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Contents

Genetic assessment of salmon and sea trout stocking in a Baltic Sea river

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ABSTRACT

Microsatellite DNA variation were used to assess the outcome of stocking salmon and trout in River Sävarå, N Sweden. No information on pre-stocking genetic composition of salmon and trout in R Sävarå was available. In two year classes of salmon smolt microsatellite data indicated that post-stocking genetic composition differed markedly (Fst = 0.048) from the main donor strain, Byskeälven salmon, and from other Gulf of Bothnia salmon stocks (Fst 0.047- 0.132). The STRUCTURE program failed to detect any sub structuring within Sävarå salmon. It was concluded that only minor introgression estimated to a proportion of 0.11 (95% CI 0.07 - 0.16) has occurred in salmon. Sea migrating trout showed overall low differentiation among populations with maximum Fst of 0.03 making analysis more cumbersome than in salmon. Still, the Sävarå trout deviated significantly from potential donor populations and structure software supported that majority of trout in Sävarå formed a distinct genetic population. Admixture was more extensive in trout and estimated to 0.17 (95% CI 0.10 - 0.25).

Key words: Salmo salar, salmo trutta, stocking, genetic introgression, genetic assignment

INTRODUCTION

Since methods for artificial reproduction of salmonids were developed more than hundred years ago stocking of natural waters with non-native strains and exotic species have taken place. Northern Sweden, which harbours the majority of Baltic Sea salmon (*Salmo salar* L.) populations, is no exception and transfers between rivers of salmon but also of anadromous trout (*Salmo trutta* L.), i.e. sea trout, have occurred extensively. Stocking was motivated by a wish to support declining populations and was facilitated by access to surplus fish from hatcheries built in connection with construction of hydro-electric power plants. Transfers were restricted when in later times awareness of importance to conserve local populations became widespread but have in some cases continued. Considering the extent to which stocking have been practiced few attempts have been undertaken to evaluate the success of these activities. We are not aware of any study on introgression resulting from stocking with non-indigenous salmon in rivers with wild population in the northern Baltic area. In Baltic sea trout Palm *et al.* (2003) reported extensive gene flow from a hatchery strain to a wild population in the river Dalälven. Outside the Baltic area several studies exist that have attempted to assess stocking impact (e.g. Hansen et al 2000; Hansen 2002; Ruzzante et al 2004; Moran et al 2005). Utter (2001) reviewed introgressive hybridization in *Oncorhynchus* and *Salmo* and noted a high variability in the literature in anticipated introgression. The development of highly variable genetic markers such as microsatellites and of new statistical methodologies for analysis of multi-locus genotype data has in recent years greatly increased possibilities to assess genetic introgression from stocking. Approaches to employ these new methods for revealing introgression even when baseline data is incomplete have given encouraging

results (Hansen *et al.*, 2001). The situation of incomplete data occurs when pre-stocking samples are missing, which is commonly the case when evaluating non-experimental releases of fish.

This study aimed at assessing the results from 17 years of stocking in a minor northern Swedish river with salmon and over shorter time with sea trout. The stocking materials used were offspring of wild salmon and sea trout obtained from parent fish caught in rivers in the same geographic region. Stockings were undertaken by a regional fisheries authority after inventories using electro-fishing that suggested poor population status. Donor populations were deliberately chosen from geographically close rivers with similar characteristics in order to increase chances of success although initially stocking material was taken from the nearby main river Ume-Vindelälven. Since reference samples of fish from Sävaråprior to stocking were not available a Bayesian statistical approach was used in order to analyse possible sub structuring of sampled fish.

MATERIALS AND METHODS

The river Sävaråis situated in Västerbotten county, northern Sweden, and reaches the Gulf of Bothnia c. 15 km north of the Ume-Vindelälven river system (Lat: N63°54’38.6”, Long: E20°33’56.5”) (Figure 1). The river Byskeälven, from which majority of stocked salmon originated, is situated approximately 200 km further to the north (Figure 1). The river Öreälven is situated 40 km south of Sävaråand sea trout from this river were used for stocking Sävaå(Figure 1). Stocking activities 1989-2005 in Sävaråare summarized in Table I. The stocking materials were first generation in hatchery of salmon and trout collected as returning spawners. The donor strains from Ume-Vindelälven, Byskeälven

and Öreälven originated from Norrfors, Abborträsk and Öreborg hatcheries, respectively. Sävaråd donor strain was roe collected in from adults in Sävaråd and hatched in Krokfors hatchery. DNA samples of Atlantic salmon were collected from outmigrating smolt from Sävaråd in 2005 (n = 48) and 2006 (n = 50). The sea trout sample (n = 49) was a mixture of smolt individuals from 2005 (n = 30) and 2006 (n =19). The smolt samples consisted mainly of three-year-old individuals, 85% and 79% for salmon and 92% and 57% for trout in 2005 and 2006, respectively. Age of smolt was determined from scale readings performed by Swedish Fisheries Board in Älvkarleby, Sweden. The smolts were captured using ”Rotary Screw Traps” (EG Solutions, Oregon, USA). Wild salmon smolt production was for 2005 estimated to 3,654 individuals (95% CI 2,857-5,069) and for wild sea trouts the estimate was 1,420 individuals (95% CI 887- 3,551) (Lundqvist et al. 2006).

Further details on data of smolt and smolt migration in Sävaråd are available in Lundqvist *et al.*, 2006. Collection of samples was done during entire run of smolt in spring and fin clips were taken and preserved in alcohol for subsequent analysis. Trout samples from Öreälven (n = 31) and Ume-Vindelälven (n = 45) were collected in 1995 and 2003, respectively, and both samples were obtained from returning spawners. Trout samples from Öreälven 1995 represent the brood stock to smolts released (table I). Trout samples from Ume-Vindelälven 2003 did not differ significantly from a sample taken 1995 (Fst = 0.004) ($P > 0.05$, exact test for population differentiation) indicating high degree of temporal stability (Östergren & Nilsson, 2006). Samples used are summarized in Table II.

