Marine fish spermatozoa are racing ephemeral swimmers: how external conditions control motility and energetic parameters.

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ABSTRACT
After a long period of spermatogenesis, marine fish spermatozoa are delivered at male spawning in sea water at the same time as ova. In most fish species, these minute unicells have to accomplish their task, that is reaching the micropyle within a very brief period (seconds to minutes), for delivery of the haploid male genome to the ova. To this goal, their highly performant flagellum must fully activate immediately on contact with sea water and propel the sperm cell at a huge initial velocity. The cost of such « hyperactivity » is a very rapid consumption of intracellular ATP which outstrips the supply. The spermatozoa become rapidly exhausted because mitochondria cannot compensate for this fast flagellar energy consumption. Therefore, most spermatozoa become immotile but should, prior to that, reach the egg micropyle within its very short motility period (0.5 to several minutes depending of species). Within this brief period following activation, successive events occur from full motility until full arrest of flagellar activity. The present knowledge allows a good description of the activation mechanism as well as that of the movement parameters characterizing the motility period: this understanding also results from in vitro experiments obtained after demembranation of flagella. In combination with the sperm energetic content (ATP), a general model is proposed which offers a guide-line for understanding of events governing the sperm life span in marine fish species with external fertilization.

Keywords
Marine fish, spermatozoa, energetics, flagella, fertilization.

1. INTRODUCTION
Marine fish spermatozoa are flagellated unicells, which have to deal with an extremely hostile environment right at delivery in seawater. During whole spermatogenesis, they have a safe environment, both surrounded and nourished by Sertoli cells and endocrine cells (Leidig cells) combined with non harmful fluid (semenal plasma). During spermatogenesis, sperm cells are prepared for accomplishing their fertilizing task for which they need to fully exploit their swimming ability immediately and as fast as possible to encounter the egg.

Most knowledge on sperm movement comes from the classical model of sea urchin spermatozoa [1]. Nevertheless, some characteristics of fish sperm show original features: motility duration [2, 3, 4], motility initiation [5, 6, 7] or flagellar motility patterns [8, 9]. Most studies dealt with fresh water fish species, but less information concerns marine sperm characteristics. In the latter, motility activation occurs immediately after contact with seawater, a high osmolarity medium compared to seminal fluid (SF). The initial velocity is very high right at activation, but motility duration lasts for short periods of time, ranging only 40 sec. to 20 min., mostly as the consequence of an energetic lack due to this high velocity.

Marine fish spermatozoa present unique features with which to study specific aspects of sperm movement: - sperm of fishes with external fertilization being immotile in the SF, transfer in a swimming competent medium fully triggers its motility, - fish sperm cells are homogenous, - their flagellum is 50-60 µm long with a ribbon shape (presence of fins) instead of cylindrical, thus, appearing brighter by dark-field and exhibiting clear wave shapes, - attenuation of waves gradually invades the whole length of the flagellum during motility, - at the last period before full stop, the waves are restricted to one third of the flagellum combined with a drastic decrease of flagellar beat frequency leading to a decrease of translation efficiency [9], - in several fish species, spermatozoa follow linear tracks: the flagellar bending is symmetrical probably because of the absence of Ca²⁺ sensitivity of the axoneme. Despite all these original features, few detailed studies on marine fish spermatozoa flagellar motility behavior were conducted. The present paper gathers most of the knowledge on sperm movement characteristics of marine fish, with special emphasis on their high velocity capacities. A relationship between ionic effects, osmolality, energetics and transience/abortion of motility is established. For general information on spermatozoa and milt of fish, the reading of some review papers such as [10 to 16] and a book, Fish Spermatology [17] are recommended.

2. RESULTS AND DISCUSSION
Spermatozoa of marine fishes reproducing by external fertilization are delivered in sea water (SW) at spawning. Obviously these individual unicells have to cope immediately with SW (a medium external to the wheeling fish fluid) which is very harmful due to its high ionic concentration constituting a high osmolarity medium compared to the sperm cytoplasm.
2.1 The seminal fluid osmolality prevents sperm motility in the genital tracks.
In marine fish, the seminal fluid (SF) osmolality is much lower than that of SW and low enough to prevent motility. The ability for spermatozoa to swim is eventually dependent of their maturation in the ducts and is hormonally controlled by adjustment of ionic concentration in the SF; such maturation can be induced in vitro [18] and in turbot and sea bass [22], it shows no dependence on sperm internal ATP stores.

2.2 Transfer from seminal fluid into seawater triggers full motility.
Hypertonicity induces the motility of spermatozoa in many marine teleosts such as cod, flounders, sea bass, sea bream, gray mullet or goby. Hypertonic sugar solutions, comprising no ions, can mimic SW in the ability to trigger sperm motility in halibut [20], turbot [21], sea bass [19, 22], tuna [23], cod and hake [24], sea bream [23] as well as in mullet [25]. Tolerance of motility towards osmolality depends on species; in this respect, motility is generally initiated in solutions which present an osmolality value higher than 300-400 mOsm./L: the osmolality gradient must be positive between outside and inside sperm cells to efficiently trigger flagellar motility.

