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Effects of Alternative Male Mating Strategies on Characteristics of Sperm Production in the Atlantic Salmon (Salmo salar): Theoretical and Empirical Investigations

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Effects of alternative male mating strategies on characteristics of sperm production in the Atlantic salmon (*Salmo salar*): theoretical and empirical investigations

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SUMMARY

Atlantic salmon (*Salmo salar*) males mature as either tiny precocious parr before seaward migration, or as older and larger anadromous males. Anadromous males dominate the spawning redds and aggressively defend females against parr intrusions. Parr gain fertilizations by sneaking in to ejaculate while anadromous males and females spawn. Such differences in mating advantage generate asymmetries in risk of sperm competition between the male strategies. Theoretical sperm competition models predict that males typically mating in disfavoured roles (here, the parr strategy) should be selected to offset this disadvantage by investing more into spermatogenesis to achieve fertilization success. First, we present a theoretical model which analyses gametic expenditure for salmon parr and anadromous male reproductive strategies. We then use the natural variance in mating pattern within this species to compare empirically how males invest in spermatogenesis. A range of reproductive traits were measured for both male strategies. Absolutely, anadromous males have larger testes and produce greater numbers of sperm than parr males. However, results show that parr invest relatively more heavily into total spermatogenesis, and have a larger gonosomatic index than anadromous males. Relative to body size, parr produced greater numbers of sperm and volumes of stripped ejaculate. There was no difference in sperm length between the two male strategies. However, more sperm were motile in parr ejaculates, and these sperm lived longer than anadromous male sperm. Our findings may explain how male parr, under elevated risks of sperm competition and occupying a disfavoured mating role (parr weigh only 0.15% of the average body mass of anadromous males) achieve disproportionately high fertilization success.

1. INTRODUCTION

Sperm competition occurs when sperm from different males compete for fertilizations (Parker 1970). It is a cryptic, yet potentially powerful, form of sexual selection, responsible for a variety of male reproductive anatomies, physiologies and behaviours in a diversity of taxa (e.g. Smith 1984). Sperm competition is a topical area of investigation, although fish have received less attention (but see Shapiro *et al.* 1994; Warner *et al.* 1995), despite exhibiting a diversity of reproductive modes and mating patterns (e.g. Breder & Rosen 1966). Because of the practical difficulties presented by experimentation at the gamete level, advances in understanding sperm competition have been made using a comparative approach (e.g. Harcourt *et al.* 1981; Møller 1988*a*, *b*; Gomendio & Roldan 1991; Harcourt 1991; Briskie & Montgomerie 1992; Svärd & Wiklund 1989; Stockley & Purvis 1993; Gage 1994). One of the most informative comparisons may be drawn between populations within a species (Harvey & Pagel 1991) which exhibit discrete alternative mating tactics presenting different risks or intensities of sperm competition. Such an approach may be instructive because intraspecific comparisons do not carry the problems associated with across-species comparisons, such as confounding effects of phylogeny (Harvey & Pagel 1991). Here we compare sperm production characteristics of two male reproductive strategies adopted by male Atlantic salmon (*Salmo salar*), which are associated with different levels of sperm competition. We set a theoretical background, and compare predictions with empirical findings.

Male Atlantic salmon adopt one of two distinct reproductive strategies at maturity (Jones 1959). Immature male parr may transform into smolts and migrate to sea for a period of intensive feeding and rapid growth. These males then migrate back to their natal river, changing into breeding condition, and spawn on the redds as dominant anadromous males. Alternatively, male parr may achieve reproductive maturity before seaward migration. These relatively tiny precocious parr sneak for matings in competition with the much larger anadromous males. Anadromous males dominate the spawning beds and aggressively drive off the smaller subordinate males (Belding 1934;

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Jones 1959), sometimes resulting in injury or death of the parr (Hutchings & Myers 1987). Large anadromous males therefore defend females and smaller males must compete to varying degrees for access to the fertilization site. Females prefer to spawn with larger anadromous males (Jones 1959; but see Myers & Hutchings 1987) and parr therefore experience greater levels of sperm competition as they sneak for matings. Anadromous males may successfully defend females to the exclusion of rival males (Belding 1934; Jones 1959) and therefore, on average, experience lower levels of sperm competition.

Theoretical analyses of sperm competition games suggest that it may be adaptive for males to play different ejaculatory strategies depending upon their 'role' in the competition (Parker 1990*a*, *b*). The models analyse sperm competition games using an evolutionarily stable strategy (ESS) approach (see Maynard Smith 1982) where stable male strategies, such that no hypothetical mutant can invade the population, are determined. In Atlantic salmon, the two male reproductive strategies provide an ideal opportunity to examine how males allocate resources to somatic and gonadal tissue, and how resources are partitioned within sperm production according to the different risks of sperm competition. Theory predicts that increased sperm competition selects for males to increase relative investment in spermatogenesis (Parker 1982, 1990*a*, *b*, 1993). This prediction is supported by comparative studies of mammals (Harcourt *et al.* 1981; Møller 1988*a*), birds (Møller 1988*b*) and butterflies (Gage 1994): in species where the mating pattern generates higher levels of sperm competition, males have evolved larger testes relative to body size. In humans, males with larger testes and greater sperm production rate are subjectively judged more likely to be involved in sperm competition (Baker & Bellis 1995). Increased investment in spermatogenesis may occur because sperm competition proceeds numerically, like a raffle (Parker 1982, 1990*a*, *b*). Thus males are driven to produce maximal numbers of minimally sized sperm to outnumber rivals. Evidence from sperm mixes indicates that sperm numbers may be important for competition success (Martin *et al.* 1974; Simmons 1987). However, there is also evidence from comparative studies that sperm competition may select for increased sperm length (Gomendio & Roldan (but see Harcourt 1991); Briskie & Montgomerie 1992; Gage 1994). Longer sperm may swim faster and more powerfully (Katz & Drobnis 1990; Gomendio & Roldan 1991), which may be advantageous if sperm competition follows the principles of a race, or where sperm must compete by swimming actively. The relations between competitive gain and investment in increasing sperm size or number may be determined by the mechanism of sperm competition (Parker 1993). The precise mode of sperm competition determines the fitness curves for investment in sperm speed, propulsive force, longevity or number. The comparison of sperm form and function across individuals within a population that consistently experience different sperm competition intensities can therefore reveal how sperm competition operates.

