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RESEARCH ARTICLE

Ovarian fluid impacts flagellar beating and biomechanical metrics of sperm between alternative reproductive tactics

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ABSTRACT

Alternative reproductive tactics (ARTs) are prevalent in nature, where smaller parasitic males typically have better sperm quality than larger territorial guard males. At present, it is unclear what is causing this phenomenon. Our objective was to gain insights into sperm form and function by examining flagellar beating patterns (beat frequency, wave amplitude, bend length, bend angle, wave velocity) and biomechanical sperm metrics (velocity, hydrodynamic power output, propulsive efficiency) of wild spawning Chinook salmon ARTs. Ovarian fluid and milt were collected to form a series of eight experimental blocks, each composed of ovarian fluid from a unique female and sperm from a unique pair of parasitic jack and guard hooknose males. Sperm from each ART were activated in river water and ovarian fluid. Flagellar parameters were evaluated from recordings using high-speed video microscopy and biomechanical metrics were quantified. We show that ART has an impact on flagellar beating, where jacks had a higher bend length and bend angle than hooknoses. Activation media also impacted the pattern of flagellar parameters, such that beat frequency, wave velocity and bend angle declined, while wave amplitude of flagella increased when ovarian fluid was incorporated into activation media. Furthermore, we found that sperm from jacks swam faster than those from hooknoses and required less hydrodynamic power output to propel themselves in river water and ovarian fluid. Jack sperm were also more efficient at swimming than hooknose sperm, and propulsive efficiency increased when cells were activated in ovarian fluid. The results demonstrate that sperm biomechanics may be driving divergence in competitive reproductive success between ARTs.

KEY WORDS: Salmon, *Oncorhynchus tshawytscha*, Spawning, Reproductive strategy, Sperm competition, Cryptic female choice

INTRODUCTION

Across a wide range of taxa, males often exhibit different tactics to increase their reproductive success: hereafter, alternative reproductive tactics (ARTs; reviewed in Oliveira et al., 2008). This is especially common in teleost fishes, where larger and more aggressive 'guard' males defend territories/nests to mate with females and, in some species, invest in parental care (Taborsky, 1998). Alternatively, smaller 'parasitic' males attempt to steal fertilizations from guard males by employing different behavioural tactics, e.g. female mimicry or sneaking behaviour, and typically invest a lot of resources in gonadal development, to maximize their reproductive success in the face of sperm competition (Taborsky, 1998; Neff et al., 2003; Fitzpatrick et al., 2016). For instance, Atlantic salmon (*Salmo salar*) parasitic parr males have a greater proportion of motile sperm and higher sperm adenosine triphosphate (ATP) content, which contributes significantly to higher fertility under competition than their larger anadromous or guard counterparts (Vladić and Järvi, 2001; Vladić et al., 2010). A similar phenomenon has been observed for other aquatic species (Oliveira et al., 2008).

Interestingly, in the ocellated wrasse (*Symphodus ocellatus*), a species with ARTs, the presence of female ovarian fluid (fluid expelled with egg batches) influenced the nature and outcome of sperm competition among ARTs by changing the relationship between sperm number and paternity in a way that shifted the fitness advantage from sperm production to velocity (Alonzo et al., 2016). Likewise, in masu salmon (*Oncorhynchus masou*), smaller sneaking parr males invested significantly more in relative gonad mass than the larger dominant anadromous males (Makiguchi et al., 2016). Additionally, it was shown that sperm velocity and motility were significantly higher, and longevity was lower in parr males than in anadromous males in river water; however, no differences between ARTs were detected when the cells were activated in ovarian fluid (Makiguchi et al., 2016). Together, these results suggest a potential for sexual selection to continue through mechanisms that enable females to bias the outcome of sperm competition, a process known as cryptic female choice (Eberhard, 1996).

During bouts of sperm competition, fertilization success is highly dependent on the ability of sperm to reach the egg micropyle within a short period of time (Yeates et al., 2007) and, as shown by Gage et al. (2004), sperm velocity represents a crucial parameter. Sperm velocity is a global factor that characterizes the movement of a sperm population, but basic information resides in the flagellum that generates motility. Therefore, studying the interaction between the sperm flagellum and its surrounding fluids (river water and ovarian fluid, in our case) is of primary importance (Rikmenspoel, 1984), especially in environments characterized by a high degree of sperm competition. Several external factors of the activation medium can control the beating characteristics of flagella, some of which are chemical, such as ions or attracting molecules (Cosson, 2015), while others are physical, such as temperature or viscosity (Holwill, 1977). In turn, beating patterns of flagella were shown to be affected by these regulating factors, including amplitude or length of waves, velocity of wave propagation and symmetry of waves (Cosson and Prokopchuk, 2014).

