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The potential direct and indirect genetic consequences for native Newfoundland Atlantic Salmon from interbreeding with European-origin farm escapes

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Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

Aquaculture usually involves the production of non-native populations and species. As well, it is increasingly focused on a relatively small number of species and a few inbred, highly selected, and domesticated strains. Although delivering economic gains, this focus can potentially lead to negative interactions and impacts on the character, abundance, and viability of native populations in farming regions. The complexity of biological systems means these risks are difficult to predict and where impacts are found to occur, they can be difficult to mitigate. This makes it imperative that risks are carefully considered in advance.

These risks are considered here in respect of a request to import genetically improved Norwegian Atlantic salmon strains to Canada for use in farms on the south coast of the island of Newfoundland. The current production in the region is *c.* 15,000 tonnes and is based on strains from the Saint John River in New Brunswick. This request occurs against a background of depressed local wild salmon stocks. These have declined in abundance by ~45% from 1996-2010, particularly near the main farming area (~70% decline in the Conne River), and are designated as “threatened” by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2010). Marine mortality is seen as the main problem, with farm-wild interactions a possible contributing factor. As such, concern exists that the use of Norwegian farmed Atlantic salmon strains may lead to further impacts, exacerbating current declines.

Available scientific evidence related to the genetic, phenotypic, and demographic consequences of indirect and direct genetic interactions of such an introduction is reviewed. It specifically considers if:

1) farmed European-origin Atlantic salmon were able to successfully breed and/or interbreed with native Atlantic salmon;
2) the likelihood that European-origin aquaculture escapes will mate successfully with native wild salmon;
3) the risks that such interbreeding would present to native populations; and
4) how risk scales with the size of the interaction.

Norwegian farmed salmon and wild, native stocks of salmon in Newfoundland are encompassed within the species *Salmo salar* L. However, a compelling body of evidence shows that they are highly genetically divergent, and probably as divergent as any two sets of populations with the species. By many experts, they are viewed as belonging to different subspecies, even considering the evidence that some wild Newfoundland stocks have a naturally mixed European and North American ancestry; this is a legacy of the period after the last glaciation during which the region’s rivers were recolonized. However, a robust and detailed understanding of the adaptive divergence of Norwegian farm and Newfoundland wild salmon is lacking.

Existing knowledge indicates that a proportion of escaped farmed Norwegian salmon would survive and enter rivers on the south coast of Newfoundland, leading to direct genetic interactions, with numbers conditioned by the magnitude and timing of escapes. If they do escape, survive, and migrate into rivers, it is likely that they will be able to breed successfully given that they have done so with native populations across their wild range, including in other parts of eastern North America. This is particularly likely where local wild populations are depressed and competition for mates is limited. Existing studies indicate interbreeding will result in lower mean population fitness, depressed abundance, altered character, and reduced viability. Through increased gene flow engendered by direct interbreeding of farm fish, genetic mixing and impacts may be extended beyond directly impacted populations and could lead to increased genetic homogenisation of populations across a region. Whether these impacts will be greater than those arising from the use of North American farm strains is uncertain and will depend on the specific nature of the differences in adaptive divergence of the respective farm strains from wild Newfoundland populations. In both cases, the size and

persistence of interactions can be expected to be crucial, with impact increasing with the amount of interbreeding and its persistence over a large number of generations. Even low levels of repeated interbreeding in already depressed populations could put local populations into an extinction vortex. The unusual phenotypic diversity of Newfoundland populations means that the consequences of impacts on salmon biodiversity may potentially be greater than elsewhere, and its preservation will be a key conservation concern.

Indirect genetic interactions may also occur. Caged or escaped farm fish can change the environment so as to alter selective pressures and long-term fitness. This can lead to decreased survival, reductions in population size, increased genetic drift, and a lowering of long-term adaptive capacity. The latter will also be the outcome of interbreeding where there is reduced hybrid fitness. This effect may be manifest beyond the first generation where some hybrids survive and lead to the introgression into wild populations of new maladaptive gene variants, changes to existing gene and genotype frequencies, and disruption of co-adaptive genomic structure, compromising the character, abundance, and viability of affected populations.

Indirect impacts from caged farm fish or freshwater rearing facilities that release waste water are of concern because of the potential for the introduction of exotic pathogens or increasing numbers of native pathogens. Again, this can cause increased or selectively-altered mortality, reducing a population’s adaptive capacity. Indirect genetic impacts can also arise if farm salmon escape as juveniles into rivers and compete with wild fish, if escaped farm adults ascend rivers and interfere with the reproduction of wild fish, reducing wild breeding success; if they spawn successfully, they may also produce offspring that compete with wild juveniles. What is not known is whether there is an increased or altered risk from using Norwegian as compared to North American farm Atlantic salmon strains. However, the risk can be expected to differ given their substantive evolutionary divergence, the presence of different pathogen strains and “species”, and host susceptibilities.

Risks of direct and indirect impacts can be expected to scale with the relative magnitude of the number of farm fish present compared to the abundance of wild populations. Small populations, or those experiencing declines or low abundance, will be more susceptible to genetic impacts than will large or healthy populations. Given the current status of populations along the south coast and near aquaculture activities, the risk of significant impact may be higher than elsewhere.

More research is required to address the knowledge gaps in current understanding. This should focus on increasing the capacity to predict impacts. Key in this respect are:

1) accurate assessments of the actual numbers of farm fish that will escape and the proportions that will enter rivers under different escape scenarios;
2) the development and refinement of genetic tools for identification farm escapes and hybridization;
3) studies of the genomic basis of adaptive divergence within wild populations and among wild and farmed strains;
4) the relative fitness of farm and wild salmon, and the various generations of hybrids, in the local environment under different conditions of wild demographics;
5) a better understanding of wild population dynamics and, in particular, the way in which density dependence affects juvenile mortality; and
6) the development of realistic, robust, virtual individual-based, stochastic population demographic models that incorporate realistic genetic models and the effects of environmental variation and change.

Conséquences génétiques potentielles directes et indirectes pour les espèces de saumon de l’Atlantique indigènes de Terre-Neuve découlant du croisement avec des saumons d'élevage fugitifs d'origine européenne

RÉSUMÉ

L'aquaculture porte habituellement sur la production de populations et d'espèces non indigènes. On a également de plus en plus recours à un nombre relativement faible d'espèces, à quelques croisements rigoureusement sélectionnés et à des souches domestiquées. Bien que cette façon de faire produise des gains économiques, elle peut aussi entraîner des interactions négatives et des répercussions sur le caractère, l'abondance et la viabilité des populations indigènes dans les régions d'élevage. La complexité des systèmes biologiques signifie que ces risques sont difficiles à prévoir et que les impacts éventuels peuvent être difficiles à atténuer. Il est donc impératif que les risques fassent l'objet d'un examen minutieux à l'avance.

Ces risques sont pris en considération dans le présent document eu égard à une demande d'importation au Canada de souches norvégiennes de saumon de l'Atlantique génétiquement améliorées aux fins d'utilisation dans les exploitations sur la côte sud de l'île de Terre-Neuve. La production actuelle dans la région est de 15 000 tonnes environ et est fondée sur des souches du fleuve Saint-Jean, au Nouveau-Brunswick. Cette demande découle de la diminution des stocks locaux de saumons sauvages. Leur abondance a connu un déclin d'environ 45 % entre 1996 et 2010, notamment près de la zone d'élevage principale (déclin d'environ 70 % dans la rivière Conne) et l'espèce est désignée comme étant « menacée » par le Comité sur la situation des espèces en péril au Canada (COSEPAC 2010). La mortalité en mer est considérée comme le principal problème, dont les interactions entre les poissons d'élevage et les poissons sauvages sont probablement un facteur. On craint donc que l'utilisation de souches norvégiennes de saumon de l'Atlantique d'élevage puisse donner lieu à d'autres impacts, qui pourraient exacerber le déclin actuel.

Ce document examine les preuves scientifiques disponibles des conséquences génétiques, phénotypiques et démographiques des interactions génétiques directes et indirectes d'une telle introduction. Plus particulièrement, il examine les éléments suivants :

1) si les saumons de l'Atlantique d'élevage d'origine européenne ont réussi à se reproduire entre eux ou avec des saumons de l'Atlantique indigènes;
2) la probabilité que les poissons d’élevage fugitifs d’origine européenne se reproduisent avec des saumons sauvages indigènes;
3) les risques que ce croisement présenterait pour les populations indigènes;
4) la proportionnalité de ce risque avec l’ampleur de l'interaction.

Le saumon d'élevage norvégien et les stocks indigènes de saumon sauvage à Terre-Neuve font partie de l'espèce *Salmo salar L.* Toutefois, des éléments de preuve convaincants indiquent qu'ils sont très divergents sur le plan génétique, et sans doute aussi divergents que n'importe quelle autre paire d'ensembles de populations de l'espèce. De nombreux experts les considèrent comme appartenant à des sous-espèces différentes, même en tenant compte des éléments qui prouvent que certains stocks sauvages de Terre-Neuve proviennent d'un mélange naturel des espèces européennes et nord-américaines; il s'agit d'un héritage de la période suivant la dernière glaciation, au cours de laquelle les rivières de la région ont été recolonisées. Cela dit, on ne comprend pas bien toute l'ampleur de la divergence adaptative du saumon d'élevage norvégien et du saumon sauvage de Terre-Neuve.

Selon les connaissances actuelles, une proportion des saumons d'élevage norvégiens fugitifs arriveraient à survivre et à pénétrer dans les rivières de la côte sud de Terre-Neuve, entraînant ainsi des interactions génétiques directes. Les chiffres dépendraient de l'ampleur et du moment de ces évasions. Si ces poissons réussissent à s'échapper, à survivre et à migrer dans les rivières, il est

probable qu'ils seront en mesure de se reproduire étant donné qu'ils l'ont déjà fait avec des populations sauvages indigènes de leur aire de répartition, y compris dans d'autres parties de l'est de l'Amérique du Nord. Ce scénario est d'autant plus probable là où les populations sauvages locales sont en déclin et où la compétition pour trouver des partenaires est limitée. Les études indiquent qu'un croisement entraînera une baisse de la valeur adaptative moyenne des populations, une diminution de l'abondance, une altération du caractère et une réduction de la viabilité. En raison de l'augmentation des flux génétiques générée par le croisement direct des poissons d'élevage, les mélanges génétiques et les impacts pourraient s'étendre au-delà des populations directement touchées et conduire à une augmentation de l'homogénéisation génétique des populations dans l'ensemble d'une région. On ignore si ces impacts seront plus importants que ceux découlant de l'utilisation de souches d'élevage nord-américaines. Cela dépendra de la nature particulière des différences dans la divergence adaptative de chacune des souches d'élevage issues des populations sauvages de Terre-Neuve. Dans les deux cas, on peut s'attendre à ce que l'ampleur et la persistance des interactions soient importantes et à ce que les impacts augmentent avec la hausse du nombre de croisements et de leur persistance au fil des générations. Même de faibles niveaux de croisement répétés dans des populations déjà vulnérables pourraient mettre les populations locales à risque d'extinction. La diversité phénotypique inhabituelle des populations de Terre-Neuve fait en sorte que les conséquences des impacts sur la biodiversité du saumon pourraient être plus grandes qu'ailleurs, et la préservation de l'espèce sera l'une des principales préoccupations en matière de conservation.

En outre, des interactions génétiques indirectes pourraient également se produire. Les poissons d'élevage en cage ou qui se sont échappés peuvent modifier l'environnement et ainsi modifier les pressions sélectives et la valeur adaptative à long terme. Cela peut entraîner une diminution de la survie, une réduction de la taille de la population, une augmentation de la dérive génétique et une diminution de la capacité d'adaptation à long terme. Cette dernière conséquence sera également le résultat des croisements intervenus là où la valeur adaptative des hybrides est réduite. Cet effet pourrait se manifester au-delà de la première génération lorsque certains hybrides survivent et entraînent l'introgression de nouvelles variantes génétiques inadaptées dans les populations sauvages, des changements de la fréquence génique ou génotypique actuelle et la perturbation de la structure génomique coadaptative, compromettant ainsi le caractère, l'abondance et la viabilité des populations touchées.

Les impacts indirects des installations d'élevage en cage ou en eau douce qui rejettent des eaux usées sont préoccupants, car ces rejets peuvent contenir des agents pathogènes exotiques ou un nombre croissant d'agents pathogènes indigènes. Encore une fois, cela peut entraîner une mortalité accrue ou modifiée de façon sélective et réduire la capacité d'adaptation d'une population. Des impacts génétiques indirects peuvent également survenir si les saumons d'élevage s'échappent dans les rivières au stade de juvéniles et entrent en compétition avec les poissons sauvages, et si les saumons d'élevage fugitifs adultes remontent les rivières et nuisent à la reproduction des poissons sauvages, réduisant ainsi le succès de la reproduction sauvage. De plus, s'ils frayent avec succès, ils peuvent produire une descendance qui fera compétition aux juvéniles sauvages. On ignore si l'utilisation de souches norvégiennes de saumon de l'Atlantique d'élevage plutôt que de souches nord-américaines se traduit par une augmentation ou une modification des risques. Toutefois, on peut s'attendre à ce que le risque diffère en raison de la divergence évolutive importante, de la présence de différentes souches d'agents pathogènes et d'« espèces », et des vulnérabilités des hôtes.

On peut également s'attendre à ce que les risques d'impacts directs et indirects augmentent avec l’ampleur relative du nombre de poissons d'élevage présents par rapport à l'abondance des populations sauvages. Les petites populations, ou celles qui connaissent un déclin ou une faible abondance, seront plus vulnérables aux impacts génétiques que les grandes populations ou les populations saines. Compte tenu de l'état actuel des populations le long de la côte sud et près des activités aquacoles, le risque de répercussions importantes peut être plus élevé qu'ailleurs.

