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Oral examination:

GENETIC ANALYSIS OF A POPULATION OF ATLANTIC SALMON (*SALMO SALAR*, L.) IN THE RHINE SYSTEM

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Atlantic salmon (*Salmo salar*, L.)

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SUMMARY

The present study was developed under the guidelines of a regional project to support the management of the anadromous salmonids in the Rhine, particularly the reintroduction of the Atlantic salmon (*Salmo salar*, L.)
The main aim was to assign the salmons ascending to the Iffezheim lock to salmons used for reintroduction in the Rhine. It was analysed if such an assignment was reasonably possible. Furthermore, we wanted to find out if an established Rhine population already exists.

The Ph.D. study started in April 2005 and was supported by voluntary field workers who sampled and checked the fish ladder at Iffezheim (Landesfischereiverband Baden).

Genotypes, based on the analysis of polymorphic microsatellite loci, of the sampled year-classes from 2002 to 2005 inclusive, have been analyzed, and referenced to suitable outgroup populations. An overall amount of 180 salmon samples have been analyzed (65 from Rhine/Germany, 22 from Burrishoole/Ireland, 50 from Allier/France, 28 from Ätran/Sweden and 15 from Lagan/Sweden).

An allozyme analysis was performed in order to identify individuals which eventually were misidentified during the sampling with brown trout or hybrids from the two species. 11 fishes out of the 304 analyzed turned out to be misidentified trouts given as salmons.

Microsatellites genotyping involved sixteen primers, two STR (Short Tandem Repeat) amplified for two loci, and nine loci (SSOSL85, SSOSL311, STR15, Ssa171, Ssa402*, Ssa402**, Ssa408, Ssa202 and Ssa411).

Genetic similarities have been evaluated by means of population genetics and forensic assignment statistics of individuals.

Analysed individuals were all in HWE (Hardy-Weinberg Equilibrium). Heterozygosity ranged from 0.60 to 0.79. Analysis of allele frequencies revealed a heterozygotes deficiency and a significant genetic drift. A Whalund effect was supposed to lie behind this homozygote excess.
By the bottleneck analysis no evidence of recent reduction in population size has been observed in the Rhine subpopulation according to TPM (two-phase model) and SMM (stepwise mutation model).

The assignment tests showed that the Rhine subpopulation shares only a small fraction of alleles with the hatchery populations. Swedish genotypes seemed to be the most representative. Swedish individuals showed the best adaptation and reproductive success with high rates of returning individuals.

Total of 118 private alleles have been found, the majority of them with a frequency equal or below 0.06. A high rate of private alleles have been found among the Rhine individuals that could be used as genetic markers in order to identify individuals of this cohort and eventually select them for a proper stocking program.

The genetic differentiation analysis showed that the Rhine subpopulations cluster together with a significant distance among the other subpopulations (F_{st} ranged from 0.079 of BUR to 0.051 of Allwild and Lagan and from 0.035 to 0.012 within Rhine subpopulations).

According to the results obtained by the neighbour-joining and the UPGMA analysis, both based on genetic distance, 4 main groups were clearly defined:

1. Rhine individuals, divided per sampled year (from 2002 to 2005);
2. Swedish individuals from hatcheries (Ätran/Lagan);
3. French individuals from hatcheries (Allhatc/Allwild);
4. Irish individuals from hatcheries (BUR).

Rhine individuals clustered together with a significant bootstrap value. Swedish and French individuals clustered together following, as expected, their geographical origins. Irish individuals were considered as the outgroup.

Swedish individuals showed the highest degree of genetic similarity to the ones of the Rhine, French and Irish individuals showed the lowest.

According to an analysis of scale patterns, Rhine individuals apparently migrate to the sea after at least two years spent in the freshwater and come back to the spawning place after one or two years (called 1 or 2 winters returning, respectively). A few individuals coming back after more than two years (called multi winter returning) have been observed, but rarely.

Some useful conclusion can be derived from the results of this study: i) a local adapted Rhine subpopulation should be considered in further projects, ii) stocking and reintroduction of individuals of this local subpopulation, besides the already existing programs, is desirable. iii) Swedish individuals (Lagan/Ätran) should be preferred for stocking programs instead of French and Irish individuals. iv) Conservation programs as “Lachs 2020” are fundamental in order to maintain the Rhine Atlantic salmon populations and to continue with the habitat restoration in order to create more suitable spawning places.

ZUSAMMENFASSUNG

Die vorliegende Promotionsarbeit wurde im Rahmen der Richtlinien eines regionalen Projektes entwickelt, welches sich mit dem Wanderlachs bzw. der Wiederansiedlung des Atlantischen Lachs (*Salmo salar*, L.) im Rhein beschäftigt.

Die zentrale Frage war, ob es bei den in der Schleuse Iffezheim gefangenen Lachse um Fische handelt, die zur Wiederansiedlung eingesetzt wurden oder ob es im Rhein bereits eine eigene Subpopulation des Lachses gibt.

Die Promotionsarbeit begann im April 2005; die Probennahme wurde von Freiwilligen vor Ort durchgeführt (Landesfischereiverband Baden), welche an der Fischtreppe in Iffezheim Fische markierten und kontrollierten.

An den beprobten Lachsen wurden eine Genotypisierung mittels Mikrosatelliten-Analyse durchgeführt. Mittels 9 polymorphen Mikrosatelliten-Loci wurden die erfassten Bestände der Jahrgänge 2002 bis 2005 analysiert und verschiedenen Populationen zugeordnet. Insgesamt wurden 180 Lachse erfasst und analysiert (65 aus dem Rhein/Deutschland, 22 aus Burrishoole/Irland, 50 aus Allier/Frankreich, 28 aus Ätran/Schweden und 15 aus Lagan/Schweden). Ein Allozym-Analyse wurde verwendet, um eventuell falsche Art-Zuordnungen zu braunen Forellen oder Hybriden der beiden Arten erkennen zu können. Insgesamt 11 der 304 beprobten Fische konnten so als Forellen identifiziert werden.

Bei der Markierung mit Mikrosatelliten wurden sechzehn Primer, zwei STR (Short Tandem Repeat) verstärkt für zwei Loci, und neun Loci verwendet (SSOSL85, SSOSL311, STR15, Ssa171, Ssa402*, Ssa402*, Ssa202 und Ssa411).

Genetische Ähnlichkeiten wurden anhand der vorhandenen genetischen und forensischen Programme evaluiert. Die Genotypen der analysierten Fische waren allesamt HWE (Hardy-Weinberg Equilibrium). Die Heterozygotie lag zwischen 0,60 und 0,79. Die Analyse der Allel-Frequenz ergab einen Mangel an Heterozygotie. Ein Whalund-Effekt wurde als Grund für den Mangel an Heterozygotie vermutet. Bei einer Bottleneck-Analyse mit TPM (two-phase model) und SMM (stepwise mutation model) konnten keinerlei Anhaltspunkte für eine Bestandsverringerung der Subpopulation im Rhein gefunden werden.

Insgesamt wurden 118 Allele festgestellt, die meisten mit einer Frequenz gleich oder niedriger als 0,06. Eine hohe Anzahl an privaten Allelen wurde im Rhein nachgewiesen. Diese können als genetische Marker verwendet werden, um Einzelfische in einem Schwarm zu identifizieren oder für ein Züchtungsprogramm zu verwenden.

Die genetische Unterscheidungsanalyse zeigte, dass die im Rhein lebenden Subpopulationen sich sehr von anderen Subpopulationen unterscheiden (F_{st} variierte von 0,079 BUR bis 0,051 zu den Allwild- und Lagan-Beständen und von 0,035 bis 0,012 zu den Rhein-Subpopulationen).

Ein Zuordnungstest mit STRUCTURE belegte, dass die Subpopulation im Rhein nur geringe Übereinstimmungen mit den Allelen der ausgesetzten Fische aufweist und dass die Rheinlachse im wesentlichen Allele der schwedischen Population besitzen. Schwedische Fische verfügten offenbar über die beste Anpassung, eine erfolgreiche Fortpflanzung sowie eine hohe Anzahl an Rückkehrern.

Nach den Ergebnissen der Distanzmethoden Neighbour-joining und die UPGMA-Analyse, konnten 4 Hauptgruppen klar definiert werden:

1. Rheinfische, unterteilt in die erfassten Jahrgänge von 2002 bis 2005;
2. Schwedische Fische aus der Aufzucht Ätran / Lagan;
3. Französische Fische aus der Aufzucht Allhatc/Allwild;
4. Irische Fische aus der Aufzucht BUR.

Rheinfische wiesen einen signifikanten Bootstrap-Wert auf. Schwedische Lachse zeigten den höchsten Grad an genetischer Ähnlichkeit mit den Rheinlachsen; die französischen und irischen Fische wiesen die niedrigste Ähnlichkeit auf.

Aus der Schuppenanalyse ergab sich, dass die Rheinfische nach spätestens zwei Jahren vom Rhein ins Meer ziehen und nach weiteren 1-2 Jahren zu ihren Laichplätzen zurückkehren. Rheinfische wiesen ein Verhalten auf, nach dem sie generell nach ein bis zwei Wintern aus dem Meer zurückkehren.

Einige hilfreiche Schlußfolgerungen können aus dieser Studie gezogen werden: i) bei zukünftigen Projekten sollten auch lokale Subpopulationen im Rhein in Betracht gezogen werden; ii) eine Züchtung bzw. Wiederansiedelung von Fischen dieser lokalen Subpopulationen zusätzlich zu den bereits bestehenden Programmen ist wünschenswert; iii) Züchtungen sollten mit schwedischen (Lagan/Ätran) und nicht mit französischen oder irischen Fischen erfolgen; iv) Programme zur Bestandserhaltung, wie das Programm „Lachs 2020“, sind grundlegend für das Überleben des Atlantischen Lachs im Rhein ebenso wie die Bewahrung oder Wiederherstellung des Habitat, um mehr geeignete Laichplätze zu schaffen.

1 INTRODUCTION

Recent studies have shown that microsatellite loci are useful markers to study genetic structuring within species (Presa, 1995) and to clarify the question of the interactions between wild and domesticated fishes that are deliberately released in the spawning rivers or escaped from hatcheries (Fritzner et al. 2001, Hansen et al 2001, 2006).
This task does not include a traditional population genetic research, but is more likely to embrace forensic biotechnology. Work does not refer to the population level but the analysis is centred on individuals.
Subject of the present study is one Atlantic salmon (*Salmon salar*, L. 1758) population in the Rhine river system.
Microsatellite method has been applied mainly for the following reasons:

- very little fish blood is needed, so the animal is not damaged and can be released after the sampling;
- the markers can be referred to literature data on other populations;
- high polymorphisms of microsatellite.

The study is supported by the Fischereiverband Baden, Landesfischereiverband Baden-Württemberg, the fisheries authorities of the relevant Regierungsbezirke on the Rhine, mainly at Karlsruhe, and in the provincial ministry at Stuttgart, the Fischereiforschungsstelle Baden-Württemberg, the Landesanstalt für Ökologie Nordrhein-Westfalen, and the natural history museums of Stuttgart and Frankfurt.

1.1 Natural History

The last glaciation, dating from ~115000 to 10000 years ago (Andersen and Borns, 1994) had a great influence on the biodiversity of northern Europe.
At that time, ice sheets covered the whole of Iceland and all but the southern extremity of the British Isles. Northern Europe was largely covered, the southern boundary passing through Germany and Poland, but not quite joined to the British ice sheet. This ice extended northward to cover Svalbard and Franz Josef Land and eastward to occupy the northern half of the West Siberian Plain, ending at the Tamyr Peninsula (Ehlers et al., 2004).
The climatic cycles associated with Pleistocene glaciations have drastically reshaped the distribution of fauna and flora in Europe. The northern regions were devastated and

recolonized according to advances and retreats of the ice sheet (Taberlet at al. 1998, Hewitt 1999, 2000).

The alternating postglacial fresh and brackish water phases of the Baltic created, for the aquatic fauna, either opportunities or barriers for the dispersal, colonization and lineages mixing (Koli, 1969).

Refugia contributing to current-day freshwater fauna have been identified in central and eastern Europe (Kontula and Väinölä, 2001), while marine and anadromous fish are most likely to have different histories due to their differing environmental requirements.

Species like Atlantic salmon are particularly difficult to understand.

The Atlantic salmon is one of the species that have been mostly influenced by the last glaciation as ice sheets covered large parts of its present distribution range both in Europe and in North America and has re-colonised north European waters following the last ice age (Tonteri et al., 2005).

Using different genetic markers it has been demonstrated that there is a division between the North American and European salmon population (Ståhl 1987, King et al. 2001).

Molecular data suggest that European salmon are further divided into 2 major groups: Atlantic salmon and Baltic salmon (Ståhl 1987, Kazakov and Titov 1991; Koljonen et al. 1999, Nilsson et al. 2001).

Nowadays, salmonid fish inhabit the regions of the Northern Hemisphere where the natural habitat has been heavily altered by human activities. Besides, salmonids have a very high standard for suitable habitats; therefore they are among the species that have mostly suffered the degradation of the aquatic environment in a great part of Europe and North America. Hydroelectric dams in many rivers have added the problems of impassable barriers for the migration between spawning areas in the fresh water and the feeding areas in the sea. Moreover, salmonid fish have been for long time the object of an intensive over-fishing for commercial and recreational fishermen.

All these factors have engraved on the drastic decrement of a great number of natural populations. As a consequence, the most common attempt to re-establish the size of the natural populations has been the introduction in nature of hatchery reared fishes. This method brought about the problem of the possible negative interaction between the wild populations and the hatchery fishes (Nelson and Soulé 1987, Hansen and Loeschcke 1994).

Besides, from the aquaculture installations of these species throughout the years occurred massive escape of fishes, which is considered the principal threat for the natural populations (Heggberget et al., 1993).

The reason for this threat is due to the different genetic structure of the two groups. The wild populations show a high level of genetic differentiation (Ryman 1983, Ståhl 1987) caused by the "homing" effect, characteristic of most of these species, so that individual population could be adapted to the specific environmental conditions of their specific habitat. On the contrary the hatchery populations often show a close relationship (Krieg and Guyomard 1985, Garcia-Marin et al. 1991, Hansen et al. 1997) caused by the founding of new hatchery from already established populations and also by the low number of breeders used to develop new generations. In addition the hatchery populations show a

sort of domestication when introduced in the wild, low fitness of them is an indication factor of this status (Hansen et al. 1995).

Taxonomy and geographic range

Atlantic salmon was classified as the species *Salmo salar* by Linnaeus in 1758.

This is one of the 20 species knows as Salmoninae, a subfamily of the Salmonidae family (Philips and Oakley, 1997).

Since Wilder (1947) showed that no evident differences in morphology and meristic character occurred between anadromous and non-anadromous forms, the species has been considered monotypic.

The historical distribution of *S.salar* is North Atlantic and its coastal drainages.

The historical range in Europe extends from Iceland in the northwest to the Barents and Kara Seas in the northeast and southward along the Atlantic coast (Fig.1).

Eastward Atlantic salmon occurred in most rivers draining into the Baltic and North Seas. However, native, wild stocks are no longer found in the Elbe and the Rhine, or in many rivers draining into the Baltic Sea, which previously had abundant salmon runs.

The species is also extinct or severely depressed in the rivers of France, Spain and Portugal, at the species' southern limit.

Over the last century the species range has generally contracted and fragmented due to industrialisation and bad water management (Parrish et al., 1998).

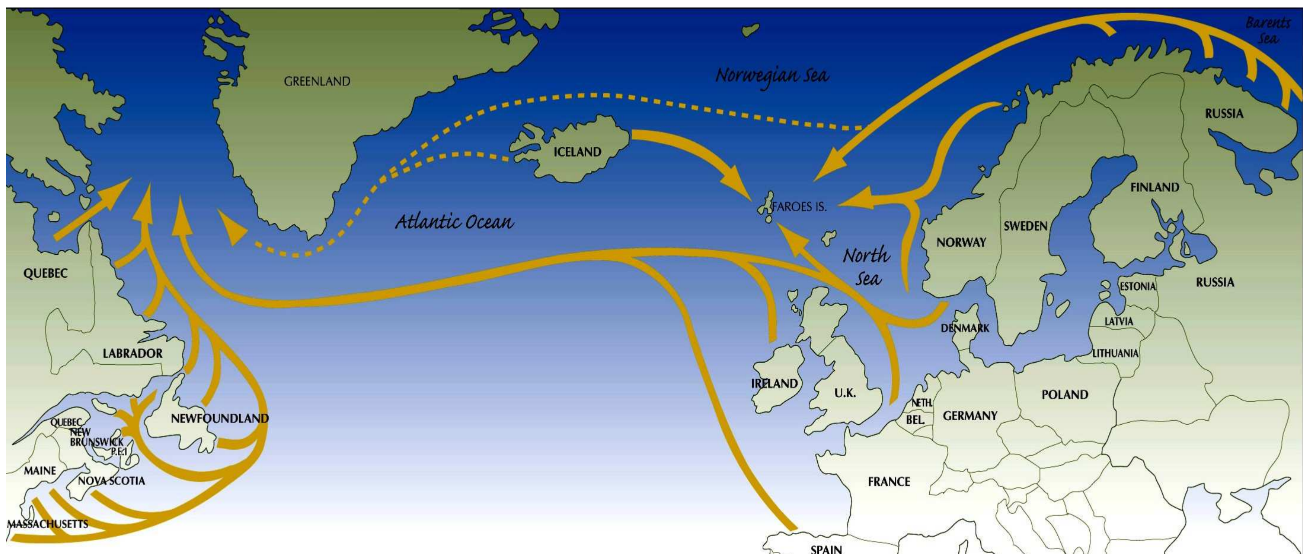


Fig.1 Atlantic salmon geographic range and migration routes (image from Atlantic Salmon Federation website http://www.asf.ca/about_salmon.php)

1.2 Atlantic Salmon life history

Atlantic salmon is mostly an anadroumus species therefore is characterised by transitional migrations between fresh water and marine habitats.

Reproduction and nursery phases occour in fresh water, followed by a feeding period in the marine environment .

The Atlantic salmon is a salmonid fish typified by laterally compressed body and dorsal adipose fin, posterior to the main dorsal fin.

Relatively large cool rivers with extensive gravelly bottom headwaters are essential during their early life.

Juvenile Atlantic salmon have one of the most norrowly defined thermal requirements for survival, feeding and growth of all the species of salmonids (Elliott, 1991). However, the range of water temperatures is highly variable and the range of thermal tolerance for the species is 0-27,8 ℃ throughout the year.

Atlantic salmon has high water chemical-physical requirements and usually occur in oligotrophic and relative unproductive streams, but in some regions (southern United Kingdom and northern Spain) salmon naturally occur in highly productive, calcium-rich systems.

Into the sea, salmon seems to prefer temperatures between 4 and 12℃. They can withstand exposure to temperatures reaching their lower lethal limit (-.7°C) and their upper lethal limit (27,8℃), but only for a short period of time (Bigelow, 1963).

Life-history stages of the species are: eggs (Fig.2), alevins (Fig.3), fry (Fig.4) and parr (Fig.5), live in the fresh water.

Fig.2 Atlantic Salmon eggs

Fig.3 Atlantic salmon alevins

Fig.4 Atlantic salmon fry

Fig.5 Atlantic salmon parr

The freshwater phases of Atlantic salmon vary between 1 to 5 years, according to river location. While the young in southern rivers, such as in the English Channel, are only one year old when they leave, the ones who live further north, such as in Scottish rivers, can be over four years old. The average age correlates to temperature exceeding 7℃.

Alevin stage: during this phase, the fish stays in the breeding ground and uses the remaining nutrients in their yolk sack. During this developmental stage, the young gills develop and become active hunters.

Once they are able to do so, they reach the fry stage. The fish grows and subsequently leaves the breeding ground in search of food. They move to areas with higher prey concentration. The final freshwater stage is when they develop into parr and prepare for the trek to the Atlantic Ocean.

During the fry age, the Atlantic salmons are very susceptible to predation. Nearly 40% are eaten by trout alone. Other predators include other fish and birds.

The older juvenile phase is characterised by vertical “parr” marks and small red spots on the sides of the body, which are lost in older fish. In the older stage, that characterises the migration phase, they are called smolts and are typically silver coloured, more elongated with darker coloured fins (Fig.6).

The parr rarely used shallow (< 10 cm) and deep (> 60 cm) stream areas, low mean water velocities (< 10 cm s-1), fine substrate (sand and finer) and stream areas without cover. However, the calculated habitat preferences were also affected by habitat availability (Heggenes,1991).

Fig.6 Atlantic salmon parr (above) and smolt (below)

Silver is the typical colour of adult salmon in the sea, but it rapidly changes after they enter the rivers and becomes even more reddish brown as they approach the spawning stage.
Males are distinguished from females by their brighter coloration, hooked lower jaw (kype) and large adipose fin.
Morphological differences between male and female have been observed only during the spawning stage, at the end of July or August, when the male lower jaw becomes as a “hook” and the female does not change in shape and colour (Fig.7).
Far from this stage the only way to distinguish male from female would be to look at the gonads.

Fig.7 Spawning female (on the left) and male “kype” (on the right) captured in the Iffezheim fishpass (image by D. Degel)

Longevity, age and body size at maturity

Like in many other salmonid species, most animals die after spawning (Patnaik et al., 1994). While most animals return to spawn in rivers, however, there are alternative life histories: parrs have a small body size and mature early. They could never migrate and

survive reproduction and breed again (Hutchings and Myers, 1994). Unverified estimates suggest that these animals may live up to 14 years

Fish may grow to a very large size and the biggest ones, which have reached up to 32Kg, are usually found in Russian and Norwegian rivers.

In Atlantic salmon dominance status and size are good predictors of the life history strategy subsequently adopted by parr within a sibling group. Small or subordinate fish has a much higher probability of adopting the strategy that leads to a cessation of growth over the following winter and a delayed migration if compared with larger or dominant individuals (Hutchings and Myers, 1994).

Dominance relations tend to be very stable (Jenkins 1969, Bachmann 1984, Abbot et al. 1985). However dominance status or ability to get food may be significant in determining life-history strategies only when conditions for growth are intermediate, because under very good conditions the majority of fish will smoltify after one year (Bagliniere and Maisse, 1985) and under very poor conditions none of them will smoltify (Metcalfe et al., 1986)

1.3 Migration: downstream migration, homing, upstream migration

Downstream migration

The downstream migration of smolts normally occurs after the juveniles have spent 1-5 years within the river (Klemetsen et al., 2003).

The migration of smolts into natural rivers is at high risk for failure, since passage of obstacles, delayed migration and predation can lead to high mortalities in the smolt-run (Hvidsten and Johnsen, 1977).

To minimize the predator pressure Ruggles (1980) suggested a smolt migration behaviour selecting high water velocities.

Reports have demonstrated that smolts generally migrate into high flow areas of the river and close to the surface (Hvidsten and Johnsen 1997, Moore et al. 1998b).

Smolts have been shown to migrate actively into lentic areas; they have high swimming capacity and can burst up to 1.95 m s^{-1} (Peake and McKinley, 1998).

Atlantic salmon smolts leave fresh water and migrate into the feeding areas of the ocean during spring and summer (Thorpe 1988, Mills 1989).

Indirect evidence shows that the permanence in the river estuary seems to be relatively short, because very few post-smolts are recorded in estuary or coastal waters during summer and autumn, besides they are already present in oceanic areas in the Northeast Atlantic (Holm et al. 2000, Holst et al. 2000).

There is strong evidence that most of the post-smolts come from rivers in southern Europe (Holst et al. 1996), but when they reach the ocean it is very difficult to keep track of their movements and migration routes. Although many countries have developed major tagging programs (West Greenland, Faroe Islands and northern Norwegian Sea), the number of fishes, which has been recaptured, is strictly linked to the fishing effort and might not represent a significant statistic value.
The migration of Atlantic salmon seems to be correlated in this phase to prominent ocean currents, continental shelf features and feeding areas.
Some possible feeding and migration routes have been detected according to these tagging programs.
Fishes from European stocks move far into the North and northeast Atlantic to get food. Salmon from southern Europe seems to contribute to the stocks of the west coast of Greenland (Hansen and Jacobsen, 2000).
Many factors can potentially influence the permanence of the salmon in the sea, such as fisheries, pollution, predation, food competition, parasites and diseases.
Natural mortality in the sea of wild and reared smolts ranges from 70% in the River Bush, Northern Ireland, to 99% in the Penobscot River, Maine, USA (Potter and Crozier, 2000).

Homing

Salmon has a great sense of smell, hearing, and taste which help them in finding food and foreseeing danger. Variation in external pressure, ad perceived by lateral line, is one of the main tools adopted by salmons in external feeling.
Atlantic salmons also use their senses to find the way back to their natal breeding habitat. Through imprinting, young fry memorize details about their home streams and they use this knowledge as adult spawners to find their way back. Scientists cannot exactly assess how salmon can complete this feat, but some hypothesis say that salmon oriented themselves by visual (uses the sun and the stars as navigational guides) or chemical cues, while others claim that these fishes have stored the taste of their home water in their brain. The general feeling is that that salmons are guided home by the characteristic odor of the parent stream which is imprinted during the smolts' migration (Maynor, 1996).
Upstream migration generally takes place after 1-3 years in the sea.
Return migration of the salmons to their natal rivers involves at first an orientation phase, from the feeding areas back to their home region and afterwards a homing phase in coastal and estuarine areas (Hawkins et al. 1979, Hansen et al. 1993).

Upstream (Spawning) migration

Most Atlantic salmons in Norway and Canada enter the rivers from May to October (Klemetsen et al. 2003), with a general tendency for large multi-sea-winter salmons to enter the rivers earlier in the season than smaller one-sea-winter fishes (Power 1981; Jonsson et al. 1990). In Scotland and other parts of the UK, salmon can enter the rivers in all months of the year, with some individuals entering more than a year prior to spawning (Klemetsen et al., 2003). In the rivers of the Kola Peninsula in Russia, such as the River Varzuga, there is a summer run of salmons spawning the same year, and an autumn run of salmons remaining in the river until the spawning period the year after (Lysenko, 1997).

Upstream migration and spawning are energetically demanding. Usually upon rivers Atlantic salmon cease feeding and again, as for the downstream migration, physiological transition between saline and fresh water is made.

Timing and patterns of the individual migration are correlated to the sex, size of the fish, river discharge and water temperature and velocity.

Maximum net ground speeds recorded during undisturbed migration was 37 km day^{-1} in the Aberdeenshire Dee, 15 km day^{-1} in the River Lærdalselva, and 49 km day^{-1} for multi-sea-winter salmon and 47 km day^{-1} for grilse in the River Tana (Hawkins and Smith 1986, Økland et al. 2001, Karppinen et al. 2004, Finstad et al. 2005). The highest migration rates were recorded early in the river migration phase and generally decreased as the fish approached the spawning ground.

Mean net ground speeds recorded in different studies generally varied between 1.6 and 31 km per day (Hawkins 1989, Heggberget et al. 1996, Gerlier and Roche 1998, Karppinen et al.2004, Johnsen et al. 1998, Økland et al. 2001, Rivinoja et al. 2001, Thorstad et al. 1998;2003b;2005b).

Timing is also related to a wide range of environmental influences (Gardner, 1976) that could affect the exposure to fisheries and predators and can therefore have an high impact on the abundance and character of the spawners (Smith et al., 1994).

Usually large females arrive earlier to the river followed by large older males, usually multi-winter-salmons, finally small males, called grisle, arrive (McKinnell 1998, Shearer 1992).

The upstream migration can be divided into two phases. The first is a slow rise through the transition between sea and river with periods of active movements alternating with stationary periods, that can be followed by a long residence in a single pool. The second phase is characterised by a rapid upstream migration to spawning sites (Hawkins and Smith 1986, Laughton 1991).

Usually fishes from the same natal source tend to regroup at or near their “born” areas prior spawning (Youngson et al., 1994).
In most situations, salmons migrating back to a natal place include individuals of different sea and freshwater age. So reproduction season involves individuals born in different years. The result is that different generations are overlapping, a behaviour that have a high impact for the genetic character of Atlantic salmon populations.

Upstream migration of released and escaped hatchery salmon
Hatchery-reared salmon returning to river as adults show more variable movement pattern than wild salmon (Power and McCleave 1980, Jonsson et al. 1990; 1991a, Potter and Russell 1994, Jokikokko 2002, Croze 2005, Jepsen et al. 2005a). The main consequence of this behaviour is that hatchery-reared fish spend a longer time than wild fish in the river before reaching the spawning area (Jokikokko, 2002). They have also been shown to go back to the hatchery where they were reared (Carr et al., 2004). Compared to wild fish, hatchery-reared salmon seem to have less chance of spawning success, they get injured more easily during the spawning period and often return to sea without having spawned (Jonsson et al. 1990, 1991a) and a higher mortality has also been recorded (Jepsen et al. 2005a).
Compared with wild salmon, escaped framed fish seem to lack river imprinting; they frequently show eroded fins (Fiske et al., 2005), seem to be physically weaker and have a higher fat content (Thorsand et al., 1997). Artificial selection makes them genetically different (Roberge et al., 2006).
This apparent physical inferiority does not affect their performance. A few studies show that both framed fish that escaped before the spawning run period and stayed for some time in nature and newly escaped fish that rapidly entered rivers migrated as fast as wild salmon and settled even further upriver (Heggberget et al. 1933a; 1996, Thorstad et al. 1998, Butler et al. 2005). A laboratory study comparing forced swim endurance of adult framed and sea ranched Atlantic salmon confirmed this (Thorstad et al., 1997). However, there is evidence that wild salmon is more capable of climbing high waterfalls than farmed fish (Johnsen et al., 1998)
Variation in water discharge seems to affect the migration of upstream wild salmon more than escaped salmon; the number of riverine movements by wild fish showed a significant increase according to changes in water flow (Thorstad et al., 1998). Farmed salmon showed no erratic movement pattern during the migration phase (Heggberget et al., 1996),

but during the spawning period they showed more and longer up- and downstream movements (Økland et al. 1995, Thorstad et al.1998).

Migration distances

The migration distance could reach approximately 3000 km. The initial migration through the marine environment may take many months. Afterwards, when they enter freshwater, Salmon begin a 'within-river' phase of migration. The amount of time needed to complete this migration and to reach their final spawning destinations depends on the time of year that fish enter the river and it may last almost a full year for early entrants.

Some records of the 60's have shown that a minimum distance travelled by a fish tagged in Canada and recaptured on the west coast of Greenland was approximately 3680 Km (Allan and Bulled, 1963).

Entering spawning river from the sea, Atlantic salmon can swim either quite short or very long distance to reach the suitable spawning area, a few km in the short Scotland rivers and several hundred Km in the central European rivers as the Rhine.

In the present project, individuals of Atlantic salmon have been shown to hatch in the Iffezheim fishpass, thus they have run at least 700km before reaching the spawning area.

1.4 Factors influencing the migration and human impact (Fisheries off-takes, Stocking)

There is evidence of physiological factors affecting migration pattern: growing stage, physical strength, hormonal control and stress level. Such inner features sometimes are generally referred to as "motivation' for migration (Johnsen et al. 1998, Thorstad et al 2005b). A few studies showed that intrinsic factors may affect migration alone or interact with other factors. For example to overcome a migration barrier a fish must reach a certain internal state but also find suitable environmental conditions.

Within the whole distribution range, Atlantic salmon populations are in decline (Parrish et al. 1998, Klemetsen et al. 2003, ICES 2006). The impacts of human activity, such as overexploitation, pollution, aquaculture and other river regulations have contributed to this decline. With a decreasing population, the last phase of the return migration and reach spawning areas is crucial. Man-made obstacles such as power station outlets, residual flow stretches, dams, weirs and fishways can influence the upstream migration, especially in long-distance river migration. Migration can be delayed for many weeks extending the exposure of fish to diseases and pollutants (Mathers et al., 2002), thus increasing mortality associated with turbine of the power-station or spillway passage (Montén 1985, Coutant

and Whitney 2000). On the other hand, laboratory studies have shown that the exposure of fish to environmental stressors may induce the ability to avoid physical and environmental stressors, as water pollution, and find areas of more favourable condition, thus affecting fish survival rates, but such avoidance behaviour has rarely been demonstrated in nature (Gray 1983; 1990, Atchinson et al. 1987, Åtland 1998).

Catch-and-release angling stress can also be considered one of the impacts of human activity that may influence migration patterns.

Atlantic salmon migrating upstream are vulnerable to delays either at little or huge man-made obstacles; a sequence of minor obstacles may reduce a fish's motivation to migrate or even make fish abandon their migration, leaving the river and entering neighbouring watercourses.

Common bypasses consist of fish-ladders. These should be adapted to the weakest swimmers in the run (Laine, 2001), and to be effective they may let pass more than 95% of the adult upstream migrants in a safe and rapid manner (Ferguson et al., 2002).

Besides, dammed reservoirs cause an increase in predators and a decrease in the migration of smolts (Mills 1965, Olsson et al. 2001).

Locally adapted behaviour may create a large individual variation in migration pattern in relation to river-specific conditions.

Salmon await falling flows before passing further upstream.

Atlantic salmon migration is also positively correlated to the increase in flows, while passage of rapids and waterfalls could be influenced by both decreased and increased flows (Trépanier et al., 1996).

Other environmental factors, besides flow, can effect salmon migration and water temperature is known to be one of the most important one in affecting fish migration speed. Maximum speeds generally positively increase with temperature (Beach, 1984).

Studies showed that Atlantic salmon passed upstream rapids in Norwegian and Scotland rivers while water temperature was increasing (Jensen et al. 1986, Gowans et al. 1996). However water temperature never exceeded 20℃ and h igh temperature effects on migration is not well studied.

Although various upper and lower temperature limits have been reported, the best thermal range for upstream migrations of Atlantic salmon differs among populations according to local adaptations (Trépanier et al 1996, Mills 1989).

Some fish migrated back and forth more than 60 Km before they advanced upriver. This up-downstream migration under relatively short time periods, called "yo-yo swimming", is

supposed to be related to water flow and speed and it is highly energy demanding (Beach, 1984). Consequently, this way of “swimming” lowers the reproductive fitness of the fish and the ones that do not find the right upstream migration route most probably stopped their migration and returned back to the sea.

Natural barriers play an important role in delaying fish migration. The delay time is quite unpredictable to humans; fish may be consistently delayed by barriers that appear easy to pass and overcome quickly barriers that appear difficult.

These are the main factors that, alone or interacting with others, influence the process of migration. How each factor affects the upstream migration is overall understood but the effects may differ among different river sections and sites. Besides, the relationship between main and a number of additional important factors is complex. The understanding of general mechanism stimulating fish within-river migration are still lacking and thus cannot be reliably predicted which conditions are essential to stimulate migration at different sites.

Fisheries off takes

The exploitation of Atlantic salmon in fresh water is probably one of the oldest kinds of fishery (Cleyet-Merle, 1990).

Spears and fixed engines have been used in lot of rivers, where the flow is more slow seines and other nets have been more common. Today rod and line is the traditional way to catch salmons.

Along the coasts, bag nets, bend nets and other fixed engine methods have century-old traditions.

In the open sea the exploitation took place from the late 1950s and the used methods were drift nets and long-line fishing operated from ocean-going vessels.

River fishing is based preferentially on sexually maturing anadromous fish. A selection may occur on run timing that varies within and between populations.

A common pattern is that early-run, large fish are more heavily exploited than late-run, smaller fish (Gee and Milner 1980, Conseugra et al. 2005a).

Catch and release methods in the river may cause a little mortality on the fish, provided water temperature are low, but could affect the behaviour after release (Dempson et al. 2002, Thorstad et al. 2003).

Open sea fishing methods are more selective on the size of the individuals, especially as a particular mesh size of the nets catches fish with certain girth size with higher probability than either larger or smaller fish.

Potential selection is stronger in marine than in fresh water habitats because both immature and maturing salmon could be the object of the fisheries.
The severe decline of salmon population from the 1980s, and the mixed stock nature of fishing in the open sea, has made necessary the introduction of a strong regulation of the fisheries both coastal and oceanic.
The North Atlantic Salmon Conservation Organization (NASCO), based on scientific advice from ICES (International Council for the Exploration of the Sea), has established since 1984 a quota of the fisheries around the Faroes and West Greenland.
Following this regulation, in-river fisheries account for an increasing proportion of the salmon catches in the North Atlantic. Another trend is increases in the use of catch and release by anglers.
In many rivers, a high proportion of the returning spawning population are exploited through angling (Mills 1991, ICES 2006), further emphasizing the importance of a strong knowledge base for management decisions concerning this migration phase.
The reported catch of Atlantic salmon in the North Atlantic had a peack at about 12.000 tonnes annually in 1973-75 and a strong decline to less than 2500 tonnes during the last few years (ICES, 2005)
This decline is partly explained by the regulation of some fisheries especially at the sea, but it also reflects lower survival rates of Atlantic salmon in the Ocean (Friedland et al., 1998), and possibly reduced smolt production caused by habitat degradation (WWF 2001).

Stocking

When Atlantic salmon populations in river system decline or have been declined, stocking and ranching have often been applied as first management option.
Stocking and ranching of Atlantic salmon have been widely used throughout the last 50 years in order to improve fishing.
Past records show that salmon has been moved, for this aim, over long distance among catchments, countries and also continent (Galvin et al., 1996) without taking into account many factors and, above all, genetic implication because all salmon populations were supposed to be functionally equivalent.
On the contrary, each river system has one or more populations, and preservation or reintroduction have to be considered in any rehabilitation efforts (Waples 1991, Youngson 2002).
Cross et al. suggest four different scenarios where stocking and ranching may be invoked always with particular regard to the wild population structure:

1) stocking where salmon populations are extinct→ reintroduction
2) stocking where native populations have a very low number or numbers approaching carrying capacity→ rehabilitation
3) stocking where numbers of salmon populations are at natural carrying capacity→ enhancement
4) Stocking where numbers are low due to human impact that cannot be removed→ mitigation

The aims of the preceding scenarios are:

1) re-establishment of healthy populations ideally self sustaining and at carrying capacity by improvement of water quality, habitat and fishery control
2) increasing of populations up to carrying capacity
3) increase population size over the natural carrying capacity to maintain a fishery at the desired level
4) compensatory fisheries production and/or biodiversity protection where problems limiting or eliminating production are unlikely to be solved in a reasonable short/medium term.

Stocked salmon have a certain impact on the wild salmon competition and interbreeding. Competition arises by adding more fish, because food and the habitat availability get reduced, thus stockings induce an increase in juvenile mortality.

Interbreeding occurs over some generations, when stocking salmon populations breed with the wild ones. Hybrids seem to have a reduced survival capability compared to wild fish and repeated stocking could induce a cumulative reduction in recruitment over generations, which could lead, in the worst case, to the extinction in vulnerable populations.

However, with correct criteria, above all genetic factors, stocking and ranching have in the last years increased salmon production.

Extensive salmon stocking is done with young fish at different stages. Smolts are stocked in some rivers, but most of the stocked fish are fry and fingerlings (Fjellheim and Johnsen, 2001).

Stocking fry or parr is only necessary and effective when natural reproduction is negatively affected (Cowx 1994; Saltveit 1998).

In the Baltic Sea, over the last 15 years the amount of annually released salmon smolts has ranged from 4,5 to 5,9 million, Finnish releases have accounted for circa 34% and

Swedish for 30% of the total smolt release (ICES, 2005). The production of hatchery smolts has been based either on captive hatchery broodstock or on catching of feral spawners from river mouths.

The stocking of Atlantic salmon in the Rhine river system has been carried out since 90's, eggs were collected from other wild salmon stocks and juveniles were released into suitable areas. Yet, the majority of the eggs come from Ireland, Sweden, France, Scotland and Denmark because probably the former native stock of Rhine salmon consisted of several different populations living in different tributaries.

From 1999 to 2003 about 20 million salmons have been released into the Rhine catchments in order to contrast the high natural mortality of young salmon, thus having sustainable populations so far. Only in the Upper Rhine, in the Rhine tributaries of the Black Forrest in Baden-Württemberg, up to 90.000 young salmons of Irish origin are annually released. (LV BW 2002, Schneider et al. 2004). The table below gives an overview over the stocking exercises from 1999-2003.

