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Proceedings of the Regional Science Peer Review of the Inner Bay of Fundy Atlantic Salmon Science Associated with the Live Gene Bank

June 13-16, 2017
Dartmouth, Nova Scotia

Chairperson: Kent Smedbol
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Foreword

The purpose of these Proceedings is to document the activities and key discussions of the meeting. The Proceedings may include research recommendations, uncertainties, and the rationale for decisions made during the meeting. Proceedings may also document when data, analyses or interpretations were reviewed and rejected on scientific grounds, including the reason(s) for rejection. As such, interpretations and opinions presented in this report individually may be factually incorrect or misleading, but are included to record as faithfully as possible what was considered at the meeting. No statements are to be taken as reflecting the conclusions of the meeting unless they are clearly identified as such. Moreover, further review may result in a change of conclusions where additional information was identified as relevant to the topics being considered, but not available in the timeframe of the meeting. In the rare case when there are formal dissenting views, these are also archived as Annexes to the Proceedings.

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SUMMARY

A Regional Peer Review meeting was held from June 13 to 16, 2017, at the Bedford Institute of Oceanography in Dartmouth, Nova Scotia to review science associated with the inner Bay of Fundy (iBoF) Atlantic Salmon Live Gene Bank (LGB) program. The focus of the meeting was to undertake a comprehensive analysis and review of the LGB program and its contribution to the recovery of the iBoF assemblages of Salmon populations. The LGB program was initiated in 1998 with the collection of juvenile iBoF Salmon to be reared in the Maritimes Region Biodiversity Facilities (Mactaquac, Mersey and Coldbrook). The objective of the LGB program is to use captive breeding and rearing technologies to conserve genetic characteristics of iBoF Salmon and maintain the population until recovery can occur.

The intent of the review was to provide an assessment of the LGB program following three generations of iBoF Salmon population restoration and maintenance. Participation in this meeting included Fisheries and Oceans Canada (DFO) Science, Species at Risk Management and Resource Management, First Nations and Aboriginal organizations, and non-DFO scientists.

This Proceedings document includes a summary of the presentations and is the record of the meeting discussions and conclusions. A Science Advisory Report and Research Documents resulting from this meeting will be published on the Fisheries and Oceans Canada (DFO) Canadian Science Advisory Secretariat's (CSAS) Website as they become available.

Compte rendu de la réunion régionale d’examen scientifique par les pairs de la banque de gènes vivants pour le saumon de l’intérieur de la baie de Fundy

SOMMAIRE

Une réunion régionale d’examen par les pairs a eu lieu du 13 au 16 juin 2017, à l’Institut océanographique de Bedford, à Dartmouth (Nouvelle-Écosse), afin d’examiner la science en rapport avec le programme de la banque de gènes vivants du saumon de l’Atlantique de l’intérieur de la baie de Fundy (IBF). La réunion avait pour objet d’entreprendre une analyse et un examen approfondis du programme de banque de gènes vivants et de sa contribution au rétablissement des assemblages de populations de saumon de l’intérieur de la baie de Fundy (IBF). Le programme de banque de gènes vivants a été lancé en 1998, par la collecte de saumons juvéniles de l’IBF en vue de les élever dans les installations de biodiversité de la région des Maritimes (Mactaquac, Mersey et Coldbrook). Le programme de banque de gènes vivants a pour objectif d’utiliser des technologies de reproduction et d’élevage en captivité pour conserver les caractéristiques génétiques du saumon de l’intérieur de la baie de Fundy et maintenir les populations jusqu’à ce qu’un rétablissement soit possible.

L’examen avait pour objet de fournir une évaluation du programme de banque de gènes vivants après trois générations de rétablissement et de maintien de la population de saumons de l’IBF. Les participants à cette réunion comprenaient des représentants du Secteur des sciences et la Gestion des ressources du MPO, de certaines Premières Nations et organisations autochtones, de l’industrie de la pêche et des scientifiques qui ne travaillent pas pour le MPO.

Le présent document comprend un résumé de la présentation et donne le compte rendu des discussions et des conclusions de la réunion. Un avis scientifique et un document de recherche découlant de cette réunion seront publiés sur le site Web du Secrétariat canadien de consultation scientifique de Pêches et Océans Canada dès qu’ils seront disponibles.

INTRODUCTION

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) identified the inner Bay of Fundy (iBoF) Atlantic Salmon (hereafter referred to as iBoF Salmon) assemblage as a Designatable Unit (DU) and assessed this population as Endangered in May 2001 (COSEWIC 2006). This population was included as Endangered on Schedule 1 of the *Species at Risk Act* (SARA) when the *Act* was passed in 2002. In 1998, prior to listing under SARA, population trends observed in several rivers of the inner Bay of Fundy prompted the collections of juveniles to be reared in the Biodiversity Facilities in the Maritimes Region (Mactaquac, Mersey, and Coldbrook biodiversity facilities) effectively initiating the present-day Live Gene Bank (LGB) programs. The objective of the LGB program is to use captive breeding and rearing technologies to conserve genetic characteristics of iBoF Salmon and maintain the population until recovery can occur (DFO 2008a). In 2008, the Recovery Potential Assessment (RPA) forecasted that this population would be extinct without the support of the LGB program (DFO 2008b; Gibson et al. 2008).

Several evaluations have been conducted to assess the scientific merit of the Live Gene Bank program. In 2004, a review was done for the Director General of Fisheries, Environment and Biodiversity Science. In 2006, COSEWIC contracted a review of the status and assessment of the iBoF Salmon population, which resulted in COSEWIC confirming the previous assessment of Endangered and, in 2008, an RPA was completed for iBoF Salmon to support recovery planning under SARA. In 2008, DFO Science also struck a national working group that produced a Science Advisory Report evaluating the contribution of captive breeding facilities to biodiversity conservation. Additionally, since its inception, yearly updates and summaries of ongoing assessment activities and genetic analyses have helped, and continue to help, adaptively manage the LGB program and guide the iBoF Salmon program through the Planning Group and Recovery Teams (DFO 2010). In addition, the approach used to conserve genetic variation and minimize loss of fitness in the LGB program has been reviewed in O'Reilly and Doyle (2007), O'Reilly and Harvie (2010) and O'Reilly and Kozfkay (2014).

However, a comprehensive analysis and review of the impact of the LGB program on the recovery of the iBoF population does not exist for the lifetime of the program. Therefore, DFO Maritimes Science requested the evaluation of iBoF Salmon science with respect to all LGB activities. As part of the Regional Peer Review process, a meeting was held from June 13 to 16, 2017, at the Bedford Institute of Oceanography in Dartmouth, Nova Scotia, to review the science associated with the LGB program.

The meeting Chairperson, Kent Smedbol, introduced himself, followed by an introduction of meeting participants (Appendix 1). The Chair thanked meeting participants for attending the DFO Regional Peer Review Process. The Chair provided a brief overview of the Canadian Science Advisory Secretariat (CSAS) peer review process and invited participants to review the meeting Terms of Reference (Appendix 2) and Agenda (Appendix 3). This Proceedings report is the record of the discussion of the meeting.

To guide discussions, three working papers were prepared, which will be produced as Research Documents upon acceptance. Concern was raised by a reviewer that, given the length of the working papers, these were not distributed with sufficient time to thoroughly review the material in advance of the meeting. The Chair proposed that this meeting be viewed as opportunity for the science leads to present their analyses and findings and that additional opportunities for review and feedback will follow the meeting. A Science Advisory Report (SAR) will also to be produced as a result of this meeting.

PRESENTATIONS AND DISCUSSION

OVERVIEW OF IBOF AND THE LGB PROGRAM

Presenter: S. Ratelle and P. O'Reilly
Rapporteur: C. Angelidis

Presentation Summary

The wild Atlantic Salmon populations in the four Designatable Units (DUs) found in the Maritimes Region are well below conservation limits. Despite a reduction or elimination of fisheries harvest, the key risk to the populations remains the rates of survival of salmon rates of survival of Salmon during the marine phase of their life history. The iBoF DU is one of four in the Maritimes Region and the only one currently listed as Endangered under SARA. The abundance indices of iBoF Salmon show a greater than 99% decline in the population since the mid-1960s where, currently, less than 200 adults inhabit the 50 iBoF rivers. Population assessments conducted in the 1990s and 2000 found that this population would go extinct in the absence of human intervention and, therefore, the 'Live Gene Bank' Programs were established at the Mactaquac, Coldbrook, and Mersey Biodiversity Facilities to preserve, and hopefully restore this distinct population.

Atlantic Salmon occupying the 50 rivers of the iBoF exhibit a number of unique phenotypic characteristics, including local migration, early maturation as adults, and greater dependency on repeat spawning for population stability. Generally speaking, the geographic distribution of these phenotypic traits is concordant with patterns of among-population genetic variation observed in the area. Important biodiversity likely exists within the iBoF assemblage of populations as well; river populations on the Chignecto Bay side of the bay cluster together, and river populations of the Minas Basin, except the Gaspereau, cluster together. This pattern of differentiation may reflect geographic isolation and expected rates of gene flow, or, potentially, ecological differences between the two sides of the inner bay, and reduced fitness of more distant migrating iBoF strays in cross-bay habitat.

The iBoF conservation program began in 1998, when initial collections of approximately 1000 wild parr (program founders) were obtained from each of the Stewiacke (STW) and Big Salmon rivers (BSR) (representative of the Minas and Chignecto Bay sides of the bay) and transferred to biodiversity facilities in DFO's Maritimes Region for captive breeding and rearing. A few years later, founders were also collected from the phenotypically distinct Gaspereau (GAK) River population. Conservation programs for the three river populations vary to some extent, with differences reflecting, in part, river infrastructure and offspring sampling capabilities. The review focused on the STW River conservation program, which, because of greater availability of information, is considered an index river for assessing rates of loss of genetic variation over time.

Prior to maturation, STW River founders (G0 generation Salmon) were genotyped at set microsatellite markers, and first-order relatedness estimated using kinship assignment methods. Initially, matings between maturing founders were prescribed so as to minimize spawnings between full siblings (full-sib) and half siblings (half-sib), thereby reducing inbreeding in the next generation. Approximately 150 to 200 crosses were carried out each year, and a small number of offspring were sampled from combined egg lots for rearing exclusively in captivity through to maturity ('captive-reared offspring'). Remaining offspring were released throughout the river for exposure to wild river conditions and natural selection for one to two years, after which a small portion (approximately 300 to 400) were caught and returned to the Coldbrook Biodiversity Facility for rearing through to maturity ('wild-exposed offspring'). Prior to maturation, captive-reared and wild-exposed offspring groups were genotyped, pedigreed and spawned in the production of the next generation of STW River salmon.

Over time, a number of program changes were implemented, including the use of Mean Kinship information to prioritize spawners and a modification to the way wild-exposed groups were managed. Beginning in 2006, a similar number of fry from each family were released into an isolated tributary (the Pembroke), for later recovery for LGB purposes (subsequent spawning in the production of the next generation). All remaining offspring (except the few not retained for exclusive captive rearing) were released throughout the STW River watershed for supplementation purposes. These different release types reflect two components of the STW River conservation program: (1) Live Gene Banking (the objective of which is to conserve genetic variation), and (2) Supplementation (the objective of which is to provide a demographic boost to the population). More recent changes to the STW River program include (a) the use of Ranked Mean Kinship breeding protocols to select and pair spawners, (b) delayed program spawning exclusively until year 5, and (c) increased spawning of wild-exposed over captive-reared salmon.

