Fisheries and Oceans Canada
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Ecosystems and Oceans Science
Sciences des écosystèmes et des océans

Newfoundland and Labrador Region

Canadian Science Advisory Secretariat
Science Response 2016/034

PROPOSED USE OF EUROPEAN-STRAIN TRIPLOID ATLANTIC SALMON IN MARINE CAGE AQUACULTURE IN PLACENTIA BAY, NL

Context

Canada has endorsed the use of sterile fish to minimize adverse effects to wild salmon from aquaculture, ranching, introduction and transfers, and transgenics within the North Atlantic Salmon Conservation Organization (NASCO) through the 1992 North American Commission Protocols for the Introduction and Transfer of Salmonids (NAC(92)24), the 2002 Oslo Resolution (CNL15.147), and the Williamsburg Resolution (CNL(06)48) (NAC/NASCO 1992).

In March 2016, Grieg NL Nurseries (hereinafter referred to as “The Proponent”) submitted a request to the Canadian Food Inspection Agency (CFIA) and Fisheries and Oceans Canada (DFO) to import triploid Atlantic Salmon eggs from Iceland. The importation has been approved by the CFIA and an import permit has been issued to the Proponent. The issuance of a CFIA import permit indicates that an export certificate will be endorsed by the Icelandic Competent Authority certifying that the disease risks associated with the import of the aquatic animal from Iceland have been mitigated. The Newfoundland and Labrador (NL) Introductions and Transfers Committee (ITC - a multi-jurisdictional committee with membership from DFO as well as the NL Departments of Fisheries and Aquaculture, and Environment and Conservation) conducted a risk assessment of the importation request under the Canadian Council of Fisheries and Aquaculture Ministers (CCFAM) National Code on the Introductions and Transfers of Aquatic Organisms, based on the outcome of the 2013 Canadian Science Advisory Secretariat (CSAS) process (DFO 2013) and recent scientific publications on performance characteristics, ecological effects, and pathogen matters using sterile salmon.

In April 2016, Aquaculture Management, Ecosystems Management Branch, NL Region requested Science Branch conduct a peer-review of the risk assessment of the ITC with respect to the following:

1. Provide a science review of the Newfoundland and Labrador Introductions and Transfers European Triploid Atlantic Salmon Risk Assessment, and scientific advice on the genetic, ecological and fish health risk from the proposed introduction of European triploid Atlantic Salmon to Placentia Bay, NL.

Specifically,

2. Characterize the effectiveness of triploid induction in fish. Provide advice on triploidy monitoring measures including sampling protocols, sample sizes required, and statistical power, to ensure triploidy induction levels are acceptable (i.e., 100%).
3. Review and provide advice on the scientific basis for predictions on impacts associated with the potential for triploid escapes to occur and subsequent impact of sterile and potential non-sterile European strain salmon on wild Newfoundland Atlantic Salmon, including genetic (direct and indirect), ecological and fish health impacts.

4. The scientific advice may also propose mitigation measures that may further reduce the risk of impacts from the introduction of European strain triploid Atlantic Salmon into NL waters.
5. To advise on any new significant information that might affect the potential for genetic and ecological effects on wild populations and that might need to be added to the existing risk assessment document.

This Science Response Report results from the Science Response Process of May 25, 2016 on the Review of the Introductions and Transfers Committee's Risk Assessment on the Proposed Use of European-strain Triploid Atlantic Salmon in Marine Cage Aquaculture in Placentia Bay, NL.

Background

In 2013, DFO Science examined the potential effects of the use of domesticated European-origin salmon on wild Atlantic Salmon and their habitats in NL waters. The review found that interbreeding between European-origin escapes and wild NL salmon is predicted to have genetic and phenotypic consequences that would likely result in a reduction in genetic diversity and reduced fitness among populations that would affect the character, abundance, and viability of the native wild populations (DFO 2013). It also found that commercial production of all-female sterile triploids would reduce the likelihood of direct genetic effects occurring between escaped and wild fish populations and have a lower rate of freshwater migration, thus reducing the proportion of escaped farmed adults returning to rivers, and decreasing the potential extent of reproductive interference. Further comparisons would be required to evaluate marine performance, pathogen resistance, disease transmission, ecological effects, costs of monitoring, and husbandry techniques to optimize performance.

Proposed Project

The Proponent is proposing an aquaculture investment in Placentia Bay, NL to annually produce 33,000 t of Atlantic Salmon. The project consists of a recirculation hatchery in Marystown, eleven marine sites in Placentia Bay, including two sites proposed for seasonal production only, and a five-year production plan with stocking levels increasing from 2 to 7 million fish per year over this period. Proposed production levels require importation of 8 million triploid eggs annually from Stofnfiskur Ltd., Iceland to ensure the required 7 million smolt for marine cage stocking because of expected egg losses during the hatchery phase (i.e., egg to smolt survival).

