

Report of the Meeting of the Stocking Guidelines Working Group

By Video Conference

5, 6, 25, 26, 27 September, 11, 28, 30 October, 7, 11, 27 November, 9 December 2024, 10, 13, 21 January and 6, 19 February 2025

1. **Opening of the Meeting**

- 1.1 The Chair Stephen Gephard (USA) opened the meeting. He welcomed new members to the Working Group: Hlynur Bárðarson as representative for Iceland, John Whitelaw as the new representative for Canada and Heidi Hansen as the new representative for Norway.
- 1.2 A list of participants is contained in Annex 1.

2. Adoption of the Agenda

2. The Working Group adopted the Agenda for the meeting, SGWG(24)05 (Annex 2).

3. Background to the Stocking Guidelines Working Group

- 3.1 The Chair reminded participants that, to mark the International Year of the Salmon (IYS), a two-day symposium titled 'Managing the Atlantic salmon in a rapidly changing environment management challenges and possible responses' was held immediately prior to the 2019 NASCO Annual Meeting. The IYS Symposium Steering Committee recommended (see <u>CNL(19)16</u>) that NASCO update the existing Stocking Guidelines in light of:
 - the advances that have been made in the last 15 years in understanding genetic effects of artificial population supplementation, i.e. stocking; and
 - the conclusions of the 2017 NASCO Special Session on Understanding the Risks and Benefits of Hatchery and Stocking Activities to Wild Atlantic Salmon Populations <u>CNL(17)61</u>.
- 3.2 The Chair also noted that the Working Group met several times from November 2023 to March 2024 to develop 'Guidelines for Stocking Atlantic Salmon' that were agreed by Council at the NASCO 2024 Annual Meeting, <u>CNL(24)61</u>. These are overarching guidelines intended to encompass other guidelines.
- 3.3 In 2024, Council considered a ten-year strategy for NASCO, together with an associated action plan, that included recommendations to update its 2004 Guidelines on the 'Use of Stock Rebuilding Programmes in the Context of the Precautionary Management of Salmon Stocks', <u>CNL(04)55</u>, and investigate scientific and management protocols for gene banking and develop associated guidelines. Council agreed 'The Future of NASCO a Ten-Year Strategy', <u>CNL(24)71rev</u>, at the 2024 Annual Meeting, which contained a high-level Action Plan. It further agreed (see <u>CNL(24)88rev</u>) that the Stocking Guidelines Working Group would reconvene to work on updating the 2004 Stock Rebuilding Programme Guidelines and consider guidelines related to gene banking. To enable this, 'Terms of Reference for the Stocking Guidelines

Working Group', <u>CNL(24)68</u>, were agreed.

4. Consideration of the Terms of Reference for the Stocking Guidelines Working Group <u>CNL(24)68</u>

- 4.1 The Working Group considered its Terms of Reference, <u>CNL(24)68</u>, which tasked the Group with:
 - 1. Considering and recommending to Council for agreement at the 2025 Annual Meeting an updated document 'Guidelines on the Use of Stock Rebuilding Programmes in the Context of the Precautionary Management of Salmon Stocks', <u>CNL(04)55</u>, which, amongst other things, indicates the need to identify and involve stakeholders and assess social and economic factors (which addresses recommendation T2 as noted in the Draft of an Action Plan for NASCO, <u>CNL(24)14</u>).
 - 2. Investigating both the scientific and management protocols for gene banking and developing guidelines in this regard (which addresses a recommendation from the External Performance Review (EPR23) and is proposed as an action in the Draft of an Action Plan for NASCO, <u>CNL(24)14</u>).

5. Working Methods

- 5.1 The Working Group noted that its Terms of Reference included a description of the Working Methods it should use as follows:
 - the Working Group should meet inter-sessionally as required, to address its Terms of Reference;
 - meetings shall be via video conference;
 - the Working Group will decide how to conduct its business to allow it to address its Terms of Reference effectively;
 - the Working Group should seek consensus in agreeing its report and in drafting updated guidelines on stock rebuilding programmes and gene banking;
 - in conducting its work the Working Group may wish to communicate with, and request information from experts in the field;
 - the Secretariat will provide logistical support and background information to the Working Group, as requested; and
 - the Working Group should submit its report to the Council of NASCO for its consideration.
- 5.2 The Chair invited views from the Group on how to approach the two Terms of Reference listed in paragraph 4.1 above. The Group agreed that the best approach would be to address guidelines on stock rebuilding programmes first and then focus on guidelines for gene banking, to produce two stand-alone documents.
- 5.3 The Group discussed recent publications relating to stock rebuilding and gene banking and agreed to invite several experts, listed below, to speak to the Group about their work:
 - Heidi Hansen (Norwegian Environment Agency, Norway). Ms Hansen spoke on the gene banking for wild Atlantic salmonids in Norway, covering frozen gene banking and cryopreservation, live gene banking, founder fish collection, reintroduction and

evaluation;

- Sten Karlsson (Norwegian Institute for Nature Research, Norway). Dr Karlsson spoke on the general principles and practice of gene banking in Norway, including some overarching guidelines;
- Kristin Bøe (Norwegian Veterinary Institute, Norway). Dr Bøe spoke on several aspects of the Norwegian gene banking programme, the collection and screening of founder fish, gene bank structure and routines, biosecurity measures and restocking practices;
- Louise de Mestral (Fisheries and Oceans Canada, Canada). Ms de Mestral spoke on the design and implementation of the Fisheries and Oceans Canada, Maritimes Region, Atlantic salmon genetic conservation programmes, including consideration of when to intervene and also subsequent monitoring relative to the programmes' objectives;
- Victoria Pritchard (Institute for Biodiversity & Freshwater Conservation, University of the Highlands and Islands, U.K.). Dr Pritchard gave an overview of Atlantic salmon gene banking in Scotland, giving background on Atlantic salmon populations and their management, including current approaches to live gene banking; and
- Colin Bull (Institute of Aquaculture, University of Stirling, U.K.). Dr Bull spoke on the 'Likely Suspects Framework Project', a salmon management decision support tool and flagship project of The Missing Salmon Alliance.
- 5.3 The Group agreed, that for the purposes of the report and proposed revised guidelines, the term 'stock rebuilding' should be defined as:

'a suite of management measures designed to restore a wild Atlantic salmon stock to sustainable levels, often determined using a defined river-specific target'.

