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Sperm quality in the alternative reproductive tactics of Atlantic salmon: the importance of the loaded raffle mechanism

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The outcome of sperm competition in species with alternative male reproductive strategies may be determined by fair or loaded raffle mechanisms. The sperm production and quality of male Atlantic salmon using alternative reproductive tactics were investigated in order to determine the relative importance of sperm quality for male reproductive success. Sexually mature resident parr males produced greater numbers of spermatozoa per millilitre of ejaculate and invested more in their gonads as a percentage of body mass than their anadromous counterparts. Parr males had greater proportions of motile spermatozoa and a greater sperm ATP content as compared with anadromous males. Parr males invested relatively more in sperm quality and sperm numbers after the effect of body size was accounted for. In fertilization experiments, parr males fertilized greater proportions of eggs than anadromous males. A polynomial model exhibited a trade-off between testes mass and ejaculate expenditure and explained 60% of the variation. These results establish that, in sperm competition with dominant males, parr males may compensate for behavioural subordinance by producing physiologically superior spermatozoa.

Keywords: sperm quality; ejaculate expenditure; gonadal investment; trade-off; Atlantic salmon

1. INTRODUCTION

The reproductive success of males in fish with external fertilization is often determined by the intensity of sperm competition, which is, in a broad sense, dependent on a male's ability to find and defend a female and on the ejaculate characteristics of competing males (Ball & Parker 1996). Sperm quality is generally found to correlate with the fertilizing ability of spermatozoa (Stoss 1983; Bencic *et al*. 1999; Levitan 2000). Beside investigations into sperm fertilizing ability (reviewed in Dziuk 1996), sperm quality has been estimated in several ways, including motility duration (Billard & Cosson 1992), morphological parameters (Holstein *et al*. 1988; Munkittrick *et al*. 1992) and metabolic rates (Kamp *et al*. 1996; Lahnsteiner *et al*. 1999). Theoretically, sperm competition may follow two mechanisms, namely a 'fair raffle' and a 'loaded raffle'. In the fair raffle, all sperm compete on equal terms, i.e. competing ejaculates are physiologically and energetically equal. Thus, only an increased number of spermatozoa in the ejaculate will yield a greater chance of success in a fair raffle (Parker 1990*a*).

Spermatozoa that are in sperm competition in a loaded raffle have unequal chances of succeeding in fertilizing an egg. Each spermatozoon will have a 'competitive weight', which is dependent on the competitive weights of the competing spermatozoa (Parker 1990*a*). A loaded raffle competition occurs when competing ejaculates display differing qualities (Parker 1990*a*,*b*). When the physiological quality of spermatozoa differs between competing males, it is expected that sperm quality will determine the outcome of competition. Ball & Parker (1996) defined ejaculate expenditure as $D = s \times r(m)$, where s is the number of sperm in the ejaculate and $r(m)$ is the sperm competitive ability as a function of sperm size (length) m. This relation can be understood as the product of the fair raffle term s multiplied by the loaded raffle term $r(m)$. The loaded raffle term $r(m)$ could be understood as any ejaculate property that is related to quality, such as sperm motility or energy resources.

Spawning salmonid males are characterized by intense intrasexual competition for access to nest-digging females. A male's proximity to a digging female determines the male's fertilization success, with males closest to the female being dominant over more distant males (Jones 1959; Fleming & Gross 1994). Theoretical models predict that a male in a subordinate role in sperm competition should invest more in sperm production than a dominant competitor male (Parker 1998).

Competition for finding and defending females in the Atlantic salmon *Salmo salar* has conferred strong selective pressure on several male traits, such as their morphology (Järvi 1990), physiology (Lahnsteiner *et al*. 1993), behaviour (De Gaudemar & Beall 1999) and life history (Jones 1959; Stearns 1992). It is typical in salmon that males adopt alternative mating tactics, with dominant anadromous males courting and defending females while younger resident parr males sneak into the spawn of the adult male and release sperm when the female expels her eggs and the dominant male ejaculates. This situation thus leads to sperm competition between parr males, whereas single dominant anadromous males more often mate in a pair with a female they monopolize, with no sperm competition taking place.