In this study we first present a theoretical background, analysing a model of gametic expenditure for the two male strategies in Atlantic salmon. We then determine sperm production characteristics of the two male types empirically. We compare gross and specific investment in spermatogenesis for both strategies. The gonosomatic index (the testes mass as a percentage of somatic mass = GSI) and the numbers, activity and morphometry of mature sperm in storage are measured to make specific comparisons. As we know that the strategies generate different risks and intensities of sperm competition (e.g. Jones 1959; Hutchings & Myers 1988), we can examine how sperm competition selects for specific investment into sperm form and function in an externally fertilizing fish.

2. THEORETICAL BACKGROUND

In the Appendix, we analyse a simple ESS (Maynard Smith 1982) model of gametic expenditure for salmon parr and anadromous males: it represents an extension of the sperm competition game models of Parker (1990*a*, *b*) in which a given male's fitness is the product of the number of spawnings achieved, and the expected progeny gained from each one. A trade-off is assumed between ejaculate effort and mate searching effort: increasing the ejaculate mass decreases the number of spawnings attained. The payoff of a given male depends on the ejaculate expenditures of the other males in the fertilization scramble. Because it is an ESS model, it is phenotypic and does not include genetics. For this purpose, parr and anadromous males can be assumed to belong to separate populations. To avoid complexity, we assume that fertilization is virtually instantaneous and that sperm size is equal for all males. In the model, at each spawning, an anadromous male is present on all occasions. With probability $(1-p)$ he is the only male present and there is no sperm competition. With probability p, N parr are also present. Thus the intensity of sperm competition on all males can be increased by varying N. (With a male-biased anadromous operational sex ratio, anadromous males may also be in competition (Jones 1959), however male parr appear to outnumber anadromous males on the redd and to avoid complexity we set the model to analyse competition between an anadromous male and N parr). We can maintain an asymmetry in the probability of sperm competition between the alternative mating strategies (through p): parr always encounter sperm competition from at least one other fish, but anadromous males occasionally do not encounter sperm competition (unless p is set to 1.0). The probability that no parr are present (zero sperm competition for anadromous males) is expected to decrease with the mean number of parr, following a Poisson probability (see Appendix).

We assume that fertilization success follows the 'raffle principle' (Parker 1990*a*) so that a given male's gains are proportional to his sperm numbers relative to the total number of sperm in competition. So that the raffle can be 'loaded' in favour of one or other male strategy (e.g. due to the ability of one or other male type to release sperm closer to the eggs); sperm from

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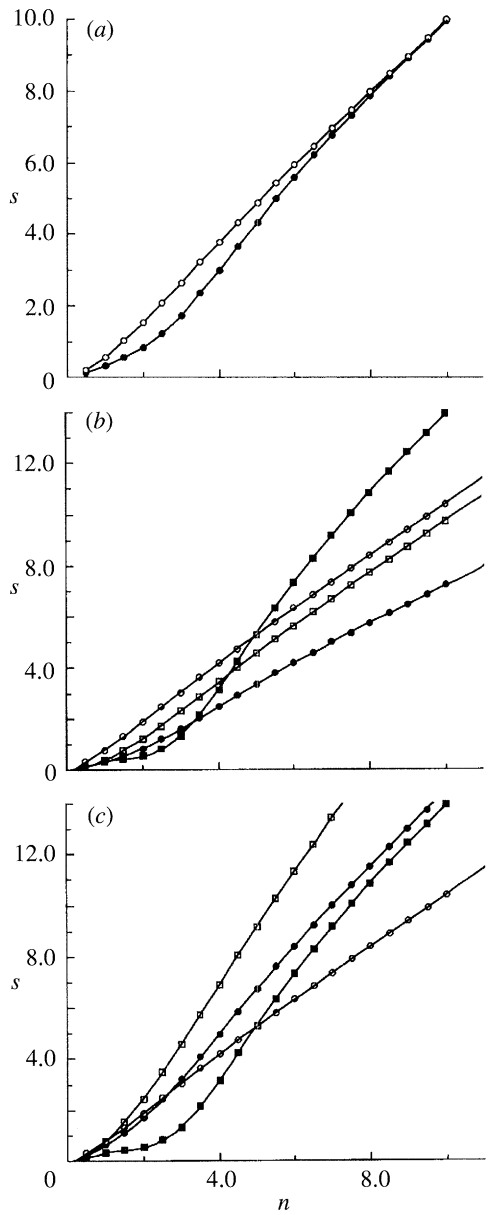