5.4 The Group agreed, that for the purposes of the report and proposed revised guidelines, the term 'gene banking' should be 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 '.

6. Stock Rebuilding Programmes in the Context of the Precautionary Management of Salmon Stocks

- 6.1 The Working Group made the following observations on determining the need for a Stock Rebuilding Programme (SRP):
 - a) the use of 'Conservation Limit', (CL) as a measure of Atlantic salmon population status and when to intervene with a Stock Rebuilding Programme (SRP) may not always be appropriate. Discrepancies exist in how a CL is calculated, with various methods used. Alternative methods or metrics could be considered to the use of CL as the sole metric for stock management, i.e. other relevant reference points (RPs), depending on available resources. Clearer guidance on the use of CLs should be considered where it continues to be used for stock management. Actions may be needed before a population reaches its CL / RP, and managers should act when stocks are at risk, not just when they fall below CLs / RPs;

- b) depleted stocks can be placed in two categories. The first category is stocks that are being exploited but are below the CL / RP, possibly with managers trying to keep them above the CL / RP. The second category is stocks so far below the CL / RP that action is required to prevent their extirpation rather than to restore them to a level to allow fishing; and
- c) the focus may need to be on both rebuilding stocks and also preserving them, especially in the context of climate change. Actions may be needed before a population reaches critical levels such as the CL / RP, in which case managers should act when stocks are at risk, not just when they fall below CL / RP.
- 6.2 The Working Group made the following observations on the initiation of Stock Rebuilding Programmes (SRPs):
 - a) climate change needs to be incorporated into the habitat and environmental strategies of SRPs, which in turn should be adaptable to changing circumstances such as the impacts of climate change. Environmental changes due to natural events need to be considered in addition to climate change. Other NASCO guidance currently available or in development and any updates in the future should also be consulted, such as 'Guidance on Best Management Practices to Address Impacts of Sea Lice and Escaped Farmed Salmon on Wild Salmon Stocks', <u>SLG(09)05</u>, and 'Management of Salmon Fisheries', <u>CNL(08)43</u>;
 - b) socio-economic considerations are important and need to be recognised in the guidelines, including the integration of Indigenous Knowledge and practices. NASCO's 'Guidelines for incorporating social and economic factors in decisions under the Precautionary Approach', <u>CNL(04)57</u>, should be taken into account;
 - c) interactions with non-native and invasive species, including pink salmon, may have significant impacts on salmon stocks and should be addressed in the guidelines;
 - d) wider interactions with native species should also be addressed, such as prey / predator interactions. Where a salmon population is heavily reduced, predation can reduce stocks so much it is difficult to maintain and rebuild them. The Group discussed the extent and control of native predator interactions, including challenges when the predator is a protected species. The use of non-lethal and lethal control methods were considered. Predator removal may be appropriate in a limited capacity, such as removal at a smolt bottleneck;
 - e) assessments of the scale of fisheries activity, from both directed wild Atlantic fisheries and catch and release fisheries, and its impact on mortality should be considered in relation to other threats such as insufficient temperature refuges. Bycatch should be considered even without data or with limited data and if shown to be having a detrimental effect then efforts should be made to eliminate it; and
 - f) gene banking is considered crucial at early stages but may be too late if stocks are severely depleted. The guidelines should clarify when and how gene banking should be applied.
- 6.3 The Working Group discussed the importance of monitoring during and after stock rebuilding. The Group made the observation that an appropriate monitoring programme to assess levels of success should be implemented with robust baseline data established prior to taking action. Parties / jurisdictions should be encouraged to adopt emerging technology and conduct research to address areas where there is a lack of data, such as bycatch fisheries.

6.4 The Working Group discussed the need for a glossary to align with NASCO's 'Guidelines for Stocking Atlantic Salmon', <u>CNL(24)61</u>, and to increase the accessibility of the SRP guidelines. A glossary was compiled and agreed by the Group to be added to the SRP guidelines.

7. Investigation of Scientific and Management Protocols for Gene Banking

7.1 Six experts presented information to the Working Group on the use of and / or positions on gene banking in Norway, Canada and Scotland, as listed in paragraph 5.3 above. The question of how to distinguish between gene banking and hatchery supplementation was raised by a member of the Working Group, such as how to ensure an action was considered gene banking rather than hatchery supplementation. Experts presented the view that the distinction mainly came from who was carrying out the action and how it was managed, with the word 'stocking' commonly associated with a legacy of fisheries supplementation orientated to produce high numbers. Gene banking is more concerned with conservation of the species and the integrity of the type of juveniles being produced, with careful management required to conserve as much genetic diversity in a population as possible.