Discussion

A reviewer sought clarification about some of the hatchery processes regarding the mixing troughs. The science lead clarified that, while not initially part of the program, beginning in 2006 the troughs were mixed thoroughly so that each individual from each family had an equal chance of being released at all locations throughout the isolated stretch of the Pembroke, a tributary of the STW River.

There was some general discussion of concerns and clarification regarding the generation time of fish in the LGB program. One reviewer was concerned that by spawning 5th year fish, the program was selecting for late maturity. The science lead clarified that Salmon that mature in their 4th year were still being spawned; however, their offspring were not incorporated into the program and, instead, were released as part of the supplementation program (none were kept in captivity). In their fifth year, these fish were spawned a second time, and offspring from this second spawning were incorporated into the program. In the past, fish were spawned in the fourth year, but this was selecting for early maturation in captive environments. By altering the program to spawn fish in their fifth year, there is less selective pressure applied to the population. The reviewer agreed that this was an effective change to the program, but still expressed concern that fish that mature in their 4th year in captivity may differ from those that would mature in their 4th in the wild and that, therefore, there exists the potential for adaptation to captivity.

There was significant discussion about balancing maximizing diversity and maximizing fitness in the program, and this topic was discussed at length in later sections and conclusions of the review. Science leads noted that both diversity and fitness were discussed at length when designing strategies for the program, and generally strategies have aimed to minimize both the loss of fitness and rates of adaptation. It was noted that there exists little to no evidence of effective strategies for minimizing the rates of domestication with the exception of minimizing the number of generations spent in captivity. The science lead noted that increasing the generation time also reduces the number of cycles of captive breeding and rearing in the program before the marine environment improves sufficiently for re-establishing wild self-sustaining populations.

One reviewer asked if any quantitative goals or metrics were built into the LGB program during the early development of LGB objectives. The science lead noted that while initially no such quantitative goals were written into the Recovery Strategy, a realistic goal would be to conserve

95% of the genetic diversity of the founder population over 20 generations. This goal was included in the working paper. Others agreed that while the program was initially set up to respond to an issue, setting and reflecting on specific goals might be helpful in the long-term.

EVALUATION OF SUCCESS OF CONSERVING GENETIC CHARACTERISTICS OF THE IBOF SALMON POPULATION ACROSS THREE GENERATIONS OF CAPTIVE BREEDING AND REARING

Presenter: P. O'Reilly
Rapporteur: C. Angelidis

Presentation Summary

The LGB program uses captive breeding and rearing technology to conserve residual populations of iBoF Salmon for future planned attempts to restore wild self-sustaining populations to rivers of the inner bay, once marine conditions improve. However, small closed populations maintained in captivity across multiple generations can be expected to change over time, and some of these changes may impact survival in the wild, adaptability of releases, and future restoration efforts. The presentation reported on rates of genetic change due to random processes, including loss of genetic variation, accumulation of inbreeding, and changes in allele frequency distributions.

Original pedigree placements of iBoF Salmon are based on kinship analyses, which appear to be about 90 percent accurate; incorrect assignments involving individuals in small half-sib groups are not expected to have a large impact on either a) initial selection of wild parr as founders, or b) rates of inbreeding in the next generation, but will have some impact on Mean Kinship estimates and future abilities of the program to minimize rates of loss of genetic variation.

The original collections of STW River wild parr were highly structured, with over half of the more than 1000 G0 Salmon clustering into as few as 10 of the largest half-sib groups. Later collections (2000 and 2001) were much less diverse than early collections (1998 and 1999) and contributed very few new half-sib families. Pedigree information accessed through the program PMx indicates that G-1 founder parent contributions into the G2 generation populations were very uneven, with a few female half-sib G-1 founder parents leaving many more descendants than both other female half-sib G-1 founder parents and all male full-sib G-1 founder parents. This unevenness in G-1 founder parent contributions, in part due to when the program was initiated, resulted in a) an increased accumulation of inbreeding in G2-G3 generation Salmon, and b) a lower overall retention of genetic variation in later generation salmon.

Specific pedigree-metrics tracked over time (across sets of Salmon spawned each year), including gene diversity, founder genome equivalents, and numbers of founder alleles, declined at a slow, moderate and rapid rate, respectively. However, when assessed by generation (as opposed to by spawner year group), rates of change were much reduced. Several metrics of molecular genetic marker variation in sets of salmon spawned in 2000 to 2015 were also assessed. The observed number of molecular genetic alleles and molecular genetic allele richness both gradually declined over time. Detailed analyses of alleles that were lost indicates that nearly all were either putative short European type alleles directly or indirectly selected against, or alleles initially present at very low frequencies (one copy in the approximately 2000 founder alleles assayed).

Observed heterozygosity generally appeared to increase from 2000 to 2013 (likely a result of the mixing of 1998-2001 families and inbreeding avoidance), but then may have begun to decline through to 2015 to levels similar to that observed in early spawner groups. The timing of this reduction coincides with the timing (offset by one generation) of marked increases in the number of G-1 half-sib female ancestors common to sets of male and female parent pairs spawned in 2010 and 2011. Expected heterozygosity changed little over the course of the study. The effective number of breeders and effective population size of sets of salmon spawned in 2000 to 2015 was estimated. Overall, generational effective population size was likely quite high and increased over time; rates of loss of genetic variation (and accumulation of inbreeding) between G0 and G3 generation salmon were expected to be modest. The effective number of breeders based on (1) all captive-reared, (2) all wild-exposed, and (3) all captive-reared and wild-exposed offspring prior to selection of spawners was also estimated. This was done to assess the impacts of proposed changes to the LGB program. Variance in family size is higher for wild-exposed compared to captive-reared offspring groups, but the differences between the two, especially from 2006 on, are modest, and result in modest differences in effective population size.

The efficacy of different founder selection regimes was explored in terms of both a) initially capturing molecular genetic variation and family diversity, and b) maintaining genetic variation and minimizing inbreeding across ten generations of captive breeding and rearing. Overall, under most conditions, Ranked Mean Kinship is expected to perform similarly or better than most other methods tested, though modelling-based analyses should be used to determine the optimal number of founders given observed levels of family structuring. The overall rate of change in allele frequency distributions over time was also assessed. After accounting for varying family representation across collection and early spawning years, drift-induced changes were small and not statistically significant. Finally, adult census population sizes made elsewhere were considered, and likely mature male parr census population sizes between 1967 and 2015, in the context of the timing of wild-parr (founder) collections, population bottlenecks, and genetic diversity going forward.

Discussion

There was some discussion among participants of the value in introgressing genes from other sources (such as the GAK River) into the STW River as a mechanism to reduce inbreeding; however, the science lead did not believe that this was warranted at this time, and indicated that doing so could potentially be deleterious given the phenotypic differences between these two river populations. He suspects that there was very little ancestral inbreeding in the STW River and to date, the LGB program has reduced the amount and rate of inbreeding (using the pedigree to avoid spawning relatives), although some level of inbreeding will be unavoidable in any captive breeding program. He further cautioned that introgressed genes from a river population that is already relatively bottlenecked could become a potential source of inbreeding in the future.

Concerns were raised about milt being carried over from the previous male to the next spawning event, despite precautions set in place to avoid contamination. Some participants suggested that there might be the possibility of something biological driving this milt carry-over effect (e.g. variable sperm mobility, sperm competition or sperm/egg compatibility). The science lead clarified that there is now a double check system in place to ensure the fish crossed are in fact the desired pair.

Several participants questioned the assumptions of the program (PMx) used for estimating pedigree-based measures of genetic variation. Of particular interest was the assumption that all founders are unrelated and all founder alleles are unique. Results presented here indicated that many founders (G0 salmon) were siblings (related individuals). Therefore, some gene variants observed in some siblings will be identical by descent. In a sense, this may result in an "over reporting" of loss of founder genetic variation, although a founder allele from individual 1 from family X may not have been successfully transmitted into a future generation, that same allele found in individual 2 also from family X, may have been. There was a suggestion from the reviewers that results from PMx may better reflect rates of change than absolute levels of genetic variation.

However, it should also be noted that the presenters used PMx to report founder parent alleles and not founder alleles, and the founder parents (having been produced years earlier when the population was larger) are likely to be much less related, and possibly unrelated.

A participant asked whether collecting the same number of founders from each river, despite differences in historical population size among the rivers, would adequately represent the genetic diversity in the population. The science lead felt that, regardless of the starting population size, the large sample size would have been sufficient to capture most diversity from each river (although he admitted that, as always, very rare alleles were likely to be missed).

There followed a lengthy discussion among all participants regarding the loss of fitness and the danger of increased domestication as a result of captive breeding and rearing. One reviewer recognized that while the LGB program has done everything possible with the level of diversity available from the initiation of the program, loss of fitness and increased domestication are inevitable. The participants were questioned on how to determine what levels of loss of fitness and adaptation to captivity would be acceptable, and they identified this as a major gap in knowledge and a key consideration when revisiting the objective of the Recovery Strategy. This led to a discussion of the current goal of the LGB program (to conserve iBoF genetic characteristics) and whether the goal should be altered to find salmon populations that can survive the inner bay as it is now (as a type of Biodiversity 2.0). The panel agreed that this would be a major shift and would acknowledge that some changes to the environment cannot be undone. A consensus was reached that ultimately this was a societal and policy decision and outside the scope of this meeting.

Some successful in river matings, including several involving salmon from lineage 3 that produced multiple (2-6) returns across at least two generations, also included a parent that appeared to exhibit European farm salmon ancestry. The science lead noted that the genomes of the two original parent populations (European and North American inner Bay) were highly divergent (close to the maximum possible across the species global distribution), that genomic diversity amongst early generation hybrids would have been very high, and that some genotypes produced may have been compatible with current marine conditions in the inner Bay of Fundy. Although the presenter cautioned against the idea of intentionally crossing European farm and wild iBoF Salmon, he did suggest that these results may provide insight into future research related to the restoration of Atlantic Salmon to rivers of the inner Bay. For example, the lack of detectable success of past hybridization experiments (involving different river populations of iBoF Salmon) may have resulted from low genetic divergence between the genomes of the original parent populations and the resulting production of similar hybrid offspring genotypes with limited genotypic diversity. Greater genotypic diversity might be expected by increasing the phylogenetic divergence of wild North American parent populations included in future hybridization experiments involving iBoF Atlantic Salmon (e.g., inner Bay X Southern Upland, or inner Bay X outer Bay, etc.). However, potential caveats include (1) favourable combinations of genes created in early generation hybrids may very well be broken up by recombination in later generation hybrids (e.g., the effects of hybridization efforts may not be long lasting) and (2) resulting hybrids may only partly represent/reflect the original genetic characteristics of iBoF Salmon; the objective of conserving the genetic characteristics of iBoF Atlantic Salmon may not be achieved. A reviewer agreed that these crosses may provide an opportunity to evaluate which European genes influence return rates. Additionally, it should also be understood that the apparent success of Atlantic Salmon from lineage 3 may be due to genetic contributions from the original iBoF G-1 ancestor (a putative half-sib female). Regardless, the exceptionally high, multi-generation performance of lineage 3 salmon is interesting and should be noted.

It was noted by one of the reviewers that, given reported introgression of non-local salmon into inner bay populations (discussed in further detail later in the document), it would be important to distinguish between demographic recovery resulting from intrinsic (from within) population growth as opposed to increases in numbers resulting from direct immigration of strays (and/or their immediate offspring).

