Multiple paternity and variance in male fertilization success within Atlantic salmon *Salmo salar* redds in a naturally spawning population

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The incidence and magnitude of multiple paternity were estimated for a natural, unmanipulated spawning population of Atlantic salmon *Salmo salar*. Egg nests were surveyed in the autumn and sub-samples were excavated the following spring. Parentage data derived from microsatellite DNA revealed an unexpectedly high level of multiple paternity. Within a single redd, females may mate with as many as 16 different males, including small mature male parr and large anadromous males. Multiple paternity was most pronounced in areas of highest redd density, corresponding with increased abundances of mature male parr. In addition, there was considerable variation in success among males, although this variability did not depend upon the number of males participating in spawning. This work underscores the value of undertaking genetic studies on the mating systems of fishes in unmanipulated, natural environments. © 2010 The Authors

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Key words: alternative reproductive strategies; mating system; mature male parr; salmonid.

INTRODUCTION

Variance in reproductive success and mate number is generally assumed to be higher among males than among females in many animals (Bateman, 1948; Shuster & Wade, 2003). Increased variance in male success is often a result of more intense sexual selection among males than females, such that more attractive or aggressive males sire a disproportionately large share of a given cohort (Clutton-Brock, 1988; Andersson, 1994). Differences in mate number are usually attributed to the fact that males of some animal species have lower gametic investment (Bateman, 1948), provide less parental care (Trivers, 1972) and consequently have higher potential reproductive rates than females (Clutton-Brock & Vincent, 1991). Many genetic studies, however, indicate that multiple mating by females may be more widespread than classical mating systems theory would predict (Hughes, 1998). In addition, males of many species adopt evolutionarily stable alternative mating strategies,

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which may increase the incidence of multiple mating and affect the variance in reproductive success among males (Shuster & Wade, 2003). Male Atlantic salmon *Salmo salar* L., for example, mature either as small mature male parr, in the absence of a seaward migration, or as large anadromous males, following migration to sea (Jones, 1959).

Multiple male matings may occur when male and female densities in spawning areas are high. During the spawning season, female *S. salar* often aggregate in or close to areas with favourable substrata for egg development (Fleming, 1996). Large males often make short-distance migrations within rivers just before the spawning period, presumably in search of females (Økland *et al.*, 2001). Males are highly receptive to female odours (Moore & Scott, 1992; Olsén *et al.*, 2002), such that a large number of males may be attracted to areas where there are many females. Female *S. salar* typically spawn asynchronously, such that the operational sex ratio (OSR: the ratio of sexually active males to mature females in a population; Emlen, 1976) is often male biased. Because male *S. salar* establish dominance relationships during spawning (Fleming, 1996; Weir *et al.*, 2004), highly aggressive males may be able to control access to females. Alternatively, when male density is high, and the OSR is heavily male biased, dominant individuals may not be able to effectively defend females (Emlen & Oring, 1977) and subordinate males may be more likely to gain access to females using alternative reproductive behaviours, such as sneaking (Fleming, 1996). Thus, it might be predicted that multiple paternity may be higher in areas with more females. Consequently, the variance in success among males should decrease as more individuals successfully fertilize eggs.

The only field study under near-natural conditions that has attempted to elucidate the genetic mating system of *S. salar* suggests that multiple male matings are common (Taggart *et al.*, 2001). Experimental (Mjølnerød *et al.*, 1998; Martinez *et al.*, 2000) and field (Taggart *et al.*, 2001) studies, however, suggest that only a small number of anadromous males may realize success during multiple male spawning events. Thus, mating events with many sires may include significant contributions from young, non-migratory male parr, who are often an order of magnitude smaller than the anadromous fish. Consequently, distinct spawning events, whereby females deposit a batch of eggs in a single nest, may involve both dominant and subordinate anadromous males, as well as mature male parr. Experimental evidence suggests that as many as 10 mature male parr can sire offspring during a single spawning event (Jones & Hutchings, 2002), a number that far exceeds the maximum number of anadromous male sires in a given spawning event (up to three large males: Mjølnerød *et al.*, 1998; Martinez *et al.*, 2000; Taggart *et al.*, 2001).

In addition to providing important information about mating systems in natural environments, this study tests the null hypotheses that: 1) the incidence of multiple paternity is independent of female density and 2) the variance in fertilization success does not change with changes in the number of sires per female. Unlike all but one study to date (Taggart *et al.*, 2001), this work was undertaken in a natural, unmanipulated population of *S. salar* whose population structure and genetic variability have not been influenced by the stocking of hatchery-reared fish.

MATERIALS AND METHODS

REDD SAMPLING

The study was conducted in Catamaran Brook, a 20 km long, third order tributary of the Little Southwest Miramichi River in north-eastern New Brunswick, Canada (Fig. 1). The system is part of a multi-year study examining ecosystem processes and the effects of logging on fish species in the catchment (Cunjak *et al*., 1993). Species in Catamaran Brook are mainly salmonids [*S. salar* and brook trout *Salvelinus fontinalis* (Mitchill)], cyprinids [blacknose dace *Rhinichthys atratulus* (Hermann) and lake chub *Couesius plumbeus* (Agassiz)], white sucker *Catostomus commersonii* (Lacépède) three-spined stickleback *Gasterosteus aculeatus* L., slimy sculpin *Cottus cognatus* Richardson, American eel *Anguilla rostrata* (Lesueur) and sea lamprey *Petromyzon marinus* L. (Cunjak *et al*., 1993). For sampling purposes, the brook has traditionally been separated into four reaches: Lower, Gorge, Middle and Upper (Fig. 1). For consistency, these designations were used when classifying redd locations.

