Coastal and freshwater pikeperch (*Sander lucioperca*) populations differ genetically in the Baltic Sea basin

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Microsatellite DNA based analysis of the pattern of genetic diversity among three coastal and five freshwater populations of pikeperch *Sander lucioperca* in the northern part of the Baltic Sea drainage basin indicated marked genetic differentiation between the coastal and lake populations. The F_{st} between these population groups was as high as 0.25 and R_{st}=0.32. In general, the lake populations showed higher genetic diversity than the coastal ones. In terms of genetic distance, the three coastal populations (Vanhankaupunginlahti, Västanfjärd and Taivassalo) grouped tightly together. The freshwater samples formed a looser group, in which the northern Lake Kemijärvi showed greater distance from the southern lakes than these did from each other. The two lake populations originally established through stockings (Lakes Painio and Averia) grouped near to their source population of Lake Lohjanjärvi and their diversity level was nearly the same. Safeguarding the unique Baltic coastal populations of *S. lucioperca* against gene flow from increasing hatchery releases using freshwater *S. lucioperca* should be a high management priority.

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The pikeperch (*Sander lucioperca*) is a Eurasian freshwater species that is widely distributed in watercourses flowing into the Baltic Sea, Caspian Sea and Black Sea. The southern limit of the species' native range extends from south of the Alps to the Pyrenees, and the western limit is the River Elbe running to the North Sea. The *S. lucioperca* did not originally occur in any rivers entering the Arctic Ocean, but has been introduced to some of the river systems of northern Russia. In western Europe, the *S. lucioperca* has been introduced in France, Belgium, the Netherlands and Great Britain. Introduced populations also live in Turkey and Morocco (Deelder and Willemsen 1964). Although the *S. lucioperca* thrives in warm freshwater, it also endures limited salinity and forms specific local populations in coastal sea areas where the salinity is not too high (about <5 psu, practical salinity units; Winkler et al. 1989; Lehtonen et al. 1996) and warm shallow spawning areas are available in the spring. Brackish water populations are thus widely distributed in the estuaries and coastal areas of the Baltic, Black and Caspian Seas.

Despite the detailed bio-geographical, and gradually accumulating genetic knowledge of the Eurasian *S. lucioperca* populations (Poulet et al. 2004, 2009; Björklund et al. 2007), no plausible hypothesis has to our knowledge been suggested concerning the potential postglacial colonization routes of the species. Although the *S. lucioperca* tolerates brackish water, re-colonization most likely started from some of the postulated freshwater refuges (Nesbø et al. 1999), together with certain other freshwater species. In a recent study of the microsatellite variation of *S. lucioperca* in Scandinavia (Björklund et al. 2007), strong genetic structuring was observed, potentially indicating long-term isolation patterns related to colonization histories.

In Finland, the *S. lucioperca* is among the economically most valuable fish species and is important for both commercial and recreational fishermen. Due to the high fishing pressures, many Finnish *S. lucioperca* populations have been subjected to growth over-fishing (Heikinheimo et al. 2006) and some of them even to recruitment over-fishing (Colby and Lehtonen 1994), despite the numerous management measures taken to regulate fishing, including minimum landing sizes, mesh-size regulations, restricted seasons and protected areas. Extensive enhancement and re-stocking programs using pond-reared juveniles have also been carried out to even out natural fluctuations in recruitment, to mitigate the effects of over-fishing or to create new fishing opportunities (Ruuhijärvi et al. 1996).

In economic terms, the stocking programs have generally been considered as sustainable: catches have increased and several new fishable populations have been established (Lehtonen et al. 1984; Ruuhijärvi et al.

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1996, 2005; Salminen and Ruuhijärvi 2004). Genetically, the releases may have been less sustainable. Due to insufficient control and planning of the releases and the low number of commercial pikeperch hatcheries, practically all stocking programs have relied on only three to four different parent populations, all of them with a southern origin. This practice has probably resulted in a large-scale loss of the original genetic diversity within the species. During the last two decades most Finnish pikeperch populations, especially those living in freshwater systems, have been subjected to the flow of foreign genes through hatcheries (Toivonen et al. 1981; Fig. 1).

The recent boom in hatchery releases has not yet reached coastal areas, mainly due to the continuously relatively high natural recruitment of the brackish water populations (Raitaniemi and Manninen 2007), promoted by the continuing eutrophication of the Baltic Sea. This offers the managers of the coastal Baltic Sea fisheries an invaluable opportunity to once more thoroughly consider the long-term sustainability of the suggested future enhancement and re-stocking programs, not only in economic but also in genetic terms. The value of the Baltic Sea as a unique environment has been widely recognized (e.g. HELCOM Baltic Sea Action Plan, <www.helcom.fi>) and preserving the genetic diversity of the fish populations adapted to the specific conditions of the Baltic Sea should be one of the main priorities at all levels of decision making.