All participants and reviewers agreed that a major concern and consideration in terms of determining achievable long-term goals for the program (with regards to the recovery of the iBoF population) is whether or not the marine environment will eventually improve. The panel agreed that the rapidly changing marine environment may result in a novel marine ecosystem, requiring new strategies from the LGB program.

One of the participants questioned whether anything in the pedigree program would preclude adaptation to marine conditions, but the reviewers noted that this is not yet a concern given that marine fitness is effectively zero (and very few fish are returning from the ocean). It becomes a concern in the future if a population begins to recover, and determining how long to continue supplementing the population with captive individuals (that may be less fit) may also be an important question to address in the future.

The discussion returned to the topic of prioritizing maximization of fitness over maximization of genetic diversity. The science lead reiterated that the most effective way to maximize fitness would be to select returning fish for spawning; however, because few fish are returning, there is little that the program can do to promote marine fitness. It was cautioned by the reviewers that by spawning new wild-origin fish, there is a danger that some of these fish have European ancestry or are strays from other Atlantic Salmon populations. For example, in the BSR River there has been a lot of effort to increase the frequency of genes that have gone to sea and returned; however; the science lead is now concerned that there is strong evidence of European genes in the BSR River population.

One of the reviewers noted that designing the program in a manner that would allow the population to evolve to match new environmental conditions would be difficult given that survival of the fish is a product of both genetics and environment (including stochastic variability). He noted that it is uncertain whether selecting and promoting the (few) returning fish in the program is selecting for more fit individuals, or selecting for individuals that survived stochastic events in the wild. Other reviewers and participants responded that this is always the case, that it is incredibly difficult to separate stochastic events from selective pressures, and that the assumption must be that returning fish have some fitness advantage.

The science lead noted that while much of our focus is directed at minimizing loss of genetic variation, several elements of the LGB program have been developed in an attempt to minimize adaptation to captivity and loss of fitness over time. The primary mechanism of minimizing fitness loss is the incorporation and prioritization of wild-exposed fish in the program. The second mechanism is to include wild-origin fish, although the science lead cautioned of the potential introgression of European farm genes. The consensus was that the program is minimizing rates of loss of genetic variation and maintaining future adaptive potential. However, attempting to increase fitness without concrete evidence of what phenotypes confer higher fitness in a changing marine environment may result in selecting less fit variants over time. The participants agreed that caution would be required when trying to improve fitness, especially given the limited understanding of the system.

Conclusions and Recommendations

This analysis evaluated only three generations, which limits the precision of estimates of rates of per generation loss of genetic variation.

Overall, the river populations in the LGB program are losing some genetic variation, but the loss is limited. In any captive breeding and rearing program, some loss of genetic diversity is inevitable. Consensus among meeting participants was that over 20 generations, if the recommended census population size of adult spawners is maintained and variance in family size is similar to that observed in the program after the 2005 brood year, it is realistic to expect that the program will be successful in conserving 95% of the Gene Diversity observed in the founders.

The current objective of the program remains to conserve the genetic characteristics of iBoF Salmon, and any drastic changes to the program goals would be policy or management decisions. A major consideration to be discussed in any future planning of the long-term objectives of the program is determining what is achievable depending on the recovery of the marine environment. At this time, introgression is not recommended as a mechanism to minimize loss of fitness. There was consensus to continue to maintain effective population size as large as possible given available resources, and to continue to isolate brood years for the purpose of possibly minimizing rates of adaptation to captivity.

INVESTIGATIONS INTO THE ORIGIN AND EXTENT OF INBREEDING IN IBOF SALMON

Presenter: P. O'Reilly
Rapporteur: C. Angelidis

Presentation Summary

The presentation reviewed the molecular genetic basis of inbreeding and inbreeding depression. Inbreeding can be thought of as an increase in the co-occurrence of alleles that are identical be descent (at a given locus) within an individual relative to some reference point (such as the level of inbreeding in a parent). Under the dominance hypothesis of inbreeding depression, inbreeding can result in little or no functional gene product being produced, with obvious implications for the survival of the individual. The magnitude of effects of various levels of inbreeding on a number of performance traits has been the subject of much research in salmonids and was recently reviewed. Inbreeding at the full-sib and half-sib level often results in reductions in trait values, and the magnitude of the effect is often higher when assessed in the wild (where conditions are more stringent) than in the captivity (where conditions are more benign). In well controlled and highly replicated studies, small populations, where inbreeding is expected to accumulate, do indeed exhibit the greatest reductions in fitness, and this is believed to be due to inbreeding depression.

Perhaps somewhat surprisingly, a lag is expected between when a bottleneck first occurs and when inbreeding might be expected. First, the initial population bottleneck results in greater genotypic similarity amongst individuals (siblings); this leads to inbreeding in the next generation. Second, if an immediate single generation bottleneck population consists of even 20 individuals and a handful of families of two or three siblings, most random crosses will be between unrelated individuals. However, if the population size remains constant across additional generations, the likelihood that two randomly paired individuals will share a common ancestor (a parent, grandparent, or great grandparent) increases dramatically; after three or four generations at this population size, inbreeding is unavoidable.

The original set of wild collected founders exhibited considerable family structuring, with more than 50 percent of all G0 salmon clustering into the 10 largest half-sib groups. These groups were likely produced by a female half-sib parent spawning with multiple mature male parr (resulting in many full-sib groups nested within larger half-sib groups). The number of crosses exhibiting some level of inbreeding increased dramatically after 2009. Detailed analyses of the ancestors common to G2 and G3 generation pairs of parents, responsible for inbreeding in G3 and G4 generation offspring, indicated that nearly all are G-1 individuals, and the vast majority of these are half-sib female parents of the 10 largest G0 half-sib groups mentioned above. Very little inbreeding was associated with common G-1 full-sib male parents or G-1 half-sib parents of small G0 half-sib groups. In other words, if the original collections had been more diverse (larger number of more similarly sized half-sib groups), both the number of inbred crosses and the average level of inbreeding observed currently would have been markedly reduced. Had the collections been initiated even two years earlier, levels of inbreeding observed currently would have been much lower. Additionally, a more diverse founder collection would have resulted in a less uneven production of G2 descendants by G-1 founder parents, more even G-1 founder parent contributions into the G2 generation, and lower rates of loss of genetic variation through time.

Analysis of the census population size of adults and parr between 1967 and 2015 suggests that this population may not have been seriously bottlenecked until 1995-1999. Given the year the large group (N=401) of founders were collected (1998), the year most were produced (1996), and the year their parents were produced (mostly 1992 and 1994), the entire sample collection may have been much more diverse if collections were initiated even two years earlier (between 1996 and 1999 instead of between 1998 and 2001). This increasing diversity would have been expected due to both (i) increasing observed (1998-2001) and expected (1996-1998) levels of family diversity in sample collections obtained earlier than later, and (ii) expected reductions in family overlap between collection year groups of founders due to expected changing proportions of virgin versus repeat spawners in the years 1995 to 2000 in the inner Bay of Fundy.

Discussion

There was a brief discussion about the value of beginning the program earlier (by collecting the founders earlier). The science lead noted that in the case of the LGB program, sampling two years earlier would have likely markedly increased family diversity. One of the participants agreed that while this was a sound practice from a scientific perspective, it may be challenging to implement for other species or populations. He further noted that action would require some evidence of negative trends in a population. Other participants questioned the role and strength of an assessment by COSEWIC in the government's decision to initiate programs; however, it was clarified that COSEWIC recommendations are not associated with any legal trigger for action or funding. The participants agreed that discussion among senior management as to when it is appropriate to initiate recovery programs would be important in future programs.

One of the participants noted that delaying the initiation of recovery programs not only reduces the likelihood of success but also increases costs. There was a question as to how earlier intervention would cut long-term costs given that the duration of the program is dictated by the quality of the environment, and not by the program design. The science lead responded that maintenance of higher levels of genetic variation through to the current generation could have been achieved by starting earlier (when levels of initial diversity were much higher) and then not genotyping salmon but maintaining large adult census and effective population sizes, compared to starting later (as was done), genotyping salmon, and following Mean Kinship breeding protocols. Another participant added that as initiation of recovery programs is delayed, the population size decreases and accordingly so do the probability of recovery and efficacy of the program.

There was some discussion around what life stages to collect for founder populations, and agreement was that adults are best suited to capture genetic variation. It was cautioned by the science lead and several participants, however, that removing adults was likely to be very costly to the productivity of the river population, and that the last remaining adults should not be removed from a population unless there is a high level of certainty that the population will not survive on its own. One of the participants questioned the merit of adding precocious parr to the founders to widen the gene pool. The science lead agreed that this could be beneficial, but cautioned that collection and use of larger numbers of parr obtained after a bottleneck event could lower effective population size, increase rates of loss of genetic variation over time, and increase inbreeding. One reviewer noted that if the population was too small to use adults, smolts could be collected as founders instead, but genotyping would have to be conducted to determine relatedness before spawning.

Conclusions and Recommendations

There was consensus that it is beneficial to initiate LGB programs earlier to capture more family diversity and that careful consideration should be given to the life stage collected. It was also agreed that adult founder collections are likely to be more diverse then smolt collections, then parr collections, and then fry collections. Although adult collections are likely to be the most variable, impacts on river productivity are likely high and should be considered only when population extirpation is highly likely or certain.

EVALUATION OF POSSIBLE INTROGRESSION OF NON-NATIVE WILD AND AQUACULTURE GENETIC MATERIAL INTO THE IBOF POPULATION

Presenter: P. O’Reilly
Rapporteur: C. Angelidis

Presentation Summary

Another potential concern for the iBoF program is the extensive introgression of non-native (non-iBoF) genes into LGB populations, and resulting changes in allele frequency distributions and, potentially, the genetic characteristics of iBoF LGB salmon. The working paper considered two nearby sources of non-iBoF Salmon: 1) wild strays from the large and nearby Saint John River, and 2) farm salmon escapes from the Passamodquoddy / Cobscook Bay area. Normally, introgression isn’t a concern for small closed captive populations; however, the management regime for the BSR River (collection and use of “in river produced” smolt obtained at the river mouth and Mean Kinship assist) not only allows for introgression, but is expected to increase rates of introgression of non-native genes into the LGB population. Demographics of the populations in the Saint John and BSR rivers were reviewed, as well as stray rates obtained from elsewhere, and expected possible proportions of Saint John versus native returns in the BSR River from the mid-1970s to 2015. Based on multiple spatial and temporal population genetic analyses, ongoing-introgression of Saint John River genes into the BSR River LGB population is likely occurring and may be fairly large in scope. Although some introgression of wild outer Bay of Fundy (oBoF) genes into the BSR River population may not be of concern (and may even be advantageous), at some point oBoF salmon may be gene banked in the inner bay, which might not be consistent with the stated objectives of conserving iBoF genetic characteristics.

