Chapter 7

Biophysics of Fish Sperm Flagellar Movement: Present Knowledge and Original Directions

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Abstract

A fish spermatozoon has a minimalist structure: head, mid-piece and flagellum with the active inner core, called "axoneme". The axoneme represents a cylindrical scaffold of microtubular doublets arranged around a pair of single microtubules and assorted along the entire length with the dynein-ATPase motors. The mechanisms of wave generation along the flagellum becomes possible due to sliding of microtubules relative to each other and their propagation is a result of a balance between mechanical constraints and intra-flagellar biochemical actors that generate force.

How fish sperm flagella mechanics adapt to external constraints, such as vicinity of surfaces or viscosity, during the very short period of motility? By use of high-speed video microscopy, stroboscopic system, modelisation and simulation approaches, we show that fish sperm flagella respond to physical and chemical signals from environment in a very brief period of time.

This review chapter presents a brief description of the biological and biochemical features that characterize fish spermatozoa. Then it describes the biophysical aspects of flagellar movement covering various topics involved in fish sperm motility and offering a compilation of the recent knowledge acquired on different physical properties, such as wave propagation, energetics, hydrodynamics, temperature, viscosity, axonemal microtubules dynamics, among other aspects.

Keywords: spermatozoon, flagellum mechanics, hydrodynamics, motility, wave propagation, fish

1. Introduction

Spermatozoa of most fish species are immotile in the genital tract due to the specific constitution of the surrounding seminal plasma [1]. Osmotic pressure, concentration of K^+ ions, as well as pH level and sucrose concentration are considered as the main factors of seminal fluid

preventing the initiation of movement of fish spermatozoa [2]. During natural spawning, ejaculated sperm cells are diluted with fresh- or seawater according to the fish habitat and right away initiate their motility by responding to changes in osmolality of the external milieu (hypo or hyper, respectively). Motility may be also induced in a laboratory designed saline solution with a certain pH, ionic and osmotic composition. Activation by the surrounding medium is immediately followed by a swimming response at full speed [3], which requires fast energy consumption by spermatozoa, thus leading to brevity of the motile period [4, 5]. In case of marine fishes, the duration of sperm motility is generally lasting for longer period as compared to freshwater species [6]. The total duration of flagellar activity of fish sperm lasts from minutes to tens of minutes [5]. However, European eel (*Anguilla anguilla*) [7] and European conger (*Conger conger*) spermatozoa [6] can swim for at least 30 min with little change in their motility characteristics such as high beat frequency, which can range up to 95 Hz. Same case for tilapia sperm where motility can last more than one hour whatever the surrounding osmolality conditions [8]. Arrest of fish sperm movement occurs partly because of rapid exhaustion of ATP by the cell and inability of the mitochondria to restore the energy content fast enough during the motility period [9], as well as due to some morphological changes mostly affecting membrane integrity and producing a curling of the flagellum [5, 10, 11]. For comparison, spermatozoa of mammals and invertebrates (e.g., oyster or sea urchin sperm) can swim for several hours [4].

It is worth to emphasize that during the swimming period, flagellar characteristics of fish spermatozoa change in many respects: wave velocity, wave amplitude, wavelength, number of waves along the flagellum length and degree of curvature of the wave [5, 12]. Whatever the wave parameter is considered, each one shows a decrease during the limited period of flagellar motility that altogether leads to a gradual but drastic lowering of the forward velocity of spermatozoa. Thus, it is clear that the behavior of the flagella is basically determining the global movement ability of the sperm cells [5, 12].

A main difficulty for observation of fish spermatozoa and quantification of their swimming parameters is that they are "fast swimmers" but for short duration [4]. This partly explains why most knowledge acquired on sperm flagellar movement comes from studies on the classical model of sea urchin sperm motility and on the mammals for more structurally complex sperm cells [13–15]. Nevertheless, fish spermatozoa are specifically interesting objects, due to their particular motility activation mode and their short motility duration that enables observation of the complete swimming period during a short time laps. Initiation of movement, the motility period and the arrest of motility of fish spermatozoa allow to develop specific studies on general understanding of regulation and signaling of sperm motility in terms of flagellar beating and wave parameters, thus leading to a better acquaintance of the fine-tuning of the internal axonemal mechanics (see [16] for a comprehensive overview on biochemical aspects of fish sperm motility).

The main aim of the present review is to describe existing methods for evaluation of the flagellum characteristics of fish sperm and present an overview of the literature embracing current understanding of their behavior from a biophysical, especially hydrodynamical, point of view.

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2. Structure of fish spermatozoa

Fish spermatozoa present the same basic structural features as most of the male germ cells of other animals though the presence of organelles in fish sperm is reduced to a minimum: a head, a mid-piece and a flagellum (**Figure 1**) [17, 18].

The head is the carrier of hereditary information, mostly the nucleus with paternal DNA material. In most fish species, the head of spermatozoa presents an almost spherical shape with diameter varying from 2 to 4 μm. However, in some cases, such as sturgeon, paddlefish and eel spermatozoa, the shape of head is elongated: up to 9 μm long and 2 μm wide [7, 19, 20]. Such variation in head shape certainly influences the swimming performance because of differences induced in the viscous friction against the aquatic milieu.

Mid-piece is a receptacle of the centrioles and the mitochondria (usually from 2 to 9 per each spermatozoon), the latter generating energy (ATP) for sperm motility [9]. In several fish families, the sperm mitochondria were found ring-shaped [17]. Even though the mitochondrial DNA is present in the sperm cells, the male mitochondrial genes are not transmitted to the progeny [21]. In a mature spermatozoon, the protein synthesis machinery is absent, and therefore, no gene expression can occur. However, the sperm epigenomic transmission of information from father to progeny is nowadays corroborated by experimental results in mammals [22], but little is known in fish species. The centriolar complex of mid-piece consists of the proximal and the distal centrioles, the latter forming the basal body of the flagellum. This complex anchors the flagellum to the head of the sperm cell and is normally located in close vicinity of the nucleus. Such mechanical anchoring is crucial for the process of wave development. It is worth mentioned still that the mid-piece of fish spermatozoa remains separated from the flagellum by the cytoplasmic canal.

The length of fish sperm flagella varies from 20 to 100 μm depending on species. Flagellar bending is generated by a highly organized cylindrical system of microtubules, called the axoneme, emanating from the basal body [23]. The basal body is a barrel-like structure made of nine triplet of microtubules strongly associated together, which reminds a cartwheel in the lumen of the proximal portion of the basal body [5, 13, 15]. As explained later, the anchoring of the flagellum to the basal body is essential for the wave generation mechanism. In turn, the canonical axoneme consists of nine pairs of peripheral microtubular doublets and one central pair of singlet microtubules. This structural arrangement is illustrated in **Figure 1**. Although the patterns adopted during flagellar movement are distinct from those of ciliary movement, and flagella are typically much longer than cilia, such basic "9 + 2" structure of the axoneme is highly conserved and almost identical among eukaryotic cilia and flagella from protozoans to human. In the axonemal structure of some species, there are some variations though, for example, in Anguilliformes and Elopiformes sperm flagella present a "9 + 0" pattern lacking central microtubules [7, 19, 24]. This specific 9 + 0 structure is probably responsible for the helical shape (3D) of flagellar waves in those species, a feature that contrasts with planar flagellar waves developed by the 9 + 2 canonical structure [7, 19].