The production strategy proposes extended hatchery rearing to stock smolt/post-smolt entries at four farm sites per year class of eggs totaling ~ 7 million fish at peak planned production. Two sites will be stocked in the spring at ~ 300 g to 450 g with up to two million fish per site; one site will be stocked in the fall at ~ 600 g with up to two million fish; and one site will be stocked the following spring at 1,500 g with up to one million fish for ongrowing without overwintering. The strategy is intended to reduce the marine rearing period and minimize holding of fish in marine sites throughout the winter periods. Use of a domesticated European-origin strain, sea cage entry of large smolt and post-smolt, and single season production is unprecedented and untested in the NL salmon aquaculture industry.

Planned start-up is fall 2016, subject to completion of the NL environmental assessment process, with stocking of marine cages beginning in the spring of 2018.

In addition to application of containment measures in the provincial Code of Containment for the Culture of Salmonids in Newfoundland and Labrador (Code of Containment), the Proponent plans to use the Aqualine Midgard cage system engineered to exceed the Norwegian technical standard for cage systems (NS 9415; Standard Norge 2009). Aqualine asserts that this system is “escape-proof” based on 450 existing installations in Scotland, Norway, Iceland, and Faroe Islands without reported escape incidents. The system is new to the NL salmon aquaculture industry and appears more robust than current cage systems according to provincial inspectors that have visited farms in Norway using the Midgard system (Elizabeth Barlow, Department of Fisheries and Aquaculture, pers. comm.). The standard requires that sites be classified with regards to environmental/oceanographic conditions, and major farm components be certified for the specific site conditions prior to installation. Introduction of the Norwegian technical standard is considered to be a major contributing factor in reducing the number of escapes and escape events in Norway since its implementation (Bridger et al. 2015).

Location of Project

Placentia Bay is a large embayment on the southeast coast of the island of Newfoundland, measuring ~ 130 km long and ~ 100 km wide at the open southern mouth of the bay (Figure 1). Water temperatures in Placentia Bay are generally suitable for salmonid aquaculture (Barton, 1996) based on long-term National Oceanic and Atmospheric Administration (NOAA) satellite daily seawater temperatures (Figure 2).

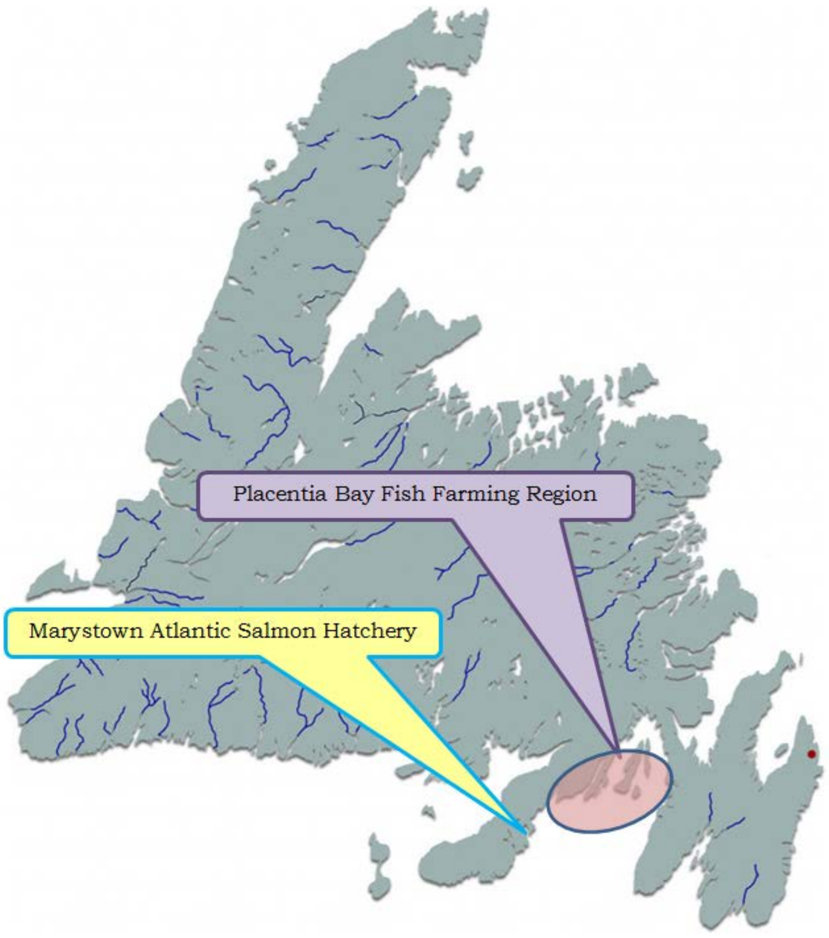

Figure 1. Location of proposed fish farms and salmon hatchery.