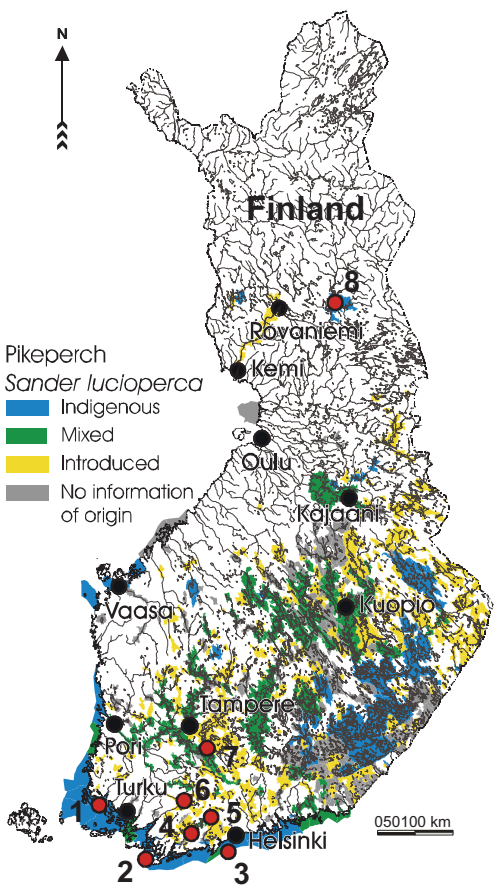

Fig. 1. The occurrence of pikeperch stocks in Finland and the locations of pikeperch sampling sites. Numbers 1–8 refer to samples described in Table 1.

Detailed knowledge of the genetic structure of the exploited fish populations is a prerequisite for their successful long-term management. The aim of this study was to provide better tools for the evaluation of different fisheries management alternatives in coastal areas by describing microsatellite DNA variation in Finnish pikeperch populations and to assess the level of genetic diversity and especially the potential differentiation between coastal and freshwater populations. The genetic structure devised from neutral marker variation indicates the general level of isolation and the time that has been available for adaptive evolution.

MATERIAL AND METHODS

Samples

Eight pikeperch populations were sampled for the genetic analyses, three of them representing the coastal and five the freshwater distribution of the species (Table 1, Fig. 1). As the aim was to investigate the natural genetic differentiation of the species, i.e. differentiation preceding the present boom in hatchery releases, and thus before major human impact, the sampled populations were chosen on the basis of their recorded management history (Halme 1961, 1962; Toivonen et al. 1981), and the availability of representative samples. For some cases, old scale samples were used to avoid the influence of recent gene flow from hatchery releases.

Coastal, brackish water populations

Taivassalo (Fig. 1, sample 1) and Västanfjärd (sample 2) are important pikeperch fishing areas in the Finnish Archipelago Sea, whereas Vanhankaupunginlahti (sample 3) is the spawning estuary of a pikeperch population in the Gulf of Finland (Fig. 1). Tagging studies have indicated that pikeperch living in the different bays, separated by chains of islands, belong to distinct spawning populations (Lehtonen 1985). Due to their relatively high recruitment during the last 15 years (Raitaniemi and Manninen 2007), the coastal populations have so far not been affected by significant hatchery releases and, therefore, fresh samples from the early 2000s could be used in the analyses.

Table 1. *Site, sample size, sampling year, year classes and origin of studied pikeperch samples.*

Site	n	Year	Year classes	Origin
Coastal samples				
1 Taivassalo	60	2001–2005	1994–1999	native
2 Västanfjärd	60	2003–2004	1995–2001	native
3 Vanhankaupunginlahti	60	2004–2005	1997–2002	native
Lake samples				
4 Lake Lohjanjärvi	60	1982–1986	1979–1981	native
5 Lake Averia	63	1984	1979–1982	introduced
6 Lake Painio	74	2006	1985–2005	introduced
7 Lake Vanajanselkä	60	1988–1991	1984–1986	native
8 Lake Kemijärvi	60	2006	1998–2003	native
All	497			

Freshwater populations

Lake Lohjanjärvi (Fig. 1, sample 4) is a medium-sized lake (8900 ha) supporting important pikeperch fisheries. The native pikeperch population of the lake (Toivonen et al. 1981) has since 1991 been stocked with hatchery fingerlings from three foreign lake populations: Lakes Averia (sample 5), Painio (sample 6), and Vanajanselkä (sample 7) (Salminen et al. 2005). Therefore, old scale samples representing the still intact year classes 1979–1981 (20 individuals from each year class) were used in the analyses.