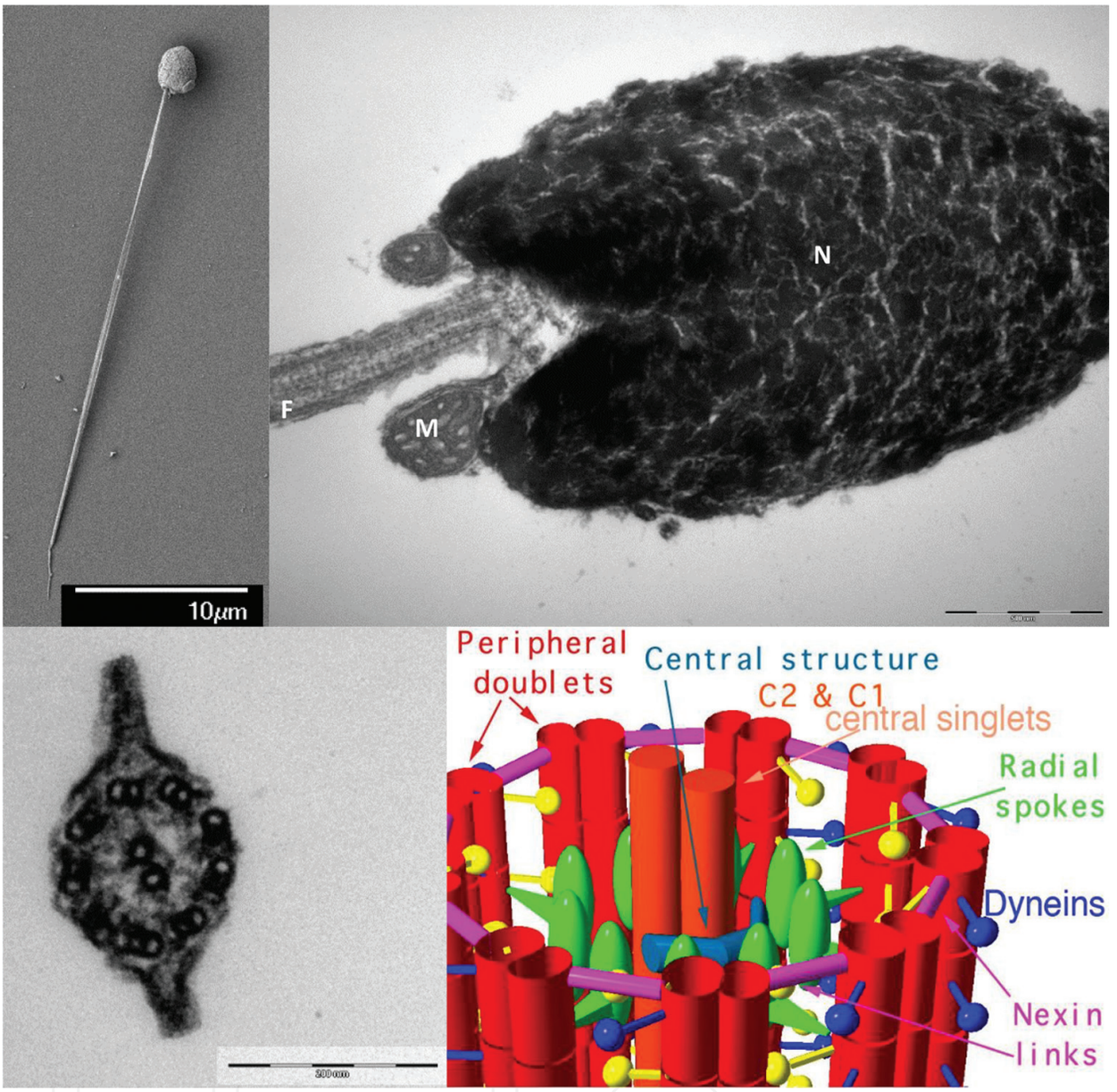

Figure 1. Morphology (top) and ultrastructure of the axoneme (bottom) of fish spermatozoon (Chinook salmon *Oncorhynchus tshawytscha*). Top: general view of spermatozoon with the ribbon-shaped flagellum and longitudinal section of the head region (N–nucleus), mid-piece with mitochondria (M) and flagellum (F) obtained by electron microscopy; scale bar = 500 nm. Bottom: A cross section of the axoneme at the distal part of the flagellum and a three-dimensional view of the arrangement of an axoneme. Microtubules are arranged according to the typical 9 + 2 structure with peripheral doublets (red), two central singlet microtubules (orange) and structures arranged around: the inner and outer dynein arms (blue and yellow), the radial spokes (green) and nexin links (pink).

The structural connections between the nine peripheral outer doublets and the sheath surrounding the central pair are named radial spokes. The central pair itself is enclosed in this sheath of proteins forming a series of projections that are well positioned to interact with each of the spoke heads and are among candidates to regulate the wave propagation [25]. Each of the outer doublets is connected to adjacent pairs of doublets by nexin links. The nexin

protein has elastic properties that allow to resist the free sliding of the microtubules with respect to each other during movement and is homologous to the dynein regulatory protein [26]. The peripheral doublets are strung with rows of dynein arms along the entire length of microtubules. These dynein arms consist of macromolecular ATPase complex [27] used as basic motor actuating the whole axoneme and extend from an outer doublet toward an adjacent doublet at regularly spaced intervals along the entire length of each A microtubules [28]. Both the spokes and the dynein complex contain different calcium-binding proteins so as for flagella to be able to respond to regulation by free calcium concentration through altering their beating pattern [29, 30]. Altogether, axonemes are composed of at least 500 different protein components [15].

The bending process in an axoneme is caused by sliding between two adjacent doublets of outer microtubules forced to slide relatively to each other by the molecular motive force, generated by dynein motor activity initially described by Gibbons and Rowe [27]. According to Ref. [31], the inner arms that are both necessary and sufficient to generate flagellar bends determine the size and shape of the waveform and the outer dynein arms add power and increase beat frequency. Due to enzymatic hydrolysis of ATP, which induces force generation of the power stroke of individual dyneins, the dynein arms interact with tubulin of the B-tubule from the adjacent doublet, causing a process of active sliding in a cooperative way [32]. The presence of inter-doublet links between peripheral microtubules and intermittent sliding between some of them creates a tension that results in flagellum oscillations [33]. Since the relative sliding of the microtubules at the proximal end (near the head) of flagellum is restricted because of the strict structural link between axonemal doublets and the basal body (see **Figure 1**), and as each microtubule doublet maintains its approximate radial position due to protein arrangement in the core of the flagellum, the filament is thus forced to bend. There are also some passive sliding that occurs in other portions of the axoneme as a consequence of the active sliding of doublets pairs [28], as well as recovery sliding due to elasticity of nexin links. To prevent sliding disintegration, dynein arms probably also act as linkers between the doublet microtubules. Apparently, the dynein arms could alternatively act either as motors or as anchors, although the separate functions of rigor formation and that of force generation could be segregated into different dynein molecules [15].

The axoneme is fully encased by the cell membrane. Often, the plasma membrane also forms one or two fin-like ridges along the fish sperm flagellar tail, which are oriented along the horizontal axis defined by the central microtubules [34–37]. The ribbon shape instead of the usual cylindrical shape of the flagellum makes it brighter when observed by dark-field microscopy and allows to better visualization and recording of wave shapes [6]. This feature of the flagellar membrane has been documented among species belonging to many fish families: Poeciliidae, Jenysiidae, Pantodontidae and Embiotocidae [17, 38–40] and was shown to potentially contribute to improve the swimming efficiency [37].

Altogether, various and original characteristics of fish spermatozoa represent attractive biological objects generating model studies for specialists in fields of physics such as hydrodynamics and fluid mechanics. Flagellar movement can be explained by various functional models that account for the presumed mechanism on a theoretical basis and include features

resulting from experiments. Such computer modeling approach aims to explain how, during the movement, several bends of opposite angular direction coexist along a flagellum and how these bends propagate along the flagellum. The precise nature of the spatial and temporal control mechanisms regulating the various flagellar and ciliary beating patterns is still not fully understood [41].

3. Physical aspects of flagellar movement

In terms of physical quantitative description, the analysis involves a viscous and incompressible fluid coupled to a single force-generating filament, the flagellum. In the past decades, several quantitative descriptions of the fluid dynamics of spermatozoa and ciliary propulsion have been attained successfully. The linear Stokes flow assumption has been used to investigate the hydrodynamic consequences of flagellar undulations taking into account the low value of the Reynolds number and the possibility to neglect inertial effects [42].

It has been hypothesized that flagellum is beating due to localized "contractions" propagated along the doublet microtubules [43]. According to the elaborated resistive force theory, active moments should balance both viscous and elastic moments present in the active filamentous flagellum. Bending waves could propagate along the flagellum if changes in length of contractile elements cause delayed changes in tension. Based on this theory, several researchers developed and explored more refined models for ciliary and flagellar motion. Using a series of photographs of cell position separated by very short periods (millisecond range) and flagellar motion parameters, Brokaw [44] was probably the first who suggested to compare computed cell trajectories and flagellar shape with experimental observations. Eventually, he proposed the model for the control of switching in which curvature controls the flagellar beat [45, 46]. The curvature control hypothesis maintains that when the flagellum bends to a sufficient curvature due to active forces, it triggers the inactivation of one set of dyneins and the activation of the set on the opposite side of the axoneme. The detachment of dynein in this case is regulated by doublet curvature [46, 47]. The degree of curvature is considered as a mechanical parameter of the axoneme that is in proportion of its resistance to bending. This model was expanded to include cross-bridge mechanics between microtubules [48]. The strain in a curved microtubule where the radius of curvature can reach up to 4 μm is very small (⇡ 1%), corresponding to strain in a tubulin dimer of angstrom range. Such a small strain is difficult to detect by an individual dynein microtubule-binding domain, except if dynein binds in a cooperative way. The degree of curvature of the axonemal microtubules could be controlled by a protein called "doublecortin." It was recently shown that this protein binds with higher affinity to curved microtubule lattices than to straight ones [49].