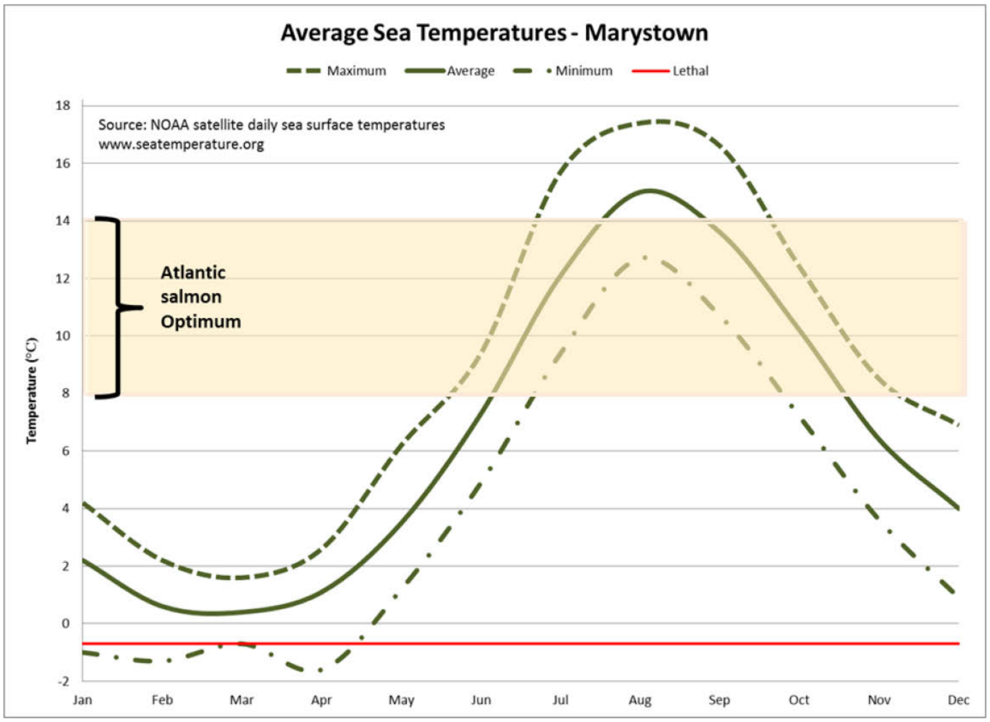

Figure 2. Average annual seawater surface temperatures at Marystown, Placentia Bay, illustrating optimum and lethal temperature limits for Atlantic Salmon. Source: World Sea Temperatures.

Proposed farms are distributed among four proposed Bay Management Areas (BMAs) (Figure 3): two seasonal sites on the eastern side of the bay near Long Harbour (Long Harbour BMA), three sites in the central channel area (Red Island BMA), three sites on the northwestern side (Merasheen BMA), and three sites on the western side of the bay (Rushoon BMA). There are currently no salmon aquaculture facilities in Placentia Bay.

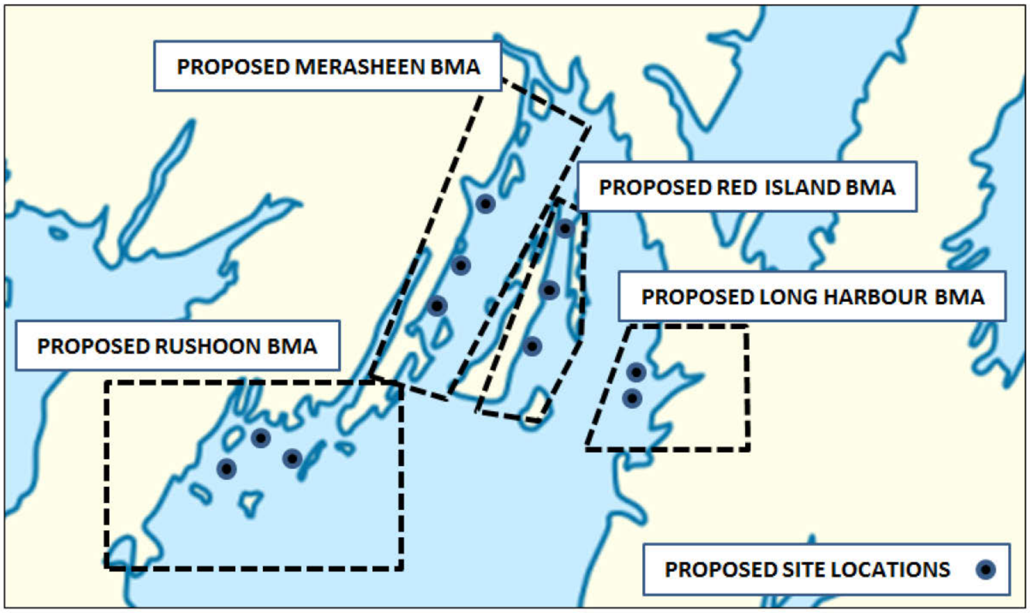

Figure 3. Proposed Placentia Bay marine cage site locations and BMAs.

Triploid Biology

Triploid organisms have three sets of chromosomes instead of the standard two (diploid). It is based on normal gametogenesis and fertilization processes that result in ovulated fish eggs retaining two sets of maternal chromosomes until after fertilization, when meiosis is completed and one set of maternal chromosomes (polar body) is ejected from the egg. If that step is blocked, the fertilized egg develops with two sets of maternal chromosomes and one set of paternal chromosomes, making the egg triploid. The application of hydrostatic pressure is currently the most common and reliable technology for triploid induction as it is much easier to ensure identical treatments in commercial applications.

