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INDIVIDUAL VARIATION IN ATLANTIC SALMON FERTILIZATION SUCCESS: IMPLICATIONS FOR EFFECTIVE POPULATION SIZE

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Abstract. Mating structure can influence the variance in individual reproductive success, which in turn has important implications for a population's effective size. Atlantic salmon (*Salmo salar*) males are characterized by alternative reproductive strategies, maturing as small parr in fresh water and/or as considerably larger anadromous males following a migration to sea. The potential for significant variation in individual reproductive success in both sexes is high. We established two experimental crosses each involving four anadromous females, four anadromous males, and 20 mature male parr in a seminatural spawning environment. We used hyper-variable microsatellite loci to identify the parents of 755 embryos and to quantify the variance in individual fertilization success. Anadromous males generally dominated fertilization success, with a mean individual fertilization success of 15.7% and 19.3% in replicates A and B, respectively, as compared with 1.9% and 1.2% for the mature male parr. There was no relationship between individual mature male parr size and individual fertilization success in any individual nest, nor was there any relationship between anadromous male size and individual fertilization success in either replicate. There was some evidence of size being an important determinant of both the frequency of spawning and the overall individual parr fertilization success among those parr identified as having spawned, although these relationships were not always significant. Both anadromous males and females were identified as having spawned with multiple partners, although the frequency of multiple anadromous males spawning simultaneously with a female was low. Fertilization of eggs by parr can significantly increase the effective number of males on a nest-by-nest basis; however, the variance in individual anadromous male fertilization success appears to have the greatest overall influence on effective population size.

Key words: effective population size; fertilization success; individual variation; mating strategies; alternative; reproductive success; *Salmo salar*.

INTRODUCTION

Effective population size, or N_e , is of considerable interest to conservation biologists because of its negative association with the rate of loss of genetic variation (Wright 1931). Mating systems can strongly influence N_e , notably by affecting the likelihood that an individual will reproduce (Nunney 1991), by influencing the variance in individual reproductive success within one or both sexes (Nunney 1993), and by affecting the number of mates per individual (Sugg and Chesser 1994). Therefore, in addition to estimating the number of adults of reproductive age of both sexes, reliable estimates of N_e require accurate estimates of the variance in individual reproductive success for both sexes within the context of an organism's typical mating structure. Increased risks of extinction faced by many populations and species underscores the need for greater empirical understanding of the influence that individual reproductive success and mating structure have on N_e .

Individuals of many species employ different tactics to obtain reproductive success. Fishes have often been

used to study such alternative reproductive strategies (reviewed by Taborsky [1994]). Salmonids, commonly exhibiting an anadromous life history (a freshwater component early in life followed by a period of rapid growth at sea and eventual return to the natal river to spawn), have been of particular interest in this field. Atlantic salmon (*Salmo salar*) is one such salmonid for which there is great interest in the influence of alternative reproductive strategies on N_e .

Atlantic salmon male parr (smaller individuals who have not migrated to sea) can mature and compete with the larger anadromous males for access to anadromous females during spawning and do fertilize some eggs (Hutchings and Myers 1988, Jordan and Youngson 1992, Morán et al. 1996, Thomaz et al. 1997, Jones and Hutchings 2001). It has been suggested that mature male parr fertilization success can increase a female's number of mates as well as potentially increasing effective population size by increasing the effective number of males and by affecting the variance in reproductive success (L'Abée-Lund 1989, Jones and Hutchings 2001). However, previous studies on Atlantic salmon reproductive success have been either limited primarily to single anadromous pair crosses (Hutchings and Myers 1988, Morán et al. 1996, Thomaz et al. 1997, Jones and Hutchings 2001) or were unable to assign

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individual reproductive success due to the limitation of the genetic markers at the time (Jordan and Youngson 1992). Such experiments, by their design, were unable to determine the influence of increased competition from anadromous males on parr mating behavior or fertilization success. Furthermore, they were unable to quantify either the actual number of mates of either sex or the variance in individual reproductive success.

Estimates of the number of anadromous salmon of reproductive age within single Atlantic salmon populations are often available. However, estimating N_e remains difficult because many factors can affect mating structure and create variability in individual reproductive success in both sexes. The sex ratio in anadromous salmon is typically female biased. However, because an individual female will spawn over 5–6 d, while an anadromous male can potentially spawn throughout the entire several-week spawning period, the operational sex ratio on the spawning grounds is typically male biased (reviewed by Fleming [1996]). For females, variation in individual size, and consequently fecundity (e.g., Thorpe et al. 1984), and variation in pre-emergence survival (e.g., Pauwels and Haines 1994) can create variation in individual reproductive success. Suitable spawning locations are often limited and a female risks lower offspring survival either by being relegated to less suitable spawning locations or by having nests dug up by subsequent spawners (Fleming et al. 1996). The potential for multiple mates also exists for both sexes. Given the extended spawning potential of anadromous males, individual males could spawn with many females. More than one anadromous male may spawn with a single female at any one time (e.g., Mjølnerød et al. 1998) and anadromous females generally spawn several times (Jones 1959).

The alternative reproductive strategy that exists within male Atlantic salmon can also lead to increased variance in individual reproductive success and have further implications for a population's effective size. Several factors can influence reproductive success in male Atlantic salmon. Total parr fertilization success within nests is dependent on the number of parr and on the order of nest construction (Hutchings and Myers 1988, Thomaz et al. 1997). Individual parr fertilization success may also depend on parr size (Thomaz et al. 1997), although this may only be true at low parr densities (Jones and Hutchings 2001). Similarly, when spawning with a single female, the body size of competing anadromous males can also be an important determinant of individual fertilization (Mjølnerød et al. 1998). A primary limitation of these studies, however, is that determinants of male fertilization success were assessed under conditions in which males had access to only one female. Thus, the degree to which these correlates of reproductive success are influenced by more natural spawning conditions, e.g., the presence

of multiple females, multiple anadromous males, and multiple parr, is not known.

