

Life histories of anadromous salmon males reveal a trade-off between primary and secondary sexual traits

by

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Abstract

The life-history trade-off between investment in somatic growth and gonadal tissue is caused by individual energy limitations and results in individuals that adopt specific tactics to achieve reproduction. Allocation in primary and secondary sexual traits in Atlantic salmon males was studied by assessing life history traits (smolt size, sea age, growth rate) based on back-calculation of scales, ejaculate energy content (sperm ATP content, mass and density) and the size of secondary sexual traits. We found that males investing less in secondary sexual traits produce ejaculates with a higher energy content. Differences were found in the investment into primary and secondary sexual traits between fish that spent one year in the sea before returning to their spawning grounds (grilse) and multi-sea-winter adults, suggesting that different energy allocation patterns in reproductive effort reflect alternative developmental pathways. These findings are consistent with the pattern where multi-sea-winter male ejaculate investment relies principally on the resource acquisition in the ocean, whereas grilse ejaculate investment relies chiefly on the resource allocation of available surplus energy, thus representing alternative male reproductive tactics.

Key words: Atlantic salmon, age at maturity, ejaculate investment, resource acquisition, resource allocation

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Introduction

The key aspect of the life history theory relates to the optimal allocation of limited energy resources into reproductive effort, which may result in trade-offs between life history traits with a complex structure (Stearns 1992; Roff 2000). After the metabolic maintenance requirements are satisfied, the surplus energy (energy above some minimum amount required for reproduction) can be used for growth, defense and reproduction. In the case of high variation in the allocation of surplus energy between life history traits, a negative correlation between these traits should be apparent (van Noordwijk & de Jong 1986).

Atlantic salmon is an anadromous species that spawns in freshwater streams; the adults return to sea and the young remain in freshwater for two or three years. When fish are about 15 cm long, young salmon (called smolt) migrate to the ocean, where they can live for one, two or more years before returning to their natal river to spawn.

During spawning migration in late summer and autumn, the salmon move into estuaries. When adults prepare for spawning, the head of the male undergoes transformation. The head elongates and the lower jaw becomes enlarged and hooked at the tip to form a kype. The actual spawning site is chosen by the female who builds a nest (redd), usually in a gravel-bottom riffle area above or below a pool. The spawning male chases away other males and intruders from the female's redd. A certain percentage of males mature precociously before migrating to the ocean. These males adopt a sneaking behavioral tactic to achieve fertilization at a size of 10–15 cm (Vladić & Petersson 2015).

Capital breeding iteroparous species (i.e. species that can spawn more than once during their lifetime), such as the Atlantic salmon, *Salmo salar* L., store energy acquired for future reproductive purposes (Sibly & Calow 1986; Stearns 1992; Jonsson & Jonsson 2005; Roff 2015). Like all teleosts, Atlantic salmon exhibit indeterminate growth, the rate of which depends on resource acquisition. A single genotype may produce different phenotypes over an environmental gradient (referred to as a reaction norm or adaptability through phenotypic plasticity; Alm 1959; Charnov & Berrigan 1991; Tomkins & Hazel 2008). Functions describing a response of an individual phenotype to environmental variability are the measure of phenotypic plasticity (Hutchings 2011). Such plastic expressions of different genotypes in a range of environments are an individual property on which selection can act. In the anadromous Atlantic salmon, most of the energy acquired before spawning is obtained during marine

life. There are two types of investment in sexual traits, which determine individual male fitness: 1) primary sexual traits comprising investment in ejaculate quality that determines individual gamete fertility and 2) secondary sexual traits that determine the outcome of intrasexual competition for access to spawning females. Ejaculate investment consists of two contributing parts, sperm quantity (count) and sperm quality (i.e. fertilization capacity or fertility; Vladić & Järvi 2001). Increased sperm density in the ejaculate ensures greater fertilization success in the Atlantic salmon (Aas et al. 1991), and behaviorally sub-ordinate precocious males, which mature in their home river without migrating to the sea, invest relatively more in gonads and sperm count per ejaculate unit (i.e. spermatocrit) compared to behaviorally dominant anadromous adults (Gage et al. 1995; Vladić 2001). Adenosine 5'-triphosphate (ATP) is the main source of energy for sperm motility, thus sperm fertility depends on the ejaculate ATP content (Christen et al. 1987; Cosson et al. 1999; Vladić & Järvi 2001).

