Agenda item 6.1
For information

Council

CNL(17)43

Approaches used to prevent the loss of Atlantic salmon populations at high risk of extinction including gene banks, adult captive rearing, smolt-to-adult supplementation - Gene banking of wild Atlantic salmonids in Norway

(Arne Sivertsen, Norwegian Environment Agency, Trondheim, Norway)

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Introduction

In 1986, the Norwegian Environment Agency established the national gene bank for wild Atlantic salmon in Norway. The main purpose of the national gene bank is to contribute to the nationwide preservation of the genetic diversity and characteristics of natural salmon stocks in the face of threats to wild populations from, *inter alia*, acidification, the parasite *Gyrodactylus salaris* and, more recently, escaped farmed salmon. This national gene bank comprises a frozen sperm-bank (established in1986) and a living gene bank (established in 1990).

As a response to inter- and intra- riverine differences in natural conditions, Atlantic salmon stocks have developed phenotypic and life-history variation among populations, some of which reflect local adaptations. Analysis of molecular genetic markers has revealed significant population genetic structuring. As a consequence, salmon stocks are managed as individual population units.

Stocking of fish in Norwegian lakes and rivers still occurs. The motivation for stocking has gradually changed from pure stock enhancement towards an increased focus on preserving biological diversity. Under certain circumstances, stocking of fish could have positive effects. However, if not conducted in a well-planned manner, that takes into account risks to the wild stocks, the unintended side-effects of stocking can result in loss of genetic variation and genetic integrity of fish populations.

Negative effects from stocking can be minimized by adherence to some general rules and guidelines cf. the policy relating to hatchery and stocking activity in Norway. However, in order to minimise the negative effects and to adapt established stocking practice accordingly it is necessary to build stocking practices upon population specific knowledge.

Objectives and strategies

During the initial developmental phases, the gene bank was based exclusively on frozen sperm. The goal was to preserve genetic material from more than 100 stocks and from at least 50 individual fish from each stock.

The Environment Agency considers live gene banks as a temporary measure to be used only in cases where salmonid stocks are threatened by extinction. At present five live gene banks are operational and one more is being planned. These live gene banks will be able to hold live fish from a maximum of 50 stocks in total.

The basic gene bank strategy for Atlantic salmon in Norway is shown in Figure 1. Within this strategy, strong emphasis is placed on measures to prevent transmission of fish disease organisms.

All parent fish are maintained in a health-control programme and protocols for how each operation (handling of the fish, transportation of ova and sperm) is to be carried out have been established by the Norwegian Veterinary Institute. Only disinfected eggs can be exported from the station in order to minimize the risk of spreading diseases to rivers. All fish production for stocking is carried out at local fish culture stations and the entire production is based on fresh water since sea water is often contaminated with pathogens.

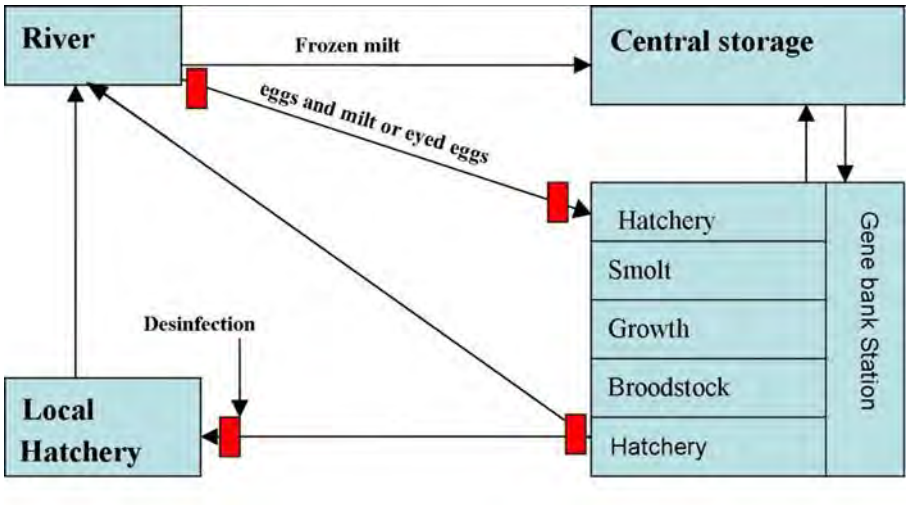

Figure 1. The gene bank strategy and transfer of biological material (red boxes - disinfected eggs) in and out of the gene bank.