In an alternative model, the control of switching where dyneins behave as slipping links was proposed. These links detach when subjected to forces acting parallel to the long axis of the microtubule doublets and thus oppose sliding [50, 51]. Appearance of sliding forces on one side of the axoneme induces detachment of the dyneins on the other side (and vice versa) meaning that opposite sides are antagonistic.

A third model for regulating flagellar and ciliary beating is the geometric clutch theory developed by Lindemann [52]. This model treats the axoneme as dynamic elastic linkages exerting force between longitudinal arrays of doublet microtubules. The hypothesis is predicated that the transverse force that develops in the plane of bending of the axoneme changes the spacing between doublet microtubules and dynein bridges pull together adjacent doublets. The force generation of the attached dyneins creates sliding and bending. This active bending increases the transverse forces that pull the doublets apart and disengage the dyneins what allows the dyneins on the opposite side of the axoneme to attach [28].

Next model for cilia and flagella incorporates discrete representations of the dynein arms, the passive elastic structure of the axoneme including the doublets and nexin links [28, 53]. In this model, dynein activation is governed by a simple curvature control mechanism [46].

Recently, some authors developed a two-dimensional mathematical model of the axoneme that can incorporate any or all of these different feedback mechanisms above [54] in order to evaluate the validity of each model. This new model includes static curvature that is responsible of asymmetric beats. Results of these authors favor the curvature control mechanism as it gives best agreement with the bending waveforms of *Chlamydomonas flagella*, and predict that the motors respond to the time derivative of curvature rather than curvature itself.

The above paragraph presents two levels of investigation for physical description of flagellar beating: *either* at the organelle level where the flagellum is considered as an active filament (without considering its internal structure), *or* at the macro-molecular level where internal components of the flagellum interact between each other in a coordinated mode.

4. Evaluation of fish sperm motility parameters

Due to the short duration of fish sperm motility, special methods for recording the sperm motion [55], and especially obtaining high-resolution flagellar images [14], have been developed. An unclassified and non-exhaustive list of variables that are commonly used to describe the motility phase of fish sperm in details includes: velocity of head displacement, percentage of motile cells, duration of motility, linearity of track of sperm heads, shape of the flagellar waves and other criteria such as wave velocity or frequency [14].

Duration of motility. The duration of motility period is estimated as the time period elapsed from activation by transfer in a swimming medium to the full arrest of progressive motility for all spermatozoa [14].

It has been demonstrated that the duration of motility is temperature dependent and species specific [56–58]. In cyprinids, it was shown that extracellular and intracellular pH, as well as the ionic composition of the swimming media, influences the initiation and duration of sperm motility [59]. As stated above, motility duration of fish spermatozoa is frequently limited by flagellar damages appearing during the motility period, mostly in relation to osmotic stress imposed at motility initiation [60].

In both freshwater and marine fishes, two main and common flagellar damages were reported: cytoplasmic blebs emerge anywhere along flagellar length during the motility period which impairs the propagation of wave [61, 62] and curling structure at flagellar tip particularly close to the end of motility period, which shortens obviously the flagellar length and leads to decrease the efficiency of axonemal beating [10, 11, 63, 64]. Damages such as blebs and curling usually result from local membrane defects caused mainly by hypo-osmotic shock, and they are usually reversed when reestablishing the osmolality of the surrounding solution to correct values [10, 65].

Duration of motility is also closely related to energy stored in fish sperm cells [16, 66, 67], as fast motility needs large rate of energy consumption that cannot be compensated by mitochondrial ATP production [4, 9]. When intracellular ATP store becomes low, flagellar dynein ATPases start to function at low rates that causes the decrease of wave amplitudes and eventually slows down the progressive motion [68–71]. Due to the decrease of the ATP store during progress of the motility phase [9, 66], the proportion of motile cells in the sperm population also decreases as a function of time after activation, which also contributes to a decrease in fertilizing ability [72]. In addition, as a consequence of ATP hydrolyses, ADP is continuously accumulating and at the end of the motility period reaches its maximal value [72]. It was shown that the presence of ADP releases the inhibitory effects of high concentration of ATP in sea urchin sperm [73, 74]. In fish sperm, a low ATP/ADP ratio would oppositely favor dynein inhibition and contribute to the decrease of flagellar beat frequency [75]. For example, in trout sperm, ATP has a K_m value of 0.2 mM [76], while the K_i for ADP is about 0.27 mM [77], and at the end of the motility period, internal ADP concentration reaches 2.28 mM, while concentration of ATP is much lower. The importance of ATP as energetic compound for sperm motility [9] is related to another major energetic compound, the creatine phosphate [78–80].

Sperm velocity. Sperm velocity represents a global combination of several parameters such as head dimension (diameter of head), beat frequency, length of flagellum and physical parameters of wave propagation like wave length and amplitude [43], which contributes differentially to energetic exhaustion. Velocities of spermatozoa are greatest immediately after activation [4], for example, in halibut 150–180 μm/s [81, 82], in fugu 160 μm/s [83], in cod 65–100 μm/s [84] or 130 μm/s [64], in hake 130 μm/s [64, 85], in tuna 215–230 μm/s [86], in turbot 220 μm/s [70, 87] and in sea bass 120 μm/s (straight line velocity) [69, 88]. High initial velocity leads to shortened total duration of motility, because fish spermatozoa mostly rely on their preaccumulated energy store for operating their propulsive motors [4, 9]. The velocity characteristics may be modulated by sperm microenvironment and particularly pH and osmolality of the swimming medium [6]. For instance, by increasing the osmolality of the activation media, the number of wave and curvatures along the flagellum will increase and this can be accompanied by a decrease in sperm velocity.

The above paragraph presents an overview of the methods and results leading to quantitative description of the fish sperm movement characteristics.

5. Flagellum wave propagation

The numerous variables developed for description of sperm motility by a follow-up of head displacement as used in CASA (Computer-assisted sperm analysis) (see above) are not independent but rather redundant. Therefore, unrelated variables were designed to describe specifically the flagellar beating through their wave properties [89]. Initial studies developed on invertebrate's sperm flagella [90] were applied to flagella of marine fish spermatozoa [14, 91, 92] such as turbot [70], sea bass [69], cod and hake [64], as well as to freshwater species such as trout [78], salmon [93], carp [60], sturgeon [3] and pike [11]. Some of the flagellar wave parameters play a critical role for the displacement efficiency of the whole sperm cell and control the forward velocity of translation, for example, the amplitude and the length of each flagellar wave, the number of bends, the curvature of the bend pattern, the wave velocity and the flagellar beat frequency.

The traits of the motility behavior of sperm flagella of fish with external fertilization are quite similar in several respects [87, 94]. Normally, wave propagation occurs from head to flagellar tip leading to forward movement of the spermatozoon with head first [5, 15]. More recently, the appearance of first bends at motility activation was described in detail [3, 95]. In most cases, the first bend initiates from the region close to the head and propagates toward the flagellar tip [3, 95]. This bend is then followed by a next one with opposite direction of curvature so that several successive bends occupy the whole flagellar length, mostly during the earliest period of fish sperm motility [5, 15]. The bend initiation mechanism itself is still not fully explained [96]. Studies demonstrated that the axoneme of demembranated spermatozoa (after removal of the membrane of flagella by application of a mild detergent) could be reinitiated to produce waves, if energy in the form of ATP was provided to the system [27, 97]. In case of rainbow trout, chum salmon or sturgeon, flagellar axonemes need to be exposed to both cyclic AMP (cAMP) and ATP to become functionally motile [61, 76, 80, 98, 99]. However, this feature is not general as the presence of cAMP does not seem to be necessary for sperm motility initiation in many other fish species [100, 101].

In all fish species studied so far, the waves propagate the whole length of the sperm flagellum when observed right after activation. However, during the motility period of fish sperm, several types of modifications of the wave pattern appear, which are paralleled by a decrease in flagellar beat frequency [6, 94]. The second part of the motility period is identified by the restriction of the waves to the third or quarter length of the flagellum near the head, leading to inefficiency of translation of the wave, decrease of velocity, and is ending up by a full stop [11, 62, 72, 78, 87]. This has been interpreted in terms of an energy transfer deficiency from the mid-piece (ATP production in mitochondria) to the distal part of the flagellum where ATP is consumed [68]. Similar problems of energy availability in trout spermatozoa were related to insufficiency of the ATP/creatine-phosphate shuttle [78].