Male secondary sexual traits are targets for female choice and are decisive in intrasexual contests between males over access to receptive females (reviewed by Andersson & Simmons 2006). There are two male life histories in the anadromous Atlantic salmon:

- 1) anadromous males that reach large body size after spending several winters in the sea, and
- 2) anadromous males that spend a single winter in the sea before returning to their spawning grounds (termed grilse), consequently with a smaller body size compared to the former type of males. The latter type of males commonly partake in sperm competition with large multi-sea-winter males (MSW males) during spawning (Mjølnerød et al. 1998).

The male's nose and kype (lower jaw) are frequently used in intrasexual contests to monopolize females on the spawning grounds. Females, on the other hand, do not enlarge these morphological traits. Prior to the breeding season, these traits are therefore regarded as male secondary sexual characters. The size of the male adipose fin is correlated with the female mate preference (Järvi 1990). In addition, salmonid females exhibit aggression toward small males during spawning (Foote 1989; Fleming 1996).

The objective of this study was to compare the patterns of investment in primary (ejaculate mass, sperm density and ATP content) and secondary sexual traits (size of sexual ornaments) by assessing the effect of life history parameters in anadromous males of the Atlantic salmon on this investment. As behaviorally subordinate fish are commonly excluded from spawning by dominant, larger and older competitors,

smaller males are confined to a subordinate role in competition for fertilization. Therefore, we hypothesized that younger grilse should allocate their surplus energy to greater ejaculate quality (i.e. primary sexual traits) instead of elaborate secondary sexual traits, such as those of older multi-sea-winter males, to increase the chances of success in sperm competition. This should be reflected as a trade-off between primary and secondary sexual traits.

Materials and methods

During the peak of the breeding seasons in 1997 (N = 9; two MSW males, seven grilse) and 1998 (N = 17; eight MSW males, nine grilse), 26 mature, adult Atlantic salmon males were sampled from the Dalälven River breeding stock at the Fisheries Research Station (Älvkarleby, eastern Sweden). The returning wild fish used in a sea-ranching program for breeding were caught in the river near the research station using a trap deployed by a hydropower dam and kept all together in a basin until spawning in October–November. In the process of fish farming, all males spent two years in a hatchery before being released into the river (see Petersson et al. 1996). During sampling at the peak of the spawning season, the experimental fish were anaesthetized with tricaine methane sulfonate (MS-222, Thompson & Joseph Ltd, Norwich, England), weighted and stripped until empty of running milt. Ejaculates were weighed to the nearest 0.1 g and divided into two lots, one for sperm density measurements and the other one for energy content measurements. Ejaculate samples used for energy content measurements were frozen at -86°C. The length of the body, lower jaw (kype), nose and adipose fin was measured to the nearest 0.5 cm using a caliper (Petersson & Järvi 1993). The animals were treated in compliance with the standards and procedures established by the Swedish Ministry of Agriculture (permission license no. 34 3632/92).

Estimation of fish age and growth

Scales from the fish were collected just above the lateral line in the middle of the body and stored in individually marked paper bags for age reading. Imprints of scales from each individual were made on plastic slides and then read in a microfilm viewer (48 × magnification). The annual increments of scales were measured from the focus to the anterior edge along the antero-posterior axis since the posterior edge of the scale is usually worn out and uncertain to interpret. The first two clearly discerned annuli, located closest to the center of the scale, show the freshwater phase, while the third annulus shows the first marine year. The first distance was measured from the focus to the third annulus (marine zone) and the next one – from the first annulus of the marine zone to the annulus indicating the beginning of the second summer zone (i.e. First Sea Winter, 1SW) after physiological adaptation to marine life (i.e. smoltification). This is a distinctive measurement that separates grilse (the first year spent in the sea) from a multi-sea-winter (several years spent in the sea) anadromous male life-history tactic. The annulus after the last winter was treated as the last season in the sea (i.e. 3SW+). The distance between annuli was used as a growth indicator, because scale inter-circuli distance is directly related to the growth of salmonids (Doyle et al. 1987). In order to compensate for the allometric growth in freshwater (Tremblay & Giguère 1992), we adjusted the direct proportional formula for the freshwater phase (Lindroth 1960):

$$L_t = 18 + \frac{[(L_c - 18)r_t]}{r_c}$$

where L_t is the estimated body length at age t , L_c is the body length at catch, r_c is the scale radius at catch and r_t the scale radius at age t . The results of ANCOVA analysis together with beta values (measure of effect sizes) and their standard errors (measure of precision) are presented in Table 1. The significance level of the ANCOVA model was set at $p < 0.05$.

