Guidelines for Gene Banking for Wild Atlantic Salmon

1. Introduction

The purpose for the guidelines outlined below is to provide best management practices[1] for gene banking to support the rebuilding of wild Atlantic salmon, hereinafter 'salmon', stocks (see NASCO's 'Guidelines on the Use of Stock Rebuilding Programmes in the Context of the Precautionary Management of Salmon Stocks', CNL(25)50). These guidelines should be considered in association with NASCO's 'Guidelines for Stocking Atlantic Salmon' (CNL(24)61).

In this document, the term *gene banking* is defined as the artificial maintenance and / or preservation of genetic variation over multiple generations of salmon populations that are currently, or anticipated to be, suffering from population decline.

Gene banking is not meant to provide large numbers of fish for ongoing stocking or enhancement purposes. Rather it is intended to preserve genetic diversity over an extended time period to allow for a defined period of re-establishment at a later time when conditions allow.

There are two types of gene banking: frozen and live.

Frozen gene banking is the process whereby milt is removed from salmon then frozen and maintained in cryopreservation facilities. When needed, the milt is thawed and used to fertilise eggs and the progeny then reintroduced into the native habitat at the appropriate time or to produce new generations in the gene bank. Frozen gene banks require eggs to be sourced from a live gene bank or from wild fish. The key advantage of frozen gene banks is the long-term retention of paternal native salmon population genetic profiles. For the same number of males, freezing milt can be more cost effective than live gene banking.

Live gene banking is the process whereby live salmon are removed from their native habitat, held and bred in captivity, and their progeny are eventually reintroduced into the native habitat at the appropriate time or to produce new generations. In most cases, live gene banking is carried out entirely in artificial environments to maintain control over the breeding and rearing processes and minimise the risk of negative impacts such as disease and genetic introgression. However, in rare instances, components of the live gene banking process take place in rivers. In those cases, juvenile salmon reared in a hatchery under live gene banking protocols are placed temporarily in the natural environment to reduce the artificial influence of hatcheries and then returned to the hatchery. In contrast to frozen gene banks, live gene banking is a short-term option for the temporary retention of a live reservoir of a representative sample of both females and males from salmon populations that are currently, or anticipated to be, suffering from population decline.

In all cases, the banked genetic material is used for future breeding to support restoration and conservation activities by enabling the reintroduction of conserved genetic material into the wild. Gene banks, therefore, help maintain the genetic diversity of salmon populations. Genetic diversity is essential for the conservation of salmon in response to anthropogenic threats, including pathogens, hydropower and climate change, as well as for the resilience and adaptability of salmon to changing environments.

[1] NASCO recognises that there may be instances where this best management practice is not applicable, e.g. where other considerations, such as 'Species At Risk' designations, may take precedence.

NASCO recommends that Parties / jurisdictions consider establishing frozen gene banks as soon as possible to ensure existing genetic diversity of salmon is preserved.

2. NASCO has adopted the following guidelines for gene banking when conserving wild Atlantic salmon.

I. Guidelines Relevant to the Design of Gene Banking Programmes

A. Gene banking should only be undertaken by competent authorities as it entails high costs, is of a long-term nature and requires specialist skills.

B. Gene banking should be designed in close consultation with geneticists.

C. Gene banking should be considered in the context of a stock rebuilding programme (SRP) (see NASCO's 'Guidelines on the Use of Stock Rebuilding Programmes in the Context of the Precautionary Management of Salmon Stocks', CNL(25)50).

D. Jurisdictions should establish a system of prioritising stocks for consideration for gene banking using region-specific criteria in the context of the available evidence.

E. When to Gene Bank

Gene banking should be initiated when evidence suggests that a population is likely to decline to the point of losing critical genetic diversity (as determined on an individual river basis). NASCO recommends strongly that the basis for the evidence should include the advice of population geneticists and not follow generic rules such as a minimum effective population size (Ne) of 50 that has been considered widely in North America in the past. NASCO considers that adherence to generic rules such as these is inappropriate. Where possible, gene banking should be considered prior to the point of decline.