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Among vertebrates, some of the most recognizable ARTs exist within the salmonids (Gage et al., 1995; Vladić and Järvi, 2001), and in this study we used Chinook salmon (*Oncorhynchus tshawytscha*) as the focal species. Chinook salmon is a semelparous (reproduce once and then die) species, where larger 'hooknose' males mature after 3–5 years on average and display many of the typical guard characters, such as exaggerated secondary sexual characteristics and male-to-male aggressive behaviour (Berejikian et al., 2010; Butts et al., 2012b). In contrast, parasitic jack males reach sexual maturity precociously, after 2 years, and try to outcompete hooknose males during the spawning season by investing disproportionately in reproductive traits, e.g. gonads and sperm swimming performance (Berejikian et al., 2010; Flannery et al., 2013; Young et al., 2013).

Our objective was to gain new and comprehensive insight into sperm form and function by examining flagellar beating patterns (i.e. beat frequency, bend length, wave amplitude, wave velocity, bend angle) and biomechanical sperm metrics (velocity, power output and propulsive efficiency) between wild spawning Chinook salmon ARTs that were activated in river water or an ovarian fluid solution.

MATERIALS AND METHODS

Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum 1792), were collected from the Credit River (43°350′N, 79°420′W), which flows into Lake Ontario (one of the Laurentian Great Lakes found in North America), from 1 to 5 October 2012 using backpack electrofishing (see Flannery et al., 2013; Butts et al., 2012b, for details; collection permit from Ontario Ministry of Natural Resources and Forestry). Chinook salmon have been stocked in Lake Ontario for over 50 years (Crawford, 2001). Based on an examination of otoliths, hooknose males and females in our study population sexually mature at 3–5 years of age whereas jacks mature at 2 years of age (T.E.P., unpublished data). The males were found haphazardly in flowing water ∼0.6–1.2 m in depth and at ∼11°C. After capture, the fish were humanely killed according to guidelines from the Ontario Ministry of Natural Resources and Forestry protocols. After drying each fish, we applied slight pressure to the abdomen to collect either milt (seminal plasma+sperm) or eggs, and accompanying ovarian fluid. The eggs collected from females were placed in a sieve and ovarian fluid was collected. Milt, eggs and ovarian fluid were kept in a cooler that was held at approximately the same temperature as the river water (∼11°C) and were transported to the lab for further analyses within 6 h of the time of gamete collection.

Eggs and their associated ovarian fluid as well as milt were collected to form a series of eight experimental blocks, each composed of ovarian fluid from a female and sperm from two males – a hooknose (n=8 in total) and a jack (n=8 in total). Males from the two ARTs were paired so that the difference in storage time of their respective milt samples was minimized. For each of these eight blocks, sperm from the hooknose and jack were activated in river water and in an ovarian fluid solution (40%) diluted in the same river water.

Flagellar beating of sperm from each male was video recorded according to Prokopchuk et al. (2015). Specifically, motility of sperm flagella was observed at ∼7 s post-activation either by 50× lens dark field or by 100× lens phase-contrast microscopy. Observations were recorded using a high-speed video camera (model i-SPEED TR, Olympus) with spatial resolution of 848×688 pixels and time resolution up to 1000 frames s^{-1} in routine recordings to obtain sharp images of flagella (Cosson et al., 1997). From these obtained images, covering one or several successive beat cycles (period between the appearance of two successive waves), detailed analysis of the flagellar beating behaviour (63.1±7.6 sperm per block, mean±s.d.) was performed using image analysis software (Olympus Micro Image 4.0.1. for Windows). Different parameters (illustrated in Fig. 1A), such as beat frequency of waves, wave amplitude, bend length, bend angle and wave velocity, were used to evaluate variation in flagellar wave patterns while sperm were swimming. Flagellar beat frequency was calculated from the time interval between two successive wave initiations occurring at the head–tail junction of a spermatozoon. Wave amplitude was determined as the perpendicular distance between the peak of the wave and a reference line drawn from the head of a spermatozoon to the tip of the flagellum through mid-points of bent regions. Bend length was measured as the distance between the two inflexion points adjacent to a bend, and bend angle was determined as the angle between tangents to the two straight segments adjacent to the bend. Wave velocity was defined by the change in distance between corresponding points along the flagellum in successive images over time.

Flagellar beating parameters evaluated from high-speed video records were then used to calculate sperm velocity, hydrodynamic power output and propulsive efficiency for jacks and hooknoses activated in river water and ovarian fluid. Sperm velocity was calculated from the resistive force theory (RFT) formula (Gray and Hancock, 1955; Eqn 1):

$$\text{Sperm velocity} = \frac{2f\pi^2 b^2}{\lambda} \left\{ \frac{1}{1+(4\pi^2 b^2/\lambda^2)-(1+(2\pi^2 b^2/\lambda^2))\frac{1}{2}(3a/n\lambda)[(\log(d/2\lambda))+1]} \right\}, \quad (1)$$

where f is beat frequency, b is wave amplitude, λ is wavelength calculated as 2×bend length, a is head radius (1.2 µm; Flannery et al., 2013), n is the number of waves along the flagellum and d is the radius of the flagellum (hooknose 0.096±0.006 µm, jack 0.094±0.007 µm).