Microsatellite variation was analyzed using standard PCR protocol with fluorescent labeled primers using a BECKMAN 800 automatic sequencer. In salmon variation at eight loci was determined: Ssa85 and Ssa289 (McConnell *et al.* 1995); Ssa171, Ssa197 and Ssa202 (OReilly *et al.*1996); SSOSL85, SSOSL417 (Slettan *et al.* 1995); and SSOSL438 (Slettan *et al.* 1996). In trout eight microsatellite loci were utilized: Str60, Str73, Str15 (Estoup *et al.*, 1993); SSOSL417 (Slettan *et al.*, 1995); SSOSL438 (Slettan *et al.*, 1996); Ssa85, Ssa197, Ssa171 (O'Reilly *et al.*, 1997).

In addition, genotypic data from previous work on salmon was used as reference. This reference data set includes all major salmon stocks, both cultivated and wild, from the Swedish coast of the Gulf of Bothnia and is described in Säisä *et al.* (2005). Reference DNA samples from the Säisä *et al.* (2005) study was analyzed together with DNA samples from Sävaråt to calibrate allele sizes with reference data set. The wild Vindelälv salmon and the Umeälv hatchery salmon, both from the same river system, was treated as a single population since they are genetically similar according to microsatellite data and extensive gene flow between them is known to have occurred.

GENEPOP3.2a was used for analysis of expected heterozygosity (He), linkage disequilibrium, conformity with Hardy-Weinberg expectations and for estimation of Fst (Weir & Cockerham, 1984) and exact test for differentiation (Raymond & Rousset, 1995). To reveal possible genetic sub structuring the software STRUCTURE (Prichard & Wen, 2004) was used applying an admixture model with allele frequencies correlated and assuming 1-5 subpopulations (K). K was determined from mean probabilities of five repeated runs with a burn in length of 100 000 and 200 000 MCMC repeats.

The population information model in STRUCTURE as described by Pritchard *et al.* (2000) was used to detect stocked individuals or individuals with part of their ancestry from stocked origin. The migration prior was varied but this had marginal effect and results using a prior of 0.10 are presented. To detect possible migrants we utilized the individual admixture coefficient (q) provided by STRUCTURE that estimates the proportion of an individuals genotype which originates from each potential parental population.

A test was made for the ability of the STRUCTURE programme to detect stocked individuals or individuals with part of their ancestry from stocked population by re-coding individuals from potential donor population and adding them to the receiving population data set. This was repeatedly done with three new individuals at a time and each time a new run of the STRUCTURE programme was performed. Individual admixture coefficients (q) for the simulated migrants were then inspected in order to obtain a rough estimate of the performance of the STRUCTURE model applied. In total 57 Byskeälv salmon was used in 19 new runs with STRUCTURE, and 45 Ume-Vindelälven salmon in 15 new runs. In trout 29 individuals from each of Ume-Vindelälven and Öreälven was used as simulated migrants in 10 new runs per reference population. The number of artificial migrants was restricted by available complete genotypes from each population.

The distribution of q values among pure Sävaråsalmon or trout was assessed by using Sävaråindividuals with $q > 0.90$ to generate pure Sävaråsalmon and trout by simulations using HYBRIDLAB (Nielsen et al. 2006) and replacing the actual Sävaådata with simulated genotypes. We adapted the cut-off point $q > 0.90$ used by Barilani et al. (2007) and Oliveira et al. (2008) to exclude non-indigenous genotypes. Re-running was then

performed in STRUCTURE using same parameters and number of genotypes as with actual data. The simulation process was repeated 10 times.

A population-level admixture estimate was obtained from mean q values and corresponding 95% confidence interval (CI) was estimated from bootstrapping as described by Hansen *et al*. 2001. When using STRUCTURE individuals with missing data from more than one locus were not included.

RESULTS

Salmon

Deviations from Hardy-Weinberg equilibrium were observed for one locus after Bonferroni correction in one of the two year classes (Table III). There was no significant linkage disequilibrium after correction for multiple tests (28 tests per year class). Without correction year class 2005 showed significant linkage disequilibrium at 0.01 level at two loci and at 0.05 at one locus and year class 2006 was significant at 0.05 level at one locus. Exact test for population differentiation did not reveal any significant differentiation between the year classes and an Fst value of 0.001 was obtained. Genetic diversity expressed as average expected heterozygosity (He) was 0.708 in yearclass 2005 and 0.745 in yearclass 2006. The range of expected heterozygosity among Bothnian sea populations was 0.632–0.754 with a mean of 0.705. Pairwise Fst values were calculated using smolt data from Sävarå and data from salmon populations along the Swedish Bothnian Sea coast (Table IV). The overall Fst for Bothnian Sea salmon was 0.060. For the Sävarå salmon lowest Fst values were obtained with the rivers Lögdeälven, Byskeälven and Ljusnan salmon. Microsatellite variation in Sävarå salmon was

markedly differentiated from Ume-Vindelälv salmon as shown by an Fst value of 0.132. Exact test for population differentiation showed Sävaå salmon to be significantly different ($P < 0.001$) from all other populations. The STRUCTURE program was used to assess possible population substructure caused by both admixture and presence of stocked smolt in the Sävaå sample. Analysis of salmon using a data set that included the two year classes from Sävaå and Byskeälven and Umeälven individuals suggested presence of three clusters, i. e. $K = 3$ had highest probability. Each cluster dominated in one of the rivers and to an approximately equal extent (Table V). The two year classes from Sävaå showed consistent results (Table V). When using only Sävaå salmon highest probability was obtained for $K = 1$. These results indicate existence of a distinct population in Sävaå.