F. Broodstock Selection

1. Collect wild salmon from targeted rivers for broodstock. Mature parr and pre-spawned adults can be for immediate use and non-mature parr, smolts or pre- or post-spawned adults can be held in captivity for future use.

2. Collect as many as possible to establish an effective population size at an appropriate level. A minimum of 200 live breeding fish has been recommended widely to conserve rare alleles. However, this number may not be appropriate in all cases and NASCO recommends strongly that the appropriate number should be determined in consultation with population geneticists. Importantly, there is a delicate balance that must be considered between collecting enough fish while leaving sufficient numbers of wild fish in the river.

3. Collect fish from as many different locations in the target river as possible.

4. Include as many observed phenotypic traits (physical, life history, behavioural) as possible.

5. Include fish from multiple year classes collected over three to five years.

6. Screen collected fish using appropriate genetic techniques and reject any fish with any evidence of ancestry that is considered undesirable in the local context (e.g. aquaculture, hybridisation or stocking); in some cases, these same techniques can be used to consider relatedness among fish to avoid retaining close relatives, especially in cases where the river population is very low.

7. Screen collected fish for disease using appropriate techniques and reject any fish that test positive for any pathogen, especially those that are transmitted vertically.

8. All fish should be individually tagged for future identification for many reasons, such as enabling rejection of fish post screening or identifying pedigree. Marking of eggs and fish and / or genetic assignment to parents in the gene bank for future identification will also assist in evaluation (see VI. Monitoring).
9. In some circumstances, genetic screening for sex determination can be carried out if deemed important. This may be especially important where the number of returning adults is low.

II. Guidelines for Frozen Gene Banking Programmes

(All guidance listed in section I pertains to this category as well)

A. Frozen gene banking should be implemented for any stocks that are likely to experience declines for many years into the future.

B. Frozen gene banking may be implemented for SRPs that may lack sufficient number of broodstock for many future years.

C. There are no concerns about the length of time frozen gene banking is conducted (as opposed to III.F.) since there are no selection pressures on frozen milt.

D. To ensure the use of established methods and protocols, consideration should be given to contracting with a commercial cryopreservation company that would store milt and manage associated data to ensure quality and proper biosecurity. However, small-scale cryopreservation may be conducted by an appropriate academic lab or agency facility.

E. The use of milt cryopreserved in the past can be used to mitigate any genetic drift that occurs in every generation of live gene banked populations in the future.

F. Cryopreserved milt in the gene bank should be supplemented from time to time to account for natural selection in the targeted population. This is relevant especially in rivers with a changing environment, e.g. as a result of climate change.

G. Prior to banking, milt should be checked for motility and viability.

H. Each wild male (F0 generation) should be stripped only once as pathogen screening requires each fish to be sacrificed to provide tissue samples. Males in the F1 generation, however, can be stripped multiple times.

I. Dependent on milt volume, milt from each male should be stored in more than one container ('straw') to allow for multiple future crosses from each male or for other applications.

J. Typically, each 'straw' may be used to fertilise around 200 eggs and each male may be used to fertilise up to 4,000 eggs.

K. Refer to NASCO's 'Guidelines for Stocking Atlantic Salmon', CNL(24)61, for additional information on breeding salmon.

III. Guidelines for Live Gene Banking Programmes (in Special Facilities)

(All guidance listed in section I pertains to this category as well)

A. Live gene banking should be used to preserve genetic variability for a restricted number of generations where stocks are in danger of decline and natural recovery is deemed unlikely. A live gene bank can be used to hold fish until conditions improve. Several populations may be kept, albeit separately, in one gene bank facility.

B. Individual families (i.e. fertilised eggs) should be held separately initially, preventing pathogens from spreading within the facility until:

1. Eggs have been proven to be pathogen free; and
2. Fish have reached a sufficient size for tagging / marking to enable identification to families.

C. Keep areas for different activities separate (e.g. hatching, rearing, broodstock) with strict disinfection and water quality protocols between each.

D. In some instances, when the number of males in the river is insufficient, or to reduce the need for males in captivity, frozen milt can be used.

