

DIVERSITY OF SPAWNING MIGRATION AND GENETIC POPULATION STRUCTURING OF ATLANTIC SALMON, *SALMO SALAR* IN THE VARZUGA RIVER

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Introduction

Over the past century, Atlantic salmon populations have been greatly affected by human activities, with many salmon populations having gone extinct and many of those that remain relying on human mediated supplementation (reviewed in Parrish et al., 1998). Reasons for these declines are many, including over-exploitation, pollution and destruction of spawning grounds due to e.g. dams or construction of hydroelectric power plants (Allendorf, Waples, 1996; Parrish et al., 1998). Anadromous fish species such as Atlantic salmon are to be especially susceptible to environmental changes due to their complex life history which is reliant on multiple habitat types (Allendorf, Waples, 1996). An additional threat to natural populations of Atlantic salmon has been the increased production of domesticated salmon, which, when they escape, may endanger the gene pools of native populations. Therefore, preserving the remaining genetic diversity of wild Atlantic salmon (*Salmo salar*) populations presents one of the greatest challenges in conservation biology.

One of the few exceptions to the general rule of population decline are the salmon populations of the Kola Peninsula in northwest Russia. Due to their remoteness, and strict fishing regulations during Soviet times, the population sizes of many rivers has not declined markedly in the past century (Kazakov, Veselov, 1998). Over the past decade, these rivers have spawned an emerging industry in the form of fishing tourism, which has provided a much needed source of income for local residents. As the annual catch numbers per river are normally low and therefore the cost per fish high (a one week fishing trip can cost over USD 8000 per rod: see e.g. www.varzina.fi), it has been recognised that fishing tourism is more likely to result in a sustainable harvesting and should therefore preferable from a conservation viewpoint compared to e.g. a traditional commercial fishery (Zubchenko et al., 1991). One of the largest such rivers is the Varzuga River, located on the south coast of the Kola Peninsula in northwest Russia (Fig. 1). Earlier ecological research of the Varzuga River has identified a number of salmon spawning and nursery areas, both in the main channel, as well as in numerous tributaries (Kazakov, 1994).

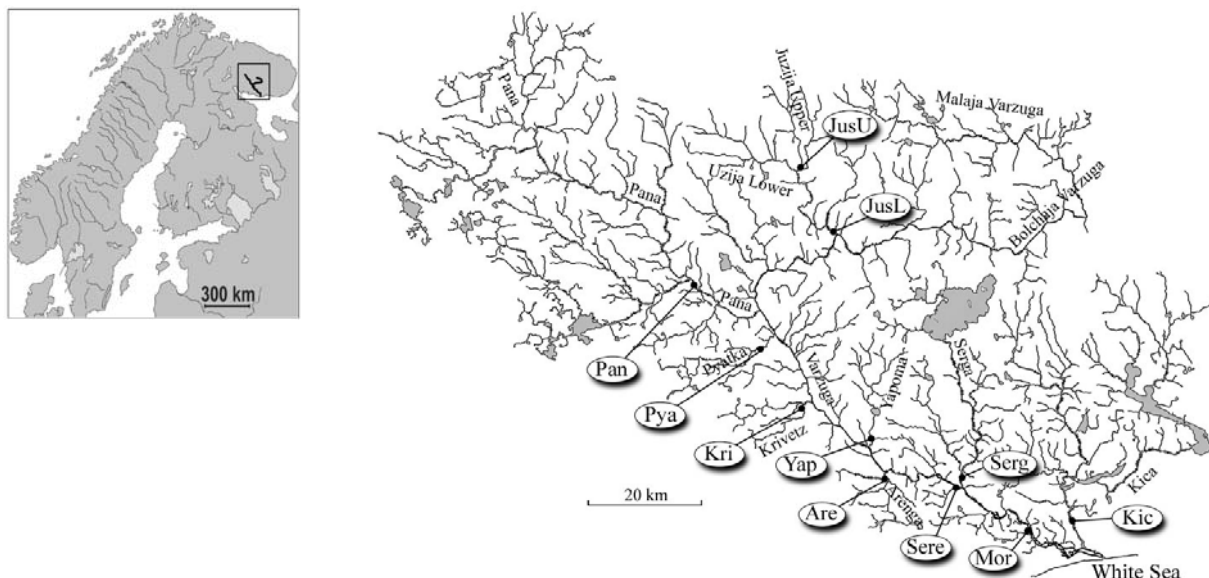


Fig. 1. Map of the Varzuga River system indicating sampling sites. Sampling site abbreviations as in Table 1

Although fishing tourism is a more preferred form of sustainable use, it is not without risk. Increased human presence in these remote regions also increases the possibility of pollution and transfer of diseases and/or parasites such as the potentially destructive *Gyrodactylus salaris*. Therefore, biodiversity assessment is warranted in order to aid in the development of management strategies aimed at ensuring the sustainable use of this valuable natural resource. As genetic variation is one of the three levels of biodiversity that the World Conservation Union (IUCN) has recommended for conservation (Reed, Frankham, 2003), assessment of the genetic diversity of salmon populations in the river system prior to any negative effects of human activity would be useful.