Hydrodynamic power output, or the power developed by the sperm flagellum against the viscous drag in the surrounding fluid, was calculated by Eqn 2 (Taylor, 1952; see also details in Dzyuba et al., 2016):

$$\text{Hydrodynamic power output} = \frac{4\pi^3 \eta f^2 b^2 l}{0.62 - \ln(2\pi a/\lambda)}, \quad (2)$$

where l is the total length of the flagellum and η is the viscosity of the swimming medium (other parameters are defined in Eqn 1). In absence of experimental determination of viscosity, the viscosity of ovarian fluid was estimated as 8 times that of river water by analogy with values measured on trout ovarian fluid at 10°C (J.C., unpublished data). This value is close to those obtained by Rosengrave et al. (2009b) at 15°C, considering that sperm movement occurs at low shearing rate (Brokaw, 1966). Therefore, a 40% ovarian fluid activation medium, as used in these experiments, leads to a viscosity value of 3.2 cP (0.0032 Pa s) relative to river water (at 11°C in our experiments). Thus, it is predicted that hydrodynamic power performance of cells is highly affected by the presence of ovarian fluid, mostly because of a higher viscosity.

Journal of Experimental Biology (2017) 220, 2210-2217 doi:10.1242/jeb.154195

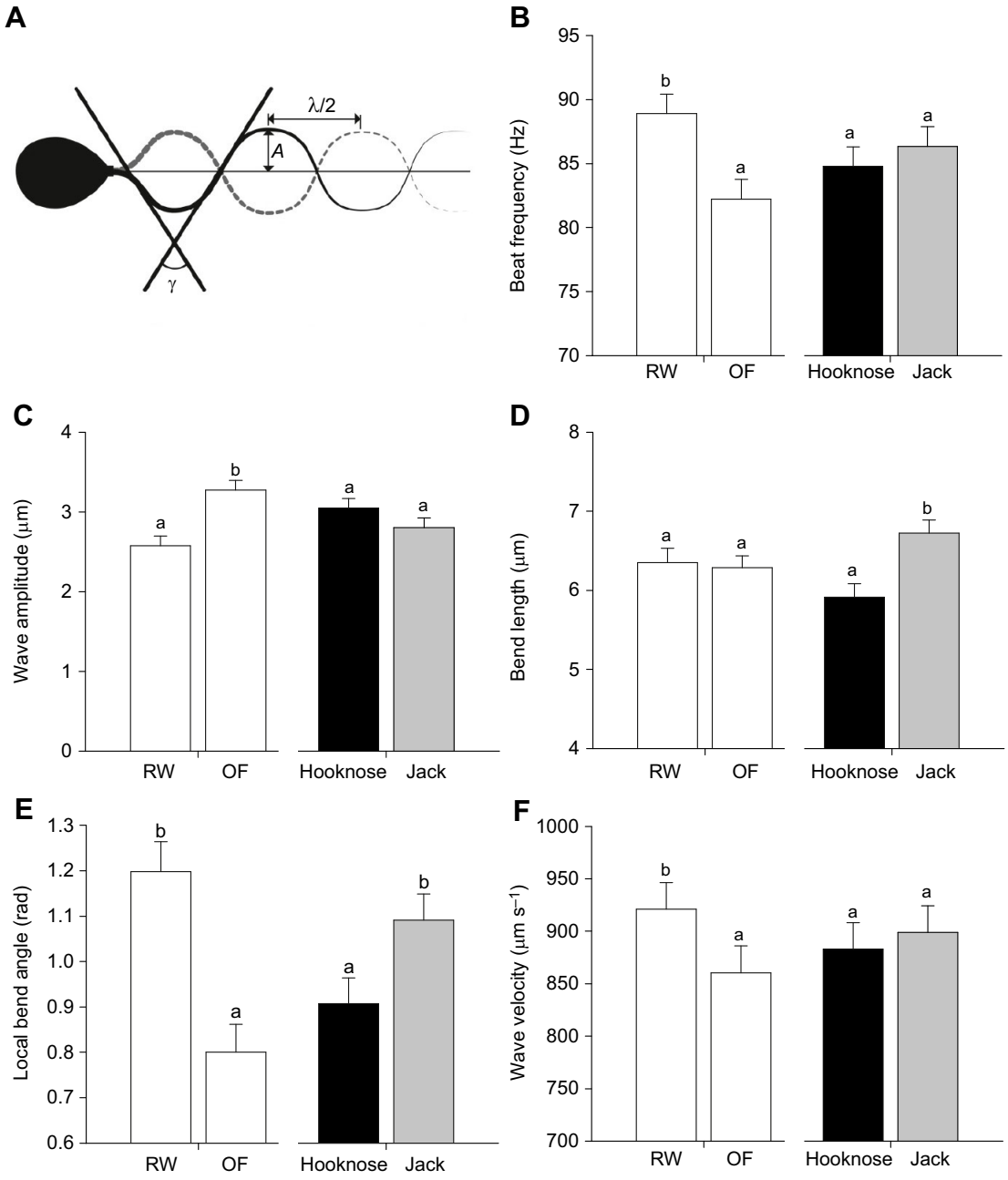

Fig. 1. Sperm flagellar beating patterns for Chinook salmon (*Oncorhynchus tshawytscha*) in each activation medium. (A) Illustration of sperm flagellar beating patterns. γ, local bend angle; λ/2, bend length; *A*, wave amplitude. (B–F) Data (means±s.e.m.) for hooknose (n=8) and jack (n=8) males in each activation medium (river water RW or ovarian fluid OF). For each metric, the alternative reproductive tactic (ART) and activation medium main effects were interpreted because of non-significant ART×activation medium interactions. Different letters indicate significant differences between the treatments (P<0.05). (B) Flagellar beat frequency was calculated from the time interval between two successive wave initiations occurring at the head–tail junction of a spermatozoon. (C) Wave amplitude was determined as the perpendicular distance between the peak of a wave and a reference line drawn from the head of a spermatozoon to the tip of the flagellum through mid-points of bent regions. (D) Bend length was measured as the distance between the two inflexion points adjacent to a bend. (E) Local bend angle was determined as the angle between tangents to the two straight segments adjacent to the bend. (F) Wave velocity was defined as the change in distance between corresponding points along the flagellum in successive images over time.

Finally, sperm propulsive efficiency was calculated using Eqn 3:

$$\text{Propulsive efficiency} = \text{Sperm velocity}/\text{Beat frequency of waves}. \quad (3)$$

Here, higher propulsive efficiency values represent more efficient swimming sperm based on the distance covered by the sperm cell during each beat cycle (Dzyuba et al., 2016).

Statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC, USA, version 9.1). Flagellar beating parameters were analysed using a series of mixed-model repeated measures ANOVA models containing the ART, activation medium (repeated factor) and the ART×activation medium interaction. ART and activation medium were considered fixed factors and male identity was considered a random factor. Variation associated with each female was controlled for statistically by incorporating female as a

Journal of Experimental Biology (2017) 220, 2210-2217 doi:10.1242/jeb.154195

random blocking factor. When interactions were detected, the saturated models were decomposed to determine the effect of activation medium for each ART and the effect of ART for each activation medium (Keppel, 1991). In the case of a non-significant interaction, main effects were interpreted. Data were transformed to meet assumptions of normality and homoscedasticity when necessary. Treatment means were contrasted using the Tukey–Kramer test. Alpha was set at 0.05 for interactions and main effects.

RESULTS

All flagellar beating patterns had a non-significant ART×activation medium interaction ($P>0.05$), therefore the ART and activation medium main effects were interpreted (Fig. 1). For beat frequency, activation medium was significant ($P<0.01$), such that beat frequency of the flagella declined in ovarian fluid (Fig. 1B). The opposite was the case for wave amplitude, such that amplitude of the flagella increased when ovarian fluid was incorporated into the activation medium ($P<0.0001$; Fig. 1C). The ART main effect was significant for bend length ($P<0.0001$), where the flagella from the jacks had a higher bend length than those from the hooknoses (Fig. 1D), while both the ART ($P<0.01$) and activation medium ($P<0.0001$) main effects were significant for bend angle, such that sperm flagella from the jacks had a higher bend angle than those from hooknoses and the angle decreased when ovarian fluid was used to activate the cells (Fig. 1E). For wave velocity, only activation medium was significant ($P<0.05$), such that wave velocity of the flagella declined in ovarian fluid (Fig. 1F). An illustration of flagellar beating patterns from hooknoses and jacks, activated in river water and ovarian fluid, is shown in Fig. 2.

The RFT formula was used to calculate velocity for individual sperm cells. Sperm from jacks swam significantly faster than sperm from hooknoses ($P<0.05$), while velocity was not impacted by either the ART×activation medium interaction ($P>0.05$) or the activation medium main effect ($P>0.05$; Fig. 3). The amount of hydrodynamic power output generated by the sperm was calculated and was found to be significantly affected by the presence of an ART×activation medium interaction ($P<0.0001$); as such, reduced models were run separately for each activation medium (Fig. 4). Interestingly, jack sperm required significantly less power output to propel themselves, when activated in either river water ($P<0.01$; Fig. 4A) or ovarian fluid ($P<0.0001$; Fig. 4B).

Both the ART ($P<0.01$) and activation medium ($P<0.0001$) main effects were significant for propulsive efficiency, such that sperm from the jacks were more efficient at swimming than those from hooknoses, and sperm from both ARTs were more efficient when activated in ovarian fluid (Fig. 5). However, the ART×activation medium interaction was not significant ($P>0.05$).