Stocking juvenile salmon in the Rhine river system 1999-2003		
Country	**River system**	**Stocking exercise**
Germany/NRW	Ruhr, Wupper, Sieg, Lahn	ca. 5,4 million
D/Rhineland-Palatinate	Sieg Ahr Saynbach Mosel/Kyll, Prum Lahn/Muhlnach	ca. 2,3 million
D/Hesse	Lahn/Dill, Weil Whisper Main/Kinzig	ca. 1 million
D/Bavaria	Main	ca. 0,2 million
D/Baden-Württemnberg	Alb Murg Rench Kinzig/Erlenbach, Gutach, Wolfach	ca. 0,3 million
Luxemburg	Sauer/Our	ca. 0,2 million
France	Old bed of the Rhine Ill	ca. 1,6 million
Switzerland	Rhine	ca. 0,3 million
D, L, F, CH	entire Rhine	ca. 11,3 million

Tab.1 Stocking in the Rhine system 1999-2003 (Data from ICPR, 2004)

A report from ICPR (International Commission for the Protection of the Rhine) in 2004 shows encouraging data about the adult returning salmons; until 2003 they were more or less 2500 individuals, and it is for sure an underestimated data, and larvae of naturally reproducing returning salmons have been observed since 1997 (Tab.2).

Stocking in the Rhine region (1999-2003)	Origin of salmons eggs importation	Returning adults
Germany/NRW	Ireland, Sweden	Yes
D/Rhineland-Palatinate	France, Sweden, Denmark, Ireland, Spain, Scotland	Yes
D/Hesse	France, Denmark, Sweden	Yes
D/Bavaria	Ireland, France	
D/Baden-Wurttemberg	Ireland, Sweden	Yes
Luxemburg	France	Yes (Moselle estuary)
France	France, Sweden	Yes
Switzerland	France	

Tab.2 Stocking and returning salmons (Data from ICPR 2004)

1.5 Behaviour ecology (Mating systems and sexual selection, Competition, Diet)

Mating system and sexual selection

Growth rate depends on food availability and quality, as well as on water temperature and photoperiods. Fish reach sexual maturity between three and seven years of age.

Adults reaching sexual maturity return to their home rivers, usually to the same areas where they were hatched and spent their initial freshwater life. Once there, the female selects a spawning site with appreciable current, according to depth (usually 0.5–3 m) and gravel size. Then she excavates a hole by turning on her side and flexing her body up and down creating a current and never touching the stones. After the female releases 8,000–26,000 eggs, the males visit the area, fertilize them, and cover the eggs. On average female deposits 600-700 eggs per Kg of her body weight. Spawning takes between two and three days. Early maturing or sneaker males return to their home stream every year, older males do so after several years in the ocean. The older males are not only larger, but also more colourful. Aggregations around a female are composed of both sneaker (smaller, younger) and older males. Once the female releases her eggs, all males release their sperm, with the greater number of eggs being fertilized by the first male that enters the nest. Young salmons fathered by precocious males grow faster than those fathered by anadromous males. Juvenile salmons (known as parr) spend most of their freshwater life in shallow riffles, mostly at the southern end of their range, until they reach 12–15 cm in length, when they transform themselves into smolt and are ready for migration in spring the first year after hatching.

During the parr phase mortality is very high, due to predators and also because piscivory and cannibalism are common in salmonids, even if strong evidence occur in brown trout and charr but little is known on Atlantic salmon (Martin and Olver, 1980; Ruggerone and Rogers, 1992; Amundsen, 1994; Griffiths, 1994)

Competition

Relatively little evidence is known to judge competitive effects at any scale.

Intraspecific competition in Atlantic salmon has been observed and recorded in more or less all the fresh-water stages of its life-history.

Competition and aggression among nesting females is rare, but can occur where spawning habitat is limited. Interactions are most common during the early phase of spawning site selection and nests construction (Webb et al., 2007).

Competition has been observed most of all among males during the reproduction stages.

Where males have to compete for females, size-related dominance hierarchies develop and as a result the smallest and subordinate males can fail to breed.

Subordinate males often adopt satellite position downstream or to one side of dominant males attempting to have access to the female trying to fertilize some eggs as they are deposited. This behaviour is commonly known as "sneaking" (Webb et al 2007, Jones and Hutchings 2001, Garant et al., 2002).

Competition for space and resources follow also among fry emerging from redds. The first individuals that emerge obtain the best habitats and a lower mortality and faster growth with later emerging fry (O'Connor et al., 2000).

Competition in this stage is known to have an important implication for the genetic character of populations and for spatial and temporal aspects of sampling of early life-history stages in genetic studies (Webb et al., 2007).

Competition among individuals is displayed also in parr phase. Parr are strongly territorial and single parr may have a territory of a few square metres within a more extensive "home range"

Territory and home range is positively connected with body size and can vary to ensure a constant supply of food and tends to increase as a fish get older (Webb et al., 2007).

Among parr the competition could be intraspecific (Grant et al., 1998) or interspecific (Fausch, 1998).

Interspecific competition influences habitat use by Atlantic salmon (Kennedy and Strange, 1986). Atlantic salmon in sympatry may adjust their habitat use due to competition from the dominant brown trout Salmo trutta (Kalleberg 1958, Nilsson 1967, Karlstrom 1977, Kennedy and Strange 1986), whereas their habitat utilization may differ in allopatry.

As result of human impact like transplantation of salmonids and hatchery another kind of competition can be observed among wild and translocated/escaped farm populations.

A transplantation of salmonids, used for re-stocking or to enlarge the resident salmon population, can fail due to “maladaptation” (Altukhov et al., 2000).
This “maladaptation” could have been caused by the superior competitive ability of the residents that have a significant competitive advantage in territorial disputes (García de Leániz et al., 2007).
Territorial and social dominance behaviour in salmonid, between cultured and wild fish, can affect both mortality and growth. Intraspecific competition may be altered in intensity when salmon from different population, wild and farm, that have not co-evolved interact, resulting in deleterious consequences (Fausch, 1988) for interspecific competition.
Native fish that compete with large, more aggressive farm fish, can suffer habitat use shifts and the mortality can increase (McGinnity et al. 1997; 2003, Fleming et al. 2000).
In addition to competition for space and territories, in some situation the rapid growth rates of farm and hybrid juveniles relative to wild juveniles may increase early maturation rates, and result in increased mating competition among early maturing male parr (Ferguson et al., 2007), and increase also breeding success and thus genetic introgression (Garant et al., 2003).
However, farm fish of another strain, under natural conditions, had substantially lower parr maturity, with hybrids being intermediate, presumably as a result of selection against parr maturity in this strain (McGinnity et al. 1997; 2003).

Diet

After hatching, young salmon begin a feeding response within a couple days. After the body absorbs the yolk sac, they begin to hunt.
Juveniles start with tiny invertebrates, but as they mature they may occasionally eat small fishes. During this time they hunt both in the substrate and also in the current. Some have been known to eat also salmon eggs. The most commonly eaten food includes caddsflies (Tricoptera), blackflies (Diptera), mayflies (Ephemeroptera) and stoneflies (Plecoptera).

In adulthood, fish feed on much larger food: Arctic squid (Teuthida), sand eels (Perciformes*: Ammodytidae*), amphipods (Amphipoda), Arctic shrimp (Decapoda, *Pandalidae*), and sometimes herring (Clupeiformes: *Clupeidae*) (Hislop and Shelton, 1993). During this feeding time the fish's size increases dramatically.

1.6 Background, aims and outline of the present project

The River Rhine is 1.320 km long and flows from the Swiss Alps through Switzerland, France, Germany and the Netherlands to the North Sea. The 225 000 km2 catchments area of the Rhine extends over parts of Switzerland, Italy, Austria, Liechtenstein, Germany, France, Belgium, Luxembourg and the Netherlands and is populated by about 54 million people. A number of industrial centres such as Basel, the Ruhr region and Rotterdam are situated along the Rhine, formerly a wild stream, meandering through a wide floodplain, today a vital shipping route. Each day approximately 450 ships pass the Rhine at Lobith - Bimmen. In the year 2000 the transport on the river at the Dutch - German border was about 162 million tonnes and is expected to rise up to approximately 199 million tonnes in 2015 (Wetzel, 2002). The river is also of importance for the water supply for agriculture and the drinking water provision for about 20 million people. Twenty-one hydropower plants on the Rhine mainstream have a total installed capacity of 2.186 MW. River Rhine has suffered severely from stream regulation and pollution.

The International Commission for the Protection of the Rhine against Pollution (ICPR; IKSR in German) was initiated by the Netherlands in the 1950s because of the concern over pollution of the Rhine and its implications for the drinking water supply. The ICPR started as a common forum of the member countries bordering the Rhine: Switzerland, France, Germany, Luxembourg and the Netherlands for periodical meetings and the formulation of pollution control agreements. On 1 November 1986, 10 to 30 tons of plant-protecting agents were discharged in fire-fighting water into the Rhine at the Sandoz plant in Schweizerhalle near Basel (Lelek, 1989). This resulted in a massive fish kill, mainly of eel, of which an estimated 200 tonnes died. With this accident the extent to which the Rhine ecosystem was endangered became apparent and this stimulated the ICPR (The International Commission for the Protection of the Rhine) to promote an international river restoration plan called the Rhine Action Programme "Salmon 2000" (IKSR - Internationale Kommission zum Schutze des Rheins -1987; Brenner 1993)

In the last 50 years, this whole massive exploitation of the Rhine and the consequent destabilization of its ecosystem caused the extinction of the salmon and a spontaneous recolonization from other rivers is highly unlikely. In the early 1990´s a “Rhine action program” of restoring the fish fauna of the Rhine status chose the Atlantic salmon as “flag species” to improve populations of certain fishes and their biotopes (“Rhine 2020”). Eggs and broodlings from adjacent populations from within the same general biogeographical

unit assumed for the extinct Rhine population continue to be imported, and the young salmons released. The construction of the fishpass in the weir at Iffezheim, which had blocked the entire stem of the Oberrhein, allowed southern communities in Baden-Württemberg and France to intensify their efforts in salmonids.

This programme is accompanied by many efforts to improve biotopes, which also benefit other species, e.g. sea trout, sea lamprey, river lamprey and shads, besides numerous invertebrates and plants. The aim of this project is to provide accessibility to, and improvement of biotopes for natural reproduction, in order to achieve a self-standing population in the long-term. Till date, a regular population of spawning migrants has been achieved and regular reproduction of salmon in tributaries of the Mittelrhein. So far two cases of spawning salmon in the Kinzig and Murg, determined by genetic markers by our group, are the first indications of reproduction in the Oberrhein area.

The present project was born with the major aim to clarify the population dynamics of the Atlantic salmon, the interactions with and within wild and hatchery populations and at least but not last, to understand by the individual assignment the existence of an own Rhine population.

The main aims of the present research can be summarised as follows:

- Genetic characterization of returning spawning migrants
- Assignment of the returners to the fish ladder at Iffezheim to the known populations used for salmon reintroduction in the Rhine
- Monitoring of the utility of different origins to recolonize the Rhine system
- Sample aging

Analysis of microsatellite data may be useful for estimating the number of post-glacial refugia that contributed to the re-colonisation of northern Europe and to understand the problem of the interaction with hatchery-reared fish.

2 MATERIAL AND METHODS

2.1 Study area and Sample material

The present research was carried out from April 2005 to May 2009.

Focus of the research was Atlantic salmon captured from 2002 to 2005 into the monitoring station of Iffezheim (Upper Rhine), for a total of 65 salmons.

The River Rhine is 1320 km long and flows from the Swiss Alps through Switzerland, France, Germany and the Netherlands to the North Sea. The 225 000 km^2 catchments area of the Rhine extends over parts of Switzerland, Italy, Austria, Liechtenstein, Germany, France, Belgium, Luxembourg and the Netherlands (Fig.8).

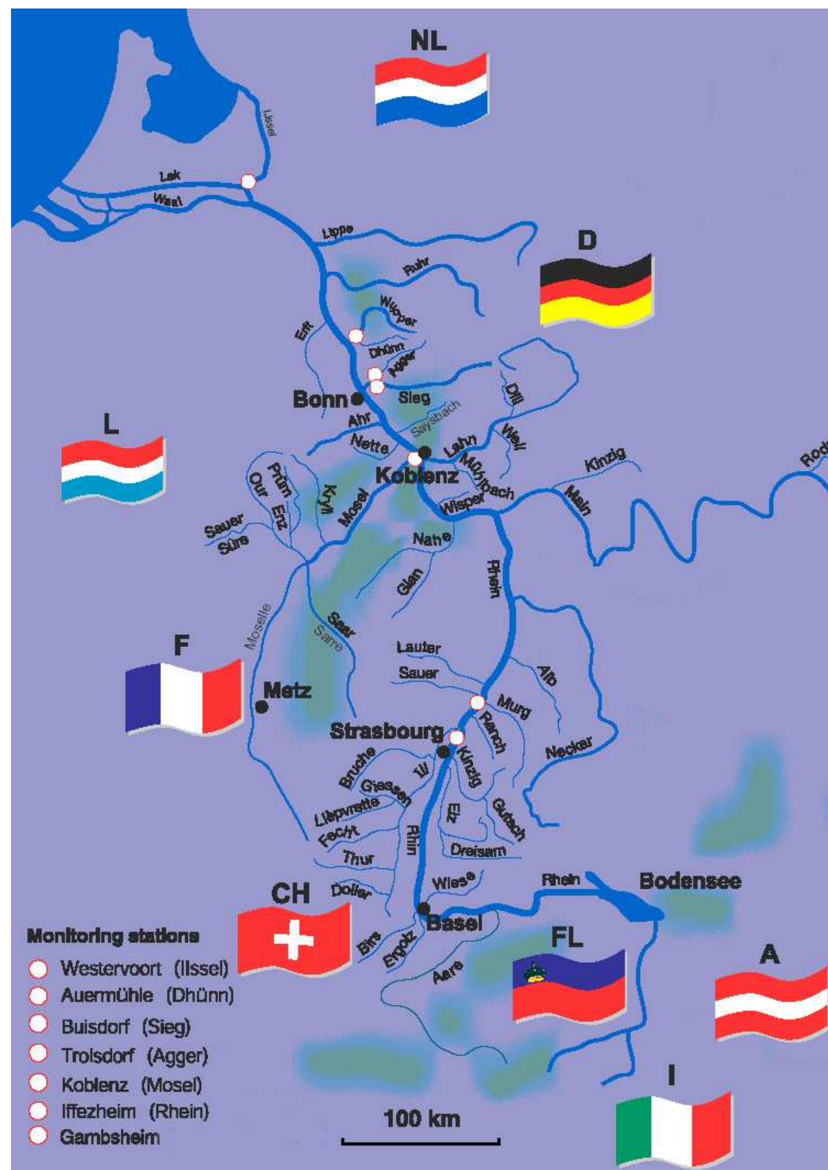


Fig.8 Rhine drainage system and monitoring stations (image from Rhine & Salmon 2020, A Programme for Migratory Fish in the Rhine System, ICPR 2004)

The minimum discharge in summer is 20 $m^3 s^{-1}$ and the maximum is about 256 $m^3 s^{-1}$. The first dam is about 700 km from the North Sea at Iffezheim 40km north of Strasbourg and has been equipped in 2000/2002 with one of the largest fish passage structures in Europe. It is a modified vertical slot pass optimised by French and German fishery and hydraulic engineering experts (Fig.9). A fish pass for the next dam upstream at Gambsheim has been constructed in summer 2006.

The mean water flow is approximately 1100 $m^3 s^{-1}$ at Gambsheim 25 Km upriver from Iffezheim; the water conductivity is in the range of 520–900 µS cm^{-1} (Gerlier and Roche, 1991). As regards the water temperature, the Rhine has shown in the past five decades an increase of about 3.5℃ due to global climate chang ing but in addition, due to anthropogenic influences (Hartmann et al., 2007).

During these sampling years the salmon number, and also other sampled species, decreased in sensitive way. The reason should be found in the normal oscillation of the Rhine productivity but also, and this is most likely the main reason, in water temperatures of summer 2003, when the Rhine water temperature reached about 27℃, too high for salmon reproduction, the survival of the eggs and the fry. Another reason could be found in the increasing fishing activity all over the coasts of the Lowlands where many salmons regularly enter the spawning rivers and the recent man made obstacles, as the sluices at the closure embankments of Haringvliet and Ijsselsea in the Rhine delta, that are accessible to a limited extent only, and free entrance for migrating fish species from the North Sea to the Lower Rhine estuary is only possible via the Nieuwe Waterweg near Rotterdam, a highly industrialised area with many harbours (IKSR 2003, Brenner et al. 2003).

Fig.9 Fishpass in Iffezheim dam. On the right the fishpass spot (Image by Mr. Degel)

In order to compare and assign “Rhine salmon” samples, salmon broodlings of the following European salmon origins (Fig.10) used for reintroducing the species in the Rhine have been included in the present study:

France: Allier
Sweden: Lagan*
Sweden: Ätran Albaum
Ireland: Burrishoole

* Lagan samples were difficult to find, and only wild released Elbe parr was obtained from Bad Schandau.

A total of 65 Atlantic salmon individuals from anadromous population (Iff) were collected, 100 hatchery individuals from different geographical origins (BUR, Allwild, Allhatc, Ätran) and 15 stocked individuals (Lagan) were analysed (Tab.3 and Fig.10).

Population	Status	Codes	n	Stade
Iffezheim	Not stocked	Iff	65	Adults
Burrishoole	Hatchery	BUR	22	Juveniles
Allier wild	Hatchery	Allwild	25	Juveniles from wild eggs
Allier hatchery	Hatchery	Allhatc	25	Juveniles
Ätran Albaum	Hatchery	Ätran	28	Juveniles
Lagan Bad Schandau	Highly stocked	Lagan	25	Juveniles

Tab.3 Populations used in this study, status, code, sample size (n) and stade (age class sampled)

2.2 Material

Analysed samples consisted of small portion of salmon adipose fin, blood and scales.
Salmon blood, scales and adipose fin samples have been provided by voluntary people in charge of monitoring and controlling for the Landfischereiverband Baden-Württemberg (Mr. D. Degel) the fish pass through the fish ladder located in the Iffezheim dam, in particular they focused on the salmon and trout returners.
The fishes have been also weighted, measured and the whole data have been recorded according to date and an individual number.

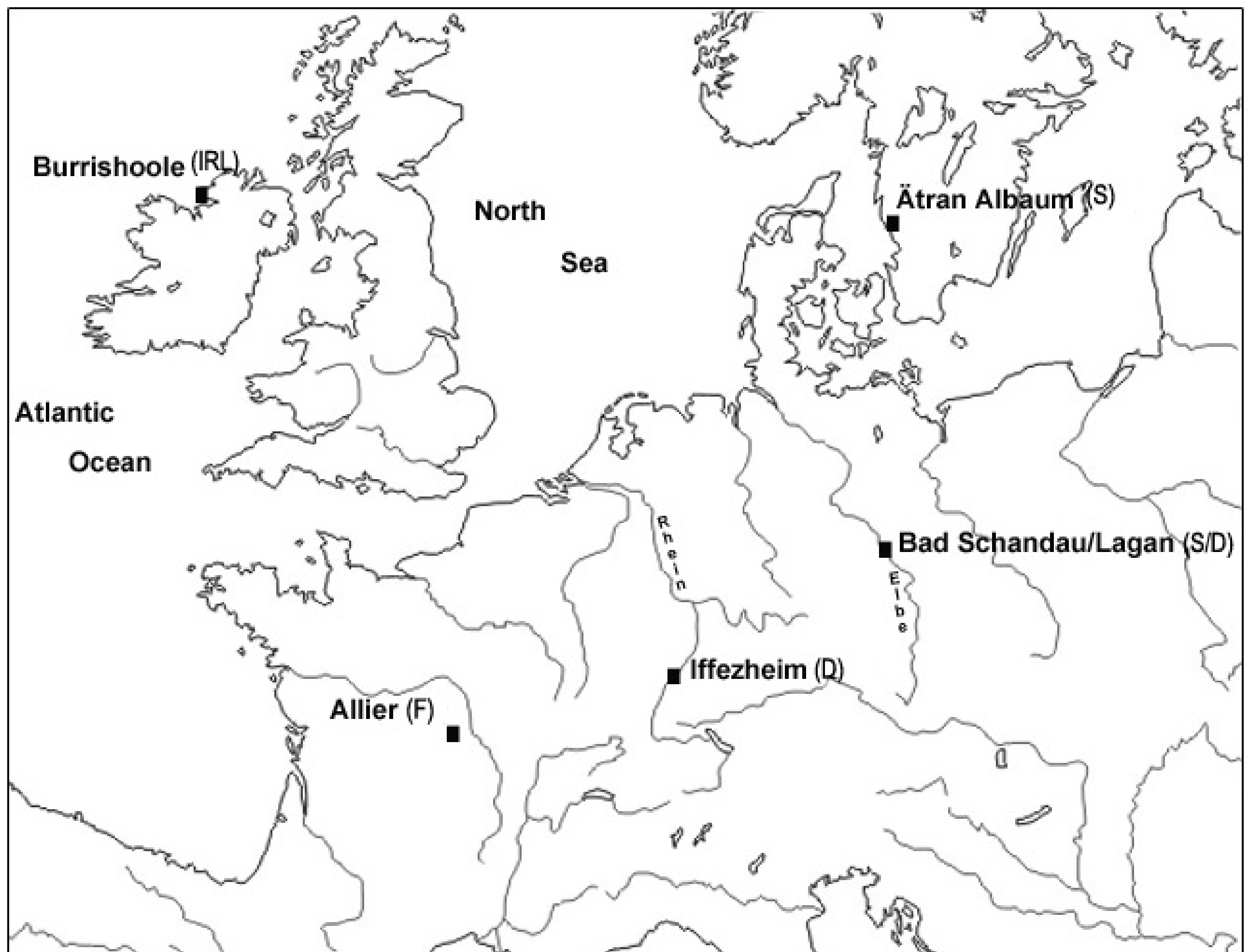


Fig. 10 European salmon populations included in the research D (Germany), F (France), S (Sweden), and IRL (Ireland)

2.2.1 Equipment

All the instruments used for laboratory analysis are listed in Table 4.

2.2.2 Solutions and chemicals

Table 5 shows a list of chemicals, enzymes and other materials used in this study and a list of buffers and solutions follows in Table 6.

2.2.3 Blood sampling

Blood was withdrawn from the caudal vein of the fish, with a syringe of 1ml, after having previously placed on the same spot ACD as anticoagulant (needle: hypodermic Luer 0,8x40).

The fish has not been damaged in any way and has been released immediately after the sampling (catch and release method).

Instruments	Company
Automated sequencer: ALFexpress	Pharmacia Bioteq
Refrigerated Centrifuge	Sorval RMC14 Du Pont
Gel chambers for agarose gel	Univ. Heidelberg
Gel chambers for allozyme determination	L.K.B. Pharmacia
Gel dryer	Memmert 30-300℃
Microcentrifuge E™	Beckman
PCR machine: Termocycler	PCR Express Hybaid
PH meter 766 Calimatic	Knick
Photometer RNA/DNA calculator	GeneQuant Pharmacia.
Pipettes: P2, P10, P20, P200,P1000	Gilson
Termocycler	Autogene II Grant
Vortex	REAX2000 Heidolph
Precision scales	CP64 Sartorius
Thermo-sheker	MR3000 Heidolph
Mikro-dismembrator	B.Braun Biotech International
Ultrasonic-cell-disruptor	Heat Systems Microson

Tab.4 Analytical instruments used in the present study

During the sampling, the fish was constantly wrapped in a wet cloth to avoid skin drying and it was held on a plastic plan.
The blood was collected in a formerly signed Eppendorf with an identification number of the sample, and it was stored in deep freezing.

2.2.4 Scales sampling

Scales have been withdrawn in variable numbers from 6 to 10 for each individual, which, according to the rules, were taken from the backside above the sideline. Scales were dried and preserved in small envelopes of paper.

Chemicals, Enzymes and other Materials	Company
Acetic acid	Merk
Acrylamide:Rotiphorese® Gel 30 (37.5:1)	Roth
Agarose SEAKEM LE	FMC Bio Products, Rockland, USA
Ammonium persulfate (APS):capsule, ≥98%	Sigma
Chloroform	Roth
dNTPs	Qbiogene
EDTA	Roth
Ethanol absolute	Merk

Tab 5 Chemicals, enzymes and other materials used in this study

Chemicals, Enzymes and other Materials	Company
Ethidium bromide	Serva
Formamide PlusOne™	Amersham Biosciences
β-mercaptoethanol	Merk
Phenol	Merk
Protease	Qiagen
QIAamp DNA Mini Kit	Qiagen
QIAquick Gel Extraction Kit	Qiagen
QIAquick PCR Purification Kit	Qiagen
Reaction tubes (0.2, 0.5, 1.5, 2 ml)	Eppendorf
Silane	Sigma
Sodium acetate	Merk
Sterile filter, 0.22µl	Millipore
Taq DNA polymerase	MP Biomedicals
TEMED (N,N,N,N,-Tetramethylendiamine) PlusOne™	Amersham Biosciences
Tris	Roth
Urea	Roth

Tab 5 (continued)

Stock solutions	
Agarose gel solution	1% agarose, 1µll/ml ethidium bromide, in distilled water
ALF stop solution	5mg/ml (20mg/ml) Dextran-blue, in formamide
Ammonium acetate	4M ammonium acetate, in distilled water
Ammonium persulfate	10% solution, in distilled water
DNA loading buffer	50% glycerol, 0.25% bromophenol blue, in distilled water
EDTA buffer	10% EDTA, 0.5%NaF, 0.5% thymol, 1% Tris (pH 7.5)
Nucleotide mix	8µM dATP, 8µM dTTP, 8µM dGTP, 8µM dCTP
Phenol/clorophorm	Phenol, Clorophorm, Isoamyl alcohol in ratio 25:24:1
PAGE solution	16ml Rothiphorese stock solution, 21.5 Urea, 6ml 10XTBE, 36.75 ml bidistilled water
TBE buffer 10X	0.89 M Tris base, 0.89 Boric acid, 2mM EDTA, distilled water ad 1l (pH 8.4)
TE buffer	10 mM Tris, 1 mM EDTA, hydrochloric acid (pH 8.0)
WB bridge buffer	250 mM Tris/60 mM citric acid, pH 8.0
WB gel buffer	WB Bridge Buffer in distilled water in a ratio 1:3, pH 8.0

Tab 6 Buffers and solution used in this study

Storing and transport of the samples

Blood and scales have been preserved frozen in loco until the end of the annual sampling, after that, they have been conveyed in styrol boxes with dry ice to prevent defrosting and then stored in lab.

2.3 Methods

Lab stocking

A stock for each blood sample has been done and stored in a deep freezer at -20-℃.
Scales have been partly frozen and partly cleaned up and put on a dia-frame, scanned and then edited one by one using the graphic software Adobe Photoshop CS to be able to see, as clear as possible, the growing circle and thus to determine the age of the fish.

2.3.1 Species confirmation applying protein electrophoresis

Genetic variation detected by protein electrophoresis arises from amino acid substitutions generated by base sequence variation (Utter et al., 1987).
These substitutions alter the charge state or conformational character of the protein and change its mobility when placed in a gel matrix subject to an electrical field.
This method has provided the first tool for meaningfully studying the genetics of species in the wild and the Atlantic salmon was one of the first target species for the study.
Protein electrophoresis has been largely applied to investigate population structure and differentiation, but it has also led to the systematic revision of the genus *Salmo* and still can be considered the primary source of insight into hybridisation in the wild with brown trout *Salmo trutta*.
The validity of the Atlantic salmon as a genetically distinct species has been demonstrated by studies of its hybridisation in the wild with *S.trutta*. Like many others congeneric salmonids the two species can produce viable hybrids.
To discriminate in a certain way, only by morphological characteristics, the two species (*S.salar* and *S.trutta*), is sometimes not so easy also for an expert eye (Fig.11).
Electrophoretic investigation of allozyme polymorphisms has contributed significantly to the solution of this problem and altogether different species-specific loci suitable for identification of hybrids have been revealed (Nyman, 1970; Guyomard, 1978; Beland *et al.*, 1981; Vuorinen and Piironen, 1984; Crozier, 1984).
Markers F1-hybrids in the Rhine system (Schreiber, unpubl. Data) have been previously documented by means of protein.
Among the proteins used to individualize the hybridisation level between the species, one that is for that purpose an unambiguous marker that can be tested even with blood samples, has been used, the Glucosephosphate Isomerase (GPI EC numbers* 5.3.1.9),

(Moss, 2006) which can also be used for screening the eggs (Mork and Heggberget, 1984). All the Iffezheim samples have been screened in order to verify the species identity and to look for the hybrids when present.
Not all DNA sequence variation leads to amino acid changes and not all amino acid changes are detectable by electrophoresis. Thus electrophoretic screening can detect only part of the amino acid sequence variation that might be present.

Fig.11 Atlantic salmon on the left side, brown trout sample on the right

Blood was diluted in 50 mM Tris/HCL pH 7.5 (including 10 mM j3-mercapto-ethanol) and applied on glass plates to 1 mm thin agarose gels (SEAKEM LE agarose, FMC Bio Products, Rockland, USA) using horizontal Multiphor electrophoresis chambers (L.K.B. Pharmacia) cooled at 4℃.
Allozyme was assayed from blood in 1% agarose gels using standard zymography (Harris and Hopkinson 1976). The alleles were designated by their electrophoretic mobility in relation to the mobility of the most frequent variant which was defined as 100%.

Buffer system to resolve GPI was the WB bridge buffer:
250 mM Tris/60 mM citric acid, pH 8.0
WB gel buffer:
WB Bridge Buffer in distilled water in a ratio 1:3, pH 8.0

The staining recipe for GPI enzyme is following given:
25ml 0.1M Tris/Hcl pH 8.0
10mg Fructose-6-phosphate
5 mg NAD (nicotinammide adenina dinucleotide)
5 mg MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2h-tetrazoliumbromide)

25ml 2% Agar noble

5 mg PMS (phenazine methosulfate)

2 µl G6PDH (glucose-6-phosphate-dehydrogenase)

* The **Enzyme Commission number** (**EC number**) is a numerical classification scheme for enzymes, based on the chemical reactions they catalyze.

2.3.2 DNA extraction

DNA has been collected by extraction from different tissues (skeletal muscle, adipose fin, scales, and gills) of same fish sample in order to verify the best DNA quality.
DNA extracted from blood gave the best DNA quality in terms of amount and suitability.

The DNA-extraction from skeletal muscle, adipose fin and blood of fish was performed with a Columnchromatography-Kit by QIAamp DNA Mini Kit of Qiagen firm following the given protocol with some changing for the blood extraction. Both protocols are described below:

Tissue: Salmonids skeletal muscle and adipose fin:

1. Cut the tissue sample in small pieces of 2-3 mm, do not use more than 25 mg of muscle. Add 180 µl of Buffer ATL (in 1.5 ml microcentrifuge-tube).

2. Add 20µl Proteinase K, mix by vortexing, and incubate at 56° C until the tissue is completely lysed. Vortex occasionally during the incubation to disperse the sample. (sometimes this step require more than an overnight staining)

3. Add 200µl of Buffer AL, mix by Pulsvortexing for 15s and incubate at 70° C for another 10 Min. Briefly centrifuge to remove drops from the inside of the lid.

4. Add 200 µl of Ethanol (96-100%) and mix by Pulsvortexing for 15s. Briefly centrifuge to remove drops from the inside of the lid.

5. Apply the mixture to a Qiamp Spin Column, without wetting the rim. Close the cap and centrifuge for 1 min. Discard the filtrate.

6. Place the Qiamp Spin Column in a clean 2 ml collection tube. Add 500µl of Buffer AW1 without wetting the rim. Centrifuge for 1 min. Discard the filtrate.

7. Place the Qiamp Spin Column in a clean 2 ml collection tube. Add another 500µl of Buffer AW2 without wetting the rim. Centrifuge for 3 min. Discard the filtrate.

1. Place the Qiamp Spin Column in a clean 1.5 ml microcentrifuge tube. Add 200 µl of distilled water. Incubate at room temperature for 5 min, centrifuge for 1 min and collect the eluate.

9. Verify the DNA concentration in the UV-Photometer at 260 nm after an overnight in the refrigerator.

Tissue: Salmonids whole blood

1. Add 20µl Proteinase K in 1.5 ml microcentrifuge-tube
2. Add 30 µl whole blood, stabilised with 170 µl TE pH 8.0.
3. Add 200µl buffer AL and mix by pulsvortexing for 15s. Incubate at 56° C for 10 Min. Briefly centrifuge to remove drops from the inside of the lid.
4. Add 200 µl of Ethanol (96-100%) and mix by pulsvortexing for 15s. Briefly centrifuge to remove drops from the inside of the lid.
5. Apply the mixture to a Qiamp Spin Column, without wetting the rim. Close the cap and centrifuge for 1 min. Discard the filtrate.
6. Place the Qiamp Spin Column in a clean 2 ml collection tube. Add 500µl of Buffer AW1 without wetting the rim. Centrifuge for 1 min. Discard the filtrate.
7. Place the Qiamp Spin Column in a clean 2 ml collection tube. Add another 500µl of Buffer AW2 without wetting the rim. Centrifuge for 3 min. Discard the filtrate.
8. Place the Qiamp Spin Column in a clean 1.5 ml microcentrifuge tube. Add 200 µl of distilled water. Incubate at room temperature for 5 min, centrifuge for 1 min and collect the eluate.
9. Verify the DNA concentration in the UV-Photometer at 260 nm after an overnight in the refrigerator.

The DNA-concentration at the end was measured after an overnight permanence at -20℃, with a UV-Photometer by 260 nm. The value is given by the machine in mg/ml and has been converted in ng/µl.

2.3.3 Microsatellite analysis background

Microsatellites are polymorphic loci consisting of short (from 2 to 5 bp) tandemly repeated arrays that appear to be widely dispersed in the eukaryote genome.
They are typically neutral, co-dominant and are used as molecular markers above all for studies of closely related organisms due to their high variability (Tautz ,1989).

The mutation rates are estimated to be in microsatellites of the order of 10^{-2} to 10^{-6}, per locus/per generation (Hancock, 1998). This instability is more often observed as changes in number of tandem repeats.

In order to explain this high rate of mutation, two models have been proposed: 1) DNA slippage, that involves slip-strand mispairing errors during the DNA replication, and 2) unequal recombination between DNA molecules (Schlötterer and Tauz 1992, Eisen 1998, Schlötterer and Pemberton 1998, Li et al., 2002).

Great advantage of these loci is that they can be investigated using the polymerase chain reaction (PCR), which is a simple technique that allows using small or degraded samples of tissue or DNA.

This process results in a production of DNA high enough to be visible on agarose or polyacrylamide gels. Only small amounts of DNA are needed for amplification as thermocycling this way creates an exponential increase in the replicated segment (Griffiths et al., 1996) and the primers that flank microsatellite loci are simple and quick to use

Another great advantage is their discrete co-dominant inheritance which makes them particularly useful for population genetic inferences that rely on estimates of heterozygosity (Schlötterer and Pemberton, 1998).

One of the few disadvantages is that the development of correctly functioning primers is often a tedious and costly process and cross-species amplification is possible only between closely related taxa.

2.3.4 Identification of primers for the present study and laboratory protocol

Several primers have been used in order to compare the results, to have a quite acceptable overview of the population structure and to verify the suitability of these primers according to the final goal.

Those primers have been selected from a great amount found in literature (Atlantic salmon and salmonids in general are and have been the object of a number of researches due to their economical value). This choice considered primers not overlapping in size, with a relative little number of alleles and the least but not the last, significant results that they have brought about in previous research projects.

2.3.5 *Laboratory protocols for microsatellite analysis*

Salmonids from Iffezheim 2002, 2003, 2005, and 2005 and stocked broodlings have been analysed. The total sample basis genotyped included 180 salmons.
To carry out statistical analysis on Iffezheim salmons two scenarios were considered: 1) a single population including the overall amount of individuals sampled from 2002 to 2005 (Tab.7a); 2) four different populations sampled every single year (2002, 2003, 2004, and 2005) (Tab.7b).

Salmon populations	**Iffezheim**	**Burrishoole**	**Allier wild eggs**	**Allier hatchery**	**Ätran**	**Lagan**	**TOT**
N° of salmon samples	65	22	25	25	28	15	**180**

Tab.7a Samples for each population involved in the molecular genetic comparison (Scenario 1)

Salmon populations	**Iffezheim 2002**	**Iffezheim 2003**	**Iffezheim 2004**	**Iffezheim 2005**
N° of salmon samples	30	11	15	9

Tab.7b Scenario 2

To optimise PCR reactions, the annealing-temperature and the number cycles were adjusted for each primer pair in a gradient PCR to minimise the stutter bands and avoid incorrect interpretation of the peaks.
PCR was performed in 20µl reaction volume containing 300ng of total genomic DNA, 1X PCR Buffer with $MgCl_2$, 1µM each Primer, 8 µM each dNTPs, 1U Taq Polymerase.
Success of PCR product was checked on 1% agarose gel.
Details of PCR profiles for each primer are listed below:

SSOSL85-SSOSL311

Cycle number	Temperature	Time(s)
1	94°	180
35	94°	40
	55°	40
	72°	40

STR15

Cycle number	Temperature	Time(s)
1	94°	180
35	94°	40
	58°	40
	72°	40

Ssa171/Ssa202

Cycle number	Temperature	Time(s)
5	94°	20
	58°	20
	72°	20
35	90°	20
	58°	20
	72°	20

Ssa402

Cycle number	Temperature	Time(s)
1	96°	180
4	95°	50
	64°	50
	72°	50
25	94°	50
	64°	50
	72°	50

Ssa411/Ssa408

Cycle number	Temperature	Time(s)
1	96°	180
4	95°	50
	62°	50
	72°	50
25	94°	50
	62°	50
	72°	50

Fifteen primers have been tested and 8 (Tab.8) of them were selected on account of the clearness of their pattern and the repeatability of the results they showed, and they were used for each individual for a total amount of more or less **1440** amplicons.
This step was the one which took the greatest amount of time because it needed a high number of repeated and crossed verifications and every single little mistake at this stage compromised the final result.
Not all the primers gave the same results in terms of readability and understanding of patterns. Some required several repetitions and comparisons between different runs.

Loci description and previous researches

Locus SS85

Source: Slettan A., Olsaker I, Lie O. (1995) Atlantic salmon (*Salmo salar*), microsatellites at the SSOSL25, **SSOSL85**, SSOSL311, SSOSL417 loci. *Animal Genetics*, **26**, 281-282

The choice for this primer was supported by the small number of alleles that previous research works have found and by the satisfactory results those works revealed.

Previous research:
Nielsen et al. (1997) have studied old scales from the Skjiern River in Denmark. The Skjiern River was repopulated in the early 90s with samples from the Swedish River Ätran, which is the geographically most proximate river with a salmon population, and from the River Conon in Scotland.
They have found at this locus out of the 4 populations observed (Skjiern River 1989, Skjiern River 1930s, Conon, Ätran) a number of alleles that ranged between 6 and 14 on 177 to 221 bp.

Tessier and Bernatchez (1999) have included this locus in their research among sympatric populations of landlocked Atlantic salmon in the 4 tributaries of the Lake St-Jean in Québec and they have made comparisons with ancient DNA sampled in the 70s in other rivers in Québec. They have observed a range of 4 to 9 alleles for the old samples with 184-206bp and for the contemporary populations a range of 5-9 alleles with 174 to 206 bp.
Säisä et al. (2005) in their research on the Atlantic salmon in the Baltic Sea have found that, out of 38 populations observed, the alleles number at this locus ranged from 1 to 15.

