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Linking individual migratory behaviour of Atlantic salmon to their genetic origin

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Abstract

Many stocks of fish consist of mixtures of individuals originating from different populations. This is particularly true for many salmon and trout stocks, where fish of different genetic background are being found in the same rivers and/or lakes due to stocking activities or straying caused by increased aquaculture activities. The interpretation of results from studies of survival and behaviour of fish from such “mixed stocks” require information of the genetic background of individual fish. We used genetic analysis combined with radiotelemetry to study upstream migration of Atlantic salmon (*Salmo salar*) in a Danish lowland river. The river has a small population of native salmon, but salmon juveniles from Irish, Scottish and Swedish populations have been stocked and return as adults. A total of 39 salmon were caught by electrofishing and tagged by surgical implantation. A tissue sample (fin clip) from each tagged salmon was analysed using microsatellite DNA analysis of 6 loci. Assignment tests were used to infer the population of origin. The results showed that the salmon run was composed of approximately 1/3 “native fish”, 1/3 foreign stocked fish and 1/3 escaped farmed salmon. The results indicate that stocked, foreign salmon had a slightly higher mortality and moved more up and down in the river than the native salmon did, but all salmon had problems passing the physical obstructions in the river. The DNA analyses enabled us to compare the behaviour of fish of different genetic origin, but the interpretation of the results was hampered by a high mortality of tagged fish. This study demonstrates that the combination of recent genetic methods and telemetry provides a potent tool for better management of mixed stock fisheries.

Introduction

In many rivers, with dwindling stocks of wild Atlantic salmon, attempts have been made to enhance populations through stocking programs. In several cases, broodstock from near or far rivers have been used. The results obtained from biotelemetric studies of the behaviour and migration of salmon are often inconclusive due to lack of knowledge of the genetic background and life-history of each tagged fish (e.g. Gerlier and Roche, 1998; Chanseau and Larinier, 2000; Thorstad *et al*., 2003). As many Atlantic salmon runs now consists of various proportions of individuals of different origin, such as: 1) wild natural born salmon, 2) hatchery reared offspring from wild (native or non-native) fish released at different life stages (½ year, yearlings or smolts), 3) escaped farmed salmon and 4) strays from hatchery reared fish from other rivers, it becomes very important for researchers to get background information of each fish’s genetic and ecological background (wild/released). Recently, the development of new and more sensitive population genetic markers, such as DNA microsatellites have been used to provide detailed information on the genetic structure of trout and salmon (see for example Nielsen *et al*., 1999; 2001a; Hansen *et al*., 2000; 2001). Not only salmonids, but also several other fish species have been shown to exhibit heterogeneous population structure. Thus, even “classical” marine fishes (i.e. wide distributions, large population sizes, high fecundity, and pelagic eggs and larvae) like cod (*Gadus morhua*) form separate populations (see for example Ruzzante *et al*., 1998; Nielsen *et al*., 2001a) that may overlap spatially at certain times, such as mixed feeding aggregations, but nonetheless form isolated spawning populations. The objective of this paper is threefold: 1) to discuss the importance of linking observed behaviour with genetic background, 2) present a method to obtain information of the genetic origin of experimental fish and 3) to present a case study where this method was used.

Materials and methods

Capture and tagging

Salmon for tagging were obtained by electric fishing from boat in the river downstream of a hydropower dam and from a trap established in one of the uppermost chambers of the fish ladder at the hydropower station. Upon capture salmon selected for tagging were anaesthetised in a 5 mgl^{-1} solution of clove oil and a radio transmitter with a trailing external antenna (ATS model F1835, 3.4 g in water) was inserted in the body cavity by standard surgical procedures (Jepsen *et al*., 2002). The incision was closed with two separate monofilament sutures and the tagged fish were released immediately after recovery. Salmon were released close to or at the position of capture, meaning that tagged salmon were released from the powerstation and approximately 10 km downstream. All salmon were measured to nearest cm and a small tissue sample (fin-clip) was taken for genetic analysis. The experimental procedures used in this study conform to the guidelines for the use of animals in research and local ethical conventions.

