Molecular Ecology (1999) **8**, 189–198

An analysis of the distribution of juvenile Atlantic salmon (*Salmo salar*) in nature as a function of relatedness using microsatellites

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Abstract

The major objective of this study was to test the hypothesis that juvenile Atlantic salmon kin occupy adjacent territories in their natural habitat in order to profit from the benefits associated with kin-biased behaviours, as has been observed under controlled laboratory conditions. Microsatellites were used to establish the relatedness of salmon fry (in their first summer of life) and parr (in their second and third summer of life) captured in adjacent territories. We did not observe a relationship between the proximity and the relatedness of either parr of the same cohort or fry in their natural habitat. Although many pairs of fry were identified as being related when sampled immediately after emergence, most family groups did not occupy adjacent territories. The high dispersal potential in rivers, the low occupation rate of the habitat and the incidence of half-sibs in nature most probably reduce the opportunity and advantage of kin-biased behaviour, in contrast to laboratory studies conducted in artificial, high-density conditions.

Keywords: juvenile Atlantic salmon, kin distribution, microsatellites

Received 10 March 1998; revision received 5 August 1998; accepted 11 September 1998

Introduction

Numerous fishes such as threespine stickleback (*Gasterostus aculeatus*) (Van Havre & FitzGerald 1988; FitzGerald & Morrissette 1992), poecilliids (Lockle *et al*. 1982), cichlids (McKaye & Barlow 1976; Barnett 1977, 1981), Atlantic salmon (*Salmo salar*) and other salmonids (*Oncorhynchus* spp.) (Quinn & Busack 1985; Quinn & Hara 1986; Olsen 1989; Brown & Brown 1992) have been shown to exhibit cooperative social behaviour towards kin. In coho salmon (*Oncorhynchus kisutch*) and Arctic charr (*Salvelinus alpinus*), the ability to discriminate kin is primarily associated with schooling behaviour that reduces the risk of predation and increases foraging efficiency (Quinn & Busack 1985; Olsen 1989). Kin-biased social behaviour of juvenile Atlantic salmon has been clearly demonstrated in the laboratory (Brown & Brown 1992, 1993b; Brown & Brown 1996a). Cooperative behaviour in groups of related juvenile Atlantic salmon was manifest as fewer aggressive interactions, the use of a greater proportion of 'threat' behaviour as opposed to fighting, smaller territory size, and improved growth (particularly in subordinates) as compared to non-kin groups (Brown & Brown 1993b; 1996a). The functional advantages of defending territories adjacent to kin include reduction of predation as less movement is required to defend the territory, reduced physical injury due to decreased agonistic encounters and the greater growth, and hence survival, resulting from such cooperative behaviours (Brown & Brown 1993b; 1996a). Cooperative behaviours among kin are selected when they enhance inclusive fitness (Hamilton 1964; Waldman 1988).

Phenotype matching is generally considered to be the mechanism employed by salmonids to recognize kin (Quinn & Busack 1985; Quinn & Hara 1986; Winberg & Olsen 1992; Brown *et al*. 1993c). Phenotype matching involves comparing the phenotype of the cue-bearer with a learned template such that no familiarity between cue-bearer and the discriminating individual is required (Lacy & Sherman 1983; Fletcher 1987). Phenotype matching is consistent with the life history of Atlantic salmon where fry (juvenile salmon in their first summer of life) hatch asynchronously and disperse following emergence from the redd (the spawning site) (Brown & Brown 1996b).

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Contribution to the programme of CIRSA (Centre Interuniversitaire de Recherche sur le Saumon Atlantique).

Active compounds produced in the liver and expelled via the intestinal tracts could mediate this recognition (Quinn & Hara 1986; Stabell 1987). As juvenile salmon occupy an array of fixed territories on the stream bed during summer months (Kalleberg 1958; Keenleyside & Yamamoto 1962), it is possible that they mark the substrate with odours that may be recognized by kin.

The selective pressure on genes which control altruism depends on the costs and benefits associated with cooperative behaviour adjusted by the degree of relatedness among individuals; $b^*r - c > 0$, where c is the cost incurred by the altruist, b is the benefit in fitness gained by the recipient of the altruistic act and r the coefficient of relatedness between the recipient and the altruist (Hamilton 1964). Thus, as relatedness increases, so does the benefit of cooperative behaviour. The benefit from these behaviours among full siblings ($r = 0.5$) should theoretically be on average at least the double of the cost incurred by the altruist. Altruism towards neighbours may evolve in populations characterized by limited dispersal (Hamilton 1964; Queller 1992) or those with kin recognition. Thus, cooperative behaviour among kin should occur in salmon natural habitat only if the proximity of kin is stable over a period of time despite the opportunity for dispersal.

Many studies of kin recognition have been conducted in artificial conditions and may or may not indicate that kin recognition is important in nature (Fletcher & Michener 1987). A major obstacle in conducting such studies in a natural habitat is the difficulty of estimating genetic relatedness among individuals of unknown pedigree (Blouin *et al*. 1996). The recent discovery of DNA microsatellites makes possible studies of parentage and kinship in the natural habitat. Microsatellites are hypervariable specific regions of genomic DNA composed of a variable number of tandem repetitions (VNTR) of two, three or four nucleotides, flanked by nonrepetitive DNA (Nakamura *et al*. 1987; Litt & Lutty 1989; Tautz 1989). Blouin *et al*. (1996) demonstrated the ability of microsatellites to discriminate unrelated individuals from full-sibs with at least 97% accuracy. However, attaining this level of accuracy necessitates using a large number of microsatellite loci.

The major objective of this study was to test the hypothesis that juvenile Atlantic salmon kin occupy adjacent territories in their natural habitat. Microsatellites were used to establish the relatedness of salmon fry and parr (juveniles in their second or third summer of life) captured on adjacent territories.