Migration into Catamaran Brook from the Little Southwest Miramichi River occurs just before spawning in early to mid-October. During their upstream migration, migratory *S. salar* were captured at a counting fence located near the mouth of the brook from 15 October 2003 until the fence was removed on 23 November 2003 (the counting fence was temporarily removed from 27 October to 3 November 2003 because of exceedingly high water levels). Fish were measured, tissues sampled for subsequent DNA analysis and scales extracted for ageing. Before release back into the brook, adults were marked with a Carlin tag. Data from an electrofishing survey, conducted yearly to assess species abundance and composition (Mitchell *et al*., 2004), were used to estimate the number of mature male parr in the system. Briefly, there were 22 electrofishing sites distributed along Catamaran Brook (Fig. 1) in riffle, run, flat and pool habitats. Each site (mean length 11·95 m, range 7·25−26·60 m; mean area 99·4 m^2, range 52·2−191·1 m^2) was blocked with barrier nets and electrofished between three and five times, using the removal system for population estimation (Zippin, 1958). Mature male parr were identified by the extrusion of milt after their abdomens had been gently squeezed. The number of redds within 1 km of each electrofishing site was used to correlate mature male parr numbers with the number of redds in an area.

Redds were surveyed from 17 October to 19 November 2003. Beginning downstream, surveys were conducted by walking along opposite banks of the brook until a redd was located. Redds were identifiable as large mounds of gravel that were cleaner than the surrounding substratum. Upon locating a redd, global positioning system (GPS) co-ordinates were recorded, using a Magellan GPS 315 hand-held unit (www.magellangps.com). Length and depth of redds were also measured and the type of substratum noted (Wentworth classification). To accurately re-locate a redd, a large metal nail was placed on both banks of the brook and the distance from each nail to the redd measured. Nearby trees were flagged for easier re-location. The continuous distance between redds was estimated using GPS co-ordinates.

From 24 April to 8 May 2004, developed eggs were retrieved from a sub-sample of redds. At this time, eggs were at the 'eyed' stage, *i.e.* between 110 and 300 degree-days old. The numbers of redds located in the Gorge and Middle reaches were 1·5 times and double that located in the Lower reach, respectively. Eighteen redds were randomly chosen for sub-sampling in proportion to the total number of redds found within the Lower ($n = 4$), Gorge ($n = 6$) and Middle reaches ($n = 8$). These redds were sampled by digging the substrate with a spade while holding a large net 1 m downstream to collect drifting eggs. Effort was made to begin at the most downstream part of each redd, such that the sampled eggs would have had a high probability of originating from the first nest deposited by each female. Excavation occurred by slowly digging in the streambed, and the net was checked for eggs after each disturbance to the substrate. Digging continued until eggs could be seen entering the net, at which point excavation ceased. The location where digging stopped was marked before counting the number of collected eggs. If the number of collected eggs was less than the desired sample size of 50, excavation continued from the marked location. A desirable sample of eggs in most redds was collected within two excavations. Although it is possible that the eggs sampled came from more than one nest, it is unlikely that more than two nests were sampled by this
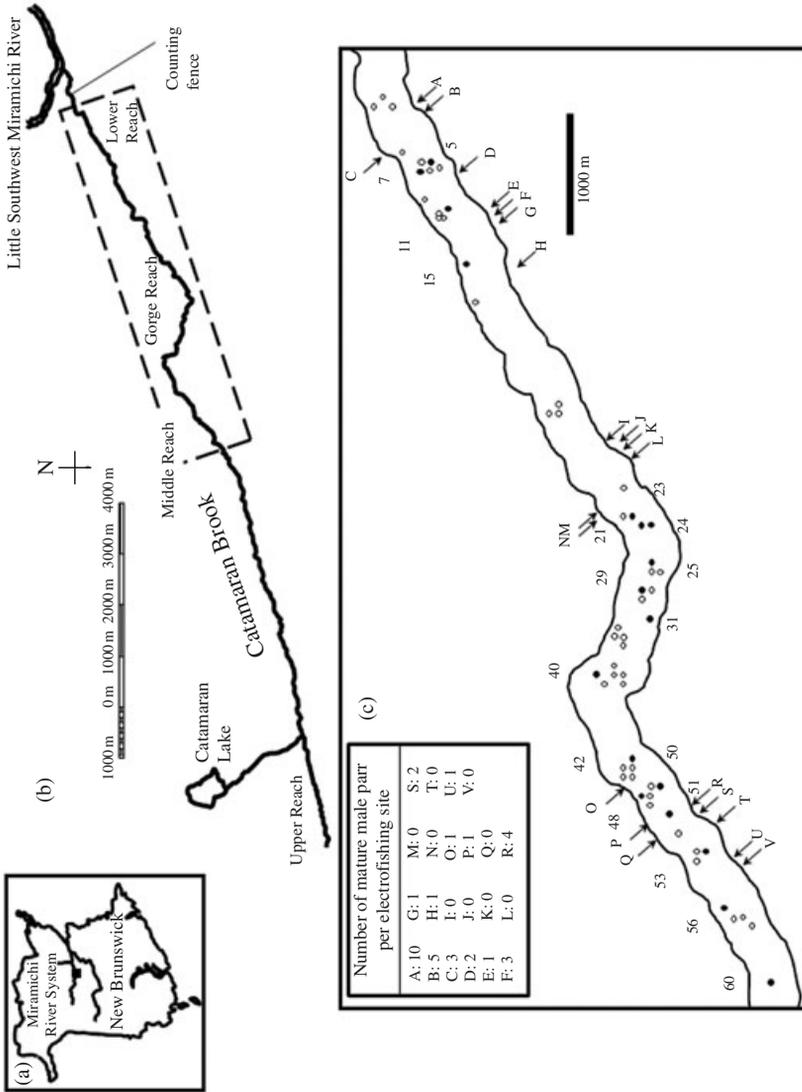
FIG. 1. (a), (b), (c) Map of the study area. (c) Sampled redds numbered sequentially upstream are indicated (●) as are approximate locations of electrofishing sites in the system (→). Denoted locations of other redds are indicated (○). The number of mature male parr found at each of the 22 electrofishing sites is shown.

method. A sample size of 50 eggs per redd was chosen. This represented a trade-off between sampling a number of eggs larger than that of the only previous study conducted on a natural population (Taggart *et al.*, 2001; *c*. 10−20 eggs per redd) and causing a destructive physical impact on the redd. Eggs from each redd were stored in 95% ethanol for subsequent genetic analysis.

