



EVALUATION OF CAPTIVE BREEDING FACILITIES IN THE CONTEXT OF THEIR CONTRIBUTION TO CONSERVATION OF BIODIVERSITY



Figure 1: Department of Fisheries and Oceans' (DFO) six administrative regions.

Context :

The maintenance of genetic diversity within populations and species is a key component of conservation biology, and acknowledged as an important goal in major international agreements such as the Convention on Biological Diversity. It is often a particular concern for species at risk, where there can be a high risk of loss of genetic diversity when populations are reduced to low numbers. Conservation biology principles encourage consideration of genetic diversity when planning and implementing recovery efforts for species at risk.

DFO maintains facilities for live-gene banking of endangered units of Atlantic salmon in Atlantic Canada, and there are discussions about the role of hatchery facilities in the Pacific Region with regard to recovery of species and population units at risk. As one component of a review of the potential costs and benefits of such programmes DFO Science struck a national Working Group to review a number of questions about the performance of captive rearing facilities with regard to maintain genetic diversity and supporting recovery of naturally breeding wild populations. The key scientific questions to be addressed were:

What is the role (if any) of hatchery facilities in conservation of biodiversity, particularly of salmonids?

- 1) Can live gene banking and supportive rearing conserve the genetic diversity within populations?
- 2) If the genetic diversity can be maintained using these approaches, what is the evidence that these lines can be reintroduced successfully as self-sustaining populations if/when the threats are removed?
- 3) Are there technical alternatives to hatchery facilities for conservation of genetic diversity and fitness?

If the answers to the above questions support a role for such facilities in conservation of genetic diversity and recovery of species at risk, there is also scope for discussing the biological rationale for maintaining multiple facilities in one area. The answers to these questions will be an important contribution to planning for conservation and recovery of aquatic species at risk.

SUMMARY

- Live gene banking programs are not a stand-alone solution to conservation of biodiversity. Threats to a wild population must be addressed effectively for the conservation of biodiversity to be achieved.
- The conclusions below were developed specifically for programs designed for severely depleted populations where there is thought to be a serious risk of extirpation. Guidelines for use of facilities in other circumstances already exist and were endorsed.
- Maintaining genetic diversity in a captive breeding program during a period of very low survival in the wild is a wise strategy whenever the low survival is due to a cause which can be addressed by management intervention, and such interventions are planned or possible to implement; or the low survival is due to environmental causes and there is an expectation that in the future conditions may return to those associated with higher survivorship. Other conditions are discussed for when captive breeding program may also be appropriate strategies.
- With careful attention to a number of aspects of the breeding program at least neutral genetic diversity within populations (and perhaps quantitative genetic diversity) can be sufficiently maintained in captivity for several generations, with loss rates estimated to be below 2% per generation. This rate is much lower than expected loss rates without attention to those aspects of the breeding program.
- Nine specific practices are listed that should be part of captive breeding programs to minimize loss of genetic diversity. These include practices applied in selecting founders, developing mating strategies, managing family sizes of progeny, protection against failures of facilities, introducing progeny in the wild, and monitoring the captive and wild populations.
- Evidence is summarized that the loss rate calculated for genetic diversity of inner Bay of Fundy salmon is well under 1%.
- The features necessary for a captive breeding program to have a high expectation of maintaining genetic diversity and the possibility of minimizing loss of fitness in the wild can be combined a number of ways, with varying implications for operation costs, likelihood of maintaining the full genetic diversity of the founder stock, and robustness to mistakes or catastrophes.
- For careful captive breeding programs to have a high likelihood of maintaining genetic diversity it is necessary to have a sufficiently large breeding population and to start the program before the wild population has declined to an extent that substantial genetic diversity has already been lost in the wild population
- Captive breeding and rearing programs should include an effective and comprehensive evaluation and monitoring component.
- Risk management and application of precaution imply that having individual genetic strains in multiple facilities is good protection against catastrophes. However, there are no compelling reasons why a single facility could not support multiple genetic strains as long as operational procedures were well designed and adhered to strictly.
- The evidence is not conclusive with regard to successful reintroduction of populations that have been maintained in captivity. Many examples of failures at re-establishing self-sustaining populations can be traced to either failures to address the threats that posed the original risk, or to captive breeding programs that did not apply appropriate measures.