Study area

Varde River is a typical Danish lowland river with a catchment area of 1090 km^2, mainly farmed land (75%). Discharge range from 10,000 – 25,000 ls^{-1}. Through the late 1800 – early 1900, the river was heavily regulated to accommodate agricultural interests and in the 1920's a hydropower station was built. This station was further developed in 1940 and gave the river its present form (Fig. 1). The main spawning areas for salmon are situated upstream of the hydropower dam, where passage is only possible through an old (chamber-type) fish ladder with approximately 150 ls^{-1}. Upstream of the dam there is a shallow reservoir (35 ha) with dense vegetation during summer.

The regulation in combination with pollution (industrial and agricultural) caused a rapid decline in the run of Atlantic salmon and after World War II, the salmon stock was considered extinct. A few salmon were still caught, but were thought to be strays from the nearby Skjern River or other rivers. In 1993 a stocking program was initiated and every year a high number of salmon juveniles from different foreign populations (Burrishoole, Corrib, (Ireland), Lagan (Sweden), Conon (Scotland)) were released in Varde River. In 1999, samples from 101 adult and juvenile salmon caught by angling and electric fishing were analysed using DNA microsattellites (6 loci) and the results compared to those from the original (indigenous) salmon population. Old scale samples from the 1910's (N=37) were analysed to give information about the genetic composition of the original Varde salmon. The comparison of genetic profiles led to the conclusion that the salmon population in Varde River had persisted (see Nielsen *et al*., 2001b for details), but at a very low level (small effective population size). This conclusion resulted in immediate management measures: cessation of stocking of salmon of foreign origin and a protection of the adult salmon in the river (only catch and release allowed). In order to facilitate additional protective/supportive measures, knowledge of the main spawning areas of the wild salmon was needed. Thus, a radiotelemetry project was planned to provide such information during the season of 2002.

Tracking

The tagged fish were tracked manually from boat or river banks approximately once a week from tagging through the spawning period. Fish posi-

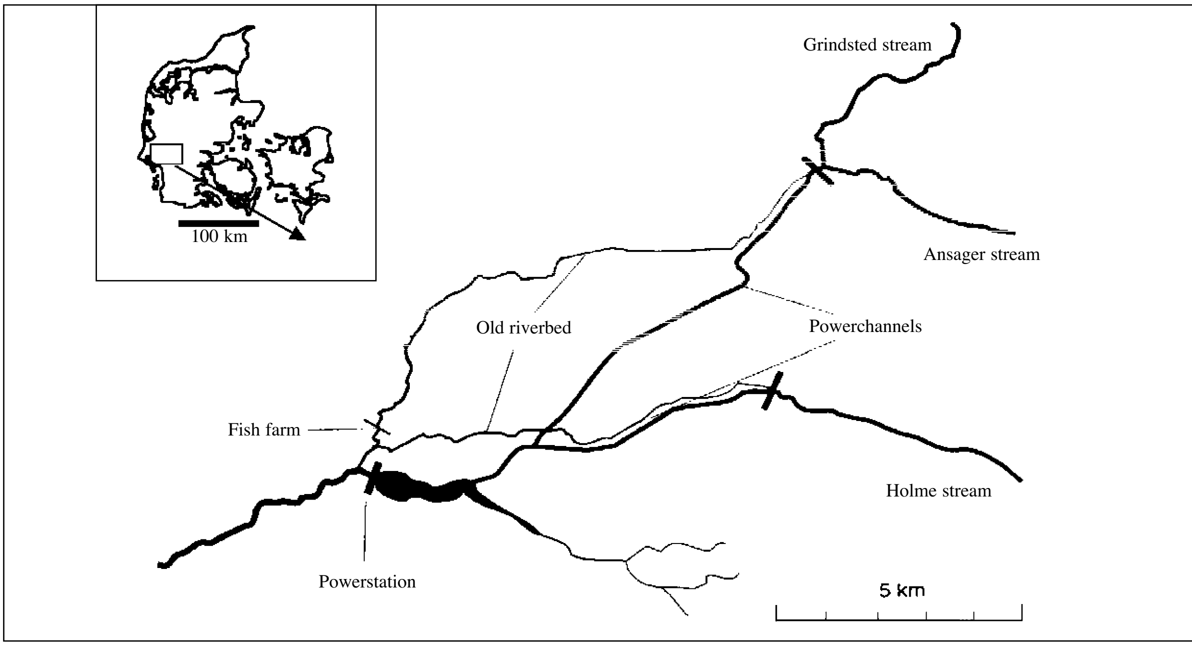

Fig 1 – Map of the study area. Vertical bars show major dams/obstacles in the system.

tions were marked by a Garmin GPS (e-trex) with fidelity of <10 m and analysed in Mapsource. Tracking was concluded in January 2003. In addition 7 automatic listening stations (ALS) were installed along the river and the tributaries.