GENETIC ANALYSES

DNA analysis of eyed eggs was completed at the Gene Probe Laboratory at Dalhousie University. Eggs were dissected and shells and yolk sacs were removed before digestion in a 96 well chimney top plate to which 100 µl of eyeball buffer (10 mM Tris, 50 mM KCl, 0·5% Tween 20) with 2 mg ml^{-1} proteinase K was added. Samples were digested overnight with light shaking at 55° C. Following digestion, plates were vortexed and then treated for 15 min at 95° C to de-activate the proteinase K. To remove impurities, samples were centrifuged at 20 000 × *g* for 2 min and subsequently stored at −20° C until polymerase chain reaction (PCR) amplification. Extracted DNA was amplified at the following microsatellite loci: *Ssa197* (O'Reilly *et al.*, 1996), *SSsp2210*, *SSsp2213*, *SSsp2215*, *SSsp2216* and *SSsp1G7* (Paterson *et al.*, 2004). The PCR programme for 10 µl volumes was as follows: denaturation at 95° C for 3 min, followed by 35 cycles of 30 s at 95° C, 30 s at 58° C, 30 s at 72° C and a final extension for 30 min at 72° C. Samples were run on 6% acrylamide gels and visualized using an FMBIO scanner (www.miraibio.com). Individuals were genotyped against standard samples of known sizes and each individual was scored three times. Samples that did not amplify for at least four loci were discarded, resulting in an average of 51 offspring per redd for analysis. Non-amplification occurred most frequently for loci that included larger alleles, a common error that may occur during amplification or because of poor DNA quality.

Genotypes of adults (32 females and 66 males) sampled at the counting fence in the autumn of 2003 were obtained from a parallel project at Concordia University (Brodeur, 2006). Tissues were extracted, using Qiagen DNeasy tissue kits (www.qiagen.com). The PCR was carried out in 24 µl volumes of PCR reaction buffer (20 mM Tris−Cl pH 9·5, 25 mM KCl, 0·05% Tween-20, 100 µg ml^{-1} bovine serum albumin and 1·5 mM MgCl$_2$), 0·2 mM dNTPs, 0·2 pmol µl^{-1} forward and reverse primers and 0·05 units µl^{-1} Tsg DNA polymerase. The following PCR thermal cycling conditions were used: initial denaturation at 96° C for 3 min, 35 cycles of 96° C for 30 s, 58° C for 30 s and 72° C for 30 s and a final extension at 72° C for 5 min. Samples were genotyped with an ABI sequencer. Genotyping was standardized by comparing the genotypes of 10 embryo samples run at both the Gene Probe Laboratory and the Concordia University and by comparing allele frequencies of captured adults with those of the genotyped embryos.

STATISTICAL ANALYSES

Paternity estimates

Direct parentage assignment was not possible due to an incomplete sample of potential parental genotypes. This sample was incomplete for two reasons. First, extremely high water levels over a 7 day period during the spawning period prevented the capture of migrating fish. Second, mature male parr were not captured or genotyped. Thus, this approach involved first identifying the maternal genotype by exclusion and then reconstructing possible paternal contributors. A single maternal genotype was easily reconstructed by recognizing the two most common alleles and verifying that at least one was present in each offspring (Feldheim *et al.*, 2004). This method was used to identify the maternal genotype in all but one redd, in which two maternal genotypes were identified using the programme COLONY 2.0.0.0 (Wang & Santure, 2009). In addition, maternal genotypes were verified using both COLONY 2.0.0.0 and PEDIGREE (Smith *et al.*, 2001).

Individual anadromous males were not included as potential parents in the COLONY analyses to ensure that the results were conservative. Thus, it is likely that the individual assignments to anadromous males captured at the fence represent an underestimation of

the actual contribution of these males. A conservative approach was adopted because the proportion of offspring in the samples that had been sired by males captured at the counting fence could not be approximated.

Full-sibling groups were assembled using two different methods, and all analyses were performed within redds ($n = 18$ separate analyses). First, the number of full-sib families within each redd was estimated, using COLONY 2.0.0.0, by specifying the maternal genotype and reconstructed potential paternal genotypes. Second, the number of full-sib families in a redd was determined using PEDIGREE (Smith *et al.*, 2001), assuming that no parental data were available. This second approach was used to corroborate the data obtained from COLONY 2.0.0.0 and to verify maternal genotypes. Data were analysed with COLONY 2.0.0.0 because this programme allows for specification of the maternal genotype in the absence of data on sires. Because the maternal genotype could be easily reconstructed by exclusion, it was used in the analysis to increase the probability of obtaining accurate full-sib groupings. The maternal genotype for each redd was entered in the programme, and paternal genotypes were reconstructed using likelihood methods. This programme is particularly powerful because it allows for specification of the rate and type of specific genotyping errors at each locus. The genotypes of each redd were run through COLONY 2.0.0.0, using error rates of 0·015, 0·025, 0·050 and 0·075. COLONY 2.0.0.0 was allowed to calculate allele frequencies in the population, mainly because the population-level allelic distributions could not be specified due to the fact that there were alleles present in the offspring that were not detected in the migrating adults (Wang, 2004).

To corroborate the COLONY 2.0.0.0 results, a second method was employed that did not allow for prior parental data to be specified. Offspring genotypes from each redd were also run through PEDIGREE (Smith *et al.*, 2001), which uses a Markov chain Monte-Carlo method and assumes no prior parental data. PEDIGREE parameters were optimized such that results from different runs were consistent. For all individual redds, a temperature and weight of 10 were used, with 24 runs of 500 000 iterations each. From these runs, those that created groups with the highest partition scores were used for subsequent analyses (Smith *et al.*, 2001).

CORRELATES OF MULTIPLE PATERNITY AND VARIANCE IN FERTILIZATION SUCCESS

The spatial dispersion of redds along the brook was assessed by the nearest neighbour method (Krebs, 1999), and the significance of deviation from randomness was determined with a z test. Redd aggregation was used as a proxy for female density. Correlates of multiple paternity (*e.g.* number of redds within 1 km and number of mature male parr in the area) were analysed using Pearson's correlations (r) and regression analyses in S-Plus 6.1 (Insightful Corp., www.tibco.com) with significance assigned at $\alpha \leq 0.05$. Paternity distribution within redds was expressed using the skew (g_1) and kurtosis (g_2) of full-sib family sizes. In addition, the coefficient of variation (C.V.) (y), calculated from $y = Z\overline{x}^{-1}$ (where $Z = $ S.D. and $\overline{x} = $ mean), of paternity among contributing paternal genotypes was used to describe the variance in fertilization success among males within redds.

