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Biocontainment measures to reduce/mitigate potential post-escape interactions between cultured European-origin and wild native Atlantic salmon in Newfoundland

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Foreword

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ABSTRACT

This paper reviews the literature and makes recommendations on biocontainment measures to reduce or mitigate potential post-escape interactions between farmed European-origin and wild native Atlantic salmon in the south coast of Newfoundland. In the absence of effective measures to prevent the escape of farmed salmon or to recapture them post-escape, the only effective method to minimize their impacts on wild populations is to ensure that farmed populations are comprised solely of sterile fish through the use of all-female triploids. The technology for producing all-female triploid populations is simple and easily applied on a commercial scale, and routinely results in populations that are entirely female and more than 98% triploid. Aside from sterility, there are no population-wide phenotypic effects of triploidy, although triploids do tend to perform less well than diploids with respect to commercial culture characteristics and, if released to the wild, are not likely to outcompete or displace native salmon. Some uncertainties do exist with respect to their disease resistance and their potential to become reservoirs for the spread of pathogens to wild populations. If the spawning potential of escaped European-origin Atlantic salmon is deemed to pose an unacceptable risk to native Atlantic salmon populations in the south coast of Newfoundland, then all-female triploid populations could be used to reduce risk. Research should continue to focus on improvement of triploid performance through breeding programs and optimization of husbandry, including nutrition, rearing environment, and fish health.

Mesures de bioconfinement visant à réduire ou à atténuer les interactions possibles après évasion entre les saumons de l'Atlantique d'élevage d'origine européenne et les saumons de l'Atlantique sauvages indigènes à Terre-Neuve-et-Labrador

RÉSUMÉ

Ce document analyse les ouvrages publiés et contient des recommandations sur les mesures de bioconfinement visant à réduire ou à atténuer les interactions possibles après évasion entre les saumons de l'Atlantique d'élevage d'origine européenne et les saumons de l'Atlantique sauvages indigènes sur la côte Sud de Terre-Neuve-et-Labrador. En l'absence de mesures efficaces pour prévenir l'évasion des saumons d'élevage ou pour les recapturer après l'évasion, la seule méthode efficace permettant de minimiser les répercussions sur les populations sauvages est de veiller à ce que les populations d'élevage comprennent uniquement des poissons stériles en utilisant des individus triploïdes entièrement femelles. La technologie utilisée pour produire des populations triploïdes entièrement femelles est simple, s'applique facilement à l'échelle commerciale et produit régulièrement des populations entièrement femelles dont 98 % sont triploïdes. Mis à part la stérilité, la triploïdie n'entraîne aucun effet phénotype à l'échelle de la population, bien que les individus triploïdes aient tendance à présenter des caractéristiques moins avantageuses que les diploïdes pour la culture commerciale et que s'ils sont relâchés dans la nature, il est peu probable qu'ils fassent concurrence aux saumons indigènes ou les chassent. Il existe certaines incertitudes concernant leur résistance à la maladie et la possibilité qu'ils deviennent des réservoirs propageant des agents pathogènes dans les populations sauvages. Si l'on juge que le potentiel de frai des saumons de l'Atlantique d'origine européenne qui se sont évadés représente un risque inacceptable pour les populations de saumon de l’Atlantique indigènes sur la côte Sud de Terre-Neuve-et-Labrador, on pourrait alors utiliser des populations triploïdes entièrement femelles pour réduire le risque. Les recherches devraient continuer de mettre l'accent sur l'amélioration du rendement des individus triploïdes grâce à des programmes d'élevage et à l'optimisation de l'élevage, notamment de la nutrition, de l'environnement d'élevage et de la santé des poissons.

INTRODUCTION

The objective of this paper is to review the literature pertaining to biocontainment measures used to reduce or mitigate potential post-escape interactions between farmed European-origin and wild native Atlantic salmon (*Salmo salar*) in the south coast of Newfoundland. More specifically, it aims to provide managers with concise, peer-reviewed, and relevant science advice with which to address two questions: (1) are there biocontainment measures that could operate subsequent to an escape event to reduce the likelihood of interaction between the escaped and wild native salmon; and (2) how could these mitigation measures result in possible reductions in genetic, phenotypic, and/or ecological risks to wild populations? Before addressing these questions in detail, a few underlying assumptions will be outlined within the context of using non-native stocks of Atlantic salmon for aquaculture in Newfoundland, where it is a native species.

Assumption 1 – some Atlantic salmon will inevitably escape from farms. Although it should go without saying, any consideration of post-escape biocontainment should always serve as a back-up to the primary goal of effective physical containment within farms. If it could be guaranteed that Atlantic salmon are farmed in such a way as to make it impossible for them to escape, then this discussion would not be necessary. From the perspectives of both environmental protection and good farming practice, it is clearly advantageous to prevent the escape of farmed fish. However, as currently practiced, Atlantic salmon farming can realistically be expected to lead to *some* degree of escapes.

Assumption 2 – not all escaped Atlantic salmon can be recovered. Given that: (1) Atlantic salmon are highly mobile and adaptable; (2) Newfoundland provides a good natural habitat for the survival of escaped Atlantic salmon; and (3) farmed Atlantic salmon are not sufficiently domesticated to prevent them from surviving and potentially even thriving in the wild (e.g., Jensen *et al.* 2013, Skilbrei 2013), it is not realistic to expect that attempts to recover or eradicate escaped farmed Atlantic salmon (e.g., by angling, netting, attraction to traps, poisoning, etc.) would be fully effective. Not only would this be a difficult undertaking, it could not likely be done without some impact on wild native Atlantic salmon populations or other species. Although this does not preclude immediate attempts to recover escaped Atlantic salmon in the event of a major release (e.g., Chittenden *et al.* 2001), it cannot realistically be expected that every escaped farmed Atlantic salmon can be recaptured or killed within a short time of its escape.

Assumption 3 – Atlantic salmon cannot be bred for failure in nature and still be used for farming. Although it is hypothetically possible to design Atlantic salmon breeding programs specifically for failure to survive outside of the farm environment, or to achieve the same through genetic modification, this has not been demonstrated for Atlantic salmon. Not only would it take considerable time to accomplish, it is not likely to yield fish well suited for commercial farming and, in the case of genetically modified fish, also may not be desired by consumers.

Given these three assumptions, the principal focus of this paper is therefore on assessing reproductive sterility as a management tool for reducing the genetic, phenotypic, and ecological risks of escaped farmed Atlantic salmon to wild native Atlantic salmon populations. Attention will also be paid to assessing the suitability of reproductively sterile (specifically triploid) Atlantic salmon for commercial production (i.e., would their use affect the biological and economic management of Atlantic salmon farming in Newfoundland?).

OPTIONS FOR ENSURING REPRODUCTIVE STERILITY OF FARMED ATLANTIC SALMON

Detailed reviews of the various approaches that have been assessed or proposed for producing reproductively sterile populations of fish are available elsewhere (e.g., Devlin and Donaldson 1992; Maclean and Laight 2000; Wong and Van Eenennaam 2008; Benfey 2009). Induced triploidy is the only one of these that is currently feasible for use in commercial Atlantic salmon aquaculture and is the focus of this paper. Other methods will be briefly outlined here.

