

Primary and secondary sexual characters in alternative reproductive tactics of Chinook salmon: Associations with androgens and the maturation-inducing steroid

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ABSTRACT

The proximate mechanisms that underlie the evolution of within-sex variation in mating behavior, sexual characters and reproductive investment patterns are still poorly understood. Species exhibiting alternative reproductive tactics (ARTs) are ideal model systems to examine these mechanisms. Chinook salmon (*Oncorhynchus tshawytscha*) exhibits two distinct ARTs: hooknoses, which are large males that establish spawning dominance hierarchies via intense male–male competition and jacks, which are smaller precocious sneaking males that steal fertilizations via sperm competition. In this study, we examine plasma testosterone (T), 11-ketotestosterone (11-KT) and maturation-inducing steroid (MIS; $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one) profiles of spawning hooknoses and jacks. Furthermore, we examine relationships between androgens and primary (gonad mass, gonadosomatic index and sperm traits) and secondary (total mass, body size, hump depth and kype length) sexual characters. Relationships between MIS and sperm traits are also examined. We found that hooknoses and jacks did not significantly differ in terms of plasma T, 11-KT or MIS concentrations. Moreover, we found significant positive relationships between levels of both androgens within each ART. There were no significant relationships between androgens, MIS and sperm traits. T and 11-KT concentrations co-varied positively with gonad investment and kype length in jacks. In hooknoses, 11-KT concentration was positively related to total mass, hump depth and condition factor. Overall, these findings suggest that there are differential androgen effects for each of the ARTs in Chinook salmon.

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1. Introduction

Among vertebrates, fishes have evolved the broadest diversity of reproductive strategies [44]. These strategies include hermaphroditism to gonochorism [14], internal to external fertilization [8,45], and sexual to asexual reproduction [65,69]. In some teleost species, discrete variation in reproductive mode exists within sexes and this is known as alternative reproductive tactics (ARTs) (see [36,51] for review). These ARTs emerge when intra-sexual competitors evolve different solutions to reproductive competition [36,72]. ARTs may be fixed for life where males form an irreversible ontogenetic sequence (e.g., bluegill sunfish, *Lepomis macrochirus*) [24] or plastic, in which case individuals switch from one reproductive tactic to another during their lifetime (e.g., initial- and terminal-phase males in the stoplight parrotfish, *Sparisoma viride*) [9]. Generally, male ARTs can be classified as either bourgeois or parasitic [71]. Bourgeois males compete for monopolization of mates and invest in behavioral (e.g., defense, courtship), physiological

and morphological characteristics (e.g., secondary sexual characters) that will allow them to gain advantaged access to females, whereas parasitic males exploit the investment of bourgeois males to fertilize eggs and invest in reproduction (e.g., spermatogenesis and testicular mass) [39,46,48,71].

Androgens, including testosterone (T) and 11-ketotestosterone (11-KT), play a vital role in the differentiation of male ARTs in fishes (see [48] for review). Furthermore, evidence suggests a role for androgens in various aspects of male reproduction including spermatogenesis [30,67], social interactions [32,50], male–male aggression [16] and also in the development of secondary sexual characters in various species with ARTs such as genital papilla and anal glands in Azorean rock-pool blenny, *Parablennius sanguinolentus parvicornis* [49], mating calls in plainfin midshipman, *Porichthys notatus* [7] and head crests in peacock blenny, *Salaria pavo* [64]. It has been demonstrated that levels of the maturation-inducing steroid ($17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one; hereafter MIS) remain low during the early to middle stages of spermatogenesis [37,68]. At later stages, MIS levels rise and peak during spermiation [37,68]. MIS has been linked to milt production [2], as well as sperm maturation; by increasing the pH of the sperm duct, which in turn elevates intra-sperm cAMP levels allowing the acquisition of sperm motility [67]. In addition, studies have shown

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that MIS plays a role in regulation of sexual behaviors and aggression [10,41]. Therefore, quantifying this steroid would provide further insights into endocrine factors regulating spermatogenesis and more specifically on sperm quality traits for species with ARTs.

Chinook salmon (*Oncorhynchus tshawytscha*) has several traits that make them an ideal system for examining the steroidal mechanisms that underlie ARTs. First, each male expresses one of two fixed alternative reproductive tactics, referred to as “hooknose” and “jack”. Hooknose males leave their natal rivers for the ocean and/or the Great Lakes at the end of their first year of life, where they mature for 3–4 years on average, and then return to natal rivers where they fight for position in a dominance hierarchy to gain closer access to spawning females [3,25,56]. This phenotype is analogous to a larger bourgeois male. Alternatively, jacks are presumably resident in their natal rivers their entire lives, reach sexual maturity precociously (after 2 years), and employ a sneaking tactic, by darting from nearby refuges, to steal fertilizations from hooknose males [3,25]. This phenotype is analogous to a smaller parasitic male. Second, salmonids develop among the most elaborate secondary sexual characters seen in breeding fishes [20], the most pronounced being the dorsal hump and the elongated snout, also known as a kype [sensu 23]. The dorsal hump is formed by a bar of cartilage under the skin and may serve as a display of status and vigor, protective shield to protect fish during bouts of male–male aggression, or barricade to block competitors from gaining access to gravid females, while the kype most likely functions as a specialized weapon for fighting [20,58]. Finally, Chinook salmon are semelparous (a single reproductive bout before death); leading to intense male–male competition for access to spawning females [20].

In this study, we examine (i) androgen and MIS profiles of wild spawning Chinook salmon ARTs, (ii) relationships between androgens and primary- and secondary sexual characters and (iii) relationships between MIS and sperm quality traits. Studying this divergence between the tactics can provide valuable insight into the proximate mechanisms that underlie the evolution of intra-sexual variation.