D'autres recherches sont nécessaires pour combler les lacunes dans les connaissances et améliorer la compréhension actuelle. Ces recherches devraient être axées sur l'augmentation de la capacité à prévoir les impacts. Les éléments clés à cet égard sont les suivants :

1) des évaluations précises du nombre réel de poissons d'élevage qui s'échapperont et des proportions qui pénétreront dans les rivières selon différents scénarios d'évasion;
2) l'élaboration et l'amélioration des outils génétiques permettant d'identifier les poissons d'élevage fugitifs et l'hybridation;
3) des études sur la génomique de divergence adaptative dans les populations sauvages et les souches sauvages et d'élevage;
4) la valeur adaptative relative du saumon d'élevage et du saumon sauvage et les diverses générations d'hybrides dans l'environnement local selon les différentes conditions des populations sauvages;
5) une meilleure compréhension de la dynamique des populations sauvages et, en particulier, l'influence de la dépendance à la densité sur la mortalité juvénile;
6) l'élaboration de modèles démographiques stochastiques réalistes, robustes, virtuels et fondés sur l'individu qui intègrent des modèles génétiques réalistes et les effets de la variation et des changements environnementaux.

INTRODUCTION

The cultivation of Atlantic salmon (*Salmo salar* L.) for food is a global industry, though most primary areas of production lie within the species' native North Atlantic range (Cultured Aquatic Species Information Programme 2013). The industry continues to expand with global production currently over 1,600,000 tonnes, with a projected further growth of ~ 50% by 2020 (Marine Harvest 2012). Industry expansion encompasses both new and existing farming regions, and is increasingly focused on a few economically improved, highly selectively bred strains developed from wild Norwegian stocks in the 1960s, though other stains are still used in some areas. This trend is driven by economics, both the need for better feed conversion efficiency, carcass quality, and disease resistance, and because improving strains is costly and takes decades.

The native ranges of many cultivated organisms such as farmed Atlantic salmon are geographically constrained at the species and/or population levels. The species in the wild is composed of highly local, genetically, phenotypically, and adaptively differentiated populations (Verspoor *et al.* 2007). Thus it is inevitable that in most salmon farming operations within the species range, the salmon used will be non-native in origin and genetically divergent. The degree of divergence will depend on the farm strain used and its selective improvements as well as the location of the farming operation. Where strains of distant geographical origin are used, concerns have been raised as to whether the risks of negative genetic interactions with escaped farm fish will be greater than if strains of more local origin are used.

The use of selectively bred, genetically improved non-native fishes in aquaculture has generally improved the economics of farming operations and contributed to local industry growth. However, a compelling body of evidence highlights the potential for negative impacts on the character, abundance, viability of native wild populations, and the productivity and stability of local ecosystems (Svåsand *et al.* 2007). Yet, the interactions underlying potential impacts can be expected to be biologically complex making risks uncertain and difficult to predict, quantify and, when they occur, to mitigate. Sustainable salmon farm development that minimises detrimental impacts to local biological communities can only be achieved with a sound understanding of the nature and extent of potential impacts, their avoidance, and their mitigation. This requires careful consideration of introductions of non-native populations, cultivated or wild, before they occur and taking into account the potential for negative impacts in introduction decisions.

The salmon farming industry has been operative in Canada since the 1970s (Knapp *et al.* 2007) and has variously lobbied for access to Norwegian farm strains based on the perception of a better economic performance that would improve the competitiveness of local farming operations. However, based on the precautionary principle, a moratorium was put in place on the use of Norwegian farm strains in eastern Canada addressing concerns that the use of more genetically divergent, non-local strains could have a higher risk of negative impacts. More recently, a ban was placed on the use of European origin salmon and European x North American hybrid fish in salmon farming operations in the adjacent US, following a federal review (National Research Council 2004). However, Norwegian farm strains dominate the salmon farming industry and are widely used (e.g., Norway, Scotland, Ireland, Iceland, Chile, and Western Canada). They are widely considered to give superior economic performance under a wide range of environmental conditions.

Salmon farming started in Newfoundland in the late 1970s and is carried out along the south coast of the island focused around the Bay d'Espoir region. Estimated production in 2011 was *c*. 14,000 tonnes (DFO 2012) and is based on Saint John River (New Brunswick) strains. However, recently, formal requests were made by two Atlantic salmon farming companies to Fisheries and Oceans Canada (DFO) for approval to import Norwegian-origin Atlantic salmon broodlines from Iceland. The initial objective for the requests was to allow studies to assess the advantages of growing Norwegian Atlantic salmon strains under environmental conditions found on the south coast of Newfoundland compared to those currently farmed. Under Canada's National Code on Introductions and Transfers of Aquatic Organisms, consideration of the request must be guided by a risk assessment.

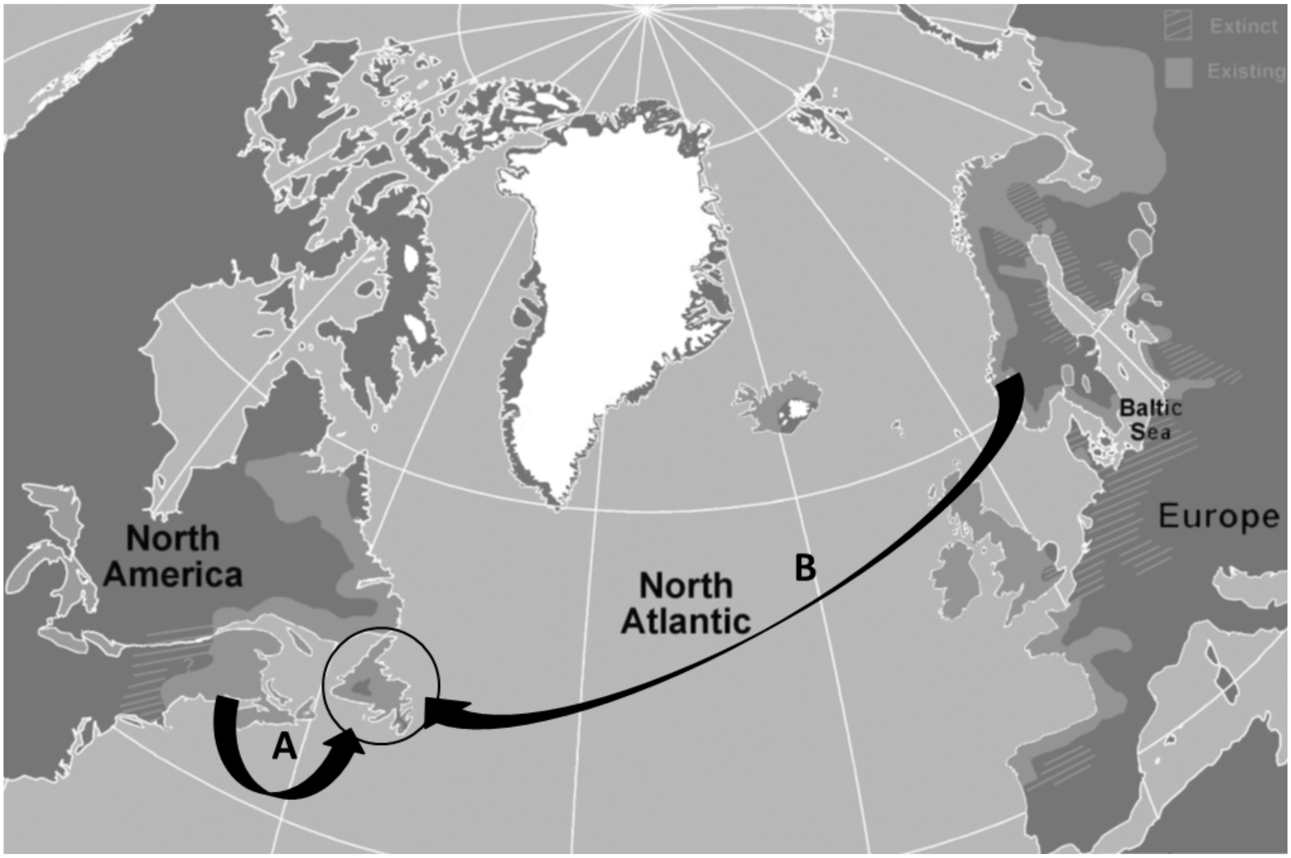

Figure 1 – A map showing the natural distribution of the Atlantic salmon and the Island of Newfoundland (circled), as well as the original geographical origins of the current (A) and proposed (B) strains used in farms in the Province; shown are the extant salmon distribution (solid light grey) and where the species has been extirpated (striped light grey).

These requests for the importation of Norwegian farm strains into Newfoundland occur against a background of locally depressed wild Atlantic salmon stocks. Stocks in the rivers on the south coast have shown a marked decline in abundance from 1996 – 2010 in the order of 45% and, particularly, in the region around the Conne River (~70%) and Bay d'Espoir, where much of the salmon farming industry is concentrated (DFO 2012). In 2010, all stocks along the south coast were designated as "threatened" (COSEWIC 2010). Projections suggest that, if current conditions remain the same, the most likely scenario is a continued overall decline of wild stocks in the region. Freshwater habitat does not appear limiting, with increased marine mortality appearing to be the main cause of the observed decline. However, the relative importance of factors such as illegal fisheries, over-exploitation in legal high seas fisheries, by-catch mortality, a changing marine environment, and negative interactions with farm salmon, is not known.

Understanding of the genetic and biological nature of Atlantic salmon has increased substantially since the 1970s when salmon farming began (Verspoor *et al.* 2007) and, in particular, over the last decade. This includes new scientific understanding relevant to defining the potential added risk of genetic and ecological interactions of local wild salmonid populations with introduced conspecific cultured and non-local salmonids. This understanding has considerable bearing on assessment of the potential risk of introducing Norwegian strains into the Newfoundland salmon farming sector, and will help clarify what is known and where knowledge gaps remain, and is critically reviewed here in this context.

PURPOSE AND SCOPE OF ASSESSMENT

With regard to the use of Norwegian farm strains of Atlantic salmon in the aquaculture industry in Newfoundland, the main identified issues of concern are:

- the increased potential for ecological effects from animals in culture
- the increased potential for direct and indirect genetic impacts from farm escapes
- the potential for preventing or reducing the likelihood of escapes from culture
- the potential for preventing or reducing post-escape interactions

The present paper addresses the second issue. It does so by critically reviewing available scientific evidence relevant to the following specific questions:

1) What could be the direct and indirect genetic and phenotypic (e.g., growth, survival) consequences to native Atlantic salmon if European-origin Atlantic salmon were to successfully breed and/or interbreed with native Atlantic salmon?
2) What is the likelihood that European-origin aquaculture escapes will mate successfully with native wild salmon?
3) What risks could such interbreeding present to native populations?
4) How might this risk to native populations scale with the size of the interaction?

GENERAL NATURE OF ISSUES RELATED TO DIRECT AND INDIRECT GENETIC IMPACTS

Advances in biological understanding make it clear that ecological and reproductive interactions with cultured salmon populations can have negative ecological, demographic, and genetic consequences for wild populations (Youngson and Verspoor 1998; Ferguson *et al.* 2007; Svåsand *et al.* 2007; Thorstad *et al.* 2008). Genetic effects can be both direct and indirect and variously affect the character, abundance, and viability of wild populations (Figure 2). Indirect effects can arise whether animals remain in containment or escape, through ecological changes (e.g., introduction of a pathogen or a therapeutant) that alter patterns of natural selection on native fish, changing gene frequencies, or reducing their abundance, so as to lead to decreased genetic diversity through inbreeding and genetic drift (Figure 2). Indirect effects may involve wild populations of any native species. Direct effects will arise only where salmon escape and involve reproductive interactions; they will be usually, but not always, confined to conspecific populations in areas where they are present. In the case of farm salmon in Europe, direct effects can also occur in respect of wild populations of the conspecific brown trout *S. trutta*. In Newfoundland, there is a potential for direct interaction with feral brown trout populations which are known to hybridize with wild Atlantic salmon (Verspoor 1986) and it is known that rates of hybridisation increase where there are escaped farm fish (see Ferguson *et al.* 2007).

The nature and extent of demographic and viability consequences caused by indirect and direct genetic effects, including both their severity and permanence, will depend on various factors. As regards indirect effects, the size of farming operations, effectiveness of containment, husbandry practises, and pathogen/parasite status of farm stocks will be of key importance. Numbers of escapes, life-history stage, time-of-year, mortality rates, reproductive competence, and phenotypic characteristics of farm stock are related to ability to compete for territories, food, and mates for both indirect and direct effects (Youngson and Verspoor 1998). The later characteristics will, in turn, be influenced by their phenotypic state when they escape, the environmental circumstances they encounter in the wild, and their phenotypic divergence from local wild populations. Phenotypic divergence will be conditioned by the nature and degree of adaptive genetic divergence of farm stocks from wild populations, in contrast to indirect impacts where this may or may not be important, as well as the nature of *genotype x environment* interactions and phenotypic plasticity.

Adaptive genetic divergence in the Atlantic salmon is highly variable and extensive, and biologically complex. The overall DNA content of the salmon genome is twice that of humans, encompassing more than six billion base pairs (Moran *et al.* 2007). Of these, more than 99.99% are found in the nuclear chromosomes, and the remainder in the mitochondria, the cell's energy producing organelles. In Atlantic salmon, mitochondrial DNA (mtDNA) is packaged into a single molecule whose size (16,665 base pairs – Hurst *et al.* 1999), represents <0.01% of the overall genome. The molecule does not appear to vary in gene organisation and varies little in size (~3 base pairs across its range – So 2006), but shows high levels of single nucleotide polymorphisms (SNPs) (Fridjonsson *et al.* 2011). However, the full extent of SNP variation remains to be resolved (Verspoor *et al.* 2012).