RESULTS

REDD SAMPLING

Sixty redds were located along a 10 km stretch of the Catamaran Brook in autumn 2003. Redds were, a mean ± S.D. 1.8 ± 0.6 m long and had a mean ± S.D. depth of 0.36 ± 0.08 m from the top of the undisturbed substratum surface. Nearest neighbour analysis indicated that the redds were clumped in space ($r = 0.61$, $|z| = 72.65$, $P < 0.001$), suggesting that some areas of the stream represented preferred spawning habitat for females.

TABLE I. Number of alleles, observed (H_o) and expected (H_e) heterozygosities for sampled offspring and captured migratory adult *Salmo salar* from Catamaran Brook, New Brunswick, in 2003 and 2004

Locus	Number of offspring alleles	H_o	H_e	Number of adult alleles	H_o	H_e
Ssa197	17	0·886	0·875	16	0·856	0·894
SSsp2210	9	0·558	0·596	9	0·588	0·581
SSsp2213	14	0·856	0·874	15	0·763	0·901
SSsp2215	18	0·861	0·839	20	0·907	0·912
SSsp2216	27	0·884	0·917	27	0·907	0·928
SSspG7	16	0·886	0·892	16	0·845	0·907
Average	17	0·822	0·832	17	0·811	0·854

GENETIC ANALYSES

Paternity estimates

Adult genotypes were in Hardy–Weinberg equilibrium for all loci (Table I); however, some deviations did occur among offspring within redds. This is not unexpected, as sampling within half-sib families violates the assumptions of random mating and large population size. These deviations, however, are not likely to affect the results because of the nature of the study. Family size and paternal distribution were relatively consistent for most redds across the four different error rates specified in COLONY 2.0.0.0 (changes in family sizes within redds across error rates ranged from 0 to 3). In cases where the number of full-sib families differed across the different error rates, they increased as error rates decreased. Paternal distribution differed markedly depending on assigned error rate for only three of the sampled redds. In these cases, the result that was most similar to that obtained by PEDIGREE was used. Consequently, all results are presented with an error rate of 0·05, such that estimates are conservative without error overestimation. To verify the accuracy of family reconstruction, results within redds were compared between COLONY 2.0.0.0 and PEDIGREE. There was generally good corroboration between the two methods, with no significant differences in the number of half-sib families (Pearson's correlation: $r = 0.979$, $n = 19$, $P < 0.001$) or overall distribution of family sizes patterns between COLONY 2.0.0.0 and PEDIGREE (Kolmogorov–Smirnov goodness-of-fit test: $ks = 0.055$, d.f. $= 1$, $P > 0.05$).

There was a considerable range in the number of males that fertilized eggs in each redd; between one and 16 paternal genotypes ($\bar{x} \pm$ s.d. $= 8.0 \pm 4.6$; Fig. 2) were reconstructed per redd, using COLONY 2.0.0.0. One redd (40) contained two maternal genotypes, and offspring of the different females were analysed separately. Reconstruction of female genotypes from offspring indicated that four of 15 identified females spawned at a minimum of two locations along the brook (Fig. 2). The distance between two redds constructed by each of these four females was 271, 490, 734 and 1050 m. This result was corroborated by reconstruction of half-sib families in PEDIGREE. In addition, seven of the reconstructed maternal genotypes could be linked to females that were caught at the counting fence. No two females at the counting fence had the same genotype at the six loci examined, and the estimated identity probability for females caught at the fence was 1.31×10^{-9}. Thus,
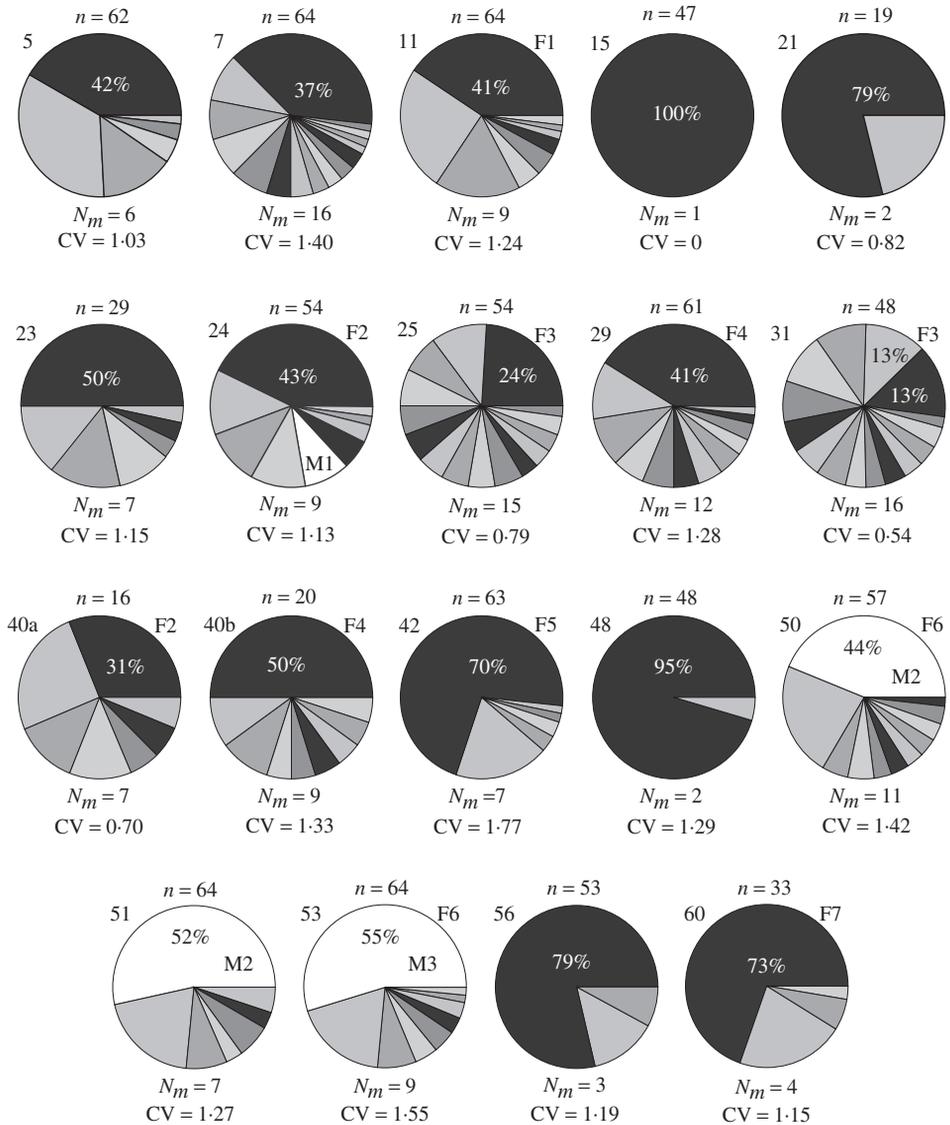
FIG. 2. Paternity distribution within redds. Numbers on upper left of each chart represent the redd numbers given in Fig. 1. *n*, the number of eggs sampled; N_m, the estimated number of males that fertilized eggs; c.v., the coefficient of variation within a given redd. Proportion of offspring for which the male is a known migratory male (○) is indicated. Females (F) and males (M) with numbers indicate arbitrarily numbered known individuals involved in spawning. Females 2, 3, 4 and 6 and male 2 each spawned in two sampled redds.