Three approaches that can be effective – but are not suitable for Atlantic salmon aquaculture – are surgical removal of the gonads, exposure to high energy radiation, and androgen treatment. Surgery is too slow, labour-intensive, and invasive to be used on a commercial scale, and the equipment needed for treating with high energy radiation is too expensive, cumbersome, and potentially dangerous to be considered. Furthermore, although permanent sterilization is possible with both surgery and irradiation, if no gonadal tissue remains after treatment, gonadal regeneration is likely with both these approaches. Androgen treatment during the period of gonadal differentiation can be highly effective for sterilization, and is both inexpensive and easily applied on a commercial scale. It has been used to produce sterile individuals and populations of several species of salmonids (Devlin and Nagahama 2002), including Atlantic salmon (Johnstone and MacLachlan 1994). The problem with this approach is that production fish are treated with a controlled substance for which there is currently no federal approval if the fish are destined for human consumption. And even if it were approved, industry is not likely to attempt marketing steroid-treated fish for risk of consumer backlash.

An alternative approach for producing reproductively sterile populations of fish is to interfere with the hypothalamic–pituitary–gonadal axis which regulates gonadal development. This is currently an active field of research, and includes techniques as varied as laser ablation of specific neurons (e.g., Abraham *et al.* 2010), vaccination against specific cell types or molecules (e.g., Sambroni *et al.* 2009), and genetic manipulations (e.g., Wong and Van Eenennaam 2008, Thresher *et al.* 2009; Hu *et al.* 2010; Xu *et al.* 2011). A common goal of all these approaches is the development of methods that are both 100% effective (i.e., no fertile animals) and also reversible. Advances in these areas should be followed, but none are likely to yield applicable results within a timeframe meaningful to this CSAS evaluation.

This leaves induced triploidy as the only approach for consideration in the current context, as a technique that is easily applied on a commercial scale, and which already has a long history of use in aquaculture and fisheries management. Successful production of triploid Atlantic salmon populations was first reported thirty years ago (Benfey and Sutterlin 1984d; Johnstone 1985) and they have been the subject of extensive basic and applied research. Furthermore, triploid Rainbow trout (*Oncorhynchus mykiss*) and Brook charr (*Salvelinus fontinalis*) are already used extensively across Canada and in other countries for aquaculture and in stocking programs for sport fishing (e.g., Kozfkay *et al.* 2006).

TRIPLOIDY AS A MANAGEMENT TOOL TO ENSURE REPRODUCTIVE STERILITY OF ATLANTIC SALMON

Effectiveness of triploidy induction methods

Triploidy refers to the condition of having three complete sets of chromosomes in the genome. Most vertebrate animals have diploid genomes (i.e., with two sets of chromosomes: one inherited from the mother and the other from the father). Triploidy can be induced in fish in three ways: (1) by duplication of the paternal genome; (2) by duplication of the maternal genome; or (3) by crossing tetraploids with diploids (Benfey 2009). Triploidy induction by duplication of the paternal genome has not been reported for Atlantic salmon, but has been achieved by fusion of sperm cells prior to fertilization in other species. Given the ease with which triploidy can be induced by duplication of the maternal genome in Atlantic salmon (Table 1), there is probably no merit to pursuing methods for duplication of the paternal genome for this purpose and it is not discussed any further in this paper.

Duplication of the maternal genome is achieved by blocking completion of the second meiotic division shortly after fertilization. When eggs are ovulated, they have not yet completed the process of meiosis, whereby the diploid chromosome complement is reduced to haploid (one set). This final step of meiosis is initiated when eggs are fertilized, and the fertilized egg therefore contains three sets of chromosomes for a short period of time after fertilization: one paternal set from the fertilizing sperm cell and two maternal sets in the egg. One maternal set then normally leaves the egg (as the 'second polar body'), but this can be blocked by various means. The first attempts to retain the second polar body in Atlantic salmon used exposure to low temperature and were largely unsuccessful (Svärdson 1945; Lincoln *et al.* 1974). Apparent successes with using cytochalasin B, a potent inhibitor of cell division, to produce triploid and mosaic polyploid Atlantic salmon (Refstie *et al.* 1977; Allen and Stanley 1979) were shown to have been ineffective when reassessed using more reliable methods for ploidy confirmation (Allen 1983; Bolla and Refstie 1985). Various anaesthetics applied under pressure have been used to induce triploidy successfully in Atlantic salmon (Johnstone *et al.* 1989), but have not been pursued for commercial application because of the proven efficacy of simple physical treatments such as heat or hydrostatic pressure for triploidy induction (Benfey and Sutterlin 1984d; Johnstone 1985; Johnstone *et al.* 1991). Both heat and pressure treatments have been used extensively for triploidy induction in Atlantic salmon (Table 1), with pressure generally preferred for two reasons:

1. it is easier to ensure that all eggs are exposed to the identical treatment in a sealed pressure vessel than it is to ensure uniform heat treatment of eggs when done on a commercial scale; and

2. the optimum heat treatment for triploidy induction depends upon pre-treatment incubation temperature, whereas the optimum pressure treatment is independent of temperature.

The equipment for pressure treating eggs is simple and is manufactured in Canada[1]. The successful production of triploids using such equipment requires optimizing a number of biotic and abiotic factors. Firstly, the treatment must begin at the correct developmental stage. Given that developmental rates are temperature dependent in fish, this means that the time after

[1] TRC hydraulics inc.

fertilization at which treatment begins must be standardized according to temperature, which is done by measuring the time interval prior to treatment in acquired thermal units (i.e., the product of temperature and time, expressed in °C-minutes). Secondly, the eggs must be held at the appropriate pressure for the appropriate length of time. There is no single 'correct' treatment in this regard; higher pressures require shorter durations than lower pressures, and there is a temporal window of opportunity for successful triploidy induction. That said, the industry standard for producing triploid Atlantic salmon is a five-minute treatment at 65,500 kPa (9,500 psi), beginning 300 °C-minutes after fertilization. Family effects represent a third factor that can influence triploidy induction success, due to true genetic effects or differences in 'egg quality' that result from variable duration of the post-ovulatory period prior to pressure treatment (Devlin *et al.* 2010).

There are two large data sets for assessing triploidy induction success in Atlantic salmon using this treatment: O'Flynn *et al.* (1997) confirmed 100% triploidy in each of 10 randomly selected individuals taken from 86 separate families spanning 5 year classes of fish, where each family represented a separate pressure-treated group of eggs, and AquaBounty Technologies Inc. demonstrated an average 99.5% triploidy induction success (range 98.9-100%) in 20 separate pressure-treated groups, representing four replicates of each of five crosses, and testing 350 individuals per replicate (Anon. 2012). The largest published data set for triploidy induction success in a salmonid species is that of Devlin *et al.* (2010) for Coho salmon (*Oncorhynchus kisutch*), who demonstrated an average 99.8% success (range 98.6-100%) when testing a minimum of 195 (and up to 1632) fish from each of 17 crosses, for a total of 15,814 individuals.

All three of the above studies were conducted by groups with extensive prior experience in the design and use of pressure systems custom-built for the production of triploid salmonids. Producers with less experience may not initially obtain such high success rates. Devlin *et al.* (2010) showed that the use of sub-optimal pressure treatments yielded a lower triploidy success rate (97.6%) which was further lowered by combining sub-optimal treatments with the use of over-ripe eggs (97.0%). And in any case, it cannot be assured that all-triploid groups will always result from even the best standard operating procedures. However, it does appear that the production of populations comprised of >98% triploids is achievable on a commercial scale. Interestingly, Devlin *et al.* (2010) found that 'failed' triploids consistently had a doubled maternal genome, as expected in triploids, but lacked most or all of the paternal genome, i.e., they were a mix of gynogenetic diploids and hyperdiploids. Gynogenetic diploids possess the correct number of chromosomes as found in normal fertile individuals, but their chromosomes are entirely of maternal origin. In Atlantic salmon, such fish develop as functional females, although the lack of the paternal genome means they are more likely to be inbred and therefore less fit in nature. Animals that lack a balanced chromosome complement, such as hyperdiploids, typically die early in development.