Lakes Averia and Painio represent typical smaller (140 ha and 780 ha, respectively) Finnish pikeperch lakes. The pikeperch populations living in these lakes are not native, but have their roots in the old introductions of the late 1800s and early 1900s (Halme 1961, 1962; Toivonen et al. 1981). Until the 1970s, pikeperch had been successfully introduced to a total of 94 lakes in southern and central Finland (Toivonen et al. 1981). In the case of Lake Painio, the pikeperch were transferred as adult fish in 1932 and as fertilized eggs in 1938 from Lake Lohjanjärvi (Halme 1961, 1962). The source population of Lake Averia is not known (introduction in 1930–1950; Toivonen et al. 1981), but Lake Lohjanjärvi is also here the most probable candidate.

Since the late 1980s, both Lake Averia and Lake Painio have been used as sources of brood fish for large-scale hatchery fingerling production, and as viable populations with continuously relatively stable recruitment they have not been affected by releases of foreign pikeperch populations. For Lake Averia, the sample represents the year classes 1979–1982 (4–35 ind./year class), and for Lake Painio, the year classes 1985–2005 (0–16 ind./year class).

Lake Vanajanselkä is the largest lake (10 300 ha) of the watercourse inhabited by native pikeperch populations (Toivonen et al. 1981). With a continuously relatively strong and stable pikeperch population, Lake Vanajanselkä supports important pikeperch fisheries and has also been used as a parent population for large-scale hatchery fingerling production (Ruuhijärvi et al. 1996). Despite the good condition of the native population, and the good availability of indigenous stocking material, large-scale hatchery releases using a foreign parent population (from Lake Painio) have recently been carried out in Vanajanselkä. Therefore, old scale samples from the intact year classes 1984–1986 (20 ind./year class) were used in the analyses.

Lake Kemijärvi (Fig. 1, sample 8) (23 000 ha) is one of northernmost native pikeperch lakes in Scandinavia (Toivonen et al. 1981). Until the 1970s the pikeperch population of Lake Kemijärvi was relatively strong and supported important local fisheries, but has since then decreased to a very low level. Despite the present poor condition of the population, no hatchery releases have so far been carried out in Lake Kemijärvi. Hence, fresh samples from the year classes 1998–2003 (1–40 ind./year class) were used in the analyses.

DNA analysis

Total genomic DNA was extracted from fin, muscle or scale samples by using a DNA DNeasy 96 Blood and Tissue Kit (Qiagen, <www1.qiagen.com>). Variation in the following 12 microstellite loci was determined: *PflaL2, PflaL3, PflaL8, PflaL9* (Leclerc et al. 2000), *Svi4, Svi6, Svi18, Svi33* (Borer et al. 1999), *SviL7, SviL8, SviL9* and *SviL11* (Wirth et al. 1999) (Table 2).

PCR were performed in a 10 µl reaction volume containing 1 × buffer for DyNAtzyme (Finnzymes, Espoo, Finland, <www.finnzymes.com>), 0.2 mM of each dNTP, 5 pmol (*PflaL3, PflaL8, PflaL9, Svi4, SviL7, SviL8, SviL9, SviL11, Svi18* and *Svi33*) or 10 pmol (*PflaL2* and *Svi6*) of each forward and reverse primer (forward primer fluorescently labeled), 0.1 U (*PflaL8, SviL9* and *Svi18*),

Table 2. *Analyzed microsatellite loci. Type of repeat sequence, number of observed alleles (N_a), allele frequency variance over samples (Nei's G_{st} and F_{st}), total heterozygosity (H_t), and mean heterozygosity (H_s) for each microsatellite locus and mean F_{st} between coastal and lake populations for each locus in Finnish pikeperch samples.*

Locus	Repeat sequency	Size	N_a	G_{st}	F_{st}	H_t	H_s	F_{st} coastal/lake
1 *PflaL2*	$(CA)_{23}$	209–229	7	0.34	0.38	0.45	0.30	0.37
2 *PflaL3*	$(TG)_{18}$	101–119	8	0.15	0.15	0.34	0.29	0.18
3 *PflaL8*	$(TG)_{39}$	167–203	16	0.30	0.32	0.72	0.51	0.42
4 *PflaL9*	$(TG)_{24}$	182–214	4	0.20	0.22	0.65	0.52	0.31
5 *Svi4*	$(AC)_{16}$	120–166	15	0.07	0.07	0.70	0.65	0.09
6 *Svi6*	$(AC)_6$	115–165	19	0.18	0.19	0.61	0.50	0.27
7 *SviL7*	$(TG)_{22}$	201–249	17	0.12	0.13	0.64	0.56	0.15
8 *SviL8*	$(TG)_{22}$	107–145	8	0.40	0.30	0.34	0.20	0.11
9 *SviL9*	$(CA)_{18}AA(CA)_3A(AC)_4$	161–223	10	0.06	0.01	0.17	0.17	0.01
10 *SviL11*	$(TG)_{26}G(TG)_8$	115–121	3	0.04	0.03	0.12	0.12	0.03
11 *Svi18*	$(AC)_{18}$	132–182	10	0.11	0.12	0.67	0.59	0.11
12 *Svi33*	$(AC)_{14}$	75–83	3	0.16	0.18	0.25	0.21	0.14
Mean allover			10	0.19	0.18	0.47	0.38	0.18
Min			3	0.01	0.04	0.12	0.12	0.01
Max			19	0.38	0.40	0.72	0.65	0.42

