Agenda item 6.2
For information

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Advances in understanding the impacts of escaped farmed salmon on the genetic integrity of wild Atlantic salmon

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Introduction

Escaped farmed Atlantic salmon have been recognized as a threat to wild salmon populations since the late 1980s when high proportions of escaped farmed salmon were found in several rivers (Gausen & Moen 1991; Hindar et al. 1991). International symposia on the potential genetic, ecological and epidemiological impacts of escaped farmed salmon, organized by NASCO, ICES and/or national authorities, have been held in 1991 (Loen, Norway; published in Hansen *et al.* 1991), 1997 (Bath, UK; published in Hutchinson, 1997), and in 2005 (Bergen, Norway; published in a conveners' report by Hansen & Windson 2006, and in a special issue of ICES Journal of Marine Science by Hutchinson 2007).

In 2016, a group of experts on interactions between aquaculture and wild salmonids, met at the ICES headquarters to summarize the state of knowledge as a response to questions posed by NASCO (ICES, 2016). The general findings of the ICES (2016) expert group report are presented at this meeting, and will not be the focus of my presentation. Rather, my aim is to present advances in the understanding of the genetic impacts of escaped farmed salmon achieved by studies after 2007, and in the following fields: (i) genetic introgression of farmed to wild Atlantic salmon, (ii) salmon biology, (iii) consequences of introgression, (iv) mechanisms of interactions, and (v) broodstock control.

Genetic introgression

Studies of gene flow from farmed to wild salmon face difficulties because the two groups belong to the same species, and the domestication process is still in its infancy (12th generation of breeding since the 1970s). Early demonstrations of gene flow were therefore opportunistic, and were most easily carried out where farmed salmon of Norwegian origin met local wild populations in e.g. Ireland and the UK.

Modern molecular genetics now employ thousands to hundreds of thousands of Single Nucleotide Polymorhisms (SNPs), and the genome sequence of Atlantic salmon was recently published in *Nature* (Lien et al., 2016). This development was used by Karlsson et al. (2011) to search for a set of SNPs that could distinguish between farmed and wild Atlantic salmon on a generic basis. They used a 7k SNP-chip to compare historical samples of wild salmon from 13 Norwegian rivers with samples of farmed salmon from the three major breeding companies, each of them represented by four year classes. A set of c. 60 SNPs was found to be collectively diagnostic for the farmed and historical wild salmon on a generic basis, even though there are genetic differences among the breeding lines of farmed salmon, as well as among wild salmon populations.

