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Reproductive investment patterns, sperm characteristics, and seminal plasma physiology in alternative reproductive tactics of Chinook salmon (*Oncorhynchus tshawytscha*)

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Although alternative reproductive tactics (ARTs) are common across a range of taxa, little is known about whether the different tactics have adapted to sperm competition risk. Chinook salmon, *Oncorhynchus tshawytscha*, have two ARTs: large males that participate in dominance-based hierarchies for access to spawning females, known as hooknoses, and small males that attempt to sneak fertilizations during spawning events from peripheral positions, known as jacks. Jacks continually face sperm competition risk because they always spawn in the presence of another male, whereas hooknoses face relatively low sperm competition risk because other males are not always present during spawning events. Based on the sneak-guard model of sperm competition this asymmetry in sperm competition risk predicts that jacks ought to invest significantly more into sperm-related traits important for sperm competition success relative to hooknoses. In the present study we report on reproductive investment patterns, sperm characteristics, and seminal plasma physiology of males that exhibit ARTs in Chinook salmon. We found that jacks invest significantly more of their somatic tissue into gonads compared with hooknoses. Sperm velocity also varied significantly between the ARTs, with jacks having significantly faster sperm than hooknoses. No significant differences in seminal plasma physiology metrics related to sperm quality were detected between the ARTs. We interpret these sperm investment patterns in light of the sneak-guard model of sperm competition that is based on differences in sperm competition risk and alternative investment possibilities among ARTs.

ADDITIONAL KEYWORDS: adenosine 5'-triphosphate – gonadosomatic index – salmon – sneak-guard hypothesis – sperm competition – sperm morphology – sperm velocity.

INTRODUCTION

Sperm competition occurs when sperm from two or more males simultaneously compete to fertilize a set of ova (Parker, 1970). This form of competition is prevalent in nature and has been observed across a variety of species (reviewed by Birkhead & Møller, 1998; Birkhead, Hosken & Pitnick, 2009). In species where sperm competition occurs, males with poor access to females (e.g. males with a smaller body size) may be selected upon for the development of different reproductive traits in order to maximize their reproductive fitness (reviewed by Taborsky, 1998, 2008). Divergence in these reproductive traits can result in the evolution of alternative reproductive tactics (hereafter ARTs). Ultimately, these tactics can manifest as size, structure, or colour polymorphisms, as well as physiological and behavioural alternatives (e.g. Brockmann, 2001; Butts *et al*., 2012). Of all vertebrates, fishes demonstrate the most widespread

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variability in ARTs for three primary reasons (reviewed by Knapp & Neff, 2008). First, the majority of fishes employ external fertilization, which creates greater opportunities for sperm competition because multiple males have access to a female's eggs. Second, fishes have indeterminate growth, which results in immense variation in body size and consequently selects for divergence in reproductive tactics. Third, fishes exhibit a highly variable distribution of parental care roles creating opportunities for males to take advantage of this variation by adopting alternative tactics (Knapp & Neff, 2008). The ARTs found in fishes generally consist of two different male morphs. There are typically larger males that employ a guarding tactic (which usually involves guarding and active courting of females) and smaller males that practice a more covert tactic of sneaking into spawning events to steal fertilizations from guard males (reviewed by Taborsky, 1997; Knapp & Neff, 2008).

Parker (1990, 1998) proposed the sneak-guard model of sperm competition to predict the different sperm investment patterns observed in situations where sperm competition risk asymmetry exists within a species, which is commonly found in species with ARTs. The model predicts that sneaker males will invest relatively more energy in spermatogenesis than guard males because they always face sperm competition risk owing to the fact that they always spawn in the presence of another male. Guard males, on the other hand, are predicted to invest relatively more in secondary sexual characteristics to secure optimal access to fertile females and relatively less in spermatogenesis than sneak males because they do not always face sperm competition risk, as sneaker males are not necessarily present for all mating attempts. For example, in bluegill sunfish *Lepomis macrochirus*, cuckolders (i.e. sneaker males) were present in only 10.3% of spawning bouts between females and parental males (i.e. guard males; Fu, Neff & Gross, 2001). Studies to date that have tested the sneak-guard model suggest that increased sperm competition risk has selected for greater investment in sperm-related traits in sneaker males compared with guard males. For example, in Atlantic salmon, *Salmo salar*, precocious parr (i.e. sneaker males) invested relatively more in their testes, their sperm density was greater, they lived longer and were more motile than anadromous males (i.e. guard males) (Gage, Stockley & Parker, 1995; Vladić & Järvi, 2001).