As an alternative to direct triploidy induction, the maintenance of tetraploid broodstock (i.e., derived from tetraploid x tetraploid crosses) and their use to produce triploids by crossing tetraploids of one sex with diploids of the other has tremendous appeal for two reasons: (1) it eliminates the need for pressure treating eggs every generation; and (2) it should theoretically yield all-triploid populations (Benfey 2009). In spite of some limited success in using this approach for Rainbow trout (Chourrout *et al.* 1986; Weber and Hostuttler 2012), there has been no published success with Atlantic salmon. However, this is a current area of research and success may be forthcoming (Runighan *et al.* 2012).

Requirement for, and effectiveness of, screening methods to confirm triploidy induction success

To date, large-scale production of triploid Atlantic salmon has been done using treatments that cannot be guaranteed to generate all-triploid populations (see: Effectiveness of triploidy induction methods), and it is therefore necessary to screen populations to confirm triploidy induction success. If the term 'batch' is used to define all the eggs that go into the pressure vessel at one time for triploidy induction, then this becomes an appropriate level of assessment (i.e., sampling a sufficient number of randomly selected eggs from any given batch should accurately predict triploidy induction success for that batch). If more than one family is represented within a batch, it is also easy to screen at the family level by using containers to separate families within the pressure vessel. The number of individuals that need to be screened from each batch depends on the degree of confidence needed for an accurate prediction of triploidy induction success. Mass screening of large numbers of individuals is possible, as demonstrated by the US Fish and Wildlife Service's certification program for stocking triploid grass carp (*Ctenopharyngodon idella*) for aquatic weed control (Zajicek *et al.* 2011). In this case, 120 randomly selected individuals from a population of no more than 6,000 fish must all be confirmed as triploid before that population is released. This sample size is based on the epidemiological principle that failure to find any 'infected' animals (i.e., diploids) in a sample of 120 individuals selected at random for a population of 6,000 individuals provides 95% confidence that the incidence rate is ≤ 2.5%. If needed, such a certification program could be developed for assessing triploidy induction success for Atlantic salmon stocks. In addition to establishing an appropriate confidence level for estimating triploidy induction success rate, it is also important to establish what the minimum acceptable triploidy induction success should be (i.e., whether it needs to be 100% or can be something lower).

Although there are many ways by which to determine ploidy in fish, the most practical ones (i.e., methods that are simple, rapid, and unambiguous) rely on measuring erythrocyte DNA content (Thorgaard *et al.* 1982; Allen 1983) or erythrocyte dimensions (Benfey *et al.* 1984) because triploid cell nuclei contain 50% more DNA than diploid nuclei and are therefore significantly larger (although not by 50%). The specific advantage of using erythrocytes is that they exist as single cells in suspension, making it easy to run them through automated particle sizing machines or flow cytometers. It is also possible to use flow cytometry to determine ploidy level at the embryo stage, making early screening of individual egg batches an option. With respect to using triploidy for producing sterile fish, a critical assumption is that fish with triploid erythrocytes also have triploid pre-meiotic germ cells (i.e., oogonia and spermatogonia). Although uncommon, it is possible for fish to contain both diploid and polyploid cells (i.e., 'mosaics'; see: Genomic stability of triploidy). Of specific concern in this regard would be fish that have triploid erythrocytes but diploid pre-meiotic germ cells, in which case they would be fertile in spite of being identified as triploids. However, given the rarity with which mosaicism occurs; the likelihood of this scenario is very small.

Requirement for, and effectiveness of, methods for producing all-female populations of triploids

Examination of gonadal development in triploids of numerous species of fish (reviewed by Benfey 1999; Maxime 2008; Piferrer *et al.* 2009; Fraser *et al.* 2012a) highlights the importance of using all-female populations if the intention is to fully suppress all aspects of sexual maturation. This is because testes in triploid males typically develop to the point of being fully functional as endocrine organs, producing sex steroids at levels no different from diploid males. As a result,

triploid males are phenotypically no different from diploid males with respect to characteristics such as external appearance at the normal age/size at maturity (i.e., secondary sexual characteristics), spawning behaviour, and mortality. Although not tested for Atlantic salmon, there is abundant evidence from other species (e.g., Benfey *et al.* 1986; Kitamura *et al.* 1991; Feindel *et al.* 2010) that triploid males can: (1) produce sperm capable of fertilizing eggs; and (2) compete effectively with diploids for access to spawning females. However, these spermatozoa are aneuploid, with a chromosome number intermediate between haploid and diploid, resulting in the production of aneuploid embryos that die early in development. This inability to produce viable offspring means that triploid males truly are sterile in a biological sense, but their use should be avoided for Atlantic salmon farming in Newfoundland. Not only are they of no advantage to fish farmers, if they do escape then they could mate with wild females, resulting in the loss of any eggs that they fertilize. Under other circumstances, the use of triploid males can be advocated as a 'feral-sterile' approach for managing invasive species (Thresher *et al.* 2014), but the use of all-female populations of triploids that do not participate in spawning would be the better approach for managing Atlantic salmon escapes in the Newfoundland context.

The production of all-female populations is relatively simple and effective in species that have a female homogametic sex determination system (equivalent to the mammalian XX-female/XY-male system), as is the case for all salmonid fishes (Devlin and Nagahama 2002). Genetic females in such species can be treated with androgens or aromatase inhibitors to allow them to develop as functional males, and these 'neomales' yield all-female offspring when crossed with normal females (Benfey 2009). Although there is only limited literature on the production of neomales in Atlantic salmon (Johnstone and MacLachlan 1994; Lee *et al.* 2004), the effectiveness of this approach for yielding all-female populations has been confirmed (Lee *et al.* 2004; Anon. 2012) and is greatly facilitated by the recent demonstration that the sex-specific marker for Rainbow trout (Yano *et al.* 2012) works equally well for salmonids of other genera, including Atlantic salmon (Yano *et al.* 2013).

Commercial-scale production of all-female triploid populations

The commercial-scale production of all-female triploid populations of Atlantic salmon requires combining the technologies described above. Neomales must be the only fish used to sire families. Until recently, the unambiguous identification of neomales was difficult and required examination of dissected testes for characteristic abnormalities (constrictions, diminished or absent sperm ducts and, ideally, the presence of some ovarian tissue with visible oocytes). The development of a reliable sex-specific marker (see: Requirement for, and effectiveness of, methods for producing all-female populations of triploids) now eliminates any risk of misidentifying neomales. Because neomales often are not 'strippable' (i.e., they do not produce free-flowing milt), their testes must be surgically removed, cut into small pieces and screened through fine mesh to obtain milt, which characteristically is very thick and should be diluted in an artificial extender prior to fertilization. Although more labour-intensive than traditional 'stripping', it is easy to do and gives high fertilization success. And, given that treatments have been optimized to produce strippable neomales in other salmonids species, it should be possible to optimize treatments to achieve this with Atlantic salmon as well. The production of all-female triploid populations then requires pressure treatment of eggs (see: Effectiveness of triploidy induction methods) following fertilization with milt from confirmed neomales.