Most paradigms on wave generation and propagation along the axoneme of flagella state that there is a clear distinction between the dynein-dependent microtubule sliding actuated by the dynein oscillatory motor and the bending mechanism that should include regulator mechanism responsible for the wave propagation. During wave propagation, a bending/relaxing

cycle propagates in register and in a frame-shifted manner with the clusters of dynein-ATPase motors operating along the axoneme [44]. The motor components and their actuating mechanism are nowadays well understood, but little is known about the elements responsible for the bending regulating [13].

The input of the above studies conducted at the intra-flagellar level shows how the coordination between all the flagellar participants is crucial for the optimization of the flagellum function.

6. Wave shape: analysis and quantification

As a general description, the wave shape of fish sperm flagella is of the arcsine type, that is, linear segments intercalated between two successive curvatures, similar to what occurs in tunicate sperm flagella or sea urchins [102].

Usually, it is assumed that flagellar waves are almost planar, that is, each sine wave is "flat" and the successive waves are coplanar. An exception to wave's flatness can be found in European eel spermatozoa, which possess a corkscrew wave shape [7, 19, 103]. However, this helical wave pattern has lower efficiency in terms of forward velocity of the spermatozoa even though flagella beats at high frequency, up to 95 Hz [7, 19]. It is also suggested that swimming in 2D partly prevents dispersion of spermatozoa far away from the egg. This hypothesis was recently tested in a simulation study [104] showing that the predicted physical advantage is related to the relative angle between sperm swimming plane and egg surface plane. In many cases and species, waves are not perfectly planar, but slightly deviate from a strict plane while successive waves are not coplanar. This feature was described for sperm flagella in several species. Such slight distortion from wave flatness would explain the ability of sperm cells to maintain swimming in the surface vicinity [105]. Actually, the majority of cells in a population of fish spermatozoa swim in the vicinity of glass surfaces [106]. Swimming in vicinity of surface is also a property that is observed in human sperm [107]. It is speculated that such an ability to swim in the vicinity of the egg surface probably represents a biological advantage for fertilization efficiency.

In addition, sperm cells may be observed rotating transiently during the motion. For example, in the case of paddlefish and sturgeon, due to the rotation of the whole sperm cell, each spermatozoon image appears alternatively with flagellar top view (waves in the plane of observation) or side view, with waves orthogonal to the observation plane [61, 93, 106]. Nevertheless, waves are not arranged according to a helical shape but rather as successive waves subscribed in different planes [105]. For sperm with quite symmetrical heads, nonplanar beating can occur with cell rolling during surface swimming, resulting in circular swimming trajectories in the direction of cell rolling, which is always the same within a species [108].

The wave shape of fish sperm flagella is affected by several factors, such as the energetic content (ATP), which controls wave amplitude [6, 72], the internal ionic concentration (ionic strength) that affects the constancy of the wave amplitude along flagellum as well as physical constraints imposed by the external milieu like viscosity and temperature [11, 12, 87, 109].

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Energetic content. The rate of energy consumption (ATP hydrolysis) by sperm flagellum determines the flagellar beat frequency and therefore velocity of forward displacement of fish spermatozoon [9, 13, 110, 111]. As already widely discussed above, there is a progressive decrease in the flagellar beat frequency in individual fish spermatozoa during the motile phase [55, 112]. Therefore, in case of fish sperm flagella, the beat frequency measurement should be associated with the precise timing after activation of that measurement because of its fast decay as a function of time [12, 14].

Internal ionic concentration. The intracellular ionic concentration is indirectly governed by the external osmolality. It was shown that the change of extracellular osmolality, which perceived by spermatozoa when transferred from seminal fluid to external milieu, causes a rapid change in intracellular ionic concentration observed during the course of the motility phase [94, 109]. As a consequence, the flagellar axonemes become exposed to a more and more drastic intracellular environment for dynein motors leading to reduce the development of waves (dampening process described above) and eventually lead to a full arrest of motility [5, 69, 72]. In some species, dampening of flagellar waves during the motility period is accompanied by asymmetry of beating. The ability to develop either symmetric or asymmetric ways of beating results in imbalanced amplitude of the bends following each other. For instance, if both bends are of equal curvature, then the symmetrical movement of sperm flagella is developed, which leads the sperm cells to describe linear tracks [13]. In case of asymmetry, sperm movement becomes consequently circular and sperm cells describe circles of corresponding diameter [113]. In cases studied in detail, asymmetry of beating is related to Ca^{2+} regulation, probably through a Ca^{2+}-calmodulin-dependent phosphorylation of some axonemal proteins [113, 114]. This Ca^{2+}-induced asymmetry also occurs in freshwater species, such as trout [115] and carp [60], where due to increased Ca^{2+} concentration spermatozoa describe circular trajectories, which become tighter with time elapsed after activation. Nevertheless, it was shown that asymmetry of the flagellar waveform can appear in the absence of any cell signaling change or flagellar heterogeneity. In physics, such phenomenon occurs commonly in elastic filament dynamics (so-called buckling instability) when passive filaments are subjected to high tangential forces. This was demonstrated also for asymmetric flagellar bending [116].

Viscosity. Sperm migrating in high viscosity fluids commonly exhibits larger numbers of waves though of lower amplitudes [86, 107]. Thus, an increase in viscosity by addition of viscous compounds in the swimming medium actually leads to a lowering of propulsive velocity of the sperm [86]. Practically, viscous medium mimics the situation occurring for sperm cells in ovary fluids or jelly-like layers that surround eggs in some fish species [117]. In addition, variations in the constitutive morphology of individual spermatozoa within the species also influence their velocity in viscous media [118]. As already mentioned, the viscosity effects are enhanced in case of the ribbon-shaped flagella like those in fish spermatozoa possessing fins, the latter greatly increasing the surface of viscous interaction with the surrounding medium [37].

Temperature effects. Fish species are adapted to a large variety of temperatures (from several degrees below 0 to 40–50°C). Low temperature could represent an adverse factor for sperm to fertilize eggs because decreasing the temperature reduces the flagellar beat frequency

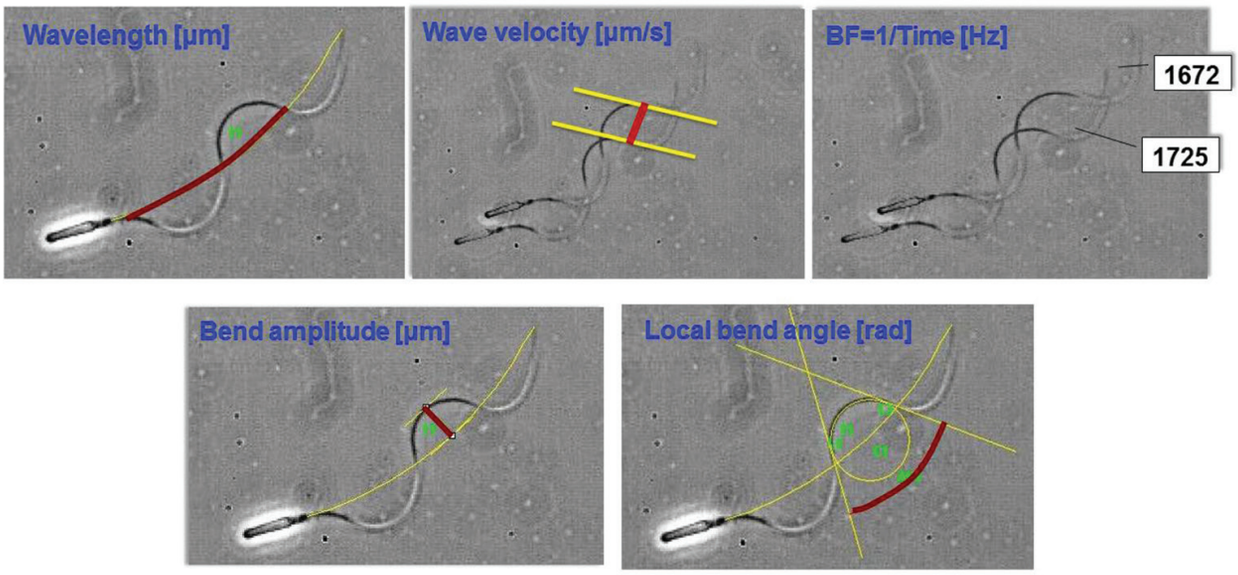

Figure 2. Series of images of sturgeon sperm illustrating the measurements of the flagellar wave parameters: wavelength, wave velocity, beat frequency, bend amplitude and local bend angle.

and consequently the sperm translational velocity [57]. This is probably compensated by the increase motility duration observed when temperature is decreased [57, 58, 119], the latter increasing statistically the chances of egg-sperm meeting. The relationship between beat frequency and temperature provides an opportunity to get access to important thermodynamic variables of the flagellar beating [120]. From a thermodynamic point of view, pressure represents an additional factor possibly acting on sperm performances, taking into account that sperm spawning occurs in deep water in some marine fish species [121]. Little is known about the pressure effects on flagellar behavior because of technical limitation for such studies [122].