In recent years, microsatellite markers have been increasingly utilised as genetic markers in conservation genetic studies as their molecular properties, including high levels of genetic variability, along with recent developments in data analysis methods offer increased power and flexibility for data analyses (Beumont, Bruford, 1999). One of the most notable conservation genetic advances precipitated by microsatellite analyses has been the possibility to study genetic relationships or populations across small geographical scales and/or at the level of the individual (e.g. Angers, Bernatchez, 1998). Numerous studies in a range of species groups have highlighted the fact that genetic structuring can occur even across very short geographic distances, which has implications for the conservation of

genetic diversity in such systems (Koskinen et al., 2001). This point has been particularly highlighted in freshwater salmonid fish populations, where high levels of genetic differentiation have been observed between populations located just tens of kilometres apart (Angers, Bernatchez, 1998; Koskinen et al., 2001, 2002) and even between individuals within populations (Carlsson et al. 1999). The majority of studies of anadromous Atlantic salmon population genetic structure have generally focused on the genetic structuring between samples from different rivers (e.g. Ståhl, 1987; Kazakov, Titov, 1991; Nielsen et al., 1996; Koljonen et al., 1999; Verspoor et al., 1999; Nilsson et al., 2001). These studies have generally detected highly significant population genetic differentiation, with genetic divergence estimates numerous times higher than normally observed in marine species (reviewed by Ward et al., 1994; DeWoody, Avise, 1999). In addition, a number of studies have observed population structuring between populations of the same continent which followed an 'isolation by distance' model, whereby genetic and geographic distance were significantly correlated (Koljonen et al., 1999; Tonteri et al., 2005). This is concordant with a scenario whereby the majority of individuals return to their natal river to spawn, with a small proportion of individuals 'straying' to spawn in nearby rivers. There are also a number of studies which have included samples from more than one spawning site per river (e.g. Ståhl, 1983; McElligott, Cross, 1991; Jordan et al., 1992; Elo et al., 1994; Garrant et al., 2000; King et al., 2001).

In many such cases, significant genetic differentiation between tributary samples has been observed, indicating that single rivers may often support more than one population. However, all studies to date have either included less than four within-river sampling sites and/or less than six polymorphic loci and hence have not been able to make detailed interpretations about within-river population genetic structuring. One of the most extensive studies is of the population structure of Atlantic salmon from seven sampling sites within the Sainte-Marguerite River in Québec, Canada (Garant et al., 2000). Perhaps the main limitation of this study was that only five microsatellite loci were analysed which could have contributed to the variance between year classes.

In this study, the population genetic structure of Atlantic salmon from 10 locations within the Varzuga River tributary system and a nearby river was assessed using genetic data from 17 microsatellite loci. Population and individual-level analyses were used to assess the presence and level of genetic differentiation within the tributary system as well as to determine if the observed genetic structuring may be associated with specific ecological characteristics of the river.

Methods

The Varzuga River

The Varzuga River (254 km in length, drainage area 7940 km², drains to the White Sea on the south coast of Kola Peninsula near the Kuzomen village (66°15' N, 36°57' W). Atlantic salmon spawning and nursery areas have been identified in eight of the more than 20 tributaries, as well as in several

regions of the main river channel (Kazakov 1994). Parr representing multiple year classes were captured from each of these regions (Fig. 1, Table 1) by electrofishing from 1998 to 2000. The average waterway distance (\pm SD) between sampling sites was 60 \pm 37km (range 5-165km). The adipose fin of each fish was preserved in 95% ethanol and the fish released. Parr density of each site was measured or taken from the literature.

Genetic analyses

DNA was extracted using a salt-based method similar to that outlined in Aljababi and Martinez (1997). In total, 17 microsatellite loci were analysed: PCR protocols for *SSOSL85*, *SSOSL311* (Slettan et al. 1995), *SSOSL438*, (Slettan et al. 1996), *Ssa85*, *171*, *197*, *202* (O'Reilly et al. 1996), were essentially as described in Primmer et al. (2000), except that 0.1 units of BioTaq DNA polymerase and PCR buffer were used. PCR protocols for *SSF43*, *Ssa412*, *SS20.19*, *SSD30* (Sanchez et al. 1997), *SLEE184*, *SLEEN82*, (ref) *SSOSL25* (Slettan et al. 1995), *Ssa14* (ref), *Ssa422* (ref), and *SS11* (ref) are as in Tonteri et al. (submitted). Loci were divided into two 'panels' (the first seven, and last 10 loci listed above) so that within each panel there was no size range overlap of loci labeled with the same fluorescent dye. This enabled all 18 loci to be electrophoresed in just two gel lanes on an ABI377 sequencer (see Primmer et al. (1999) for electrophoresis details). The locus *Ssa289* was also analysed, however as this marker has been shown to be physically linked to the locus *Ssa422* at a distance of <10cM, this marker was excluded from all analyses as it was the less polymorphic of the two.