As population sizes decline, quantifying the mating structure and variation in individual reproductive success, as well as factors associated with this variation, is critical. In addition, understanding the influence alternative reproductive strategies have on effective population size is of central importance to the conservation of genetic diversity in many species. We use Atlantic salmon as a model species to address the following objectives: (1) to quantify individual fertilization success, to identify the factors associated with individual fertilization success, and to describe the mating structure of anadromous males and females and mature male parr in a semi-natural environment; and (2) to quantify the influence that variation in individual fertilization success and the influence alternative reproductive strategies have on effective population size.

MATERIALS AND METHODS

Field experiment

Experiments were undertaken in outdoor flow-through raceways (14.6 × 1.2 m cement channels with controlled water flow) at the Margaree Fish Hatchery, Nova Scotia (46°21' N, 60°58' W), in the autumn of 1996. Substrate suitable for spawning was added to the raceways to a depth of 30 cm. Mature male parr were electrofished from nearby tributaries of the Margaree River one week prior to the experiment. Grilse males (i.e., males that spent one winter at sea before returning to spawn) were obtained from the fish trap on Lake O'Law River, the largest of the Margaree River's tributaries. Multi-sea-winter (MSW) males and females were obtained by seining the Margaree River. All fish were in spawning condition but had not yet been involved in spawning at the time of their collection.

We established two replicates of experimental crosses each having 4 MSW females, 2 MSW males, 2 grilse males and 20 mature male parr of different sizes (range 68–123 mm). The parr were divided into four size classes: 69–80 mm (75.3 ± 3.2 mm [mean ± 1 SD]), 81–90 mm (84.9 ± 2.7 mm), 91–110 mm (97.9 ± 5.7 mm), and 111–123 mm (116.9 ± 3.7 mm). Parr were placed in the raceways prior to the addition of the anadromous salmon (body size data, by replicate, are presented in Table 1). The raceways were covered with netting anchored by cinder blocks. We measured the length and mass of each fish and collected a fin sample from each fish for microsatellite DNA analysis after spawning had been completed in December 1996.

Eyed embryos were collected from 8 and 10 nests from replicates A and B, respectively, in March 1997. While the number of nests each female will make depends on habitat quality and the degree of competition from other females (Fleming 1996), similar-sized females (~4 kg) in Europe created an average of ~6 nests. We thus sampled an estimated 37% of all the

TABLE 1. Lengths of anadromous males (δ_i – δ_{iv}), anadromous females (φ_i – φ_{iv}), and number of parr from each size class in each replicate raceway (Rep.).

Rep.	Anadromous salmon lengths (cm)								No. parr from each size class (mm)			
	δ_i	δ_{ii}	δ_{iii}	δ_{iv}	φ_i	φ_{ii}	φ_{iii}	φ_{iv}	70–80	81–90	91–110	111–122
A	80.0	77.7	63.2	52.0	82.2	81.2	77.8	74.1	8	7	4	1
B	90.0	78.0	59.5	58.9	90.3	83.5	81.6	77.3	8	7	4	1

nests constructed. Parr and embryo collection were facilitated by the ability to lower the water levels in the raceways. To sample nests, markers were placed every 1.3 m along the length of the raceways. Beginning at the center of the raceway along the transect of the downstream marker, 10–15 cm of substrate were dug away. The area cleared expanded outwards from the center point until a nest was found. This procedure was then repeated at the next upstream marker transect. In the B raceway, nests were not found near three transects, necessitating a search below the first (downstream) transect to increase the number of nests sampled.

Genetic analysis

DNA was extracted from all potential parents and from 60 offspring from each of six nests per raceway. To identify the major contributors to the remaining nests in each raceway, 10 embryos from each of these remaining nests (nests 7–10 in raceway A, nests 7–8 in raceway B) were also examined. Each dissected embryo head or small subsample of fin tissue (~50 mg) was digested in 100 μ L of eyeball buffer (10 mmol/L Tris [Tris(hydromethyl)aminomethan $C_4H_{11}NO_3$], 50 mmol/L KCl, 0.5% Tween20 [polyoxyethylene (20) sorbitan monolaurate]) and proteinase K (0.1–0.4 μ g) in a 500- μ L tube and incubated between 4 and 16 h at 45–55°C. Samples were vortexed 2–3 times during this digestion. Samples were heated at 94°C for 5–10 min to kill the proteinase K, frozen at –80°C (for times varying from overnight to several months), thawed, centrifuged at 14 000 rpm (Eppendorf [Hamburg, Germany] microcentrifuge) for 5 min and then diluted 100 \times . Two μ L of this DNA (approximate concentration 3–300 nanograms) were used for microsatellite analysis. The tetranucleotide microsatellite loci *Ssa171*, *Ssa197*, and *Ssa202* and the dinucleotide locus *Ssa85* were run on all samples following the methods described by O'Reilly et al. (1996). In the cases where these loci proved insufficient in determining parentage (141 of 755 embryos genotyped, 35 of 392 from raceway A and 106 of 363 from raceway B; see Table 1 for replicate designations), additional loci were examined until paternity could be unambiguously established. In descending order, these dinucleotide loci were *Ssa12* (O'Reilly 1997), *Omy105*, and *Omy38* (Heath et al. 2001). To eliminate bias, microsatellite analyses were repeated and/or extended to all individuals that had partial genotype information but for which

parentage could not initially be assigned unambiguously. No further attempts were made to genotype those embryos that had not amplified at any locus if the number of embryos from a nest that could be assigned parentage was greater than 50. Thus all embryos for which any microsatellite information exists were unambiguously assigned parentage. For the nests in which only 10 embryos were examined, only one attempt to genotype embryos was made; all embryos with any genotype information in these nests had sufficient data to unambiguously assign parentage.