In addition, measurement of flagellar parameters including beat frequency at different temperatures allows better understanding of energetic constraints involved in sperm movement [4, 56, 57]. The behavior of fish sperm flagella presents various and original interests as they are able to convert chemical energy into mechanical to generate movement [120]. Due to biophysical methods, such as hydrodynamic analyses of the beat patterns, it become possible to estimate the minimum intracellular consumption of chemical energy that needed for flagellar motion [16].

Some fish species reproduce at temperatures definitely lower than room temperature, and therefore, motility parameters should be measured at lower temperatures. As an option observation can be carried out in a temperature-controlled room. In addition, it is possible to control the temperature on the microscope itself, but this often leads to problem of condensation on the condenser and objective lenses. The temperature control of the glass slide can be simply set due to the contact with a cooling micro-Peltier plate. This also allows to measure local temperature with a micro-thermistor immersed in the observation drop [14].

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Successive positions of flagellar waves can be observed in series of video frames obtained at several time intervals during the motility phase. This allows measurement of flagellar wave parameters (**Figure 2**).

The above paragraph shows that internal parameters (energy content as example) act in synergy with external parameters (viscosity as example) and complement each other in the signaling processes that allow fish spermatozoa to rapidly adapt to a large diversity of situations they are confronted to during their short-term motility.

7. Conclusions

The main aim of this review is to describe features of fish sperm motility, flagellar mechanics and different characteristic determining movement and show the interest of studying them from a biophysical point of view. To assess motility of fish sperm, a lot of variables, such as velocity of head displacement, percentage of motile cells, duration of motility, linearity of track of sperm heads, are commonly used to estimate the ability of a sperm population to achieve optimal fertilization. All these parameters describe the whole cell movement through the head displacement, but data that are more informative can be obtained from observation of flagellar behavior, as the flagellum is the actual source of movement generation. At present, most investigations on the mechanisms of flagellar beating and propulsion of spermatozoa were developed due to studies on mammalian and sea urchin flagella. Nevertheless, detailed records of fish sperm flagella from different species offer a unique opportunity to observe successive stages in the swimming period: activation step of motility itself, motility period and the gradual decrease leading to the end of the motility period. Due to the fact that fish spermatozoa swim at high speed and possess short period of motility, it has been historically difficult to observe their flagellar behavior. Therefore, most of knowledge about fish sperm motility was initially obtained from studies of the head movement using, for instance, CASA. However, additional methods to describe the details of wave flagellar movement, such as high magnification microscopy combined with stroboscopic illumination and high-speed video microscopy, were developed recently and become accessible. First attempts to obtain detailed description of fish flagellar behavior already reveal to be helpful for basic understanding of mechanistic and hydrodynamic aspects of their motile function and its adaptability. A further challenge will be to integrate the understanding of these basic mechanisms to the diversity of patterns exhibited during spermatozoa movement in different swimming fluids and under various signaling processes.

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References

[1] Morisawa S, Morisawa M. Acquisition of potential for sperm motility in rainbow trout and chum salmon. J. Exp. Biol. 1986;**126:**89–96. PMID: 3806005

[2] Alavi SMH, Cosson J. Sperm motility in fishes. (II) Effects of ions and osmolality: a review. Cell Biol. Int. 2006;**30:**1–14. doi:10.1016/j.cellbi.2005.06.004

[3] Prokopchuk G, Dzyuba B, Bondarenko O, Rodina M, Cosson J. Motility initiation of sterlet sturgeon (*Acipenser ruthenus*) spermatozoa: describing the propagation of the first flagellar waves. Theriogenology. 2015;**84:**51–61. doi:10.1016/j.theriogenology.2015.02.011

[4] Cosson J. Frenetic activation of fish spermatozoa flagella entails short-term motility, portending their precocious decadence. J. Fish Biol. 2010;**76:**240–279. doi:10.1111/j.1095-8649.2009.02504.x

[5] Cosson J. The motility apparatus of fish spermatozoa. In: Alavi SMH, Cosson J, Coward K, Rafiee G, editors. Fish Spermatology. Oxford: Alpha Science International Ltd; 2008. p. 281–316.

[6] Cosson J, Groison AL, Suquet M, Fauvel C, Dreanno C, Billard R. Studying sperm motility in marine fish: an overview on the state of the art. J. Appl. Ichthyol. 2008;**24:**460–486. doi:10.1111/j.1439-0426.2008.01151.x

[7] Gibbons BH, Baccetti B, Gibbons IR. Live and reactivated motility in the 9 + 0 flagellum of Anguilla sperm. Cell Motil. 1985;**5:**333–350. doi:10.1002/cm.970050406

[8] Legendre M, Alavi SMH, Dzyuba B, Linhart O, Prokopchuk G, Cochet C, Dugué R, Cosson J. Adaptations of semen characteristics and sperm motility to harsh salinity: extreme situations encountered by the euryhaline tilapia *Sarotherodon melanotheron heudelotii* (Dumeril, 1859). Theriogenology. 2016. doi:10.1016/j.theriogenology.2016.04.066

[9] Cosson J. ATP: The sperm movement energizer. In: Kuester E, Traugott G, editors. Adenosine Triphosphate: Chemical Properties, Biosynthesis and Functions in Cells. New York: Nova Publisher Inc; 2013. p. 1–46.

[10] Perchec G, Cosson MP, Cosson J, Jeulin C, Billard R. Morphological and kinetic changes of carp (*Cyprinus carpio*) spermatozoa after initiation of motility in distilled water. Cell Motil. Cytoskeleton. 1996;**35:**113–120. doi:10.1002/(SICI)1097-0169(1996)35:2<113::AID-CM4>3.0.CO;2-B

[11] Alavi SM, Rodina M, Viveiros AT, Cosson J, Gela D, Boryshpolets S, Linhart O. Effects of osmolality on sperm morphology, motility and flagellar wave parameters in Northern pike (*Esox lucius* L.). Theriogenology. 2009;**72:**32–43. doi:10.1016/j.theriogenology.2009.01.015

[12] Cosson J, Prokopchuk G. Wave propagation in flagella. In: Rocha L, Gomes M, editors. Wave Propagation. Cheyenne: Academy Publish; 2014. p. 541–583.

[13] Gibbons IR. Cilia and flagella of eukaryotes. J. Cell Biol. 1981;**91:**107–124. doi:10.1083/jcb.91.3.107s

[14] Cosson J. Methods to analyse the movements of fish spermatozoa and their flagella. In: Alavi SMH, Cosson J, Coward K, Rafiee G, editors. Fish Spermatology. Oxford: Alpha Science International Ltd; 2008. p. 63–102.

[15] Cosson J, editor. Flagellar Mechanics and Sperm Guidance. Bentham Books Publisher; 2015. p. 424. doi:10.2174/97816810812811150101

[16] Dzyuba B, Bondarenko O, Fedorov P, Gazo I, Prokopchuk G, Cosson J. Energetics of fish spermatozoa: the proven and the possible. Aquaculture. 2016. doi:10.1016/j.aquaculture.2016.05.038

[17] Lahnsteiner F, Patzner RA. Sperm morphology and ultrastructure. In: Alavi SMH, Cosson J, Coward K, Rafiee G, editors. Fish Spermatology. Oxford: Alpha Science International Ltd; 2008. p. 1–61.

