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Channel catfish ovarian fluid differentially enhances blue catfish sperm performance

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ABSTRACT

For externally fertilizing fishes, interactions between male and female gametes have been shown to have remarkable impacts on sperm performance. Ovarian fluid (OF) and its ability to alter the swimming behavior of fish sperm makes it a determining factor of fertility. With the expansion of channel catfish (*Ictalurus punctatus*) ♀ × blue catfish (*Ictalurus furcatus*) ♂ hybrid aquaculture, it is essential to understand the impacts during fertilization and the magnitude such gametic interactions have on sperm performance and subsequent male fertility potential. This study was conducted to address the following: 1) activate blue catfish sperm with/without channel catfish OF to determine impacts on sperm performance and 2) assess if sperm behave differently when activated in the OF from individual females. Sperm (n = 4 males) were activated without OF (control) and with diluted OF from unique females (n = 6), creating 24 experimental crosses. Sperm motility (%), velocity (VCL), and longevity were analyzed using computer assisted sperm analyses software. With OF incorporated in the activation media, sperm velocity was significantly higher than the control at 10, 20, and 30 s post-activation. OF did not have an impact on motility for any females at 10 s and 20 s post-activation but became significantly higher than the control at 30 s. In all cases, OF treatments greatly increased longevity. Male × female interactions were highly significant, such that motility, velocity, and longevity were dependent on specific male-female pairs. This information shows that OF should be incorporated in aquatic media to simulate natural spawning conditions and accurately assess the fluid mechanics of sperm propulsion for each male. Additionally, there are mechanisms that drive gamete interactions that need to be explored further, which may improve selection of male-female pairs for *in-vitro* fertilization. On a broad scale, our results also help to shed light on the complexities of fertilization and fish reproduction overall, which may have implications for recruitment variability and recovery strategies of threatened and/or endangered freshwater species.

1. Introduction

Fish sperm are dormant in the male reproductive tract, and activation of sperm occurs after release from the genital pore into an aquatic environment (reviewed by Alavi and Cosson [1]). The duration of sperm motility varies widely among fishes, though generally it is briefer in freshwater than marine species (~1–2 min) [2,3]. During this critical window, sperm swimming velocity and motility are imperative for successful fertilization because each sperm cell has limited time to locate an egg and subsequently penetrate the micropyle to achieve fertilization [4]. For externally fertilizing fishes, sperm motility strongly depends on the physical and biochemical properties of the activation environment, including but not limited to temperature, pH [5], osmolality and ionic composition [1], and viscosity [6]. Notably, the presence of ovarian fluid (OF) may considerably alter these parameters, affecting sperm swimming behavior and fertility outcomes [7–10].

OF accumulates before ovulation and is stored inside the

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coelomic cavity with the eggs [11]. When the egg batch is released during spawning, this maternal fluid adheres closely to the outer membrane of the egg surface. In addition to OF having important biological functions, prior studies have shown that it may alter sperm behavior and swimming trajectories both positively and negatively. In freshwater fishes, many studies have reported enhanced sperm performance with OF present in the activation media. Many of these cases demonstrated extended longevity, faster swimming velocities, and higher percent motility [6,12–14]. From these results, sperm may be affected because of the ability of OF to act as a chemoattractant that stimulates biochemical signaling and inter-gametic communication. Evidence of chemotaxis has been found for a diverse array of freshwater and marine species, but how this occurs is still a topic of investigation [15]. It has been hypothesized that factors such as specific herring sperm-activating proteins (HSAPs) and sperm motility initiating factors (SMIFs), enzymes, metabolites, and ions are involved in these processes, depending on the OF concentration and sperm proximity to the egg surface [16–18].

Not only does the presence/absence of OF impact sperm performance and fertility but its properties also vary among species and even by individual females [19]. This may be due to intrinsic differences in female egg quality, egg ripeness, or differences in OF composition. It remains contested exactly how OF enhances sperm motility and behavior, but the physical and biochemical properties are candidate factors, as they alter the activation micro-environment [20,21]. Ovarian fluid is unique in its viscosity, pH, osmolality, organic constituents (i.e. proteins, metabolites, enzymes), and ionic composition [22]. When all these different factors are incorporated in the activation media, OF becomes a platform for chemical signaling and male × female interactions. For example, in chinook salmon, *Oncorhynchus tshawytscha,* the ions Ca^{2+} and Mg^{2+} in OF strongly regulate sperm motility [18]. Additionally, Na^{+}, Cl^{-}, and K^{+} have also been identified as important ions affecting motility and that OFs with different concentrations of these ions altered sperm performance [23–25]. Some of these chemical constituents improved energy production and duration in sperm by influencing adenine triphosphate (ATP) metabolism that fuels motility [6,26].

Differing OF composition between females (which may be related to increased ATP efficiency and other drivers of sperm motility) creates a unique fertilization micro-environment and allows intraspecific sperm selection at the gametic level [27]. This selection may even lead to a phenomenon termed “cryptic female choice” [28], which dictates that females select for specific genotypes or phenotypes that increase genetic quality of their progeny and give sperm from certain males a competitive advantage [29]. Hence, the ability to control reproductive success at microscopic scales may be one of the most powerful selection pressures driving reproduction and subsequent evolutionary processes [18]. Accordingly, previous studies have explored male × female gamete interactions and have found remarkable variability in sperm swimming behavior of specific males when sperm was activated in OF from different females [12,30]. Evolutionarily, these changes in gamete behaviors may have developed to prevent the mating of closely related individuals in some fishes [31]. Alternatively, sperm containing nuclear DNA of more related genotypes (both intraspecific and interspecific) are sometimes favored as well [8]. These interactions, which are important in swaying fertility outcomes, are yet to be explored for certain cultured fishes.