0.2 U (*PflaL2, PflaL3, PflaL9, Svi4, Svi6, SviL7, SviL8* and *SviL11*) or 0.3 U (*Svi33*) of DyNAzyme II DNA Polymerase (Finnzymes, Espoo, Finland) and 15–25 ng of genomic DNA.

PCR were carried out in a thermal cycler (MJ Research) and the temperature profile of the PCR program was at 94°C for 4 min, followed by 30 cycles at 94°C for 1 min, the locus-specific annealing temperature for 1 min, elongation at 72°C for 1 min, and a final elongation step at 72°C for 10 min. Locus-specific annealing temperatures were 50°C for *Svi6*, 53°C for *PflaL2, PflaL3, PflaL8, SviL7, SviL8* and *SviL11,* 58°C for *PflaL9* and *SviL9*, and 60°C for *Svi4, Svi18* and *Svi33*. An ABI 3130 Genetic Analyzer was used for genotyping and allele sizes were determined with GeneMapper 4.0 software (Applied Biosystems, <www3.appliedbiosystems.com/AB_Home/index.htm>).

Statistical analysis

The numbers of alleles in samples were compared by a rarefaction-based allelic richness measure (A_r; El Mousadik and Petit 1996; Petit et al. 1998) that was calculated with FSTAT software ver. 2.9.3 (Goudet 2001). The program calculates allelic richness for the smallest number of individuals typed for any locus, which in the present study was 31 individuals for 11 loci (locus *SviL9* excluded). Allele frequency variances over samples were estimated as G_{st} (Nei 1973), and heterozygosity with the aid of DISPAN software (Ota 1993).

Exact tests for the Hardy–Weinberg (H-W) equilibrium (Guo and Thompson 1992) and population differentiation were analyzed with the GENEPOP 3.2 software package (Raymond and Rousset 1995) with Markov chain parameters, 300 batches and 3000 iterations. Probabilities of H-W equilibrium tests for samples were adjusted over loci using the sequential Bonferroni procedure for multiple tests (Rice 1989).

The expected heterozygosity level in each sample was calculated using Popgene ver. 1.32 (Yeh and Boyle 1997). Analysis of the differences between samples was based on allele frequency differences, using pairwise F_{st} values (Weir and Cockerham 1984), which were estimated with FSTAT ver. 2.9.3 (Goudet 2001). Standard deviations and confidence intervals were estimated through bootstrapping. R_{st} values and an allele size randomisation test (Hardy et al. 2003) implemented in the program SPAGeDi 1.1.b (Hardy and Vekemans 2002) was used to test whether stepwise mutations have contributed to the genetic differentiation among populations, i.e. whether $R_{st} > F_{st}$. A significant outcome of the test suggests that populations have diverged for a sufficiently long time for mutations to have contributed significantly to differentiation, which could be the case if populations have originated from different glacial refugia. Genetic distances between samples were calculated using Nei's D_A distances (Nei et al. 1983). A phylogenetic tree was constructed using a neighbour joining (NJ) algorithm (Saitou and Nei 1987) with DISPAN software (Ota 1993). Bootstrapping with 1000 replicates was used to test the statistical strength of the branches. The file was converted into New Hampshire format with NJBAFD (Takezaki 1998) and the tree was drawn with TreeView ver. 1.6.1 (Page 2000). To describe the level of

genetic differentiation in all populations and in the case when only brackish and freshwater samples were treated separately a GeneClass self-assignment test was done (Cornuet et al. 1999), with leave-one-out procedure and Bayesian option.

RESULTS

Amount of genetic diversity

The amount of genetic diversity was in general relatively high. The number of alleles in the studied loci varied from 3 to 19, with an average of 10 alleles/locus (Table 2). The diversity of the loci varied considerably, with G_{st} over all samples being ten times higher for *SviL8* (0.40) than for *SviL11* (0.04). G_{st} over loci was above the mean value at four loci: *PflaL2, PflaL8, PflaL9* and *Svi8L*. Total diversity (H_t) among loci varied between 0.12 (*SviL11*) and 0.72 (*PflaL8*). The differentiation between coastal, brackish water and lake population samples was clearest at four loci, *PflaL2, PflaL8, PflaL9* and *Svi6* (Table 2), from which *PflaL2* and *PflaL8* especially differentiated these two forms.