2. Methods

2.1. Fish collection

Male Chinook salmon ($n = 41$ hooknoses and 13 jacks) were collected during the spawning season using backpack electrofishing from 3 to 6 October 2010 in the Credit River (43°35'N, 79°42'W), which flows into Lake Ontario. Chinook salmon have been stocked in Lake Ontario for over 40 years [12]. Males were selected to encompass a wide range in size to ensure that discrete age classes were collected. After capture, the fish were humanely killed with a rapid blow to the head. Blood samples were collected from the caudal vein using 3 mL heparin-rinsed syringes equipped with a 23-gauge needle. Blood collection occurred within 2 min from the time of capture to minimize hormonal variation due to handling stress. All blood samples were kept on ice until they were returned to the lab (within 5 h), where they were centrifuged for 10 min at 2500 rpm to separate the blood plasma. Plasma was then stored at -80°C until androgen and MIS assays were conducted.

Total mass (± 10 g) was recorded for each male at the time of collection. In addition, measurements (± 1 mm) of fork length (distance from tip of snout to center of the fork in the caudal fin), hump depth (distance from the lateral line to the highest point on the dorsal surface) and kype length (distance from middle of eye to the tip of the snout) were obtained from each male. Fish were dissected and their testes were removed and weighted (± 5 g). Gonadosomatic index (GSI = testes mass/(total mass – testes mass) $\times 100$) and Fulton's

condition factor ($((\text{total mass}/\text{fork length}^3) \times 10^5)$) were later calculated.

2.2. Androgen and MIS assays

Plasma levels of androgens and MIS were determined using commercial Enzyme-linked-Immunoabsorbent Assays (ELISA; Cayman Chemicals, Ann Arbor, MI, USA) following company instructions. Assay sensitivity for T was 3.9 through 500 pg/mL, for 11-KT 0.78 through 100 pg/mL, and for MIS 1.95 pg/mL through 250 pg/mL. Prior to assaying individual samples, an optimization protocol was conducted for each hormone-strategy combination to determine the optimal dilution and whether plasma should be extracted prior to assaying. A plasma pool was created from 10 individuals for each strategy and both ether extracted [6] and non-extracted plasma pools were assayed at eight different dilutions for each hormone to confirm linearity with the assay's standard curve and to detect the optimal dilution allowing the pool to fall on the middle of the linear portion of the standard curve. Extraction did not increase our ability to determine androgen or MIS levels, and optimal dilutions for both strategies were determined as 1:1000 dilution for T, 1:12000 dilution for 11-KT and 1:2,000 for MIS. For each hormone, plasma was assayed in triplicate across four (T), three (11-KT) or four (MIS) assay plates yielding an intra- and inter-assay variation of 2.42% and 12.67% for T, 3.93% and 14.38% for 11-KT, and 4.8% and 9.6% for MIS, respectively. Relevant cross-reactivity for the T assay included 5α -dihydrotestosterone (27.4%), 5β -dihydrotestosterone (18.9%), methyltestosterone (4.7%), androstenediol (3.7%), for the 11-KT assay: testosterone ($<0.01\%$); 11-KT: adrenosterone (2.9%), and for the MIS assay: 20 β -hydroxyprogesterone (0.1%).

2.3. Sperm quality assessment

Milt (<0.2 μL) was micropipetted into a chamber of a 2X-CEL glass slide (Hamilton Thorne, MA, USA) and covered with a coverslip (22 \times 22 mm). Sperm were then activated with 15 μL of activation media (river water at 11 $^{\circ}\text{C}$). Sperm were video-recorded using a CCD black and white video camera (XC-ST50, Sony, Japan) module at 50 Hz vertical frequency, mounted on an external phase contrast microscope (CX41 Olympus, Melville, NY, USA) with a 10 \times negative-phase magnification objective [55]. Once recorded, sperm traits were analyzed using the HTM-CEROS sperm analysis system (version 12, CEROS, Hamilton Thorne Biosciences, Beverly MA, USA) set at the following parameters: number of frames = 60, minimum contrast = 11, photometer = 55–65, minimum cell size = 3 pixels. Male salmon have a very short opportunity to fertilize eggs once their gametes are released from the genital pore. For instance, in Sockeye salmon, *Oncorhynchus nerka*, 80% of the eggs are fertilized within 5 s of gamete activation [28], while in Atlantic salmon *Salmo salar*, a 2 s delay in sperm release reduced fertilization success to 30% from an expected 50% [74]. Therefore, to simulate a more natural spawning event we analyzed average path velocity (V_{AP}), straight-line velocity (V_{SL}), curvilinear velocity (V_{CL}) and percent motile sperm at 5 s post-activation. Each video recording was manually checked for quality control. 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 sperm swam out of the field of view before adequately being assessed.

2.4. Sperm density

Sperm density was counted under a Zeiss Axiostar compound microscope at 400 \times magnification using an improved Neubauer haemocytometer (see [55] for details). In brief, milt (1.5 μL) from each male was first diluted in 500 μL of Cortland's saline solution

(7.25 g/L NaCl; 0.38 g/L KCl; 0.47 MgSO₄ × 7H₂O; 0.4 g/L Na₂HPO₄ × H₂O; 1.0 g/L NaHCO₃; 0.22 g/L MgCl₂; 1.0 g/L C₆H₁₂O₆; adjusted to pH 7.8) that does not activate the sperm. To obtain homogenous solutions samples were mixed thoroughly. The sperm suspension (10 µL) was then micropipetted onto a haemocytometer that had been pre-covered with a coverslip and was left for ~10 min to allow sperm sedimentation. The sperm were counted in 5 of the 25 squares on the grid. The average number of sperm in the 5 squares was multiplied by 25 (to estimate the number of sperm in all squares), then by 10 (depth of the chamber in µm) and finally by 500 (to get the original volume of the sample in µL). Densities were expressed as the number of sperm cells per mL of milk.