Table 1. Sampling and genetic diversity details of sampling sites in the Varzuga River tributary system, and g

№	Site	Abbrev.	Coordinates	Distance from		Parr density (m ²)	n	Genetic diversity indices	
				river mouth	main channel			A _r	H _E
1	Juzija Upper	JusU	67°00'N, 36°20'E	140	23	0,2	21	6.4	0.62
2	Juzija Lower	JusL	66°58'N, 36°20'E	129	2	0,3	39	6.6	0.65
3	Pana	Pan	66°53'N, 35°54'E	106	9	0,8	34	7.1	0.66
4	Pyatka	Si	66°50'N, 35°57'E	93	1		40	7.1	0.67
5	Krivec	MB	66°41'N, 36°00'E	78	1		40	7.2	0.67
6	Yapoma	Ya	66°36'N, 36°10'E	64	2		37	7.1	0.67
7	Arenga	A	66°32'N, 36°11'E	57	5		30	6.5	0.68
8	Serezhnyi	Sp	66°28'N, 36°28'E	37	0	0,4	35	7.1	0.66
9	Serga	Se	66°28'N, 36°29'E	40	4	0,2	46	6.9	0.66
10	Morskoi	SK	66°23'N, 36°40'E	26	0	0,1	26	6.2	0.63
11	Kica	Kic	66°21'N, 36°53'E	32	28	0,3	44	6.9	0.68

FSTAT v.2.9.3 (Goudet, 2001) was used to calculate unbiased gene diversity (H_E) and allelic richness (allele number corrected for sample size using the rarefaction method outlined in (El Mousadik, Petit, 1996)) averaged across loci within each population. GENEPOP version 3.4 (Raymond, Rousset, 1995) was utilised to estimate deviations from Hardy-Weinberg (H-W) equilibrium across populations (within loci) and across loci (within populations) using the probability test, and to estimate deviations from genotypic linkage equilibrium (L-E) across all populations. Corrections for multiple significance tests were performed using Fisher's method, as implemented in GENEPOP, and applying a sequential Bonferroni type correction (Rice, 1989). Tests for statistically significant genic differentiation between populations were conducted using GENEPOP (corrections for multiple tests were performed as indicated above). GENEPOP was also used to estimate interpopulation F_{ST} values, i.e. variance in allele frequencies (Weir, Cockerham, 1984).

Genetic distances between populations were estimated using the infinite allele model (IAM) based D_A distance (Nei et al., 1983). Due to the low geographical distances between sampling sites, it is unlikely that mutations have contributed to population divergence and hence genetic distances based on the stepwise mutation model were not applied. The resulting D_A genetic distance matrices were used to construct a Neighbor-Joining (N-J) phylogram and confidence estimates on the tree topology was attained by resampling over loci with 5000 bootstrap replicates. The genetic distance estimation, bootstrapping, N-J phylograms and consensus trees construction procedures were carried out using the POPULATIONS 1.2.28 (Langella, 1999) computer program.

Spatial genetic structure was assessed at both the population and individual levels. At the population level, the association between pairwise estimates of population genetic differentiation (F_{ST}) and natural logarithm of waterway distances between sampling sites were assessed using a Mantel test of matrix correspondence (Mantel 1967), using the procedure of Smouse et al. (1986) which estimates matrix correspondence using a measure (r_{xy}) analogous to an auto-correlation co-efficient. The statistical significance of the values were obtained via 999 random permutations. F_{ST} , rather than $F_{ST} / (1 - F_{ST})$, was used as it is more appropriate for a one-dimensional stepping stone model (Rousset, 1997). Spatial genetic structure at the individual level was assessed using a microspatial autocorrelation