Introduction

- 7.2 The Working Group made the following observations on the background and definition of 'gene banking':
 - a) gene banking should be defined in a way that can be broadly understood and also allows for specific nuances in different contexts, such as the differences between practices in Canada and Norway. 'Gene banking' falls into two broad categories, that of 'frozen gene banking', where milt is removed from salmon then frozen and maintained in cryopreservation facilities, and 'live gene banking', where live salmon are removed from their native habitat, held and bred in captivity, and their progeny reintroduced into the native habitat;
 - b) practices do not always fit conventional 'gene banking', for example Canada's use of two types of 'live gene banking' in either a 'captive live gene bank' or 'in-stream live gene bank' may not be viewed as conventional gene banking;
 - c) in-stream live gene banking is done in isolated sections of stream separate from wild salmon populations, and differs from stocking through its consideration of genetic variation;
 - d) a Scottish Government discussion paper on gene banking (Gilbey 2024¹) highlighting the importance of gene banking in preserving genetic material for threatened stocks included key points:
 - the main purpose of gene banking is to create a living reservoir of genetic material for the re-establishment or enhancement of threatened salmon stocks;
 - the preservation of live fish in living gene banks is a measure used for the most seriously threatened salmon stocks that are no longer capable of surviving in their natural habitats before the danger has been mitigated;

¹ Gilbey, J. 2024. The conservation of Atlantic salmon in Scotland through gene banking: principles and considerations. Scottish Marine and Freshwater Science, 15, 41 pp. doi: 10.7489/12528-1

- cryopreservation of milt is a method used to preserve genetic profiles and maintain genetic integrity;
- frozen gene banking helps prevent the loss of genetic material from threatened populations and allows for future re-establishment, requiring technical expertise in genetics, reproductive physiology, and data management; and
- the main benefit of frozen gene banking is its cost-effectiveness in preserving genetic material;
- e) the timescales of live gene banking versus frozen gene banking require consideration when defining and planning a programme. Live gene banking was deemed, in theory, to have a short timescale and frozen gene banking a longer time scale.

Guidelines Relevant to the Design of Gene Banking Programmes

- 7.3 The Working Group made the following observations on the design of gene banking programmes:
 - a) 'critical genetic diversity' should be defined and included, for example, based on recommendations from the presentations given to the Group by the six experts, as listed in paragraph 5.3 above. 'Critical genetic diversity' should be calculated on an individual river basis;
 - b) gene banking should be considered prior to the point of decline where possible;
 - c) collecting fish from wild populations and ensuring genetic diversity while avoiding negative impacts on natural stocks presents practical challenges. The minimum number of fish to collect for a gene banking programme to ensure the preservation of rare alleles requires informed consideration, especially in situations where populations are very low. Geneticists should be consulted to determine how many fish should be collected from natural populations while ensuring natural spawning is not disturbed;
 - d) gene banking programmes should be implemented alongside stock rebuilding programmes;
 - e) a wide range of factors could be considered in the prioritisation of rivers on which to conduct gene banking programmes. Stocks under consideration for gene banking programmes should be prioritised under region specific criteria; and
 - f) the steps required for broodstock selection in gene banking programmes have considerable overlap with NASCO's Guidelines for Stocking Atlantic Salmon, <u>CNL(24)61</u>. The extensive information specific to gene banking provided by the six experts, listed in paragraph 5.3, was fundamental in informing the guidelines.

Guidelines for Frozen Gene Banking Programmes

- 7.4 The Working Group made the following observations on frozen gene banking programmes:
 - a) stocks are now declining to the extent that gene banking is imperative. Any stocks that are likely to face future decline should be considered for frozen gene banking at as early a stage as possible;

- b) the use of commercial cryopreservation services is preferable where resources allow. Such services have established methods and protocols and therefore could be more effective than small academic laboratories or agency facilities;
- c) the screening of milt for pathogens before its use for frozen gene banking is of great importance and necessary for fish health. However, due to limitations in current technology, pathogen screening often requires killing the fish, particularly for testing for viral infections. As a result a male can only be stripped of milt once.
- d) if a male contributes a lot of milt in one stripping, it can be stored in several containers ('straws') and frozen for subsequent use. The milt from one male can be used more than once in one generation but it has to be in the right proportion and in connection with the size of the stock etc.
- e) genetic analysis is often used before adding fish to the gene bank to avoid closely related individuals being included. This ensures the preservation of genetic diversity in the gene bank; and
- f) the guidelines for broodstock mating are relevant to both frozen gene banking and live gene banking. They should, therefore, be included in both sections.

Guidelines for Live Gene Banking Programmes (in Special Facilities)

- 7.5 The Working Group made the following observations on live gene banking programmes (in special facilities):
 - a) frozen gene banking is seen as a long-term solution, while live gene banking is considered for short-term use. A live gene bank in its purest form would be just holding that population in captivity until conditions improve in its native environment. For the purposes of these guidelines, a live gene bank should ideally involve both males and females, not just females with frozen milt;
 - b) in terms of broodstock mating, Norway uses a special data programme to avoid crossbreeding close relatives. Careful consideration is required when using males from different year classes or frozen milt from different years, and how this affects genetic diversity;
 - c) broodstock may be used for multiple years; however, the number of years should be limited. The number of years would vary between gene banking programmes depending on the predefined criteria and objectives of the programme;
 - d) maintaining high water quality in live gene banking facilities is important, each family within a gene bank should have its own water supply to prevent cross-contamination; and
 - e) the guidelines should address pathogen screening for fish entering gene banks and how to handle infected fish. A gene bank should have much stricter control than a hatchery. Gene banks in Norway have strict biosecurity protocols, ensuring all eggs are disinfected. Milt from different males is stored in separate containers to prevent cross-contamination.

