Genetic variation in asp (Aspius aspius) in Lake Mälaren and effects of turbidity and oxygen depletion in asp roe

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Abstract

This thesis concerns three aspects of reproduction in asp (*Aspius aspius*). Migrating fish species often have a homing behavior and form subpopulations which are reproductively isolated from each other. Microsatellite analysis in three loci revealed no genetic differentiation between the asp populations of River Örsundaån and River Sävjaån. The result signifies that the asp population of Lake Ekoln, a sub basin of Lake Mälaren, should be managed as one population. A preference for clear water is often associated with the asp, yet rivers outside Uppsala where asp is known to spawn have a high turbidity. Egg preference for water quality with regard to turbidity was tested by adding two different amounts of clay (60 mg l⁻¹ and 220 mg l⁻¹) into aquaria containing asp roe. There was no effect of suspended silt particles on the survival of the eggs. The reason for fish to migrate upstream to spawn is unclear. One reason for the migration could be the high oxygen saturation in fast flowing streams caused by the constant mixing of the water column. The third part addresses the question whether low oxygen saturation affects egg survival. The question was tested by placing roe in aquaria which were subsequently bubbled with nitrogen gas 6-10 hours every day to lower the oxygen saturation of the water. Even though the oxygen saturation did not go below 25.5 % all the eggs were dead after one week, suggesting that egg survival is dependent on high oxygen saturation.

Introduction

Stream spawning species of fish are known to have an ability to ‘home’ – return to their natal river to spawn. The ability to home has been extensively studied in salmonid species. In fisheries it has been known for some time that a population may be composed of several reproductive isolated subpopulations, corresponding independently to harvest and managing activities. The homing behaviour limits gene flow between populations, promoting the formation of genetically distinct populations. Selection experienced by subpopulations may cause them to form local adaptations, both genetic and phenotypic, to the environmental conditions in natal rivers (Wenburg, 1998). The ability to home has also been demonstrated in the cyprinid roach (*Rutilus rutilus*). The species homed with a high precision, minimizing migration between subpopulations (Labée-Lund et al., 1985). It is not unlikely that the riverine cyprinid species asp has a homing behaviour like the roach. The asp is classified as vulnerable (VU) by Artdatabanken. However, if the species consist of several smaller populations restricted to different tributaries the species might be even more threatened because of the frequent bottlenecks experienced due to regular extinction and recolonization events (Frankham et al., 2004). A small subpopulation holds a smaller genetic variation than a large population and the lack of genetic variation may lead to inbreeding depression which can increase the extinction rate. Smaller populations are also generally more sensitive to environmental changes and if the spawning fails due to detrimental environmental changes during a short period of time the population might possibly go extinct. If the asp has a homing behaviour it is important to create migration routes along all streams where asp is known to spawn in order to retain and strengthen the metapopulation of the fish in Lake Mälaren. Since a larger number of subpopulations can hold a greater genetic variation it is valuable to protect subpopulations of a differentiated species. I tested whether the asp in Lake Mälaren has a homing behaviour with the H₀ hypothesis that there is no genetic differentiation between populations.
High turbidity is often a threat to the survival of eggs and fry in salmonid species. In running water high turbidity is normal at times with high precipitation but the turbidity is also dependent on the management of fields, clear-cut forest and ditching. These are activities that can severely threaten the health of fish (Degerman et al, 1998). Silt is known to lower the survival of salmon eggs by preventing oxygen flow through the chorion of the eggs (Soulsby et al, 2001). For the asp a preference for clear water is mentioned in the literature (Curry-Lindahl, 1985, Art databanken, 2006). This however is contradicted by observations made during inventories of asp in Uppsala where asp were seen spawning in water with high turbidity. I tested in a laboratory study the effects of different levels of turbidity on egg survival. The hypothesis was that the presence of inorganic clay particles suspended in the water would lower the percentage of eggs hatched.