Hybrid catfish, the cross between channel catfish, *Ictalurus punctatus*, females and blue catfish, *Ictalurus furcatus*, males now account for a large percentage of total US aquaculture production because they outcompete both of the parent species in pond aquaculture [32]. Reproductive isolating mechanisms (e.g. behavioral incompatibility, preferences in spawning environments, timing of spawning) between the two species prevent natural spawning, and thus, *in-vitro* fertilization is applied in which sperm and eggs are stripped and mixed together manually [36]. With hybrid catfish aquaculture steadily growing, research is focusing on ways to maximize fertility outcomes during artificial fertilization. One way of accomplishing this is studying gamete interactions that occur during the short window of contact in order to understand the overarching mechanisms of sperm performance and fertility, which may improve artificial fertilization techniques. As mentioned previously, the activation period for many freshwater species (including blue catfish) is very short when analyzed by typical sperm assessment methods (described by Fauvel et al. [33]). However, previous quantifications of sperm activity for this species have not simulated natural conditions because they lacked OF in the activation media. The ability for OF to interact with sperm and possibly enhance its performance indicates that the fertilization capacity for each male may be much greater than what has been previously observed because OF may shorten or lengthen the sperm activity window. The impacts of gamete interactions found in other species give reason to believe that specific male-female pairs may also lead to differential sperm behavioral interactions in relation to sperm motility, velocity, and longevity metrics [12,34].

The objectives of this study are to determine 1) if sperm motility, swimming velocity, and longevity of blue catfish are impacted by the presence of channel catfish OF and 2) if sperm from specific males behave differently when activated in the OF from individual females to identify potential gamete interactions. By addressing these specific questions, our aim is to highlight the importance of simulating natural spawning conditions when making deductions about male fertility potential. With little known on exactly how OF alters sperm performance and with no previous research done for ictalurid catfishes, it is important to determine if male × female interactions impact artificial fertilization. Our conclusions can be applied to hybrid catfish aquaculture while simultaneously progressing current knowledge of how these processes occur from both aquacultural and ecological perspectives.

2. Materials and methods

2.1. Ovarian fluid collection

All fish used for this experiment originated from agricultural research ponds at the EW Shell Fisheries Center at Auburn University, AL, USA. Female channel catfish (n = 6; mean ± SEM weight = 0.87 ± 0.06 kg) were transferred to 260 × 72 × 50 cm flow-through holding tanks and held in soft mesh bags. Temperatures were 28–30 °C, pH was between 6.8 and 7.2, and water flow was 1.5–2 L/s. Prior to collection, fish were fed once daily with 35% protein pelleted catfish feed until satiation. Females were induced to spawn with 2 intraperitoneal injections of luteinizing hormone-releasing hormone analogue (priming dose of 20 μg/kg female body weight and a resolving dose of 80 μg/kg), LHRHa (Syndel International Inc., Ferndale, WA, USA) following protocols by Dunham & Masser [36]. Starting at 36 h post-injection, fish were checked routinely every 4–6 h for ovulation. Fish were removed from the bags when eggs were seen adhered to the mesh. At that time, they were sedated in tanks with 200 ppm MS-222 (tricaine methanesulphonate; Argent Laboratories Inc., Redmond, WA, USA) buffered with 400 ppm sodium bicarbonate to minimize stress during handling. Care was taken to dry the vent to avoid contamination from urine or feces. The eggs were stripped from the body cavity and filtered through a 1 mm mesh screen over a 250 mL beaker to separate the OF from the eggs. The volume of the collected OF (~0.5 mL per female) was then pipetted from the

bottom of the beaker and transferred to 1.5 mL microcentrifuge tubes. To remove egg debris, blood, and other residue, OF was centrifuged (Mikro 200, VWR, Radnor, PA) at 4 °C at 5000 rpm for 10 min [7], and only the supernatant was used for experiments. The OF pH was obtained directly by a B10P Benchtop pH meter (VWR, Radnor, PA, USA). Osmolality of the fluid was obtained from each individual using a Vapro 5600 osmometer (Wescor Inc, Logan, UT, USA), and the mean was taken of two replicates. A summary of the OF properties for each female are provided in Table 1. OF pH ranged from 7.3 to 8.5 and osmolality from 171 to 243 mOsm/kg among the six females in this study.

2.2. Sperm collection

Blue catfish males (n = 5; mean weight ± SEM = 3.53 ± 0.18 kg) were euthanized following industrial protocols, and testes were dissected from the body cavity using forceps and surgical scissors. The whole testes were rinsed with 1X PBS, drained, and then finely macerated through a mesh screen. For microscopic examination, it is recommended to use a predilution step in a medium that does not initiate motility in order to reduce sperm solution viscosity and better enable it to mix with the activation media [20,35]. Therefore, collected milt was further diluted to 8–10 mL/g of testes weight in non-activating 1X PBS to a density of ~6.5×10^7 sperm/mL, which is standard for use in artificial fertilization of catfish [36]. Sperm solutions were kept at 4 °C, and all trials were conducted within 18 h of processing to ensure there was no degradation of sperm quality/viability due to prolonged storage time.