2.5. Statistical analysis

Independent sample *t*-tests were used to compare androgen levels, MIS, primary and secondary sexual characters between the ARTs (v. 9.1; SAS Institute Inc., Cary, NC, USA). Next, linear regressions were used to relate (i) levels of androgens and MIS, (ii) androgens and primary sexual characters, (iii) MIS and primary sexual characters and (iv) androgens and secondary sexual characters (both relative and absolute). Relationships between androgens, MIS and the primary sexual characters, sperm motility and velocity, were also analyzed using non-linear regression. Relative hump depth was estimated from the residuals of the regression between hump depth and log₁₀ body mass, while relative kype length was estimated from the residuals of the regression between kype length and log₁₀ body mass. Principle components analysis (PCA) was used to summarize variation in the three sperm velocity metrics (*V*_{AP}, *V*_{SL} and *V*_{CL}) (v. 9.1; SAS Institute Inc., Cary, NC, USA). One informative PC axis was extracted that explained 95.2% of the variation.

Regression analyses were run separately for each ART because our primary interests were to examine relationships within hooknoses and jacks. One of the underlying assumptions of standard ordinary least squares regression is that the independent variable, or X-axis, is measured with no error [75]. Because androgens and MIS were measured with possible error, we analyzed the data using reduced major axis regression (model II regression) (RMA, v. 1.17, <http://www.bio.sdsu.edu/pub/andy/rma.html>) [4]. Residuals were tested for normality (Shapiro–Wilk test) and homogeneity of variance (plot of residuals vs. predicted values). Total body mass was log₁₀ transformed to meet assumptions of normality and homoscedasticity.

3. Results

Descriptive statistics for androgens, MIS, primary and secondary sexual characteristics are shown in Table 1. Testes mass (*t*₅₂ = 7.59, *p* < 0.0001), total mass (*t*₅₂ = 50.60, *p* < 0.0001), fork length (*t*₅₂ = 13.64, *p* < 0.0001), absolute hump depth (*t*₅₂ = 11.42, *p* < 0.0001) and absolute kype length (*t*₅₂ = 13.85, *p* < 0.0001) of hooknoses were significantly larger than those of jacks, whereas GSI of jacks was significantly larger than that of hooknoses (*t*₅₂ = 3.31, *p* = 0.002; Table 1). Condition factor did not differ between the tactics (*t*₅₂ = 0.87, *p* = 0.388; Table 1).

Plasma concentrations of T (*t*₅₂ = 0.34, *p* = 0.735), 11-KT (*t*₅₂ = 0.46, *p* = 0.647) and the 11-KT:T ratio (*t*₅₂ = 1.26, *p* = 0.213) did not differ between hooknoses and jacks (Table 1, Fig. 1). In addition, concentrations of MIS were not significantly different between the tactics (*t*₅₁ = 1.10, *p* = 0.276; Table 1). Regression analyses demonstrated a significant positive relationship between T and 11-KT concentrations for hooknoses (*R*² = 0.15, *F*_{1,40} = 6.66, *p* = 0.014; Fig. 1A) and jacks (*R*² = 0.71, *F*_{1,12} = 27.25, *p* < 0.001; Fig. 1B). Plasma concentrations of T and 11-KT were not significantly related to MIS for either hooknoses (T: *R*² = 0.04, *F*_{1,39} = 1.52, *p* = 0.225, 11-KT: *R*² = 0.03, *F*_{1,39} = 1.21, *p* = 0.279) or jacks (T: *R*² = 0.02, *F*_{1,12} = 0.26, *p* = 0.623, 11-KT: *R*² = 0.05, *F*_{1,12} = 0.60, *p* = 0.456).

Regression analysis demonstrated a significant positive relationship between T concentration and testes mass in jacks (*R*² = 0.55, *F*_{1,12} = 13.31, *p* = 0.004; Fig. 2A) but not for hooknoses (*R*² = 0.07, *F*_{1,40} = 2.88, *p* = 0.098; Fig. 2A). Both ARTs showed significant positive relationships between 11-KT concentration and testes mass (hooknoses: *R*² = 0.23, *F*_{1,40} = 11.80, *p* = 0.001, jacks: *R*² = 0.39, *F*_{1,12} = 6.95, *p* = 0.023; Fig. 2B). For jacks, T (*R*² = 0.66, *F*_{1,12} = 20.97, *p* = 0.001; Fig. 2A) and 11-KT (*R*² = 0.34, *F*_{1,12} = 5.61, *p* = 0.037; Fig. 2C and D) plasma concentrations were both positively related to GSI, however for hooknoses both these relationships were non-significant (T: *R*² = 0.09, *F*_{1,40} = 3.66, *p* = 0.063, 11-KT: *R*² = 0.08, *F*_{1,40} = 3.22, *p* = 0.080; Fig. 2C,D).