approach developed for multi-allelic co-dominant loci (Smouse, Peakall, 1999; Peakall et al., 2003). These multivariate procedures strengthen spatial signal by combining alleles and loci, hence reducing stochastic noise (Smouse, Peakall, 1999). The autocorrelation co-efficient generated, r , is closely related to Moran's-I. Twenty randomly chosen individuals per population were included in the individual-level analyses. Individual genetic distances were estimated using the squared distances measure (PhiPT) outlined in Peakall et al. (2003). Waterway distances (in km) were used, with all individuals from the same pair of sampling sites being assigned the same value and individuals from the same population being assigned a value of zero. Two descriptors of spatial genetic autocorrelation were employed: in the first, a correlogram was plotted to assess the genetic correlation as a function of distance between genotypes. The autocorrelation coefficient r , was plotted as a function of seven discrete distance classes (10, 20, 40, 60, 80, 100, 120 or 160km) with individuals separated by 0-10km being included in the first class, individuals separated by 11-20km included in the second class etc. The second genetic autocorrelation analysis, a multi-distance class (MDC) analysis, utilised the same distance classes, however rather than assessing genetic autocorrelation in six discrete groups, individuals from more distant groups are added to the previous groups i.e. individuals separated by 0-10 km are included in the first class, individuals separated by 0-20km included in the second class etc. The first analysis provides an estimate of the extent of non-random genetic structure, while the second enables a more accurate estimation of scale across which spatial genetic structure can be detected (Peakall et al., 2003). 95% confidence intervals (CI) about the null hypothesis of no spatial genetic structure were determined from 999 permutations, and the 95% CI of r estimates were calculated by bootstrapping pairwise comparisons (1000 replicates) with replacement. Significant genetic autocorrelation was concluded when the CIs of r and those expected under the null hypothesis did not overlap (see Peakall et al. 2003 for more details). All spatial genetic structure analyses were performed using the GenALEX v5.1 software package (Peakall, Smouse, 2001).

Results

The dynamics of spawning migrations of the summer- and autumn run salmon in River Varzuga

is known to have a complex structure related to different timing of the upstream migrations and the distance to the spawning grounds. The spawning and nursery areas in River Varzuga are mostly situated in the mainstream and in 1st-order tributaries: Juzia, Kichesara, Pana, Pyatka, Krivets, Japoma, Aren'ga, Serga and Kitsa, and not so often – in 2nd-order tributaries, e.g. Indel' or Mel'ga. This is a feature distinguishing it from large rivers such as Pechora, Mezen', Ponoj and many other rivers of Europe and North America, where the mainstream serves only as a transit way for the salmon travelling to spawning tributaries (Zubchenko et al., 2002).

Summer run fish in River Varzuga demonstrate five types of migration differing in the timing of migration peaks (Fig. 2).

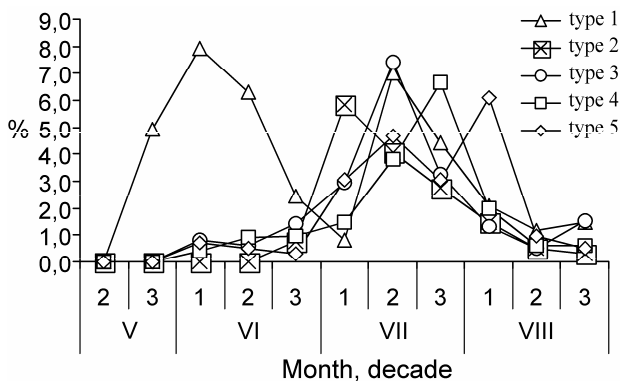


Fig. 2. Structure of spawning migrations of the summer run salmon in the Varzuga River watershed in 1977-2001

The structure of spawning migrations of autumn run fish is more diverse due to a complex dynamics of hydrological and meteorological conditions in the autumn season. Six types of migration are distinguished for fish of this biological group (Fig. 3), also depending on the timing of massive upstream migration.

Immediately after ice drift (between 1st-20th of May), large fish including 1SW (96% of all fish), 2SW (3.8%) and singular 3SW spawners ascend the river (data from 1992-2002). Among these, females prevail (58.0%) (Mel'nikova, 1962; Lysenko, Berestovsky, 1999). According to our data and data by Kuz'min (1975) and Kazakov et al. (1992), this is fish from the previous year's autumn run which had overwintered in the downstream of the river and resume the spawning migration in spring after ice drift. Fish of this group accounted for 8.3% of all migrants between 1988 and 1997. They continue migrating until late June. In the second ten days of June, summer run fish begin ascending the river.

The first ones to arrive are males (51%) and females (49%) aged 2SW and 3SW (1-3%). They contributed 0.7% to the total 1992-2002 spawning stock and migrated ca. until July 10. Starting the third ten days of June, grilse begin their upstream migration, males accounting for 93%. The proportion of grilse among spawning migrants in 1988-1997 reached 26.1%.