A model with prior population information was in a subsequent analysis used in order to identify stocked individuals among Sävaå salmon by inspection of individual admixture coefficients (q). Specifying a migration prior of 0.10 showed that 52 individuals out of 72 had q values > 0.90 but also revealed presence of some individuals with intermediate values and lowest q obtained was at 0.167. One individual with $q = 0.216$ had Umeälven as likely origin but other individuals with intermediate values showed more resemblance with Byskeälv salmon.

A test for the ability of the programme to detect migrants when using prior population information was made by re-coding Byskeälven salmon individuals to Sävaå and re-running the analysis. A wide range of q values (0.002 – 0.879) with mean 0.343 was obtained for these simulated migrants. This was compared with the distribution of q values among simulated pure Sävaå salmon. From 10 repeated simulations and re-

running in structure of pure Sävarå salmon a lowest q value of 0.299 was obtained. Combining the highest q value obtained from simulated migrants with the lowest q value obtained for simulated pure Sävarå salmon gave an interval 0.299 - 0.879 that provides an estimate of the range of overlapping q values of stocked Byskeälv salmon and Sävarå salmon.

In the simulations of pure Sävarå salmon on average 10.8 % of q values, corresponding to 7.8 individuals at a sample size of n = 72, fell into this overlapping range. Analysis of sampled individuals from Sävarån showed one individual with q = 0.167 and 15 individuals with q values in range 0.299-0.879 suggesting an excess of approximately 7 individuals in the overlapping range compared to expected number. These 7 individuals together with one that had a q value lower than the overlapping range and the single individual assigned to Umeälven was used to estimate the proportion of stocked fish in the Sävarå sample to 12.5 %.

This correspond well with the estimate of introgression obtained from the mean q value of Sävarå salmon of 0.111 (95% CI 0.070 - 0.159).

Sea trout

Deviations from Hardy-Weinberg equilibrium (homozygote excess) were observed for Ume-Vindelälven at two loci, for Sävarå at one loci and at none in Öreälven after Bonferroni correction. A significant deficit of heterozygotes (Fis = 0.106; $P < 0.01$) was obtained with Sävarå trout but not in the other two populations. For Sävarå trout observed and expected heterozygosities per locus are given in Table (III). Significant genotypic disequilibrium was, after correction for multiple tests, observed for one locus pair in Ume-Vindelälven sample only. Uncorrected disequilibrium tests revealead three

significant locus pairs at 0.05 level, one at 0.01 and one at 0.001level in Ume-Vindelälven. For Sävaråfive locus pairs showed significance at 0.05 and one at 0.01 level and for Öreälven there was two significances at 0.05 level. Exact test for population differentiation revealed significant differentiation ($P < 0.001$) in all pair wise comparisons. The lowest Fst value was obtained between Ume-Vindelälven and Öreälven (Fst = 0.023) while the Fst between Sävaråand Ume-Vindelälven was 0.033 and Sävaråand Öreälven was 0.026, respectively. The global Fst was 0.028. Genetic diversity expressed as average expected heterozygosity was 0.69 in all three trout populations. The STRUCTURE program was used to detect possible population substructure caused by both admixture and presence of stocked sea trout smolt in the sample from Sävaråe. When Sävaråtrout was run separately STRUCTURE suggested presence of two clusters as K = 2 had highest probability, with 29 and 20 individuals in the two clusters, respectively (Figure 2). Using data from the two potential donor populations and data from Sävaråhighest probability was obtained with four clusters (Table VI). Each of three clusters was dominated by one population , while a fourth cluster contained fewer individuals in total and more or less equal numbers from each population (Table VI). As illustrated in Figure 2 the larger cluster of Sävaråtrout was rather stable between these two cluster analyses while individuals from the smaller cluster were split into the four clusters in the second analysis. As with salmon, a model with prior population information was then used in order to identify stocked individuals among Sävaråsea trout from individual admixture coefficients (q). Setting the migration prior to 0.10 resulted in detection among 49 Sävaråtrout of 19 individuals with q values < 0.90. All but one of these 19 individuals was assigned to the smaller of the two clusters suggested for Sävaråe. Consequently, 30

individuals exceeded 0.90. When testing the ability of STRUCTURE to detect migrant trout among Sävarå samples a wide range of q values was obtained for simulated migrants (Umeälven 0.128 – 0.951 with mean 0.659, Öreälven 0.002-0.940 with mean 0.547) . When compared with the distribution of q values among simulated pure Sävarå trout (10 repeated simulations and re-running in STRUCTURE) a lowest q value of 0.460 was obtained. Accordingly, when combining the results from simulated migrants and simulated pure Sävarå trout there was an overlapping range of q values of 0.460-0.951. In simulations of pure Sävarå trout on average 35 % of q values, corresponding to 16.8 individuals at a sample size of n=49, fell into this overlapping range. Analysis of sampled individuals from Sävarån showed 24 individuals with q values in range 0.460-0.951 suggesting an excess of approximately 7 individuals in the overlapping range compared to expected number. These 7 individuals together with 6 that had a q value lower than the overlapping range was used to estimate the proportion of stocked fish in the Sävarå sample to 26.5 %.

This is a higher value than the estimate of introgression obtained from the mean q value of Sävarå trout of 0.173 (95% CI 0.10 - 0.25).