Chinook salmon, *Oncorhynchus tshawytscha*, have large guard-type males known as 'hooknoses' (*sensu* Gross, 1985; derived from the exaggerated snout, which develops at maturity) and small sneaker-type males known as 'jacks' (Healey, 1991; Heath *et al*., 1994; Butts *et al*., 2012). Chinook salmon are external fertilizers, semelparous, and exhibit a promiscuous non-resource-based mating system (Healey, 1991; Fleming & Reynolds, 2004). Mating occurs seasonally in streams where females compete for oviposition sites in order to dig nesting areas using an oscillating motion with their tails (Healey, 1991; Berejikian, Tezak & LaRae, 2000). Once the digging is complete females deposit their eggs in a series of nests comprising a single redd (Berejikian *et al*., 2000; Berejikian, Tezak & Schroder, 2001). Females appear to exhibit choice for larger males by delaying their spawning in the presence of relatively small males (Berejikian *et al*., 2000). Males provide only their sperm (i.e. genes); they do not provide parental care to offspring, and they provide no material benefits to females (Berejikian & Tezak, 2005). Hooknoses mature several years after leaving their natal streams (age-3 and age-4, Berejikian *et al*., 2010) and are characterized by a larger body size and hooked snout (Fleming & Reynolds, 2004). Hooknoses have primary access to females, they enter the nesting area first during spawning events, exhibit courtship behaviours, and chase off other males that come near spawning females. Jacks develop precociously and reach sexual maturity after a year of leaving their natal stream (age-2, Berejikian *et al*., 2010). Jacks have been observed to have similar coloration to females during spawning (Berejikian *et al*., 2010; T. E. Pitcher, unpubl. data), they hold positions upstream (or downstream) of the courting pair, they are often chased off and attacked by hooknoses (occasionally resulting in death for the jack, T. E. Pitcher, unpubl. data), and sneak into the nesting area from satellite positions when spawning occurs between a female and a hooknose. In a recent study examining Chinook salmon spawning behaviour in semi-natural spawning channels containing hooknoses, jacks, and females, 40% of the spawning events involved only one hooknose male, while the rest of the spawning events included two to five males (including both hooknoses and jacks) (Berejikian *et al*., 2010). In addition, hooknoses had superior access and position during spawning because they entered the nest first during spawning events (i.e. prior to jacks) and jacks participated almost exclusively in spawning events by sneaking into the nest from satellite positions and sired approximately 20% of all progeny (Berejikian *et al*., 2010).

In the present study we first tested the predictions of the sneak-guard model by measuring reproductive investment patterns (gonad size) and sperm traits (sperm velocity, motility, longevity, morphology, and number) important for sperm competition success in salmonids (Vladić, Afzelius & Bronnikov, 2002; Gage *et al*., 2004; Tuset *et al*., 2008a) for wild-caught spawning jacks and hooknoses. Given that jacks

almost always spawn in the presence of a hooknose, and that they are usually not ideally positioned to release sperm during a spawning event, we predicted that jacks would invest relatively more resources into gonad development and sperm quality metrics than hooknoses. Finally, we measured physiological characteristics of milt (i.e. adenosine 5′-triphosphate, proteins, antioxidant capacity), all of which have been linked to sperm quality in fishes (reviewed in Alavi *et al*., 2008), to further our understanding on potential mechanisms that may be driving the predicted divergence in sperm characteristics between the tactics.

MATERIALS AND METHODS

Fish collection and body size measurements

Chinook salmon were collected during two spawning seasons (2–6 October 2010 and 4–6 October 2011) using backpack electrofishing from a winter run in the Credit River (Mississauga, Ontario, Canada, 43°35′N, 79°42′W), which flows into Lake Ontario (which has been stocked for over 40 years) (see Crawford, 2001; Pitcher & Neff, 2006, 2007; Butts *et al*., 2012). Individuals collected in 2010 were used to examine gonad investment patterns and sperm characteristics between the ARTs, whereas individuals collected in 2011 were used to examine the seminal plasma physiology between the ARTs. It was not possible to collect gonad measures and sperm characteristics from the individuals collected in 2011 due to the time spent preparing seminal plasma samples in the field. In both years we considered males less than 675 mm in fork length to be jacks based on length–age relationships derived from otolith assessment for our population from the same spawning season in 2010. All males less than 675 mm were age 1+ and males above this size threshold were age 2 + (750–850 mm) or age 3 + (900–950 mm) (Ontario Ministry of Natural Resources, unpubl. data). In addition, all fish less than 675 mm in fork length in our population were morphologically characteristic of jacks (e.g. no pronounced secondary sexual characters and coloration similar to that of females).