reconstructed genotypes were probably from the same individual and not from two individuals possessing the same genotype. Similarly, the genotypes of three males that were captured at the counting fence were recognized, one of which was identified in two different redds (50 and 51) 380 m apart. In the case where the identified

male fertilized a small proportion of eggs (*e.g.* redd 24; Fig. 2), however, there is less certainty that the fish is indeed an individual identified at the counting fence because particular paternal genotype reconstruction is less reliable when the number of offspring available to reconstruct paternity is low (*i.e.* the full allelic composition of potential fathers is incomplete or poorly supported). In all other cases, individuals fertilizing the highest proportion of eggs were identified as anadromous males and the reconstructed paternal genotype was the most likely one (redds 50, 51 and 53; Fig. 2). In one case, two males caught at the counting fence possessed the same genotype, were similar in size (58 and 57 cm fork length) and were captured 30 days apart first going upstream and then downstream. Thus, it is possible that this was the same individual tagged twice.

CORRELATES OF MULTIPLE PATERNITY AND VARIANCE IN FERTILIZATION SUCCESS

The number of eggs sampled differed among redds, but this did not affect estimates of the number of males involved ($r = 0.340$, $n = 19$, $P > 0.05$) or the proportion of eggs fertilized by the most successful male ($r = -0.128$, $n = 19$, $P > 0.05$). Not surprisingly, the number of eggs fertilized by the most successful male decreased as the number of males fertilizing eggs increased ($r = -0.866$, $n = 19$, $P < 0.001$; Fig. 3). On average, eight males fertilized eggs in a redd with the most successful male obtaining a predicted mean of 41% fertilization success.

Paternity distribution was positively skewed in 11 of the 19 redds and significantly leptokurtic in 10 of the 11 redds where the distributions were significantly skewed (Fig. 2). Variance in male mating success (approximated by the c.v. in numbers of eggs fertilized per male in a given redd) was not associated with the number of males that fertilized eggs ($r = 0.166$, $n = 19, P > 0.05$). The degree of multiple paternity, however, was correlated with the density of redds {*i.e.* number located within 1 km of a focal redd [$r = 0.577$, $n = 19$, $P < 0.05$; Fig. 4(a)]}. In addition, electrofishing data indicated that mature male parr were more commonly found in areas with multiple redds [$r = 0.551$, $n = 22$, $P < 0.05$; Fig. 4(b)]. Approximate population size was estimated, using Petersen's estimate (Krebs, 1999), to obtain a total anadromous population size of 162 individuals. The sex ratio at capture was 2·1 males per female. Thus, there was a minimum of 109 and 52 migratory males and females, respectively, in the adult population. These numbers should be considered underestimates because of the flood conditions that affected the mark-recapture data. Estimates from electrofishing data indicate that *c*. 500 mature male parr were present in the system.

DISCUSSION

One other study to date has assessed the genetic mating system of *S. salar* under natural conditions (Taggart *et al.*, 2001). The present study serves to both complement and expand the results of this previous work. Notwithstanding the fact that this research was undertaken in a different system, it differs from that of Taggart *et al.* (2001) in two important ways. First, within-redd sample sizes were increased five-fold in the present study, such that a better estimate of the magnitude of
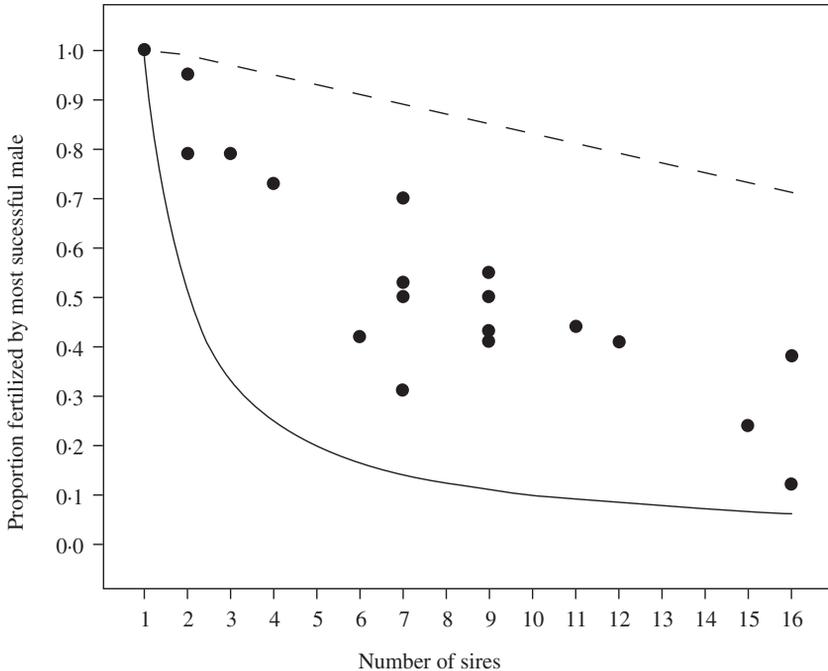
FIG. 3. Relationship between the proportion of eggs fertilized by the most successful male in a redd and the number of males participating in spawning (●): predicted paternity distribution if males fertilize eggs with equal success (—) and the maximum possible fertilization probability of the most successful male, given the number of males contributing in a nest (- - -).