Many species of fish migrate from lakes to spawn in streams. The reason for this migration is uncertain. Two explanations could be that predation pressure is lower in streams, or that oxygen demand of the roe is too high for the eggs to survive in lakes where oxygen saturation can be lower at times with high respiration rate or standing water. Therefore a small stream where the water is turbulent and the gas exchange with the atmosphere is high might be a suitable place for fish to spawn (Allan, 1995). The demand of dissolved oxygen levels varies among life stages of a species. The fish embryo consumes the most oxygen just before hatching (Czerkies, 2002). A study in embryonic rainbow smelt (osmerus mordax) showed that oxygen levels below 1,9 mg L⁻¹ reduced the survival of the embryo to 0 %. In Carp (Carpus carpio) a oxygen level of 6 mg L⁻¹ was sufficient to reduce the survival to zero (Fuda et al, 2007). To test the oxygen demand of embryonic asp laboratory experiments were carried out. The hypothesis was that egg survival would be lower in oxygen depleted aquaria than in aquaria with high oxygen saturation.

In order to strengthen the asp population it is important to know about factors affecting recruitment and differentiation of the population. Factors affecting the environment in the spawning locations are likely to affect recruitment. If the environmental conditions in the streams are too poor for egg development it can have detrimental effects on recruitment and thereby the size of the population. Fundamental facts like homing behaviour is important to know to facilitate conservation and management of a threatened species.

**Methods**

**Study species**

The asp in Sweden is vulnerable to changes in the environment since it is living on the edge of its geographical range. It is one of the largest species of the cyprinid family with a maximum length exceeding 1 m. In contrast to other cyprinids, the asp is piscivorous. When the fish has reached a size of around 20-30 cm it switches from a diet consisting of plankton and benthic fauna to one consisting mainly of fish. The asp is fairly similar to other cyprinid species but can be recognised by its large mouth and under bite. Although it feeds on fish the asp has no teeth. The scales are silvery, often darker closer to the body, and shift in colour towards golden as the fish ages. The pectoral, pelvic and anal fins have a reddish tone. The cordal peduncle is sturdy and the tailfin is large.

Lake Mälaren is known to be inhabited by asp. In spring during spawning the fish can be seen upstream in several of the inlets. The spawning starts when the temperature of the water has reached about 6°C (Berglund, 2006). A single female can release 50 000-500 000 eggs into
the streaming water. The roe then attaches to the bottom substrate consisting of stones and gravel or macrophytes like common water moss (Fontinalis sp.). The roe hatches after 2-3 weeks, depending on the water temperature. The asp have been thought also to spawn on shoals in lakes (Artdatabanken, 2006), however, this has yet to be confirmed.

During the last 50 years a reduction in the asp population has been observed in Sweden, probably due to habitat degradation and obstructions in streams preventing the asp from reaching spawning locations further upstream (Artdatabanken, 2006). In recent years the population seems to have stabilised however, reduced in size. Perhaps due to the low commercial value, knowledge about the species is scarce. Therefore, it is also placed in the category data deficient (DD), meaning that knowledge about the species is too scarce for any accurate estimation to be made about the risk of extinction the species might be facing. Therefore, it is important that the knowledge increases about the species such that we would know how it can be efficiently protected it in the future.

Area description

Five spawning locations in two streams in Uppsala: River Örsundaån and River Sävjaån, were studied (Figure 1). The River Örsundaån drains an area of 734 km² consisting mainly of forest (52 %) and arable land and meadows (42 %). Due to the sparse population of the area River Örsundaån is largely affected by nutrients from discharge of individual sewage (Mohlin, 2005). The spawning location is the longest in Uppsala, stretching 250 m downstream from the dam in Vånsjöbro (Berglund 2006). The river bed of the upper section of the stream is covered with large boulders and stones while the river bed in the lower section is covered in gravel and clay. There is some cover of water moss on the stones and common club-rush (Schoenoplectus lacustris) on the edges of the stream. The location is partly shaded by trees.