Chinook salmon were located upstream in turbid water ranging from 2 to 4 feet in depth. Water temperature at the time of collection was ~11 °C. We humanely killed the fish with a sharp blow to the head with a blunt instrument, and obtained milt samples by applying pressure on the abdomen of each fish. The initial male ejaculate was discarded in a standardized manner and the external urogenital pore was wiped dry to avoid contamination from water, urine, faeces, and blood. Milt samples were collected into 532-mL clear Whirl-pak sample bags (Nasco, Newmarket, ON, Canada) and were placed in coolers (~2–3 h) for sperm characteristic analyses (see below) or seminal plasma physiology analyses (see below). Total body mass (±10 g), testes mass (±5 g), and fork length (±1 cm) were recorded for each male.

Reproductive investment patterns and sperm characteristics

Gonadosomatic index

Soma mass (body mass – testes mass) is a more useful measure than body mass in allometry analyses, particularly because the gonads represent a significant proportion of the total body mass (Stoltz, Neff & Olden, 2005). Gonadosomatic index (GSI = testes mass/soma mass) was calculated and used as a metric for male reproductive investment (see Statistical analyses below for details).

Sperm characteristics

A milt sample (1.5 μL) from each individual was micropipetted into a chamber of a 2X-CEL glass slide (Hamilton Thorne, Beverly, MA, USA), covered with a glass coverslip (22 × 22 mm), and activated with 15 μL of 11.0 °C river water (the approximate temperature of the river during spawning). Water temperature was maintained at 11.0 ± 0.5 °C using an HEC-400 Heat Exchanger, a BC-110 Bionomic Controller, and an AS-3001 Stage Cooler (20/20 Technology Inc., Wilmington, NC, USA). Activated sperm were video recorded using a CCD B/W video camera module (XC-ST50, Sony, Japan) at 50 Hz vertical frequency, mounted on a microscope (CX41 Olympus, Melville, NY, USA) that was equipped with a 10× negative-phase objective (see Pitcher *et al*., 2009). Video-recordings were analysed using the HTM-CEROS sperm tracking software package (CEROS version 12, Hamilton Thorne). We used the following recording parameters: number of frames captured in sequence within 1 s = 60 Hz; total number of sequential images captured for analysis = 60; minimum contrast = 11; minimum number of pixels that an object must be in order to be counted = 3. The following parameters were measured for each male's sperm: average path velocity (average velocity on the smoothed cell path), straight-line velocity (average velocity on a straight line between the start and end points of the track), curvilinear velocity (average velocity on the actual point-to-point track followed by the cell), and motility (proportion of motile sperm in the field of view showing propulsive motility) at 5, 10 and 15 s post-activation. These estimates correspond to the mean of all motile cells analysed; that is, for each male, the velocity and motility of each individual sperm cell were measured but the estimate used in our final analyses corresponds to a mean across

all individual sperm cells. Results were qualitatively similar for all three sperm velocity estimates (data not shown) and as such we present only results for straight-line velocity, hereafter referred to as sperm velocity. Sperm longevity was estimated as the time from sperm activation until ~95% of the sperm cells, within the field of view, were no longer exhibiting progressive forward motion (Gage *et al*., 2004). Two observers, with no knowledge of the male's identities, measured longevity with high repeatability between measures for all of the males examined ($r^2 = 0.91$, $P = 0.001$, $N = 64$). Thus, we used the mean longevity of both observers in all analyses.