Guidelines for In-stream Live Gene Banking Components

- 7.6 The Working Group made the following observations on in-stream live gene banking components:
 - a) live in-stream gene banking applications are where fish are placed temporarily in the wild to maintain a genetic representation until they are later recaptured. The fish are returned to the gene bank facility after their exposure in the wild;
 - b) this strategy attempts to reduce the artificial influence of hatcheries and preserve both genetic diversity and adaptive traits suited to the wild;
 - c) biosecurity may be a concern, especially with potential wild exposure of live gene bank fish. Such concerns may be addressed by selecting isolated sites or stretches of river that can be physically separated from wild populations using natural or artificial barriers (like waterfalls or dams);
 - d) the recapture process involves health screening of fish several weeks before recapture to ensure they are pathogen-free. Fish that test positive to pathogens would not be retained. The ideal scenario is to have a facility to quarantine recaptured fish until a clean bill of health is given before returning them to the gene bank;
 - e) careful consideration should be given to the escape of surplus fish to the wild in view of biosecurity. Fish should be released in carefully planned scenarios to ensure minimal risk;
 - f) the optimal time of exposure of fish to wild conditions will vary by definition due to variation in river, environmental conditions, and the specific goals of individual gene bank programmes; and
 - g) in-stream live gene banking aims to continue growing fish out and rearing them to maturity. After reaching maturity, these fish could be bred within the associated live gene bank programme, allowing for the process to be repeated as described in the preceding section on guidelines for live gene banking programmes.

When to Start Releases from a Gene Bank

- 7.7 The Group invited Sten Karlsson (Norwegian Institute for Nature Research, Norway) to speak on when to start releases from a gene bank as an expert in the field. Dr Karlsson gave a presentation on 'Advice for gene banking' which included four common scenarios for considering the introduction of fish produced through gene banking into a river.
- 7.8 The Working Group made the following observations on when to start releases from a gene bank:
 - a) monitoring is required to establish if conditions are suitable to release fish produced through gene banking techniques. Scenarios in which releases should be considered include indications that the threat has gone or will be gone soon and environmental conditions are improving, but the remaining population is of unsuitable genetic variation and / or is at a critically low level; and
 - b) monitoring is essential to establish if conditions are unsuitable for the release of fish produced through gene banking techniques. Scenarios in which releases should not be considered include indications that the threat and environmental conditions are

poor and are not expected to improve soon, or that the threat has gone and the remaining population has reached a sustainable level with suitable genetic variation.

Monitoring

- 7.9 The Working Group made the following observations on monitoring:
 - a) monitoring of the fish in the gene banks is important, to ensure the required level of genetic variation and integrity is achieved and to adjust the parameters of the gene banking programme as needed. Criteria to be monitored may include for example the life stages and the number of fish released or where in the watercourse the releases take place;
 - b) monitoring of the fish released into the stream is important, from the survival and growth of the fish to competition between the offspring of the fish from the gene bank and wild fish. This information is required to demonstrate whether the releases are successfully increasing the population, both in terms of its size and its genetic diversity. Caution is needed when a river still has wild fish so that the gene banked offspring will not outcompete or be outcompeted by the wild fish;
 - c) continuous monitoring of the habitat the fish produced by the gene banks are released into is essential to ensure the environmental conditions are suitable to support the released fish. It is important to establish whether the factors that led to the decline of the population remain and continue to pose a threat; and
 - d) monitoring will enable the assessment of whether the overall goals of the programme have been achieved and the releases should be continued or not. It is desirable to terminate any gene banking programme as soon as possible.

Retention of Gene Banks

- 7.10 The Working Group made the following observations on the retention of gene banks:
 - a) once monitoring has indicated that releases from a live gene bank should not continue, the live gene bank should cease operating;
 - b) the milt of any male fish remaining in a live gene bank that are not included in the frozen gene bank should be sampled and frozen before ending the operation of the live gene bank;
 - c) the domestic protocols of individual Parties / jurisdictions should be used to determine the treatment of any fish remaining in the live gene bank. This may vary from releasing the fish into an appropriate waterbody to terminating them; and
 - d) any frozen gene bank samples should be retained for possible future use.

8. **Recommendations to Council**

a) Revisions to the 'Guidelines on the Use of Stock Rebuilding Programmes in the Context of the Precautionary Management of Salmon Stocks'

8.1 The Group considered the current structure of the 'Guidelines for Stocking Atlantic Salmon', <u>CNL(24)61</u>, that were agreed by Council at the NASCO 2024 Annual Meeting, as a template for the updated guidelines on the use of stock rebuilding programmes in the context of the precautionary management of wild Atlantic salmon. The Group developed 'Draft Guidelines on the Use of Stock Rebuilding Programmes in the Context of the Precautionary Management of Salmon Stocks', SGWG(24)06, (Annex 3), and recommends that Council adopt these Draft Guidelines.

