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Sperm motility parameters and spermatozoa morphometric characterization in marine species: A study of swimmer and sessile species

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ABSTRACT

The biodiversity of marine ecosystems is diverse and a high number of species coexist side by side. However, despite the fact that most of these species share a common fertilization strategy, a high variability in terms of the size, shape, and motion of spermatozoa can be found. In this study, we have analyzed both the sperm motion parameters and the spermatozoa morphometric features of two swimmer (pufferfish and European eel) and two sessile (sea urchin and ascidian) marine species. The most important differences in the sperm motion parameters were registered in the swimming period. Sessile species sperm displayed notably higher values than swimmer species sperm. In addition, the sperm motilities and velocities of the swimmer species decreased sharply once the sperm was activated, whereas the sessile species were able to maintain their initial values for a long time. These results are linked directly to the species-specific lifestyles. Although sessile organisms, which show limited or no movement, need sperm with a capacity to swim for long distances to find the oocytes, swimmer organisms can move toward the female and release gametes near it, and therefore the spermatozoa does not need to swim for such a long time. At the same time, sperm morphology is related to sperm motion parameters, and in this study an in-depth morphometric analysis of ascidian, sea urchin, and pufferfish spermatozoa, using computer-assisted sperm analysis software, has been carried out for the first time. A huge variability in shapes, sizes, and structures of the studied species was found using electron microscopy.

1. Introduction

A wide diversity of reproductive strategies and fertilization methods can be found in marine fauna [1]. External fertilization (broadcast spawning) is the most common reproductive strategy in aquatic environments, and is generally thought to be ancestral to internal methods of reproduction. However, several factors are involved in this kind of reproductive strategy, including the evolution of both male and female gametes in the aquatic environments. Regarding eggs, features such as cell size, the size and shape of accessory structures, and the chemoattractants of some marine species have already been documented [2]. Regarding male gametes, external fertilization involves a fundamental process known as sperm competition [3]. This mechanism, defined as when sperm from two or more males compete for the fertilization of eggs, can lead to a wide range of morphologic and physiological adaptations and, over time, reproductive traits such as spermatozoa morphology, swimming period, or swimming speed [4] have been filtered by natural selection. Therefore, it seems reasonable to suppose that species

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sharing similar reproductive strategies might also share similar spermatozoa features, whereas species with radically different reproductive strategies might display differences both in morphologic and in kinetic sperm parameters.

In terms of sessile species, which show limited or even no movement over the substrate, one factor in particular is fundamental in the fertilization of their gametes: the proximity of the congeners usually ensures reproductive success, whereas isolation can lead to reproductive failure. With the aim of reducing this issue, the eggs of some marine species are able to release specific substances called chemoattractants. These can be detected by the spermatozoa that orient their swimming direction up the concentration gradient toward the oocyte [5–7].

On the other hand, marine fish can swim and both males and females are able to come together to reproduce and, for this reason, fish sperm does not need to swim for a long time to find the female gametes. In addition, in some fish species the egg micropyle closes quickly after contact with seawater (SW), and the spermatozoa must find the micropyle within a short time ranging from several seconds to a few minutes [8]. This means that fish spermatozoa exhibit high motility at the beginning of sperm activation, and the kinetic traits of marine fish sperm are based on these biological and behavioral premises.

Thus, the main goal of this study was to use a computer-assisted sperm analysis (CASA) system and assisted sperm morphometry analysis software, to evaluate the kinetic and morphometric sperm parameters of some marine species belonging to different taxa. The data obtained were used to compare the main differences between the species, and to inquire into some theories to explain the biological reasoning for the size, shape, and motion of the spermatozoa. Scanning electron microscope pictures were captured to evaluate the shape of the sperm cells.

2. Materials and methods

2.1. Animal handling, sperm collection, and sampling

All the trials were carried out in accordance with the animal guidelines of the University of Tokyo on Animal Care and, the eels specifically, were handled in accordance with the European Union regulations concerning the protection of experimental animals (Dir 86/609/EEC).

The pufferfish (*Takifugu niphobles*) displays an unusual spawning behavior at Arai Beach near the Misaki Marine Biological Station (Japan). Large schools of fish arrive to the beach with the spring tide, around the time of the new moon, and spawning takes place repeatedly from 2 hours before sunset until sunset. During that time, pufferfish males were caught and moved to the Misaki Marine Biological Station facilities. To collect the sperm, the genital area was cleaned with freshwater to avoid contamination of the samples by feces, urine, or SW. The sperm was collected by applying gentle pressure and then diluted (1:50) in a seminal plasma-like solution [9].

Eel males (*Anguilla anguilla*) were moved from the Valenciana de Acuicultura, S.A. fish farm (Spain) to the aquaculture facilities at the Universitat Politècnica de València (Spain). The fish were gradually acclimatized to artificial SW and maturation was induced through weekly intraperitoneal hormone injections over the course of 11 weeks (hCG; human chorionic gonadotropin; 1.5 IU/g fish). The sperm samples were collected by abdominal pressure 24 hours after the hormone injection [10,11] and after the genital area had been cleaned to avoid contamination by feces, urine, or SW. The sperm was collected by applying gentle pressure and then diluted (1:50) in a P1 medium [9].

Ascidians (*Ciona intestinalis*) were collected in Aburatsubo Bay (Kanagawa, Japan) and kept in constant light to prevent spontaneous spawning. Having removed the tunic and opened the body with scissors, the oviduct was punctured with forceps to remove the eggs. The sperm was obtained by puncturing the sperm duct with forceps and extracting the sperm using a Pasteur pipette. The sperm was then diluted (1:50) in SW (see Section 2.2).

Sea urchins (*Anthocidaris crassispina*) were also collected in Aburatsubo Bay (Kanagawa, Japan), and the sperm was obtained by the administration of an intracoelomic injection of 1 mL of acetylcholine solution (0.5 M) into the soft tissue of the oral surface of the animal. The animal was gently shaken after the injection to distribute the acetylcholine to all the gonads. The sperm was then collected using a pipette and diluted (1:50) in a seminal plasma-like solution.

All the sperm samples (n = 8; for each species) were kept in their diluted form at 4 °C until both the motility and morphometric analyses were carried out.

2.2. Assessment of sperm motility parameters

The diluted sperm was activated by mixing 0.5 μL with 4 μL of artificial SW consisting of 460 mM of NaCl, 10 mM of KCl, 36 mM of $MgCl_2$, 17 mM of $MgSO_4$, 9 mM of $CaCl_2$, and 10 mM of HEPES, with 1% BSA (wt/vol) and a pH adjusted to 8.2. In the case of the ascidian, sperm motility was initiated by SW containing SAAF (sperm-activating and attracting factor), prepared from the unfertilized eggs as per the method described previously by Yoshida, et al. [12].

The sperm-SW mix was examined using a SpermTrack-10 chamber (Proiser R + D, S.L., Spain). Video sequences were recorded (at 50 fps) using a high-sensitivity video camera mounted on a phase contrast microscope with a 10× objective lens. All the motility analyses were performed in triplicate using the motility module of Integrated Semen Analysis System (ISAS) software (Proiser R + D, S.L.). All the sperm samples were evaluated in 2 hours that followed extraction.

The following parameters were examined in this study: total motility (TM, %), defined as the percentage of motile cells; progressive motility (PM, %), defined as the percentage of spermatozoa which swim in an essentially straight line; curvilinear velocity (VCL, μm/s), defined as the average velocity of a spermatozoa head along its actual curvilinear trajectory; straight line velocity (VSL, μm/s), defined as the time/average velocity of a spermatozoa head along the straight line between its first