Sperm density was counted under a Zeiss Axiostar compound microscope (Carl Zeiss Vision Inc., Toronto, ON, Canada) at 400× magnification using an improved Neubauer haemocytometer (see Pitcher *et al*., 2009; Butts *et al*., 2012). A milt sample (1.5 μL) from each male was first diluted in 500 μL of Cortland's saline solution (7.25 g L^{-1} NaCl; 0.38 g L^{-1} KCl; 0.47 $MgSO_4.7H_2O$; 0.4 g L^{-1} $Na_2HPO_4.H_2O$; 1.0 g L^{-1} $NaHCO_3$; 0.22 g L^{-1} $MgCl_2$; 1.0 g L^{-1} $C_6H_{12}O_6$; adjusted to pH 7.8). To obtain homogenous milt-diluent solutions, samples were mixed thoroughly using a pipettor. A sample of the sperm suspension (10 μL) was then micropipetted onto a haemocytometer that had been pre-covered with a coverslip. The number of sperm in each of the five larger squares on the haemocytometer was counted. There are 25 of these large squares on the haemocytometer and each of these large squares has 16 smaller squares within it. Sperm were counted in the four large corner squares and the large centre square. The mean number of sperm per large square count (i.e. mean of the five counts) was multiplied by 25 (to obtain the mean per 5 × 5 large-square grid), by 10 (the depth of the chamber in μm), and then by the initial volume of the sample to estimate the sperm density. Sperm densities are expressed as the total number of sperm per mL of a male's ejaculate.

Sperm morphology was quantified for a subset of jacks and hooknoses ($N = 14$ hooknoses and 16 jacks) collected from the 2010 spawning season. Sperm morphology smears were prepared by diluting 1.5 μL of milt in a solution composed of Cortland's saline (200 μL) and glutaraldehyde (125 μL; G7526; Sigma-Aldrich, St. Louis, MO, USA). For each male, 5 μL of this sperm solution was then pipetted onto a frosted tip microscope slide and prepared using Diff-Quick (Thermo Electron, Pittsburgh, PA, USA) (Tuset *et al*., 2008a). We prepared three separate smears for each male. The smears were allowed to air dry, then permanently sealed with Permount mounting medium (SP15; Fisher Scientific, Fair Lawn, NJ, USA) and topped with a coverslip. Digital images of sperm heads and flagella were captured using an Olympus BX51 microscope equipped with an Olympus DP72 digital camera (Olympus Inc., Melville, NY, USA). One hundred sperm heads were measured haphazardly for each smear with an oil immersion objective (100× magnification). Morphometric analyses of sperm heads (width and length) were performed using ImageJ analysis software (V. 1.41; developed by W. Rasband, National Institutes of Health, Bethesda, MD, USA) and an ImageJ plug-in (Butts *et al*., 2011b). The mean sperm head trait (width or length) value from the three independent smears was used for statistical analyses. In addition, flagellum length ($N = 30$ sperm per male) was manually measured from its insertion in the head to the end of the filament using ImageJ analysis software.

Physiological characteristics of milt

In October 2011, fresh milt from 37 hooknoses and 24 jacks was centrifuged for 15 min at 8000***g*** (accuSpin Micro 17, Fisher Scientific). The supernatant was then pipetted into 2-mL cryovials and flash frozen in liquid nitrogen. Seminal plasma samples were transported to Olsztyn, Poland, via a dry shipper for physiological analyses. We measured total protein concentration (mg mL^{-1}), lactate dehydrogenase (LDH) activity, total antioxidant capacity (expressed as an equivalent of the μmol concentration of Trolox solution), superoxide dismutase activity (one unit is defined as the quantity of SOD required to produce 50% inhibition of this reaction), anti-trypsin activity (expressed as U L^{-1} of trypsin inhibited), pH, and osmolality (mOsmol kg^{-1}) as per the methods outlined by Butts *et al*. (2011a). The mean of two or three measurements per male was used for statistical analysis.

A subset of males was used to measure ATP (adenosine 5′-triphosphate) of sperm cells ($N = 17$ hooknoses and 19 jacks from the October 2011 collection). Five microlitres of milt was micropipetted into 16 × 125-mm Pyrex culture tubes containing 4995 μL of boiling lysing buffer (25 mM HEPES, 10 mM magnesium acetate, 2 mM EDTA, 3 mM sodium azide, pH 7.75; Perchec *et al*., 1995) and left for 2–3 min at 98–100 °C. The sperm suspension was then centrifuged for 15 min at 16 000***g***. The supernatant was removed and stored in liquid nitrogen until analysis. ATP content was determined using an ATP Bioluminescent Assay Kit (Sigma-Aldrich, product number FL-AA). Luminescence was read with a multifunctional microplate reader. ATP content of each sperm sample was calculated using the standard method provided in the kit manual and was reported as nanomoles ATP per 10^9 sperm. The mean of the two measurements per male was used for statistical analysis.

Statistical analyses

Data were analysed using SAS statistical analysis software (v.9.1; SAS Institute Inc., Cary, NC, USA). Residuals were tested for normality (Shapiro-Wilk test) and homogeneity of variance (plot of residuals vs. predicted values). As needed, data were transformed to meet the assumptions of normality and homoscedasticity. Data are presented as mean ± SEM.