Figure 1. Some numerical results of the theoretical model. The ESS ejaculate expenditure for anadromous males, s_a^*, is given in solid symbols; that for parr, s_p^*, is given in open symbols. (*a*) A case where the only asymmetry between the two mating strategies is that anadromous males compete against N parr with probability p, whereas parr always compete against an anadromous male and $N-1$ other parr. Other parameters are symmetric ($r = 1.0$, $C/D = 1.0$ for both mating strategies). The fact that anadromous males are sometimes free of sperm competition causes their ESS sperm expenditure to be below that of parr, especially at low N where the probability of zero sperm competition is highest. (*b*) The same asymmetry applies as in (*a*) (anadromous males are free of sperm competition with probability $(1-p)$, and $C/D = 1.0$ for both mating strategies. However, r, the relative value of parr sperm is not equal to 1.0, so that the fertilization raffle is loaded. When $r = 2.0$ (squares), a parr sperm has twice the chance of fertilizing as an anadromous sperm. This causes anadromous males to increase their ejaculate expenditure beyond that for parr at high N values, though at low N values this effect is overridden by the high probability of zero sperm competition for anadromous males. When $r = 0.5$ (circles), a parr sperm has only half the chance of fertilizing as an anadromous sperm. This adds to the 'zero sperm competition' effect, so that parr should have higher sperm expenditure across the entire range of N. (*c*) The same asymmetry applies as in (*a*) (anadromous males are free of sperm competition with probability $(1-p)$, and all sperm have equal prospects ($r = 1.0$). However, C/D (the cost of gaining a mating relative to the cost of producing sperm) is unequal for the two mating strategies. When $C/D_{\text{parr}} = 2.0$ and $C/D_{\text{anadromous}} = 1.0$ (squares), parr can produce each unit of sperm twice as cheaply (relative to obtaining matings) as anadromous males, and hence have much higher sperm production – this escalates the sperm levels of both strategies. In the reverse case where $C/D_{\text{parr}} = 1.0$ and $C/D_{\text{anadromous}} = 2.0$ (circles), the opposite applies, though this effect is overridden at low N by the 'zero sperm competition' effect.

each strategy are assigned different 'competitive masses'. Gains to a male are proportional to the number of his sperm multiplied by their competitive mass, divided by the sum of all sperm in competition multiplied by their competitive masses. The competitive mass of anadromous sperm is standardized as 1.0; parr sperm have a relative competitive mass of r. Thus if $r = 0.5$, each parr sperm has only half the chance of fertilizing an egg as each anadromous sperm; if $r = 2.0$, each parr sperm has twice the chance of each anadromous sperm. The cost of obtaining a mating (C), and the cost of producing a sperm (D) are also allowed to differ for the two mating strategies.

Conclusions can be summarized as follows. Consider first a symmetric scramble in which all males face equal circumstances: p is set to 1.0 for equal sperm competition; r is set to 1.0 so that all sperm fare equally; and the ratio of the two forms of costs (C/D) is equal for parr and anadromous males. This case generates the result that sperm expenditure should rise linearly with the number of fish present minus one (N ; see also Parker & Ball 1995).

Biologically, however, there are always likely to be asymmetries between parr and anadromous males, and numerical solutions for such cases can be iterated from equations (6*a*), (6*b*) of the Appendix. Computations show that there are three biological effects which act to increase expenditure on sperm, so that the effects of asymmetries between the two mating strategies can be predicted from the following.

(*a*) *Higher intensity of sperm competition*

Parr always face sperm competition, whereas anadromous males are entirely free from sperm competition with a probability p. This means that the sperm competition faced by parr always exceeds that faced by anadromous males, but decreasingly so as parr become commoner because p declines with N. This causes sperm effort from parr to increase beyond that of anadromous males. The nature of the difference depends on the form of p (N). In the Appendix, p (N) is an approximation of a Poisson process for the distribution of parr among spawning sites. It generates the intuitively appealing result that the biggest difference between parr and anadromous expenditures occurs when N is low so that p is high. At large N, p approaches 1.0 and expenditures converge if other aspects (r, C/D) are also equal (see figure 1*a*). There is

little information regarding the 'normal' number of precocious parr present in competition at spawning. It seems likely that numbers are highly variable both temporally and spatially, given that the proportion of the male parr population that reach sexual maturity varies by year and population (Jones 1959). Over 80% of some male parr populations may develop testes (Myers *et al.* 1986), and some spawning populations may exceed ratios of 20 mature parr per anadromous male (Hutchings 1986). At this ratio, parr fertilization success (relative to one anadromous male) reaches an asymptote (Hutchings & Myers 1988).

(*b*) *Disfavoured role*

The disfavoured role is modelled by r, the weighting of sperm in the 'loaded raffle'. Following Parker (1990*a*), we find that competitors in the disfavoured role are pushed into a higher sperm expenditure (see figure 1*b*). Parr appear to reside in the disfavoured role for mating. Females prefer to spawn with anadromous males (Jones 1959) and anadromous males aggressively defend ripe females and drive parr away. Anadromous males therefore have the 'information advantage' and are closer to the female when spawning begins.