Farm salmon escapes from nearby marine net pen sites are another potential source of non-local genes. Farm salmon production increased markedly from the mid-1980s through to 2000 in both Maine and in the Maritimes. In 1989, European farm eggs and milt were imported into Maine, and soon permeated 30-50 percent of broodstock (Baum 1998). European farm salmon are highly genetically divergent from North American salmon. This extent of divergence represents both an increased risk to native iBoF Salmon, but also an increased ability to detect farm salmon genes in wild populations. European farm salmon ancestry has been detected, with a high degree of certainty, in juvenile salmon obtained from the Upper Salmon, BSR, and STW rivers, and indicate the presence and spawning of pure European farm salmon escapes spanning the period 1997-2007. Additional analyses of patterns of inheritance, effects of linkage between the European type Ssa202 alleles and the sex determining locus in salmon indicates that the spatial and temporal influence of European farm escapes (or their hybrid offspring) in the inner bay may be larger. Also of concern are observations of short European type Ssa202 alleles in nearly all sample collections (often at moderate frequencies) of “in river” produced smolt obtained on the BSR River in the years 2003 to 2015; if these alleles reflect the presence of European ancestry in F2-F4 hybrids, the actual prevalence of lower levels of European ancestry in these collections may be higher (possibly 10-25%). Also reviewed was the prevalence of suspected European farm ancestry in STW and GAK LGB populations. It was also noted that one lineage of BSR River adult returns, exhibiting a short Ssa202 allele, also produced multiple adult returns across two generations, though this may reflect the genetic effects of the original non-European female half-sib parent or possible novel genotypes associated with the mixing of two highly divergent genomes. Finally, although the number of crosses and overall sample sizes were small, there was a trend towards lower early juvenile survival of European/STW River hybrids in the wild compared to pure STW River families monitored in the same environment at the same time.

Discussion

There was a lengthy discussion of the potential consequences of introgression of non-native salmon into the iBoF Salmon population. The science lead noted that the introgression of European origin genes or oBoF genes into iBoF populations is facilitated by the design of certain elements of the LGB program. All adults observed in the BSR River are allowed to spawn in the river; they are not collected for spawning in the LGB program. However, their offspring are collected in a smolt wheel located near the mouth of the river. The science lead noted that BSR River smolt that do not assign to any known LGB cross would be identified as a new founder, assigned a low Mean Kinship value, and be prioritized high for spawning. This is part of the Mean Kinship program intended to increase the frequency of rare alleles. Reproductive contributions of smolt produced in the wild (by wild returning salmon) have been increased further, both to (a) decrease rates of adaptation to captivity, and (b) to increase the frequency of genes that have successfully survived current marine conditions. There is a concern that if some of these smolt were indeed descendants of either European or local (Saint John River) origin farm salmon, increasing their reproductive contributions in the BSR River LGB population would not be recommended.

One of the participants asked whether it would be possible to differentiate between wild oBoF strays and farm salmon escapes of Saint John River origin. Both the science lead and one of the reviewers were skeptical that current markers were capable of differentiating local farmed salmon from native Saint John River fish, but both recognized that this is an important recommendation for future investigation. One of the reviewers suggested that individual assignment tests based on historical BSR and Saint John River samples could be used to establish a “cut off” value for exclusion of Saint John River strays. Single nucleotide polymorphism (SNP) markers might be more informative than microsatellite loci in identifying non-local salmon. It was recognized that this would require significant SNP analyses and that funding might not be sufficient for such an endeavor, but this might be an important decision for management in the future.

The participants discussed methods of identifying, tracking, and managing strays. It was suggested that SNP chip panels could be used to identify fish from different sites, but it was noted that SNP panels are often proprietary to aquaculture companies. One reviewer noted that the tools exist to improve identification of strays, but the major difficulty is in acquiring the samples to test the tools. It was noted that identification of possible European/iBoF hybrid individuals in iBoF LGB populations was based on one or more of the following: 1) observation of putative European alleles at multiple marker loci; 2) observation of a single short European-type Ssa202 allele; or 3) identification of individuals related to fish of known European ancestry.

There was some discussion around how compelling the evidence was for European farm ancestry in iBoF rivers, including the BSR and GAK Rivers. In both river populations, there is evidence of introgression of European farm salmon, including the presence of the short European type Ssa202 alleles. The science lead noted that in particular in the BSR River, the frequency of short (European-type) Ssa202 alleles appeared to be increasing over time. The clustering of the short European type alleles across multiple loci within individuals and the observed inheritance patterns suggested that this was the result of recent hybridization between European and North American salmon. The science lead clarified that there were likely two main proximate sources of European alleles: 1) mature male parr produced by early spawnings of European farm salmon residing in iBoF rivers; and 2) repeated spawnings of ongoing farm salmon escapes in iBoF rivers. He further noted that after two or three rounds of backcrossing to the North American parental population, the European genes would be dispersed in the population and become more diluted within individuals. There was agreement among the participants and reviewers that if European genes still have an impact in F2 and F3 generation descendants, even low levels of European ancestry should be considered a serious concern.

Given the extent of the dispersion of European alleles in the BSR River population, one reviewer questioned 1) how the introgressed alleles could be removed from the population, and 2) how one would decide what level of EU ancestry would be acceptable The science lead noted that the introgression of European alleles into the BSR River was so pervasive that if all fish of European ancestry were removed from the population, a large percentage of the original founder genetic variation would be lost from the population. He suggested that removal of European alleles from the BSR River should not be attempted, but instead there should be efforts to minimize further introgression. He also recommended that in the future any smolts collected in the BSR River be tested against all known parents in the system. If the new smolts do not match one of the known LGB crosses, they should be assumed to originate from outside the LGB program. To prevent further introgression, only fish that type back to a known BSR River cross (or for which there is a high degree of certainty that they are native to the BSR River) should be integrated into the program. The science lead and reviewers noted that while this recommendation would reduce future introgression into the BSR River, there would be associated consequences to the approach: 1) it would eliminate introgression of wild-origin salmon into the LGB program (typically encouraged to minimize domestication) and 2) it would reduce the introgression of fit alleles from fish that have been to sea.

While there was evidence that BSR River/European hybrids exhibiting short European markers appear to have performed remarkably well (having produced one or more adult returns, including one lineage that produced six adult returns, and adult returns across two generations), the science lead reiterated that there is much uncertainty regarding the reasons for this superior performance, and whether observed high at-sea survival will continue into the future. He emphasized that the improved fitness of this one BSR River lineage could have been the result of high performing genes associated with one particular early half-sib female ancestor, or the European/inner bay hybridization itself. To tease apart these effects, he recommends experimental work and replication. One of the reviewers also noted that the increase in returns could also have been the result of a more favourable marine environment that year. The discussion went on further to caution that different families will perform differently in different years depending on environmental conditions. There was consensus that maintaining genetic variability and family diversity is important to ensure high productivity in variable marine environments, and provides the population with greater adaptive potential for it to respond to future changes in marine conditions.

In response to the earlier discussion on the timing of initiating LGB programs, one of the participants asked about whether any analysis was being undertaken for the oBoF or Southern Uplands populations and whether there has been any evidence of European introgression in those populations. The science lead clarified that fish are being genotyped in the Southern Uplands, but aside from some evidence of introgression in the Saint Mary’s River, there is limited evidence of extensive introgression in other Southern Uplands populations. The science lead explained that oBoF populations are likely less vulnerable to the effects of introgression of European farm salmon genes than are inner bay populations. Because of the size of many oBoF populations, European farm escapes likely represented a small proportion of reproducing salmon in any given year, limiting rates of introgression of European genes.

Conclusions and Recommendations

There has likely been limited introgression of European genes into the STW River LGB population, a moderate amount into the GAK, and potentially significantly more in the BSR River population. While it may be possible to cull the European genes from the STW River, it is unlikely to be feasible for the BSR River. No consensus was reached on how to address the issue in the GAK River. Further analyses are needed to assess whether or not attempts should be made to cull European farm ancestry from the GAK LGB population.

The management recommendation for the BSR River LGB population was to limit future introgression of new European farm genes by spawning only wild-collected smolt of known origin (i.e., LGB produced smolt released into the river as fry, or “in river” produced smolt produced by known BSR parents). The unintended consequence of this strategy would be to markedly reduce the incorporation of genes that survived current marine conditions back into the program (a large proportion of “in river” produced smolt captured each year are offspring of parents of unknown origins).

Additional genetic analyses, in particular SNP analyses, and analysis of historical samples could contribute to differentiating Saint John River strays from BSR River natives. It was recognized that resources are insufficient to allow additional genotyping of every fish in each captive breeding program and that management would have to allocate extra resources to address this problem. A discussion about how to allocate resources was recommended.

MONITORING – SUMMARY OF THE DFO LED MONITORING ACTIVITIES ON IBOF SALMON RIVERS IN COLLABORATION WITH MANY PARTNERS

Presenter: S. Ratelle and R. Jones
Rapporteur: C. Angelidis

Presentation Summary

Smolt biological characteristics are collected on the Big Salmon River (BSR), Gaspereau River, and Stewiacke River, whereas genetic analysis at time of capture is only determined for smolts captured on the Big Salmon River. The biological characteristics include general age and length info among the rivers and detailed BSR comparisons by genetic origin. As comparative multi-year and genetic information are not available, the length of the Gaspereau River wild or LGB unfed fry smolts (2016) was compared to the length of the Stewiacke River (2014-2016) and of the BSR smolts (2016) from the same groups. Gaspereau River smolts are significantly larger than Big Salmon River smolts, which are significantly larger than Stewiacke River smolts, but smolts from all three stocks are primarily Age 2 dominant at time of migration. Interestingly, the growth rates of the Age 2 smolts from the cross-breeding experiment, which included individuals from the aforementioned three main river stocks, showed the same trend despite being reared in a similar environment, the Pollet River (Petitcodiac River tributary). The progeny from the Stewiacke x Stewiacke parents were the smallest, and the smolts that originated from Gaspereau x Gaspereau river parents origin smolts were the largest, while the Big Salmon x Big Salmon smolts were an intermediate size, which corresponds with the river specific data collected from 2014 to 2016.

The BSR smolts were further scrutinized to investigate differences among different origins (wild, LGBfry, and LGBparr) that could guide stocking strategies efforts. The length of the Age 2 smolts was not significantly different between Wild and LGBfry although the length of wild Age 3 was significantly longer than LGBfry. The LGBparr were smaller at Age 2 and Age 3. The age proportion was not significantly different among origins except for a higher proportion of Age 1 LGBparr. The proportion of females between wild and LGBfry were not significantly different where on average 58% of the smolts were female. There is a significant difference in the average emigration day of wild, LGBfry, and LGBparr smolts where 50% (median) of the wild smolts emigrated sooner (Julian Day 136 or May 16th) then the LGBfry (Julian Day 140 or May 20th) and lastly the LGBparr (Julian Day 145 or May 25th). The trend of wild smolts emigrating earlier than their LGB counterparts is consistently observed throughout the 12 years of available data.

The Stewiacke Electrofishing survey in 2013 and the Broadscale Electrofishing survey in 2014 were designed to investigate the return of sea run spawners into rivers of both LGB-supported (Stewiacke in 2013 and Salmon River [Truro] in 2014) and most importantly currently unsupported rivers (32 iBoF rivers). The genetic analysis of the juveniles caught in the Stewiacke survey detected an estimated presence of two ‘unknown’ female spawners although these could also be from ungenotyped salmon in the system. The minimal presence of juveniles in the 33 rivers surveyed in 2014 and the multiple genetic analyses that determined the introgression of non-iBoF sources yet again suggest the minimal or lack of returning truly wild iBoF Salmon and low returns of LGB sea run adults.

Discussion

In summarizing the biological characteristics of the GAK River population, it was found that very little historical data was available. One of the participants suggested that data may be available in the archives from brood stock assessments conducted in the 1980s at White Rock. One of the participants noted that the observed differences in the lengths of smolts between the GAK and STW rivers could be the result of the environment or the fish, and noted that the STW population is extremely slow-growing.

One of the reviewers highlighted that it is important to remember that the objective is to conserve the entire DU of iBoF Salmon. He suggested that a key finding was that the broadscale survey of 19 rivers in the inner bay only found a few fish, which indicates that there is a significant amount of vacant habitat.

