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Spatial and temporal genetic composition of Atlantic salmon (*Salmo salar*) in River Elliðaár, Iceland

Leó Alexander Guðmundsson

45 eininga ritgerð til M.S. prófs

Júní 2007

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By Leó Alexander Guðmundsson

MSc degree research project (45 units)

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Rannsókn á erfðasamsetningu lax (*Salmo salar*) í árkerfi Elliðaáa í tíma og rúmi og möguleg áhrif eldislax á erfðasamsetningu villta stofnsins

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45 eininga ritgerð til M.S. prófs

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ABSTRACT

Atlantic salmon (*Salmo salar*) in the Elliðaár river system (Reykjavík, Iceland) was analyzed with a spatial and temporal genetic approach, using 7-8 microsatellite markers. The Elliðaár river system is a small system which comprises one main river and two upper rivers. Shortly after and during influx of farmed salmon into the river system, the native stock declined, juvenile density dropped as well as changes in life-history characteristics occurred. Genetic analysis was performed on parr samples (N=398) collected in all rivers with temporal replicates and temporal adult samples (N=584) collected in the main river in 1948-2005, of which 171 were farmed salmon. Allelic variation of spatial and temporal parr samples was F_{ST}=0.015, most pairwise F_{ST} comparisons were significant but there was a lack of consistency in the genetic divergence between rivers. Furthermore, there was a significant negative correlation between the effective number of breeders (N_b) and F_{ST} (Pearson R=-0.57, N=21, P<0.01), suggesting that the observed divergence was not biologically meaningful. For temporal adult samples, allelic variation (F_{ST}=0.006) was rather low and genetic diversity was stable and introgression of farmed salmon was not detected with a Bayesian assignment method. Consequently, spatial and temporal heterogeneity of parr samples might be associated with the "Allendorf-Phelps effect" and salmon therefore panmictic in the river system. Furthermore, the observed biological changes that have occurred in the salmon population are neither due to outbreeding depression, resulting from hybridization with farmed fish, nor due to inbreeding depression of isolated breeding units.

ÁGRIP

Erfðasamsetning lax (*Salmo salar*) í árkerfi Elliðaáa var könnuð í tíma og rúmi með 7-8 „microsatellite“ erfðamörkum. Laxastofn Elliðaáa hefur verið í lægð á undanförnum árum. Seiðaframleiðsla í efri ám vatnakerfisins hefur til að mynda minnkað mikið og lífssögu þættir breyst. Þar sem mikið af eldislaxi gekk í árnar á tíunda áratug síðustu aldar óttuðust menn að rekja mætti hnignun stofnsins að hluta til innblöndunar við eldislax. Jafnframt var hugsanlegt að efri árnar hefðu áður hýst sérstaka undirstofna sem nú væru að mestu horfnir. Við greiningu á stofngerð voru seiði rannsökuð úr Elliðaá, Hólmsá og Suðurá frá 1990-91 og 2002 (N=398). „Langtíma“ stöðugleiki Elliðaárstofnsins og möguleg blöndun við eldislax var könnuð með að greina sýni úr fullorðnum laxi, sem veiddur var í Elliðaá, á tímabilinu 1948-2005 (N=584), þar af 171 eldislaxi. Erfðamunur milli áa var á bilinu 0.2-3.6% (F_{ST}) og flestir samanburðir voru martækir. Hins vegar var erfðamunur ekki stöðugur í tíma og marktækt neikvætt samband var á milli náttúrulegs fjölda hrygningarfisks (N_b) og F_{ST} (Pearson R=-0.57, N=21, P<0.01). Því er líklegt að Elliðaárstofninn greinist ekki í undirstofna. Erfðasamsetning fullorðinna laxa á tímabilinu 1948-2005 var stöðug m.t.t. F_{ST} og erfðabreytileika. Innblöndun við eldislax greindist ekki. Lítil seiðaframleiðsla í Hólsmá og Suðurá orsakast því ekki af hnignun undirstofna og blöndun við eldislax skýrir ekki þær breytingar sem hafa orðið á laxastofni Elliðaáa.

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INTRODUCTION

Genetic methods are becoming more important in conservation and management of fish species (Ryman & Ståhl 1981; Youngson et al. 2003). Conservation of genetic diversity within populations is necessary since it allows populations to evolve in response to changing environmental conditions and heterozygosity is correlated to population fitness (Reed & Frankham 2003). In addition IUCN, the World conservation Union, recognizes the need to conserve biodiversity at three levels: genetic, species and ecosystem diversity (McNeely et al. 1990). It is a common view that effectively small and isolated populations are more vulnerable to the detrimental effects of inbreeding and loss of genetic diversity, which may lead to extinction (Keller & Waller 2002). Therefore, defining populations and understanding the forces that shape their genetic structure is important in any conservation and management scheme. Nowadays, genetic resources of many salmonid populations are protected in order to preserve the long-term evolutionary potential of the species, including Atlantic salmon (*Salmo salar*) (Waples 1995; Lage & Kornfield 2006).

Atlantic salmon belongs to the well studied genus Salmo in the salmonid subfamily, Salmoninae, which comprises 30 species (ITIS 2006). The native range includes the rivers and Northern Atlantic Ocean bound by North America, Scandinavia, and Europe (MacCrimmon & Gots 1979). Salmon are iteroparous (may spawn more than once) and display an anadromous life cycle. After a juvenile period in fresh water, salmon migrate to sea (Menzies & Shearer 1957; Hansen & Jacobsen 2003), where they reach maturity and return to their natal river to spawn (Quinn & Dittman 1990). The homing ability of salmon promotes formation of isolated breeding units, which enables local adaptation by minimizing gene flow among populations (Taylor 1991).

Indeed, it is within local populations that adaptive evolution takes place through systematic changes in allele frequencies (Hartl & Clark 1997). Salmon populations exploit a diverse range of environments and display considerable variability in life-history characters and phenotypic plasticity (Klemetsen et al. 2003), which is important for their stability and persistence (Saunders & Schom 1985; Taylor 1991).

For decades, genetic studies have shown that salmon populations are highly structured both between and within rivers systems (Ståhl 1987; Daníelsdóttir et al. 1997; Garant et al. 2000; King et al. 2001; Primmer et al. 2006) as well as being temporally stable (McElligott & Cross 1991; Jordan et al. 1992; Moffett & Crozier 1996). Most work on differentiation and structure of salmon population has been based on protein polymorphism of allozyme loci (Verspoor et al. 2005). These allozymes have generally lower resolution power than the more recently developed methods using nuclear DNA markers, e.g. microsatellites (O'Connel & Wright 1997; Estoup et al. 1998). Studies using highly polymorphic DNA markers have supported previous findings, demonstrating that salmon populations in river systems are highly structured (Galvin et al. 1996; Garant et al. 2000; Primmer et al. 2006). These studies have also provided evidence that isolation by distance processes may act in large river systems (Primmer et al. 2006) and stability of the structure may depend on environmental stability (Garant et al. 2000). However, the spatial boundaries of population structure in river systems are yet to be defined (Verspoor et al. 2005).

In contrast to allozymes, DNA markers do not require fresh tissue samples and PCR (polymerase chain reaction) technology enables one to amplify markers with minute amount of DNA. As a result, historical analysis can be performed on archived samples

of scales, otoliths and bones (Nielsen et al. 1997; Ruzzante et al. 2001; Consuegra et al. 2002). Furthermore, powerful statistical methods have been developed for highly polymorphic markers, such as microsatellites, that use multilocus genotype information to assign individuals to its population of origin (or exclude) and assess admixture proportions (e.g. Cornuet et al. 1999; Pritchard et al. 2000). Using these techniques, temporal stability of salmon populations have been assessed over several decades (e.g. Nielsen et al. 1997; Lage & Kornfield 2006) and fish of native origin has been detected in a historically stocked river, previously thought to be extinct (Nielsen et al. 2001).

A general decline and extinction of salmon populations has occurred throughout their native range (MacCrimmon & Gots 1979). One third of the remaining wild populations are either endangered or close to extinction (WWF 2001). The causes are due to multiple factors, including habitat destruction, construction of dams, overfishing, pollution, changes in the marine environment and aquaculture (Heggberget et al. 1993; Parrish et al. 1998). Production of farmed salmon has grown immensely during the last three decades and aquaculture is now considered as one of the major threats facing wild salmon populations (Hindar et al. 1991; WWF 2001). Farm salmon may compete with wild fish for resources, introduce diseases and pathogens and interbreed with native conspecifics (Hindar et al. 1991; Heggberget et al. 1993; McGinnity et al. 2003; Naylor et al. 2005). Artificial selection and domestication in hatchery have been shown to influence fitness related traits among farmed fish populations resulting in faster growth, greater aggressiveness and earlier smolting (Einum & Fleming 1997; McGinnity et al. 2003; Lacroix & Stokesbury 2004). Thus, the potential negative effect on locally adapted salmon populations

caused by introgression of farmed fish has long been addressed (Behnke 1972; Saunders 1981; Ståhl 1987). Now, evidence support that farmed fish may spawn in the wild (Sægrov et al. 1997) and interbreed with native fish (Crozier 1993; Clifford et al. 1998). Furthermore, in an experimental field study, hybridization of wild and farmed salmon resulted in fitness reduction of the wild population (McGinnity et al. 2003).

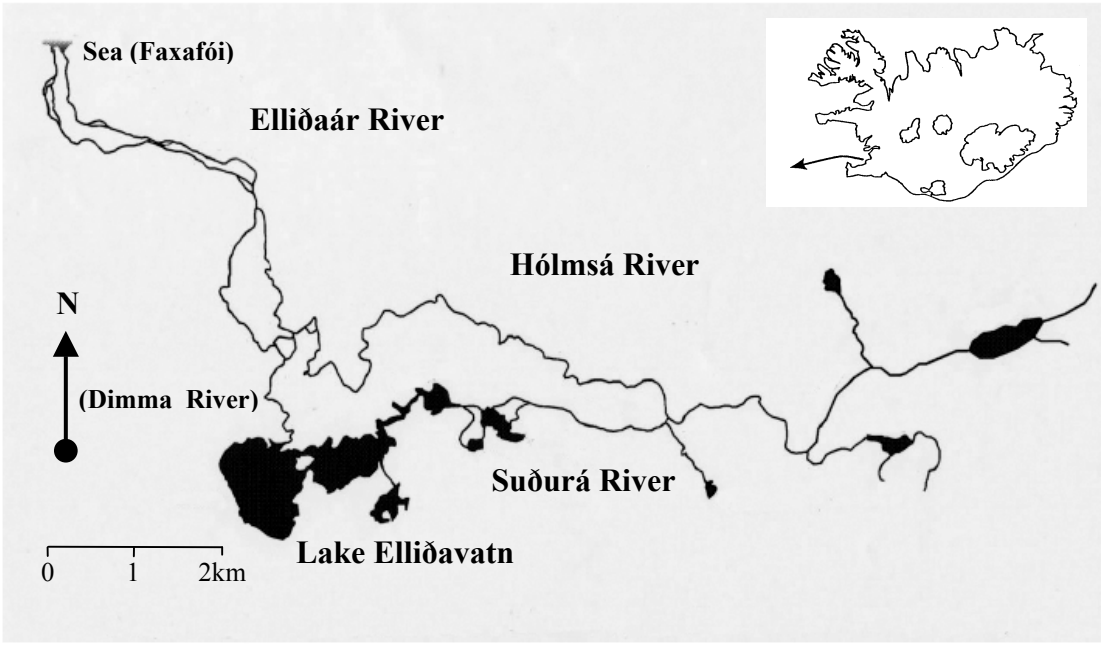
In present study, salmon in the Elliðaár river system in SW Iceland was investigated with spatial and temporal genetic methods. This river system has suffered from human mediated disturbances for decades, including damming of main river (Elliðaár River), and influx of hatchery fish for more than a decade. Shortly after the influx commenced, number of returning adults declined, parr production dropped and life-history characteristics of juveniles changed (resulting in faster growth and earlier smolting). Concurrently, a similar trend in individual abundance and earlier smolting was observed in River Ewe (Scotland), which received influx of farmed fish, although possible introgression was not assessed (Butler et al. 2005).

The main objective of this study was to map the genetic variation and structure of the salmon population in the Elliðaár river system in SW Iceland. Establish the temporal stability of this structure as well as to explore the effects of influx of farmed salmon into the Elliðaár river system on the native gene pool.

BACKGROUND

Study site

The Elliðaár river system is located in southwestern Iceland (64°06'N, 21°51'W) and flows into the Gulf of Faxaflói, draining a catchment area of 286km^2 (fig. 1) (Antonsson & Guðjónsson 2002). The outflow of Lake Elliðavatn (2.0km^2) forms the main river, Elliðaár River (6km), which is divided into two interconnected branches that run through the city of Reykjavík. Upstream, the river is called the Dimma River and the branches below are named by how they wind according to the cardinal points. Two tributaries run into Lake Elliðavatn, Hólmsá (11km) and Suðurá River (4km), connected upstream in one location. The river system is spring fed meaning that water reaches the surface mainly through porous lava. Some surface runoff enters the system and discharge peaks during spring melt (Antonsson & Guðjónsson 2002). In general, flow and thermal regimes are relatively stable throughout the year. The spring water is rich in minerals and has relatively high pH and thus low concentration of CO_2, as characterizes many spring fed rivers (Guðjónsson 1990). Average annual flow is 4.86m^3/s in Elliðaár River and it can drop below 3.0m^3/s in dry years (Birgisson et al. 1999). Average discharge is considerable lower in Hólmsá and Suðurá River, 2.26m^3/s and 0.38m^3/s, respectively (Birgisson et al. 1999). In 2003-2004, average temperature in Elliðaár River was 6.5°C (0.7-15.7) and pH 8.46 (7.71-9.82) (Þórðarson 2004). During the same period, Hólmsá and Suðurá River were colder than Elliðaár River and pH values lower, 5.2°C (0.0-13.6), pH 8.12 (7.70-8.98), and 5.0°C (-0.3-11.1) and pH 8.12 (7.50-8.80), respectively (Þórðarson 2004). Distance from the main water sources and the effect of the lake can explain the thermal and pH differences between the upper and lower rivers, since Lake Elliðavatn is very shallow (mean depth of 1 m) with high primary production.



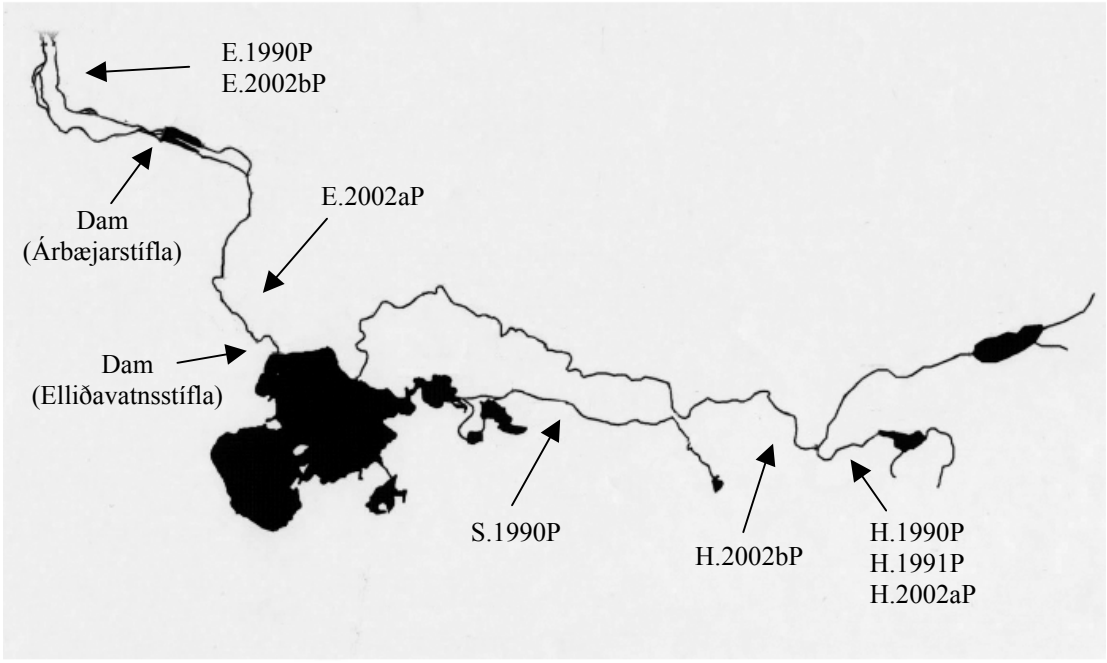


Figure 1 Map of the Elliðaár river system before and after damming of Elliðaár River (upper and lower map respectively) (see text for detailed description). Sampling sites of parr samples are given in the lower map. The contemporary parr sample from Suðurá River (S.2002P; not on map) was collected from the entire river due to scarcity of fish.

Heavy spring floods are relatively rare. The highest discharge recorded was 217m^3/s in 1968, when the dam at Lake Elliðavatn burst in a spring flood (Birgisson et al. 1999).

The river substrate is heterogeneous, ranging from sand to solid rock bottom. Areas of gravel, cobbles and boulders create desirable spawning substrates and nursery environments for juvenile salmon, especially in Elliðaár and Hólmsá River (Antonsson & Guðjónsson 1998). The only natural barrier impassable for migrating salmon is a large waterfall downstream in one branch of Elliðaár River. Salmon can pass other natural obstacles such as small waterfalls and steep riffles that are mainly located in Elliðaár River. Salmon spawn both in the upper and lower rivers. Salmon cohabits with brown trout (*Salmo trutta*) in the river system but dominates in Elliðaár River. Other fish species present in the river system are arctic charr (*Salvelinus alpinus*), threespined stickleback (*Gastersteus aculeatus*) and European eel (*Anguilla anguilla*).

Anthropogenic disturbance

The Elliðaár river system has been subjected to anthropogenic disturbances in the past century. Hydroelectric facilities, developments in relation to water withdrawal and other urban disturbances have all played a significant role in its ecology. It has been estimated that only 57% of the rivers are in its original state as other parts have been altered (Antonsson & Guðjónsson 1998).