(*c*) *Higher relative sperm costs*

The C/D ratio: (cost of obtaining a spawning)/(cost of producing a sperm) exerts an important effect on relative sperm expenditure. The higher this ratio, the lower the costs of sperm relative to the costs of obtaining matings. Higher C/D ratios favour higher sperm expenditure (see figure 1*c*; see also Parker 1990*b*; M. A. Ball & G. A. Parker, unpublished data) Thus the strategy with the higher C/D ratio will have (if all other aspects are equal) the higher sperm expenditure. However, it is intuitively obvious that the C/D ratio must depend on body size: parr are approximately 0.15% the body size of anadromous males, and are therefore not able to support testes of equivalent size to anadromous male gonads. We suggest that the C/D ratio is directly related to body size. Because of the constraints of body size, the cost of producing 1 absolute unit of sperm will increase with body size (smaller male body size may constrain ejaculate size and recovery time for further ejaculate production, see for example Simmons 1995). The costs in obtaining a spawning (by parr or anadromous tactics) may not be as different with respect to body size: both male strategies incur temporal costs as they wait on the redds for spawning opportunities and both strategies may generate mortality costs. Parr suffer risk of mortality from anadromous male aggression on the redds (Hutchings & Myers 1987) whereas anadromous males suffer mortality risks during the period of migration and feeding at sea (Mills 1989). More data on the risks presented to either male strategy are required to evaluate our assumptions. Because male body size may be negatively related to sperm production costs, we therefore expect C/D to increase approximately in direct proportion to body size. Because we have set C/D to 1.0, the relations demonstrated in figure 1 are interpreted as relative, not absolute, sperm expenditures. The actual sperm expenditure is Ds^*, where D scales directly with body size. Figure 1*c* is therefore to be interpreted as the effect of deviations from the situation where C/D increases in direct proportion to body size.

3. MATERIALS AND METHODS

We sampled reproductively mature male Atlantic salmon that had been trapped in the North Tyne system, U.K. and were maintained in all-male holding tanks at Kielder salmon hatchery.

(*a*) *GSI*

Anadromous and parr males in breeding condition were randomly selected for GSI measurement after one stripping. Males were killed, weighed, and the testes dissected out of the abdominal cavity and weighed. GSI was determined by calculating testes mass as a percentage of body mass (de Vlaming *et al.* 1982).

(*b*) *Sperm counts*

All dissociated mature sperm in storage in each male were stripped into plastic tubes by experienced fish culturists. The area around the vent of each fish was dried to prevent contamination by mucus and water, and care was taken to avoid urine contamination. Males had not been stripped for at least seven days to allow their sperm reserves to fill. The mass of the male and the volume of the stripped ejaculate were recorded. Strip samples were returned to the laboratory under refrigeration. Sperm were distributed evenly in each stripped sample by gentle stirring and a subsample of each strip was taken by Gilson autopipette and diluted with distilled water. Dispersed sperm in these dilutions were counted twice for each male using an 'improved Neubauer chamber' haemocytometer. Number of sperm in each stripped ejaculate was determined by multiplying mean sperm count by the sample's dilution factor and volume.

(*c*) *Sperm activity*

Strip samples that had not been contaminated with urine or water during stripping were used for sperm behaviour analysis. One ml subsamples from each strip sample were sealed in sterile eppendorf tubes, refrigerated at 2 °C, and returned to the laboratory for analysis. Sperm behaviour analyses were run at 4–6 h and 24–26 h after stripping. Samples were selected randomly for analysis so that potentially confounding effects of time since stripping were controlled. Furthermore, we detected no changes in sperm activity across the two hour periods of analysis (unpublished data). One microlitre sperm subsamples were dispersed and activated by adding to Tyne river water at 11 °C on a glass slide. Sperm behaviour was recorded by video camera at × 100 magnification under dark-field phase microscopy. Two measures of sperm activity were determined by a 'blind' subject: (i) the percentage of sperm in the field of view (range 500–3000 sperm) which showed propulsive motility at 10 sec

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after activation; and (ii) the duration that at least 5% of the sample showed propulsive motility. Activity levels were measured twice at both times after stripping. Before full analysis, both measures of sperm activity (% of sperm motile and duration of motility) were checked for repeatability across a range of sperm activities. Both measures showed high repeatability within samples (% of sperm motile: $R = 0.9$, $p < 0.001$, $n = 57$ paired measures; duration of motility: $R = 0.9$, $p < 0.001$, $n = 57$ paired measures).

(*d*) *Sperm morphometry*

50 µl sperm subsamples were dispersed in 500 µl of buffer (modified Barth solution, Gurdon 1991) and fixed with 25 µl of formaldehyde. Subsamples of these solutions were dispersed in distilled water on glass slides and allowed to dry. This method of preparation ensured that sperm lay flat on the slide and presented a two-dimensional image for measurement. Sperm were magnified ×400 through phase contrast and a video link to a flat-screen monitor. Sperm heads and tails were measured on this monitor without knowledge of the sample's identification. Sperm were analysed from 25 males (14 anadromous and 11 parr) and flagellar lengths were measured from 50 sperm from each male. Head length measurements were made on 30 sperm from 10 males (5 anadromous and 5 parr).

4. RESULTS

(*a*) *GSI*

Anadromous male testes were absolutely heavier than parr ($U = 127$, $p < 0.001$). Anadromous ($r_{sp} = 0.77$, $p = 0.005$, $n = 12$) and parr ($r_{sp} = 0.85$, $p = 0.0016$, $n = 11$) testes mass scale positively with body mass (see figure 2) and there was a significant difference between the two slopes ($t = 2.11$, $p = 0.048$). Male parr GSI was significantly larger than anadromous GSI (Mann-Whitney $U = 123$, $p < 0.001$, see figure 3. Anadromous GSI: $x = 2.33$ (± 0.243 s.e.) $n = 12$; parr GSI: $x = 4.65$ (± 0.35 s.e.) $n = 11$), and parr therefore invest more heavily in gonadal tissue per unit body mass than anadromous males.

