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Genetic characteristics of broodstock collected from four Norwegian coastal cod (*Gadus morhua*) populations

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The aquaculture industry in Norway is now focused on developing economically viable farming based on the Atlantic cod, *Gadus morhua*. Extensive research has been carried out on this species for the past two decades, much of it in connection with stock enhancement. Until now, most of the intensive cage culture has been based on wild broodstock. However, a future cod aquaculture industry must be based on a domesticated broodstock, and the initial selection of wild cod becomes an important issue. Genetic differentiation between coastal cod populations in Norway has been reported, and it is of interest to evaluate offspring from some of these populations under farmed conditions. Live mature cod were collected at four selected spawning sites along the Norwegian coast (Porsangerfjord, Tysfjord, Herøy/Helgeland, and Øygarden). The fish were transported to Parisvatnet, a cod aquaculture facility west of Bergen, where they were kept in net pens. Individual tagging and extensive sampling (blood, white muscle, and fin clips) for genetic characterization were carried out. Each potential broodstock fish was genotyped at the haemoglobin and pantophysin I loci in addition to five allozyme (*LDH-3**, *GPD**, *IDH-2**, *PGM**, *PGI-1**) and ten microsatellite loci (*Gmo2*, *Gmo3*, *Gmo8*, *Gmo19*, *Gmo34*, *Gmo35*, *Gmo36*, *Gmo37*, *Gmo132*, *Tch11*). Comparison of allele frequencies revealed significant genetic differences among some of the coastal cod samples, and offspring performance of the broodstock is now being compared under farmed conditions. The overall test revealed significant genetic differences among the coastal broodstocks, with the *HbI*, *Pan*I and the microsatellite *Gmo132* loci being most informative.

Keywords: Atlantic cod (*Gadus morhua* L.), coastal cod populations, cod farming, genetic profiling.

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Introduction

The Atlantic cod (*Gadus morhua*) is an important commercial species in the Northeast Atlantic and is now considered to be one of the most promising new species for future aquaculture. After the breakthrough in the production of juvenile cod in 1983 (Øiestad *et al.*, 1985), extensive studies of cod juvenile production as well as stock enhancement were not carried out (for summary see Svåsand *et al.*, 2000). In recent years, the interest has moved in the direction of commercial net pen farming of this species, as discussed in Kvenseth *et al.* (2000). During the last few years, there have been substantial private investments in cod juvenile hatcheries as well as in net pen rearing of cod to market size. If successful, the industry plans to imply a steady increase in farmed cod production to about 100 000 t in the next ten years.

The future success of cod farming is clearly dependent on optimal use of the genetic resources present in wild cod populations. The importance of starting with a broad genetic base is illustrated by the experiences with Atlantic salmon (*Salmo salar*), where offspring from about 40 river populations were tested initially under farmed conditions. After years of domestication and selective breeding, existing strains are dominated by only a few of the original founding populations (Gjedrem *et al.*, 1991; Gjøen and Bentsen, 1997), i.e. these populations might be more suited for domestication than the other initial populations.

Previous studies, based on immunogenetic methods and polymorphism in blood proteins (Møller, 1966, 1968), demonstrated genetic differences among the migratory stock of northeast Arctic cod and various groups of non-migratory cod stocks distributed in fjords and in Norwegian coastal areas. Much less differentiation was found in the interpretation

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of patterns of geographic variation when allozymes were used in genetic studies (Jørstad, 1984; Mork *et al.*, 1985; Jørstad and Nævdal, 1989). Recent studies using various genetic markers have revealed large genetic differences among stocks of cod, such as for the *Pan*I locus in samples from northern Norway (Fevolden and Pogson, 1995, 1997) and elsewhere (cf. Imsland and Jonsdottir, 2002, and references therein). New studies on cod population structure using microsatellite loci have generally revealed a more detailed and complicated population structure both in the western Atlantic (Bentzen *et al.*, 1996; Ruzzante *et al.*, 1998) and in the eastern Atlantic (Hutchinson *et al.*, 2001; Nielsen *et al.*, 2001; Jonsdottir *et al.*, 2002; Knutsen *et al.*, 2003).