Locus	Repeat unit	Sequence	Annealing-Temp.℃	Number of Cycles	Allele size range bp	Reference
SSOSL 85f*	$(GT)_{22}$	TGT GGA TTT TTG TAT TAT GTT A	55°	36	154-222	Slettan et al., 1995a
SSOSL 85r*		ATA CAT TTC CTC CTC AAT CAG T				
SSOSL311f*	$(TG)_{38}$	TAG ATA ATG GAG GAA CTG CAT TCT	55°	36	124-179	Slettan et al., 1995a
SSOSL311r*		CAT GCT TCA TAA GAA AAA GAT TGT				
STR15f*	$(GT)_{13}$	TGC AGG CAG ACG GAT CAG GC		36	197-252	Estoup et al., 1993
STR15r*		AAT CCT CTA CGT AAG GGA TTT GC				
SSa171f	$(TGTA)_{14}(TG)_7$	TTA TTA TCC AAA GGG GTC AAA A	58°	40	197-249	O' Reilly et al., 1996
SSa171r*		GAG GTC GCT GGG GTT TAC TAT				
SSa202f	$(CA)_3(CTCA)_{17}$	CTT GGA ATA TCT AGA ATA TGG C	58°	40	223-268	O' Reilly et al., 1996
SSa202r*		TTC ATG TGT TAA TGT TGC GTG				
SSa402f	$(GA)_{55}$	GCT TTG GCA ATG CAT GTG GTA AT	64°	30	150-183	Cairney et al., 2000
SSa402r*/1 L /2 L #		CCT ATC CCT GTT GTT GCT GAC			190-296	
SSa408f	$(GACA)_{27}$	AAT GGA TTA CGG GTA CGT TAG ACA	62°	30	208-322	Cairney et al., 2000
SSa408r*		CTC TTG TGC AGG TTC TTC ATC TGT				
SSa411f	$(CT)_{70}(GT)_1$	TCC GCA CAG ACC AGA AGA ACG	62°	30	256-283	Cairney et al., 2000
SSa411r*		CAC CCC TCC GTT TTA TCA C				

Tab.8 Summary of the primers and conditions used for the amplification of nine *S.salar* microsatellite loci. # Two loci detected- presumed duplicate pair reflecting the tetraploid origin of the salmonid genome (Ohno, 1970). * marks primers used with CY5-labelling for detection of PCR fragments on the Alf Express

Tonteri et al. (2005) have observed a total of 19 alleles across the populations in a size range of 178-226 bp out of 23 populations observed in the Atlantic Ocean, White, Baltic and Barents Sea of anadromous and non-anadromous Atlantic salmons.

Locus SS311

Source: Slettan A., Olsaker I, Lie O. (1995) Atlantic salmon (*Salmo salar*), microsatellites at the SSOSL25, SSOSL85, **SSOSL311**, SSOSL417 loci. *Animal Genetics*, **26**, 281-282

The choice for this primer was supported by the small number of alleles that previous research works have found and by the satisfactory results those works revealed

Previous research:
Säisä et al. (2005) in their research on the Atlantic salmon in the Baltic Sea have observed that, out of 38 populations, the alleles numbers at this locus ranged from 3 to 24.

Tonteri et al. (2005) have observed a total of 31 alleles across the populations in a size range of 120-186 bp. Out of 23 populations in the Atlantic Ocean, White, Baltic and Barents Sea of anadromous and non-anadromous Atlantic salmon.

A comparison between old scales samples and recent samples was done by Nielsen et al. (1997) in the Skjiern River (Denmark) population. The Skjiern River was repopulated in the early 90s with samples from the Swedish River Ätran, which is the geographically most proximate river with salmon populations, and from the River Conon in Scotland.
They have found at this locus, out of the 4 populations observed (Skjiern River 1989, Skjiern River 1930s, Conon, Ätran), a number of alleles that ranged between 10 and 19 on 126 to 170 bp.

Locus Ssa171/Ssa202
Source: O'Reilly P.T., Hamilton L.C., McConnell S.K. and 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 Science* **53**, 2292-8
The choice for this primer was supported by the small number of alleles that previous research works have found and by the satisfactory results those works revealed

Previous research:
O'Reilly et al (1996) have isolated 4 microsatellites from Atlantic salmon to evaluate the genetic variation among populations of 3 rivers in Nuova Scotia, Canada for a total of 109 individuals. They have observed at this locus a number of alleles ranging between 14-18 for a total of 25 alleles for a size range of 214-278 bp.

Tessier and Bernatchez (1999) have included this locus in their research among sympatric populations of landlocked Atlantic salmon in the 4 tributaries of the Lake St-Jean in Québec and they have made a comparison with ancient DNA sampled in the 70s in other rivers in Québec. They have observed a range of 6 to 13 alleles for the old samples with

237-265bp and for the contemporary populations a range of 9-14 alleles with 231 to 265 bp.

Säisä et al. (2005) in their research on the Atlantic salmon in the Baltic Sea have found that, out of 38 populations, the alleles numbers at this locus ranged from 3 to 16.

Tonteri et al. (2005) have observed a total of 25 alleles across the populations in a size range of 206-260 bp out of 23 populations on Atlantic Ocean, White, Baltic and Barents Sea of anadromous and non-anadromous Atlantic salmon.

Locus STR15

Source: Estoup A., Presa P., Krieg F., Vaiman D., and 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

Previous research:

Estoup et al. based their research on four populations of 10 individuals for each:

two stocks of hatchery-reared trout (Gournay, French und Cuneo, Italy), one Atlantic wild population (River Bresles, Normandy, French) and one Mediterranean wild population (River Artesiaga, Ebro basin, Spain).

The individuals were male trouts, rainbow trouts and female trouts.

Locus Ssa402/Ssa408/Ssa411

Source: Cairney M., Taggart J.B. and Høyheim B. (2000) Characterization of microsatellite and minisatellite loci in Atlantic salmon (*Salmo salar* L.) and cross-species amplification in other salmonids. *Molecular ecology*, **9**, 2155-2234.

The choice for this primer was supported by the small number of alleles, by the possibility to have with one primer two good no overlapping loci that previous research work has found and by the satisfactory results this work revealed.

Previous research:

Cairney et al. (2000) have studied different size-selected Atlantic salmon genomic libraries employing microsatellite enrichment methology.

Characterization of primer sets involved no isotopic and isotopic screening.
Level of variability at each identified locus was assessed in 21 wild adult salmon.
Polymorphic loci were also screened in 2 Atlantic salmon families each consisting of 2 parents plus 46 progeny.
Cross-species amplification was assessed in 7 other salmonid species: *Salmo trutta*, *Oncorhyncus mikyss*, *O. clarki*, *O. nerka*, *Salvelinus alpinus*, *Coregonus lavaretus*, *Thymallus thymallus* with 2 individual for each species.
Out of 164 clones sequenced, 144 had identifiable repeat motifs.

42 primer sets could be designed that flanked micro-minisatellite sequences.
22 sets gave discrete products on no isotopic testing with Atlantic salmon samples and were further optimised for isotopic screening.
Out of 25 loci amplifies, 20 were detected as being polymorphic.
Many of the primer sets are potentially informative for other salmonid species.

2.3.6 Fragment analysis -recognition of alleles and genotypes

Formamide and Dextran blau (30%) have been added to the amplicons and for length determination, 2µl of each amplicon was separated in a 5% polyacrylamide gel on an ALF Express II genetic analyser and the alleles were visualized in a graphic form using Alfwin^{TM} Sequence Analyser Software. Both, instrument and software are by Amersham Pharmacia Bioteq, Freiburg.
For the first four primers (SSOSL85, SSOSL311, STR15, Ssa171) 5 lanes of external were loaded onto each gel (STR System HumFIBRA-FGA and the STR System HumVWA-vWA by the Serac Company).
For the further four primers (Ssa202, Ssa408, Ssa402, Ssa411), since the former allele-ladder was no more available, 3µ of blue194bp obtained with a knowing size sequence excided from the vector “Bluescribe KS+” was added at each amplicon as internal standard and 5 lines of external standard, GE healthcare (50-500 bp), were loaded onto each gel. Both standards, blue194bp and GE healthcare (50-500 bp), have been tested and calibrated to completely fit with the former standards system used and in order to have comparable results (description of this steps is given in the result section).
From the comparison with known size external allele ladder, alleles of every single locus have been identified and typified by graphics models and frequency tables.

2.3.7 Reference and Marker

For the DNA-Typisierung, as Allele-ladder, the STR System HumFIBRA (FGA) and the STR System HumVWA (vWA) by the Firm Serac have been used.
Short Tandem Repeats (STR) are short polymorphic pieces of DNA (150-350 Basepare), in which tandem of basepares (from 2 to 7) are repeated. The number of repetitions in a certain locus could be highly variable, so it is likely to get more alleles with different possible length.
During the primer screening, these allele ladders were no more available from the Serac. A new one from Amersham Biosciences, covering the size range from 50 to 500bp with a distance of 50bp, was used after having tested it with back regression to verify the compatibility among this and the old one in the alleles value determination. Besides, it was also used an internal marker blue194bp obtained with a knowing size sequence excided from the vector “Bluescribe KS+” following the protocol of the human genetic labour.
PCR was performed in 50µl reaction volume using as template 10ng Bluescribe KS+ with the 194bp insert (provided by the Human Genetic Labour) , 1X PCR Buffer with $MgCl_2$, 1µM each Primer (provided by the Human Genetic Labour) , 8 µM each dNTPs, 1U Taq Polymerase and 5µ DMSO (Dimethyl Sulfoxide).

PCR cycles were performed as follows:

Cycle number	Temperature	Time(s)
1	94°	300
50	94°	45
	60°	45
	72°	45
1	72°	420
1	4°	300
1	20°	300

2.4 Data Analysis

In the following paragraphs data analysis methods used in the present study will be explained by giving background information and a rough outline of the procedure.

2.4.1 Population genetic analysis of microsatellite

To perform a reliability microsatellite data analysis it is important to assume an appropriate evolutionary model.
Four different models can be assumed:

1. the infinite allele model (IAM, Kimura and Crow, 1964): a mutation involves the change of any number of tandem repeats and result always in an allelic state that was not previously encountered in the population.
2. The stepwise mutation model (SMM, Kimura and Otha, 1978): restricted mutation losing or gaining a single repeat.
3. The two-phase model (TPM, Di Rienzo et al., 1994): the state of the mutating allele changes by an absolute number of repeats unit with the highest probability, usually assigned to mutation steps, of one tandem repeat and lower probability assigned to mutation steps of more than one tandem repeat.
4. The K-allele model (KAM, Crow and Kimura, 1970): assumed exactly K possible allelic states and any allele has constant probability of mutating towards any of the other K-1 allelic states.

Under this assumption for each population was measured genetic polymorphism as the mean number of alleles per Locus (A), observed heterozygosity (H_{obs}) and unbiased estimate of expected heterozygosity from Hardy-Weinberg assumptions (H_{exp}) using GENETIX 4.05 software (Belkhir et al., 2000).
The number of allele independent of sample size, allelic richness, was performed by FSTAT 2.9.3 software (Goudet, 2001).
The most important concept for classical population genetic analysis is probably the assumption of hierarchical F-statistic (Wright, 1951).
This defines the fixation indices that equal the reduction in heterozygosity expected with random mating at any hierarchical level of a population relative to another more inclusive level of the hierarchy.

The two most common indices are F_{is} and F_{st}. The F_{is} index describes the reduction in heterozygosity that usually occurs when in a subpopulation the inbreeding takes place (mating between close relatives). This index becomes 0 when there is no inbreeding and the frequencies of the genotype are in Hardy-Weinberg equilibrium and it becomes 1 when there is a complete inbreeding, that means that the entirely subpopulation consists of homozygotes.

F_{is} (Weir and Cockerham, 1984) was calculated for each population using GENETIX software. Deviations from Hardy-Weinberg equilibrium (HWE) were tested using the exact probability test (Guo and Thompson, 1992) with GENPOP 4.0.9 (Raymond and Rousset, 1995) using the following Markov chain parameters for all tests:

Dememorization: 10000

Batches: 20

Iterations per batch:5000

Significance levels have been calculated at each locus, for each population and over all loci for each population.

Genes not in random association are called in linkage disequilibrium (Hartl and Clark, 1997).

For example, this situation could happen if two loci are in the same chromosome in a relative small physical distance, so that there is a high probability that they could segregate together. If this is the case, the two loci are not independent, therefore one locus should be excluded from further analysis.

Linkage disequilibrium was tested in the present study between all loci pairwise using FSTAT software.

2.4.2 Spatial structure

Several tests were carried out to analyse the spatial structure of the salmon population.

(i) Pairwise homogeneity tests of allele frequencies were performed using Fisher's exact test implemented in GENPOP 4.0.9, assuming that significant differences in the distribution of the allele frequencies is indicative of populations reproductively isolated.

Another fixation index was used measuring the heterozygote deficit, F_{st}, in a subdivided population relatively to its expectations under Hardy-Weinberg equilibrium (Hartl and Clark, 1997). This index is for this reason useful to determine the population differentiation.

When the index value is ranging from 0 to 0.05 there is little genetic differentiation, from 0.05 to 0.15 a moderate genetic differentiation, from 0.15 to 0.25 a great genetic differentiation and when it is above 0.25 a huge genetic differentiation (Hartl and Clark, 1997).

The most widely used method to estimate F-statistic (F_{is} and $F_{st)}$ is the one suggested by Weir and Cockerham (1984) based on a conventional analysis of variance framework.

Often, microsatellites appear to follow a stepwise mutation model, therefore, Slatkin (1995) suggested another estimation method that takes into account this model and the variance in allele size, the R_{st} value.

(ii) In the present study both indices were applied. F_{st} index was calculated using permutation procedure in GENETIX and R_{st} index was implemented by GENPOP 4.0.9 (Raymond and Rousset, 1995).

(iii) Factor analysis of correspondences was implemented with Genetix software. Correspondence analysis is an exploratory data analytic technique designed to analyze simple two-way and multi-way tables containing some measure of correspondence between the rows and columns. Exploratory data analysis is used to identify systematic relations between variables when there are no (or rather incomplete) a priori expectations as to the nature of those relations.

A population is a cloud of points (individuals) adding to each point contributes inertia to the cloud minimizes the space between points.

Inertia is a term borrowed from the "moment of inertia" in mechanics. A physical object has a center of gravity (or centroid). Every particle of the object has a certain mass *m* and a certain distance *d* from the centroid. The moment of inertia of the object is the quantity md^2 summed over all the particles that constitute the object.

$$\text{Moment of inertia} = \sum md^2$$

This concept has an analogy in correspondence analysis. There is a cloud of profile points with masses adding up to 1. These points have a centroid (i.e., the average profile) and a distance (Chi-square distance) between profile points. Each profile point contributes to the inertia of the whole cloud.

0 for the absence, 1 for the presence of the allele with the heterozygote state, and 2 for the homozygote state represent each individual. Inertia values determine where the dots lay by consistency between themselves in the data.

(iv) An individual assignment test was carried out to assign the individuals to populations in which the likelihood of their genotype is highest. The assignment test was performed with GENAIEX 6.2 software (Peakall and Smouse, 2001).

A Markov chain Monte Carlo clustering approach (MCMC) was implemented with the program STRUCTURE 2.1 (Pritchard et al., 2000) to assign individuals to K subpopulations, or cluster, based on their multilocus genotypes.
Individuals were assigned in a way that minimized the amount of HWE or gametic disequilibrium occurred within populations.

(v) The Structure program was ran according to the two different scenarios described above (see Tab.4a/Tab.4b). For each scenarios the program was ran three times, fitting K from 1-9 for the first and from 1-12 for the second.
In both cases the runs used a burning period of 50 000 iterations and a period of data collection of 50 000 iterations.
Initially parameters assumed were the admixture model and correlated alleles frequencies, after the HWE analysis and under the hypothesis of a Wahlung effect, the analysis was repeated assuming a no admixture model that fit better with the studied situation.

(vi) Genetic distance between populations was estimating using Cavalli-Sforza, Edward's genetic distance (Cavalli- Sforza and Edwards, 1967) and Nei's genetic distance (Nei, 1972) as implemented in GENETIX 4.0.5 (Belkhir et al., 2000).
Both measurements assume that all differences populations arise from genetic drift.
Nei's distance is formulated for an infinite isoalleles model of mutation, in wich there is a rate of neutral mutation and each mutant is to a completely new alleles. It is assumed that all loci have the same rate of neutral mutation, and that the genetic variability initially in the population is at equilibrium between mutation and genetic drift, with the effective population size of each population remaining constant.

Nei's distance is:

$$D = -\ln\left(\frac{\sum_m \sum_i p_{1mi}\, p_{2mi}}{[\sum_m \sum_i p^2_{1mi}]^{1/2}\ [\sum_m \sum_i p^2_{2mi}]^{1/2}}\right)$$

where m is summed over loci, i over alleles at the m-th locus, and where p_{1mi} is the frequency of the i-th allele at the m-th locus in population 1.
Subject to the above assumptions, Nei's genetic distance is expected, for a sample of sufficiently many equivalent loci, to rise linearly with time.

The Cavalli-Sforza's chord assumes that there is no mutation, and that all gene frequency changes are by genetic drift alone. However it doesn't assumes that population sizes have remained constant and equal in all populations. It copes with changing population size by having expectations that rise linearly not with time, but with the sum over time of 1/N, where N is the effective population size. Thus if population size doubles, genetic drift will be taking place more slowly, and the genetic distance will be expected to be rising only half as fast with respect to time.

Cavalli-Sforza's chord distance is given by

$$D^2 = 4\sum_m [\ 1 - \sum_i p_{1mi}^{1/2}\, p_{2mi}^{1/2}\ ] \ /\ \sum_m (a_m - 1)$$

where m indexes the loci, where i is summed over the alleles at the m-th locus, and where a is the number of alleles at the m-th locus. It can be shown that this distance always satisfies the triangle inequality. Note that as given here it is divided by the number of degrees of freedom, the sum of the numbers of alleles minus one. The quantity which is expected to rise linearly with amount of genetic drift (sum of 1/N over time) is D squared, the quantity computed above, and that is what is written out into the distance matrix.

(vii) A bootstrap analysis was performed by first generating 100 distance matrices performed with Cavalli-Sforza, Edward's genetic distance and Nei's genetic distance, which were then used to generate 100 trees with neighbour-joining method (Saitou and Nei, 1987) and with UPGMA method (Michener and Sokal, 1957) with Seqboot, Gendist and Neighbour program in PHYLIP 3.68 (Felsenstein, 1981).

The neighbour-joining method is a special case of the star decomposition method. The raw data are provided as a distance matrix and the initial tree is a star tree. Then a modified distance matrix is constructed in which the separation between each pair of nodes is adjusted on the basis of their average divergence from all other nodes. The tree is constructed by linking the least-distant pair of nodes in this modified matrix.
Among hundred different trees those numbers indicate how many times this relative position has occurred. Low numbers are indicative of a less robustness of the clusters; vice versa high numbers indicate the robust reliability of them.

The unweighted pair-group method with arithmetic mean (UPGMA) is a popular distance analysis method and hierarchical clustering.
It was originally developed for constructing taxonomic phenograms, i.e. trees that reflect the phenotypic similarities between populations, but it can also be used to construct phylogenetic trees if the rates of evolution are approximately constant among the different lineages.
UPGMA employs a sequential clustering algorithm, in which local topological relationships are identified in order of similarity, and the phylogenetic tree is build in a stepwise manner.
Given a set of pairwise distances, assume that the two closest taxa *i* and *j* are actually sisters of each other on the tree, join them into a single node, whose distance to *i* or *j* is $d_{ij}/2$, and recompute distances to everything else, then repeat this, until everything joined together.
The great disadvantage of UPGMA is that it assumes the same evolutionary speed on all lineages, for example the rate of mutations is constant over time and for all lineages in the tree. This is called a “molecular clock hypothesis”. And this method turns out to be very good when data are close to a molecular clock.
On the other hand, this would mean that all leaves (terminal nodes) have the same distance from the root and in reality the individual branches are very unlikely to have the same mutation rate. Therefore, UPGMA frequently generates wrong tree topologies.

A consensus tree was then implemented with Consense program in PHYLIP.
It carries out a family of consensus tree methods called the M_l methods (Margush and McMorris, 1981) by producing a composite tree as a result of a consensus among all those trees, including those linked by strict consensus and majority rule consensus. In a strict consensus, all conflicting branching patterns among the trees are resolved by making

those nodes multifurcating. In a majority-rule consensus, conflicting branching patterns are resolved by selecting the pattern seen in more than 50% of the trees.
Basically, the consensus tree consists of monophyletic groups that occur as often as possible in the data. If a group occurs in more than a fraction *l* of all the input trees it will definitely appear in the consensus tree.

Population differentiation can be evaluated from microsatellites in a number of ways.
Two or more statistics may depend on many factors, usually difficult to quantify.
(viii) GENAIEX 6.2 (Peakall and Smouse, 2001) was used to quantify genetic variability at different hierarchical levels of the population substructure including AMOVA method (Excoffier et al., 1992).

2.4.3 Isolation by distance

To determine the congruency between geographical and genetic divergence of the populations Mantel test was performed using the program GENAIEX 6.2 (Peakall and Smouse, 2001). Statistical significance was tested with 999 random permutations.
Information on within-population diversity was used to detect recent population bottlenecks (Cornuet and Luikart 1996, Luikart et al. 1998a, Luikart et al. 1998b) and recent migration among populations by testing for excess in heterozygosity using the program BOTTLENECK 1.2.0.2 (Pyri, Luikart and Cornuet 1999). Three models were assumed: the IAM (Infinite allele model), the SMM (stepwise mutation model) and theTMP (two-phase model) with 10% multistep changes and variance of 10. Due to small number of loci analysed (n=9), a Wilcoxon sign-rank test was used.
A second method was also used, the graphical representation of the mode-shift indicator originally proposed by Luikart et al. (1998) and performed with Bottleneck software. Loss of rare alleles in bottlenecked populations is detected when one or more of the common allele classes have a higher number of alleles than the rare allele class (Luikart et al. 1998).

All the genetic software need a specific input-file, it is, thus, recommended the use of a file converter in order to have the proper format for each software. In the present study the software FORMATOMATIC 0.8.1 (Manoukis, 2007) has been applied and it is available at web site: http://taylor0.biology.ucla.edu/~manoukis/Pub_programs/Formatomatic/

In all the statistics, when applicable, significance values were adjusted using sequential Bonferroni correction (Rice, 1989).

$1\text{-}(1\text{-}\alpha)^{1/n}$(corrected for *n* comparisons)

In statistics, the **Bonferroni correction** states that if an experimenter is testing *n* dependent or independent hypothese on a set of data, then one way of maintaining the familywise error rate is to test each individual hypothesis at a statisticsn significance level of $1/n$ times what it would be if only one hypothesis were tested (α/n).
The Bonferroni correction is a safeguard against multiple tests of statistical significance on the same data falsely giving the appearance of significance, for example as 1 out of every 20 hypothesis-tests is expected to be significant at the $\alpha = 0.05$ level purely due to chance. Furthermore, the probability of getting a significant result with *n* tests at this level of significance is $1–0.95^n$ (1–probability of not getting a significant result with *n* tests).

2.5 Scale reading

Salmon scales are often used in age and growth studies because they reflect growth at the different stages of life of the fish (Tesch, 1968). Growth patterns have been used to distinguish between different groups of salmons, for example, to recognize the salmons of European and North American origin in the high seas salmon fishery at West Greenland (Reddin et al., 1987).
Scalimetry is controversial and needs careful application, because many scales lack the central core (replacements scales), and because the fish can remineralize elements and materials from the scale bone, so to interfere with additive growth. Accordingly ageing can be misleading.
The results on salmon scales age determination in Lund and Hansen (1991) confirm these difficulties. There was a great variation in the mis-ageing rate of the farmed salmons of known freshwater age among the different farms. Only averages of 27% of the fishes were aged correctly. All the mis-aged fishes were given a higher smolt age.
Also the sea age of the fishes from different farms showed considerable variations in the mis-aging rate, and only an average of 53% of the fishes were aged correctly. The main part of the mis-aged fishes had been over aged (a year older).

According to ageing and growth analysis, Lund and Hansen (1991) have identified 6 scales characters that could point out the differences between fish farm released and escapees in nature and wild salmons:

1) Smolt size.
2) Smolt age.
3) Transition from fresh water to salt water.
4) Sea winter band.
5) Summer check.
6) Replacement scales.

Scales from Ifezzheim subpopulations have been cleaned and mounted on dia-frames for age determination by the growth rings reading.
Statistics evaluation have been carried out using a software written for this purpose by a former member of the working group GenoAssign 1.0 (M.Wang, 2002) for the age estimation and migration behaviour of the referees individuals.

3 RESULTS

3.1 Species confirmation

Iffezheim blood samples for the years 2002-2003-2004-2005 have been tested with the diagnostic allozymes in order to verify the identity of salmons and to find possible misidentifications and hybrids with trout.

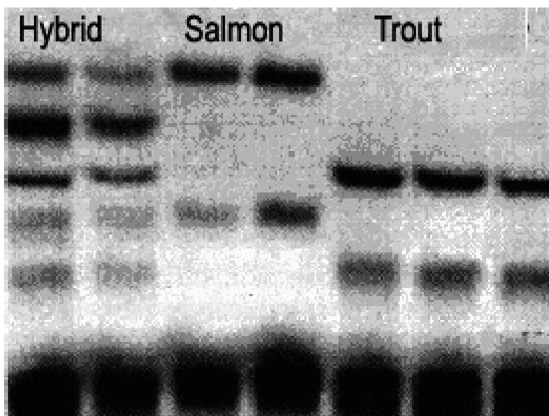


Fig.12 Electrophoretic patterns of glucose phosphate isomerase (GPI) for Atlantic salmon, brown trout and their hybrids

Atlantic salmon displayed an invariant symmetrical three-banded pattern showing two GPI loci (GPI-1* and GPI-3*) along with a single interlocus heterodimer band (GPI-2*). Only a single band is common to both species, the brown trout GPI-2 homodimer (Fig.12).
The pattern of brown trout showed the same pattern (three bands) with exclusive alleles but occasional variant phenotypes were observed.
This enzyme thus allowed positive identification of hybrid fish, which, without exception, displayed perfect summation of parental patterns *S.salar* and *S.trutta*.

Only 11 fishes out of the 304 analysed, have proved to be misidentified trouts given as salmons.

Misidentification throughout the four years:
2002 1 misidentification
2003 4 misidentification
2004 4 misidentification
2005 2 misidentification
People in charge of the monitoring correctly identified the 89% of the salmons sampled.

3.2 Quality control and calibration of the size reference

For the screening of the loci Ssa202, Ssa402, Ssa408, Ssa411, the size marker used as reference for the microsatellites size determination has been changed, because the former markers, STR System HumFIBRA (FGA) and the STR System HumVWA (vWA) by the Firm Serac, were no more available.

This new marker from Amersham Biosciences (GE healthcare) covers the size range from 50 to 500bp with a distance of 50bp.

The new ladder has been tested in an acrylamide gel in order to calibrate it completely. According to the protocol supplied by the company, 10 clear picks would have been expected, but only 9 clear picks were observed and the remaining one was not perfectly matching the expectation.

To verify the correct performance of the ladder, these 9 picks, size and time (Tab.9), have been tested with a linear regression function performed with the Statistical Programme Package for the Social Sciences software (SPSS Inc., Chicago, IL, USA).

The variants were completely explained by the curve (Fig.13/Tab.10), R-Quadrat=1.000.

The “time” variant is 42 times the base pair variant (b1=42.98) plus the constant, K=3238.3.

This function, in the end, sufficiently described the performance of the ladder.

Pick(bp)	Time(s)
100.00	7451.00
150.00	9624.00
200.00	11797.00
250.00	14000.00
300.00	16202.00
350.00	18401.00
400.00	20490.00
450.00	22578.00
500.00	24577.00

Tab.9 Picks size of the ladder (50-500bp Amersham Biosciences) and time read in the acrylamide gel

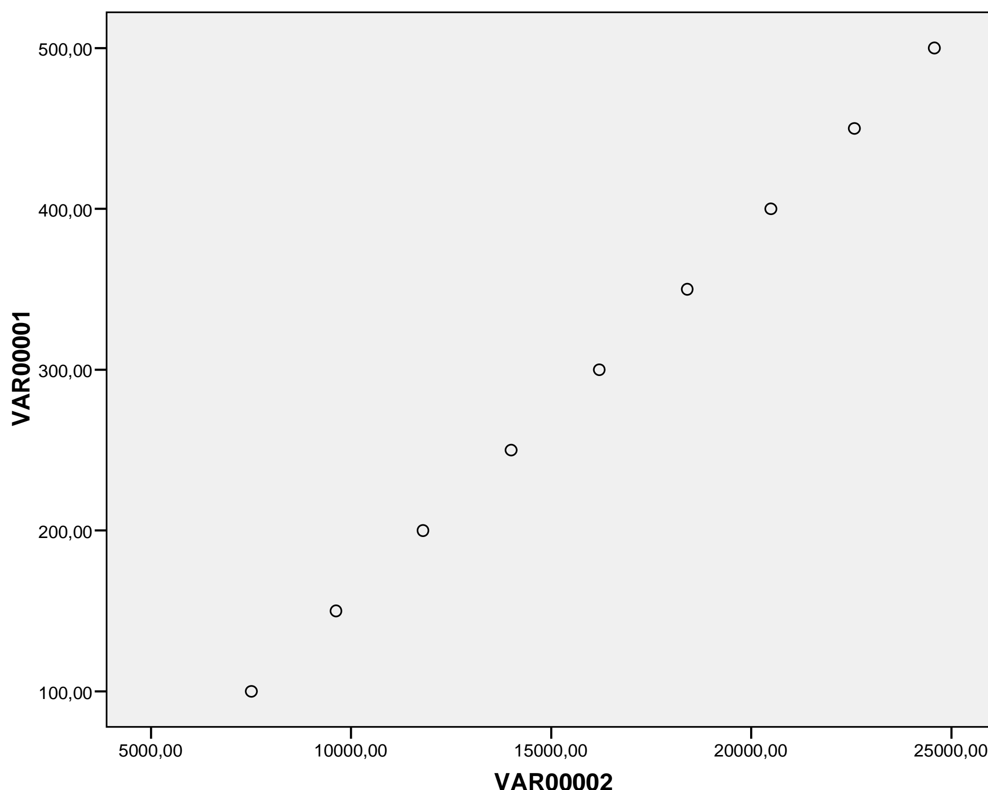


Fig.13 Linear regression describing the GE healthcare ladder performance. Var00001=Bp, Var00002=Time

Dependent Variable: VAR00002 = Time

Equation	Model summary					Statistic parameter	
	R-Quadrat	F	Degrees of freedom 1	Degrees of freedom 2	Sig.	Constant	b1
Linear	1.000	37401.644	1	7	.000	3238.278	42.975

Independent Variable VAR00001 = Bp

Tab.10 Table summarizes the regression model and parameters of the linear regression representation (Fig.11)

In order to have also an internal marker to apply together with the sample and the external ladder, the blue194bp marker obtained with a knowing size sequence excided from the vector “Bluescribe KS+” has been tested.

Acrylamide gel with this internal size marker and the ladder from GE healthcare (50-500 bp) was run.

Some problems in the resolution of the GE healthcare ladder picks have occurred, the first two picks (50 and 100bp), as well as the last two (450 and 500bp), were not enough clear. Using the clear picks (150 to 400bp/Tab.11a), the internal size marker (194bp/Tab.11b) has been tested performing a linear regression.

The internal marker has performed a linear behaviour fitting the GE healthcare ladder as shown in the graphic (Fig.14) and in the statistical table (Tab.12).

Picks(Bp)	Time(s)
150.00	10179.00
200.00	12496.00
250.00	14810.00
300.00	17187.00
350.00	19437.00
400.00	21627.00

Tab.11a Picks size of the ladder GE healthcare and time read in the acrylamide gel

Picks(Bp)	Time(s)
194.00	12161.00

Tab.11b Pick size of the ladder blue194 (Internal size marker) and time read in the acrylamide gel

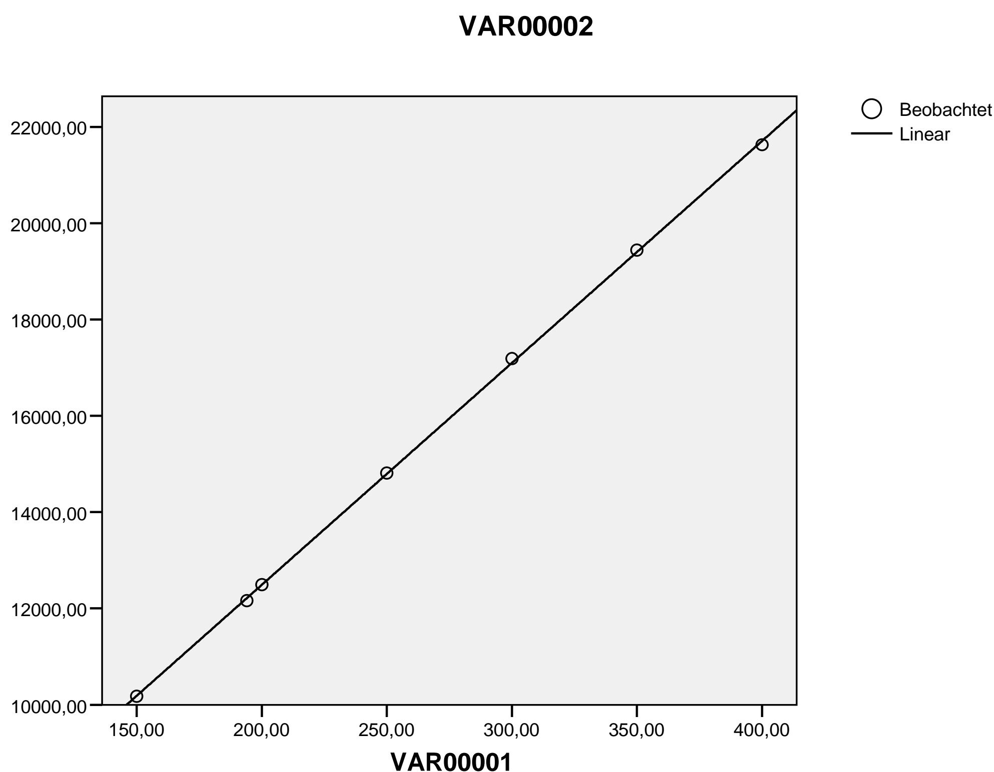


Fig.14 Linear regression describing the GE healthcare ladder together with the blue194bp performance. Var00001=Bp, Var00002=Time

Dependent Variable: VAR00002 = Time

Equation	Model summary					Statistic parameter	
	R-Quadrat	F	Degrees of freedom 1	Degrees of freedom 2	Sig.	Constant	b1
Linear	1.000	28364.988	1	5	.000	3278.586	46.067

Independent Variable VAR00001 = Bp

Tab.12 Table summarizes the regression model and parameters of the linear regression representation (Fig.14)

The internal size marker blue194bp and another internal size marker, 200bp from GE healthcare, together with the 50-500bp ladders from the same company, have been tested in order to verify the suitability of those markers as „internal and external size marker" in the microsatellites system of this study (Tab.13).

A difference in the running velocity was observed in the acrylamide gel. The first twelve lines ran faster than the other lines, so the time of the picks from the 13th line to the 40th is much more homogenous and thus more easily comparable with the reference ladder and within the sample picks themselves.

The time of the picks of the ladder was bookmarked among the range 150-300bp, then the difference in time between one pick and the other, the average time for 50bp, 6bp and for 1bp (Tab.14) were calculated, and then the internal markers were inserted in this reference system.

By combining those values in a linear regression, the fitting of the internal size markers in the referee system has been verified (Tab15, Fig.15).

P	Time(s)	Marker
150.00	8836.00	GE healthcare 50-500bp ladder
194.00	**10615.00**	**Internal size marker Blue194**
200.00	10895.00	GE healthcare 50-500bp ladder
200.00	**10883.00**	**Internal size marker GE healthcare 200bp**
250.00	13014.00	GE healthcare 50-500bp ladder
300.00	15133.00	GE healthcare 50-500bp ladder

Tab.13 Picks size and time read in the acrylamide gel of the markers.

Bp	**Time(s) mean**
50	2099
6	252
1	42

Tab.14 Time average extrapolated from the test to better calculate the allele size in this reference system

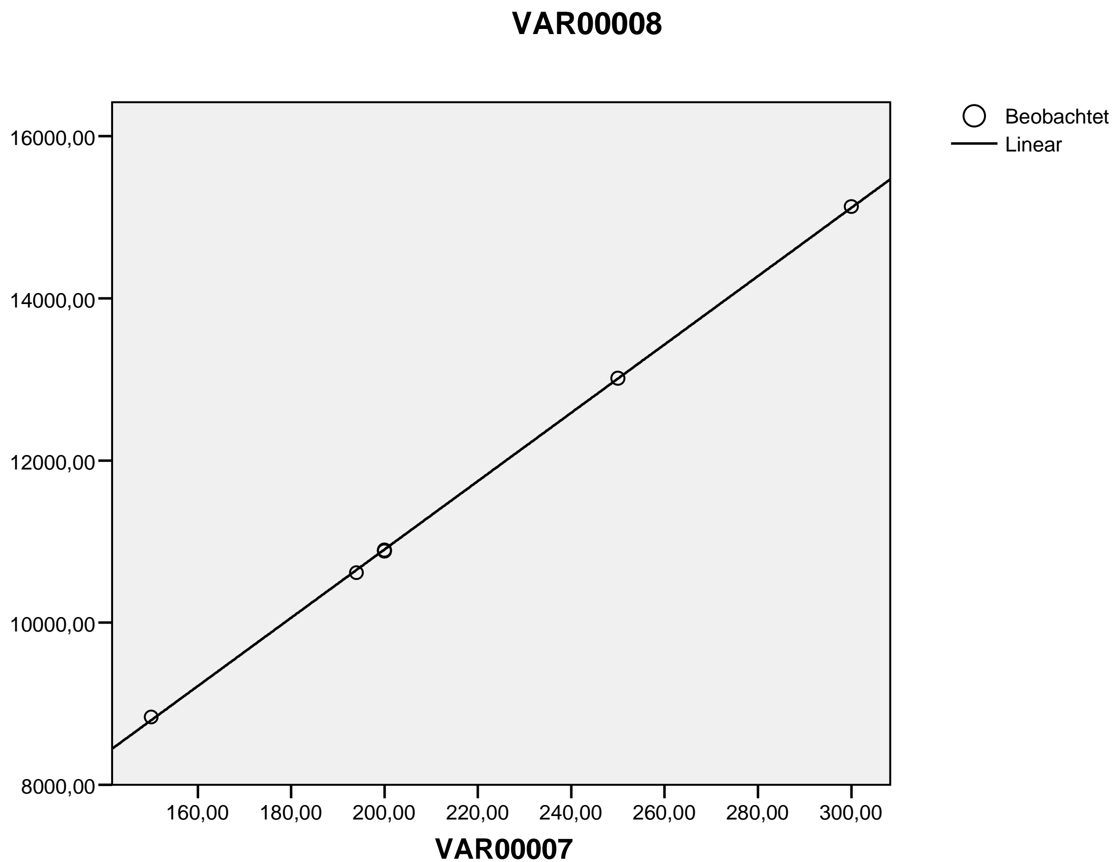


Fig.15 Linear regression describing the GE healthcare ladder 50-500bp together with the blue194bp and 200bp GE healthcare performance. Var00008=Bp, Var00007=Time. The circle representing the 200bp is in bold because the external markers and the internal one of GE healthcare overlap, thus confirming the perfect linearity of the system.

Dependent Variable: VAR00008 = Time

Equation	Model summary					Statistic parameter	
	R-Quadrat	F	Degrees of freedom 1	Degrees of freedom 2	Sig.	Constant	B1
Linear	1.000	26990.049	1	4	.000	2473.512	42.144

Independent Variable VAR00007 = Bp

Tab.15 Table summarizes the regression model and parameters of the linear regression representation (Fig.15)

Due to the overlapping of the internal markers with allele of some loci, it was not possible to mix them with the sample, in those cases the marker was used as external reference.

3.3 Choice of the polymorphic gene markers

Sixteen loci have been tested: SSOSL85, SSOSL311 (Slettan et al., 1995), Ssa402*/Ssa402**, Ssa408, Ssa410, Ssa411, Ssa412, Ssa413, Ssa422 (Cairney et al., 2000), Ssa171, Ssa197, Ssa85, Ssa202 (O´Reilly et al., 1996), STR15, STR60 (Estoup et al., 1993). From them 9 loci were suitable and have been selected for the salmon screening (SSOSL85, SSOSL311, Ssa171, STR15, Ssa402*/Ssa402**, Ssa408, Ssa202 and Ssa411). A table of the resulting genotype (Appendix 1, Tab. 16a/16b) has been produced.

Locus SSOSL85
Salmon screening:
Dinucleotide locus
38 Alleles observed in range 154-222bp
Alleles frequencies are given in Appendix 2, Tab.17.

The allele base pair was calculated by direct comparison with known size DNA marker: STR System HumFIBRA (FGA 176-224 bp).

The interpretation of the curves and therefore the analysis of the alleles at this locus were not complicated. The pattern was immediately clear and did not require so many repetitions to have all the samples screened.