The percentage of motile sperm (mean ± SD) for the hooknoses was 83.69 ± 17.51%, while for the jacks the percentage of motile sperm (mean ± SD) was 87.98 ± 11.20%. Plasma concentrations of androgens and MIS were not significantly related to the percentage of motile sperm for either hooknoses (all *R*² ≤ 0.05, *F*_{1,39} ≤ 1.89, *p* ≥ 0.178) or jacks (*R*² ≤ 0.24, *F*_{1,12} ≤ 3.51, *p* ≥ 0.088; Fig. 3A–C). Mean (±SD) *V*_{AP}, *V*_{SL} and *V*_{CL} for the hooknoses were 83.0 ± 21.2, 55.1 ± 21.2 and 106.3 ± 25.2 µm/s and for the jacks, mean (±SD) *V*_{AP}, *V*_{SL} and *V*_{CL} were 102.1 ± 24.2, 73.8 ± 22.5 and 127.4 ± 26.1

Table 1

Testosterone (T), 11-ketotestosterone (11-KT), 11-KT/T ratio, 17,20β-dihydroxypregn-4-en-3-one (MIS), primary and secondary sexual characters reported for alternative reproductive tactics (ARTs) in Chinook salmon, *Oncorhynchus tshawytscha*. The sample size (n), mean, standard deviation (SD), minimum (min) and maximum value (max) are reported for each variable. Independent sample *t*-tests were used to compare T, 11-KT, 11-KT/T ratio, MIS, primary- and secondary sexual characters between the ARTs.

Indices	Hooknose (n = 41)				Jack (n = 13)				T-test	
	Mean	SD	Min	Max	Mean	SD	Min	Max	<i>t</i>	<i>p</i>
T (ng/mL)	72.69	32.08	9.96	170.89	69.34	25.29	31.53	122.10	0.34	0.73
11-KT (ng/mL)	170.62	84.77	34.07	361.78	157.54	105.81	54.10	375.81	0.46	0.65
11-KT/T ratio	2.66	1.47	0.71	6.86	2.11	0.84	1.09	3.90	1.26	0.21
MIS (ng/mL)	80.40	46.39	20.64	176.75	65.18	30.24	36.17	145.51	1.10	0.27
<i>Primary sexual characteristics</i>										
Testes mass (g)	402.6	145.5	110.0	680.0	179.2	67.4	65.0	295.0	7.59	<0.0001
Gonadosomatic index (%)	5.5	1.8	1.9	9.1	8.3	3.3	3.3	17.2	3.31	0.001
<i>Secondary sexual characteristics</i>										
Kype length ^a (mm)	109.4	14.5	82.0	142.0	66.8	7.6	55.0	81.0	13.85	<0.0001
Hump depth ^a (mm)	114.2	18.8	81.0	165.0	71.3	8.4	58.0	90.0	11.42	<0.0001
Total mass (g)	7871.49	1973.96	4224.08	12842.33	2346.47	488.18	1700.97	3260.20	50.60	<0.0001
Fork length (mm)	832.8	65.3	702.8	949.0	551.8	62.8	398.1	638.9	13.64	<0.0001
Condition factor	1.34	0.13	1.02	1.60	1.51	0.70	0.74	3.59	0.87	0.40

^a Absolute measure; *t* = *T*-statistic; *p* = *p*-value; condition factor = (total mass/(fork length)³) × 10⁵.

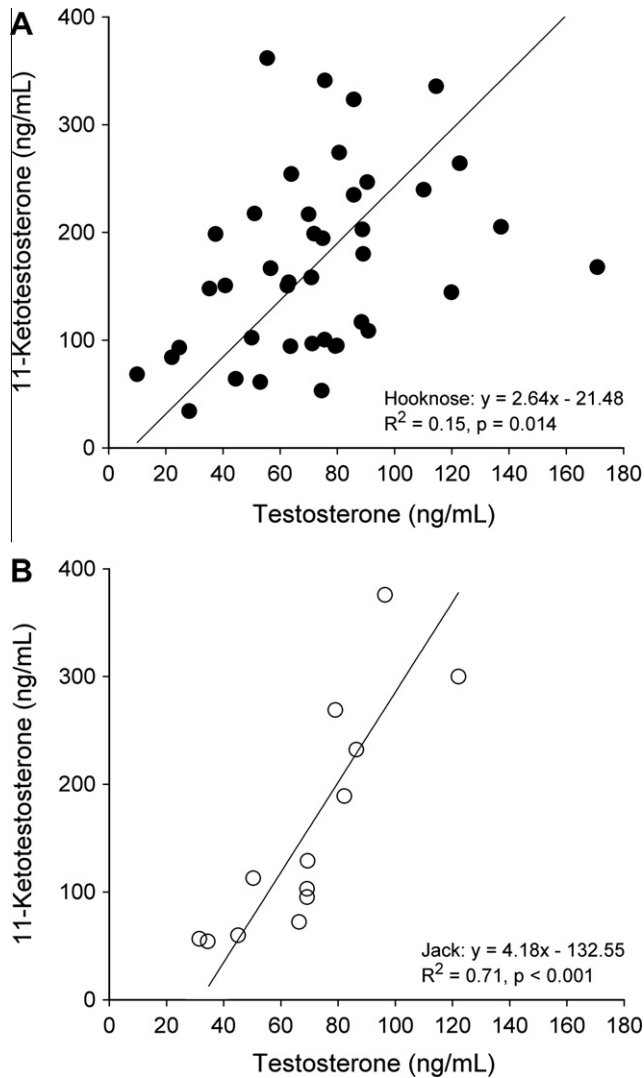


Fig. 1. Relationships between testosterone and 11-ketotestosterone for alternative reproductive tactics in Chinook salmon, *Oncorhynchus tshawytscha*. Hooknoses are represented by filled circles and jacks are represented by open circles. Solid lines show significant linear relationships.