INTRODUCTION

A peer review and advisory meeting was held in March, 2008, to address the questions posed in the Context. The meeting was informed by a major literature review and working papers summarizing the results of the live-gene banking project in Atlantic Canada. This CSAS Science Advisory Report is the product of the scientific review and deliberations at that meeting.

1. This Science Advisory Report is specifically about live gene banking and more generally captive rearing as a conservation measure for populations at risk of extinction. It is stressed that even live gene banking programs are not a stand-alone solution to conservation of biodiversity. Wherever there are threats to a wild population, including but not exclusively habitat loss, barriers to access to suitable habitat, and direct or indirect harvesting, the threats must be addressed effectively for the conservation of biodiversity to be to be achieved.

FRAMEWORK FOR ASSESSMENT

The role of hatchery facilities:

2. There are a range of programs that involve breeding salmon in captivity, as illustrated in Figure 2. Programs and measures necessary for conservation of biodiversity are different from top to bottom, and goals for hatcheries as a component of those programs will change correspondingly. The conclusions below were developed specifically for programmes designed for severely depleted populations where there is thought to be a serious risk of extirpation.

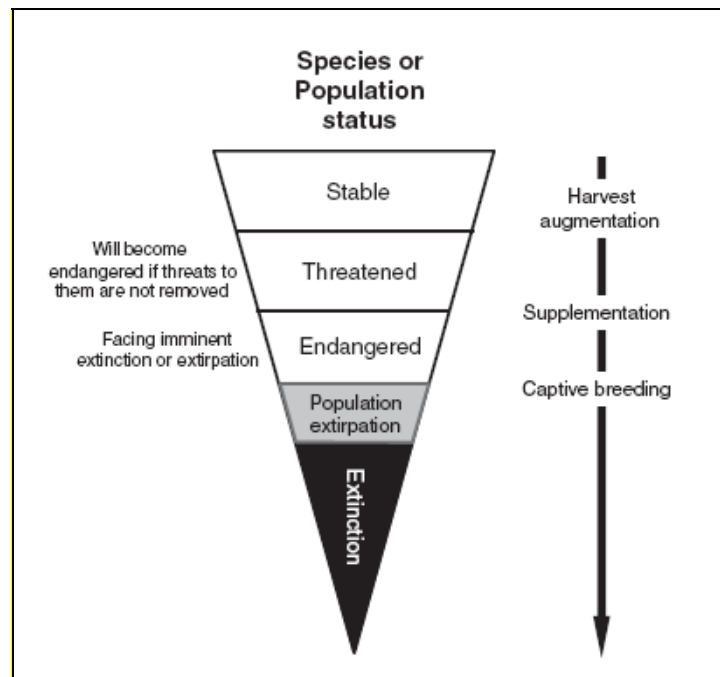


Figure 2: The continuum of different types of hatchery programs ('harvest supplementation', 'supplementation', and 'captive-breeding') in relation to the status of a species or population. The designation of different programs to specific points along the continuum is not intended to be prescriptive. After Fraser, D.J. (2008).

3. There are excellent guidelines for hatchery operations for harvest augmentation and these were reaffirmed at this meeting.
4. When a population is considered in need of rebuilding but not at imminent risk of extinction, priority should be given to addressing known threats to the population. However, in some cases supplementation with hatcheries might play a constructive role in assisting in recovery, as a complement to addressing the threats with effective management actions. In these cases there are also excellent guidelines for using hatcheries in the supplementation role, and these were also endorsed at the meeting.
5. The additional guidelines in this SAR with regard to captive breeding programs apply to stages in Figure 2 where risk of extirpation is high and the management measures that have been implementing do not ensure rapid and secure recovery.

When captive breeding programs should be considered for implementation

6. Maintaining genetic diversity in a captive breeding program during a period of very low survival in the wild is a wise strategy whenever:
 - a. the low survival is due to a cause which can be addressed by management intervention, and such interventions are planned or possible to implement; OR
 - b. the low survival is due to environmental causes and there is an expectation that in the future conditions may return to those associated with higher survivorship.
7. Even when the conditions in 6 are not met (for example the future environmental conditions may be unknown but there is no reason to assume that they will be similar to past conditions when survivorship was higher), maintaining genetic diversity in a captive breeding program when survival in the wild is low can be a wise strategy because:
 - a. There may have been inherent value in the traits which made a population distinct to begin with; loss of such traits is irreversible;
 - b. There may have been inherent value in the traits which made a population distinct, and these traits may be useful in helping this or other populations adapt to future challenges;
 - c. The original population in an area may still be the best starting point for a population that has the capacity to adapt to the different current or future conditions, because it was better adapted to the local conditions than any other lineage;
 - d. These populations may be a source of traits needed for future human uses that are currently unforeseen (e.g. resistance to a disease that in future could threaten aquaculture).