The nuclear genome is far more complex and remains poorly understood, though much of it has been recently sequenced (Davidson *et al.* 2010). Chromosome numbers can vary among populations (Moran *et al.* 2007) but the extent of any variation within the chromosome and gene structure is largely unknown. However, in some species, these genome features do vary substantively among individuals and populations. What is known is that, as in other species, the nuclear genome contains large numbers of SNPs (Lien *et al.* 2011; Bourret *et al.* 2012), with millions expected to be present within genes and in non-coding regions. However, although the functional role may be identified, their adaptive relevance remains uncertain. In only a few cases is there a compelling case for their adaptive relevance (e.g., MEP-2 – Verspoor and Jordan 1989; MHC-II – Dionne *et al.* 2007), despite the fact that all adaptive differentiation at the population level is ultimately defined by DNA variation.

Most published estimates of genetic divergence among populations of Atlantic salmon do not address adaptive divergence directly and are based on molecular variation in relatively small numbers of individuals, populations, and arbitrary mitochondrial or nuclear loci in the genomes (King *et al.* 2007). They are also of limited use as a proxy for adaptive divergence. A general correlation between molecular and adaptive divergence is apparent at the interspecific and inter-generic levels but not among populations within species (Reed and Frankham 2001). The actual relationship between measures of molecular and phenotypic divergence is complex (Reed and Frankham 2003; McKay and Latta 2002) and even low levels of molecular divergence can be associated with high levels of adaptive divergence. The same is true of molecular divergence and pre- and post-zygotic reproductive isolation (Edmands 2002). In general, where high levels of molecular divergence are found, they have been associated with high levels of adaptive evolutionary divergence, often among populations within designated species but adaptive divergence appears to often be heterogeneously distributed across the genome (Nosil *et al.* 2009) such that it may not be reflected in general molecular surveys of variation. However, recent advances in molecular genetics, now being applied to the study of adaptation in Atlantic salmon (Bourret *et al.* 2012) and other species (Bradbury et al 2010; Poulsen *et al.* 2011), are providing increasingly accurate molecular assessments of adaptive divergence at the population level within species. These studies confirm that arbitrary estimates of molecular divergence often underestimate adaptive divergence.

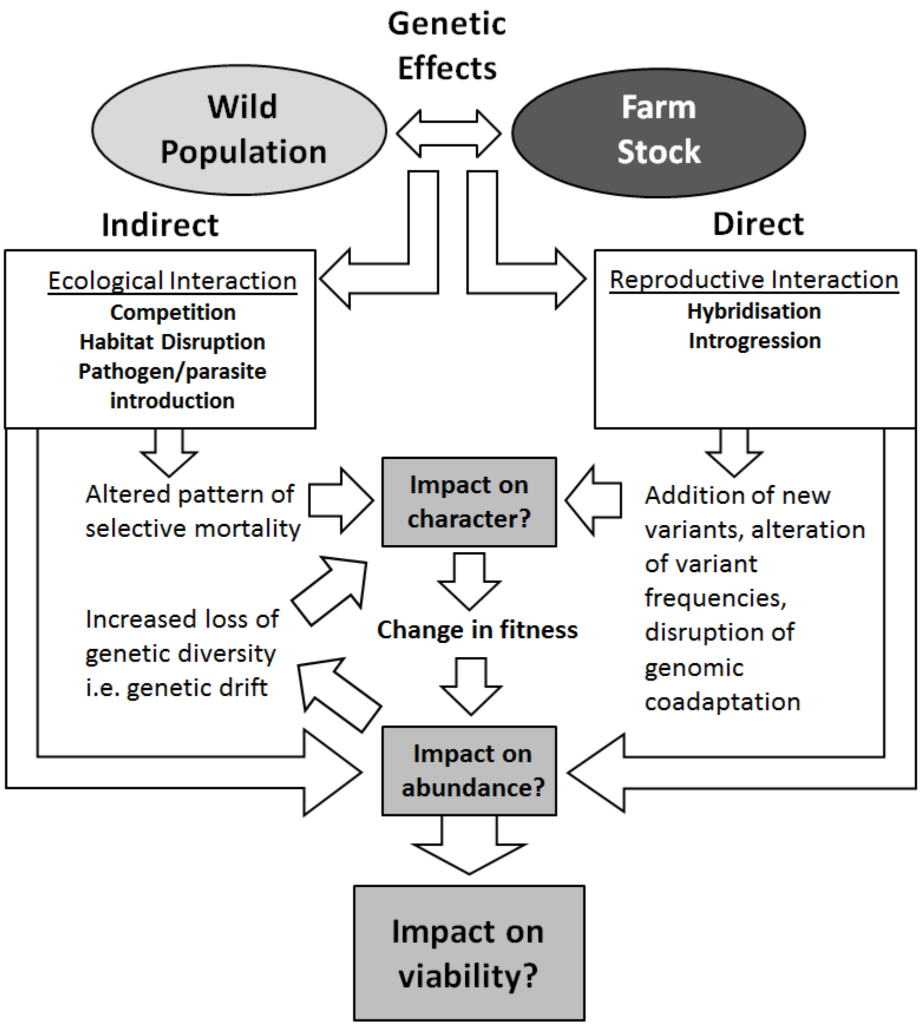

Figure 2 - Diagrammatic overview of pathways of direct and indirect genetic effects arising from interactions between wild and farm populations.

Molecular markers remain the main source of insight into the evolutionary divergence of populations within most species. Given observations in other species, in Atlantic salmon they support the view that substantive and significant adaptive divergence among populations occurs, for which there is already a compelling, if circumstantial, case (Garcia de Leaniz *et al.* 2007). However, they are of limited use as a basis for drawing robust specific inferences about the genetic, phenotypic, and demographic consequences of interactions between farmed and wild fish. Of more value are studies of quantitative and phenotypic variation in performance-related traits that address issues such as *genotype x environment* interactions and phenotypic plasticity. In this regard, assessments of Darwinian fitness derived from reciprocal transplants or common garden experiments, which compare the phenotypes and performance of wild and farm fish and different generations of hybrid offspring, are particularly informative (e.g., McGinnity *et al.* 1997, 2003). However, to assess the implications of observed adaptive differences requires consideration of the interaction of a complex set of genetic, phenotypic, and demographic parameters. This is something that requires the use of sophisticated predictive modelling approaches (Gilbey *et al.* 2007).

GENETIC DIFFERENTIATION OF NORWEGIAN FARMED AND NEWFOUNDLAND WILD SALMON

Adaptive divergence between Norwegian farm and Newfoundland wild populations will have two components:

1) naturally evolved evolutionary differences between wild Newfoundland and wild Norwegian populations; and
2) man-mediated genetic changes caused by domestication and artificial selection on Norwegian farm salmon (Figure 3).

These are likely to overlap to some degree but also to be different as the selective processes and time frames involved not the same. Short-term evolutionary processes are expected to be fundamentally different than long-term processes (e.g., Barrett and Schluter 2008). At the same time, while differences associated with domestication and artificial selection may be shared across farm strains, significant differences can be expected in selective responses where the wild genomes on which stains are based are co-adaptively divergent. No studies directly comparing the genetics of Newfoundland wild populations and Norwegian farm strains have been carried out but what indirect evidence is available is reviewed.

(MIS)PERCEPTIONS FROM TAXONOMY

Taxonomy gives a simplistic view of genetic diversity within and among populations and, as a consequence, there is a serious risk of misperceptions regarding the divergence of Norwegian farm and Newfoundland wild salmon given their assignment to the same designated Linnaean species, *Salmo salar* L. This is because, unfortunately, as “Once a Latin binomial or trinomial is in the literature the group of organisms to which it refers almost automatically assumes an aura of reality that may or may not be commensurate with the taxon’s true evolutionary distinctiveness” (Avise and Hamrick 1996). Hand in hand with this, there is a general tendency, even amongst biologists, to assume a taxon once defined is monotypic and effectively invariant. In turn, there is the assumption that all populations with the species can be crossed successfully without incurring outbreeding depression. At the same time, as a legacy of agricultural genetics and observed heterosis when inbred cultured lines are crossed, it is often assumed that crossing different populations will invariably lead to heterosis. In the case of the Atlantic salmon, this view is encouraged by the relative morphological uniformity of populations and widespread success in producing first generation interpopulation hybrids under benign, artificial culture conditions.

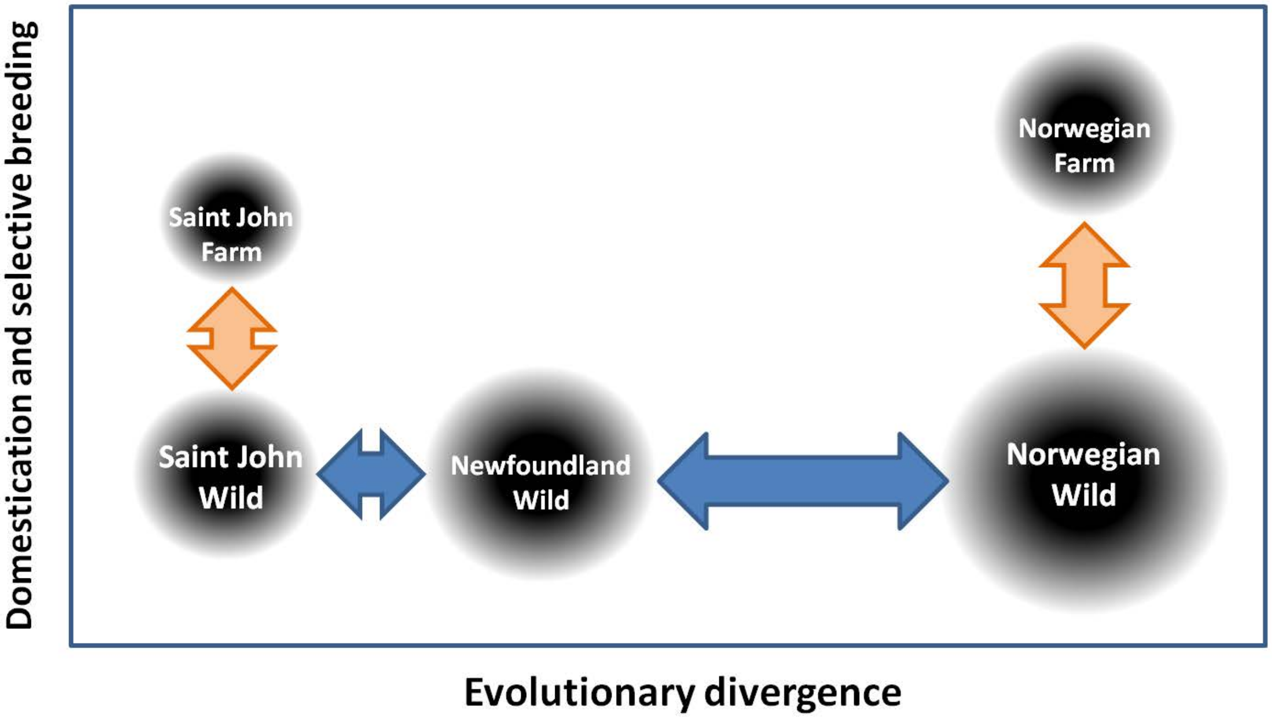

Figure 3 - Conceptual view of divergence between wild Newfoundland salmon populations and Norwegian farm strains compared to the Saint John River farm strain.

Species designations often fail to reflect evolutionary diversity (e.g., Hey 2006) as morphological divergence, at the heart of most designations, is not inextricably linked with it. Though morphology has proven a good proxy in many cases, often it has not. In many cases the absence of morphological distinctiveness among populations is not correlated with an absence of reproductive isolation and genetic studies are revealing an increasing number of "cryptic species" (Bickford *et al.* 2007). The reality is that there is a continuous spectrum of evolutionary divergence among populations and a variable relationship of morphological distinctiveness with evolutionary divergence. As such, reproductive isolation between some designated species may be incomplete. However, within some designated species it may be highly evolved between some populations, as may adaptive divergence. Thus, species designations must be considered to be working hypotheses of evolutionary divergence.

The history of salmon population classifications illustrates our uncertainty regarding their evolutionary status. The first populations designated as *S. salar* were Swedish salmon stocks by Carl von Linnaeus in 1758. Prior to 1947, however, fish now considered as *S. salar* were variously classified on the basis of morphology and biology as distinct species and subspecies (McCrimmon and Gots 1979). Fish in Iceland were initially designated as *S. nobilis* (1774), and some in the British Isles as *S. gracilis* (1865), in the White Sea *S. brevipes* (1882), while anadromous stocks in North American were generally considered from the mid-19th century to be *S. salar*. In Europe, the names *Trutta salar relicta* (1863) and *S. hardinii* (1866) were applied to nonanadromous salmon in Lake Ladoga, Russia and Lake Vanern, Sweden, respectively. In North America, nonanadromous forms in the eastern USA and Canada were assigned to *S. sebago* (1854) or *S. gloveri* (1856), with more northerly nonanadromous forms in Quebec given the subspecies designation of *S. salar ouananiche* (1896). However, more extensive and comprehensive comparative morphological assessment indicated that, at least on the basis of morphology, the designation of these various forms as distinct species or subspecies was unsustainable. Yet subspecific designations such as *S. s. sebago* for nonanadromous forms are still occasionally applied by some biologists (e.g., Vuorinen 1982) and can still be found in current popular science accounts of the species.

The modern view of the species as evolutionarily cohesive, homogeneous, and monotypic is attributable to Wilder (1947). He carried out a superficial morphological assessment of both anadromous and non-anadromous "salmon" from a small number of broadly representative North American and European rivers and found no evidence of consistent morphological divergence. However, increasingly biological studies show populations currently assigned to *S. salar*, while monophyletic, to be biologically and evolutionarily diverse (King *et al.* 2007; Webb *et al.* 2007). In particular, despite superficial morphological similarities, North American and European populations clearly represent distinct evolutionarily groups justifiably designated as different subspecies (King *et al.* 2007), a view increasingly accepted (e.g., Brenna-Hansen *et al.* 2012), within which there is less marked, but still significant, evolutionary divergence. More controversially, based on divergence from wild stocks due to artificial selection and domestication, it has been proposed that farm salmon also be viewed as a distinct, polyphyletic subspecies *S. salar domesticus* (Gross 1998). Given the dramatic morphological, behavioural, and genetic divergence of cultivated species from wild progenitors (e.g., dogs), such a view, while contentious, has arguable biological merit.