Five alleles were observed for the homozygotes: 190bp, 192bp, 194bp, 199bp e 201bp and the pattern was clear and unambiguous.

The pattern of the heterozygotes was not as simple as the one of the homozygotes. Different combinations of curves and complex picks was observed and, above all, a high number of alleles. Out of 6 populations, assuming Iffezheim sample as a population by itself, with a total number of 180 individuals, 31 different alleles ranged among 176-205bp could be found.

Alleles that differed for one or two base pair were found. In this case the identity of the uncertain allele was assigned by observing which allele was more represented.

So far, this primer did not imply many problems of interpretation. The high number of alleles could be a negative criterion of choice to identify and allocate the screened salmon samples as belonging to a specific population.

Locus SSOSL311
Salmon screening:
Dinucleotide locus
54 Alleles observed in range 124-179bp
Alleles frequencies are given in Appendix 2 Tab.18.

Reference marker for this locus was STR System HumVWA (vWA 127-171 bp).

The interpretation of the curves and therefore the analysis of the alleles at this locus were not complicated. The pattern was immediately clear and did not require so many repetitions to have all samples screened.

Five alleles were observed for the homozygotes: 125bp, 126bp, 127bp, 128bp and 131bp and the pattern was clear and unambiguous.

The pattern of the heterozygotes was more complex. Composite picks patterns and a significant number of alleles have been observed.

Locus Ssa171
Salmon screening:
Tretranucleotide locus
46 Alleles observed in range 197-279bp
Alleles frequencies are given in Appendix 2, Tab.19.

The primer has initially introduced some problem of interpretation and individualization of the correct picks. Therefore, different repetitions and cross checks have been required with internal marker.

Being a tretranucleotide locus, the pattern of the homozygote was composed by two picks, having more or less the same magnitude, usually separated by two to four base pair and

the pattern of the heterozygote was composed by four picks, two for each allele, also separated by several base pairs.

Locus STR15
Salmon screening:
Dinucleotide locus
33 Alleles observed in range 197-252bp
Alleles frequencies are given in Appendix 2, Tab.20.

This primer is born in literature as profit in the analysis of genetics of the population of trouts. Assuming that the genetic distance between trout and salmon is practically void and therefore supposing that the sequence of DNA amplified by this primer is more or less identical, it has been experimented on the salmons and the results are completely comparable, as expected.
The primer has initially introduced some problems of interpretation and individualization of the correct picks. Therefore, different repetitions and cross checks have been required with internal marker.

The pattern was constituted by complex curves that made it difficult to distinguish clearly the different alleles: the homozygotes and the heterozygotes.
Once understood the pattern, this primer has proved to be rather reliable and useful for the following genetic analysis of the population.

The primer showed a small number of alleles and combinations and proved to be a good candidate together with the SSOSL85 primer for the definition of the genetic profile of the examined population.

Locus Ssa402
Salmon screening:
Dinucleotide locus.
The primers amplify for two different no overlapping loci, Ssa402* and Ssa402**.
The allele base pair was found by direct comparison with known DNA marker GE healthcare 50-500 bp combined with a single fragment digested from the pBluescript II KS(-) phagmid vector (194 bp).

Ssa402*:
22 Alleles observed in range 150-183bp
Alleles frequencies are given in Appendix 2, Tab.21.

The interpretation of the curves and therefore the analysis of the alleles at this locus were not complicated. The pattern was immediately clear and did not require many repetitions to have all the samples screened.

Thirteen alleles were observed for the homozygotes: 163bp, 164bp, 166bp, 167bp 168bp, 169bp, 170bp, 171bp, 172bp, 173bp, 174bp, 176bp and 183bp all with a clear and unambiguous pattern.
The pattern of the heterozygotes was also of quite simple comprehension. By the graphic point of view the pattern showed 2 clear different picks separated by a minimum of 1bp to a maximum of 10bp.
So far, this primer did not imply many problems of interpretation, and the relative low number of alleles could be a positive criterion of choice to identify and allocate the screened salmon samples as belonging to a specific population.

Ssa402**:
40 Alleles observed in range 190-296bp
Alleles frequencies are given in Appendix 2, Tab.22.

The interpretation of the curves was not immediately clear due to some stutter picks near the main one. This problem was solved with the help of the previous research that gave indication of the size range and the number of alleles, by reading the profile of several samples and by increasing the annealing temperature in the pcr to decrease the stutter picks.

Eight alleles were observed for the homozygotes: 203bp, 204bp, 205bp, 207bp, 211bp, 212bp, 214bp and 217bp.

The pattern of the heterozygotes was of quite simple comprehension once understood which was the pattern with the main picks. By the graphic point of view the pattern showed 2 clear different picks separated by a minimum of 1bp to a maximum of 20bp.

Even if this locus showed a high polymorphism, the great part of the genotypes is placed in the middle of the observed range.

Only some Iffezheim sample showed very different alleles size.

So far, this primer did not imply many problems of interpretation, and the relative low number of alleles could be a positive criterion of choice to identify and allocate the screened salmon samples as belonging to a specific population.

Locus Ssa411
Salmon screening:
Dinucleotide locus.
16 Alleles observed in range 256-283bp
Alleles frequencies are given in Appendix 2, Tab.23.
The interpretation of the curves and the analysis of alleles were not complicated. The pattern was immediately clear and did not require repetitions at all.

16 alleles were observed at this locus and all of these alleles were represented in the homozygote genotypes.

This locus is highly oligomorphic, it is characterized by a few numbers of alleles and a highest number of homozygote genotypes instead of heterozygotes, therefore this locus could be considered strongly conservative.

The pattern of the heterozygotes was very clear, as well as the homozygote one, with two main picks separated by a minimum of 1bp to a maximum of 5bp.

So far this primer did not imply many problems of interpretation, and the low number of alleles could be a really positive criterion of choice to identify and allocate the screened salmon samples as belonging to a specific population.

Locus Ssa408
Salmon screening:
Tetranucleotide locus
92 Alleles observed in range 208-322bp
Alleles frequencies are given in Appendix 2, Tab.24.

The interpretation of the curves was not immediately clear, due to the tetranucleotide nature of this locus, so the right alleles are already graphically constituted by two curves the homozgotes and by four curves the heterozygotes, plus intermediary curves and some stutter picks near the main one. The problem of the individuation of the main picks representing the alleles was solved with the help of the previous research, which gave indication of the size range and the number of alleles and by reading the profile of several samples. The problem of the stutter picks was partially solved by increasing the annealing temperature in the pcr to decrease their number.

Twenty alleles were observed for the homozygotes: 221bp, 238bp, 239bp, 242bp, 254bp, 256bp, 264bp, 265bp, 280bp, 282bp, 284bp, 290bp, 292bp, 296bp, 300bp, 301bp, 307bp, 308bp, 319bp.

The pattern of the heterozygotes was of also not easy to define because of the curves number, but after the analysis of several samples it was digested and understood. By the graphic point of view, the pattern showed 4 more or less clear different picks, separated by a minimum of 4bp to a maximum of several base pair.

This locus showed a high polymorphism and would possible be a really negative marker to identify and allocate the screened salmon samples as belonging to a specific population.

Locus Ssa202
Salmon screening:
Tetranucleotide locus
30 Alleles observed in range 223-268bp
Alleles frequencies are given in Appendix 2, Tab.25.

The interpretation of the curves and therefore the analysis of the alleles at this locus were not complicated. The pattern was immediately clear and did not require so many repetitions to have all the samples screened.

Eleven alleles were observed for the homozygotes: 229bp, 237bp, 239bp, 240bp, 244bp, 245bp, 246bp, 247bp, 248bp, 251bp and 256bp.

The pattern of the heterozygotes was simple and did not show particular interpretation problems.

Sometimes the picks curves were almost overlapped, outdistanced for less than 2 base pair, the pattern in such cases was not so clear. Samples of this kind of pattern needed to be repeated until the picks were clear and well separated.

The high number of alleles at this locus could be a negative criterion of choice to identify and allocate the screened salmon samples as belonging to a specific population. Although, being clear and easy to read, this primer could be considered a good candidate as genetic marker.
Primers for Ssa411, SSa408, SSa202, SSa402 loci have been tested also for some trout samples and three of them (SSa408, SSa202, SSa402) have given good signals.
Examples of the loci alleles profiles are given in the following graphic (Fig.16).

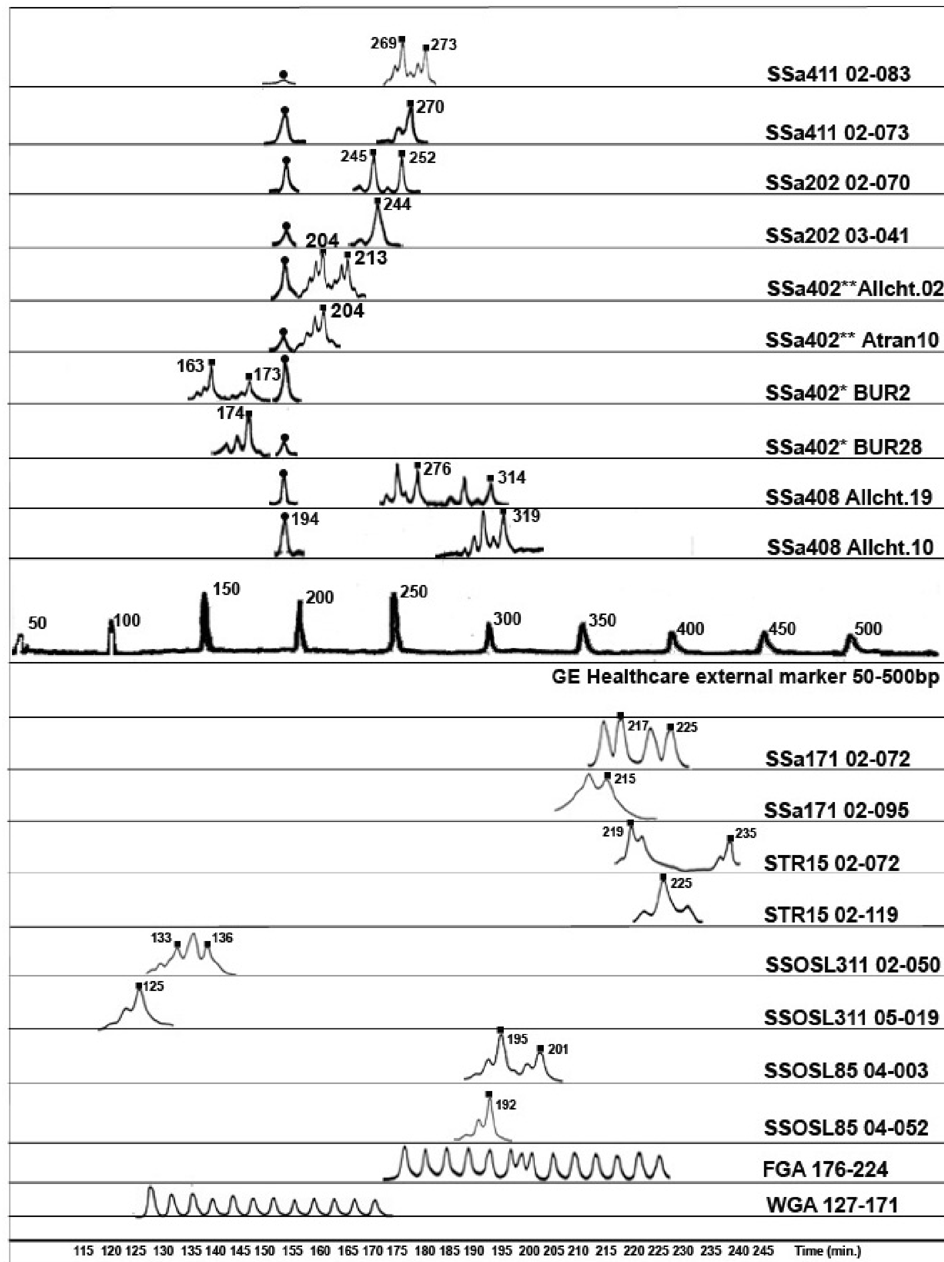


Fig.16 Analysis of microsatellite fragment lengths on Alf Express automated fragment analyser. Example of homozygotes and heterozygotes profile for each locus. Genetic external markers are FGA-WGA from Serac and 50-500bp from GE Healthcare. Peaks marked with circles represent internal standards, those with squares the sample fragments

3.4 Population genetic analysis of microsatellite

Loci analysis

The total number of alleles and observed heterozygosity for each locus ranged from 8 to 21 (mean = 15 assuming Iffezheimtot as a whole population, mean = 14 assuming each Iffezheim population a single one) and 0.79 to 0.90 (mean= 0.85) as shown in Tab.26

	Hexp,	**H n,b,**	**Hobs,**	**P(0.95)**	**P(0.99)**	**Mean number of Alleles per Locus**
Iff02	0.90	0.91	0.74	1.00	1.00	21
S.D. :	0.05	0.05	0.24			
Iff03	0.85	0.89	0.78	1.00	1.00	11
S.D. :	0.08	0.09	0.21			
Iff04	0.81	0.84	0.60	1.00	1.00	13
S.D. :	0.17	0.18	0.31			
Iff05	0.79	0.84	0.64	1.00	1.00	8
S.D. :	0.11	0.12	0.28			
Ifftot	0.90	0.90	0.70	1.00	1.00	30
S.D. :	0.08	0.08	0.22			
BUR	0.81	0.83	0.71	1.00	1.00	13
S.D. :	0.14	0.14	0.27			
Allwild	0.87	0.89	0.67	1.00	1.00	15
S.D. :	0.05	0.05	0.23			
Allhatc	0.87	0.89	0.72	1.00	1.00	14
S.D. :	0.05	0.05	0.22			
Ätran	0.86	0.88	0.73	1.00	1.00	16
S.D. :	0.09	0.09	0.18			
Lagan	0.85	0.87	0.66	1.00	1.00	12
S.D. :	0.09	0.09	0.29			
Mean	0.85	0.87	0.70	1.00	1.00	15

Tab.26 Mean number of alleles and heterozigosity at each locus per population. Observed (H_{obs}) and expected heterozygosity with (H_{exp}), and without ($H_{n.b}$) bias (Nei, 1978), p(0.95)/p(0.99) probability that the locus is considered as polymorph if the most frequent allele does not exceed 95 % (respectively 99 %). GENETIX 4.05

All the considered loci are polymorphic in each assumed populations as p(0.95) and p(0.99) values shown in Tab.27/Tab.28.

Percentage of Polymorphic Loci	
Population	**%P**
Iff02	100.00%
Iff03	100.00%
Iff04	100.00%
Iff05	100.00%
Ifftot	100.00%
BUR	100.00%
Allwild	100.00%
Allhatc	100.00%
Ätran	100.00%
Lagan	100.00%
Mean	100.00%
SE	0.00%

Tab.27 Percentage of polymorphic loci per population GENAIEX 6.2

Number of individuals analysed, number of alleles, H_{obs}, $H_{exp,}$ Weir and Cockerham (1984) and Robertson and Hill (1984) F_{is} and allelic richness for each locus within each sample site are resumed in Tab.28.

	Iff. 02	Iff.03	Iff.04	Iff.05	Iff.tot	Bur	Allwild	Allhatch	Ätran	Lagan
No. Individuals	30	11	15	9	65	22	25	25	28	15
Locus										
SSOSL85										
Na	24	12	19	10	33	15	14	10	16	10
H_{exp}	0.92	0.88	0.74	0.83	0.95	0.89	0.94	0.94	0.95	0.84
H_{obs}	0.67	0.91	0.8	0.67	0.74	0.59	0.68	0.88	0.89	0.27
Fis(W&C)	**0.3**	0.03	**0.17**	**0.28**	**0.23**	**0.35**	**0.65**	**0.62**	**0.38**	**0.42**
Fis(R&H)	0.17	0	0.1	0.12	0.12	0.24	0.51	0.47	0.3	0.4
SSOSL311										
Na	21	11	9	9	34	17	21	22	28	13
H_{exp}	0.92	0.88	0.74	0.83	0.83	0.89	0.94	0.94	0.95	0.84
H_{obs}	0.93	0.91	0.33	0.22	0.74	0.91	0.68	0.88	0.89	0.27
Fis(W&C)	0.01	0.02	**0.57**	**0.76**	**0.25**	0	**0.3**	0.08	0.08	**0.49**
Fis(R&H)	-0.01	-0.01	0.3	0.59	0.11	0.09	0.19	0.08	0.05	0.46
STR15										
Na	11	7	7	4	15	6	11	11	11	9
H_{exp}	0.79	0.72	0.63	0.61	0.61	0.5	0.86	0.87	0.87	0.8
H_{obs}	0.67	0.64	0.33	0.44	0.44	0.32	0.64	0.68	0.61	0.53
Fis(W&C)	**0.17**	**0.16**	**0.5**	**0.33**	**0.26**	**0.39**	**0.27**	**0.24**	**0.32**	**0.36**
Fis(R&H)	0.05	0.16	0.36	0.17	0.1	0.38	0.34	0.19	0.37	0.27

Tab.28 Number of observed alleles (Na), observed (H_{obs}) and expected (H_{exp}) heterozygosity, Fis calculated at each population for the 9 microsatellite used in this study. GENETIX 4.05. Allelic richness FSTAT 2.9.3.

	Iff. 02	Iff.03	Iff.04	Iff.05	Iff.tot	Bur	All wild	All hatchery	Ät	Lag
No. Individuals	30	11	15	9	65	22	25	25	28	15
Locus										
SSa171										
Na	22	14	16	8	38	15	18	25	24	20
H_{exp}	0.92	0.9	0.92	0.84	0.84	0.9	0.91	0.93	0.94	0.94
H_{obs}	0.73	0.64	0.73	1	1	1	0.92	0.96	0.75	1
Fis(W&C)	**0.22**	**0.33**	**0.24**	-0.13	**0.22**	-0.09	0.01	-0.01	**0.22**	-0.03
Fis(R&H)	0.13	0.2	0.17	-0.07	0.15	-0.05	0.01	0.02	0.18	-0.02
SSa402*										
Na	14	7	10	3	16	8	8	10	13	11
H_{exp}	0.87	0.79	0.86	0.65	0.65	0.78	0.8	0.85	0.87	0.88
H_{obs}	0.77	0.73	0.33	0.33	0.33	0.55	0.64	0.92	1	0.87
Fis(W&C)	**0.13**	**0.13**	**0.64**	**0.53**	**0.31**	**0.32**	**0.22**	-0.06	-0.14	0.05
Fis(R&H)	0.05	0.14	0.47	0.49	0.18	0.37	0.19	0	-0.07	0.08
SSa402**										
Na	25	12	13	10	36	6	16	12	7	10
H_{exp}	0.92	0.9	0.88	0.86	0.86	0.69	0.88	0.87	0.83	0.78
H_{obs}	0.97	1	0.87	1	1	0.95	0.84	0.84	0.71	0.8
Fis(W&C)	-0.03	-0.07	0.05	-0.11	-0.03	-0.36	0.07	0.06	**0.16**	0.01
Fis(R&H)	-0.02	-0.05	0.02	-0.06	-0.01	-0.16	0.1	0.02	0.19	0.01
SSa202										
Na	19	13	15	10	33	14	11	8	11	13
H_{exp}	0.91	0.9	0.93	0.86	0.94	0.92	0.84	0.81	0.83	0.89
H_{obs}	0.87	0.82	0.93	0.67	0.85	0.95	0.8	0.84	0.79	0.93
Fis(W&C)	0.07	**0.14**	0.03	**0.28**	**0.1**	-0.02	0.07	-0.02	0.08	-0.02
Fis(R&H)	0.07	0.11	0.04	0.16	0.12	0	0.01	-0.03	0.08	-0.02
SSa411										
Na	11	6	5	6	14	9	8	7	7	6
H_{exp}	0.86	0.71	0.44	0.72	0.82	0.82	0.8	0.83	0.66	0.66
H_{obs}	0.17	0.36	0.13	0.56	0.25	0.32	0.28	0.44	0.43	0.2
Fis(W&C)	**0.81**	**0.52**	**0.72**	**0.28**	**0.7**	**0.63**	**0.66**	**0.49**	**0.37**	**0.72**
Fis(R&H)	0.77	0.32	0.7	0.17	0.72	0.48	0.41	0.5	0.26	0.44
SSa408										
Na	39	15	24	15	61	23	25	21	22	19
H_{exp}	0.97	0.92	0.95	0.93	0.98	0.93	0.93	0.93	0.93	0.93
H_{obs}	0.9	1	0.93	0.89	0.92	0.77	0.88	0.64	0.86	0.8
Fis(W&C)	0.09	-0.04	0.05	**0.1**	0.06	**0.2**	0.07	**0.33**	**0.1**	**0.18**
Fis(R&H)	0.07	-0.03	0.04	0.06	0.06	0.09	0.01	0.2	0.08	0.13
Allelic richness (mean)	10.86	9.71	9.92	8.44	11.12	8.55	9.48	9.21	9.50	9.56
Mean H_{exp}	0.90	0.84	0.79	0.79	0.83	0.81	0.88	0.89	0.87	0.84
Mean H_{obs}	0.74	0.78	**0.60**	**0.64**	0.70	0.71	0.71	0.79	0.77	**0.63**
Multilocus Fis										
All loci	**0.19**	**0.13**	**0.32**	**0.25**	**0.22**	**0.15**	**0.25**	**0.18**	**0.16**	**0.23**
All loci (except STR15, Ssa402* and Ssa411)	**0.11**	**0.07**	**0.19**	**0.2**	**0.14**	0.01	**0.19**	**0.18**	**0.17**	**0.17**

Tab.28 (continued)

Levels of genetic diversity (H_{exp}) were similar in all populations but did not match the observed values showing a diffuse heterozygosity deficiency. One locus showed a significant deviation from the expectation: the Ssa411. At this locus all the observed heterozygosity is much lower than the expected one, the reduced genetic diversity could mean the relative conservation level of this locus within populations.

By the analysis of the fixation index F_{is} all populations seemed to have a homozygote excess with a significant value of heterozygotes deficiency. The basis for heterozygote deficiency in populations has been theoretically and experimentally explored and has been shown to be caused by inbreeding, by positive assortative mating, by pooling populations with different allele frequencies (the Wahlund effect) or due to one or more non-amplifying alleles (null allele-tab.29).

	Iff02	Iff03	Iff04	Iff05	Iff	Bur	Allwild	Allhatc	Ätran	Lagan
SS85	0.13	0.00	0.06	0.21	0.12	0.15	0.30	0.27	0.17	0.19
SS311	0.00	0.00	0.22	0.33	0.10	0.03	0.15	0.04	0.03	0.31
STR15	0.08	0.06	0.19	0.07	0.10	0.15	0.14	0.10	0.16	0.14
SSa171	0.10	0.13	0.14	0.00	0.11	0.00	0.00	0.00	0.10	0.00
SSa402*	0.21	0.05	0.41	0.54	0.28	0.15	0.09	0.00	0.00	0.02
SSa402**	0.01	0.00	0.00	0.00	0.01	0.00	0.04	0.00	0.07	0.00
SSa408	0.04	0.00	0.00	0.00	0.03	0.07	0.02	0.15	0.04	0.07
SSa202	0.03	0.05	0.00	0.09	0.05	0.00	0.00	0.00	0.04	0.07
SSa411	0.39	0.19	0.24	0.10	0.33	0.27	0.28	0.22	0.14	0.28

Tab.29 (Locus by population) table of estimated null allele frequencies

However, 63 out of 90 Hardy-Weinberg exact tests by locus within populations were nominally significant ($p<0.05$) and 45 were significant after the Bonferroni correction ($p<0.0006$) and by populations within loci 66 ($p<0.05$) and 53 after the correction.

Totally within all loci and all populations X^2 calculated with 92 degree of freedom was nearly infinite and the probability to be in HWE was highly significant for all loci over all the populations.

Highly considerable is the evaluation of the private alleles among the population. A Total of 118 private alleles have been found, the majority of them with a frequency equal or below 0.06 with the following exception: Iff02-STR15-235=0.083; Iff03-SSOSL311-148=0.136, Iff03-STR15-236=0.091, Iff03-Ssa202-244=0.091; Iff05-SSOSL85-222=0.111, Iff05-SSOSL311-131=0.111, Iff05-Ssa411-256=0.222; BUR-Ssa408-307=0.091; Allwild-STR15-

214=0.080, Allwild-Ssa408-319=0.200; Ätran-STR15-211=0.107, Ätran-Ssa402**-201=0.089, Ätran-Ssa408-237=0.089; Lagan-Ssa202-268=0.100.

The basic rationale underlying Slatkin's (1985) method is that private alleles are likely to attain high frequency only when Nm (number of migrants) is low.
Nm is the expected number of migrants exchanged among populations each generation. Three regression lines are provided performing GENPOP software (Tab.30). Regression curve of migrant numbers on generations (oy: over years) (Barton and Slatkin, 1985) and a corrected estimate were provided using values from the closest regression line. [migration rate, m = (1-Fst)/2Fst].

Mean sample size: 24.5
Mean frequency of private alleles p(1)= 0.0444805
Number of migrants for mean N=10: 6.60227*
Number of migrants for mean N=25: 2.62932*
Number of migrants for mean N=50: 1.7051*
Number of migrants after correction for size= 2.68298**

Tab.30 Estimation of Nm by GENEPOP 4.0.9 * 3 regression lines. ** Corrected estimate using values from the closest regression line which measures the relationship between two variables using the least squares method (a technique that constructs a graph based on the equation which best fits the data-best fitting curve)

In the following evaluation two scenarios are going to be described:

1) Iffezheim population splitted in the different sampled years
2) Iffezheim population assumed as a single one

Scenario 1:
mean number of private and common alleles is shown in table 31 and their graphical distribution in Fig.17

Even with relative low number of sampled individuals, the Iffezheim assumed populations showed a high numbers of private alleles that could support the genetic diversity of these individuals, thus assigning them to an own population.

Mean Allelic Patterns Across Populations

Mean values									
Population	**Iff02**	**Iff03**	**Iff04**	**Iff05**	**BUR**	**Allwild**	**Allhatc**	**Ätran**	**Lagan**
Na	20.667	10.778	13.111	8.444	12.556	15.000	13.889	15.556	12.333
Na Freq. >= 5%	7.000	5.333	6.889	8.444	5.222	6.333	6.556	7.556	7.000
Ne	12.840	7.891	9.465	6.211	7.708	9.176	9.284	9.918	8.532
I	2.646	2.136	2.191	1.855	2.101	2.352	2.309	2.357	2.204
No. Private Alleles	3.444	0.778	1.889	1.667	1.000	1.667	1.111	1.222	0.333
No. LComm Alleles (<=25%)	2.222	1.778	1.444	1.111	1.889	2.333	2.444	3.111	1.889
No. LComm Alleles (<=50%)	6.111	4.222	4.556	3.444	4.778	5.556	5.333	6.333	5.222

Tab.31 Mean of allelic patterns across populations. Na=number of different alleles, Na (Freq >= 5%) = Number of Different Alleles with a Frequency >= 5%, Ne = Number of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), No. Private Alleles = Number of Alleles Unique to a Single Population, No. LComm Alleles (<=25%) = Number of Locally Common Alleles (Freq. >= 5%) Found in 25% or Fewer Populations, No. LComm Alleles (<=50%) = Number of Locally Common Alleles (Freq. >= 5%) Found in 50% or Fewer Populations. GENAIEX 6.2

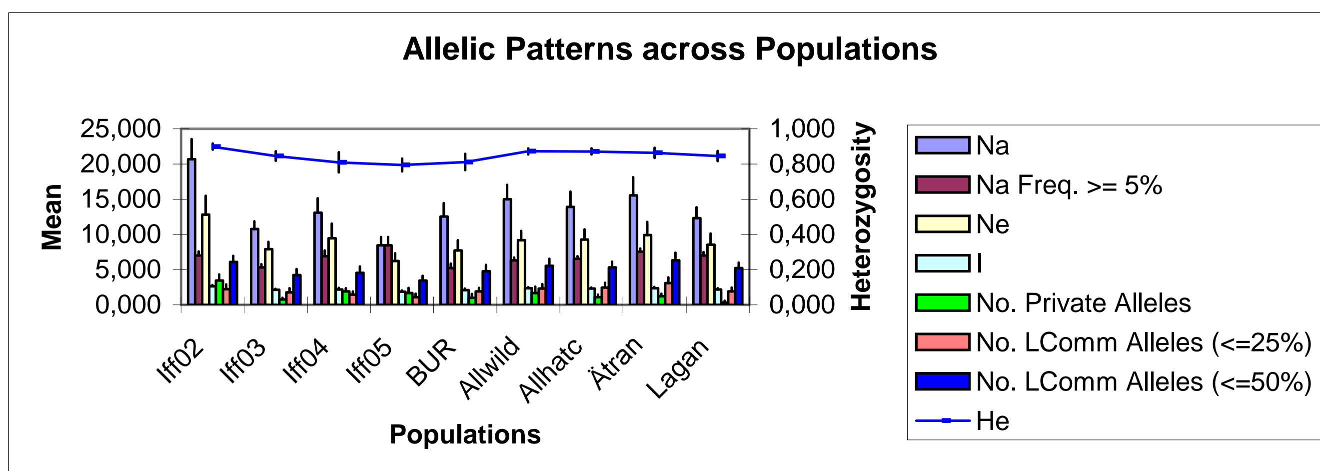


Fig.17 Allelic patterns across populations (s. Tab.30) GENAIEX 6.2

Scenario 2:

mean number of private and common alleles is shown in table 32 and their graphical distribution in Fig.18.

The question still difficult to solve is if the Iffezheim “population” is a wild one or the result of numerous reintroduction into the Rhine river system throughout the years.

Mean Allelic Patterns Across Populations

Mean values						
Population	**Ifftot**	**BUR**	**Allwild**	**Allhatc**	**Ätran**	**Lagan**
Na	30.111	12.556	15.000	13.889	15.556	12.333
Na Freq. >= 5%	5.111	5.222	6.333	6.556	7.556	7.000
Ne	15.817	7.708	9.176	9.284	9.918	8.532
I	2.841	2.101	2.352	2.309	2.357	2.204
No. Private Alleles	11.222	1.000	1.667	1.111	1.222	0.333
No. LComm Alleles (<=25%)	0.000	0.000	0.000	0.000	0.000	0.000
No. LComm Alleles (<=50%)	5.111	4.000	4.778	4.667	4.556	4.444

Tab.32 Mean of allelic patterns across populations. Na=number of different alleles, Na (Freq >= 5%) = Number of Different Alleles with a Frequency >= 5%, Ne = Number of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), No. Private Alleles = Number of Alleles Unique to a Single Population, No. LComm Alleles (<=25%) = Number of Locally Common Alleles (Freq. >= 5%) Found in 25% or Fewer Populations, No. LComm Alleles (<=50%) = Number of Locally Common Alleles (Freq. >= 5%) Found in 50% or Fewer Populations. GENAIEX 6.2

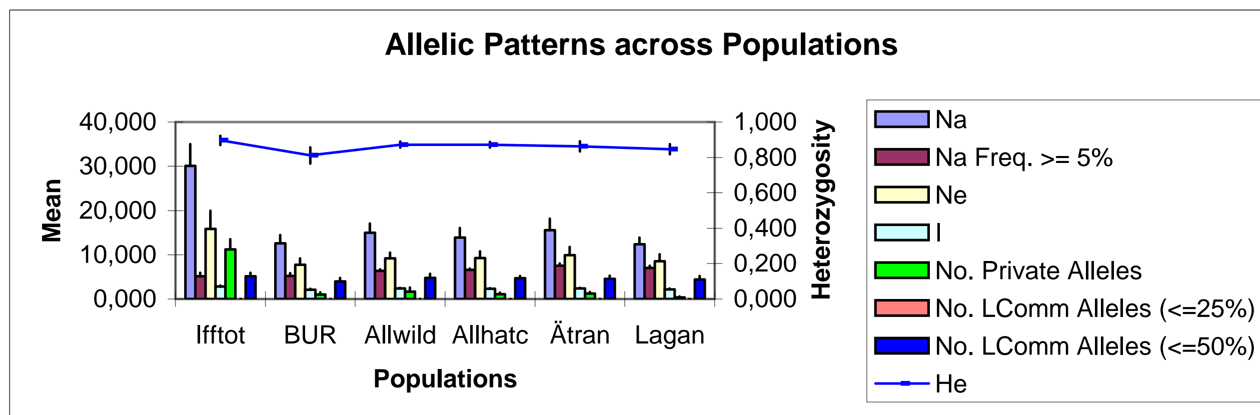


Fig.18 Allelic patterns across populations (s. Tab.31) GENAIEX 6.2

According to the data in the table and the graphic below (Tab.33/Fig.19), it is evident that not many common alleles have been found among the Iffezheim "population" and the stocking population. All the possible combinations have been taken into account (n!).

Common alleles	SSOSL85	SSOSL311	STR15	SSa171	SSa402*	SSa402**	SSa408	SSa202	SSa411	Tot Loci
Bur/Allhatc/Allwild/Ät/Lag	3	1	0	0	1	3	0	2	4	14
Bur/Allhatc/Allwild/Ät	0	2	0	0	1	0	0	0	0	3
Bur//Allhatc/Allwild/Lag	1	0	0	2	1	0	0	0	1	5
Allhatc/Allwild/Ät/Lag	1	1	0	1	1	0	0	0	0	4
Bur/Allhatc/Ät/Lag	0	0	0	0	1	1	0	0	0	2
Bur/Allwild/Ät/Lag	2	2	0	2	1	1	0	1	1	10
Allhatc/Allwild/Lag	0	1	0	1	0	2	0	0	0	4
Allhatc/Allwild/Ät	0	1	1	2	1	1	0	0	0	6
Bur/Allhatc/Allwild	1	1	0	0	0	0	1	1	0	4
Bur/Allhatc/Lag	0	0	0	0	0	0	0	1	0	1

Tab.33 presence of common alleles among Iffezheim and all the possible combinations of stocking populations

common alleles	SSOSL85	SSOSL311	STR15	SSa171	SSa402*	SSa402**	SSa408	SSa202	SSa411	tot Loci
Allhatc/Ät/Lag	1	0	0	1	4	0	1	0	0	7
Bur/Allwild/Lag	0	0	0	0	0	1	1	1	0	3
Bur/Allhatc/Ät	0	1	0	0	0	0	1	0	1	3
Allwild/Ät/Lag	1	0	0	1	0	0	0	1	0	3
Bur/Allwild/Ät	1	0	0	1	0	0	0	1	1	4
All hat/All wil	0	0	0	4	0	3	3	0	0	10
Bur/Ät/Lag	0	0	0	0	0	0	0	1	0	1
Allhatc/Ät	0	0	0	0	0	0	0	0	0	0
Allhatc/Lag	0	0	0	2	0	0	0	0	0	2
Bur/Allhatc	1	1	0	1	0	0	1	1	0	5
Allwild/Lag	0	0	0	0	0	0	1	0	0	1
Allwild/Ät	1	1	0	0	0	0	0	1	0	3
Bur/Allwild	0	0	0	0	0	0	1	0	0	1
Ät/Lag	0	6	0	1	1	0	4	0	0	12
Bur/Ät	3	0	0	1	0	0	0	1	0	5
Bur/Lag	1	0	0	0	0	0	0	2	0	3
Allhatc	0	0	0	3	0	0	4	2	0	10
Allwild	1	0	0	0	0	3	2	1	0	7
Bur	1	1	0	0	1	0	3	0	0	6
Ät	1	3	1	2	0	0	10	0	0	17
Lag	1	1	0	0	0	1	3	2	0	8

Tab.33 (continued)

From the analysis of the previous data, it is likely to assess that the Iffezheim cohort has a different origin or, even more likely, a mix-stocking origin with a relevant component of “Rhine” genotype.

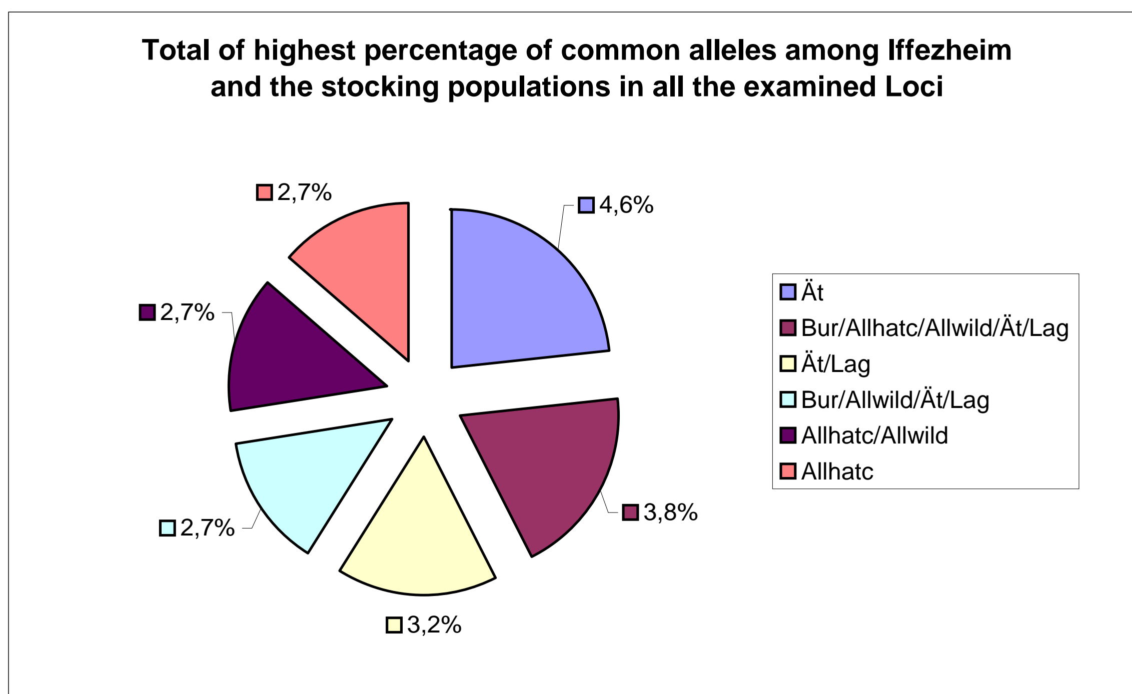


Fig.19 Highest percentage of common alleles among Iffezheim and stocking populations

The test for genotypic disequilibrium for each pair of 9 microsatellites loci over all populations showed that eleven of the 360 comparisons were significant ($P<0.05$). After Bonferroni correction for multiple tests, four combinations were significant at $P<0.0002$.

3.5 Population structure

Clear genic and genotypic differentiation was revealed among the studied populations. Heterogeneity in allele frequencies was highly significant for all the loci (Fisher's method, d.f. =18 P<0.0001).

Pairwise Fst values for the usual two scenarios ranged from 0.012 for the assumed Iffezheim (from now on called "Rhine population"/Germany) populations to 0.081-0.111 among Burrishoole (Ireland) population and the Rhine population (Tab.34a/34b). Genetic differentiation is more pronounced between Rhine population and the other referees populations. The populations from the same geographical origin, Allwild/Allhatc, Ätran/Lagan are also much more closely related as expected.

The Burrishoole population is the most different one clustering as an out-group.

Applying the PCA (Principal coordinates analysis) to the Fst results, the internal structure of analysed populations was clearly showen (Fig.20a/20b).