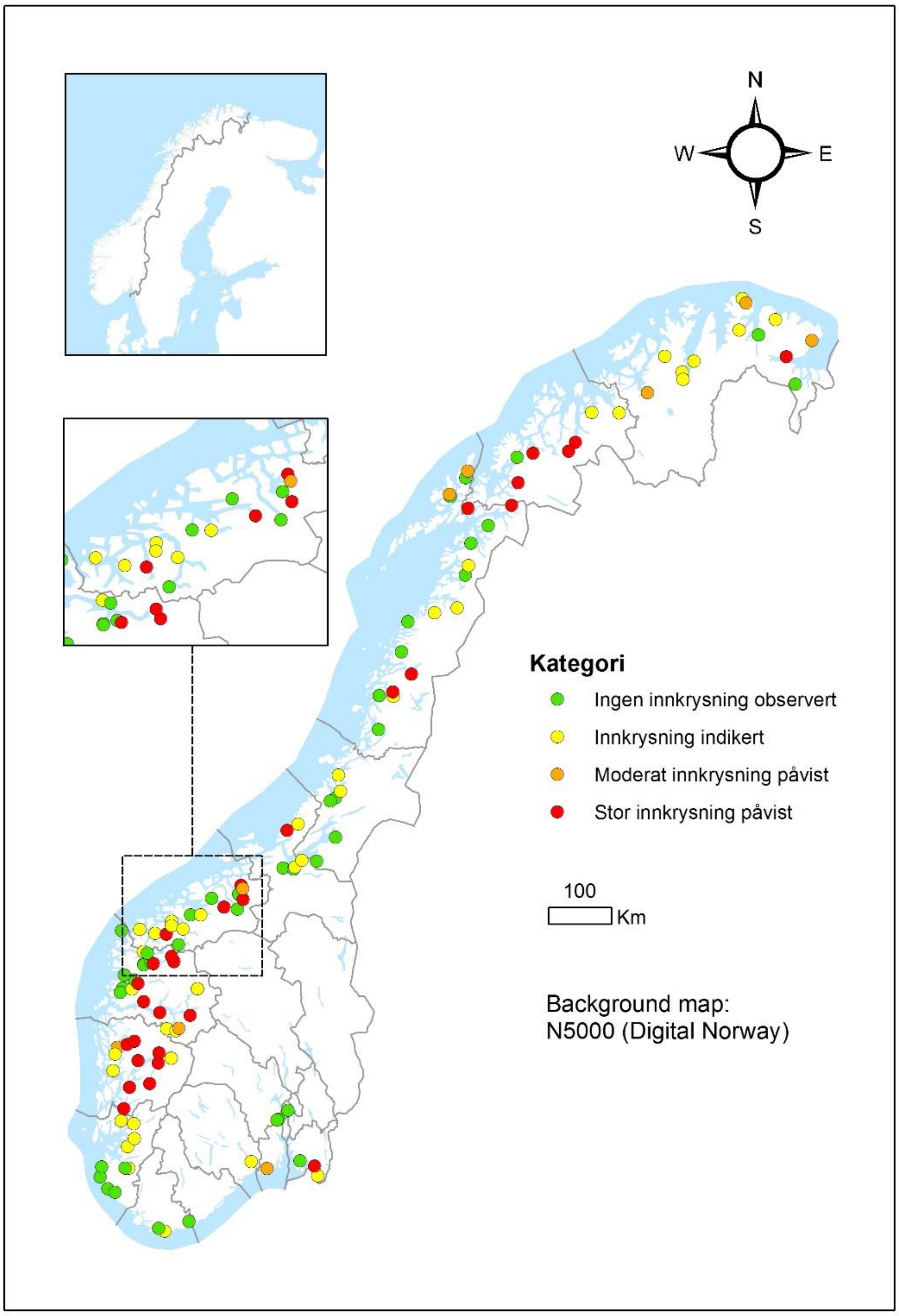
This set of SNPs (or a slight modification of it) was used by Glover et al. (2013) to study levels of genetic introgression in 20 Norwegian Atlantic salmon populations where a historical sample of the population could be compared with a modern sample. In the cases where a genetic change was found, they checked whether the change was best explained by introgression of farmed salmon or by genetic exchange with neighbouring wild populations. They found levels of genetic introgression from farmed to wild salmon ranging from 0 to 47%.

At the same time, Karlsson et al. (2014) developed an alternative approach for analysis that was not dependent on a historical sample for assessing genetic introgression in a modern sample. First, they calculated a genetic centre point for wild and farmed salmon, respectively, to which any individual could be compared with respect to probability of belonging (e.g. probability of being wild, or proportion of wild genome; *P(Wild)*. Then, from analysing a large set of historical wild salmon and salmon from the dominating breeding nuclei, they defined an expected distribution of P(Wild) estimates for pure wild salmon individuals and for pure farmed salmon individuals. Because analyses were done at the individual level, the obtained probability distribution includes all evolutionary processes that act on the genetic composition of the individual, including genetic drift and gene flow between wild populations (Karlsson et al. 2014).

This approach enables quantification of farm genetic introgression from a contemporary sample without having historical samples from this particular population, as every individual can be compared to the probability distribution of wild salmon. Local historical samples will increase the precision as long as they constitute a good representation of the spawning population before any impact of escaped farm salmon. Simulations demonstrated that the method gives a precise estimate of P(Wild) at the population level (Karlsson et al. 2014), although not necessarily for individual fish.

Analyses of Norwegian population samples, using this method, are soon available from more than 20,000 individual Atlantic salmon hatched in the wild from more than 100 rivers representing three quarters of the total number of Atlantic salmon spawning in Norwegian rivers (Karlsson et al., in revision). In this study, the range of population estimates of farmed to wild introgression, varied from 0 to more than 40%, and statistically significant introgression was found in one half of the populations studied.

Upon request from Norwegian authorities, the studies by Karlsson et al. (in revision) and Glover et al. (2013) were recently combined by researchers from NINA and from the Institute of Marine Research (IMR) to classify 125 populations with respect to their genetic integrity (Anon. 2016). The researchers established four categories of introgression: green = no genetic changes observed; yellow = weak genetic changes indicated but less than 4% farmed salmon introgression; orange = moderate genetic changes documented 4-10% farmed salmon introgression; red = large genetic changes demonstrated >10% farmed salmon introgression. Based upon these analyses, 44, 41, 9 and 31 of the populations studied fell into categories green-yellow-orange-red, respectively. In Figure 1, these 125 populations are shown on a map of Norway.