DISCUSSION

Sperm movement is the result of forward thrust due to the propagation of backward flagellar waves combined with the resistance of the fluid (i.e. river water, ovarian fluid) in which the cell is swimming (Cosson and Prokopchuk, 2014). The resulting velocity can be evaluated by global head displacement of the cell population, as used by computer-assisted sperm analysis (CASA) algorithms, or alternatively by evaluation of individual cell velocity obtained from flagellar parameters (i.e. wave amplitude, beat frequency of waves, bend length) extracted from high-speed camera recordings with high resolution, as performed in our study. We detected no interactive effects between the ARTs and activation media for the five sperm flagellar parameters that we measured (see Fig. 1). Together, this suggests that the sperm flagella from both jacks and hooknoses did not beat differently when activated in river water or ovarian fluid, suggesting no biased ovarian fluid selection between the tactics for these specific flagellar parameters.

In contrasts, there were numerous ovarian fluid-based responses, indicating that flagellar parameters for both tactics were impacted, either positively or negatively, when this maternally induced substance was added to the activation medium. This is not surprising, considering that ovarian fluid is a significant portion (10–30%) of the egg mass in salmonid spp. (Wojtczak et al., 2007). In turn, ovarian fluid has been shown to directly impact the outcome of a fertilization event by modifying sperm performance, i.e. motility and velocity (Urbach et al., 2005; Rosengrave et al., 2009a; Diogo et al., 2010; Gasparini et al., 2012; Galvano et al., 2013; Alonzo et al., 2016). This is related to changes in viscosity (Turner

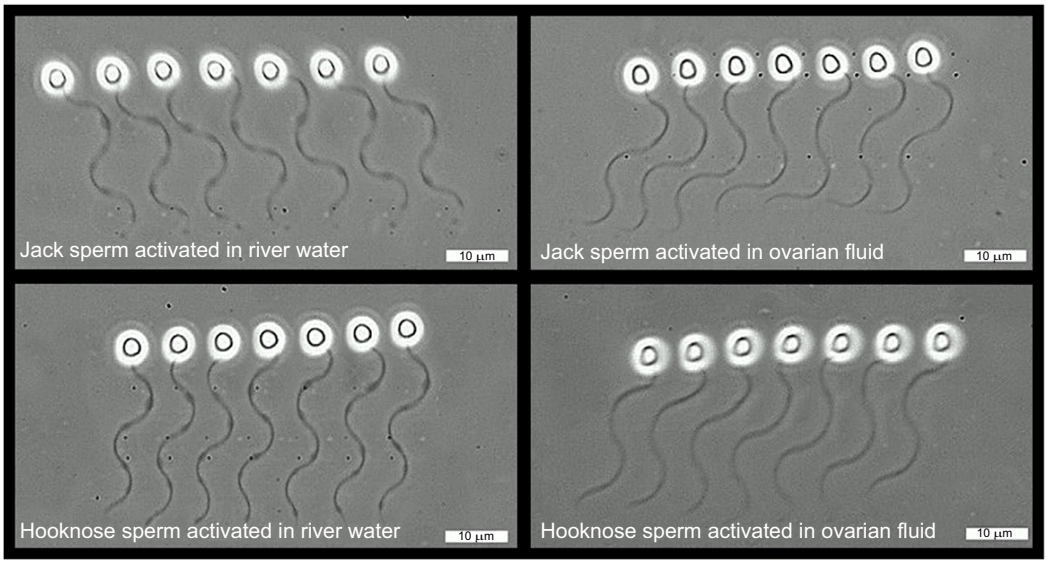

Fig. 2. Successive snapshots of flagellar beating patterns for Chinook salmon hooknose and jack males activated in river water or ovarian fluid. Snapshots were obtained every 0.002 s by high-speed video microscopy using a 100× magnification phase-contrast objective.

Journal of Experimental Biology (2017) 220, 2210-2217 doi:10.1242/jeb.154195

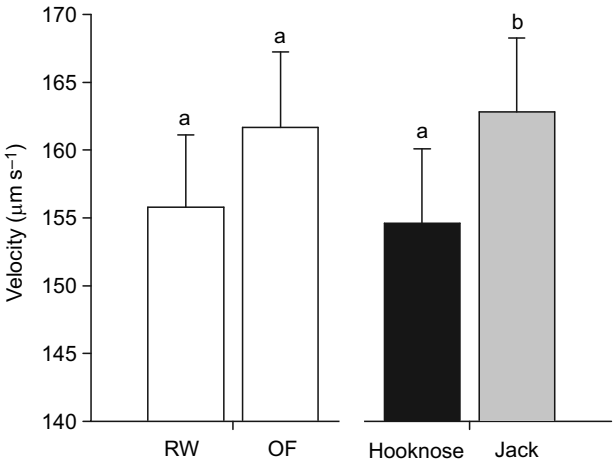

Fig. 3. Sperm velocity of Chinook salmon in each activation medium. Velocity (mean±s.e.m.) was calculated from the resistive force theory formula (Gray and Hancock, 1955) for hooknose (n=8) and jack (n=8) males in river water or ovarian fluid. For sperm velocity, the ART and activation medium main effects were interpreted because of a non-significant ART×activation medium interaction. Different letters indicate significant differences between the treatments (P<0.05).