In this study, we tested the prediction that resident Atlantic salmon parr males invest proportionally more in sperm production and quality at the peak of the spawning season than anadromous males. In addition,

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the relationship between investment in the testes and ejaculate expenditure was investigated.

2. MATERIAL AND METHODS

(a) *Collection of fish and treatment of gametes*

All fish were sampled between 1 and 20 November 1998 at the National Board of Fisheries' Research Station, Älvkarleby, Sweden. The research station is located in the lower part of the River Dalälven, which flows into the Gulf of Bothnia, eastern Sweden (60°34′ N, 17°27′ E). The natural migration routes for returning salmonids in Älvkarleby were blocked after the hydroelectric plant was built in 1915. Due to destruction of the migration routes for spawning, the salmon population has been maintained by the propagation of fry reared artificially until the smolt stage, which are then released downstream from the dam as two-year old smolt. Twenty sea-ranched Atlantic salmon anadromous males and females were sampled from the home-returning stock trapped at the research station. Twenty sexually mature parr males from the hatchery were also sampled at the same time. All fish were anaesthetized with tricaine methane sulphonate (MS-222, Thomson and Joseph Ltd, Norwich, UK) before taking body measurements (weight and total length) and stripping the fish for milt and eggs. Care was taken to avoid contamination of the milt with urine and faeces. Prior to the fertilization experiments, the gametes were held in plastic beakers at 1–2 °C for no more than 3 h. After the experiments, the fish were sacrificed and the weight of their gonadal tissue was measured. Their total gonadal expenditure was measured as their testes mass as a percentage of their somatic body mass, i.e. the Gonado-somatic Index (GSI).

(b) *Sperm quantity estimation*

The stripped milt weight was obtained by stripping each anaesthetized male until it was empty of running milt and by weighing it to the nearest 0.1 g. Sperm concentrations per millilitre of ejaculate (spermatocrits) were assessed by centrifugation in a micro-haematocrit centrifuge (2000 *g*) for 5 min and reading the sperm cell density against a calibrated scale. The spermatocrit has been assessed as a reliable and sufficient method for estimating sperm concentration in Atlantic salmon ejaculates (Aas *et al*. 1991). The stripped sperm mass in the whole milt was calculated as the spermatocrit multiplied by the stripped milt weight. Since the stripped milt weight was positively correlated with body weight, the residuals of milt weight on body weight were taken in the calculation of sperm quantity in order to control for an allometric relationship between the body mass and stripped milt mass.

(c) *Sperm quality estimation*

Theoretically, each motile sperm cell has a chance of fertilizing an egg. The duration of a sperm cell's motility reflects sperm viability and correlates with the sperm fertilization capacity (Stoss 1983). Sperm motility was determined by microscopic observations under a Nikon phase-contrast light microscope (Shiyoda-ku, Tokyo, Japan) at ×100 magnification in triplicate. No cover slide glass was applied. Motility was initiated by suspending a drop of ejaculate in motility activation buffer (20 mM Tris–HCl, pH 8.0, 125 mM NaCl) (Moss *et al*. 1991) at a dilution ratio of 1:500 at 4 °C. This temperature has been previously determined as optimal for Atlantic salmon sperm motility (Vladić & Järvi 1997). Since we were interested in determining which components of sperm motility behaviour are of significance for sperm quality, sperm motility was assessed in three ways, i.e. as longevity, proportions and velocity. Sperm longevity was defined as the time-period until all spermatozoa in the sample observed under the microscope had stopped their forward movements, which characterize motile spermatozoa. Zero sperm motility in a given ejaculate commenced after the last spermatozoon in the visual field had started to vibrate, thereby marking the end of propulsive sperm motility, and other spermatozoa were immobile. The mean of three measurements was calculated for each fish. Sperm longevity was measured for 29 individuals (15 anadromous males and 14 parr males). In addition, sperm motility was video recorded on a VHS tape and replayed at 12 frames s^{-1} in order to determine the proportion of motile spermatozoa and the average velocity of the spermatozoa at three time-intervals, namely (i) 10 s after the initiation of motility, (ii) one-third of the total sperm longevity time and (iii) two-thirds of the total sperm longevity time. As each motile spermatozoa theoretically has a chance of fertilizing an egg, this was done in order to look at the variances in sperm longevity, with an emphasis on looking at the upper limit values. The videotape images were traced on transparent plastic sheets using a marker and the proportion of motile spermatozoa was calculated by dividing the number of progressively moving spermatozoa by the total number of spermatozoa on the screen. Sperm velocity was estimated by measuring the distances between sperm head positions at 12 time-steps over a time-period of 1 s. Sperm proportions were measured for 29 individuals (15 anadromous males and 14 parr males) and sperm velocity for 24 individuals (12 anadromous males and 12 parr males).