$\mu\text{m/s}$. All relationships between androgens, MIS and sperm velocity were non-significant (hooknoses: $R^2 \leq 0.07$, $F_{1,40} = 2.81$, $p \geq 0.102$, jacks: $R^2 \leq 0.12$, $F_{1,12} = 1.55$, $p = 0.240$; Fig. 3D–F). Mean (\pm SD) sperm density was $5.07 \times 10^7 \pm 1.44 \times 10^7$ sperm/mL for the hooknoses and $5.31 \times 10^7 \pm 1.49 \times 10^7$ sperm/mL for the jacks. There were no significant relationships between T (hooknoses: $R^2 < 0.01$, $F_{1,40} = 0.19$, $p = 0.663$, jacks: $R^2 = 0.02$, $F_{1,12} = 0.17$, $p = 0.688$), 11-KT (hooknoses: $R^2 < 0.01$, $F_{1,40} = 0.04$, $p = 0.838$, jacks: $R^2 = 0.04$, $F_{1,12} = 0.48$, $p = 0.503$) and MIS (hooknoses: $R^2 = 0.02$, $F_{1,39} = 0.87$, $p = 0.357$, jacks: $R^2 = 0.01$, $F_{1,12} = 0.09$, $p = 0.773$) and sperm density for either of the ARTs.

No significant relationships were detected between plasma T concentration and total mass for either of the ARTs (hooknoses: $R^2 = 0.01$, $F_{1,40} < 0.01$, $p = 0.963$, jacks: $R^2 = 0.05$, $F_{1,12} = 0.53$, $p = 0.483$; Fig. 4A). Regression analysis demonstrated a significant positive relationship between plasma 11-KT concentration and total mass in hooknoses ($R^2 = 0.12$, $F_{1,40} = 5.29$, $p = 0.027$; Fig. 4B) but not for jacks ($R^2 = 0.12$, $F_{1,12} = 1.50$, $p = 0.246$; Fig. 4B). Plasma T concentration was not significantly related to absolute (hooknoses: $R^2 = 0.04$, $F_{1,40} = 1.73$, $p = 0.196$, jacks: $R^2 = 0.25$, $F_{1,12} = 3.57$, $p = 0.086$; Fig. 4C) or relative hump depth (hooknoses: $R^2 = 0.08$, $F_{1,40} = 3.44$, $p = 0.071$, jacks: $R^2 = 0.24$, $F_{1,12} = 3.57$, $p = 0.086$) for

either ART, however, 11-KT concentration was positively related to absolute ($R^2 = 0.33$, $F_{1,40} = 18.92$, $p < 0.0001$; Fig. 4D) and relative hump depth ($R^2 = 0.22$, $F_{1,40} = 10.76$, $p = 0.002$, $y = 0.16x - 27.32$) for hooknoses and absolute hump depth for jacks ($R^2 = 0.32$, $F_{1,12} = 5.12$, $p = 0.045$; Fig. 4D). Plasma concentrations of T ($R^2 = 0.46$, $F_{1,12} = 9.41$, $p = 0.011$; Fig. 4E) and 11-KT ($R^2 = 0.59$, $F_{1,12} = 15.72$, $p = 0.002$; Fig. 4F) were positively related to absolute kype length for jacks, however these relationships were non-significant for hooknoses (T: $R^2 = 0.01$, $F_{1,40} = 0.17$, $p = 0.679$, 11-KT: $R^2 = 0.08$, $F_{1,40} = 3.33$, $p = 0.076$; Fig. 4E and F). Androgens were not significantly related to relative kype length for either ART (hooknoses: $R^2 < 0.02$, $F_{1,40} \leq 0.71$, $p \geq 0.405$, jacks: $R^2 \leq 0.13$, $F_{1,12} \leq 1.46$, $p \geq 0.254$).

There were no significant relationships between androgens and fork length ($R^2 \leq 0.04$, $F \leq 1.46$, $p \geq 0.234$). In addition, testosterone concentration was not significantly related to condition index for either ART (hooknoses: $R^2 < 0.01$, $F_{1,40} = 0.005$, $p = 0.943$, jacks: $R^2 < 0.01$, $F_{1,12} = 0.03$, $p = 0.865$; Fig. 3G), however, 11-KT concentration was positively related to condition index for hooknoses ($R^2 = 0.20$, $F_{1,40} = 9.93$, $p = 0.003$, $y = 1.51e - 3x + 1.08$) but not for jacks ($R^2 = 0.04$, $F_{1,12} = 0.51$, $p = 0.489$).

4. Discussion and conclusions

In this study we report several key findings: (1) androgen and MIS concentrations did not differ between hooknoses and jacks, (2) T concentration was positively related to 11-KT concentration within each ART, (3) T and 11-KT concentrations were both positively related to gonad investment in jacks, (4) there were no significant relationships between androgens, MIS and sperm traits, (5) 11-KT concentration was positively related to total mass and condition for hooknoses and hump depth for both ARTs, and (6) T and 11-KT concentrations were both positively related to kype length in jacks.