2.3. CASA sperm motility assessment

Sperm samples from all males were assessed to ensure sufficient quality immediately after collection using computer-assisted sperm analysis (CASA), CEROS II software (Hamilton Thorne Biosciences, Beverly MA, USA), and a A×10 Lab. A1 microscope (Carl Zeiss Meditec Inc., CA, USA) equipped with a 10× magnification negative phase objective. Sperm (<0.1 μL) was activated in an 80 μm 2X-CEL chamber with a 22 × 22 mm glass coverslip (Hamilton Thorne Biosciences, Beverly MA, USA). Each treatment was done in triplicate. Videos were taken at 10 s post-activation and every 10 s afterwards until cessation, defined as the point when motility dropped to <5%. Percent motility and curvilinear velocity (VCL) are commonly used indicators of sperm performance [2,37,38] and were analyzed for each activation. CASA sperm detection parameters were optimized on the CEROS II software based on the recommended default settings and after manual adjustments. Camera settings were as follows: images were taken at with a capture speed of 60 frames per second, exposure was set at 4 ms, camera gain at 300, and the integration time at 500 ms. For cell detection, cells were tracked with sizes between 1 and 8 μm, minimum cell brightness was set at 45, and the photometer range of the illumination fields were between 20 and 30. Each recorded video frame was checked manually for tracking accuracy. Sperm tracks were removed from analyses if the software incorrectly combined crossing tracks of multiple sperm, split the track of a single sperm, or if a cell exited the observation window before being adequately tracked [39].

Table 1
Ovarian fluid properties of the individual channel catfish, *Ictalurus punctatus*, females used in this study (n = 6), including pH and osmolality (mOsm/kg) taken from the fluid during egg collection.

Female ID	Weight (kg)	Ovarian Fluid	
		pH	Osmolality
1	0.9	8.5	241
2	0.94	8.2	227
3	0.86	7.3	221
4	0.98	8.1	171
5	0.63	8.1	229
6	0.96	7.9	243

2.4. Experimental design

Sperm were activated without OF (control) and with OF from 6 unique females. All trials were conducted at room temperature. For the control solution, 15 μL of distilled water was used (pH = 8, osmolality = 6 ± 1.3). For the treatments, OF was diluted with an equal volume of the control solution to get a concentration of 50%. OF percentages as little as 5–10% [14,40–42] and up to 100% [25,34,43] have been commonly used to assess sperm performance across fish species depending on the species and the amount of fluid expelled with each egg batch. The amount of OF encountered by sperm varies in the activation environment, whether it be natural or artificial fertilization (reviewed by Zadmajid et al. [22]). Due to this study being the first of its kind on blue catfish sperm and channel catfish OF, we conducted preliminary testing with 25% OF and 50% OF. We observed no discernible differences between the two concentrations and therefore used 50% OF to match methodology from previous studies [6,10,18]. A full-factorial design was implemented by crossing 4 males and 6 females, resulting in 24 unique sperm-OF combinations. For each trial, sperm from each male was activated in 15 μL of the diluted OF solution from each female, and videos were taken starting at every 10, 20, and 30 s. Because longevity outlasted the control in the OF treatments, videos were further obtained at 40 and 60 s and then at every 15 s afterwards until cessation (when motility dropped <5%).

2.5. Statistical analyses

All data were analyzed using SAS statistical analysis software (v.9.1; SAS Institute Inc., Cary, NC, USA). Residuals were evaluated for normality (Shapiro–Wilk test) and homoscedasticity (plot of residuals vs. predicted values) to ensure they met model assumptions. VCL data were $\log_{(10)}$ transformed and motility data were arcsine square root transformed to meet these assumptions. Alpha was set at 0.05 for testing main effects and interactions. Variance components (% of overall variation due to random effects) were constructed using the restricted maximum likelihood (REML) method, and least squared means (LSMs) and standard errors were reported.

We utilized two different statistical approaches to address our objectives. First, we wanted to assess if OF has on impact on sperm performance. Here, we ran a series of one-way ANOVA models at each post-activation time (10, 20, and 30 s) to compare sperm motility and velocity (n = 4 replicate males) when sperm was activated with (from n = 6 females) and without (control) OF. A posteriori analyses were performed using Dunnett's multiple comparisons method where sperm activated with each female's OF was compared to the control. Second, motility and velocity were analyzed using a full-factorial ANOVA with post-activation time as a fixed factor and male, female, and all associated interactions as random effects. With this mixed model, denominator degrees of freedom for all F-tests were approximated using the Kenward-Roger procedure. Variance components (VCs) were constructed as percentages to represent the overall variability in the data due to each random effect. To test for significant variability among VCs greater than zero in the PROC MIXED model, likelihood ratio statistics were generated (Littell et al., 1996) from the −2[Res]tricted

log-likelihood estimate of the full model and then with each VC held to 0 using the PARMS statement. The probabilities were halved to account for the one-tailed probability, and the significance level (p-value) for each random VC was obtained [44–46].

3. Results

OF from each of the six females did not have an impact on percent motility at 10 s ($F_{6,21} = 1.00$, $P = 0.450$; Fig. 1A) and 20 s post-activation ($F_{6,21} = 0.95$, $P = 0.483$; Fig. 1B). However, a significant effect became apparent at 30 s as motility for the control treatment neared cessation ($F_{6,21} = 0.12$, $P < 0.0001$). Unlike the control trials, motility for the OF treatments did not significantly decrease at 30 s (Fig. 2), and mean sperm motility was 2–3 times higher at 30 s for all females (Fig. 1C). Results from the Tukey's post-hoc tests revealed notable improvements in motility for three of the six females when compared to the control. For velocity, OF had notable impacts on sperm performance for each female and time when compared to the control (Fig. 1D–F). Specifically, at 10 s post-activation, sperm velocity between the control and OF treatments was improved by as much as 61% ($F_{6,21} = 3.31$, $P = 0.0187$; Fig. 1D), while at 20 s and 30 s improvements in velocity were as high as 53% when compared to the control for 20 s ($F_{6,21} = 10.07$, $P < 0.0001$) and 30 s, ($F_{6,21} = 9.38$, $P < 0.0001$; Fig. 1E–F).