These rationales also apply for units of biodiversity above the population level.

Maintenance of Genetic Diversity in Facilities

8. Over time, closed, captive populations of salmon simply kept and bred in captivity are likely to lose genetic diversity, measured from neutral markers, both due to random

processes and possibly to adaptation to domestic culture. (Rates of loss of genetic markers vary greatly but values of 10-20% per generation have been estimated for the early period of captive breeding.)

9. The rates of loss of non-neutral (adaptive in the wild) genes must be at least as great and may be greater if they are selected against during domestication. The rate of loss of genetic diversity of species with life histories different from salmon might be different from the rates of loss estimated for salmon.
10. With careful attention to a number of aspects of the breeding program at least neutral genetic diversity within populations (and perhaps quantitative genetic diversity) can be sufficiently maintained in captivity for several generations, with loss rates estimated to be below 2% per generation.
11. In general practices to be applied in a captive breeding program for conservation of biodiversity should include:
 - a. Founders should be sampled from the remaining wild population in such a way that best represents all known components of that population, and minimizes selection for any subset of the range of trait variation observed.
 - b. An adequate number of individuals (200-300) should be obtained from as many geographically varied sites as possible.
 - c. Offspring should be analyzed at 10 or more variable molecular genetic markers. This information should be used to estimate first-order relatedness (kinship), and in planning for recovery of founder diversity.
 - d. Mating strategies should be employed that minimize (1) loss of genetic variation, (2) accumulation of inbreeding over time, and (3) adaptation to captivity.

In the absence of any pedigree information, the first two can be minimized by maximizing the effective population size through equalizing sex ratios among spawners, minimizing fluctuations in population size across generations, and minimizing variance in family size of offspring. This is particularly important at the adult stage when they are ready to be spawned in the production of the next generation (i.e., producing as close as possible two mature adult spawners for every set of parents spawned in the previous generation; note that "family" in the absence of pedigree information is the offspring from each spawning);

- e. Minimizing variation in family size will also serve to reduce (halve) adaptation to captivity. Steps that can be taken to achieve this, in order of increasing effort and expense, include:
 - i. sample representatives of families prior to combining for communal rearing;
 - ii. select the appropriate number of offspring so that, to the extent possible, two individuals from each cross survive through to maturity;
 - iii. where possible, either a) rear families in isolation until individuals are large enough to tag for either individual or family identification, or b) genotype offspring physically tagged at a later stage so as to assign offspring to family of origin; and
 - iv. A step that technically is not minimizing variation in family size but does take additional effort is to maintain multigenerational pedigrees and employ

minimization of Mean Kinship techniques to minimize loss of genetic variation and accumulation of inbreeding over time.

- f. In order to minimize catastrophic loss of populations, groups of families should be reared in at least two separate locations in a facility with independent fail-safe protocols including independent water supplies and, preferably, in two different and isolated facilities.
 - g. Milt and tissue should be preserved from the founder generations of salmon, by storage at appropriate temperatures, for later use in minimizing genetic change in captive populations or, possibly, use at a later time in reconstituting wild populations, when and if appropriate technologies are developed.
 - h. Populations should be exposed to as natural environmental conditions as possible, including attempts to simulate natural conditions in the hatchery environments (use of gravel substrates, mid-water feeding, etc) or, preferably, exposure of families to wild native river environments for as much of their life cycle as circumstances permit.
 - i. Populations should be monitored over time to assess
 - i) that the prescribed rearing/mating strategies are being achieved,
 - ii) rates of loss of genetic variation,
 - iii) rates of adaptation to captive conditions and loss of wild fitness, and
 - iv) program efficacy in achieving stated goals and objectives.
12. Through applying an effective mix of the above components, the loss rate calculated for inner Bay of Fundy salmon is well under 1%, as illustrated by several results from that work.