Reproductive potential of triploid females

Given the underlying premise for their use for biocontainment, it is critical that triploid female Atlantic salmon not be able to reproduce. Triploid males routinely reach spermiation and are able to produce functional (but aneuploid) spermatozoa (see: Requirement for, and effectiveness of, methods for producing all-female populations of triploids) but triploid females generally do not reach ovulation. This is because most triploid oogonia fail to proceed to the oocyte stage and, as a result, there are very few (if any) ovarian follicles that develop to a stage of functional steroid biosynthesis. Triploid females therefore retain the endocrine profiles of early juvenile fish and, as a consequence, do not produce sufficient vitellogenin for oocytes to develop to a stage necessary for the production of viable eggs. And any eggs that do complete vitellogenesis will not be released due to the lack of endocrine signalling for ovulation.

The above typical scenario notwithstanding, there have been a number of reports of triploid females occasionally ovulating mature eggs. It should be recognized that although this is rare, it is a real event that requires assessment within the context of the use of female triploid Atlantic salmon for biocontainment. Johnstone *et al.* (1991) estimated that one individual in a thousand female triploid Atlantic salmon (0.1% of the population) ovulates eggs. However, these eggs are variable in size and, when fertilized with sperm from diploid males, yield aneuploid embryos that die early in development. The same observation has been made for female triploid Brook charr (Benfey 1996). Thus, it appears that both triploid males and females are capable of producing post-meiotic germ cells, but that these cells are aneuploid. The number of germ cells that progress through meiosis is greatly reduced in both sexes compared to diploids, and in triploid females their subsequent progression through to maturity (as ovulated oocytes) is further hindered by the lack of an appropriate endocrine environment. It is this last point that explains why triploid males are more likely to reach spermiation than triploid females are to reach ovulation.

From the perspective of using female triploids for minimizing the impacts of escaped farmed Atlantic salmon on wild populations, this means that potentially one fish in a thousand will spawn with wild males, removing those males from the effective spawning population for those specific spawning events. Given that wild males will spawn more than once in a given season, this would have negligible impact on native population structure. In the event that mixed-sex populations of triploids were to be used rather than all-female populations, then the possibility exists of triploid males mating with these occasional ovulated triploid females, an event that would likely lead to a significant proportion of their offspring themselves being triploid due to the combination of aneuploid gametes having a modal chromosome number midway between haploid and diploid. Not only has such a theoretical scenario never been shown to have occurred, it can be discounted as a way by which triploids could become established in the wild because of the rarity of triploid females reaching ovulation. Furthermore, the effective use of triploids for aquaculture requires them to be used as all-female populations, meaning that there should be no triploid males to escape in the first place.

Genomic stability of triploidy (possibilities for ploidy mosaics or reversion to diploid state)

Concerns are raised occasionally about presumed triploid individuals actually having a mix of ploidy levels among different cells (i.e., mosaic animals) or reverting to a fully diploid state. Having diploid pre-meiotic germ cells (oogonia or spermatogonia) in a presumed triploid individual would invalidate the use of triploidy for ensuring reproductive containment because such fish would likely produce normal haploid gametes. Mosaics were reported to result from

triploidy induction attempts in Atlantic salmon (Allen and Stanley 1979), based on variability in erythrocyte size measurements, but this was subsequently shown to be incorrect (Allen 1983). Occasional mosaic individuals resulting from heat treatments for the production of triploid Atlantic salmon were deformed and died early in development (Fox *et al.* 1986). Mosaics have been observed in other fish species following triploidy induction (Ewing *et al.* 1991; Teplitz *et al.* 1994; Goudie *et al.* 1995) or without any specific attempt to manipulate chromosome number (Yamaki *et al.* 1999, 2006). The latter study showed that haploid-diploid and diploid-triploid mosaic Amago salmon (*Oncorhynchus masou ishikawae*) produce viable haploid gametes; this may explain the production of euploid spermatozoa from presumed triploid testes in other species (Van Eenennaam *et al.* 1990; Kawamura *et al.* 1992). In the event that mosaics existed in presumed all-triploid populations of Atlantic salmon, the use of all-female populations would reduce the likelihood of any fish producing viable gametes.

Full reversion of confirmed triploid individuals to diploidy has never been reported in fish but does occur in at least one species of bivalve: the Pacific oyster (*Crassostrea gigas*) (Allen *et al.* 1996, 1999). There are two theoretical routes to such an outcome: the loss of one or a few chromosomes at a time, presumably through errors in cell division, or the loss of an entire haploid chromosome set in a single event (Benfey 2009). The first scenario is highly unlikely because it would result in aneuploid cells that would have to survive and replicate through ever declining chromosome numbers until reaching the diploid state. The second scenario is a more plausible pathway to diploidy and applies to rare examples of self-sustaining 'species' which reproduce by gynogenesis, such as the Amazon molly (*Poecilia formosa*). All such naturally-occurring cases are actually hybrids of two closely related species that require males of one of the progenitor species to activate development in their eggs, and the haploid chromosome set that is lost is the paternal set in the fertilizing spermatozoon. This can also result from artificial hybridization. Hybrids between female Atlantic salmon and male Brown trout (*Salmo trutta*) are the most likely source of such an outcome for Atlantic salmon (i.e., a triploid hybrid that becomes diploid due to the loss of the Brown trout genome). Such fish would be gynogenetic diploid Atlantic salmon, and would therefore develop as fertile females. Within the context of biocontainment of farmed Atlantic salmon, this would require a failure to exclude Brown trout from the breeding program providing the fish, which is both a highly unlikely event and something which can be confirmed easily through genotyping of the broodstock.

IMPACTS OF TRIPLOIDY ON PHENOTYPIC CHARACTERISTICS RELEVANT TO GENETIC AND ECOLOGICAL RISKS OF ESCAPED ATLANTIC SALMON TO WILD NATIVE ATLANTIC SALMON POPULATIONS

A great deal of research has been conducted on triploid Atlantic salmon to determine how they differ from diploids, largely focused on identifying any unique culture requirements (Table 1). Where comparable data exist, triploid Atlantic salmon show the same general characteristics as do triploids of other species (Benfey 1999; Maxime 2008, Piferrer *et al.* 2009; Fraser *et al.* 2012a). The following discussion outlines phenotypic effects of triploidy (as compared to the diploid phenotype) within the context of their ability to escape from fish farms and, subsequently, to survive and disperse in the natural environment.

Anatomy and morphology

As juveniles, triploid salmonids (including Atlantic salmon) of both sexes have a lower condition factor than diploids, a characteristic that is also clearly apparent when comparing sexually mature

female diploids to sibling female triploids. Female triploid salmonids retain the appearance of juveniles throughout their lives and therefore do not develop secondary sexual characteristics. Aside from these maturation-related effects, triploids are generally indistinguishable from diploids based on their external appearance. Although the frequency of lower jaw, gill, and skeletal deformities is higher in triploid populations of Atlantic salmon than in diploid populations (Sutterlin *et al.* 1987; Sadler *et al.* 2001; Pepper *et al.* 2004; Lijalad and Powell 2009; Powell *et al.* 2009; Fjelldal and Hansen 2010; Leclercq *et al.* 2011), most triploid Atlantic salmon do not exhibit such abnormalities. However, when present, they can affect swimming performance, metabolic scope for activity, and the ability to recover from exhaustive exercise (Lijalad and Powell 2009; Powell *et al.* 2009). Triploid Atlantic salmon post-smolts are also more prone to develop cataracts (Wall and Richards 1992; Leclercq *et al.* 2011). The underlying causes for these abnormalities are currently unknown, but they could potentially arise from one or more genotypic, environmental, or nutritional effects that are exacerbated by triploidy due to altered gene dosage or increased cell size.

