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Stock transfer in vendace: an evaluation using microsatellite markers

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Lake Höytiäinen in eastern Finland has suffered from several prolonged vendace (*Coregonus albula* (L.)) recessions during the last decades. To strengthen the spawning stock, about 2.6 million vendace were transferred from lake Suvasvesi into lake Höytiäinen in late winter 2000. The success of stock transfer was studied with the help of microsatellite genetic markers. Newly hatched larvae were collected in two years preceding and two years after the first reproduction of the transferred fish in lake Höytiäinen. Microsatellite DNA variation of larvae was assessed and the genotypes were compared with the genotypes of the two original populations and known hybrids of these two populations. An assignment test showed that a majority of the sampled larvae originated from the indigenous lake Höytiäinen population, and stock transfer had only minor effects on the population structure of lake Höytiäinen.

Introduction

Stock transfers of vendace (*Coregonus albula* (L.)) have been common in Finland for centuries (Lahti 1987, Jurvelius *et al*. 1995). Usually vendace have been transferred to small lakes with no original vendace stock and the success of the operation has been easy to determine. A few transfers have also been made to larger lakes containing sparse original populations (Lahti 1987, Jurvelius *et al*. 1995). In this situation, if vendace stock improves, it is much more difficult to assess the success of the transfer because natural variation of year-class strength of vendace is high in most lakes.

Marking of stocked fish provides a method to quantify the contribution of stocking to a fish population. By using otolith marking, even the contribution of larval stocking can be monitored (e.g. Eckmann *et al*. 1998, Secor & Houde 1998). This method, however, is restricted to evaluation of the success of stocked individuals. It gives very little information about the impact of the stocking on future generations of the species. If the stocked and native fish differ in genetic properties, the impact of their offspring on total stock size may be detected for several generations. Koljonen and Jurvelius (1995) evaluated the success of vendace transfer into lake Puruvesi in eastern Finland (described in Jurvelius *et al*. 1995) by enzyme electrophoresis and RAPD markers. Modern molecular techniques provide higher resolution to population analysis due to higher levels of genetic variation (e.g. Smith &

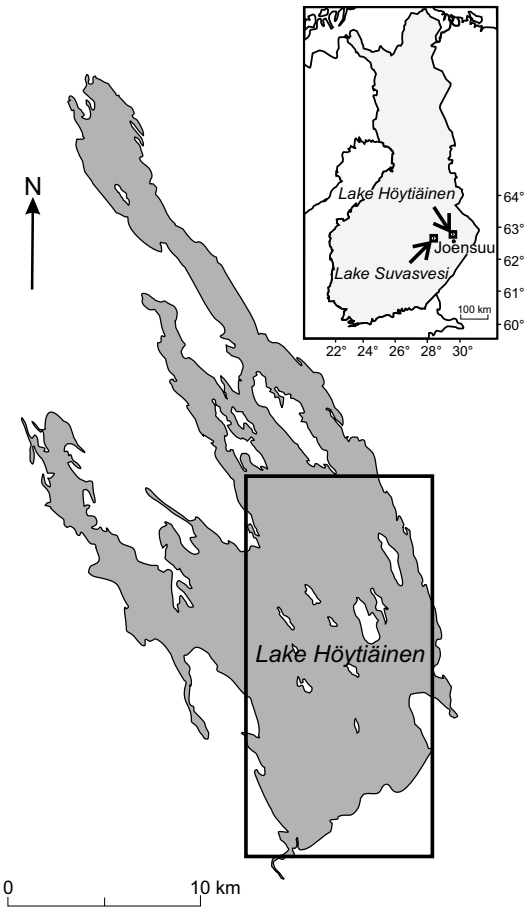

Fig. 1. Location of lakes Höytiäinen and Suvasvesi. Larval sampling area in lake Höytiäinen is indicated with a rectangle.

Wayne 1996, O'Connell & Wright 1997). Microsatellite DNA loci have proven to be effective in studies for identification of indigenous populations and estimating stocking effects on fish populations. This method has been applied to many salmonid populations (e.g. Atlantic salmon (*Salmo salar*), Martinez *et al*. 2001, Nielsen *et al*. 2001; brown trout (*Salmo trutta*), Fritzner *et al*. 2001, Hansen 2002; grayling (*Thymallus thymallus*), Koskinen *et al*. 2002) but, to our knowledge, not to coregonids.