Materials and methods

Field work

Field work was conducted on the Trinité River, situated on the north shore of the St Lawrence River, supporting a run of ≈ 2000 adults per year (Caron *et al*. 1992). Juvenile salmon were sampled with a diver-operated electrofishing device (James *et al*. 1987) modified to run on a generator mounted on a backpack. This system produces a local electric field and permits a diver to capture parr or fry in shallow water directly on their territories without disturbing adjacent individuals.

Five areas of 100 m^2 (5×20 m) were sampled. A diver moving upstream, followed by an assistant carrying the generator/backpack, captured all juvenile salmon encountered in the area. The position of each fish was marked immediately after capture with a numbered lead marker. After the capture of all fish in the sampling area, we mapped the positions of the lead markers corresponding to the territories of individual fish. To do so simply in the field, we measured distances among markers to create a mosaic of juxtaposed triangles. The juxtaposition of all triangles, using a simple graphics program (CORELDRAW), permitted the geographical position of every individual to be established in each 5×20 m area. Sampled fish were frozen in liquid nitrogen within 90 min of capture. In the laboratory, fish were weighed (± 0.005 g), measured (0.05 mm) and their DNA extracted using a standard phenol–chloroform protocol (Sambrook *et al*. 1989).

Fry and parr were sampled in two kinds of habitat due to their different habitat requirements. Three areas in the main stem of the river (designated 1, 2 and 3) were sampled 1 week before the emergence of fry in habitat characterized by heterogeneous substrate sizes (10 cm to 1 m) and relatively strong currents that corresponded to the highest density of parr. Two additional areas (designated 4 and 5) were sampled in typical spawning habitat characterized by small substrate sizes (5–20 cm) and shallow depths that corresponded to the highest density of fry. The first sampling of fry occurred at emergence (end of June). As yolk-sac fry remain in the gravel in the vicinity of the redd for ≈ 15–20 days following hatching, sibs and half-sibs should be most clustered at emergence, possibly reflecting limited dispersal rather than deliberate association. Nevertheless, sampling immediately after emergence served as a test of our ability to identify kin. Fry were again sampled 10 days later.

High densities of juvenile fish indicated high-quality habitat that may be associated with a high rate of agonistic behaviour among juveniles to maintain their territories. We presumed that biased distributions among related parr and fry would be most evident in these habitats (Brown & Brown 1993a;b).

Microsatellite protocol

Due to their high number of alleles and high heterozygosity, microsatellites and minisatellites are the best tools to determine relatedness of individuals in the absence of

information on pedigree (Blouin *et al*. 1996). These characteristics influence directly the probability that two individuals share, by chance, a certain number of alleles and the probability of falsely classifying individuals as unrelated or related. This probability can be reduced by using highly variable microsatellites or by adding additional microsatellites as they become available. In this study, we used four polymorphic, yet easy-to-score, microsatellites to avoid any ambiguities in scoring gels. Microsatellites with tetranucleotide repetitions provided the least ambiguous results.

Genetic relatedness between individuals was analysed on the basis of alleles detected at four microsatellite loci. Pairs of primers composed of complementary DNA of the flanking regions were used to amplify the microsatellites Ssa202, 197, 171 (O'Reilly *et al*. 1996) and SSOSL85 (Slettan *et al*. 1995) loci using PCR. The PCR reactions were performed in 10 mL volumes: 1 mL of buffer (Boehringer Mannheim), 200 mM for each dNTP, 0.5 U *Taq*, 4.5 pmol and 0.5 pmol end-labelled (α^{32}P) of one primer, 5 pmol of the other primer, ≈ 60 ng of DNA and water to a volume of 10 mL. All PCR amplification cycles were preceded with 5 min of denaturation (94 °C) and ended with an extension (72 °C) of 10 min. The denaturing/annealing(x-temperature)/extension cycle times were 30/30(58 °C)/30 s, respectively. The SSCSL85 PCR conditions are available in Slettan *et al*. (1995). The reaction products were resolved on a 6% denaturing polyacrylamide sequencing gel visualized by autoradiography. The allelic size of PCR products was determined by comparison with a standard M13 sequence (USB, Sequenase Version 2.0 DNA Sequencing Kit). All microsatellites were highly polymorphic. The Ssa loci can be amplified in the same reaction and electrophoresed together on the same gel. However, we ran locus Ssa171 separately for clearer results.

Statistical analysis

Expected heterozygosity (H_E), allelic correlation within individuals (F_{IS}, Wright 1951), departure from Hardy–Weinberg equilibrium and genotypic linkage disequilibrium for each locus of the population were calculated using the GENEPOP computer package version 1.2 (Raymond & Rousset 1995). To test the alternative hypotheses of deficiency or excess of heterozygotes, this program uses the Markov chain method to obtain unbiased estimates of the exact Fisher test (1000 iterations).

We calculated the coefficient of relatedness (r_{xy}) between the members of all possible pairs (dyads) of fish within a sampling area according to the method of Queller & Goodnight (1989) using the allele frequencies observed in the Trinité population (reported in Fontaine *et al*. 1997). No statistical relationship was observed between r_{xy} and the geographical distance separating pairs of fish (data not shown). We thus proceeded to quantify all pairs of fish in each sampling area as unrelated, half-sibs or full-sibs using the protocol and program of Blouin *et al*. (1996). The identification of half-sibs was important given the high level of multiple paternity in wild Atlantic salmon (see the Discussion). We then determined whether pairs of fish classified as full-sibs occupied adjacent territories.

Using the observed allelic frequency distributions observed in the river, we randomly generated 10 000 pairs of unrelated individuals, 10 000 pairs of half-sibs and 10 000 pairs of full-sibs (Fig. 1). These simulated distributions were used to assign all pairs of sampled juvenile salmon to one of the three types of relationship. Increasing the number and variability of loci used will reduce the overlap of these theoretical distributions due to the lower probability that two unrelated individuals share common alleles or that two related individuals do not share alleles.