River Sävjaån is situated east of Uppsala and runs west where it flows into River Fyrisån and further into Lake Ekoln, a sub-basin of Lake Mälaren (Figure 1). Due to forest and discharge of private sewage in the catchment area the river is eutrophic. In the spawning location in Funbo the bottom substrate consist of stones and gravel. The stones are often covered with common water moss (Fontinalis sp.) Funboån is the only river in the River Fyrisån catchment area that does not have obstructions in the stream (Länsstyrelsen, 2004). There are 15 spawning locations along the River Sävjaån (Berglund, 2006) of which four were chosen for sampling of eggs for testing of homing ability. At Funbo kyrka the spawning location starts under the bridge and extends 100 m downstream. The location is not shaded by trees and the vegetation consists mainly of reed and common club-rush. The Spångtorp location is situated downstream from Funbo kyrka. The Spawning area is 50 m long and the river bed is covered with stones and gravel that in some places are covered by common water moss. The vegetation also consists of common club-rush but no trees shade the water. Further downstream from Spångtorp is Falebro, close to a road bridge. The river bed is covered with stones and boulders and the vegetation around the stream consists of reed, common club-rush and common water moss. The location is only 20 m in length. Just downstream from Falebro is the fourth location, situated under a railway bridge in Åby. There is some shading from the bridge and the vegetation is similar to Falebro. The spawning location is 25 m long.

Genetic variation
Roe was collected from River Örsundaån (Vånsjöbro) 25th April 2007 and from the four spawning sites in River Funboån: Funbo kyrka (18th April 2007), Spångtorp (26th April 2007), Falebro and under the railway bridge in Åby (27th April 2007).

Figure 1: Map showing the spawning locations in River Örsunda and river Sävjaån.

The roe was collected from the bottom substrate by picking up stones and plants to which the roe had adhered. The sampling stations were spread out along the stream about 10 m apart to ensure that roe was collected from several individuals. Several eggs were collected from each sampling station. From the Spawning location in River Örsundaån 31 eggs were analysed, 8 eggs were analysed from the Funbo location, 12 from Spångtorp, 5 from Falebro and 8 from Åby. Eggs were stored in eppendorf tubes with 95% ethanol. The species of the eggs was determined by comparing the gene ATP 6 and 8 of the eggs with other cyprinid species native to Sweden. DNA was then analysed for kinship between individuals using four microsatellite markers.

To prepare the eggs for sequencing, DNA was extracted using the chelex method. Asp roe was homogenized in an eppendorf tube together with a 5% chelex solution and then incubated at 56˚C for 45 minutes in a water bath. The tubes were then vortexed for 10 seconds and then heated at 98˚C in a constant temperature block for 15 minutes. The samples were vortexed again before being centrifuged at 13000 rpm for 5 minutes to pellet chelex beads and excess waste from the eggs. The supernatant was transferred to a clean tube and stored at 4˚C. For the amplification of the locusts ATP 6 and 8 the PCR program the TCON22 (Eppendorf) was used. The program consisted of 3 min denaturation at 94˚C followed by 38 cycles of: 15 seconds at 94˚C, 15 seconds at 51˚C and 45 seconds at 72˚C. The reaction finally ended with 3 minutes at 72˚C. The reaction was carried out using a primer pair: TLF (10 µM) and TC550R (10 µM) which gives 500 base pair-fragments of DNA. The PCR product was sequenced by Macrogen Inc. of South Korea. The DNA-sequences were analysed using the software Sequencing analysis 3.3 and Auto assembler 2.1. The DNA-sequences was transferred to a text file and then compared with other cyprinid species using the software DAMBE.exe.

Four microsatellites were isolated from the asp roe. For the amplification of locusts with the primers mfw 1, 7, 10 and 18 the following amplification programme was carried out: 10 minutes at 95˚C followed by 35 cycles of 20 seconds at 95˚C, 45 seconds at the primer specific temperature 51˚C for mfw1, 53˚C for mfw 7 and 10 and 47˚C for mfw 18, 90 seconds
at 72°C and finishing with extension for 10 minutes at 72°C. 1 µl of the PCR-product was mixed with 0.25 µl Tamra 500 (Applied biosystems) and 0.90 µl Formamide loading dye (Amersham Biosciences) and then separated on a 5 % acryl amide gel on an ABI 377 sequencer (Applied biosystems). The microsatellite sequences were then analysed using the computer software Gene scan 3.2.1 and Genotyper 2.1.0.