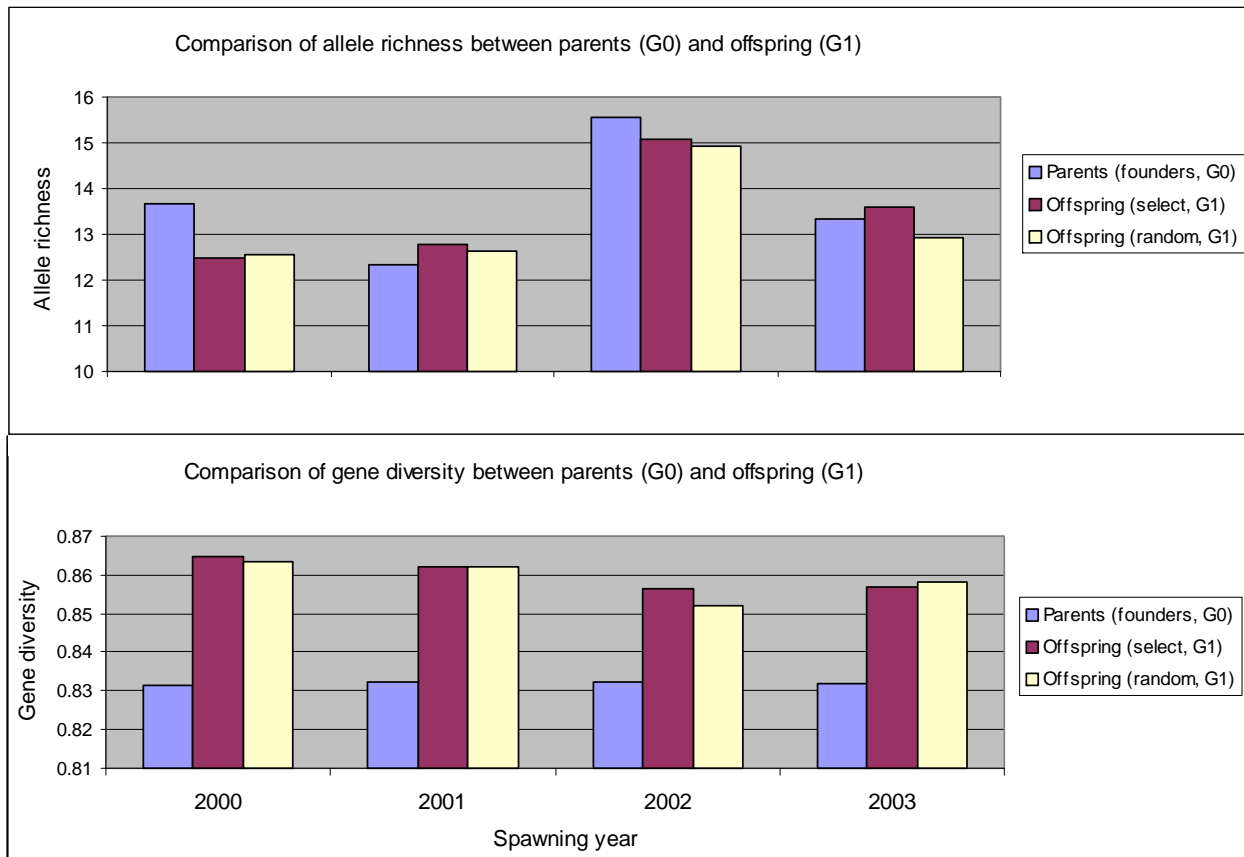


Figure 3: Allele richness (upper graph), as one measure of genetic diversity, was compared across 4 years using two different strategies, selected mating vs. simulated random mating. Results indicated that allele richness was quite a bit lower in the offspring relative to the parents in 2000, slightly lower relative to the parents in 2002, but slightly higher in the selected offspring relative to the parents in 2001 and 2003. Allele richness was generally higher in the offspring selected for spawning (select, G1) compared to a similar number of randomly chosen G1 salmon. The breeding strategies resulted in gene diversity (lower graph) that was consistently higher in the offspring relative to their respective parents.