and Montgomerie, 2002), pH (Wojtczak et al., 2007) and/or ionic composition of activation media (Rosengrave et al., 2009b). Ovarian fluid has even been found to differentially enhance sperm based on genetic relatedness of mates (Gasparini and Pilastro, 2011; Butts et al., 2012a) or spawning origin (i.e. wild versus farmed males; Beirão et al., 2014). Thus, when testing further ART paradigms, we strongly suggest a shift from the classic sperm activation medium (water only) to more natural spawning media, encompassing interactions between gametes and their associated fluids (Fig. 6). This is particularly important for broadcast spawning teleost fishes, such as salmonids, as each female creates a unique spawning environment by simultaneously expelling ovarian fluid along with an egg batch (Lahnsteiner et al., 1995; Johnson et al., 2014).

Application of the RFT, initially developed by Gray and Hancock (1955), enabled velocity evaluation based on Eqn 1, and sperm velocity values corresponding to the different experimental situations described in this paper are presented in Fig. 3. Surprisingly, these calculated sperm velocity values were not enhanced when the cells were activated in ovarian fluid, as would be expected (see above), but as predicted the jacks had superior sperm performance (i.e. higher sperm velocity) when compared with hooknose males, which further solidifies previous findings that jacks invest disproportionately in reproduction in order to increase their chances of fertilizing more eggs during periods of intense sperm competition (Flannery et al., 2013).

An estimation of the hydrodynamic power output generated by the flagellum in various swimming situations was obtained from Eqn 2 and these values are presented in Fig. 4 (Taylor, 1952; Holwill, 1977). It should be noted that, in Eqn 2, the viscosity of the medium (η) in which the sperm cells were swimming is included. Viscosity is known to greatly influence flagellar wave properties (Rikmenspoel, 1984; Lauga, 2007) and, therefore, modifies sperm velocity. In two pioneering papers, Brokaw (1965, 1966) investigated the effects of viscosity on the sperm flagellar parameters of three different marine species and demonstrated that beat frequency and wave velocity are mainly affected while other parameters such as wavelength or bend angle are only slightly changed in the range of viscosity investigated in our paper. Another study (Rikmenspoel, 1984) confirms in bull sperm that beat frequency decreases while wavelength remains constant with increasing viscosity (as we observed in salmon) but, in contrast to our results, wave amplitude decreases, as was also observed by Brokaw (1965, 1966). Such species specificity may reflect differences in the energetic content (ATP) as the power output is also affected oppositely by viscosity in salmon spermatozoa compared with those of other species.

The effects of viscosity have also been predicted by theoretical physics (mostly hydrodynamics) applied to biology. The results from Lauga (2007) put forward some predictions. (1) Compared with a Newtonian fluid of the same viscosity, it is energetically advantageous to swim in a fluid that has some elasticity. This is the