The general lack of significant differences in anatomy and morphology suggests that triploid Atlantic salmon will not be much different from diploids in their abilities to escape from fish farms and to survive and disperse in the natural environment. Their lower condition factor is likely too slight to make it easier for them to escape from land-based facilities or net pens, but does suggest that they may have lower energy reserves and therefore may not live as long if unable to forage successfully once no longer offered pelleted feed. Deformed/blind individuals are certainly less likely to survive in the wild, but their incidence in triploid Atlantic salmon populations is low and likely to decrease with improvements in diet formulation and breeding programs designed to optimize triploid performance.

Cardiovascular function and metabolic scope

As is characteristic of triploid fish in general, triploid Atlantic salmon have larger red blood cells than diploids, but their numbers are reduced to maintain an equivalent haematocrit and blood haemoglobin concentration (Benfey and Sutterlin 1984c; Sadler *et al.* 2000a; Cogswell *et al.* 2002). Triploid Atlantic salmon do not differ from diploids in their oxygen consumption rate (Benfey and Sutterlin 1984b; Lijalad and Powell 2009), haemoglobin-oxygen binding affinity (Sadler *et al.* 2000a), aerobic swimming ability (Cotterell and Wardle 2004; Lijalad and Powell 2009), or stress response (Sadler *et al.* 2000b). However, they have less endurance in prolonged swimming tests (Cotterell and Wardle 2004), suggesting a reduced anaerobic capacity. Furthermore, as already noted, deformities in triploid Atlantic salmon can affect swimming performance, metabolic scope, and the ability to recover from exhaustive exercise (see section: Anatomy and morphology).

One of the more commonly identified limitations of triploid salmonids is a reduced ability to survive chronic exposure to what would be considered sub-lethally high (for diploids) temperature (e.g., Pepper *et al.* 2004). This may reflect lower blood oxygen carrying capacity in triploid Atlantic salmon (Graham *et al.* 1985), as is also apparent for Chinook salmon (Bernier *et al.* 2004). This would become problematic at higher temperatures due to increased metabolic oxygen demand concomitant with decreased oxygen solubility, and may therefore explain why triploid Atlantic salmon (and Brook charr) have lower thermal optima than diploids (Atkins and Benfey 2008). Verhille *et al.* (2013) have shown that high temperature disruption of cardiac function begins at lower temperatures in triploid Rainbow trout than in diploids, further supporting the concept of a lower thermal optimum for triploids.

Based solely on reduced anaerobic capacity and a lower optimum temperature, triploid Atlantic salmon will not be much different from diploids in their ability to escape from fish farms and to survive and disperse in the natural environment under most conditions. However, they will be at a disadvantage if the receiving environment is prone to high temperature and/or low dissolved oxygen events such as are most likely to occur in the summer.

Nutrition

Research on triploid nutrition is limited and has almost always used commercial diets formulated for diploids. The only study to have looked at nutrient utilization by triploid Atlantic salmon fed non-commercial formulated diets is that of Burke *et al.* (2010), who varied dietary phosphorus levels in a controlled tank experiment with freshwater pre-smolts. Independent of diet, triploids had higher specific growth rates than diploids in this experiment. They did not differ in food conversion efficiency, indicating that the better growth of triploids was due to greater feed intake. Triploids also had higher whole-body energy and lipid content as well as higher energy and nitrogen retention efficiencies than diploids, but they did not differ from diploids in whole-body protein content. Triploidy did not affect the apparent digestibility coefficients for dry matter, ash, or phosphorus, but triploids appeared to be less able to cope with high dietary phosphorus levels, as indicated by a declining growth advantage over diploids as dietary phosphorus levels increased. Bone ash content and phosphorus within the bone ash did not differ significantly between ploidies at the beginning or end of the experiment, but initial plasma phosphorus levels (before fish were switched from a commercial diet to the formulated test diets) were significantly lower in triploids than in diploids. More recently, in an experiment that varied dietary L-carnitine levels in diploid and triploid Rainbow trout, Ozório *et al.* (2012) found no effect of ploidy on growth or proximate composition (whole body, muscle, and liver), but triploids had lower liver fatty acid levels than diploids, independent of L-carnitine level.

There is currently insufficient information to predict whether effects of triploidy on nutrient requirements – if any – will affect the ability of triploid Atlantic salmon to utilize natural food sources any differently from diploids.

Disease/parasite resistance

Many fish farmers feel that triploid Atlantic salmon are more susceptible to disease than diploids, but there is no published scientific evidence to support this from challenges with virulent pathogens. However, research on complement activity in triploid Atlantic salmon suggests that they may be slightly disadvantaged compared to diploids in their ability to withstand exposure to bacterial pathogens (Langston *et al.* 2001). A more recent study of immune function in triploid Atlantic salmon also found them to have a lower relative abundance of B-cell lymphocytes than in diploid controls (Fraser *et al.* 2012c). B-cells are an important part of the adaptive immune response, being responsible for carrying antibodies that bind to antigens on the surface of potential pathogens and thereby targeting them for phagocytosis. Further, supporting the possibility of reduced immunocompetence in triploid Atlantic salmon, Ozerov *et al.* (2010) found them to have higher loads of the freshwater monogenean parasite *Gyrodactylus salaris* than diploids (median counts of 65 and 46 per fish, respectively) in a survey of parasites at an Atlantic salmon hatchery in Estonia. In this case, triploids were of unknown origin and were presumed to have arisen ‘spontaneously’.

The only published study of disease resistance in triploid Atlantic salmon found them to be no different from diploids in their susceptibility to bacterial kidney disease, but this study was flawed

by using triploids and diploids from different families (Bruno and Johnstone 1990). Studies with other salmonid species have generally found no effect of triploidy on complement and phagocyte activity, vaccine efficacy, or disease resistance, although Jhingan *et al.* (2003) showed triploid Coho salmon to be less resistant than diploids to vibriosis caused by *Vibrio anguillarum*. If triploid Atlantic salmon are less resistant to pathogens, there is the risk that they can become sources of infection to other fish due to the accumulation of higher pathogen loads and/or changes in their behaviour which make them more likely to spread the pathogen. There has been some suggestion of this from cage culture trials in Newfoundland, where it was observed that mortality from unintended infection by bacterial pathogens occurred first among triploids and then spread to adjacent cages with diploids (Pepper *et al.* 2004). Although this is a concern, the fact that triploids are reproductively sterile at least eliminates any risk of direct genetic effects on wild populations due to the introduction (or loss) of genes affecting immune function and disease resistance.

In the absence of properly controlled and executed studies exposing triploid Atlantic salmon to virulent pathogens or parasites, it is difficult to predict how they would compare to diploids in terms of their susceptibility to diseases encountered in farms prior to escape or in the wild after escape. From a biocontainment perspective, lessened disease/parasite resistance could be both beneficial (leading to higher mortality and/or a shorter time to death for escaped fish) and detrimental (leading to infected and moribund fish becoming a reservoir for pathogen exposure to wild fish). This latter point is clearly a concern, and further research on how triploidy affects immune response and disease resistance in Atlantic salmon is warranted.

Cognitive ability and behaviour

Laboratory studies

Overall brain size is not affected by triploidy in Atlantic salmon, but the cerebellum and telencephalon have been shown to be larger than in diploids (Fraser *et al.* 2012b). As noted by the authors of this study, triploids may therefore have enhanced cognitive capacity related to foraging and migration, which could increase their chances for survival in the wild but would also make them more vulnerable to predation. This same study found triploids to have smaller olfactory bulbs than diploids, which may reduce their ability to find food in the wild. However, any predictions of cognitive and sensory capacity based on these data must be viewed with caution, since brain cell size has been shown to be larger in triploids than in diploids in a number of species, including Atlantic salmon (Small and Benfey 1987). Given that overall brain size is no larger in triploids, this suggests that brain cell numbers may be reduced. Increased cerebellum and telencephalon size in triploids may therefore simply be a compensatory mechanism to allow for equivalent – rather than enhanced – cognitive capacity for functions related to these brain centres. The true nature of cognitive capacity related to survival skills in the wild cannot be understood without first determining how the triploid brain accommodates changes in cell size and number. The only true behavioural test of cognitive capacity in triploid fish found no difference from diploids in learning ability in Brook charr (Deeley and Benfey 1995).