E. To minimise the deleterious influence of hatcheries on the live gene-banked offspring (i.e. to be stocked in the relevant river), NASCO's 'Guidelines for Stocking Atlantic Salmon', CNL(24)61, should be followed.

F. Limit the duration and number of generations in gene banks to minimise deleterious effects of captive broodstock.

G. Broodstock can be used for multiple years until such time as they are too old to meet the pre-defined criteria for the programme, for example, eye-up rates are no longer acceptable.

H. If any broodstock test positive for a pathogen while in the gene bank facility, they must be destroyed.

I. Broodstock Mating (Steps in Chronological Order)

1. Perform genetic analyses to determine relatedness among broodstock and avoid mating close relatives. Genetic analyses for relatedness should also be considered when selecting new fish to be used in the gene bank broodstock population by comparing their relatedness to fish already in the gene bank.
2. Ensure that equal numbers of males and females in each population are crossed, i.e. the contribution of each broodstock to the mating scheme should be equal.
3. Fertilise each egg batch from a single female with milt from a single male (i.e. no pooled milt which may result in sperm competition).
4. Supplement with frozen milt, for example, when the number of live males in the population is insufficient, or to reduce the need for males in captivity, or to allow for crossing of males in year classes absent from the live gene bank.
5. Refer to NASCO's 'Guidelines for Stocking Atlantic Salmon', CNL(24)61, for more general advice on broodstock mating.

J. Management of Offspring Within the Gene Bank

1. Include equal numbers of offspring (eggs or fry) from each crossing in the gene bank. When a crossing produces a number of offspring in excess of that required for the gene banking programme, the surplus offspring produced should be removed from the gene bank.
2. Perform fish health screening on broodstock after spawning and offspring to screen for vertically transmissible diseases, including PCR analysis. Fish, or offspring from fish, that test positive to any pathogen should be removed from the gene bank.
3. Establish and maintain a database of pedigrees to inform future crossings.

IV. Guidelines for In-River Live Gene Banking Components

(All guidance listed in sections I and III pertains to this category as well)

In-river live gene banking has been used alongside live gene banking. It is an approach that seeks, temporarily, to maintain a representation of the offspring of live gene bank crosses within their natural river environment. This strategy attempts to reduce the artificial influence of hatcheries and preserve both genetic diversity and adaptive traits suited to the wild. The process described in A – F below is envisaged to occur annually to produce continual generations for reintroduction into the river until such time monitoring suggests it be discontinued. This ensures the continuity of the in-river live gene bank population while minimising the loss of genetic diversity and family representation. However, this approach has the risk of reduced biosecurity because it relies on the retention of live fish from the wild environment in the gene bank.

A. Hatchery Rearing

1. Rear salmon in hatchery under live gene banking protocols (refer to section III).
2. Maintain some offspring in the live gene bank (the 'captive' fish). Others are allocated for release into the river at the earliest stage possible (the 'wild-exposed' fish). Ensure equal representation of individual families is designated for both locations.

B. Site Selection for Release

1. Locate rearing site in the contributing population's watershed.
2. Select site with optimal rearing conditions to support released fish.
3. Ensure the site is free of anadromous salmonids.
4. Prevent inward migration by choosing a site isolated from existing wild populations by artificial or natural barriers, e.g. waterfalls, steep cascades, counting fences / dams, to protect genetic integrity and avoid hybridisation / competition and help minimise disease risks.
5. In some jurisdictions the prevention of outmigration will be necessary and if this cannot be practically achieved the use of in-river gene banking must be reconsidered. Best management practice would be to select sites that allow for as much control of outmigration as possible.
6. Avoid selection of sites with known pathogen risk.

C. Release of Offspring (Wild-Exposed Fish)

1. Prior to release, define the optimal length of time for wild exposure of the released offspring.
2. Equal numbers of offspring from each family should be released.
3. Due to the need to account for natural mortality, the absolute number of offspring released could be more than the number retained in the captive group. Equalised family representation should be achieved across both the captive live gene bank and the wild-exposed live gene bank, even if the absolute number of offspring differs (e.g. five eggs per family in the captive group and 400 eggs per family in the released group).
4. When a crossing produces a number of offspring in excess of that required for the gene banking programme, the surplus offspring produced should be removed from the gene bank.