Independent sample *t*-tests were used to compare soma mass and testes mass between hooknoses and jacks. Next, soma mass and testes mass were $\log_{10}$-transformed and then testes mass was regressed onto soma mass using a reduced major axis regression, or otherwise called model II linear regression, to obtain regression equations. Model II linear regression was used, rather than standard ordinary least squares regression, because both the *x*- and *y*-variables were measured with possible error (Zar, 1996). The regression equations for each ART were obtained using RMA statistical software (using RMA, v. 1.17, http://www.bio.sdsu.edu/pub/andy/rma.html; Bohonak, 2004). The use of ratios between soma mass and gonad mass (e.g. GSI) is straightforward and common in fish studies to examine relative investment into sperm production and appears to control for differences in body size and hence an individual's overall energy budget (Stoltz *et al*., 2005). It is important to note that GSI will control only for differences in body size if gonad mass scales linearly for body mass. Other studies where this assumption is not met should use an ANCOVA method (Tomkins & Simmons, 2002) to test for differences in gonad investment between ARTs (e.g. Marentette *et al*., 2009). Because gonad mass scales linearly for body mass in both ARTs in our study (see Fig. 1), an independent sample *t*-test was used to compare GSI between the two tactics (see Neff, Fu & Gross, 2003; Marentette *et al*., 2009). Finally, model II linear regressions were used to determine the relationships between absolute and relative testes mass (GSI) and sperm density for both tactics.

Temporal changes in sperm velocity and motility were analysed using mixed-model repeated measures ANOVAs. Models contained the two main effects, ART (jack and hooknose) and post-activation time (5, 10 and 15 s post-activation), as well as the accompanying first-order ART × post-activation time interaction. ART and post-activation time were considered fixed factors in the repeated measures models, whereas male identity was considered a random factor. Treatment means were contrasted using the Tukey–Kramer method.

Independent sample *t*-tests were used to compare longevity, density, head length, head width, flagellum length, ATP content, and seminal plasma physiology traits between ARTs. Furthermore, within each ART, linear regressions were used to relate male body size with these metrics.

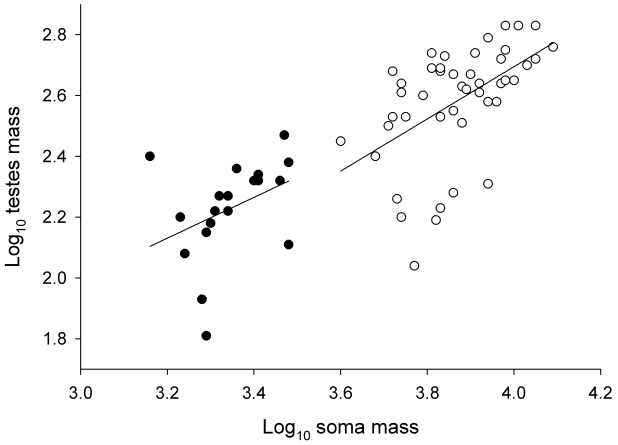

Figure 1. Relationships between testes mass and soma mass for alternative reproductive tactics (jack = closed circles and hooknose = open circles) in Chinook salmon, *Oncorhynchus tshawytscha*. For hooknoses, there was a significant relationship ($y = 1.67x - 3.88$, $r^2 = 0.27$, $P < 0.001$), whereas for jacks, a non-significant positive was found ($y = 1.77x - 3.68$, $r^2 = 0.15$, $P = 0.106$).

RESULTS

Soma mass (hooknose: 7610 ± 290 g, jack: 2258 ± 109 g, $t_{62} = 17.87$, $P < 0.001$) and testes mass (hooknose: 413 ± 22 g, jack: 180 ± 13 g, $t_{62} = 7.13$, $P < 0.001$) were significantly larger for hooknoses than for jacks (Fig. 1). For hooknoses, there was a significant positive linear relationship between testes mass and soma mass ($y = 1.67x - 3.88$, $r^2 = 0.27$, $F_{1,44} = 15.86$, $P < 0.001$), whereas for jacks, a non-significant positive relationship was found ($y = 1.77x - 3.68$, $r^2 = 0.15$, $F_{1,18} = 2.92$, $P = 0.106$). The GSI of jacks was significantly greater than that of hooknoses (hooknose: 5.51 ± 0.26%, jack: 8.11 ± 0.66%, $t_{62} = 4.28$, $P < 0.001$). There were no significant relationships between sperm density and absolute gonad size (jacks: $r^2 = 0.01$, $F_{1,18} = 0.09$, $P = 0.767$; hooknoses: $r^2 = 0.04$, $F_{1,44} = 1.66$, $P = 0.204$) or relative gonad size (jacks: $r^2 = 0.16$, $F_{1,18} = 3.19$, $P = 0.092$; hooknoses: $r^2 = 0.01$, $F_{1,44} = 0.48$, $P = 0.491$).