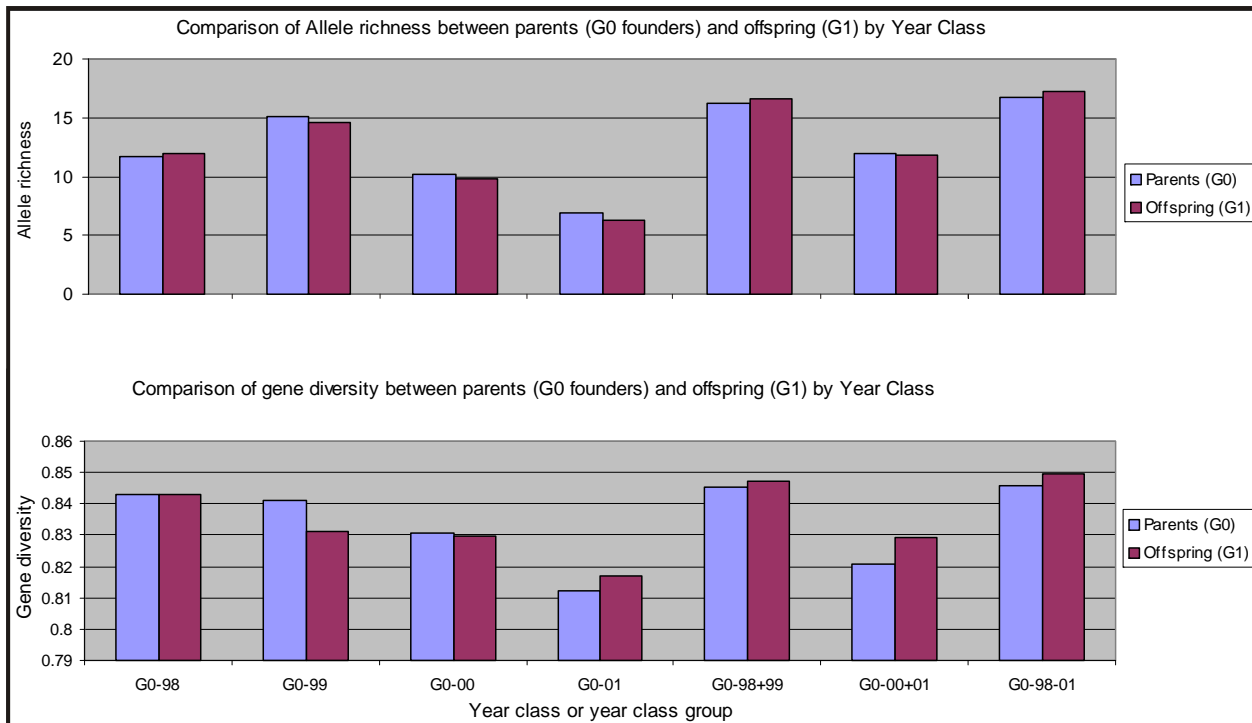


Figure 4: When evaluated by single year-class (eg. G0-98, G0-99, etc.), and groups of combined year classes (G0-98+G0-99, etc) allele richness (upper graph) and gene diversity (lower graph) varied much more between parents from different year classes than between parents and offspring (G1) from within any single year class. Overall, allele richness was similar in the parents and offspring from within a given year class or year class group and gene diversity estimates were generally as high or higher in offspring relative to parents from a given year or year class group. Small observed reductions in allele richness in offspring relative to respective parental groups were often associated with the high frequency of cross-year or cross-generation spawnings.

Genetic variation will be recovered in resulting offspring, but this cannot be included in any single-year tabulation of offspring allele richness or gene diversity, and therefore these values represent a minimal estimate of recovery of genetic diversity.



Figure 5: Comparing wild collections of Stewiacke River juveniles (wild juveniles) from different year classes, the subset of founders chosen from these wild collections of juveniles using selection criteria employed in the inner Bay of Fundy work program (founders, selected), and the same number of hypothetical founders randomly sampled (founders, random), allele richness (upper graph) and gene diversity (lower graph) estimates varied much more across year classes of wild juveniles than between wild juveniles and either selected or randomly sampled founders. Overall, gene diversity and allele richness estimates were similar between wild collections of juveniles and their respective founder groups, but allele richness estimates were often slightly higher in the founders, selected groups and significantly so in several comparisons (*). Markedly higher levels of genetic variation observed in the selected, founder group in the G0-01 class likely reflects extensive levels of family structuring observed in this last collection of wild Stewiacke River juveniles. These results indicate that the use of Mean Kinship information in the selection of G0 spawners increased the recovery of founder diversity relative to what would have been expected if a similar number of broodstock had been chosen at random.