The overall mean heterozygosity (H_e) over loci and samples was 0.39 and allelic richness (A_r) 5.3 (Table 3). The most variable was the Lake Vanajavesi pikeperch sample, with a heterozygosity of 0.46 and average allelic richness of 5.5. This southern Lake Vanajavesi population also had the highest number (9) of private alleles (alleles that are present in only one population). The least variable was the coastal Taivassalo sample, with a mean heterozygosity of 0.30 and an average allelic richness of 3.3. Statistically significant differences in heterozygosity were observed between the Lake Vanajavesi sample and the coastal samples of Taivassalo and Västanfjärd ($p < 0.05$). Statistically significant deviations in Hardy–Weinberg equilibrium occurred in three samples. In Painio sample there was a deficiency of heterozygotes ($F_{is} = 0.03$), and in coastal samples Taivassalo ($F_{is} = –0.10$) and Västanfjärd ($F_{is} = –0.12$) excess of heterozygotes, indicating possibly subpopulation structure in the Painio sample and mixing of breeding populations in the coastal samples.

The average mean heterozygosity for the coastal samples was lower (0.34) than for the lake samples (0.42) (Table 3), but the difference was not significant. The allelic richness of coastal samples was on average lower (3.6) than that of the lake samples (4.6). This difference was also statistically significant ($p < 0.01$).

Table 3. *Mean sample size for 12 loci, mean heterozygosity (H_e), its standard error (SE), and average allelic richness based on 11 loci and 31 individuals (A_r) of the studied Finnish pikeperch populations.*

	Mean N	H_e	SE	A_r/11 Loci
Coastal				
1 Taivassalo	58.1	0.30	0.06	3.3
2 Västanfjärd	57.6	0.34	0.06	3.9
3 Vanhankaupunginlahti	55.6	0.37	0.07	3.4
Mean		0.34		3.6
Lake				
4 Lake Lohjanjärvi	53.7	0.40	0.07	4.3
5 Lake Averia	51.4	0.41	0.07	4.3
6 Lake Painio	68.8	0.36	0.08	4.2
7 Lake Vanajanselkä	51.8	0.46	0.08	5.5
8 Lake Kemijärvi	56.3	0.45	0.06	4.0
Mean		0.42		4.6
Overall mean		0.39		5.3

Genetic differentiation

Genetic differentiation was statistically highly significant between all other pairs of samples except for the geographically close coastal populations of Taivassalo and Västanfjärd. According to genetic distances, the samples grouped into two main groups: lake populations and coastal populations (Fig. 2). The average genetic distance between lake and coastal samples was 0.19, and the corresponding F_{st} was 0.25 and R_{st} as high as 0.32 (Table 4). Genetic distances within these groups were smaller, the coastal populations in particular forming a homogeneous group with the average distance among them being only 0.07 and F_{st} 0.11 (R_{st} only 0.03). R_{st} was higher than F_{st}, in all other cases, indicating mutation possibly playing a role behind the population structure, which can be regarded as sign of polyphyletic origin. According to the permutation test

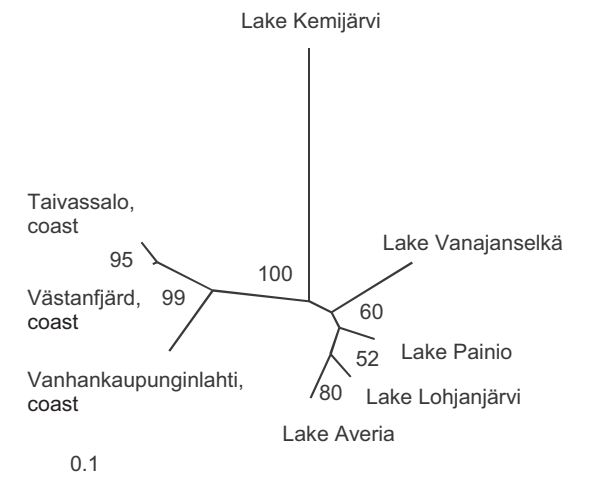

Fig. 2. Unrooted tree of genetic distances between the studied Finnish pikeperch populations based on 12 microsatellite DNA loci.

Table 4. *F_{st} and R_{st} estimates and D_A distances for Finnish pikeperch population groups.*

Groups	F_{st}	R_{st}	D_A
Between coast and lakes	0.25	0.32	0.19
within coast	0.11	0.03	0.07
within lakes	0.11	0.16	0.15
Between northern and southern lakes	0.19	0.26	0.22
within southern lakes	0.06	0.09	0.08

for allele sizes, statistically significant difference could be observed for all populations (P = 0.04). On average, introduced populations from Lakes Painio and Averia had a low F_{st} (0.03) and small genetic distance (0.06) from their likely source population of Lake Lohjanjärvi and also from each other. The smallest F_{st} and genetic distance within the lake samples was observed between Lake Lohjanjärvi and Lake Painio (0.01 and 0.05 respectively; Table 5).