The main source of chemical energy for sperm motility is obtained from adenosine 5′-triphosphate (ATP), which is produced by the sperm mitochondria during the process of oxidative phosphorylation (Christen *et al*. 1987). Bencic *et al*. (1999) showed that the ATP content in Chinook salmon seminal fluid was only 0.06% of the ATP content in whole semen samples. The majority of seminal ATP is thus present in sperm cells. An ATP assay was conducted in order to estimate the ATP content in the milt from 17 anadromous males and 17 parr males. The ATP assay was conducted by the luminometric method according to Hampp (1985). Phosphoenolpyruvate was obtained from Sigma-Aldrich AB (Stenheim, Germany). The ATP monitoring reagent (containing firefly luciferase and D-luciferin) and ATP standard were obtained from Bio-Orbit (LKB, Wallac, Turku, Finland). All measurements were performed at room temperature in duplicate and the mean value of the two measurements was used. The ATP concentrations were expressed as nanomoles per millilitre of spermatozoa.

(d) *Sperm fertilization ability*

Laboratory experiments investigating the ability of anadromous male and parr male spermatozoa to fertilize eggs were performed in a spawning channel (18 cm × 10 cm) with river water running at 0.2 m s^{-1} at a temperature of 4–5 °C between 4 and 24 November 1998. A pair of males (one parr male and one anadromous male) was tested against one female in each fertilization experiment. During the experiment, 50 eggs from one of 20 females were applied in a batch and fertilized by one male only. The sperm mass from stripped parr males was on average 15% of the anadromous male sperm mass. In total there were 20 anadromous males, 20 parr males and 20 females. The parr males had consistently greater spermatocrit values than the anadromous males (table 1), so 200 µl of parr male milt was

Table 1. Summary of the data compared between anadromous males and parr males.

(*n* is the number of males contributing to the mean. Ejaculate weight is the residuals of stripped milt weight on body weight. Stripped sperm mass is the product of the spermatocrit and stripped milt weight. Sperm quantity is the product of the spermatocrit and stripped milt weight residuals on body size. Factor 1 (sperm quality) is the PCA factor scores first axis (see § 2e, § 3 and table 2). Ejaculate expenditure is sperm quantity × sperm quality. Factor 2 (velocity) is the PCA factor scores second axis with sperm velocity as the highest loading (see table 2).)

	mean value		s.d.					
variable	anadromous male	parr male	anadromous male	parr male	F	H	n	p
body weight (g)	7695.00	50.14	5837.31	13.10	—	29.27	40	0.000
total gonad weight (g)	262.96	5.04	153.66	1.84	—	29.27	40	0.000
GSI (%)	4.08	11.22	1.06	3.08	—	28.40	40	0.000
ejaculate weight (g)	4.23	−4.23	21.82	1.29	—	7.32	40	0.007
spermatocrit	0.43	0.76	0.18	0.15	42.27	—	40	0.000
stripped sperm mass	17.18	1.91	10.13	0.96	—	29.27	40	0.000
sperm quantity	2.04	3.18	2.62	0.30	—	8.67	24	0.003
ATP (nmol ml^{-1})	0.54	8.66	1.38	0.87	48.59	—	34	0.000
ATP residual spermatocrit	1.68	4.09	5.12	3.71	10.53	—	34	0.003
longevity (s)	781.40	208.10	740.90	111.70	—	2.33	29	0.127
proportion motile sperm								
10 s after initiation	0.41	0.90	0.41	0.19	—	9.40	29	0.002
one-third of total longevity time	0.01	0.41	0.02	0.42	—	12.06	29	0.001
two-thirds of total longevity time	0.01	0.06	0.01	0.18	—	6.53	29	0.011
sperm velocity (mm s^{-1})								
10 s after initiation	0.11	0.11	0.01	0.01	0.59	—	24	0.443
one-third of total longevity time	0.04	0.05	0.03	0.03	0.37	—	24	0.543
two-thirds of total longevity time	0.04	0.04	0.01	0.02	1.28	—	24	0.258
number of fertilized eggs	18.25	35.30	11.68	11.10	22.38	—	40	0.000
factor 1 (sperm quality)	0.55	0.79	0.27	0.11	—	5.07	24	0.024
ejaculate expenditure	0.89	2.50	2.17	0.38	—	12.40	24	0.000
factor 2 (velocity)	−0.03	0.03	1.12	0.92	0.03	—	24	0.869