DNA analysis

Six microsatellite loci were assayed: *SsoSL 85, SsoSL 311, SsoSL 417, SsoSL 438* (Slettan *et al*., 1995), *Ssa 85* and *Ssa 202* (O'Reilly *et al*., 1996). "Selfclassification tests", i.e., the assignment of individuals of known origin in baseline samples, to determine assignment power among populations and assignment of present population samples of unknown origin, were done using the program GeneClass (Cornuet *et al*., 1999). We used the "Bayesian method" option (modified from Rannala and Mountain, 1997). The probability of correct assignment depends largely on the relatedness of the different populations. The percentage of correctly assigned foreign individuals was rather high (98.8–99%), whereas the probability of distinguishing between individuals from the closely related Varde and Skjern populations was rather low, so we chose to group these together in Fig. 2. For further details on the assignment of individuals to populations of origin in Varde River or exogenous populations, see Nielsen *et al*. (2001b).

Results

From June 18 to September 17, a total of 59 salmon (56–102 cm Total Length) were caught by electric fishing in the lower river and in the fish ladder trap. Of these, 39 (35 electro-fished, 4 trap-caught) were radio-tagged by surgical implantation (Table 1). The results of the DNA analysis (Fig. 2) showed that nearly one third of all salmon caught were most likely of Varde/Skjern origin and most likely wild fish.

Nineteen salmon were tagged from mid-June to mid-July, where the water temperature rose to above 15 ^{0}C and we decided to postpone further tagging until the temperature decreased. The tagged fish generally moved little in the lower river and none approached the dam in attempt to move upstream during the first weeks. Most fish moved downstream after release and one of the tagged salmon apparently left the river and was not recorded again. In late July, water temperatures rose to above 20 ^{0}C and stayed very high for an unusually long period of time (6-7 weeks). During the first week of August we concluded that 13 of the tagged salmon had died. We managed to recover seven dead fish and found 4 transmitters on the bank, whereas the remaining two transmitters were irretrievable from the bottom of the river. The dead salmon and the transmitters were found close to the place of last location from tracking. Some of the dead fish were still quite fresh and examination of these revealed no apparent cause of death. One of the dead salmon had been tagged 42 days before and the incision was fully healed and the sutures shed. Two tagged salmon disappeared and were not later recorded during the period with high mortality. In September when water temperatures decreased to about 15 ^{0}C, we resumed tagging and in the period from 17 to 24 September 20 salmon were tagged. Of these, three died within few days after tagging, while the remaining were tracked for a long period through the spawning season.

The results from the tracking of the remaining 20 tagged salmon (3 surviving fish from the early tagging + 17 from the later tagging) showed several major problems for upstream migration in the river. Only seven tagged salmon used the fish ladder to enter the reservoir and the upper river (Fig.3). Five tagged salmon entered the old river channel that has a very limited flow and only gives access to a few spawning areas. Of these five, three got into a fish farm, despite 30 mm fencing. Later we electrofished a part of the outlet channel of the fish farm and estimated that 15-25% of the total spawning run of salmon was trapped there. Of the seven fish, that passed the power station, two (one Varde/Skjern 272, one foreign 083; Fig 4) moved relatively directly up to known spawning areas and stayed there during the spawning season (Nov.-Dec.). The remaining five (two Varde/Skjern, three foreign) moved in a more erratic manner and were recorded in two or three different tributaries. Two of these moved down through the fish ladder again and one later entered the fish farm, whereas the other jumped out of the fish ladder and died.

Some (3) of the tagged salmon that did not pass the hydropower station, left the lower river (to enter other rivers?), one was caught by an angler (released, but found dead six days later) and some (4) stayed in the lower river throughout the spawning period.