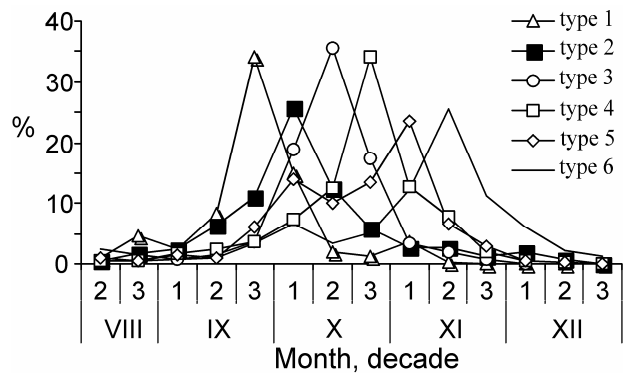


Fig. 3. Structure of spawning migrations of the autumn run salmon in the Varzuga River watershed in 1977-2001

The autumn run continues from the end of the second ten days of August to the first ten days of December. Autumn run salmon contribute 64.9%. The proportion of 1SW fish among them varies from 70 to 90% in different years, 3SW fish are very rare, and females account for 71.0%.

Thus, the migration peak of summer run salmon in River Varzuga occurs between 1st and 20th of July, and that of autumn run salmon – in October-November (Fig. 4).

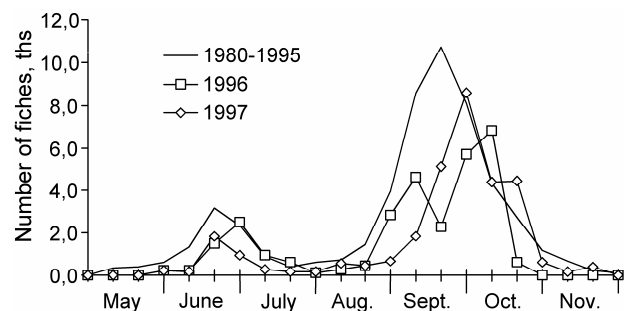


Fig. 4. Dynamics of spawning migrations of the summer- and autumn run salmon in River Varzuga (1980-1997)

One can therefore assume that the variation of the time at which spawners enter the river contributes to the formation of the complex genetic structure of salmon populations.

The number of alleles at any single locus across populations ranged from two to 30, with a mean of 13. The mean number of alleles per locus (across the 17 microsatellite loci) within populations ranged from 6.6 to 8.9, however the range in allelic richness (average allele number within populations, corrected for differences in sample size) was smaller, being 6.2-7.2 (Table 1). Average observed heterozygosity was relatively similar for each population, varying from 0.60 to 0.69, as was expected gene diversity (0.61-0.68) (Table 1).

No significant ($P < 0.05$) deviations from Hardy-Weinberg equilibrium were identified for any population or locus. Genotypic linkage equilibrium tests across populations suggested significant ($P < 0.05$) linkages only between the loci *Ssa197* and *SSOSL85*, after correcting for multiple tests, which is not more than expected due to *type I* error. Highly significant genic differentiation ($P < 0.0001$) was observed between the populations and, consequently, the null-hypothesis of random mating between all sampling sites in the Varzuga tributary system could be rejected. In addition, random mating could be rejected for 46 of the 55 possible population pairs following sequential Bonferroni correction (Table 2).

The level of genetic differentiation between sampling locations, as estimated by F_{ST} , ranged between 0.006 and 0.07 (Table 2), with the global F_{ST} being 0.014.

The neighbour-joining D_{CE} phylogram revealed several groups of populations with moderate to high bootstrap support (Fig. 5). These were normally populations located close to each other (e.g. Jusija Upper - Jusija Lower, Morskoi - Kica, Arenga - Japoma), however there were exceptions, with

some samples from nearby locations not sharing a high genetic affinity (e.g. Serga-Serezhnji) and relatively distant populations being genetically similar (e.g. Krivec - Jusija Upper and Lower).

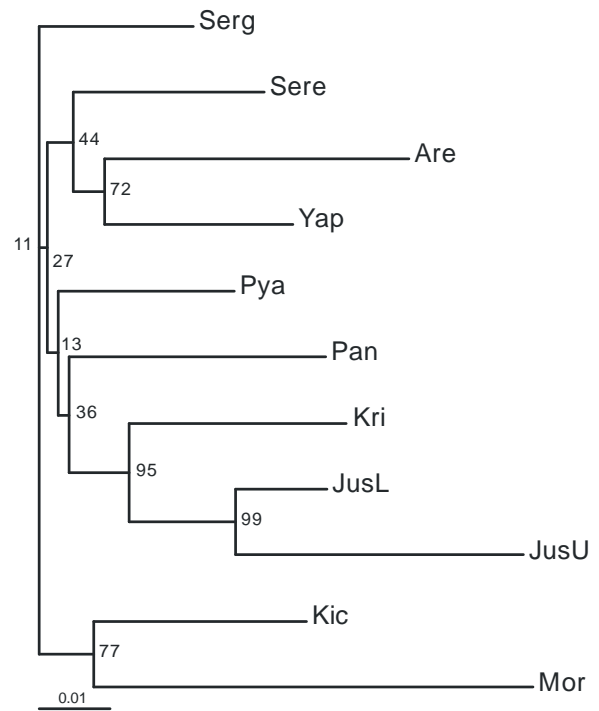


Fig. 5. Genetic relationships of Varzuga River Atlantic salmon as resolved by microsatellite analyses. The population phylogram was constructed using the N-J method and is based on the D_A genetic distance. Numbers beside the nodes of the tree represent D_A bootstrap support (in percent) based on 5000 replicates of re-sampled loci.