DISCUSSION

Stocking of Sävarå with non-indigenous salmon over a 17 year long period has not resulted in replacement or any extensive introgression. The salmon sampled from Sävarå was found to be genetically distinct from the donor populations and from other Bothnian Sea populations. There was no indication of excess of homozygotes or of linkage disequilibrium after Bonferroni corrections, which are signs of mixing and admixture,

and a single cluster was suggested by STRUCTURE. Taken together this suggests the Sävaå salmon to be a population which is distinct from other populations and since the amount of variation was comparable to other salmon populations in the region also has a reasonable good genetic status. Using the present genetic composition to assess the result of stocking is obviously challenging when original pre-stocking data is not available. Accordingly, it is not possible to state that our samples represented a native unaffected stock. The estimated introgression rate of 0.11 may lead a strict conservationist to regard the population as too affected by non-indigenous populations to have any value in conservation. As there exists no strict cut-off point when a population can be declared as lost the value of present Sävaå salmon population for conservation is a matter of opinion. In any case, before further assessments have been done the practical management of the population should assume that it is a distinct group and avoid further human mediated introgression from non-indigenous salmon.

Low Fst in all pair-wise comparisions made evaluation of possible introgression in sea-trout less conclusive. Still, it was noted that the Sävaå sea trout was significantly differentiated from the potential donor populations in Öreälven and Ume-Vindelälven, a result that was supported from clustering obtained using the STRUCTURE software.

In salmon the analysis detected a few individuals that likely represented surviving stocked Byskeälv salmon and possibly some of hybrid origin. However, the wide range of q values obtained with simulated migrants from Byskeälven points out that only detection of individuals with pure origin in the stocked population can be done with reasonable certainty. Vähä and Primmer (2006) reported that accurate detection of hybrids would require a much larger number of markers than used here for populations

with Fst in the order of 0.05. The higher Fst between Sävaråand Umeälven should make it easier to detect hybrids between these two populations. As salmon from Umeälven was used only in the first years of stocking the detection of one such individual may represent natural straying from that river to Sävaråå.

Significant deficit of heterozygotes was observed in Sävaråå trout and could be explained by mixing of native with stocked trout. The suggestion from the structure analysis of two clusters in Sävaråå and the observation that individuals with admixture coefficient below 0.90 almost exclusively belonged to the smaller of the clusters also indicate that mixing has occurred. Alternatively, deficit of heterozygotes may also have resulted from differentiation within Sävaråå. Local differentiation of trout within a watershed has been documented repeatedly (Hansen & Mensberg, 1998; Carlsson & Nilsson 2001; Östergren & Nilsson, 2006). Heterozygote deficiency may also be influenced by year-class in the presence of temporal genetic variation or due to varying stocking proportions. It was not possible to support or reject such hypothesis due to lack of individual data on year-class, however the dominance of 3-year-olds in the sample material would have been more apparent in the cluster analyses if year-class was the basis to the STRUCTURE results. Yet, influence of year-class on heterozygote deficiency can not be ruled out. The reported difference between Sävaråå sea trout and the donor populations suggests that at least a considerable part of its genetic composition reflects a unique Sävaråå sea trout population but likely some introgression has occurred. The low differentiation observed among sea trout populations in this study makes it difficult to separate stocking effects from naturally occurring gene flow. There have been few genetic studies on Sea trout

populations in rivers in Northern Baltic and the low differentiation among our rivers could be an effect of stocking but alternatively be a characteristic in this region.

The low number of stocked fish among the out-migrating salmon smolt is indicative of poor effect from stocking and could suggest low survival in the freshwater phase of stocked fish. One possible reason for low survival among stocked salmon could be that the status of the Sävaråsalmon stock, which was assessed by electrofishing, was underestimated before stocking was initiated. If most habitats suitable for parr were occupied when the stocking material was introduced the stocked fish may have had poor chances of surviving. Underestimation of the native population size and stocking too few for any detectable effect may also be an explanation. Another possibility is that Byskeälv salmon has genetically based adaptations that are not compatible with conditions in Sävaråsalmon. The Byskeälven stock was chosen as it represented a stock from a river with some ecological similarities with Sävaråsalmon, the two rivers have a similar flow regime as they receive their water from the inland forest area as opposed to for example Ume-Vindelälven which is largely mountain fed. However, the importance of local adaptation in salmon is poorly understood.

Stocking of Sävaråsalmon with salmon continued until 2005 and with trout until 2004. Accordingly, there is still a possibility that introgression will occur in the future. Also, there may exist cohorts of introduced fish that have contributed little with offspring to the two year classes that were studied. Ryman (1997) showed that a pulse of introgression may cause wide fluctuation in allele frequencies that persist over long time in populations with overlapping generations. Continued genetic monitoring of Sävaråsalmon and trout should therefore be undertaken. Considering that many Baltic Sea salmon and sea trout

populations have been lost during the 20th century the value of each remaining wild population for conservation should be high. Even if such a population is small it has a value as an independent genetic unit (Hilborn *et al.* 2003). Other salmon and sea trout populations in the Bothnian Sea area may be in a similar situation as salmon in Sävarå. It could be that evaluations based on insufficient population data has led to erroneous assumption that populations were too weak to recover which in turn motivated stocking with non-indigenous material. As shown here stocking may not always be efficient and there can be a number of rivers that still hold viable wild populations which are not extensively introgressed. All major salmon populations in the Baltic have been genetically characterized with different marker types (Koljonen *et al.* (1999), Nilsson *et al.* (2001), Säisä *et al.* (2005). Still, some minor populations have not been studied, often because stocking activities have suggested them not to represent native populations and accordingly to be of little value in conservation. In contrast with salmon little is known on genetic structuring in Baltic sea trout and no comprehensive overview exists. Reports from previous studies on introgression of stocked anadromous trout and salmon in wild populations indicate varying outcomes. Skaala *et al.* (1996) reported gene flow from hatchery trout to anadromous trout in a Norwegian river and extensive gene flow from sea-ranched to wild sea trout was observed by Palm *et al.* (2003) in Swedish River Dalälven. In Danish waters Hansen *et al.* (2000) found little contribution of hatchery trout to an anadromous population and Ruzzante *et al.* (2004) noted absence of hatchery sea trout among spawners. Suggested explanations for poor stocking success in trout include local adaptations and domestication effects (Hansen, 2000), competitive

interactions (Borgstrom *et al.* 2002) and poor survival in the sea phase (Ruzzante *et al.* 2004).