Data analyses

Gene diversity at each locus in salmon from the Margaree River (sample sizes were 157, 166, 168, and 131 salmon for *Ssa202*, *Ssa197*, *Ssa171*, and *Ssa85*, respectively) was calculated as the heterozygosity expected under Hardy-Weinberg equilibrium using TFGA (tools for population genetic analyses; Miller 1997). Parentage was determined by the program PROBMAX (Danzmann 1997), which assigns progeny to parents from a mixture of potentially contributing parents when parental genotypes are known. This program also allows manipulation of the data set to identify potential mis-genotyping, such as failure to recognize a heterozygote due to stuttering or mis-genotyping by one allele length (see PROBMAX program manual).

To determine the power of these microsatellite loci to assign parentage with increased sample sizes, all embryos with complete 4-locus genotype information from each embryo data set (for raceways A and B) were combined. Parentage was then assigned by PROBMAX for each embryo data set, using the corresponding parental data set. The parental data sets were then combined, approximately doubling the number of potential parents, and the analysis rerun.

Estimating N_e

Following Lande and Barrowclough (1987), the effective number of males was calculated as

$$N_{em} = (N_m \bar{k}_m - 1) / [\bar{k}_m + (\sigma_{k_m}^2 / \bar{k}_m) - 1]$$

where N_m is the actual number of males, and k_m and $\sigma_{k_m}^2$ represent the number of offspring and associated variance, respectively, produced by an individual male in its lifetime. The equivalent method was used to calculate the effective number of females. We used the determined value of individual fertilization success as

TABLE 2. Individual parr fertilization success (as percentages) in each nest. Also reported are mean individual parr success for nests 1–6 (\bar{X}_{1-6}) and for all nests (\bar{X}_{all}), the sample size (n), and total parr success (Total (%)) for each nest. Samples sizes for the means refer to the number of nests.

Parr†	Parr length (mm)‡	Individual parr fertilization success (%)										\bar{X}_{1-6}	\bar{X}_{all}	
		1	2	3	4	5	6	7	8	9	10			
Individual fertilization success														
a2	77	0	0	0	0	0	0	0	0	10.0	0	0.0	1.0	
a4	79	0	0	0	0	0	0	0	0	20.0	0	0.0	2.0	
a5	80	0	0	0	0	0	15.3	0	0	0	0	2.6	1.5	
a7	82	0	0	0	0	0	1.7	0	0	0	0	0.3	0.2	
a8	83	0	0	0	0	0	11.9	0	0	0	0	2.0	1.2	
a9	83	0	0	0	0	0	3.4	0	0	0	0	0.6	0.3	
a11	88	0	0	0	0	0	13.8	0	0	0	0	2.3	1.4	
a12	89	0	0	0	0	0	5.1	0	0	0	12.5	0.9	1.8	
a13	89	0	0	0	0	0	25.4	0	0	0	0	4.2	2.5	
a14	90	0	0	0	0	0	8.5	0	0	0	37.5	1.4	4.6	
a15	91	0	0	0	1.7	74.6	0	0	0	0	0	12.7	7.6	
a17	99	0	0	0	0	0	8.5	0	0	0	0	1.4	0.9	
a18	107	0	1.7	44.6	0	0	0	44.4	0	0	12.5	7.7	10.3	
a19	107	0	0	0	0	0	6.8	0	0	0	37.5	1.1	4.4	
b6	78	0	0	0	0	33.3	0	0	0	n/a	n/a	5.6	4.2	
b7	79	0	0	0	1.9	0	1.8	0	0	n/a	n/a	0.6	0.5	
b11	87	0	0	13.3	0	0	0	0	0	n/a	n/a	2.2	1.7	
b13	89	0	1.8	0	3.8	0	0	0	0	n/a	n/a	0.9	0.7	
b17	106	0	0	0	11.3	66.7	1.8	0	0	n/a	n/a	13.3	10.0	
Sample size and total parr success														
Replicate A														
n		60	58	56	60	59	59	9	9	10	8	6	10	
Total (%)		0	1.7	44.6	1.7	74.6	100	44.4	0	30.0	100	37.1	39.7	
Replicate B														
n		59	56	60	53	60	56	9	10	n/a	n/a	6	8	
Total (%)		0	1.8	13.3	17.0	100	3.6	0	0	n/a	n/a	22.6	17.0	

† Entry symbol key: “a2” means “parr 2 from Raceway A”. Only parr identified as having fertilization success are included in table; no fertilization success was detected for parr in Raceway A of 74, 77, 81, 85, 93 and 122 mm or for parr in Raceway B of 70, 73, 75, 77, 78, 82, 83, 84, 88, 91, (95), (100), 109, 114, and 118 mm. (Sizes in parentheses are approximate, because parr were decomposing at the time of collection.)

‡ Note that length frequencies differ from those presented in Table 1 as a result of parr growth over the course of the experiment.

our measure of lifetime success both from each nest individually as well as for the entire raceway by weighting each nest equally.