8.2 The Group noted that there would be an element of overlap between these Guidelines and the 'Guidelines for Stocking Atlantic Salmon', <u>CNL(24)61</u>. The nature of required or desired action to address a threatened salmon stock may require both documents to be consulted for guidance.

b) Scientific and Management Protocols for Gene Banking

- 8.3 The Group considered the current structure of the 'Guidelines for Stocking Atlantic Salmon', <u>CNL(24)61</u>, as a template for the guidelines on gene banking. The Group developed 'Draft Guidelines for Gene Banking for Wild Atlantic Salmon', SGWG(24)07, (Annex 4), and recommends that Council adopt these Guidelines.
- 8.4 The Group noted that there would be an element of overlap between these Guidelines and both the 'Guidelines for Stocking Atlantic Salmon', <u>CNL(24)61</u>, and the proposed 'Draft Guidelines on the Use of Stock Rebuilding Programmes in the Context of the Precautionary Management of Salmon Stocks', SGWG(24)06. The nature of required or desired action to address a threatened salmon stock may require all three documents to be consulted for guidance.
- 8.5 The Group raised that it was important to note that genetic experts should be consulted in any consideration of gene banking wild Atlantic salmon populations.

c) Any Other Recommendations

- 8.6 The Group noted that the experts consulted in the development of the gene banking guidelines made an urgent call for the widespread cryopreservation of milt in frozen gene banks as soon as possible given the severe population decline of wild Atlantic salmon across its range.
- 8.7 The Group recommends, therefore, that Council urges Parties / jurisdictions to establish frozen gene banks as soon as possible to ensure existing genetic diversity of wild Atlantic salmon is preserved.

9. Other Business

9.1 There was no other business.

10. Report of the Meeting

10.1 The Group agreed the report of its meeting.

11. Close of the Meeting

11.1 The Chair thanked participants for their contributions and closed the meeting.

Stocking Guidelines Working Group Meeting – List of Participants

Hlynur Bárðarson	Marine Research Institute, Iceland
Steve Gephard (Chair)	Fisheries Consultant, United States
Heidi Hansen	Norwegian Environment Agency
Sarah McLean	Loughs Agency, European Union
Steve Sutton (NGO)	Atlantic Salmon Federation, Canada
Simon Toms	Environment Agency, UK
John Whitelaw	Parks Canada
Emma Hatfield	Secretary, NASCO
Clare Cavers	Assistant Secretary, NASCO

Annex 2

SGWG(24)05

Meeting of the Stocking Guidelines Working Group to Revise Stock Rebuilding Programmes

5, 6, 25, 26, 27 September, 11, 28, 30 October, 7, 11, 27 November, 9 December 2024, 10, 13, 21 January and 6, 19 February 2025

By Video Conference

Agenda

- 1. Opening of the Meeting
- 2. Adoption of the Agenda
- 3. Background to the Stocking Guidelines Working Group
- 4. Consideration of the Terms of Reference for the Stocking Guidelines Working Group, <u>CNL(24)68</u>
- 5. Consideration of Working Methods
- 6. Consideration of Stock Rebuilding Programmes in the context of the Precautionary Management of Salmon Stocks
- 7. Investigation of Scientific and Management Protocols for Gene Banking
- 8. Recommendations to Council
 - a) Revisions to the 'Guidelines on the Use of Stock Rebuilding Programmes in the Context of the Precautionary Management of Salmon Stocks'
 - b) Scientific and Management Protocols for Gene Banking
 - c) Any Other Recommendations
- 9. Other Business
- 10. Report of the Meeting
- 11. Close of the Meeting

Annex 3

SGWG(24)06

Draft Guidelines on the Use of Stock Rebuilding Programmes in the Context of the Precautionary Management of Salmon Stocks

1. Introduction

This document provides guidance on the process of establishing Stock Rebuilding Programmes (SRP) for wild Atlantic salmon stocks, in relation to the Precautionary Approach as adopted by NASCO in 1998². It also references several other guidance documents developed by NASCO and should be read in conjunction with those documents. These Guidelines replace a previous set of NASCO Guidelines adopted in 2004 and incorporate current concerns in the context of the overarching threat of climate change.

An SRP is likely to be a suite of management measures designed to restore a wild Atlantic salmon stock to a sustainable level as measured using a defined river-specific target. The nature and extent of the programme will depend upon the status of the stock and the pressures that it is facing.

While the short-term response to a stock failing to achieve its river-specific target may be to reduce or eliminate exploitation, there will generally be a need to identify and address the causes of the stock decline along with other actions.

2. Determination of the Need for a Stock Rebuilding Programme

I. Consult Stakeholders³

Stakeholder groups and Indigenous Peoples need to be consulted when SRPs are being considered and kept informed when action is planned. Wherever possible, they should be involved from the earliest stages in the development of an SRP. Benefit may be gained from their general experience of wild Atlantic salmon management and their specific knowledge of the stock(s) in question.

Consideration also needs to be given to the potential incidental effects of an SRP on other users or those with interests in other parts of the ecosystem that may be affected. Early involvement may also help to secure the buy-in of groups that may be affected by proposed measures.

The responsibilities of different groups and organizations in the SRP must be clearly defined.

Consideration should be given to the development of education material for dissemination to interested groups and the wider public.

II. Evaluate Status of Stock

A. When to Develop Stock Rebuilding Programmes

It is recommended that SRPs be developed for all stocks that are failing to exceed their Conservation Limits (CLs) or other relevant reference points (RP). Parties / jurisdictions may also consider SRPs where the long-term viability of the stock is at risk of failing to exceed its CL. Most NASCO Parties / jurisdictions have established CLs for many of their salmon stocks, based at a national, regional, river or population level according to their management objectives. There may be some Parties / jurisdictions that use alternative means for

² Agreement on Adoption of a Precautionary Approach, <u>CNL(98)46</u>

³ Guidelines for incorporating social and economic factors in decisions under the Precautionary Approach, <u>CNL(04)57</u>

determining if a stock is healthy and these Guidelines are also applicable in those cases. However, assessing the status of the stock requires more than simply determining whether the escapement has fallen below the CL, and a range of other factors will influence management decisions on the nature and extent of the SRP. As Parties / jurisdictions identify a stock that is in need of rebuilding by an SRP, baseline data need to be documented to assist determining the impact made by an implemented SRP.