Generally the foreign stocked salmon moved longer distances than native salmon did (Table 1) and three of the tagged fish (Fig. 4, Fish 161) classified as Varde/Skjern, stayed in the lower river

Table 1 – Comparison of mortality (number and percentage) and migration (average and range) of foreign and native salmon (mean Total Length and range are given) The group of foreign fish are composed of 9 Burrishoole, 9 Corrib, 5 Conon, 1 Lagan and 1 farmed salmon. Mortality includes fish that disappeared from the river before the spawning period. There was statistical significant difference in measured migration length between the groups (Mann-Whitney U-test; W=7.5; p=0.02)

Origin	N	Total Length (cm)	Mortality (before November)	Measured migration (km)
Foreign	25	69 (56–89)	16 (64%)	49.8 (4–90)
Native (Va/Sk)	14	77 (58–102)	8 (57%)	22.0 (4–40)

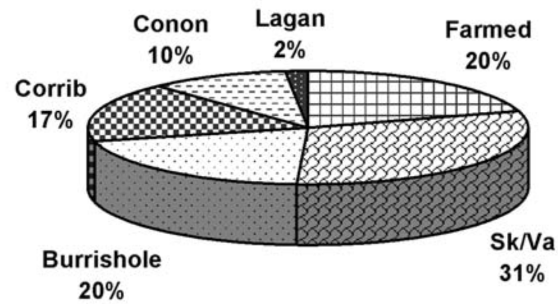

Fig. 2 – The genetic distribution resulting of the assignment test of samples from 59 adult salmon caught in Varde River in 2002.

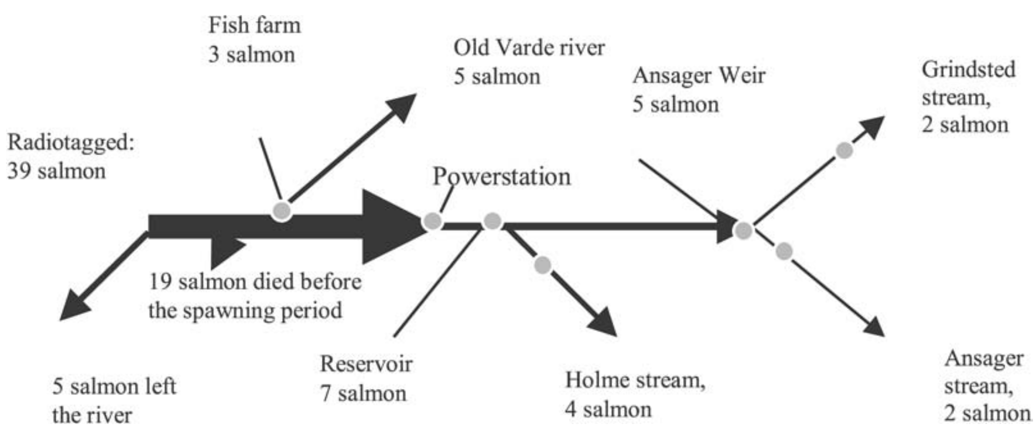

Fig. 3 – Schematic view of the spatial distribution of radio-tagged salmon in the Varde River system. The circles show position of Automatic listening stations (ALS). Note that numbers don't add up because some of the tagged fish were observed in several tributaries (see text).

and did not approach the dam at any time. During the spawning period these individuals showed clear preference for a certain area, where there was good current and coarse gravel on the bottom. During very low flow, it became clear that some fish had actually spawned there as reds were visible. Earlier this area was not judged as suitable spawning area because of depths of >1 m, but attempts will be made to find juveniles in the area using electric fishing.

Discussion

The expected difference in behaviour between native and introduced fish was not documented by the present results. This was partly due to the high mortality of the fish and to the many problems caused by man made obstacles in the river system. However, the fact that only one of the tagged native fish showed distinct homing behaviour to a tributary, whereas three presumably spawned in