Utter (2001) noted that translocation of anadromous salmonid populations was more difficult than of freshwater ones. He attributed this difference to the more complex life-history of anadromous populations but restricted the difficulties to translocation between lineages. Although northern Baltic salmon is proposed to be a mixture of two glacial lineages (Nilsson *et al.* 2001; Säisä *et al.* 2005) it is not anticipated that this has hindered introgression. However, our results add to difficulties in translocation of anadromous salmonids. That introgression is possible even when there is a long geographic distance, and likely considerable ecological differences, between donor population and receiving population is indicated by work of Moran *et al.* (2005) on Spanish rivers stocked with Scottish salmon.

Our results indicate that stocking natural waters with non-indigenous salmon or anadromous trout should be performed only when population loss has been thoroughly documented. Even in such cases it is doubtful if stocking is a good strategy. Vasemägi *et al.* (2001) showed that wild salmon, although outnumbered many times by hatchery salmon, were more effective in re-colonizing a Baltic river. Perhaps allowing for natural re-colonization or recovery should be considered as a first option.

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REFERENCES

Barilani, M., Sfougaris, A., Giannakopoulos, A., Mucci, N.,Tabarroni, C., & Randi, E. (2007). Detecting introgressive hybridisation in rock partridge populations (Alectoris graeca) in Greece through Bayesian admixture analyses of multilocus genotypes. *Conservation Genetics* **8**, 343-354. doi: 10.1007/s10592-006-9174-1

Borgstrom, R., Skaala, O. & Aastveit, A. H. (2002). High mortality in introduced brown trout depressed potential gene flow to a wild population. *Journal of Fish Biology* **61**, 1085-1097. doi: 10.1006/jfbi.2002.2112

Carlsson, J., & Nilsson, J. (2001). Effects of Geomorphological Structures on Genetic Differentiation among Brown Trout Populations in a Northern Boreal River Drainage. *Transactions of the American Fisheries Society* **130**, 36-45.

Efron, B. (1983). Estimating the error rate of a prediction rule: improvement of cross-validation. *Journal of the American Statistic Society* **78**, 316-331.

Estoup, A., Presa, P., Krieg, F., Vaiman. D. & Guyomard, R. (1993). (CT)n and (GT)n microsatellites: a new class of genetic markers for Salmo trutta L. (brown trout). *Heredity* **71**, 488-496.

Hansen, M. M., Nielsen, E. E., Bekkevold, D. & Mensberg, K-L. D. (2001). Admixture analysis and stocking impact assessment in brown trout (Salmo trutta), estimated with

incomplete baseline data. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 1853-1869.

Hansen, M. M., Ruzzante, D. E., Nielsen, E. E. & Mensberg, K-L. D. (2000). Microsatellite and mitochondrial DNA polymorphism reveals life-history dependent interbreeding between hatchery and wild brown trout (Salmo trutta L.) *Molecular Ecology* **9**, 583-594. doi:10.1046/j.1365-294x.2000.00898.x

Hansen, M. M. & Mensberg, K. L. (1998). Genetic differentiation and relationship between genetic and geographic distance in Danish sea trout (Salmo trutta L.) populations. *Heredity* 81, 493-504.

Hansen, M. M. (2002). Estimating the long-term effects of stocking domesticated trout into wild brown trout (Salmo trutta) populations: an approach using microsatellite DNA analysis of historical and contemporary samples. *Molecular Ecology* **11**, 1003-1015. doi:10.1046/j.1365-294X.2002.01495.x

Hilborn, R., Quinn, T. P., Schindler, D. E. & Rogers, D. E. (2003). Biocomplexity and fisheries sustainability. *Proceedings of the National Academy of Sciences* **100**, 6564-6568.

Koljonen, M.-L., Jansson, H., Paaver, T., Vasin, O. & Koskiniemi, J. (1999). Phylogeographic lineages and differentiation pattern of Atlantic salmon (Salmo salar) in the Baltic Sea with management implications. *Canadian Journal of Fisheries and Aquatic Sciences* **56,** 1766-1780.

Lundqvist, H., Öhlund, G., Karlsson, L., Larsson, S., Carlsson, U., Alanärä, A., Kiessling, A., Eriksson, L-O., Leonardsson, K., Östergren, J. and Nilsson, J. 2006. Salmon and sea trout migration in Sävaråå 2005: Study to determine Sävaråå as a potential

index river for Swedish forest rivers. Report from Department of aquaculture, SLU 2-13. Working paper no 11 WGBAST (Baltic salmon and trout assessment working group), (ICES CM 2006/ACFM:21. 209 pp).

McConnell, S. K., OReilly, P., Hamilton L., Wright, J. N. & Bentzen P. (1995). Polymorphic microsatellite loci from Atlantic salmon (Salmo salar): genetic differentiation of North American and European populations. *Canadian Journal of Fisheries and Aquatic Sciences* **52,** 1863-1872.