multiple paternity could be obtained and offspring could be assigned to parents more accurately. While a sample size of 50 eggs per redd may represent a small proportion of the total number of eggs produced per female in a spawning season (*c*. 5000 eggs; Randall, 1989), females typically lay their eggs in a series of discrete nests (Fleming, 1996). The sampling regime used here probably captured only one or two spawning events, or nests, such that the sample was *c*. 10% of eggs per nest. Despite this, the magnitude of multiple paternity in many samples was remarkably high, such that larger sample sizes should increase the variance and magnitude of multiple paternity. Thus, the results of this study might conservatively underestimate the variability and number of sires participating in spawning. Second, the use of microsatellites rather than minisatellites allowed for greater resolution of full-sib families, as suggested by Taggart *et al.* (2001). As such, the number of males participating in spawning could be estimated with more confidence, as could the distribution of paternity in single spawning events. Thus, this study provides additional and valuable information about the frequency and magnitude of multiple male spawnings in *S. salar*, as well as new information relevant to the testing of hypotheses regarding changes in paternity distribution with fish density.

  Three primary conclusions can be drawn from this field study of naturally occurring *S. salar*: 1) multiple paternity occurs frequently under natural conditions, whereby some females may spawn with up to 16 different males, 2) females have
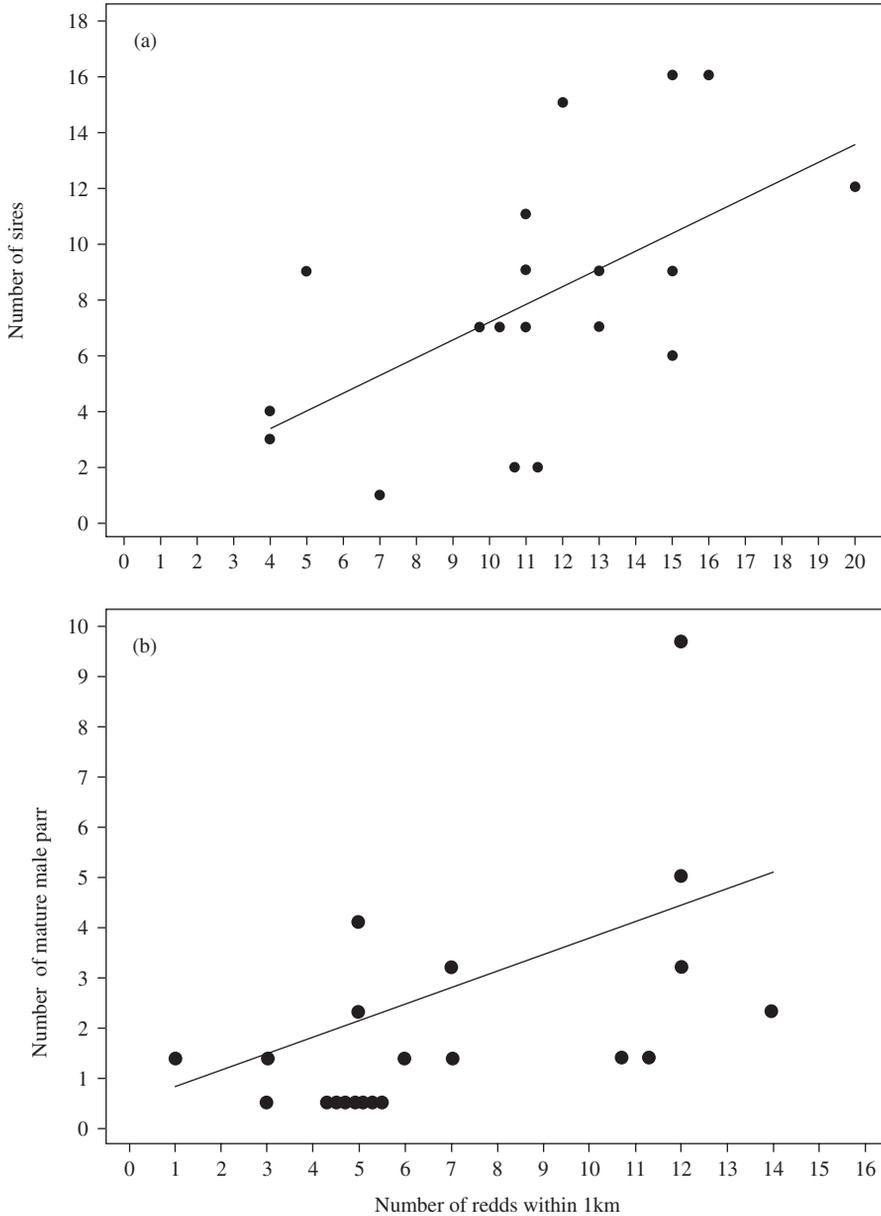
FIG. 4. Relationship between density of redds (number within 1 km) and (a) the number of males fertilizing eggs in a redd and (b) the number of mature male parr sampled during electrofishing. The curves were fitted by: (a) $y = 0.89 + 0.63x$ and (b) $y = -0.88 + 0.37x$.

preferred spawning areas and the frequency and magnitude of multiple paternity tends to be higher in these areas and 3) there is considerable variance in fertilization success among males that does not depend on the magnitude of multiple paternity.

