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Short communication

Ovarian fluid influences sperm performance in lake trout, *Salvelinus namaycush*

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ABSTRACT

The objectives of this study were to determine whether (i) the presence and concentration of ovarian fluid (OF) affects sperm performance traits, and (ii) variation in sperm performance traits is due to male identity, female identity, and/or male × female interactions in lake trout, *Salvelinus namaycush*. Spermatozoa from four males were activated in river water and OF from four females at two concentrations (10 and 15%). Presence of ovarian fluid influenced sperm traits; no differences were detected between 10 and 15% OF. Sperm traits varied depending on parental identity, such that sperm of some males perform better in the OF of all females and that in OF of some females sperm traits are higher for all males.

1. Introduction

In externally fertilizing teleosts, each female creates a unique spawning environment by simultaneously expelling her ovarian fluid (OF), along with an egg batch [1–3]. Different concentrations of OF have been shown to influence sperm performance traits [4–6] and when activated in OF, sperm performance can depend upon individual male by female interactions, suggesting a possible role in cryptic female choice [3,7,8]. Within this context, ionic [8], biochemical [9], and genetic components (i.e. genes of the major histocompatibility complex) [10] of the OF have played a key role in stabilizing the micro-environment around the micropyle [1], which in turn has increased the fertilizing ability of sperm [11,12].

Thus, the objectives of this study were to determine whether (i) the presence and concentration of OF affects sperm performance traits, and (ii) variation in sperm performance traits is due to male identity, female identity, and/or male × female interactions in lake trout, *Salvelinus namaycush*. Lake trout spawn in nocturnal aggregations, where several males spawn simultaneously with a female, thus intense sperm competition and gamete selection are probable in the wild [13].

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2. Materials and methods

Sperm traits were measured in lake trout from the Ontario Ministry of Natural Resources Codrington Fisheries Research Facility. The origin of broodstock and rearing conditions prior and during this study are reported in Butts et al. [7]. Gametes and their associated fluids were collected from four males and four females in November 2011, which represents peak spawning [14]. Mean (±SEM) fork length and body weight were 684.5 ± 12.3 mm and 4231.3 ± 423.9 g for the males and 606.3 ± 17.9 mm and 2953.3 ± 271.4 g for the females, respectively. Age of these fish ranged from 9 to 11 years. The fish were anaesthetized with MS-222 prior to stripping of gametes. The urogenital pore was wiped dry to avoid contamination. Pressure was then applied to the abdomen and milt was collected using sterilized pipettes. Egg batches, along with the OF, were collected and OF was then separated from the eggs with 1 mm mesh. Gametes were stored in a cooler filled with ice packs (4–6 °C) until further analyses (sperm were activated within 5 h). Sperm performance traits were analyzed at 5, 10, and 15 s post-activation (PA) using methods detailed in Butts et al. [7]. In brief, sperm was activated with inflowing river water at ~7.0 °C and pH 7.0. The following sperm traits were evaluated: average path velocity (VAP, hereafter referred to as sperm velocity), linearity, percent motility, and longevity [15].

For each male ($n = 4$), sperm was activated in water (0% OF) and OF from each female ($n = 4$) at 10 and 15% OF concentrations. Two replicate sperm-activations were conducted for each treatment combination; the mean of these two independent activations was used for statistical analyses. We used 10 and 15% OF concentrations in the activation media as it seems likely that sperm would encounter relatively low concentrations of OF during a spawning event, as OF comprises only 5–20% of the total egg volume in lake trout [7]. Data were analyzed using one-way mixed-model ANOVAs. The independent variable was OF concentration (fixed effect), and male was considered a random factor. Models were run separately at each PA time using SAS statistical analysis software (v.9.1; SAS Institute Inc., Cary, NC, USA).

Furthermore, sperm from each male was activated in each female's OF (four males × four females × two replicate sperm-activations; the mean of the two independent activations was used for statistical analyses). No significant differences in sperm performance traits were detected between 10 and 15% OF concentrations (see below); thus sperm were therefore activated in 10% OF. Sperm performance traits were analyzed using a factorial ANOVA containing the male (random effect) and female (random effect) main effects as well as the male × female interaction. Models were run separately at each PA time.