For sperm velocity, the first-order ART × post-activation time interaction was not significant ($F_{2,120} = 1.28$, $P = 0.281$). However, significant effects were detected for ART ($F_{1,63.9} = 4.17$, $P = 0.045$) and post-activation time ($F_{2,120} = 93.72$, $P < 0.0001$) such that mean sperm velocity of jacks was significantly higher than that of hooknoses, and sperm velocity

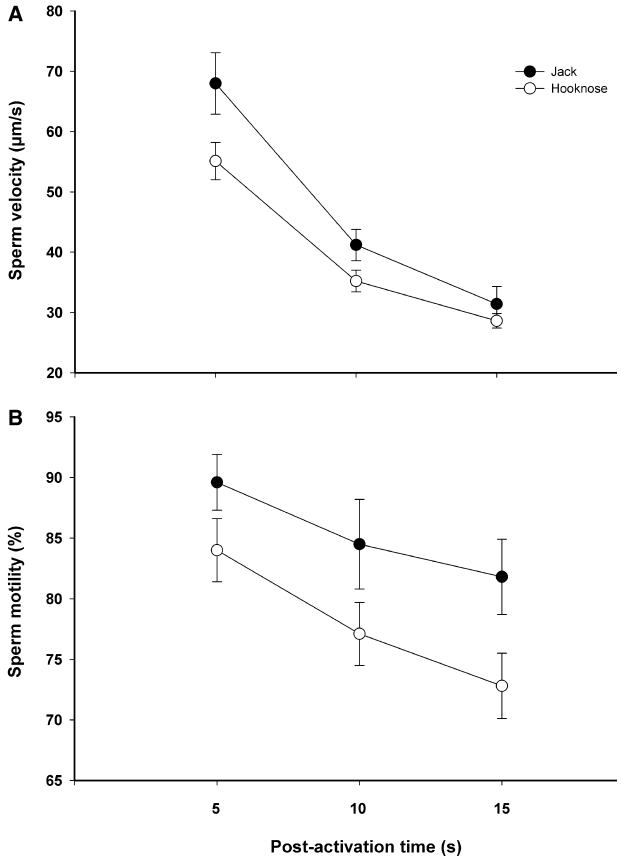

Figure 2. Straight-line velocity (A) and motility (B) of sperm (mean ± SEM) are reported at 5, 10 and 15 s post-activation for alternative reproductive tactics (ARTs; jack = closed circles and hooknose = open circles) in Chinook salmon, *Oncorhynchus tshawytscha*.

decreased significantly with time post-activation (Fig. 2A). The first-order ART × post-activation time interaction was not significant for sperm motility ($F_{2,123} = 0.41$, $P = 0.667$; Fig. 2B). In addition, the ART main effect was not significant ($F_{1,64.8} = 3.71$, $P = 0.059$), while there was a significant effect of post-activation time ($F_{2,123} = 18.56$, $P < 0.0001$) such that motility decreased significantly with time post-activation (Fig. 2B). There were no significant differences in sperm longevity (hooknose: 20.7 ± 0.8 s, jack: 22.6 ± 0.9 s, $t_{62} = 1.34$, $P = 0.185$), sperm density (hooknose: $4.9 \pm 0.2 \times 10^7$ sperm mL^{-1}, jack: $5.3 \pm 0.3 \times 10^7$ sperm mL^{-1}, $t_{62} = 1.02$, $P = 0.314$), sperm head length (hooknose: 2.82 ± 0.02 μm, jack: 2.84 ± 0.02 μm, $t_{28} = 0.71$, $P = 0.486$), sperm head width (hooknose: 2.30 ± 0.01 μm, jack: 2.32 ± 0.02 μm, $t_{28} = 0.94$, $P = 0.357$), or flagellum length (hooknose: 26.97 ± 0.31 μm, jack: 26.21 ± 0.36 μm, $t_{28} = 1.57$, $P = 0.127$) between the hooknoses and jacks. Male body mass was not significantly related to sperm characteristics for either hooknoses ($R^2 \leq 0.06$, $F_{1,44} = 2.85$, $P \geq 0.099$) or jacks ($R^2 \leq 0.11$, $F_{1,17} = 2.00$, $P \geq 0.176$).