Lake Höytiäinen in eastern Finland has suffered from several prolonged vendace recessions during the last decades, and low spawning stocks have been strengthened by stock transfers between 1978 and 1980, and in 2000. A few years after the earlier transfer, the vendace stock recovered, but whether this was due to the transferred fish is not known because no detailed monitoring of stocking success occurred (Korhonen & Turunen 1995). The stock transfer in 2000 was closely monitored from the very beginning, and the monitoring programme included estimation of transfer mortality, determination of age and size distribution of stocked fish, estimation of larval production before and after the stocking, and hydroacoustics study of the biomass of vendace in the pelagic area (Huuskonen *et al*. 2001). One of the main objectives of our study was to determine the genetic origin of the new year class after the transferred fish had reproduced in lake Höytiäinen. For this, we analysed genetic differences between the original and stocked vendace using microsatellite DNA markers. Here, we report the results from population assignment tests and assess the success of the stock transfer by comparing the genotypes of newly hatched larvae collected after the first reproduction of the transferred fish with the genotypes of the two original populations and known hybrids of these two populations.

Material and methods

Study area

Lakes Höytiäinen and Suvasvesi are large, oligotrophic lakes in eastern Finland (Fig. 1). In late winter 2000, about 2.6 million vendace were transferred from lake Suvasvesi into lake Höytiäinen. Over 80% of the fish were age 2, the rest were primarily age 1 (Huuskonen *et al*. 2001). According to the results obtained by experimental caging, mortality of the stocked fish was high (range 61%–97%; Huuskonen *et al*. 2001), and hence the actual number of stocked fish that survived in the lake is much smaller than the original number stocked. The study area (153.9 km^2) was the southern part of the lake (Fig. 1) where the introductions were done.

Larval sampling

The total number of newly hatched vendace larvae was estimated using a stratified random

sampling design. Samples were collected from four depth strata: zone 1 (shoreline to 0.5 m), zone 2 (0.5–1 m), zone 3 (1–2 m), and zone 4 (> 2 m). Within each sampling stratum, samples were randomized around the lake. Sampling procedures and equipment are given in detail by Karjalainen *et al.* (1998). In zone 1, samples were gathered with a tube-net pushed by a wader parallel to the shore. In zones 2 and 3, a jet-driven 5-m boat with Bongo nets attached to the front was used. To ensure good coverage of the stratum, the samples were hauled along a systematic, zigzag route between the inner and outer borders of each sampling zone. In zone 4, 2 km × 2 km randomly chosen squares determined the borders of each tow. In all zones, the volume of each haul was measured with a flowmeter and catches were converted to sample abundances. The total number of larvae was expressed as individuals per hectare.

In 1999, vendace larvae were sampled in the first week after ice-out, whereas in 2000–2002 the samples were taken during the first and third week after ice-out. All captured fish larvae were preserved in the field with 94% ethanol and then taken to the laboratory, where they were identified and counted. The genetic analysis was carried out using 93 randomly selected vendace larvae sampled in 2001. Reference information was obtained by analysing 50 adult individuals from both original populations and 57 known hybrids of these populations. Hybrids were obtained by artificial fertilization of the gametes of individuals from the populations and incubating the eggs in the laboratory until hatching.

Molecular methods

DNA was Chelex® extracted from a whole larva or from approximately 1 mm^3 of a caudal fin from an adult fish according to the method of Estoup *et al.* (1996). A total of six microsatellite markers that were isolated from whitefish were tested in vendace. Of these marker loci, five loci amplified well in vendace and were chosen for the analysis: *BWF-1* (Patton *et al.* 1997), *BWF-2* (Patton *et al.* 1997), *C2-157* (Turgeon *et al.* 1999), *COCL23* (Bernatchez 1996), and *SsBgIII.26* (sequences provided by L. Bernatchez laboratory at the Université Laval in Québec, Canada). All 10 μl PCR reactions were performed in a buffer concentration of 10 mM Tris-HCL (pH 8.3), 50 mM KCL, 1.5 mM $MgCl_2$, 250 μM dNTPs, 0.05U *Ampli-Taq* polymerase, 0.1 to 0.4 μM of each primer (one of each pair being labelled with the fluorescent label, FAM, HEX or NED), and 0.2% of the original Chelex® extracted DNA. The annealing temperature for all loci was 56 °C. Following PCR, semi-automated microsatellite analysis was carried out by analysing all five loci in one run with an ABI377 sequencer (Applied Biosystems). Genotypes were scored with the aid of the Genotyper 2.0 program (Applied Biosystems).