B. Uncertainty in Assessments of Stock Status

Information on the stock may be limited, so there may be uncertainties about both the CL/RP and the current stock status. In addition, the numbers of salmon returning to spawn can be highly variable, and so the stock will sometimes fluctuate around the CL/RP simply as a result of natural variation. These uncertainties must be taken into account in the decision-making process.

C. Nature of Stock Decline

Both the duration and degree of the stock decline are relevant to the determination of the need for an SRP. The further a stock falls below its CL / RP and the more years for which it does this, the greater the need for management action. The nature of the stock decline (e.g. timing and severity of decline) may also be informative in identifying the main causes. However, if the stock has been well above the CL / RP in recent years, this may suggest that the current management practices are appropriate under most normal circumstances.

D. Stock Diversity

Consideration must also be given to other stock criteria, such as age structure, run timing, fecundity and genetic diversity. A minor overall shortfall in egg deposition, for example, may mask an underlying problem with one or more stock components.

III. Evaluate Causes of Stock Decline and Threats to Stock

Proposals for remedial measures should be developed on the basis of a full assessment of the pressures faced by the stock. Stocks may fall below their CL/RP as a result of reduced production and / or increased mortality, and these can result from either natural or anthropogenic factors (including fishing). The exact reasons for the stock decline may be unknown and multifaceted, but possible causes and potential threats should be described and evaluated. The following categories of factors may be considered:

A. Climate Change

(including more intense and more frequent short-term weather events such as storms, flood and drought conditions and heat waves, as well as long-term changes in sea level, warming rivers and oceans and associated changes in predator and prey distributions)

Climate change is an overarching, anthropogenic-induced change that will directly affect wild Atlantic salmon and exacerbate the many existing threats that create additional challenges for the survival of wild Atlantic salmon at all life history stages. Remedial measures will be needed to address the rapid changes in conditions that are likely to be brought about due to climate change.

B. Environmental Change

(including rainfall and river flow patterns, river temperatures, changes in water pH)

Short-term environmental change often occurs as a result of distinct natural events such as fires, storms or landslides. Long-term environmental change often occurs as a result of ongoing anthropogenic activities not associated with climate change, such as pollution,

hydropower operations, clear-cutting forests, etc. Appropriate management actions will need to take account of best predictions of the likely duration and extent of the environmental change.

C. Habitat Degradation

(including water quality, water chemistry, water quantity caused by man-made structures or extractions, sedimentation, factors affecting food production, obstructions and impediments to juvenile or adult migration)

Habitat degradation often results in harmful reduction of population productivity levels and carrying capacity. It is important to identify areas of degraded habitat and determine whether the causes are natural or man-made and whether or not the impact is reversible.

D. Impacts of Salmonid Farming

(including escaped fish, genetic introgression in wild salmon stocks, transfer of sea lice and other pathogens from farmed salmon to wild salmon)

The open pen rearing of Atlantic salmon and other salmonids has been shown to have deleterious impacts on wild Atlantic salmon stocks. It is important to assess the extent of the impact of such fish farms on wild stocks.

E. Species Interactions

(including predator and prey interactions, diseases and parasites, competition with native species, interactions with non-native species including intentional introductions and dispersal of previously introduced species into new regions, such as pink salmon)

Issues discussed in this section are considered separate to interactions related to impacts of salmon farming as discussed in 2.III.D above. The potential impact of all trophic level interactions should be assessed taking into account known characteristics such as salmon and predator / prey ecology and any changes in stocks of other species. Other species may carry pathogens that could affect wild Atlantic salmon. The stocking of other fish species could introduce a source for competition and alter predator / prey interactions.

F. Fisheries

(including catches of wild Atlantic salmon in all directed fisheries in marine, estuarine and freshwater habitats, bycatch of all life stages of wild Atlantic salmon, non-catch fishing mortality, harvesting of prey species)

There are directed fisheries that harvest adult wild Atlantic salmon; studies have shown that even catch and release recreational fishing is a source of mortality. There is evidence of bycatch of wild Atlantic salmon in some marine fisheries, e.g. in mackerel fisheries, but the full scale is unknown. Bycatch is likely in fisheries that overlap with wild Atlantic salmon migrations. Fisheries that harvest forage species may reduce the amount of available food for wild Atlantic salmon.

G. Stock Component Effects

(including sea-age groups, size classes, tributary populations, etc.)

The threats listed above may affect stock components differently. It is important to identify those components in greatest need of protection or restoration in each instance. For example, age groups may be differentially affected by fisheries that are size-selective, and tributary populations may be differentially affected by water quality problems.

H. Loss of Genetic Diversity and Integrity

(inbreeding, loss of rare alleles, reduced effective population size)

When salmon stocks decline there is often a loss of genetic diversity that leads to inbreeding, loss of rare alleles and a reduced effective population size. This in turn leads to reduced fitness and performance and thus survival. Stock rebuilding programmes often include the stocking of hatchery-reared fish, which can cause or accelerate negative genetic impacts (see 'Guidelines for Stocking Atlantic Salmon', <u>CNL(24)61</u>).

I. Research Needs

The lack of sufficient data can be an obstacle to an appropriate evidence base to inform stock rebuilding programmes.