To explore potential evidence for gamete interactions between specific male-female pairs, we ran a series of mixed model ANOVAs. Here, as expected, both sperm motility ($F_{14,322} = 80.72$, $P < 0.0001$) and velocity ($F_{14,42} = 31.92$, $P < 0.0001$) were significantly impacted by time post-activation. Control treatments, in general, showed the highest motility at 10 s post-activation but then decreased at each time point until cessation, which was never longer than 30 s (Fig. 3). In all cases, OF treatments always induced increased longevity beyond that of the control. Sperm activated in OF had a much longer activity window, and decreases in motility were less defined, often remaining constant for multiple time points. Additionally, motility significantly varied among each male ($P = 0.011$; VC = 21%) and there was a highly significant male × female interaction ($P < 0.0001$; VC = 26%), showing that channel catfish OF differentially enhances blue catfish sperm performance (Fig. 3, Table 2). For example, sperm from Male 1 lasted 210 s with OF from

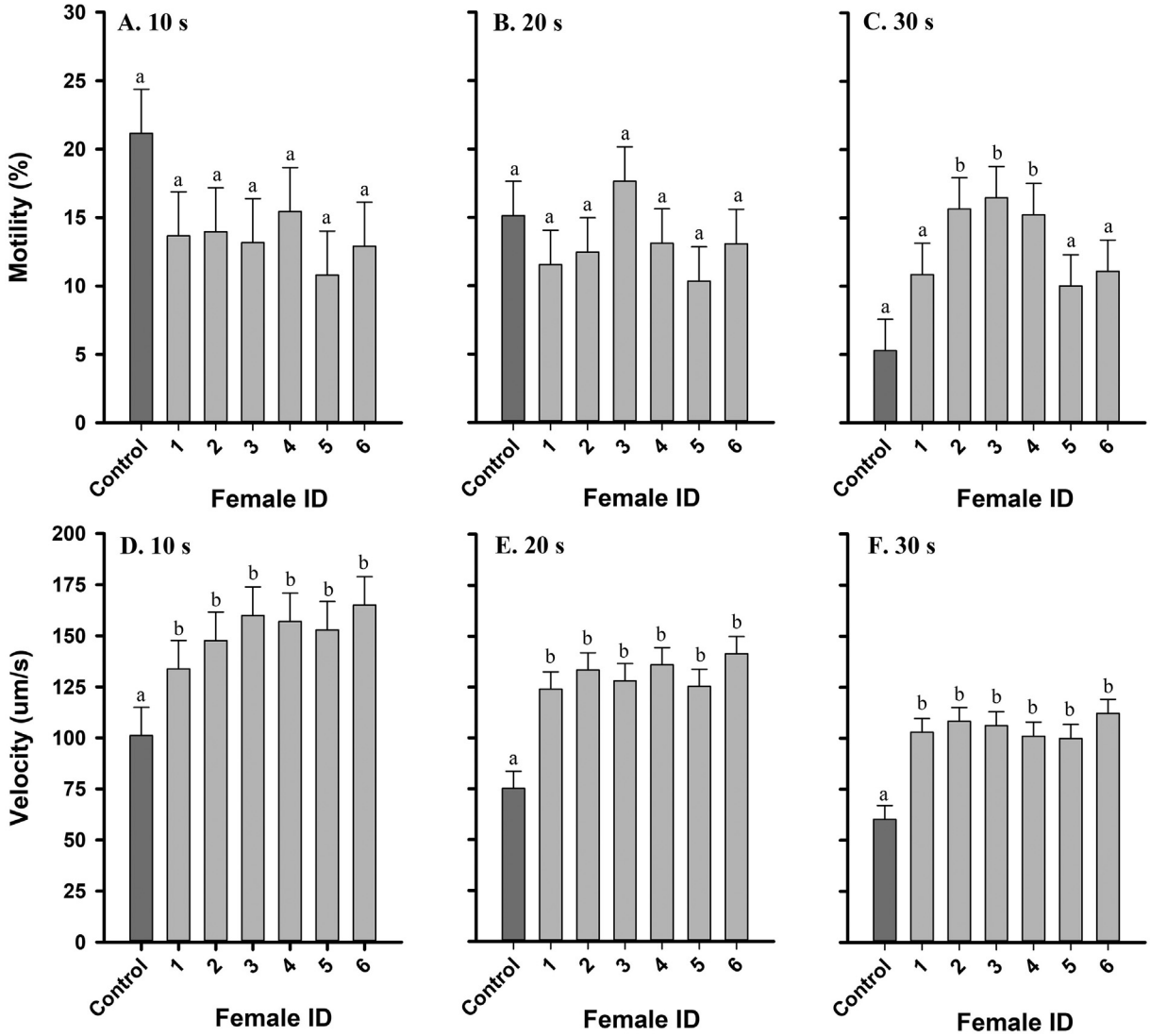

Fig. 1. Percent motility and velocity (VCL) of sperm from blue catfish, *Ictalurus furcatus*, males (n = 4) activated without ovarian fluid (control) and in ovarian fluid from channel catfish, *I. punctatus*, females (n = 6) at 10, 20, and 30 s post-activation. Each bar represents the least square means +SEM. Different letters represent a significant difference from the control as determined from the Dunnett's test at alpha = 0.05.