EVALUATION OF THE EFFECTS OF THE OVERALL PROGRAM AND SPECIFIC MANAGEMENT STRATEGIES EMPLOYED WITHIN, ON FITNESS-RELATED TRAITS IN IBOF SALMON

Presenter: P. O'Reilly and S. McWilliam-Hughes
Rapporteur: C. Angelidis

Presentation Summary

Adaptation to captivity, expected to impact the survival and/or reproductive success (fitness) of individuals released into the wild during future population restoration attempts, is one of the primary concerns of many captive breeding and rearing programs. A number of measures, based on reviews of the available literature, have been recommended to mitigate effects of adaptation to captivity including a) minimizing the number of generations individuals are maintained in captivity; b) fragmentation of populations; and c) minimizing the strength of selection for captive conditions by naturalizing rearing environments.

One of the main elements of the iBoF LGB program is the release of early juveniles into wild native river habitat, where they reside for one to two years exposed to natural selection, and subsequent capture for reintegration into the LGB population. Recent research by Evans et al. (2014) involving BSR LGB salmon suggests that exposure of parents to wild river conditions as early juveniles increases survival of offspring by approximately two times relative to offspring of exclusively captive reared salmon. Possible mechanisms responsible for this effect include genetic changes associated with the different selective regimes, and non-genetic (e.g., epigenetic) transmission of environmentally influenced traits.

The performance (survival and size at age) of offspring of various cross types (different combinations of captive-reared and wild-exposed male and female parents) was compared to assess the generality of results reported by Evans et al. (2014) and to shed light on the mechanisms responsible for transgenerational effects on survival as observed for BSR salmon (e.g., genetic, epigenetic, and maternal or egg effects). There was no effect seen of parent (including maternal versus paternal) rearing environment on offspring survival, including from release to Age 1 in the wild. There was also no effect of cumulative ancestral (parent and grandparent) rearing environment on offspring (and/or grand offspring) survival in the wild. However, there was a possible effect of parental rearing environment on offspring size at Age 1 in the wild.

Several other predictor variables (all associated with different management strategies) were also tested for possible effects on offspring survival, including: a) Mean Kinship, b) parent family size or survival in the wild, c) level of pedigree inbreeding, d) level of molecular genetic variability, e) outbreeding, f) generations of captive breeding and rearing, and g) both male and female parent age. There was no effect detected of (a) to (d) on either survival or size at age in the wild, but there was a possible effect of outbreeding (e) and generations in captivity (f) on survival in the wild in one of several year groups tested. However, female (but not male) parent age, before and sometimes after controlling for egg size effects, appeared to have a large and consistent impact on both offspring size (at various ages) and survival in the wild, with performance of offspring of Age 5 female parents increasing relative to performance of offspring of Age 4 salmon (for some metrics, performance of offspring produced by parents increased through to female parent Age 6). These results, especially the latter, have implications for recent program modifications: delaying reproduction for the Live Gene Bank program until year 5 in an attempt to minimize the number of generations of captive breeding and rearing. In other words, we do not expect the implemented delay in reproduction to negatively impact offspring survival in the wild.

A number of traits were monitored, including size at various life stages and ages, survival at different life stages, incidence of deformity at the fry stage by family, and age at first maturation as adults. No discernable (directional) trends were detected in assessed traits across years, with a few exceptions. Survival from fertilization to shock at first appeared to increase over time, although this probably reflects higher fertilization success across years. Many instances of what appeared to be higher mortality in particular families was probably poor initial fertilization success of eggs in early years (it is challenging to distinguish between a non-fertilized egg and a fertilized egg that died early in development). This difference likely reflects procedural changes rather than biological differences in produced eggs.

Discussion

The science lead emphasized that the groups for which data are available have been maintained in captivity for only two generations and, therefore, any effects are expected to be small. He first discussed a previous study by Salinas and Munch (2012) that found that offspring performed better if reared under the same temperature regime as their parents, an example of transgenerational effects and possible epigenetic inheritance. The science lead then reviewed a paper by Evans et al. (2014) involving BSR LGB salmon, where offspring of wild-exposed salmon exhibited higher early juvenile survival in the wild compared to offspring of captive-reared salmon in the same environment. The science lead believes that this study provides evidence that wild-exposure in the LGB program is beneficial and may improve offspring survival in the wild, despite the fact that a similar study conducted on STW LGB fish found no effect of parental rearing environment (wild-exposure) on early juvenile survival in the wild. The science lead noted that the STW “wild-exposure effects” experimental design was not ideal and the sample sizes were small, a result of “piggy-backing” research onto previously existing projects.

There was also some discussion regarding the collection of smolt samples and the cost of genotyping additional samples (in addition to wild-exposed parr). While in the past collections in the STW River were mostly parr, the new smolt wheel that is operated on the STW River by the Mi’kmaw Conservation Group could facilitate collection of smolts for the LGB program. Several participants noted that collecting and spawning smolts in the program is difficult as it requires more genotyping and the cost of genotyping is of concern. It was agreed, however, that while expensive, genotyping is necessary and that the large number of samples to be genotyped highlighted the necessity for high throughput workflows. One of the reviewers noted that this decision would depend on the long-term goals of the program, although reiterated that genotyping would be necessary to ensure that the right fish are included in the program.

Some participants questioned the usefulness of including adult returns into the program in the STW River. Largely, the consensus was that the usefulness depends on the fish. In situations where the fish returned because of higher fitness, then there was value in introducing those more fit genotypes into the program. However, the panel recognized that a return may also simply be the result of stochastic events at sea and may not represent selection by the marine environment; however, separating these two processes is virtually impossible. One of the reviewers noted that if several returns originated from the same lineage, it may be possible to evaluate if there exists something significant or consistent in the lineage, with the potential for selecting for increased survival at sea. However, she also noted that the major challenge arises in deciding how to apply such information.

The science lead presented results from an experiment testing the effects of cumulative ancestral rearing environments (parents and grandparents) on offspring / grand-offspring survival in the wild in the wild, from release to Age 1. In most years tested, cumulative ancestral rearing environment was not associated with survival in the wild. However, a possible positive association (with survival increasing with cumulative captive rearing) was observed in one year (2011). These results were unexpected and could reflect sample size limitations, small cross numbers for some treatments, and possible family effects (the chance partitioning of high performing families in treatment groups exhibiting more cumulative captive rearing). Not all reviewers were convinced that the results presented were indeed significant. The lead also noted that results from this one year conflict with program design and direction. Some of the participants questioned how large of an effect the strategy of exposing early juveniles to wild river conditions would have on the viability of the STW River population. The science lead did not think that wild exposure of juveniles would itself lead to population viability, especially given current low marine survival, but indicated that the strategy may be important in minimizing cumulative adaptation to captive conditions and loss of fitness in future populations intended for release.

There was a question about the possibility of crossing GAK with STW fish, with the suggestion that if the crosses did poorly they could be culled, and if they demonstrated increased performance introgression could be continued. The science lead indicated that crosses have already been carried out between STW and GAK River salmon and results on survival from release to Age 1 in the wild are pending, which will help inform decisions on whether or not to introgress GAK genes into the STW LGB populations. The science lead was skeptical that there would be a pronounced positive effect, because there appears to be little evidence of high levels of cumulative inbreeding in the STW River population. Several participants did not expect there to be a benefit from incorporating such crosses in the program. The science lead also indicated that there had been substantial outcrossing already in the inner bay, mostly on the Chignecto Bay side (likely involving oBoF salmon from another DU), and little effect had been observed to date. He did not expect that outcrossing with the GAK would improve performance in the STW unless the STW was significantly more inbred than he believed. Another participant noted that while GAK River fish do experience increased survival at sea (compared to those in the STW), this could be a function of life history (e.g. length of migration or proximity to the iBoF/oBoF boundary). The general consensus was that there was likely no harm in crossing GAK with STW and releasing the offspring without incorporating them into the LGB program, but that it was unlikely to be beneficial either.

There was some discussion over the significance of results that suggested an increase in offspring survival with female spawner age. One of the reviewers was not convinced that the difference was significant and noted that the difference observed was small compared to the natural variations observed in the wild. The science lead responded that he felt the effect was real, given that the same trend was observed across all metrics testing for effect of parent age on offspring performance, and across all years assessed. This result supports the change that was made to spawn at Age 5 instead of the previous spawning at Age 4. The consensus among participants was that the methods applied in the program have the ability to detect moderate effects, which should give confidence when no effects are detected in future studies.

In regards to the trait monitoring work, one of the participants noted that in the size-at-stage analyses, 2005 appeared to be an anomaly. He suggested removing the year from the analyses to see if it removes the noise from the results. The participants were unsure what may have caused the anomaly in 2005. Another participant proposed that the decrease in egg area variance observed between G3 and G4 may have been the result of the switch to using image software and suggested that the trend observed is likely an artefact. It was recommended that to test whether there was a real trend in egg size variance, the old mechanical method (using a v-ruler) could be used to measure egg area previously measured by the image software, and the results could be compared.

The presence of European ancestry in some of the families released into the Pembroke River (as part of the Wild-exposed offspring group) provided the opportunity to assess the relative survival of European/Stewiacke hybrids compared to pure Stewiacke River Salmon. The percentage of the offspring population comprised of European/Stewiacke hybrids declined from release to Age 1, and from Age 1 to Age 2, consistent with increased mortality of hybrids relative to pure Stewiacke X Stewiacke Salmon during the freshwater phage of their life cycle. Other participants noted that differences in family size between STW and STW/EU crosses at Age 1 in the wild, reported as reflecting differences in survival, might actually be due to differences in migration timing between the two groups; offspring of STW/EU Salmon may simply leave the area (Pembroke) earlier (prior to sampling) at Age 1.

Conclusions and Recommendations

The participants and reviewers agreed that the program should continue with the wild-exposure protocol and recommended conducting new experiments to test for the effects of cumulative ancestral rearing with larger sample sizes and controlling for family differences (to minimize genetic effects and reduce noise). It was also recommended that in future discussion, the use of smolt wheels for sample collection be examined.

ASSESSMENT OF THE STATUS OF ATLANTIC SALMON IN THE IBOF DU BASED ON DFO INFORMATION, AND WHERE POSSIBLE, EVALUATION OF THE DIFFERENT RELEASE STRATEGIES OF THE LBG PROGRAM

Presenter: R. Jones and S. Ratelle
Rapporteur: C. Angelidis

Presentation Summary

As a part of the LGB review process, this Working Paper is a synopsis of the iBoF DU Atlantic Salmon monitoring activities, undertaken from 2001 to 2016, associated with the LGB Program. All of the assessment activities heavily incorporate genetic analyses to evaluate the success of the LGB in preventing the extirpation of this endangered Salmon population.

Annual mark-recapture estimate of adults returning to the Big Salmon River and annual counts of adult Salmon returning to the Gaspereau River are evaluated against conservation requirements that were determined for each index river based on accessible habitat area and the biological characteristic information of the returning adult Salmon. Estimates of emigrating juvenile Salmon (i.e., smolts) using rotary screw traps and surface bypasses are assessed against reference levels and used to determine survival rates for juveniles released from the LGB program as well as the smolt to adult return rates. Results of a crossbreeding experiment on a tributary (Pollet River) of the Petitcodiac River are also presented.