Table 2. Pairwise F_{ST} values (above diagonal) and waterway distances (km) between population pairs (below diagonal). All F_{ST} values not significant following Bonferroni correction are highlighted in bold

	A	JusL	JusU	Kic	MB	Pa	Se	Si	SK	Sp	Ya
A	0	0.019	0.039	0.021	0.017	0.010	0.011	0.013	0.042	0.006	0.007
JusL	82	-	0.007	0.013	0.013	0.014	0.012	0.006	0.049	0.006	0.010
JusU	93	11	-	0.026	0.016	0.017	0.024	0.017	0.072	0.019	0.022
Kic	81	153	164	-	0.015	0.015	0.011	0.009	0.026	0.007	0.012
MB	30	52	63	101	-	0.010	0.014	0.004	0.038	0.007	0.008
Pa	59	41	52	130	29	-	0.004	0.004	0.038	0.007	0.006
Se	25	97	108	54	45	74	-	0.006	0.021	0.004	0.007
Si	45	37	48	116	15	14	60	-	0.032	0.003	0.004
SK	31	103	114	50	57	80	14	66	-	0.029	0.029
Sp	20	92	103	61	40	69	5	55	11	-	0.001
Ya	17	69	80	88	17	46	32	34	38	27	-

Tests for associations between genetic divergence (F_{ST}) and geographic distance at the population level revealed a near significant association ($r_{xy} = 0.33$, $p = 0.05$, $r^2 = 0.11$) when all 11 samples were included (Fig. 5), however closer inspection of the pairwise F_{ST} values indicated that pairs including one particular population (Morskoi), were generally much higher, relative to geographic distance, than those of other population pairs (Fig. 5), which may have affected the Mantel analysis. Accordingly, a highly significant isolation by distance effect was revealed when the Morskoi population was excluded from the Mantel test ($r_{xy} = 0.53$, $p = 0.002$, $r^2 = 0.28$; Fig. 5). It is worth noting however that genetic divergence also increased with waterway distance between pairs including the Morskoi population (Fig. 5), but the level of genetic divergence was much higher.

The genetic characteristics of the Morskoi population were also unusual in other ways. For example, there was a trend for genetic diversity (H_E) to be negatively associated with distance from the river mouth in all populations except Morskoi (Fig. 7). This trend was statistically significant if Morskoi was excluded from the analysis.

Tests for spatial genetic structure at the individual level revealed that the genetic autocorrelation coefficient, r , was significantly positive for the 10 km and 20 km size classes, intercepting the x-axis at 34 km (Fig. 8a). Contrastingly, significantly negative genetic autocorrelation was observed for the 120 km size class (Fig. 8a). The genetic correlation for increasing waterway distance classes indicated that r was significantly positive for waterway distance classes up to 100 km, with the level of the autocorrelation decreasing with each addition of more distantly located individuals (Fig. 8b).

When individuals sampled from locations more than 120 km apart were included, positive genetic autocorrelation was no longer detectable. The above tests were conducted using individuals from all 11 sampling sites. Exclusion of the Morskoi population, which displayed an unusually high level of genetic divergence with other populations (see above) did not change the conclusions of the correlogram analysis (Fig. 8a), but for the MDS analysis (Fig. 8b), the largest distance class for which r was significantly positive was 80 km rather than 100 km.

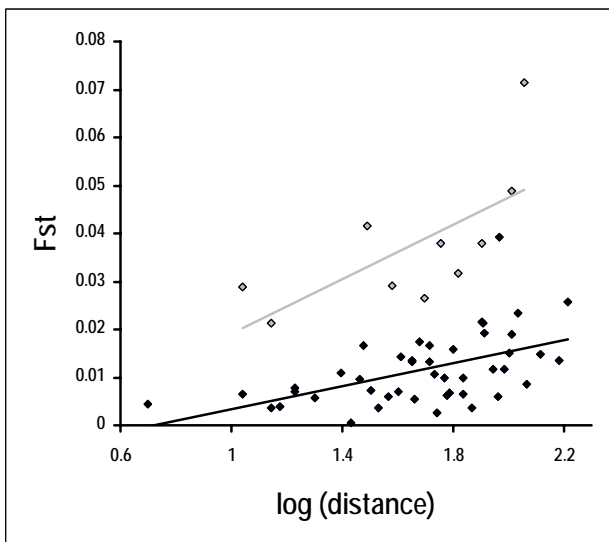


Fig. 6. Mantel test indicating the observed isolation by distance signal. Grey dots and dashed trend line are for the Morskoi population. Black dots and solid trend line are for all other populations.