Scenario 1:

	Iff03	Iff04	Iff05	BUR	Allwild	Allhatc	Ätran	Lagan
Iff02	**0.021**	**0.034**	**0.025**	0.081	0.052	0.053	**0.042**	**0.042**
Iff03		**0.026**	**0.035**	0.085	0.049	0.065	0.067	0.067
Iff04			**0.012**	0.105	0.072	0.091	0.092	0.080
Iff05				0.111	0.079	0.084	0.091	0.085
BUR					0.076	0.082	0.096	0.086
Allwild						0.048	0.055	0.051
Allhatc							0.065	0.051
Ätran								**0.034**

Tab34a Pairwise Fst between the sampled populations GenAlEX 6.2

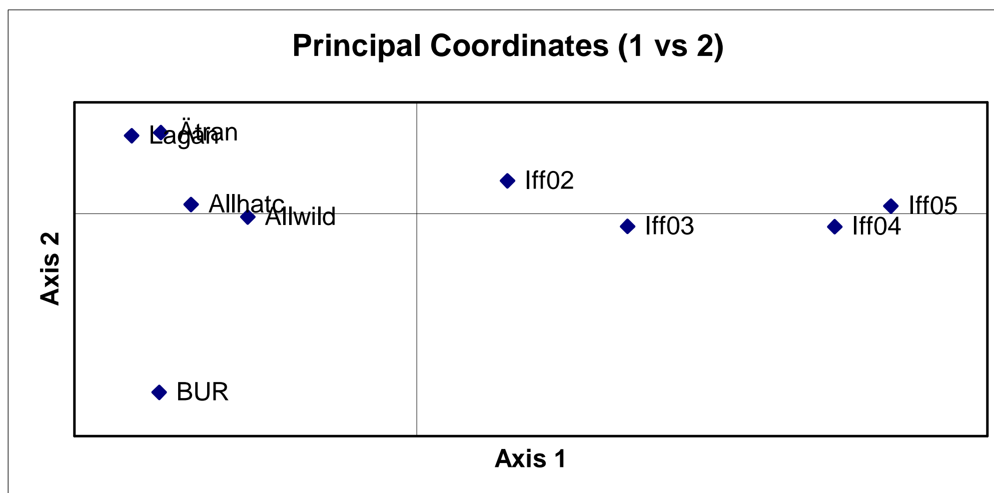


Fig.20a PCA of Fst values. The first axis represents the greatest variance by any projection of the data called the first principal component; the second axis is the second greatest variance. GenAlEX 6.2

Scenario 2:

	BUR	Allwild	Allhatc	Ätran	Lagan
Ifftot	0.07944	0.05145	0.05879	0.05475	0.05148
BUR		0.07632	0.08249	0.09582	0.08617
Allwild			0.04805	0.05526	0.05114
Allhatc				0.06503	0.05069
Ätran					0.03435

Tab.34b Pairwise Fst between the sampled populations GenAIEX 6.2

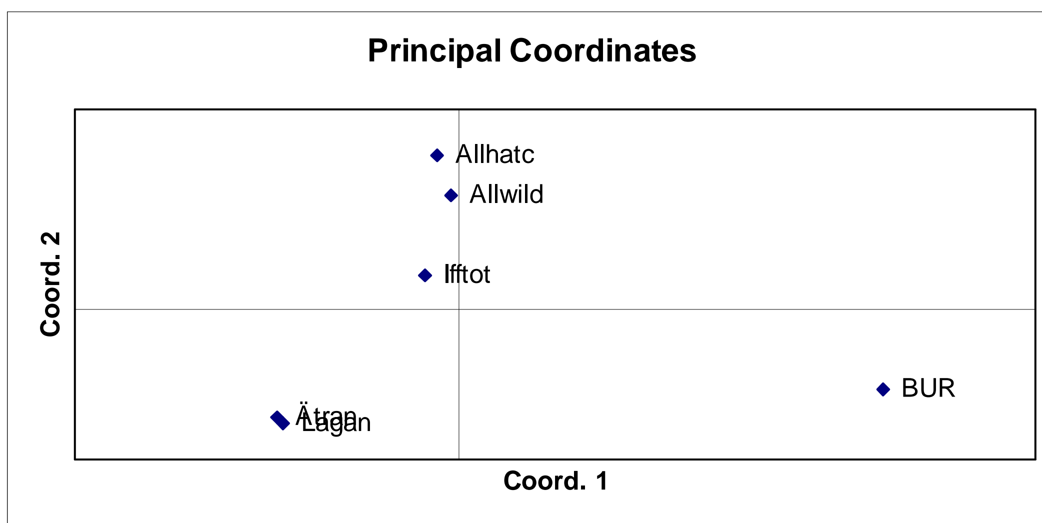


Fig.20b PCA of Fst values. The first axis represents the greatest variance by any projection of the data called the first principal component; the second axis is the second greatest variance. GenAIEX 6.2

Long (1986) and Smouse and Long (1988) have shown that the "trace" of the AFC (Factor analysis of correspondences) can be assimilated to the estimator of Fst. Robertson Hill and Guinand (1996) showed that in this case the values of inertia along each axis could be regarded as combinations of linear values of Fst monolocus.

The image below shows the internal structure of the studied populations applying the AFC analysis (Genetix 4.0.5). Correspondences between diploid genotypes are graphically depicted in 3D.

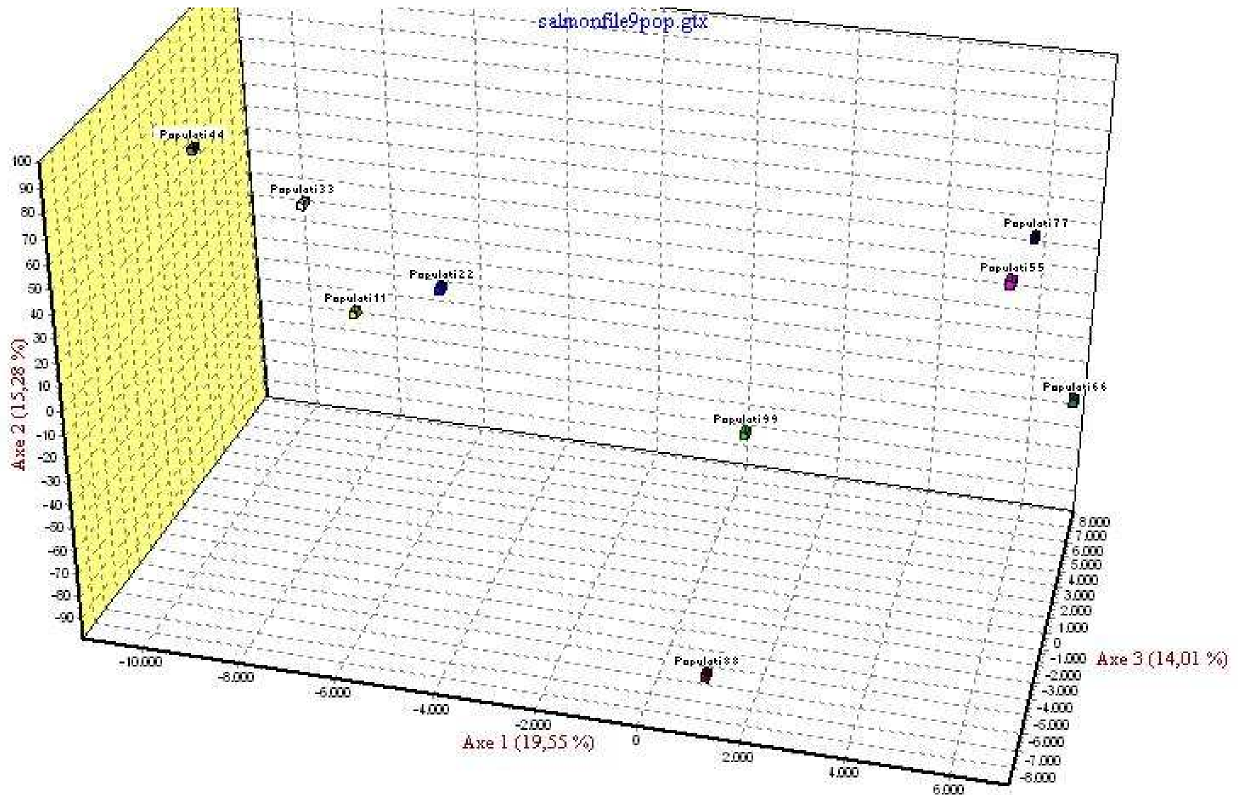


Fig.21 AFC analysis. Genetix 4.0.5 Populations 11-22-33-44 represent Iff02-Iff03-Iff04-Iff05, Population 55, 66, 77, 88, 99 are respectively BUR, Allwild, Allhatc, Ätran and Lagan

Also in this analysis the Rhine populations cluster together and the other populations cluster according to their geographical origins.

Analysis of molecular variance (AMOVA) showed that the highest percentage of molecular variance for genotypic distance was displayed within populations (Fig.22) and for allelic distance based on F-statistic within individuals (Fig.23).

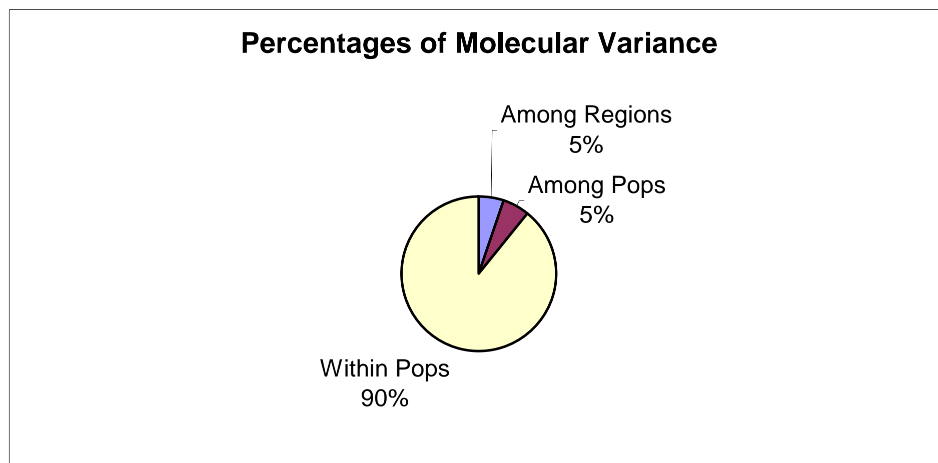


Fig.22 Input as Codominant Genotypic Distance Matrix for Calculation of PhiPT (PhiPT = (AP + AR) / (WP + AP + AR) = (AP + AR) / TOT in which AR = Estimated Variance. Among Regions, AP = Est. Var. Among Pops, WP = Est. Var. Within Pops)

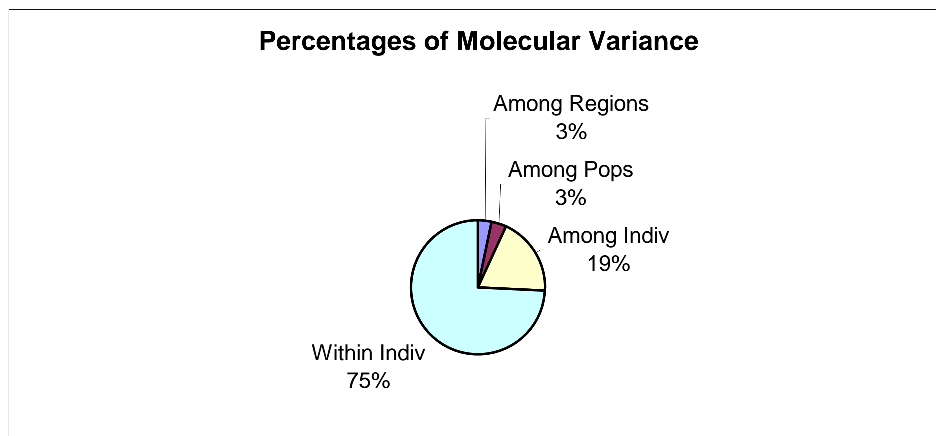


Fig.23 Input as Allelic Distance Matrix for F-Statistics Analysis. Probability, P(rand>=data), for Frt, Fsr, Fst, Fis and Fit is based on permutation across the full data set

Amova results indicated that, even if the variance between Rhine subpopulations and the other referee subpopulations was significant (F_{st}<0.05), it was not as significant as the variance within populations and individuals. This could be well explained by the high polymorphism of the examined loci and by the moderate rate of panmixis. All the Fst values between Rhine subpopulations and the other ones ranged between 0.05-0.15.

Assignment result

Marked genetic differentiations were also supported by assignments tests. The 89% of the individuals were correctly assigned to their geographical native population (Tab. 35).

The lowest levels of correct assignment were found in the Lagan subpopulation, where one third of the individuals have been assigned to other subpopulations. This result was not completely unexpected, because this referee subpopulation was the only one caught in the wild, thus the risk to have a non "pure" subpopulation was high, while the other referees populations directly came from hatchery.

The only one incorrectly assigned individual of Allhatc was an Allwild, so this subpopulation could be considered completely correctly assigned because of the common geographical origin.

The other percentage of incorrect assignments, including the one referred to the Rhine subpopulation, was comparable.

Assignment results showed that the contribution of the stocking referee subpopulations to the Rhine assumed subpopulation had different percentage according to the analysed years. There was a high presence of Sweden individuals that probably won the competition with other stocking/wild subpopulations and had a more stabile reproductive

success in this area. However, data also showed that this contribution to the Rhine subpopulation was less significant than expected, and this evidence could support the initial idea that a possible own salmon subpopulation was present and more or less stabile in the Rhine.

Summary of Population Assignment Outcomes to 'Self' or 'Other' Population (With Leave One Out Option)			
Pop	**Self Pop**	**Other Pop**	**Percent of not correct assignment**
Ifftot	57	8	12.31%
BUR	21	1	4.55%
Allwild	22	3	12.00%
Allhatc	24	1	4.00%
Ätran	26	2	7.14%
Lagan	10	5	33.33%
Total	160	20	
Percent tot	89%	11%	

Tab.35 Populations assignment

Considering the Rhine subpopulation, only eight individuals could not be identified as belonging to that subpopulation throughout the four sampled years. The graphic below (Fig.24) shows in percentage how those 8 individuals could be assigned to the other referee subpopulations.

In 2002, five out of 30 sampled individuals could be assigned to different origin, 1 of BUR, 1 of Allwild, 2 Lagan and 1 Ätran.

In 2003 only one individual out of 11 was assigned to Ätran subpopulation.

In 2004 two individuals out of 15 were assigned to Ätran and to Lagan, and in 2005 all of the nine individuals were assigned to the Rhine subpopulation.

A complete overview of the assignment result is given in Appendix 3, Tab.36.

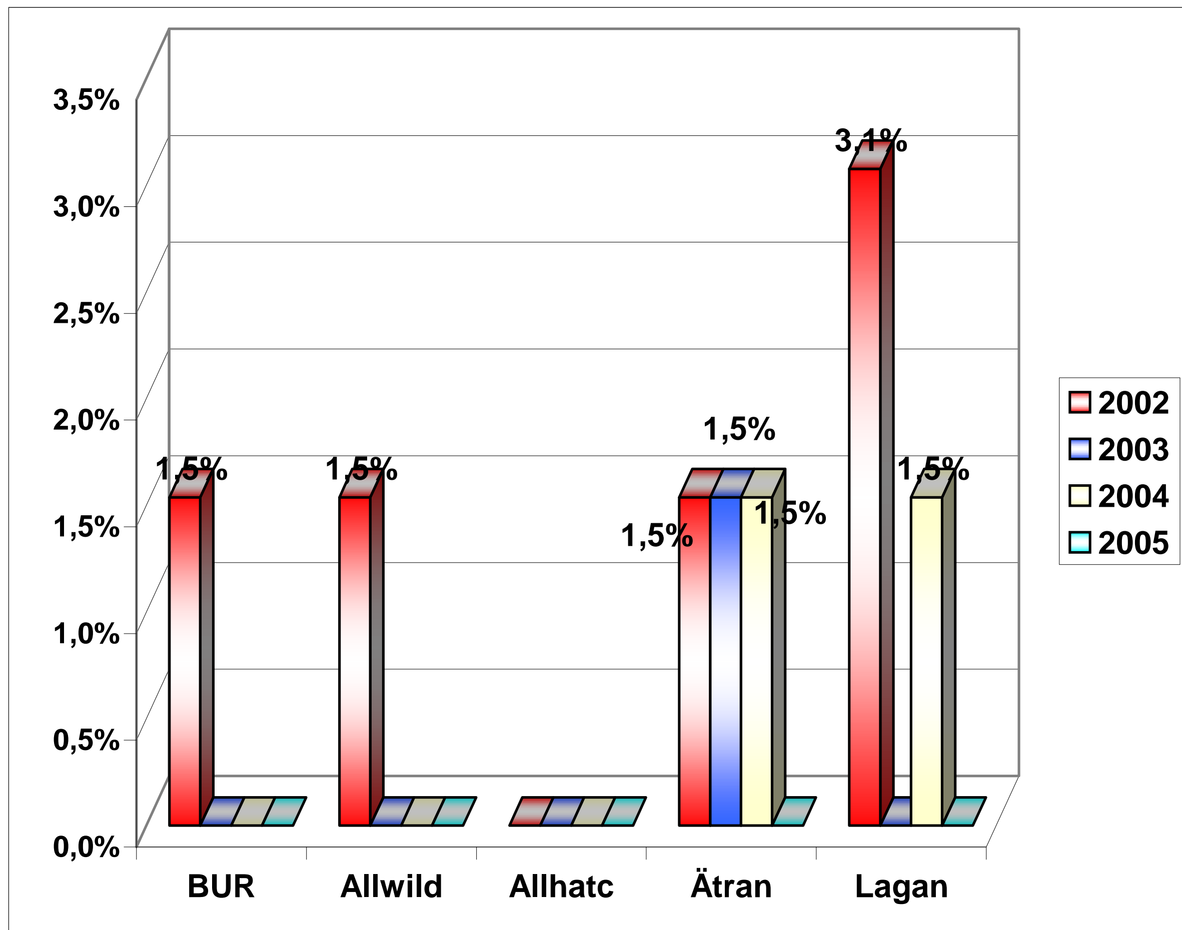


Fig. 24 Percentage of assignment of the 8 non-Iffezheim individuals among the other referee subpopulations

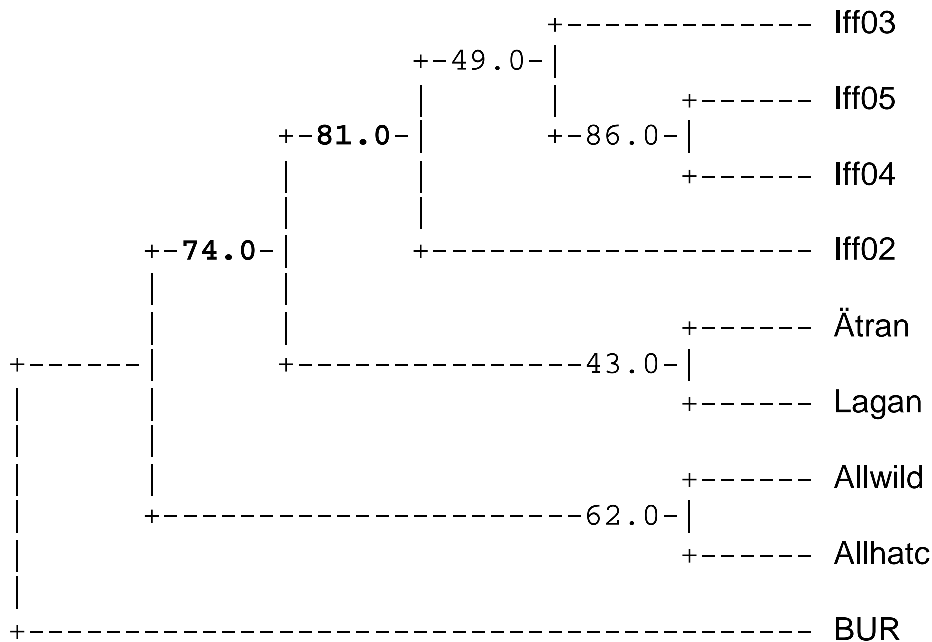
The neighbour-joining trees, giving the Burrishoole subpopulation as outgroup, showed a quite defined structure with two main clusters: Iffezheim-broodlings.

In this case, the highest robustness was given by the Rhine subpopulation, although, the individuals sampled in 2003 had a non-significant relation with the individuals sampled in the other years. This is probably because of the little number of individuals sampled in that year.

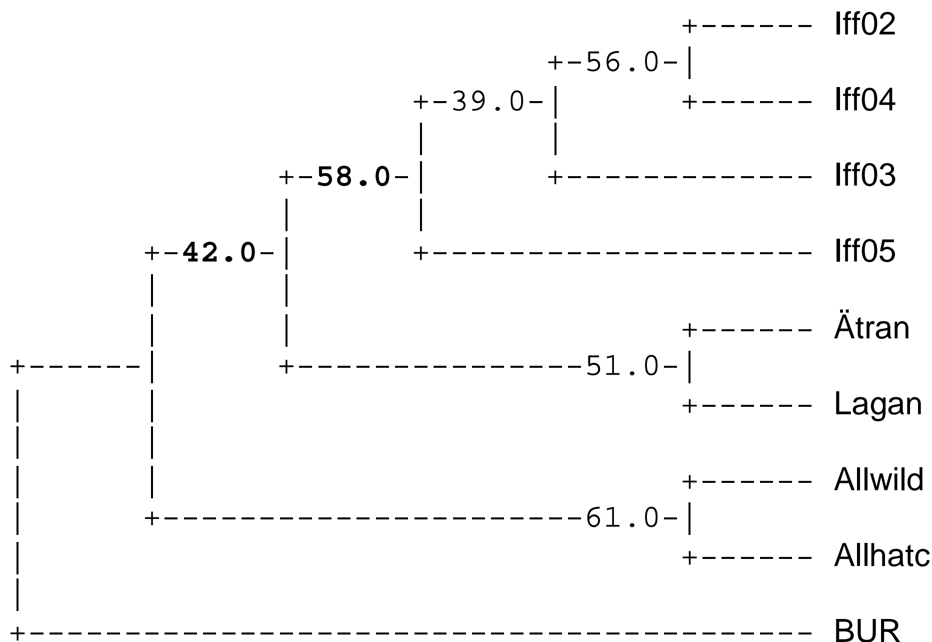
However, the “broodlings cluster” was, as expected, much weaker and with less similarities than the “Iffezheim cluster”.

Both Neighbour-joining and UPGMA methods have been applied performing Cavalli-Sforza and Nei’s genetic distance, obtaining more or less the same clustering.

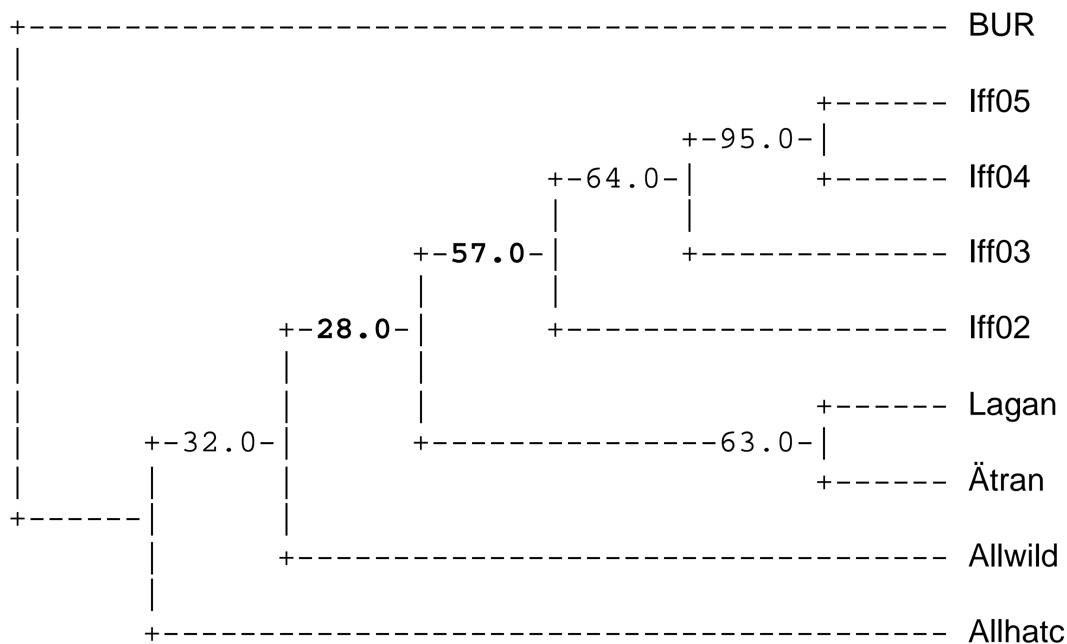
Consensus neighbour-joining method tree, Cavalli-Sforza's genetic distance (DC):



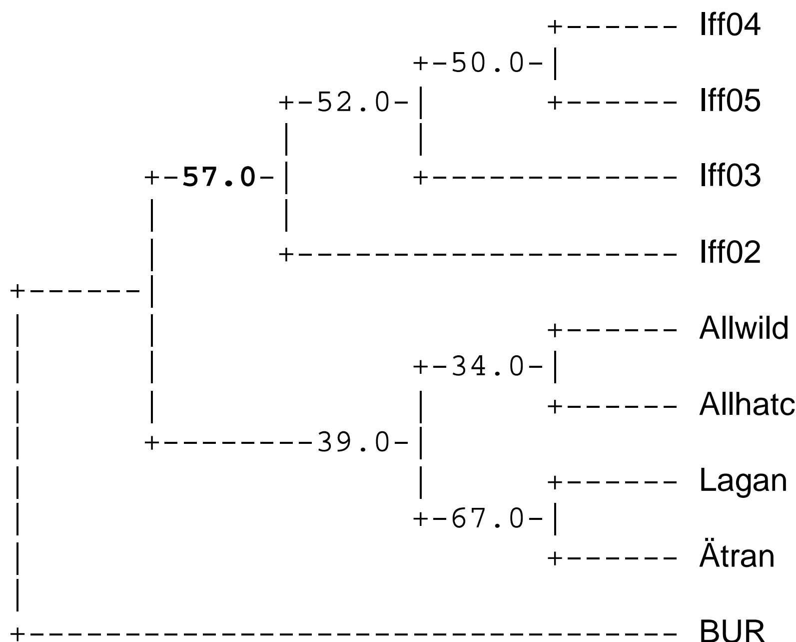
Consensus UPGMA method tree, Cavalli-Sforza's genetic distance (DC):



Consensus tree, neighbour-joining method, Nei’s genetic distance (DA):



Consensus tree UPGMA method, Nei’s genetic distance (DA):



The numbers on the branches indicate the number of times the partition of the species into the two sets which are separated by that branch occurred among the trees, out of 100 trees.

The representation of the phenograms clearly depicted a situation where the Rhine subpopulations always cluster together and the other referee subpopulations cluster according to their geographical origin.

The Swedish individuals seem to be the closest to the Rhine subpopulation.

The Structure software showed the highest posterior probability for K=2 when performed with the admixture model assumption, grouping together Rhine (Iffezheim)/Swedish (Ätran, Lagan) individuals and French (Allier)/Irish (Burrishoole) individuals (Fig.24).

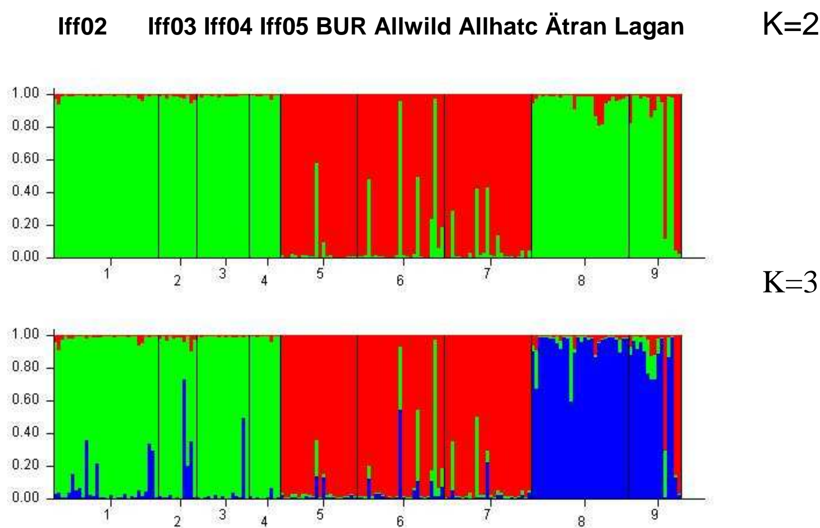


Fig.25 Result of the assignment test with Structure 2.1 admixture model. Each individual is represented by a vertical column, subdivided into k coloured segments according to the estimated membership to the k fractions.

For k=3 assuming admixture model, the Rhine subpopulation showed a major fraction (green) which could be rarely found in the other individuals except for one Allwild individual that shared almost completely the same Rhine alleles, and a little fraction of Swedish alleles (blue).

Irish and French individuals shared more or less the same alleles pattern (red).

Applying the no admixture model, that is the most informative for the present study, the structure changed revealing substructure not clearly visible in the previous model. The highest posterior probability was K=2 whit a high value was observed also for K=3-5 (Fig.26).

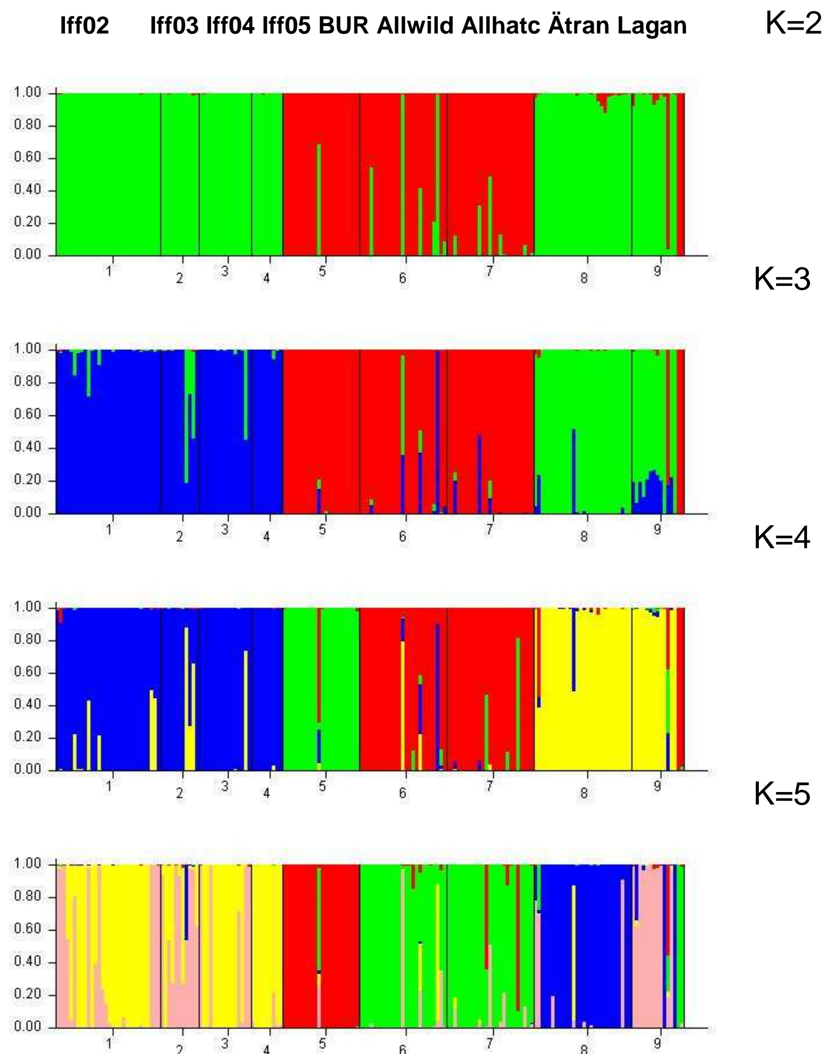


Fig.26 Result of the assignment test with Structure 2.1 admixture model. Each individual is represented by a vertical column, subdivided into k coloured segments according to the estimated membership to the k fractions.

In this case, however, K=4 showed the most informative structure for this study expectations, where the Rhine individuals showed an almost unique blue fraction, with 6 individuals displaying a little fraction of Swedish alleles, and 2 individuals an almost complete Swedish pattern. The other individuals were almost completely assigned to their geographical origin, Ireland, France and Sweden and K=5 showed a quite similar situation with more Rhine and Lagan individuals sharing the same genetic pattern.

The pattern of genetic composition was similar to the clustering analysis and in some case less complex. (K=2).

The most reliable structure was comprehensive of 4 salmon subpopulations, where the different geographical origins are displayed with a more or less unique genetic pattern.

This result confirms the reliability of the stocking station, because almost all the tested individuals caught in the fish hatcheries were “pure”, on the other hand, it encourages the hypothesis of a noticeable and stabile Rhine subpopulation.

3.6 *Isolation by distance*

Mantel tests detected significant associations with the IAM (Infinite allele model) model between both genetic and geographical distance (r2=0.0302, P=0.001) as Fig.27 shows.

These results indicate an isolation-by-distance pattern.

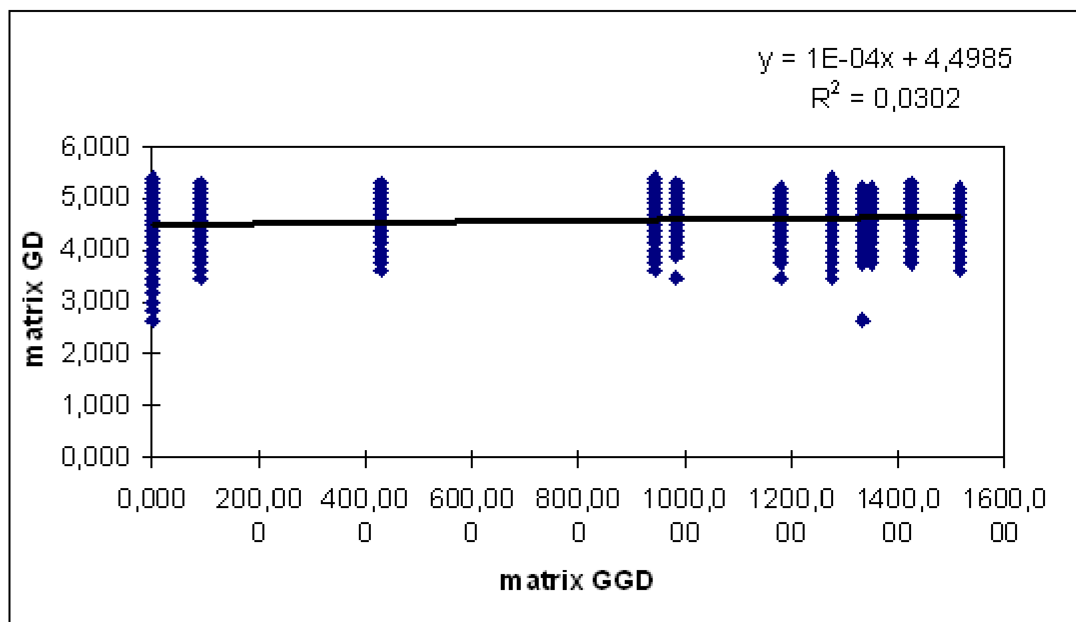


Fig.27 Mantel Results for matrix of geographic distance (GGD) vs matrix of genotypic distance (GD)

After a bottleneck event, the observed number of alleles is lower than the number predicted from the Hardy-Weinberg heterozygosity, under the assumption that population is at mutation-drift equilibrium (i.e. its effective size has remained constant in the past) (Nei et al. 1975, Watterson 1984). Populations after a recent bottleneck should have significant heterozygosity excess if compared to that based on the observed number of alleles. Thus, bottlenecks can be studied by comparing expected gene diversities (based on number of alleles) and observed gene diversities (Watterson 1978, 1986).

Iff04, Ätran and Lagan subpopulations showed a non-significant heterozygosity excess even under the IAM model.

Under the SMM (stepwise mutation model) and TPM (two-phase model), none of the other subpopulations showed evidence of recent bottleneck (p>0.05) (Tab.37a). Thus, populations have probably reached new lower mutation-drift equilibrium after the bottlenecks, but they have widely spread after those events (Maruyama and Fuerst 1984).

At equilibrium, SMM and TPM should have reasonable contiguous allelic states. If gaps that follow the bottleneck are progressively “filled in” by mutations, there can be a transient excess of alleles (i.e. deficiency of heterozygosity) (Cornuet and Luikart 1996). Luikart *et al.* (1998) concluded in their simulation studies that a bottleneck size likely to be detectable is approximately N_e = 20 and a power of the test depends on the generations since bottleneck happened. Only Iff05 subpopulation showed heterozygosity excess under the IAM and TPM, but this could be due to the little numbers of sampled individuals.
Evaluating the scenario 1, most of the Rhine subpopulations do not fill this criterion and the bottleneck might be so recent that there have not been enough generations to show any traces of bottleneck.

Populations	IAM	TPM	SMM
Iff02	0.010	0.326	0.997
Iff03	0.019	0.326	0.990
Iff04	**0.150**	0.500	0.976
Iff05	0.007	0.019	0.213
BUR	0.014	0.285	0.993
Allwild	0.014	0.326	0.995
Allhatc	0.003	0.064	0.993
Ätran	**0.102**	0.590	0.999
Lagan	**0.180**	0.410	0.981

Tab.37a Wilcoxon rank test (probability of heterozygosity excess) for null hypothesis under three microsatellite evolution models (scenario 1)

When Rhine subpopulations were considered as a whole (scenario 2/Tab.37b), the bottleneck size criterion was matched and no recent bottlenecks were detected.

Population	IAM	TPM	SMM
Iff tot	0.007	0.367	0.997

Tab.37b Wilcoxon rank test for scenario 2

Discrepancy between the IAM test and the TPM and SMM tests comes out as a consequence of the different heterozygosity expectations at mutation equilibrium (Shriver et al. 1993; Valdes et al. 1993; Luikart and Cornuet 1997b). Given that microsatellite mutation is thought to occur largely through the stepwise process, a combination of the SMM and the IAM is expected to provide the best estimate of heterozygosity equilibrium for the bottleneck analysis (Di Rienzo et al., 1994). The absence of heterozygosity excess, using both the strict SMM and the mixed TPM, suggests that the contemporary population is at mutation-drift equilibrium.

The Mode-shift indicator test was also used as a second method to detect potential bottlenecks, as the nonbottleneck populations that are close to mutation-drift equilibrium are expected to have a large proportion of alleles with low frequency. This test discriminates many bottle necked populations from stable populations (Luikart 1997; Luikart and Cornuet 1997). A graphical representation using allelic class and proportion of alleles showed a normal 'L' shaped distribution (Fig.28). This distribution clearly reinforces the result that the studied subpopulations had not experienced a recent bottleneck.