All the southern lake populations were relatively similar, with an average distance of 0.08 and F_{st} 0.06, but the sample of northernmost lake population (Lake Kemijärvi) differed strongly from the southern lake populations, with F_{st} 0.19 and a D_A distance of 0.22. In general it was still more similar to lake than coastal populations (Table 5). In the dendrogramme, all the bootstrap values were over 50% and the distinction between lake and coastal samples had 100% bootstrap support (Fig. 2). If the three most variable loci, *PflaL2*, *PflaL8* and *SviL8*, were excluded from the distance analysis the grouping into two main groups remained the same and the grouping probability was still as high as 97%. When the GeneClass self-assignment test was done for all populations, the percentage of correct assignment was 72.93%, but when only coastal and lake populations were considered separately, as many as 98.59% of the individuals were correctly assigned to its source of origin, either lake or coast.

DISCUSSION

Differences between coastal and lake populations

The main finding of this study was the marked genetic differentiation between coastal and lacustrine pikeperch populations. In general, the lake populations showed higher genetic diversity than the coastal ones. The three coastal populations (Vanhankaupunginlahti, Västanfjärd and Taivassalo) also grouped tightly together in terms of genetic distance. The five lake samples formed a looser group, where the sole northern population in our study (Lake Kemijärvi) showed a greater distance from the other four.

Björklund et al. (2007) recently examined the genetic diversity of pikeperch populations within about the same geographical area as we did (i.e. around northern Baltic Sea), and there are many similarities between their results and ours. For instance, the observed allele sizes were quite similar in both studies, and the overall level of genetic diversity was also about the same. The most important finding of Björklund et al. (2007) was the variability of the populations along the north-south axis, with the northern populations being in general more diverse than the southern ones. However, the authors did not specifically mention the difference between lake and coastal populations, which we consider crucial from both scientific and management perspectives. Nevertheless, in their data the lake populations also had a higher allelic richness (average 4.7 alleles) and genetic diversity (0.55) than the coastal populations (3.5 alleles and 0.50).

The observed differentiation level between pikeperch ecotypes is higher than that of conspecific fish populations on average. The F_{st} of 0.25 is very high compared to the F_{st} among anadromous and freshwater whitefish types (*Coregonus lavaretus*), the mean of which was reported to be only 0.03 and 0.42 for two species, *Coregonus peled* and *C. lavaretus*, in a five-loci microsatellite data set (Säisä et al. 2008). It is also high when compared to the genetic diversity among Atlantic salmon

Table 5. *Estimates of D_A distances (above diagonal) and F_{st} values (below diagonal) between pairs of Finnish pikeperch populations.*

	Taivassalo	Västanfjärd	Vanhankaupunginlahti	Lake Lohjanjärvi	Lake Averia	Lake Painio	Lake Vanajanselkä	Lake Kemijärvi
Taivassalo	*	0.02	0.10	0.20	0.20	0.17	0.18	0.25
Västanfjärd	0.01	*	0.09	0.19	0.19	0.15	0.16	0.25
Vanhankaupunginlahti	0.17	0.13	*	0.17	0.18	0.16	0.17	0.28
Lake Lohjanjärvi	0.30	0.27	0.20	*	0.05	0.06	0.11	0.20
Lake Averia	0.30	0.27	0.22	0.01	*	0.07	0.11	0.22
Lake Painio	0.29	0.25	0.20	0.04	0.03	*	0.09	0.21
Lake Vanajanselkä	0.22	0.19	0.17	0.09	0.09	0.09	*	0.24
Lake Kemijärvi	0.30	0.27	0.25	0.18	0.16	0.21	0.19	*

(*Salmo salar*) populations, which similarly are depended on freshwater in their reproduction, and for which the polyphyletic origin is observed. The overall F_{st} for all studied European populations was 0.14 at 9 microsatellite loci (Säisä et al. 2005). The F_{st} for all Baltic Sea drainage populations, including three colonization lineages, was 0.11. In the data of Björklund et al. (2007) the mean F_{st} for pikeperch populations was 0.17. The high R_{st} values also support the hypothesis of polyphyletic origin. A strong genetic structuring is evident in pikeperch populations from the Baltic Sea area, either as result of selective forces or long-term isolation patterns related to colonization histories.

The northern Lake Kemijärvi was also sampled in the study of Björklund et al. (2007), and hence offers an interesting opportunity for a closer comparison of the two studies. The diversity levels observed for Lake Kemijärvi by Björklund et al. (2007), measured either as allelic richness (4.2/4.0) or as gene diversity (0.48/0.46), were in good accordance with our own observations (values from Björklund et al. and our study, respectively). However, in contrast to Björklund et al. (2007), the Lake Kemijärvi population was in the present study no more diverse than the other four (southern) lake populations. This might indicate a higher genetic diversity in Finnish, more eastern, than Swedish pikeperch populations.