The mean, SEM, minimum, and maximum values for ATP content, protein concentration, lactate dehydrogenase activity, anti-oxidant capacity, superoxide dismutase activity, anti-trypsin activity, pH, and osmolality are reported in Table 1 for hooknoses and jacks. No significant differences were detected between the ARTs for any of these physiological characteristics (all $P \geq 0.200$; Table 1). All relationships between male body mass and milt characteristics were non-significant within ARTs (hooknoses: $R^2 \leq 0.12$, $F_{1,32} = 4.01$, $P \geq 0.054$; jacks: $R^2 \leq 0.14$, $F_{1,23} = 3.44$, $P \geq 0.077$).

DISCUSSION

The sneak-guard hypothesis predicts that males employing the sneaking tactic will invest relatively more energy into spermatogenesis and will have higher sperm quality than males employing the guarding tactic (Parker, 1990; Parker, 1998). Overall, our data provide some support for this hypothesis as we found that jacks invest proportionately more of their somatic tissue into gonads compared with hooknoses and sperm velocity varied significantly between the ARTs, with jacks having faster sperm than hooknoses. We also found that sperm motility, longevity, density, and head morphology all tended to be greater (albeit not significantly) in jacks compared with hooknoses.

Consistent with the sneak-guard hypothesis we found that GSI was ~47% greater in jacks than in hooknoses. Our finding that jacks had significantly larger relative investment in gonads is consistent with other studies of fishes with ARTs, including Atlantic salmon (Gage *et al*., 1995; Vladić & Järvi, 2001), bluegill sunfish (Neff *et al*., 2003), combtooth blenny, *Scartella cristata* (Neat, Locatello & Rasotto, 2003), round goby, *Apollonia melanostoma* (Marentette *et al*., 2009), and a shell-brooding cichlid, *Lamprologus callipterus* (Schütz *et al*., 2010). The difference in relative testes investment probably relates to trade-offs with other investments, such as spawning hierarchy defence, which is often related to body size investment. For example, larger hooknose males can obtain better spawning positions than smaller hooknose males, thereby reducing their sperm competition risk by having more favourable spawning positions in terms of excluding other males and improving their proximity to females during spawning (Berejikian *et al*., 2010). In contrast, it is unlikely that jacks investing more into body size would benefit in terms of mating access to females, and thus investing more energy into testes may improve their success at sperm competition, if larger

Table 1. Adenosine 5'-triphosphate (ATP), and seminal plasma biochemistry [protein concentration, lactate dehydrogenase activity (LDH) activity, anti-oxidant capacity, superoxide dismutase activity (SOD), anti-trypsin activity, pH, and osmolality] are reported for alternative reproductive tactics (ARTs) in Chinook salmon, *Oncorhynchus tshawytscha*

	Hooknoses		Jacks			*t*-test	
Sperm quality trait	Mean (SEM)	Range (min.–max.)	Mean (SEM)	Range (min.–max.)	d.f.	*t*	*P*
ATP, nM ATP per 10^9 sperm	39.43 ± 6.90	4.92–103.97	48.53 ± 8.16	2.73–109.93	34	0.84	0.406
Protein concentration (mg mL^{-1})	1.38 ± 0.10	0.63–3.42	1.32 ± 0.14	0.42–3.34	59	0.30	0.763
LDH (U L^{-1})	323.37 ± 44.34	21.01–1269.41	388.40 ± 71.61	104.25–1515.70	59	0.82	0.418
Anti-oxidant capacity*	0.30 ± 0.03	0.08–0.68	0.28 ± 0.02	0.11–0.52	59	0.60	0.554
SOD activity (U mL^{-1})	2.60 ± 0.34	0.00–9.05	2.68 ± 0.45	0.00–10.01	59	0.16	0.873
Anti-trypsin activity†	382.50 ± 40.24	91.96–1308.51	386.63 ± 48.37	92.04–1163.07	58	0.07	0.948
pH	8.15 ± 0.04	7.17–8.51	8.20 ± 0.03	8.00–8.50	59	0.82	0.414
Osmolality (mOsmol kg^{-1})	284 ± 7	143–336	280 ± 7	177–333	59	0.33	0.746