Statistical analysis

We calculated genetic diversity indices, including observed number of alleles (A), observed number of heterozygotes (H_o), and expected level of gene diversity (H_e). As there were differences in the number of successfully scored individuals among loci and populations, the allele number was adjusted to a common sample size of 50 individuals using the formula of Ewens (1972). The level of genetic differentiation between populations was analysed by calculating the pairwise F_{ST} (Weir & Cockerham 1984). To explore the origin of the larvae in lake Höytiäinen, an individual assignment test was applied using the software GeneClass 1.0.02 (Cornuet *et al.* 1999). We used direct estimation of frequencies with 10 000 simulated individuals and a threshold probability of 0.0100. Other genetical analyses were conducted using GENEPOP 3.1b (Raymond & Rousset 1995).

Results

Larval abundance

The density of newly hatched vendace larvae in lake Höytiäinen was about 2200 ind. ha^{-1} in the two years preceding the transfer, and 4200 ind. ha^{-1} after the first reproduction of the transferred fish in 2001 (Fig. 2). In 2002, the larval density was 3400 ind. ha^{-1}.

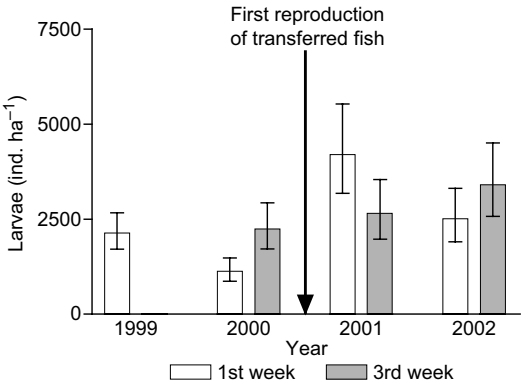

Fig. 2. Density of vendace larvae in lake Höytiäinen in 1999–2002. Abundance at the first and third week after ice-off are given separately. In 1999 there was only one sampling occasion. Vertical bars indicate 95% confidence limits.

Reference populations

Across all five loci, on average 28.6 (Höytiäinen) and 48.4 (Suvasvesi) individuals were scored successfully. The low number of successfully scored Höytiäinen-individuals was due to low amplification success of this population over all the loci. There were no differences in the genetic diversity indices between the populations: the adjusted mean numbers of alleles were 17.2 (S.D. = 8.1) in Höytiäinen and 16.4 (S.D. = 7.1) in Suvasvesi; the observed heterozygosities were 0.57 (S.D. = 0.29) and 0.56 (S.D. = 0.16); and the expected gene diversities were 0.80 (S.D. = 0.14) and 0.73 (S.D. = 0.18), respectively. Thus, the level of genetic diversity appeared to be similar in both populations (Mann-Whitney U-test for differences in number of alleles, observed and expected gene diversities: $U = 11.0$, $p = 0.84$; $U = 8.0$, $p = 0.42$; and $U = 11.0$, $p = 0.84$; respectively). A heterozygote deficit was observed in both populations. F_{is} values were positive over all loci (mean $F_{is} = 0.153$ for Höytiäinen and 0.114 for Suvasvesi) and highly significant (infinite X^2 value). This may be a result of earlier mixing of gene pools. Null alleles (non-amplifying alleles) were not detected in the data of known hybrid families. An exact differentiation test showed that populations were genetically different: four out of five loci showed significant differentiation in allele frequencies, and the combined test (Fisher's method) yielded an infinite X^2 value, which was statistically highly significant. However, the level of differentiation appears to be not very high: F_{ST} value (allele identity) between the populations was 0.021 (± 0.001; 95% confidence interval).

Sampled larvae

The mean number of alleles among sampled larvae was 15.4. The observed heterozygosity was 0.64 and expected gene diversity was 0.74. Thus, the level of genetic diversity was rather similar to the reference populations, although the mean number of alleles was slightly lower and observed heterozygosity was somewhat higher among the larvae than in the reference populations.

Of the 93 larvae, five were scored unsuccessfully and were omitted from the analysis. An assignment test was thus applied to 88 larvae. Of these, 61 individuals (69.3%) were assigned to the original Höytiäinen population and 10 individuals (11.4%) to the Suvasvesi population. Four larvae (4.5%) were assigned to the reference group of known hybrids and 13 (14.8%) could not be assigned to any of the groups using a probability threshold of 0.01.