Factors affecting differentiation

The potential mechanisms behind the observed genetic differentiation of pikeperch populations include postglacial colonization history, gene flow and genetic drift. The variation in neutral markers is usually not assumed to be directly linked to potential local adaptations, i.e. the ability of the populations to cope with their specific biological and physical environment. In this respect, the observed differentiation between the sea and freshwater forms of pikeperch was somewhat surprising, as in other species the ecotypic differences have not usually been reflected in the genetic distances of neutral markers. In brown trout (*Salmo trutta*), for example, the most important differentiating factor is the river system, followed by the difference between marine and freshwater ecotypes (Ryman 1983). In our study, some of the loci showed especially high divergence simply between populations of different environments, such as *Pfla2* (F_{st} 0.37) and *Pfla8* (F_{st} 0.42), possibly indicating some connection to adaptively important genes. However, the colonization history and migration pattern of brown trout and pikeperch are assumed to differ markedly. Migration and gene flow between freshwater and marine forms of brown trout is also more constant than between pikeperch ecotypes.

The differences between the freshwater and coastal populations may also stem from their different initial postglacial colonization history. The habitat requirements of pikeperch are similar to its relative, the perch (*Perca fluviatilis*). As these two percids are basically freshwater species, but tolerate brackish water, it is likely that their postglacial colonization routes have been at least partly the same. Most freshwater species have re-colonised Scandinavia from some of the large refuge lakes or lake areas, into which they were forced to withdraw during the glaciations (Hewitt 1999). Threespined stickleback has probably been an exception from this as it more readily spawns even in marine environment (Mäkinen et al. 2006). At least four potential refugia have been proposed for freshwater fishes (Nesbø et al. 1999), and the Baltic Sea drainages have been assumed and found to be a contact zone in the eastern and western distribution of several species, such as Atlantic salmon (*Salmo salar*) (Koljonen et al. 1999; Säisä et al. 2005), brown trout (García-Marín et al. 1999), grayling (*Thymallus thymallys*) (Koskinen et al. 2000) and bullhead (*Cottus gobio*) (Kontula and Väinölä 2001).

The four refugia proposed as sources of contemporary perch populations are: the Danubian area (1), the Black Sea (2), western Europe (3), and eastern Europe (4), with the Danubian population being the oldest. However, the perch populations in Fennoscandia probably only originate from the three latter refugia (2–4; Nesbø et al. 1999). When the native distribution area of pikeperch is compared to the proposed colonization routes of perch, it seems possible that pikeperch initially colonized the Baltic Sea drainage area from only the two eastern refugia, i.e. from eastern Europe and the Black Sea area. If this is actually the case, these two phylogenetic lineages may still underlie the present genetic diversity in Scandinavian pikeperch, potentially including the observed differences between the coastal and lake populations.

In addition to the initial post-glacial re-colonization that potentially already occurred during the first Baltic Sea stage, the Baltic Ice Lake (about 15 000 – 11 600 BP; Myrberg et al. 2006), the underlying differences in the genetic constitution of the coastal and lake populations may also relate to the later stages of the Baltic Sea with their different salinities, temperatures and water levels. Instead of the relatively cold Baltic Ice Lake, or the saline Yoldia Sea (about 11 600 – 10 800 BP), the original distribution of pikeperch in northern Europe has been suggested to be related to the Lake Ancylus (freshwater) stage (Lehtonen et al. 1996), which apparently provided the species a favorable habitat from 10 800 to 9 000 BP and a distribution channel to areas covered by the former lake, i.e. up to 100 – 150 meters above the present water level of the Baltic Sea. The history of the coastal distribution may, however, be shorter, due to the high salinity (up to 20 psu) of the next stage of the Baltic Sea, the Litorina Sea (8000 – 4000 BP), which probably limited the favorable

habitat for pikeperch in coastal areas. Salinities over 5 psu increase the mortality of pikeperch eggs and early larvae (Winkler et al. 1989), although adult pikeperch tolerate exposure to gradually rising salinity peaking at 29–33 psu (Brown et al. 2001). The salinity of the present Baltic Sea varies from 2 psu in the north to 20 psu in the south.

As the geographical distribution of our sampling was limited, no final conclusion can yet be drawn about the colonization lineages of pikeperch in our waters. More extensive sampling of pikeperch populations throughout the Fennoscandian distribution of the species should be organized. The significant differentiation between the freshwater and brackish water populations, however, indicates a deeper genetic cleavage than simply postglacial population differentiation.