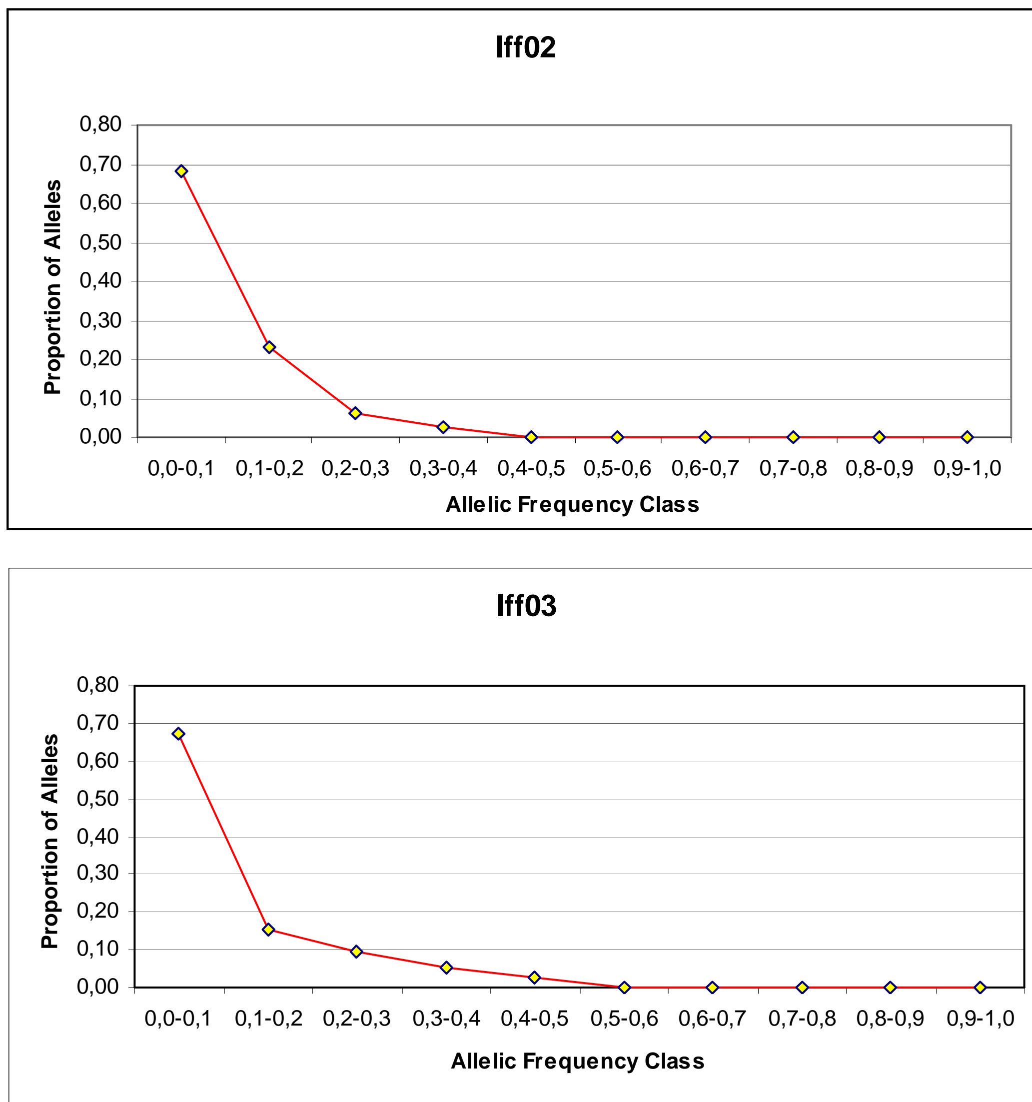
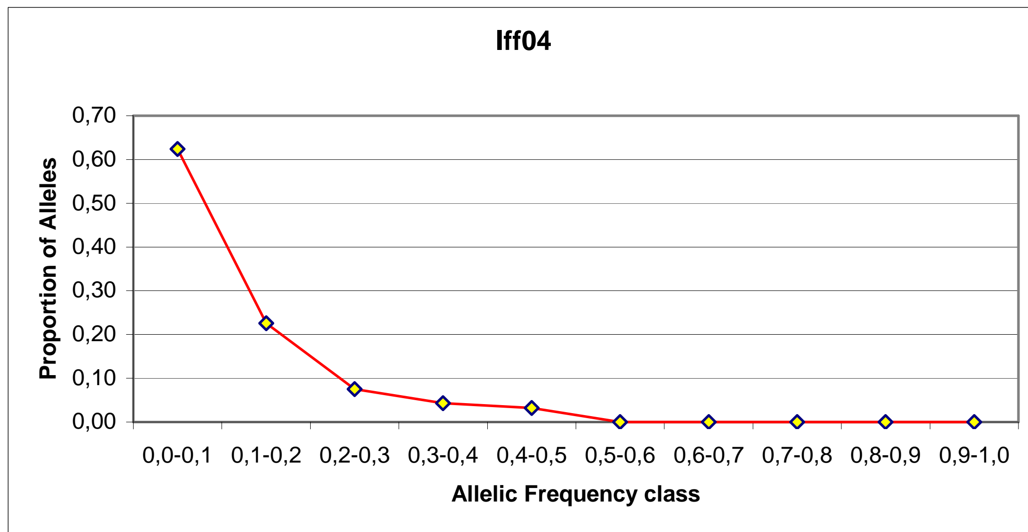
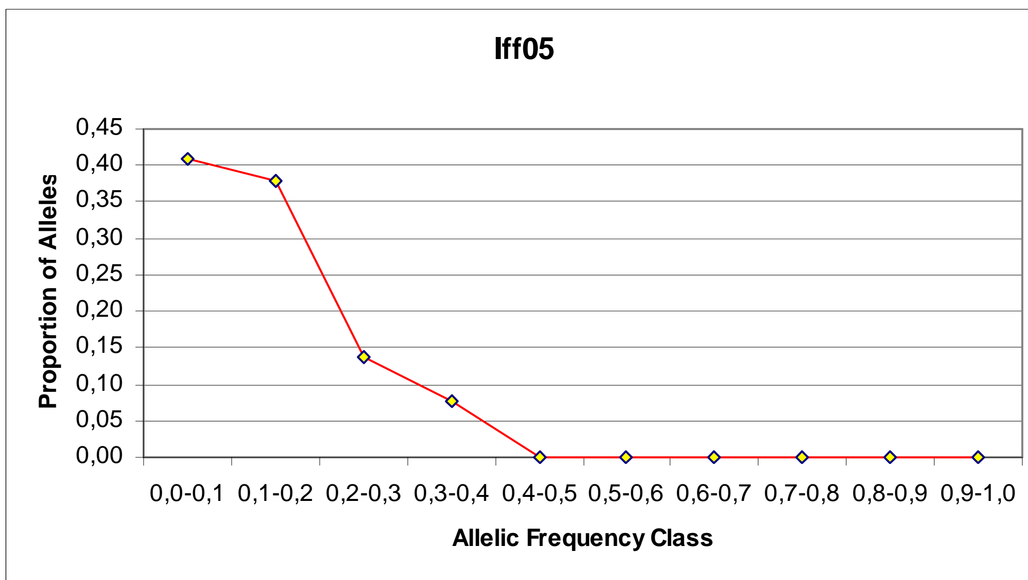


Fig.28 Graphic representation of proportion of alleles and their distribution in salmon subpopulations





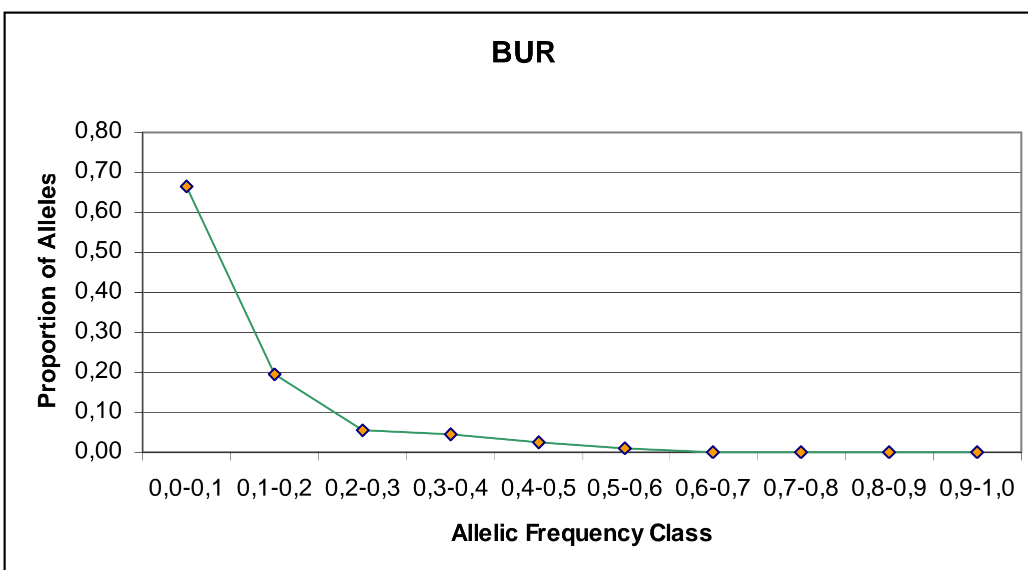
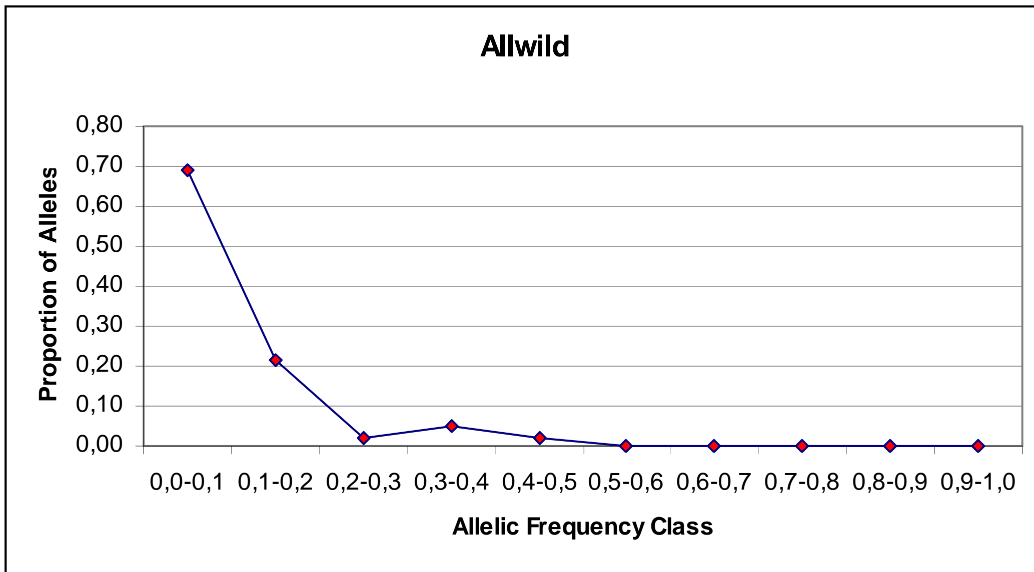
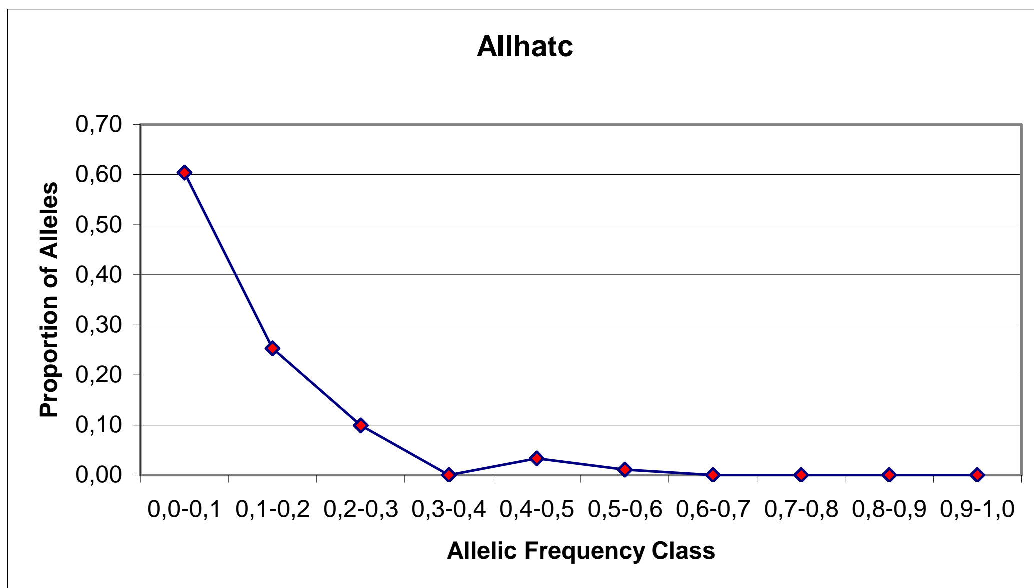


Fig.28 (continued)





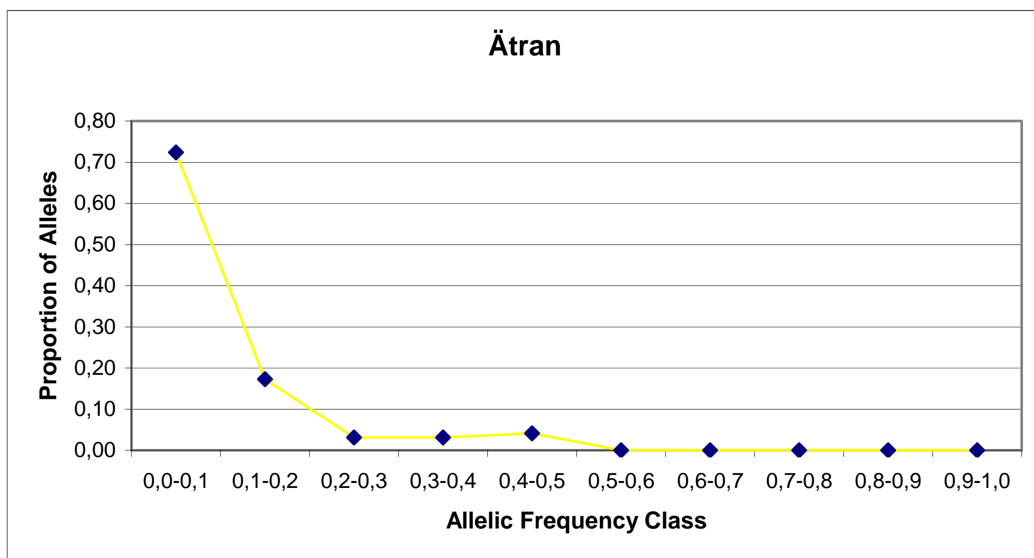


Fig.28 (continued)

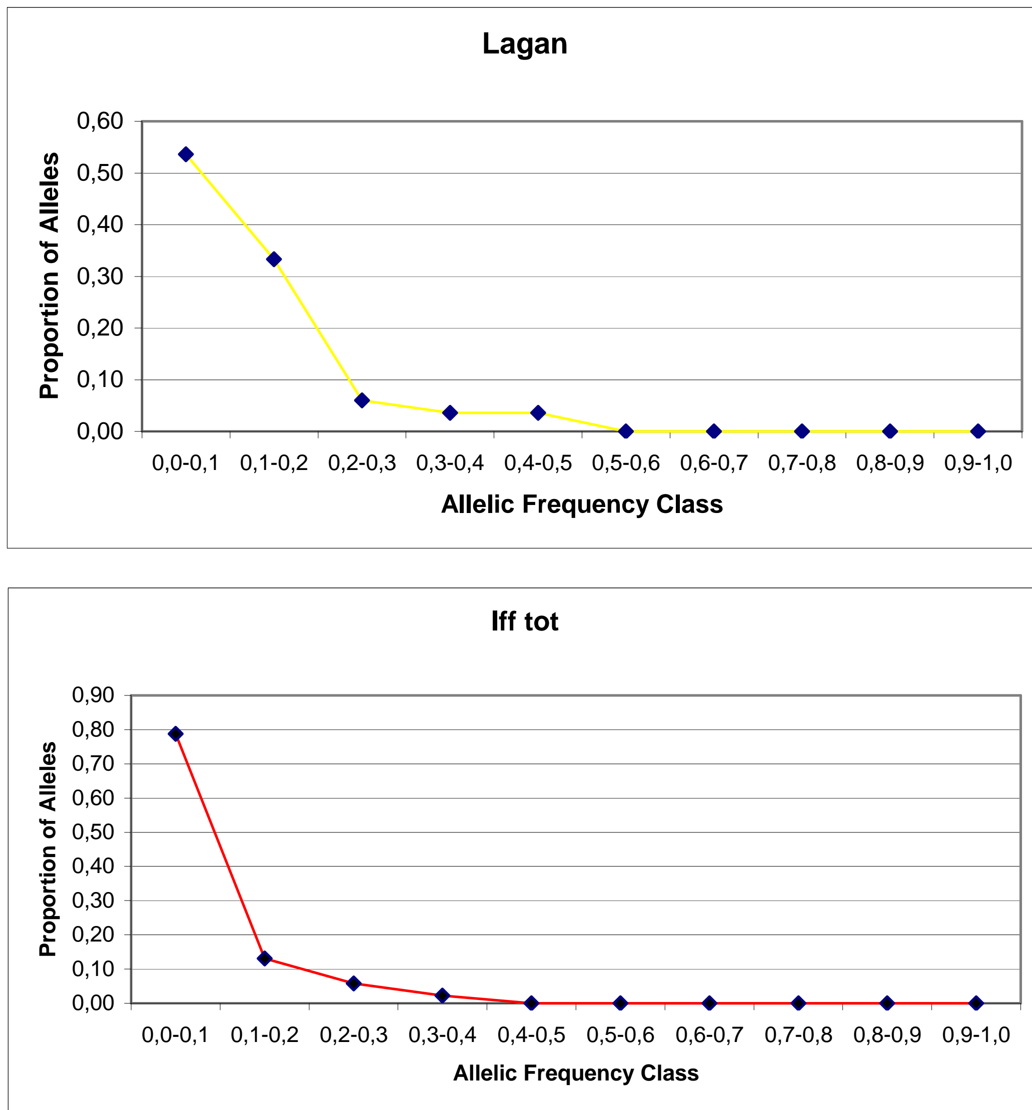


Fig.28 (continued)

3.7 Scale reading

By reading the Atlantic salmon scale (Fig.29) the following results have been obtained:

- they spent at least two years in the river from their birth before entering the sea, the great part of the Iffezheim salmons were multi-winter returners (Fig.30),
- they have spent at least 4 years in the sea before coming back in the Rhine, but the majority of the sampled individuals came back after 1-2 years (Fig.31).

Table 38 is a summary of the freshwater/marine permanence and age determination inferred by the scale reading.

Fig.29 Salmon scales with clearly distinct marine/freshwater stage, winter and summer bands

By comparison of the assignment test results (GenoAssign 1.0), a prospect of the age and migration behaviour of the referee stocking subpopulations has been evaluated (Tab.39). The highest percentage of the sampled Rhine individuals assigned to the other referee populations displayed more or less the same migration behaviour of the Rhine individuals. They came back to the river after at least 3 years spent in the sea, but the majority were 1-winter returners.

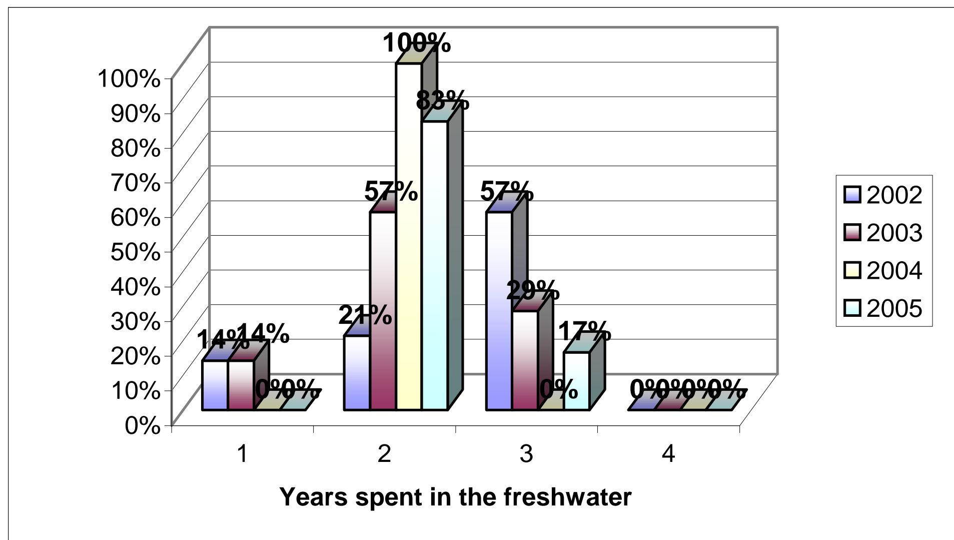


Fig.30 Percentage of Rhine individuals with different freshwater permanence sampled in the 4 analysed years. The percentage has been calculated considering only the individuals with unambiguous age determination

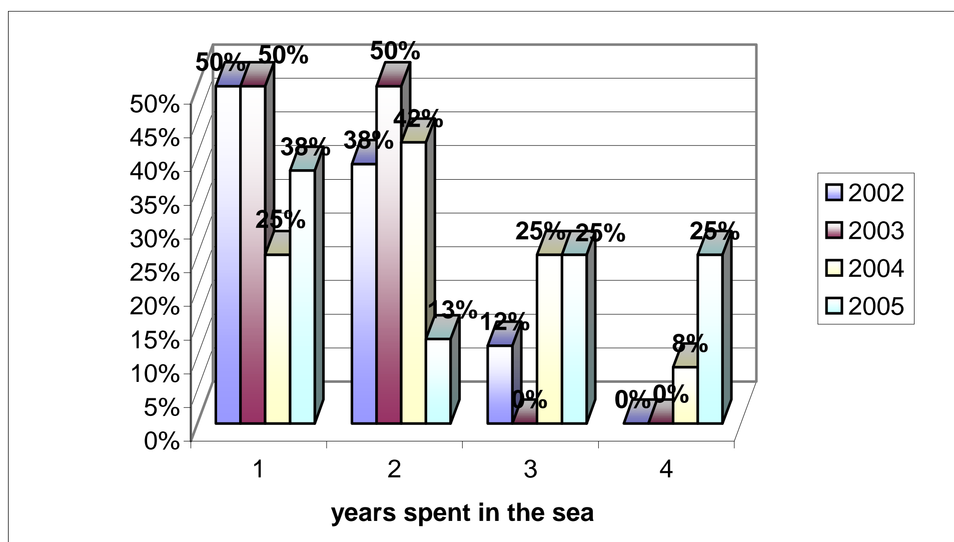


Fig.31 Percentage of Rhine individuals with different marine permanence sampled in the 4 analysed years. The percentage has been calculated considering only the individuals with unambiguous age determination

A graphical (Fig.32) overview, comprehensive of all the Rhine individuals, showed that the highest number of individuals after a freshwater permanence of 2 years migrated as smolt and stayed into the sea for other 2 years before coming back to the Rhine to spawn.

These results support the hypothesis that the Rhine individuals behaved as multiwinter salmon, in 2004/2005 the percentage of individuals which stayed into the sea for 3-4 years was much higher than in 2002/2003.

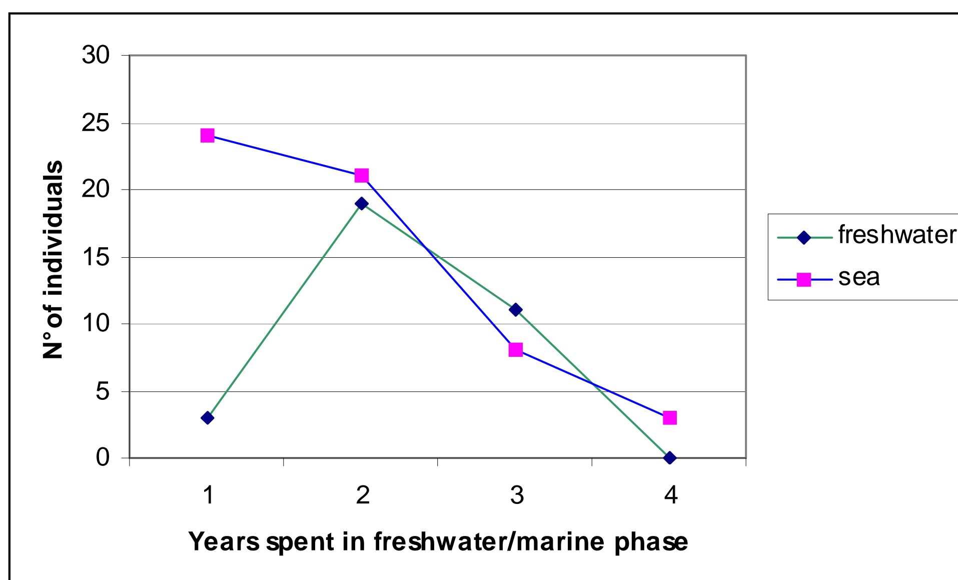


Fig.32 Estimated permanence of Rhine individuals during the freshwater/marine phase

	Freshwater permanence	Marine permanence	Sampling date	Possible year of birth	Possible entry into the sea
Iff 2002					
Fish					
18	3	2	25/07/2002	1996	2000
20	2	1	26/07/2002	1998	2001
29	?	1	29/07/2002	?	2001
33	1	1	29/07/2002	?	2001
40	2	2	02/08/2002	1997	2000
48	2	2	04/08/2002	1997?	2000
57	2?	2	07/08/2002	1997?	2000
58	?	1	08/08/2002	?	2001
62	3	2	10/08/2002	1996	2000
65	no scales		17/08/2002	?	?
66	?	3	18/08/2002	?	1999
69	?	1	20/08/2002	?	2001
70	1?	1	20/08/2002	?	2001
72	3	2	21/08/2002	1996?	2000
80	3	1	30/08/2002	1997?	2001
81	3	1	02/09/2002	1998?	2001
83	3	1	13/09/2002	1996/1997	2001
85	3	1	15/09/2002	1998?	2001
89	?	?	29/09/2002	?	?
90	?	1	29/09/2002	?	2001
93	3?	1	02/10/2002	?	2001
95	3	2	03/10/2002	1997	2000
96	?	?	04/10/2002	?	?
99	no scales		12/10/2002	?	?

Tab.38 Age determination and freshwater/marine permanence inferred by scale reading, of the Rhine individuals

	Freshwater permanence	Marine permanence	Sampling date	Possible year of birth	Possible entry into the sea
100	4?	2	16/10/2002	1997	2000
106	1?	2	18/10/2002	1997?	2000
114	?	2	01/11/2002	?	2000
116	?	1	01/11/2002	?	2001
119	?	3	03/11/2002	1996?	1999
Iff. 2003					
1	2	2	06/07/2003	1998?	2001
4	?	2	14/09/2003	?	2001
6	3	1	16/09/2003	1998?	2002
10	1?	1	03/10/2003	1999	2002
11	2	1	04/10/2003	1998	2002
12	2	1	04/10/2003	1999	2002
19	2	1	16/10/2003	1999?	2002
21	1?	2	21/10/2003	1998?	2001
41	no scales		04/11/2003	?	?
48	3	2	11/11/2003	1997	2001
51	1	2	12/11/2003	?	2001
Iff. 2004					
5	1?	3	06/07/2004	1998?	2001
6	2	1	06/07/2004	1999?	2003
7	2	2	06/07/2004	1999	2002
Iff. 2004					
13	?	3	10/07/2004	?	2001
17	2	2	15/07/2004	1998?	2002
18	2	4	15/07/2004	1996?	2000
30	2	1	22/07/2004	2000	2003
36	?	1	25/07/2004	?	2003
37	2	3	27/07/2004	1997	2001
39	no scales		09/09/2004	?	?
41	no scales		04/10/2004	?	?
46	?	?	16/10/2004	?	?
48	2	2	17/10/2004	1998?	2002
52	3?	2	27/10/2004	1996	2002
53	2?	2	01/11/2004	1999	2002
Iff. 2005					
5	2	3	05/07/2005	1999	2002
14	2?	4	05/08/2005	?	2001
18	2	2	04/09/2005	?	2003
19	2	1	16/09/2005	2001	2004
20	3	?	20/09/2005	1998	2002/2003
21	2?	4	26/09/2005	1997	2001
22	2	1	29/09/2005	2001	2004
40	1?	3	16/11/2005	2000?	2002
46	2	1	28/11/2005	2001?	2004
50	1	3	04/08/2002	?	1999

Tab 38 (continued)

	Sea age	Allhatc	Allwild	Ätran	Lagan	BUR
2002	1	10	2,5	1	3	1,5
	2	3	5	0,5	1,5	1
	3	1	0	1	0	1
	4	0	0	0	0	0
2003	1	2	2,5	0,5	0,5	0,5
	2	2,5	0,5	0	0,5	2,5
	3	0	0	0	0	0
	4	0	0	0	0	0
2004	1	0,5	1,5	0	0	2,5
	2	0	4	0	0	1
	3	0	1	0	0	1
	4	0	1	0	0	0
2005	1	1	2	0	0	0
	2	0	0	0	0	0
	3	0	0	1	0	1
	4	0	0	0	0	2

Tab.39 Estimation of the sea permanence of the referees stocking subpopulations by assignment test results (GenoAssign 1.0)

Estimation results about the sea permanence of the referee subpopulations were comparable with the data given in literature (i.e. Piggins, 1980, Prouzet, 1990, McGinnity et al., 2003) and they displayed more or less the same migration behaviour of the Rhine individuals.

Assignment results by GenoAssign 1.0 software are given in Appendix 3, Tab.40.

4 DISCUSSION

The main aims of the present research could be summarised as follows:

- Assignment of the Atlantic salmon returners to the fish ladder at Iffezheim to the known populations used for salmon reintroduction in the Rhine
- Monitoring of the utility of different Atlantic salmon origins to recolonize the Rhine system
- Management of restocking and recolonization of the Atlantic salmon in the Rhine river system
- Sample aging

This study has combined classical lab work and lab analysis with relative new analysis and conception about how to consider a mixed stock-analysis, because this case study focused on the individual level and not on the classical level of population or groups.

Isoenzyme approach was not possible because at the bottom of this project there was the safety of the animals and to proceeds with this analysis the death of the animal would have been necessary.
This study showed again that GPI, one of the few enzymes working with blood, remains the faster and most informative method to recognize misidentification between brown trout and Atlantic salmon, and hybrids from the two species.

Taking into account the safety of the animals, I have proceeded with microsatellites analysis. This kind of approach brings about the problem of high numbers of alleles pro locus.

Thus, for a relative small sample a huge “Sampling-Bias” is to be taken into account.

Keeping in mind all the preceding assessments, significant and useful results have been achieved mainly following this kind of approach.

4.1 Genetic diversity and heterozygotes deficiency

This study showed a significant genetic diversity among the Rhine subpopulations and the one used for reintroduction. The expected heterozygosity is a little bit higher than the

observed one, even if this last ranged from 0.60 to 0.79, being thus completely comparable to other European studies (King et al 2001, Saisa et al 2005).

A diffuse deficiency in heterozygosity is evident in all the examined populations even the stocked ones but, however, the rate of genetic diversity is remarkable and supports the hypothesis already assumed by Grandjean et al. (2009) that a coordinated selection program in the progenitor choice can avoid the decrease in genetic diversity of stock populations.

The basis for heterozygote deficiency in populations has been theoretically and experimentally explored and it has been shown to be caused by inbreeding, by positive assortative mating, by pooling populations with different allele frequencies (the Wahlund effect) under-dominant selection. Heterozygote excess in populations is not as common and therefore it has not been as fully theoretically explored.

Overdominant selection favouring, associative overdominance (Nei, 1987), and negative assortative are common textbook explanations for observed heterozygote excess and are generally used to explain heterozygote excess in natural populations.

The Wahlund effect is probably in this case the most plausible reason for the heterozygotes deficiency caused by subpopulation structure. Namely, if two or more subpopulations have different allele frequencies, then the overall heterozygosity is reduced, even if the subpopulations themselves are in a HWE, as clearly showed by this study results, all the subpopulation are indeed under HWE.

The underlying reasons for this population subdivision could be geographic barriers to gene flow but this is not the case.

In this case study there are no geographical or physical barriers other than those of the origin of samples used for the reintroduction, but we could have secondary barriers as reproductive barrier, mating selection and reproductive success, some kind of “sympatric speciation”.

Plausible is a process of genetic drift in the subpopulations due to intensive annual salmon reintroduction in all the Rhine segments.

Genetic drift may cause alleles to disappear completely, and thereby reduce genetic variability.

Genetic drift is one of several evolutionary processes that lead to changes in allele frequencies over time. In contrast to natural selection, the changes due to genetic drift are

not driven by environmental or adaptive pressures, and may be beneficial, neutral, or detrimental to reproductive success.
The effect of genetic drift in this case study could be more relevant because of the small examined populations.
Despite heterozygotes deficiency by the bottleneck analysis, no evidence of recent reduction in population size has been observed in the Rhine subpopulation according to TPM (two-phase model) and SMM (stepwise mutation model).
The impact of reintroduction has not heavily influenced the structure of the Rhine subpopulation, neither a reduction in the number of alleles has been observed due to reintroduction programs.

By the assignment tests, indeed, it is clearly shown how the Rhine subpopulation shares only a small fraction of alleles with the hatchery ones and the Swedish alleles seem to be most representative even if the same consideration could be vice versa made, a small alleles fraction of Rhine alleles is present in the Sweden subpopulations.
Swedish, Irish for the great part, but also French individuals have been spreadly used in the last years for reintroduction in the Baden-Württemberg Rhine segment, in order to sustain the Atlantic salmon population but the first one has the great part of common alleles with the one of the Rhine, showing a plausible best adaptation and reproductive success with high rates of returning individuals.

Evidence obtained from the present study shows that the Irish stocking population is the most different from the one of the Rhine. The reason could be find in the restocking program exercises in the Rhine throughout the last few years (ICPR data). The Irish population was the less used, eggs and smolts from Sweden and France were more spreadly used.
A high rate of private alleles has been found among the subpopulations and the one of the Rhine shows the highest presence of private alleles that could be used as genetic markers, in order to identify individuals of this cohort and eventually select them for a proper stocking program.

In this research is quite impossible to talk about “native” population, and thus, of the impact of stocking on it, because Rhine population was formally said to be extinct for at

least a century, so in theory what is now sampled and analysed should be the result of years of stocking programs.

Despite of this consideration the results obtained in the present project depict a quite clear situation where a significantly different population could be differentiate from the stocking ones.

However, annual intensive stocking could have a high impact on native stock and cause the disappearance of the wild stock (Grandjean et al. 2009, Vasemägi et al. 2001).

4.2 Population structure

The genetic differentiation shows a quite clear situation where the Rhine subpopulations cluster together and show a significant distance among the other subpopulations (ranged from 0.079 of BUR to 0.051 of Allwild and Lagan).
Thus, gene flow is not sufficient to overcome the barriers raised, even in a sympatry situation, by geographical origin. One could assume that in nature individuals tend to mate preferentially within those of the same geographical origin and therefore sharing the same genetic pattern.
Differences produced by genetic drift are not covered by gene flow.
Homing effects prevent this species from indiscriminate assortment, because same origin individuals go back to the same natal river to reproduce, preserving the genetic pool of this single subpopulation.
Atlantic salmon is known by literature to form, for each spawning river, a subpopulation different from neighbour subpopulations of the same river basin (Sanchez et al. 1996, Koljonen et al. 1999, Verspoor et al. 1999, King et al. 2001).

Even if Rhine is intensively annually restocked the F_{ST} value among the sampled individuals throughout four years was always significant.
F_{IT} (inbreeding coefficient of an individual relative to the total population) can be partitioned into F_{ST} (effect of subpopulations compared to the total population) due to the Wahlund effect and F_{IS} (inbreeding coefficient of an individual relative to the subpopulation) due to inbreeding.
Normally the effective population size (the number of breeding individuals in an idealized population that would show the same amount of dispersion of allele frequencies under

random genetic drift or the same amount of inbreeding Wright 1931, 1938) is used to determine these probabilities.

For Atlantic salmon the effective number of breeders per year should not be less than 150 to avoid inbreeding effect, so the effective population size should be kept as large as possible, artificial selection and unnatural migration rates should be avoided (Consuegra and Nielsen, 2007).

According to the results obtained by the neighbour-joining and the UPGMA analysis, both based on genetic distance, 4 main groups were clearly defined:

1. Rhine individuals, divided per sampled year (from 2002 to 2005);
2. Swedish individuals from hatcheries (Ätran/Lagan);
3. French individuals from hatcheries (Allhatc/Allwild);
4. Irish individuals from hatcheries (BUR).

Rhine individuals clustered together with a significant bootstrap value. Swedish and French individuals clustered together following, as expected, their geographical origins. Irish individuals were considered as the outgroup.

The bootstrap values even if significant for the Rhine individuals do not clearly support the robustness of the derived trees.

The neighbour-joining method based on DC (Cavalli-Sforza) genetic distance, gives the most informative phenogram, where a bootstrap value of 74.0 presides over the bifurcation between the Rhine individuals and the other referee individuals, and the value of 81.0 at the bifurcation of the cluster merging together the Rhine individuals.
Individuals genotypically appearing once more to be closer to the ones of the Rhine, are the Swedish individuals (Ätran/Lagan).
French (Allier) and Irish (Burrishoole) individuals show the highest degree of genetic diversity.

Also the assignment tests support this evaluation, showing the highest percentage of shared alleles between Rhine and Swedish individuals instead of French and Irish.

Although, we must consider that even if the percentage of alleles shared between these populations is high, it is never high enough to hide the clear identification of groups genetically different and distinguishable as the Rhine group.
This strong phylogeographic structuring in the Atlantic salmon comes from the paleogeographic events, as the postglacial recolonization (Consuegra et al., 2002), and ecological species behaviour as the homing effect (Stewart et al 2003, Saunders and Bailey 1978).
Thus, contemporary gene flow among populations is limited even among tributaries within rivers.
In the present study the genetic difference among the studied subpopulations can hardly been explained by gene flow from other different populations, except for those used for reintroduction.
One explanation could be spontaneous recolonization from different wild cohort that has, throughout the years, established a local stabile subpopulation returning every year to spawn.

4.3 Scales analysis

Age determination by scale reading showed some significant information about the migration behaviour of the Rhine salmons. Rhine individuals seem to migrate to the sea after at least two years spent in the freshwater and to come back to the spawning place after one or two years.
Rhine individuals generally displayed behaviour of 1-2 sea- winter returning.
This migration model is not so different from the behaviour of the other referee populations in the wild, and so far, it seems to be the mostly used by the Atlantic salmon.

Scale reading is, however, a time demanding and quite subjective method.
Furthermore, there is often a high probability to get replacement scales either without core or unable to let us identify sea/freshwater rings unambiguously.
A high rate of replacement scales could also be symptomatic of farmed individuals who lost their scales much more frequently than the wild ones, due to rubbing caused by the high density of fishes in the hatchery.

Despite some important results, the error rate in the interpretation is so high that this method should be considered not totally reliable.

The mineral analysis of the scale could be much more informative.
Presence of defined values of Strontium and Magnesium can really help in the determination of the marine/freshwater permanence of the fish.

4.4 Conservation and management

Fundamental for the Atlantic salmon management is to understand that every river and even every tributary can support their own different cohort even with small but significant genetic difference from the stocking individuals. The structure of these cohorts could appear very complex among and within rivers.
Phylogeographic difference is also to be taken into account, because from several molecular studies significant differences have been revealed between Eastern and Western Atlantic salmon populations (McGinnity et al. 2004, Youngson et al. 2003), but even within Eastern populations themselves among and within Baltic and Atlantic Ocean drainages (Ståhl 1987, Verspoor et al. 1999, Koljonen et al. 2002).

The most suitable management for the Atlantic salmon reintroduction, when possible, should be to avoid transfer and translocation of individuals from different geographic origin or to limit, as much as possible, this practice by supporting the stocking and reintroduction of native individuals to sustain the local population.

The Atlantic salmon populations have local adaptation but should not have to be considered as isolated units. In order to maintain genetic differentiation, gene flow should be supported by maintaining the population size at their largest sustainable size.

Stocking with non-native fish should be taken into account when the native is inept to sustain self-breeding and therefore is endangered of going extinct. In this case, supportive breeding has to be considered until the wild/hatchery reaches again a high effective population size.
The number of breeders has to be high and individuals per generation should not be less than 50-500 (Consuegra et al., 2007).

The stocking exercise in the Rhine begun after the native population was said to be extinct.

But nowadays evidence of a stabile, if not a real, population, different from the stocking ones, has to be considered.
I would suggest that, in order to avoid the encouraging of an own population with fixed alleles frequencies, the Rhine Atlantic salmon should be still supported with stocking exercise, but selecting the right source population is the most important issue for salmon management.

By the evidence of the present study, the Swedish individuals seem to be the most appropriate for a stocking exercise in the Rhine because they are the most genetically similar to the Rhine individuals and seem to have the best adaptation to the local habitat conditions and also a better fitness than the French and the Irish individuals.

Besides I would suggest to select appropriate breeders of local cohort and to establish a stocking exercise with those individuals.

Exercise of brown trout introgression, should be further limited where Atlantic salmon recolonization occurs, because the hybridisation between the two species significantly reduces the population fitness.
For this reason, the escape of farmed individuals, brown trout but even Atlantic or even more Pacific salmon must be absolutely avoided.

In our case study, fortunately, no hybrids have been observed in the sampled Rhine individuals due to the correct reintroduction exercises and probably to the favourable proportion of Atlantic salmon breeders.

This project proves once again how genetic analysis in the study of Atlantic salmon are the most appropriate tool to understand the population structure and to provide in fine scale useful management suggestions and it should be more widely used for exploitation regulatory and for farming, stocking and reintroduction.
In particular, microsatellite analysis is the less expensive lab method, the most informative and faster to be developed. It is advisable, however, to use more than ten loci for a proper and reliable genetic analysis, especially in the presence of a few individuals per populations.

F_{st} index remains the most informative value to estimate fine population structure in mix-stocked population.
Besides, assignment method is even more extremely useful for genetic identification even with a small population size.

An appropriate program of environmental rehabilitation, of development of suitable reproductive habitat and of stocking regulatory is strongly recommended to be continued. Projects “Lachs 2000” before, and “Lachs 2020” now, promoted by the “Internationale Kommission zum Schutz des Rheins (IKSR), are the base for this significant result of Atlantic salmon restoration in the Rhine drainage system and for the establishment of a probably local population favoured by the many fishpass, that over the years have made again the Rhine a suitable route for fish migration.