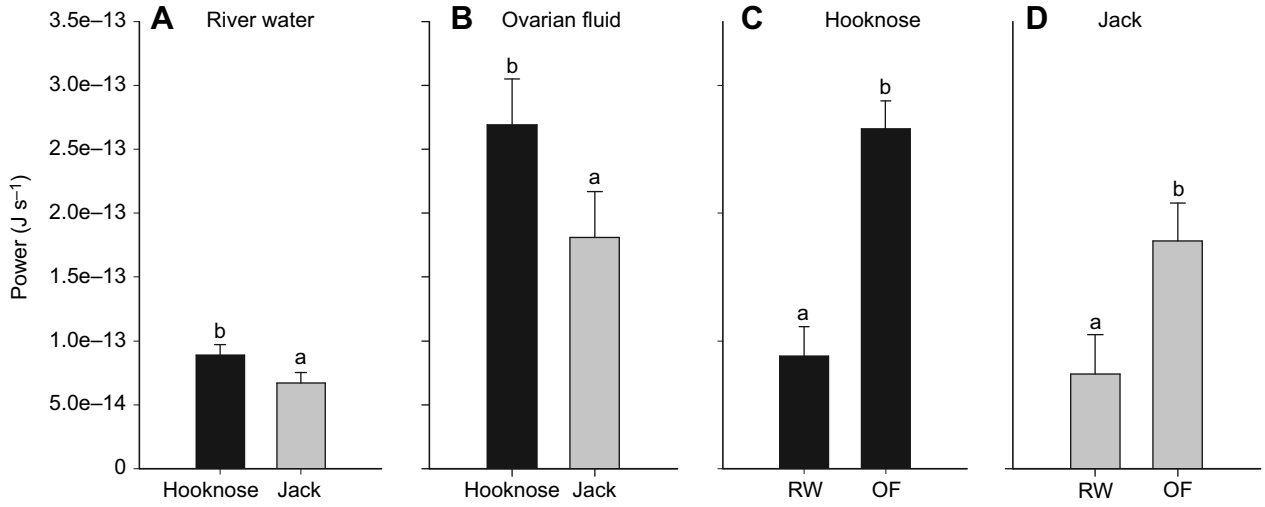

Fig. 4. Hydrodynamic power output of Chinook salmon in each activation medium. Power per individual spermatozoon (mean±s.e.m.) was calculated (Taylor, 1952; Holwill, 1977) for hooknose (n=8) and jack (n=8) males in river water or ovarian fluid. The statistical analysis was first run as a saturated model and when a significant interaction was detected, decomposed models were run separately for each activation medium (A, river water; B, ovarian fluid) to facilitate the interpretation of ARTs and for each ART (C, hooknose; D, jack) to facilitate the interpretation of activation medium. Different letters indicate significant differences between the treatments (P<0.05).

Journal of Experimental Biology (2017) 220, 2210-2217 doi:10.1242/jeb.154195

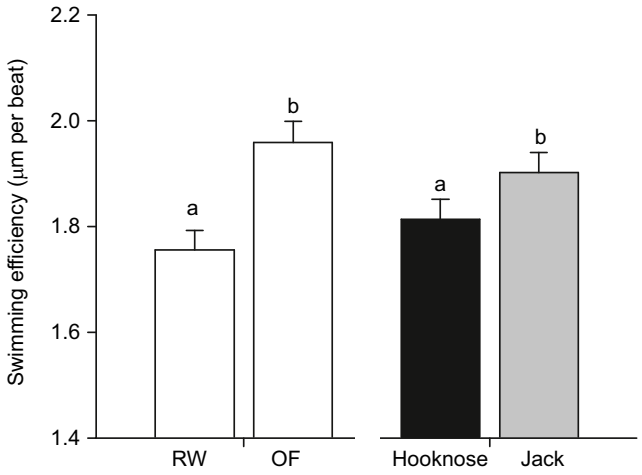

Fig. 5. Sperm swimming efficiency for Chinook salmon in each activation medium. Efficiency (mean±s.e.m.) was calculated by dividing sperm velocity (Gray and Hancock, 1955) by beat frequency of waves for hooknose (n=8) and jack (n=8) males in river water or ovarian fluid. The ART and activation medium main effects were interpreted because of a non-significant ART×activation medium interaction. Different letters indicate significant differences between the treatments (P<0.05).

case for ovarian fluid, considered as a non-Newtonian (viscoelastic) fluid. (2) A non-Newtonian fluid could be exploited by biological systems to their advantage because viscoelastic stresses allow a fine-tuning of the kinematics of transport and locomotion in a manner that is not possible with a Newtonian fluid. This could explain how some females could favour a particular male's spermatozoa based only on the viscoelastic properties of their ovarian fluid. In this respect, it is remarkable that the ovarian fluid viscosity presents a large variability (Rosengrave et al., 2009b), which could reflect such cryptic choice between females.

In our experiments, we could not obtain a direct measurement of the viscosity of ovarian fluid; therefore, we used an estimation of viscosity and applied it to Eqn 2. It is likely that actual ovarian fluid viscosity varied among individual females (Rosengrave et al., 2009b) and could have added some experimental noise. Regardless, such data would only result in a bulk value of ovarian fluid viscosity, whereas in fact local viscosity near the micropyle plays a more crucial role during a spawning event (Yanagimachi et al., 1992). Here, our calculation of hydrodynamic power output clearly shows that jack sperm required less power to propel themselves through the water column when activated in river water or ovarian fluid. In parallel with this reduced power output, sperm from jacks swam faster than sperm from hooknoses and were more efficient swimmers. Additionally, our earlier work showed no difference in sperm ATP content between the tactics (Flannery et al., 2013). Together, this suggests that jacks are probably more effective at utilizing their metabolic resources (i.e. ATP) for power output or are more bio-mechanically adapted to efficiently propel themselves to the micropyle, compared with hooknoses. Our calculation of hydrodynamic output also clearly shows that when ovarian fluid is incorporated into the swimming medium, the sperm require further power output for movement, which can probably be attributed to increased viscosity (Turner and Montgomerie, 2002) and/or alterations in biochemical composition (i.e. ions, proteins as discussed below) of the activation media (see Wojtczak et al., 2007; Hatef et al., 2009; Rosengrave et al., 2009b, among others). The main protein composition of salmon ovarian fluid was investigated by Johnson et al. (2014): their proteomic analysis led to identification of 174 proteins of interest, among which 26 are either involved in the hypoxia pathway or are chemical stimuli, some of the latter probably being related to control of sperm function near the egg.