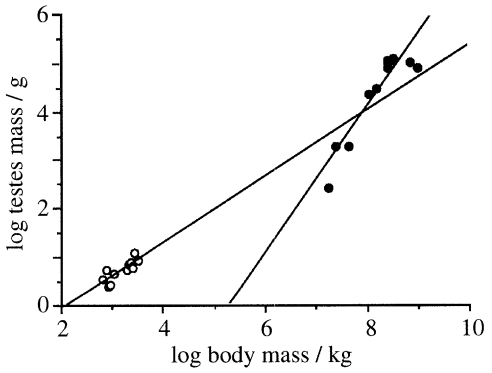

Figure 2. Positive significant allometry between body mass and testes mass across anadromous and parr males ($n = 12$ and 11 respectively). There is a significant difference between the slopes (see §4): parr slope: $y = -1.425 + 0.68x$; anadromous slope: $y = -8.2 + 1.507x$.

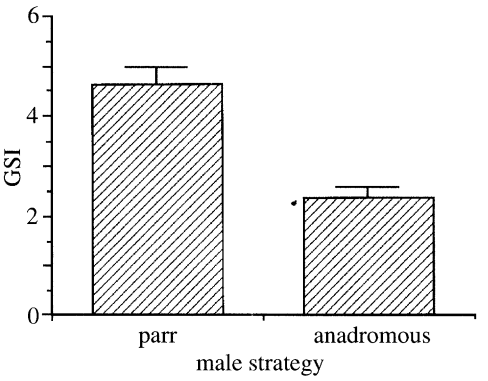

Figure 3. Male parr GSI is significantly greater than anadromous male GSI ($n = 12$ and $n = 11$ respectively). GSI is testes weight as a % of body weight.

(*b*) *Sperm numbers*

Anadromous males had absolutely greater stripped ejaculate volumes ($U = 424$, $p < 0.001$) and more sperm per strip ($U = 400$, $p < 0.001$) than parr. (Strip volumes: anadromous: $\bar{x} = 25.3$ ml (± 0.22 s.e. $n = 66$), parr: $\bar{x} = 1.6$ ml (± 0.15 s.e. $n = 11$). Strip sperm numbers: anadromous: $\bar{x} = 1.539 \times 10^{11}$ (1.7×10^{9} s.e. $n = 66$), parr: $\bar{x} = 2.1 \times 10^{10}$ ($\pm 5.61 \times 10^{8}$ s.e. $n = 11$)). However, per unit body mass, stripped ejaculate volume ($U = 303$, $p < 0.001$) and sperm number ($U = 303$, $p < 0.001$) were relatively greater in parr than anadromous males. (relative strip volumes: anadromous: $\bar{x} = 4.356$ ml kg^{-1} (± 0.06 s.e. $n = 66$), parr: $\bar{x} = 68.3$ ml kg^{-1} (± 1.0 s.e. $n = 11$). Relative sperm numbers: anadromous: $\bar{x} = 1.54 \times 10^{11}$ kg^{-1} ($\pm 1.7 \times 10^{9}$ s.e. $n = 66$), parr: $\bar{x} = 9.2 \times 10^{11}$ kg^{-1} ($\pm 2.44 \times 10^{10}$ s.e. $n = 11$)).

There was no relation between anadromous male body mass and either stripped ejaculate volume ($r_{sp} = 0.21$, $p = 0.09$, $n = 66$) or sperm number ($r_{sp} = 0.02$, $p = 0.85$, $n = 66$). Parr body mass did not correlate with sperm numbers ($r_{sp} = 0.53$, $p = 0.094$, $n = 11$) but mass was significantly positively associated with stripped ejaculate volume ($r_{sp} = 0.76$, $p = 0.008$, $n = 11$).

(*c*) *Sperm activity*

Parr sperm were more active than anadromous males' sperm. Parr sperm showed greater % motility than anadromous males' sperm at both analysis times after stripping (4–6 h: $U = 123$, $p < 0.0001$; 24–26 h: $U = 127$, $p < 0.0001$; see figure 4*a*). Parr sperm were motile for longer periods after activation than anadromous male sperm (4–6 h: $U = 116$, $p < 0.002$; 24–26 h: $U = 121$, $p = 0.0006$; see figure 4*b*). Anadromous male sperm activity declined between the two analysis times for both measures of sperm motility (% sperm motile: $Z = -3.06$, $p = 0.002$, see figure 4*a*; motility duration: $Z = -2.76$, $p = 0.006$, see figure 4*b*). There was a decline in % motility of parr sperm between the two measurement times ($Z = -3.16$, $p = 0.001$, see figure 4*a*). However, parr sperm showed no significant decline in the durations of propulsive motility between the two analyses times after stripping ($Z = -1.6$, $p = 0.11$, see figure 4*b*).

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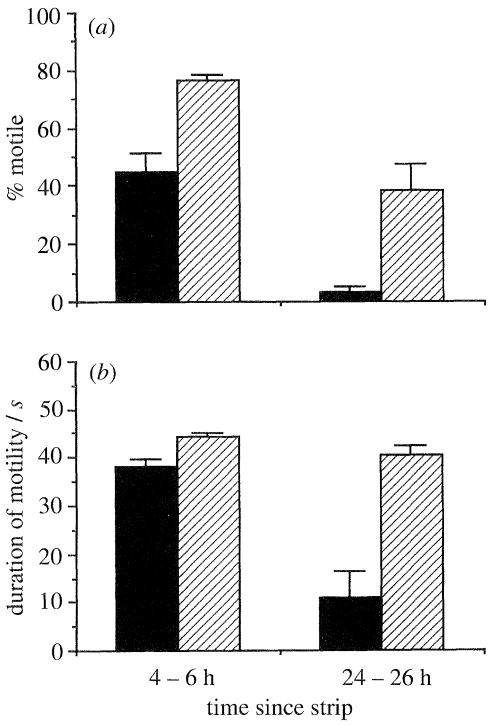

Figure 4. Parr sperm ($n = 11$ males) are more active than anadromous male sperm ($n = 12$ males). (*a*) The percentage of sperm that are motile at 10 s after activation is significantly higher in parr stripped ejaculates than anadromous male ejaculates. The % motility of both males' sperm declined with time since stripping. (*b*) Parr sperm were progressively motile for longer periods than samples from anadromous males. Samples from anadromous males declined in activity with time since stripping, however there was no decline in the duration of parr sperm activity over the 24–26 h period after stripping.