The effective population size for each raceway was calculated after Wright (1938) as

$$N_e = 4N_{em}N_{ef}/(N_{em} + N_{ef})$$

where N_{em} and N_{ef} are the effective number of males and females, respectively.

RESULTS

As previously reported (Jones and Hutchings, 2001), there was substantial allelic diversity among the wild Margaree River salmon at the four microsatellite loci examined (18, 16, 27, and 9 alleles at *Ssa202*, *Ssa197*, *Ssa171*, and *Ssa85*, respectively). Gene diversity (H_e , expected heterozygosity) was similarly high (0.91, 0.90, 0.92, and 0.76 at *Ssa202*, *Ssa197*, *Ssa171*, and *Ssa85*, respectively). These levels of variation resulted in a high success rate in parental determination for each embryo with just these four loci (357 of 392 embryos from raceway A and 279 of 363 from raceway B). Most initially unassigned embryos had insufficient infor-

mation to distinguish between two, and occasionally three, potential fathers. When only those embryos with complete four-locus genotype information were used in the analysis, the number of complete parental assignments was 311 of 341 embryos from raceway A (16 with two and 14 with three potential fathers) and 262 of 336 embryos from raceway B (74 embryos with two potential fathers). When the potential parental contribution was artificially increased by combining the parental data sets, the number of times these four loci were unable to distinguish between the true father and other potential fathers of a given embryo increased by 14 in raceway A (to 20 with two, 23 with three, and 1 with four potential fathers) and by 24 in raceway B (to 71 with two, and 27 with three potential fathers). In all cases, additional information from other loci allowed unambiguous parental assignment.

Effect of size on male reproductive success

Overall, size was clearly an important determinant of fertilization success. The total anadromous male mean fertilization success was higher than the total parr mean fertilization success per nest (62.9% and 77.0%

TABLE 3. Individual anadromous female (Anad. ♀) involved, individual anadromous male fertilization success (Anad. ♂ success), total parr fertilization success, and the number of embryos for which parentage was assigned (*n*) for each nest.

Nest	<i>n</i>	Anad. ♀ †	Anad. ♂ success (%)	Total parr success (%)
A1 _{ii}	59	♀ Aii	♂ Aii, 100	0
A1 _{iii}	1	♀ Aiii	♂ Aii, 100	0
A2	58	♀ Aiii	♂ Aii, 94.8; ♂ Aiii, 3.4	1.7
A3	56	♀ Aiii	♂ Aiv, 53.6; ♂ Aiii, 1.8	44.6
A4	60	♀ Aii	♂ Aii, 98.3	1.7
A5	59	♀ Aii	♂ Aiii, 25.4	74.6
A6 _{vi}	58	♀ Aiv	0	100
A6 _{ii}	1	♀ Aii	0	100
A7	9	♀ Aiii	♂ Aiv, 55.6	44.4
A8	9	♀ Aii	♂ Aii, 100	0
A9	10	♀ Aiii	♂ Aiii, 70	30
A10	8	♀ Ai	0	100
B1	59	♀ Biv	♂ Bii, 98.3; ♂ Bii, 1.7	0
B2 _i	54	♀ Bi	♂ Biii, 100	0
B2 _{ii}	2	♀ Bii	♂ Biii, 50	50
B3 _i	58	♀ Bi	♂ Biii, 87.9	12.1
B3 _{ii}	2	♀ Bii	♂ Biii, 50	50
B4	53	♀ Bi	♂ Biii, 83	17
B5	60	♀ Biii	0	100
B6	56	♀ Biii	♂ Biii, 96.4	3.6
B7	9	♀ Biv	♂ Bii, 100	0
B8	10	♀ Biv	♂ Biii, 100	0

Note: The subscripts refer to raceways A and B and individual fish identification numbers are as in Table 1.

† When embryos from more than one female were found in a nest, the nest was subdivided based on the anadromous female contribution.

for the four anadromous males vs. 37.1% and 23.0% for the 20 parr in raceways A and B, respectively, averaged over nests 1 to 6; Tables 2 and 3). Mean individual fertilization success was thus 15.7% and 19.3% for the anadromous males in raceways A and B, re-

spectively, and 1.9% and 1.2% for the parr (averaged over nests 1 to 6; similar results were obtained when the data from all nests were included). While mean anadromous male fertilization success was generally high, there were some nests in which parr obtained substantial success.

Despite the apparent importance of size among all males, there was little evidence of such an overall association within life-history type. Individual mature male parr size was independent of individual fertilization success within (Table 2; all *r*² were <0.2 and their associated *P* values were all >0.1) and among nests in each raceway (Fig. 1). Similarly, there was no apparent

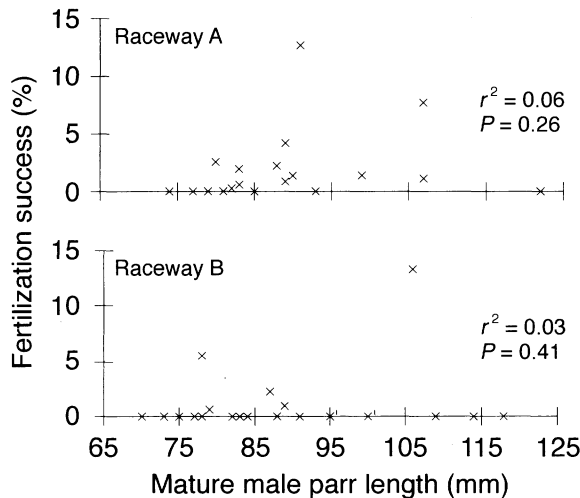


FIG. 1. Individual parr fertilization success averaged over six nests (×) vs. parr length for each raceway (A and B). Length frequencies differ from those presented in Table 1 as a result of parr growth over the course of the experiment. In raceway B, the “×” parr lengths were from decomposed parr and are approximate. The *r*² and associated *P* values refer to the linear regressions between individual parr fertilization success and parr body size.