In terms of their behaviour, juvenile triploid Atlantic salmon were found to be less aggressive than diploids in one study (Carter *et al.* 1994) but not in another (O'Keefe and Benfey 1997), likely due to differences in fish size and experimental design. Both studies tested fish in small tanks. Of greater relevance to this report, Ratelle (2006) studied competitive interactions between juvenile triploid and diploid Atlantic salmon reared in a semi-natural system consisting of replicate stream channels with natural substrate and using barriers to control fish emigration. Regardless of

whether they were reared in mixed ploidy groups (diploids and triploids) or as all the same ploidy, ploidy did not significantly affect specific growth rate when fish were unable to emigrate or emigration patterns once barriers were removed (i.e., average day of emigration, percent fish emigrated and cumulative emigration). Independent of ploidy, slower growing fish tended to be the ones that emigrated from the competition arena. Studies with other salmonid species have failed to find a consistent effect of triploidy on aggressiveness or the establishment of feeding hierarchies (O'Keefe and Benfey 1999; Garner *et al.* 2008).

Field trials

There have been several experimental releases of triploid Atlantic salmon smolts specifically to determine return rates for scenarios where fish might be intentionally released (for sea ranching) or might escape from aquaculture cages (Cotter *et al.* 2000; Wilkins *et al.* 2001). In the first study, diploids and triploids were tagged and released on three separate occasions, one mimicking a sea-ranching program and the two others mimicking cage escapes, using both mixed-sex and all-female populations of fish (Cotter *et al.* 2000). Adult triploid return rates to the coast and subsequently to the river of origin were approximately 25% that of diploid returns for the sea-ranched fish, independent of whether they were mixed-sex or female-only populations. Adult triploid return rates to the coast from mimicked cage escapes were only 22% of diploid return rates for mixed-sex populations and even lower (15 - 17%) for all-female populations. None of the cage 'escapees' returned to the river where their hatchery of origin was located, but two triploids did enter other rivers. The second study followed essentially the same protocol and yielded similar results (Wilkins *et al.* 2001). Although triploids clearly had lower return rates than diploids in both studies, the lack of high-seas fisheries prevented the collection of data for non-returning fish and it is therefore unknown whether 'missing' triploids had died or remained at sea. However, the fact that the triploids that did return were similar in size to returning diploids suggests that surviving triploids fed and grew well in the ocean (Wilkins *et al.* 2001). Also unknown is why triploid females, which had much smaller ovaries than diploid females, returned at all; the authors suggested that they were perhaps schooling with adult diploids on the feeding grounds and subsequently followed these fish as they returned to fresh water for spawning (Cotter *et al.* 2000; Wilkins *et al.* 2001).

Although no data are available on the fate of female triploid Atlantic salmon that remain at sea, similar trials conducted with sterile Coho salmon (sterilized by androgen treatment during early development) that were released as smolts for recovery in marine fisheries showed that some fish lived longer and grew larger at sea than the fertile fish which returned to fresh water for spawning (Hunter and Donaldson 1983; Donaldson and Hunter 1985). A similar study that released androgen-sterilized Coho and Kokanee salmon (*O. nerka*) into lakes that lacked access to the sea also found that small numbers of fish lived longer than fertile fish but, in contrast to the earlier studies into the ocean, sterile fish did not grow larger than fertile fish in spite of living up to twice the normal lifespan (Parkinson and Tsumura 1988; Johnston *et al.* 1993). Longer-lived animals may consume prey not normally associated with the species, especially if they grow to a larger size, making ecological impacts in freshwater and marine environments difficult to predict. However, it is important to note that, unlike Pacific salmon such as Coho and Kokanee, Atlantic salmon are repeat spawners. Because of this life-history characteristic, female triploids that remain at sea (or in lakes, in the case of landlocked populations) will not necessarily live longer or grow larger than fertile fish which survive to return repeatedly to feeding grounds after spawning. Although repeat spawners represent a minority of the adult Atlantic salmon population, they would nevertheless likely outnumber female triploids that escape from aquaculture.

Early evidence that triploid Rainbow trout could survive and disperse in Newfoundland waters came from the observation and capture (by angling) of large Rainbow trout in the Conne River during Atlantic salmon population surveys between 1990 and 2000 (Dempson *et al.* 1998; Dempson *et al.* 2000; Porter 2000; Dempson *et al.* 2001; van Zyll de Jong *et al.* 2004). Although their ploidy was not confirmed, these fish were likely escaped triploids, given that Rainbow trout culture in the region was restricted to triploids prior to 1999 (Geoff Perry, Fisheries and Oceans Canada, pers. comm.). An experimental release of tagged fish in a study designed to model the movements of escaped triploid Steelhead trout from sea cages in the same region showed that most fish initially stayed near the site of release but then gradually dispersed (Bridger *et al.* 2001).

A number of studies have been conducted in the USA on the suitability of triploidy to provide 'catchable' Rainbow trout for stocking into freshwater systems where the introduction of fertile fish is not desired. The first of these (Simon *et al.* 1993) found stocked triploids to have reduced growth and survival compared to stocked diploids, but this was attributed to having stocked the fish in a "marginal" environment (i.e., high temperature and low dissolved oxygen) and subsequent trials found little or no difference in dispersal, growth, or survival between stocked diploid and triploid Rainbow trout (Dillon *et al.* 2000; Teuscher *et al.* 2003; Kozfkay *et al.* 2006; Wagner *et al.* 2006; High and Meyer 2009; Koenig *et al.* 2011; Koenig and Meyer 2011). Unfortunately, none of these studies examined the diet of the stocked triploids, which would give a good indication of whether they might displace wild fish.

Although there is extensive stocking of triploid Brown trout in southern England, there are no peer-reviewed publications on their performance. However, it has been reported (Solomon 2003; Chatterji *et al.* 2007) that female triploid Brown trout show better over-winter survival than diploids, presumably due to the absence of spawning mortality. Stomach content analysis showed that they have the same diet as wild trout, with little fish predation. Tracking studies showed only "very limited" movement of female triploids into spawning areas and no observable interaction with wild fish during spawning. These studies concluded that female triploids are a good choice for anglers, with no apparent effects on wild trout densities or growth rates. Similarly, field studies have shown that female triploid Brook charr do not exhibit spawning migration while male triploids do (Warillow *et al.* 1997). A more detailed study by Budy *et al.* (2012) found no difference between stocked diploid and triploid Brook charr with respect to their growth, catch-per-unit-effort or, importantly, diet (based both on stomach content and stable isotope analysis).

Taken together, these results from laboratory studies on cognitive ability and behaviour and from field trials suggest that escaped triploid Atlantic salmon, if free of obvious deformities, will not be much different from diploids in their abilities to forage, escape predation, and disperse in the wild in freshwater environments. Given that they would most likely be used as all-female populations, triploid Atlantic salmon that escape from sea cages will exhibit low rates of entry into rivers. Those that remain at sea may live longer and grow larger than most fertile diploids, but likely will be no longer-lived or larger than diploid repeat spawners.