WILD NEWFOUNDLAND STOCKS: POPULATION STRUCTURING AND GENE FLOW

In Newfoundland, salmon encompass both anadromous and nonanadromous populations, with both types found in most rivers, except in the southwest,and with non-anadromous populations in lakes and rivers both above and below impassable falls including on the south coast. Molecular studies show that they have a polyphyletic origin. In most river systems, they have evolved *in situ* from anadromous colonisers through the development of transient or permanent barriers to migration (Verspoor 1994; Verspoor *et al.* 2005) overlaid by populations of anadromous salmon established by subsequent waves of anadromous colonists. These forms are highly divergent both genetically and phenotypically (King *et al.* 2007; Webb *et al.* 2007; Verspoor pers. comm.[1]) and often have evolved reproductive isolation allowing them to exist in sympatry (Birt *et al.* 1991; Verspoor 1994; Verspoor and Cole 1989, 2005). However, they appear to have the capacity to hybridise successfully in culture (Hutchings and Myers 1985), though survival to maturity and reproductive fitness of F_1 and backcross hybrids is unknown. Thus, where nonanadromous populations are not landlocked, there is a potential for indirect and direct genetic interactions with farm fish.

A variety of molecular marker studies of anadromous populations confirm the existence of extensive inter-river population structuring in anadromous Newfoundland salmon. This was initially indicated by allozyme studies (Verspoor *et al.* 2005). More recently, Palstra and Ruzzante (2010), using microsatellite markers, found that despite documented demographic instability, population structure remained temporally stable over a period of six decades (1951-2004), a stability also seen as regards protein variation (Møller 2005). Stability was also indicated in respect of effective population size in most rivers where reliable estimates could be obtained (Palstra *et al.* 2007). Furthermore, the data indicate that contemporary gene flow is low, and, where it occurs, is often asymmetric, intermittent, and linked to changes in population dynamics. Additionally, directionality of migration appears to vary over time, with gene flow from large to small populations over contemporary timescales, and the converse over longer evolutionary timescales. The patterns of variation imply that gene flow is not sex-biased but is regionally variable and linked to the demographic and life-history characteristics of populations. As well, they suggest that factors impacting the demographics of individual populations can affect the genetic stability of populations on a broader geographical scale. This indicates a need

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for caution in making generalisations about what happens as regards impacts on structure and gene flow in a given region based on findings elsewhere. It also highlights the need for independent, locally based studies if perturbations affecting population demographics and the genetic integrity of populations in a region are to be accurately assessed.

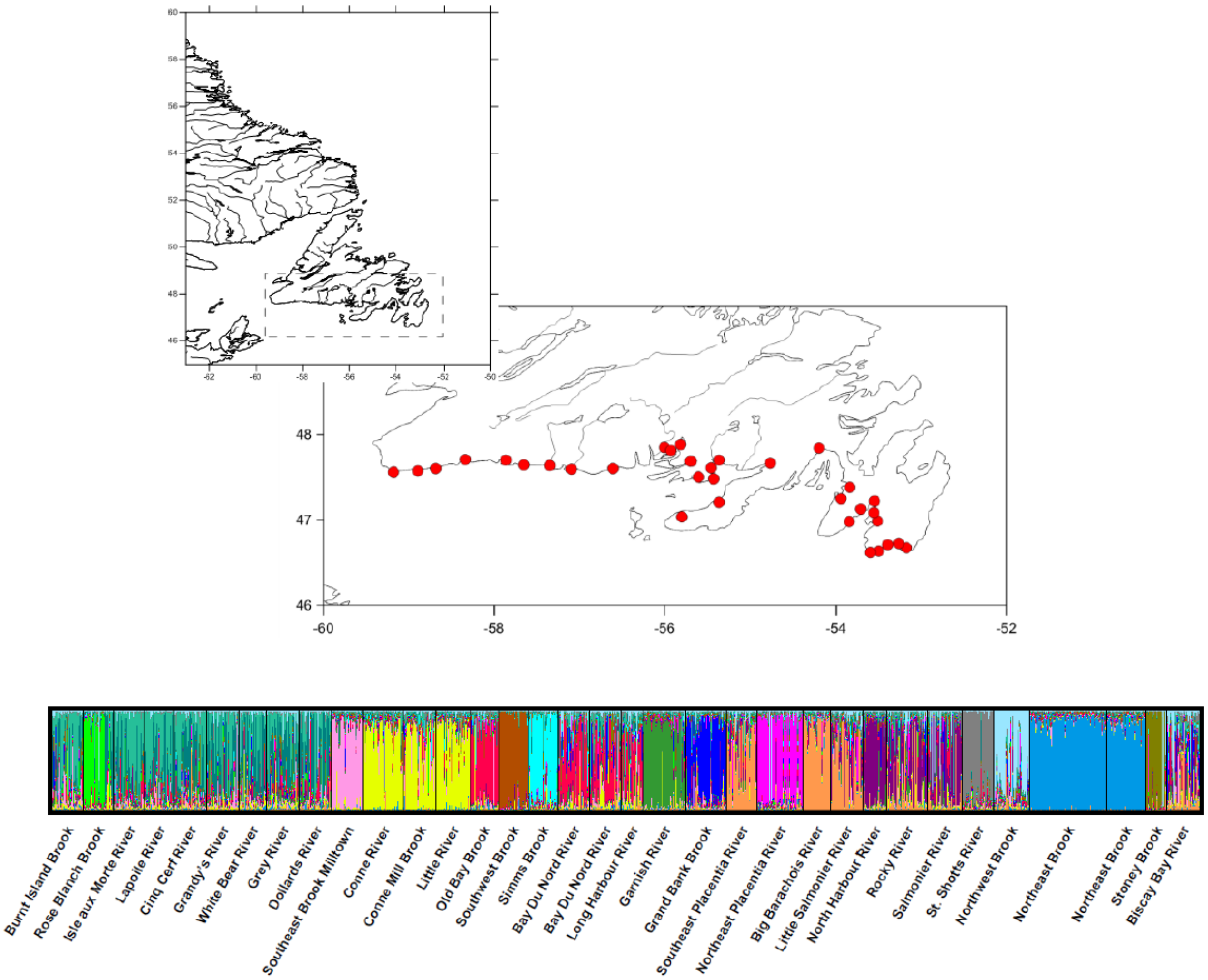

Figure 4 – Visualisation of genetic relatedness of salmon populations in rivers along the south coast of Newfoundland based on a suite of 15 microsatellite marker loci derived using the programme STRUCTURE. The coloured lines between the black lines represent the genetic character of individuals. The more similar the patterns for a given population, the more closely related they are. Note that most populations are highly divergent. (Modified from Bradbury et al. 2014).

Most recently, Bradbury *et al.* (2014) further examined microsatellite variation in Newfoundland and Labrador in relation to landscape features and reported significant associations with both climate (temperature, snowfall, and pH) and habitat area. These associations suggest strong influences of both genetic drift and adaptation to climate on local gene flow and metapopulation structure. The largest subdivision among populations was observed between those on the island and on mainland Labrador, consistent with life history and phenotypic differences. Among populations on the island, the south coast was characterized by the largest population structuring both among individual rivers and larger regions. This view is reinforced by mitochondrial DNA data (Verspoor pers. comm.[1]). At Northeast Brook, Trepassey, on the Newfoundland south coast, Johnstone *et al.* (2013) examined demographic and genetic estimates of effective size and concluded that counts of anadromous individuals are inadequate to explain genetic effective size, highlighting the large contribution of early male maturation in freshwater in this region. Taken together, this existing work on salmon population structure in Newfoundland indicates significant structuring is present, particularly along the south coast, where structuring is temporally stable over decades and is associated with fine geographic scale local adaptation and habitat characteristics. Studies of landscape, climatic, or demographic correlates of spatial genetic connectivity can provide insight into processes which regulate diversity and, in particular, on adaptive divergence. However, it is difficult to conclusively demonstrate the relative importance of local selection, dispersal, and genetic drift. These results echo early work on allozymes (Verspoor *et al.* 2005) and associations of variation with life history and environmental parameters such as proportion of grilse (Verspoor 1986) and lake-use (Verspoor pers. comm.[1]).

This general view of adaptive divergence is supported by observations of phenotypic variation, though in most cases, studies of the heritability of phenotypic traits are lacking. Within stocks of nonanadromous salmon, populations in Newfoundland show considerable behavioural variation. Some populations are stream resident (potamodromous) and others lake resident (limnodromous), both showing little migration during their life cycle. However, others spawn and spend part of their juvenile phase in a tributary stream and then migrate into a lake where they feed, mature, and live as adults (adfluvial) (Gibson and Haedrich 2006), many showing high levels of iteroparity. Among anadromous stocks, phenotypic population differentiation is seen in respect of life history traits such as smolt length, smolt age, egg to smolt survival, size at maturity, sea age at maturity, marine survival, and incidence of male parr maturation in freshwater (Dalley *et al.* 1983; Hutchings and Jones 1998). For instance, average smolt age in anadromous populations across the south coast of Newfoundland has been shown to vary from 3.1 to 4.0 years (Bradbury pers. comm.[2]). However, it is difficult to draw any inferences regarding the extent to which differences reflect underlying genetically-based adaptive differentiation in these traits as variation in many traits is under both genetic and environmental control (Webb *et al.* 2007). One aspect of the behaviour of many Newfoundland salmon populations is the migration of young juveniles into lakes to feed for a number of years before smolting and undergoing their marine migration. Such behaviour has not been generally reported for European populations (Klementsen *et al.* 2003) and has been argued to represent an adaptive behaviour in Newfoundland salmon (Hutchings 1986) that increases growth and survival.

GENETIC DIVERGENCE OF WILD NEWFOUNDLAND AND WILD NORWEGIAN SALMON

Genetic divergence among populations has many manifestations. At its most basic level, it relates to genomic differences with regard to the size, structure, organisation, and compartmentalisation of the DNA into chromosomes and functional regions referred to as genes. However, it is the expression of these genes in interaction with the environment that defines whole organism characteristics related to gene translation, transcription, development, physiology, and morphology (i.e., the phenotype). It is this latter facet that is crucial to defining individual fitness and the character, abundance, and viability of populations at the local level. What is known about the divergence of wild Newfoundland and Norwegian populations is reviewed.

Consideration of this divergence in the current context must take into account the diversity among wild populations in each of the two regions, particularly as comparisons in the literature are often based on an arbitrary or biased selection of populations. This is most important in respect of wild populations in Newfoundland given that these are the focus of concern, but also because the populations used for the establishment of Norwegian farm strains are not generally amongst those included in comparative studies of divergence. Additionally, the nature and extent of adaptive structuring and gene flow

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among wild Newfoundland populations will be an important factor conditioning the consequences of indirect and direct genetic interactions.

Genomic divergence

Mitochondrial: No variation is seen in mitochondrial DNA (mtDNA) gene order or structure at the interspecific level and, based on a lack of inter-generic variation in this character it is highly unlikely that the two types of salmon (i.e., wild south coast Newfoundland and wild Norwegian) differ in respect of organisation of their mtDNA genomes. A minor divergence in respect of genome size is indicated (So 2006). Though based on the analysis of a few individuals, it suggests that the mtDNA genome of Newfoundland salmon is 3 bp larger than European salmon but no Norwegian salmon were included. This arises from a 2 bp insertion/deletion in the D-loop region and a 1 bp insertion/deletion in the tRNA tyrosine. However, these differences are associated with a 0.6-0.9% divergence between Europe and North America in mtSNP variation; based on general rates of mtDNA evolution, this translates into their having diverged in the order of 500,000 years before present (yrs bp) (King *et al.* 2007). Most of this divergence has no protein coding implications but it cannot be ruled out that other variants affect genome function and relate to co-adaptation with nuclear variation. More wide-ranging restriction enzyme analysis shows that both "European" and "North American" haplotypes are present in some resident and anadromous populations in Newfoundland as well as Labrador and Northern Quebec (Birt *et al.* 1991; King *et al.* 2000; Verspoor pers. comm.[1]). This suggests that some reproductive mixing has occurred, most likely in the early stages of post-glacial recolonisation of the region following the last glacial maximum *c.* 18,000 years ago (King *et al.* 2007). "North American" haplotypes also occur at low frequencies in Northern Europe in rivers of the Kola Peninsula in Russia (Makharov *et al.* 2005). However, populations in Norway and Newfoundland remain highly divergent (Figure 5).

Nuclear: No direct comparison has been carried out between wild Newfoundland and wild Norwegian genomes as regards their nuclear organisation but the general genetic differences between North American and European salmon were recently reviewed by King *et al.* (2007). Based on general cytological comparisons of populations in the two regions, Norwegian and Newfoundland populations are likely to differ in their chromosome numbers. The possibility that some populations in Newfoundland have typical European or intermediate chromosome numbers cannot be ruled out however, given the evidence that the region was colonised by both European and North American salmon following the last ice age (King et al. 2007). The extent of this post-glacial mixing appears to be limited but variable and is associated largely with nonanadromous populations (Verspoor pers. comm.[1]). Studies at the GH1 growth hormone gene found major differences between European and North American populations to be greater than within each region (Ryynänen and Primmer 2004), with some evidence of a low level of post-glacial historical rather than contemporary gene flow.

Recent molecular marker studies of genomic organisation show that in two cases two linkage groups in Scottish salmon found on two chromosomes were combined into single linkage groups in Saint John River salmon (Lubieniecki *et al.* 2010). The same study also suggested there were some additional chromosomal rearrangements but finer scale mapping using SNPs is needed for their definitive resolution. Thus, there is, as yet, no information available in respect of differences in gene ordering or structure within chromosomes in transcribed or untranscribed regions, though differences have been noted between individuals from the two sides of the Atlantic. However, in some well-studied model species, such differences are seen among populations and are often are associated with adaptive population differentiation (e.g., Richards *et al.* 2005).