Triploid Atlantic Salmon that are effectively induced are sterile in the true biological sense (i.e., inability to produce viable offspring). In triploid females, egg development generally stops early in gametogenesis, resulting in functional sterility and retention of endocrine profiles like juvenile fish. However, it has been estimated that 0.1% of female triploids will produce variable size eggs and that these eggs may be capable of being fertilized with sperm from diploid males, but this results in organisms that are aneuploid (i.e. have a chromosome number that is not an exact multiple of haploid) and therefore are likely to die in early development.

Triploid males are quite different. Spermatogenesis can progress through meiosis and these fish are capable of developing functional testes and expressing endocrine profiles identical to maturing diploid males, but they produce aneuploid sperm. These cells are functional and capable of fertilizing eggs, but the resulting embryos are themselves aneuploid, and are likely to die early in development.

There are no population-wide phenotypic effects of triploidy other than sterility, although triploid commercial culture characteristics are more variable than diploids and, if released to the wild, triploids are less likely to outcompete or displace native salmon given physiological and behavioural differences associated with triploidy. Some uncertainties do exist with respect to disease resistance and potential to become reservoirs for the spread of pathogens to wild populations.

Analysis and Response

The 2013 DFO Science peer review reported the potential effects of the use of domesticated European-origin salmon on wild Atlantic Salmon and their habitats in Newfoundland and Labrador (NL).The current analysis reviews the NL-ITC European Triploid Atlantic Salmon Risk Assessment, to provide scientific advice on the direct and indirect genetic, ecological and fish health risks to wild Atlantic Salmon from the proposed introduction of European triploid Atlantic Salmon to Placentia Bay, NL. The review is categorized into the four specific objectives outlined in the Terms of Reference for this Science Response Process.

Characterize the effectiveness of triploid induction in fish. Provide advice on triploidy monitoring measures including sampling protocols, sample sizes required, and statistical power, to ensure triploidy induction levels are acceptable (i.e., 100%).

The proposed use of sterile (triploid) European-origin fish in marine cages in Placentia Bay significantly reduces the number of reproductively viable fish that, if escaped, could have direct genetic consequences to wild Atlantic Salmon populations. Commercially produced triploid salmon eggs can have an induction success rate near 100% subject to standard operating procedures (SOPs) and monitoring protocols.

Demonstration of 100% triploidy induction is not practical given the requirement to test every individual and the destructive nature of verification methods at embryo-larval stages. The egg supplying facility produces triploid families by application of hydrostatic pressure to fertilized eggs from individual females and then samples to verify triploidy induction using flow cytometry. This is the most reliable approach and based on expected fecundity, egg batch size is expected to be approximately 8,000 eggs per female. **The SOPs for triploidy induction and verification should be submitted to permit rigorous assessment.** Confidence in asserted triploidy rates close to 100% will require testing of each family and with sample sizes greater than that currently proposed by the egg supplying facility.

The minimum sample size required for a given probability of finding at least one failed triploid at specific triploidy effectiveness rates can be calculated using the Poisson approximation to the binomial distribution based on:

$$n = -\frac{\ln(1-\gamma)}{p}$$

Where, γ = probability of finding at least one defect or triploid failure and

p = estimated proportion defective or failed triploids in the population.

Sample sizes are provided in Table 1. **Flow cytometry is currently the preferred method for confirmation as it yields unambiguous results.**

Table 1. Sample size required for given probability of finding at least one failed triploid per batch (~ 8,000 eggs) at different presumed triploidy effectiveness levels.

Presumed Triploid Effectiveness Rate	***p***	γ**=0.5**	γ**=0.9**	γ**=0.95**	γ**=0.98**	γ**=0.99**
98%	0.02	35	115	150	196	230
99%	0.01	69	230	300	391	461
99.9%	0.001	693	2303	2996	3912	4605

If an alternative sampling regime and sample size is suggested, it should be accompanied by a statistical explanation of the methods chosen.

It is important to consider what other products of triploid induction exist and their potential influences. One product of failed triploidy induction is aneuploid embryos, which likely die early in development. Therefore, their contribution to genetic or ecological risk would be low.

If, during triploid induction, the second maternal polar body is not retained, the result can equally be diploid females or males, since only one set of chromosomes from each parent is combined in the fertilized egg. It is expected that such diploid fish would have the same reproductive potential as diploid males and females produced without pressure shocking, as reported in the 2013 CSAS process (DFO 2013).

Incomplete triploid induction can also produce eggs with only maternal chromosomes (gynogens), as there has not been any paternal genetic contribution. These gynogens can develop as functional females and can be capable of reproduction; however, they have been reported as having lower survival (Pepper et al. 2004) and potentially lower reproductive potential (Piferrer et al. 1994). The risk assessment assumes, however, that they have the same reproductive potential as diploid females. While this does provide the worst-case scenario in the assessment of risk, it is very likely that such an outcome would result in overall reduced viability, but some individuals would be very viable as regular diploids.

Although triploid Atlantic Salmon are sterile in the true biological sense, some indirect genetic risk does exist by triploid males which have the capacity to produce aneuploid sperm, however the resultant offspring would also be aneuploid. The potential reduction in wild egg abundance removed through triploid male activation may be a significant factor. However, other influences (discussed later in the next section) will likely reduce this possibility.