4.5 Conclusion

Some important issues have been obtained by the end of this project and our conclusion could be summarised as follows:

1. Classical genetic approach could be an important support in this kind of project but not sufficiently informative and sometimes impossible to apply when the safety of the animal is fundamental.

2. Modern genetic approaches are preferable as the most informative in the study of population genetic and population structure of Atlantic salmon. In this contest, microsatellite analysis is highly recommended as extremely informative as the mtDNA method, but much faster, less expensive and highly repeatable.

3. F_{st} index shows that Rhine individuals are the most genetically similar and can be clustered in a different group as the genetic distance based phenogramms show. Swedish individuals show the highest degree of genetic similarity to the ones of the Rhine, French and Irish individuals show the lowest.

4. A selection of source population should be probably reviewed according to the results obtained in this study. Swedish individuals (Lagan/Ätran) should be

preferred for stocking programs instead of French (Allwild/Allhatc) and Irish (BUR) individuals.

5. A local adapted Rhine subpopulation has to be considered in further research and restocking projects. Stocking and reintroduction of individuals of this local subpopulation should be desirable. Current stocking programs, with opportune source population, have to be continued in order to support the population size as well as social use.

6. Regarding Atlantic salmon conservation, the present study showed how habitat restoration could be decisive to recreate “new” populations in rivers, in this case the Rhine, where salmon had disappeared and may encourage natural recolonization.

7. In order to have a complete overview of the population structure of the Rhine individuals, a more intensive comparison should be performed with more individuals and more genetic markers. Keeping in mind this aim, a comparison with old samples is also desirable in order to verify the “wild” pattern of the Rhine Atlantic salmon and then compare it with the stocking individuals.

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7 APPENDIX

7.1 Appendix-1

Individuals genotype for each locus

Charge	Fisch		SSOSL85		SSOSL311		STR15		SSa171	
2002	18	1	194	200	135	138	220	221	213	217
2002	20	1	190	192	135	139	220	221	223	231
2002	29	1	199	199	147	150	234	252	217	234
2002	33	1	199	199	126	130	219	235	217	234
2002	40	1	195	210	142	144	219	219	219	219
2002	48	1	208	210	126	127	219	219	213	228
2002	50	1	203	207	133	136	219	219	212	230
2002	57	1	182	191	139	143	220	220	212	212
2002	58	1	195	195	126	129	219	220	212	225
2002	62	1	183	190	139	143	220	222	212	217
2002	65	1	197	201	127	127	219	220	212	212
2002	66	1	194	194	134	139	220	220	217	221
2002	69	1	199	199	124	127	223	225	217	221
2002	70	1	184	197	126	129	219	235	217	221
2002	72	1	199	199	127	130	219	235	217	225
2002	80	1	189	199	139	143	219	219	217	236
2002	81	1	197	201	126	126	219	221	217	232
2002	83	1	201	201	125	127	219	235	220	220
2002	85	1	191	205	142	143	219	219	237	246
2002	89	1	191	195	125	127	220	225	225	225
2002	90	1	207	207	125	127	220	225	214	226
2002	93	1	182	188	126	129	219	221	214	219
2002	95	1	191	191	125	127	219	235	215	215
2002	96	1	195	197	125	127	219	219	215	238
2002	99	1	191	195	155	159	221	224	219	238
2002	100	1	183	190	155	159	221	224	215	223
2002	106	1	177	178	155	159	219	219	214	236
2002	114	1	181	181	138	152	225	227	225	225
2002	116	1	193	210	128	150	224	227	225	225
2002	119	1	176	184	128	150	225	225	240	242
2003	1	1	191	195	154	158	220	236	215	230
2003	4	1	188	205	143	146	219	219	226	226
2003	6	1	183	187	163	166	219	220	210	230
2003	10	1	193	210	143	148	219	219	223	229
2003	11	1	190	190	132	135	223	223	210	212
2003	12	1	190	206	144	148	220	236	203	231
2003	19	1	183	190	163	166	219	220	215	215
2003	21	1	186	187	144	148	219	219	221	224
2003	41	1	186	195	143	146	216	219	215	215
2003	48	1	183	193	154	157	219	220	209	209
2003	51	1	188	203	143	143	225	227	216	225
2004	5	1	181	182	127	127	226	228	229	229
2004	6	1	199	199	127	127	219	219	214	238
2004	7	1	188	205	173	175	219	219	214	232
2004	13	1	154	163	173	175	225	225	229	229

Tab.16a Individuals genotype of the loci SSOSL85, SSOSL311, STR15, Ssa171

Charge	Fisch		SSOSL85		SSOSL311		STR15		SSa171	
2004	17	1	184	190	127	127	219	221	225	232
2004	18	1	200	203	127	127	219	219	225	232
2004	30	1	194	200	127	127	219	219	236	249
2004	36	1	185	205	127	127	219	219	230	242
2004	37	1	194	209	140	143	219	219	214	223
2004	39	1	176	184	127	127	219	221	223	233
2004	41	1	190	190	140	143	219	219	214	222
2004	46	1	197	199	163	167	221	221	218	244
2004	48	1	183	201	128	128	219	221	220	220
2004	52	1	192	192	128	128	220	220	230	242
2004	53	1	184	190	132	132	222	225	239	239
2005	5	1	195	195	127	127	220	226	205	217
2005	14	1	178	222	131	131	224	226	214	226
2005	18	1	185	195	140	143	219	219	197	205
2005	19	1	197	201	125	125	220	220	214	219
2005	20	1	199	199	128	128	219	219	205	223
2005	21	1	195	222	127	127	219	226	214	226
2005	22	1	185	186	142	142	219	219	207	211
2005	40	1	188	198	127	127	219	219	205	223
2005	46	1	199	199	134	138	219	220	205	223
Burrishoole	1	2	194	196	151	154	207	207	213	217
Burrishoole	2	2	183	184	153	155	207	209	210	213
Burrishoole	4	2	184	184	153	155	207	209	210	213
Burrishoole	6	2	188	188	153	156	207	207	213	217
Burrishoole	7	2	184	184	153	156	207	207	211	217
Burrishoole	8	2	184	184	153	156	207	207	203	213
Burrishoole	9	2	197	199	153	156	207	207	211	215
Burrishoole	10	2	188	199	155	158	207	207	206	209
Burrishoole	11	2	194	195	153	155	207	209	213	216
Burrishoole	12	2	184	194	174	177	209	209	209	213
Burrishoole	13	2	186	189	142	142	212	213	208	236
Burrishoole	14	2	188	195	160	163	206	206	210	213
Burrishoole	19	2	182	183	143	143	207	209	208	210
Burrishoole	21	2	183	187	152	155	208	208	212	213
Burrishoole	22	2	185	185	152	155	207	207	214	217
Burrishoole	23	2	184	187	162	166	207	207	210	219
Burrishoole	24	2	183	184	150	153	207	207	209	210
Burrishoole	25	2	184	184	153	156	207	209	216	219
Burrishoole	26	2	193	193	153	156	207	207	209	217
Burrishoole	28	2	193	194	171	174	207	207	206	212
Burrishoole	29	2	193	193	152	156	207	208	212	214
Burrishoole	30	2	190	190	160	163	207	207	204	219
Allier wild	1	2	194	194	165	168	214	215	216	219
Allier wild	2	2	194	194	149	151	213	215	204	220
Allier wild	3	2	191	191	161	164	212	213	213	221
Allier wild	4	2	186	187	142	142	213	214	201	207
Allier wild	5	2	190	190	155	158	213	214	209	220
Allier wild	6	2	194	194	143	143	213	213	215	219
Allier wild	7	2	195	199	149	152	212	213	209	213
Allier wild	8	2	193	195	140	140	212	213	211	211

Tab.16a (continued)

Charge	Fisch		SSOSL85		SSOSL311		STR15		SSa171	
Allier wild	9	2	193	193	161	165	212	213	204	219
Allier wild	10	2	195	195	140	140	212	213	203	209
Allier wild	11	2	189	189	154	157	206	208	204	219
Allier wild	12	2	191	191	165	168	206	206	207	225
Allier wild	13	2	178	179	127	144	216	218	204	211
Allier wild	14	2	200	200	149	152	216	216	204	211
Allier wild	15	2	193	194	171	173	206	208	204	210
Allier wild	16	2	193	194	155	179	207	207	204	206
Allier wild	17	2	200	200	152	156	215	215	204	225
Allier wild	18	2	201	201	127	127	216	216	211	217
Allier wild	20	2	193	195	161	165	212	213	213	220
Allier wild	21	2	196	196	140	140	213	214	211	220
Allier wild	23	2	193	193	145	145	212	213	210	219
Allier wild	24	2	200	200	154	157	215	217	209	209
Allier wild	26	2	190	190	127	127	215	215	203	214
Allier wild	27	2	190	190	144	156	216	216	203	217
Allier wild	29	2	190	196	142	147	197	197	203	222
Allier hatchery	1	2	192	192	155	156	216	216	227	231
Allier hatchery	2	2	192	192	152	156	197	197	216	233
Allier hatchery	3	2	195	195	154	157	207	208	205	205
Allier hatchery	4	2	192	194	161	164	197	198	207	216
Allier hatchery	5	2	192	192	156	170	197	198	220	230
Allier hatchery	6	2	192	194	140	144	207	208	204	211
Allier hatchery	7	2	192	192	140	144	197	198	219	223
Allier hatchery	8	2	194	194	150	152	197	198	219	223
Allier hatchery	9	2	192	194	141	155	207	208	204	223
Allier hatchery	10	2	189	189	127	127	197	198	223	240
Allier hatchery	11	2	189	189	141	152	198	199	211	223
Allier hatchery	12	2	166	168	153	156	197	198	219	236
Allier hatchery	13	2	166	166	150	152	198	199	231	234
Allier hatchery	14	2	192	192	155	171	198	198	215	223
Allier hatchery	15	2	192	192	142	142	206	206	218	223
Allier hatchery	16	2	190	191	164	164	200	202	223	229
Allier hatchery	17	2	192	192	162	164	201	203	221	231
Allier hatchery	18	2	193	193	152	155	206	206	216	235
Allier hatchery	19	2	191	195	154	157	207	207	215	219
Allier hatchery	20	2	193	195	142	145	200	202	203	209
Allier hatchery	21	2	193	197	162	165	207	207	211	223
Allier hatchery	22	2	195	195	165	166	206	207	220	225
Allier hatchery	23	2	193	193	157	160	202	203	227	230
Allier hatchery	24	2	190	190	152	155	206	206	221	231
Allier hatchery	26	2	193	193	165	168	206	208	214	229
Ätran Albaum	1	2	180	188	151	154	198	200	217	218
Ätran Albaum	2	2	186	186	142	146	198	200	213	234
Ätran Albaum	3	2	185	193	135	139	199	199	208	222
Ätran Albaum	4	2	193	195	155	158	211	212	209	226
Ätran Albaum	5	2	193	193	133	137	219	219	208	228
Ätran Albaum	6	2	186	186	128	136	199	200	225	237
Ätran Albaum	8	2	192	195	169	172	211	212	210	212
Ätran Albaum	10	2	199	200	126	126	211	212	212	217

Tab.16a (continued)

Charge	Fisch		SSOSL85		SSOSL311		STR15		SSa171	
Ätran Albaum	11	2	188	201	143	146	211	212	206	225
Ätran Albaum	12	2	187	193	144	144	212	212	222	222
Ätran Albaum	13	2	182	182	143	146	217	217	223	223
Ätran Albaum	14	2	191	194	139	143	219	219	208	232
Ätran Albaum	15	2	185	193	165	168	217	217	219	232
Ätran Albaum	16	2	186	186	133	138	216	216	204	204
Ätran Albaum	17	2	188	194	135	138	219	219	204	217
Ätran Albaum	18	2	180	180	135	135	215	215	203	203
Ätran Albaum	19	2	185	195	137	140	215	215	233	233
Ätran Albaum	20	2	186	186	164	167	215	215	208	208
Ätran Albaum	21	2	192	194	146	147	211	212	220	231
Ätran Albaum	22	2	196	196	155	157	211	213	215	224
Ätran Albaum	24	2	194	194	144	147	212	213	212	221
Ätran Albaum	25	2	186	187	155	157	212	213	212	228
Ätran Albaum	26	2	200	200	146	147	212	213	212	228
Ätran Albaum	27	2	186	186	144	147	212	213	212	212
Ätran Albaum	28	2	186	192	144	154	212	213	215	225
Ätran Albaum	29	2	195	198	156	160	216	218	222	234
Ätran Albaum	30	2	195	195	154	156	212	213	222	225
Ätran Albaum	31	2	186	192	166	169	212	213	212	225
Lagan Bad Schandau	1	2	193	193	128	128	198	198	221	227
Lagan Bad Schandau	2	2	187	187	127	127	198	200	219	224
Lagan Bad Schandau	3	2	181	195	130	130	198	198	199	204
Lagan Bad Schandau	5	2	187	193	127	127	200	200	199	204
Lagan Bad Schandau	6	2	181	183	128	128	198	200	211	223
Lagan Bad Schandau	7	2	183	183	126	128	198	200	204	223
Lagan Bad Schandau	8	2	181	193	128	128	207	207	205	217
Lagan Bad Schandau	9	2	183	183	143	143	197	199	202	211
Lagan Bad Schandau	10	2	199	199	128	128	198	198	208	217
Lagan Bad Schandau	11	2	200	200	136	140	212	213	203	209
Lagan Bad Schandau	12	2	195	195	145	145	197	197	213	216
Lagan Bad Schandau	14	2	181	191	127	127	198	198	215	227
Lagan Bad Schandau	16	2	192	195	158	158	212	213	209	222
Lagan Bad Schandau	17	2	187	190	135	138	212	213	207	213
Lagan Bad Schandau	18	2	187	191	142	146	208	209	203	214

Tab.16a (continued)

Charge	Fisch	SSa402*		SSa402**		SSa408		SSa202		SSa411	
2002	18	163	172	205	212	221	221	245	251	266	266
2002	20	168	170	212	215	249	292	239	242	268	268
2002	29	173	176	190	216	220	284	245	251	265	265
2002	33	172	176	215	217	243	289	245	251	267	267
2002	40	163	172	203	205	240	281	241	251	267	272
2002	48	169	171	204	212	282	282	242	246	275	275
2002	50	170	171	202	204	262	290	229	245	270	270
2002	57	170	171	202	213	255	260	229	232	270	270
2002	58	170	171	204	206	244	287	232	251	270	270
2002	62	162	170	193	225	267	274	236	242	269	269
2002	65	183	183	202	204	254	260	236	236	268	268
2002	66	162	170	209	216	243	289	240	256	268	268
2002	69	169	169	210	212	248	290	239	243	267	267
2002	70	170	171	205	212	241	291	245	251	267	267
2002	72	183	183	212	212	242	286	245	251	266	267
2002	80	183	183	211	212	220	248	242	252	270	270
2002	81	170	172	203	205	240	268	229	248	272	272
2002	83	183	183	204	218	266	275	239	242	269	273
2002	85	183	183	205	209	238	242	248	248	270	270
2002	89	170	171	203	204	240	247	232	239	283	283
2002	90	168	169	202	213	248	257	236	248	267	267
2002	93	170	172	204	207	262	293	248	255	268	275
2002	95	163	170	203	206	258	268	229	236	268	272
2002	96	170	172	203	208	262	293	235	238	267	267
2002	99	169	172	204	205	265	265	251	251	270	270
2002	100	183	183	204	205	244	256	229	249	269	269
2002	106	170	172	206	213	248	263	236	239	267	267
2002	114	151	152	165	266	214	228	247	247	266	266
2002	116	150	151	288	296	217	247	229	240	266	266
2002	119	161	162	255	275	241	246	229	232	280	280
2003	1	170	172	205	214	254	286	232	245	265	265
2003	4	170	172	205	213	241	244	235	235	268	273
2003	6	163	172	208	227	268	286	245	251	268	268
2003	10	170	171	204	212	259	271	234	254	268	273
2003	11	171	173	203	211	275	289	232	254	265	265
2003	12	172	172	206	226	234	241	232	248	268	268
2003	19	164	170	204	212	254	269	232	245	270	270
2003	21	164	164	208	218	254	278	236	246	267	272
2003	41	171	171	212	226	246	278	244	244	268	268
2003	48	169	170	203	205	250	278	235	255	265	270
2003	51	170	172	212	214	250	291	229	242	268	268
2004	5	164	164	252	255	231	251	238	252	268	268
2004	6	172	172	204	208	290	297	245	245	268	268
2004	7	171	171	204	204	256	289	249	254	268	268
2004	13	169	176	252	255	238	242	236	239	264	264
2004	17	167	168	203	213	243	281	223	255	268	268
2004	18	163	170	203	206	254	270	235	252	268	273
2004	30	183	183	207	214	266	290	232	235	268	268
2004	36	170	172	203	205	243	262	239	242	268	272
2004	37	183	183	204	213	262	293	229	254	268	268

Tab.16b Individuals genotype of Ssa402*, Ssa402**, Ssa408, Ssa202, Ssa411

Charge	Fisch	SSa402*		SSa402**		SSa408		SSa202		SSa411	
2004	39	183	183	205	207	243	259	236	255	268	268
2004	41	167	167	202	203	296	296	241	255	273	273
2004	46	172	172	205	205	245	270	232	242	268	268
2004	48	170	170	203	204	269	275	229	232	268	268
2004	52	168	171	203	205	244	278	235	267	267	267
2004	53	171	171	259	270	216	221	238	241	268	268
2005	5	183	183	254	256	230	252	238	238	266	271
2005	14	183	183	278	282	219	235	236	239	281	281
2005	18	183	183	202	204	263	276	245	245	268	273
2005	19	172	172	203	204	240	240	248	248	268	273
2005	20	183	183	204	205	261	297	248	255	268	268
2005	21	170	172	203	206	252	271	235	245	268	273
2005	22	170	172	203	206	250	269	235	245	268	272
2005	40	170	172	300	303	209	225	232	238	256	256
2005	46	170	170	202	204	281	297	223	233	268	268
Burryshole	1	163	163	203	204	278	299	223	233	271	273
Burryshole	2	163	173	203	204	277	298	246	249	266	266
Burryshole	4	172	173	203	204	277	304	245	249	268	268
Burryshole	6	172	172	203	204	285	292	233	239	268	268
Burryshole	7	172	172	203	204	282	299	223	245	273	273
Burryshole	8	164	172	203	204	300	306	239	245	268	268
Burryshole	9	164	175	203	204	279	300	248	248	270	270
Burryshole	10	164	172	203	204	290	316	240	246	268	268
Burryshole	11	164	173	205	206	265	300	230	232	271	273
Burryshole	12	173	173	204	205	283	290	230	240	268	273
Burryshole	13	176	176	204	205	300	300	229	245	268	268
Burryshole	14	161	164	204	205	301	301	245	249	270	270
Burryshole	19	164	164	204	205	288	294	243	250	268	272
Burryshole	21	173	174	204	205	281	308	223	242	269	270
Burryshole	22	164	164	204	205	308	308	223	242	268	268
Burryshole	23	164	174	204	204	208	301	229	248	266	266
Burryshole	24	164	164	204	205	307	307	230	249	259	259
Burryshole	25	164	164	204	212	280	301	230	242	266	266
Burryshole	26	164	174	205	212	280	307	229	242	266	270
Burryshole	28	161	164	205	212	294	307	239	242	270	270
Burryshole	29	164	173	202	204	280	280	243	250	267	267
Burryshole	30	174	174	204	205	265	300	246	250	267	272
Allier wild	1	171	173	205	214	295	318	243	256	267	272
Allier wild	2	161	170	204	213	295	3118	239	249	268	268
Allier wild	3	170	172	217	217	299	314	246	253	268	268
Allier wild	4	170	173	208	213	305	322	236	243	268	268
Allier wild	5	172	173	205	213	319	319	239	245	268	268
Allier wild	6	172	173	205	208	299	319	246	256	267	267
Allier wild	7	166	166	216	224	299	316	243	253	267	267
Allier wild	8	164	173	205	213	312	319	246	246	267	267
Allier wild	9	165	173	205	216	306	3116	239	246	269	269
Allier wild	10	164	173	205	216	266	306	249	256	266	270
Allier wild	11	173	173	205	214	319	319	243	249	269	274
Allier wild	12	173	173	204	207	276	314	243	253	268	273
Allier wild	13	169	171	202	205	250	282	240	246	268	268

Tab.16b (continued)

Charge	Fisch	SSa402*		SSa402**		SSa408		SSa202		SSa411	
Allier wild	14	172	172	219	224	299	320	249	252	266	270
Allier wild	15	169	172	214	214	319	319	246	256	266	270
Allier wild	16	172	172	205	205	306	319	246	250	274	274
Allier wild	17	170	170	204	215	283	293	246	250	269	269
Allier wild	18	170	170	202	204	294	300	240	250	269	269
Allier wild	20	173	173	202	205	272	316	256	256	268	268
Allier wild	21	171	172	208	214	305	319	246	256	269	269
Allier wild	23	172	172	202	205	278	293	246	246	274	274
Allier wild	24	170	171	211	213	291	302	246	246	268	268
Allier wild	26	170	171	206	209	258	296	256	256	268	268
Allier wild	27	172	173	205	212	250	295	243	246	268	268
Allier wild	29	170	171	211	211	290	296	249	252	269	274
Allier hatchery	1	170	172	214	219	291	298	245	249	269	269
Allier hatchery	2	269	170	207	207	266	294	243	250	269	274
Allier hatchery	3	168	170	205	206	296	296	242	255	269	269
Allier hatchery	4	162	168	203	207	290	290	248	248	269	274
Allier hatchery	5	169	171	204	204	279	290	245	249	269	274
Allier hatchery	6	169	170	204	213	292	292	245	245	269	274
Allier hatchery	7	169	171	207	207	266	290	242	249	269	274
Allier hatchery	8	163	170	215	230	291	297	249	255	269	274
Allier hatchery	9	170	172	207	213	292	292	242	249	269	269
Allier hatchery	10	169	171	207	215	287	294	248	255	268	268
Allier hatchery	11	170	172	204	213	292	300	242	255	268	272
Allier hatchery	12	161	163	204	213	290	299	242	248	267	267
Allier hatchery	13	168	170	205	214	265	293	245	245	267	267
Allier hatchery	14	162	171	207	207	287	293	245	248	271	271
Allier hatchery	15	170	172	214	219	294	300	245	255	271	271
Allier hatchery	16	159	162	205	207	265	291	242	245	271	271
Allier hatchery	17	161	163	208	216	267	295	245	248	266	266
Allier hatchery	18	171	172	203	215	284	284	242	245	271	271
Allier hatchery	19	171	172	205	213	286	293	248	255	266	271
Allier hatchery	20	171	172	205	213	264	264	245	248	266	266
Allier hatchery	21	171	172	208	213	288	301	245	248	266	266
Allier hatchery	22	171	173	205	208	264	264	245	255	267	271
Allier hatchery	23	170	171	205	208	286	292	248	248	272	272
Allier hatchery	24	173	173	205	208	264	264	243	249	267	272
Allier hatchery	26	171	171	205	214	265	265	245	247	267	271
Ätran Albaum	1	162	171	203	205	259	265	233	249	267	267
Ätran Albaum	2	169	170	214	214	275	296	236	243	268	273
Ätran Albaum	3	162	170	203	203	262	267	236	239	267	267
Ätran Albaum	4	163	169	201	202	252	270	229	236	267	267
Ätran Albaum	5	163	172	201	202	255	274	239	239	267	267
Ätran Albaum	6	167	171	205	205	242	273	243	249	267	267
Ätran Albaum	8	166	169	201	202	255	271	229	239	267	267
Ätran Albaum	10	169	171	204	204	240	255	240	243	267	267
Ätran Albaum	11	163	173	201	203	262	262	236	256	267	267
Ätran Albaum	12	167	169	203	206	252	256	236	243	267	267
Ätran Albaum	13	162	170	203	203	255	267	236	243	267	272
Ätran Albaum	14	162	170	205	205	239	239	229	239	273	273
Ätran Albaum	15	167	170	204	206	238	267	239	249	272	272

Tab.16b (continued)

Charge	Fisch	SSa402*		SSa402**		SSa408		SSa202		SSa411	
Ätran Albaum	16	170	171	201	204	238	238	240	240	272	272
Ätran Albaum	17	160	162	204	206	254	267	236	240	267	271
Ätran Albaum	18	169	171	202	203	237	268	236	239	267	271
Ätran Albaum	19	165	169	214	214	239	274	236	236	267	267
Ätran Albaum	20	163	170	204	204	255	268	230	236	267	267
Ätran Albaum	21	171	173	204	205	253	262	236	250	267	272
Ätran Albaum	22	164	171	204	205	237	265	236	240	267	272
Ätran Albaum	24	164	170	202	204	265	265	236	239	267	272
Ätran Albaum	25	171	172	204	205	265	271	237	237	266	270
Ätran Albaum	26	169	172	203	205	265	271	230	240	267	271
Ätran Albaum	27	169	171	202	205	255	271	237	250	272	272
Ätran Albaum	28	169	172	203	205	255	271	230	243	267	268
Ätran Albaum	29	168	171	203	205	237	249	236	239	266	266
Ätran Albaum	30	169	170	202	204	237	252	236	236	266	270
Ätran Albaum	31	169	170	205	206	237	252	239	239	266	271
Lagan Bad Schandau	1	168	174	203	205	274	280	229	238	266	266
Lagan Bad Schandau	2	162	171	205	205	256	256	229	268	267	267
Lagan Bad Schandau	3	168	170	205	205	244	248	242	268	267	267
Lagan Bad Schandau	5	162	172	205	212	270	283	249	268	267	267
Lagan Bad Schandau	6	162	170	203	205	254	254	229	238	266	266
Lagan Bad Schandau	7	168	171	205	226	260	279	223	235	266	266
Lagan Bad Schandau	8	168	168	203	204	267	276	235	239	266	266
Lagan Bad Schandau	9	163	172	203	205	264	270	235	239	267	267
Lagan Bad Schandau	10	165	171	205	207	270	282	239	242	267	267
Lagan Bad Schandau	11	171	172	202	204	267	273	229	229	267	272
Lagan Bad Schandau	12	163	172	204	212	242	242	232	239	268	268
Lagan Bad Schandau	14	169	170	205	206	270	283	223	236	270	272
Lagan Bad Schandau	16	167	170	204	204	248	254	229	239	267	267
Lagan Bad Schandau	17	166	166	216	224	299	316	246	249	267	267
Lagan Bad Schandau	18	165	171	205	207	264	277	243	250	269	270

Tab.16b (continued)

7.2 Appendix 2

Alleles frequencies per locus

- SSOSL85

Alleles(N)	Iff 02	Iffi 03	Iff 04	Iff 05	BUR	Allhatc	Allwild	Ätran	Lagan
154	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
163	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
166	0.000	0.000	0.000	0.000	0.000	0.060	0.000	0.000	0.000
168	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000
176	0.017	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
177	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
178	0.017	0.000	0.000	0.056	0.000	0.000	0.020	0.000	0.000
179	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000
180	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.054	0.000
181	0.033	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.133
182	0.033	0.000	0.033	0.000	0.023	0.000	0.000	0.036	0.000
183	0.033	0.137	0.033	0.000	0.091	0.000	0.000	0.000	0.167
184	0.033	0.000	0.101	0.000	0.273	0.000	0.000	0.000	0.000
185	0.000	0.000	0.033	0.111	0.045	0.000	0.000	0.054	0.000
186	0.000	0.091	0.000	0.056	0.023	0.000	0.020	0.232	0.000
187	0.000	0.091	0.000	0.000	0.045	0.000	0.020	0.036	0.167
188	0.017	0.091	0.033	0.056	0.091	0.000	0.000	0.054	0.000
189	0.017	0.000	0.000	0.000	0.023	0.080	0.040	0.000	0.000
190	0.050	0.183	0.134	0.000	0.045	0.060	0.140	0.000	0.033
191	0.100	0.045	0.000	0.000	0.000	0.040	0.080	0.018	0.067
192	0.017	0.000	0.067	0.000	0.000	0.360	0.000	0.071	0.033
193	0.017	0.091	0.000	0.000	0.114	0.140	0.160	0.107	0.133
194	0.050	0.000	0.067	0.000	0.091	0.100	0.160	0.089	0.000
195	0.099	0.091	0.000	0.221	0.045	0.120	0.100	0.107	0.133
196	0.000	0.000	0.000	0.000	0.023	0.000	0.060	0.036	0.000
197	0.066	0.000	0.033	0.056	0.023	0.020	0.000	0.000	0.000
198	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.018	0.000
199	0.150	0.000	0.101	0.221	0.045	0.000	0.020	0.018	0.067
200	0.017	0.000	0.067	0.000	0.000	0.000	0.120	0.054	0.067
201	0.066	0.000	0.033	0.056	0.000	0.000	0.040	0.018	0.000
203	0.017	0.045	0.033	0.000	0.000	0.000	0.000	0.000	0.000
205	0.017	0.045	0.067	0.000	0.000	0.000	0.000	0.000	0.000
206	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000
207	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
208	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
209	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
210	0.050	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000
222	0.000	0.000	0.000	0.111	0.000	0.000	0.000	0.000	0.000

Tab.17 Alleles frequencies of SSOSL85 locus

	Iff 02	Iffi 03	Iff 04	Iff 05	BUR	Allhatc	Allwild	Ätran	Lagan
N	24	12	19	10	15	10	14	16	10
H_{exp}	0.923	0.88	0.74	0.827	0.885	0.938	0.939	0.951	0.844
$H_{n.b.}$	0.938	0.922	0.766	0.876	0.906	0.957	0.958	0.968	0.874
H_{obs}	0.667	0.909	0.8	0.667	0.591	0.88	0.68	0.893	0.267

Tab.17 (continued) N. of observed alleles (N), observed (H_{obs}) and expected heterozygosity with (H_{exp}), and without ($H_{n.b}$) bias (Nei, 1978), GENETIX 4.05 software

- SSOSL311

Alleles(N)	Iff 02	Iffi 03	Iff 04	Iff 05	BUR	Allhatc	Allwild	Ätran	Lagan
124	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
125	0.083	0.000	0.000	0.111	0.000	0.000	0.000	0.000	0.000
126	0.117	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.033
127	0.166	0.000	0.466	0.332	0.000	0.040	0.100	0.000	0.200
128	0.033	0.000	0.133	0.111	0.000	0.000	0.000	0.018	0.300
129	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
130	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067
131	0.000	0.000	0.000	0.111	0.000	0.000	0.000	0.000	0.000
132	0.000	0.045	0.067	0.000	0.000	0.000	0.000	0.000	0.000
133	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000
134	0.017	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
135	0.033	0.045	0.000	0.000	0.000	0.000	0.000	0.071	0.033
136	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.033
137	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000
138	0.033	0.000	0.000	0.056	0.000	0.000	0.000	0.036	0.033
139	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000
140	0.000	0.000	0.067	0.056	0.000	0.040	0.120	0.018	0.033
141	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000
142	0.033	0.000	0.000	0.111	0.045	0.060	0.060	0.018	0.033
143	0.067	0.228	0.067	0.056	0.045	0.000	0.040	0.054	0.067
144	0.017	0.091	0.000	0.000	0.000	0.040	0.040	0.089	0.000
145	0.000	0.000	0.000	0.000	0.000	0.020	0.040	0.000	0.067
146	0.000	0.091	0.000	0.000	0.000	0.000	0.000	0.089	0.033
147	0.017	0.000	0.000	0.000	0.000	0.000	0.020	0.071	0.000
148	0.000	0.137	0.000	0.000	0.000	0.000	0.000	0.000	0.000
149	0.000	0.000	0.000	0.000	0.000	0.000	0.060	0.000	0.000
150	0.050	0.000	0.000	0.000	0.023	0.040	0.000	0.000	0.000
151	0.000	0.000	0.000	0.000	0.023	0.000	0.020	0.018	0.000
152	0.017	0.000	0.000	0.000	0.068	0.120	0.060	0.000	0.000
153	0.000	0.000	0.000	0.000	0.227	0.020	0.000	0.000	0.000
154	0.000	0.091	0.000	0.000	0.023	0.040	0.040	0.054	0.000

Tab.18 Alleles frequencies of SSOSL311 locus

Alleles(N)	Iff 02	Iffi 03	Iff 04	Iff 05	BUR	Allhatc	Allwild	Ätran	Lagan
155	0.050	0.000	0.000	0.000	0.136	0.100	0.040	0.054	0.000
156	0.000	0.000	0.000	0.000	0.159	0.080	0.040	0.036	0.000
157	0.000	0.045	0.000	0.000	0.000	0.060	0.040	0.036	0.000
158	0.000	0.045	0.000	0.000	0.023	0.000	0.020	0.018	0.067
159	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
160	0.000	0.000	0.000	0.000	0.045	0.020	0.000	0.018	0.000
161	0.000	0.000	0.000	0.000	0.000	0.020	0.060	0.000	0.000
162	0.000	0.000	0.000	0.000	0.023	0.040	0.000	0.000	0.000
163	0.000	0.091	0.033	0.000	0.045	0.000	0.000	0.000	0.000
164	0.000	0.000	0.000	0.000	0.000	0.080	0.020	0.018	0.000
165	0.000	0.000	0.000	0.000	0.000	0.060	0.080	0.018	0.000
166	0.000	0.091	0.000	0.000	0.023	0.020	0.000	0.018	0.000
167	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.018	0.000
168	0.000	0.000	0.000	0.000	0.000	0.020	0.040	0.018	0.000
169	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000
170	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000
171	0.000	0.000	0.000	0.000	0.023	0.020	0.020	0.000	0.000
172	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000
173	0.000	0.000	0.067	0.000	0.000	0.000	0.020	0.000	0.000
174	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000	0.000
175	0.000	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000
177	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000
179	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000
N	21	11	9	9	17	22	21	28	13
H_{exp}	0.92	0.88	0.74	0.83	0.89	0.94	0.94	0.95	0.84
$H_{n.b.}$	0.94	0.92	0.77	0.88	0.91	0.96	0.96	0.97	0.87
H_{obs}	0.93	0.91	0.33	0.22	0.91	0.88	0.68	0.89	0.27

Tab.18 (continued) N. of observed alleles (N), observed (H_{obs}) and expected heterozygosity with (H_{exp}), and without ($H_{n.b}$) bias (Nei, 1978), GENETIX 4.05

- Ssa171

Alleles(N)	Iff 02	Iffi 03	Iff 04	Iff 05	BUR	Allhatc	Allwild	Ätran	Lagan
197	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
199	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067
201	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000
202	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033
203	0.000	0.045	0.000	0.000	0.023	0.020	0.080	0.036	0.067
204	0.000	0.000	0.000	0.000	0.023	0.040	0.160	0.054	0.100
205	0.000	0.000	0.000	0.277	0.000	0.040	0.000	0.000	0.033
206	0.000	0.000	0.000	0.000	0.045	0.000	0.020	0.018	0.000

Tab.19 Alleles frequencies of Ssa171 locus

Alleles(N)	Iff 02	Iffi 03	Iff 04	Iff 05	BUR	Allhatc	Allwild	Ätran	Lagan
207	0.000	0.000	0.000	0.056	0.000	0.020	0.040	0.000	0.033
208	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.089	0.033
209	0.000	0.092	0.000	0.000	0.091	0.020	0.100	0.018	0.067
210	0.000	0.092	0.000	0.000	0.136	0.000	0.040	0.018	0.000
211	0.000	0.000	0.000	0.056	0.045	0.060	0.120	0.000	0.067
212	0.117	0.045	0.000	0.000	0.068	0.000	0.000	0.143	0.000
213	0.033	0.000	0.000	0.000	0.205	0.000	0.060	0.018	0.067
214	0.050	0.000	0.133	0.166	0.045	0.020	0.020	0.000	0.033
215	0.067	0.228	0.000	0.000	0.023	0.040	0.020	0.036	0.033
216	0.000	0.045	0.000	0.000	0.045	0.060	0.020	0.000	0.033
217	0.166	0.000	0.000	0.056	0.114	0.000	0.040	0.054	0.067
218	0.000	0.000	0.033	0.000	0.000	0.020	0.000	0.018	0.000
219	0.066	0.000	0.000	0.056	0.068	0.080	0.100	0.018	0.033
220	0.033	0.000	0.067	0.000	0.000	0.040	0.080	0.018	0.000
221	0.050	0.045	0.000	0.000	0.000	0.040	0.020	0.018	0.033
222	0.000	0.000	0.033	0.000	0.000	0.000	0.020	0.089	0.033
223	0.033	0.045	0.067	0.166	0.000	0.180	0.000	0.036	0.067
224	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.018	0.033
225	0.133	0.045	0.067	0.000	0.000	0.020	0.040	0.089	0.000
226	0.017	0.092	0.000	0.111	0.000	0.000	0.000	0.018	0.000
227	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.067
228	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.054	0.000
229	0.000	0.045	0.134	0.000	0.000	0.040	0.000	0.000	0.000
230	0.017	0.091	0.067	0.000	0.000	0.040	0.000	0.000	0.000
231	0.017	0.045	0.000	0.000	0.000	0.060	0.000	0.018	0.000
232	0.017	0.000	0.100	0.000	0.000	0.020	0.000	0.036	0.000
233	0.000	0.000	0.033	0.000	0.000	0.020	0.000	0.036	0.000
234	0.033	0.000	0.000	0.000	0.000	0.020	0.000	0.036	0.000
235	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000
236	0.033	0.000	0.033	0.000	0.023	0.020	0.000	0.000	0.000
237	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000
238	0.033	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
239	0.000	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000
240	0.017	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000
242	0.017	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000
244	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
246	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
249	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
N	22	14	16	8	15	25	18	24	20
H_{exp}	0.92	0.90	0.92	0.84	0.90	0.93	0.91	0.94	0.94
$H_{n.b.}$	0.93	0.94	0.95	0.89	0.92	0.95	0.93	0.95	0.97
H_{obs}	0.73	0.64	0.73	1.00	1.00	0.96	0.92	0.75	1.00

Tab.19 (continued) N. of observed alleles (N), observed (H_{obs}) and expected heterozygosity with (H_{exp}), and without ($H_{n.b}$) bias (Nei, 1978), GENETIX 4.05

- STR15

Alleles(N)	Iff 02	Iffi 03	Iff 04	Iff 05	BUR	Allhatc	Allwild	Ätran	Lagan
197	0.000	0.000	0.000	0.000	0.000	0.160	0.040	0.000	0.100
198	0.000	0.000	0.000	0.000	0.000	0.200	0.000	0.036	0.367
199	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.054	0.033
200	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.054	0.167
201	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000
202	0.000	0.000	0.000	0.000	0.000	0.060	0.000	0.000	0.000
203	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000
206	0.000	0.000	0.000	0.000	0.045	0.160	0.080	0.000	0.000
207	0.000	0.000	0.000	0.000	0.682	0.160	0.040	0.000	0.067
208	0.000	0.000	0.000	0.000	0.068	0.080	0.040	0.000	0.033
209	0.000	0.000	0.000	0.000	0.159	0.000	0.000	0.000	0.033
211	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.107	0.000
212	0.000	0.000	0.000	0.000	0.023	0.000	0.140	0.250	0.100
213	0.000	0.000	0.000	0.000	0.023	0.000	0.260	0.143	0.100
214	0.000	0.000	0.000	0.000	0.000	0.000	0.080	0.000	0.000
215	0.000	0.000	0.000	0.000	0.000	0.000	0.140	0.107	0.000
216	0.000	0.045	0.000	0.000	0.000	0.040	0.140	0.054	0.000
217	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.071	0.000
218	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.018	0.000
219	0.383	0.456	0.567	0.555	0.000	0.000	0.000	0.107	0.000
220	0.183	0.227	0.067	0.222	0.000	0.000	0.000	0.000	0.000
221	0.100	0.000	0.167	0.000	0.000	0.000	0.000	0.000	0.000
222	0.017	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
223	0.017	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000
224	0.050	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
225	0.100	0.045	0.100	0.000	0.000	0.000	0.000	0.000	0.000
226	0.000	0.000	0.033	0.167	0.000	0.000	0.000	0.000	0.000
227	0.033	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000
228	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
234	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
235	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
236	0.000	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000
252	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	11	7	7	4	6	11	11	11	9
H_{exp}	0.79	0.72	0.63	0.61	0.50	0.87	0.86	0.87	0.80
$H_{n.b.}$	0.80	0.75	0.66	0.65	0.51	0.88	0.87	0.88	0.83
H_{obs}	0.67	0.64	0.33	0.44	0.32	0.68	0.64	0.61	0.53