PATERNITY ESTIMATES

Results of this study indicate that multiple male spawnings are common in *S. salar*; findings that are consistent with those reported by Taggart *et al.* (2001). Other studies under natural or near-natural conditions have shown that there might be many males siring offspring (*S. salar*, Garant *et al.*, 2001; *Oncorhynchus mykiss* (Walbaum), Seamons *et al.*, 2004; Kugliowski *et al.*, 2005; *Oncorhynchus tshawytscha* (Walbaum), Bentzen *et al.*, 2001), suggesting that male dominance is not absolute in many systems. This may be associated with the fact that external fertilization in salmonids can allow for subordinate males to adopt sneaking tactics during mating. Experimental evidence suggests that the incidence of multiple paternity by large males during the same spawning event is limited to a maximum of two or three males (Mjølnerød *et al.*, 1998; Taggart *et al.*, 2001). This assertion is supported by the experimental work of Martinez *et al.* (2000) who found that multiple paternity attributable to many adult male contributions was not common. Thus, it is unlikely that the number of sires in a given nest is due only to contributions from large males, such that much of the paternity is attributable to participation by large numbers of mature male parr.

Given these observations, it can be conservatively estimated that parr contribution in this study range from 22%, if the three most successful males are assumed to be anadromous, to 46% in spawning events involving only one anadromous male. Under these assumptions, the latter result is consistent with that of Taggart *et al.* (2001; 40–50% of offspring sired by anadromous males in 91% of the sampled redds). It is possible, however, that individual parr may be the most successful males in some spawning events, as individual parr are capable of fertilizing a large number of eggs (Myers & Hutchings, 1987), although the incomplete sampling of potential parents in this study precludes accurate estimates of fertilization success of anadromous males and mature male parr.

Based on previous experimental and field studies, anadromous males were expected to be present in most nests and fertilize a large number of eggs. Only three males, however, could be identified as having sired offspring in the nests sampled, in contrast with 11 of 19 females that could be assigned to particular redds. One possibility for this discrepancy between the present results and previous observations is that mature male parr were the dominant fertilizers in this study. Poor assignment to captured anadromous males may also be affected by the accuracy of genotype reconstruction and the criteria for accepting parentage assignment, differences in migratory cues and timing between males and females, and the structure of the mating system.

CORRELATES OF MULTIPLE PATERNITY AND VARIANCE IN FERTILIZATION SUCCESS

The significant spatial clumping of redds in Catamaran Brook is indicative of areas of high female density. These locations also produced redds with the most pronounced degree of multiple paternity, suggesting that male density in these areas was also high relative to other spawning sites. Consequently, dominance and monopolization by one or two individuals may not have been energetically feasible or physically possible (Brown, 1964; Emlen & Oring, 1977). Under these circumstances, smaller anadromous males using alternative reproductive behaviour are likely to be successful at fertilizing eggs (Fleming, 1996). Furthermore, mature male parr were more abundant in areas of increased female activity, and contributions from parr in these

locations were probably substantial. This result is consistent with the prediction that the magnitude of multiple paternity should increase in areas of high fish density.

Contrary to the prediction that the variance in fertilization success should decrease with an increase in number of sires, the variance in success among paternal males was generally high across redds. While the variance in success among males cannot be calculated due to the fact that only successful males were sampled, variance in reproductive success among males in a single spawning event is expected. Under experimental conditions that included only large males, Mjølnerød *et al.* (1998) found that the most successful male fertilized an average of 91% of sampled eggs. In the present study, the most successful male fertilized an average of 41% of sampled eggs, which may be due to the natural conditions under which spawning occurred and the presence of mature male parr in the system. In addition, the proportion of eggs fertilized by the most successful male decreased linearly as the number of contributing males increased. This may indicate that fertilization ability is not equal among males when fertilization success decreases as a function of the number of males contributing paternity. Sperm competition among many males may result in a skew in fertilization success among males if they vary in sperm characteristics and sperm competitive ability (Gage *et al.*, 1995, 1998).

In summary, this examination of the paternal contributions to eggs by male *S. salar* in a wholly natural system reveals an unexpectedly high level of multiple paternity. Within a single redd, females may mate with as many as 16 different males and variance in fertilization success within redds is high regardless of the number of sires detected. In addition to strong inferential evidence that females preferentially select nest sites, the abundance of mature male parr, as well as the incidence of multiple paternity, increases with redd density. This work emphasizes the importance of conducting and repeating genetic studies on the mating systems of fishes in unmanipulated, natural environments and suggests that the distribution of multiple paternity may be predicted by ecological factors.

References

Andersson, M. (1994). *Sexual Selection*. Princeton, NJ: Princeton University Press.

Bateman, A. J. (1948). Intra-sexual selection in *Drosophila*. *Heredity* **2,** 349–368.

Bentzen, P., Olsen, J. B., Seamons, T. R. & Quinn, T. P. (2001). Kinship analysis of Pacific salmon: insights into mating, homing, and timing of reproduction. *Journal of Heredity* **92,** 127–136.

Brodeur, N. N. (2006). Dispersion patterns of kin in young-of-the-year Atlantic salmon (*Salmo salar* L.) in Catamaran Brook, New Brunswick. MSc Thesis, Concordia University, Montréal, QC, Canada.

Brown, J. L. (1964). The evolution of diversity in avian territorial systems. *Wilson Bulletin* **76,** 160–169.

Clutton-Brock, T. H. (1988). *Reproductive Success: Studies of Individual Variation in Contrasting Breeding Systems*. Chicago, IL: The University of Chicago Press.

Clutton-Brock, T. H. & Vincent, A. C. J. (1991). Sexual selection and the potential reproductive rate of males and females. *Nature* **351,** 58–60.

Cunjak, R. A., Caissie, D., El-Jabi, N., Hardie, P., Conlon, J. H., Pollock, T. L., Giberson, D. J. & Komadina-Douthwright, S. (1993). The Catamaran Brook (New-Brunswick) habitat research project: biological, physical, and chemical conditions (1990-1992). *Canadian Technical Report in Fisheries and Aquatic Sciences* **1914**.

Emlen, S. T. (1976). Lek organization and mating strategies in the bullfrog. *Behavioural Ecology and Sociobiology* **1,** 283–313.

Emlen, S. T. & Oring, L. W. (1977). Ecology, sexual selection and the evolution of mating systems. *Science* **197,** 215–233.