Mean standard error (SEM), minimum (min.) and maximum (max.) value are reported for each sperm quality trait. Independent sample t-tests were used to compare ATP, and seminal plasma biochemistry between the ARTs.
*Expessed as μmol Trolex equivalents per 1 mL.
†Expressed as U L^{-1} of trypsin inhibited.

investment in gonads led to more sperm. However, based on a post-hoc analysis examining the relationship between absolute and relative testes mass (GSI) and sperm density for both tactics, we found no evidence suggesting sperm density covaries with absolute gonad size or relative gonad size. Instead, investment in gonads may relate to the ability of males to replenish their sperm stores and seminal plasma after several matings, a hypothesis that could be tested using a new method to measure ejaculate expenditure in salmonids (see Fitzpatrick & Liley, 2008).

Sperm velocity appears to be the primary determinant of competitive fertilization success in salmonids (e.g. Gage *et al*., 2004; Rudolfsen *et al*., 2006). In our study, sperm velocity was higher in the sneaking tactic relative to the guarding tactic. Similar findings have been reported for other fish species with ARTs, including bluegill sunfish (Burness *et al*., 2004), black goby, *Gobius niger* (Locatello *et al*., 2007), and a shell-brooding cichlid, *Telamatochromis vittatus* (Fitzpatrick *et al*., 2007). Male salmon have a very short opportunity to fertilize eggs once their gametes are released into the aquatic environment. For example, in Sockeye salmon (*Oncorhynchus nerka*) 80% of the eggs are fertilized within 5 s of gamete activation (Hoysak & Liley, 2001) and a 2-s delay in sperm release significantly reduced fertilization success to 30% from an expected 50% in Atlantic salmon (Yeates *et al*., 2007). Therefore, in a competitive sperm–egg environment it would be advantageous to have faster swimming sperm, especially for jacks because of their suboptimal spawning position relative to the hooknoses (Berejikian *et al*., 2010). Size and shape of sperm have been associated with velocity and fertilization ability in fishes (Tuset, Trippel & de Monserrat, 2008b). In our study, sperm morphology metrics did not differ between the hooknoses and jacks and this result is consistent with the majority studies which have compared sperm morphology between species with ARTs such as Atlantic salmon (Gage *et al*., 1995; Vladić *et al*., 2002), bluegill sunfish (Leach & Montgomerie, 2000), round goby (Marentette *et al*., 2009), black goby (Locatello *et al*., 2007), grass goby (Locatello *et al*., 2007), and the shell brooding cichlid (Fitzpatrick *et al*., 2007).

To better understand the nature of these observed differences in sperm velocity we further explored the ATP content of sperm and physiological characteristics of milt, all of which have been linked to sperm function and fertilizing ability in teleost fishes (reviewed by Alavi *et al*., 2008). Activity of sperm is dependent both on ATP stored prior to ejaculation and that synthesized during the motility phase (Christen, Gatty & Billard, 1987; Vladić & Järvi, 2001). In turn, ATP levels in sperm have been correlated with velocity and/or fertilizing ability (Lahnsteiner *et al*., 1998; Bencic *et al*., 1999; Zilli *et al*., 2004). In bluegill sunfish, a species with ARTs, sneaker males had 1.5 times more ATP in their sperm than parental males at times when sperm had significantly higher velocity (Burness *et al*., 2004), while in Atlantic salmon, smaller parr males have a greater proportion of motile sperm and greater sperm ATP content than

their larger anadromous counterparts (Vladić & Järvi, 2001). We did not find such a trend as no significant difference in sperm ATP content was detected between the hooknoses and jacks. This does not mean that ATP is an unimportant energy source in ARTs of Chinook salmon. Rather, it means that inter-individual variation in ATP levels may not be the primary determinant of variation in sperm swimming velocity among individual males (Burness, Moyes & Montgomerie, 2005). To our knowledge, this is the first study that examines seminal plasma characteristics in a fish species with ARTs. Recently, Butts *et al*. (2012) found that hooknoses and jacks did not differ in terms of plasma androgen (testosterone, 11-ketotestosterone) or maturation-inducing steroid (MIS; 17α,20β-dihydroxy-4-pregnen-3-one) levels, which in turn may reflect the lack of significance in the seminal plasma characteristics we measured because androgens regulate the secretory activity of the spermatic duct, which influences seminal plasma composition (Nagahama, 1994).

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