Adult fish caught in individual rivers are kept in fish tanks at local stations for a few months until they become sexually mature. The sperm is collected from these fish, frozen and then transported to a central storage facility. In addition, fresh milt and ova are transported to a disinfection facility. After disinfection, the fertilized ova (single pair mating) are transported to the regional gene bank station where ova from each female are kept in quarantine in separate hatching cylinders. Ova are removed from the quarantine when diseases which could be transmissible to the interior of the eggs are discovered.

Within the gene bank station, each family group is maintained in separate tanks until the fish can be marked. Thereafter, the families are pooled and each stock is kept in separate tanks throughout their lifecycle. The station is divided into four sections: (1) hatchery and initial feeding stage; (2) parr; (3) smolt; and (4) older fish. These divisions facilitate disease management and the management of family groups and stocks.

Each of the captive stocks spends two generations in the station. The production is based on the following guidelines aimed at retaining genetic diversity of the stocks:

- maximum survival;

- long generation time;
- identification;
- equal size of family groups;
- a minimum effective population size of 50 for each generation;
- surplus fish-production for safety; and
- mating schemes including the use of frozen sperm.

Sperm-bank – method of cryopreservation and sampling strategy

Cryopreservation (deep freezing) of sperm enables the preservation of genes for a virtually unlimited period.

A new cryopreservation method, developed by Cryogenetics AS in Norway, was adopted for the gene bank program in 2010. Larger volumes of sperm, more suitable for broodstock production, can now be preserved:

- one sample stored in liquid nitrogen contains enough sperm cells to fertilize approximately 4,000 ova with an expected fertilization percentage of > 90 %. (average numbers from Atlantic salmon);
- gonad extraction of sperm significantly increases the amount of sperm available for freezing in situations where regular stripping of fish is difficult or not possible.

The following sampling strategy has been employed by the gene bank programme:

- sperm from at least 50 individuals from each stock is frozen. Since the sampling cannot be carried out on identifiable stocks, each river is considered a sampling unit. large tributaries are sampled separately. This choice of strategy is based on the empirical evidence that genetic differentiation along a single watercourse is minor compared to the among-river variation;
- sampling is carried out for a period of at least two years in each river to reduce the chances of gross over-representation of a single year-class;
- emphasis is placed on sampling from stocks representing a wide geographical and ecological range;
- stocks which are threatened by extinction are given priority over other stocks;
- stocks which are of particular scientific value, or valuable for fishing purposes, are also given priority;
- autopsy of the brood is conducted to identify any diseases; and
- genetic testing is conducted.

The Norwegian salmonid gene bank now contains material from more than 6,000 wild salmon individuals from about 200 distinct stocks. It also contains genetic material from 26 different anadromous trout stocks and 2 stocks of anadromous Artic charr. The aim of the gene bank is to collect sperm from at least 50 individuals from each stock. The collection is carried out over several seasons, years, and in different parts of the watercourse, to avoid collecting material from fish that are closely related.

The sperm-bank also contains sperm from 6,300 individuals from 35 populations of self-produced brood from the live gene bank.

Live gene banking

Administration of genes and fish in the live gene-bank
FAGER is the management system and database. FAGER is the operational tool for control and overview of kinship in the facilities practical activities, where individual-based historical data are significant. PIT tags are applied to all fish and provide an effective and unintrusive way of handling fish and optimal control of kinship.

When handling, each fish is registered and selected data are stored in the database. Pedigree is available for each individual and constitutes a basis for the determination of new combinations in production or to new generations in the gene bank or other stocking activity.

At the age of 7-10 years, selected fish are, if necessary, used for production of new generations of brood stock. To protect the genetic variation that exists between individuals in the same family group, the crossing regime shown in Figure 2 is applied. This gives 32 different crossing combinations of the new family group.