The development of Elliðaár River for hydroelectric power had the single most effect. Because of this development, the morphology of the river system drastically changed

by flooding and temporally drying large river stretches in addition to change the hydrologic characteristics both in Elliðaár River and in Lake Elliðavatn. In 1921, one branch in Elliðaár River was dammed, creating the Árbæjarlón reservoir. The dam (Árbæjarstífla) was extended in 1929, thus blocking the upstream passage of salmon (fig. 1). Migrating salmon were therefore caught in traps below the station and transported upriver in small water tanks (Ingólfsson 1986). From 1960 onwards, the dam has been opened during upstream migration of adult salmon (Hjartarson et al. 1998). Until recently, one of the branches between the dam Árbæjarstífla and the power station frequently dried up in winters with the following loss of juvenile salmon. Additionally, both branches sometimes dried up in the early years of the dam. Now, however, water flows in both branches of Elliðaár River between the dam and station year around (Antonsson et al. 2006). A second reservoir was created in 1926, when Dimma River was dammed upstream (dam Elliðavatnsstífla). As a result, the area of Lake Elliðavatn was almost doubled in size, flooding large parts of Dimma and Hólmsá River, which might have been significant spawning and nursery grounds for salmon. Salmon could migrate past the dam to the upper rivers, also when the larger dam was created after the spring flood in 1968.

Additionally, supposedly important spawning and nursery grounds were lost following the constructions of water pipes in the early 20th century and afterward developments connected to water withdrawal. As a result, a section of Hólmsá River dried up and parts of downstream Suðurá River was altered (Antonsson & Guðjónsson 1998). However, the average discharge of the river system has not been affected as water withdrawal occurs from groundwater that is not part of the Elliðaár system.

Street runoff entering the river system and estuary, bridges crossing the rivers and nearby traffic, expanding settlement as well as land fillings and a harbor close to the estuary, add to the environmental stress (Antonsson & Guðjónsson 1998). Recently, some measures have been taken to prevent pollution from street runoff (Antonsson et al. 2006) and new buildings are prohibited closer than 150m from the rivers or lake.

Salmon in the Elliðaár river system

Long-term monitoring programs of salmon abundance and characteristics of returning adults (71 yr and 57 yr, respectively) and juvenile density and characteristics (18 yr) have been carried out. Statistics of catches from angling fisheries have been collected almost continuously since 1907, as well as statistics of coastal fisheries near the estuary from the late 20th century and of stocking activities from 1925 to present. In addition, estimates of the proportion of farmed salmon entering Elliðaár River are available.

Life cycle

Salmon in the Elliðaár river system display a typical anadromous life cycle. After reaching maturity at sea, it migrates into Elliðaár River. River ascent begins in mid June, peaks in July and ends in mid September (Antonsson et al. 2006). Spawning activities take place in November and migration to the upper rivers commences shortly before spawning. Fry hatch in April and the juvenile period lasts two to four years before transition to the smolt stage. The smolt run (juvenile river descent) commences in late May and finishes in June (Antonsson & Guðjónsson 2002). The majority of the salmon reaches maturity after one-year growth period at sea and some

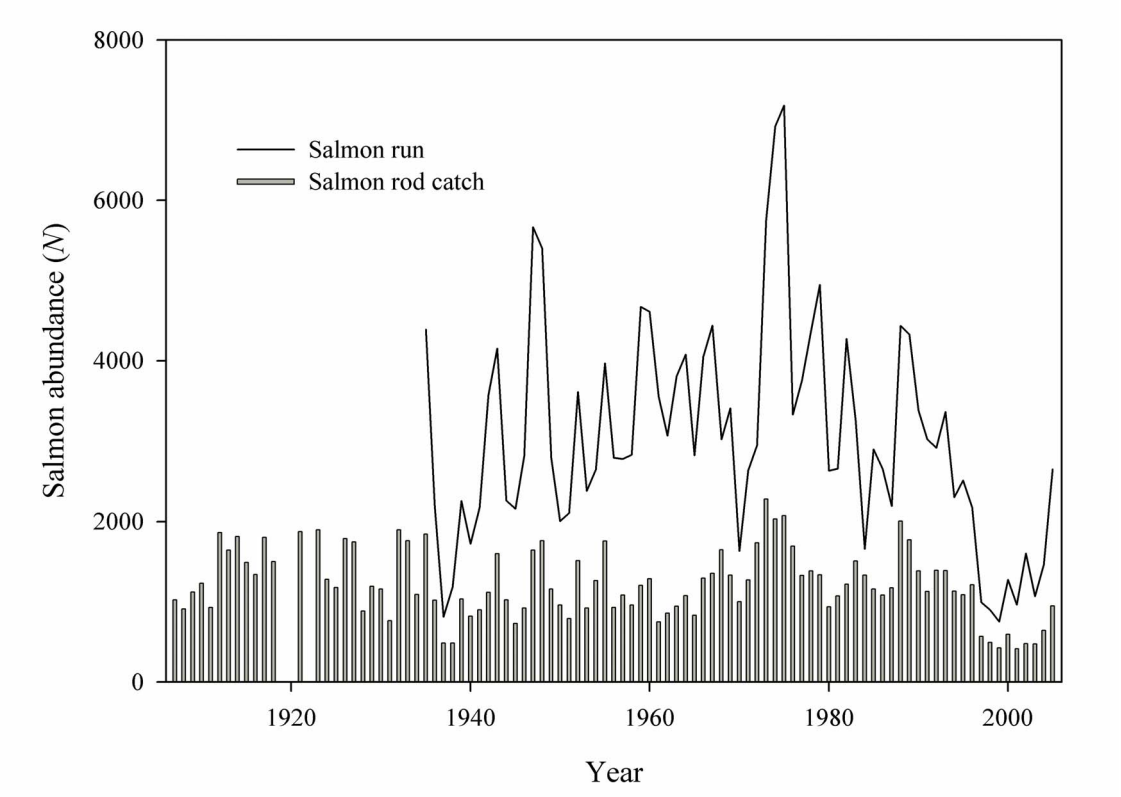


Figure 2 Salmon abundance measured as the number of adults in the run and rod catch in 1935-2005 and 1907-2005, respectively. Catch data from 1919, 1920 and 1922 is not available (Antonsson et al. 2006 and references therein).

males become sexually mature before seaward migration (precocious males). Most salmon die after spawning, either in the river or at sea, while some may spawn twice and rarely three or four times (multiple spawners). Additionally, a number of precocious males display seaward migration and return as adult spawners (Sigurður Guðjónsson, personal information). Thus in any given year, several age groups represent the spawning stock in the Elliðaár river system.

Adult abundance and characteristics of the spawning stock

Despite the small size of the Elliðaár river system, it has a record of large salmon runs. Based on data from the Institute of Freshwater Fisheries, adult abundance estimates in 1935-2005 ranged from 813 to 7184 individuals (median = 2822) (fig. 2). A significant decline in abundance occurred in 1997-2004, where the run fluctuated

around 1000 individuals. A decline of similar degree, though less persistent, was observed in 1937-1938.

Grilse or one-sea-winter fish (1SW) characterizes the run, whereas two-sea-winter fish (2SW) and multiple spawners occur to some extent. In recent years, numbers of 2SW fish have steadily declined and are now almost absent in the run. Previously, it represented 5-10% of spawning adults. Sex ratio of returning adults is female biased, 55-60% on average, and has increased in recent years, e.g. exceeding 65% in 1997 and 2003 (Antonsson et al. 2006 and references therein).

Juvenile density and characteristics

Historically, Elliðaár River is among the most productive rivers in Iceland, with respect to juvenile density. Density has frequently exceeded 150 individuals per $100m^2$ in single pan electrofishing and even reached 380 close to the outlet of Lake Elliðavatn (Antonsson 2002). The upper rivers have always displayed considerably less juvenile production, even though density has occasionally been more on average (fig. 3). That is because of the superior growth conditions in Elliðaár River, which normally result in one-year earlier smolting of juveniles. By standardized samplings in 1987-2005, average juvenile (fry and parr) density per $100m^2$ ranged from 14.2 to 173.2 in Elliðaár River (four sampling sites) and from 1.4 to 97.0 in the upper rivers combined (four sampling sites). A permanent shift towards lower density occurred in 1988-1989, when it dropped from 173.2 to 28.3 in Elliðaár River and from 97.0 to 33.7 in the upper rivers. Since then, juvenile density in Elliðaár River has remained relatively stable whilst a steady decline has taken place in the upper rivers (Antonsson

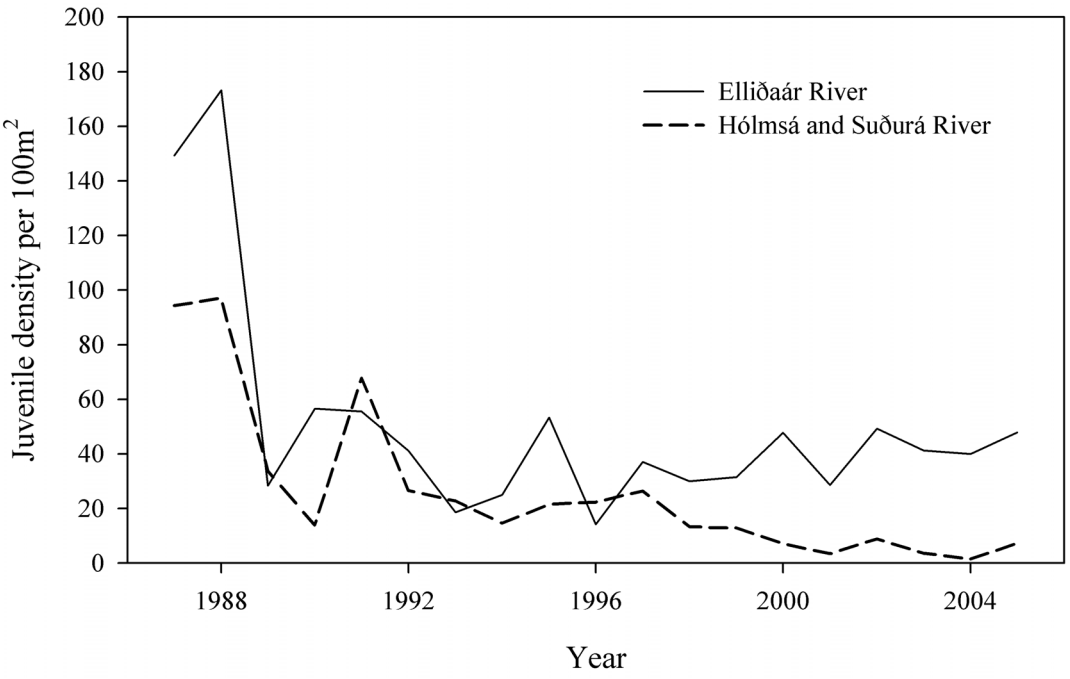


Figure 3 Juvenile salmon density per 100 m^2 in Elliðaár River (solid line) and in Hólmsá and Suðurá River (broken line) in 1987-2005. In 2002, stocking of juveniles commenced in Hólmsá and Suðurá River (Antonsson et al. 2006 and references therein).

et al. 2006 and references therein). The two years showing the highest juvenile production (1987 and 1988) are likely not abnormally high, since the rivers have history of large adult runs and high juvenile density was also recorded in an earlier study in Elliðaár and Hólmsá River (Garðarsson 1983). From 2002 to present, the upper rivers have been stocked with parr (Elliðaár origin).

The different trend observed in the upper and lower rivers is somewhat reflected in the age composition of juveniles. Additionally, the life history characteristics, age of smolting, growth rate and weight, have changed during the sampling period. In 1988-1994, 3+ fish dominated the smolt run and 4+ was generally more abundant than 2+ fish (fig. 4). In 1995, the proportion of 2+ increased significantly and has since been more abundant than other age groups in the run. In only eighteen years, the proportion of 2+ in the smolt run has increased from below 20% to over 65% (Antonsson et al.

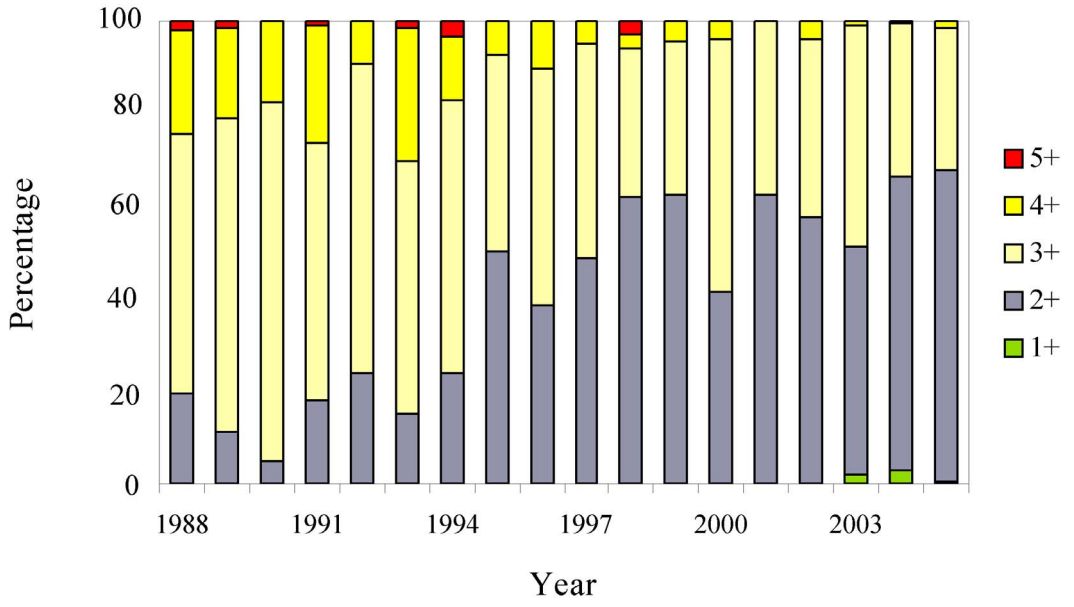


Figure 4 Proportion of different age-classes in the smolt run in 1988-2005 (Antonsson et al. 2006 and references therein).

2006 and references therein). Although the causes of this phenomenon have not yet been studied, it is obviously connected to several factors, e.g. smaller proportion of the smolt run originating from the upper rivers, where juveniles generally reach higher age than in Elliðaár River, and increased growth rate in the rivers, revealed by larger and heavier juveniles within age groups (Antonsson et al. 2004).

Fisheries and stocking

Given the fact that adults ascend the upper rivers shortly before spawning, salmon angling fisheries occur only in Elliðaár River and not in Hólmsá and Suðurá River. In 1907-2005, angling catches ranged from 485 to 2276 individuals (median = 1177) (fig. 2; Antonsson et al. 2006 and references therein). In 1937-1938 and 1997-2004, annual catches barely exceeded 500 individuals, which coincide with the low abundance of returning adults in those years. Compared to other salmon rivers in Iceland, fishing effort in Elliðaár River is quite low, generally around 30-50%

(Antonsson et al. 1998; Guðjónsson et al. 1996). From 1932, salmon fisheries at sea have been prohibited by law in Iceland (Ísaksson et al. 1997). However, few coastal trap nets were allowed, two of which were located close to the estuary of Elliðaár River. The limited available information suggests that the coastal fisheries had most often insignificant effect on the salmon run, since the annual catch generally remained below five hundred (Antonsson et al. 1998) and migrating salmon from nearby rivers must have entered the traps as well. Coastal fisheries near the estuary ceased in 1980 (Antonsson et al. 1998).

Stocking activities have been carried out in the rivers since 1925. In 1925-1931, the rivers were stocked with alevins from the Alviðra River stock, located in south Iceland (Ingólfsson 1986). Since then, native fish has been used in the stocking process, using alevins, parr and smolts (Ingólfsson 1986; Antonsson et al. 1998). Broodstocks have varied from tens to thousand individuals. Effect of the early stocking remains controversial while no correlation exists between run size and stocking intensity in 1934-1998 (Antonsson et al. 1998). In recent years, however, tagging studies show that via improved stocking techniques it can contribute up to 20% of the catch (Antonsson et al. 2005).

Influx of farmed salmon

In the eighties and nineties, a large-scale production of salmon in aquaculture was carried out in southwestern Iceland. Consequently, hatchery salmon of at least two strains (reared salmon from sea cages and strayers from sea ranching stations) were caught in Elliðaár River and other rivers in the area (Guðjónsson 1991). These farmed

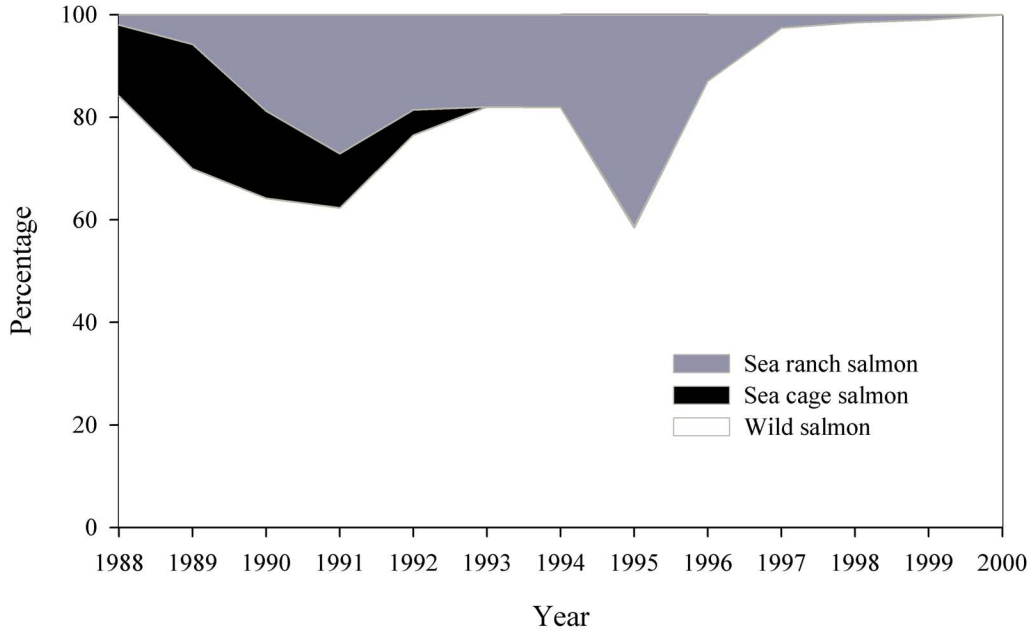


Figure 5 Proportion of wild and farmed (sea cage and sea ranch; both strains of Icelandic origin) salmon in Elliðaár River from 1988 to 2000. Estimates are based on the anglers catch. Influx of farmed salmon into the Elliðaár river system began in 1984 (see text; Antonsson & Guðjónsson 2001 and references therein).

fishes were of Icelandic origin but composed of a mixture of several populations (Guðjónsson 1989; Sigurður Guðjónsson, personal information). In 1988-1995, farmed salmon constituted generally between 15-35% of the catch in Elliðaár River and the proportion decreased sharply after that, last detected in 1999 (fig. 5; Antonsson & Guðjónsson 2001 and references therein). Data on the early influx in 1984-1987 is not available for Elliðaár River. However, from 1986 and onward, the proportion of farmed salmon was assessed in Leirvogsá River, a nearby salmon river. The estimates in 1988 and later on were very similar to those of Elliðaár River, thus the estimates of 2% influx in 1986 and 10% in 1987 (Viðarsson & Guðjónsson 1991) may reflect the proportion in Elliðaár River in those years. However, it is likely, that due to the limited time of sampling, the estimates in Elliðaár River underestimated the proportion of farmed fish during the spawning season. The estimates were based on

fish caught in the angling season, from mid June to mid September, while the influx of farmed fish peaked in August and continued after the salmon angling season (Viðarsson & Guðjónsson 1991). In addition, because of the limited time of sampling, it is unknown if farmed salmon migrated to the upper rivers. However, it is likely that most farmed salmon stayed downstream in Elliðaár River, since Árbæjarstífla dam always closed in mid September. The farmed salmon that entered Elliðaár River was sexually mature and one had spawning marks from previous spawning. However, spawning success of the farmed fish is unknown (Guðjónsson 1991).