2.3 Immediately after activation, marine fish sperm swim with very high efficiency.
Right after activation by transfer into SW, all live spermatozoa appear very « fast » swimmers. Initial sperm speed of forward displacement in various species confirms a high velocity (150-300 µm/sec) which results from a high flagellar beat frequency (50-70 Hertz), itself highly ATP consuming as detailed later.

2.4 The motility period is limited to a short duration for marine fish’s sperm.
The duration of motility is usually short, ranging minutes to tens of minutes, and in addition it is temperature dependent and species-specific; the motility duration is also limited by damage appearing during the motility period, by contact with SW, (blebs or curling resulting from local membrane defects engendered by osmotic stress) [26].

2.5 Most motility parameters are decreasing during the motility period.
Most parameters decline within tens of seconds to few minutes depending on species, which leads to full arrest [26]. Despite differences between species in the time scale, the percentage of motile cells, the beat frequency and the velocity all exhibit a general tendency in all cases: a high initial value followed by a decline during the motility period.

2.6 Wave shape is changing during the motility period.
In fish spermatozoa, the wave shape is composed of curvatures intercalated between segments leading (sine shape, see figs 1 a to c). During the motility period a dampening process appears (see figs 1 d to f), accompanied eventually (example in sea bass) by asymmetry of beating, both evolving towards full immotility (fig. 1g). The wave amplitude (WA) of the flagellum also drops down, first in the distal portion as it was clearly demonstrated in turbot [26].

2.7 The regulation of axonemal motility by ionic concentration can be observed in vitro
Experiment using demembranated sperm models show that in vitro waves pattern can mimic those of in vivo flagella depending of the general and non specific ions concentration so called ionic strength [23].

2.8 The energy available in marine fish spermatozoa is rapidly exhausted.
A common feature is the decrease in sperm ATP content during the motility period: in sea bass, initial ATP values of 90 nmole/10⁹ spermatozoa [19] and of 1.22 µmol/mg of protein [27] were observed. In turbot, the ATP content is dependent of an ageing phenomenon related to the maturity period [28], ranging 200 nmole.10⁹ spermatozoa [29]; inhibitors of respiration (KCN) or of ATP synthesis (oligomycin) have little effects on the internal ATP concentration.

2.9 The ultimate task for sperm: meeting an egg.
How does the egg attract sperm and guide it to micropyle? In fish, there are very few examples of the demonstration of such phenomenon, so called chemotaxis, except in the pacific herring (Clupea pallasi): spermatozoa become active only when they reach the egg chorion, i.e. the vicinity of the micropyle [30 to 32] which delivers some components [33], such as the HSAP (Herring Sperm Activating Peptide) and the SMIF (Sperm Motility Initiation Factor) [34]. As a complement, in most fish species, physicotaxis, a tendency for spermatozoa to swim along any surface including that of an egg [35] contributes to sperm attraction due to the presence of guidance grooves located on surface of some eggs [36, 37] converging towards the micropylar funnel [35].

2.10 Towards a global paradigm including the osmolality control of motility
Osmolality effects on fish motility have been studied by [6] and [38] in marine and fresh water species. The model presented in

Figure 1. Swimming turbot sperm at high magnification: in a to c, three successive video images (3 flashes per image) of a spermatozoon activated since 23 sec; in d to f, same at 63 sec. with the dampening of the waves appearing progressively during the motility period leading to full stop in g at 3 min. Bar scale at bottom g represents 10 µm.
Mechanical activation represents the second signal in response to the first (osmotical) signal via the stretch activated channels located in the sperm membrane. A specific and reversible inhibitor of the stretch activated channels (SAC), gadolinium, is active on carp spermatozoa [40] and sea bass, turbot and tuna [30] sperm but inactive on sperm of other species apart from fishes [30, 40, 41]. In addition, the putative presence (and effects on motility) of water channels, aquaporins, comes from observations where sperm motility, in turbot and sea bass, is sensitive to very low concentrations of inhibitors of aquaporins such as HgCl₂ and other mercury derivatives [26, 42].

3. CONCLUSIONS

We propose a paradigm involving SACs and aquaporins in the signaling pathway of fish sperm activation (Figure 2). In fine, the local stretching of membranes would be the signal perceived by the axoneme because of the mechanosensitivity of this micromachine. As complementary information, fish sperm motility videos are available at: http://biodev.obs-vlfr.fr/~cosson/fishsperrn/

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5. REFERENCES


Figure 2. Activation process and signal transduction in marine spermatozoa: general schematic representation of the interacting processes occurring during the motility period of a spermatozoon. Sea water is of much higher osmolality compared to SF: the osmolality jump induces an osmolality reaction at the sperm membrane level; water exits the sperm cell, a process accelerated by aquaporins. As a consequence of water exit, internal ionic concentration increases and reaches optimal values for dynein motors activity. At this time point, beating of flagella is at maximal velocity but decreases with time because of 2 reasons: the ionic concentration becomes too high to sustain correct dynein activity and ATP concentration declines and becomes limiting for flagellar beating. After some period, flagellar activity stops because of these unfavourable conditions.