3. Initiation of Stock Rebuilding Programmes

I. Plan and Prioritise Management Actions

The SRP should be developed to address the causes of stock decline and threats that have been identified in section 2.III. Efforts should be made to ensure all activities are consistent with the Precautionary Approach. The SRP should include an agreed series of aims and objectives, within a defined timeline, against which stock recovery should be monitored and assessed, as well as both interim and final targets. Where several causes of stock decline have been identified, prioritisation of actions may assist in planning the SRP.

A. Climate Change Mitigation

When a specific impact can be attributed to climate change, special actions may be possible. For example, if stream water temperature is exceeding the species' tolerance, the identification and protection of cold-water refuges could be considered. Cold water refuges can also be achieved by creating shade through the planting of trees in the riparian zone. At locations and times when fish are likely to be stressed by high water temperatures, fisheries, including catch-and-release, should be suspended to minimise the risk of increased mortality.

B. Environmental Change Mitigation

If a distinct natural event is the cause of stock decline, remedial action should be considered to reduce the impact and build resilience. For example, in the case of a landslide, any blockages resulting in migratory barriers should be removed and damaged habitat restored. Environmental change caused by anthropogenic activities can often be mitigated through the termination of the harmful activity (such as pollution), the reversal of the impacts (such as liming acidified streams) or compensation of the impact (such as fish passage at migration barriers). All new proposals need to be appropriately assessed and regulated to minimise the risk of harmful environmental change.

C. Habitat Restoration

Degraded habitat can often be improved by using commonly accepted restoration practices such as removal of barriers, reconstruction of original stream channel and the addition of habitat complexity such as creating stream meanders, in-stream wood structures and boulder clusters. Further guidance is provided in document $CNL(10)51^4$, which provides a framework for use by jurisdictions that have responsibility for activities involving wild Atlantic salmon habitat.

⁴ NASCO Guidelines for the Protection, Restoration and Enhancement of Atlantic salmon Habitat, <u>CNL(10)51</u>

D. Reducing the Impacts of Salmonid Farming

NASCO provides guidance on best management practices (BMPs) in salmonid farming in document $\underline{SLG(09)5}^5$, which are intended to reduce the impacts of salmonid farming. If such BMPs are not implemented or are followed but are ineffective, eliminating specific open pen farms and transitioning to closed containment facilities may be advisable.

E. Mitigating Species Interactions

These actions will depend on the involved species and the nature of the interaction with wild Atlantic salmon (e.g. competition or predator / prey interactions). If the interacting species is non-native, its elimination, reduction or containment should be considered, if feasible. The effects of programmes introducing and / or stocking other species should be evaluated for negative interactions with wild Atlantic salmon and curtailed if necessary. Non-lethal deterrence of native wild Atlantic salmon predators could also be considered, where feasible. In limited cases, where deterrence methods are not appropriate or have failed, lethal methods of control of native wild Atlantic salmon predators might be considered when carried out under appropriate legal constraints. Issues created as a result of negative species interactions could be mitigated through the restoration of native habitat suitable to improve conditions favourable for wild Atlantic salmon. Possible sources of pathogens affecting wild Atlantic salmon should be investigated and appropriate management measures implemented.

F. Fishery Management

Assessments at the relevant scale of how aspects of fisheries activity are contributing to stock decline and long-term sustainability should be conducted based on the best available data. Reducing the mortality in directed wild Atlantic salmon fisheries is often the first response to a decline in stocks since it is likely to have the most immediate effect on the spawning escapement. However, reducing mortality alone is unlikely to have long-term benefits for recovery if other issues are the root cause of the decline. Therefore, reducing harvest and unwanted mortality, such as in catch and release fisheries, should be seen in the context of other measures that may be taken, such as reductions in unreported catch and bycatch, improving and restoring habitat and reducing the impacts of salmonid farming. Reductions in fisheries mortality may only be required while other problems / threats are remedied. However, if long-term changes in production are expected, there may be a need for a modified harvest strategy. Ideally, such responses should be based upon approved plans. If bycatch of wild Atlantic salmon or the harvest of wild Atlantic salmon forage species are shown to be having a detrimental impact to any stock rebuilding programme, efforts should be made to eliminate these threats. The NASCO Decision Structure⁶ provides further guidance on the decision-making process for determining appropriate management measures in targeted fisheries.

G. Conserving Diversity of Stock Components

Management decisions must take all components of the stock into consideration. For example, if a fishery is killing only early-run fish, that harvest must either be eliminated or the mortality should be distributed throughout the entire time frame of the run. Furthermore, if there is a size-selective fishery, size-at-harvest and / or gear regulations can be adjusted to reduce such selectivity. Additionally, if broodstock collection for any SRP does not sample all stock components adequately, such practices need to be modified.

⁵ Guidance on Best Management Practices to address impacts of sea lice and escaped farmed salmon on wild salmon stocks, <u>SLG(09)5</u>

⁶ Decision Structure For Management of North Atlantic Salmon Fisheries. 2002. <u>CNL31.332</u>

H. Conserving and Restoring Genetic Diversity and Integrity

Conserving genetic variation and integrity will improve the chances that a stock can be rebuilt. Gene banking can be an important tool in conserving and restoring genetic diversity such as through the conservation of important and rare alleles and traits. Further guidance on gene banking can be found in document CNL(25)XX (also see section 3.II.C. below). Further guidance on stocking can be found in NASCO's 'Guidelines for Stocking Atlantic Salmon', <u>CNL(24)61</u>.

I. Research Needs

Where there is insufficient information of the nature and / or extent of the threats facing the stock, the management plan needs to include a provision for further research.