MATERIALS AND METHODS

Samples

During this study, the available samples of Atlantic salmon were used for three distinct but non-exclusive approaches of its genetic structure in the Elliðaár river system. First, a population structure analysis was based on available samples of wild parr that were collected from Elliðaár, Hólmsá and Suðurá River in 1990-91 and in 2002 (N=398). Second, the temporal stability of the main river (Elliðaár River) was assessed by analyzing a series of adult samples from 1948 to 2005 (N=413). Third, possible introgression of farmed fish was assessed by comparing the genetic composition of farm (N=171) *versus* wild adult samples, caught before, during and after influx. Sample characteristics are listed in table 1. Wild samples from 1948 and 1962 will often be referred herein as old samples, samples in 1989-1992 as recent and samples from 2002 and 2005 as contemporary.

Table 1 Sample information. Sample code, collection site, year of sampling, life stages (A=adult, P=parr), sample size, origin (W=wild, F=farmed) and tissue type (M=muscle, L=liver, E=eye).

Sample code	Collection site	Year	Life stage	Sample size	Origin	Tissue
E.1948A	Elliðaár R.	1948	A	51	W	Scale
E.1962A	Elliðaár R.	1962	A	43	W	Scale
E.1989A	Elliðaár R.	1989	A	97	W	Scale
E.1990P	Elliðaár R.	1990	P	39	W	M,L,E
E.1991A	Elliðaár R.	1991	A	38	W	M,L,E
E.1992A	Elliðaár R.	1992	A	90	W	Scale
E.2002aP	Elliðaár R.	2002	P	48	W	Fin clip
E.2002bP	Elliðaár R.	2002	P	51	W	Fin clip
E.2005A	Elliðaár R.	2005	A	94	W	Scale
H.1990P	Hólmsá R.	1990	P	34	W	M,L,E
H.1991P	Hólmsá R.	1991	P	40	W	M,L,E
H.2002aP	Hólmsá R.	2002	P	46	W	Fin clip
H.2002bP	Hólmsá R.	2002	P	53	W	Fin clip
S.1990P	Suðurá R.	1990	P	69	W	M,L,E
S.2002P	Suðurá R.	2002	P	18	W	Fin clip
K.1989	Elliðaár R.	1989	A	96	F	Scale
HB.1992	Elliðaár R.	1992	A	75	F	Scale

Parr

Parr samples were collected from Elliðaár and Suðurá Rivers in 1990 and from Hólmsá River during two consecutive years (1990 and 1991). These are the samples which have been used for the allozyme study of (Daníelsdóttir et al. 1997). Parr were sacrificed and tissues (muscle, liver and eye) were immediately frozen and kept at -75°C. In 2002, only fin clips were taken and preserved in 95% ethanol. Fork length was measured and precocious males identified before being released at the location of capture. Age of individuals was not estimated particularly. However, in 1990-91, only 1+ to 3+ parr were sampled (Sigurður Guðjónsson, personal information), whereas it can be estimated based on the length distribution (data not shown), that each sample in 2002 comprised of juveniles up to four year-classes (0+ to 3+). Samples were collected by electrofishing 100-300m river stretches during a single day in August

(1990 and 1991) and during a week in October (2002). Recent and contemporary samples were collected approximately from the same locations, although in 2002, the whole Suðurá River was sampled due to the scarcity of fish in the river. In addition, a contemporary sample from upstream Elliðaár River was added to the study. As mentioned earlier, in recent years, the upper rivers have been stocked with parr. Salmon caught in Elliðaár River was used as broodstock. However, all parr analyzed in present study were naturally spawned, since sampling took place before the stocking.

Adult

Scales were collected from adult salmon that were caught by anglers along the entire Elliðaár River in 1948 and 1962 (June and July), and in 1989, 1992 and 2005 (July and August). Additionally, in 1991, fishes were caught with gill nets in a single day in August and, as for the recent parr samples, tissues were originally collected for the allozyme study of Daníelsdóttir et al. (1997). Farmed fishes were removed from the wild samples of 1989, 1991 and 1992 by identifying differences in scale characteristics between farmed and wild fish (Lund & Hansen 1991). However, discriminating between farmed and wild fish, by means of scale characteristic analysis, is not always an accurate technique, particularly when farmed fish originate from sea ranching and were released as smolt (Lund & Hansen 1991). Therefore, some farmed individuals might still be present in the wild samples. Applying the same technique, the origin of farmed fish in 1989 and 1992 was determined. The farm sample from 1989 composed of cage rearing salmon and the 1992 sample of sea ranch salmon. Scales were stored at room temperature in paper bags and tissues were kept frozen at -75°C.

DNA extraction

Fresh tissue

DNA from recent (adult and parr) and contemporary (parr) tissue samples was extracted using 10% Chelex®-100 resin (Bio-Rad Laboratories). Approximately 10mg of tissue and 100µL of Chelex resin were placed in a 96-well PCR tray and, heated for 60min at 95°C. Products were centrifuged at 3500 RPM (Rotates Per Minutes) for 10min. Supernatants (DNA) were collected with wide bore pipette tips and transferred to a new 96-well PCR tray.

DNA from some recent tissue samples was particularly difficult to extract (yielded low-quality DNA). In these cases, a phenol/chloroform extraction method was employed in order to obtain sufficient DNA of good quality for amplification (described below).

Scales

DNA from adult scales was isolated with a modified phenol/chloroform protocol of Taggart et al. (1992). In addition, old scale DNA was purified and concentrated with Microcon® YM-50 (Millipore) centrifugal filter tubes, as recommended by Nielsen et al. (1997; 1999a). Depending on sampling date and size of scales, four to eight non-cleaned dried scales were placed in a 1.5ml microfuge tube with 490µl STE buffer (0.1M NaCl, 0.05M Tris-HCL pH 8.0, 0.01M Na_2EDTA pH 8.0), 20µl Proteinase K (20mg/ml) and 20µl SDS (10%). They were then incubated for 15h at 37°C. 5µl of RNase A (10mg/ml) was then added and tubes were kept at 37°C for 1h. Non-digested material was spun down for 5min at 13000RPM. 250µl phenol (pH 8.0) was then added and tubes were shaken vigorously for few seconds, followed by gentle

mixing for 5min. 250µl chloroform (isoamyl alcohol (24:1)) was added and tubes were shaken vigorously for few seconds, gently mixed for 2min and centrifuged for 5min at 13000RPM. The upper water-face was carefully removed (wide bore pipette tip) and placed in a new tube. In the next step, 500µl chloroform was added and products were spun down for 5min at 13000RPM. Again, the top aqueous layer was carefully removed and put in a new tube. 1000µl of -20°C ethanol (96%) was added and tubes were kept at -80°C for 1h (DNA precipitation phase). Then, tubes were spun down for 15min at 13000RPM and supernatants removed. Pellets (DNA) were washed with 150µl -20°C ethanol (70%), centrifuged for 2min at 13000 RPM and supernatants removed. Pellets were subsequently cleaned with 150µl 96% ethanol at room temperature, centrifuged for 2min at 13000 RPM and supernatants removed again. Finally, the pellets were dissolved with 100µl (contemporary DNA) and 60µl (recent and old DNA) of distilled water (dH_2O).

Following extraction, old scale DNA was purified and concentrated with Microcon tubes. Although Nielsen et al. (1997) recommended to use Microcon tubes instead of ethanol precipitation, DNA was filtered after ethanol precipitation, which might have caused poorer yield of DNA templates. This, however, is not clear since the manufacturer of Microcon noted that some organic chemicals, such as chloroform, might cause leaching from component parts. This was not particularly investigated. DNA was filtered twice with Microcon and concentrated templates diluted with 20µl dH_2O. To confirm the reproducibility of results and the fact that many PCR reruns were needed, two extraction rounds of old scale DNA were required.

Table 2 Primer names (multiplexes denoted in parenthesis) and sequences, repeat motif of microsatellites (di- or tetra- nucleotide; RM), number of alleles (N_A), allelic size range in base pairs, expected heterozygosity (H_E) and F_{ST} of each locus, calculated over all samples (N=982), except H_E of Ssa405 was only based on parr samples. Departure from annealing temperatures of multiplexes are given in parenthesis (see text). *Rundown of annealing temperature for PCR with old scale DNA: Ssa202 (56; 52; 48), Ssa404 (59; 55; 51) and SSOSL25 (59; 58).

Locus	Primer sequences (5'-3')	RM	N_A	Allelic range	H_E	F_{ST}	Annealing temp. (°C)
Ssa85 (III)	ACC CGC TCC TCA CTT AAT C AGG TGG GTC CTC CAA GCT AC	di	18	119-159	0.708	0.016	58 (-2)
Ssa197 (I)	TGG CAG GGA TTT GAC ATA AC GGG TTG AGT AGG GAG GCT TG	tetra	13	167-219	0.827	0.016	58 (-2)
Ssa202 (I)	TTC ATG TGT TAA TGT TGC GTG CTT GGA ATA TCT AGA ATA TGG C	tetra	9	240-272	0.697	0.015	58 (-2)*
Ssa404 (III)	ATG CAG TGT AAG AGG GGT AAA AAC CTC TGC TCT CCT CTG ACT CTC	tetra	27	189-305	0.920	0.015	58 (+1)*
Ssa405	CTG AGT GGG AAT GGA CCA GAC A ACT CGG GAG GCC CAG ACT TGA T	tetra	19	300-408	0.888	-	61
SSOSL 25	ATC TAC ACA GCT CCT GGT GGC AG CAT GTA ATG GGT CGA GAG AAG TG	di	9	145-171	0.681	0.017	58*
SSOSL 85 (II)	TGT GGA TTT TTG TAT TAT GTT A ATA CAT TTC CTC CTC ATT CAG T	di	15	181-223	0.571	0.016	56
SSOSL 311 (II)	TAG ATA ATG GAG GAA CTG CAT TCT CAT GCT TCA TAA GAA AAA GAT TGT	di	21	121-175	0.809	0.013	56

Microsatellite techniques

The genetic variability of parr and adult samples was analyzed at seven and eight microsatellite loci, respectively, four dinucleotides and three to four tetranucleotides (table 2). Because quality of DNA varied greatly between samples and tissue types, several PCR methods were applied.

Initially, using high quality DNA, eleven microsatellites were optimized in four multiplexes (M); M1) Ssa171, Ssa197 and Ssa202 (O'Reilly et al. 1996), M2) SSOSL85 and SSOSL311 (Slettan et al. 1995), M3) SSOSL25 (Slettan et al. 1995),

Ssa85 (O'Reilly et al. 1996) and Ssa404 (Cairney et al. 2000) and M4) Ssa405, Ssa407 and Ssa408 (Cairney et al. 2000), where Ssa407 was amplified separately. Forward primers were end-labeled with NED™, 6-FAM™ or HEX™ dyes (Applied Biosystems). Pull-up between dyes of SSOSL25 (HEX) and Ssa85 (6-FAM) affected the ease of typing alleles at these loci (selecting the right allele peak). SSOSL25 was therefore removed from M3 and amplified and run separately. Multiplexes were sensitive to DNA quality and successful amplification was only achieved with DNA extracted from contemporary tissue. The yield of PCR products in multiplexes decreased with DNA from recent tissues, hence non-amplified or ambiguous samples were frequent and reruns often required. Amplification was thus repeated until all genotypes could be accurately determined. However, despite numerous reruns of Ssa171, Ssa407 and Ssa408 (M1 and M4) with recent tissue DNA, many samples did not amplify and these loci were therefore excluded from the study.

Amplification in multiplexes with recent and old scale DNA yielded little or no PCR products. A combination of several factors probably contributed to these technical problems, e.g. poor quality of DNA, too small PCR reaction volumes, primer concentrations and annealing temperatures of multiplexes deviated frequently from the optimal temperature of each primer. PCR reactions were therefore optimized specifically for each primer, using DNA from recent scale samples. However, as was later discovered, the quality of old scale DNA was so low that some critical changes were made to the protocols. In general, all PCR reactions generally contained 2.0µL of DNA, 250µM of each dNTP, 1× reaction buffer (10mM Tris-HCl, pH 8.8, 1.5mM $MgCl_2$, 50mM KCl and 0.1% Triton X-100), DyNAzyme™ DNA Polymerase (Finnzymes) and distilled water.

Multilocus amplification

Multiplex PCRs were carried out in 10µL reaction volumes. Concentration of primers (forward and reverse) and polymerase were as follows: M1) 0.60µM Ssa171, 0.25µM Ssa197, 0.80µM Ssa202 and 0.4U polymerase; M2) 0.3µM SSOSL85, 0.5µM SSOSL311 and 0.4U polymerase; M3) 0.2 µM Ssa85, 0.5µM Ssa404, 0.4µM SSOSL25 and 0.4U polymerase; M4) 0.30µM Ssa405, 0.3µM Ssa408 and 0.6U polymerase (0.7µM Ssa407 and 0.4U polymerase). All multiplexes contained 1.5mM $MgCl_2$ except M1, which performed better with 2.0mM. Thermal cycles were conducted in GeneAmp®2700 thermal blocks and conditions were as follows: 3min denaturing step at 95°C, 5 cycles of 20s at 94°C, 20s at annealing temperature and 20s at 72°C, 30 cycles of 20s at 90°C, 20s at annealing temperature and 20s at 72°C and a final 7min extension at 72°C. The annealing temperature of M1, M2 and M3 was 58°C and 61°C for M4. Additionally, each step in M4 was increased to 50s instead of 20s.

Single locus amplification

Single locus PCRs with contemporary and recent scale DNA were performed in 10µL reaction volumes. Concentrations of primers were 1.0µM (Ssa202, Ssa197, SSOSL311, Ssa404 and Ssa85) and 0.5µM (SSOSL25 and SSOSL85) and each reaction contained 0.6U of polymerase and 2.0mM $MgCl_2$ (1.5mM in SSOSL311). Thermal cycling conditions were as followed: 3min at 95°C, 5 cycles of 20s at 94°C, 20s at annealing temperature and 20s at 72°C, 30 cycles (+5 cycles with recent scale DNA) of 20s at 90°C, 20s at annealing temperature and 20s at 72°C and a final 7min extension at 72°C. Annealing temperatures were 56°C (Ssa197, Ssa202, SSOSL85, SSOSL311 and Ssa85), 59°C (Ssa404) and 59°C-58°C (5, 30-35 cycles) (SSOSL25).

SSOSL25 was amplified with contemporary and recent tissue DNA using the same protocol as described for contemporary scale DNA.

Old scale DNA was amplified in 25µL volumes that contained the same concentration of reagents as the single locus 10µL volume reactions, except primer concentration of Ssa404, Ssa197 and Ssa85 that was reduced to 0.5µM. Additionally, 0.5µg/µl of BSA was added to reduce effects of inhibitor substances in the PCR, which resulted in stronger amplification. Thermal cycling conditions of all loci, except for SSOSL25, were modified: Ssa197, Ssa85, SSOSL311 and SSOSL85) 3min at 95°C, 5 cycles of 20s at 94°C, 30s at 56°C and 40s at 72°C, 35 cycles of 20s at 90°C, 30s at 56°C and 40s at 72°C and a final 10min extension at 72°C; Ssa404 and Ssa202) 3min at 95°C, 10 cycles of 20s at 94°C, 30s at annealing temperature and 30s at 72°C, 10 cycles of 20s at 90°C, 30s at annealing temperature and 30s at 72°C, 20 cycles of 20s at 90°C, 30s at annealing temperature and 30s at 72°C and a final 10min extension at 72°C. Annealing temperatures of Ssa404 and Ssa202 decreased between cycling rounds: Ssa404) 59°C, 55°C and 51°C; Ssa202) 56°C, 52°C and 48°C.

Gel loading

Prior to loading on gels, PCR products were generally diluted with distilled water and mixed with a loading solution. Multiplex PCR products were diluted 1:3, while single locus PCR products, which were run as in multiplexes (gelplex) and therefore combined, were diluted 1:1. However, old scale PCR products were run separately, since amplifications were often weak. This enabled more of each product to be loaded on a gel, which increased the possibility of detecting poorly amplified alleles. Ssa85 and SSOSL25 were diluted 1:3, Ssa197 1:2, SSOSL311 and SSOSL85 1:1, Ssa404

and Ssa202 were not diluted since some individuals contained weak bands that otherwise would have not been detected. Opposed to other non-diluted PCR products, this did not affect the banding pattern of Ssa404 and Ssa202, except for few individuals that amplified very strongly. Products of those individuals were diluted 1:1 or 1:2, which cleared the patterns. Other old scale samples, with known genotypes, typed from non-diluted products, were also diluted and run as control samples. The control samples confirmed that allele sizes did not change between runs of diluted and non-diluted products. PCR products were then mixed 1:1 with a loading solution containing deionized formamide, loading buffer and size standard (GeneScan^{TM} 350 or 500 ROX^{TM}, Applied Biosystems) in the ratio 0.2:0.15:0.65. PCR products were then denaturized for 3min at 95°C in a thermal block and 2µl loaded on a 5% acrylamide gel and run for two and a half hour by an ABI PRISM® 377 DNA sequencer (Applied Biosystems). Typing was performed with the software GeneMapper^{TM} vers. 3.0 (Applied Biosystems). Unfortunately, since the sequencer broke down before running of the contemporary scale sample (E.2005A), the sample was run in a capillary sequencer in the Prokaria lab. To standardize between the two sequencers, 30 individuals (parr from Elliðaár River in 2002) with known genotypes, were run in the Prokaria lab.

