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The effects of rival seminal plasma on sperm velocity in the alternative reproductive tactics of Chinook salmon

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ABSTRACT

Sperm competition is prevalent and intense in many animal mating systems, and is a major force driving evolution of such mating systems. The objective of this study was to determine the effect of seminal plasma on sperm velocity of male Chinook salmon (*Onchorhynchus tshawytscha*), which possesses a mating system with male alternative reproductive tactics and intense sperm competition. Male Chinook salmon either adopt a small, precocious sneaking tactic (jack) or a large, dominant tactic (hooknose). To test whether the seminal plasma can effect sperm velocity amongst sperm competitors, two experiments were done whereby males were paired based upon the alternative tactic each male adopted, with the first experiment consisting of jack-hooknose pairs (N = 16) and the second experiment consisting of jack-jack and hooknose-hooknose pairs (N = 12 and 14, respectively). Within each pair, milt of each male was manipulated such that seminal plasma was removed and swapped between the males in each pair and sperm velocity was measured. Jack seminal plasma caused a significant decrease (~11.9%) in hooknose sperm velocity while causing a significant increase in jack sperm velocity (~7%), while alternatively, hooknose seminal plasma had no affect on sperm velocity of jack or other hooknose males. This study shows that rival seminal plasma may affect the outcome of sperm competition between males; males adopting a sneaking tactic, that spawn in a disadvantageous mating position, may be able to compensate for this deficit by being more competitive through the effects of their seminal plasma on their competitor's sperm velocity.

1. Introduction

Sperm competition occurs when sperm from multiple males compete to fertilize a female's eggs [1]. This form of post-copulatory competition is a taxonomically widespread phenomenon and a powerful evolutionary force that has shaped the evolution of male mating behaviour, morphology and physiology [2–4]. Sperm competition is especially prevalent in species in which male alternative reproductive tactics are present due to males from each tactic having unequal opportunities to fertilize eggs (e.g. Refs. [5–7]). In such species, the males often have different traits, that can take the form of morphological, behavioural, and life history differences, selected to maximize reproductive success [8].

The most prevalent alternative reproductive tactics across taxa is the existence of the sneak-guard dichotomy in males (see Ref. [9] for a taxonomic review). Sneaker males usually have small body size and use covert techniques to sneak into mating events between guard males and females to obtain reproductive opportunities. Whereas guard males are typically large in body size and have more pronounced secondary sexual characteristics to aid in asserting behavioural dominance over other males and females, including fighting off other males while protecting and monopolizing females. Parker [10] developed mathematical models for sneak-guard mating systems to help explain their evolution in the context of sperm competition. Those models assume that there is a difference in sperm competition risk and perception of such risk between the two alternative tactics. Sneaker males are presumed to have high sperm competition risk and accurate 'knowledge' of this risk because every time they mate there will be at least one other male (i.e. guard male(s)) present. Whereas the guard males are presumed to have lower sperm competition risk because sneakers do not participate in all mating events and their "knowledge" of risk is less reliable because they are often unaware of the presence of sneaker males. These models have been supported in a number of

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empirical studies. For example, in Atlantic salmon (*Salmo salar*), precocious parr (sneaker male) had larger testes, ejaculate volume, and number of sperm cells (all relative to body size) in addition to having more motile, longer living sperm [11,12], which were shown to provide greater fertilization success per spawning event than the anadromous (guard) males [12].

Most of the studies to date that examine sperm competition dynamics have focused on either differences in sperm number or sperm quality [2]. However, sperm only make up a portion of the ejaculate and other components, such as seminal plasma (or fluid) can have effects on the outcome of sperm competition. For example, in the stalk-eyed fly (*Cyrtodiopsis whitei*), Fry & Wilkinson [13] found that males had a dramatic decrease in fertilization success in the presence of the seminal plasma from other males. It has also been shown that male *Drosophila melanogaster* can alter the amount of seminal plasma in an ejaculate depending on the level of sperm competition risk [14]. It is important to note that most of this evidence stems from studies done on insects, but there is little known about whether seminal plasma can have similar effects in other taxa.

In fishes, there are only two studies that examine the effects on seminal plasma on the outcome of sperm competition [15,16], furthermore, only one of these studies examine the effects in a mating system that exhibits alternative reproductive tactics [15]. Within male Arctic charr (*Salvelinus alpinus*), it was found that the percentage of motile sperm was significantly higher in the presence of another male's seminal plasma, than in the male's own seminal plasma, however there was no such effect on sperm velocity [16]. This result may have little biological relevance however, because sperm velocity, and not percent motility, is the best predictor of fertilization success in Arctic charr [17] and other salmonid species [18–20]. Locatello et al. [15] showed that in the grass goby (*Zosterisessor ophiocephalus*), a species with a sneak-guard alternative reproductive tactic mating system, there was a tactic-specific effect of seminal plasma on a rival male's sperm performance. In the seminal plasma of the guard males, sneaker males showed an increase in sperm velocity of approximately 9%, which consequently resulted in a 10% increase in their fertilization success. Conversely, the presence of sneaker seminal plasma decreased the sperm velocity of guard males by approximately 7%, which in turn caused a 9% reduction in fertilization success.