Major differences have been found between wild Newfoundland and Norwegian populations in the frequencies of gene (i.e., allelic) variants in respect of allozymes, and non-transcribed minisatellite and microsatellite loci (King *et al.* 2007). As Figure 5 shows, as with mtDNA variation, populations are generally much more closely related to other populations on their respective sides of the Atlantic, something also paralleled in respect of nuclear SNPs (Bourret *et al.* 2012). Comparison of the signals from mtDNA and nuclear DNA analysis indicate European genetic types observed at nuclear loci in Newfoundland occur at lower frequencies and are less widespread than is the case in respect of mtDNA variation and are usually uncorrelated (Verspoor pers. comm.[1]).

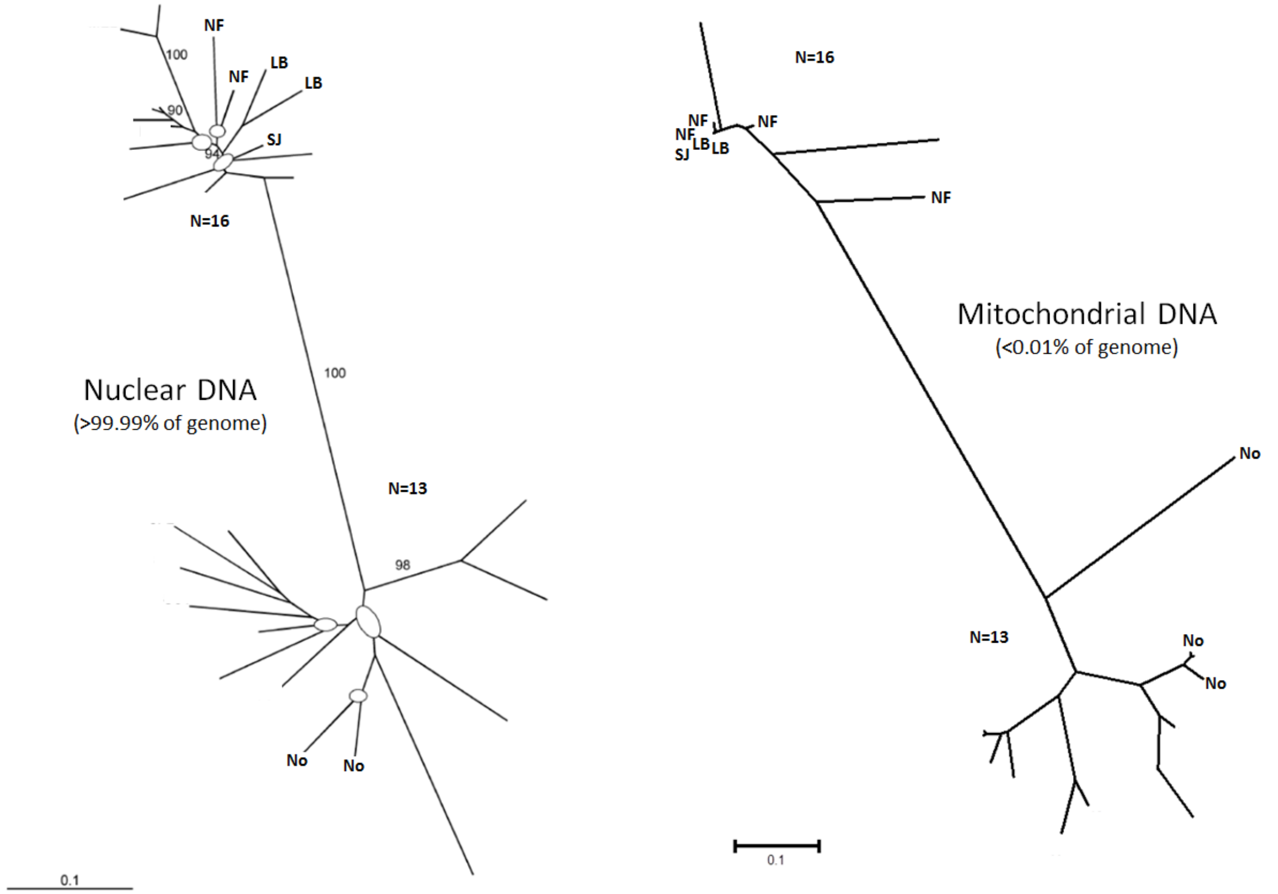

Figure 5 – Comparison of the nearest neighbour joining trees for microsatellite (left) and mitochondrial (right) DNA differentiation based on Nei's genetic distance metric Da. Microsatellite data for 29 populations from King et al. 2001 for 12 microsatellites; mitochondrial DNA data from restriction fragment analysis of the ND1 gene region data from Verspoor (pers. comm.[1]) for a comparable set of 29 populations including many of the same as used in the microsatellite study; NF – Newfoundland, LB – Labrador, No – Norway, SJ – Saint John River.

Phenotypic and adaptive divergence

Anadromous Atlantic salmon populations, at least superficially, appear to be the same across the species range with anadromous as well as non-anadromous populations showing substantive overlap with regard to standard morphological body traits (Wild 1947; Claytor *et al.* 1991). Where less obvious specific morphological and meristic differences occur among populations, local environmental factors, such as water temperature and flow, appear more important than continent-of-origin in explaining variation (Claytor *et al.* 1991). However, adaptive divergence among different populations can occur in the absence of morphological divergence. For example, odd and even year runs of pink salmon *Oncorhynchus gorbuscha* are morphologically more or less indistinguishable but reproductively distinct and highly divergent at molecular loci (Beacham *et al.* 2012). At the same time, hybridisation of distinct populations from the same or different rivers is associated with strong outbreeding depression (Gharrett *et al.* 1999; Wang *et al.* 2007). Equally, similarities in superficial character can have a different genetic basis (e.g., flesh colour in anadromous and non-anadromous sockeye *O. nerka* – Craig and Foote 2001).

Thus morphological assessments can be poor predictors of adaptive divergence. There is often a high degree of heritability of trait differences seen when two populations are reared in a common environment but the character of a population for a given trait may differ across environments due to complex *genotype x environment* interactions during development. This conditioning of phenotype by the environment is known as phenotypic plasticity and means that the character of a population can alter when it is placed in a new environment and revert to a previous state when placed in its old environment. Phenotypic plasticity is often observed with regard to life history traits, such as smolt age, growth rate, age of maturity, proportion of early maturing male parr, etc., though most of these also have a heritable aspect to their variation (Webb *et al.* 2007). Some life history traits, such as anadromy, seem to be expressed independently of environment provided that free movement to the sea is allowed.

These biological realities mean that the prediction of phenotype, performance, and fitness of a population in a new environment is difficult based on population behaviour in its original environment, and confounds comparisons of phenotypic performance between populations in different environments. For this reason, it is difficult to draw any conclusions regarding the known phenotypic differences between wild Norwegian and Newfoundland salmon, as regards obvious characteristics such as size at age, smolt age, sea age, age of maturity, etc. (Webb *et al.* 2007), without undertaking common garden or reciprocal transplant experiments. However, salmon in Newfoundland are generally characterised by a high frequency of small grilse (DFO and MNRF 2008) and those in Norway by a high incidence of large multi-sea winter fish; both traits with a known strong genetic component but also subject to major environmental modification (Webb et al*.* 2007). Additionally, Newfoundland anadromous populations are characterised by a high proportion of males that mature in freshwater prior to any marine migration (Dalley *et al.* 1983) and a sex ratio among returning adults strongly skewed towards females.

Another major difference between wild populations in Newfoundland and Norway that is often forgotten, is the incidence of wild populations that remain resident in freshwater systems throughout their lifecycle. In Newfoundland, nonanadromous populations occur in most river systems and often have free passage to and from the sea (Scott and Crossman 1973; Webb *et al.* 2007). In contrast, such populations are rare in Norway and where they are found are landlocked by impassable falls that allow only emigration (Berg 1985). Such freshwater residency is most likely the legacy of post-glacial landscape development and environmental change, and is clearly heritable, reflecting a major, though not diagnostic, phenotypic and adaptive difference between populations in the two regions.

There is no direct evidence relating to the degree of adaptive genomic divergence of wild Newfoundland populations from those in Norway, as is the case in respect of Atlantic salmon populations found in most regions of the species' range (Garcia de Leaniz *et al.* 2007). This probably reflects both the difficulty in undertaking studies of adaptive differentiation as well as the absence of reasons for doing so, particularly in respect of populations from different sides of the Atlantic Ocean. Yet, there is a compelling case that adaptive differentiation among populations occurs at the tributary level within rivers, between rivers within regions, and between regions on both sides of the Atlantic (Garcia de Leaniz *et al.* 2007), although the full extent of this differentiation and the full spectrum of traits to which it relates has only begun to be understood. It is clear, however, that adaptive divergence increases with geographical distance, both in Atlantic salmon as well as other salmonid species (Fraser *et al.* 2011). The differences between anadromous and non-anadromous populations found in the same rivers in Newfoundland (Verspoor and Cole 1989; 2005) illustrate the extent of adaptive divergence even on small geographical scales. Equally, McGinnity *et al.* (2004) showed that the non-native wild salmon from a neighbouring river had only 30-40% the life-time fitness of the native stock in the Burrishole River in western Ireland.

Support for the view that very substantive co-adaptive genomic divergence exists between Newfoundland and Norwegian salmon comes from the study of Cauwelier *et al.* (2012) where the impact of mixing salmon genomes from the Eastern and Western Atlantic was assessed. They examined the survival to hatch of F_1 and backcross hybrids between Newfoundland resident salmon and an anadromous Scottish population under benign hatchery conditions. While egg survival in the F_1 families was similar to pure parental crosses, in the backcross of F_1 males to Newfoundland females egg mortality was 100% whereas egg mortality in backcrosses to Scottish females was more or less normal. This type of fitness depression is more typical of that observed in interspecific salmonid crosses and is consistent with genomic disruptions known as Dobzhansky-Muller incompatibilities caused by the mixing of different co-adapted genomes. Thus the study clearly points to a major adaptive divergence that may relate, at least in part, to the substantive evolved genomic divergence between European and North American populations. However, it may also relate to incompatibilities arising from mixing adaptively divergent anadromous and non-anadromous populations. In any case, developmental stability appears to be strongly linked to genomic coadaptation (Clarke 1993). That the latter may be important is suggested by the fact that Boulding *et al.* (2008) report successfully producing backcrosses between Norwegian and Canadian salmon under hatchery conditions, though this involved farm salmon strains. However, Whether some compromise or asymmetry in backcross viability was observed, it was not reported. What it does suggest is that the extent of adaptive divergence may be highly dependent on the populations compared and not strictly related to evolutionary divergence measured by arbitrary molecular markers. Furthermore, the observed maladaptation can be expected to be more apparent when hybrids are reared under more challenging natural conditions.

GENETIC DIVERGENCE OF FARMED NORWEGIAN STRAINS

Differences between farmed strains and their wild populations of origin can have three causes:

1) relaxation of natural selection on some traits due to a more benign environment allowing phenotypes that might otherwise have died to survive resulting in a shift in population means;
2) domestication selection response to the culture environment; and
3) selection for economic traits.

The genetic character of Norwegian farm strains has been reviewed by Bentsen and Thodsen (2005), Ferguson *et al.* (2007), and Thorstad *et al.* (2008). Genetic differences between farm strains and wild salmon stocks are defined, in the first instance, by the wild origin of the farm strain and chance genetic changes related to the use of small numbers of brood fish for strain foundation and perpetuation. Secondarily, it may also be conditioned by the selective use of one component of a wild stock with regard to age cohort or phenotype. This is usually associated with a loss of genetic diversity, both neutral and potentially adaptive. Once established, further genetic differences can arise from both intentional and unintentional domestication selection, as well as artificial selection for performance traits (e.g., higher growth rates, feed conversion efficiency, disease resistance, body shape, and fat content). Current Norwegian strains are derivatives of two main breeding stocks, Mowi and Sunndalsøra, with the latter giving rise to a second, Kyrksæterøra. The Mowi strain, representing ~25% of eggs used in the industry since the middle of the last decade, was established mainly using fish from three rivers in central Norway famous for their large size and late maturity. The Sunndalsøra and Kyrksæterøra strains, currently part of the Aquaforsk breeding programme, were established from 40 Norwegian and one Swedish river in the early 1970s, with the contribution of the majority of the rivers dramatically reduced after only 4 generations, with fish from only one or two rivers dominating in each year class (Gjedrem *et al.* 1991; Gjøen and Berntsen 1997). The original four breeding lines (four distinct cohorts with a four year life cycle) are in the process of being integrated into a single strain (Thorstad *et al.* 2008). This narrowing of the genetic diversity is attributed to founder effects and major genetic differences among the original stocks in key performance traits such as growth rate, age of maturation, and size at time of slaughter.

Genomic divergence

The few tens of generations that separate farm strains from their wild stocks of origin suggests that there is unlikely to be any major divergence of farm stocks from their wild progenitor populations in respect of basic genomic organisation (e.g., chromosome structure or physical gene linkage on chromosomes) though this has not been explicitly examined. The differences that have been reported relate to allelic variation at protein genes, at mitochondrial and nuclear DNA loci in respect of microsatellites (Ferguson *et al.* 2007; Thorstad *et al.* 2008), and more recently in respect of nuclear SNPs (Karlsson *et al.* 2011). What these studies show are general reductions in allelic variation and in mean levels of heterozygosity up to 50% and the differentiation between strains and wild founder populations are two to six times higher than among wild populations (Thorstad *et al.* 2008). However, interestingly, a recent study found higher levels of mtDNA diversity than in wild stocks (Karlsson *et al.* 2010), most likely because of the use of larger numbers of females per male in mating schemes and the use of a large number of founder populations in some strains.