Table 1

Analysis of covariance (separate slopes model) of log10 SST and log ejaculate investment in the male age tactic with the covariate log soma mass and after the removal of the interaction term (reduced model)

Source	df	Mean square	F	Beta (tactic x soma)	SE Beta	p
Model SST	3	0.071	109.75	6.485	0.848	0.000
Model EI	3	5.844	3.759	-2.428	2.756	0.026
Age x soma	4	0.999	17.62	(0.133; 0.318)	1.061; 3.448)	0.000
Error SST	22	0.0001				
Error EI	22	1.555				

SST = Secondary Sexual Character (Trait) Index; EI = Ejaculate Investment; Age x soma = the interaction term; df = degrees of freedom. Beta values indicate effect sizes; in Tactic x Soma column; the first values applies to Beta for SST and the second value applies to Beta for EI. SE beta indicates the measurement precision (N = 26).

Measurement of sperm quantity and ejaculate quality

The ejaculate quality was estimated by measuring sperm density per ml ejaculate and ATP concentration per ml of ejaculate as previously described (Vladić et al. 2002). Spermatocrit was assessed as a reliable and sufficient measure of the sperm quantity in Atlantic salmon ejaculates (Aas et al. 1991). In short, spermatocrit was estimated as a percentage of sperm per ml of milt by centrifugation in a haematocrit centrifuge (2000 g) for five minutes immediately after stripping the fish, reading the spermatocrit values against a calibrated scale. ATP in the samples (a proxy for ejaculate quality) was measured by the luminometric method according to Hampp (1985) at pH = 7.75.

Analysis of life history traits

The analyzed life history traits were defined as follows (log-transformation was used to reduce the variability within the samples, thus fulfilling the ANOVA assumption of homogeneity of variance):

Adult body size is the natural logarithmic value of somatic weight (i.e. weight without gonadal weight and soma size).

Smolt body size was estimated by back-calculation from scale reading (see above). Logarithmic values were used.

Annual sea growth rate is calculated as: $\ln(\text{body length}) - \ln(\text{smolt length})$ divided by the number of years spent by a given fish in the sea.

Sea age is the number of winters spent by a male in the sea before returning to the natal river to spawn.

We performed Principal Component Analysis on the above life history traits to reduce the number of dimensions in the analysis of the above-measured life history traits and to create fewer variables containing measured life-history variables that could affect individual investment into primary (ejaculate investment index) and secondary (sexual traits index) characters. By doing this, we have obtained two principal variables that represent the variation caused by the life history traits. PC1 had the highest factor loadings for the sea age and adult size, i.e. log soma (hereinafter referred to as ALLOCATION; abbreviated ALL), whereas PC2 had the highest factor loadings for the first sea year growth rate and smolt size, which had

opposite signs (hereinafter referred to as ACQUISITION; abbreviated AQS). These two individual PC factor scores are used in the non-linear estimation models presented below (Table 2). The plotted factor loadings depicted the positions of the loadings in the analytical space (Table 2 and Fig. 2). The number of principal components (PC) to be retained in the analysis was decided according to the Kaiser criterion, i.e. PCs with an eigenvalue > 1 after varimax normalization. The first PC explained 55% of the co-variation among the traits (see above) and exhibited equal signs between significant traits, i.e. log soma and sea age, which is therefore defined as ALLOCATION. The second Principal Component explained an additional 34%

Table 2

PC of the life history traits used to determine the allocation and acquisition of energy for ejaculate quality and secondary sexual traits

Life history trait	PC 1	PC 2
SEA AGE	0.98	-0.07
SMOLT SIZE	-0.45	-0.78
GROWTH RATE	-0.12	0.96
<i>logSoma</i>	0.88	0.23
Eigenvalue	2.12	1.37
% of variance	54.7	34.2

The variables are Varimax normalized and arranged according to their PC loadings on the first axis. The eigenvalues indicate the proportion of variance extracted by the factors. Red bold fonts indicate values with loadings greater than 0.7. Eigenvalues greater than 1 are retained in the analysis (according to the Kaiser criterion). The first axis score (SEA AGE) and the second axis score (GROWTH RATE) are used as ALLOCATION and ACQUISITION trade-off, respectively. Cumulative percentage of variance explained: Factor 1 = 54.7%; Factor 2 = 89.0% (N = 26).