13. The features necessary for a captive breeding program to have a high expectation of maintaining genetic diversity and the possibility of minimizing loss of fitness in the wild, should include the development and use of breeding protocols, and measures to minimize adaptation to captive rearing, such as those illustrated in Figure 6. These features can be combined a number of ways, with varying implications for operation costs, likelihood of maintaining the full genetic diversity of the founder stock, and robustness to mistakes or catastrophes.

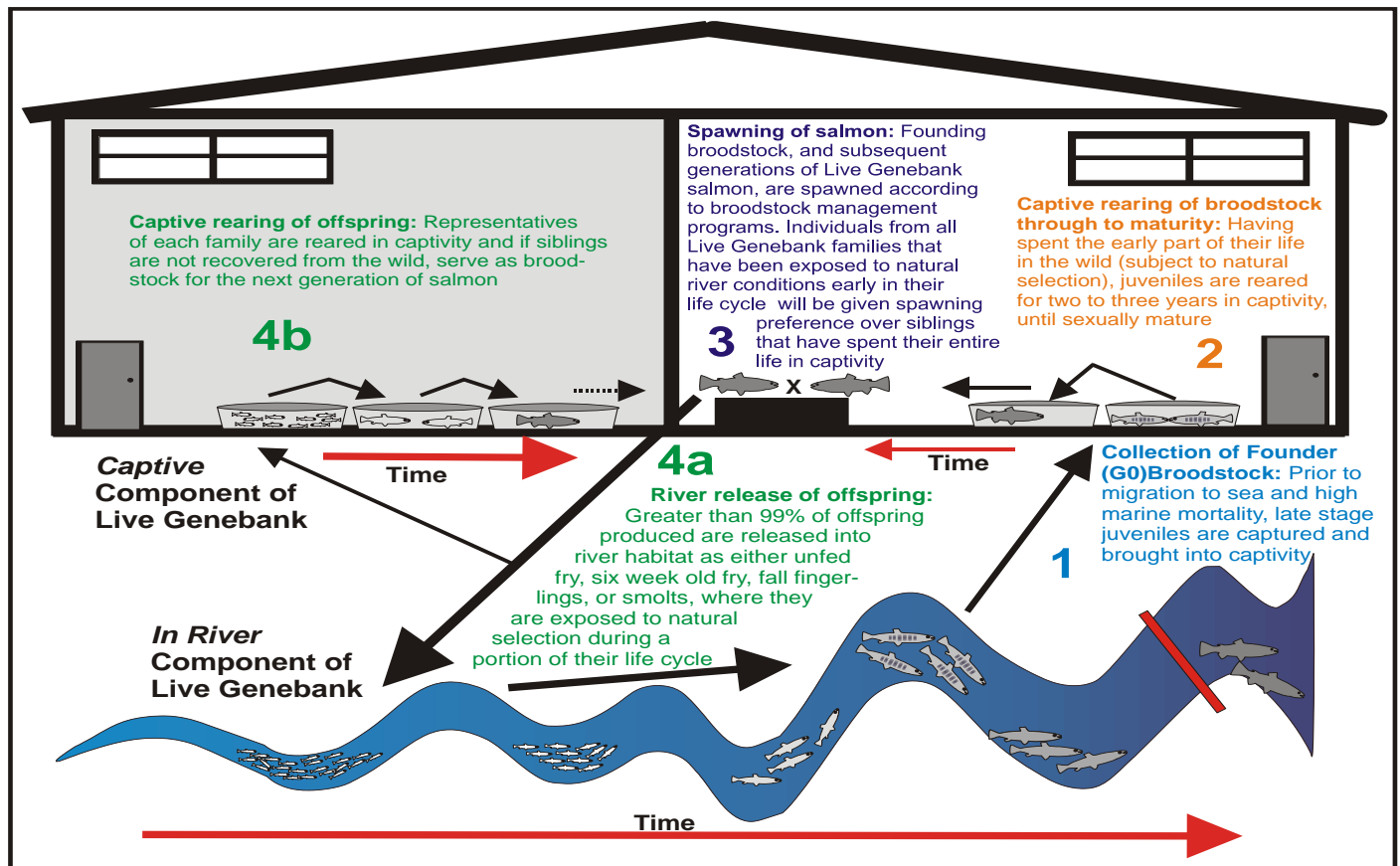


Figure 6. Schematic depicting the wild and captive components of the iBoF LGB program, including the use of breeding programs designed to minimize loss of genetic variation and wild fitness, and the preferential spawning of wild-exposed salmon.