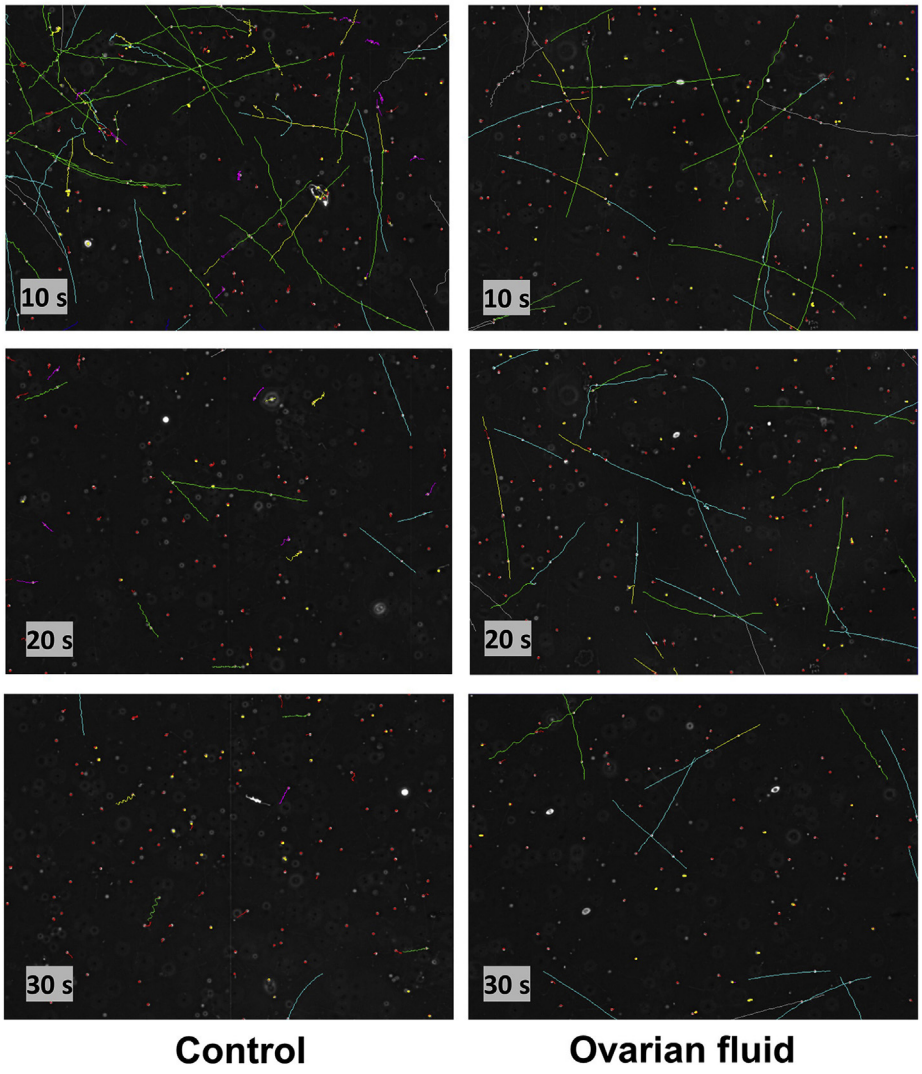

Fig. 2. Still frames obtained from CASA sperm analysis software of blue catfish, *Ictalurus furcatus,* sperm taken at 10 s, 20 s, and 30 s post-activation in control solutions without ovarian fluid and with ovarian fluid from channel catfish, *I. punctatus*, females. Individual sperm cells were tracked for 1 s at a rate of 60 frames/s at each time. Cells were classified by the computer tracking software based on movement speeds during each capture frame. Rankings are listed from the most to least active as follows: blue = progressive movement, green = standard motile movement, yellow/purple = slow movement, and red = static/immotile. White tracks represent cells that were removed due to errors in tracking accuracy. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

Females 4 and 6 but only lasted 105 s–120 s when activated in OF from Females 3 and 5, respectively. For sperm velocity, the interaction term was also highly significant and accounted for 23.1% of the variability (Table 2), and no other random effects were significant (VC = ≤ 4%). Similar to motility, control treatments had the highest sperm velocity at 10 s that gradually decreased at 20 s and 30 s post-activation, but this effect was mitigated with OF from all females (Fig. 4). There were also large differences among females within each male, such that in one example, sperm from Male 4 swam for 75 s in OF from Females 3 and 5 but up to 135 and 150 s for Females 1 and 2, respectively (Fig. 4B). Similar cases of different sperm longevity between females can be observed for the other males in this study.

4. Discussion

With hybrid catfish aquaculture steadily growing, it has become essential to understand gamete interactions and the mechanisms of fertility that may help to improve artificial fertilization. This study was the first of its kind to analyze the impacts of channel catfish OF on blue catfish sperm performance, simulating natural spawning conditions by incorporating OF in the sperm activation media. When sperm were activated without OF, motility duration was remarkably short (~30 s), which is characteristic of freshwater species [20,35]. Interestingly, inclusion of 50% OF in the activation media enhanced sperm performance considerably beyond 30 s, highlighting a phenomenon that has been observed in several other hatchery-reared fishes [7,18,47]. Previously, reports of sperm motility from the extracted testes of blue catfish encompass a wide range with values between 26 and 69% [48,49]. In this study, we observed motility on the lower end of this spectrum (<30%). Highly variable motility percentages may be due to the necessary lethal methods of extraction and processing as well as the status of gonadal development. It is not surprising, then, that motility is lower compared to fish in which manual, non-lethal stripping of mature sperm cells can be employed. Thus, for species with low initial sperm quality (such as blue catfish), incorporating OF may be even more important since it enhances critical sperm performance