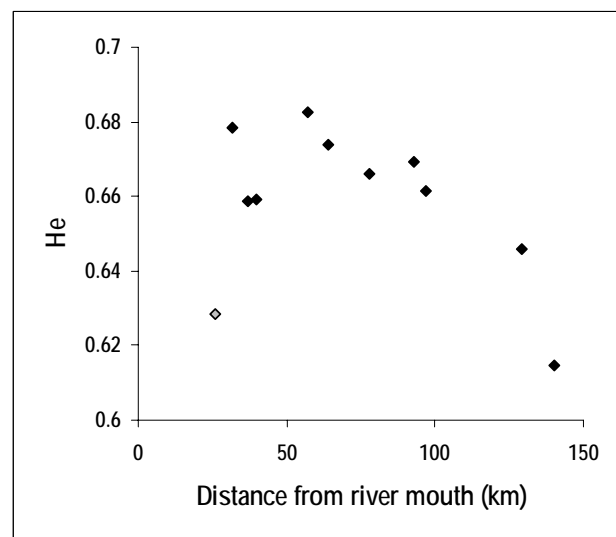


Fig. 7. Association between the distance of sampling site from the river mouth and genetic diversity (H_E). The Morskoi population is indicated by a grey dot.

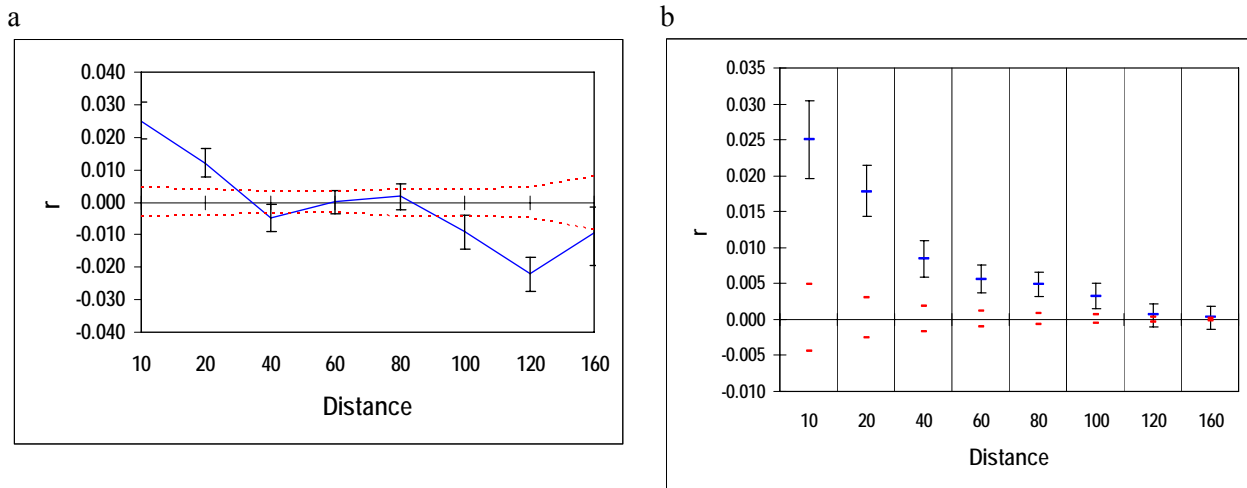


Fig. 8. Genetic autocorrelation analyses of Varzuga salmon genetic structure. a - correlogram showing the genetic correlation, r , as a function of distinct distance classes. Dotted lines indicated 95% CI about the null hypothesis of no genetic structure and error bars about r indicate 95% CI as determined by bootstrapping. b- The genetic correlation r , for increasing distance classes. Red bars indicate 95% CI about the null hypothesis of no genetic structure and error bars about r indicate 95% CI as determined by bootstrapping.

Discussion

Substantial genetic differentiation was identified between Atlantic salmon sampled from the tributaries and main stream of the Varzuga river system. Significant genic differentiation was observed over distances as short as 11km, with the null hypothesis of random mating being rejected for all pairwise samples separated by a waterway distance of >69 km (Table 2). The global level of genetic differentiation between the 11 sampling sites, as estimated by F_{ST} was 0.014. This value is at the lower end of the range observed in earlier studies of within river genetic differentiation in Atlantic salmon using allozymes ($F_{ST} = 0.007-0.067$; McElligot & Cross, 1991; Elo et al., 1994) or microsatellites (0.011-0.082; Beacham, Dempson 1998; Garant et al., 2000; Spiddle et al., 2001). In contrast to other studies, which included samples from seven or more sampling sites from the same river system (McElligot, Cross 1991; Elo et al., 1994; Garant et al., 2000, relatively clear genetic structuring was observed in the Varzuga tributary system. The neighbour-joining phylogram revealed several clusters of populations that were supported by moderate to high bootstrap support (Fig. 5). These were normally populations located close to each other (e.g. Jusija Upper - Jusija Lower, Morskoi - Kica, Arenga - Japoma), however there were exceptions, with some samples from relatively distant populations being genetically similar (e.g. Krivec - Jusija Upper and Lower) and other samples from nearby locations not sharing a