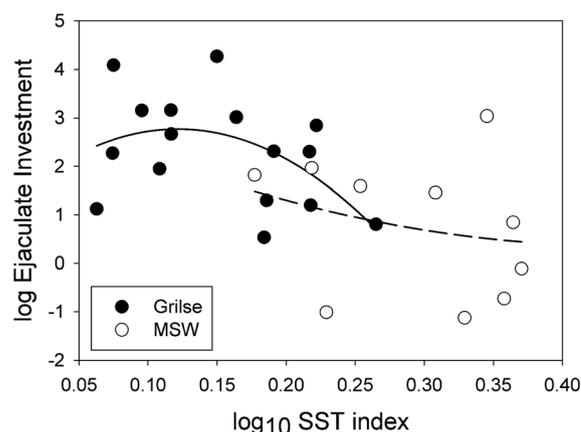


Figure 1

Polynomial form of the trade-off between primary and secondary sexual traits

ANCOVA, log Ejaculate investment in depending on \log_{10} SST index with strategy (grilse vs multi-sea-winter) as a covariate. Grilse least square mean EI = 1.97, multi sea-winter least square mean EI = 1.31; Model: $r^2 = 0.36$, $F = 6.345$, $p = 0.006$

of the co-variation by the first sea year growth and smolt size, which had opposite signs, indicating that body growth in the sea and smolt size are affected by resource intake, which is therefore defined as ACQUISITION trade-off.

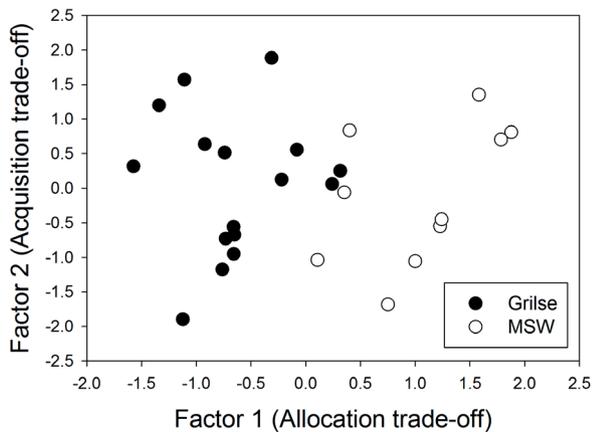


Figure 2
PC results for the correlation matrix of the four life history variables (sea age, smolt size, growth rate and log soma)

Secondary sexual traits index (SST) is the logarithm of the sum of the values for the kype, nose and adipose fin, divided by three to obtain the SST index, because there was no indication of a negative relationship, instead a positive relationship was found between the log adipose fin (independent variable) and the log kype and log nose (dependent variables; multiple regression: $r^2 = 0.883$, $F_{2,23} = 86.52$, $p < 0.001$).

Ejaculate investment index (EI) was calculated as the log weight of the stripped ejaculate multiplied by sperm quantity, i.e. spermatocrit (sperm concentration in the volume of 1 ml of semen) and quality, i.e. ATP concentration per ml of ejaculate.

Statistical analysis

We first tested whether slopes of the ejaculate investment on the body size functions of the two migratory life history tactics (i.e. grilse or multi-sea-winter males) differed in the homogeneity of variance. In the case of differences, the cause-effect relationship of the investment in ejaculate quality between grilse and multi-sea-winter males could not be easily distinguished from the body size allometry (as discussed by Tomkins & Simmons 2002).

There was no difference in the body length between the two years of collection (Kruskal-Wallis

test, $H = 0.036$, $p = 0.085$, $N = 26$), thus indicating that we could merge the data from these two years. To control the body size allometry, Analysis of Covariance (ANCOVA) on Secondary Sexual Traits (SST) and Ejaculate Investment (EI) was performed with the body length as a covariate. After testing for the interaction effect in the separate slopes model ANCOVA, the reduced ANCOVA model was used to test for differences between grilse and multi sea-year males in the SST and EI indices. To assess the relationship between the secondary and primary sexual traits, a linear regression between the \log_{10} transformed variables was performed. The significance level of these models was set at $p < 0.05$.

Following de Jong (1995), our next step was to search for function coefficients that are used as traits in the life history analysis. Therefore, we attribute the change in primary and secondary sexual traits to the life history, which consists of the developmental threshold (i.e. smolt size) and the environmental opportunity (i.e. sea growth and sea age). To understand how such conjugated life history traits affect the primary and secondary sexual traits, we designed user-defined non-linear models of the Taylor expansion type:

$$y = a + b1 \times ALL + b2 \times AQS + c1 \times ALL^2 + c2 \times AQS^2 + d1 \times (ALL \times AQS)$$