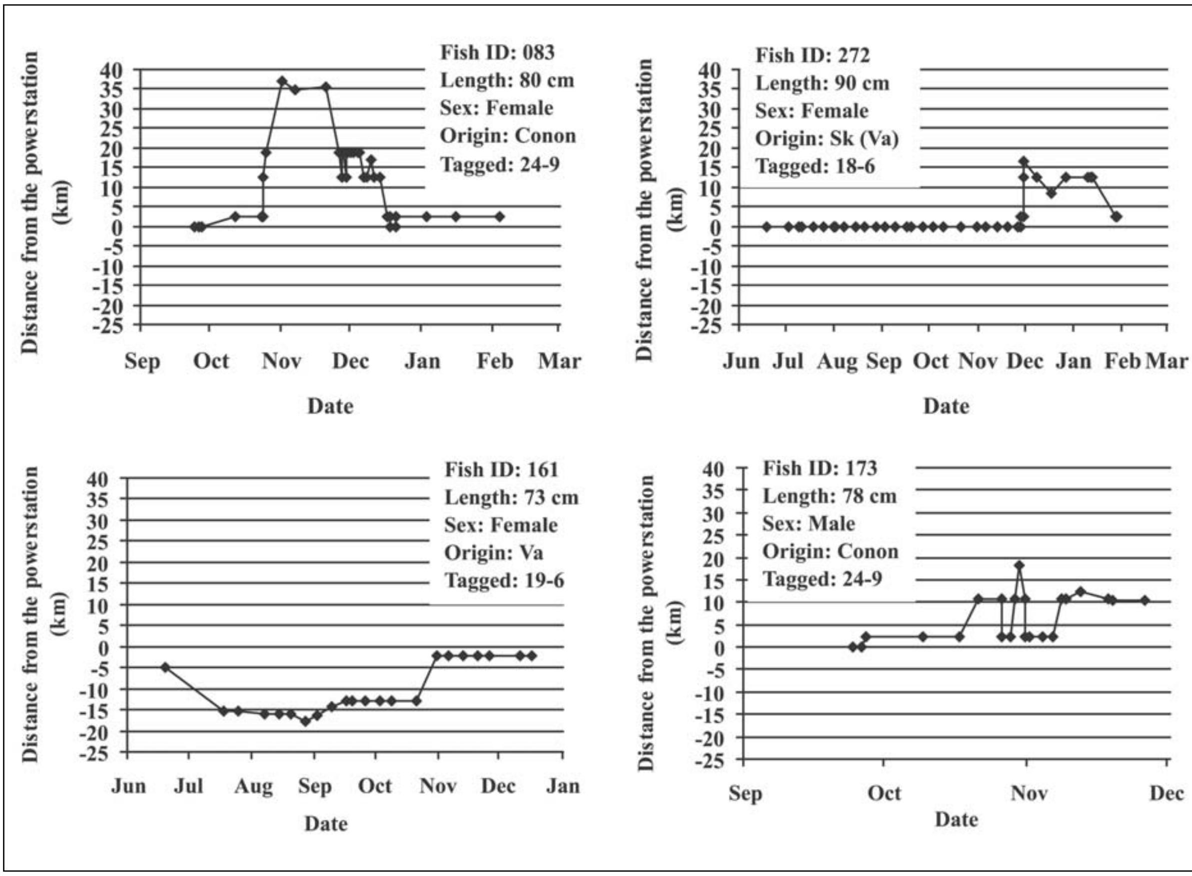

Fig. 4 – Examples of migration of radio-tagged salmon in Varde River.

the river downstream of the power station, indicate that this area may be very important for the persistence of Varde salmon as long as the obstacles exist.

The use of genetic analyses proved to be very valuable in the study of the salmon in Varde River, but even with sophisticated analyses like this one, the picture was not black and white. The genetic profiles can give information (with a certain probability) of what population each fish belongs to, but not about whether it is hatchery reared for supportive breeding or wild fish. During the last three years the salmon used for stocking in Varde River are all offspring from broodstock that was genetically tested and assigned to be Varde/Skjern salmon (supportive breeding), so we could never be 100% certain that a tagged fish was indeed a wild indigenous salmon. A comparison of the behaviour of native *vs*. foreign fish reveals that in both groups there are fish that move erratically up and down the river and also (fewer) fish that show a much more determined migration. However, the comparison of distance moved (Table 1) indicate that the foreign, stocked fish show more of this up and downstream movement than the native fish. This is in accordance with the results from a study of stocked salmon in River Gudenaa, Denmark where most salmon showed extensive erratic up- and downstream movements (Aarestrup *et al*., 2000).