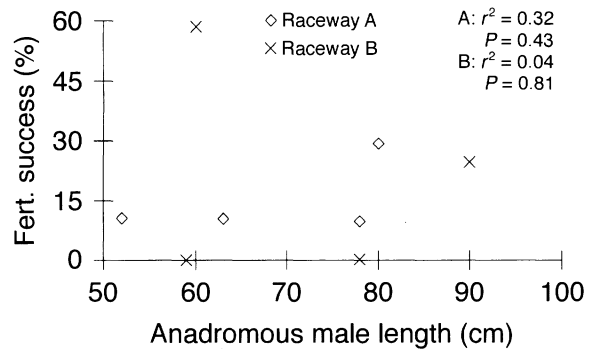


FIG. 2. Individual anadromous male fertilization success vs. anadromous male length averaged over six nests for raceways A and B. The *r*² and associated *P* values refer to the linear regressions between individual anadromous male fertilization success and anadromous male body size.

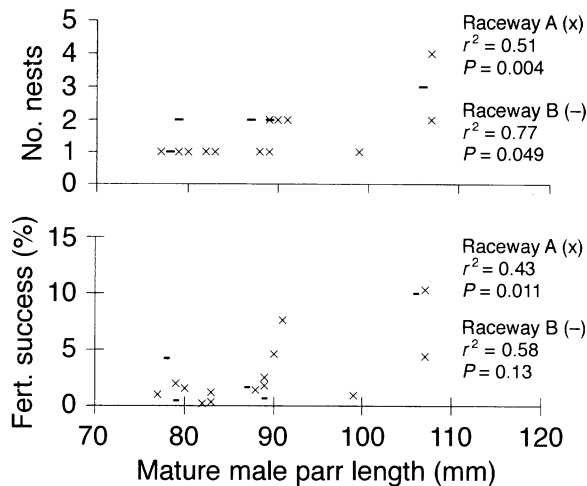


FIG. 3. Number of nests in which an individual mature male parr was identified as having spawned and mean individual parr fertilization success vs. mature male parr length for those parr who were identified as having spawned in any of the 10 nests in raceway A and eight nests in raceway B. The r^2 and associated P values refer to the linear regressions between the respective y variables and mature male parr length.

relationship between anadromous male size and individual fertilization success among nests within raceways (Fig. 2).

Although there was no evidence of an association between individual fertilization success and parr size when all parr were included in the analysis, there was some evidence of such a relationship among only those parr identified as having spawned. In raceway A, when all 10 nests were weighted equally, there was a significant relationship between parr size and the number of nests in which a parr was identified as having spawned ($r^2 = 0.51$, $P = 0.004$; $n = 14$ parr; Fig. 3) and between parr size and mean individual fertilization success ($r^2 = 0.43$, $P = 0.011$; $n = 14$ parr; Fig. 3), although the latter association was not significant when the analysis was restricted to the 6 nests for which sample sizes exceeded 50 ($r^2 = 0.18$, $P = 0.17$; $n = 12$ parr for parr size and number of times an individual was identified as having spawned and $r^2 = 0.06$, $P = 0.42$; $n = 12$ parr for parr size and mean fertilization success). In raceway B in which parr were only identified as having spawned in the nests in which sample sizes exceeded 50, there was a significant relationship between parr sizes and the number of nests a parr was identified as having spawned ($r^2 = 0.77$, $P = 0.049$; $n = 5$ parr; Fig. 3) but not parr size and mean individual fertilization success ($r^2 = 0.58$, $P = 0.13$; $n = 5$ parr; Fig. 3).

Spawning associations

The incidence of successful multiple anadromous spawnings within nests was low. In the three nests in which more than one anadromous male had spawned

with the same anadromous female (nests A2, A3, and B1; Table 3), fertilization success of the second anadromous male was $<4\%$. Among nests in which more than one female had spawned, (nests A1, A6, B2, and B3; Table 3), the second female contributed $<4\%$ of the eggs. In two of these four instances (nests B2 and B3), the same anadromous male was involved in spawning with both females.

In contrast to the low number of multiple anadromous partners within nests, multiple partnering at locations throughout the raceways appeared to be common (Table 3, Fig. 4). For example, in raceway A, ♀_{ii} and ♀_{iii} spawned with anadromous males in four and five nests, respectively, and both had spawned with three different anadromous males. Females in raceway B also had multiple anadromous male partners, although to a lesser degree (Table 3, Fig. 4). Anadromous males also spawned with multiple partners (Table 3, Fig. 4); in raceway B, ♂_{iii} was identified as having spawned in six nests and spawned with all four anadromous females. Similar to the anadromous salmon, individual parr also appeared to participate in spawning with different females and along the entire length of the raceways (Table 2; Fig. 4). In addition to spawning with multiple partners, the anadromous salmon of both sexes and the mature male parr also spawned along the entire length of the raceways (Fig. 4).

Effective population size

Multiple anadromous males spawning simultaneously with the same anadromous female had little influence on the effective number of males ($N_{e\text{♂}}$) per nest, increasing it 3–8% above the value expected had only one anadromous male been involved in spawning at that nest (Table 4). Similarly, the occurrence of embryos from multiple females in a nest also only resulted in an increase in the effective number of females ($N_{e\text{♀}}$) per nest by 3–8% over the value expected had only one anadromous female contributed to that nest (Table 4). Both occurrences were also relatively rare, further diminishing their effect on N_e .