Moran, P., Perez, J., Dumas, J., Beall, E., & Garcia-Vazquez, E. (2005). Stocking-related patterns of genetic variation at enzymatic loci in south European Atlantic salmon populations. *Journal of Fish Biology* **67** (Supplement A), 185-199. doi:10.1111/j.1095-8649.2005.00847.x

Nielsen, E. E., Bach, L.A. & Kotlicki, P. (2006). Hybridlab (version 1.0): a program for generating simulated hybrids from population samples. *Molecular Ecology Notes* **6**, 971-973. doi: 10.1111/j.1471-8286.2006.01433.x

Nilsson, J., Gross, R., Asplund, T., Dove, O., Jansson, H., Kelloniemi, J., Kohlmann, K., Löytynoja, A., Nielsen, E. E., Paaver, T., Primmer, C.R., Titov, S., Vasemägi, A., Veselov, A., Öst, T. & Lumme, J. (2001). Matrilinear phylogeography of Atlantic salmon (Salmo salar L.) in Europe and postglacial colonisation of the Baltic Sea area. *Molecular Ecology* **10**, 89-102. doi:10.1046/j.1365-294X.2001.01168.x

Oliveira, R., Godinho, R. Randi, E., Ferrand, N., & Alves, P. C. (2008). Molecular analysis of hybridisation between wild and domestic cats (Felis silvestris) in Portugal: implications for conservation. *Conservation Genetics* **9**, 1-11. doi: 10.1007/s10592-007-9297-z

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

Palm, S., Dannewitz, J., Järvi, T., Petersson, E., Prestegaard, T. & Ryman, N. (2003). Lack of molecular genetic divergence between sea-ranched and wild sea trout (Salmo trutta). *Molecular Ecology* **12(8),** 2057-2071. doi:10.1046/j.1365-294X.2003.01892.x

Pritchard, J. K. & Wen, W. (2004). Documentation for STRUCTURE software: version 2. Avaliable from http://pritch.bsd.uchicago.edu.

Pritchard, J. K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* 155, 945-959.

Raymond, M. & Rousset, F. (1995). GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86,** 248-249.

Ruzzante, D. E., Hansen, M. M., Meldrup, D. & Ebert, K. M. (2004). Stocking impact and migration pattern in an anadromous brown trout (Salmo trutta) complex: where have all the stocked spawning sea trout gone? *Molecular Ecology* **13(6),** 1433-1445. doi:10.1111/j.1365-294X.2004.02162.x

Ryman, N. (1997). Minimizing adverse effects of fish culture: understanding the genetics of populations with overlapping generations. *ICES Journal of Marine Science* **54,** 1149-1159.

Skaala, O., Jorstad, K. E. & Borgstrom, R. (1996). Genetic impact on two wild brown trout populations after release of non-indigenous hatchery spawners. *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 2027-2035.

Slettan, A., Olsaker, I. & Lie, Ø. (1995). Atlantic salmon, *Salmo salar*, microsatellites at the SSOSL25, SSOSL85, SSOSL311, SSOSL417 loci. *Animal Genetics* **26**, 277-285.

Slettan, A., Olsaker, I. & Lie, Ø. 1996. Polymorphic Atlantic salmon, *Salmo salar* L., microsatellites at the SSOSL438, SSOSL439 and SSOSL444 loci. *Animal Genetics* **27,** 57–58.

Säisä, M., Koljonen, M.-L., Gross, R., Nilsson, J., Täthinen, J., Koskiniemi, J. & Vasemägi, A. (2005). Population genetic structure and postglacial colonization of Atlantic salmon (*Salmo salar*) in the Baltic Sea based on microsatellite DNA variation. *Canadian Journal of Fisheries and Aquatic Sciences* **62**, 1887-1904.

Utter, F. (2001). Patterns of subspecific anthropogenic introgression in two salmonid genera. *Reviews in Fish Biology and Fisheries* **10**, 265-279.

Vähä, J.-P. & Primmer C. R. (2006) Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. *Molecular Ecology* **15**, 63-72. doi:10.1111/j.1365-294X.2005.02773.x

Vasemägi, A., Gross, R., Paaver, T., Kangur, M., Nilsson, J. & Eriksson L.-O. (2001). Identification of the origin of an Atlantic salmon (*Salmo salar* L.) population in a recently recolonized river in the Baltic Sea. *Molecular Ecology* **10**, 2877-2882. doi: 10.1046/j.1365-294X.2001.t01-1-01407.x

Weir, B. S. & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358-1370.

Östergren, J. & Nilsson, J. (2006). Population genetic structure of brown trout (Salmo trutta L.) with different life histories in two northern Swedish rivers. In Östergren J. 2006. Migration and genetic structure of anadromous Salmo salar & Salmo trutta in

northern Swedish rivers. Doctoral thesis 2006:112. ISSN: 1652-6880. ISBN: 91-576-7261-X. Department of Aquaculture, Swedish University of Agricultural Science, Umeå, Sweden.