applied and the volume of anadromous male milt was adjusted according to its spermatocrit value relative to the parr male in the pair. The resulting sperm quantities applied were therefore the same for each pair of males in the fertilization experiments (paired *t*-test for dependent samples of paired males, $t = 0.941$ and $p = 0.358$). Directly after the eggs had been placed at the proximate end of the channel, 200 µl of parr male stripped milt or, alternatively, the calculated milt volume from one anadromous male was applied in a standardized fashion using a laboratory pipette at the rear end of the spawning channel. The eggs were collected 10 min after fertilization and carefully rinsed with Buffodine (Evans Vanodine Int. PLS, Preston, UK) in order to prevent bacterial infection, and then eventually transferred to one of the 40 incubation holders containing running groundwater at 6–7 °C. Fertilized eggs were monitored weekly during development and alevines were sacrificed in April 1999.

(e) *Data analysis*

Sperm competitive ability was estimated as the factor score for the sperm physiological traits that are assumed to determine the sperm competitive weight in a principal components analysis (PCA). The traits determining the sperm competitive weight, i.e. $r(m)$, namely the logarithm of sperm longevity, the proportion of motile sperm and sperm velocity 10 s after activation, were included in the PCA together with ATP concentrations controlled for spermatocrit. It was possible to measure all these sperm traits reliably for 24 individuals, i.e. 12 anadromous males and 12 parr males. The factor scores from the first axis of these 24 individuals were taken as an estimate of sperm quality ($r(m)$). The sperm quantity (s) in the milt from these 24 individuals was estimated by taking natural logarithm values of the spermatocrit multiplied by the residuals from the linear regression of milt weight on body weight brought to positive values. A relative sperm quantity by body weight value was calculated in order to control for the body size disparity between alternative male phenotypes. We used the Kaiser criterion (i.e. eigenvalues > 1) in deciding on the number of factors retained in the PCA. The ejaculate expenditure (D) was calculated as the product of sperm quantity (s) and sperm quality ($r(m)$).

The frequency distributions of all variables were tested for normality using the Kolmogorov–Smirnov test. The comparisons between strategies for the variables measured were performed by analysis of variance (ANOVA). Regression lines between sperm quality and fertilization success were compared between alternative males using analysis of covariance. The variances were homogenized by using a natural logarithmic transformation. Kruskal–Wallis non-parametric ANOVA was performed for the comparison in the case of heteroscedastic variances. The covariation between testes mass and ejaculate expenditure was examined by a third-order polynomial regression model. The model was $y = a + b \times x + c \times x^2 + d \times x^3$, where y is the normalized ejaculate expenditure, x is the natural logarithm of testes weight and a, b, c and d are parameters. The significant values were set at $p \leqslant 0.05$. The statistical software used was Statistica (StatSoft Inc. 1999).

Table 2. PCA of the sperm physiological traits determining sperm competitive ability.

(The variables are Varimax normalized and arranged after their PCA loadings in the first axis. The eigenvalues relate to the variance extracted by the factors. Values with loadings greater than 0.7 are indicated with asterisks. The criterion used for retaining the principal components was the Kaiser criterion (eigenvalues > 1). The first axis scores for each individual are used as the sperm quality ($n = 24$).)

component	factor 1	factor 2
percentage of motile sperm after 10 s	0.838*	0.172
ATP concentration	0.715*	0.162
sperm velocity at 10 s	0.223	0.874*
log sperm longevity	0.665	−0.522
eigenvalues	1.724	1.073
percentage of the variance	43.110	26.826
cumulative percentage	43.110	69.937

3. RESULTS

The parr males produced gonads that were only 2% of the weight of the testes produced by the anadromous males. However, expressed as a percentage of body mass, the parr males had greater GSI values, with the anadromous males investing on average only 36% of the parr male gonadal investment (Kruskal–Wallis ANOVA, $p < 0.001$). The parr males had on average 1.8 times greater spermatocrit values. Nevertheless, the average estimated stripped sperm mass was absolutely greater in the anadromous males (table 1).