Overall, the available data on Salmon in iBoF DU indicates that populations are showing no signs of recovery and are persisting at low abundance levels mainly because of the ongoing LGB program. Since 2003, the first year returns from the LGB program were expected, adult abundance estimates to the Big Salmon River have averaged 46 fish, ranging from 16 (2004) to 118 (2011). For those years when small Salmon have been sampled on the Big Salmon River, progeny of LGB fry and parr releases have represented 21.4% of the total returns. Since 2001, the annual egg deposition estimates on the Big Salmon River have been below 10% of the conservation egg requirement in 14 of the 16 years assessed and averaging about 5% over the time-series. Adult returns to the Gaspereau River are monitored by counting the small and large Salmon captured in a pool and weir fishway designed to bypass the White Rock Dam. The mean count over the past decade has been seven fish ranging from 2 to 16 and since the LGB program was initiated on the Gaspereau River, most of the returning adults are assigning to LGB progeny. From 2005 until 2014, 69% of the small and 76.5% of the large returns are progeny of LGB releases. Since 2001, considering the potential eggs from all returning anadromous adults, the egg deposition estimates on the Gaspereau River, upriver of White Rock Dam have been below 10% of the conservation egg requirement in 14 of the 16 years assessed

Summing the number of Age 2, Age3, and Age 4 across multiple smolt classes (a 3-year period), the total smolt production from the LGB unfed fry releases has ranged from 1,948 to 6,947 fish. The percentage of released unfed fry surviving to the smolt stage has ranged from 1.0 to 2.5% with a 1.8% mean survival rate over the time series (release year; 2001 to 2012). Similarly, total smolt output by release year can be calculated for the LGB adipose fin clipped juveniles, primarily released as Age 0+ fall fingerlings, with the total smolt production estimates ranging from 1,067 to 9,755 fish from 2001 until 2011. Mean survival to the smolt stage for the LGB progeny released as parr over the time-series has been 6.6% (ranging from 2.5 to 10.6%). A time-series of freshwater survival rates for unfed fry and parr releases to smolt stage on the Gaspereau is being developed.

The annual small Salmon abundance estimates from the Big Salmon River from 2002 to 2016 combined with the smolt abundance estimates from 2001 to 2015 were used to determine the annual smolt to small Salmon return rates. Combining smolts and small Salmon produced from wild spawners, LGB fry, and LGB parr, the smolt to small Salmon return rate has averaged 0.32% ranging from (0.05% to 0.69%) over the time-series. The mean smolt to small Salmon return rate for the smolts that originated from LGB unfed fry is 0.18% or one small salmon return for every 561 smolts emigrating and ranged from 0 to 0.35%. This is about three times better than the mean return for LGB parr of 0.06% or one small Salmon return for every 1,622 smolts. The annual smolt abundance estimates from the Gaspereau River upriver of White Rock Dam from 2007 to 2015 combined with the small Salmon returns to the fishway from 2008 to 2016 were used to determine the annual smolt to small Salmon return rates. Combining smolts and small Salmon by origin, the mean smolt to small Salmon return rate has averaged 0.18% while ranging from (0% to 0.43%) over the time-series. With the addition of the large Salmon returns the following year the mean value increases to 0.25% and ranging from (0% to 0.64%). There were no small or large returns from the approximately 2,000 smolts that emigrated in 2012. It is important to note that smolt to adult survival rates would be considered a minimum estimate as generally more than half of the emigrating smolts above White Rock Dam are exposed to the negative impacts (i.e. acute turbine mortality, delayed mortality) of migrating through turbines.

In 2010, there were about 120 crosses between fish from the NS Live Gene Bank program at CBF and about 30 crosses of BSR LGB fish at MBF for the cross breeding experiment. About 73% of the unfed fry released into the Pollet River in 2011 were from the NS crosses. In 2011, all crosses were conducted from the NB BSR and Point Wolfe River LGB programs as Salmon from the NS LGB program were not available. Unfed fry to smolt survival for the 337,622 (combination NS and BSR LGB crosses) unfed fry released in 2011 was estimated to be 0.6%. Based on the genetic analysis of the 2013 smolts, the unfed fry to smolt (Age 2) survival for the BSR crosses (0.92%) was 2.3 times better than the survival for the NS crosses (0.40%). In comparison the unfed fry to smolt for BSR LGB fish released into the Big Salmon River was 1.2% for the Age 2 smolts and 1.3% when combining all age classes. The survival of the 2012 unfed fry (n=37,246) to the smolt stage was 3.9% which was double that observed from unfed fry released in the Big Salmon River that same year. Four sections of the Pollet River were surveyed by divers in late September in 2014, 2015 and 2016. These sections surveyed for possible returning adult Salmon from the crossbreeding experiment included more than 20 km of habitat and contain the known major holding pools within the river. Very few fish were observed in all three years so no seining or sampling activities were conducted to investigate whether any of these returns were progeny of the cross breeding experiment.

Discussion

There was a brief discussion about the capture and marking of fish in the BSR River for smolt assessment. One participant sought clarification on the mark and capture scheme as it switched to only four days per week. The science lead clarified that using catch data (from collections five days per week) he can apply the marking to a five-day estimate. It was suggested that he should consider a four-day estimate and apply a methodology such as an area under the curve method. He was willing to discuss and explore other options for estimating smolt assessments.

One participant questioned whether enough fry were marked to actually see them in the smolt wheel (considering similar results from the Fundy National Park where only 1% of fry survive to parr). He further questioned whether the lower number of parr experienced better survival. The science lead admitted that it was not clear if there was a signal for better survival given lower number of fry released, but recognized that it was reasonable to expect higher survival with lower stocking densities (empty habitat symptoms).

There was some discussion about the efficiency of bypasses and one of the participants recommended acquiring generation status from Nova Scotia Power to confirm whether bypasses are less efficient when Nova Scotia Power is operating turbines. The participants and reviewers agreed that this knowledge could inform future operations. One of the objectives was to identify and remedy anthropogenic threats limiting survival and/or recovery of iBoF Salmon in freshwater environments. One of the participants noted that more consideration should be given to how to identify and qualify good quality freshwater habitat.

PERSPECTIVE OF MARINE SALMON ECOLOGIST ON THE RECOVERY OF IBOF SALMON

Presenter: M. Trudel
Rapporteur: C. Angelidis

Presentation Summary

Dr. Trudel presented his perspective on the accomplishments of the LGB program to maintain the genetic diversity of iBoF Salmon. Despite this effort, iBoF Salmon continue to show no sign of recovery, with either no or few LGB salmon returning to their natal river after over 15 years of effort. Without the LGB program, this population is at risk of extinction, indicating the necessity of maintaining the LGB program for iBoF Salmon. Additional effort is needed to reach the interim recovery target of 9,900 adults. It was suggested that over 3 million smolts are currently needed on an annual basis to reach that interim target at the low marine survival rates observed during the last two decades, and possibly more if survival rates are lower at low Salmon abundance (i.e. depensation), as in the Snake River Sockeye Salmon population. Various strategies were proposed for producing adult Salmon (fry release vs smolt release vs smolt-to-adult supplementation) based on available stage-specific survival rates. Few adult iBoF Salmon are expected to return using the current practice of releasing unfed fry, which may lead to further losses in genetic diversity. A smolt-to-adult supplementation (SAS) program could rapidly produce large numbers of iBoF Salmon, and may be an approach that could assist in the recovery of iBoF Salmon.

Discussion

A reviewer discussed the challenge of marine survival and understanding marine threats. His major suggestion was to increase the number of smolts released into the system based on the argument that the ocean conditions may never improve. Some of the participants did not agree and noted that to produce a sustained wild population the rate of survival and returns had to increase. The science lead did not feel that producing more smolts would help reach that objective unless it also increased diversity in the population. Several other participants agreed that given the target of recovering a wild, sustained population stocking would not achieve that goal, but that increasing the return rate was essential. The reviewer clarified that he was not suggesting changing the LGB program dramatically (and recognized that the program has been effective at maintaining genetic diversity), but reiterated that to achieve a self-sustaining population the rate of survival at sea had to change. Until that point, he suggested that something must be done to increase the number of returns.

Several participants noted that it remained largely unknown as to which characteristics affect survival rate in the ocean. A reviewer was surprised by the recommendation to ignore ocean conditions and the statement that conditions would never improve. Another reviewer responding that the primary contributing factor to returning fish is the number of smolts released. While he acknowledges that ocean survival is an important factor, he cautioned that it may not be the only factor limiting returns. The third reviewer felt that the largest threat to the iBoF Salmon was the loss of marine selection during the most dramatic effects of climate change. He noted that the lack of fish out in the Bay of Fundy precluded any adaptation to these new and changing conditions. Participants and reviewers agreed that it was essential to get fish into the ocean in order for selection to act on them. From this discussion, participants agreed that a monitoring program to evaluate survival among the different LGB rivers would be important to implement and maintain in the long-term.

There was a brief discussion around the merit of a sea-cage approach, and some participants felt that the current sea-cage programs had not been supported sufficiently to evaluate its potential as a recovery tool.

Conclusions and Recommendations

Given the target of recovering a self-sustained wild population, it was felt that flooding the system with smolts (stocking) would not be sufficient but that, instead, increasing the return rate of fish was essential. It was recognized that a major limitation of the program is the fact that so few fish are surviving in the marine environment in such a critical period of climate change. It was recognized that more fish need to be in the ocean for selection to act on the population.

DOCUMENTS

There was consensus that the three working papers will be published as Research Documents. There was a recommendation from one reviewer regarding consistency in language and terminology used in the working papers. The reviewer expressed confusion surrounding the different use of the term “population” and recommended defining key terminology and remaining consistent with the language used in SARA. Further, it was suggested that the working papers should more clearly explain that the LGB programs are different in each of the three rivers (STW, GAK and BSR rivers); the analysis and discussion should be separated for the different river programs.

SCIENCE ADVISORY REPORT

The Chair acknowledged that a draft Science Advisory Report (SAR) had not been prepared for review at the meeting. It was proposed that meeting participants discuss the key analyses, conclusions and recommendations that should be included in the SAR. The draft SAR would then be circulated to meeting participants following the meeting for their review and, if necessary, a follow-up meeting by teleconference would be scheduled. There was agreement from all participants with the proposed approach.

Objective 1: Evaluate the success of conserving genetic characteristics of the iBoF Salmon population across three generations of captive breeding and rearing

Key Observations and Analyses

The science lead confirmed that estimates of kinship among founders were not completely accurate, with degrees of accuracy varying with family size and structure. He expected accuracy to be approximately 90%. There was considerable family structure in all founder populations, which resulted in the overrepresentation of some families and reduced the variability of the founder population. The reviewers and participants agreed that initiating collections of adults earlier, before the level of family structure reached the level it had attained for the implementation of the initial LGB program, would be highly beneficial in maintaining long-term population diversity and in reducing inbreeding accumulation over time. One of the reviewers emphasized that while initiating the program earlier would have reduced the accumulation of inbreeding, levels of inbreeding in the program are still very low as a result of the spawning procedures in place in the LGB.

One of the major gaps in knowledge arises because the program is not able to calculate the increased loss of variation due to selection. The science lead noted that the original founder selection regimes would have resulted in the loss of a few percent of the initial genetic variation. Alleles that were lost from the populations were alleles that were initially rare. A reviewer noted the large potential gains from proper operational management. A key procedure that the participants recommended continuing was to not combine the families before producing fry (as this would have resulted in the loss of significant diversity). It was also recognized that molecular data collected was useful in revealing and resolving problems in the biodiversity facilities. The participants and reviewers felt that this demonstrated the value of monitoring the LGB program.