Triploid females are almost invariably sterile, with a very small percentage able to produce eggs of varying size; however, resulting embryos die early and therefore present little risk to wild populations. Notwithstanding the low likelihood of escaped triploid salmon having contact with wild spawning salmon with resulting genetic consequences, the use of all-female triploid Atlantic Salmon would completely exclude males and thereby eliminate their potential to activate wild eggs, reducing the number available to their wild male counterparts. Consequently, the recommendation of the DFO 2013 process regarding **the use of all-female triploid Atlantic Salmon is reaffirmed.**

It is unlikely that the hydrostatic pressure methodology will consistently achieve 100% triploidy induction success. As such, a low number of diploids will be present in batches derived from this method. Therefore, the main issue is whether the number of diploids in an escaped population is high enough to interbreed with local populations and impact on fitness. Thus, examining escape data with triploidy success and local population size is a critical assessment to consider. Low numbers of diploids entering a large wild population will probably have minimal effect (the population may sort out maladaptive genotypes in the few hybrids in the next generation). However, introduction of a large number of escaped diploids into a small population, or even a few escaped diploids into a very small population, could extirpate the adaptive structure of that population very quickly.

Review and provide advice on the scientific basis for predictions on impacts associated with the potential for triploid escapes to occur and subsequent impact of sterile and potential non-sterile European strain salmon on wild Newfoundland Atlantic Salmon, including genetic (direct and indirect), ecological and fish health impacts.

Notwithstanding the level of triploidy induction, the proportion of reproductively viable escaped fish that would have the potential to have direct genetic consequences would be dependent on a number of variables, including distribution of farmed fish (number of sites and cages per site), mechanism of escape, volume of escape, time of year, location of site of escape, life stage, survivability, access and proximity to spawning habitat, access to spawning wild salmon, and survivability of offspring.

The use of triploid European- or North American-origin salmon considerably removes or reduces direct genetic impacts and was identified during the 2013 DFO CSAS process as a possible mitigation measure (Figure 4; Verspoor et al. 2015). Indirect genetic and ecological impacts would be further reduced by the use of all-female triploids. As triploid and European-origin salmon have not previously been used in the NL aquaculture industry, the ecological and indirect genetic risks relative to diploids are largely unknown.

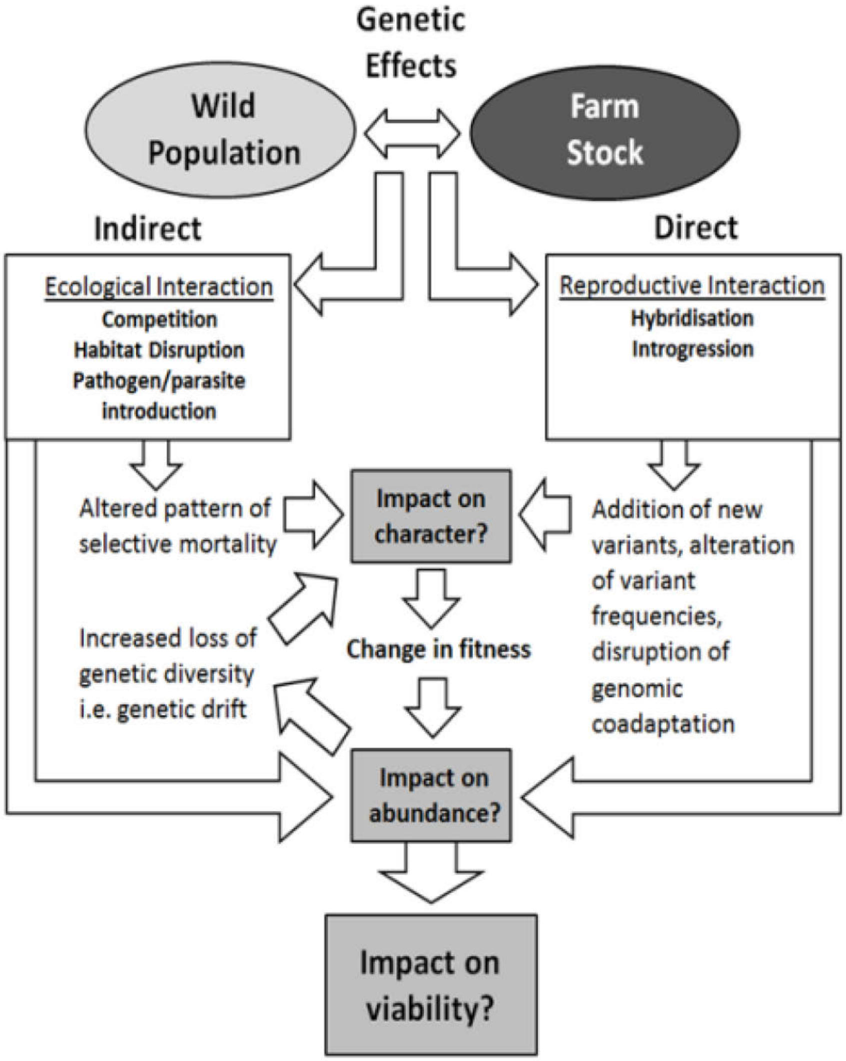

Figure 4. Pathways of direct and indirect effects (source: Verspoor et al. 2015).