[18] Jamieson BGM, Leung LKP. Fish Evolution and Systematics: Evidence from Spermatozoa: with a Survey of Lophophorate, Echinoderm, and Protochordate Sperm and an Account of Gamete Cryopreservation. Cambridge: Cambridge University Press; 1991. 334 p. doi:10.1046/j.1420-9101.1992.5040721.x

[19] Gibbons BH, Gibbons IR, Baccetti B. Structure and motility of the 9 + 0 flagellum of eel spermatozoa. J. Submicr. Cytol. 1983;**15:**15–20. PMID: 6842644

[20] Linhartova Z, Rodina M, Nebesarova J, Cosson J, Psenicka M. Morphology and ultrastructure of beluga (*Huso huso*) spermatozoa and a comparison with related sturgeons. Anim. Reprod. Sci. 2013;**137:**220–229. doi:10.1016/j.anireprosci.2013.01.003

[21] DeLuca Steven Z, O'Farrell Patrick H. Barriers to male transmission of mitochondrial DNA in sperm development. Dev. Cell. 2012;**22:**660–668. doi:10.1016/j.devcel.2011.12.021

[22] Casas E, Vavouri T. Sperm epigenomics: challenges and opportunities. Front. Genet. 2014;**5:**330. doi:10.3389/fgene.2014.00330

[23] Inaba K. Molecular architecture of the sperm flagella: molecules for motility and signaling. Zool. Sci. 2003;**20:**1043–1056. doi:10.2108/zsj.20.1043

[24] Mattei C, Mattei X. Spermiogenesis and spermatozoa of the *Elopomorpha* (teleost fish). In: Afzelius B, editor. The Functional Anatomy of the Spermatozoon. Oxford: Pergamon Press; 1975. p. 211–221.

[25] Smith EF, Yang P. The radial spokes and central apparatus: mechano-chemical transducers that regulate flagellar motility. Cell Motil. Cytoskeleton. 2004;**57:**8–17. doi:10.1002/cm.10155

[26] Heuser T, Raytchev M, Krell J, Porter ME, Nicastro D. The dynein regulatory complex is the nexin link and a major regulatory node in cilia and flagella. J. Cell Biol. 2009;**187:**921–933. doi:10.1083/jcb.200908067

[27] Gibbons IR, Rowe AJ. Dynein: a protein with adenosine triphosphatase activity from cilia. Science. 1965;**149:**424–426. doi:10.1126/science.149.3682.424

[28] Dillon RH, Fauci LJ. An integrative model of internal axoneme mechanics and external fluid dynamics in ciliary beating. J. Theor. Biol. 2000;**207:**415–430. doi:10.1006/jtbi.2000.2182

[29] Gibbons BH, Gibbons IR. Calcium-induced quiescence in reactivated sea urchin sperm. J. Cell Biol. 1980;**84:**13–27. PMID: 7350165

[30] Eshel D, Brokaw CJ. New evidence for a "biased baseline" mechanism for calcium-regulated asymmetry of flagellar bending. Cell Motil. Cytoskeleton. 1987;**7:**160–168. doi:10.1002/cm.970070208

[31] Brokaw CJ. Control of flagellar bending: a new agenda based on dynein diversity. Cell Motil. Cytoskeleton. 1994;**28:**199–204. doi:10.1002/cm.970280303

[32] Sale WS, Satir P. Direction of active sliding of microtubules in Tetrahymena cilia. Proc. Natl. Acad. Sci. U.S.A. 1977;**74:**2045–2049. PMID: 266725

[33] Satir P. The present status of the sliding microtubule model of ciliary motion. In: Sleigh MA, editor. Cilia and Flagella. New York: Academic Press; 1974. p. 131–141.

[34] Nicander L. Comparative studies on the fine structure of vertebrate spermatozoa. In: Baccetti B, editor. Comparative Spermatology. Rome: Academic Press; 1970. p. 47–56.

[35] Matos E, Santos MNS, Azevedo C. Biflagellate spermatozoon structure of the hermaphrodite fish *Satanoperca jurupari* (Heckel, 1840) (Teleostei, Cichlidae) from the Amazon River. Braz. J. Biol. 2002;**62:**847–852. doi:10.1590/s1519-69842002000500014

[36] Suquet M, Dorange G, Omnes MH, Normant Y, Roux A, Fauvel C. Composition of the seminal Fluid and ultrastructure of the spermatozoon of turbot (*Scophthalmus maximus*). J. Fish Biol. 1993;**42:**509–516. doi:10.1111/j.1095-8649.1993.tb00355.x

[37] Gillies EA, Bondarenko V, Cosson J, Pacey AA. Fins improve the swimming performance of fish sperm: a hydrodynamic analysis of the Siberian sturgeon *Acipenser baerii*. Cytoskeleton. 2013;**70:**85–100. doi:10.1002/cm.21093

[38] Dadone L, Narbaitz R. Submicroscopic structure of spermatozoa of a cyprinodontiform teleost, *Jenynsia lineata*. Z. Zellforsch. Mikrosk Anat. 1967;**80:**214–219. doi:10.1007/BF00337457

[39] Stanley HP. An electron microscope study of spermiogenesis in the teleost fish *Oligocottus maculosus*. J. Ultrastruct. Res. 1969;**27:**230–243. doi:10.1016/S0022-5320(69)80014-6

[40] Lahnsteiner F, Berger B, Weismann T, Patzner RA. Sperm structure and motility of the freshwater teleost *Cottus gobio*. J. Fish Biol. 1997;**50:**564–574. doi:10.1111/j.1095-8649.1997.tb01950.x

[41] Brokaw CJ. Simulating the effects of fluid viscosity on the behaviour of sperm flagella. Math. Meth. Appl. Sci. 2001;**24:**1351–1365. doi:10.1002/mma.184

[42] Brennen C, Winet H. Fluid mechanics of propulsion by cilia and flagella. Ann. Rev. Fluid Mech. 1977;**9:**339–398. doi:10.1146/annurev.fl.09.010177.002011

[43] Gray J, Hancock GJ. The propulsion of sea-urchin spermatozoa. J. Exp. Biol. 1955;**32:**802–814.

[44] Brokaw CJ. Bending moments in free-swimming flagella. J. Exp. Biol. 1970;**53:**445–464. PMID: 5529723

[45] Brokaw CJ. Bend propagation by a sliding filament model for flagella. J. Exp. Biol. 1971;**55:**289–304. PMID: 5114025

[46] Brokaw CJ. Computer simulation of flagellar movement. Biophys. J. 1972;**12:**564–586. doi:10.1016/s0006-3495(72)86104-6

[47] Morita Y, Shingyoji C. Effects of imposed bending on microtubule sliding in sperm flagella. Curr. Biol. 2004;**14:**2113–2118. doi:10.1016/j.cub.2004.11.028

[48] Brokaw CJ, Rintala DR. Computer simulation of flagellar movement. III. Models incorporating cross-bridge kinetics. J. Mechanochem. Cell Motil. 1975;**3:**77–86. PMID: 1214108

[49] Bechstedt S, Lu K, Brouhard Gary J. Doublecortin recognizes the longitudinal curvature of the microtubule end and lattice. Curr. Biol. 2014;**24:**2366–2375. doi:10.1016/j.cub.2014.08.039

[50] Brokaw CJ. Molecular mechanism for oscillation in flagella and muscle. Proc. Natl. Acad. Sci. U.S.A. 1975;**72:**3102–3106. PMID: 1059095

[51] Camalet S, Jülicher F. Generic aspects of axonemal beating. New J. Phys. 2000;**2:**24–24. doi:10.1088/1367-2630/2/1/324

[52] Lindemann CB. Testing the geometric clutch hypothesis. Biol. Cell. 2004;**96:**681–690. doi:10.1016/j.biolcel.2004.08.001

[53] Dillon RH, Fauci LJ, Omoto C. Mathematical modeling of axoneme mechanics and fluid dynamics in ciliary and sperm motility. DCDIS Ser A Math Anal. 2003;**10:**745–757.

[54] Sartori P, Geyer VF, Scholich A, Jülicher F, Howard J. Dynamic curvature regulation accounts for the symmetric and asymmetric beats of *Chlamydomonas flagella*. Elife. 2016;5. doi:10.7554/eLife.13258

[55] Billard R, Cosson MP. Measurement of sperm motility in trout and carp. In: De Pauw N, Jaspers EJ, Ackefors H, Wilkins NP, editors. Aquaculture: A Biotechnology in Progress. Bredene: European Aquaculture Society; 1989. p. 499–503.