Several statistics software were used to analyse the results given by the microsatellite analysis:

Genepop on the web 3.4:
- The microsatellite length was first transferred into Genepop on the web 3.4 to compare observed and expected heterozygosity in each population. Alleles which were only present in one or two individuals were discarded from the data set. The results were then used to calculate if the population was in Hardy Weinberg equilibrium by using a Chi square test.
- The populations were checked for linkage disequilibrium using Fisher’s method,
- Population differentiation. An unbiased estimate of the p-value of a log-likelihood (G) based exact test was performed. All pairs of populations were compared for all loci. The null hypothesis tested was: "the genotypic distribution is identical across populations" (Table 2).
- Population fragmentation (FST). The degree of inbreeding was used to measure the degree of differentiation between the fragments. Reproductively isolated subpopulations that are differentiated from the total population can be analysed using FST (Table 4). FST can take on values between 0 and 1 and the value is inversely related to dispersal ability of the population. According to this method one single migrant is sufficient to prevent a complete differentiation of populations. This conclusion assumes that migrants and residents are equally likely to produce offspring. This is often not the case since the migrant have a much lower fitness than the residents of the area. In nature about 10 migrants per generation is required to prevent differentiation (Frankham et.al.).
- Migration between spawning areas. Migration was determined by Nm which is the number of migrants successfully entering a population per generation.

Microchecker 2.2.3:
- Null alleles were distinguished in the populations as a result of heterozygote deficiency.

PCA-GEN 1.2.1:
- PCA analysis (Principal Components Analysis) shows the relative genetic differences between the spawning areas. The technique reduces a multidimensional data set to lower dimensions by projecting the plotted data onto a one dimensional plane. A coordinate system was produced in which the populations were displayed relative to each other. The data was transformed so that it was expressed in terms of the patterns between them. Populations which were situated closer to each other in the coordinate system were genetically similar and vice versa (Smith, 2002, Figure 3).

Effects of turbidity

Roe was sampled on the 26th of April 2007 from River Funbo (Figure 2). 15 stones with adhered roe was picked up and brought back to the lab to be placed in 12 aquariums holding 30 L of tap water. The aquaria were exposed to fluorescent lights controlled by a timer to simulate night and day. All of the aquaria were bubbled with air to make sure that oxygen saturation was high. The water was 90 % saturated with oxygen. The aquaria were divided
into three different treatments with four replicates for each treatment: Clear water, low turbidity and high turbidity. In the turbid aquaria silt particles (red clay commonly used in ceramics) were added to tap water in two different concentrations; 60 mg l\(^{-1}\) ww. and 225 mg l\(^{-1}\) ww. respectively, hereafter referred to as treatments 1 and 2. The eggs on the stones were counted shortly after introduction into the aquaria, and were then monitored throughout the experiment until all live eggs had hatched. The amount of vegetation covering the stones was estimated and varied between none to up to 90 % vegetation coverage. On the stones with extensive vegetation cover some or all of the eggs were attached to the algae or moss. The survival rate was defined as the proportion of eggs hatched as compared to the total number of eggs introduced into each aquarium. The proportions hatched between the different groups, clear, low turbidity and high turbidity was arc sin-transformed and the difference in survival was analysed by ANOVA. ANOVA assumes a normal distribution and equal variance of the populations.