3. Results and discussion

The presence of OF had a significant effect on sperm velocity at 5 ($p < 0.01$), 10 ($p < 0.05$), and 15 s ($p < 0.05$) PA; sperm motility at 5 ($p < 0.001$), 10 ($p < 0.01$), and 15 s ($p < 0.01$) PA; and linearity at 10 ($p < 0.05$), and 15 s PA ($p < 0.05$) (Fig. 1). Moreover, the presence of OF had an effect on longevity ($p < 0.001$), such that sperm swam longer at the 10% (17.78 ± 0.55 s), and 15% OF concentrations (18.84 ± 0.55 s), when compared to 0% OF (11.00 ± 0.79 s). For all of the sperm performance traits, no significant difference was found between the 10% and 15% OF concentrations. Overall, these results suggest that sperm performance traits are enhanced in the presence of OF and that these effects represent OF concentrations at realistic spawning levels. A similar effect has been previously demonstrated in fishes, i.e. sperm swimming speed increased in OF when compared to sperm activated in water alone [4,5]. This has implications for relative reproductive success among males, as sperm velocity and

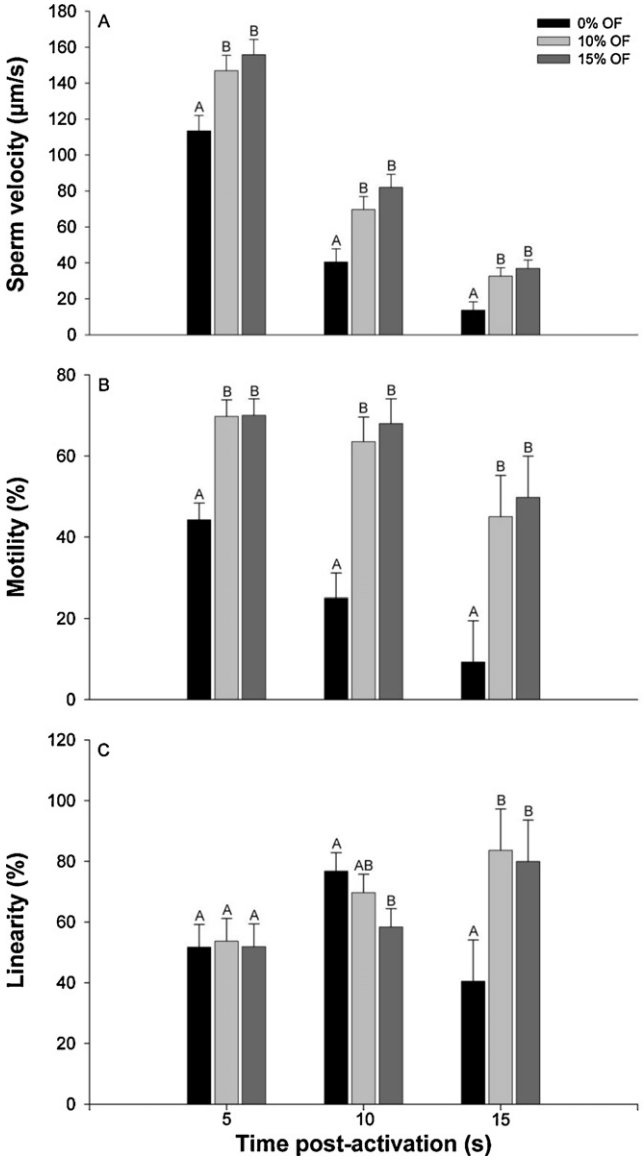

Fig. 1 – The effect of ovarian fluid concentrations (0, 10 and 15%) on average path velocity (A), percent motility (B), and linearity (C) at 5, 10, and 15 s post-activation of sperm in lake trout, *Salvelinus namaycush*. Error bars represent standard errors. Bars without a common superscript differed significantly.

motility (among other performance traits) has been shown to correlate with competitive fertilization success in fishes [15,16]. Thus, our results further strengthen the importance of incorporating OF into sperm-activation and fertilization media to represent more natural spawning environments, particularly if cryptic female choice is a possibility.

For all sperm performance traits there were non-significant male × female interactions ($p > 0.05$), therefore male and female main effects were interpreted. For sperm velocity there was a significant male effect at 5 ($p < 0.05$), and 10 s PA ($p < 0.05$; Fig. 2A), and a significant female effect at 15 s PA ($p < 0.01$; Fig. 2B), while for sperm motility a significant male effect was detected at 15 s PA ($p < 0.01$; Fig. 2C). A significant male effect was detected for linearity at 5 ($p < 0.01$), and 10 s PA ($p < 0.001$; Fig. 2E), and a significant female effect was detected at 15 s PA ($p < 0.05$; Fig. 2F). Ovarian fluid from the different females affected longevity ($p < 0.01$), while the male effect was non-significant. Together, these findings suggest that some males perform better in the OF of all females and that some females have OF in which sperm traits are higher for all males. On the contrary, we found no interaction effect and therefore no evidence of a potential mechanism of cryptic female choice (male × female interaction) as detected in other fish species [3,8]. Thus, further investigation is required to understand the mechanisms by which OF influences sperm performance in lake