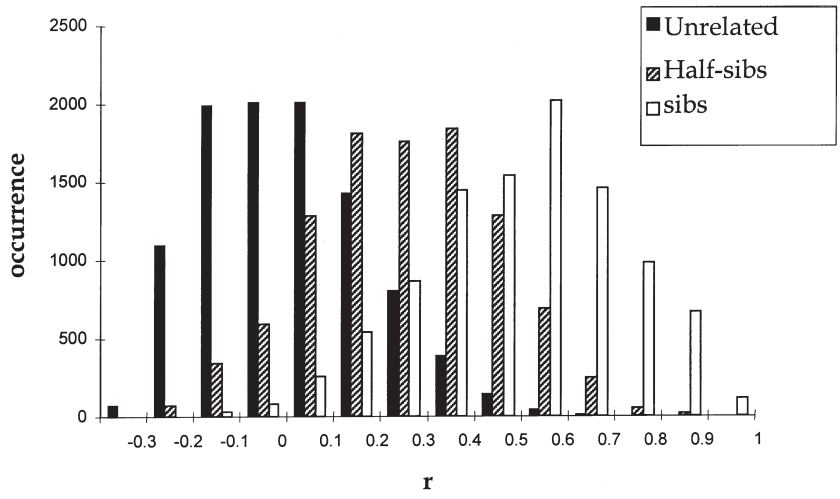

Fig. 1 Theoretical distribution of r_{xy} values from 10 000 unrelated, half-sib and full-sib dyads. Means and standard deviation were 0.050 ± 0.17, 0.248 ± 0.19 and 0.497 ± 0.21, respectively.

To determine the probability that a pair of fish (dyad) of one type of relationship would be misclassified as belonging to some other type of relationship, we used the midpoint between the means of any two simulated distributions of r_{xy} (unrelated vs. full-sibs, half-sibs vs. full-sibs), as the threshold value for classification (Blouin *et al*. 1996). These threshold values were r_{xy} = 0.25 between unrelated and full-sibs and r_{xy} = 0.38 between half-sibs and full-sibs. For example, to distinguish unrelated dyads and full-sib dyads, a dyad that falls to the right of the threshold value of 0.25 would be classified as belonging to the full-sib category (Fig. 2). The percentage of randomly generated unrelated dyads falling to the right of the threshold value is the empirically determined type I error and the percentage of randomly generated full-sibs that fall to the left of the threshold value is the type II error (Fig. 2).

In distinguishing unrelated and full-sibs, the threshold value of 0.25 resulted in an unacceptably high type I error (≈ 10%, see the Results). In order to reduce the number of unrelated pairs misclassified as full-sibs, we increased the threshold value in order to create type I errors of 5% (r_{xy} = 0.31) and 1% (r_{xy} = 0.47). A threshold value of 0.47 was also used to estimate the probability of false inclusion of half-sib dyads as full-sib dyads (type I error) at the most conservative threshold level used to distinguish unrelated and full-sib dyads.

By reducing the level of type I error, we increased the level of type II error and would thus misclassify a certain number of full-sib dyads as unrelated. In order to minimize type II error, we reconstructed the family structure of dyads classified as full-sibs by adding nonselected dyads if they respected the following condition: two individuals A and B were considered to be kin, even if their r_{xy} value was lower than the threshold, if both shared a common sib C within a selected dyad (r_{xy} higher than the threshold) (Rico *et al*. 1992). A second reason for reconstructing families was to determine the most acceptable threshold value to determine full-sib dyads. If we consider that members of the reconstructed family are full-sibs, the mean r_{xy} of all pairwise comparisons inside full-sib groups should tend towards a value of 0.5. However, if the level of misclassification in the family groups is high due to the false inclusion of unrelated or half-sib dyads, this should reduce the mean value of r_{xy} towards zero. Considering the possibility that unrelated and particularly half-sibs may be present in the reconstructed family groups, principally in the case of the lower threshold values, a mean r_{xy} value was calculated for these new family groups to assess the effect of misclassification and to determine the most acceptable threshold for identifying full-sib dyads.

Finally, we tested whether fish of the same age class classified as kin were bigger than pairs of nonrelated fish. Although increased growth rate is but one of the functional advantages of defending territories adjacent to kin (Brown & Brown 1993b; 1996a), it is the simplest and most direct method of detecting the beneficial effects of kin recognition.

Results

The four loci used were highly variable as revealed by levels of heterozygosity which ranged from 0.81 to 0.94. F_{IS} values were low and no loci showed significant departures from Hardy–Weinberg equilibria (Table 1) or genotypic linkage disequilibrium. These results showed the absence of assortative mating, subdivision or dependent segregating loci in our system.

The r_{xy} values of randomly generated pairs of unrelated, half-sibs and sibs are shown in Fig. 1. The mean values of r for the unrelated, half-sibs and sibs were 0.005, 0.248