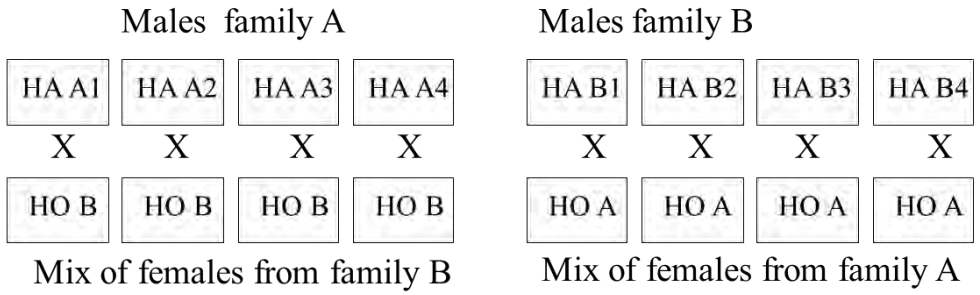

Figure 2. Creating new generations. Every male fish from family A is mated with a mixture of eggs from four females from family B, etc. The quantity of eggs from each female are standardized. After fertilization is over, all eggs are gathered into a single unit that will constitute the new family group.

With individual marking and family structure, mating can be controlled so that genetic breadth is maintained over time in a gene bank. Active mate choice is not permitted in the gene bank. In the materials returned to rivers, all family groups, genders and individuals should be the most equally represented. The crossing regime is, to the extent practicable, intended to maximise the number of variants, which mitigates the negative effects of lack of mate choice. The probability of losing rare alleles is reduced by crossing individuals which are potentially related. This is evaluated by assessment of relatedness among new individuals from the founding population with the help of molecular genetic markers and full pedigree information for each offspring (brood stock).

Unintentional selection (domestication) is counteracted by limiting mortality. Domestication may also be counteracted by supplementing with new families from the founder population or by supplementation with frozen sperm from previously captured wild fish and first generation broodfish.

Restoration of fish stocks in use of Live gene banks

Most of the anadromous fish restoration projects are related to the eradication programmes for *Gyrodactylus salaris* in which the national gene banks play a major role. The main goal of the restocking is to establish sustainable populations in the affected watercourses. The likelihood to withstand threats and challenges in the natural environment increases the amount of genetic variation. To maximise maintenance of this variation, material from the live gene bank is reintroduced to the rivers in as many genetic combinations as possible from the brood stock (F1 generation in the gene bank). Rebuilding stocks is, therefore, a long-term process with a time horizon of five to ten years. The return of offspring from the gene bank does not end until all the family groups and available individuals in the gene bank have contributed and the number of adult fish in the stock has reached the natural spawning target estimated for the specific river.

Re-establishment of anadromous salmonid stocks from the national gene banks is mainly achieved by planting ova. Other life stages including smolts may be used in restocking in the first years of the re-establishment to catalyse the process and also to avoid, displace and minimize negative genetic effects of the presence of individuals of unwanted origin e.g. farmed salmon and strayers.

After the release of offspring has commenced in the different watercourses, the local river-specific selection starts again and nature decides which variations are viable.

Evaluation of results

The marking method
Since 1993, the Norwegian gene banking programme has used Alizarin otolith group marking of Atlantic salmon. This approach has enabled group marking of a high number of individuals in a fast, simple, secure and affordable way. For code marking, repeated marking at different ontogenetic stages of the eyed ova can be employed. In the period 2005-2015, more than 25 million eyed ova were marked in this way.

Organization of the Gene bank – programme
The gene-banking programme for salmonids in Norway is administered by the Norwegian Environment Agency. The practical field-work is planned in cooperation with the National Veterinary Institute, local county administrators and other local contacts.

The National Veterinary Institute is the National Competence Center for all practical implementation in the field and standardisation of routines at the gene-bank facilities.

The permanent sperm bank is run by Cryogenetics AS and divided into two equal separate units located in two geographical parts of the country.

The live gene bank stations are owned and operated by private companies, hydropower boards, research institutes and others. Contracts govern the relationship between the Environment Agency and the owners. All production costs and investments are financed by the Agency. The Environment Agency decides upon all issues concerning the use of the stations' facilities and also provides instructions.

Acknowledgements:
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