**Effects of oxygen deficiency**

Roe was collected from River Funbo (figure 2), 26\(^{th}\) April 2007, by collecting stones from the stream to which eggs had adhered. The eggs were put into 4 aquariums each containing 20 L of oxygenated tap water. The eggs were left over night to acclimatise and the experiment started on the 27\(^{th}\) April. To see if the roe could survive in oxygen depleted environments the oxygen content was lowered in the four aquariums by bubbling the water with nitrogen gas. The oxygen content was decreased to around 30 % saturation 6-10 hours per day. The water was then bubbled with air thus increasing the oxygen saturation. The aquaria were monitored throughout the experiment and the number of eggs adhered to the stones was observed. The number of white (dead), moldy or empty eggs were counted. The aquaria contained tap water and were bubbled continuously with air. Due to lack of room the control aquaria were situated in a separate room from the oxygen depleted aquaria. Unlike the test group the control group did not receive any daylight but was exposed to light from fluorescent lamps controlled by a timer to simulate night and day. The oxygen content of the water was measured to be of 90 % saturation. Treatment differences were compared using Mann Whitney U-test. The Mann
Whitney U-test is non-parametric and compares medians of two unmatched samples. The U-test was chosen because it is suitable for small sample sizes and may be used for as few as four observations in each sample (Fowler et al. 1998). Moreover, the test can be used for data which are not normally distributed. As a comparison between egg survival in streams and lakes 6 stones was placed in the spawning location in Funbo a few days before the spawning. After the spawning the stones with adhered roe were placed in cages to protect the eggs from predation. The stones were then moved to Lake Funbosjön and placed in a wind sheltered area close to the shore. The stones were monitored with regard to egg survival.

**Results**

*Genetic variation*

The sequencing of the ATP 6 and 8 gene revealed the presence of one ide (*Leuciscus idus*) roe. All other eggs collected correlated well with the asp when analysed in DAMBE.exe. The microsatellite analysis showed that the DNA fragments obtained from the primer pair mfw 18 were too short. Instead of the reported length of 204 base pairs (Crooijmans, 1997) the lengths obtained were 80 – 90 base pairs long. The results from the primer pair mfw 18 were therefore not included in the study.

A Chi square test showed that the population deviated from the Hardy Weinberg equilibrium (n=4, $\alpha$=7.81). Null alleles were detected in several loci as suggested by the excess of homozygotes for most allele size classes. Null alleles were detected in all loci in the populations Funbo, Falebro and Örsunda. In the Spångtorp population null alleles were present in the mfw 7 loci. In the Åby population null alleles were present in the mfw 10 loci. No linkage disequilibrium was found in any population pair. No genetic difference could be seen between eggs collected in different spawning locations. Table 1 shows pairwise F<sub>ST</sub>-values in the lower part of the table and pairwise p-values in the upper part of the table. None of the population pairs showed any genetic differentiation from each other (p>0.05). The number of migrants between spawning locations was found to be 0.98 individuals per generation.

<table>
<thead>
<tr>
<th>Location</th>
<th>Funbo</th>
<th>Spångtorp</th>
<th>Falebro</th>
<th>Åby</th>
<th>Örsunda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Funbo</td>
<td>-</td>
<td>p&gt;0,05</td>
<td>p&gt;0,05</td>
<td>p&gt;0,05</td>
<td>p&gt;0,05</td>
</tr>
<tr>
<td>Spångtorp</td>
<td>0,0157</td>
<td>-</td>
<td>p&gt;0,05</td>
<td>p&gt;0,05</td>
<td>p&gt;0,05</td>
</tr>
<tr>
<td>Falebro</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>p&gt;0,05</td>
<td>p&gt;0,05</td>
</tr>
<tr>
<td>Åby</td>
<td>0,0022</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>p&gt;0,05</td>
</tr>
<tr>
<td>Örsunda</td>
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<td>0</td>
<td>0,0119</td>
<td>0,0045</td>
<td>-</td>
</tr>
</tbody>
</table>

The PCA analysis shows genetic difference between sampling sites (Figure 3). The further apart the two locations are in the coordinate system the more genetically differentiated are the populations. The percent inertia was for x 41.56 and for y 29.49 meaning that the graph explains a large part of the genetic variation within the populations.
The same analysis was conducted dividing the samples into only two populations – The Örsunda population and the Sävja population, containing the populations Åby, Falebro, Spångtorp and Funbo(07). The two populations showed no genetic differentiation ($F_{ST}=0.0068$, $p = 0.122$). The number of migrants entering a population increased to 1.8 individuals per generation.