The high mortality of the tagged fish came very suddenly and unexpectedly. It was probably caused by unusual weather conditions. The fact that the majority of the fish died within a short period of few days, despite the fact that they were tagged 5-42 days earlier, indicates that it was unlikely that handling and tagging was directly responsible for the deaths. In the same period we did find dead, untagged salmon and trout in the lower river. Electric fishing in the river after the deaths, provided very poor catches (of sea-trout and salmon) compared to earlier and knowing that no fish had passed upstream, we concluded that untagged salmon and trout either had died or left the river. However, we cannot rule out the possibility that the combined effect of capture, handling and tagging made the tagged fish more vulnerable to hostile environmental conditions.

Despite the fact that the high mortality left us with few fish to analyse, the results have highlighted several important problems to be solved in order to secure the existence of salmon in Varde River. The study also provided an indication of how the salmon population may have been able to persist by utilising the lower river for spawning. The current data cannot be used as documentation of successful spawning in unexpected areas, but future research may provide that. The results have directly effected management decisions regarding the monitoring of escaped farmed salmon in several rivers, monitoring the environmental conditions during summer in the lower Varde River and increased the political pressure to secure passage at the power station. In addition it has been decided to focus more research on how to prevent fish from entering into fish farms.

References

Aarestrup, K., Jepsen, N., Rasmussen, G., Økland, F., Thorstad, B. & Holdensgaard, G. 2000. Prespawning migratory behaviour and spawning success of sea-ranched Atlantic salmon (*Salmo salar* L.) in the River Gudenaa, Denmark. *Fish. Mgnt. Ecol.*, 7: 387-400.

Chanseau, M. & Larinier, M. 2000. The behaviour of returning adult Atlantic Salmon (*Salmo salar* L.) in the vicinity of a hydroelectric plant on the Gave de Pau river as determined by radiotelemetry. *In* A. Moore & I. Russel, eds. *Advances in Fish Telemetry*, 257-264 pp. Lowestoft, Suffolk, UK, CEFAS. 264 pp.

Cornuet, J.M., Piry, S., Luikart, G., Estoup, A. & Solignac, M. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics*, 153: 1989-2000.

Gerlier, M. & Roche, P. 1998. A radiotelemetry study of the migration of Atlantic salmon (*Salmo salar* L.) and sea trout (*Salmo trutta trutta* L.) in the upper Rhine. *Hydrobiologia*, 371/372: 283 – 293.

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 trout and wild brown trout (*Salmo trutta* L.). *Mol. Ecol.*, 9: 583-594.

Hansen, M.M., Kenchington, E. & Nielsen, E.E. 2001. Assigning individual fish to populations using microsatellite DNA markers: Methods and applications. *Fish and Fisheries*, 2: 93-112.

Jepsen, N., Koed, A., Thorstad, E.B. & Baras, E. 2002. Surgical implanting of telemetry transmitters in fish. How much have we learned? *Hydrobiologia*, 483: 239-248.

Nielsen, E.E., Hansen, M.M. & Loeschcke, V. 1999. Genetic variation in time and space: Microsatellite analysis of extinct and extant populations of Atlantic salmon. *Evolution*, 53: 261-268.

Nielsen, E.E., Hansen, M.M., Schmidt, C., Meldrup, D. & Grønkjær, P. 2001a. Population of origin of Atlantic cod. *Nature*, 413: 272.

Nielsen, E.E., Hansen, M.M. & Bach, L. 2001b. Looking for a needle in a haystack: Discovery of indigenous salmon in heavily stocked populations. *Cons. Gen.,* 2: 219-232.

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. *Can. J. Fish. Aquat. Sci.*, 53: 2292-2298.

Rannala, B., Mountain, J.L. 1997. Detecting immigration by using multilocus genotypes. *Proc. Ntl. Aca. Sci. USA*, 94: 9197-9201.

Ruzzante, D.E., Taggart, C.T. & Cook, D. 1998. A nuclear DNA basis for shelf- and bank-scale population structure in Northwest Atlantic cod (*Gadus morhua*): Labrador to Georges Bank. *Mol. Eco.*, 7: 1663-1680.

Slettan, A., Olsaker, I. & Lie, Ø. 1995. EMBL, GenBank, DDBJ databases. Accession number: Z48596, Z48597, Z48578 and Z49134.

Thorstad, E.B., Økland, F., Kroglund, F. & Jepsen, N. 2003. Upstream migration of Atlantic salmon at a power station in the River Nidelva, Southern Norway. *Fish. Mgnt. Ecol.*, 10: 1-8.