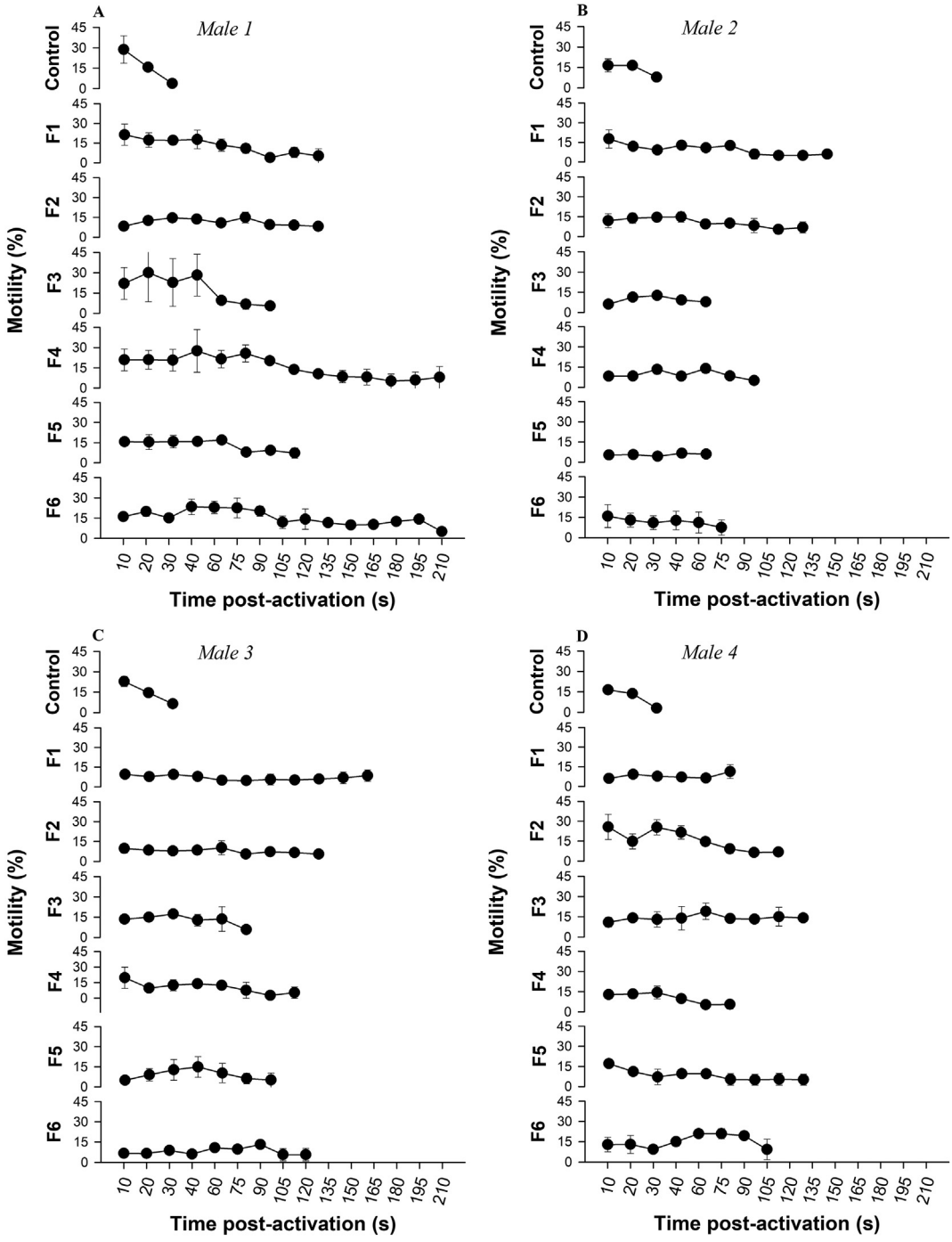

Fig. 3. Duration of sperm motility for each blue catfish, *Ictalurus furcatus,* male activated without ovarian fluid (control) and in ovarian fluid from channel catfish, *I. punctatus*, females (n = 6). There were 24 crosses in total, and male × female interactions were responsible for differences in longevity observed between pairs. Each point represents the motility (%) ± SEM at each time until cessation, defined as the point in which sperm motility dropped to < 5%.

traits and increases the fertilization capacity of each male.

Female OF played a remarkable role in enhancing sperm motility and velocity, both of which are positively correlated with fertility [37]. Sperm competition is introduced when sperm is pooled from multiple males for artificial fertilization, as is common practice in catfish hatcheries. Among males, it would be expected that those with higher sperm motility and velocity would have the capacity to fertilize more eggs, but differential responses to OF may cause deviations from expected fertilization outcomes [6]. In this study, it was hypothesized that motility, velocity, and longevity would be higher in OF than in water alone. Some studies have shown improvements in sperm performance with OF immediately after sperm activation [8,50]. Alternatively, there are also accounts of delayed responses where sperm traits were not immediately improved until later during activation and sperm exhibited greater longevity [7,51]. In this case, differences in sperm motility were observed as time progressed and as the control neared cessation. Although motility did not show immediate positive effects (improvements observed at 30 s), the differences between the control and OF treatments were immediate for velocity. Freshwater sperm,

Table 2
Restricted Maximum Likelihood (REML) variance component (VC) percentages from the mixed model factorial ANOVAs for sperm motility (%) and velocity (VCL) of blue catfish. *Ictalurus furcatus,* (n = 4) crossed with ovarian fluid from channel catfish, *I. punctatus*, females (n = 6). Variance components of the random effects were classified into female, male, interactions terms, and residual error. Significant variance components are denoted by * at alpha = 0.05.