The sperm ATP concentrations were positively correlated with the spermatocrit values, indicating that greater sperm densities hold greater ATP reserves ($F_{1,32} = 15.43$, $r = 0.570$ and $p < 0.001$). Therefore, the sperm ATP content was compared between strategies using the residuals from the linear regression of the sperm ATP concentrations on the spermatocrit values. This test revealed a significant difference in ATP content between the sperm of the anadromous males and parr males (table 1).

No difference could be detected in the mean sperm longevity time between the anadromous males and parr males. Although most spermatozoa in a drop of ejaculate stopped the characteristic forward movement of motile spermatozoa within 2 min, single anadromous male spermatozoa were observed to be motile even at 20 min after the initiation of motility at 4 °C. The proportion of motile spermatozoa was consistently greater in the parr male ejaculates for all three of the time-steps analysed, namely 10 s after sperm motility initiation and one-third and two-thirds of the total longevity time (table 1). Sperm velocity declined over the course of time, from 0.11 mm s^{-1} at 10 s after motility initiation to 0.043 mm s^{-1} after one-third of the ejaculate lifetime, although the sperm velocity did not differ between the anadromous males and parr males (ANOVA repeated measurements, $F_{2,44} = 0.28$ and $p < 0.757$) (table 1).

The first axis of the PCA scores the most clearly reflected association between ATP concentrations and the proportion of motile sperm 10 s after the initiation of motility, whereas the second axis was the most strongly related to sperm velocity. The first PCA axis explained 43% of the variance and the second axis explained 27% (table 2 and figure 1). The factor scores of the first axis corresponding to sperm quality were Varimax normalized and standardized to values between zero and unity, and the sperm competitive weight was compared between alternative male mating tactics. One-way ANOVA yielded a significantly greater sperm quality, i.e. $r(m)$, for the parr males. Sperm quantity, i.e. s, was also greater in the parr males after taking a body size allometry into account (table 1). Consequently, the total ejaculate expenditure (D) was greater in the parr males (table 1). The second PCA axis loaded most heavily upon sperm velocity. No difference was detected between strategies in the second PCA axis (table 1 and figure 1).

When the anadromous males and parr males did supply equal numbers of spermatozoa for fertilizing the eggs (paired t-test, $t = 0.941$ and $p > 0.358$), the parr males fertilized a significantly greater proportion of eggs (table 1). Sperm quality, as derived from the factor scores of the first PCA axis, was positively correlated with fertilization success when the strategy effect was controlled for

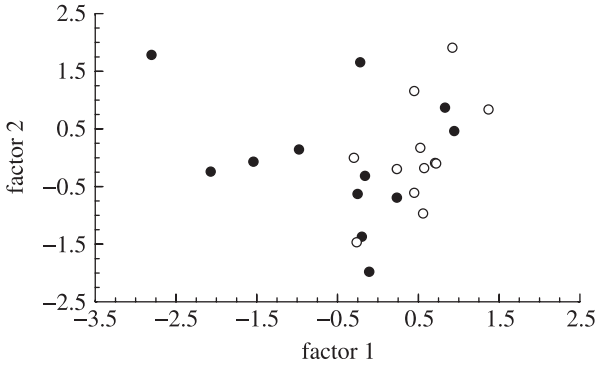

Figure 1. Results of the PCA of the correlation matrix of the four sperm physiological variables: ATP concentration (residuals of the spermatocrit), percentage motile sperm 10 s after motility initiation, sperm velocity 10 s after motility initiation and sperm longevity. The rotation was performed by Varimax normalization. The loadings for the variables are presented in table 2. Solid circles, anadromous males; open circles, parr males.