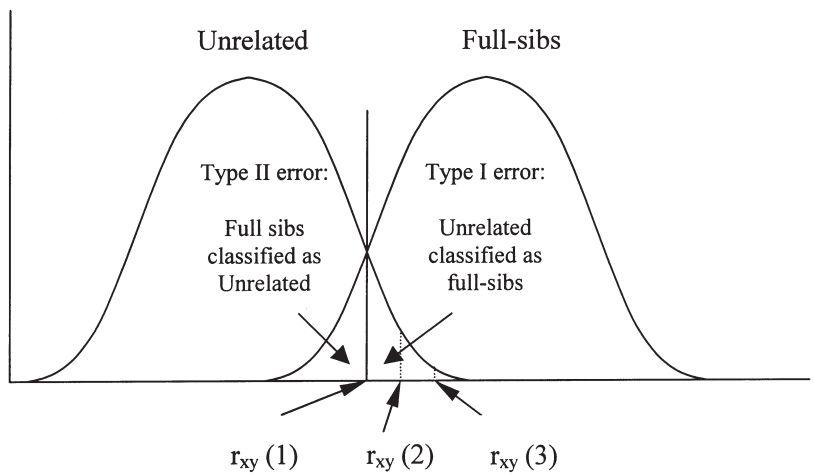

Fig. 2 Example of the criterion of false inclusion rate of unrelated as full-sibs (type I error) or full-sibs as unrelated (type II error). The threshold value corresponding to r_{xy} (1) is the midpoint between the means of the theoretical distributions of both categories. The thresholds r_{xy} (2) and (3) were set to reduce the probability of false inclusions of unrelated as full-sibs (type I error) at 5% and 1%, respectively. All thresholds values are shown in Table 2. Modified from Blouin *et al*. (1996).

Table 1 Expected heterozygosity (H_E), fixation index (F_{IS}) and Hardy–Weinberg equilibrium (P-values) for all areas and loci

		Loci											
		Ssa171			Ssa197			Ssa202			Ssosl85		
	n	H_E	F_{IS}	H-W (P)	H_E	F_{IS}	H-W (P)	H_E	F_{IS}	H-W (P)	H_E	F_{IS}	H-W (P)
Area 1	16	0.94	−0.07	0.54	0.83	0.04	0.68	0.83	0.09	0.74	0.83	−0.06	0.61
Area 2	16	0.94	0.14	0.08	0.86	0.13	0.38	0.92	0.12	0.13	0.85	0.19	0.07
Area 3	13	0.96	0.04	0.12	0.84	0.09	0.52	0.79	−0.06	0.97	0.79	0.23	0.38
Area 4	71	0.94	0.01	0.31	0.88	0.01	0.98	0.88	−0.03	0.53	0.85	−0.04	0.27
Area 5	47	0.91	−0.02	0.32	0.88	0.03	0.08	0.88	−0.04	0.41	0.75	0.07	0.06
Mean		0.94	0.27	0.13	0.86	0.06	0.54	0.86	0.02	0.66	0.81	0.08	0.27

and 0.497, respectively, and agree with theoretical distributions (Queller & Goodnight 1989; Blouin *et al*. 1996).

Numbers of kin

The r-values were calculated only between parr of the same age class due to the presence of different cohorts in the same area. The numbers of pairs of fish classified as kin after applying the different threshold values are presented in Table 2. Only area 3 had a dyad of related parr (1-year old or greater) identified at all the threshold values. Twenty-eight dyads of fry in area 4 and 16 in area 5 had higher r-values than the most conservative threshold (r_{xy} = 0.47, type I error (unrelated as full-sib) = 1% (half-sib as full-sibs) = 13.6%). The observed distributions of r are represented in Fig. 3. The theoretical distribution of r_{xy} of unrelated dyads (Fig. 1) and the observed distributions of r_{xy} calculated for parr and fry dyads (Fig. 3) were similar with a mean around zero. However, the skewed shape of the distribution of observed r_{xy} values for fry dyads to the right indicates a greater incidence of relatives among fry than parr.

In the areas where fry were captured, a large number of dyads were classified as kin at different threshold values. We reconstructed families from dyads identified at the more-conservative threshold levels. The threshold of 5% probability of false inclusion of unrelated fish as full-sibs (type I error) created large family groups with a mean r_{xy} of 0. This indicates that a large number of unrelated individuals were included in the reconstructed families and, thus, this threshold was rejected. The reconstructed family groups from the highest threshold of r_{xy} = 0.47 (1% type I error) had a mean r-value of 0.40 (Table 3). We kept this most-conservative threshold to identify family groups. Therefore, even if the rate of false inclusion of full-sibs as unrelated (type II error) is high (42.1%) using this threshold, the incorporation of nonselected dyads in reconstructed family groups decreased the probability of type 2 error by increasing the number of related dyads in areas 4 and 5 by 93% and 112%, respectively (Table 3). We

Table 2 Percentage of error of type 1 and 2 and number of dyads ($n(n-1)/2$) classified as kin (yearly age classes 0–3+) according to thresholds of the different scenarios. The threshold values were calculated as the midpoint between the means of the simulated distributions of r_{xy} of unrelated and full-sibs (1), the r_{xy} value where the proportion of error of type 1 was 5% (2), and the r_{xy} value where the proportion of error of type 1 was 1% (3). Finally, the threshold level between half-sibs and full-sibs was set at the most-conservative threshold level of r_{xy} (0.47) used to distinguish unrelated and full-sib dyads (4). n is the number of each age class sampled at each of the five sites (A1 to A5). The mean value r_{xy} is given for the total of dyads of the different age classes

			A1			A2		A3		A4	A5		
Scenario	Threshold r_{xy}	% of error Type (1); (2)	1+	2+	3+	1+	2+	1+	2+	0+	0+	1+	2+
(1) U v/s S	0.25	10.3; 13.5	2	1	0	0	5	3	1	322	108	2	0
(2) U v/s S	0.31	5.0; 19.9	1	1	0	0	1	1	0	167	74	1	0
(3) U v/s S	0.47	1.0; 42.1	0	0	0	0	0	1	0	28	16	0	0
(4) H v/s S	0.47	13.6; 42.1	0	0	0	0	0	1	0	28	16	0	0
n			5	9	2	6	9	7	5	71	36	9	2
Dyads			10	36	1	15	36	21	10	2485	630	36	1
Mean r_{xy}			0.10	0.00	0.04	0.00	0.00	0.07	0.02	0.04	0.06	0.06	0.08