**Effects of turbidity**

On the 1\textsuperscript{st} of May the eggs begun to hatch and movement could be observed inside the eggs. The effect of clay on the survival of the roe was small and not statistically significant (treatment 1: $F_2=2.34$ n=4 p=0.160, treatment 2: $F_2=0.51$ n=4 p=0.493). When a smaller amount, 60 mg l\textsuperscript{-1} ww. of clay, was added the median survival (63 % hatched) seemed to increase as compared to the aquaria with no clay added (25 % hatched) (Figure 4). Median survival was also higher than the control group in aquariums with 225 mg l\textsuperscript{-1} of clay (49 % hatched). Treatment 1 contained the highest observed proportion of eggs hatched in aquarium number 10 where 92 % of the eggs hatched.
Figure 4: Median and quartiles of proportions of eggs hatched in aquariums with no clay added (0), 60 mg l\(^{-1}\) clay added (1) and 255 mg l\(^{-1}\) clay added (2).

**Effects of oxygen deficiency**

At the beginning of the experiment (day 1) all eggs in the oxygen free aquariums were alive (Figure 5). One week after the eggs were brought into the aquaria all eggs attached to the stones in the oxygen depleted aquaria were dead or empty. Live eggs were identified by the hard shell and clear content. White, moldy and empty eggs were considered dead. Empty shells would normally be considered as hatched eggs, although no larvae were observed during the entire experiment. On day 4 the number of eggs alive dropped dramatically in the aquaria. On day 7 there were no live eggs left in the oxygen depleted aquaria and the stones were only occupied by white, moldy and empty eggs, no fry were observed in any of the aquaria. A Mann Whitney U-test showed a lower survival in the oxygen depleted aquaria (z = -1.984 n=4 p=0.0472). Survival in the control aquaria was 0, 3, 46 and 70 %. During the incubation of asp roe in Lake Funbosjön the cages and roe was covered in silt but nonetheless some of the eggs hatched and at the end of the monitoring movement could be seen inside the eggs indicating that they were alive.
Discussion

The microsatellite analysis showed no genetic difference between the two tributaries River Örsundaån and River Sävjaån indicating that the asp does not have a homing behaviour. Judging from the number of migrants between River Örsundaån and River Sävjaån (1.8 individuals per generation) the gene flow between the rivers is small. The results indicate that the asp population of the sub basin Ekoln can be considered as one population. This does not rule out the possibility that the asp population of the entire Lake Mälaren has a sub-population structure. This study was made on only a small part of the asp population, the asp population of Ekoln, which may be differentiated from asp in other sub basins of Lake Mälaren. However, the relatively large distance between the Örsundaån population and the other populations strongly indicate that there is a mixing of the asp populations in Lake Ekoln. There was a lack of heterozygosity in the eggs sampled which could be a result of the presence of null alleles. This means that some of the informaion was lost during the analysis of the microsatellites as some of the samples which was thought to be homozygous could in fact have been heterozygous. The results from the PCA test showed some genetic difference between eggs from the different spawning locations (Figure 3). 71 % of the variation was explained by the x and y axes (percent inertia: 41.56 and 29.49). Yet the analysis showed no logic differentiation between the spawning locations. Eggs from two of the spawning locations in River Sävjaån were more similar to eggs from River Örsundaån than to eggs from the other locations in River Sävjaån. The F_{ST} test showed no genetic differentiatation of populations within River Sävjaån. The results are supported by Wolter et al. (2003) who...
found no sub-population structure of the asp in River Elbe. The home range for the entire population was thought to include more than the 120 km Elbe stretch that was studied.

Many of the primers of Croijmans (1997) were not successful in amplifying the microsatellites in asp. In many cases several fragments appeared and in some cases none. If further research is to be done on migration in asp I recommend that new primers are designed as the mfw-primers do not seem to fit the genome of the asp. In this study only three primers were used, which is below minimum. At least five microsatellite markers should be used to obtain reliable results. The spawning locations analysed here only represent a small part of the total asp population in Lake Mälaren and the sample sizes were in some cases too small. In future studies the number of samples in each location should be at least 30.