Positive control samples were applied in all PCRs and negative controls were included in the complete protocol process of old scale DNA. To avoid contamination of old scale DNA, the work was conducted separately from recent and contemporary sample processes. Furthermore, preparation of old scales PCR products was conducted in a laminar flow cabinet with UV light for sterilization. Control samples confirmed that allele sizes varied neither between different PCR protocols nor

between different preparations of PCR products. Because of the risk of cross/aerosol contamination of old scale DNA and poor PCR amplification in some cases, reproducibility of results was obtained by performing a second round of amplification and scoring of up to 100% per loci per sample.

Genetic analysis

Samples of different life-stages, parr and adult (wild and farm), were analyzed separately. For each sample, allele numbers, allele frequencies, observed (H_O) and expected (H_E) heterozygosity and F_{IS} (the inbreeding coefficient within subpopulations according to Weir & Cockerham (1984)) was calculated in GENETIX vers. 4.05.2 (Belkhir 2004). To minimize bias due to uneven sample sizes, genetic diversity was further quantified with Nei's unbiased diversity (H_S, average expected heterozygosity) and allelic richness (A_R) in FSTAT vers. 2.9.3 (Goudet 2001). Tests of differences in H_S and A_R among sample pairs (or groups) were performed by averaging the estimates over loci for each sample and significance was assessed by 5000 permutations in FSTAT. When groups contained more than one sample, estimates were averaged over samples and loci within each group. All probability tests were one-sided. A_R values of samples presented in the text were averaged over loci.

Each sample was tested for conformation to Hardy-Weinberg expectations (HWE) and for linkage disequilibrium (LDE) by exact tests in GENEPOP vers. 3.4 (Raymond & Rousset 1995b). In the same software, population differentiation was assessed by exact tests of homogeneity in allele (genic) and in genotypic frequencies. However, since results of the two tests were almost identical, only the former was presented. Unbiased *P*-values of the exact tests for each locus (or pairs for LDE analysis) in each

sample were calculated using default settings of the Markov chain. Global tests combined *P*-values across loci or samples using Fisher's method. Extent of overall allelic variation and pairwise genetic differentiation among samples was estimated with F_{ST} (Weir & Cockerham 1984) as implemented in GENETIX and in Arlequin vers. 2.000 (Schneider et al. 2000), respectively. Significance of pairwise values was estimated by 10,000 permutations in Arlequin. Sequential Bonferroni adjustments were used to determine statistical significance of multiple comparisons (Rice 1989).

Plausible causes for deviation from HWE were tested with MICRO-CHECKER vers. 2.2.3 (van Oosterhout et al. 2004). By utilizing information about size distribution and repeat motif of microsatellites, the software determined if deviation might be due to null alleles (mutation in flanking region), short allele dominance (large allele dropout) or mis-scoring of stutter peaks (van Oosterhout et al. 2004).

Several tests were used specifically for the analysis of population structure with parr samples. A hierarchical analysis of molecular variance (AMOVA) was performed in Arlequin to determine the extent of spatial structure compared to temporal allelic variation. Significance of variance components ascribed to 1) among rivers, 2) among temporal samples within rivers and 3) among individuals within samples was estimated by 10,000 permutations of a non-parametric approach (Excoffier et al. 1992).

Number of possible subpopulations were determined by the Bayesian method of Pritchard et al. (2000), as implemented in STRUCTURE vers. 2.0. This cluster method was chosen because it may detect cryptic population structure, whereas it does

not need a priori information about samples. Methods that compare a priori samples (e.g. the assignment method of Rannala & Mountain (1997), see below) cannot detect hidden structure within samples (Waples & Gaggiotti 2006). By setting different numbers of *K* (number of genetically distinct clusters), the most likely value was assessed by comparing the posterior probability (Ln P(D)) of the data for a given *K*, as recommended in Pritchard et al. (2000). Loosely, the method assigns individuals (based on multilocus genotype data) completely or partially to *K* clusters with respect to Hardy-Weinberg equilibrium and linkage equilibrium within each cluster (Pritchard et al. 2000). Calculations were performed with a Burnin period of 200,000 followed by 1,000,000 Markov Chain Monte Carlo (MCMC) iterations. Default settings of the admixture model and the model of correlated allele frequencies were applied. Five simulations were carried out for each *K* value tested, which ranged from one to seven. Graphical representation of individual assignment proportions was performed with the software DISTRUCT (Rosenberg 2004).

Effective number of breeders (N_b) was estimated for each parr sample. More accurately, some combination of N_b in several previous years was estimated because the parr samples consisted of juveniles of different ages (Robin Waples, personal information). N_b was estimated in the program LDN_E (Waples and Do, unpublished), which uses the linkage disequilibrium method of Waples (2006). Alleles at lower frequencies than 0.02 were eliminated, which has provided a good balance between precision and bias (Robin Waples, personal information), and 95% parametric CIs was calculated. Finally, correlation between N_b and F_{ST} was estimated according to Garant et al. (2000). As discussed later in more detail, it was performed to assess if low N_b values could have inflated differentiation estimates, since lower within group

variance may increase among group variance. A pairwise table of harmonic means of N_b was constructed and corresponding F_{ST} estimates were used in the correlation analysis.

For the analysis of possible introgression of farmed fish, the Bayesian assignment method of Rannala & Mountain (1997) was used, as implemented in GeneClass2 (Piry et al. 2004). Evaluation of different assignment methods available in GeneClass2 have shown that the Bayesian method may have the best performance (Pinto et al. 2005), even when the assumptions of HWE and linkage equilibrium were not met (Cornuet et al. 1999). Furthermore, simulations have shown that the Bayesian method outperformed the method implemented in STRUCTURE, i.e. with the same settings as used in present study, under various scenarios (Waples & Gaggiotti 2006; but see Hauser et al. 2006). First, to evaluate the quality of the genetic baseline, recent and contemporary wild adult samples were assigned to the two baseline samples (E.1948A and E.1962A) separately. Then, recent and contemporary wild adult samples were assigned to the baseline samples and the two farm strains. Finally, all wild adult samples from 1962-2005 and farm samples were assigned to E.1948A. The assignment probability was assessed by the Monte-Carlo resampling method of Paetkau et al. (2004) with 100,000 iterations.

RESULTS

Amplification

A total of 982 salmon in seventeen samples were analysed (table 1). Eight microsatellite loci were amplified for parr samples (N=398) and seven microsatellites for samples of wild adults (N=413) and farmed fish (sea ranching, N=75; sea cages,

N=96). Amplification of old scale samples was not very successful. Of 74 individuals tested from 1948, only 51 amplified at five or more loci, which was the criterion for including an individual for subsequent analysis. In 1962, amplification was more successful (43 individuals of 53) and almost all individuals in the recent and contemporary samples that were tested amplified successfully. At the genotype level, amplification of contemporary and recent microsatellite DNA was generally highly successful (Appendix A and B), with over 99% scoring of all samples after the possible salmon/trout hybrids had been removed (see below). Conversely, amplification of old scale DNA was not as successful, 95.0% (E.1948A) and 93.7% (E.1962A), whereas loci with larger alleles were generally harder to amplify (Appendix B). Degradation of the old DNA probably resulted in fewer large amplifiable fragments (large allele drop-out) (Nielsen et al. 1997; 1999a). However, other factors affecting the quality of the DNA or PCR optimization problems are also plausible, since the loci with largest alleles, Ssa404, amplified better than Ssa202.

Exclusion of trout or salmon/trout hybrids

Prior to the statistical analysis, few individuals in the wild samples were excluded from the study. They were identified as possible brown trout or hybrids of brown trout and salmon. Some of their alleles deviated from the common allelic ranges of wild and farmed fishes, the most extreme case being a 40bp deviation of the $Ssa197^{131}$ allele. To verify this, three brown trout from Elliðaár River were analyzed (Appendix C). As a result, five parr and three adults were excluded; four individuals from S.2002P, two from E.2005A and one from each of H.1991P and E.1989A.

Microsatellite loci characteristics

The eight microsatellites analyzed showed high levels of variability and a total of 130 alleles were identified for salmon (table 2). The number of alleles at each locus (N_A) ranged from 9 (Ssa202 and SSOSL25) to 27 (Ssa404) and expected heterozygosity (H_E) ranged from 0.571 (SSOSL85) to 0.920 for Ssa404 (table 2). The four tetranucleotide loci (values of Ssa405 were only based on parr samples) exhibited slightly higher variability on average and higher level of heterozygosity than the four dinucleotides (N_A 17.0 vs. 15.5 and H_E 0.833 vs. 0.692, respectively). Allele sizes ranged from 119 (Ssa85) to 305bp (Ssa404) for adults (wild and farmed) and to 408bp (Ssa405) for parr samples. Allelic size range at individual loci varied from 26 base pairs (SSOSL25) to 116 (Ssa404) (table 2).

Hardy-Weinberg equilibrium

Departures from Hardy-Weinberg expectations (HWE) of each locus in each sample were detected in 24 tests, 14 of 72 tests in parr samples and 10 of 56 tests in adult samples. Only Ssa197 in H.2002bP did not fit HWE after sequential Bonferroni correction (72 comparisons). Over all loci, departure from HWE was detected in E.2002bP, H.2002bP, E.1948A, E.1962A, E.1989A and E.2005A. After sequential Bonferroni correction for nine and eight comparisons, respectively, heterozygote excess was detected in one parr sample, H.2002bP, and heterozygote deficiency in the adult samples, E.1948A, E.1962A and E.1989A (table 7 for adult samples; H.2002bP is not in any statistical table since the sample was later modified). However, E.1962A and E.1989A were only marginal significant after correction for multiple tests. No deviation from HWE was detected in the farm samples.

The heterozygote excess in H.2002bP might be attributed to a recent reduction in population size, with loss of rare alleles (Cornuet & Luikart 1996) or few parents contributed to the sample (Pudovkin et al. 1996). The heterozygote deficiency in the adult samples could be due to Wahlund effect (more than one population was sampled), inbreeding (Hartl & Clark 1997) or the presence of null alleles (Jarne & Lagoda 1996), as suggested by analyzing the data in MICRO-CHECKER. However, as only the three oldest samples deviated significantly from HWE, among which two (more recent samples) were only marginal significant, technical problems might have caused this, e.g. related to poor DNA quality. Although large allele dropout was not detected with MICRO-CHECKER, DNA quality differed greatly between individuals within E.1948A and E.1962A, thus large allele dropout might have contributed to the observed excess of homozygote of relatively small alleles in some individuals. Departures from HWE due to heterozygote deficiency in historical samples have previously been recorded for Atlantic salmon and brown trout (Nielsen et al. 1997; 1999b; Hansen et al. 2002; Lage & Kornfield 2006), which suggests that poor DNA quality might be a common problem. However, Ryman (1997) has proposed that this might be expected in taxa showing overlapping generations or demographically stochastic reproduction. In this study, the deviations were not considered to affect the outcome of the analysis, since the samples displayed temporal stability in genetic composition.

Linkage disequilibrium

Linkage disequilibrium (LDE) was detected in all samples, except in E.1962A and E.1991A. In general, LDE was more frequent in parr than in adult samples, 44 of 252 comparisons and 15 of 168 comparisons, respectively. In parr samples, almost half of

the observed LDE was attributed to one sample, H.2002bP, 19 pairs of which five were significant after sequential Bonferroni correction (252 comparisons). Significant LDE was also detected in one pair in H.1991P after sequential Bonferroni correction. In adult samples, eight of the 15 pairs in LDE were detected in the two farm samples, six in K.1989 and two in HB.1992. However, only one pair in HB.1992 was significant after sequential Bonferroni correction (168 comparisons) and none in the wild adult samples.

It is unlikely that the observed LDE in this study was due to physical linkage of loci on the same chromosome (or at least not closely linked on the same chromosome) (Tonteri et al. 2005; Gilbey et al. 2004; Cairney et al. 2000). The LDE could have resulted from admixture of populations with different allele frequencies (Hartl and Clark 1997), such as admixture of subpopulations or introgression. This explanation is likely in the case of the farm populations, which were created by mixing of several populations (Guðjónsson 1989; Sigurður Guðjónsson, personal information). However, the difference in the numbers of LDE between wild adult and parr samples indicated that sibling groups might have been present in the parr samples. The observed LDE was not thought to affect the analysis, except in H.2002bP, which was modified before further analysis.

Population structure (parr samples)

As mentioned above, the significant heterozygote excess and LDE in H.2002bP might indicate the presence of a sibling group. Analysis with the STRUCTURE software supported that, as it clustered 24 of the 53 individuals of H.2002bP together. Only few other individuals in the study (wild and farmed, data not shown) assigned strongly to

this group when analyzed with a potential number of populations of four or more (K=4; fig. 8). This was also supported by the length distribution of these 24 individuals which varied from 5.7-7.5cm, indicating that they probably belonged to the same year class. The possibly sibling group was removed from the sample and the remaining individuals were then pooled with H.2002aP, which was sampled in the vicinity. Although the two samples were genetically different after removal of the possibly sibling group (F_{ST}=0.0270, P<0.0001), the difference was not thought to reflect real population structure (see discussion). The pooled sample fitted HWE and no significant LDE was detected after sequential Bonferroni correction. Abbreviation of the pooled sample is H.2002P.

Overall genetic variability of parr samples, as measured by gene diversity (H_S) and allelic richness (A_R), was 0.737 and 7.153, respectively. In 1990-91, H_S was highest in Elliðaár River (0.769) and lowest in Hólmsá River in 1990, 0.728 (table 3). However, H_S was almost the same in Elliðaár River in 1990 and in Hólmsá River in 1991. In 2002, H_S was highest in the Elliðaár River samples (0.753 in E.2002aP and 0.748 in E.2002bP) and lowest in Suðurá River (0.673). Nevertheless, no significant differences in H_S were observed among samples, among rivers (temporal samples combined) or periods (rivers combined). As in H_S, A_R estimates in 1990-91 were highest in Elliðaár River (7.323) and lowest in Hólmsá River in 1990, 6.494 (table 3). Again, there was almost no difference between Elliðaár River in 1990 and Hólsmá River in 1991. In 2002, A_R was highest in Elliðaár River (E.2002aP, 6.717) and lowest in Suðurá River (5.125). Significant difference in A_R was only detected among periods (P=0.0084). The observed differences and similarities between the 1990 sample in

Table 3 Allelic richness (A_R), F_{IS}, observed and expected heterozygosity (H_O and H_E), gene diversity (H_S) and effective number of breeders (N_b) of parr samples. No significant departures from HWE were detected after sequential Bonferroni correction (8 comparisons).

Parr	A_R	F_{IS}	H_O	H_E	H_S	N_b with 95% CI
E.1990P	7.323	-0.024	0.7875	0.7594	0.7691	130.4 (65.7-908.4)
E.2002aP	6.717	-0.017	0.7656	0.7451	0.7528	39.7 (29.8-55.6)
E.2002bP	6.416	-0.051	0.7868	0.7415	0.7484	22.3 (17.4-29.1)
H.1990P	6.494	0.021	0.7132	0.7175	0.7284	27.5 (19.6-40.9)
H.1991P	7.232	0.036	0.7372	0.7549	0.7650	51.4 (35.0-86.0)
H.2002P	6.533	-0.006	0.7150	0.7063	0.7111	65.9 (48.8-94.5)
S.1990P	6.939	-0.015	0.7609	0.7444	0.7499	76.8 (55.3-116.4)
S.2002P	5.125	-0.048	0.7054	0.6502	0.6731	221.8 (19.6-infinite)

Elliðaár River (E.1990P) and the samples in Hólmsá River in 1990-91 (H.1990P and H.1991P) indicated that sampling variance was considerable.

Overall allelic frequencies variation was F_{ST}=0.015 and most pairwise F_{ST} comparisons were significant (table 4). Non-significant F_{ST} comparisons were observed within rivers (H.1990P-H.1991P and E.1990P-E.2002aP) and among rivers (E.1990P-H.1991P). After sequential Bonferroni correction (28 comparisons), the within river (E.1990P-E.2002bP) and among river comparisons (H.2002P-S.2002P, E.1990P-H.2002P, and E.1990P-H.1990P) were non-significant. Test for homogeneity in allele frequencies revealed more significant comparisons and only E.1990P-S.2002P was not significant and E.1990P-H.1991P and H.2002P-S.2002P were non-significant after sequential Bonferroni correction (table 4). However, non-significant comparisons involving S.2002P could be the result of little statistical power due to small sample size. All within river comparisons were significant according to the homogeneity tests. There was no obvious pattern in pairwise F_{ST} estimates, except that almost all comparisons involving E.1990P were non-significant

Table 4 Pairwise F_{ST} values (above diagonal) and significance of homogeneity tests (below diagonal) for parr samples. Values in bold were significant after sequential Bonferroni correction (28 comparisons). Significance level for F_{ST} values, * P<0.05, ** P<0.001 and *** P<0.0001.