D. Recapture of Wild-Exposed Fish

1. After the optimal wild exposure time period has lapsed, use appropriate methods to collect a targeted number of late parr (electrofishing) or out-migrating smolts (traps).

2. Transport the collected fish back to the live gene bank facility.
3. Quarantine the collected fish and screen for vertically transmissible diseases (e.g. PCR analysis). Only fish with clear health results should be transferred out of quarantine into general rearing at the live gene bank facility. Remove fish that test positive for pathogens.
4. Individually tag and genotype healthy recaptured fish before reintroduction into the live gene bank facility.

E. Rear the wild-exposed and captive fish to maturity.

F. Breeding

1. Breed the wild-exposed fish following the protocols under section III.
2. Incorporate the captive fish into the breeding programme only where family representation is missing from the wild-exposed fish.

V. When to Start Releases from a Gene Bank

The decision to start releasing fish from a gene bank will be site-specific depending on local conditions as determined by monitoring, see section VI.

There are four common scenarios for considering introducing gene bank fish into the river.

A. Scenario1: when monitoring indicates that the threat that caused the stock decline is gone, and the in-river population has grown to a sustainable level and has been determined to represent suitable genetic variation and integrity, no releases from the gene bank are recommended.

B. Scenario 2: when monitoring indicates that the threat is gone, and the environmental conditions are improving but the remaining population in the river has been determined to represent unsuitable genetic variation and integrity and / or is at a critical low effective population size, engage in 'large-scale' releases of fish from gene bank for many years to re-establish the population. 'Large-scale' is relative to the existing population in the river and it is important for the gene bank fish to be dominant, for example by a ratio of 4:1. Consider removal of fish with unsuitable genetic variation and integrity from the river prior to release of fish from the gene bank.

C. Scenario 3: when monitoring indicates that the threat will be gone in the near future (mitigations have been / will be done) and the remaining population in the river has been determined to represent unsuitable genetic variation and integrity and / or is at a critical low effective population size, initiate gene bank releases. At an early stage, release a restricted amount to help maintain some level of suitable genetic variation and integrity in the population and to keep the population above the critically low effective population size. The purpose is to ensure river fish with unsuitable genetic variation and integrity do not dominate the river prior to the subsequent re-establishment releases. When the threat is gone, start re-establishment with 'large-scale' releases from the gene bank for many years. Consider removal of fish with unsuitable genetic variation and integrity from the river prior to release of fish from the gene bank.

D. In the case of Scenarios 2 and 3, releases may be discontinued when monitoring indicates that:

- the threat is gone, and the population has good possibilities for natural production;

- the genetic variation and genetic integrity conserved in the gene bank has been transferred to the population (dominating with origin in fish from gene bank); and
- positive demographic trends and management targets have been reached.

E. Scenario 4: when monitoring indicates that the threat and the environmental conditions are poor and will remain poor in the unforeseen future, do not make gene bank releases. Maintain the genetic material in frozen and live gene banks, i.e. make sure there is enough material left when / if conditions improve to re-establish the population. However, consideration may be given to establishing in-river gene banking, see section IV.

VI. Monitoring

Monitoring is required in the gene banks themselves, in the release environment and of the released fish. Monitoring of genetic variation and integrity in the gene bank will be very important, not only to measure achievement but also as a basis for adjusting the form, scope and time horizon of the programme. For example, an assessment of different release life stages, the number of releases, and where in the watercourse, and whether the releases can be terminated or need to be continued.

A. Ongoing monitoring of the river and marine habitats is needed to determine if factors that led to the decline of salmon are still present or if the habitats remain suitable for supporting salmon.

B. The performance and traits (e.g. egg condition and size, fecundity, survival at life stages, and size at age) of the gene banked fish and the in-river population should be monitored to determine if the gene banking programme is resulting in unintentional and deleterious changes in the genome.