We report, for the first time, levels of the androgens T and 11-KT, as well as MIS, in spawning ARTs of Chinook salmon from a wild population. Only one other study thus far has examined androgen levels for male Chinook salmon and their work focused on plasma concentrations of triiodo-L-thyronine, T, and growth hormone in captive-bred one-year-old jacks and non-maturing fish during the months prior to maturation [26]. Our plasma T concentrations are in general agreement with those findings as well as those reported for other salmonids such as sockeye salmon [13,29], coho salmon, *Oncorhynchus kisutch* [18], and rainbow trout, *Oncorhynchus mykiss* [17,38], whereas concentrations are higher than those reported for Atlantic salmon [40,70]. Plasma concentrations of 11-KT and MIS did not follow the same trend, with our values being higher than those reported for the majority of salmonids, such as Atlantic salmon [33,40], masu salmon, *Oncorhynchus masou* [42], brown trout, *Salmo trutta*, and brook trout, *Salvelinus fontinalis* [10]. Nevertheless, we did notice some similarities between our 11-KT levels and those previously reported for chum salmon, *Oncorhynchus keta* [52] and pink salmon, *Oncorhynchus gorbuscha* [15]. As well, our MIS levels are similar to values reported for chum salmon [52] and coho salmon [18]. Within the literature, 11-KT and MIS data is highly variable for rainbow trout [60,68]; therefore making comparisons with our values is difficult. Regardless of intra- and inter-species trends, we still have to be cognizant of maternal influences (i.e., female-male sex ratios and pheromones) [38], male-male interactions [50], the stage of maturation [40], seasonal variations [17], abundance of hormone receptors [34], hormone metabolizing enzymes [34] and assay-to-assay variation (ELISA vs. radioimmunoassay) when interpreting such data as all these factors can have an influence on the final concentration of a specific hormone.

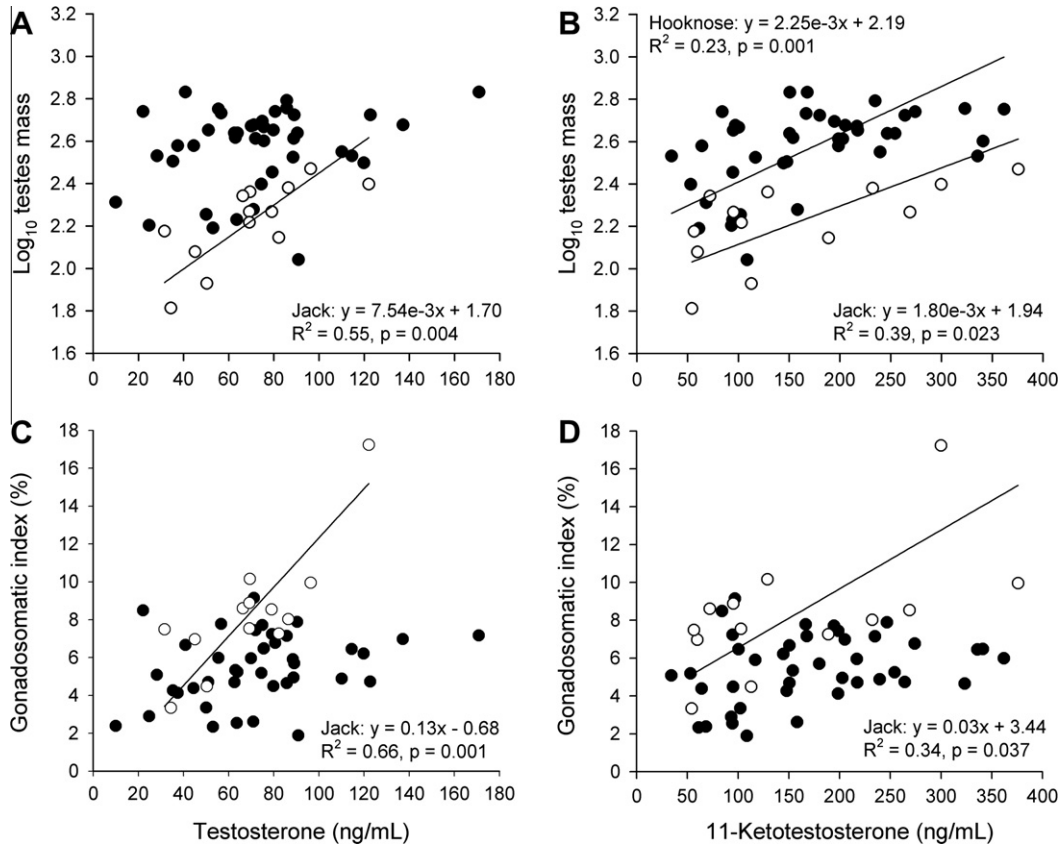


Fig. 2. Relationships between androgens (testosterone and 11-ketotestosterone), testes mass and gonadosomatic index in alternative reproductive tactics of Chinook salmon, *Oncorhynchus tshawytscha*. Hooknoses are represented by filled circles and jacks are represented by open circles. Solid lines show significant linear relationships.