	E.1990P 1	E.2002aP 2	E.2002bP 3	H.1990P 4	H.1991P 5	H.2002P 6	S.1990P 7	S.2002P 8
1	-	0.0042	0.0062	0.0114	0.0022	0.0065	**0.0078***	**0.0176***
2	**<0.05**	-	**0.0191*****	**0.0237*****	**0.0101***	**0.0144***	**0.0140*****	**0.0259***
3	**<0.001**	**<0.0001**	-	**0.0340*****	**0.0120***	**0.0261*****	**0.0331*****	**0.0347****
4	**<0.0001**	**<0.0001**	**<0.0001**	-	0.0053	**0.0128***	**0.0206*****	**0.0269***
5	0.012	**<0.0001**	**<0.0001**	**<0.05**	-	**0.0086***	**0.0122*****	**0.0286***
6	**<0.001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.001**	-	**0.0113*****	0.0128
7	**<0.001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	-	**0.0341*****
8	0.078	**<0.001**	**<0.0001**	**<0.0001**	**<0.001**	0.018	**<0.001**	-

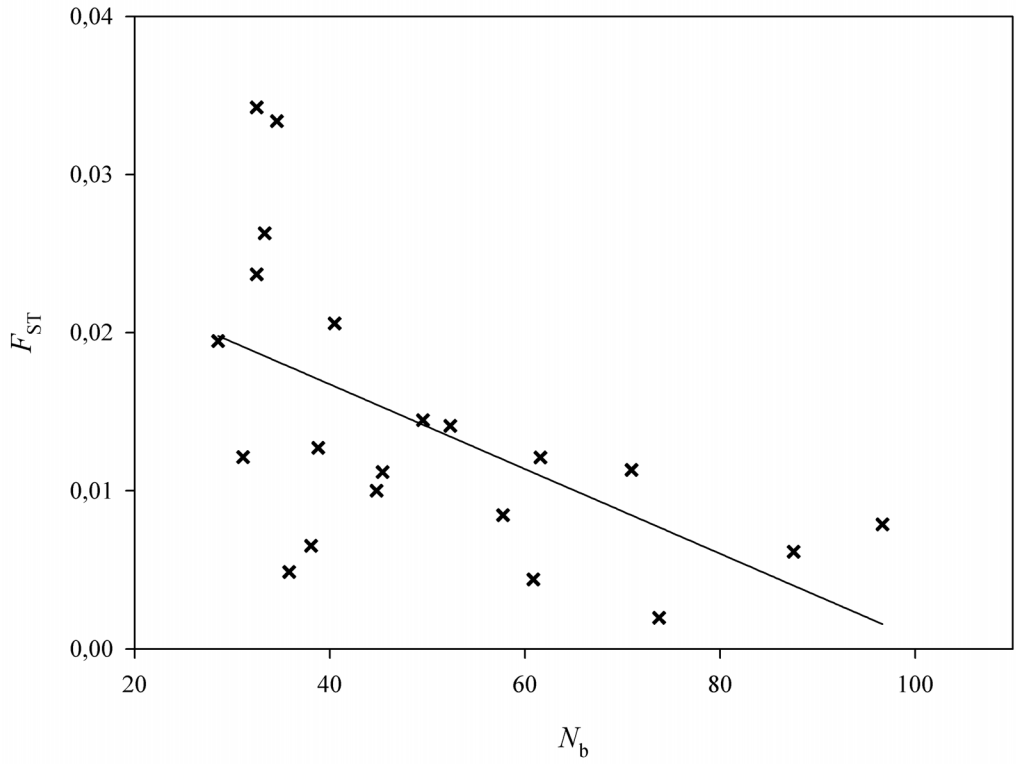


Figure 6 Relationship between level of genetic differentiation among samples (F_{ST}) and the number of breeders (N_b) in the parr samples (Pearson R=-0.57, N=21, P<0.01).

Table 5 Hierarchical analysis of molecular variance (AMOVA) of allele frequency. Organization of parr samples in analysis: Elliðaár River (E.1990P, E.2002aP and E.2002bP), Hólmsá River (H.1990P, H.1991P and H.2002P) and Suðurá River (S.1990P and S.2002P).

Variance component	d.f.	% total variance	*F*-statistic	*P*
Among rivers	2	0.38	0.00383	0.00356
Among years within sampling sites	5	1.28	0.01282	0.00000
Within samples	730	98.34	0.01660	0.00000

and the rate of differentiation increased in 2002. Average F_{ST} values in 1990-91 were 1.0% and 2.1% in 2002. In 1990-91, N_b estimates ranged from 28 in H.1990P to 130 in E.1990P (table 3). In 2002, the estimates ranged from 22 in E.2002bP to 66 in H.2002P (excluding the abnormal value of 222 in S.2002P). N_b estimate of S.2002P was only based on 14 individuals and therefore not reliable. A significant negative relationship between pairwise F_{ST} values and effective number of breeders (N_b) was detected (Pearson R=-0.57, N=21, P<0.01; fig. 6). The AMOVA ascribed more genetic variance to the temporal component than to spatial structure, 1.23% (P<0.0000) and 0.38% (P=0.00356), respectively (table 5). The software STRUCTURE detected the highest probability of one population, since the Ln P(D) was highest for K=1 in five trials (fig. 7). That was further supported by the equal assignment proportions of each sample for K=2, 3 (table 6). However, although no overall subdivision was detected with STRUCTURE, some individuals showed strong assignment (>0.80) to different clusters when K=4 (fig. 8), or with higher K's. That might reflect deviation from random mating (Falush et al. 2003), e.g. evidenced by sampling of siblings or families (Aspi et al. 2006).

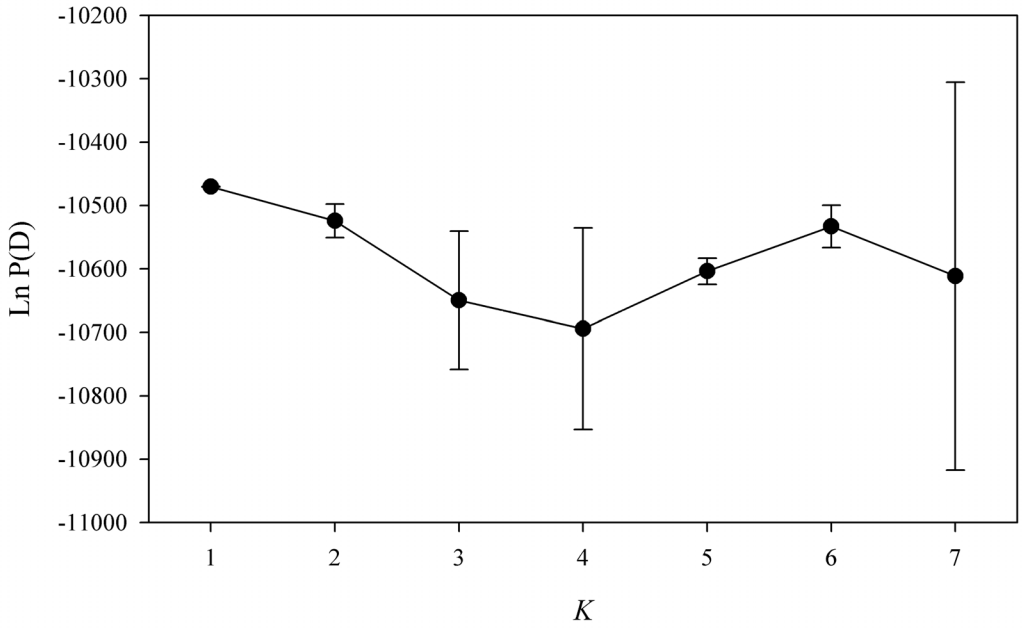


Figure 7 Ln P(D) values of parr samples, calculated with the software STRUCTURE. *K* represents the number of possible genetical distinct clusters. Standard deviation was estimated from five simulations.

Table 6 Assignment proportions of each parr sample for *K*=2 to 3 using the software STRUCTURE. *K* represents the number of possible genetical distinct clusters.

Parr samples	Assignment proportions				
	K=2		*K*=3		
	1	2	1	2	3
E.1990P	0.593	0.407	0.349	0.285	0.366
E.2002aP	0.459	0.541	0.333	0.377	0.299
E.2002bP	0.739	0.261	0.394	0.172	0.434
H.1990P	0.385	0.615	0.319	0.383	0.298
H.1991P	0.495	0.505	0.335	0.323	0.342
H.2002P	0.408	0.592	0.327	0.371	0.302
S.1990P	0.428	0.572	0.300	0.389	0.311
S.2002P	0.443	0.557	0.343	0.358	0.299

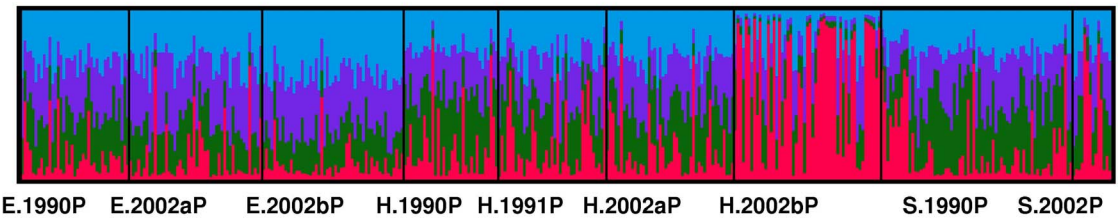


Figure 8 Graphical presentation of assignment proportions of each parr sample for K=4 (where K is pre-defined possible number of genetic clusters), calculated in STRUCTURE. The strong assignment observed in H.2002b was probably due to a sibling group present in the sample (see text).

Table 7 Observed and expected heterozygosity (H_O and H_E), gene diversity (H_S), F_{IS} and allelic richness (A_R) for wild adult and farm samples. Significant departures from HWE after sequential Bonferroni correction (8 comparisons) are denoted in bold.

Adult	H_O	H_E	H_S	F_{IS}	A_R
E.1948A	0.693	0.734	0.742	**+0.067**	8.349
E.1962A	0.651	0.727	0.738	**+0.118**	8.787
E.1989A	0.723	0.736	0.740	**+0.022**	8.661
E.1991A	0.711	0.749	0.760	+0.065	7.301
E.1992A	0.767	0.725	0.729	-0.052	8.597
E.2005A	0.723	0.725	0.728	+0.008	8.056
K.1989	0.753	0.762	0.766	0.017	10.078
HB.1992	0.762	0.753	0.758	-0.005	9.842

Temporal stability and possibly impact of farmed fish (adult samples)

Adult samples revealed no significant differences in genetic variability from 1948 to 2005, with respect to H_S and A_R. H_S varied from 0.728 in E.2005A to 0.760 in E.1991A and A_R varied from 7.301 in E.1991A to 8.787 in E.1962A (table 7; fig. 9). Although not significantly different from other estimates, H_S was higher and A_R lower in E.1991A. This could be the result of reduction in the population size, causing loss of rare alleles, followed by a temporary increase in H_S. That explanation was not supported by biological data (fig. 2). Sampling variance in E.1991A might have contributed to this, as estimates of the more recent and contemporary sample (E.1992A and E.2005A) were similar to the older samples (E.1948A, E.1962A and

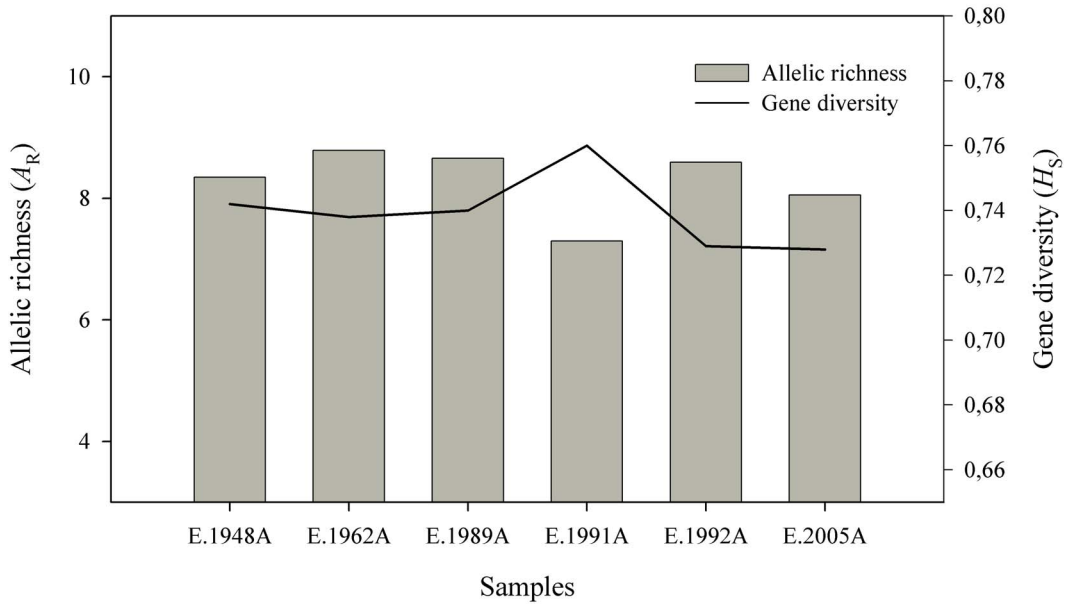


Figure 9 Genetic diversity of wild adult salmon samples.

E.1989A). Genetic variability of the farm samples was higher than variability of the wild samples, although not significantly.

Pairwise F_{ST} values of wild samples ranged from negative (which can be viewed as little or no differentiation) to less than 1% (table 8). Ten of fifteen comparisons were non-significant and only one was significant after sequential Bonferroni correction (28 comparisons). Comparisons of the contemporary sample, E.2005A, and E.1962A, E.1992A, revealed negative F_{ST} values and other comparisons involving E.2005A were not significant after sequential Bonferroni correction. Most tests of homogeneity in allele frequencies were non-significant after sequential Bonferroni correction or significant at the $P<0.05$ level (table 8). The most significant comparisons involved E.2005A, E.1948A-E.2005A and E.1989A-E.2005A ($P<0.001$, respectively). However, E.2005A was not significantly different from E.1992A and after sequential Bonferroni correction, it differentiated from E.1962A and E.1991A at the $P<0.05$

Table 8 Pairwise F_{ST} values (above diagonal) and significance of homogeneity tests (below diagonal) for wild adult and farm samples. Values in bold were significant after sequential Bonferroni correction (28 comparisons). Significance level for F_{ST} values, * P <0.05, ** P <0.01 and *** P <0.0001.

	E.1948A 1	E.1962A 2	E.1989A 3	E.1991A 4	E.1992A 5	E.2005A 6	K.1989 7	HB.1992 8
1	-	0.0009	0.0021	-0.0022	0.0021	0.0039	**0.0198*** **	-0.0008
2	0.317	-	0.0008	-0.0058	0.0007	-0.0023	**0.0210*** **	-0.0029
3	0.016	**<0.05**	-	**0.0097***	0.0037	0.0053	**0.0368*** **	**0.0088****
4	0.036	0.043	**<0.01**	-	0.0047	0.0056	**0.0264*** **	0.0060
5	0.016	**<0.05**	0.067	**<0.05**	-	-0.0005	**0.0238*** **	**0.0053***
6	**<0.001**	**<0.05**	**<0.001**	**<0.05**	0.023	-	**0.0265*** **	**0.0057***
7	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	-	**0.0144*** **
8	**<0.05**	**<0.001**	**<0.0001**	**<0.05**	**<0.0001**	**<0.0001**	**<0.0001**	-

level. Differentiation between wild and farm samples varied with respect to the origin of the farmed fish. F_{ST} values of fish originating from sea cages (K.1989) and wild adult samples were approximately 2-2.5% and all highly significant (P<0.0001) (table 8). Highly significant differentiation was also revealed by all homogeneity tests (P<0.0001). However, the sea ranch sample (HB.1992) displayed very low differentiation when compared to the wild samples. Two F_{ST} values were negative and other less than 1%, same as measured between years within the wild samples. However, most tests of homogeneity of allele frequencies were highly significant after sequential Bonferroni correction (P<0.0001), although two comparisons were significant at the P<0.05 level. The wild contemporary sample, E.2005A, did not differentiate less than other wild samples from the two farm strains.

Assignment of recent and contemporary wild samples to the historical samples with the method of Rannala & Mountain (1997), revealed that probability of individuals assigning to E.1948A and E.1962A was highly correlated (R=0.84, R^2=0.71, P=0.0000). The high correlation supported that both old scale samples were

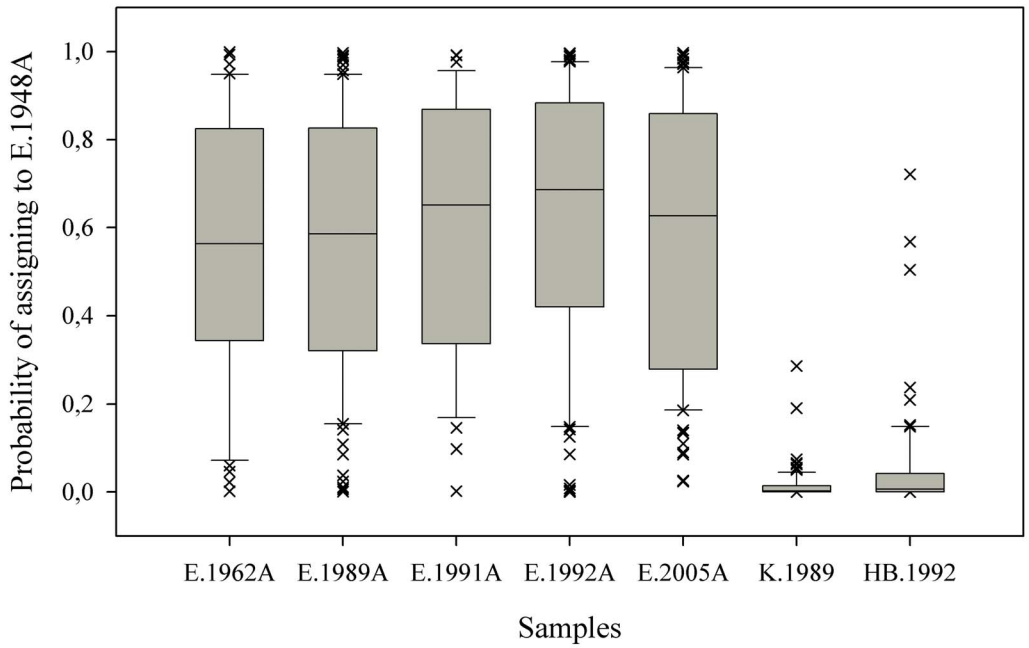


Figure 10 Boxplot showing probability of individual in each wild adult sample (from 1962-2005) and in the two farm strains assigning to the oldest wild adult sample (E.1948A). Median probability of each sample is denoted with a black line. Calculated in GeneClass2 with the method of Rannala & Mountain (1997).

representative of the Elliðaár salmon. Only few wild individuals assigned to the farm samples when baseline samples were selected E.1948A, K.1989 and HB.1992 and in those cases, assignment to E.1948A was generally much stronger. Individuals in E.2005A did not assign more to the farm samples than other wild samples furthermore, assignment tests showed that the probability of wild salmon assigning to the oldest sample, E.1948A, was stable from 1962 to 2005 and the median probability was almost always more than 0.6 (fig. 10). The probability of the farmed individuals assigning to E.1948A was very low, even for most individuals in HB.1992, which differentiated very little from the wild samples, as measured by F_{ST}.