Variance component	Motility	Velocity
	%VC	%VC
Female	0	0
Male	21.02*	4.00
Female × Male	25.74**	23.07**
Time × Female	0	0
Time × Male	0	3.04
Time × Female x Male	0	0.14
Residual Error	53.24	69.75
* P < 0.05; ** P < 0.0001		

powered by ATP stored in the cells prior to activation, use up their energy reserves rapidly after activation, causing motility and velocity to decline as sperm lose propulsive power [52,53]. For the control, this happens within 30 s, at which there are few sperm cells left exhibiting movement. However, when OF was present, many more cells surpassed this time frame and were still actively swimming at higher speeds, perhaps due to factors of the OF that create a more favorable environment for the sperm [54]. Future research on identifying these sperm-enhancing factors presents the next step to understanding the complex underlying mechanisms of fertility.

In this study, it was evident that male × female interactions altered sperm motility and velocity and that longevity within each male was highly variable depending on the specific female OF. In some cases, motility duration from one female was almost double than that of another (120 s as compared to 210 s). These results offer the first clear evidence of gamete interactions between channel catfish and blue catfish because sperm responded more favorably to the cues of specific OFs better than others. Thus, it is imperative to look beyond the scope of individual maternal and paternal effects and consider male-female pairs together. Looking beyond the species used in this study, gamete interactions also shape reproduction in natural spawning environments. For fish that spawn in large aggregations in the wild, gametes endure high levels of competition. Only sperm that reach the micropyle first can achieve fertilization, making the ability for eggs to attract different sperm types a remarkably powerful selective force [56,57]. Gamete interactions between individual males and females and their effects on sperm performance have been observed in laboratory experiments for several other externally fertilizing species including Arctic charr, *Salvelinus alpinus* [12], chinook salmon [18], lake trout, *Salvelinus namaycush* [8], ocellated wrasse, *Symphodus ocellatus* [29], and zebrafish, *Danio rerio* [55], among others. Results are primarily derived from fish species with similar reproduction strategies in the wild, in which males and females gather and release massive amounts of gametes freely into the water column. However, this phenomenon has also been identified in species that undertake internal fertilization such as the guppy, *Poecilia reticulata* [58,59]. The common theme to the development of gamete interactions appears to be some degree of male competition, whether it be fish that are artificially spawned in hatcheries or those that spawn in natural environments. It may be difficult to determine to what degree sperm are influenced by OF in the natural environment, but OF undeniably performs critical functions in reproduction processes. Results from sperm activation studies, including ours, point to the fact that incorporating OF typically enhances sperm performance and that gamete interactions are prevalent across diverse fish groups. However they occur, the ways in which gamete interactions alter fertility and offspring production in the hatchery setting should be assessed for any fish of interest.

There are still questions surrounding why sperm from certain males performs better in specific OFs, but biochemical and physical properties of the OF may be responsible [18]. Variation in physical and biochemical properties of the OF between females alters the activation micro-environment and sperm behavior [20]. Compositions of ions, proteins, and other components have been previously quantified in fishes, but such detailed analyses have not been assessed for channel catfish. Knowledge of these factors may establish links to specific OF characteristics and improvements in sperm performance that can be tested prior to spawning. Future research can then study OF constituents as indicators of egg quality in order to improve selection of females for reproduction. However, it must not be overlooked that characteristics of the sperm may also cause differential responses to OF during activation and should also be assessed. From the genetic standpoint, it has been hypothesized that sexual selection in fishes occurs through major histocompatibility complex (MHC) genes [60]. Supporting this claim, Yeates et al. [61] found that Atlantic salmon, *Salmo salar*, fertility promoted males with similar MHC genes over males that were more genetically distinct. In another example, fertilization success was positively correlated with specific MHC class II genes in chinook salmon [62]. Analyzing genetic relatedness and/or phenotypes of individual fish may determine if underlying genetic complexes are also responsible for why sperm from certain males were favored over others. In future works, it would also be worth investigating if sperm from channel catfish would outcompete blue catfish sperm under the same experimental conditions. These results would broaden our understanding of the evolutionary selective forces governing gamete interactions and how they occur between different species.

Some studies have also documented differential sperm performance between low and high OF concentrations [63,64], and in many cases they share a positive relationship. As mentioned previously, ovarian fluid percentages as little as 5–10% and as high as 100% have been commonly used in sperm motility studies. Some species including Atlantic cod, *Gadus morhua*, and rainbow trout, *Oncorhynchus mykiss,* produce large amounts of OF relative to the total egg volume (10–30%) [41,65] while others produce considerably less. In accordance with the latter, we observed that channel catfish produced small volumes of OF relative to the egg mass, although this may also be affected by small female size and that fish were spawned later in the season. Given that these were laboratory trials, we were cognizant of the effects different OF concentrations could have on sperm motility metrics, leading us to conduct exploratory trials prior to experimentation with 25% OF and 50% based on OF concentrations used in other sperm activation studies. We observed that sperm activity was higher than the control at both concentrations. The OF concentration in the activation environment changes with distance, increasing as sperm approach the egg surface and the micropyle [66,67]. The concentration would be higher around the micropyle than in the surrounding solution, making it likely that sperm encounter a flexible range of concentrations during fertilization events. Therefore, with more experimentation, the optimal OF concentration can be determined from the natural spawning environment and for sperm motility assessments, which may be more or less than the concentrations tested in this study. Activation solutions for the sperm containing water and a specific percentage of OF can be then be created artificially for more accurate assessments of sperm performance traits from blue catfish males before fertilization takes place. Utilizing this