Reproduction between triploid females and triploid males has never been observed in salmonids, therefore it is very unlikely that such an activity would impact natural spawning of wild salmon. Any reproduction between a diploid and a triploid salmon (male or female) would result in aneuploid offspring which would likely die early in development.

Indirect genetic impacts are reduced by several factors. For example, where triploids enter rivers, their distribution in the lower reaches is typical of immature diploid escapees (Glover et al. 2016). It is unlikely that triploids would attempt to participate in and therefore disrupt wild fish spawning.

The results of triploid-diploid escape genetic characterization (Glover et al. 2016) coupled with studies involving release of triploid and diploid smolts and adults (Cotter et al. 2000, Wilkins et al. 2001) suggest that commercial production of triploids will limit genetic introgression and significantly reduce the frequency of escapes entering rivers thereby reducing ecological interactions by limiting spawning competition and potential disease transmission.

Should the Aqualine Midgard system prove more effective than current containment practices, the incidence and rate of escape would also be expected to be reduced. However, this cage system is new to the provincial aquaculture industry and has not been evaluated in the NL environment, and therefore **sea trials with the Aqualine Midgard system are recommended prior to full-commercial operations.**

The Risk Assessment asserts that the number of escapes present in the environment is expected to be no greater than reported escapes already observed in the existing Atlantic Salmon aquaculture industry in the province. There are two types of escape incidents:

1. Infrequent acute releases of large numbers of fish usually associated with extreme weather events, equipment failures and/or human-error (detected and reported releases);
2. Frequent releases of small numbers of fish during farm operations (underreported or undetected releases).

The most significant reported escape incident in the provincial salmon aquaculture industry occurred in September 2013, which resulted in over 20,000 diploid salmon being released into the wild within six weeks of the normal spawning period for wild salmon. For the same amount of viable salmon to escape in the context of the current proposal, it is suggested that all 7 million salmon would have to escape. Modelling studies have shown that frequent small-scale losses may be more problematic than sporadic large-scale escapement events since they have the potential to reduce fitness of wild populations over several generations (Baskett et al. 2013). It is not known how many salmon have migrated into rivers in Fortune Bay, however, new evidence shows successful spawning among escaped farmed salmon, and successful hybridization of escaped farmed salmon with wild fish in a number of Fortune Bay rivers (Ian Bradbury DFO, pers. comm.).

Farm-to-salmon river separation distance criteria of 20-30 km have at times been proposed as a measure to reduce wild-farmed salmon interactions. According to the risk assessment, 8 of the 20 salmon rivers managed for salmon production in Placentia Bay, are within the criteria, and one river (Bay de L’Eau) has good spawning and rearing habitat near the river mouth which would increase the likelihood of wild-farmed salmon interactions in the event of escape incidents from farms.

In 2010, the Southern Newfoundland Designatable Unit (DU) salmon population was designated as ‘Threatened’ by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) based on three threats: fisheries, aquaculture, and changes in the marine environment resulting in lower marine survival. In 2015 an updated review was carried out on the status of salmon populations along Southern Newfoundland (DU4). For Salmon Fishing Areas (SFAs) 11 and 12, abundance of salmon declined by more than 30% during the previous three decades. Results from several individual rivers show declines of over 70% at Conne River, and at Little River returns have fallen by more than 80% since 1996. Northeast River Placentia in Placentia Bay (SFA 10), although not monitored from 2003-14, showed 21% decline in small salmon returns and 9% decline in large salmon when compared to the 1992-2002 mean (DFO 2016). Reasons for these declines are not clear.

According to the risk assessment, experience in monitoring of escaped diploid Atlantic Salmon in NL waters reinforces a low risk of ecological impacts, due to the lack of evidence of feeding in freshwater systems, and previous reports suggesting unlikely competition in the marine environment between wild and farmed salmon (DFO 2013). **Any releases of triploid Atlantic Salmon should be followed with a mandatory monitoring program** to provide empirical data to test the predictions made in Benfey (2015).

With the possible exception of sea lice (*Lepeophtheirus salmonis*), there are few reliable data sets on the distribution of fish pathogens in wild populations, and the knowledge of interactions with wild pathogen reservoirs is limited. Regardless, the farmed fish would be regularly monitored by provincial aquaculture veterinarians through disease and pathogen screening, once marine sites are established.

Triploids will reduce the likelihood of direct genetic impacts, and may reduce but not eliminate interactions with wild salmon in freshwater, but according to the review by Thorstad et al. (2008) their use may have little if any effect on reducing the transmission of disease or parasites and these authors recommended that studies designed to examine ecological interactions be carried out before undertaking large scale use of triploids.