DISCUSSION

Population structure based on parr samples

In present study the observed genetic heterogeneity among samples of juvenile Atlantic salmon was in congruence with earlier studies on population structure within river systems and drainages (Ståhl 1987; Crozier & Moffett 1989; McElligott & Cross 1991; Verspoor et al. 1991; Jordan et al. 1992; Hurrell & Price 1993; Elo et al. 1994; Galvin et al. 1996; Daníelsdóttir et al. 1997; Beacham & Dempson 1998; Garant et al. 2000; Primmer et al. 2006). The Elliðaár river system is perhaps the smallest river system, in which salmon population structure has been investigated, although genetic differentiation over similar distances have been reported; within a single river (Heggberget et al. 1986) and between tributaries in river systems (Crozier & Moffett 1989; Hurrell & Price 1993; Garant et al. 2000; Primmer et al. 2006). Unlike most previous studies, however, temporal samples were included in this study to get a more accurate picture of the spatial structure (Waples 1998; Garant et al. 2000). Accordingly, a panmictic population is probably the most likely description of the genetic pattern observed in this small river system and the observed heterogeneity does not reflect a biologically significant structure. As discussed below, the heterogeneity might be explained by the "Allendorf-Phelps effect."

Overall allelic variation in the Elliðaár river system was F_{ST}=0.015 (both periods combined). This variation falls within the previously reported range of variation among samples within river systems and drainages, 0.7-6.7% (Ståhl 1987; McElligott & Cross 1991; Verspoor et al. 1991; Jordan et al. 1992; Elo et al. 1994; Galvin et al. 1996; Garant et al. 2000; Primmer et al. 2006). Interestingly, overall variation doubled between the two periods studied, F_{ST}=0.010 in 1990-91 and F_{ST}=0.021 in 2002. Thus,

variation among rivers was less in 1990-91. The sample from Elliðaár River in 1990 (E.1990P) did not differ significantly from the two Hólmsá River samples (H.1990P and H.1991P), although these sites were separated by the greatest distance in the study (~15 km). In contrast, the two samples from Elliðaár River in 2002 (E.2002aP sampled upstream and E.2002bP downstream) differentiated, although they were separated by only ~5 km (fig. 1). Both samples differed from the two upper rivers and at a rate that was about two to four times higher than corresponding estimates in 1990-91, measured by F_{ST}. A similar shift in allelic variation can be viewed in the microsatellite study of Garant et al. (2000). In that study, salmon fry was sampled during two consecutive years from seven locations in the considerably larger river system of Sainte-Marguerite River (Canada). Overall F_{ST} value was 3.4% and average values within years changed from 2.7% to 4.1% (Garant et al. 2000). As in the Elliðaár river system, isolation by distance (the tendency of individuals who stray to reproduce in neighbouring subpopulations) did not explain the genetic pattern in the Sainte-Marguerite River (Garant et al. 2000).

Temporal stability was observed in Elliðaár River, where E.1990P did not differ from the two contemporary samples (E.2002aP and E.2002bP), as measured with F_{ST}. Although temporal stability was not detected in other rivers, it is noticeable that E.1990P did not vary from the contemporary Hólmsá River sample (H.2002P). Indeed, temporal changes in allele frequencies within a river were more pronounced than spatial structure among the three rivers. According to the hierarchical analysis of molecular variance (AMOVA), the temporal component explained 1.28% of the variance, whereas the spatial component explained only 0.38%. In Sainte-Marguerite River, the temporal component explained 2.5% of the variance and 0.9% could be

ascribed to the spatial component (Garant et al. 2000). The difference in levels of F_{ST} between the two studies might be related to different sizes of the river systems or to the different life stages sampled during the two studies (parr during present study and fry in Garant et al. (2000)). The latter is probably a more likely explanation since in a microsatellite study analyzing salmon parr samples from the huge river system of Varzuga River (Russia), overall F_{ST} was almost the same as in the Elliðaár river system (Primmer et al. 2006). Interestingly, the distribution of variance was similar in the present study compared to the study of Garant et al. (2000), i.e. the temporal component was about three times higher than the spatial component. In Sainte-Marguerite River, non-significant variance was attributed to the grouping of samples by river branches, indicating that structure was not on river basis. On the other hand, the relatively weak but significant spatial component was among other thought to reflect population structure based on spawning/nursery habitat (Garant et al. 2000). In the present study, the weak but significant spatial component was not thought to reflect population structure.

Indeed, as pointed out by Waples (1998), significant genetic differences may routinely occur between geographical samples given enough data, since departure from complete panmixia may generally occur. He also pointed out that to test whether genetic differentiation reflected real population structure rather than some artifact (e.g. random sampling error and/or stochastic changes in allele frequencies), the best approach was to replicate sampling. Then, if spatial structure is consistent in time, it can be determined with much more confidence that the signal is of some biological importance and that it reflects real population structure. If, however, the spatial structure is not consistent, it may indicate that the population is not structured into

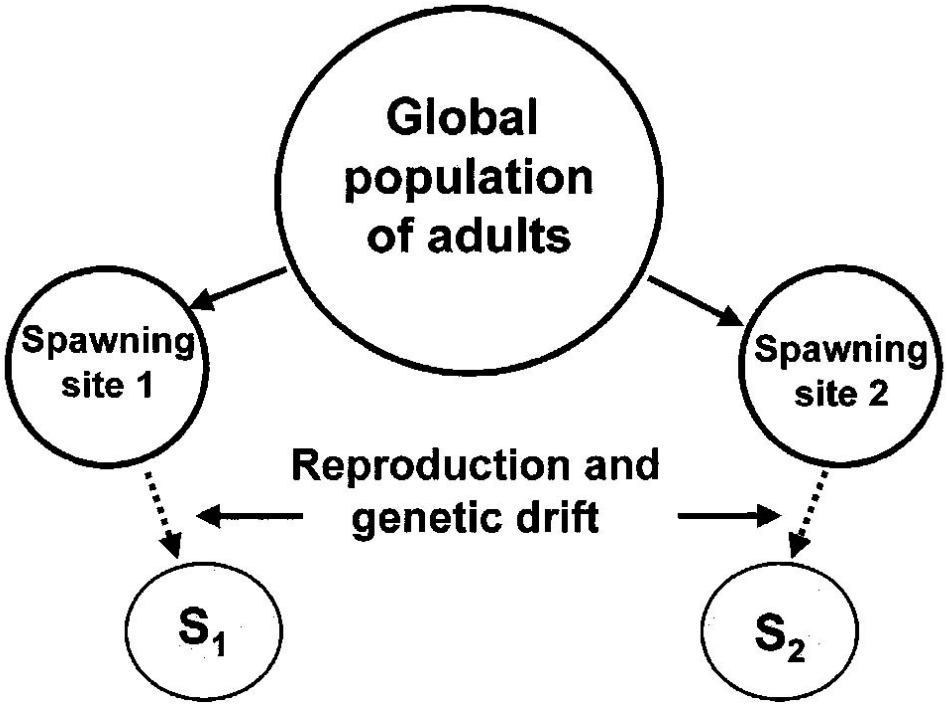


Figure 11 A schematic diagram of how the "Allendorf-Phelps effect" may occur. The diagram illustrates how genetic heterogeneity may arise due to an episode of founder effect/genetic drift in a sampling scheme where progeny from two different locations (S_1 and S_2) are sampled and the adults are panmictic (see text). Figure from Waples (1998).

isolated breeding units or that the biology of the species is poorly understood. A special case of departure from panmixia was addressed in Allendorf and Phelps (1981) and later discussed by Waples (1998). Namely, that by sampling spatially (or temporally) separated juveniles rather than breeders, the chance of detecting genetic divergence among samples will be inflated. This is because the juvenile samples do not conform to the assumption of being randomly sampled from the global population. Therefore, although studying a panmictic population, one might detect genetic divergence among rivers or within river, due to stochastic changes in allele frequency between generations (genetic drift/founder effect) and the chance of detecting divergence increases as the number of spawners becomes smaller (Allendorf & Phelps 1981). This has been named the "Allendorf-Phelps effect" (fig. 11; Waples 1998). To avoid this possibility, Allendorf & Phelps (1981) stated that the correct way was to

sample adults. However, since the chance of detecting differentiation between juvenile samples increases as N_b becomes smaller (since within group variance decreases) it is possible to estimate if the "Allendorf-Phelps effect" is influencing the data by exploring the relationship between differentiation (e.g. F_{ST}) and N_b (Waples & Teel 1990; Waples 1998; Garant et al. 2000).

Indeed, in the present study spatial structure was observed to some extend but structure was not consistent in time. This may indicate that the population was either not structured or that something significant in the biology of salmon in the river system was unknown. The first possibility seems more likely since the Bayesian clustering method in the STRUCTURE software detected the highest likelihood of one population. However, Waples & Gaggiotti (2006) demonstrated that the use of the Structure software to detect the number of populations was limited when gene flow was high or moderate, as one might expect to be the case in such a small river system. However, the negative relationship between N_b and F_{ST} for the parr samples provided a plausible explanation for the instability in observed allelic frequencies. According to this, the population is most likely not structured and the genetic pattern probably results from the "Allendorf-Phelps effect."

It could be argued that the lack of population subdivision in the Elliðaár river system might have resulted from anthropogenic influences, i.e. homogenization of subpopulations due to human mediated factors disturbing the otherwise naturally stable environment. There have been no catastrophic events in the system due to pollution and influx of farmed salmon was probably mainly limited to Elliðaár River (discussed later in more detail). The most pronounced event that could have affected

population structure was the extension of the dam in 1929, which prevented natural upstream migration of spawning salmon. Fortunately, after construction of the dam, salmon was caught in traps just below the dam and transported upstream. If the population was structured, some mixing of subpopulations would probably have resulted from this, since salmon was confined below or above the dam during the spawning season. However, the largest and most productive spawning grounds for salmon in Elliðaár River are located above the dam and numerous salmon were transported upriver each year. Therefore, it is likely that most of the salmon that was transported upriver had the possibility to spawn in its river of origin. Since 1960, spawning salmon has been able to move freely upriver past the dam. Thus, if there is propensity for structure formation in the river system and given that the dam might have caused some breakdown of population structure, it is likely that structure should have reappeared after 30-40 years of free passage and selection of spawning sites. By using a hypothetical example of a salmonid species founding new tributaries in a river system, Wang et al. (2002) demonstrated that only few generations were needed for population subdivision. The example assumed that N_e of the colonizers in each tributary was low and accurate homing (little or no gene flow). Although this extreme scenario is somewhat unrealistic for the Elliðaár salmon, it showed that population subdivision might occur fast in small and isolated breeding units due to drift.

The general view on populations structure of Atlantic salmon in river systems is that populations are divided into subpopulations, although the scale at which structuring occurs and its nature is less understood (Beacham & Dempson 1998; Garant et al. 2000; Primmer et al. 2006). This study provides valuable information on that subject and it is possibly the first study on salmon demonstrating that heterogeneity in a

whole river system might simply be due to the "Allendorf-Phelps effect." In some sense, comparison with previous studies is difficult, e.g. sampling schemes may differ as well as the number and type of loci. Also, it has been shown that earlier studies might have overestimated differentiation (Garant et al. 2000). Despite these anomalies, there may be some signs in earlier studies implying that the "Allendorf-Phelps effect" is not only limited to geographically small scale studies, i.e. not only the degree of differentiation has been overestimated but also the degree of structuring.

Compared to other studies on population structure of salmon in river systems, the sampling scheme of Garant et al. (2000) is the most similar to present study. In that study, temporal stability was detected at four sampling sites and instability at three sites. The genetic pattern was explained by two alternative evolutionary models: the member-vagrant hypothesis of Iles & Sinclair (1982) predicting that adaptation of juveniles to specific spawning/nursery habitats might enable precise homing of adults and result in reproductive isolation. However, in an unstable environment, population structure may be too short-lived for the formation of locally adapted gene pools. Extinction-recolonization processes according to the metapopulation model might then characterise the system (Garant et al. 2000). Their hypothesis was supported by temporal instability at one site that experienced displacement of spawning/nursery habitat during a summer flood. Interestingly, temporal stability at the four sampling sites was practically only supported by results of a neighbour-joining phenogram constructed from chord distances (D_{CE}). Temporal stability was not supported by pairwise D_{CE} and F_{ST} values. Furthermore, by comparing all pairwise F_{ST} values and N_b estimates presented in Garant et al. (2000), a highly significant negative relationship was detected (Pearson R= -0.41, N=91, P<0.01). However, the authors

did not detect a significant relationship between these estimates, perhaps because only unstable sites were included in the analysis. Results of the present study and the study of Garant et al. (2000) are in many ways similar. Although not refuting their proposals, it might be possible that the "Allendorf-Phelps effect" was responsible for the observed genetic heterogeneity in larger river systems, such as in Sainte-Marguerite River, and the degree of structuring might have been overestimated for salmon in some river systems.

Temporal stability based on adult samples

Analysis of a series of adult salmon samples from Elliðaár River over a period of 5 decades revealed very low variation in genetic variability and in genetic composition. The Elliðaár salmon population has therefore been genetically stable despite some reduction in run size and juvenile production in recent years. This is in accordance with some previous studies on temporal genetic variability of Atlantic salmon over several decades (Nielsen et al. 1999b; Tessier & Bernatchez 1999; Säisä et al. 2003; Consuegra et al. 2005; Skaala et al. 2006). In this study, genetic variability was estimated by gene diversity and allelic richness. Gene diversity was stable over the study period and allelic richness decreased slightly in E.2005A (however not significantly), which might indicate that the decreased salmon run had resulted in some loss of genetic variation. It has been shown both theoretically and empirically that number of alleles is a much more sensitive estimator of loss of genetic variability than mean heterozygosity (Nei et al. 1975; Waples 1990; Luikart et al. 1998; Spencer et al. 2000; Koljonen et al. 2002; Ardren & Kapuscinski 2003). This results from the fact that heterozygosity mostly reflects high frequency alleles whereas low frequency alleles contribute little to heterozygosity and are more susceptible to loss due to drift

(Nei et al. 1975; Wang et al. 2002). Since reduction in effective population size increases the rate of drift, it will affect the number of alleles more severely than mean heterozygosity, especially apparent for microsatellites when allele numbers are high (Koljonen et al. 2002). In present study, the lower allelic richness in E.2005A was not statistically significant, which is perhaps due to lack of statistical power since only seven microsatellites were studied (Säisä et al. 2003). However, sampling variance might have caused this weak signal, e.g. variation was higher between the two consecutive sampling years, E.1991A and E.1992A (A_R=7.30 and A_R=8.60, respectively) than between the two oldest samples and the contemporary, E.1948A (A_R=8.35), E.1962A (A_R=8.79) and E.2005A (A_R=8.06).

Several factors other than sampling variance might have biased the results of genetic variability. Perhaps, allelic richness was downwardly biased in the two oldest samples due to large allele dropout. Allelic richness may have been overestimated in the recent samples (especially in E.1989A and E.1992A) due to the presence of farmed escapees in the samples. As discussed earlier, discrimination between wild and farmed salmon was achieved by determining scale characteristics. The method has some limitations and therefore some farmed fishes, possibly with alleles not found in the wild population, may have been present in the wild samples. Indeed, few alleles were identified in E.1989A (three alleles) and E.1992A (four alleles) that were present in either farm sample and not in other wild samples (including parr samples). This indicates that the discrimination by scale characteristics was not perfect. However, it cannot be excluded that due to high microsatellite polymorphism, the alleles were simply not detected in other samples or they may have appeared in the samples via introgression. By assigning wild adult salmon to wild baseline samples (E.1948A and

E.1962A) or farm samples (HB.1992 and K.1989) and consequently excluding wrongly classified fish, it was possible to correct for some of the bias. Furthermore, by applying the same method, possible strayers from neighbouring rivers (or farmed fish with unknown origin) were excluded if individuals in wild adult samples did not assign to the baseline samples (E.1948A and E.1962A) or by self-assignment of baseline samples (Fillatre et al. 2003). Thus, after sorting out possible farmed fish or strayers from the wild adult samples, allelic richness became more temporally stable. Additionally, gene diversity became very stable, measured 0.73 in all samples, except in E.1991A (0.75). In E.1948A, E.1989A, E.1992A and E.2005A, allelic richness ranged from 7.90 (E.1948A) to 8.16 (E.1989A). After the adjustments, allelic richness was still highest in E.1962A (8.51) and lowest in E.1991A (7.12). However, the relatively high allelic richness in E.1962A and low in E.1991A was mainly due to one locus in each case, SSOSL311 and Ssa404, respectively. By excluding e.g. SSOSL311 from the analysis, allelic richness became almost the same in E.1948A and E.1962A (7.40 and 7.49, respectively) and it became slightly higher and very stable in E.1989A, E.1992A and E.2005A (7.91, 7.93 and 7.91, respectively). Thus, by accounting for the possibility of farmed fish and/or strayers in the samples and not excluding the possibility of some large allele dropout and sampling variance, it can be concluded that there are no signs of reduced genetic variability in the Elliðaár salmon population.

Stability in genetic composition was observed with F_{ST}, as all pairwise comparisons except one were non-significant and most estimates ranged between negative values and 0.6%. The exact tests of homogeneity in allele frequencies had more power in detecting significant differentiation than permutation of F_{ST} values. In a study using

simulated and real data, the power of the exact test outperformed permutation under similar conditions, i.e. with low differentiation, uneven sample sizes and multiple comparisons (Ryman et al. 2006). The exact tests were non-significant in all comparisons involving E.1948A, except when compared to E.2005A (P<0.001). Conversely, all comparisons involving E.2005A were significant (P<0.05, 0.001), except in the comparison of E.1992A. At individual loci, significant differentiation between wild adult samples was only observed at the two most polymorphic loci, SSOSL311 and Ssa404, in ten of 30 comparisons. That was perhaps not surprising as it has been shown that power of exact tests increase with higher number of alleles (Raymond & Rousset 1995a; Rousset & Raymond 1995). There was no consistent pattern in what comparisons were significant between loci and all samples were at least once involved in a significant comparison. Only the comparison of E.1948A and E.2005A was significant at both loci. Therefore, the two informative loci may reflect either genetic drift or sample variance. It is hard to distinguish between the two possibilities and perhaps they are both right to some extend. If drift was acting on the population, most difference should normally be viewed between the samples most separated in time. However, that was not observed with F_{ST} and the level of significance of the exact test was equal among E.1948A and E.1989A in the comparison of E.2005A. Therefore, it is probably impossible to distinguish between the two possibilities in this study. However, if genetic drift has acted on the population, its effect has been small and genetic composition has essentially been stable during the study period.