(*d*) *Sperm morphometry*

Sperm lengths showed significant normal distributions (Filliben plots of normality (Aitken *et al.* 1989) showed $R = 0.98$ and $R = 0.96$ across anadromous and parr males respectively ($p < 0.01$)). ANOVA tests were used to identify sites of variance, treating males (not sperm) as levels of independence.

Sperm head length did not differ between male strategy ($F_{1,8} = 0.04$, $p = 0.84$) or between males ($F_{8,289} = 1.1$, $p = 0.34$) (anadromous: $\bar{x} = 3.88$ μm ($\pm .036$ s.e. $n = 150$ sperm from five males), parr: $\bar{x} = 3.9$ μm ($\pm .013$ s.e. $n = 150$ sperm from five males).

There was no difference between the flagellar lengths of sperm produced by parr and anadromous males ($F_{1,23} = 0.546$, $p = 0.47$; anadromous: $x = 33.39$ μm ($\pm .063$ s.e. $n = 700$ sperm from 14 males, parr: $x = 33.73$ μm ($\pm .066$ s.e., $n = 550$ sperm from 11 males)).

5. DISCUSSION

In the Atlantic salmon, precocious parr appear to occupy a disfavoured mating role. Parr are attacked by anadromous males and are not attractive to females as mates (Jones 1959). Parr may suffer high mortality from the aggression of male conspecifics (Jones 1959; Hutchings & Myers 1987). In a similar mating system in the related chinook salmon (*Oncorhynchus tshawytscha*), almost half of the dead parr on the spawning redds had been killed by adult conspecifics, probably males (Gebhards 1960). Despite these disadvantages, precocious maturity in salmon parr is a persistent reproductive strategy, sometimes adopted by over 80% of the male parr population (Jones 1959; Myers *et al.* 1986). The precocious parr strategy may be advantageous because males suffer reduced time costs and risks of mortality before reproduction. Smoltification and seaward migration enable rapid growth, but carry significant risks of mortality (Mills 1989). Males must trade-off mortality risks against the predicted reproductive pay-offs from either strategy. Those spending more time at sea achieve a larger body size and dominate the redds at spawning, but also incur higher pre-reproductive mortality risks.

External fertilization, where females may have less control over paternity, may enable the evolution of sneak and satellite mating tactics. Although dominant males attempt to monopolize females in fish, sneak and satellite tactics are common (reviewed by Taborsky 1994). The persistence of disfavoured mating strategies suggests some reproductive success. In a Newfoundland population of *S. salar*, single male parr fertilized an average of 5% of the eggs in a redd (Hutchings & Myers 1988). In our study, anadromous males averaged 16.25 kg (± 0.31 s.e., $n = 66$) whereas parr averaged only 0.024 kg (± 0.002 s.e., $n = 11$). Parr body size therefore represents only 0.15% of anadromous male size, a much smaller fraction than the fertilization rate in competition with anadromous males. Our data on relative (to body size) investment by either male strategy into gross spermatogenesis, sperm numbers and sperm activity may explain how precocious parr, despite mating in a disfavoured role and under increased intensity of sperm competition, achieve a disproportionately high fertilization success (Hutchings & Myers 1988). Male Atlantic salmon parr fertilize more eggs, for their body size, than other salmonid satellite males such as chum salmon (*Oncorynchus keta*). This success may be due to disruptive selection for small male body size for fertilization success in a satellite role (Gross 1985). Alternatively or additionally, fertilization success may be enhanced by increased relative investment in sperm numbers, activity and competitiveness. These findings are consistent with our theoretical predictions that parr may be disfavoured in sperm competition and that their adaptive strategy is to 'spend' relatively more on sperm (Parker 1990*a*, *b*; Stockley & Purvis 1993).

There may also be asymmetries in mating role within the anadromous male population. Smaller and younger grilse may be at a competitive disadvantage to the larger 'hooknose' males that have spent two or more winters at sea (Belding 1934). We find that stripped ejaculate sperm number and volume are not correlated with body size across 47 anadromous males (despite a six-fold range in body mass from 3.5–22.5 kg). Although there was a trend towards a positive body size: stripped ejaculate volume relation ($p = 0.09$), there was no relation between body size and stripped ejaculate sperm number across anadromous males. A similar situation appears to apply in trout

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(Linhart 1984) where smaller trout produce relatively (to body size) larger volumes of stripped ejaculate. Across anadromous salmon, we find the relation describes a straight line, where smaller anadromous grilse produce similar numbers of stripped mature sperm as larger, multi-sea-winter fish. As there is a strong relation between testes size and body size across males (see figure 2), it seems strange that smaller grilse should produce as many sperm as larger hooknose males. In a similar manner to parr, disfavoured male grilse may be selected to invest relatively more heavily in the ejaculate (Parker 1990*a*, *b*; Stockley & Purvis 1993). Furthermore, grilse may trade ejaculate sperm number against mating frequency and may therefore have longer intercopulatory intervals to recover their sperm stores, as predicted in the theoretical background §2.