14. For careful captive breeding programs to have a high likelihood of maintaining genetic diversity it is necessary to have a sufficiently large breeding population and to start the program before the wild population has declined to an extent that substantial genetic diversity has already been lost in the wild population. A “sufficiently large breeding population” will depend partly on the objectives of the program, but generally at least 100 breeders (50:50 sex ratio) would be a minimum and a few hundred are generally adequate to maintain neutral variation in the short term (tens of generations) if appropriate mating strategies are employed. Loss of adaptive genetic variation is independent of population size, so even a large breeding stock does not ensure maintenance of adaptive genetic variation. Some genetic variation that is exposed to selection in the wild may be neutral (or nearly neutral) in captivity, particularly when mortality is low. Such variation would be expected to be lost at a rate that is dependent on population size.
15. A minimum number of spawners below which a captive breeding program would not be expected to maintain genetic diversity was not determined. However, there is a large body of scientific literature that could form the basis for advice on how risk would vary with the starting population size, the differences in selection intensity between the hatchery and wild conditions, and how long the program would be maintained in captivity at a given size.

16. Captive breeding and rearing programs should include an effective and comprehensive evaluation and monitoring component. First, program objectives, and strategies for achieving those objectives, should be reviewed by an independent group of recognized authorities in salmonid captive breeding and rearing, including fish culturalists, geneticists, and field biologists. Second, program hatchery operations should be evaluated from time to time to ensure that procedures and operations were carried out as originally specified. Third, rates of change in (a) neutral genetic marker variation, (b) fitness in the wild, and (c) quantitative traits, particularly those that have a direct role in fitness, should be assessed on an ongoing basis, every two to three years. Fourth, the efficacy of individual programs in restoring wild self-sustaining populations should be evaluated, and this information used both to adapt individual programs, but also to inform management as to the performance of captive breeding and rearing technology in the recovery of declining salmonid populations in Canada.
17. The current strategy uses genes from as many founder families as possible. This does not assume that all founder families contain genes that will be well adapted to the future conditions under which self-sustaining populations may be re-introduced into the wild. Rather, it assumes that by conserving genes from as many families as possible, the suite of families in each captive generation will have the greatest likelihood of producing some genotypes capable of breeding successfully at the time of re-introduction, and forming a basis for a re-established population.
18. One of the key features necessary for a captive breeding program to maximize the likelihood of maintaining genetic diversity is to equalize family size prior to breeding. Equalization of family sizes is expected to halve the adaptation of the captive population to the conditions which prevailed before the equalizing took place (and functionally doubles the effective population size relative to random mating at that step). Such a reduction in adaptation to captive breeding conditions is important, to reduce the effects of selection for domestication.
19. Applying the same strategy of equalizing family size to a captive-bred population following a time at liberty in the wild will increase neutral genetic diversity in the subsequent breeding population, but also may reduce the local adaptation that has occurred during the period in the wild conditions.
20. For a number of years the program for inner Bay of Fundy salmon equalised family size before release into river environments and again after collecting older parr or smolts from river habitat to use in breeding. The first equalization is considered essential, to reduce the effects of domestication prior to release into freshwater. The contribution of the second equalisation to minimizing loss of genetic diversity is unclear. It is uncertain if this step is optimal, and this aspect of the program is under review.
21. This trade-off between allowing adaptation (if it is observed) to prevailing conditions to be represented in the breeding population and maintaining genetic diversity for future “choice” is logically inescapable, and the optimal balance is likely to be case-specific.
22. For some types of captive breeding programs, valuable information can be obtained if captive bred fish are periodically released into the wild, to test whether the strains in culture produce genotypes capable of surviving and breeding in the wild. However such releases should be planned carefully, to ensure that they can provide the desired information and that the released fish do not pose a risk to the survival or reproduction of

any wild fish that are still in the freshwater or marine habitats where the releases may occur. Otherwise don't just mess around.