The models, defined as Model 1 (M1) and Model 2 (M2), assessed the amount of variation in SST and EI, respectively, where y is the amount of variation in SST (M1) or EI (M2), ALL stands for PC1 axis scores (allocation trade-off), AQS stands for PC2 axis scores (acquisition trade-off), a is the intercept, and b , c and d are constants (function coefficients). The form of these functions (concave or convex) can provide information on how individuals invest their surplus energy into primary and secondary sexual traits. The loss function was used [$L = (\text{observed} - \text{predicted})^2$]. The Gauss-Newton method was used as an estimation method. The significance of these models was tested according to critical values for correlation coefficients from Statistical Tables (Table R; Rolf and Sokal 2012, pp. 123–125). All statistical analyses were performed using the Statistica software (StatSoft Inc. 2014).

Results

Trade-off between primary and secondary sexual traits

No significant interaction effect of somatic mass on the migratory tactic was observed after testing

for the index of secondary sexual traits, SST (ANCOVA homogeneity-of-slopes model, interaction term log soma x tactic: $F_{1,22} = 0.015$, $p = 0.904$) and for the Ejaculate Investment index, EI (ANCOVA homogeneity-of-slopes model, interaction term log soma x tactic: $F_{1,22} = 0.001$, $p = 0.983$). Therefore, we could exclude the interaction effect from ANCOVA. The reduced ANCOVA model yielded a significant effect of the soma size allometry on the investment in both primary and secondary sexual traits and a significant effect of sea age x somatic mass interaction (Table 1). As expected, males that spent more than two years in the marine environment were significantly longer than grilse males (grilse males: $\mu = 76.91$ cm, $N = 16$; MSW males: $\mu = 108.15$ cm, $N = 10$; In model, $F_{1,24} = 37.81$, $p < 0.0001$). If males of the Atlantic salmon endure a trade-off when allocating the surplus energy in primary and secondary sexual traits, we should expect a negative linear regression after removing the allometric effect of the correlated body size. This theoretical prediction was corroborated (Fig. 1).

Trade-off between resource allocation and acquisition

we have examined the effect of body weight and sea age (PC 1 axis hereafter referred to as ALLOCATION trade-off) and the ability to acquire resources for growth during the first year in the ocean: smolt size and growth rate (PC 2 axis: hereafter called

ACQUISITION trade-off) on the primary and sexual male traits that oppose each other (Fig. 1). Therefore, we correlated PC1 and PC2 with the SST and EI indices, respectively, in order to discern the effect of the above life history traits on the development of individual primary and secondary sexual characters. A significant difference was found in ejaculate investment between grilse and multi-sea-winter males (reduced ANCOVA model, log Ejaculate Investment: $r^2 = 0.302$, $F_{2,23} = 4.965$, $p = 0.016$). Fisher's LSD post-hoc comparison revealed that grilse males invest more energy in each ejaculate than MSW adults (grilse males: $\mu = 2.311$; multi-sea-winter males: $\mu = 0.773$; $p < 0.01$; Fig. 3a). In addition, we found no differential effect of log soma on the SST index in grilse and multi-sea-winter males (ANCOVA, interaction term life history x log Soma mass, $F_{1,22} = 0.015$, $p = 0.904$). No deviations from the assumption of homogeneity of variance were detected (Levene's test, log₁₀ SST: $F_{1,24} = 0.432$; $p > 0.05$; log soma: $F_{1,24} = 0.462$, $p > 0.05$). A significant difference in log₁₀ SST investment between grilse and multi-sea-winter males was found (reduced ANCOVA model, log₁₀ SST investment $r^2 = 0.746$, $F_{2,23} = 33.91$, $p < 0.001$). Fisher's LSD post-hoc comparison revealed that grilse males invest less energy in secondary sexual traits than MSW adults (grilse males: $\mu = 0.153$; multi-sea-winter males: $\mu = 0.295$, $p < 0.001$) (Fig. 3b).

Different user-defined non-linear models were applied with only PC1 (ALL trade-off) and PC2 (AQS trade-off) as explanatory variables. Both model 1