Discussion

Although there was an increase in the number of newly hatched larvae in lake Höytiäinen after the transfer, only a small proportion of these can be attributed to the transferred fish. The majority (69%) of the analysed larvae were assigned to the indigenous lake Höytiäinen population. Only 11% may have originated from the stocked Suvasvesi individuals, and 4.5% were likely hybrids between the two populations. Overall, the assignment probabilities were quite low; only 10 individuals exceeded a probability level of 0.5 and 34 individuals exceeded the level of 0.1. These low probabilities are a natural consequence of quite low levels of differentiation between the reference populations and the analysis of only five microsatellite loci. In spite of this, the results give a quite clear indication that the majority of the larvae actually originated from the indigenous population of lake Höytiäinen.

Nevertheless, the transferred vendace reproduced successfully in lake Höytiäinen and hence stock transfer seemed to have some effect on the population structure. Precision of the assignment method (higher probability values) could be increased by analysing more microsatellites and thus more reliable estimates of the success of the stock transfer would be obtained.

We were able to analyse 93 larvae in addition to 100 adults and 57 hybrids. This was only about one percent of all vendace larvae caught during the first sampling occasion in 2001. For the reliability of the results it is therefore essential that the analysed fish were a representative sample of the larval population. While we can be reasonably confident that the samples are representative of the population in the lake (*see* Karjalainen *et al*. 1998), we have to accept that our sample size was small and hence, instead of using exact percentage values, it is better to conclude that the transferred fish made a minor contribution to larval production in lake Höytiäinen.

The spawning stock size of the indigenous vendace population in lake Höytiäinen was very small in autumn 2000. According to an echo-survey in August 2000, the biomass of indigenous vendace was well below 0.5 kg ha^{-1}. The stocked biomass was about 1 kg ha^{-1} so the contribution of the stocked fish to the 2001 year class was clearly lower than what would be expected. There are several possible reasons for the low abundance of offspring that originated from the lake Suvasvesi vendace stock. First, and in our opinion most importantly, high mortality associated with transfer simply reduced the number of this stock of vendace spawning in autumn 2000 (Huuskonen *et al*. 2001). Second, natural mortality of vendace may have been high during their first summer in lake Höytiäinen, resulting likewise in a low number of spawners. This, however, does not seem to be likely because abundance of possible predators was not very high. Stockings of brown trout had been reduced for several years before the transfer, and although the stock of pikeperch (*Sander lucioperca*) was high, vendace were not an important food item for them (H. Huuskonen *et al*. unpubl. data). Also, fishing of vendace was forbidden after the transfer. Third, the reproductive output (proportion of mature females and/or gonadosomatic index) may have been low among the transferred fish as observed in stock transfer into lake Puruvesi (Jurvelius *et al*. 1995). Finally, it is possible that the spawning behaviour of the transferred fish differed from that of original fish. Obviously, the original fish stock would be expected to be better adapted to local lake conditions and thus have higher reproductive fitness.

Transfer of fish is always a threat to an indigenous population. Mixing of gene pools, as has happened in lake Höytiäinen, may result in reduction of fitness due to out-breeding depression and loss of local adaptations (Avise & Hamrick 1996). In the case of lake Höytiäinen, however, this may not be so critical. The level of genetic difference between lakes Höytiäinen and Suvasvesi was low and, more importantly, due to earlier stock transfers, the vendace stock of lake Höytiäinen was already a "mixture" of several populations and therefore not indigenous in the literal sense. This is also indicated by the positive F_{is} values of our study. There is no information available on the genetic effects of earlier transfers, but these could be studied by analysing old scale samples of the indigenous lake Höytiäinen population before any transfer actions had taken place, and comparing the genetic structure with the current population.

Karjalainen *et al*. (2000) analysed abundance data of vendace larvae and recruits in several Finnish lakes with productive vendace fisheries, and concluded that high larval abundance is generally needed to produce a high number of recruits. According to the scale by Karjalainen *et al*. (2000), abundances of newly hatched vendace larvae between 1999 and 2002 in lake Höytiäinen have been moderate, i.e. 1200 to 6500 ind. ha^{-1}. Based on this view, no high recruitment of vendace would be expected even if mortality during the first summer was low. Because the mortality of vendace in lake Höytiäinen has on the contrary been high, possibly due to increased predation pressure by perch (*Perca fluviatilis*), the recovery of vendace stock by means of stock transfer seems to be unlikely (Huuskonen *et al*. 2001). This should not, however, be taken as an evidence of general unprofitableness of vendace transfers because high transfer mortality can be substantially reduced by improving handling and transport techniques.

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