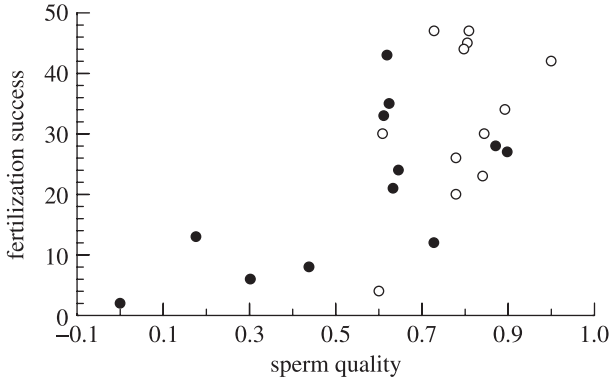

Figure 2. Relationship between sperm quality and fertilization success for the anadromous males and parr males (see table 2 for the components defining sperm quality). The principal components factor scores of the first axis are standardized between zero and unity (see § 3). ANCOVA within-cell regression, $F_{1,21} = 8.846$ and $p < 0.01$. Solid circles, anadromous males; open circles, parr males.

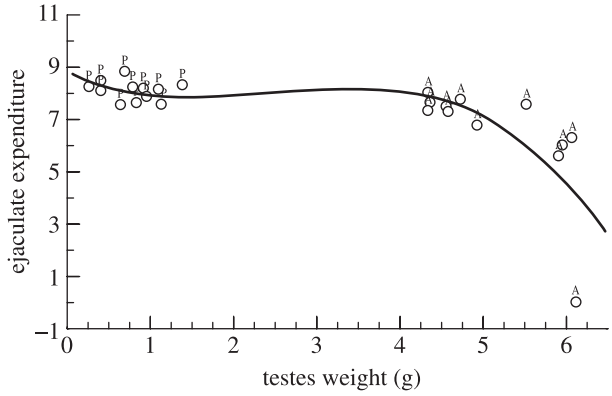

Figure 3. A general polynomial model applied in order to reveal the relationship between the logarithm of testes mass and ejaculate expenditure. The model was $y = a + b \times x + c \times x^2 + d \times x^3$. The simplex estimation method reveals that the parameters $a = 8.84$, $b = -1.55$, $c = 0.74$ and $d = -0.10$ minimize the residual variance around the regression lines (loss function $=$ (observed $-$ predicted)^2). The model explained 60.0% of the variance. P, parr males; A, anadromous males.

by an ANCOVA (ANCOVA within-cell regression, $F_{1,21} = 8.846$ and $p < 0.01$) (figure 2).

The polynomial model describing the covariation between testes mass and ejaculate expenditure explained 60% of the variance in ejaculate expenditure (figure 3). As illustrated in figure 3, male life histories are separated in the model, exhibiting a negative association between testes mass and ejaculate expenditure.

4. DISCUSSION

This study found that the parr males sampled invested approximately two times more in their gonadal tissue as a percentage of their somatic tissue and in their spermatocrit than the anadromous males sampled. These results agree with the prediction derived from the loaded raffle hypothesis, namely that males that are more likely to experience sperm competition are selected for greater investment in sperm production (Parker 1990*a*,*b*). However, this effect does not imply that individual parr males are able to compete with anadromous males solely on the basis of sperm numbers because of the disproportionately greater adult size and, consequently, greater numbers of sperm per ejaculate. Existing evidence suggests that individual precociously mature Atlantic salmon parr males are capable of fertilizing 5–26% of eggs in a redd (Hutchings & Myers 1988; Thomaz *et al.* 1997).

Several studies have reported a greater sperm longevity time in Atlantic salmon parr males than in anadromous males (Daye & Glebe 1984; Gage *et al.* 1995; Vladić 2000). We could not confirm a longer period of sperm motility duration in parr males at an optimal temperature in this study (see Vladić & Järvi 1997). A possible explanation for this discrepancy in the duration of sperm motility may be the effect of greater spermatocrit values in parr males. The duration of sperm motility may be masked by the greater spermatocrit values of parr male ejaculates. Since parr males have greater spermatocrit values, the chance of observing some spermatozoa still moving is greater for parr male than anadromous male spermatozoa. However, a very long sperm motility duration, as observed in the anadromous males in this study, is not expected to confer any significant advantage to such a male, since eggs are fertilized within several seconds after the expulsion of gametes (Ginsburg 1968) and the chance of fertilizing eggs will increasingly diminish after emitting gametes into freshwater because of the osmotic shock on sperm membranes (Perchec *et al.* 1996). Thus, sperm vigour, i.e. the proportion of motile sperm within an ejaculate, is a more important trait than sperm longevity in fish reproduction (Cosson *et al.* 1999).