Gene flow between populations may be mediated by natural mechanisms (migrations from one water body to another) or by man. In the case of Fennoscandian, and especially Finnish pikeperch populations, human-induced gene flow through early introductions and later enhancement and re-stocking activities should not be underestimated. The history of population transfers is over 100 years long (Halme 1961, 1962), and during the latest boom in enhancement and re-stocking programs, only a few of the original freshwater populations of pikeperch have probably remained totally intact from human-induced gene flow from other populations. According to the current stock registry database of the Finnish Game and Fisheries Research Institute, only 7% of a total of 880 reported sites of pikeperch occurrence could be regarded as indigenous. All other populations are known to have been targets of supplementary juvenile releases or are the result of population introductions (Fig. 1). Indigenous populations mainly remain in the eastern and coastal areas.

During the last 25 years in many water bodies, massive and continuing releases of pond-reared 1-summer-old juvenile pikeperch (totalling 5–10 million individuals per year; Anonymous 2001) have probably markedly influenced the genetic diversity of the species. Taking into account the usually very small number of source populations, the limited number of breeders and the practices of producing very large quantities of individuals per family, the likely direction of this change has been from a higher original to a reduced present species level diversity.

When selecting pikeperch populations for our study we used all available knowledge on management actions (Halme 1961, 1962; Toivonen et al. 1981; Lehtonen et al. 1984) to avoid the influence of recent gene flow from hatchery releases on our results. Therefore, historical samples were used in some of the cases (Lakes Lohjanjärvi and Vanajanselkä). However, in addition to six indigenous populations (three coastal, three freshwater) we also sampled two populations (Lakes Painio and Averia) that were originally established through introductions, although in this case already in the first half of the 20th century. These two populations were selected because they have been - and still are - among the most common hatchery populations in Finland, and hence sources of past and future human-induced gene flow, not only in freshwater systems but also in potentially increasing future releases in coastal areas.

The pikeperch populations of Lakes Painio and Averia grouped close to their likely source population of Lake Lohjanjärvi and, somewhat surprisingly, their diversity level was nearly the same. This suggests that at least in these two cases the old practice of introducing pikeperch to new water bodies by several replicate transfers of adult individuals and/or fertilized eggs has not created a significant bottleneck, and also that the recruitment has been continuously strong enough to maintain the originally transferred diversity. Strong and stable recruitment is also the reason for their present use as source populations for hatchery production.

Management implications

Safeguarding the genetically unique Baltic coastal pikeperch populations from gene flow from the looming hatchery releases using lake pikeperch should be a high management priority at all levels of decision making. This is especially important taking into account the ecological change in the marine environment that is continuously challenging the ecological adaptability of all organisms living in the Baltic Sea (see HELCOM Baltic Sea Action Plan, <www.helcom.fi>). Factors such as continuing eutrophication and pollution, invasive species, decreasing salinity and increasing fishing pressures may all markedly influence future recruitment and habitat conditions of pikeperch in the coastal areas of the Baltic Sea. In this situation, the potentially very high environmental stress on the species' adaptive potential should not be intentionally increased by haphazardly introducing foreign genes to the populations. The application of the *precautionary approach* would imply that instead of releases that may lead to mal-adaptation and reduction of the viability of the costal pikeperch populations, priority should be given to more sustainable long-term management approaches, especially to sound regulation of the fisheries.

The two principal source populations (Lakes Averia and Painio) of recent Finnish hatchery production of pikeperch juveniles appeared genetically surprisingly diverse, taking into account their background as human-introduced populations. Despite this fact, their extensive and continuous use as all-round populations in multiple releases in freshwater systems all over the country is questionable and threatens the still existing genetic diversity and potential local adaptations of the species. The long genetic distance between the northernmost (Lake

Kemijärvi) and the other lake populations suggests that long transfers from the south to the north, or vice versa, should particularly be avoided. If supportive releases appear absolutely necessary in some special circumstances, local indigenous populations should be preferred as source populations and rearing should generally aim at producing juveniles from a larger number of breeders with a lower number of individuals per family.

Detailed knowledge of the variability and population structure of exploited fish populations is a prerequisite for their sustainable long-term management. In the case of the pikeperch populations, the analysis of 12 microsatellite loci appeared to be able to produce invaluable new information on their past and present genetic variability and differences between populations. Even from the oldest samples of this study (from the early 1980s) it was possible to get DNA-results for the analysis. The possibility of extracting the DNA from catch (scale) samples originally collected for other purposes improved the cost-effectiveness of the analysis.

In this study we investigated the indigenous genetic diversity of pikeperch populations, which in the case of most Finnish freshwater systems has already been lost because of the apparently unlimited faith in the benefits of hatchery releases among local managers and water owners. Much more research and communication is clearly needed if the old management principles are to be changed. The next step towards more sustainable management could be the mapping and thereafter strict protection of the remaining genetically intact indigenous freshwater populations. It would also be crucial to know how the most heavily stocked indigenous pikeperch populations have adapted to the pressure from the continuous flow of foreign genes: what is left of their original genetic structure?

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