It is known that farmed salmon can get diseases from wild fish, triploids can be more susceptible than diploids, and disease outbreaks can occur on farms that could potentially impact wild fish. Pathogens, especially from more susceptible triploids, can spread to surrounding waters where wild fish can be exposed and become infected. While it is difficult to accurately predict susceptibility of triploid European salmon to endemic pathogens in NL environmental conditions, the use of appropriate husbandry, nutrition, surveillance, vaccination and treatment, coupled with the avoidance of sites with suboptimal conditions, would contribute to minimizing risks to wild salmon populations. The ultimate impact on wild fish populations, however, remains unknown. **More research on ecological interactions is still warranted regardless of whether farmed salmon are triploids or diploids.** Research specific to European strains would also be beneficial, especially regarding the susceptibility to endemic pathogens. At a minimum, the Proponent should ensure that these studies are carried out concurrently with the proposed phased production plan for this undertaking, to obtain at least preliminary results, prior to full scale operations.

The scientific advice may also propose mitigation measures that may further reduce the risk of impacts from the introduction of European strain triploid Atlantic Salmon into NL waters.

Should this proposal be approved, the following mitigation measures were identified to further reduce the risk of impacts from the introduction of European strain triploid Atlantic Salmon into NL waters:

1. **Production of all-female triploids instead of mixed sex triploids is strongly recommended** as it eliminates triploid males and diploid males from farm populations, thereby eliminating direct and indirect genetic and ecological risks associated with expected reproductive behaviours of triploid or diploid male farm escapes.
2. The proponent proposes to use a new-to-NL marine cage system called the Aqualine Midgard system which has been designed against a Norwegian technical standard that has been viewed as effective at reducing escape incident rates in other jurisdictions. **Consideration should be given to mechanisms to apply and evaluate the Norwegian technical standard to installations of the Aqualine Midgard systems in the province prior to stocking, with a view to confirming the integrity of this system in the NL environment.** The Norwegian standard requires certification of cage systems design and installation with the site-specific environmental conditions of the farm site.
3. It is understood that containment measures of the “Code of Containment” are mandatory and applied as conditions of licence in federal and provincial aquaculture-related licences. It is recommended that the **measures in the Code of Containment be reviewed against saltwater operations containment measures identified in the DFO 2013 process**

(Bridger et al. 2015) and identified gaps adequately addressed to reduce risks of escapement due to structural and operational failures. This may include re-examination of surface collars, net standards, moorings, inventory handling practices, inspection and maintenance, record keeping, staff training, and recapture methods.

4. Given the uncertainty regarding dispersal and survival of farm escapes, **application of DNA methods to enable genetic identification of individual families and forensic investigation of escapes back to the farm of origin** is recommended.
5. The Province has a continuous aquatic animal health surveillance program for hatcheries producing smolts for marine cage aquaculture that requires regular fish health testing. **Regular Fish health sampling should be maintained through all life history stages prior to authorization of entry to sea cages. Additionally, confirmatory triploid validation testing should be undertaken prior to authorization of entry to sea cages**.
6. Standard Operating Procedures (SOPs) are considered essential to maintaining triploidy induction success rates as close to 100% as possible. **SOPs for triploidy induction and verification from the egg supplying facility should be submitted to the Minister of DFO** as part of the information required in applications for transfer licences so that they may reviewed as part of the application review process.
7. The proposed project will be developed over a five year planning period. This would enable **scientific studies to be initiated either before or concurrent with the phased production plan** to obtain preliminary results prior to full-scale commercial operations. It is recommended that the Proponent ensure that scientific investigations of triploid performance and triploid-wild salmon interactions be conducted prior to commencement of commercial operations.
8. Consideration should be given to **collecting appropriate performance-based measures of health, survival and biological performance to facilitate evaluation of triploid performance in NL conditions**. Results of this performance-based monitoring could then be used to assist in decision-making re: import of additional eggs and/or stocking additional fish and/or additional sites. This would require establishment of agreed upon performance standards/thresholds.

To advise on any new significant information that might affect the potential for genetic and ecological effects on wild populations and that might need to be added to the existing risk assessment document.

The egg supplying facility has indicated it pressure shocks eggs from each female separately, and it would sample 10 eggs from each of these “batches” to verify triploidy. The average number of eggs per batch is 8,000, resulting in an approximate sample percentage of 0.125%. Confidence in effective triploidy induction close to 100% will require further analysis to determine appropriate sample size and statistical power required to ensure acceptable triploid induction levels. **The current level of sampling to determine success of triploid induction is insufficient** (i.e., 10 eggs/ batch).

The application of hydrostatic pressure is the most common technology for triploid induction as it is much easier to ensure identical treatments in commercial applications. Other technologies exist, but are not commercially feasible at this time. **As new technologies and methods to verify triploidy induction become available, current protocols should be revisited and revised accordingly.**

Nutrition research, the development of triploid specific commercial rations, and improvements in husbandry and genetics have augmented triploid growth rates and decreased incidences of cataracts and skeletal deformities in post-smolts (Benfey 2015). **The NL commercial aquaculture operations should remain up-to-date in these areas**.