Genetic divergence at regulatory or structural gene loci, will be partially conditioned by selection for heritably variable economic traits, and thus, can be expected to be higher overall (Merilä and Crnokrak 2001). In line with this, Vasemägi *et al.* (2012) in a comparison of three independent domesticated/captive Atlantic salmon strains and their wild progenitor populations, found ten genomic regions which showed signs of directional selection. One identified gene was the same as a gene indicated in the Roberge *et al.* (2005) study to show a shift in farm strains using a different approach. For the remaining genomic regions examined Vasemägi *et al.* (2012) found little evidence of parallel changes in the three study farm-wild pairs. These studies seem to suggest that some genetic differences will be shared across farm strains but that others will be strain specific. The latter may occur if selective responses are conditioned by the overall co-adaptive nature of the genomes of the wild progenitors, each of which will, to some extent, be unique such that the genetic response in each case may be different even though the traits selected for will, to a large extent, be the same.

The often unique genetic changes that may evolve in farm strains may be of two types. In respect of genes controlling quantitative traits such as growth and body shape, they are likely to involve allelic changes that have additive effects. Thus in respect of such traits, the expectation when farm escapes interbreed with wild fish, is that the performance of hybrids and backcrossed individuals will be intermediate, proportional to the relative parental genomic content. However, many traits will be non-quantitative and involve regulatory genes where the control of gene transcription is often non-additive, such that phenotype and fitness of hybrids may be non-intermediate and difficult to predict.

Roberge *et al.* (2007) compared genome-wide gene transcription profiles of Norwegian wild salmon with second generation wild backcrosses and found that > 6% of the detected genes exhibited significantly different transcription levels, whose differences exceeded those between pure farmed and wild strains. This observation is consistent with non-additive gene interactions and suggests that artificial selection has led to changes in genomic control of gene transcription involving epistatic interactions. Transcription shifts outside the parental range could be particularly maladaptive and, in contrast to additive gene effects, lead to even higher levels of mortality in second generation hybrids, giving a lagged effect on population demographics. With such effects the outcome of reproductive mixing farm and wild genomes becomes much more difficult to predict. This unpredictability will be compounded by non-Mendelian epigenetic changes (e.g., by DNA methylation, induced by the rearing environment) (Li and Leatherland 2012). Such epigenetic changes can be transmitted from parent to offspring but may alter again after over a few generations when the genome finds itself in a new environment.

Thus what is known shows it is difficult to predict both the phenotypic and fitness consequences of hybridisation. Both can be expected to vary depending on the specific farm strains and wild populations involved, and with the environment in which the interaction occurs. For this reason it is important to assess phenotypic and fitness consequences on a situation specific basis.

PHENOTYPIC AND ADAPTIVE DIVERGENCE

No direct comparisons have been reported of Norwegian farm strains and wild Newfoundland populations either in culture or in the wild; neither is there any reported study comparing the performance and adaptive divergence of Newfoundland and North American farm strains in the wild in Newfoundland. Yet such studies are essential to address issues of *genotype x environment* interaction and phenotypic plasticity, and to evaluate the implications of direct genetic interactions. However, given the diversity of wild populations in Newfoundland, undertaking a representative and informative range of comparisons would require a large number of common garden studies. In the absence of such studies, it is only possible to draw general inferences from work undertaken in other contexts, recognising their limitations and considering how these will be extended by the naturally evolved divergence of wild Newfoundland and Norwegian populations.

Observed heritable divergence has been documented in relation to traits such as growth rate, body size, survival, delayed maturity, stress tolerance, temperature tolerance, disease resistance, flesh quality, and egg production. Unintentional correlated changes also occur for fitness-related traits (e.g., survival, deformity, spawning time, morphology, aggression, risk-taking behaviour, sea water adaptation, and growth hormone production) (Jonsson and Jonsson 2006; Ferguson *et al.* 2007; Thorstad *et al.* 2008; Solberg et al. 2013). The extent of divergence is variable. For example, genetic gains of >100% have been found for growth, and 20% for feed conversion efficiency, after five to six generations such that in general farm salmon outgrow wild salmon both in culture and the wild (Ferguson *et al.* 2007). Some trait variation observed also appears to reflect phenotypic plasticity (e.g., head, fin and caudal peduncle size) (Fleming *et al.* 1994). There also appears to be differences in behaviour in the wild in terms of key biological factors such as spawning time, mating success, distance of spawning migration in rivers, though the extent to which these are conditioned by genetic divergence is unclear. In the case of escaped juvenile fish, phenotypic differences in size compared to wild fish will reflect both the culture environment as well as the escapee's heritable capacity for faster growth. Where overall fitness comparisons have been made, wild local salmon populations show superior survival to Norwegian farm strains, be in it Ireland (McGinnity *et al.* 2003) or Norway (Fleming *et al.* 2000; Skaala *et al.* 2012).

More recently, phenotypic studies have been extended from morphological and behavioural traits to the direct analysis of gene expression. Roberge *et al.* (2005) studied the transcription profiles of 3557 genes in the progeny of farmed and wild Atlantic salmon from Norway and Canada grown in controlled conditions. These farm fish had been subject to five to seven generations of artificial selection. They found heritable changes in gene transcription profiles of 20% for ~1.5% of the expressed genes, with 16% of genes exhibiting parallel changes showing significant differences in both strains, strongly suggesting a common directional selection was responsible. Genes coding for enzymes involved in energy metabolism (e.g., glycolysis, citric acid cycle, and oxidative phosphorylation) were all under-transcribed in farmed salmon of both strains while in other cases, genes only showed under- or over-regulation in one farm strain. This led the authors to suggest that pre-existing genetic differences between populations and how they regulate metabolic processes can cause differences in the way farm strains respond to selection.

In other work, Solberg *et al.* (2012) found farm salmon to have elevated to IGF-1 expression, a gene linked to growth. Fraser *et al.* (2008) found wild salmon from acidified rivers in Nova Scotia survived better under acid conditions in culture than farm fish but there was no difference under non-acid conditions. Differences in gene expression have also been found under common conditions in respect of gene transcription profiles (Normandeau *et al.* 2009). There is also considerable evidence that many of these differences are underpinned by both additive as well as non-additive, epistatic gene effects (Roberge *et al.* 2007; Normandeau *et al.* 2009; Debes *et al.* 2012), and support the view that differentiation is partially co-adaptational. The role of epigenetic changes associated with processes such as DNA methylation has not been explored but it seems likely such changes will occur in farm stocks, as there is a suggestion that these may be manifest in the culture environment (e.g., Christie *et al.* 2012a), and contribute to adaptive divergence.

POTENTIAL FOR AND RISKS OF INTERACTION CONSEQUENCES

Genetic interactions can potentially impact the character or abundance of wild populations in ways that affect their evolutionary legacy, intrinsic socio-economic value, and viability. A potential for negative consequences to wild populations exists in any situation where farmed strains are introduced outside of a system of complete isolation and containment, unless:

1) there is no co-introduction of exotic pathogens or parasites;
2) the presence does not lead to amplified levels in the wild of native parasites and pathogens; and
3) they are genetically identical to local wild populations.

However, none of these is the case in the current context. This potential is considered in more detail below in relation to indirect and direct genetic effects.

On the south coast of Newfoundland, concern focuses on anadromous salmon populations (DFO 2012). However, nonanadromous populations should also be included where they are not isolated from interactions by impassable barriers to upstream migration. The occurrence of such nonanadromous populations has been little studied but they appear to be widespread, having been found in rivers on Cape Race and along the Northeast coast of Newfoundland, and may well occur along the south coast.

INDIRECT GENETIC EFFECTS

Indirect genetic interactions can have negative consequences on wild native populations in two ways:

1) by changing the environment so as to differentially alter the mortality or reproductive success among genetic types in the wild population; and
2) by depressing abundance and reducing adaptive genetic diversity.

Changes to the selective environment could arise through an induced increase in a naturally-occurring pathogen or parasite. Changes in the genetic variability of MHC genes (major histocompatibility complex) involved in immunity have been observed in wild brown trout in Ireland following the establishment of salmon farms. This was thought to be the result of stronger selection in favour of particular alleles and against others (e.g., Coughlan *et al.* 2006; De Eyto *et al.* 2007; De Eyto *et al.* 2011) due to changes in phenotypic resistance. In North America, MHC II variation has been implicated in natural temperature-related pathogen resistance (Dionne *et al.* 2007). In the case of the MHC genes, lost variants are likely to be associated with resistance to other pathogens to which the population may be naturally exposed from time to time. As such the result of this loss would be a change in the longer-term adaptive capacity of the population. Such changes may also occur in respect of newly evolved strain characteristics in a natural pathogen. For example, it is known that sea lice can evolve resistance to chemotherapeutants and this can lead to elevated sea lice levels despite treatment (Denholm *et al.* 2002; Jones *et al.* 2006). Further research has also shown that *in situ* evolution of virulence is possible (Pulkkinen *et al.* 2010).

However, the situation is likely to be more complex than described as our understanding of variation within named pathogens is limited and a pathogen that appears to be common between two areas may in fact be genetically divergent in ways that affect interaction dynamics. Differences in genetics and virulence are seen among VHSV strains from different regions (Benmansour *et al.* 1997), as has also been found with respect to ISAV (Ritchie *et al.* 2009). Furthermore, wild salmonids may not have inherent resistance to different or newly-evolved strains, as illustrated by the susceptibility of non-Baltic Atlantic salmon populations in Europe to the mongenean skin parasite, *Gyrodactylus salaris*, with the result that when exposed, susceptible populations can go extinct. Given the high level of divergence between Norwegian farmed and Newfoundland wild populations, such problems have a high risk of affecting interaction dynamics. This may be a particular issue as regards highly divergent non-anadromous populations. The extent to which this might be the case in the current context is unknown.

Overall strain variation in pathogens and parasites is poorly understood even though a great deal of research has been carried out on salmonid diseases. Little systematic work has been done on population level interactions between wild populations and farmed stocks (Bergh 2007). A review carried out by leading fish disease specialists across Europe (Raynard *et al.* 2007) concluded that current understanding of disease interactions between wild and selectively-bred aquaculture populations, and its impacts on biodiversity, is inadequate for drawing robust conclusions. They also raise the issues discussed above, that the geographical scope of many existing studies is too limited, and that disease presence may be very contingent on local conditions relating to the environment (e.g., temperature, water quality, etc.), as well as host population size and density, both in culture and in the wild. They state that some exchange of pathogens is inevitable, particularly in net-pen culture, though the risk and extent can be minimised if husbandry practises are based on sound epidemiological principles for disease control. They also conclude "... that infected farm populations pose a significant infection risk to wild populations". This suggests that the greatest concern is the introduction of exotic pathogen strains and the local evolution of virulence under conditions conducive to disease outbreaks and high pathogen loads (e.g., high rearing densities and high stress levels). In this context, the loss of genetic diversity in wild populations can make them more vulnerable.

The problems of exotic disease may be attenuated over time by selection for resistance. However, this is likely to take decades, at best, to be realised; it also assumes that local wild populations are not first driven to extinction. The latter has happened with regard to *G. salaris* when it was introduced outside of its native range in the early 1970s, most probably as a result of transfers of Baltic salmon to hatcheries in Norway (Johnsen *et al.* 1999). However, the impact of *G. salaris* introductions to river systems outside of its native range has been variable (Olstad 2013). While the reasons for this are not clear, it is most probably due to variable combinations of differences in the genetic character of the introduced parasite and the salmon populations, as well as differences among the environments in which the interactions occurred.

The impacts of disease interactions will not necessarily scale with numbers of farmed fish and may occur where small numbers of farm fish are present if they locally amplify numbers of native parasites (e.g., sea lice). However, impacts will also be conditioned by whether farm fish remain confined to cages or come into close proximity to wild fish as a result of the escape of juveniles into rivers from juvenile rearing facilities, or of adults into the marine environment and their ascendance into rivers to spawn. Multiple factors will interact to condition pathogen transmission and disease development. With the possible exception of sea lice, little is known about distribution of fish pathogens in wild populations or of host resistance and susceptibility, limiting our capacity to predict interactions between farmed and wild Atlantic salmon. However, it is reasonable to expect that disease threats exist and are enhanced by increased farmed fish abundance, all else being equal.

Reductions in wild Atlantic salmon population size from indirect interactions could arise where there is increased mortality of fish through disease or competitive interactions with escaped farm fish or their offspring (e.g., during the juvenile phase of the life cycle in relation to food and territory). These reductions could also occur due to interference with wild spawning by mature escaped fish (e.g., disturbing redds and increasing egg mortality) (Ferguson *et al.* 2007). This will lead to increased inbreeding and genetic drift, and a loss of genetic variation (Youngson and Verspoor 1998) if there is a reduction in population size as has occurred from *G. salaris* introductions in Norwegian rivers. While much of the variation lost may not be adaptively relevant, some will be associated with long-term adaptive capacity to sporadic and transient selective pressures (e.g., extreme environmental conditions or occasional disease outbreaks).

At least initially, both types of introduction inevitably affect population density, altering the frequency of competitive interactions, levels of food availability, or functional responses of predators. Competition for food can be expected as farmed and wild salmon diets will overlap in the wild and the larger farmed juveniles typically outgrow their wild counterparts (McGinnity *et al.* 1997, 2003; Fleming *et al.* 2000; Skaala *et al.* 2012). The size difference increases their food requirements and gives them a competitive edge for territory over wild juveniles. This view is reinforced by farmed fish having heritably greater aggression and risk-taking (Fleming and Einum 1997; Fleming *et al.* 2002); hybrid juveniles often demonstrating trait expression intermediate between farm and wild juveniles (McGinnity *et al.* 1997, 2003; Fleming *et al.* 2000; Skaala *et al.* 2012). Fleming *et al.* (2000) showed that the productivity of the native juvenile salmon population was depressed by more than 30% in the presence of farmed and hybrid juveniles, a general view shared by other studies (Ferguson *et al.* 2007, Skaala *et al.* 2012). The impact of such competitive interactions can be expected to scale with the number and frequency of interactions (e.g., McGinnity *et al.* 2009).