[56] Dadras H, Dzyuba V, Cosson J, Golpour A, Dzyuba B. The in vitro effect of temperature on motility and antioxidant response of common carp *Cyprinus carpio* spermatozoa. J. Therm. Biol. 2016;**59:**64–68. doi:10.1016/j.jtherbio.2016.05.003

[57] Dadras H, Dzyuba B, Cosson J, Golpour A, Siddique MAM, Linhart O. Effect of water temperature on the physiology of fish spermatozoon function: a brief review. Aquacult. Res. 2016. doi:10.1111/are.13049

[58] Billard R, Cosson MP. Sperm motility in rainbow trout *Parasalmo mykiss*: effect of pH and temperature. In: Zohar Y, Breton B, editors. Reproduction in Fish: Basic and Applied Aspects in Endocrinology and Genetics. Paris: INRA; 1988. p. 161–167.

[59] Marian T, Krasznai Z, Balkay L, Emri M, Tron L. Role of extracellular and intracellular pH in carp sperm motility and modifications by hyperosmosis of regulation of the Na^+/H^+ exchanger. Cytometry. 1997;**27:**374–382. doi:10.1002/(SICI)1097-0320(19970401)27:4<374::AID-CYTO9>3.0.CO;2-C

[60] Perchec Poupard G, Gatti JL, Cosson J, Jeulin C, Fierville F, Billard R. Effects of extracellular environment on the osmotic signal transduction involved in activation of motility of carp spermatozoa. J. Reprod. Fertil. 1997;**110:**315–327. doi:10.1530/jrf.0.1100315

[61] Cosson J, Linhart O, Mims SD, Shelton WL, Rodina M. Analysis of motility parameters from paddlefish and shovelnose sturgeon spermatozoa. J. Fish Biol. 2000;**56:**1348–1367. doi:10.1111/j.1095-8649.2000.tb02148.x

[62] Alavi SMH, Hatef A, Pšenicka M, Kašpar V, Boryshpolets S, Dzyuba B, Cosson J, Bondarenko V, Rodina M, Gela D, Linhart O. Sperm biology and control of reproduction in sturgeon: (II) sperm morphology, acrosome reaction, motility and cryopreservation. Rev. Fish Biol. Fish. 2012;**22:**861–886. doi:10.1007/s11160-012-9270-x

[63] Tsvetkova LI, Cosson J, Linhart O, Billard R. Motility and fertilizing capacity of fresh and frozen-thawed spermatozoa in sturgeons *Acipenser Baeri* and *A. ruthenus*. J. Appl. Ichthyol. 1996;**12:**107–112. doi:10.1111/j.1439-0426.1996.tb00071.x

[64] Cosson J, Groison AL, Suquet M, Fauvel C. Motility characteristics of spermatozoa in cod (*Gadus morhua*) and hake (*Merluccius merluccius*). Cybium. 2008;**32:**176–177.

[65] Linhart O, Alavi SMH, Rodina M, Gela D, Cosson J. After finishing of motility, common carp (*Cyprinus carpio*) sperm is able to re-initiate a second motility period and to fertilize eggs. Cybium. 2008;**32:**187–188.

[66] Billard R, Cosson MP. The energetics of fish sperm motility. In: Gagnon C, editor. Controls of Sperm Motility: Biological and Clinical Aspects. Boca Raton: CRC Press; 1990. p. 153–174.

[67] Ingermann R. Energy metabolism and respiration in fish spermatozoa. In: Alavi SMH, Cosson J, Coward K, Rafiee G, editors. Fish Spermatology. Oxford: Alpha Science International Ltd; 2008. p. 241–266.

[68] Perchec G, Jeulin C, Cosson J, Andre F, Billard R. Relationship between sperm ATP content and motility of carp spermatozoa. J. Cell Sci. 1995;**108:**747–753. PMID: 7769016

[69] Dreanno C, Cosson J, Suquet M, Cibert C, Fauvel C, Dorange G, Billard R. Effects of osmolality, morphology perturbations and intracellular nucleotide content during the movement of sea bass (*Dicentrarchus labrax*) spermatozoa. J. Reprod. Fertil. 1999;**116:**113–125. PMID: 10505062

[70] Dreanno C, Cosson J, Suquet M, Seguin F, Dorange G, Billard R. Nucleotide content, oxidative phosphorylation, morphology, and fertilizing capacity of turbot (*Psetta maxima*) spermatozoa during the motility period. Mol. Reprod. Dev. 1999;**53:**230–243. doi:10.1002/(SICI)1098-2795(199906)53:2<230::AID-MRD12>3.0.CO;2-H

[71] Billard R, Cosson J, Fierville F, Brun R, Rouault T, Williot P. Motility analysis and energetics of the Siberian sturgeon *Acipenser baerii* spermatozoa. J. Appl. Ichthyol. 1999;**15:**199–203. doi:10.1111/j.1439-0426.1999.tb00234.x

[72] Chauvaud L, Cosson J, Suquet M, Billard R. Sperm motility in turbot, *Scophthalmus marimus*: initiation of movement and changes with time of swimming characteristics. Environ. Biol. Fishes. 1995;**43:**341–349. doi:10.1007/bf00001167

[73] Kinoshita S, Miki-Noumura T, Omoto CK. Regulatory role of nucleotides in axonemal function. Cell Motil. Cytoskeleton. 1995;**32:**46–54. doi:10.1002/cm.970320106

[74] Omoto CK, Yagi T, Kurimoto E, Kamiya R. Ability of paralyzed flagella mutants of Chlamydomonas to move. Cell Motil. Cytoskeleton. 1996;**33:**88–94. doi:10.1002/(sici)1097-0169(1996)33:2<88::aid-cm2>3.0.co;2-e

[75] Omoto CK. Mechanochemical coupling in cilia. Int. Rev. Cytol. 1991;**131:**255–292. doi:10.1016/s0074-7696(08)62021-5

[76] Cosson MP, Cosson J, Andre F, Billard R. cAMP/ATP relationship in the activation of trout sperm motility: their interaction in membrane-deprived models and in live spermatozoa. Cell Motil. Cytoskeleton. 1995;**31:**159–176. doi:10.1002/cm.970310208

[77] Penningroth SM, Peterson DD. Evidence for functional differences between two flagellar dynein ATPases. Cell Motil. Cytoskeleton. 1986;**6:**586–594. doi:10.1002/cm.970060607

[78] Saudrais C, Fierville F, Loir M, Le Rumeur E, Cibert C, Cosson J. The use of phosphocreatine plus ADP as energy source for motility of membrane-deprived trout spermatozoa. Cell Motil. Cytoskeleton. 1998;**41:**91–106. doi:10.1002/(SICI)1097-0169(1998)41:2<91::AID-CM1>3.0.CO;2-I

[79] Fedorov P, Dzyuba B, Fedorova G, Grabic R, Cosson J, Rodina M. Quantification of adenosine triphosphate, adenosine diphosphate, and creatine phosphate in sterlet spermatozoa during maturation. J. Anim. Sci. 2015;**93:**5214. doi:10.2527/jas.2015-9144

[80] Dzyuba V, Dzyuba B, Cosson J, Rodina M. Enzyme activity in energy supply of spermatozoon motility in two taxonomically distant fish species (sterlet *Acipenser ruthenus*, Acipenseriformes and common carp C*y*prinus *carpio*, Cypriniformes). Theriogenology. 2016;**85:**567–574. doi:10.1016/j.theriogenology.2015.09.040

[81] Billard R, Cosson J, Crim LW. Motility of fresh and aged halibut sperm. Aquat. Living Resour. 1993;**6:**67–75. doi:10.1051/alr:1993008

[82] Vermeirssen ELM, de Quero CM, Shields RJ, Norberg B, Kime DE, Scott AP. Fertility and motility of sperm from Atlantic halibut (*Hippoglossus hippoglossus*) in relation to dose and timing of gonadotropin-releasing hormone agonist implant. Aquaculture. 2004;**230:**547–567. doi:10.1016/s0044-8486(03)00414-9

[83] Takai H, Morisawa M. Change in intracellular K^+ concentration caused by external osmolality change regulates sperm motility of marine and freshwater teleosts. J. Cell Sci. 1995;**108:**1175–1181. PMID: 7622603

[84] Trippel EA, Morgan MJ. Sperm Longevity in Atlantic Cod (*Gadus morhua*). Copeia. 1994;**1994:**1025. doi:10.2307/1446727

[85] Groison A-L, Suquet M, Cosson J, Le Coz J-R, Jolivet A, Garren F. Biological characteristics of European hake (*Merluccius merluccius*) sperm. Cybium. 2008;**32:**178.