FIGURES

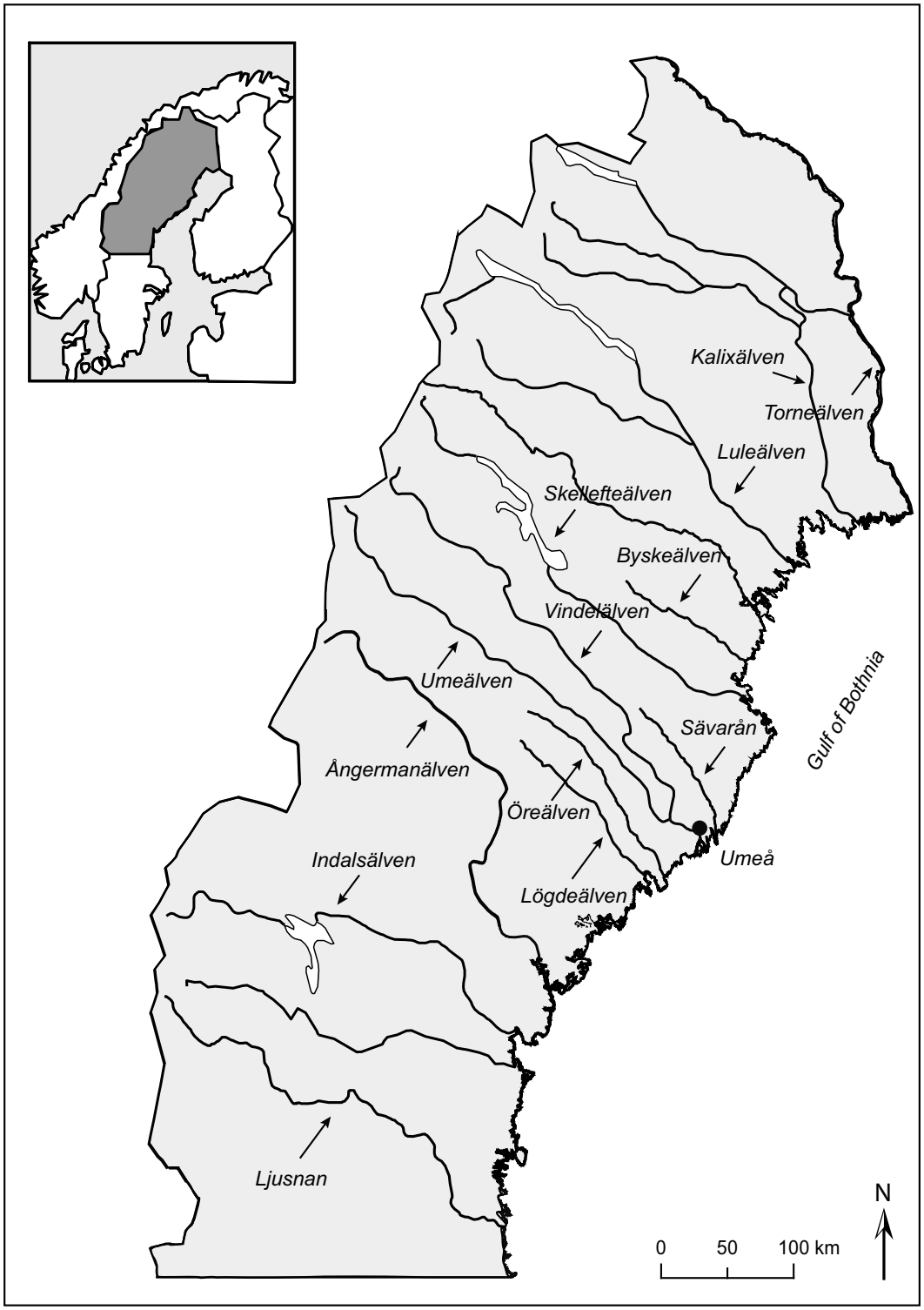

Figure 1. Map of N Sweden showing location of river Sävarån and other rivers referred to.

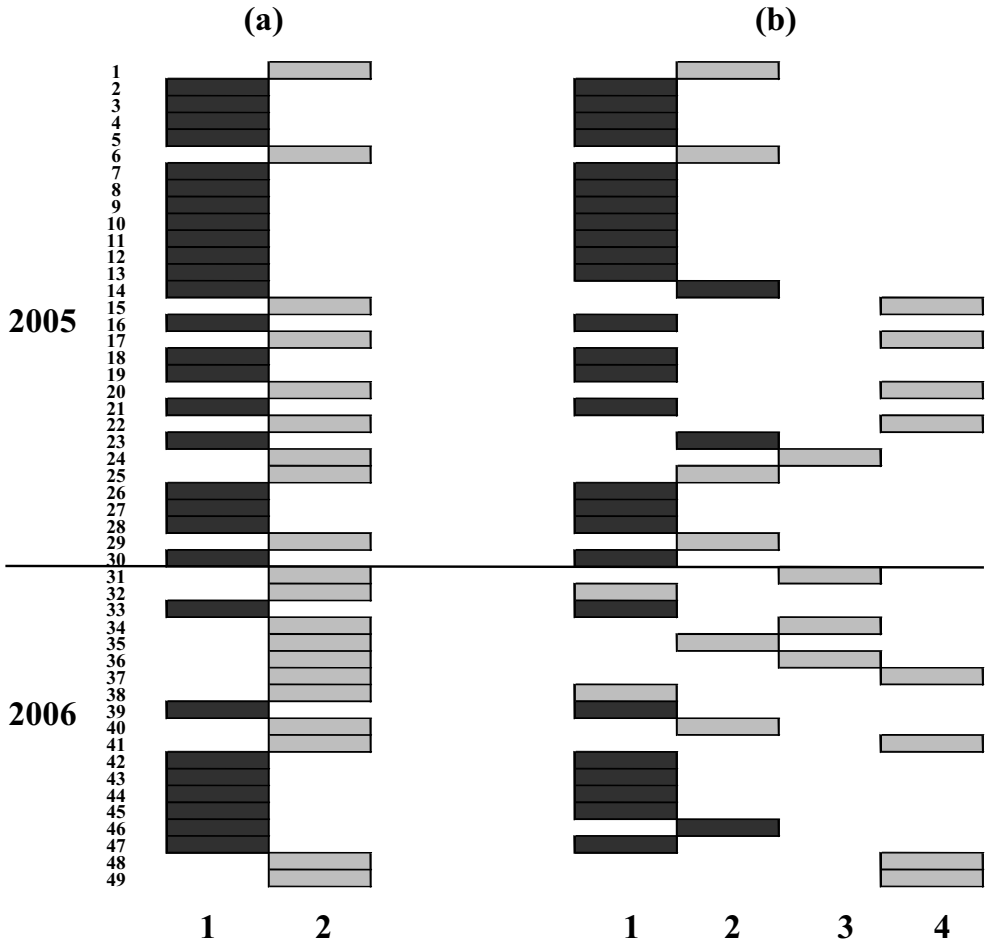

Figure 2. The relationship between clusters obtained by STRUCTURE. Samples from Sävar trout analysed alone (A) and together with donor populations (B). Each rectangle represent one individual (total n = 49). Sampling year and individual numbering is indicated to the left. Cluster nr in B corresponds to cluster nr in table VI.