Sources of Uncertainty

The following sources of uncertainty specific to the project and NL environment have been identified:

- Although all-female triploids are preferred, it is uncertain whether the applicant plans to use all-female or mixed-sex triploid eggs.
- Specific to this proposal, confidence in effective triploidy rates close to 100% will require consultation with the egg-supplying facility and further analysis to determine appropriate sample size and statistical power required to ensure acceptable triploid induction levels. **Additionally, triploidy rates should be re-assessed prior to fish being transferred to sea cages**.
- Performance of the new proposed cage systems in southern Newfoundland is unknown, and though it is expected to perform as well if not better than existing systems, no data exists on its performance.
- Status of wild populations in Placentia Bay is uncertain as there is only one assessment facility and existing baseline data are limited. Risk is elevated for small depressed populations.
- Given reduced tolerance to sublethal stressors in triploids and the European-origin of the farmed strain, the effect of any differences in endemic pathogen susceptibility is unknown.
- The relative performance of European-origin strains versus the existing available North American-origin strains (i.e., Saint John, Gaspé, and Penobscot) in aquaculture in the NL environment is unknown and should be evaluated in view of the likely cold environment fish would experience.

While there have been research and pilot-scale studies of triploid Atlantic Salmon, commercial culture of triploid salmon has been limited. Consequently, relative to diploids, there are knowledge gaps around direct and indirect genetic and ecological interactions between triploids and wild Atlantic Salmon populations. In particular, the following sources of uncertainty have been identified:

- There is uncertainty around fitness differences between farm, wild and farm-wild hybrids in the wild; the extent of competitive interactions between farm and wild fish in the wild; their effect on the survival of wild fish; and, the impact of local population demographics on interaction outcomes.
- The cumulative effects of chronic, low-level escapes are unknown and difficult to assess because low-level escapes are difficult to identify and monitor at individual sites, but modelling studies have shown that low-level escapes can be more problematic than single, large-scale losses.
- There is uncertainty regarding the fate of escaped farm-origin fish in the marine and freshwater environments, including post-escape dispersal patterns, survival, feeding, and their movements into wild salmon rivers, timing of maturation and maturation success**. It is recommended that the Proponent ensure baseline studies to characterize the genetic**

structure of existing salmon populations in Placentia Bay are carried out prior to commercial production.

- Potential reproductive success of wild salmon and diploid European-origin farm escapes (i.e. “failed” triploids) and the strength of selection against hybrids is unknown. This will determine the demographic cost to hybridization and the time for farm-origin alleles to be purged from wild populations.

Conclusions

Consistent with current literature and the 2013 DFO CSAS process, the commercial production of all-female triploid (sterile) salmon is an effective means of significantly reducing direct genetic impacts to wild Atlantic Salmon populations, but it is uncertain as to whether it would reduce the indirect genetic, ecological and/or fish health impacts. The use of mixed-sex triploids does not reduce the indirect genetic or ecological risks to wild Atlantic Salmon populations to the same extent as all-female triploids. The use of all-female triploid Atlantic Salmon would completely remove triploid and diploid males from the farm population and eliminate genetic and ecological risks associated with reproductive behaviours of escaped farm-origin males, but other ecological impacts could still exist. Any triploid Atlantic Salmon releases should be followed up with a monitoring program to provide empirical data to test the predictions made in Benfey (2015), originating from the 2013 DFO CSAS process.

It is unlikely that current methods of triploid induction will achieve 100% success (i.e., no diploids) in all families. The current level of sampling by the egg-supplying facility to determine success of triploid induction is insufficient. An alternative sampling protocol with appropriate sample sizes and levels of significance need to be established to confirm the acceptable triploid induction level, with possible follow-up sampling prior to fish being transferred from the hatchery to sea cages. The sample sizes required to detect diploids in presumed triploid families at 98-99.9% triploidy induction efficacies was a product of this CSAS process (see Table 1).

To reduce risks of escapement due to structural and operational failures, “Code of Containment” measures should continue to apply and be reviewed against saltwater operations containment measures identified in the DFO 2013 process (Bridger et al. 2015). Should the Aqualine Midgard system be demonstrated to be more effective than current containment practices, the incidence and rate of escape would also be expected to be reduced. However, this cage system is new to the provincial aquaculture industry and has not been evaluated in the NL environment, and therefore sea trials are recommended prior to full-scale operations.

The ITC risk assessment is thorough to the extent possible, based on the information currently available; however, uncertainty and knowledge gaps remain relating to the impacts of European-origin triploid Atlantic Salmon on wild NL Atlantic Salmon. The current advisory process identified several recommendations and mitigations to minimize ecological and genetic consequences of wild-farmed salmon interactions. Scientific studies are recommended to address these unknowns.

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June 9, 2016

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This Report is Available from the

Center for Science Advice (CSA)
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E-Mail: DFONLCentreforScienceAdvice@dfo-mpo.gc.ca
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ISSN 1919-3769

Correct Citation for this Publication:

DFO. 2016. Proposed Use of European-Strain Triploid Atlantic Salmon in Marine Cage Aquaculture in Placentia Bay, NL. DFO Can. Sci. Advis. Sec. Sci. Resp. 2016/034.

Aussi disponible en français :

MPO. 2016. Utilisation proposée de saumons de l’Atlantique triploïdes de souche européenne des cages d’aquaculture en milieu marin dans la baie Placentia (T.-N.-L.). Secr. can. de consult. sci. du MPO, Rép. des Sci. 2016/034.