23. It should be possible to monitor losses of natural fitness (if any) in the freshwater and marine phase of the life cycle. This would involve using the so called "animal model" to estimate genetic trends in survival in each phase and, possibly, genetic trends in reproductive success as well. The information required to do this consists of pedigree and survival records on a sufficient number of animals in each phase accumulated over two or more generations. These data presently exist for fresh water for the Inner Bay of Fundy salmon live gene bank program but the number of identified returns from the sea is not yet sufficient. These data would be analysed statistically, using established methods, to estimate genetic breeding values and trends of breeding value over time.

OTHER CONSIDERATIONS

Considerations about Facilities for Captive Breeding Programs

24. Risk management and application of precaution imply that having individual genetic strains in multiple facilities is good protection against catastrophes (disease, mechanical failures in the facility, etc). It is also possible that operating several moderate-sized (e.g. a couple hundred spawners) captive populations would produce additional benefits in reducing adaptation to captivity and providing more opportunities for F2 matings to produce greater expressed genetic variance, when compared to a single much larger program. The evidence is not available currently to evaluate these possibilities for salmonids, but the topic warrants directed research.
25. It is also a consideration that different existing facilities have different strengths and weaknesses in terms of supporting different life history stages. Developing programs that take advantage of existing facilities instead of building new ones need to take these properties of the facilities into account.
26. However, there are no compelling reasons why a single facility could not support multiple genetic strains, as long as operating protocols at the facility ensured no chance of errors in mixing populations, or transmission of disease and appropriate fail-safe protocols were in place against catastrophic loss.
27. Live gene banking, or any program for the maintenance of genetic diversity during a period of high risk to the wild population, intrinsically requires a long-term commitment of resources, for the program to achieve its goals. Moreover these programs have high initial infrastructure costs, unless facilities are already available. Success of any program for the long-term conservation of genetic diversity will require secure long-term funding. There could be very large gains in efficiency of these programs if there were a national or zonal strategy for addressing infrastructure and operating requirements.
28. Costs of maintaining the facilities for captive breeding should be evaluated in the context of the research opportunities provided, as well as in the contexts of the population being conserved, and with respects to salmonid and conservation biology in general. Inner Bay of Fundy captive breeding and rearing activities, and associated molecular genetic and pedigree information, directly supports a very large body of conservation research

involving four Canadian universities with in-kind contributions totaling over 1 million dollars.

Considerations Regarding Technical Alternatives and Reintroduction of Self-Sustaining Populations in the Wild

29. The evidence is not conclusive with regard to successful reintroduction of populations that have been maintained in captivity, although there are a number of documented failures of efforts to re-establish self-sustaining populations. Many examples of failures at re-establishing self-sustaining populations can be traced to either failures to address the threats that posed the original risk, or to captive breeding programs that did not apply the appropriate measures for maintaining genetic diversity or for ensuring that genotypes capable of adapting to the threats are available.
30. Low marine survivorship has become a major factor in declines of many Atlantic and Pacific salmon populations. It has been difficult to identify the proximate causes of the low marine survivorship, in many cases making it unfeasible to address all of the threats and maximize the likelihood of success at reintroduction in the short term. Research on causes of the low marine survivorship, feasibility of sustaining smaller populations in freshwater habitats, and research on genotypes that are adapted to marine conditions would all be valuable, as components of programs intended to reintroduce self-sustaining salmon populations in the future.
31. As potential technical alternatives to conserving salmonid genetic diversity, surrogate broodstock technologies hold promise, but as yet have not been tested in a real world conservation situation. Thus, for practical reasons, cryopreserved sperm may be a more useful means of retaining genetic diversity. However, both surrogate and cryopreservation methods require some level of captive breeding and therefore cannot be viewed as a replacement for captive breeding. Other alternatives include translocations to new habitats, which may be available in some cases but for several biological reasons must also be considered with caution.
32. Notwithstanding the limitations above that mean cryopreservation is not currently a stand-alone solution to the conservation of genetic diversity, cryopreservation of milt and tissue is considered an important measure for future recovery of endangered or extirpated populations and provision of genetic resources for other human uses. This field of technology is developing rapidly, and a working group with appropriate experts should be tasked with developing a departmental strategy for current departmental practices that are well positioned for yet-to-be-developed but foreseeable future developments.

SOURCES OF INFORMATION

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