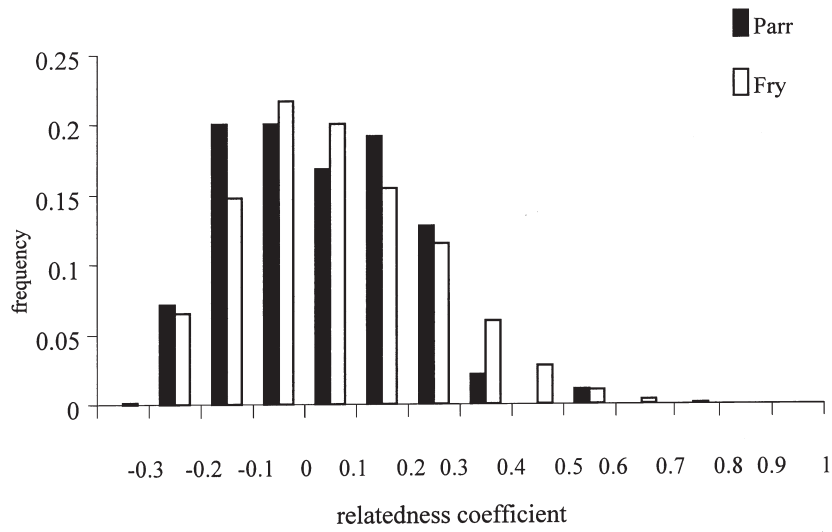

Fig. 3 Mean distribution of r_{xy} values from all dyads of parr (areas 1–5) and fry (areas 4 and 5).

thus recovered most of the full-sib dyads that were misclassified before family reconstruction. The level of *r* from these reconstructed family groups (0.4) was lower than the theoretical mean value of 0.5 for full-sibs. This probably reflects the presence of half-sibs in the reconstructed family groups caused by the 13.6% misclassification rate of half-sibs as full-sibs at the $r_{xy} = 0.47$ threshold.

Spatial distribution of kin

Fifty-four per cent (38 of 71 fry) and 56% (20 of 36 fry) of all fry sampled were involved in kin groups in areas 4 and 5, respectively (Table 3). In area 4, the 38 related fry were composed of six family groups of three or more fry and six related pairs, for a total of 12 families. In area 5, the 20 related fry dyads were composed of three family groups of three or more fry and two related pairs, for a total of five families (Table 4). However, the family groups of fry were not predominantly located in adjacent territories suggesting that distributions were not based on kin relationships (Fig. 4a,b). The low number of sibs detected precludes the analysis of aggregation to assess whether sibs are clustered.

However, some related fry in area 5 were identified as potential neighbours as no intervening fish separated them and we tested whether these fish grew more quickly than pairs of nonrelated fish. The mean weight (0.27 ± 0.02 g) and length (30.6 ± 1.2 mm) of the 10 related dyads composed of neighbours were not significantly different from that of 10 randomly selected dyads composed of unrelated neighbours (0.27 ± 0.03 g; 30.4 ± 1.5 mm) (Table 4). This suggests no detectable advantage at these ages associated with having kin for neighbours. Only four pairs of parr, in areas 1, 2 and 3, were identified as related with a threshold value corresponding to the 5% probability of false inclusion of unrelated as full-sibs (type I error) and just one in the area 3 for the 1% level of probability. A distance of more than 4 m separated the pair identified as full-sibs at a threshold of 5% of false inclusion, and a distance of 17.8 m for the dyad identified as full-sibs at a threshold fixed of 1%. No overlapping or juxtaposition among territories of

Table 3 Families of fry composed of three or more members (F) and the number of related pairs found in areas 4 and 5 with coefficient of relatedness higher than the threshold of $r_{xy} = 0.47$ (1% of type I error). New nonselected dyads were added to a family if they respected the following condition: two individuals were considered as kin if both shared the same brother in a selected dyad ($r_{xy} > 0.47$)

	A4								A5				
	F1	F2	F3	F4	F5	F6	Related pairs	Total	F1	F2	F3	Related pairs	Total
No. of dyads $r_{xy} > 0.47$	7	5	3	3	2	2	6	28	6	4	3	2	15
New dyads added	8	10	3	3	1	1	–	26	9	6	3	–	18
No. of fry implicated	6	6	4	4	3	3	12	38	6	5	4	4	20
Mean of r_{xy}	0.39	0.32	0.46	0.41	0.40	0.53			0.33	0.33	0.44		

Table 4 Number of reconstructed families in areas 4 and 5, the total numbers of related dyads included in these families and the number of those involving neighbours (see Fig. 4a,b)

Area of fry	No. of families	No. of related dyads	No. of related dyads that were neighbours
4	12	54	1
5	5	33	7

these fish was possible. Their spatial position, the heterogeneity of the substrate, territory sizes of ≈ 1 m^2 (Keeley & Grant 1995) and the presence of other parr among them offered little chance of interaction among kin.

Discussion

The results of this study did not reveal kin-biased distribution among juveniles of Atlantic salmon sampled in their natural habitat. Despite previous experimental

