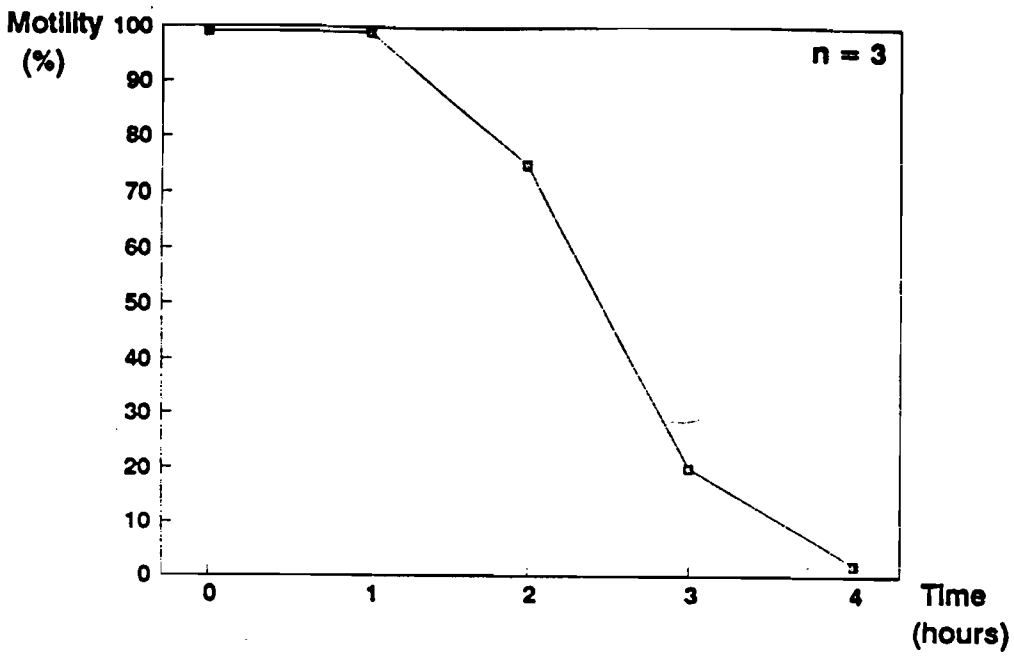

Fig. 3. Variation in time of scallop sperm motility after seawater dilution at ambient temperature (20°C).

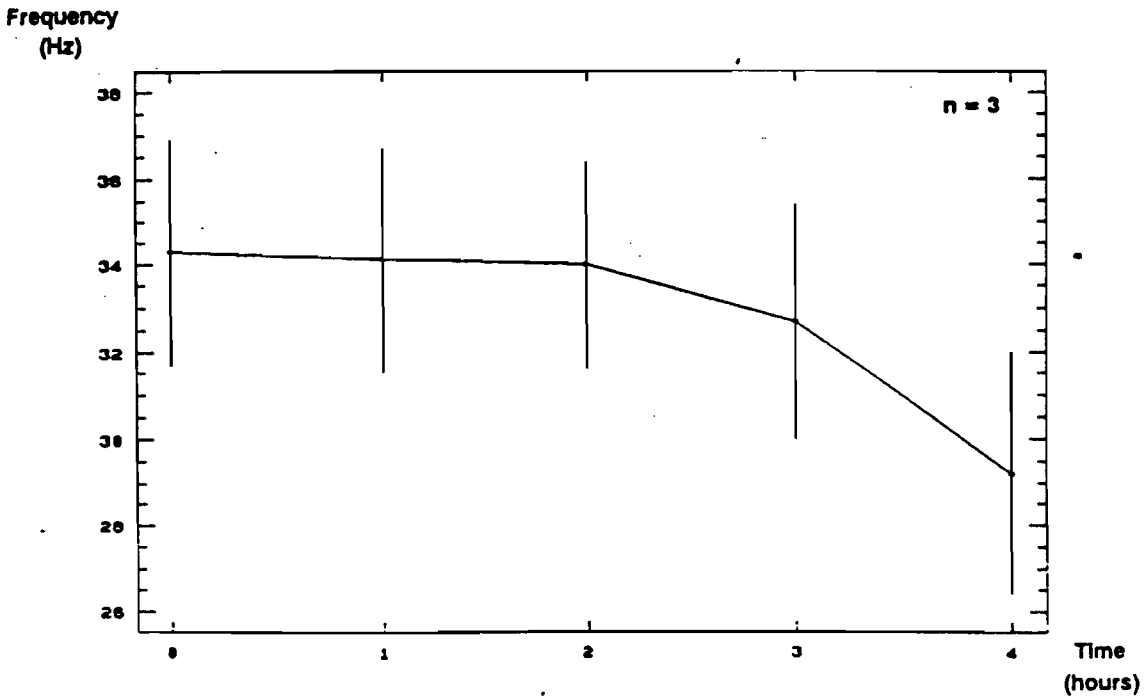

Fig. 4. Variation in time of scallop sperm flagellar beat frequency after seawater dilution at ambient temperature (20°C).

Table 1. Student T-test on scallop sperm fertility based on the stimulation mode and the sperm collecting site. Values reported are t calculated (least significant difference) with df = 16 and p = 0.001. (NS) = no significant difference; *** = highly significant difference.

		Sea water release	Anterior kidney		Gonad		
		Thermal shocks	Thermal shocks	Serotonin injection	Thermal shocks	Serotonin injection	No stimulation
Gonad	No stimulation	-43.0***	-41.5***	-36.0***	-9.3***	-11.1***	/
	Serotonin injection	-37.0***	-35.5***	-30.9***	-2.2 (NS)	/	
	Thermal shocks	-31.8***	-30.5***	-27.1***	/		
Anterior kidney	Serotonin injection	-1.8 (NS)	-0.7 (NS)	/			
	Thermal shocks	-1.1 (NS)	/				
Sea water release	Thermal shocks	/					