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**Title:** Genetic assignment as a tool to identify the origin for Atlantic cod (*Gadus morhua*) escapees in Norwegian fjords

The Atlantic cod (*Gadus morhua* L.) represents one of the most economically important fishes in the north Atlantic. However, serious declines in abundance have been observed in many wild stocks, a situation which has stimulated the increasing global interest for cod farming. In Norway, which is one of the largest producing countries, current aquaculture production is approximately 7500 tons. This industry has considerable potential to expand, and the Norwegian government has issued sufficient farming licenses to enable a commercial production of approximately 300 000 tons.

A major challenge for aquaculture is containment. In Norway, the reported numbers of farmed escaped cod have ranged from 20 000 – 290 000 individuals yearly in the period 2004-2008, although the real figure is probably much higher due to underreporting. There is universal concern over the potential for negative genetic interactions between farmed escaped fish and their wild counterparts. The Institute of Marine Research has established protocols for genetic assignment of escaped Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*), but this study represents the first molecular genetic characterization of Atlantic cod reared on marine farms, and the assignment of escapees in a fjord system.

Samples consisting of approximately 47 fish each were taken from nine cages located on four commercial farms throughout Norway. In addition, 28 farmed escapees were recaptured in the sea. Nine microsatellite loci and the *Pan I* gene were analysed, revealing a total of 181 alleles (range = 78-114 per sample). Comparing allelic variation with published data for wild cod suggests that lower genetic variation exists within single cages than in wild populations. Significant linkage disequilibrium was observed among pairs of loci in all samples, suggesting a low number of contributing parental fish. Global  $F_{ST}$  was 0.049, and the highest pair-wise  $F_{ST}$  value was 0.085. For single loci, the *Pan I* gene was the most diagnostic, displaying a global  $F_{ST}$  of 0.203. Simulations among the samples collected on farms revealed an overall correct self-assignment percentage of 75%, demonstrating the possibility to identify individuals to cage of origin. Identification of the 28 escapees revealed a single cage as the most likely source of origin for half of the escapees, whilst the remaining fish were assigned to a mixture of samples suggesting more than one source of escapement.

Three major genetic clusters were identified. Bayesian clustering of the data revealed significant structure, which displayed concordance with the three major lineages identified by UPMGA diagram including data from all loci. An overall correct self-assignment percentage of 75% was observed among the 9 baseline samples. This remained almost unchanged when the locus *Pan I* was excluded from this analysis (74%), and only minor differences in pattern of miss-assignment were observed. Correct self-assignment ranged from 51-89% for individual samples, and the

pattern of miss-assignment varied, and among-sample miss-assignment tended to reflect genetic similarity. All genetic assignment methods implemented identified the most likely origin for 13-15 of the 28 escapees. The remaining escapees were directly assigned to a mixture of the baseline samples, with a maximum of 4 escapees being directly assigned to any one alternative sample.

This study demonstrated that highly significant genetic differentiation exists among groups of cod reared in production cages, both in and between farms. Through a combination of genetic analyses, in addition to real-life assignment of 28 unknown escapees, it has been demonstrated that genetic assignment may be used to identify the source of farmed escaped cod, as has previously been described for Atlantic salmon, and rainbow trout.