As a result of the artificial environment, domestication of any wild organism will introduce selection, both intentional and unintentional, and this is likely to cause genetic changes. For domestication of a new species, such changes are fundamental to the adaptation to farming conditions, and selective breeding methods in fish (Gjedrem, 2000) are being applied to develop high performance strains under farmed conditions. The knowledge of performance traits that are under genetic control is essential to the development of a selective breeding strategy for developing highly productive farmed strains for the future. Such information is also of vital importance to the evaluation of the potential genetic impact on wild populations owing to possible large-scale escapement of farmed cod. As in the Atlantic salmon industry, escapees represent a potential threat to the genetic integrity of wild populations (see Hutchinson, 1997, and references therein; Bekkevold *et al.*, 2006). However, the genetic influence depends on the magnitude of genetic differences between wild and farmed populations, especially with regard to life history characteristics. Recent studies of salmon have demonstrated a decrease in the fitness of hybrid offspring between the two groups (Fleming *et al.*, 1996, 2000; McGinnity *et al.*, 1997). The long-term salmon studies (McGinnity *et al.*, 2003) in Ireland clearly demonstrated that interbreeding between wild and farmed salmon can cause dramatic reductions in population fitness.

As farming of Atlantic cod has just started, information about potential effects of interbreeding, or any other domestication-induced effect between farmed and wild cod, is lacking. Such information, however, is urgently needed to develop an appropriate breeding strategy associated with minimal negative environmental impacts. Although a number of genetic studies have been carried out in connection with stock enhancement (Jørstad *et al.*, 1994; Otterå *et al.*, 1999) and specific farming conditions (van der Meeren *et al.*, 1994; Svåsand *et al.*, 1996; Hansen *et al.*, 2001; van der Meeren and Jørstad, 2001), we need more comprehensive and detailed information about the genetic profiles of local coastal cod populations as well as knowledge of differences between populations in important biological parameters. In 2002, a new program was initiated to compare the fitness of offspring from different coastal cod populations. The present genetic screening results of broodstock collected from four different coastal cod populations are part of this larger programme. This genetic screening comprises six polymorphic proteins, ten microsatellite loci, and the *Pan*I loci in all 16 loci.

Material and methods

Collection of broodstock

Broodstock were collected during the 2002 spawning season. Four areas, representing a broad range of environmental conditions, were selected, ranging from Porsangerfjord in Finnmark in the north to Øygarden in Hordaland in the south (Figure 1). The RV "Fangst" operated in March/April in the northern region in cooperation with the commercial Danish seine fishing vessel "Brenningen" to collect broodstock in Porsangerfjord. The abundance of spawners at the local spawning site in Smørfjord (Jørstad, 1984; Jakobsen, 1987) was limited, and only a few fish were caught in this area. Larger catches of migrating spawners were obtained in the outer/middle part (east Magerøysund) of this fjord system. Potential future spawners (size range 55–65 cm) were selected and transferred to holding tanks on RV "Fangst" and transported to a marine holding facility outside Hammerfest where they were kept in net pens. In early July, the spawners were transported in tanks with running seawater on RV "Michael Sars" from Hammerfest to Parisvatnet Field Station in Øygarden west of Bergen.

Fishing and collection of samples of spawners in Tysfjord in April 2002 suggested that this coastal cod stock, characterized by skin pigmentation, large size, and high condition factor, could be interesting to test under farmed conditions. Practical considerations prevented the collection of broodstock in April. Potential spawners were collected during October 2003 using RV "Fangst". Fishing was based mainly on traps and hand gear, and fish were kept in seawater tanks aboard the vessel. About 170 fish, including immature cod, were caught and transported to Parisvatnet for rearing as broodstock. Additional spawners (40 fish) were collected during the 2003 spawning season by a local fisherman in Tysfjord and also transported to Parisvatnet for use in the 2004 spawning season.

Spawners from the Helgeland region were provided by a local fisherman in Herøy on the coast of Helgeland. These cod were caught in the Herøy area in autumn 2001 and kept in net pens for sale as local broodstock to the cod farming industry. About 170 fish of appropriate size (see above) were transferred to RV "Fangst" in early April 2002 and transported in tanks with running seawater to Parisvatnet.

Wild cod (about 80 fish) from selected localities in the Øygarden area were caught during spring 2002 with cod traps and hand gear. This fishery was conducted by personnel from the Parisvatnet Field Station, and the fish were kept in net pens and raised as broodstock at the station.