The influence of mature male parr fertilization success on $N_{e\text{♂}}$ per nest varied considerably but tended to be greatest when total parr fertilization success was high. For example, when total parr success was 100% in a nest in raceway A, rather than an expected $N_{e\text{♂}}$ value of 1 had the female spawned with a single anadromous male, $N_{e\text{♂}}$ per nest increased to 4.7 (nest A10, calculation not shown) and 7.7 (Table 4), although in raceway B, $N_{e\text{♂}}$ was only 1.8 at the nest with 100% parr success (Table 4). Low levels of total parr fertilization success (2–4%) had little influence on $N_{e\text{♂}}$, with $N_{e\text{♂}}$ per nest increasing only 3–8% due to the parr fertilization success. At moderate levels of total parr fertilization success (30–45%), $N_{e\text{♂}}$ per nest was slightly greater than 2.

Restricting the analysis to the six nests with the largest sample sizes from each raceway, mean increases in

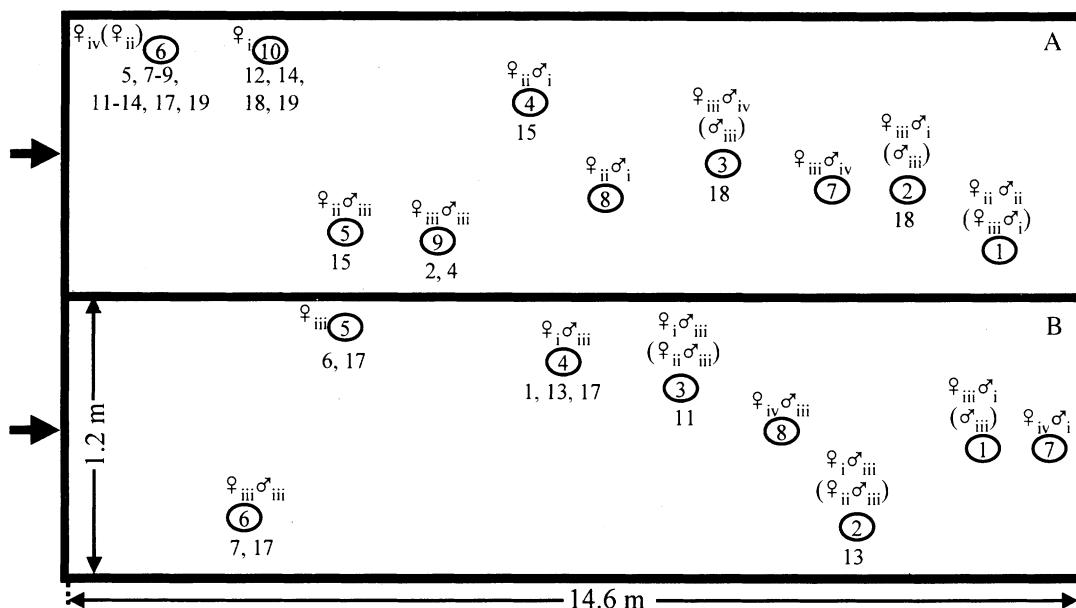


FIG. 4. Anadromous males (σ_i – σ_{iv}), anadromous females (φ_i – φ_{iv}), and parr (1–20) involved in spawning at each nest in both raceways (A and B). The numbers in ellipses refer to the nest numbers designated in Tables 2–4. Anadromous fish with minimal contribution (1–2 embryos) at a nest are indicated in parentheses. Arrows at left indicate location of water inflow; individuals spawned along the entire length of the raceways. Note that the raceway lengths and widths are not to scale and that the nest sizes are exaggerated.

$N_{e\delta}$ per nest were ~ 2.3 and 1.3 times the value expected had only one anadromous male been involved in spawning at each nest in raceways A and B, respectively. These increases were mostly the result of parr fertilization success. If the nests in which parr obtained 100% success had been an artifact of the raceway structure and were excluded from the analysis, mean $N_{e\delta}$ in both raceways declined to 1.18 times the values expected had only one anadromous male been involved in spawning at each nest.

When the variance in individual reproductive success was considered over the entire raceway, $N_{e\delta}$ was 6.02 and 2.41 in raceways A and B, respectively, rather than an expected $N_{e\delta}$ of 4 had only the anadromous males spawned. The $N_{e\varphi}$ was 2.59 and 2.66 in raceways A and B, respectively, rather than an expected $N_{e\varphi}$ of 4 . The overall N_e was 7.24 and 5.06 in raceways A and B, respectively, a decrease from an expected N_e of 8 had only the four anadromous males and the four anadromous females spawned and had all spawned with the variance and means in individual fertilization equal as expected under idealized conditions (Hartl and Clark 1989:89).

If the nests in which parr had 100% fertilization success were excluded from the analyses, the preceding values all decrease. The $N_{e\delta}$ decrease to 4.26 and 1.76 in raceways A and B, respectively, when nests A6 and B5 are excluded. The $N_{e\varphi}$ decrease to 1.94 and 2.37 in raceways A and B, respectively. The overall N_e declines to 5.32 and 4.04 in raceways A and B, respectively. If the total parr contribution is artificially re-

duced to 0% (by assigning all parr fertilization success to the anadromous male that dominated at each nest), the $N_{e\delta}$ declines to 3.63 and 1.48 at raceways A and B, respectively, resulting in N_e values of 5.05 and 3.64 . This represents a decline in N_e of $<10\%$ over their preceding values.