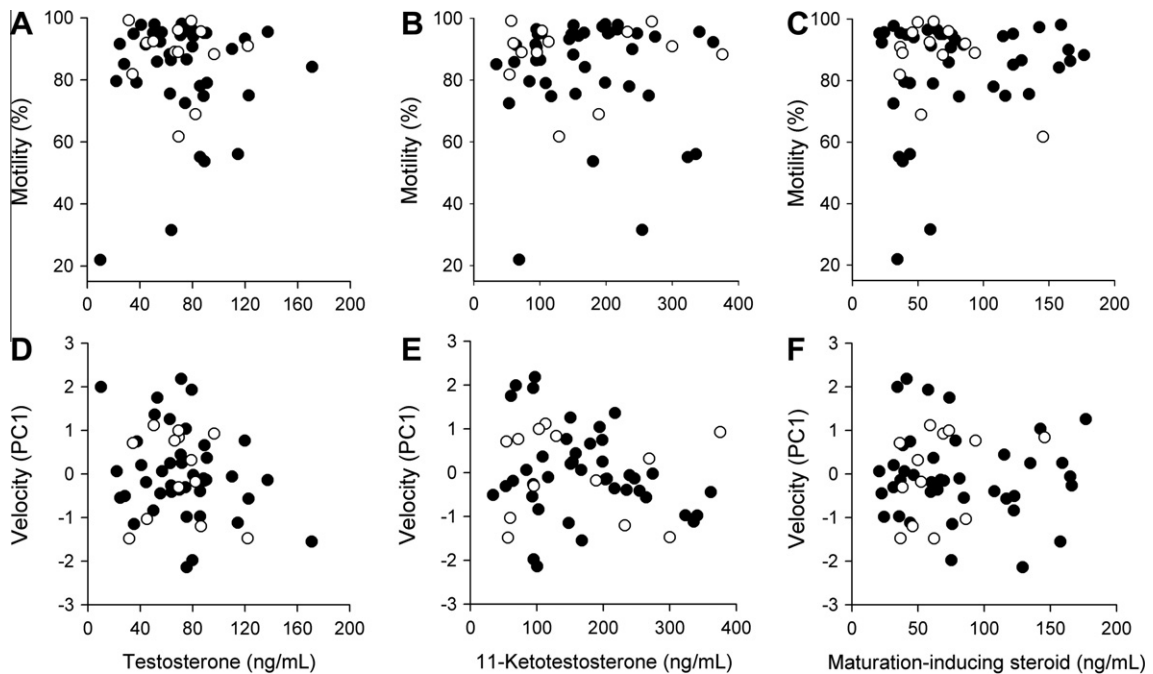


Fig. 3. Relationships between testosterone, 11-ketotestosterone, maturation-inducing steroid ($17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one) and sperm traits (motility and velocity at 5 s post-activation) in alternative reproductive tactics of Chinook salmon, *Oncorhynchus tshawytscha*. Hooknoses are represented by filled circles and jacks are represented by open circles. Positive sperm velocity scores indicate faster sperm velocity.

Plasma levels of androgens have been measured in species with ARTs [7,35,48,63]. No consistent pattern emerges from a comparative survey of T levels between the tactics. For example, in the

peacock blenny, *S. pavo* [46], rock-pool blenny [61] and Atlantic salmon [70] plasma levels of T are higher in bourgeois than parasitic males, whereas the opposite is the case for the plainfin mid-

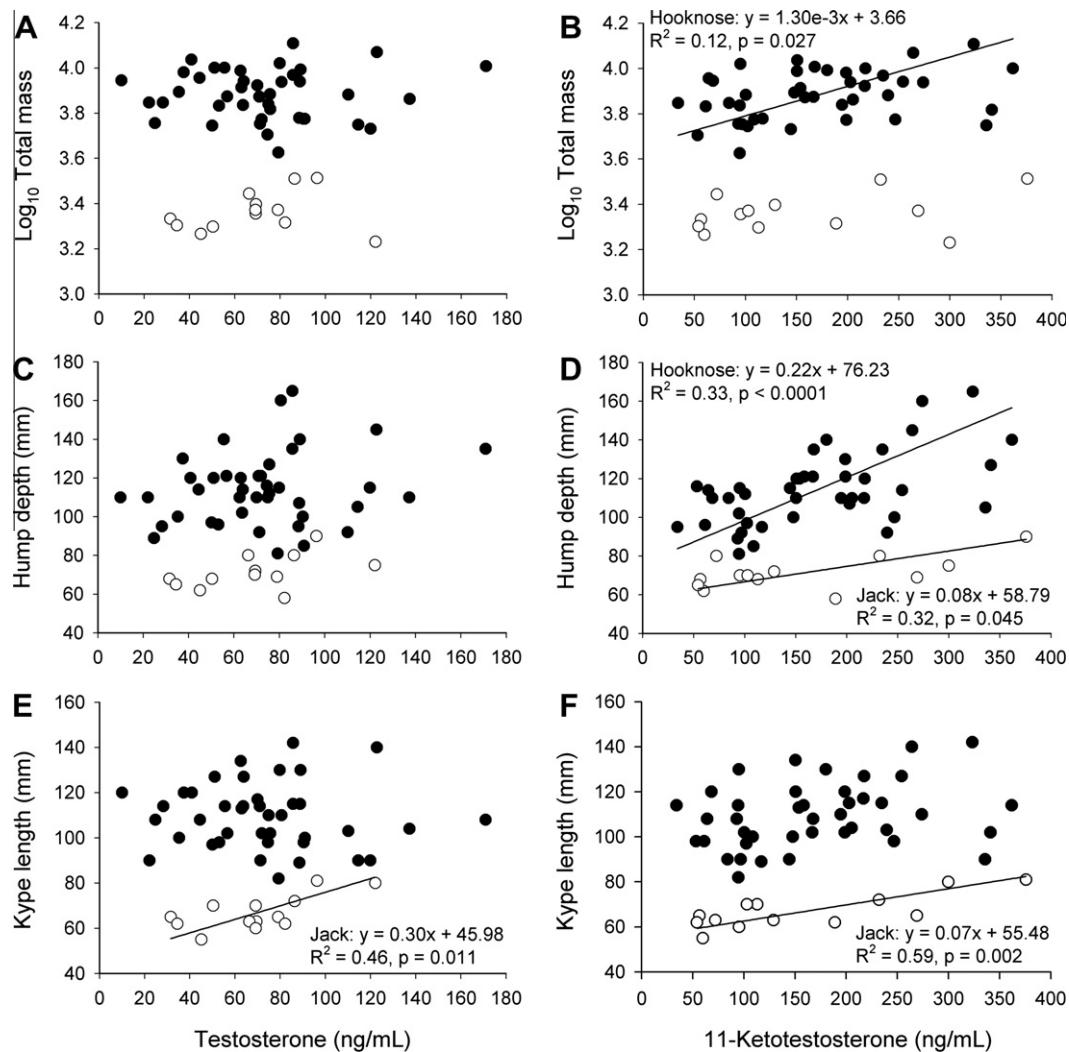


Fig. 4. Relationships between androgens (testosterone and 11-ketotestosterone), total mass, absolute hump depth and absolute kype length. Hooknoses are represented by filled circles and jacks are represented by open circles. Solid lines show significant linear relationships.