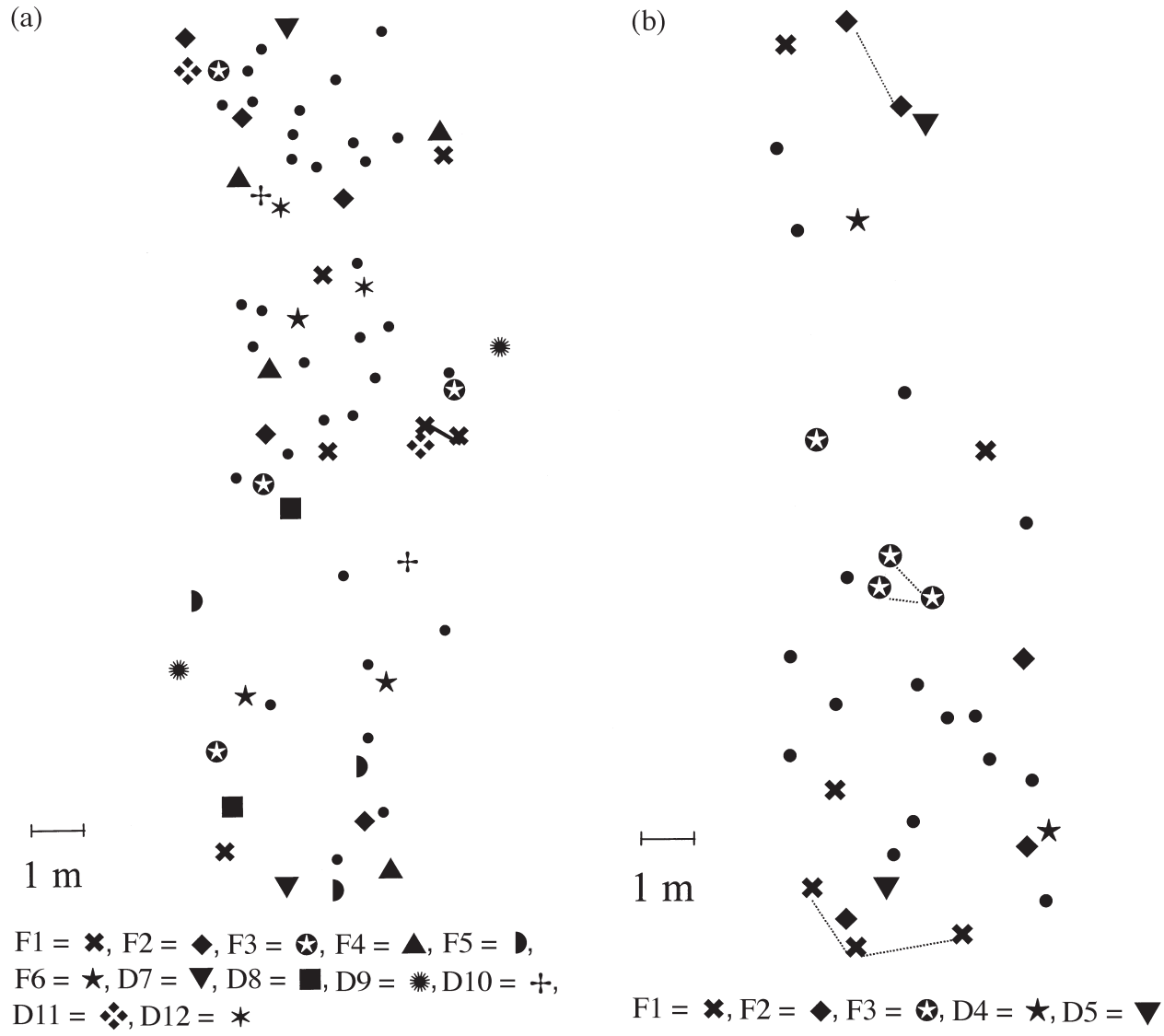

Fig. 4 (a) Fry distribution in area 4. Symbols indicate the kin relationship ($r > 0.47$) among members of the different reconstructed families (F) or related dyads (D) which are composed of three or more individuals and of two fry, respectively (see Table 3). Dots are individuals not related to any other fry. Full sibs connected by lines are those considered to be neighbours. (b). Fry distribution in area 5. Symbols are associated to the kin relationship ($r > 0.47$) among members of the different reconstructed families (F) or related dyads (D) which are composed of three or more individuals and of two fry, respectively (see Table 3). Dots are individuals not related to any other fry. Full-sibs connected by lines are those considered to be neighbours.

demonstrations of kin discrimination in Atlantic salmon and of the fitness benefits associated with establishing territories next to kin (Brown & Brown 1996a), we found little evidence of siblings defending adjacent territories. From the 166 possible dyads of parr, only one satisfied the criterion for full-sibs with a coefficient of relatedness of 0.65. The great distance between the two parr did not meet the essential requirement of proximity needed to benefit from the advantages of kin-biased behaviour. Although 12 family groups were identified among fry in area 4, little kin-biased distribution was seen immediately following emergence despite the identification of many related individuals. In addition, neither the weight nor the length of the few neighbouring kin differed significantly from that of unrelated neighbours occupying the same habitat.

It is unlikely that the lack of relatedness seen among the great majority of neighbouring salmon parr was due to the inability of our procedures to detect siblings. One objective of sampling fry immediately after emergence was to test our ability to detect kin because family groups would be clustered relatively close to their natal redds at this time. The high proportion of fry dyads identified as kin-related confirmed the ability of our procedures to identify full-sibs despite the small number of microsatellites and the use of a conservative value of the coefficient of relatedness to minimize the classification of unrelated fish as full-sibs. The high probability of type II error (42%) that resulted from using a high threshold value to minimize type I error was corrected by recovering kin-related dyads through the process of family reconstruction.

We propose three factors that may account for the difference between the findings of Brown & Brown (1996b) and the results presented here: (i) critical biotic and abiotic differences between natural and artificial habitats; (ii) specific environmental conditions that influence the distribution and abundance of fish; and (iii) the presence of a high proportion of half-sibs in natural populations which reduces the fitness benefits of cooperative behaviour by lowering the coefficient of relatedness.