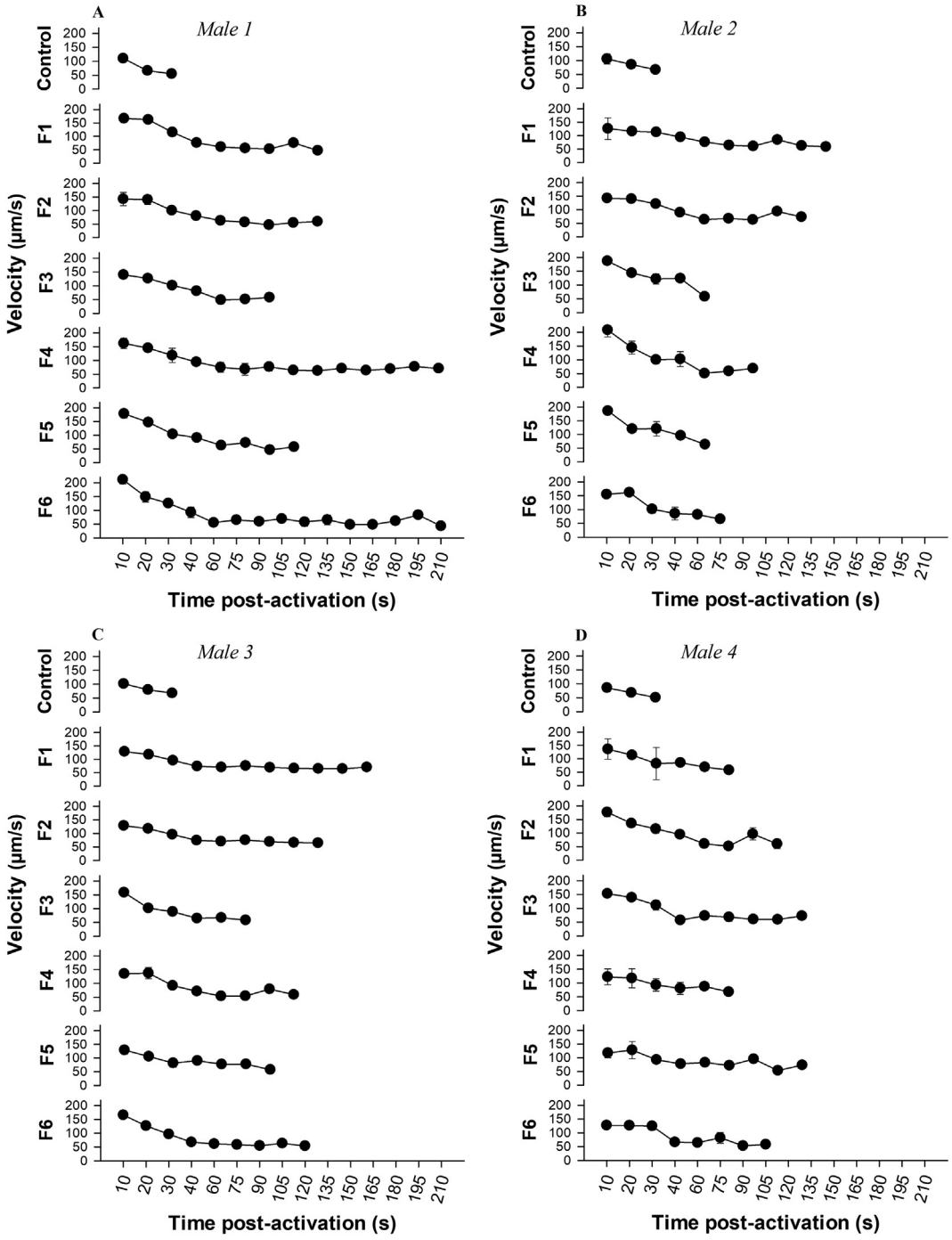

Fig. 4. Sperm velocity and longevity for each blue catfish, *Ictalurus furcatus,* male activated without ovarian fluid (control) and in ovarian fluid from channel catfish, *I. punctatus*, females (n = 6), with 24 crosses total. Male × female interactions were largely responsible for the differences in longevity between specific pairs. Each point represents the velocity (VCL) ± SEM at each time until cessation, defined as the point in which sperm motility dropped to < 5%.

practice for hybrid catfish spawning will further standardize hatchery operations.

5. Conclusions

Overall, results from our sperm activation trials show not only that OF improves sperm motility, velocity, and longevity, but also that OF from individual channel catfish females differentially enhances behavior of blue catfish sperm. From this study, we have contributed valuable information that confirms the importance of female OF for sperm quality assessment in the lab, although there are still questions to be answered regarding the underlying mechanisms behind sperm × OF interactions. On a broader scale perspective, this knowledge can be applied to improve aquaculture for hybrid catfish as well as other species whose production relies on artificial fertilization. By implementing more accurate, standardized methods of sperm quality assessment of individual males, we gain greater understanding of mechanisms that potentially impact fertilization. Finally, these results have added to the growing knowledge base of the overarching mechanisms of reproduction in

freshwater fishes and how gamete interactions have transcended the diverse array of teleosts present today.

CRediT authorship contribution statement

J.N. Myers: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. **A.J. Bradford:** Investigation. **V.S. Hallas:** Investigation, Writing - review & editing. **L.L. Lawson:** Investigation, Writing - review & editing. **T.E. Pitcher:** Writing - review & editing. **R.A. Dunham:** Resources, Writing - review & editing. **I.A.E. Butts:** Validation, Supervision, Funding acquisition, Project administration.

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