Analytical or Methodological Concerns

No major analytical or methodological concerns were identified at this time. There was a discussion of improving the methods using SNP analyses to produce more data quickly. This discussion is outlined in the recommendations below.

Recommendations

Reviewers and science leads discussed the potential for new genotyping methods with faster and cheaper throughput that would be worth the investment to allow the program to proceed. The science lead reiterated that SNP analyses provide a lot of data quickly, although was unsure of the cost, but did express concern that in SNP data provided by other groups all loci were in Hardy-Weingburg disequilibrium. He expressed concern that this might have been the result of a genotyping error. The reviewers agreed that this was a fair concern and agreed that it would take time to develop a reliable technique, although one reviewer was optimistic that given the progress in developing SNP panels for Coho Salmon on the west coast, reliable SNPs for Salmon would be developed soon. Other participants also commented that the cost of SNP genotyping was similar to the cost of a microsatellite run. It was also recommended by a reviewer that a later discussion should evaluate ways to identify European farm fish.

One reviewer noted that 500 SNP loci are approximately equivalent to 10-15 microsatellite loci, and that in many cases SNPs are performing better than microsatellites in stock identification. She recommended identifying key fish in the program and conducting genome-wide screening to identify markers for identifying European ancestry.

Another reviewer felt that it was important to capture the change in approach for estimating kinship (from Mean Kinship to Ranked Mean Kinship) as this played a key role in maintaining genetic diversity. It was encouraged to continue building monitoring into the LGB and other program work plans, where methodologies and performance are evaluated.

Other Discussions

One of the participants noted that wild genes from wild-origin fish are being integrated into the BSR River, but the same opportunity is not present in the STW River as, until recently, the program lacked the methods to integrate genes. She recommended that in a new program with Mi'kmaw Conservation Group (MCG), there be an opportunity for integrating wild-origin genes. She further noted that if this opportunity became of interest, there was a fish passage issue in the GAK and a potential opportunity in the STW with the upcoming MCG program.

It was noted that it will be important to emphasize that the three rivers are three different LGB programs. There was also a recommendation for including a section in the Science Advisory Report with a flow chart of various management options and how to balance trade-offs.

Objective 2: Investigations into the origin and extent of inbreeding in iBoF Salmon

Key Observations and Analyses

The science lead noted that an earlier start would have reduced the level of inbreeding and family structure in the founders, but emphasized that some level of inbreeding accumulation is unavoidable in a captive breeding and rearing program. The participants and reviewers agreed that it is advantageous to select adults as they provide more diversity than smolts. The science lead noted that the potential cost in terms of using adults is that it may be challenging or dangerous to remove the adults from the population unless it is certain that the population is incapable of recovery on its own. If there is uncertainty of coming extirpation, smolts could be collected along with a few adults. The major advantage of sampling adults is that there is guaranteed sampling of each family and no concern of sampling the offspring of adults in a manner to capture all variability from all families. Smolts provide more diversity than fry as a result of the multiple year classes. Smolt wheel collections are superior to fry collections as they collect samples from the whole river. The consensus was that smolts are the safest samples to collect, as there is a benefit to allowing adults to reproduce in the wild, but under circumstances where the population is expected to become extirpated, collecting adults will benefit the long-term diversity of the program.

Based on the analyses of males and females and inbreeding, it was agreed that a key finding was that the absolute number of parents and the quality of males and females influences the conservation of genetic variation. Of particular importance is the role of females, as adding more females has a larger effect (as a result of the high level of family structure). However, it was agreed that nothing will better mitigate the problem of loss of genetic diversity and inbreeding than beginning sampling earlier with similarly sized families.

Analytical or Methodological Concerns

One reviewer suggested that the work on tracing inbreeding back was a very helpful analysis. She also suggested that whatever life stage is used in the future (depending on circumstance), it will be most effective to collect when adult numbers are high.

One reviewer felt there was a missed opportunity to screen European alleles. Several recommendations regarding this are outlined below.

Recommendations

One reviewer suggested continued verification of fish until the threat of European introgression is removed, emphasizing that the source of the European farm fish must be identified and cut off to eliminate introgression long-term. He further suggested cross-breeding experiments in the available vacant habitat and felt that it was a major institutional hurdle that fish cannot be moved between two provinces despite the fact that they are a part of the same DU. The reviewers and participants agreed that there was no scientific basis for such isolations, and feared that they may lead to impediments to the program in the future. The reviewer felt that such cross-breeding experiments could increase the overall diversity of the iBoF Salmon, but at the expense of local conserved diversity (within rivers).

The science lead expressed concern that in an effort to incorporate at-sea fitness by increasing the contributions of fish that went to sea and returned, with the idea that wild-origin fish reduce domestication and presumably possess fitter genotypes that are able to survive at sea, the program inadvertently increased European introgression. He warns that there is always some level of complexity that has not been accounted for that presents unforeseen challenges to the program. He feels that while similar problems can be minimized in the future, European genes have already introgressed and become diffuse in the current river populations. As had been discussed at length previously, he reiterated that culling individuals with European genes from the BSR River would likely remove the majority of individuals from the population. While one reviewer noted that, given past experience, it is now possible to screen new fish entering the program for European or other introgressing fish or strays that are not native to the BSR River, the science lead cautioned that it remains challenging to differentiate Saint John River Salmon from BSR River fish as a result of significant gene flow between the systems until very recently.

The science lead recommended screening Salmon at additional loci, but recognized that this would require additional funding. One of the reviewers noted the danger of being “penny-wise versus pound-foolish” and felt the additional screening would be worth the extra effort and cost. She noted that the alternative could be to eliminate integrating wild-origin fish into the program, but that while this would reduce (and perhaps eliminate) the risk of introgression, there would also be harm in the loss of fitness and diversity that otherwise would have been gained by including new founders into the program.

The science lead recommended that should funds be unavailable for additional screening, new fish that cannot be conclusively identified as being native BSR River fish should not be included in the program. Further, he suggested that if more screening confirms with reasonable certainty that the new fish is not of European or farm origin, they should be included in the program. He felt confident that while the program has likely captured the majority of existing BSR River variation through smolt collections over the past 15 years, there was still value in including new founder variation as a mechanism of introducing at-sea fitness to the program.

Consensus was reached among the reviewers and participants that given the ability to screen, continued screening of non-native fish is recommended. It was also agreed that there was still value in sampling BSR River returns as a mechanism for introducing wild-origin genes (capturing selection in the marine environment) even if it is no longer useful in capturing more diversity. Consensus was that the only reason to exclude wild-origin fish would be if there was concern they were of farm or European origin. It was also agreed that beyond simply screening for European alleles, the source of the European introgression should be identified and cut off.

Objective 3: Evaluation of the effects of the overall program and specific management strategies employed within, on fitness-related traits in iBoF Salmon

Key Observations and Analyses

There was little evidence of parental or grand-parental effect on offspring survival, but there was an effect on offspring size. There was also an effect of environment on offspring survival and some effect of mean kinship on survival in the wild (although it was noted that this was correlated with the cycles of captive breeding and should be interpreted with caution). To date, there was no evidence of a relationship between pedigree inbreeding and fitness, but it was noted by several participants and reviewers that the data only capture three generations which may not be sufficient to identify fitness effects.

There was some discussion surrounding the option of spawning at the fifth year exclusively. The implications of such a change would be the creation of five separate populations running in parallel, which would be a large departure from the current program. The result would be switching from a single large effective population size to create five moderate effective population sizes. One reviewer noted that in model organisms, an effective population size of 250-300 individuals is sufficient to avoid inbreeding, but that in these smaller populations the rates of adaptation to captivity will be higher. It was agreed that the advantages to isolating brood years would include: 1) moving from a more complex overlapping-generation system to a simpler system with discrete generations (making it simpler to estimate effective population size), 2) facilitate estimation of effective population size and provide more confidence in results, and 3) increase retention of genetic variation. The major limitations of such a system were identified as: 1) the increased rate of inbreeding accumulation in each brood stock over time, and 2) the danger of losing a lot of fish if there is a failure in the program. It was recommended that the risk of inbreeding accumulation could be mitigated by allowing some gene flow between brood years, and that milt should be cryopreserved as a backup in the event of a catastrophic loss of part of the population.

There was also some discussion among the reviewers and science leads regarding the risk of selecting for late maturation by forcing fifth year spawning, but it was agreed that this would not be a major concern as long as fourth year fish were still given the opportunity to spawn, but their offspring would be released as supplementation and would not be included in the program. The only major concern would occur under the situation where an individual that matured in the fourth year but did not survive to reproduce a second time in the fifth year. Reviewers and participants agreed that while this was a concern, most fish survived to spawn twice.

Analytical or Methodological Concerns

No major analytical or methodological concerns were identified.

Recommendations

Reviewers agreed that more data over a longer time period would be helpful in evaluating the program. One of the key limitations in assessing the effectiveness of the program was identified as being the relatively small sample size across most analyses.

Objective 4: Assessment of the status of Atlantic Salmon in the iBoF DU based on DFO information, and where possible, evaluation of the different release strategies of the LBG program

Key Observations and Analyses

The reviewers and participants identified the status of rivers in reference to the LGB as a key observation regarding the evaluation of LGB strategies. There is evidence of extirpation of Salmon in rivers that are not supported by the LGB, and conversely Salmon populations are persisting in populations that are supported by the LGB. It was noted that while the objective of the LGB was not to swamp every river with LGB fish, some consideration could be given to expanding the LGB program into rivers where suitable freshwater habitat is available by increasing the unfed fry releases. It was suggested that for long-term self-sustaining populations to be re-established, more habitat may need to be seeded in the future. It was identified by one of the participants that freshwater habitat is also a major concern for the success of the program, particularly surrounding issues with aboiteaux, causeways, and overpasses.

It was recognized by the reviewers that the BSR River program includes a very thorough monitoring program that facilitated separating out origins by life stage. It was noted that neither the STW or GAK programs had the same level of monitoring and, as a result, it was not possible to tease out information for assessing viability because the data were collected in the context of the LGB. The reviewers agreed that assessing viability is only possible in the BSR River.

One reviewer noted that monitoring and having genetic data made possible the evaluation of different release strategies, but also that the monitoring has not been evenly weighted across the rivers. He further noted that the current release strategies do not produce enough smolts to incorporate fitness elements into the program at this time. The consensus among the reviewers and participants was the number of smolts released is not currently enough to capture fitness.

Analytical or Methodological Concerns

No major analytical or methodological concerns were identified.

Recommendations

The first major recommendation identified by the reviewers was that there was potential room to increase the amount of support to the supplementation component of the program to evaluate its potential to contribute to the establishment of a sustainable population. It was argued that the supplementation program is no less important than the primary objective of the program, and instead is just a separate component of the same goal. However, it was emphasized by numerous participants that in order to achieve a self-sustaining population, consideration of the habitat issue is essential (both marine and freshwater). The reviewers recommended increasing the use of available habitat (of extirpated rivers). There was again consensus that more habitat should be seeded in order to produce reasonable smolt returns.

While improving habitat was identified as a key recommendation, reviewers questioned how to take advantage of the habitat already available and recognized its potential use for experimental work. It was recommended that more experimental work be conducted, and that population analyses conducted by the National Oceanic and Atmospheric Administration (NOAA) on the west coast be reviewed for advice on conservation (including abundance, trends, spatial distribution, and diversity analyses).

One participant expressed concern that recommendations for the program be consistent with the Recovery Strategy and Action Plan for the species. It was noted that the Recovery Strategy identifies 10 priority rivers. It concluded that the current recommendations and structure of the program are consistent with the Action Plan.