high genetic affinity (e.g. Serga-Serezhnji). In contrast, inter-tributary comparisons of Sainte-Marguerite salmon (seven sampling sites) revealed bootstrap support lower than 50%, despite higher overall genetic differentiation, (Garant et al., 2000). A further notable difference from earlier studies was the strong isolation by distance signal observed between Varzuga tributary populations (Fig. 6). The only other study to assess this did not observe any significant association between genetic divergence and waterway distance between population pairs (Garant et al., 2000).

Given the partially contrasting genetic structuring of tributary samples observed between studies, how can these observations be interpreted in light of the alternative evolutionary models (the member vagrant model and the metapopulation model) summarised by Garant et al., (2000) One of the key differences between the models is the relative level of genetic structuring expected. The member-vagrant model (Garant et al., 2000) predicts that population structure evolves as a consequence of selective forces promoting precise homing, which would result in locally adapted gene pools and hence, strong genetic structure among populations. A significant isolation by distance signal would also support this model (Garant et al., 2000). On the other hand, the metapopulation model (reviewed by Rieman, Dunham, 2000) predicts that given unstable environmental conditions, the evolution of locally adapted gene pools is restricted due to recurring local extinctions. In this scenario, a lower level of genetic structuring

would be expected (McQuinn, 1997; Rieman, Dunham, 2000) nor would an isolation by distance effect (Garant et al., 2000). In light of these predictions under the alternative models, the relatively high level of genetic structuring (Fig. 5) and significant isolation by distance signal (Fig. 6) observed in the Varzuga tributaries are concordant with the predictions of the member-vagrant evolutionary model. This implies that a number of relatively stable discrete breeding units occur within the Varzuga tributary system, with the significant association between genetic divergence and waterway distance (Fig. 6) implying that migration is more likely to occur between nearby populations. It should be pointed out however that the current data-set is not sufficient to test an additional prediction of the member-vagrant model, namely that the genetic structuring should also be temporally stable (Garant et al., 2000). This would be an interesting avenue for future research.

Based on the lack of temporal stability observed at some sampling sites, and the absence of a strong isolation by distance signal, Garant et al. (2000) concluded that the member-vagrant model was too rigid to predict the extent of genetic divergence among Sainte-Marguerite River salmon populations, and that unpredictable environmental change, resulting in extinction - re-colonisation processes more consistent with the metapopulation model were also important. This study highlighted a direct observation of such an extinction - re-colonisation event caused by flooding, which resulted in a high level of genetic differentiation between samples collected in different years.

Although the member-vagrant model appears to predict the genetic structuring of Varzuga Atlantic salmon populations quite well, there is nevertheless indirect evidence that extinction - re-colonisation processes may also be important in this system. For example, the unusually high level of genetic divergence observed between the Morskoi population and all other populations, combined with the low level of genetic diversity in this population is consistent with this population being subjected to a harsh genetic bottleneck at some time in the past, with genetic drift explaining the high level of inter-population divergence. However, the member-vagrant model still appears to fit this population, as interpopulation divergence is still associated with waterway distance (Fig. 6). Accordingly, the most likely source of migrants contributing to the re-establishment of this population is the nearby Kica population given the strong population affinities observed between these two populations (Fig. 5).

Is it possible to resolve the differences in population structure observed between this and other studies? At least some of the contrasts could be explained differences in sampling strategies between studies which could potentially effect direct comparisons of the results. Firstly, Garant et al. sampled fry (age 0+), while the current study analysed parr (age 1+ - 3+). As it is likely that the dispersal level from natal nursery areas is higher for parr than for fry, this difference could explain the lower level of genetic divergence between sampling sites observed in the current study. Secondly, the geographic scale of the current study (average (\pm SD) interpopulation waterway distance = 60 ± 37 km (Table 2) being several times larger than that in Garant et al. (2000). This may explain the lack of an observable isolation by distance effect in Sainte-Marguerite salmon.

From a conservation perspective, it is clear that the Varzuga river salmon do not represent a single panmictic population. The spatial autocorrelation analyses indicated that gene flow between locations separated by >100 km is limited, with the majority of migration within the tributary system occurring between nearby populations (Fig. 7). This implies that the conservation of multiple spawning and nursery areas is important for the long-term preservation of this salmon population.

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