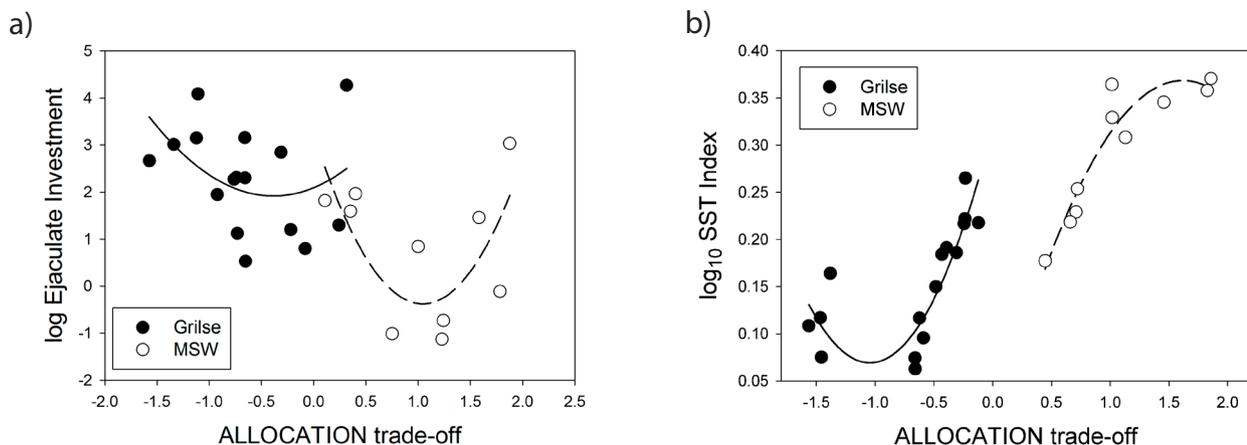


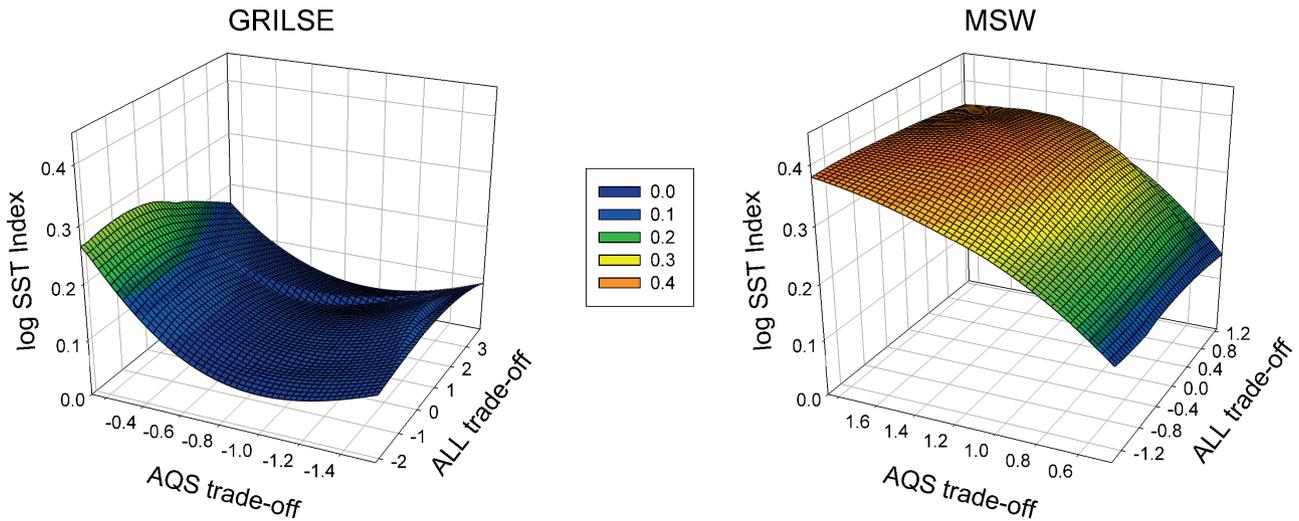
Figure 3

a. Polynomial function slopes depicting the effect of somatic condition/growth rate (ALLOCATION trade-off) on ejaculate investment in grilse and anadromous adult males (see Table 2 for the components in the column PC1). ANCOVA reduced model, $r^2 = 0.301$, $F_{2,23} = 4.965$, $p = 0.016$; within-cell regressions, strategy, $F_{1,23} = 1.012$, $p > 0.05$; SMOLT condition by fish age, $F_{1,23} = 0.668$, $p > 0.05$; **b.** Polynomial function slopes depicting the relationship between smolt size/growth rate on log₁₀ SST investment in grilse and anadromous adult males (see Table 2 for the components in the column PC1). ANCOVA reduced model, $r^2 = 0.747$, $F_{2,23} = 33.90$, $p < 0.001$; within-cell regressions, strategy, $F_{1,23} = 0.175$, $p > 0.05$; SMOLT condition by fish age, $F_{1,23} = 17.720$, $p < 0.01$