The phenomenon of differential investment in spermatogenic tissue according to male mating strategy and sperm competition is recognised in other fishes. In the bluegill sunfish *Lepomis macrochirus* (Gross 1982), and the ocellated wrasse *Symphodus ocellatus* (Taborsky 1994), males adopting sneak and satellite strategies develop significantly heavier GSIs than the larger territorial males. Furthermore, in the bluehead wrasse (*Thalassoma bifasciatum*) males release more sperm under situations of elevated sperm competition (and also with larger females and with more eggs released (Shapiro *et al.* 1994)). We find that parr spend relatively more on spermatogenesis for their body size (see figure 3), and that this investment is made specifically in sperm numbers and sperm activity. Where the sperm competition mechanism follows the principle of a pure raffle (Parker 1982, 1990*a*, *b*), fertilization success is achieved by males that ejaculate the most numbers of sperm into the competition (e.g. Martin *et al.* 1974; Simmons 1987). This mechanism of competition may explain why sperm are often numerous and tiny (Parker 1982). Reproductive success in external fertilization is less influenced by factors such as sperm storage, female manipulation, sperm removal and displacement, and the timing of insemination relative to fertilization. External fertilization with synchronous release of gametes may, therefore, equate closely to a raffle where selection drives the production of maximal numbers of minimally sized sperm. This may explain why external fertilizers produce smaller sperm than internal fertilizers (Jamieson 1991; Stockley *et al.* 1995).

We found no differences in sperm lengths between the anadromous and parr strategies (and perhaps therefore swimming velocity, Katz & Drobnis 1990). Sperm competition may select for increased sperm length where the sperm competition mechanism follows the principles of a race, or where sperm are selected to compete actively (Gomendio & Roldan 1991; Briskie & Montgomerie 1992; Gage 1994). However, our findings for salmon are consistent with a raffle-based sperm competition mode where numerical superiority is the adaptive strategy (Parker 1993). When the benefit of increasing sperm numbers rises linearly for most of the parameter space, but competitive value of sperm size increases with diminishing returns, sperm size is predicted to be optimized after the marginal value theorem (Charnov 1976; Parker & Stuart 1976) and is independent of sperm competition risk (Parker 1993). Such a situation applies in a pure raffle, and this may be approached in nature under external fertilization.

Although we found no difference in flagellar length between the male strategies, there were clear differences in sperm motility. A greater proportion of parr sperm were motile, and these were motile for longer periods (see figures 4*a*, *b*). These findings suggest that sperm activity, as well as sperm number, is an important factor for sperm competition in external fertilization. During spawning, salmon sperm are extremely active for a brief period (see figure 4*b*) and achieve high velocities (Terner 1986), necessary for encountering ova in the turbulent and competitive environment of the spawning redd. Males in disfavoured mating roles may redress their disadvantages by increasing ejaculate competitiveness through producing greater numbers of more active sperm. Our findings confirm earlier indications that sperm motility differs between parr and anadromous male salmon (Kazakov 1981). Similar differences in sperm motility associated with alternative male strategies, and/or male age have also been reported in rainbow trout (*Salmo gairdneri*, Linhart 1984) and stickleback (*Gasterosteus aculeatus* de Fraipoint *et al.* 1993). Our theoretical predictions suggest that salmon parr, if mating in a disfavoured role, should be selected to 'spend' relatively more on sperm. We find that parr have a larger gonosomatic index, and produce larger numbers of sperm relative to body size. There is no difference in sperm length between the two male strategies, however, parr sperm exhibit greater activity. In conclusion, these gamete characteristics may explain how parr achieve relatively high fertilization success for their tiny body size when in competition with anadromous males (Hutchings & Myers 1988).

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APPENDIX

An ESS model of sperm expenditure in salmon alternative mating strategies

A given male's fitness is the product of n, the number of spawnings achieved, and v, the expected progeny gained in each one. The fitness of a rare mutant ejaculation strategy J in either a parr or anadromous population playing strategy I is:

$$W(\mathrm{J},\mathrm{I}) = n(\mathrm{J}\,\mathrm{I}).\ v(\mathrm{J}\,\mathrm{I}). \qquad (1)$$

Fertilization is instantaneous and sperm size is constant for all males. We consider deviations in ejaculate effort

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by allowing males to vary sperm number, s. A much more complex approach is required for a process of continuous fertilization and where sperm size (which affects survivorship during fertilization) is allowed to deviate; the results of such a model indicate that much of the parameter space, sperm size would be fairly constant (M. A. Ball & G. A. Parker, unpublished data) approximating to the solution given in Parker (1993). Thus mutant strategies are continuous deviations in $s_a \neq s_a^*$ for anadromous males, and $s_p \neq s_p^*$ for parr. As in the above papers, increasing s unilaterally is assumed to decrease n (fewer spawnings are achieved if more sperm are ejaculated at each one), and to increase v (under sperm competition, the gain from each spawning increases as more is spent on each ejaculate).