INDUSTRY ACCEPTANCE OF TRIPLOID ATLANTIC SALMON

Results of commercial-scale trials

Pilot-scale evaluation of triploid Atlantic salmon for aquaculture began around the same time in Atlantic Canada (Friars and Benfey 1991; Sutterlin and Collier 1991; McGeachy *et al.* 1995; O'Flynn *et al.* 1997; Benfey 2001; Friars *et al.* 2001; Pepper *et al.* 2004), Scotland (Johnstone *et al.* 1991; Johnstone 1993; McCarthy *et al.* 1996), Tasmania (Jungalwalla 1991), and the west-coast USA (Galbreath *et al.* 1994; Galbreath and Thorgaard 1995b). Similar pre-commercial trials were later undertaken in Ireland (Cotter *et al.* 2002) and Norway (Oppedal *et al.* 2003). Results from these trials were mixed and are difficult to compare due to differences in strains of fish, husbandry methods, and environmental conditions, as well as limitations in experimental design. On the whole, triploids tended to grow well, even occasionally outperforming diploids when the latter started to mature, but often had reduced survival compared to diploids. Mortalities were highest during egg incubation and first feeding, when they have minimal economic impact, and during the marine phase when economic impact is greatest. As a result, salmon farmers in North America and Europe showed no interest in adopting triploidy as a management tool, and the use of triploidy in commercial farming of Atlantic salmon is currently limited to Tasmania where there is a high incidence of early sexual maturation of farmed fish as grilse.

Two more recent projects have re-addressed the culture characteristics of triploid Atlantic salmon, building on these earlier studies and taking advantage of advances in salmon husbandry and the availability of top performing industry strains. One project was funded from 2008 to 2010 through the European Union's 7th Framework Programme (*Salmotrip: Feasibility Study of Triploid Atlantic Salmon Production*) and the other is currently underway (2012 to 2015) with funding from the Norwegian Research Council (*Solving Bottlenecks in Triploid Salmon Production – A Way to Strengthen the Sustainability of the Salmon Aquaculture Industry*). A number of publications have already come from the *Salmotrip* project and are summarized below. These papers, as well as research yet to be published (Taranger and Trippel 2012), will provide a more realistic view of triploid performance from an industry perspective.

Four papers from the *Salmotrip* project examine culture characteristics of triploid Atlantic salmon, focusing on freshwater production, smolt characteristics, and post-smolt performance in sea water (Fjelldal and Hansen 2010; Taylor *et al.* 2011; Leclercq *et al.* 2011; Taylor *et al.* 2012). Although triploids were smaller than diploids at hatch, they had superior growth rates through the freshwater phase, with no difference in survival after the eyed-egg stage. This superior growth carried over through smolting, as indicated by triploids attaining smolt status four weeks earlier than diploids. Triploids maintained their size advantage as post-smolts in sea water, although their growth rates were initially the same as for diploids (first 90 days in sea water) and ultimately lower than for diploids (first year in sea water). Deformities were rare during the freshwater phase (< 2% of the population) and were equally prevalent in diploids and triploids. However, the incidence of spinal deformity, twisted, or shortened jaws and cataracts was higher in triploid smolts and post-smolts than in diploids.

Triploidy did not affect smolt quality, based on their appearance, gill Na^+/K^+-ATPase activity, plasma osmolality, and seawater survival in comparison to diploid controls for both 0+ and 1+ out-of-season smolts. The authors suggest that earlier conclusions about reduced smolt quality (and subsequent seawater survival) for triploid Atlantic salmon may have been the result of not ensuring that triploids had attained sufficient energy reserves to truly complete the parr-to-smolt transformation. There is some merit to this suggestion, considering that triploid Atlantic salmon

repeatedly have been shown to have a lower condition factor than diploids, including in these studies. Current smolt production strategies for Atlantic salmon may therefore need to be modified for the production of top quality triploid smolts that will thrive in the marine environment, based on the accumulation of sufficient energy reserves and redefining smolt selection criteria.

The authors of these *Salmotrip* papers suggest that the higher prevalence of deformities and cataracts in triploid Atlantic salmon can likely be addressed through improvements in basic husbandry (i.e., optimizing the rearing environment), nutrition, and selection, as demonstrated when similar problems were initially encountered with diploid Atlantic salmon when there was less knowledge and experience with this species in aquaculture. These are no longer issues of concern in commercial salmon farming precisely because of genetic and husbandry improvements that have been made as the industry has matured. The current research on triploid Atlantic salmon in Norway, which has strong industry support, focuses on nutrition (especially with respect to histidine and phosphorus supplementation to address cataracts and skeletal deformities, respectively), selection for triploid performance, and optimization of rearing conditions (focusing especially on temperature and dissolved oxygen levels) (Taranger and Trippel 2012).

Strain selection and breeding programs for enhancing triploid performance

It is unclear how a second maternal set of chromosomes affects the ability to select for improved production traits in triploids. Given that they are sterile, triploids themselves cannot be used in breeding programs. Although it would be easiest to select based on diploid performance (i.e., making triploids from the same parents that give the best performing diploids), a study with triploid Atlantic salmon concluded that selection programs based on diploid performance may not yield the best performing triploids (Friars *et al.* 2001). This conclusion is not supported by research with other salmonids, which have generally found little evidence of family X ploidy interactions (Blanc and Vallée 1999; Johnson *et al.* 2004; Shrimpton *et al.* 2007; Chiasson *et al.* 2009). However, the doubled maternal genome does apparently affect phenotypic expression of important production traits in unpredictable ways that may still affect selection programs (Johnson *et al.* 2007). More information is needed on family effects and correlated responses with respect to triploid performance and how well it can be predicted by diploid performance. Breeding programs for triploid performance should be modelled on those already used to protect broodstock for biosecurity reasons in commercial aquaculture. This approach retains broodstock in onshore facilities where pathogen exposure is minimized, and bases broodstock selection on the performance of their siblings through the full production cycle. In the case of breeding programs for triploid success, the same principle would apply, except that diploids from each family would be retained in the onshore facilities for selection as broodstock depending on the performance of their triploid siblings in commercial production. Such a study would also allow the identification of genetic loci that have predictive value for triploid performance, with such loci then included in breeding and cryopreservation programs.

Selecting the best strains for triploid performance is equally as critical as within-strain selection programs. There has been only a single study to date that has compared the growth of Atlantic salmon triploids of different strains under identical conditions (Sacobie *et al.* 2012). In this case, triploid Mowi strain (European origin) and Cascade strain (North American origin) fish were shown to have very different growth characteristics as post-smolts in a 12-week tank trial. Under less controlled conditions, Pepper *et al.* (2004) also showed markedly different performance between triploid Newfoundland and Cascade strain salmon in cage trials in Bay d'Espoir, Newfoundland and Labrador.

FINAL ANALYSIS, CONCLUSIONS AND RECOMMENDATIONS

Summary of the pros and cons of using all-female triploid populations from the perspectives of reducing impacts on wild native populations and costs to farmers

Given that the stock or strain of origin of escaped farmed Atlantic salmon is unlikely to influence the fact that escaped fish will survive and disperse in the wild in native habitats for this species, the only effective way to reduce or mitigate potential post-escape interactions between farmed European-origin and wild native Atlantic salmon in Newfoundland is to ensure that the farmed fish cannot reproduce. Although this does not eliminate ecological risks, it at least assures that any direct ecological impacts will be limited to the lifetime of the escaped fish. The use of sterile fish eliminates the risk of genetic introgression by the escaped fish into the native population structure.