Feldheim, K. A., Gruber, S. H. & Ashley, M. V. (2004). Reconstruction of parental microsatellite genotypes reveals female polyandry and philopatry in the lemon shark, *Negaprion bervirostris*. *Evolution* **58,** 2332–2342.

Fleming, I. A. (1996). Reproductive strategies of Atlantic salmon: ecology and evolution. *Reviews in Fish Biology and Fisheries* **6,** 379–416.

Gage, M. J., Stockley, P. & Parker, G. A. (1995). Effects of alternative male mating strategies on characteristics of sperm production in the Atlantic salmon (*Salmo salar*): theoretical and empirical investigations. *Philosophical Transactions of the Royal Society B* **350,** 391–399.

Gage, M. J. G., Stockley, P. & Parker, G. A. (1998). Sperm morphometry in the Atlantic salmon. *Journal of Fish Biology* **53,** 835–840.

Garant, D., Dodson, J. J. & Bernatchez, L. (2001). A genetic evaluation of mating system and determinants of individual reproductive success in Atlantic salmon (*Salmo salar* L.). *Journal of Heredity* **92,** 137–145.

Hughes, C. (1998). Integrating molecular studies with field methods in studies of social behavior: a revolution results. *Ecology* **79,** 383–399.

Jones, J. W. (1959). *The Salmon*. London: Collins.

Jones, M. W. & Hutchings, J. A. (2002). Individual variation in Atlantic salmon fertilization success: implications for effective population size. *Ecological Applications* **12,** 184–193.

Krebs, C. J. (1999). *Ecological Methodology*, 2nd edn. New York, NY: Addison Wesley Longman Inc.

Kugliowski, D. R., Ford, M. J. & Berejikian, B. A. (2005). Breeding structure of steelhead inferred from patterns of genetic relatedness among nests. *Transactions of the American Fisheries Society* **134,** 1202–1212.

Martinez, J. L., Moran, P., Perez, J., de Gaudemar, B., Beall, E. & Garcia-Vasquez, E. (2000). Multiple paternity increases effective size of southern Atlantic salmon populations. *Molecular Ecology* **9,** 293–298.

Mitchell, S., Fraser, A., Hardie, P. & Cunjak, R. A. (2004). Electrofishing data summaries for Catamaran Brook, New Brunswick, 1990-1992. *Canadian Data Report of Fisheries and Aquatic Sciences* **1130**.

Mjølnerød, I. B., Fleming, I. A., Refseth, U. H. & Hindar, K. (1998). Mate and sperm competition during multiple-male spawnings of Atlantic salmon. *Canadian Journal of Zoology* **76,** 70–75.

Moore, A. & Scott, A. P. (1992). 17a,20fi-dihydroxy-4-pregnen-3-one 20-sulphate is a potent odorant in precocious male Atlantic salmon *(Salmo salar* L.) parr which have been pre-exposed to the urine of ovulating females. *Proceedings of the Royal Society B* **249,** 205–209.

Myers, R. A. & Hutchings, J. A. (1987). Mating of anadromous Atlantic salmon, *Salmo salar* L., with mature male parr. *Journal of Fish Biology* **31,** 143–146.

Økland, F., Erkinaro, J., Moen, K., Niemelä, E., Fiske, P., McKinley, R. S. & Thorstad, E. B. (2001). Return migration of Atlantic salmon in the River Tana: phases of migratory behaviour. *Journal of Fish Biology* **59,** 862–874.

Olsén, K. A., Johansson, A.-K., Bjerselius, R., Mayer, I. & Kindhal, H. (2002). Mature Atlantic salmon (*Salmo salar* L.) male parr are attracted to ovulated female urine but not to ovarian fluid. *Journal of Chemical Ecology* **28,** 29–40.

O'Reilly, P. T., Hamilton, L. C., McConnell, S. K. & Wright, J. M. (1996). Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Canadian Journal of Fisheries and Aquatic Sciences* **53,** 2292–2298.

Paterson, S., Piertney, S. B., Knox, D., Gilbey, J. & Verspoor, E. (2004). Characterization and PCR multiplexing of novel highly variable tetranucleotide Atlantic salmon (*Salmo salar* L.) markers. *Molecular Ecology* **4,** 160–162.

Randall, R. G. (1989). Effect of sea-age on the reproductive potential of Atlantic salmon (*Salmo salar*) in eastern Canada. *Canadian Journal of Fisheries and Aquatic Sciences* **46,** 2210–2218.

Seamons, T. R., Bentzen, P. & Quinn, T. P. (2004). The mating system of steelhead, *Oncorhynchus mykiss*, inferred by molecular analysis of parents and progeny. *Environmental Biology of Fishes* **69,** 333–344.

Shuster, S. M. & Wade, M. J. (2003). *Mating Systems and Strategies*. Princeton, NJ: Princeton University Press.

Smith, B. R., Herbinger, C. M. & Merry, H. R. (2001). Accurate partition of individuals into full-sib families from genetic data without parental information. *Genetics* **158,** 1329–1338.

Taggart, J. B., McLaren, I. S., Hay, D. W., Webb, J. H. & Youngson, A. F. (2001). Spawning success in Atlantic salmon (*Salmo salar* L.): a long-term DNA profiling-based study conducted in a natural stream. *Molecular Ecology* **10,** 1047–1060.

Trivers, R. L. (1972). Parental investment and sexual selection. In *Sexual Selection and the Descent of Man 1871-1971* (Campbell, B., ed.), pp. 136–179. Chicago, IL: Aldine Publishing Company.

Wang, J. (2004). Sibship reconstruction from genetic data with typing errors. *Genetics* **166,** 1963–1979.

Wang, J. & Santure, A. W. (2009). Parentage and sibship inference from multi-locus genotype data under polygamy. *Genetics* **181,** 1579–1594.

Weir, L. K., Hutchings, J. A., Fleming, I. A. & Einum, S. (2004). Dominance relationships and behavioural correlates of spawning success in farmed and wild male Atlantic salmon (*Salmo salar*). *Journal of Animal Ecology* **73,** 1069–1079.

Zippin, C. (1958). The removal method of population estimation. *Journal of Wildlife Management* **22,** 82–90.