Tab.20 Alleles frequencies of STR15 locus. N. of observed alleles (N), observed (H_{obs}) and expected heterozygosity with (H_{exp}), and without ($H_{n.b}$) bias (Nei, 1978), GENETIX 4.05

- Ssa402*

Alleles(N)	Iff 02	Iffi 03	Iff 04	Iff 05	BUR	Allhatc	Allwild	Ätran	Lagan
150	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
151	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
152	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
159	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000
160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000
161	0.017	0.000	0.000	0.000	0.045	0.040	0.020	0.000	0.000
162	0.050	0.000	0.000	0.000	0.000	0.060	0.000	0.089	0.100
163	0.050	0.045	0.033	0.000	0.068	0.060	0.000	0.071	0.067
164	0.000	0.136	0.067	0.000	0.386	0.000	0.040	0.036	0.000
165	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.018	0.067
166	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.018	0.067
167	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.054	0.033
168	0.033	0.000	0.067	0.000	0.000	0.060	0.000	0.018	0.167
169	0.083	0.045	0.033	0.000	0.000	0.100	0.040	0.214	0.033
170	0.217	0.273	0.133	0.278	0.000	0.200	0.200	0.179	0.133
171	0.100	0.182	0.167	0.000	0.000	0.240	0.120	0.179	0.167
172	0.133	0.273	0.167	0.278	0.159	0.160	0.240	0.071	0.133
173	0.017	0.045	0.000	0.000	0.159	0.060	0.280	0.036	0.000
174	0.000	0.000	0.000	0.000	0.114	0.000	0.000	0.000	0.033
175	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000
176	0.033	0.000	0.033	0.000	0.045	0.000	0.000	0.000	0.000
183	0.200	0.000	0.200	0.444	0.000	0.000	0.000	0.000	0.000
N	14	10	7	3	8	10	8	13	11
H_{exp}	0.87	0.79	0.86	0.65	0.78	0.85	0.80	0.87	0.88
$H_{n.b.}$	0.88	0.83	0.89	0.69	0.80	0.87	0.82	0.88	0.91
H_{obs}	0.77	0.73	0.33	0.33	0.55	0.92	0.64	1.00	0.87

Tab.21 Alleles frequencies of Ssa402* locus. N. of observed alleles (N), observed (H_{obs}) and expected heterozygosity with (H_{exp}), and without ($H_{n.b}$) bias (Nei, 1978), GENETIX 4.05

- Ssa402**

Alleles(N)	Iff 02	Iffi 03	Iff 04	Iff 05	BUR	Allhatc	Allwild	Ätran	Lagan
190	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
193	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
200	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
201	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.089	0.000
202	0.067	0.000	0.033	0.111	0.023	0.000	0.080	0.125	0.033
203	0.083	0.091	0.200	0.222	0.182	0.040	0.000	0.196	0.133
204	0.150	0.091	0.167	0.222	0.455	0.100	0.080	0.214	0.167

Tab.22 Alleles frequencies of Ssa402** locus

Alleles(N)	Iff 02	Iffi 03	Iff 04	Iff 05	BUR	Allhatc	Allwild	Ätran	Lagan
205	0.117	0.136	0.167	0.056	0.250	0.180	0.260	0.232	0.400
206	0.050	0.045	0.033	0.111	0.023	0.020	0.020	0.071	0.033
207	0.017	0.000	0.067	0.000	0.000	0.200	0.020	0.000	0.067
208	0.017	0.091	0.033	0.000	0.000	0.100	0.060	0.000	0.000
209	0.033	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000
210	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
211	0.017	0.045	0.000	0.000	0.000	0.000	0.060	0.000	0.000
212	0.133	0.182	0.000	0.000	0.068	0.000	0.020	0.000	0.067
213	0.050	0.045	0.067	0.000	0.000	0.140	0.100	0.000	0.000
214	0.000	0.091	0.033	0.000	0.000	0.080	0.100	0.071	0.000
215	0.033	0.000	0.000	0.000	0.000	0.060	0.020	0.000	0.000
216	0.033	0.000	0.000	0.000	0.000	0.020	0.060	0.000	0.033
217	0.017	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000
218	0.017	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000
219	0.000	0.000	0.000	0.000	0.000	0.040	0.020	0.000	0.000
224	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.033
225	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
226	0.000	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.033
227	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000
230	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000
252	0.000	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000
254	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
255	0.017	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000
256	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
259	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
265	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
266	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
270	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
275	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
278	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
282	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
288	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
296	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	25	12	13	10	6	12	16	7	10
H_{exp}	0.92	0.90	0.88	0.86	0.69	0.87	0.88	0.83	0.78
$H_{n.b.}$	0.94	0.94	0.91	0.91	0.71	0.89	0.90	0.84	0.81
H_{obs}	0.97	1.00	0.87	1.00	0.95	0.84	0.84	0.71	0.80

Tab.22 (continued) N. of observed alleles (N), observed (H_{obs}) and expected heterozygosity with (H_{exp}), and without ($H_{n.b}$) bias (Nei, 1978), GENETIX 4.05

- Ssa411

Alleles(N)	Iff 02	Iffi 03	Iff 04	Iff 05	BUR	Allhatc	Allwild	Ätran	Lagan
256	0.000	0.000	0.000	0.222	0.000	0.000	0.000	0.000	0.000
259	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000	0.000
264	0.000	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000
265	0.033	0.227	0.000	0.000	0.000	0.000	0.000	0.000	0.000
266	0.117	0.000	0.000	0.056	0.159	0.140	0.100	0.089	0.267
267	0.233	0.045	0.067	0.000	0.068	0.140	0.140	0.536	0.500
268	0.133	0.455	0.733	0.444	0.318	0.060	0.340	0.036	0.067
269	0.083	0.000	0.000	0.000	0.023	0.240	0.200	0.000	0.033
270	0.200	0.136	0.000	0.000	0.182	0.000	0.060	0.036	0.067
271	0.000	0.000	0.000	0.056	0.045	0.220	0.000	0.071	0.000
272	0.067	0.045	0.033	0.056	0.045	0.080	0.020	0.179	0.067
273	0.017	0.091	0.100	0.167	0.114	0.000	0.020	0.054	0.000
274	0.000	0.000	0.000	0.000	0.000	0.120	0.120	0.000	0.000
275	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
280	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
283	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
N	11	6	5	6	9	7	8	7	6
H_{exp}	0.86	0.71	0.44	0.72	0.82	0.83	0.80	0.66	0.66
$H_{n.b.}$	0.87	0.74	0.46	0.76	0.84	0.85	0.81	0.67	0.69
H_{obs}	0.17	0.36	0.13	0.56	0.32	0.44	0.28	0.43	0.20

Tab.23 Alleles frequencies of Ssa411 locus. N. of observed alleles (N), observed (H_{obs}) and expected heterozygosity with (H_{exp}), and without ($H_{n.b}$) bias (Nei, 1978), GENETIX 4.05

- Ssa408

Alleles(N)	Iff 02	Iffi 03	Iff 04	Iff 05	BUR	Allhatc	Allwild	Ätran	Lagan
208	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000
209	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
214	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
216	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
217	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
219	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
220	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
221	0.033	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
225	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
228	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
230	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
231	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
234	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Tab.24 Alleles frequencies of Ssa408 locus

Alleles(N)	Iff 02	Iffi 03	Iff 04	Iff 05	BUR	Allhatc	Allwild	Ätran	Lagan
235	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
237	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.089	0.000
238	0.017	0.000	0.033	0.000	0.000	0.000	0.000	0.054	0.000
239	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.054	0.000
240	0.050	0.000	0.000	0.111	0.000	0.000	0.000	0.018	0.000
241	0.033	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000
242	0.033	0.000	0.033	0.000	0.000	0.000	0.000	0.018	0.067
243	0.033	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000
244	0.033	0.045	0.033	0.000	0.000	0.000	0.000	0.000	0.033
245	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
246	0.017	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000
247	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
248	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067
249	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000
250	0.000	0.091	0.000	0.056	0.000	0.000	0.040	0.000	0.000
251	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
252	0.000	0.000	0.000	0.111	0.000	0.000	0.000	0.071	0.000
253	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000
254	0.017	0.136	0.033	0.000	0.000	0.000	0.000	0.018	0.100
255	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.000
256	0.017	0.000	0.033	0.000	0.000	0.000	0.000	0.018	0.067
257	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
258	0.017	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000
259	0.000	0.045	0.033	0.000	0.000	0.000	0.000	0.018	0.000
260	0.033	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.033
261	0.000	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
262	0.050	0.000	0.067	0.000	0.000	0.000	0.000	0.071	0.000
263	0.017	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000
264	0.000	0.000	0.000	0.000	0.000	0.120	0.000	0.000	0.067
265	0.033	0.000	0.000	0.000	0.045	0.080	0.000	0.107	0.000
266	0.017	0.000	0.033	0.000	0.000	0.040	0.020	0.000	0.000
267	0.017	0.000	0.000	0.000	0.000	0.020	0.000	0.071	0.067
268	0.033	0.045	0.000	0.000	0.000	0.000	0.000	0.036	0.000
269	0.000	0.045	0.033	0.056	0.000	0.000	0.000	0.000	0.000
270	0.000	0.000	0.067	0.000	0.000	0.000	0.000	0.018	0.133
271	0.000	0.045	0.000	0.056	0.000	0.000	0.000	0.089	0.000
272	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000
273	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.033
274	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.033
275	0.017	0.045	0.033	0.000	0.000	0.000	0.000	0.018	0.000
276	0.000	0.000	0.000	0.056	0.000	0.000	0.020	0.000	0.033
277	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000	0.033
278	0.000	0.136	0.033	0.000	0.023	0.000	0.020	0.000	0.000
279	0.000	0.000	0.000	0.000	0.023	0.020	0.000	0.000	0.033

Tab.24 (continued)

Alleles(N)	Iff 02	Iffi 03	Iff 04	Iff 05	BURr	Allhatc	Allwild	Ätran	Lagan
280	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.000	0.033
281	0.017	0.000	0.033	0.056	0.023	0.000	0.000	0.000	0.000
282	0.033	0.000	0.000	0.000	0.023	0.000	0.020	0.000	0.033
283	0.000	0.000	0.000	0.000	0.023	0.000	0.020	0.000	0.067
284	0.017	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000
285	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000
286	0.017	0.091	0.000	0.000	0.000	0.040	0.000	0.000	0.000
287	0.017	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000
288	0.000	0.000	0.000	0.000	0.023	0.020	0.000	0.000	0.000
289	0.033	0.045	0.033	0.000	0.000	0.000	0.000	0.000	0.000
290	0.033	0.000	0.067	0.000	0.045	0.100	0.020	0.000	0.000
291	0.017	0.045	0.000	0.000	0.000	0.060	0.020	0.000	0.000
292	0.017	0.000	0.000	0.000	0.023	0.120	0.000	0.000	0.000
293	0.033	0.000	0.033	0.000	0.000	0.060	0.040	0.000	0.000
294	0.000	0.000	0.000	0.000	0.045	0.060	0.020	0.000	0.000
295	0.000	0.000	0.000	0.000	0.000	0.020	0.060	0.000	0.000
296	0.000	0.000	0.067	0.000	0.000	0.040	0.040	0.018	0.000
297	0.000	0.000	0.033	0.111	0.000	0.020	0.000	0.000	0.000
298	0.000	0.000	0.000	0.000	0.023	0.020	0.000	0.000	0.000
299	0.000	0.000	0.000	0.000	0.045	0.020	0.080	0.000	0.033
300	0.000	0.000	0.000	0.000	0.136	0.040	0.020	0.000	0.000
301	0.000	0.000	0.000	0.000	0.091	0.020	0.000	0.000	0.000
302	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000
304	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000
305	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000
306	0.000	0.000	0.000	0.000	0.023	0.000	0.060	0.000	0.000
307	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.000	0.000
308	0.000	0.000	0.000	0.000	0.068	0.000	0.000	0.000	0.000
312	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000
314	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000
316	0.000	0.000	0.000	0.000	0.023	0.000	0.060	0.000	0.033
318	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000
319	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.000	0.000
320	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000
322	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000
N	39	15	24	15	23	21	25	22	19
H_{exp}	0.97	0.92	0.95	0.93	0.93	0.93	0.93	0.93	0.93
$H_{n.b.}$	0.99	0.96	0.98	0.98	0.96	0.95	0.95	0.95	0.97
H_{obs}	0.90	1.00	0.93	0.89	0.77	0.64	0.88	0.86	0.80

Tab.24 (continued) N. of observed alleles (N), observed (H_{obs}) and expected heterozygosity with (H_{exp}), and without ($H_{n.b}$) bias (Nei, 1978), GENETIX 4.05

- Ssa202

Alleles(N)	Iff 02	Iffi 03	Iff 04	Iff 05	BUR	Allhatc	Allwild	Ätran	Lagan
223	0.000	0.000	0.033	0.056	0.091	0.000	0.000	0.000	0.067
229	0.117	0.045	0.067	0.000	0.068	0.000	0.000	0.054	0.200
230	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.054	0.000
232	0.067	0.182	0.100	0.056	0.023	0.000	0.000	0.000	0.033
233	0.000	0.000	0.000	0.056	0.045	0.000	0.000	0.018	0.000
234	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000
235	0.017	0.136	0.100	0.111	0.000	0.000	0.000	0.000	0.100
236	0.100	0.045	0.067	0.056	0.000	0.000	0.020	0.304	0.033
237	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.054	0.000
238	0.017	0.000	0.067	0.167	0.000	0.000	0.000	0.000	0.067
239	0.083	0.000	0.067	0.056	0.068	0.000	0.060	0.196	0.167
240	0.033	0.000	0.000	0.000	0.045	0.000	0.040	0.107	0.000
241	0.017	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000
242	0.083	0.045	0.067	0.000	0.114	0.140	0.000	0.000	0.067
243	0.017	0.000	0.000	0.000	0.045	0.040	0.120	0.107	0.033
244	0.000	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000
245	0.100	0.136	0.067	0.222	0.114	0.300	0.020	0.000	0.000
246	0.017	0.045	0.000	0.000	0.068	0.000	0.300	0.000	0.033
247	0.033	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000
248	0.083	0.045	0.000	0.167	0.068	0.220	0.000	0.000	0.000
249	0.017	0.000	0.033	0.000	0.091	0.120	0.100	0.054	0.067
250	0.000	0.000	0.000	0.000	0.068	0.020	0.060	0.036	0.033
251	0.150	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000
252	0.017	0.000	0.067	0.000	0.000	0.000	0.040	0.000	0.000
253	0.000	0.000	0.000	0.000	0.000	0.000	0.060	0.000	0.000
254	0.000	0.091	0.067	0.000	0.000	0.000	0.000	0.000	0.000
255	0.017	0.045	0.100	0.056	0.000	0.140	0.000	0.000	0.000
256	0.017	0.000	0.000	0.000	0.000	0.000	0.180	0.018	0.000
267	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
268	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100
N	19	13	15	10	14	8	11	11	13
H_{exp}	0.91	0.90	0.93	0.86	0.92	0.81	0.84	0.83	0.89
$H_{n.b.}$	0.93	0.94	0.96	0.92	0.94	0.82	0.86	0.85	0.92
H_{obs}	0.87	0.82	0.93	0.67	0.95	0.84	0.80	0.79	0.93

Tab.25 Alleles frequencies of Ssa202 locus. N. of observed alleles (N), observed (H_{obs}) and expected heterozygosity with (H_{exp}), and without ($H_{n.b}$) bias (Nei, 1978), GENETIX 4.05

7.3 Appendix 3

Assignment

Fisch	Pop	Ifftot	BUR	Allwild	Allhatc	Ätran	Lagan	Assigned Pop	
18	**Ifftot**	23.157	22.842	24.832	25.886	24.851	23.149	2	BUR
20	**Ifftot**	21.970	26.849	26.701	22.485	26.372	24.335	1	Ifftot
29	**Ifftot**	27.894	28.251	29.861	29.831	30.701	30.898	1	Ifftot
33	**Ifftot**	22.078	26.940	27.812	27.840	26.213	25.551	1	Ifftot
40	**Ifftot**	20.560	24.370	25.270	23.738	21.497	24.000	1	Ifftot
48	**Ifftot**	24.361	27.790	27.451	29.065	26.687	25.363	1	Ifftot
50	**Ifftot**	19.990	25.979	27.548	27.132	22.253	26.330	1	Ifftot
57	**Ifftot**	20.857	27.446	26.849	29.764	23.266	25.682	1	Ifftot
58	**Ifftot**	18.363	26.854	26.150	26.849	22.350	24.335	1	Ifftot
62	**Ifftot**	24.097	27.660	27.037	26.527	25.335	24.335	1	Ifftot
65	**Ifftot**	17.233	27.753	26.723	30.734	24.454	26.233	1	Ifftot
66	**Ifftot**	24.244	27.558	23.582	27.655	26.169	28.551	3	Allwild
69	**Ifftot**	22.724	27.173	25.733	27.095	24.266	22.062	6	Lagan
70	**Ifftot**	19.567	25.790	25.698	24.205	23.701	23.551	1	Ifftot
72	**Ifftot**	18.484	27.065	28.338	28.919	28.049	24.000	1	Ifftot
80	**Ifftot**	20.716	26.702	28.849	31.844	28.766	27.603	1	Ifftot
81	**Ifftot**	19.447	25.639	27.990	26.081	21.422	24.097	1	Ifftot
83	**Ifftot**	22.168	29.235	26.599	28.259	28.552	29.102	1	Ifftot
85	**Ifftot**	21.754	27.595	28.939	29.175	27.498	28.250	1	Ifftot
89	**Ifftot**	19.814	29.128	25.724	26.724	24.037	24.205	1	Ifftot
90	**Ifftot**	22.223	30.378	27.493	26.431	26.021	25.057	1	Ifftot
93	**Ifftot**	20.060	25.289	25.971	23.236	25.331	26.603	1	Ifftot
95	**Ifftot**	19.338	27.736	26.448	26.219	22.432	22.830	1	Ifftot
96	**Ifftot**	17.779	28.393	25.238	24.131	22.539	22.347	1	Ifftot
99	**Ifftot**	22.479	23.476	24.832	24.344	23.884	25.603	1	Ifftot
100	**Ifftot**	22.695	25.224	25.923	24.162	27.652	23.460	1	Ifftot
106	**Ifftot**	21.432	27.685	25.335	26.046	21.911	24.631	1	Ifftot
114	**Ifftot**	30.546	31.258	30.513	29.919	30.139	28.870	6	Lagan
116	**Ifftot**	28.272	28.586	28.882	29.192	27.049	27.138	5	Ätran
119	**Ifftot**	28.356	30.253	33.592	31.609	31.961	29.592	1	Ifftot
1	**Ifftot**	22.009	27.853	25.087	23.362	26.199	24.921	1	Ifftot
4	**Ifftot**	19.634	27.420	26.663	28.509	24.468	25.648	1	Ifftot
6	**Ifftot**	22.250	23.731	27.166	26.071	28.139	27.551	1	Ifftot
10	**Ifftot**	20.370	26.829	26.369	26.071	22.901	25.603	1	Ifftot
11	**Ifftot**	26.723	28.092	27.995	29.877	28.584	29.756	1	Ifftot
12	**Ifftot**	23.367	25.923	25.117	25.700	28.470	27.080	1	Ifftot
19	**Ifftot**	19.367	22.540	27.180	27.831	27.012	24.761	1	Ifftot
21	**Ifftot**	25.361	27.023	26.879	29.639	22.639	27.057	5	Ätran
41	**Ifftot**	23.186	27.914	25.020	27.293	23.946	25.539	1	Ifftot
48	**Ifftot**	22.213	25.030	24.184	25.101	23.952	23.398	1	Ifftot
51	**Ifftot**	20.996	24.032	23.837	25.925	25.961	24.876	1	Ifftot
5	**Ifftot**	25.594	27.959	27.626	30.530	31.731	28.296	1	Ifftot
6	**Ifftot**	16.286	23.289	23.786	23.372	28.389	25.205	1	Ifftot
7	**Ifftot**	21.369	25.600	25.865	26.798	23.901	25.789	1	Ifftot
13	**Ifftot**	29.454	32.703	32.212	31.990	29.107	31.102	5	Ätran

Tab.36 Assignment Values (With Leave One Out Option) GenAIEx 6.2 Log Likelihoods shown as positive the lowest value indicates the most likely population

Fisch	Pop	Ifftot	BUR	Allwild	Allhatc	Ätran	Lagan	Assigned Pop	
17	**Ifftot**	19.469	26.219	26.082	26.381	27.980	25.426	1	Ifftot
18	**Ifftot**	18.925	28.885	26.175	28.627	23.562	22.921	1	Ifftot
30	**Ifftot**	19.583	29.160	25.246	26.627	28.796	27.074	1	Ifftot
36	**Ifftot**	17.202	25.327	25.186	24.897	22.388	22.620	1	Ifftot
37	**Ifftot**	18.348	26.423	25.439	26.555	25.630	27.330	1	Ifftot
39	**Ifftot**	19.086	28.355	27.114	26.175	27.853	26.171	1	Ifftot
41	**Ifftot**	24.000	28.438	27.511	29.384	24.995	28.664	1	Ifftot
46	**Ifftot**	21.722	23.207	25.540	26.272	27.943	24.097	1	Ifftot
48	**Ifftot**	18.861	26.423	25.519	27.530	24.992	22.250	1	Ifftot
52	**Ifftot**	23.043	29.813	29.407	24.522	24.663	20.403	6	Lagan
53	**Ifftot**	26.478	29.396	28.127	29.400	30.886	29.057	1	Ifftot
5	**Ifftot**	22.890	30.265	28.893	28.042	29.047	25.222	1	Ifftot
14	**Ifftot**	27.808	32.703	32.513	33.893	31.167	31.926	1	Ifftot
18	**Ifftot**	19.475	25.538	26.971	27.178	25.685	28.108	1	Ifftot
19	**Ifftot**	19.384	23.402	27.397	25.817	28.175	28.330	1	Ifftot
20	**Ifftot**	17.352	26.586	28.813	26.337	27.373	24.375	1	Ifftot
21	**Ifftot**	18.382	25.845	25.175	25.344	23.941	23.620	1	Ifftot
22	**Ifftot**	21.000	24.872	24.636	24.691	23.177	24.955	1	Ifftot
40	**Ifftot**	20.885	30.417	29.513	28.025	27.201	25.523	1	Ifftot
46	**Ifftot**	20.068	25.886	26.121	26.877	25.266	24.210	1	Ifftot
1	**BUR**	27.245	20.539	26.015	25.081	24.261	25.256	2	BUR
2	**BUR**	26.577	14.794	24.180	24.256	25.698	22.307	2	BUR
4	**BUR**	23.845	13.075	23.214	23.691	26.795	25.256	2	BUR
6	**BUR**	23.678	15.752	23.804	24.237	24.521	23.733	2	BUR
7	**BUR**	24.558	15.306	25.237	24.316	27.423	24.733	2	BUR
8	**BUR**	23.810	12.769	22.600	23.839	26.407	24.557	2	BUR
9	**BUR**	25.020	18.709	27.344	23.710	28.554	25.858	2	BUR
10	**BUR**	23.629	16.752	21.077	24.821	25.261	23.608	2	BUR
11	**BUR**	26.832	17.228	24.436	22.746	23.662	26.824	2	BUR
12	**BUR**	26.614	17.698	22.662	27.002	25.595	26.727	2	BUR
13	**BUR**	24.820	24.560	23.190	25.242	24.590	25.153	3	Allwild
14	**BUR**	25.784	19.366	23.929	24.390	26.595	26.949	2	BUR
19	**BUR**	25.116	19.156	23.971	26.770	23.490	23.636	2	BUR
21	**BUR**	26.064	18.998	24.084	24.147	27.101	22.937	2	BUR
22	**BUR**	24.449	16.403	24.723	25.050	26.972	25.682	2	BUR
23	**BUR**	26.199	16.813	26.678	24.044	27.040	23.682	2	BUR
24	**BUR**	28.874	16.113	27.166	27.548	28.731	27.153	2	BUR
25	**BUR**	26.863	13.775	27.280	25.365	28.322	25.557	2	BUR
26	**BUR**	25.885	13.863	23.980	24.024	26.148	20.501	2	BUR
28	**BUR**	25.926	17.772	24.424	25.198	26.188	25.000	2	BUR
29	**BUR**	27.097	19.527	20.895	22.422	21.852	22.840	2	BUR
30	**BUR**	26.892	20.512	22.172	22.312	25.701	22.539	2	BUR
1	**Allwild**	27.628	25.435	18.425	21.562	21.618	26.676	3	Allwild
2	**Allwild**	25.053	23.793	18.166	23.703	22.863	25.852	3	Allwild
3	**Allwild**	26.363	26.472	20.398	26.821	27.171	24.256	3	Allwild
4	**Allwild**	25.901	26.342	22.170	25.652	24.893	26.284	3	Allwild
5	**Allwild**	23.221	21.376	16.764	23.317	26.383	24.904	3	Allwild
6	**Allwild**	24.631	22.399	17.058	24.159	21.539	22.631	3	Allwild
7	**Allwild**	29.271	25.422	21.670	27.937	24.563	20.636	6	Lagan
8	**Allwild**	27.534	23.232	15.988	23.979	23.449	21.506	3	Allwild

Tab.36 (continued)

Fisch	Pop	lfftot	BUR	Allwild	Allhatc	Ätran	Lagan	Assigned Pop	
9	**Allwild**	28.971	24.185	16.485	23.805	24.584	21.858	3	Allwild
10	**Allwild**	27.002	22.586	18.176	24.766	23.016	21.751	3	Allwild
11	**Allwild**	31.278	24.669	18.569	19.540	26.847	28.676	3	Allwild
12	**Allwild**	27.814	25.602	22.892	23.663	27.487	28.108	3	Allwild
13	**Allwild**	24.233	25.970	20.451	25.214	23.750	23.904	3	Allwild
14	**Allwild**	28.989	25.865	20.363	24.994	26.609	24.353	3	Allwild
15	**Allwild**	27.922	24.043	19.026	23.522	25.283	26.824	3	Allwild
16	**Allwild**	28.283	19.442	19.508	20.902	25.812	24.222	2	BUR
17	**Allwild**	27.194	27.708	20.110	23.390	24.357	25.460	3	Allwild
18	**Allwild**	24.214	27.072	22.414	23.566	25.591	24.983	3	Allwild
20	**Allwild**	27.088	22.937	15.856	25.861	23.648	24.824	3	Allwild
21	**Allwild**	28.670	29.718	17.067	24.362	27.966	27.409	3	Allwild
23	**Allwild**	27.718	23.822	19.010	24.282	25.509	22.955	3	Allwild
24	**Allwild**	27.935	28.150	21.039	25.615	24.963	24.955	3	Allwild
26	**Allwild**	23.149	29.105	20.410	26.293	26.964	25.284	3	Allwild
27	**Allwild**	24.310	21.133	17.769	23.930	25.042	25.256	3	Allwild
29	**Allwild**	28.239	29.892	23.983	21.805	27.246	25.807	4	Allhatc
1	**Allhatc**	27.576	27.572	22.511	19.081	25.806	28.205	4	Allhatc
2	**Allhatc**	30.360	28.996	23.964	18.304	27.215	25.761	4	Allhatc
3	**Allhatc**	24.681	26.633	23.263	21.809	26.926	23.511	4	Allhatc
4	**Allhatc**	27.600	27.678	26.821	18.250	28.788	25.066	4	Allhatc
5	**Allhatc**	25.603	25.991	24.316	17.379	24.854	24.225	4	Allhatc
6	**Allhatc**	25.935	24.414	20.732	16.617	26.243	26.284	4	Allhatc
7	**Allhatc**	26.310	30.031	24.344	15.354	26.313	22.850	4	Allhatc
8	**Allhatc**	26.706	27.502	23.520	18.584	27.498	26.385	4	Allhatc
9	**Allhatc**	26.247	24.134	22.094	15.309	27.243	24.432	4	Allhatc
10	**Allhatc**	24.151	28.984	24.740	21.130	29.400	23.987	4	Allhatc
11	**Allhatc**	25.170	23.821	23.812	18.182	26.516	23.913	4	Allhatc
12	**Allhatc**	25.971	21.823	25.687	20.615	26.592	23.714	4	Allhatc
13	**Allhatc**	25.388	27.170	26.203	19.605	23.896	24.760	4	Allhatc
14	**Allhatc**	27.700	29.443	30.706	15.908	25.936	25.104	4	Allhatc
15	**Allhatc**	27.656	26.500	26.247	17.854	26.812	29.330	4	Allhatc
16	**Allhatc**	27.046	27.754	28.923	19.597	26.801	26.250	4	Allhatc
17	**Allhatc**	27.978	27.753	28.354	21.677	27.820	27.302	4	Allhatc
18	**Allhatc**	28.606	22.555	25.538	18.979	26.599	27.426	4	Allhatc
19	**Allhatc**	23.408	22.428	21.503	17.396	24.947	23.575	4	Allhatc
20	**Allhatc**	24.440	23.315	21.230	18.649	24.674	18.978	4	Allhatc
21	**Allhatc**	25.229	20.990	22.964	18.394	28.051	24.273	4	Allhatc
22	**Allhatc**	24.803	24.583	21.813	17.843	24.373	24.648	4	Allhatc
23	**Allhatc**	25.631	26.688	26.008	19.906	24.956	25.523	4	Allhatc
24	**Allhatc**	27.842	22.988	20.101	20.759	26.051	25.807	3	Allwild
26	**Allhatc**	25.793	24.674	22.454	19.326	22.264	25.153	4	Allhatc
1	**Ätran**	25.694	24.266	26.300	22.495	20.810	20.811	5	Ätran
2	**Ätran**	25.596	27.996	24.017	24.821	22.359	25.720	5	Ätran
3	**Ätran**	24.325	26.803	28.016	25.909	17.178	20.113	5	Ätran
4	**Ätran**	24.815	25.381	24.240	25.937	18.558	20.478	5	Ätran
5	**Ätran**	24.502	26.000	25.955	27.812	18.451	22.886	5	Ätran
6	**Ätran**	25.704	27.401	24.709	24.824	20.196	19.409	6	Lagan
8	**Ätran**	25.843	26.918	25.666	27.689	17.746	23.153	5	Ätran
10	**Ätran**	22.617	24.993	23.605	26.919	18.121	21.567	5	Ätran

Tab.36 (continued)

Fisch	Pop	Ifftot	BUR	Allwild	Allhatc	Ätran	Lagan	Assigned Pop	
11	**Ätran**	24.500	26.300	25.344	29.141	19.480	25.676	5	Ätran
12	**Ätran**	27.125	28.128	24.316	27.289	17.086	21.141	5	Ätran
13	**Ätran**	24.717	28.456	29.162	25.749	20.203	22.733	5	Ätran
14	**Ätran**	20.579	25.647	26.274	28.303	22.296	24.472	1	Ifftot
15	**Ätran**	25.967	26.224	24.696	24.918	21.617	24.335	5	Ätran
16	**Ätran**	28.556	29.383	24.705	27.201	21.635	27.358	5	Ätran
17	**Ätran**	22.611	26.100	27.328	27.121	19.683	23.932	5	Ätran
18	**Ätran**	27.314	29.841	25.587	28.422	19.887	24.965	5	Ätran
19	**Ätran**	27.983	31.813	24.627	27.414	21.654	26.835	5	Ätran
20	**Ätran**	26.523	25.993	25.900	27.521	17.664	25.437	5	Ätran
21	**Ätran**	24.749	25.393	22.366	22.490	18.133	23.631	5	Ätran
22	**Ätran**	25.584	23.582	22.766	24.624	19.037	24.454	5	Ätran
24	**Ätran**	23.463	23.188	21.484	24.532	15.907	24.233	5	Ätran
25	**Ätran**	25.286	22.520	22.669	24.925	18.834	22.949	5	Ätran
26	**Ätran**	26.349	24.421	23.426	26.139	16.752	23.347	5	Ätran
27	**Ätran**	26.490	27.197	24.748	27.849	15.359	26.153	5	Ätran
28	**Ätran**	23.399	23.696	21.167	23.064	16.013	22.824	5	Ätran
29	**Ätran**	25.885	25.180	24.668	24.097	21.717	22.177	5	Ätran
30	**Ätran**	23.169	25.831	21.135	25.481	16.559	22.432	5	Ätran
31	**Ätran**	24.635	26.051	23.952	25.112	16.461	23.426	5	Ätran
1	**Lagan**	25.993	24.475	28.371	23.353	24.480	18.178	6	Lagan
2	**Lagan**	24.125	27.350	24.992	23.824	22.280	17.165	6	Lagan
3	**Lagan**	24.792	27.962	25.867	22.183	23.937	17.430	6	Lagan
5	**Lagan**	24.625	25.708	22.795	23.370	22.815	15.879	6	Lagan
6	**Lagan**	22.711	26.683	28.399	24.029	23.860	15.319	6	Lagan
7	**Lagan**	24.635	26.503	28.195	24.125	25.754	18.290	6	Lagan
8	**Lagan**	24.418	22.562	27.502	24.529	25.056	19.618	6	Lagan
9	**Lagan**	22.941	23.110	25.443	24.398	22.637	19.595	6	Lagan
10	**Lagan**	23.108	26.156	26.823	24.518	22.830	18.206	6	Lagan
11	**Lagan**	25.092	25.490	20.478	26.754	18.496	22.625	5	Ätran
12	**Lagan**	23.173	22.790	22.883	24.310	26.543	26.984	2	BUR
14	**Lagan**	22.293	28.549	25.388	23.520	23.641	20.568	6	Lagan
16	**Lagan**	23.968	24.502	22.552	25.689	17.676	20.666	5	Ätran
17	**Lagan**	29.184	26.080	19.469	28.840	24.438	26.591	3	Allwild
18	**Lagan**	26.876	23.763	22.470	23.106	25.726	26.070	3	Allwild

Tab.36 (continued)

		Allhatc	Allwild	Ätran	Lagan	BUR
2002	Fish	AIc	AIc	AIc	AIc	AIc
Iffezheim	18	-0.2139	0.0325	-0.3078	0.0373	-0.0903
Iffezheim	20	0.4330	0.1096	-0.3082	-0.4253	0.5400
Iffezheim	29	0.9020	-0.2432	-0.7820	-0.2132	-0.2465
Iffezheim	33	-0.0684	-0.8884	-0.3278	-0.2118	-0.6824
Iffezheim	40	-0.0811	0.3718	-0.1677	-0.1083	-0.1253
Iffezheim	48	0.6895	0.1297	-0.0233	-0.3797	-0.4063
Iffezheim	50	1.0816	0.0228	-0.3814	-0.2056	0.1568
Iffezheim	57	0.7311	0.3041	-0.3022	0.3084	-0.0339
Iffezheim	58	0.3386	-0.1424	-0.1075	-0.3751	-0.5062
Iffezheim	62	0.3902	0.9149	0.1626	-0.0492	0.0520
Iffezheim	65	-0.1498	-0.0089	0.0028	-0.5037	-0.2429
Iffezheim	66	0.0166	0.0255	-0.2744	-0.2588	0.1887
Iffezheim	69	0.5757	-0.1405	-0.1653	0.0378	-0.1139
Iffezheim	70	0.6079	-0.0399	0.2168	0.2100	-0.4383
Iffezheim	72	0.7194	-0.9524	-0.3542	-0.2012	-0.7757
Iffezheim	80	0.9067	-0.1775	-0.3451	0.4823	-0.0563
Iffezheim	81	0.2710	0.2066	-0.0895	-0.4288	-0.1145
Iffezheim	83	0.4766	-0.0137	-0.4326	0.7781	-0.2961
Iffezheim	85	-0.0180	-0.3731	-0.6847	-0.1391	-0.2723
Iffezheim	89	-0.0376	0.4736	0.3466	0.3186	0.1292
Iffezheim	90	0.5345	0.4283	0.3335	0.5606	0.1533
Iffezheim	93	0.3758	0.1481	0.1732	-0.1507	0.0036
Iffezheim	95	-0.3679	0.1813	-0.2447	-0.2400	-0.8675
Iffezheim	96	0.2591	0.7782	0.1320	0.5224	-0.2349
Iffezheim	99	0.3471	-0.1787	-0.0354	-0.2801	0.6566
Iffezheim	100	0.4703	0.6966	0.0480	-0.2689	-0.4010
Iffezheim	106	-0.3022	-0.1840	0.2542	0.1928	0.0820
Iffezheim	114	-0.4431	-0.1295	-0.3593	-0.0284	0.4861
Iffezheim	116	-0.0496	-0.4441	-0.3715	-0.0322	0.0778
Iffezheim	119	0.1895		0.8794	0.4471	0.5346
2003						
Iffezheim	1	0.3627	0.1714	-0.1845	0.3905	0.0655
Iffezheim	4	0.3849	0.1814	-0.4317	0.1342	0.6379
Iffezheim	6	0.4204	0.7307	-0.0636	-0.2752	-0.1850
Iffezheim	10	0.4832	-0.3226	-0.3425	0.0307	0.0436
Iffezheim	11	-0.4318	0.4725	0.4774	-0.2876	0.0623
Iffezheim	12	-0.1033	0.3552	-0.9917	-0.8577	-0.0800
Iffezheim	19	0.6523	-0.3100	-0.3859	-0.2686	-0.5047
Iffezheim	21	-0.1741	-0.5651	-0.3414	-0.0260	0.2185
Iffezheim	41	-0.0495	-0.1574	-0.2077	-0.3611	-0.1859
Iffezheim	48	0.1124	0.3607	0.1773	0.1085	0.3823
Iffezheim	51	0.3486	-0.2152	0.0733	-0.3285	0.1873
2004						
Iffezheim	5	-0.6605	-0.0033	-0.7302	-0.4023	0.5491
Iffezheim	6	-0.2312	-0.3873	-1.0394	-0.3727	-0.1022
Iffezheim	7	-0.4347	-0.0706	-0.1169	-0.4274	0.3669
Iffezheim	13	-0.4912	-1.0213	0.0550	0.5825	0.6974

Tab.40 individuals assignment used for age interpretation (GenoAssign 1.0, M.Wang)

		Allhatc	Allwild	Ätran	Lagan	BUR
2004	**Fish**	**Alc**	**Alc**	**Alc**	**Alc**	**Alc**
Iffezheim	17	-0.0671	0.8732	-0.9006	-0.1297	0.5915
Iffezheim	18	-0.6173	0.3765	-0.3931	0.0849	-0.3829
Iffezheim	30	-0.2771	0.0309	-0.3645	-0.0881	0.8924
Iffezheim	36	-0.0189	0.4732	0.1469	0.0533	0.2275
Iffezheim	37	0.1850	0.5490	-0.2546	-0.6248	0.6991
Iffezheim	39	-0.0065	0.2676	-0.4163	-0.2338	1.0985
Iffezheim	41	0.0067	-0.5930	-0.3492	-0.8213	-0.3671
Iffezheim	46	-0.0110	0.4937	-0.0052	0.2005	-0.2072
Iffezheim	48	-0.4232	0.4634	-0.3088	-0.1171	0.0322
Iffezheim	52	0.0898	0.7438	-0.1947	0.1817	0.0139
Iffezheim	53	-0.0025	0.5047	-0.8203	-0.1842	0.4272
2005						
Iffezheim	5	-0.1663	0.4100	0.5075	-0.0707	0.0449
Iffezheim	14		-1.0213	0.7647	-0.1766	1.0807
Iffezheim	18	0.8701	0.0467	-0.3265	0.4304	0.0028
Iffezheim	19	-0.1590	0.2587	-0.2441	-0.1507	-0.0949
Iffezheim	20	0.1519	-0.1277	-0.4889	-0.1386	0.0156
Iffezheim	21	-0.0439	0.1923	-0.0181	-0.0006	0.3488
Iffezheim	22	-0.1134	0.1543	-0.2724	-0.5218	0.0294
Iffezheim	40	0.2278	0.3587	0.2596	0.1570	0.6591
Iffezheim	46	0.3081	-0.2266	-0.0624	-0.3271	-0.2923

Tab.40 (continued)