Chinook salmon (*Oncorhynchus tshawytscha*) exhibit the sneak-guard alternative reproductive tactic in males, where the large, dominant hooknoses (i.e. guards) have priority in mating positions with females, while the small, precocious jack males (i.e. sneakers) adopt the sneaking tactic [21–23]. This alternative reproductive tactic mating system with external fertilization allows females to mate with multiple males simultaneously and thus promotes intense sperm competition between males. It has been shown that in ~40% of spawning events, only one hooknose is present, while in the other ~60% there is anywhere from 2 to 5 males present, including both jacks and hooknoses [24]. Previous work has shown that jacks have relatively larger testes and their sperm swims faster in river water compared to hooknose sperm [25], which supports the theoretical work done by Parker [10], suggesting that the sneaker (jack) should invest more into spermatogenesis instead of other traits. However, energetic investment into testes (as the model predicts) does not necessarily mean investment into just sperm cells; it could also be an investment into other components of the ejaculate, such as the seminal plasma.

The objective of this study is to examine whether sperm competition is influenced by seminal plasma by examining sperm velocity, an important metric for competitive fertilization success (e.g. Refs. [18,19]). Based on sperm competition theory [10], it can be hypothesized that, due to the asymmetry in sperm competition risk between tactics, jacks should be selected to be more competitive, which can happen in a number of ways: (1) jack seminal plasma decreases hooknose sperm velocity, (2) hooknose seminal plasma increases jack sperm velocity, and/or (3) jack seminal plasma increases another jack's sperm velocity. We tested these hypotheses using two experiments, the first used pairs of males that adopted different tactics (between-tactic) and the second used pairs of males adopting the same tactic (within-tactic). In both of these experiments, seminal plasma was swapped between males in each pair to examine the effect of seminal plasma on sperm velocity of other males.

2. Materials and methods

2.1. Fish collection

Male Chinook salmon from both alternative reproductive tactics were collected, using standard electroshocking techniques, from the Credit River (Mississauga, Ontario, Canada; 43°35′N, 79°42′W) between September 30 and October 11 in 2013 (experiment one; Hooknose: N = 16, mean ± S.E. mass = 7.7 kg ± 0.5 kg, range = 5.1–11.0 kg; Jack: N = 16, mean ± S.E. mass = 2.2 kg ± 0.2 kg, range = 1.3–3.6 kg) and September 29 and October 9 in 2014 (experiment two; Hooknose: N = 28, mean ± S.E. mass = 8.0 kg ± 0.3 kg, range = 4.6–11.4 kg; Jack: N = 24, mean ± S.E. mass = 2.0 kg ± 0.1 kg, range = 0.4–3.4 kg).

2.2. Milt collection

Milt (sperm and seminal plasma) was collected from all males in 532-mL clear whirl-pak sample bags (Nasco, Newmarket, ON, Canada) by gently applying abdominal pressure on the fish, being careful there was no contamination by water, urine or feces. The milt was then placed in a cooler at the river water temperature (~11 °C) until analysis took place (2 to 3 h later).

2.3. Experimental design

There are three treatment groups for each of these experiments: (1) control, (2) sham control and (3) tactic-swap. The control treatment is milt that has not been centrifuged, while the sham control treatment is milt that has been centrifuged, but the resulting separate sperm cells and seminal plasma were immediately recombined. By comparing these two treatments, the effect of centrifugation on the sperm cells can be determined. The tactic-swap treatment is the main experimental treatment in which seminal plasma is swapped between males in each pair, which for experiment one contained a jack male and a hooknose male, therefore deemed the between-tactic swap experiment, and for experiment two contained males from the same tactic, both jack-jack pairs and hooknose-hooknose pairs, therefore deemed the within-tactic swap experiment. For experiment one, N = 16 jack-hooknose pairs were used, and for experiment two, N = 12 jack-jack pairs and N = 14 hooknose-hooknose pairs were used. For both experiments, males were only used once, so each pair contains a unique set of males.

2.4. Treatment preparation

To separate the milt into its components of sperm cells and seminal plasma, 1000 μL of milt was placed in a 1.7 mL Eppendorf tube and centrifuged (accuSpin Micro 17, Fisher Scientific) at 300 × g for 10 min [26]. The resulting separate seminal plasma and sperm components were carefully pipetted out and placed in separate Eppendorf tubes in a chilling block set at 11 °C