and 2 explained a significant proportion of variation in energy investment in SST (82.0% vs 63.3% for $p < 0.01$, cf. Rolf & Sokal 2012) and EI (58.2% vs 55.3% for $p < 0.05$, cf. Rolf & Sokal 2012; Fig. 4). However, SST was significantly affected by the parameter a and the allocation parameter b1 (a; $t = 12.961, p < 0.001$; b1; $t = 8.490, p < 0.001, df = 20$), whereas EI was affected

in a complex manner by parameters: a, the allocation parameter b1, the quadratic acquisition parameter c2 and interaction between the allocation and acquisition parameters d1 (a: $t = 2.533, p = 0.02$; b1: $t = -3.860, p = 0.001$, c2: $t = 2.796, p = 0.011$, d1: $-2.087, p = 0.05$; $df = 20$; Fig. 4).

a)



b)

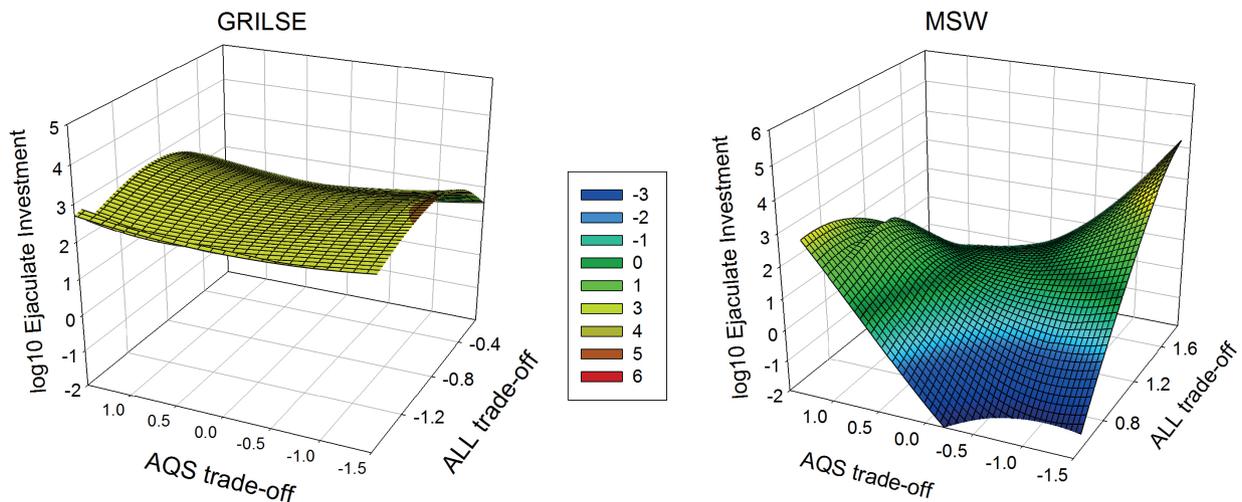


Figure 4

a. Model 1) The Model 1 (M1) of the form of equation: $y = a + b_1 \times ALL + b_2 \times AQS + c_1 \times ALL^2 + c_2 \times AQS^2 + d_1 \times (ALL \times AQS)$ assessed the amount of variation in SST (y) in relation to the allocation (ALL) and acquisition (AQS) trade-offs. Proportion of variance accounted for: 81%, $R = 0.90$ vs 0.57 for $p = 0.001$ (Rolf and Sokal 2012; Table R); **b.** Model 2) The Model 2 (M2) of the form of equation: $y = a + b_1 \times ALL + b_2 \times AQS + c_1 \times ALL^2 + c_2 \times AQS^2 + d_1 \times (ALL \times AQS)$ assessed the amount of variation in EI (y) in relation to the allocation (ALL) and acquisition (AQS) trade-offs. Proportion of variance accounted for: 51%, $R = 0.71$ vs 0.57 for $p = 0.001$ (Rolf & Sokal 2012; Table R)

Discussion

The evolutionary trade-off between the investment in secondary and primary sexual characters is a well-established phenomenon (for a review, see Simmons & Emlen 2006). This study reaffirms the ecological constraints that select for male differential allocation in reproduction by exhibiting a trade-off between primary and secondary sexual traits. The findings of this study could not reject our hypothesis that grilse males allocate their surplus energy into higher ejaculate quality instead of secondary sexual traits as a way to increase the success in sperm competition with older multi-sea-winter males. Therefore, the results presented here provide an indication that the strategy for allocating the surplus energy in the investment in primary and secondary sexual traits in the two male life histories is distinct, thus supporting our hypothesis that Atlantic salmon males partition the investment of surplus energy into these two components of male reproductive investment. In addition, we show that grilse and multi-sea-winter males represent alternative allocation and acquisition tactics of the Atlantic salmon.