The ESS, I, is characterized by the strategy pair $\{s_a^*, s_p^*\}$, and the conditions for I to be as ESS against mutants deviating as $s_a \neq s_{aq}^*$ are:

$$\left|\frac{\mathrm{d}W(s_a, \mathrm{I})}{ds_a}\right|_{s_a = s_a^*} = 0$$
$$\left|\frac{\mathrm{d}^2W(s_a, \mathrm{I})}{ds_a^2}\right|_{s_a = s_a^*} < 0 \qquad (2)$$

(see e.g. Maynard Smith 1982), and from (1) and (2), local stability of s_a* requires (Parker 1993):

$$\left|-\frac{n(s_a^*, \mathrm{I})}{n'(s_a^*, \mathrm{I})}\right|_{s_a = s_a^*} = \left|\frac{v(s_a^*, \mathrm{I})}{v'(s_a^*, \mathrm{I})}\right|_{s_a = s_a^*}; \qquad (3)$$

where the prime denotes the first derivative with respect to s_a: n' is negative and v' positive. Analogous conditions to (2) and (3) apply for the stability of the ESS ($\mathrm{I} = s_a^*, s_p^*$) against mutants deviating as $s_p \neq s_p^*$.

Consider the function $n(\mathrm{J}, \mathrm{I})$. Energy spent on the ejaculate at each spawning is traded off against energy spent on obtaining spawnings. We assume that each male has a fixed total energy budget, R_a or R_p, and that the cost of obtaining each spawning (finding a female etc) is C_a or C_p. The cost of each sperm is D_a or D_p, so that the cost of the ejaculate is $D_a s_a$ or $D_p s_p$. Note that R, C, and D are all expressed in the same units. The ESS number of matings per male is thus $R/(C + Cs^*)$, whereas a mutant playing $s \neq s^*$ achieves $R/(C + Ds)$ matings. Hence the number of matings gained by a mutant anadromous male playing $s_a \neq s_a^*$, relative to the population average for anadromous males, is:

$$n(s_a, \mathrm{I}) = (C_a + D_a s_a^*)/(C_a + D_a s_a),$$

and similarly for parr. Note that the absolute amount of resources, R_a or R_p, that a male has at its disposal will not affect its ESS sperm expenditure (see also M. A. Ball & G. A. Parker, unpublished data).

We therefore obtain:

$$\left|-\frac{n(s_a^*, \mathrm{I})}{n'(s_a^*, \mathrm{I})}\right|_{s_a = s_a^*} = \frac{C_a}{D_a} + s_a^*, \qquad (4a)$$

$$\left|-\frac{n(s_p^*, \mathrm{I})}{n'(s_p^*, \mathrm{I})}\right|_{s_p = s_p^*} = \frac{C_p}{D_p} + s_p^{**}, \qquad (4b)$$

respectively for anadromous males and parr.

Constuction of the function $v(\mathrm{J}, \mathrm{I})$ is more complex. In nature, it appears that a typical spawning consists of usually one (and occasionally more) anadromous males and a variable number of parr (i.e. against a frequency distribution). In the interests of mathematical tractability, we simplify by assuming that with probability p a single anadromous male plays against N parr. With probability $(1-p)$, the anadromous male has no competitors (sperm competition is absent). The probability of zero parr will be a declining function of the mean number of parr when parr are present. Specifically, we assume that the probability of there being zero parr follows a random (Poisson) process so that $(1-p) = \exp(-N)$, where N is the mean of the Poisson distribution.

Fertilization success follows the 'raffle principle' (Parker 1990*a*) and the raffle can be 'loaded' in favour of one or other male strategy allowing different competitive masses as explained in the text. (The competitive mass of anadromous sperm is standardized as 1.0; parr sperm have a relative competitive mass of r). Thus the fertilization gains are:

$$v(s_a, \mathrm{I}) = (1-p) + p[s_a/(s_a + Nrs_p^*)],$$

for an anadromous male mutant deviating as $s_a \neq s_a^*$, and:

$$v(s_p, \mathrm{I}) = rs_p/[s_a + (N-1)rs_p^* + rs_p],$$

for a mutant parr deviating as $s_p \neq s_p^*$.

We can now otain:

$$\left|\frac{v(s_a^*, \mathrm{I})}{v'(s_a^*, \mathrm{I})}\right|_{s_a = s_a^*} = \frac{(s_a^* + Nrs_p^*)[s_a^* + Nrs_p^*(1-p)]}{pNrs_p^*}; \qquad (5a)$$

$$\left|\frac{v(s_p^*, \mathrm{I})}{v'(s_p^*, \mathrm{I})}\right|_{s_p = s_p^*} = \frac{(s_p^*(s_a^* + Nrs_p^*)}{s_a^{**} + (N-1)rs_p^*}; \qquad (5b)$$

respectively for anadromous males and parr.

Following equation (3), we use equations (4) and (5) to obtain:

$$\frac{s_a^{*2}}{Nrs_p^*} + (1-p)(2s_a^* + Nrs_p^*) = p\left(\frac{C_a}{D_a}\right); \text{ from (4}a\text{) and (5}a\text{);} \qquad (6a)$$

$$s_a^* = rs_r^*\left(\frac{D_p s_p^*}{C_p} - (N-1)\right); \text{ from (4}b\text{) and (5}b\text{).} \qquad (6b)$$

Note that cost parameters are always expressed in terms of the ratios C_a/D_a, C_p/D_p. Unfortunately, we cannot obtain explicit solutions for s_a^*, s_p^*. Substitution of (6b) into (6a) yields a complex polynomial in s_p^*, which can nevertheless be solved numerically by computer iteration (see figure 1).

However, we can obtain a solution for the game without any inequalities between parr and anadromous males, by assuming that on all occasions, there is just one anadromous male and N parr (i.e. $p = 1.0$), that $C_a/D_a = C_p/D_p$, and that $r = 1$. Under the assumption of symmetry, it is easy to show that $s_a^* = s_p^* = NC/D$; i.e. sperm expenditure should rise linearly with N, which is the total number of competing fish minus one (see also M. A. Ball & G. A. Parker, unpublished data).