Background map:
N5000 (Digital Norway)

Fig. 1. Categorisation of 125 salmon populations in Norway with respect to farmed to wild salmon genetic introgression. The categories used are green = no introgression observed, yellow = introgression indicated, orange = moderate introgression demonstrated, red = high introgression demonstrated (from Anon. 2016, Glover et al. 2013 and Karlsson et al., in revision).

The study shows that only one third of Norwegian Atlantic salmon populations are without signs of genetic introgression in these samples, which for the large majority of rivers are based on samples of adult Atlantic salmon that have spent their entire life in the wild. The highest genetic introgression is found in the fish farming regions along the west coast of Norway, and there is a highly significant correlation between genetic introgression and the the long-term average proportion of escaped farmed salmon in the rivers (Karlsson et al., in revision).

Salmon biology

Knowledge about the biology of Atlantic salmon has recently been summarised in the books by Jonsson & Jonsson (2011) and Aas et al. (2011), and in the ICES Journal of Marine Science issue from the symposium Salmon at Sea in 2012 (Chaput 2012). One finding is a much wider oceanic distribution area of Atlantic salmon than previously mapped, especially to the north and northeast (beyond the archipelago of Svalbard and far into the Barents Sea). Incidentally, escaped farmed salmon are found alongside wild salmon near the coast of Spitsbergen at 80 ^{o}N (Jensen et al. 2013).

The 7k SNP-chip has been used to describe the large-scale population genetic structure of Atlantic salmon (Bourret et al. 2013), confirming earlier studies showing distinct groups in Europe and North America (Ståhl 1987) and also showing a clear distinction of wild salmon between the eastern Atlantic Ocean, the Barents-White Sea, and the Baltic Sea (Bourret et al. 2013). As a consequence of this, the genetic contrast between farmed Atlantic salmon and historical wild salmon populations along the European coast must be established separately for Atlantic Ocean populations and Barents-White Sea populations (Karlsson et al., in revision). Interestingly, both of these phylogeographic groups were represented among the wild source populations that gave rise to farmed Atlantic salmon, but only Atlantic Ocean populations were represented by generation three in the Norwegian breeding programme (Gjøen & Bentsen 1997).

More than 220,000 SNPs studied in 1500 salmon from 57 rivers in Fennoscandia were used recently to unravel the genetic basis for sea-age (and thereby body size) at maturity in Atlantic salmon (Barson et al. 2015). The study indicated that 39% of the genetic variation in sea-age at maturity could be ascribed to a single gene. Moreover, the study demonstrated a new mechanism for maintenance of genetic variation in a major-effect gene, as a fish with both the early- and the late-maturing allele became large if it was a female and remained small if it was a male (Barson et al. 2015). This finding is a leap forward in our knowledge about the ecological genetics of Atlantic salmon and may have implications for management of both wild and farmed salmon. In a parallel study finding the same major gene effect, Ayllon et al. (2015) proposed that targeted selection in farmed strains could be used to reduce the incidence of early maturation in aquaculture.

In April 2016, the complete DNA sequence of the Atlantic salmon genome was published (Lien et al. 2016). This, and the above studies, demonstrates that salmon biology is entering the genomic era, and that we can expect major leaps of knowledge of both wild and farmed Atlantic salmon biology in the future.

Consequences of introgression

Whole-river controlled experiments in Ireland (Burrishoole river system) and Norway (River Imsa) led McGinnity et al. (1997; 2003) and Fleming et al. (2000) to conclude that intrusion of escaped farmed salmon into natural rivers could lead to lowered fitness and productivity, with repeated escapes causing cumulative fitness depression and potentially an extinction vortex in vulnerable populations (McGinnity et al. 2003). This conclusion was based on the results from following mixed populations from natural spawning or experimental crosses through one or two generations, and chacterising the growth rate, survival, life history and reproductive capacity relative to the native population.