DISCUSSION

Effect of body size on male reproductive success

Although we documented high variation in individual fertilization success, the considerably larger anadromous males obtained much greater success than parr. This result is consistent with all previous studies of alternative reproductive success in Atlantic salmon (Hutchings and Myers 1988, Jordan and Youngson 1992, Morán et al. 1996, Thomaz et al. 1997, Jones and Hutchings 2001). Parr need not have as high fertilization success as anadromous males to obtain similar fitness because they reproduce at a younger age and avoid the high mortality associated with anadromy (Hutchings and Myers 1994).

Although we found mean individual parr fertilization success to be ~ 13 times lower than that of the mean individual anadromous male fertilization success, we may still have overestimated individual parr fertilization success. In natural situations the ratio of mature male parr to anadromous males appears to be higher than that in our experiment (e.g., L'Abée-Lund 1989), which, at similar total parr fertilization success levels, would result in lower mean individual parr fertilization

TABLE 4. Actual ($N_{a\delta}$) and effective ($N_{e\delta}$) number of males, actual ($N_{a\varphi}$) and effective ($N_{e\varphi}$) number of females, and effective population size (N_e) calculated for each nest in raceways A and B.

Nest or raceway	$N_{a\delta}$	$N_{e\delta}$	$N_{a\varphi}$	$N_{e\varphi}$	N_e
Nest					
A1	2	1.03	2	1.03	2.07
A2	3	1.11	1	1	2.11
A3	3	2.10	1	1	2.71
A4	2	1.03	1	1	2.03
A5	2	1.63	1	1	2.48
A6	10	7.65	2	1.04	3.66
B1	2	1.04	1	1	2.03
B2	2	1.04	2	1.08	2.11
B3	2	1.31	2	1.07	2.35
B4	4	1.43	1	1	2.36
B5	2	1.82	1	1	2.58
B6	3	1.08	1	1	2.07
Raceway					
$A_{\bar{x}}$	3.67	2.43	1.33	1.01	2.85
A_{overall}	16	6.02	3	2.59	7.24
$A_{\text{not } 6}$	6	4.26	2	1.94	5.32
$A_{\text{no-parr}}$	4	3.63	2	1.94	5.05
$B_{\bar{x}}$	2.50	1.29	1.33	1.03	2.29
B_{overall}	8	2.41	4	2.66	5.06
$B_{\text{not } 5}$	7	1.76	4	2.37	4.04
$B_{\text{no-parr}}$	3	1.48	4	2.37	3.64

Notes: Mean effective numbers (\bar{x}) were calculated as the mean of the $N_{e\delta}$ and $N_{e\varphi}$ from each nest in a raceway; N_e was calculated from these resulting means. The overall effective numbers were calculated by using the overall individual fertilization success values from each raceway (weighting each nest equally at $n = 50$). Similar calculations were made excluding the nests in which parr obtained 100% fertilization success ($A_{\text{not } 6}$ and $B_{\text{not } 5}$). The influence of parr on effective sizes was removed by assigning all parr fertilization success to the dominant male at that nest (no-parr).

success. Our test of the potential power of these genetics markers to distinguish among greater numbers of potential parents suggests that successful parentage assignment can be achieved with greater numbers of potential contributors. The most frequent cases of unassigned parentage in our simulation involved the inability to distinguish between two or three potential fathers and generally involved the same individuals in each raceway. Prescreening of individuals to be used in experiments could allow for the exclusion of those individuals sharing alleles at multiple loci, preventing such analytical difficulties. Alternatively, as demonstrated in this experiment (e.g., with two males in raceway B that required an additional three loci to unambiguously determine paternity), additional loci can be used to distinguish between potential parents. It is thus possible in future studies to increase the number of parr present to more natural ratios.

The influence of increased anadromous male competition on total parr success is difficult to ascertain. Single anadromous pair cross experiments, albeit with variation within and among studies, have typically found total parr fertilization success per nest to be ~30% (Hutchings and Myers 1988 [20%], Morán et al. 1996 [51%], Thomaz et al. 1997 [30%], Jones and

Hutchings 2001 [31%]), while the only multiple-pair cross to date revealed considerably lower total parr success (Jordan and Youngson 1992, 11% mean total parr success). The results from our experiment suggest higher total parr success (30%) in multiple anadromous pair crosses. However, if the three nests in which parr obtained 100% success were an artifact of the raceway structure (e.g., nests constructed immediately adjacent to the walls) and are excluded, total parr success would be considerably lower (16%), supporting the hypothesis that increased anadromous male competition results in lower total parr fertilization success. We recognize that our findings remain somewhat inconclusive as the raceway walls could function in a manner similar to large boulders found in natural systems. Thus, the results obtained in nests adjacent to the raceway walls may indeed reflect natural conditions.

During spawning, mature male parr are arranged in a hierarchical manner with the largest male nearest the female (Myers and Hutchings 1987). At low parr densities (six parr), one study found a relationship between individual parr size and reproductive success when parr were competing with one anadromous male for fertilization opportunities with one anadromous female (Thomaz et al. 1997). The cost of maintaining this improved access to the female is expected to increase with larger numbers of competitors to a point at which body size may have no influence on fertilization success (Hutchings and Myers 1994); Jones and Hutchings (2001) found some evidence supporting this. In the present experiment, only 2 of 18 nests showed evidence of more than two parr successfully spawning and many parr were found not to have fertilized any eggs at all. It is thus not surprising that no relationship between parr size and individual reproductive success was found either within or among nests. Larger parr might be expected to spawn more frequently and thus have lower variance in mean reproductive success (Jones and Hutchings 2001). Although the low detected success rate precludes a testing of this hypothesis in our experiment, the apparent relationship between parr size and number of nests in which an individual spawned, among those parr who were detected to have spawned, provides some support for it.