The velocity of the attachment of spermatozoa to an egg membrane has been suggested as a major factor in determining success in heterospermic mammalian inseminations (Dziuk 1996). In the present study, the sperm velocity declined from 0.11 to 0.037 mm s^{-1} over time, ranging from 10 s to two-thirds of the time since the initiation of sperm motility in accordance with depletion of sperm ATP stores. There was no indication that sperm velocity differs between alternative reproductive tactics. However, the proportions of motile spermatozoa were consistently greater in the parr males. This suggests that parr males compensate for their behavioural subordinance by producing numerous spermatozoa with greater vigour. This result agrees with the work on a teleost fish, i.e. the turbot, by Dréanno *et al.* (1999) who found that the percentage of motile sperm was a more important trait for achieving fertilization than sperm velocity.

In a situation where dominant behaviour results in the exclusion of subordinate anadromous males, an alternative, sneaking tactic may become selectively favoured (Fleming & Gross 1994). Parr males that sneak into the spawn of anadromous fish should invest more in sperm physiological competitiveness than anadromous adults, which invest in secondary sexual traits. This situation creates intense sperm competition in the spawning ground. In accordance with this, the consistently greater proportions of motile spermatozoa in the parr male ejaculates, which indicated greater sperm vigour, may be explained by the greater ATP concentrations. In addition, the differences in sperm ATP concentrations between the anadromous males and parr males are consistent with the results from fertilization experiments. When both types of males had an equal sperm quantity applied in the experiments, the parr males fertilized a significantly greater proportion of eggs. Thus, although parr males are unable to deliver similar numbers of spermatozoa in an ejaculate due to the correlated discrepancy in body size between the strategies (Gross 1985), they are capable of compensating for this disadvantage by producing metabolically superior spermatozoa.

It was assumed that the association between the sperm physiological properties in the PCA would yield the sperm quality. In doing so, sperm quality was quantified as being composed of several physiological traits. In the first PCA axis, the degree of association factor score was the strongest between the proportion of motile sperm at the beginning of sperm motility and ATP concentration. A significantly positive correlation between sperm quality, as quantified by the first PCA axis, and fertilization success implies that such sperm physiological traits as

ATP concentration and sperm vigour are appropriate traits for determining sperm quality. Moreover, it was found that the parr males invested more in sperm quantity after accounting for the effect of body size. Thus, the total ejaculate expenditure was greater in the parr males. This result thus implies that, for parr males, sperm quality plays a significant additive role in determining the outcome of the sperm competition in a numerical raffle with anadromous males.

An interesting finding in the current study was the response of the anadromous males and parr males to the function of ejaculate expenditure on testes mass. Both types of male showed decreasing ejaculate expenditure with an increase in testes mass. Thus, such a physiological trade-off implies that investment in relatively large gonads is energetically costly.

In summary, this study showed that behaviourally subdominant parr males invest more in their gonads and in more dense ejaculates with spermatozoa containing greater ATP concentrations than anadromous males. The latter finding has two consequences. First, the greater ATP content in parr male spermatozoa allows their gametes to be of greater vigour and, second, high-quality spermatozoa make parr males capable of gaining increased reproductive success when the effect of loading in sperm competition is included. The trade-off between ejaculate expenditure and investment in the testes indicated the cost of maintaining large testes. This study was designed to investigate the relative effect of sperm quality in order to clarify the proximate mechanism of sperm competition between the alternative reproductive tactics of Atlantic salmon. We conclude that males that are more likely to experience sperm competition increase their competitive ability by producing metabolically superior spermatozoa. In the light of this conclusion, a question of significant interest, which was not studied here, relates to male ejaculation tactics in direct sperm competition under natural spawning conditions.

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