Similar studies have now been performed in a second Norwegian stream (River Guddalselva; Skaala et al. 2012), where a family-based analysis provided qualitative support for the early studies in the Burrishoole and the Imsa, although with some quantitative differences. Skaala et al. (2012) also found an effect of density on the relative performance of farmed offspring (which was reduced with higher density), and an effect of egg size (with larger eggs leading to higher offspring performance).

In Canada, experiments across two generations with one farmed and two wild populations (Fraser et al. 2010a; 2010b) suggested that farmed-to-wild population crosses could differ substantially and under some conditions be sufficiently mismatched to prevailing environmental conditions that they would have reduced survival in the wild. They concluded that repeated farmed–wild interbreeding could adversely affect wild populations, reaffirming conclusions from previous experimental studies.

The number of controlled experiments is limited and they are extremely time- and manpower-demanding. The advance in characterising individual fish by their P(Wild) provides another way of testing whether introgression of farmed to wild salmon has an effect on the ecology and life history of wild salmon. Preliminary analyses by Geir Bolstad, NINA, and co-workers, of non-introgressed and introgressed adult wild salmon from more than 50 populations suggest that ecological and life-history changes are widespread in Atlantic salmon populations.

Mechanisms of interactions

Artificial selection for increased growth rate in farmed Atlantic salmon doubled the capacity for growth over the first five generations of the breeding program (Thodesen et al. 1999). Experiments with families of farmed salmon, wild salmon and their crosses, now show that growth rate in a hatchery is a “perfect marker” in that all families of farmed salmon grew faster than all families of farmed-x-wild salmon which grew faster than all families of wild salmon (Solberg et al. 2013).

In the wild, however, a higher growth capacity may be a mixed blessing, as energy-rich food is not easily available throughout the year, and as the search for food may render the fish vulnerable to predators. The earliest free-living life stage may be one where increased growth capacity may be particularly advantageous, because in late spring/early summer growth conditions may be at their best. Experiments with offspring of wild and farmed salmon in stream channels at Ims, Norway, showed a higher realised growth in farmed than in wild juveniles, and that the presence of farmed juveniles reduced the survival of wild juveniles in confined enclosures (Sundt-Hansen et al. 2015). This may be one mechanism explaining how farmed juveniles could have reduced survival compared with wild juveniles (Fleming et al. 2000; McGinnity et al. 2003), and still reduce the survival of wild juveniles in whole-river experiments.

Broodstock control

The ability to distinguish farmed offspring from wild offspring by molecular genetic methods is now being actively used in Norwegian management. Stock enhancement of wild Atlantic salmon has a long history, and is actively used as a compensation for reduced smolt productions caused by hydropower regulation. Moreover, populations in danger of extinction are being propagated in live gene banks until the man-made factor that threaten these populations is controlled. In either case, it would be unfortunate if propagation of wild fish in reality was propagating fish of farmed heritage.

Norwegian management authorities have therefore requested that all wild broodstock are checked with respect to their genetic origin (i.e. after fish that are escaped farmed salmon have been excluded by scale reading). In autumn 2014 and 2015, all broodstock were tested genetically with the set of SNPs that distinguish between farmed and wild salmon (Karlsson et al. 2011), and with the analytical method of Karlsson et al. (2014). A fixed P(Wild) was set to exclude all pure farmed salmon and only a small percentage of pure wild salmon. In 2014 and 2015, respectively, 14% and 18% of all broodstock were excluded as parents for stock enhancement and gene banking because of their likely farmed heritage (Karlsson et al. 2016).

Sterile salmon in fish farming has been advocated as one way to reduce genetic interactions. Sterility on a large scale can be easily induced by triploidy, and triploid families of farmed salmon are commercially available today. Triploid salmon may however develop secondary sexual characteristics. Experiments have now been conducted with triploid (sterile) males (Fjelldal et al. 2014). Qualitative observations demonstrated that triploid male Atlantic salmon displayed the full range of spawning behaviors of wild males, and stimulated the wild female to spawn in the absence of wild males. The authors maintain, however, that quantitative data are needed before suggesting triploidy as a mitigative action.

Spontaneous triploid salmon are found at low frequencies in farmed salmon. A comparison of large numbers of farmed salmon in aquaculture, with escaped farmed salmon in the wild, suggest that only a fraction of triploid farmed fish may enter rivers (Glover et al. 2016). This is an interesting observation, as it in addition to the reduction of genetic interactions, may implicate that the transfer of disease agents from farmed to wild fish may be reduced with a reduction in farmed fish seeking fresh water.

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