A second participant recommended further research into understanding the marine mortality and to identifying where there are any mitigation steps that can be applied to improve the marine survival rate. There was some discussion as to how such research would fit into the LGB program given its goal of maintaining iBoF genetic characteristics. It was recognized that understanding the marine environment is essential to the long-term objective of establishing self-sustaining river populations in the iBoF; however, mitigating the marine challenges is not within the scope of the LGB program. Another participant recommended exploring further steps that could improve or increase marine exposure to allow selection a longer time to act on fish that are being incorporated into the system.

In response to the agreement that the number of released smolts is insufficient to capture the element of fitness, the reviewers recommended producing more smolts. There was some discussion on the best way to produce more smolts, as production in a hatchery may not produce high quality smolts. It was recommended that this discussion be explored further in the future.

A final recommendation was made by one of the participants to include electrofishing effort in analyses of electrofishing success.

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APPENDICES

APPENDIX 1: LIST OF MEETING PARTICIPANTS

Review of the Inner Bay of Fundy Atlantic Salmon Science Associated with the Live Gene Bank

Regional Advisory Process - Maritimes Region

June 13 - 16, 2017
Dartmouth, Nova Scotia

Chairperson: Kent Smedbol

Name	Affiliation
Angelidis, Christine	Dalhousie University, Department of Biology
Burbidge, Chris	DFO Maritimes / Species at Risk Management
Carr, Jonathan	Atlantic Salmon Federation (ASF)
Clarke, Corey	Parks Canada Agency, Fundy National Park
de Mestral, Louise	DFO Maritimes / Population Ecology Division
Elhaimer, Elias	DFO Maritimes / Species at Risk Management
Epworth, Wendy	Fort Folly Habitat Recovery Program
Gibson, Jamie	DFO Maritimes / Population Ecology Division
Giffin, Geoff	Atlantic Salmon Federation (ASF) - NB Salmon Council
Hardie, Dave	DFO Maritimes / Population Ecology Division
Harris, Lei	DFO Maritimes / Population Ecology Division
Harvie, Carolyn	DFO Maritimes / Population Ecology Division
Jones, Ross	DFO Maritimes / Population Ecology Division
Kavanagh, Sana	Confederacy of Mainland Mi’kmaq (CMM)
Kocik, John	National Oceanic and Atmospheric Administration (NOAA) - USA
Lenentine, Beth	DFO Maritimes / Population Ecology Division
Levy, Alex	DFO Maritimes / Population Ecology Division
London, Evelyn	Oromocto First Nation / Fisheries
MacDonald, Jennifer	DFO Maritimes / Centre for Science Advice Maritimes
Martin, Tim	Native Council of Nova Scotia
Mason, Taylor	Confederacy of Mainland Mi'kmaq (CMM)
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Name	Affiliation
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Withler, Ruth	DFO Pacific / Science

APPENDIX 2: TERMS OF REFERENCE

Review of the Inner Bay of Fundy Atlantic Salmon Science Associated with the Live Gene Bank

Regional Advisory Process - Maritimes Region

June 13 - 16, 2017
Dartmouth, Nova Scotia

Chairperson: Kent Smedbol

Context

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) identified the inner Bay of Fundy (iBoF) Atlantic Salmon assemblage as a Designatable Unit (DU) and assessed this population as Endangered in May 2001 (COSEWIC 2006). Furthermore, this population was included as Endangered on Schedule 1 of the *Species at Risk Act* (SARA) when the Act was passed in 2002. In 1998, prior to listing under SARA, population trends observed in several rivers of the inner Bay of Fundy prompted the collections of juveniles to be reared in the Biodiversity Facilities in the Maritimes Region (Mactaquac, Mersey, and Coldbrook biodiversity facilities) effectively initiating the present-day Live Gene Bank (LGB) programs. The objective of the LGB program is to use captive breeding and rearing technologies to conserve genetic characteristics of iBoF Salmon and maintain populations until recovery can occur (DFO 2008a). In 2008, the Recovery Potential Assessment (RPA) forecasted that this population would be extinct without the support of the LGB program (DFO 2008b; Gibson et al. 2008).

Several evaluations have been conducted to assess the scientific merit of the Live Gene Bank program. In 2004, a review was done for the Director General of Fisheries, Environment and Biodiversity Science. In 2006, COSEWIC contracted a review of the status and assessment of the iBoF Salmon population, which resulted in COSEWIC confirming the previous assessment of endangered and, in 2008, an RPA was completed for iBoF Salmon to support recovery planning under SARA. In 2008, DFO Science also struck a national working group that produced a Science Advisory Report evaluating the contribution of captive breeding facilities to biodiversity conservation. Additionally, since its inception, yearly updates and summaries of ongoing assessment activities and genetic analyses have helped, and continue to help, adaptively manage the LGB program and guide the iBoF Salmon program through the Planning Group and Recovery Teams (DFO 2010).

However, a comprehensive analysis and review of the LGB program on the recovery of the iBoF population does not exist for the lifetime of the program. Therefore, DFO Maritimes Science requested the evaluation of iBoF Salmon science with respect to all LGB activities. The intent of this review is to provide an assessment of the LGB program following three generations (i.e., 15 years) of iBoF Salmon population restoration and maintenance as population recovery has yet to occur. This review will direct the development of an updated 5-year plan for the LGB program.

Objectives

The purpose for this meeting is to evaluate the contribution of the LGB program in achieving two key objectives of the iBoF Salmon Recovery Strategy: 1) to conserve the genetic characteristics of iBoF Salmon and 2) to help re-establish wild self-sustaining populations of iBoF Salmon.

More specifically, the objectives of the meeting are to:

- Evaluate the success of conserving genetic characteristics of the iBoF Salmon population across three generations of captive breeding and rearing,
- Investigate the origins and levels of inbreeding in iBoF Salmon,
- Evaluate the effects of the overall program (multiple generations of captive breeding and rearing), and specific management strategies employed within, on fitness-related traits in iBoF Salmon, and
- Assess the status of Atlantic Salmon in the iBoF DU based on DFO information, and where possible, evaluate the different release strategies of the LGB program.

In addition, the meeting will also report recent findings of possible introgression of non-native wild and aquaculture genetic material into iBoF populations, a new potential threat to the conservation of iBoF genetic characteristics.

Expected Publications

- Research Documents (3)
- Proceedings
- Science Advisory Report

Participation

- DFO Science
- DFO Species at Risk Management Division
- DFO Fisheries & Aquaculture Management
- Parks Canada Agency- Fundy National Park
- Aboriginal Organizations and First Nations
- NGOs
- Technical expert reviewers

References

COSEWIC (Committee on the Status of Endangered Wildlife in Canada). 2006. COSEWIC Assessment and Update Status Report on the Atlantic Salmon Salmo salar (Inner Bay of Fundy Populations) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. viii + 45 pp.

DFO (Fisheries and Oceans Canada). 2008a. Evaluation of Captive Breeding Facilities in the Context of their Contribution to Conservation of Biodiversity. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2008/027.

DFO (Fisheries and Oceans Canada). 2008b. Recovery Potential Assessment for Inner Bay of Fundy Atlantic Salmon. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2008/050.

DFO (Fisheries and Oceans Canada). 2010. Recovery Strategy for the Atlantic Salmon (*Salmo salar*), Inner Bay of Fundy Populations [Final]. In: Species at Risk Act Recovery Strategy Series. Ottawa: Fisheries and Oceans Canada. xiii + 58 pp. + Appendices.

Gibson, A.J.F., Bowlby, H.D., Bryan, J.R., and Amiro, P.G. 2008. Population Viability Analysis of Inner Bay of Fundy Atlantic Salmon With and Without Live Gene Banking. DFO Can. Sci. Advis. Sec. Res. Doc. 2008/057.

APPENDIX 3: MEETING AGENDA

Review of the Inner Bay of Fundy Atlantic Salmon Science Associated with the Live Gene Bank

Regional Advisory Process - Maritimes Region

June 13 - 16, 2017

Lewis King Boardroom
Bedford Institute of Oceanography
Dartmouth, Nova Scotia

Chairperson: Kent Smedbol

DAY 1 (Tuesday, June 13, 2017)

Time	Topic	Leads
09:00 – 09:30	Welcome and Introductions	Kent Smedbol
09:30 – 10:30	Overview of inner Bay of Fundy Atlantic Salmon and the Live Gene Bank Program	Patrick O'Reilly and Stephanie Ratelle
10:30 – 10:45	***Break*** *(Coffee/tea provided)*	
10:45 – 12:00	Objective 1: *Evaluation of the success of conserving genetic characteristics of the iBoF Salmon population across three generations of captive breeding and rearing*	Patrick O'Reilly
12:00 – 13:00	***Lunch*** *(Not provided – cafeteria is on-site)*	
13:00 – 14:30	Objective 1: discussion cont'd	Patrick O'Reilly
14:30 – 14:45	***Break*** *(Hospitality not provided)*	
14:45 – 16:00	Objective 1: discussion cont'd	Patrick O'Reilly

DAY 2 (Wednesday, June 14, 2017)

Time	Topic	Leads
09:00 – 09:15	Recap of Previous Day	-
09:15 – 10:30	Objective 2: *Investigations into the origin and extent of inbreeding in iBoF Salmon*	Patrick O'Reilly
10:30 – 10:45	***Break*** *(Coffee/tea provided)*	
10:45 – 12:00	Objective 2: discussion cont'd	Patrick O'Reilly

Time	Topic	Leads
12:00 – 13:00	***Lunch** (Not provided – cafeteria is on-site)*	
13:00 – 14:30	Evaluation of possible introgression of non-native wild and aquaculture genetic material into iBoF populations	Patrick O'Reilly
14:30 – 14:45	***Break** (Hospitality not provided)*	
14:45 – 16:00	Objective 3: *Evaluation of the effects of the overall program and specific management strategies employed within, on fitness-related traits in iBoF Salmon*	Patrick O'Reilly Sherisse McWilliam- Hughes

DAY 3 (Thursday, June 15, 2017)

Time	Topic	Leads
09:00 – 09:15	Recap of Previous Day	-
09:15 – 10:30	Objective 3: discussion cont'd	Patrick O'Reilly Sherisse McWilliam- Hughes
10:30 – 10:45	***Break** (Coffee/tea provided)*	
10:45 – 12:00	Objective 4: *Assessment of the status of Atlantic Salmon in the iBoF DU based on DFO information, and where possible, evaluation of the different release strategies of the LGB program*	Ross Jones Stephanie Ratelle
12:00 – 13:00	***Lunch** (Not provided – cafeteria is on-site)*	
13:00 – 14:30	Objective 4: discussion cont'd	Ross Jones Stephanie Ratelle
14:30 – 14:45	***Break** (Hospitality not provided)*	
14:45 – 16:00	Introduction to the Science Advisory Report (SAR)	-

DAY 4 (Friday, June 16, 2017)

Time	**Topic**	**Leads**
09:00 – 09:15	Recap of Previous Day	-
09:15 – 10:30	Review of the Science Advisory Report (SAR)	-
10:30 – 10:45	***Break*** *(Coffee/tea provided)*	
10:45 – 12:00	Review of the SAR cont'd	-
12:00 – 13:00	***Lunch*** *(Not provided – cafeteria is on-site)*	
13:00 – 14:30	Review of the SAR cont'd (*as needed*)	-
14:30 – 14:45	***Break*** *(Hospitality not provided)*	
14:45 – 16:00	Review of the SAR cont'd (*as needed*)	-