II. Interim Measures

Where stocks are severely depleted, full recovery is likely to take several generations; therefore, there may be a need to develop a staged approach to any SRP and to adopt certain interim measures, such as, and in no particular order:

A. Interim Reference Points

Where stock rebuilding is likely to take several generations it may be appropriate to define intermediate 'recovery' reference points. This may assist in tracking stock recovery over a longer period.

B. Stocking

NASCO considers that where integrity (i.e. evolutionary and ecological naturalness) of the wild stock is a management priority, stocking should not be considered as a remediation measure. However, consideration may be given to the need for interim stocking of hatchery products as an emergency stock protection measure. Stocking may be used to circumvent bottlenecks in production while other actions are taken to address the cause of the stock decline. Further guidance is provided in NASCO's 'Guidelines for Stocking Atlantic Salmon', <u>CNL(24)61</u>.

C. Gene Banking

This can be considered another means of protecting valuable stock traits when the total loss of a stock is possible. However, it should be noted that gene banking should be implemented at an early stage of the SRP. Further guidance is provided in NASCO's 'Guidelines for Gene Banking for Wild Atlantic Salmon', CNL(25)XX.

III. Assess Social and Economic Factors

Consider the social and economic consequences of different management options including the possible impacts on other users and other activities that may affect success⁷. NASCO developed its 'Guidelines for Incorporating Social and Economic Factors in Decisions under the Precautionary Approach', <u>CNL(04)57</u>, to provide a framework for incorporating social and economic factors into decisions that may affect wild Atlantic salmon and the environments in which it lives.

These factors include, for example:

• Indigenous Peoples' knowledge / rights / customs;

⁷ It is worth noting that NASCO has agreed, as part of its Ten-Year Strategy, <u>CNL(24)71rev</u>, that any of its Resolutions, Agreements and Guidelines to be updated from 2025 onwards will include socio-economic considerations

- whether there is a need to permit a residual fishery to continue (e.g. subsistence fishing);
- whether the fishery itself has an intrinsic value (e.g. cultural and / or heritage values of specific methods); or
- whether certain lower impact fishing activities (e.g. catch and release angling) may be allowed with the aim of retaining public interest in wild Atlantic salmon conservation.

IV. Monitor and Evaluate Stock Recovery

Monitoring should be conducted until the aims and objectives of the SRP are reached, to permit appropriate evaluation of the stock recovery. Objectives, and the strategies to achieve them, should be reviewed, and revised if necessary, at regular intervals during the recovery process.

The stock recovery assessment must reference the appropriate baseline (see section 2.II.A), against which success should be measured. This should also help identify any failures in the SRP that would need to be addressed.

Consideration should be given to post-project evaluation (after the original aims and objectives have been met) to assess the long-term efficacy of the SRP.

Efforts should be made to disseminate the outcome of any SRP, including both successes and failures, to facilitate knowledge-sharing.

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.

Conservation Limits (CLs) – reference points or thresholds used to define the minimum number of spawning adults required for a healthy, sustainable salmon population in a specific river or population.

Broodstock – an individual or a group of mature individuals 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.

Escapement – the number of adult salmon that return to their spawning grounds, typically used as an indicator of stock health.

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

Gene banking – 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. Typically, a gene bank is cryopreserved samples of milt from targeted salmon populations. The samples can be thawed and used in the future to culture individuals as part of a recovery or restoration program.

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.

Reference Points (RPs) – benchmarks used to guide management decisions regarding the status of a salmon stock, such as target population levels or biomass thresholds.

Sea-age groups – subdivisions of a salmon population based on the number of years spent at sea before returning to fresh water to spawn, influencing the timing and management of fisheries.

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

Stock diversity – the variety within a stock of salmon, including factors like age structure, run timing, and genetic diversity, all of which contribute to the resilience and sustainability of the stock.

Trophic interactions – the relationships between organisms within an ecosystem, including predator-prey dynamics, competition for food, and other interspecies interactions that can affect salmon populations.

SGWG(24)07

Draft Guidelines for Gene Banking for Wild Atlantic Salmon

1. Introduction

The purpose for the guidelines outlined below is to provide best management practices⁸ 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)XX). 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 streams. 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,

⁸ 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.

including pathogens, hydropower and climate change, as well as for the resilience and adaptability of salmon to changing environments.

NASCO recommends that Parties / jurisdictions establish 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)XX).
- 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 stream 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 are inappropriate. Where possible, gene banking should be considered prior to the point of decline.

- F. Broodstock Selection
 - 1. Collect wild salmon from targeted streams for broodstock. Mature parr and prespawned adults can be for immediate use and non-mature parr, smolts or pre- or postspawned 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 stream.
 - 3. Collect fish from as many different locations in the target stream 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 stream population is very low.

- 6. Screen collected fish for disease using appropriate techniques and reject any fish that test positive for any pathogen, especially those that are transmitted vertically.
- 7. 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).
- 8. 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 streams 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', <u>CNL(24)61</u>, 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 stream 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 stream), NASCO's 'Guidelines for Stocking Atlantic Salmon', <u>CNL(24)61</u>, 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', <u>CNL(24)61</u>, 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-stream Live Gene Banking Components

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

In-stream 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 stream 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 stream until such time monitoring suggests it be discontinued. This ensures the continuity of the in-stream 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 stream at the earliest stage possible (the 'wild-exposed' fish). Ensure equal representation of individual families are 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-stream 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 program, 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 stream.

- A. Scenario1: when monitoring indicates that the threat that caused the stock decline is gone, and the in-stream 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 stream 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 stream 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 stream 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 stream 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 stream fish with unsuitable genetic variation and integrity do not dominate the stream 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 stream 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-stream 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 stream 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-stream 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 stream, 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 stream 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.