Artificial habitats may engender biological phenomena that may be relatively rare in the natural environment. The limited movements of fish confined to stream channels at high densities will artificially increase the number of aggressive interactions between fish. Based on a regression between fish length and territory size (Keeley & Grant 1995), we calculated the territory size normally occupied by the number and size of parr used in the two experiments of Brown & Brown (1993b, 1996a). The total territory size calculated for the parr in these two experiments was 0.45 m^2 and 10.52 m^2. However, the experimental stream tanks measured 0.53 m^2 and 1.12 m^2 resulting in occupation rates of 85% and 99%, respectively, values much higher than generally found in nature. Salmon juveniles generally occupy less than 20% of the available area in nature (Allen 1969). In the present study, only 5% of the sampled areas was occupied by juvenile salmon. This will surely influence the frequency of aggressive behaviours, which was higher than 90 per hour in Brown & Brown's (1993b) study in comparison to 8 per hour observed in natural habitat (Keeley & Grant 1995). High densities of fish in artificial conditions may thus amplify differences in the frequency of aggressive behaviour, and thus growth, between kin and non-kin groups which may not operate in natural habitat. Finally, the relatively short duration of controlled experiments conducted in the confines of a stream tank greatly limits the potential for dispersal that dominates the fluvial environment. The physical force of dispersion most surely acts to separate siblings following emergence. This was reflected in our data as the vast majority of related dyads and families were observed among fry shortly after emergence with few identified amongst older parr (Table 2).

It seems plausible that the selective advantage of kin-biased distribution may only be manifest under specific sets of environmental conditions. Habitats such as tributaries or small streams may offer conditions of high density and low dispersal that are more conducive to the establishment of kin-biased distribution than the low-density, highly dispersive environment of larger rivers. In addition, the relatively low numbers of spawning fish in small streams may result in the production of large numbers of individuals from relatively few families, thus increasing the probability of the occurrence of neighbouring kin. Thus, the nature of kin recognition in salmon may be context dependent (Waldman 1988), reflecting a conserved adaptation that is expressed in particular conditions of density, dispersal and genetic diversity and that suffers no negative selection if not expressed. Studies of kin distribution should be conducted in other populations to provide insight into the selective pressure associated with the evolution of kin recognition in different and fluctuating environments (Blaustein 1987).

The complex life history of the Atlantic salmon tends to create large numbers of half-sibs. Dominant anadromous males may fertilize several nests made by several different females. One female may deposit eggs in several nests, reproducing with several anadromous males. The participation of precocious male parr in reproduction is not negligible with up to 20% of the eggs in a nest fertilized by several precocious parr (Hutchings & Myers 1988; Morán *et al*. 1996; Thomaz *et al*. 1997; P. M. Fontaine *et al*., unpublished). The coefficient of relatedness of half-sibs is, on average, 0.25 in comparison to 0.50 for full-sibs. Thus, the benefit in fitness gained by the recipient of the altruistic act should be on average at least four-times greater than the cost incurred by the altruist, twice that in the case of full-sibs. Finally, although some studies have revealed

a capacity of animals to discriminate half-sibs (coho salmon, Quinn & Busack 1985; frog tadpoles, Blaustein & O'Hara 1982; ground squirrels, Holmes & Sherman 1982), we do not know if this occurs in Atlantic salmon. To conclude, our results do not disprove the existence of kin recognition and its potential benefits as revealed in laboratory conditions. However, they provide evidence that the advantage of such behaviours may be limited in nature due to environmental and demographic conditions that influence fish density, the dispersal of relatives and their degree of relatedness.

Acknowledgements

The authors thank Louis Bernatchez for his advice and comments on the manuscript, Françoise Colombani for assistance and direction in the laboratory, François Caron of the Ministère de l'Environnement et de la Faune du Québec who provided facilities on the Trinité River, M. S. Blouin for his advice and statistical program on relatedness, and Daniel Laroche for field assistance. This research was supported by grants from the Natural Sciences and Engineering Research Council (NSERC) research grants program to J.D. P.-M.F. was supported by funds from GIROQ (Groupe Interuniversitaire de Recherches Océanographiques du Québec), CIRSA (Centre Interuniversitaire de Recherche sur le Saumon Atlantique), the F. de B. Gourdeau Foundation (Federation Québecoise pour le Saumon Atlantique) and by an Olin fellowship (Atlantic Salmon Federation).

References

Allen KR (1969) Limitations on production in salmonid populations in stream. In: *Symposium on Salmon and Trout in Streams*. (ed. Northcote TG), pp. 3–18. Institute of Fisheries, University of British Colombia, Vancouver, BC, Canada.

Barnett C (1977) Chemical recognition of the mother by the young of the cichlid fish *Cichlasoma citrinellum*. *Journal of Chemical Ecology*, **3**, 461–466.

Barnett C (1981) The role of urine in parent–offspring communication in a cichlid fish. *Zeitschrift Fur Tierpshychologie*, **55**, 173–182.

Blaustein AR (1987) Kin recognition in vertebrates (excluding primates): mechanisms, functions, and future research. In *Kin Recognition in Animals*. (eds Fletcher DJC Michener CD), pp. 333–357. John Wiley and Sons, Chichester.

Blaustein AR, O'Hara RK (1982) Kin recognition in *Rana cascadae* tadpoles: maternal and paternal effects. *Animal Behaviour*, **30**, 1151–1157.

Blouin MS, Parsons M, Lacaille V, Lotz S (1996) Use of microsatellite loci to classify individuals by relatedness. *Molecular Ecology*, **5**, 393–401.

Brown GE, Brown JA (1992) Do rainbow trout and Atlantic salmon discriminate kin? *Canadian Journal of Zoology*, **70**, 1636–1640.

Brown GE, Brown JA (1993a) Do kin always make better neighbours?: the effects of territory quality. *Behavioral Ecology and Sociobiology*, **33**, 225–231.

Brown GE, Brown JA (1993b) Social dynamics in salmonid fishes: do kin make better neighbors? *Animal Behaviour*, **45**, 863–871.

Brown GE, Brown JA (1996a) Does kin-biased territorial behavior increase kin-biased foraging in juvenile salmonids? *Behavioral Ecology*, **7**, 24–29.