The role of ovarian fluid during the fertilization process was experimentally investigated by Yeates et al. (2013) and their main conclusion was that it is responsible for chemotaxis, a mechanism that allows attraction of the sperm to the eggs, more specifically to the micropyle, and strongly increases the chances of fertilization (reviewed in Cosson, 2015). In our experiments, the role played by chemo-attraction during fertilization is difficult to integrate because our sperm velocity values were obtained in a free situation on a glass slide rather than near the egg micropyle where local properties of the fluid are not defined. As emphasized above, the physical properties (viscosity) near the egg are certainly peculiar. Additionally, this

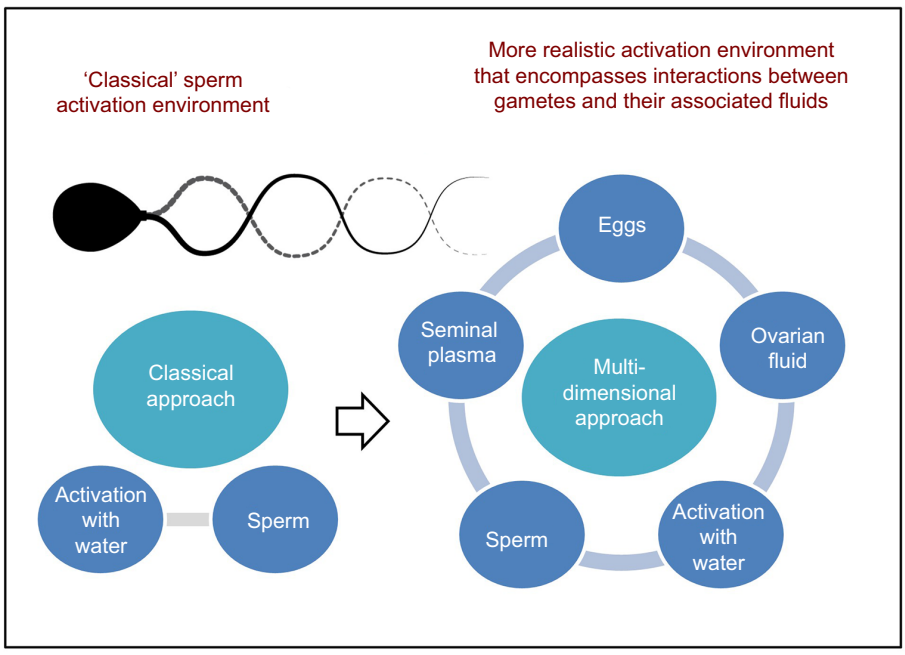

Fig. 6. Proposed paradigm shift for sperm activation in fishes: classical approach to a multi-dimensional framework.

local biochemical environment contains either substances released by the egg micropyle (i.e. herring sperm-activating proteins from eggs of Pacific herring, *Clupea pallasii*; Oda et al., 1995) or molecules involved in egg–sperm recognition, such as major histocompatibility complex (MHC) proteins (Yeates et al., 2009).

The local ionic concentration may also play a role in the behaviour of sperm cells. For example, Ca^{2+} ions affect linearity of sperm tracks (Butts et al., 2013) via changes in the symmetry of beating flagella (Cosson et al., 1989; Alavi et al., 2008, 2011) and it was shown in externally fertilizing fish species, including salmonids, that sperm are sensitive to external and internal Ca^{2+} ion concentrations (Cosson et al., 1989; Bondarenko et al., 2014) and that the response to such signalling processes is responsible for sperm guidance to the egg micropyle (Yanagimachi et al., 2013). This phenomenon may be involved in salmon reproduction, especially for sperm–egg recognition, as sperm motility (mostly its linearity) was shown to be Ca^{2+} sensitive in salmonid sperm (Okuno and Morisawa, 1989; Dziewulska and Domagała, 2013). The circularization of the swimming path may well favour the spiralling of sperm when swimming close to and inside the egg micropyle, as this has been shown to be part of the guidance process in other fish species (Yanagimachi et al., 2013). In this respect, a differential responsiveness of sperm cells from jacks versus hooknoses to Ca^{2+} could explain part of our results. Further experiments in this direction could shed some light on this, thus warranting additional investigation.

Conclusions

In conclusion, our findings add significantly to the growing body of literature on reproductive competition by furthering our understanding of mechanisms that may be driving the predicted divergence in sperm traits between ARTs. Most notably, we clearly show: (i) deviations between the ARTs for flagellar beating parameters; (ii) that jack sperm swim faster than hooknose sperm and require less power to propel themselves through the water column; and (iii) that jack sperm are more efficient at swimming than hooknose sperm and swimming efficiency increases when the cells are activated in ovarian fluid. Together, these results are not merely of theoretical interest, i.e. for sexual selection and sperm competition theory, but of practical importance. For instance, knowledge of gamete quality is a prerequisite for developing techniques to successfully fertilize, cryopreserve and rear embryos. Thus, these results have implications for farming, breeding and managing salmon stocks.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

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Data availability

The datasets supporting this article are available on request from the corresponding author.

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