In contrast to the number of studies that have examined the factors influencing individual parr reproductive success, less attention has been directed to examining the factors influencing individual reproductive success among anadromous males. We found no evidence of an influence of size on anadromous male reproductive success. The high individual reproductive success by a smaller male in raceway B contrasts somewhat with Mjølnerød et al.'s (1998) finding that size was often an important determinant of dominance and individual fertilization success. Jones (1959:101), however, noted that the dominance of an anadromous male in a group "is certainly not dependent on size" and

suggested that dominance may be related to an individual's readiness to spawn.

Spawning associations

Both anadromous males and females spawned with multiple mates. Females clearly spawn with different males in successive spawning events and males do spawn with different females. The results from this study may even underestimate the number of potential spawning partners because the maximum number of potential anadromous mates was limited to four. Anadromous females lay many nests (e.g., Jones (1959), up to 8; Fleming (1996), 5 to 7) and can spawn with multiple males each time (e.g., Mjølnerød et al. 1998). Mjølnerød et al. (1998) described using one anadromous male in 31 different spawning trials. Although information regarding this male's involvement in his earlier trials (1–27) was not presented, he was still obtaining some fertilization success as late as the 29th event while another male was dominating fertilization success as late as his 15th trial.

We observed little evidence of multiple anadromous males being involved in spawning within nests. Among the few cases we detected, the second anadromous male achieved very little fertilization success. Although single-pair matings are common, matings involving multiple males are reportedly of a higher frequency than observed in our experiment (13–63%, Fleming 1996). Further, when multiple male fertilization events occur, the second male has been reported to obtain slightly higher fertilization success than that reported here (up to 16%, Mjølnerød et al. 1998). The occurrence of embryos from multiple females in the same nest suggests that some degree of nest superimposition occurred in our experiment, although the extent of complete nest displacement cannot be assessed.

Effective population size

The results of our experiment suggest that variance in anadromous male individual fertilization success has the greatest overall influence on effective population size (N_e) while parr individual fertilization success is of only moderate influence. The effective number of males, both within nests and raceways, was lower than the number of males identified as having any fertilization success and was much lower than the number of males available for spawning (24 males were present in each raceway; the overall $N_{e\sigma}$ was 6.02 and 2.41 in raceway A and B, respectively).

Because estimates of the number of anadromous males often exist while usually little is known about the number of mature male parr present at spawning, the influence of mature male parr fertilization success, in addition to that of the anadromous males, is of great interest. Within individual nests, the influence of parr fertilization success can increase the effective number of males several times that which would be expected had only one anadromous male spawned with the fe-

male. From such a finding alone or by extrapolating from previous single-pair cross experiments, one might conclude that parr fertilization success would profoundly affect N_e . However, due to their greater overall fertilization success, anadromous males have a greater potential influence on N_e than parr. This potential is realized because there is great variation in individual anadromous male fertilization success. Indeed, variance in both anadromous male and female success resulted in a decrease in N_e over the value expected under idealized conditions by up to 46% and 26%, respectively, while parr success may increase N_e by as little as 5% (Table 4). We suggest that a critical parameter to quantify in the wild is variance in anadromous salmon reproductive success, and that parr fertilization success, as a determinant of N_e , is likely to be of secondary import.

Assumptions made in our calculations of N_e can result in some biases. For all calculations of N_e we weighted each nest equally. This could result in an overestimation of N_e for two reasons. Firstly, the number of eggs per nest, spawned by a single female, tends to decline with each subsequent nest constructed by that female (Fleming et al. 1996). If some males are more successful in spawning with females when females first spawn, there will be a higher variance in individual male fertilization rate, resulting in a lower $N_{e\sigma}$. Secondly, nests can be lost (Barlaup et al. 1994) and loss may depend on factors such as spawning time, or the ability of females to obtain desirable spawning locations (Fleming et al. 1996). Nest loss will result in an increase in the variance of individual female reproductive success, resulting in a further decrease in $N_{e\sigma}$. While these factors may bias our calculations of N_e , our overall conclusions remain robust.

In reviewing the factors that influence the ratio of effective to actual population sizes in nature, Frankham (1995) concluded that variance in family size and unequal sex ratios were of prime importance. However, of greater importance was the effect of fluctuations in population size; this was not addressed in our study. Over the short term, N_e is the harmonic mean of the annual effective population size and is thus most influenced by the smallest annual N_e (Wright 1938). When populations are at their lowest abundance, fewer anadromous males will be present on the spawning grounds. Simultaneously, the number of mature male parr per female will remain constant or possibly increase; when parr densities are lower, growth rates increase and faster-growing individuals tend to have a higher probability of maturation (e.g., Thorpe 1986). Thus, at low population sizes, mature male parr may have their highest fertilization success and may have their greatest within-generation influence on N_e .

Our findings have many general implications to natural populations of other species. The different findings in our two replicates emphasize not only the clear need for replication in such experiments but also how, even

in populations of stable size, annual differences in N_e will likely exist due to interannual variation in individual reproductive success. Again, because N_e is the harmonic mean of the annual effective population size and is thus most influenced by the smallest annual N_e , this can be of great concern to threatened or endangered species for which population sizes are already small. When quantification of N_e is desirable to aid in the conservation of natural populations, quantification of the variance in individual reproductive success, and the potential degree of interannual variability in this estimate, is essential.

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