DIRECT GENETIC EFFECTS

Direct interactions arise if farmed juveniles or adults escape and interbreed with wild salmon or other species. Interbreeding has been observed in all studied areas that have farming operations and native salmon populations (Ferguson *et al.* 2007; Glover et al. 2012; Coulson 2013). Breeding among farmed fish in the wild has been observed even outside the species' native range (e.g., Volpe *et al.* 2000). Breeding with wild brown trout *S. trutta* has also been observed (Ferguson *et al.* 2007). Furthermore, Bentsen and Thodesen (2005) concluded that no commercial aquaculture system will be entirely secure, and that some level of escapes will always occur and scale with the size of the operation.

Interbreeding can have direct genetic consequences in two ways. First, like indirect interactions, it can induce reductions in population size with a resulting non-specific loss of both adaptive and non-adaptive diversity. The second occurs through the introgression of new adaptively-relevant gene variants, the alteration of existing gene and genotype frequencies, and the disruption of co-adaptive genomic structure. In principal, these may lead to a positive outcome as regards population character, abundance, and viability (heterosis), or a negative outcome (outbreeding depression), though the latter is most generally the case due to the local adaptation of populations. The population-level consequences from direct interactions will depend on the extent of reproductive interactions and the fitness of hybrid offspring.

Continuous interbreeding between wild Newfoundland salmon populations and Norwegian farmed Atlantic salmon escapes, which have a narrow genetic base, is expected to significantly reduce overall levels of genetic variability, if the escape events are sufficiently large and frequent. This effect has been observed in other salmonid species in relation to supplementation programs (e.g., anadromous steelhead trout, *Oncorhynchus mykiss*; Christie *et al.* 2012b). They found a substantial Ryman–Laikre[3] effect whereby the total effective combined population size of wild and hatchery fish was one-

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[3] The "Ryman–Laikre" effect represents the disproportionate contribution of a small number of broodstock (i.e., from hatchery fish) to the combined wild and hatchery fish population genetic diversity. This will produce in a decrease in the effective genetic size of the wild population, resulting in increased probabilities for inbreeding and a decrease in overall wild population fitness.

third of the original size for wild fish alone, even though the added hatchery fish doubled the total number of spawners. Thus, abundance alone cannot be used as a measure of impact. In respect of Atlantic salmon, a recent review of genetic change in Norwegian salmon populations (Glover *et al.* 2012) found that despite significant levels of farm escapes and known interbreeding, historic wild population structure and divergence was largely retained. This indicates that farmed escapees have had generally limited success in the wild (i.e., cumulative survival and reproduction). In 15 of 21 populations with farmed salmon observed on spawning grounds, no temporal genetic changes were detected. Furthermore, where introgression was noted, these levels were linked to the density of the wild population. The low direct impact was attributed to the reduced spawning success of farmed fish and the reduced fitness of any hybrids that were produced. Despite a lack of alteration in the gene pool, research did not examine whether the interbreeding depressed population abundance through reduced hybrid fitness. Some genetic alteration may also arise from the straying of hybrid fish from one river to another, as well as from direct farm escapes. Maintenance of genetic diversity among wild populations appears to be important for the maintenance of overall wild population abundance as not all populations perform equally well in a given year due to differences in adaptive capacities. If this is so, it is important to conserve natural population diversity. This “portfolio effect” appears to be important in maintaining regional abundance in Alaskan salmon populations (Hilborn *et al.* 2003; Schindler *et al.* 2010). However, its strength and biological significance may be variable depending on the species, location, and status of populations (e.g. Carlson and Satterthwaite 2011). The extent to which it is possible to restore/rescue genetically homogenised populations once their genetic character has been altered is uncertain and most probably far from straight forward (Tallmon *et al.* 2004). In many cases, it must be accepted that the unique diversity of native populations may be permanently lost where they are subject to introgression of genes from non-native fish.

No direct studies of Norwegian farmed salmon reproduction in Newfoundland rivers have been reported. However, where studied, Norwegian farmed salmon are known to breed successfully in the wild in all environments that contain native salmon populations and at least transient feral populations have been found outside the native range in British Columbia (Volpe *et al.* 2000) and South America (Soto *et al.* 2001; Pascual *et al.* 2009). Furthermore, they are even capable of interbreeding with brown trout, suggesting a strong inclination to breed in the wild (Ferguson *et al.* 2007). This demonstrates that there is a high level of phenotypic plasticity as regards reproductive life-history traits, such that it is reasonable to conclude that they will be able to breed in rivers in Newfoundland if they escape from farms. It is also reasonable to conclude that there will be a high probability of interbreeding with wild Atlantic salmon populations. This will be favoured by the fact that runs of adult fish in Newfoundland rivers tend to be dominated by females, reducing male competition, and increasing the role of precocious parr in fertilisation, a situation in which farmed origin parr have been shown to dominate in experimental studies (Weir *et al.* 2005; Ferguson *et al.* 2007). At the same time, both large anadromous and early-maturing male parr tend to stay in spawning condition for a much longer period, increasing the likelihood of a temporal overlap in spawning period between farm and wild populations. However, even if there is no spatial or temporal overlap of breeding, feral populations of farm fish could be established and compete with native populations. This could even occur if the fitness of farm-wild hybrids is depressed compared to both parent populations, particularly if the feral farmed population is reinforced by a continuous input of escapes.

Where interbreeding does occur, and the fitness of hybrids is depressed, overall abundance may decrease due to reduced mean survival rates. The mean lifetime fitness of wild eggs can be expected to decrease and may not be compensated for by the increased deposition of farmed salmon eggs, whose lifetime fitness may be even lower (McGinnity *et al.* 2003; Ferguson *et al.* 2007; Skaala *et al.* 2012). This consequence may involve first generation hybridization as well as backcrossing of hybrids back into the wild population. A recent study by Fraser *et al.* (2010) found developmental rates in embryos of farmed salmon and farmed–wild hybrids (i.e., F_1, F_2, wild backcrosses) to be reduced compared to those of two regional wild populations. They concluded that hybrid developmental rates were sufficiently mismatched to prevailing environmental conditions that they would result in reduced survival in the wild such that repeated farmed–wild interbreeding could adversely affect wild populations. Thus, there is an increasing body of knowledge that suggests that this is a general phenomenon, though some studies have assessed performance in artificial environments where conditions are more benign, giving a false impression of actual fitness differentials.

Available information indicates fitness depression is dependent on the exact nature and extent of the adaptive divergence among stocks, the environment in which the interaction occurs, and the traits being examined (Fraser *et al.* 2010). Where hybrids have depressed fitness, the effective number of wild breeders may be depressed and it is expected that there will be an elevated non-specific loss of both adaptive and non-adaptive variation through increased genetic drift. The loss of the latter may affect long term population adaptive capacity viability, particularly in strongly fluctuating environments or in the face of strong directional shifts in environmental conditions (e.g., climate change) (McGinnity *et al.* 2009).

Where a proportion of F_1 hybrids survive and reproduce, new genetic variants can be introduced into wild populations where farm stocks are of non-local origin, with the likelihood of introduction positively correlated with evolutionary divergence of the farm stock and wild population; the greater the divergence, the greater the number of unique variants the stock will contain. Where farm stocks are of local origin and only a few generations removed from the wild, differentiation will be almost exclusively in respect of gene frequencies, and the loss of variation through genetic drift, as the time will have been insufficient for new mutations to have accumulated. Where farm stocks are non-local in origin, they will contain mutational or recombinational gene variants absent in local wild populations. Dramatic shifts in genetically-based fitness can occur even in a single generation (Christie *et al.* 2012a).

New gene variants can also potentially occur in cultivated stocks through epigenetic gene modification, even after a single generation (Li and Leatherland 2012). These can be induced by the culture environment being different from the wild environment (e.g., in respect of food abundance and quality) and could transfer to a wild stock by interbreeding. In so far as these are induced and encouraged by the culture environment, such variation is likely to be maladaptive in the wild. Though this type of genetic change is poorly understood, it may be reversed in subsequent offspring in the wild.

Major changes to the genetic structure of a wild population can also occur, even where farm and wild populations share the same variants. These changes may occur simply through alterations to the frequencies of existing variants (Figure 6), with even small allele frequency changes dramatically changing genotype frequencies. In so far as this involves adaptive variation, this will further erode mean population fitness until natural selection restores the optimal variant frequencies. How long this takes will depend on the duration and severity of selection, how the genetic variation is expressed phenotypically, and whether this expression is controlled by additive gene effects, gene interactions (i.e., epistasis), or by genes controlling multiple traits (pleiotropy). Compared to wild Newfoundland stocks, the available evidence for Norwegian farmed salmon supports the fact that a high proportion of loci show major differences in their allele frequencies (Verspoor *et al.* 2005; King *et al.* 2001; Bourret *et al.* 2012), at least some of which will be adaptively relevant (e.g., Verspoor and Jordan 1989; Dionne *et al.* 2007).

The nature and extent of epistatic interaction among genes and overall genomic architecture is poorly understood but its existence is supported by a compelling body of evidence (e.g., West-Eberhart 2003). In the few species where it has been studied, it is known to vary among evolutionarily divergent populations and is expected to increase with evolutionary divergence. It is also likely to involve physical and functional interactions and, in so far as it is adaptive, genetic co-adaptation, can evolve in unique and independent ways in each population through selection and genetic drift, depending on its unique historical and environmental circumstances. Where such populations interbreed, the co-adaptation can break down in the hybrid offspring, though the resulting outbreeding depression may only become apparent after the first generation of hybridisation and be asymmetrical in backcrosses (e.g., Cauwelier *et al.* 2012).

All these genetic changes can potentially lead to outbreeding depression where adaptive genomic structure is involved. In general, in the wild, reproductive mixing with non-native populations where populations are locally adapted, will lead to maladaptive genetic changes and outbreeding depression; in contrast to the outbreeding heterosis that is seen in culture when inbred lines are crossed. The severity of outbreeding depression will be dictated by the degree of adaptive and co-adaptive divergence among populations, the amount of interbreeding and whether interbreeding is regular or sporadic. Outbreeding depression in a population will be countered by natural selection and, provided the population does not go extinct first, local adaptation will be gradually restored. However, the more severe the outbreeding depression and the smaller the affected population, the more likely that the fitness depression will decrease population abundance to a point where survival is insufficient to sustain the population in the face of other factors that may naturally depress survival unpredictably.

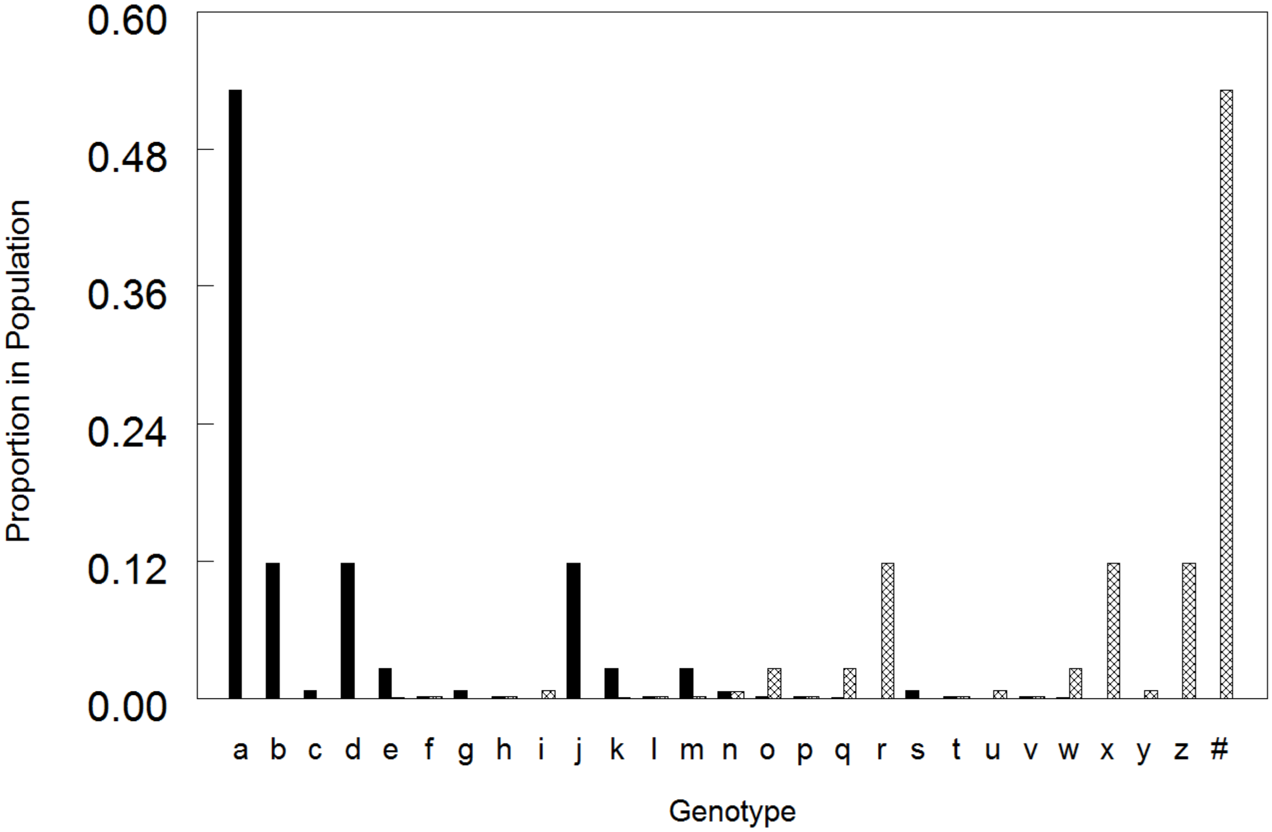

Figure 6 - The extent of genotypic differentiation that will exist between a population before and after the frequencies of its two gene variants at each of three genes have been shifted with the common variant reduced from 0.9 to 0.1, and the rare variant conversely increased. If so, the shift in the relative frequencies of the 27 different possible genotypes are shown. Once the gene frequencies have been shifted, a genotype that only occurred in 1 in 500,000 individuals becomes the dominant genotype and the previously common genotype is only seen in 1 in 500,000 individuals. Thus, in a normally sized wild population of a few hundreds or thousands of individuals, the post-interaction population would, in practice, have few or no genotypes in common with the pre-interaction population. If these genotypes are adaptively different, this could have a major impact on population fitness.