Multi-sea-winter males have higher reproductive success than grilse males (cf. Jordan et al. 2007) and research on sperm competition between anadromous males found rather small contribution of competing males as the dominant single males fertilize more than 80% of eggs in a batch (Mjølnørød et al. 1998). The difference between grilse and multi-sea-winter males in investment into primary and secondary sexual traits found in this study indicates that these life histories are indeed alternative life-history paths, although they are usually presented as continuous life histories overlapping each other and resulting in variable mixed proportions of grilse, several multi-sea-winter males, and precocious parr in the population (Thorpe et al. 1998). This is indeed crucial, because environmental and maternal effects are more important than additive paternal genetic effects for the choice of male tactics governed by genetic monomorphism rather than polymorphism (Paez et al. 2010; reviewed in Vladić & Petersson 2015). Grilse may have better acquisition of resources in the first sea year so they can undertake return migration earlier than multi-sea-winter males, which need to stay longer in the ocean to reach the corresponding condition (cf. Salminen 1997). However, recent studies identified a strong genetic effect of the vestigial like-family member 3 gene across 57 wild populations of the Atlantic salmon, which promotes earlier sexual maturation in males but later sexual maturation in females, explaining 39% of the phenotypic variation (Barson et al. 2015). In another

system with alternative male reproductive tactics, the Chinook salmon (*Oncorhynchus tshawytscha*), Lehnert et al. (2018) found distinct alleles at the Major Histocompatibility I (MH I α_1) locus in anadromous and precocious jack males, which plays a role in the immune response.

An important effect of trade-offs is that they enhance individual fitness by affecting the shape of trade-off functions, driving the compromise between the investment into primary and secondary sexual traits. Convex trade-offs should select for semelparity, when all individuals postpone reproduction to the last phase of life (Michod 2007). Concave trade-offs should select for iteroparity when there is no “big-bang” reproduction; instead this would prolong the expected reproduction toward repeated spawning events (Michod 2007). This indicates that both grilse and MSW males exhibited concave functions reflecting the effect of allocation and resource acquisition on secondary sexual characters, but these have proven to be tilted toward opposite sides of the coordinate space (Fig. 4). Ejaculate investment was complexly affected by the functions modelled: grilse ejaculate investment was intrinsically greater than in the case of MSW males, as evidenced by the form of the function (positive floating), whereas MSW male ejaculate investment depended more on the prolonged resource acquisition in the sea to reach the spawning condition (concave-up, increasing; Fig. 4b). Together our results show that the interaction of smolt size and growth rate (i.e. acquisition trade-off) affects the secondary sexual traits in a quadratic fashion in both male types (concave-down form; Fig. 4a) and ejaculate investment in a multiplicative fashion in multi-sea-winter males, thus emphasizing that the ejaculate evolution should be understood in a complex, multivariate way (concave-up form; Fig. 4b; see also Lüpold 2013). From a biological perspective, this means that the Atlantic salmon grilse and older males may follow different developmental pathways. This could be attained via genotype-by environment interaction (Garant et al. 2003; Vladić et al. 2010) that affects the developmental canalization, so that reorganization of gene regulatory networks affecting body growth and gamete quality, and thereby social status, can be attained to achieve evolutionary innovation (so called modularity; see Holo 2015). While pointing to the high costs associated with achieving a high social status, Rudolfson et al. (2006) found that when males of Arctic charr, *Salvelinus alpinus*, reach a dominant position in the spawning hierarchy, they trade off this behavioral trait by producing ejaculates with a reduced sperm count, and that the gametes swim at a reduced velocity compared to ejaculates from subordinate

males. A trade-off between secondary sexual traits and spermatozoa quality is also found in guppy (Pitcher et al. 2003), bluegill sunfish (*Lepomis macrochirus*) (Neff et al. 2003), cichlid *Lamprologus callipterus* (Schütz et al. 2010) and chinook salmon *Oncorhynchus tshawytscha* (Flannery et al. 2012). In guppies, such a trade-off has a genetic basis (Evans 2010). Therefore, this trade-off seems to be a common feature across fish taxa. In conclusion, our study provides evidence that grilse and multi-sea-winter anadromous salmon represent alternative male reproductive tactics, which attain reproductive success by means of a trade-off in allocation and acquisition in primary and secondary sexual traits.

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