Induced triploidy is currently the only method available for rendering large populations of farmed Atlantic salmon sterile, and a great deal of research has been conducted on the basic biology and culture characteristics of triploid Atlantic salmon. It is relatively easy and inexpensive to produce all-female triploid populations on a commercial scale, although invariably attaining 100% triploidy induction success is unlikely using current methods. However, with experience and adherence to good farm practice and standard operating procedures, it should be possible to routinely attain >98% induction success. By using all-female triploid populations, approximately 99.9% of the farmed population should never reach sexual maturity.

Based on all the information currently available, it would appear that the use of triploid Atlantic salmon would have no impact on the rate of fish escape from farms, but that it may reduce their survival and dispersal in the receiving habitat. Triploid Atlantic salmon are more likely to have deformities and, if reared for a significant time in sea water, cataracts. They are also less likely to survive in habitats that are relatively warm or low in dissolved oxygen. If they are indeed less resistant to pathogens and parasites, then this will further limit their survival in the wild but adds the risk that they may become reservoirs for disease transmission to wild salmon populations. Triploid Atlantic salmon are likely to be effective competitors with wild diploids for food and space but, assuming all-female populations, they will show greatly reduced migration to fresh water if they escape from seawater sites.

From the perspective of the fish farmer, triploids may demonstrate inferior performance but this does not seem to be an insurmountable problem. Higher pre-hatch mortality represents little economic loss and can be accommodated by incubating larger numbers of eggs. Freshwater production data indicate no problems with triploids in terms of survival or growth, and they produce high quality smolts. However, reduced growth and a higher incidence of deformities and cataracts in post-smolts are of concern. The fact that similar issues with diploid Atlantic salmon earlier in the development of this industry are no longer encountered, due to improvements in husbandry and genetics, suggests that this can be addressed for triploids as well. In this regard, developments from recent and current trials in Scotland and Norway should be followed.

In conclusion, a review of the literature indicates that there are no dramatic effects of triploidy on Atlantic salmon production traits in aquaculture, aside from their sterility. Their oft-reported reduced performance may reflect sub-optimal rearing conditions or the need to use different strains of fish. For all the research done evaluating triploid Atlantic salmon in Atlantic Canada, there is no information on how triploids of the best-performing diploid strains compare to their diploid siblings. Future research should evaluate the performance of triploids derived from the

best-performing production strains, and should focus on defining the optimum rearing environment for triploids.

RECOMMENDATIONS

1. If the spawning potential of escaped European-origin Atlantic salmon is deemed to pose an unacceptable risk to native Atlantic salmon populations in Newfoundland, then all-female triploid populations could be used as an alternative to further reduce that risk.
2. Populations that are verified pure Atlantic salmon (i.e., no Brown trout hybrids) derived from crosses between confirmed neomales and normal females (i.e., all-female offspring), with an acceptably high triploidy induction success (>98%), would provide the greatest reduction in risk.
3. Research should continue to evaluate and focus on improvement of triploid performance through breeding programs and optimization of husbandry, including nutrition, rearing environment, and fish health.
4. Characterization of the immune response and disease resistance of triploid Atlantic salmon should be a research priority, with a focus on predicting whether their escape would pose a greater risk of pathogen amplification and disease transfer to wild fish than that posed by escaped diploids of the same strain.
5. Given the one-generation lead time needed to create all-female populations using neomales, if it is at all likely that triploid European-origin Atlantic salmon will be allowed in Newfoundland on a trial basis, then the production of neomales of the appropriate European strain(s) should be a priority.
6. A pilot-scale evaluation in Newfoundland of the culture performance of disease-free all-female triploid European-origin and Saint John River (industry select) strains of Atlantic salmon would assist in the determination of whether the former will outperform the latter. In order to provide relevant information, such a study should use current husbandry standards, with no mixing of diploids and triploids within cages.
7. Any releases of triploid Atlantic salmon should be followed with a monitoring program to provide empirical data to test the predictions made in this paper.
8. Research developments elsewhere in the world should be followed, especially with respect to new information from *Salmotrip* and current research in Norway.

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TABLE

Table 1. *Publications on triploid Atlantic salmon (chronological order).*

Reference	Triploidy induction [1]	Triploidy verification [2]
Svärdson 1945	C, spont	karyo
Lincoln *et al.* 1974	C	karyo
Refstie *et al.* 1977	CB	karyo
Allen and Stanley 1979	CB	RBC size, karyo
Allen 1983	CB, spont	fcm
Benfey and Sutterlin 1984d	H, P	CC
Benfey and Sutterlin 1984c	H	RBC size, CC
Benfey and Sutterlin 1984b	no info	no ID
Benfey and Sutterlin 1984a	H	CC
Benfey *et al.* 1984	H	RBC size, CC, den
Bolla and Refstie 1985	CB	fcm, karyo
Graham *et al.* 1985	H	fcm
Johnstone 1985	H	RBC den
Fox *et al.* 1986	H	RBC den
Johnstone 1987	H, P	RBC den
Small and Benfey 1987	P	CC
Sutterlin *et al.* 1987	H	CC
Johnstone *et al.* 1989	A	RBC den
Quillet and Gaignon 1990	H	RBC size, karyo
Johnstone *et al.* 1991	H, P, A	?
Wall and Richards 1992	H, P	?
Carter *et al.* 1994	P	ovarian development
Galbreath *et al.* 1994	H	fcm
Galbreath and Thorgaard 1995a	H	fcm
Galbreath and Thorgaard 1995b	H	fcm
McGeachy *et al.* 1995	P	fcm
Wilkins *et al.* 1995	H	?
McCarthy *et al.* 1996	P	ovarian development
O'Flynn *et al.* 1997	P	fcm
O'Keefe and Benfey 1997	P	fcm
Johnston *et al.* 1999	P	nucleoli, fcm
Cotter *et al.* 2000	P	RBC size
Sadler *et al.* 2000a	P	RBC size
Sadler *et al.* 2000b	P	RBC size
Benfey 2001	(review)	
Friars *et al.* 2001	P	fcm

Reference	Triploidy induction [1]	Triploidy verification [2]
Langston *et al.* 2001	P	no ID
Wilkins *et al.* 2001	P	RBC size, den
Sadler *et al.* 2001	P	RBC size
Cogswell *et al.* 2002	P	RBC size
Cotter *et al.* 2002	P	RBC size
Oppedal *et al.* 2003	P	RBC size
Bjørnevik *et al.* 2004	P	?
Cotterell and Wardle 2004	P	RBC size
Pepper *et al.* 2004	P	RBC size, fcm
Atkins and Benfey 2008	P	RBC size
Lijalad and Powell 2009	P	RBC size
Powell *et al.* 2009	P	RBC size
Burke *et al.* 2010	P	RBC size
Fjelldal and Hansen 2010	P	RBC size
Ozerov *et al.* 2010	spont	genotyping
Cantas *et al.* 2011	P	RBC size
Leclercq *et al.* 2011	P	RBC size
Taylor *et al.* 2011	P	RBC size
Fraser *et al.* 2012b	P	RBC size
Fraser *et al.* 2012c	P	RBC size
Sacobie *et al.* 2012	P	fcm
Taylor *et al.* 2012	P	RBC size

[1] Triploidy induction methods: **A** = anaesthetic, **C** = cold, **CB** = cytochalasin B, **H** = heat, **P** = pressure, **spont** = spontaneous.
[2] Triploidy verification methods: **CC** = erythrocyte size from automated particle size analyzer, **fcm** = erythrocyte DNA content by flow cytometry, **genotyping** = microsatellite analysis, **karyo** = karyotyping, **nucleoli** = number of nucleoli, **RBC den** = erythrocyte DNA content by microdensitometry, **RBC size** = erythrocyte dimensions from blood smears.