[86] Cosson J, Groison AL, Suquet M, Fauvel C, Dreanno C, Billard R. Marine fish spermatozoa: racing ephemeral swimmers. Reproduction. 2008;**136:**277–294. doi:10.1530/REP-07-0522

[87] Cosson J, Billard R, Cibert C, Dreanno C, Linhart O, Suquet M. Movements of fish sperm flagella studied by high speed videomicroscopy coupled to computer assisted image analysis. Pol Arch Hydrobiol. 1997;**44:**103–113.

[88] Abascal FJ, Cosson J, Fauvel C. Characterization of sperm motility in sea bass: the effect of heavy metals and physicochemical variables on sperm motility. J. Fish Biol. 2007;**70:**509–522. doi:10.1111/j.1095-8649.2007.01322.x

[89] Cosson J. Flagella parameters used as descriptors of fish spermatozoa motility. Anim. Reprod. Sci. 2016;**169:**128–129. doi:10.1016/j.anireprosci.2016.03.078

[90] Brokaw CJ. My favourite cell: the sea urchin spermatozoon. BioEssays. 1990;**12:**449–452. doi:10.1002/bies.950120910

[91] Cosson J. The ionic and osmotic factors controlling motility of fish spermatozoa. Aquacult. Int. 2004;**12:**69–85. doi:10.1023/B:Aqui.0000017189.44263.Bc

[92] Ishijima S. Comparative analysis of movement characteristics of lancelet and fish spermatozoa having different morphologies. Biol. Bull. 2012;**222:**214–221. PMID: 22815370

[93] Cosson J, Lahnsteiner F, Prokopchuk G, Valdebenito II. Initiation, prolongation, and reactivation of the sperm motility. In: Vladic T, Petersson E, editors. Evolutionary Biology of the Atlantic Salmon. Boca Raton: CRC Press; 2015. p. 63–107. doi:10.1201/b18721-7

[94] Cosson J, Dreanno C, Billard R, Suquet M, Cibert C. Regulation of axonemal wave parameters of fish spermatozoa by ionic factors. In: Gagnon C, editor. The Male Gamete: From Basic Science to Clinical Applications. Montréal: Cache River Press; 1999. p. 161–186.

[95] Dzyuba V, Cosson J. Motility of fish spermatozoa: from external signaling to flagella response. Reprod. Biol. 2014;**14:**165–175. doi:10.1016/j.repbio.2013.12.005

[96] Gibbons IR. Transient flagellar waveforms during intermittent swimming in sea urchin sperm. II. Analysis of tubule sliding. J. Muscle Res. Cell Motil. 1981;**2:**83–130. doi:10.1007/bf00712063

[97] Summers KE, Gibbons IR. Adenosine triphosphate-induced sliding of tubules in trypsin-treated flagella of sea-urchin sperm. Proc. Natl. Acad. Sci. U.S.A. 1971;**68:**3092–3096. PMID: 5289252

[98] Morisawa M, Okuno M. Cyclic AMP induces maturation of trout sperm axoneme to initiate motility. Nature. 1982;**295:**703–704. doi:10.1038/295703a0

[99] Morisawa M, Okuno M, Suzuki K, Morisawa S, Ishida K. Initiation of sperm motility in teleosts. J. Submicrosc. Cytol. 1983;**15:**61–65. PMID: 6302300

[100] Cosson M-P, Gagnon C. Protease inhibitor and substrates block motility and microtubule sliding of sea urchin and carp spermatozoa. Cell Motil. Cytoskeleton. 1988;**10:**518–527. doi:10.1002/cm.970100408

[101] He S, Jenkins-Keeran K, Woods LC. Activation of sperm motility in striped bass via a cAMP-independent pathway. Theriogenology. 2004;**61:**1487–1498. doi:10.1016/j.theriogenology.2003.08.015

[102] Brokaw CJ. Microtubule sliding in swimming sperm flagella: direct and indirect measurements on sea urchin and tunicate spermatozoa. J. Cell Biol. 1991;**114:**1201–1215. doi:10.1083/jcb.114.6.1201

[103] Woolley DM. Studies on the eel sperm flagellum. 2. The kinematics of normal motility. Cell Motil. Cytoskeleton. 1998;**39:**233–245. doi:10.1002/(sici)1097-0169(1998)39:3<233::aid-cm6>3.0.co;2–5

[104] Ishimoto K, Cosson J, Gaffney EA. A simulation study of sperm motility hydrodynamics near fish eggs and spheres. J. Theor. Biol. 2016;**389:**187–197. doi:10.1016/j.jtbi.2015.10.013

[105] Cosson J, Huitorel P, Gagnon C. How spermatozoa come to be confined to surfaces. Cell Motil. Cytoskeleton. 2003;**54:**56–63. doi:10.1002/cm.10085

[106] Boryshpolets S, Cosson J, Bondarenko V, Gillies E, Rodina M, Dzyuba B, Linhart O. Different swimming behaviors of sterlet (*Acipenser ruthenus*) spermatozoa close to solid and free surfaces. Theriogenology. 2013;**79:**81–86. doi:10.1016/j.theriogenology.2012.09.011

[107] Smith DJ, Gaffney EA, Blake JR, Kirkman-Brown JC. Human sperm accumulation near surfaces: a simulation study. J. Fluid Mech. 2009;**621:**289. doi:10.1017/s0022112008004953

[108] Woolley D. Motility of spermatozoa at surfaces. Reproduction. 2003;**126:**259–270. doi:10.1530/rep.0.1260259

[109] Inaba K, Dreanno C, Cosson J. Control of flatfish sperm motility by CO_2 and carbonic anhydrase. Cell Motil. Cytoskeleton. 2003;**55:**174–187. doi:10.1002/cm.10119

[110] Holwill ME. Kinetic studies of the flagellar movement of sea-urchin spermatozoa. J. Exp. Biol. 1969;**50:**203–222. PMID: 4237926

[111] Denehy MA. The propulsion of nonrotating ram and oyster spermatozoa. Biol. Reprod. 1975;**13:**17–29. PMID: 1222179

[112] Cosson MP, Cosson J, Billard R. Synchronous triggering of trout sperm is followed by an invariable set sequence of movement parameters whatever the incubation medium. Cell Motil. Cytoskeleton. 1991;**20:**55–68. doi:10.1002/cm.970200107

[113] Brokaw CJ. Calcium sensors in sea urchin sperm flagella. Cell Motil. Cytoskeleton. 1991;**18:**123–130. doi:10.1002/cm.970180207

[114] Morita M, Takemura A, Nakajima A, Okuno M. Microtubule sliding movement in tilapia sperm flagella axoneme is regulated by Ca^{2+}/calmodulin-dependent protein phosphorylation. Cell Motil. Cytoskeleton. 2006;**63:**459–470. doi:10.1002/cm.20137

[115] Cosson MP, Billard R, Letellier L. Rise of internal Ca^{2+} accompanies the initiation of trout sperm motility. Cell Motil. Cytoskeleton. 1989;**14:**424–434. doi:10.1002/cm.970140312

[116] Becker LE, Shelley MJ. Instability of elastic filaments in shear flow yields first-normal-stress differences. Phys. Rev. Lett. 2001;87. doi:10.1103/PhysRevLett.87.198301

[117] Yeates SE, Diamond SE, Einum S, Emerson BC, Holt WV, Gage MJG. Cryptic choice of conspecific sperm controlled by the impact of ovarian fluid on sperm swimming behavior. Evolution. 2013;**67:**3523–3536. doi:10.1111/evo.12208

[118] Tuset VM, Trippel EA, de Monserrat J. Sperm morphology and its influence on swimming speed in Atlantic cod. J. Appl. Ichthyol. 2008;**24:**398–405. doi:10.1111/j.1439-0426.2008.01125.x

[119] Alavi SMH, Cosson J. Sperm motility in fishes. I. Effects of temperature and pH: a review. Cell Biol. Int. 2005;**29:**101–110. doi:10.1016/j.cellbi.2004.11.021

[120] Cosson J . Fish spermatozoa motility: physical, and bio-energetic interactions with their surrounding media. In: Morisawa M, editor. Sperm Cell Research in the 21st Century: Historical Discoveries to New Horizons. Tokyo: Adthree Publishing Ltd; 2012. p. 152–156.

[121] Cosson J, Groison AL, Fauvel C, Suquet M. Description of hake (*Merlucius merlucius*) spermatozoa: flagellar wave characteristics and motility parameters in various situations. J Appl. Ichthyol. 2010;**26:**644–652. doi:10.1111/j.1439-0426.2010.01563.x

[122] Holwill ME. Hydrodynamic aspects of cilia and flagella. In: Sleigh MA, editor. Cilia and Flagella. London: Academic Press; 1974. p. 143–175.

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