shipman, *P. notatus* [7] and cockwing wrasse, *Symphodus melops* [73]. Contrary to the studies mentioned, we found no significant difference in plasma T concentrations between ARTs. Similar findings have been reported for the Lusitanian toadfish, *Halobatrachus didactylus* [43] and rock-pool blenny [47]. The finding that 11-KT was the androgen found in higher concentrations than T concurs with results reported for most teleosts [5]. In the plasma, 11-KT is usually found in higher concentrations in bourgeois than parasitic males [7,48], and such cases have been reported specifically for salmonids [40,70]. Surprising, we did not find such a trend as 11-KT did not differ between the ARTs in our study.

Data are also available which compare MIS profiles between male tactics [27,62]. For example, plasma $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one (MIS measured in this study) levels did not differ significantly between reproductive tactics in St. Peter's fish, *Sarotherodon galilaeus* [62], saddleback wrasse, *Thalassoma duperrey* [27] and Atlantic salmon [40]. In the belted sandfish, *Serranus subligarius*, $17\alpha,20\beta,21$ -trihydroxy-4-pregnen-3-one (20 β S), rather than $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one, is linked to body size and mating tactic [11]. Plasma $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one levels were undetectable in the Lusitanian toadfish, while 20 β S levels increased immediately before spawning in both bourgeois and parasitic male tactics [43]. In our study, we also detected no

difference in $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one levels between spawning jacks and hooknoses.

Overall, these results, which compare androgens and MIS between the tactics, are interesting when discussed in the context of salmonid biology. For instance, Gross [23], shows that salmon ARTs are evolutionary favored by frequency-dependant disruptive selection on spawning grounds; larger hooknoses and smaller jacks gain closer access to females and thus have a higher chance of fertilizing eggs [23]. To overcome these challenges, jacks specialize at sneaking fertilizations by darting from nearby refuges into ongoing spawning events and invest disproportionately in gonads, whereas hooknose males compete for access to females by fighting for ranks in spawning hierarchies and invest in body attributes [3]. Therefore, both ARTs are exposed to challenges, which may help explain, our elevated 11-KT levels and why no differences in androgen and MIS profiles were detected between the tactics; as for the majority of species it is difficult to disentangle potential effects of MIS on male courtship behavior from effects on sperm maturation [48].

We report relationships between plasma T and 11-KT for each ART. The association was stronger in the jacks than hooknoses (R^2 of 0.71 vs. 0.15, respectively). One possible explanation is that aromatase, a key enzyme that converts androgens such as T to estrogens (including estriol and estradiol) [34] is presumed to

regulate expression of divergent male phenotypes in at least one species with alternative reproductive tactics [66], may differ significantly between hooknoses and jacks. For example, Schlinger et al. [66] found that in midshipman fish, aromatase activity in the central nervous system vocal center is significantly higher in the parasitic males as compared to the bourgeois males.

We found that jacks had a higher reproductive investment in gonads than hooknose males. Furthermore, research has recently shown that jacks have faster swimming sperm [19]. In the fish biology literature, sperm velocity is considered, for the most part, the primary determinant of competitive fertilization success [22]. Taken together our results support predictions of sperm competition theory in that males employing the sneaking tactic will invest more resources into spermatogenesis than males employing the guarding tactic as a result of intense sperm competition [53,54]. Additionally, we showed that plasma T and 11-KT concentrations co-varied positively with GSI in jacks.

In salmonids, androgens (such as T and 11-KT) are necessary for regulating spermatogenesis (sperm production), while MIS regulates the maturation and quality of sperm [67]; therefore, not detecting significant relationships between plasma concentrations of T or 11-KT and sperm quality as measured by motility/velocity was not surprising. In addition, no relationships were detected between sperm quality metrics and MIS levels. One possible reason, for not detecting such relationships, between sperm metrics and MIS, is that circulating levels of MIS in the plasma might not reflect the hormonal environment present during sperm development in the testes and spermatic duct [1].

The results from our study and others [48] indicate that androgens are likely related to the development of secondary sexual characters in fishes. Our work also shows a more consistent pattern between 11-KT levels and secondary sexual characters, particularly total mass, hump depth (both relative and absolute) as well as condition factor, in hooknose males than in jacks. This was not unexpected as hooknose males use secondary sexual characters to gain access to gravid females [20]. For instance, the mass of a male has previously shown to be positively correlated with spawning-site occupancy [58]. In addition, males with larger humps (measured as absolute size) are more likely to be found as a breeding partner than males with smaller humps [58]. Dorsal humps also serve as a shield to protect spawning fish during bouts of male-male aggression while fighting for female proximity [57], while condition factor provides a general indication of overall fish health [59]. Absolute kype length was also linked to plasma T and 11-KT concentrations, but only for jacks. Gross [23] found that Coho salmon jacks in high-density situations used their kypes for gaining closer proximity to females prior to spawning.

In conclusion, our correlational findings suggest that there are differential androgen effects for each of the fixed ARTs in Chinook salmon. In order to assess the potential causal roles that steroids have in the development of primary and secondary sexual characters in ARTs there is a need for studies using “phenotypic engineering” [31]; i.e., alter hormone levels experimentally and then compare primary and secondary sexual characters of altered and unaltered individuals. Several potential mechanisms linking sexual characters and hormones exist, including the hypothesis that androgens have a dualistic effect; they stimulate the development of sexual characters important for sexual selection while reducing immunocompetence [21].

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