C. The survival and growth of salmon released into the river, including competition between the gene banked offspring and the wild fish, should be monitored to confirm that the releases are resulting in increased riverine populations and establishing suitable genetic variation and integrity in those populations.

D. Adaptive management should be practiced if monitoring demonstrates that changes to the gene banking or the use of its products are warranted.

VII. Retention of Gene Banks

After releases have been terminated, operation of live gene banks is typically ceased and fish are either released into the river or sacrificed according to appropriate, domestic protocols. Males in the live gene bank that are not represented in the frozen gene bank should be incorporated into the frozen gene bank prior to termination of the live gene bank. It is recommended that any frozen gene bank associated with the live gene bank is maintained into the future.

Glossary

Allele – a variant form of a gene. For example, humans have two copies of most genes (one from each parent): the copies are alleles of that gene.

Broodstock – a group of mature individuals or an individual used in aquaculture / hatcheries for breeding purposes. Broodstock can be a population of salmon maintained in captivity as a source of progeny used in stocking.

Critical Genetic Diversity – the minimum level of genetic variation necessary to maintain the population's adaptive potential, reproductive success and long-term viability in a changing environment.

Cryopreservation – the process of preserving cells, tissues, or organs by freezing them at very low temperatures.

Effective Population Size – the number of individuals that effectively participate in producing the next generation. Generally, the effective size of a population is considerably less than the census size and is calculated by this formula: $Ne=(4*Nm*Nf)/(Nm+Nf)$; where:

- **Ne** = effective population size
- **Nm** = the number of breeding males
- **Nf** = the number of breeding females

A geneticist is consulted to calculate the effective population size of a salmon population.

Eye-up rate – the rate at which salmon embryos develop eyes expressed as a percentage.

Families – in the context of salmon breeding, this refers to the offspring of one mating (one female, one male)

F0 fish – wild collected adults, from which subsequent generations are denoted as F1, F2, F3 etc.

Fecundity – the reproductive capacity of an organism, often measured as the number of eggs produced by a female fish, which influences stock sustainability.

Genetic diversity – the biological variation of genes that occurs within species or a local population of the species. High levels of genetic diversity (many different genes or alleles) are considered favourable to help the species to adapt to changing conditions such as climate change. Low levels of genetic diversity (fewer different genes or alleles) can lead to fish with reduced fertility and resilience in the wild.

Genetic introgression – the incorporation of novel genes or alleles from one group of fish (e.g. hatchery-origin salmon) into the gene pool of a second, distinct group of fish (e.g. wild-origin salmon) via mating.

Genetic drift – the random fluctuations in the numbers of gene variants (genotypes) in a population over time that occurs in the river / live gene bank, i.e. due to the chance disappearance of particular genes as individuals die or do not reproduce.

Genome – the complete set of genetic information in a species or local population of a species.

Genotype – to determine the genetic makeup or genotype of an organism, essentially analysing its DNA to identify specific gene variations; the act of doing this is called ‘genotyping’.

Motility – the ability of sperm to swim and effectively move and access eggs.

PCR analysis – method using the Polymerase Chain Reaction, a technique used by molecular biologists for making copies of specific pieces of DNA to target specific parts of the genome to look for variation, for example between individuals.

Pedigree – a representation of the genetic relatedness among individuals across generations; shows how genes have been inherited and may include traits or genetic disease.

Phenotypic trait – a measurable characteristic of an individual such as length, disease resistance, or behaviour. A phenotypic trait is the product of the expression of genes in an environment; most traits are controlled by many genes.

SNP markers – SNPs (pronounced ‘snips’) are single nucleotide polymorphisms – i.e. single letter differences in the DNA sequence. SNPs are commonly used to measure genetic variation and relatedness and can be used as ‘markers’ for the identification of species and their hybrids.

Stock – a management unit comprising one or more salmon populations. This would be established by managers, in part, for the purpose of regulating fisheries.

Stock Rebuilding Programme (SRP) – a suite of management measures designed to restore a salmon stock to sustainable levels, often determined using a river-specific target.

Vertically transmitted disease – a disease transferred from parent to offspring, which may be acquired from either parent.