Indeed, in order to assess if the decline in the Elliðaár population has resulted in accelerated drift, temporal changes in N_e have to be estimated. Unfortunately, only

preliminary estimates of N_e are currently available. By using the salmon temporal method (Waples et al. 2007), N_b was estimated 188 between E.1948A-E.2005A and 251 between E.1962A-E.2005A. According to Waples (1990), it can be translated into N_e of 752 and 1004, respectively, since generation time of salmon in Elliðaár River is approximately 4 years. Although the estimates are just preliminary, they might be reasonable. In the much larger Teno River, N_e was estimated 1412 for salmon between 1939 and 1995 (Säisä et al. 2003). In the same study, genetic variability and allele frequencies were stable. In a study on the anadromous brown trout in Karup River (Denmark), Hansen et al. (2002) estimated N_e to be 671 between 1912 and 1996. Genetic variability was stable the same period and differentiation was measured the same as between 1948 and 2005 in present study. The authors concluded that N_e of that size indicated that neither drift nor migration from genetically different stocks (including stocked hatchery trout) had strongly affected the genetic composition (Hansen et al. 2002). Although the salmon model used in the preliminary analysis is best used for samples spanning no more than a decade or two (Robin Waples, personal information), it seems likely that the long-term N_e is somewhat larger than 500 for salmon in the Elliðaár river system. N_e of 500 has been proposed to be the minimum size required for maintaining the evolutionary potential of populations (Franklin 1980).

Possible impact of farmed fish

First indications of non-successful invasion of farmed salmon were gained by the observed temporal stability in genetic variation and composition of the wild population. The two farm strains that entered the rivers and were analyzed in this study were both more variable than the wild salmon with respect to gene diversity and

allelic richness, although not significantly. That indicates that the broodstocks consisted of many individuals and/or confirms the knowledge that the broodstocks consisted of individuals from several populations (Guðjónsson 1989; Sigurður Guðjónsson, personal information). The two farm strains differed genetically, as measured with F_{ST}=0.014 (P<0.0001) and the homogeneity test (P<0.0001).

By identifying "diagnostic" marker alleles, it has been possible to infer about potential introgression in salmonid species due to influx of farmed fish or restocking with foreign strains. By exploring presence or absence of alleles, evidences of hybridizations between conspecific indigenous and non-indigenous salmonids have been identified with markers such as allozymes (Crozier 1993; Skaala et al. 1996), mtDNA (Clifford et al. 1998; Hansen et al. 2000; Marzano et al. 2003; van Houdt et al. 2005) and microsatellites (Hansen et al. 2000; Martinez et al. 2001). By applying this method, three different alleles were detected in three individuals that were present in either farm strain but not in other wild samples; one allele in each of the contemporary samples E.2002P1 ($Ssa85^{129}$), S.2002P ($SSOSL311^{135}$) and E.2005A ($Ssa85^{119}$) (Appendix C). In this survey, the recent adult samples (E.1989A, E.1991A and E.1992A) were excluded, due to reasons mentioned earlier, and both the old scale samples and recent parr samples were considered as the genetic baseline. The alleles were present in very low frequencies, one or two copies in each sample. Therefore, in this study, the "diagnostic" alleles alone did not provide evidence for the occurrence of introgression. It is likely, that due to the high microsatellite polymorphism, the rare alleles were not detected in other wild samples. Considering the parr samples from 1990 (N=142) and 1991 (N=40) as genetic baseline might seem questionable. However, the youngest fish in 1990 and 1991 were spawned in 1988 and 1989,

respectively, and the main influx commenced after that. Interestingly, two of the three "wild" individuals with possible farm alleles were previously identified as possible salmon/trout hybrids. One individual in S.2002P and E.2005A contained one or more alleles that in this study were diagnostic for brown trout. As previously mentioned, the possible hybrids were not included in any statistical analysis.

Slightly more linkage disequilibrium (LDE) was detected in the contemporary adult sample (E.2005A), than in other wild adult samples. Three locus pairs in E.2005A were in LDE, two in E.1992A and one in E.1948A and E.1989A. LDE can result from several factors such as real or artificial population admixture (Hartl and Clark 1997). Compared to tests of Hardy-Weinberg equilibrium (HWE), LDE is a more sensitive measure of possible introgression, since only one generation of random mating (when generations are non-overlapping) is required to attain HWE after mixture. In contrast, LDE will dissipate at a much slower rate determined by the recombination fraction (Hartl and Clark 1997). Therefore, given the short time from the influx of farmed fish, if LDE would have accumulated due to introgression it should probably have prevailed in the population in 2005. However, although higher LDE was detected in E.2005A, with respect to number of pairs and level of significance, none of the pairs were significant after correction for multiple tests. In Crozier et al. (1993) and Skaala et al. (2006), LDE in wild juvenile salmon samples were believed to result from introgression of farmed fish. However, locus pairs in LDE were significant in both studies unlike what observed in this study.

The sea ranch strain (HB.1992) differed very little from the wild adult samples in allele frequencies. The rate of differentiation was similar to within comparisons of

wild samples, with F_{ST} values ranging from negative to 0.9% (P<0.01). Lack of differentiation resulted from the fact that about 50% of the broodstock used in the hatchery experiments originated from the Elliðaár population (Guðjónsson 1989) and drift had probably been weak due to the supposedly large broodstock. Conversely, the sea cage salmon (K.1989) differentiated from F_{ST}=0.02-0.037 from wild adult samples and all comparisons were highly significant. One of the consequences of introgression with farmed fish is homogenization of genetic composition (van Houdt et al. 2005), i.e. the genetic composition of a wild population will resemble that of the farm strain more after introgression (Koskinen et al. 2002). This was not observed in the present study (F_{ST} values and exact tests), as the contemporary sample (E.2005A) did not differentiate less from the farm strains than older adult samples.

To investigate this further, multilocus genotypes were explored by assigning recent and contemporary adult samples to the baseline samples and the farm strains. In short, few individuals of the recent samples assigned with more probability to either of the farm strain than to the baseline samples, probably because of wrong classification, as discussed earlier. However, none of the individuals in E.2005A assigned to the farm strains with more than 10% probability and in those cases, assignment to the baseline samples normally exceeded 80%. Furthermore, assignment of adult samples to the oldest sample (E.1948A) was stable from 1962 to 2005 (fig. 10), opposed to what might be expected if introgression had affected significantly and increased the multilocus complexity (Pinto et al. 2005). Overall, absence of "diagnostic" alleles and significant LDE, temporal stability in allele frequencies and in multilocus genotypes support that influx of farmed fish did not affect the genetic integrity of the Elliðaár population. Therefore, introgression is probably not responsible for changes in life-

history characteristics, e.g. earlier smolting of juveniles (fig. 4). In the experimental field study of McGinnity et al. (2003), hybridization of farmed and wild salmon did not result in earlier smolting, although hybrids grew faster. Therefore, since introgression was not detected in the Elliðaár salmon, it is most likely that the faster growth rate of juveniles is due to density dependent and/or environmental factors. These results are in congruence with the results of Skaala et al. (2006), which showed that despite extensive influx of farmed salmon in some Norwegian rivers, evidence of introgression was not always detected.

CONCLUSIONS

This study adds to the growing number of genetic studies utilizing historical and spatial samples to describe and/or investigate the potential consequences of biological phenomenon. Importantly, present study is probably the first empirical study on salmon, which shows that the "Allendorf-Phelps effect" might have caused within river system heterogeneity and that salmon in a whole (but very small) river system might be panmictic. Perhaps these results reflect the small size of the river system and/or the approach of combining spatial and temporal samples. Thus, the study contributes to the growing knowledge of salmon population structuring, especially in defining the lower limits of structure within river systems. It cannot be excluded that the outcome of the study would have been different if a pristine river system of similar size was investigated. However, for future studies on related subjects, the temporal method applied in this study and in Garant et al. (2000) is highly recommended. Furthermore, sampling scheme based on sampling of juveniles may produce significant heterogeneity that has no biological meaning. Therefore, if juveniles and not adults are sampled, large river stretches and multiple age groups

should normally be sampled. Present study has valuable management implications, e.g. stocking in the Elliðaár river system should not be on river basis. However, these results ought not to be transferred to other river systems for management purposes. More studies are needed to reveal the extent and nature of salmon population structure in river systems.

What might have caused the apparent changes observed in the river system if neither outbreeding depression due to hybridization with farmed salmon nor inbreeding depression of isolated populations in the upper river were causing them? Although introgression of farmed fish was not detected, it cannot be excluded that farmed fish may have affected indirectly, e.g. by disturbing spawning activities of wild salmon. However, that does probably not explain the steady drop in juvenile density in the upper rivers, since the distribution of farmed fish was probably more or less restricted to Elliðaár River. Furthermore, Leirvogsá River, which experienced influx of the same farm strains and of similar degree, did not display less juvenile production during that period. Interestingly, the dramatic decline in salmon juvenile density in the Elliðaár river system in 1988-89 was also observed for juvenile brown trout in Hólmsá and Suðurá River and for juvenile salmon in Leirvogsá River (Antonsson 2002; Antonsson 2006). An especially cold spring in 1989 might have caused this. However, low juvenile density of brown trout and salmon in Leirvogsá River was only limited to one or two years and was not the beginning of a general trend. It is likely that multiple factors have contributed to the changes in abundance and life-history characteristics of salmon in the system, e.g. infection of the bacteria *Aeromonas salmonicida* in 1994-95, appearance of the freshwater diatom *Didymospheenia geminata* in 1994, urban pollution, influx of farmed fish and lack of

spawners in the upper rivers. However, discussion on that subject is still only speculative. Perhaps, these changes might be related to a much larger phenomenon affecting life in the river system and elsewhere. For example, concomitant changes in density and in life-history characteristics of salmon, a shift in dominance was observed in Lake Elliðavatn. Proportion of brown trout steadily increased from 30% to 90% between 1984 and 2004 at the expense of artic charr (Antonsson et al. 2005). Indeed, temperature changes have been recorded in recent years. Perhaps climatic changes coupled with density dependent factors may explain the faster growth of juveniles, earlier smolting and skewed sex ratio of returning adults in recent years. Today, earlier smolting of Atlantic salmon has been observed in few rivers in Britain (Butler et al. 2005; Cragg-Hine et al. 2006) and climatic changes are thought to be a key factor influencing production of Pacific salmon (Noakes et al. 2000). This, however, needs further investigation. In conclusion, although temporal stability in genetic composition of Elliðaár salmon was observed in 1948-2005 and preliminary results indicate that effective population size is large enough for maintaining its evolutionary potential, the very low juvenile production in the upper rivers is worrying. If this condition prevails, it might result in loss of genetic diversity in the long run.

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APPENDIX A

Sample size (N), allelic richness (A_R), observed and expected heterozygosity (H_O and H_E) and F_{IS} at eight microsatellite loci for parr samples. No deviation from HWE was detected after sequential Bonferroni correction (64 comparisons).

		Ssa85	Ssa197	Ssa202	Ssa404	Ssa405	SSOSL 25	SSOSL 85	SSOSL 311
E.1990P	N	39	39	39	39	39	39	39	39
	A_R	3.994	7.702	5.135	11.221	10.730	4.815	7.234	7.756
	F_{IS}	-0.034	+0.004	+0.021	+0.031	+0.026	-0.137	-0.128	-0.023
	H_O	0.718	0.821	0.718	0.872	0.872	0.795	0.711	0.795
	H_E	0.686	0.813	0.724	0.888	0.883	0.691	0.623	0.767
E.2002aP	N	48	48	48	48	48	48	48	48
	A_R	4.553	6.507	4.821	9.751	9.595	4.249	6.095	8.162
	F_{IS}	-0.129	-0.005	+0.146	-0.070	-0.058	+0.096	-0.061	-0.041
	H_O	0.729	0.792	0.604	0.958	0.938	0.625	0.667	0.813
	H_E	0.640	0.779	0.699	0.887	0.878	0.683	0.622	0.773
E.2002bP	N	51	51	51	51	51	51	51	51
	A_R	4.907	6.422	4.825	9.849	9.237	4.484	5.204	6.398
	F_{IS}	-0.090	+0.007	-0.079	-0.067	-0.077	-0.167	+0.122	-0.017
	H_O	0.824	0.804	0.745	0.922	0.922	0.824	0.471	0.784
	H_E	0.749	0.801	0.684	0.855	0.848	0.700	0.530	0.764
H.1990P	N	34	34	34	34	34	34	34	34
	A_R	5.201	7.688	3.823	11.696	9.351	4.688	4.468	5.035
	F_{IS}	+0.131	-0.105	+0.158	-0.008	-0.022	-0.105	+0.142	+0.051
	H_O	0.647	0.912	0.559	0.912	0.882	0.765	0.441	0.588
	H_E	0.732	0.814	0.653	0.891	0.851	0.683	0.506	0.610
H.1991P	N	39	39	39	39	39	39	39	39
	A_R	5.320	7.752	4.434	10.708	11.251	4.815	5.319	8.258
	F_{IS}	-0.050	+0.068	-0.004	-0.018	-0.016	+0.014	+0.200	+0.146
	H_O	0.795	0.769	0.666	0.923	0.923	0.692	0.462	0.667
	H_E	0.748	0.814	0.656	0.895	0.897	0.693	0.568	0.769
H.2002P	N	75	75	75	75	75	75	75	75
	A_R	3.972	6.116	4.192	11.168	10.499	4.359	4.954	7.002
	F_{IS}	+0.042	+0.027	-0.238	+0.006	-0.052	+0.129	+0.064	+0.014
	H_O	0.627	0.733	0.840	0.893	0.933	0.587	0.360	0.747
	H_E	0.649	0.749	0.675	0.893	0.882	0.669	0.382	0.752
S.1990P	N	69	69	69	69	69	69	69	69
	A_R	4.366	6.862	5.274	11.127	9.170	4.226	5.997	8.488
	F_{IS}	-0.118	+0.027	-0.020	-0.010	+0.065	-0.043	-0.023	-0.028
	H_O	0.725	0.739	0.768	0.913	0.812	0.681	0.594	0.855
	H_E	0.644	0.754	0.747	0.898	0.862	0.648	0.577	0.826
S.2002P	N	14	14	14	14	14	14	14	14
	A_R	4.000	5.000	4.000	6.000	10.000	4.000	2.000	6.000
	F_{IS}	+0.060	+0.086	+0.100	+0.139	-0.106	-0.420	-0.130	-0.222
	H_O	0.643	0.643	0.643	0.714	1.000	0.929	0.288	0.786
	H_E	0.658	0.676	0.686	0.796	0.875	0.640	0.245	0.625

APPENDIX B

Sample size (N), allelic richness (A_R), observed and expected heterozygosity (H_O and H_E) and F_{IS} at seven microsatellite loci for adult samples. Bold value showed significant departure from HWE after sequential Bonferroni correction (56 comparisons).

		Ssa85	Ssa197	Ssa202	Ssa404	SSOSL25	SSOSL85	SSOSL311
E.1948A	N	51	51	41	46	50	49	51
	A_R	7.186	9.073	4.926	13.410	4.577	7.738	11.531
	F_{IS}	-0.024	+0.107	+0.259	+0.018	-0.128	+0.010	**+0.188**
	H_O	0.706	0.745	0.488	0.891	0.720	0.592	0.706
	H_E	0.683	0.826	0.648	0.898	0.633	0.592	0.859
E.1962A	N	43	43	30	39	43	41	43
	A_R	4.698	9.192	5.000	16.448	4.000	7.392	14.779
	F_{IS}	-0.050	+0.126	+0.218	+0.206	+0.034	+0.072	+0.155
	H_O	0.674	0.721	0.533	0.718	0.628	0.537	0.744
	H_E	0.636	0.815	0.668	0.890	0.642	0.571	0.869
E.1989A	N	96	96	96	96	96	96	96
	A_R	5.518	9.119	6.214	15.143	4.779	9.538	10.314
	F_{IS}	-0.035	+0.058	+0.076	+0.045	-0.065	-0.075	+0.112
	H_O	0.677	0.781	0.656	0.865	0.708	0.688	0.688
	H_E	0.651	0.825	0.706	0.901	0.662	0.636	0.770
E.1991	N	38	38	38	38	38	38	38
	A_R	4.992	8.914	4.999	11.526	4.958	6.947	8.772
	F_{IS}	+0.094	+0.110	+0.169	-0.063	-0.012	+0.162	+0.034
	H_O	0.658	0.763	0.605	0.921	0.711	0.500	0.816
	H_E	0.715	0.845	0.717	0.856	0.693	0.587	0.833
E.1992A	N	90	90	90	90	90	90	90
	A_R	7.095	9.192	6.143	15.454	6.203	7.085	9.009
	F_{IS}	-0.047	+0.034	-0.157	-0.030	-0.065	-0.138	-0.014
	H_O	0.756	0.800	0.756	0.944	0.756	0.578	0.778
	H_E	0.718	0.823	0.650	0.912	0.706	0.505	0.763
E.2005A	N	92	92	92	90	92	92	91
	A_R	6.592	9.245	5.652	13.881	4.987	7.436	8.600
	F_{IS}	-0.023	+0.052	-0.022	-0.030	+0.054	+0.053	-0.012
	H_O	0.728	0.772	0.707	0.933	0.663	0.500	0.758
	H_E	0.708	0.809	0.687	0.902	0.697	0.525	0.745
K.1989	N	96	96	96	96	96	96	96
	A_R	8.905	10.477	8.075	19.865	5.506	7.364	10.353
	F_{IS}	+0.083	+0.059	-0.060	+0.042	-0.048	+0.001	+0.016
	H_O	0.698	0.813	0.729	0.896	0.708	0.667	0.760
	H_E	0.757	0.859	0.685	0.930	0.672	0.664	0.768
HB.1992	N	75	75	75	74	75	75	75
	A_R	7.028	10.360	7.841	17.683	5.512	8.255	12.216
	F_{IS}	-0.096	+0.013	+0.008	+0.018	-0.094	+0.064	+0.035
	H_O	0.747	0.853	0.693	0.919	0.733	0.560	0.827
	H_E	0.677	0.859	0.694	0.929	0.666	0.594	0.851

APPENDIX C

Allele frequency table of each locus and each sample, including a sample of 3 brown trout and possible salmon/trout hybrids in few samples (see text). *N* denotes sample size and NA stands for not available.