Brown GE, Brown JA (1996b) Kin discrimination in salmonids. *Reviews in Fish Biology and Fisheries*, **6**, 201–219.

Brown GE, Brown JA, Crosbie AM (1993c) Phenotype matching in juvenile rainbow trout. *Animal Behaviour*, **46**, 1223–1225.

Caron F, Shields M, Fournier D (1992) *Registre de l'exploitation du saumon au Québec, 1984–92*. Ministère du Loisir, de la Chasse et de la Pêche, Direction de la gestion des espèces et des habitats, Service de la faune aquatique, Québec.

FitzGerald GJ, Morrissette J (1992) Kin recognition and choice of shoal mates by threespine sticklebacks. *Ethology, Ecology and Evolution*, **4**, 273–283.

Fletcher DJC (1987) The behaviour analysis of kin recognition: Perspectives on methodology and interpretation. In: *Kin Recognition in Animals*. (eds Fletcher DJC Michener CD), pp. 19–54. John Wiley and Sons, Chichester.

Fletcher DJC, Michener CD (1987) Introductory remarks. In: *Kin Recognition in Animals*. Edited by. (eds Fletcher DJC Michener CD), pp. 1–6. John Wiley and Sons, Chichester.

Fontaine PM, Dodson JJ, Bernatchez L, Sletan A (1997) A microsatellite assesment of metapopulation structure in Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 2434–2442.

Hamilton WD (1964) The genetical evolution of social behaviour. I. II. *Journal of Theoretical Biology*, **7**, 1–52.

Holmes WG, Sherman PW (1982) The ontogeny of kin recognition in two species of ground squirrels. *American Zoologist*, **22**, 491–517.

Hutchings J, Myers RA (1988) Mating success of alternative maturation phenotype in male Atlantic salmon, *Salmo salar*. *Oecologia*, **75**, 169–174.

James PW, Stuart CL, Zale AV, Maughan OE (1987) Diver-operated electrofishing device. *American Journal of Fisheries Management*, **7**, 597–598.

Kalleberg H (1958) Observations in a stream tank of territoriality and competition in juvenile salmon and trout (*Salmo salar* L. & *S. trutta*). *Report of the Institute of Freshwater Research, Drottninholm*, **39**, 55–98.

Keeley ER, Grant WA (1995) Allometric and environmental correlates of territory size in juvenile Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 186–196.

Keenleyside GJA, Yamamoto FT (1962) Territorial behaviour of juvenile Atlantic salmon (*Salmo salar* L.). *Behaviour*, **19**, 139–169.

Lacy RC, Sherman PW (1983) Kin recognition by phenotype matching. *American Naturalist*, **121**, 489–512.

Litt M, Lutty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics*, **4**, 397–401.

Lockle DM, Madison DM, Christian JJ (1982) Time dependency and kin recognition of cannibalistic behavior among poeciliid fishes. *Behavioral and Neural Biology*, **35**, 315–318.

McKaye KR, Barlow GW (1976) Chemical recognition of young by the Midas Cichlid, *Cichlasoma citrinellum*. *Copeia*, **1976**, 276–282.

Morán P, Pendás AM, García-Vázquez E (1996) Genetic assessment of the reproductive success of Atlantic salmon precocious parr by means of VNTR loci. *Heredity*, **77**, 655–660.

Nakamura Y, Leppert M, O'Connell P *et al.* (1987) Variable numbers of tandem repeat (VNTR) markers for human gene mapping. *Science.*, **235**, 1616–1622.

O'Reilly PT, Hamilton LC, McConnell SK, Wright JM (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.

Olsen KH (1989) Sibling recognition in juvenile Arctic charr, *Salvelinus alpinus* (L.). *Journal of Fish Biology*, **34**, 571–581.

Queller DC (1992) Does population viscosity promote kin selection? *Trends in Ecology and Evolution*, **7**, 322–324.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

Quinn TP, Busack CA (1985) Chemosensory recognition of siblings in juvenile coho salmon (*Oncorhynchus kisutch*). *Animal Behaviour*, **33**, 51–56.

Quinn TP, Hara TJ (1986) Sibling recognition and olfactory sensitivity in juvenile coho salmon (*Oncorhynchus kisutch*). *Canadian Journal of Zoology*, **64**, 921–925.

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): Population genetics software for exact test and ecumenism. *Journal of Heredity*, **86**, 248–249.

Rico C, Kuhnlein U, FitzGerald GJ (1992) Male reproductive tactics in the threespine stickleback, an evaluation by DNA fingerprinting. *Molecular Ecology*, **1**, 79–87.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, New York.

Slettan A, Olsaker I, Lie Ø (1995) Atlantic salmon, *Salmo salar*, microsatellites at the SSOSL25, SSOSL85, SSOSL311, SSOSL417 loci. *Animal Genetics*, **26**, 281–282.

Stabell OB (1987) Intraspecific pheromone discrimination and substrate marking by Atlantic salmon parr. *Journal of Chemical Ecology*, **13**, 1625–1643.

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research.*, **17**, 6463–6471.

Thomaz D, Beall E, Burke T (1997) Alternative reproductive tactics in Atlantic salmon: factors affecting mature parr success. *Proceeding of the Royal Society of London B*, **264**, 219–226.

Van Havre N, FitzGerald GJ (1988) Shoaling and kin recognition in the threespine stickleback (*Gasterosteus aculeatus* L.). *Biological Behavior*, **13**, 190–201.

Waldman B (1988) The ecology of kin recognition. *Annual Reviews of Ecological Systematics*, **19**, 543–571.

Winberg S, Olsen KH (1992) The influence of rearing conditions on the sibling odour preference of juvenile Arctic charr, *Salvelinus alpinus* L. *Animal Behaviour*, **44**, 157–164.

Wright S (1951) The genetical structure of populations. *Annals Eugenics*, **15**, 323–354.

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