The extent to which direct genetic interactions will cause changes to the character of a population, reducing population fitness and abundance and threatening the viability of local wild populations, will depend on the interplay of a multitude of genetic, demographic, and environmental factors, of which one is adaptive genomic divergence. Predicting the effects of exogenous stocks on local populations requires that the factors relevant to fitness and their interactions, and their local status are known. The reality is that even basic life-cycle and demographic parameters, such as natural mortality rates and effective population sizes, are poorly understood, as is the genetic basis for variation in most fitness traits related to growth, development, maturation, and fecundity. Thus, there is often little knowledge of the extent to which most genes are subject to selective pressures, fitness differences, and genetic drift.

The only available solution to this problem is to use the results of empirical studies to develop computer-based simulation models. Empirical studies comparing the fitness of wild and escaped cultured stocks, whilst essential for providing baseline measurements of individual and population level fitness are very expensive and time consuming to perform; this is particularly the case given the many native species now cultured in Europe. It is also difficult to extrapolate from the limited experimental studies to different mixing and local scenarios. For example, although it resulted in invaluable data, the common garden experiment carried out in Ireland on farmed and wild salmon, and hybrids, under natural conditions, took ten years to complete but only studied impacts over two generations of introgression for a single species and a single site. In contrast, computer-based models of population mixing can easily be used to simulate ecological and genetic interactions between cultured and wild stocks for a complete range of conditions. Thus, in principal, modelling can provide a comprehensive insight into the potential consequences for population character and abundance. However, for the modelling to be informative, it needs to contain a realistic representation of both demographic and genetic processes and parameters.

A full range of interaction outcomes is possible in principle and modelling suggests that what actually happens will depend critically on the magnitude and frequency of escapes, the degree to which the escapees survive, mature, and interbreed, as well as the nature and extent of their adaptive divergence from the wild populations (Hindar *et al.* 2006; Gilbey and Verspoor submitted). This view is supported by the recent work of Glover *et al*. (2012) (see also Verspoor *et al.* 2009), though these only address the impact on the genetic character of populations and not whether interbreeding will have depressed population abundance through depressed fitness of hybrids.

In general, where large numbers escape relative to wild population abundance, and wild populations are already depressed, as is the case of populations along the south coast of Newfoundland, there will be a greater depression of fitness. There will also be a greater threat to wild populations if farm fish and hybrids have superior performance as juveniles in freshwater but are inferior in their ability to survive and return to natal rivers to spawn. However, the actual impacts will also depend on other variables as well, which may vary with the combination of farm strain, wild population and from one year or location to the next. The nature and extent of consequences, and their permanence, will depend on factors such as the numbers of escapes, when they escape, their longevity, reproductive competence, competitive ability, and pathogen/parasite burden. Furthermore, the consequences will be conditioned by the degree of adaptive genetic divergence of the farmed fish compared to the wild stock, the physical and disease state of escaped fish, and environmental conditions in culture and in the wild. Nonetheless, huge challenges in parameterizing these types of models exist and would require significant field, genomic, and experimental work to develop for the region in question.

CRITICAL KNOWLEDGE GAPS

Required knowledge is lacking on:

- Adaptive population structuring in Newfoundland salmon stocks, particularly on the south coast, including non-anadromous stocks where these are in accessible reaches of rivers.
- Differences in genomic architecture among European and North American / Newfoundland salmon.
- Strain differentiation in relation to virulence in respect of pathogens and parasites and regional distribution of strains.
- Resistance and susceptibility of wild Newfoundland populations to different pathogen and parasite strains.
- Phenotypic differences in reproductive behaviour of escaped farmed salmon and their feral offspring and farm-wild hybrids, compared to wild populations.
- Fitness differentials among farmed, wild and farmed-wild hybrids in the wild.
- Extent of competitive interactions among farm and wild fish in the wild and their effect on the survival of wild fish, and the impact of local population demographics on interaction outcomes.
- Influence of colonization history on susceptibility to genetic interactions with Norwegian farmed fish.

CONCLUSIONS

The general body of knowledge on indirect and direct genetic interactions between genetically divergent conspecific populations has expanded over the last decade. This has strengthened the case that there is a significant risk of substantive phenotypic and genetic consequences for native populations when a non-native conspecific population (e.g., an exotic farm strain) is introduced deliberately or inadvertently which can affect their character, viability, and abundance in management time frames (i.e., decades and centuries). However, despite the Atlantic salmon being one of the best characterised species from a demographic, phenotypic, and genetic perspective, it is insufficiently well characterised to provide the necessary information required to allow a robust prediction of the consequences for wild populations of indirect and direct genetic interactions with Norwegian farmed strains. The biological complexity of indirect and direct genetic interactions, and the importance of situation specific conditions, means that obtaining a robust assessment of risk is non-trivial.

Though specific studies are lacking in relation to farmed Norwegian strains and wild Newfoundland populations, what is known strongly supports that they are highly adaptively divergent. However, the precise nature and extent of this divergence is uncertain. The divergence can be expected to be variable depending on the wild populations considered, given the diversity of wild Newfoundland populations. This view is not diminished by some Newfoundland populations retaining a legacy of natural historical mixing of European and North American salmon during the early post-glacial recolonisation of the region.

There is a biological potential for indirect genetic impacts from farmed fish kept in open containment in fresh or salt water. This impact is associated with the release of exotic pathogen/parasite strains or the increase in the incidence of native pathogens/parasites. Should this lead to increased or selectively altered mortality, it can alter gene frequencies and reduce genetic diversity, compromising a population’s capacity to deal with environmental change in the long term. Insufficient knowledge exists regarding whether there is a greater risk from Norwegian farm salmon compared to North American farm strains. However, the risk from Norwegian strains of farmed Atlantic salmon is arguably greater given their more substantive evolutionary divergence and the restricted regional distribution of many pathogen and parasite strains. The lack of knowledge regarding local adaptation to pathogens and parasites in wild salmon populations, and variation in virulence of pathogens and parasites, precludes predicting the additional risk of using Norwegian stocks. However, there may be an increased risk due to a higher evolved resistance to sea lice of Norwegian strains.

Indirect genetic impacts will almost certainly occur where Norwegian farmed salmon are reared in open systems in freshwater (juveniles) or salt water (post-smolts and adults). Norwegian farmed salmon can be expected to be relatively maladapted to wild Newfoundland conditions. Juvenile escapees will also be expected to compete with wild juveniles. Additionally, a proportion that have escaped from marine cages can be expected to survive, mature, and enter rivers to spawn. These will have the potential to interfere with the reproduction of wild fish, reducing wild breeding success, and to breed successfully and produce offspring that compete with wild juveniles. Both may alter selective pressures, change gene frequencies, or depress wild abundance increasing the loss of variation through genetic drift.

Direct genetic impacts from escaped Norwegian farmed salmon will occur where they mature, enter rivers, and reproduce successfully with wild fish. Interbreeding can be expected, though its precise extent is difficult to predict due to complications associated with *genotype x environment* interactions and phenotypic plasticity in reproductive traits. The performance of pure Norwegian and hybrid offspring can be expected to be significantly depressed compared to wild populations. Although the extent of this fitness depression is unknown and cannot be predicted, it is likely to be greater than would occur from interbreeding with a local or North American farmed strain. It will also be dependent on the ecological circumstances, the amount of interbreeding, and the demographic status of the wild populations involved.

Based on available evidence, a significant risk of negative consequences to the character, abundance, and viability of native populations cannot be dismissed. This risk could be better informed by genetically-based demographic risk assessment models that can evaluate the simultaneous effects of different parameter states and interaction conditions and provide a more robust perspective on likely impacts (Gilbey *et al.* 2007; Fraser *et al.* 2010). However, these models will only be informative if they are underpinned by situation- and context-specific experimental work and by a sound understanding of the biology and population dynamics of affected salmon populations.

RECOMMENDATIONS

The following key recommendations are made in respect of scientific work needed to better inform the risk and severity of consequences from indirect and direct genetic interactions with wild south coast Newfoundland populations in respect of the introduction of farmed Norwegian strains into the aquaculture industry:

- Quantify the frequency and distribution of farmed escapes of Saint John River strain in coastal southern Newfoundland and refine assessments of abundance of local stocks in monitored and non-monitored populations.
- Instigate studies into the nature and extent of strain variation in known salmon pathogens and parasites and assess the resistance and carrier status of Norwegian farm salmon in comparison with wild Newfoundland stocks for different pathogen and parasite strains.
- Quantify the fitness differences of pure parental Norwegian farm strains, wild Newfoundland strains at risk, and their hybrid progeny including F_1 and BC individuals at different life history stages, using controlled common garden experiments, and evaluate the extent of genotype-environment interactions and phenotypic plasticity; compare to local farm strains.

- Develop hypotheses for genomic regions and pathways of potential impact from introgression using genome sequencing / scanning in wild Newfoundland and both North American and Norwegian farmed Atlantic salmon.
- Evaluate risk using biologically-realistic stochastic interaction models parameterized using the information collected in relation to the previous recommendations under different interaction scenarios as regards different pathogen and parasite status, numbers of escapes, and demographic status of wild populations.

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APPENDIX 1: ESSENTIAL DEFINITIONS

Commonly used biological terms can have a wide range of meanings, both among the general public and within biology itself, something which can lead to misunderstanding. Thus it is essential that there is clarity with regard to what is meant by a term to understanding the underlying science related to a given biological context; in the current context of population genetics, the use of the term population is arguably the most problematic. The definitions applied to key technical words in this document are set out below:

Congeneric – individuals belonging to different species placed within the same genus; these are in principle so designated due to their having diverged relatively recently from a common ancestor (e.g., Atlantic salmon *Salmo salar* and brown trout *S. trutta*).

Conspecific – individuals assigned to the same designated Linnaean species; as such, in so far as species designations are working hypotheses, the assignment may be subject to change as biological knowledge changes and species designations are revised.

Epigenetic - heritable changes in an organism's phenotype induced by the environment that are not underpinned by DNA base sequence changes but rather by process such as the methylation of the bases.

Extinction vortex – a term used to describe a situation where a population is put into a state that leads increasingly to its extinction as a result of a negative feedback loop driven potentially by factors such as increased inbreeding due to smaller population size or reduced reproductive success due to a relatively reduced chance of finding a suitable mate or of mating at all as chances of encountering potential mates decline.

Genome – the sum of all DNA contained in the cell of an organism that is inherited from its parents and passed on to its offspring.

Genotype environment interactions – interactions between the genome and the environment that underpin an organism's development and determine its character and fitness, including its capacity to leave viable offspring.

Introgression – the transfer of genes from one breeding population into another following hybridisation where there is interbreeding of hybrids with individuals from one or other parental population and the production of viable, fertile offspring leading to incorporation of the foreign genes into the affected population.

Heritability – the degree to which a particular phenotypic trait or value is passed on from parents to offspring; this measure is often dependent on the environment in which the trait values of the parents and offspring are measured due to genotype environment interactions and phenotypic plasticity.

Heterosis – increased performance or fitness which may occur where genetically divergent individuals interbreed, e.g., when they derive from two genetically distinct population; see also **Outbreeding Depression**.

Hybridisation – the interbreeding of individuals from two evolutionarily distinct populations which may be from the same or a different designated Linnaean species; such interbreeding may or may not lead to introgression.

Hybrids – the individuals produced by hybridisation.

Metapopulation structure – the evolutionary organisation of individual breeding populations within a species into larger population groups, within which some level of regular or intermittent gene flow occurs among populations.

Mutation – the alteration of the DNA base sequence in the absence of recombination, usually by the chance replacement of one DNA base (A, C, T, or G) by another during DNA replication.

Outbreeding depression – at the individual level, depressed performance or fitness of hybrids caused by allele frequency differences among populations or differences in the co-adaptive structure of the mixing genomes; at the population level, the resulting collective reduction in overall survival or reproductive success.

Phenotype – the organismal manifestation of the interaction between a genome and the environment, either with respect to all traits or an individual trait (e.g., growth phenotype).

Phenotypic plasticity – the manifestation by a given genotype of different phenotypes under different environmental conditions; phenotypic changes in response to environmental change.

Population – a group of individuals and their offspring within which mating occurs more or less at random, but where mating with other such groups is more or less absent; alternative terms: genetic population, deme.; not to be confused with the use of the term in a demographic or statistical sense; see Waples and Gaggiotti (2006) for discussion of differences in use of the word population in a biological context.

Recombination – the process during gamete production which generates new genetic variants or composite genotypes among genes that are physically linked by the crossing over and mixing of the two DNA strands of which a chromosome is composed.

Salmon – *Salmo salar* L.; while also commonly used to refer to Pacific salmon, this use is excluded here.

SNPs – (single nucleotide polymorphisms) locations in the genomic DNA where the nucleotide can vary heritably between the maternal and paternal chromosome or between individuals.

Species – a set of one or more populations, which, by virtue of their perceived sharing of a particular set of biological characteristics and an ability to inherently produce viable, fertile offspring, are deemed to be distinct from other populations, and are given a common and unique Latin binomial (e.g., *Salmo salar*).

Stock – an arbitrary group of individuals defined, usually for purposes of management, in respect of some characteristic such as all being found in a particular area (e.g., a river or marine zone) or all possessing a specific biological trait (e.g., age of maturity, time of return to freshwater); such a group may or may not be coincident with a genetic population and may rather correspond to part of a genetic population or to encompass all or part of a number of genetic populations.

Strain – a reproductively isolated breeding lineage of animals that have been maintained in culture for two or more generations and subject to both domestication and artificial selection for particular traits.

Subspecies – a set of one or more populations within a species sharing a particular set of biological characteristics and some degree of inherent reproductive or physical isolation that are not sufficiently distinct from other species populations to be considered to be distinct at the species level.