Mills (1981) noted that the survival of dace eggs decreased as the percentage of fine sand and silt increased, probably as a response to lower oxygen conditions at the surface of the eggs. The results from the turbidity preference investigation did not confirm this. There was no difference in survival in the different treatments. There was a trend towards an increased survival in the aquaria containing silt particles, with the highest observed proportion of roe at 92 % survival. Median survival was highest in aquaria with 60 mg l⁻¹ clay added tightly followed by the survival in aquaria with 255 mg l⁻¹ clay added. Surprisingly, the median survival was lowest in the aquaria with no clay added (Figure 4). Oxygen consumed by the egg is only available within the boundary layer of water at the egg surface. Clay particles deposited on the egg surface can cause the oxygen concentration gradient between the inside of the egg and the boundary layer to decrease, thereby restricting the oxygen supply to the egg. Greig et al. (2005) found a decrease in oxygen consumption in salmon eggs in the presence of clay particles. They also hypothesised that clay particles could infiltrate and clog the respiratory canals in the egg’s chorion through which the embryo is supplied with oxygen. During the building of the bridge in Öresund the turbid water with a silt content of 3 mg l⁻¹ was enough to scare off fish (Degerman et al., 1998). The survival of roe can depend on several other factors. Infection by fungus kills eggs and can be transferred among eggs in the same aquarium, speeding up the mortality rate. The age of the eggs could have varied at the point of sampling, giving different percentage of survival on different stones. For some of the eggs the earlier stages of embryogenesis could have passed where as some eggs were still in the earlier and more critical stages of their development when sampled.

It was hypothesised that one reason for fish to spawn in streams was due to the high levels of oxygen in the water and that a drop in oxygen saturation would affect the survival of the eggs. The statistical test showed a significant difference in survival between the oxygen depleted aquaria and the control. The results points to a sensitivity of the eggs towards low oxygen levels since the oxygen saturation was only lowered to around 30 %. On the other hand higher temperatures cause stronger response to hypoxia in the embryos due to the increased metabolic activity. The temperature in the aquariums was held at approximately 20°C. Czerkies (2001) found that low oxygen saturation triggered the hatching process in eyed eggs of whitefish (Coregonus albula), enabling the embryo to escape from the anoxic conditions. Extended exposure to low oxygen saturation disturbed the course of hatching and the embryos were not capable of rupturing the chorion. The drop in dissolved oxygen which can occur in slow flowing waters as a result of high metabolism might be a reason for the fish to choose to migrate up to streaming waters to spawn. Eggs incubated in Lake Funbosjön hatched successfully despite of being placed in a wind sheltered bay. In such habitats, drops in oxygen saturation during night might occur if the respiration exceeds photosynthesis. The oxygen deficiency experienced by the eggs during night was counteracted by the lowered metabolic
activity due to low water temperatures in the lake. Differences in temperature between the lake and aquaria in the laboratory was probably responsible for the difference in survival of the eggs in the different experiments.

**Conservation and management**

Considering the results from the microsatellite analysis the asp populations in River Örsunda and River Sävjaån should be managed as one population. The fact that the asp population of Lake Ekoln is not differentiated into several subpopulations is a finding that strengthens the situation for the asp in Lake Ekoln. Metapopulations formed by homing behaviour are more vulnerable because of the extinction and recolonization events often experienced by the subpopulations. The populations’ genetic variation decrease with extinction rate. Therefore a large population does not have the same problem because of gene flow between populations, and thus, it is also more resilient towards environmental changes. The finding also indicates that recolonisation of new spawning areas in new tributaries can occur without any need of human interference like stock enhancement, since the individuals are not tied to a specific stream by their homing behavior. The highest priority when conserving a threatened species is to remove the threatening factor responsible for the decline in population size. River Fyrisån is known to have been inhabited by asp in the past but the fish has not been able to migrate upstream Islandsfallet since 1810 when the weir was built (Johansson et al. 2002). There are several potential spawning locations around Lake Mälaren, apart from the locations in River Fyrisån, which are currently not accessible for the asp due to obstructions in the streams. If the obstructions, which compose the biggest threat to the species, were to be removed a stronger population of asp with potentially greater resistance towards environmental changes would inhabit Lake Mälaren. The clearing of migration routes have been made a priority in Uppsala. This will not only help the asp in regaining its former population size but will also help several other species and markedly increase the biological diversity of the streams in Uppsala.

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