TABLES

Table I. The number and origin (donor strain) of different year classes of stocked Atlantic salmon and sea trout juveniles in the river Sävarån

	Year	Fry	One summer- old juveniles	One year-old juveniles	Two year-old juveniles	Donor strain
Salmo salar	1989	100 000			5 000	Ume-Vindelälven
	1990				6 000	Ume-Vindelälven
	1992			8 350		Byskeälven
	1994		24 263			Sävaråå & Byskeälven
	1995		35 000			Sävaråå & Byskeälven
	1996		20 000			Byskeälven
	1997		40 000			Byskeälven
	1998		50 000	28 000		Byskeälven
	1999		89 500			Byskeälven
	2000		52 000			Byskeälven
	2001		77 000			Byskeälven
	2002	30 000				Byskeälven
	2003		33 600			Byskeälven
	2004		37 500			Byskeälven
	2005	174 500	60 000		1 100	Byskeälven
Total		304 500	518 863	36 350	12 100	
Salmo trutta	1992		7 310	4 240		Ume-Vindelälven
	1993		13 700			Ume-Vindelälven
	1994		4 300			Ume-Vindelälven
	1996		3 500			Sävarån
	1997		1 000			Sävarån
	1998		1 200			Sävarån
	1999		2 700			Sävarån
	2000		6 000			Öreälven
	2002		6 000			Öreälven
	2004		6 000			Öreälven
Total			51 710	4 240		

Table II. Samples used in the study and their origin (river), number per species and year of sampling.

River	Salmon (n)	Sea trout (n)	Year
Sävaråå	48	30	2005
Sävaråå	50	19	2006
Ume- Vindelälven	47	-	1995
Ume- Vindelälven	-	45	2003
Byskeälven	69	-	1999
Öreälven	-	31	1995

Table III. Summary of microsatellite data: P-values for excess of homozygosity in H-W tests with significance level (* = P< 0.05,** = P< 0.01) after Bonferroni correction for 8 tests, expected heterozygosity (He), observed heterozygosity (Ho) and sample size (n).

Salmo salar				*Salmo trutta*		
Locus		2005	2006	Locus		
Ssa85	H-W test	0.001**	0.250	Str60	H-W test	0.251
	He	0.740	0.830		He	0.457
	Ho	0.625	0.750		Ho	0.408
	n	40	40		n	49
Ssa171	H-W test	0.309	0.956	SsOsl438	H-W test	0.093
	He	0.753	0.794		He	0.665
	Ho	0.735	0.878		Ho	0.609
	n	34	41		n	46
Ssa197	H-W test	0.830	0.940	Ssa85	H-W test	0.168
	He	0.887	0.888		He	0.680
	Ho	0.936	0.932		Ho	0.612
	n	47	44		n	49
Ssa202	H-W test	0.853	0.145	Ssa197	H-W test	0.120
	He	0.664	0.726		He	0.612
	Ho	0.767	0.675		Ho	0.587
	n	30	40		n	46
Ssa289	H-W test	0.488	0.758	Str73	H-W test	0.173
	He	0.698	0.704		He	0.638
	Ho	0.696	0.744		Ho	0.490
	n	46	43		n	49
SsOsl85	H-W test	0.046	0.144	SsOsl417	H-W test	0.930
	He	0.845	0.812		He	0.804
	Ho	0.765	0.795		Ho	0.857
	n	34	39		n	49
SsOsl417	H-W test	0.276	0.432	Str15	H-W test	0.013
	He	0.710	0.709		He	0.750
	Ho	0.634	0.795		Ho	0.592
	n	41	39		n	49
SsOsl438	H-W test	0.206	0.988	Ssa171	H-W test	0.005*
	He	0.364	0.495		He	0.894
	Ho	0.319	0.587		Ho	0.783
	n	47	46		n	46

Table IV. Pair-wise F_{st} between Sävarån and Gulf of Bothnia *Salmo salar* stocks from eight microsatellite loci. *** significance at 0.001 from exact test for differentiation.

Gulf of Bothnia stock	F_{st}
Torneälv	0.072***
Kalixälv	0.092***
Luleälv	0.070***
Skellefteälv	0.117***
Byskeälv	0.048***
Ume-Vindelälv	0.132***
Lögdeälv	0.047***
Ångermanälv	0.081***
Indalsälv	0.111***
Ljusnan	0.059***

Table V. Proportion of membership of each *Salmo salar* population in each of three clusters.

	Clusters			
Population	1	2	3	n
Byskeälv	0.789	0.111	0.100	69
Sävaråå 2005	0.133	0.828	0.039	33
Sävaråå 2006	0.158	0.758	0.084	39
Ume-Vindelälven	0.092	0.029	0.879	48

Table VI Proportion of *Salmo trutta* in four clusters as determined by STRUCTURE.

	Inferred clusters				
Given population	1	2	3	4	Number of individuals
Sävaråå	0.461	0.183	0.133	0.223	49
Ume-Vindelälven	0.120	0.203	0.419	0.257	45
Öreälven	0.098	0.498	0.146	0.259	31