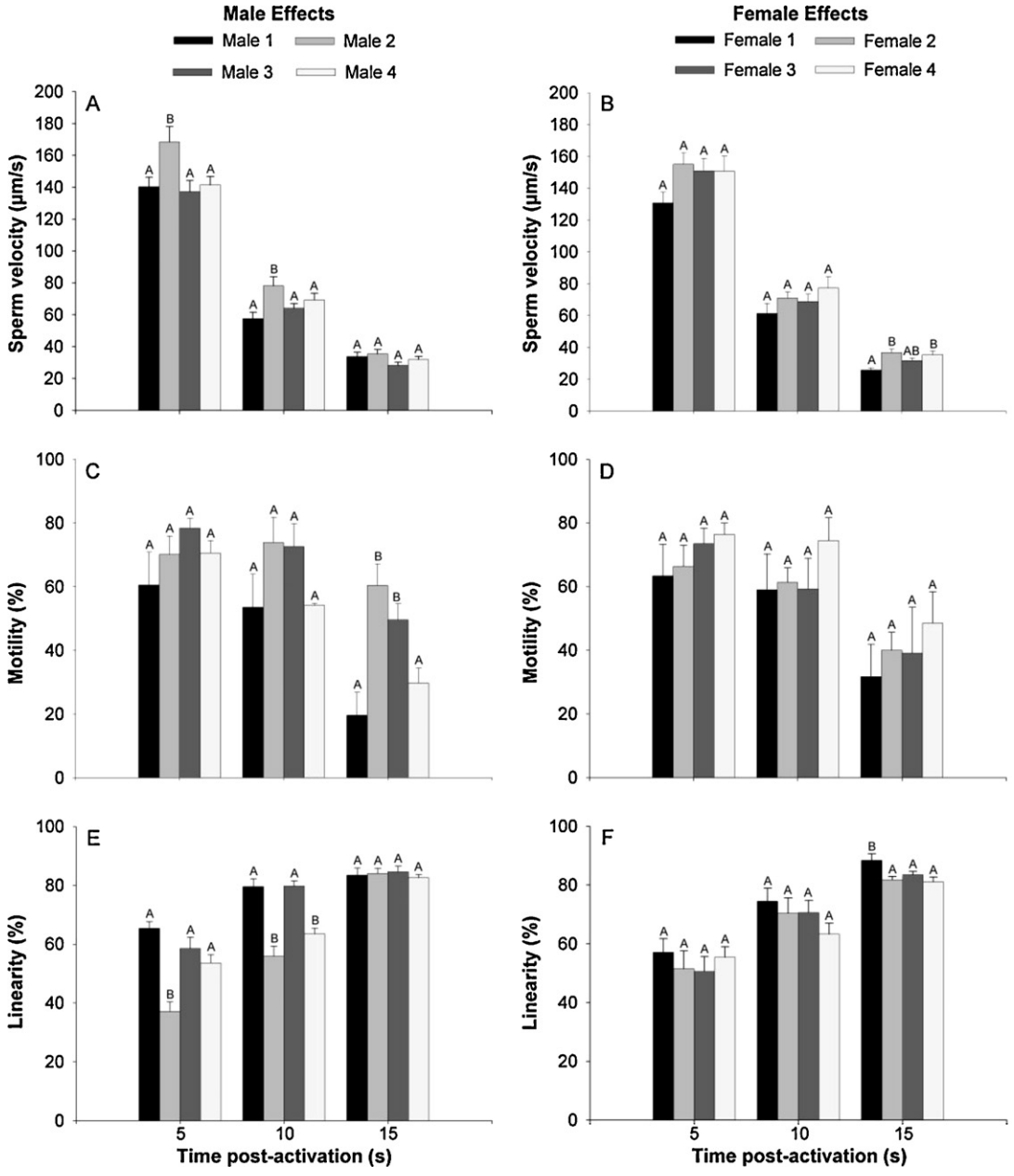

Fig. 2 – The effects of male identity, female identity, and the male × female interaction on average path velocity, percent motility, and linearity in lake trout, *Salvelinus namaycush*. Sperm traits were activated in 10% ovarian fluid. For all sperm traits there were non-significant male × female interactions, therefore the male (A, C, and E) and female (B, D, and F) main effects were interpreted. Error bars represent standard errors. Bars without a common superscript differed significantly.

trout. For instance, it has been shown that the ionic composition of OF enhances sperm performance in Chinook salmon, *Oncorhynchus tshawytscha* [8], while sperm-activating proteins enhance sperm performance in Pacific herring, *Clupea pallasii* [9]. Future studies should also investigate how OF impacts fertility as it has been suggested that sperm released closer to an egg will be exposed to higher concentrations of OF and possibly gain a fertilization advantage [5].

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