Locus	E.1962A		E.1991A		E.2005A		E.2002aP		H.1990P		H.2002aP		S.1990P		K.1989		Trout
	E.1948A		E.1989A		E.1992A		E.1990P		E.2002bP		H.1991P		H.2002bP		S.2002P		HB.1992	
SSOSL25																		
(*N*)	50	43	97	38	90	94	39	48	51	34	40	46	53	69	18	96	75	3
121	0	0	0	0	0	0	0	0	0	0	0.013	0	0	0	0.028	0	0	0
123	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.167
125	0	0	0.005	0	0	0.005	0	0	0	0	0	0	0	0	0.028	0	0	0.5
127	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.056	0	0	0.333
133	0	0	0	0	0	0.005	0	0	0	0	0	0	0	0	0.056	0	0	0
145	0	0	0	0	0.006	0	0	0	0	0	0	0	0	0	0	0	0	0
149	0.14	0.081	0.124	0.092	0.144	0.096	0.077	0.146	0.196	0.044	0.063	0	0.028	0.015	0.028	0.052	0.08	0
151	0.31	0.442	0.268	0.316	0.333	0.372	0.346	0.427	0.333	0.309	0.375	0.261	0.453	0.442	0.194	0.406	0.393	0
153	0.5	0.384	0.485	0.421	0.394	0.372	0.410	0.323	0.382	0.441	0.375	0.544	0.396	0.377	0.444	0.380	0.407	0
155	0.04	0.093	0.093	0.145	0.078	0.090	0.103	0.094	0.059	0.059	0.075	0.109	0.094	0.094	0.167	0.120	0.08	0
157	0	0	0	0	0.006	0	0	0	0	0	0	0	0	0	0	0	0	0
165	0.01	0	0.026	0.026	0.028	0.059	0.064	0.010	0.029	0.147	0.1	0.087	0.028	0.073	0	0.026	0.027	0
167	0	0	0	0	0.006	0	0	0	0	0	0	0	0	0	0	0	0	0
171	0	0	0	0	0.006	0	0	0	0	0	0	0	0	0	0	0.016	0.013	0
SSOSL85																		
(*N*)	49	41	97	38	90	94	38	48	51	34	40	46	53	69	18	96	75	NA
179	0	0	0	0	0	0	0	0	0	0	0.013	0	0	0	0.028	0	0	
181	0.031	0	0.026	0.026	0.039	0.037	0.013	0.010	0.010	0.015	0.038	0.011	0	0.015	0.028	0.016	0.027	
183	0.602	0.634	0.588	0.618	0.689	0.676	0.592	0.594	0.657	0.677	0.613	0.717	0.764	0.630	0.722	0.531	0.613	
185	0	0	0.021	0	0.006	0	0.013	0.010	0.010	0	0	0.011	0	0.094	0	0	0.013	
187	0	0	0.005	0	0	0	0	0	0	0	0	0	0	0	0	0.010	0	
189	0.061	0.073	0.062	0.053	0.089	0.075	0.053	0.125	0.020	0.015	0.063	0.098	0.009	0.073	0	0.104	0.033	
191	0.194	0.110	0.083	0.118	0.089	0.096	0.079	0.073	0.177	0.118	0.15	0.065	0.132	0.080	0.111	0.172	0.1	

APPENDIX C continued

193	0	0	0.026	0.040	0.017	0.021	0.040	0.115	0	0.029	0	0	0.009	0.015	0	0.109	0.12
197	0	0.024	0	0	0	0	0.013	0	0.010	0	0	0	0	0	0	0.010	0
203	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.028	0	0
207	0	0.012	0	0	0	0.005	0	0	0	0	0	0	0	0	0.056	0	0
211	0.010	0	0.026	0	0	0.016	0.040	0	0	0	0.013	0.044	0	0.065	0	0	0.013
213	0	0.012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
215	0	0	0	0	0	0	0	0	0	0	0.013	0	0	0	0	0	0
217	0.041	0.061	0.052	0.092	0.022	0.016	0.053	0.021	0.069	0	0.013	0.022	0	0	0	0.021	0.02
219	0.010	0	0.036	0	0.006	0.005	0	0	0.010	0	0	0	0.047	0	0.028	0	0.007
221	0.010	0	0	0	0	0	0	0	0	0	0	0	0.009	0	0	0	0
223	0.041	0.073	0.077	0.053	0.044	0.053	0.105	0.052	0.039	0.147	0.088	0.033	0.028	0.029	0	0.026	0.053
SSOSL311																	
(*N*)	51	43	97	38	90	93	39	48	51	34	40	46	53	69	18	96	75	3
121	0	0.012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
125	0.020	0.012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
127	0.206	0.151	0.134	0.171	0.25	0.194	0.154	0.052	0.216	0	0.05	0.174	0.076	0.065	0.111	0.427	0.193	0
129	0.088	0.163	0.052	0	0.05	0.054	0.051	0.052	0.010	0.147	0.138	0.065	0.094	0.101	0	0.031	0.073	0
131	0.118	0.093	0.057	0.132	0.05	0.027	0.039	0.042	0.059	0.044	0.113	0.044	0.009	0.080	0.028	0.037	0.067	0
133	0.039	0.035	0.021	0.105	0.017	0.054	0.026	0.021	0.039	0	0.013	0.022	0	0.007	0	0.005	0.013	0
135	0	0	0	0	0.006	0	0	0	0	0	0	0	0	0	0.028	0	0.007	0.333
143	0	0.023	0	0	0	0	0	0.010	0	0	0.013	0	0	0	0	0.010	0	0
145	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.111	0	0	0.167
146	0	0	0.005	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
147	0	0.023	0	0	0.006	0.005	0	0	0	0	0	0	0	0	0.028	0.042	0.02	0.333
149	0.039	0.023	0.062	0.040	0.05	0.011	0.039	0.021	0.128	0.015	0.1	0.065	0.038	0.058	0.056	0.047	0.033	0
151	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.167
153	0.059	0.035	0.026	0.053	0	0.016	0	0.135	0.010	0	0.013	0.022	0	0.036	0.028	0.021	0.013	0
155	0	0.012	0	0	0	0	0	0	0	0.015	0.013	0	0	0	0	0	0.007	0
157	0.128	0.151	0.160	0.184	0.167	0.194	0.205	0.125	0.147	0.191	0.088	0.130	0.217	0.181	0.111	0.078	0.167	0
159	0.010	0.012	0.005	0	0	0.005	0.039	0.021	0	0	0	0	0	0.065	0	0	0.007	0
161	0.049	0.012	0.036	0.040	0.017	0.032	0.039	0.104	0.010	0.015	0.038	0.054	0.179	0.073	0.056	0.057	0.067	0
163	0.010	0.012	0.016	0.013	0.006	0.005	0.013	0	0	0	0.013	0	0	0	0	0.083	0.06	0
167	0.235	0.221	0.412	0.263	0.378	0.403	0.397	0.417	0.382	0.574	0.413	0.424	0.377	0.326	0.444	0.162	0.253	0
169	0	0.012	0.010	0	0.006	0	0	0	0	0	0	0	0.009	0.007	0	0	0.013	0

APPENDIX C continued

171	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.007	0
175	0	0	0.005	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ssa85																	
(*N*)	51	43	97	38	90	94	39	48	51	34	40	46	53	69	18	96	75	3
111	0	0	0	0	0	0	0	0	0	0	0.013	0	0	0	0.056	0	0	0.833
115	0	0	0.005	0	0	0	0	0	0	0	0	0	0	0	0.056	0	0	0
117	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.083	0	0	0.167
119	0	0	0	0	0	0.005	0	0	0	0	0	0	0	0	0	0.005	0	0
121	0	0.012	0.005	0	0	0.005	0	0	0	0	0	0	0	0	0	0	0	0
123	0.010	0	0.016	0	0.028	0.016	0	0	0	0	0	0	0	0.007	0	0.005	0.007	0
127	0	0	0	0.040	0.006	0	0	0.010	0	0.015	0.025	0	0	0	0	0.042	0.007	0
129	0	0	0	0	0	0	0	0.010	0	0	0	0	0	0	0	0.010	0	0
131	0.010	0	0	0	0.006	0.011	0	0	0	0	0	0	0	0	0	0	0	0
133	0.5	0.547	0.523	0.434	0.444	0.457	0.462	0.542	0.343	0.368	0.363	0.5	0.340	0.529	0.361	0.380	0.507	0
135	0.010	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.047	0.02	0
137	0.128	0.128	0.170	0.211	0.211	0.160	0.231	0.1771	0.186	0.309	0.25	0.185	0.302	0.217	0.111	0.271	0.127	0
139	0.020	0	0.010	0	0.006	0.016	0	0	0.078	0.118	0.038	0	0	0.007	0	0.005	0.007	0
141	0.177	0.151	0.108	0.171	0.156	0.170	0.167	0.156	0.284	0.044	0.2	0.217	0.217	0.109	0.083	0.100	0.12	0
143	0	0	0	0	0.006	0	0	0	0	0	0	0	0	0	0	0.016	0	0
145	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.007	0
147	0.010	0	0	0	0.006	0	0	0	0	0	0	0	0	0	0	0	0.013	0
149	0.137	0.163	0.160	0.145	0.122	0.154	0.141	0.104	0.108	0.147	0.113	0.098	0.142	0.130	0.25	0.104	0.187	0
153	0	0	0	0	0.011	0.005	0	0	0	0	0	0	0	0	0	0	0	0
155	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.005	0	0
159	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.010	0	0
Ssa197																		
(*N*)	51	43	97	38	90	94	39	48	51	34	40	46	53	53	18	96	75	3
131	0	0	0	0	0	0.005	0	0	0	0	0.013	0	0	0	0.056	0	0	0.333
139	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.111	0	0	0.667
167	0	0	0.005	0	0.006	0	0	0	0	0	0	0	0	0	0	0.010	0.02	0
171	0	0	0	0	0	0	0	0	0	0.015	0	0	0	0	0	0	0	0
179	0.010	0.012	0.016	0.079	0.017	0.059	0	0.010	0.039	0	0.025	0	0.009	0.009	0	0.129	0.093	0
183	0.216	0.279	0.227	0.211	0.178	0.197	0.167	0.25	0.245	0.088	0.238	0.098	0.321	0.321	0.111	0.047	0.12	0

APPENDIX C continued

187	0.039	0.035	0.016	0.053	0.044	0.037	0.103	0.031	0.010	0.044	0.088	0	0.019	0.019	0	0.010	0.007	0
191	0.118	0.023	0.098	0.026	0.089	0.059	0.051	0.083	0.098	0.162	0.05	0.130	0.028	0.028	0.056	0.125	0.16	0
195	0.177	0.186	0.088	0.145	0.094	0.101	0.090	0.052	0.108	0	0.038	0.076	0.028	0.028	0	0.073	0.127	0
199	0.010	0.023	0.010	0.026	0.006	0.021	0.039	0.021	0	0.088	0.013	0	0.009	0.009	0.056	0.037	0.047	0
203	0.088	0.163	0.165	0.171	0.189	0.154	0.180	0.260	0.235	0.206	0.188	0.152	0.274	0.274	0.389	0.120	0.12	0
207	0.049	0.012	0.036	0	0.028	0.016	0.026	0	0	0.029	0.013	0	0	0	0	0.104	0.027	0
211	0.265	0.198	0.273	0.211	0.294	0.325	0.321	0.281	0.245	0.309	0.275	0.522	0.293	0.293	0.222	0.266	0.24	0
215	0.029	0.070	0.067	0.079	0.056	0.027	0.026	0.010	0.020	0.059	0.063	0.022	0.019	0.019	0	0.078	0.033	0
219	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.010	0.007	0
Ssa202																		
(*N*)	41	30	97	38	90	94	39	48	51	34	40	46	53	69	18	96	75	NA
240	0	0	0.005	0	0	0	0	0	0	0	0	0	0	0.073	0.056	0.026	0.013	
244	0	0	0.005	0	0.006	0	0	0	0	0	0	0	0	0	0.056	0.016	0.013	
248	0	0	0.005	0	0.006	0	0	0	0	0	0.013	0	0	0	0	0.041	0.047	
252	0.024	0.033	0.072	0.053	0.017	0.043	0.077	0.031	0.108	0	0.013	0.022	0	0.036	0	0.109	0.053	
256	0.134	0.2	0.263	0.171	0.211	0.181	0.192	0.302	0.088	0.235	0.188	0.207	0.123	0.290	0.361	0.037	0.133	
260	0.488	0.483	0.433	0.408	0.522	0.489	0.397	0.417	0.422	0.471	0.45	0.391	0.708	0.261	0.333	0.510	0.493	
264	0.049	0.05	0.046	0.092	0.044	0.069	0.051	0.063	0.029	0.015	0.013	0.087	0.009	0.036	0	0.057	0.04	
268	0.305	0.233	0.165	0.276	0.172	0.202	0.269	0.177	0.343	0.265	0.313	0.294	0.160	0.304	0.194	0.188	0.193	
272	0	0	0.005	0	0.022	0.016	0.013	0.010	0.010	0.015	0.013	0	0	0	0	0.016	0.013	
Ssa404																		
(*N*)	46	39	97	38	90	92	39	48	51	34	40	46	53	69	18	96	74	NA
189	0	0	0	0	0.006	0	0	0	0	0	0	0	0	0	0	0	0	
205	0	0.013	0.010	0	0.006	0	0.013	0	0.029	0	0	0	0	0	0	0.057	0.054	
209	0	0	0.005	0	0	0	0	0	0	0	0	0	0	0	0	0.021	0.007	
213	0.033	0.039	0.016	0.066	0.044	0.076	0.064	0.125	0.020	0.059	0.05	0.065	0.038	0.073	0.111	0.057	0.047	
217	0	0	0.005	0	0.006	0.005	0	0	0	0.044	0	0	0	0	0	0.026	0.014	
221	0	0	0	0	0.011	0.022	0	0	0	0.015	0.025	0.011	0.019	0	0	0	0	
225	0.011	0.013	0.005	0	0	0	0	0	0	0	0	0	0	0	0	0.042	0.007	
227	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.028	0	0	
229	0.152	0.231	0.103	0.158	0.111	0.163	0.192	0.115	0.265	0.044	0.113	0.109	0.094	0.058	0	0.125	0.101	
233	0.130	0.064	0.098	0.092	0.094	0.033	0.064	0.052	0.039	0.029	0.088	0.044	0.208	0.109	0.139	0.037	0.047	
237	0	0.013	0.036	0	0.039	0.022	0.026	0.063	0	0.044	0	0.033	0.009	0.022	0	0.047	0.034	

APPENDIX C continued

241	0.011	0.013	0.021	0	0.028	0	0.026	0.010	0	0	0.063	0.022	0	0.065	0	0.042	0.041
245	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.014
249	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.005	0
253	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.021	0
257	0	0.026	0	0	0	0.011	0	0	0	0	0	0	0	0	0	0.021	0
261	0.011	0.064	0.036	0.013	0.028	0.092	0.026	0.125	0.078	0.132	0.15	0.054	0.104	0.015	0	0.151	0.014
265	0.120	0.115	0.186	0.053	0.117	0.130	0.064	0.146	0.039	0.118	0.163	0.141	0.066	0.087	0	0.026	0.088
269	0.076	0.026	0.041	0.053	0.033	0.005	0.077	0	0	0.059	0	0.033	0.076	0.015	0.111	0.021	0.041
273	0.109	0.115	0.072	0.276	0.144	0.092	0.167	0.156	0.177	0.044	0.025	0.065	0.057	0.058	0.25	0.042	0.122
277	0.076	0.064	0.134	0.105	0.106	0.098	0.141	0.063	0.078	0.221	0.1	0.217	0.142	0.181	0.167	0.021	0.081
281	0.033	0	0.031	0.040	0.017	0.038	0.013	0.010	0.049	0.044	0.038	0.011	0.038	0.065	0.056	0.026	0.061
285	0.011	0.026	0.016	0	0.044	0.005	0.013	0	0.010	0	0.013	0.011	0	0.015	0	0.083	0.041
289	0.098	0.051	0.103	0.026	0.067	0.054	0.051	0.031	0.049	0.088	0.1	0.152	0.104	0.073	0.139	0.057	0.095
293	0.109	0.103	0.067	0.105	0.089	0.125	0.064	0.104	0.147	0.059	0.075	0.011	0.047	0.152	0	0.021	0.068
297	0	0.013	0.005	0	0	0	0	0	0.010	0	0	0	0	0	0	0	0
301	0.022	0.013	0.010	0.013	0.006	0.027	0	0	0.010	0	0	0.022	0	0.015	0	0.037	0.020
305	0	0	0	0	0.006	0	0	0	0	0	0	0	0	0	0	0.016	0.007
Ssa405																	
(*N*)	NA	NA	NA	NA	NA	NA	39	48	51	34	39	46	53	69	14	NA	NA	NA
300							0	0	0	0.015	0	0	0.009	0	0			
316							0	0	0	0	0	0	0.009	0	0			
344							0	0.010	0.029	0	0	0.011	0	0	0			
348							0.090	0.156	0.118	0.044	0.039	0.152	0.009	0.130	0.071			
352							0.115	0.104	0.069	0.044	0.128	0.130	0.340	0.138	0.071			
356							0.180	0.167	0.226	0.074	0.115	0.109	0	0.109	0.143			
360							0.051	0.010	0.039	0.118	0.103	0.011	0.019	0.036	0.107			
364							0.013	0.010	0	0	0.013	0.011	0	0	0			
368							0.103	0.094	0.029	0.206	0.141	0.185	0.264	0.246	0.143			
372							0.013	0.031	0.020	0	0	0	0.076	0	0.036			
376							0.077	0.135	0.216	0.118	0.103	0.130	0.038	0.073	0.071			
380							0.013	0	0	0	0.039	0.022	0.028	0	0.071			
384							0.039	0.031	0.020	0.015	0.013	0	0	0.015	0			
388							0.026	0.010	0.020	0.029	0.026	0	0	0.007	0			
392							0.013	0	0.020	0	0.013	0.011	0	0.007	0			

APPENDIX C continued

396	0.192	0.156	0.177	0.25	0.154	0.109	0.151	0.138	0.071
400	0.064	0.073	0.020	0.015	0.039	0.076	0.028	0.044	0.214
404	0	0.010	0	0	0.051	0.022	0	0.015	0
408	0.013	0	0	0.074	0.026	0.022	0.028	0.044	0