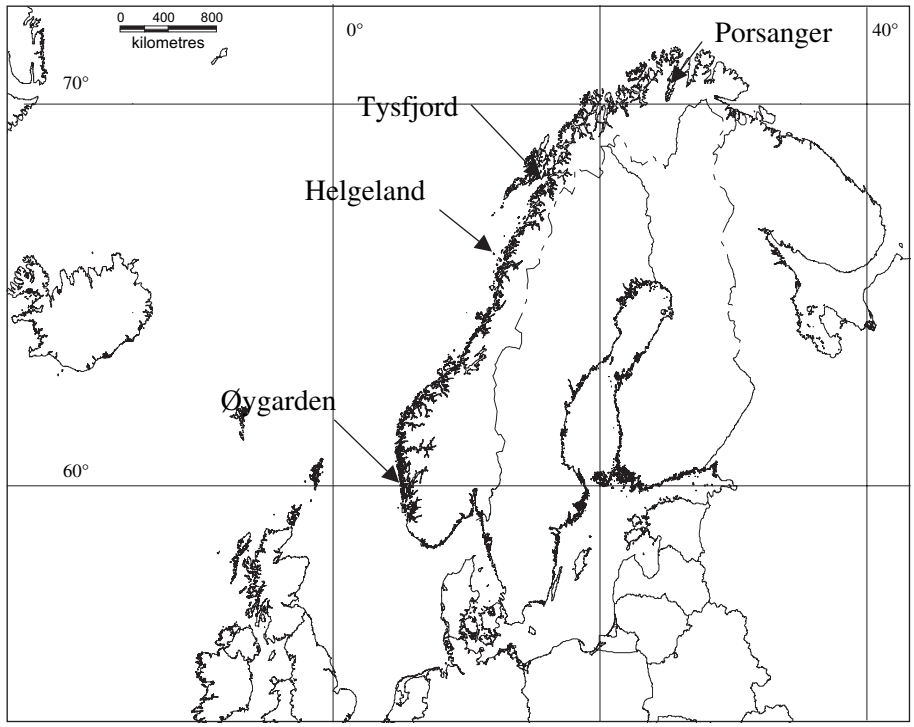

Figure 1. Collection of broodstock of cod from four costal cod locations along the Norwegian coast. The fish were transported alive to Parisvatnet Field Station in Øygarden where sampling was conducted in November 2002.

Initially, the broodstock collected from the different regions were kept separately in net pens. High summer temperatures in 2002 caused substantial mortality in all groups, and individual tagging and sampling were not possible until the temperature was appropriate to fish handling. High temperatures combined with inadequate weaning to commercial dry feed (DanFeed) were the main cause of mortality.

Sampling and genetic analyses

After the period of adaptation in cages, all surviving fish from the different regions were carefully anaesthetized and measured (length, weight). In addition, they were individually tagged (PIT tags), and samples (blood, white muscle, and fin clips) were taken for various genetic analyses.

Blood samples (about 100 µl) were drawn from the large blood vessel in the gill of each fish by use of sterile syringe. The samples were immediately mixed with one drop of heparin and kept on ice until analyses could be conducted in the laboratory in Bergen, one to three days after collection. They were analysed using agar gel electrophoresis (Jørstad, 1984, modified from Sick, 1961) to identify the different haemoglobin (*HbI*) genotypes. The different banding patterns corresponding to the most common genotypes have been described (Sick, 1961; Dahle and Jørstad, 1993). Small samples of white muscle tissue were taken by biopsy from each fish, kept on ice during sampling, and later frozen at low temperature (−80°C). These samples were analysed as soon as possible (within two weeks) by starch gel electrophoresis (Jørstad, 1984). Five different tissue enzymes were analysed, including lactate dehydrogenase (LDH), isocitrate dehydrogenase (IDH), glycerophosphate dehydrogenase (GPD), phosphoglucomutase (PGM), and phosphoglucose isomerase (GPI), all known to be polymorphic in cod (Jørstad *et al.*, 1980; Mork *et al.*, 1985).

For DNA extraction, fin clips (dorsal fin) were taken from each fish and put in 100% ethanol. The DNA extractions were carried out using a DNA isolation kit (Qiagen Dneasy®96 Tissue Kit), and all extractions were routinely checked after isolation by 6 min of electrophoresis on agarose gels (Ready-To-Run Agarose Gel 1.2%, Amersham Biosciences) to make sure that the isolated DNA was of high molecular weight and concentration. Ten microsatellite loci used in a number of other population studies of cod were analysed (see Table 1). Labelling of primers was carried out according to ABI 3100 requirements and the potential of multiplexing. However, some of the microsatellites were amplified separately in the PCR to ensure sufficient amplification, and mixed prior to fragment separation. Fragment sizing was conducted using the ABI Genotyper computer program package. The pantophysin locus (*Pan*I) was produced by PCR amplification followed by digestion with the restriction enzyme *Dra*I, as described by Fevolden

Table 1. Microsatellite loci used for analyses of broodstock collected from four coastal cod populations in Norway. H_{exp} is the expected and H_o is the observed heterozygosity.

Locus	Annealing temperature (°C)	Size range	No. of alleles	F_{st}	H_{exp}	H_o
*Gmo2**	56	102–138	14	0.0001	0.851	0.608
Gmo3†	56	179–207	6	0.0001	0.191	0.198
Gmo8†	56	120–208	21	0.0009	0.906	0.743
Gmo19†	56	120–226	33	0.0000	0.940	0.807
Gmo34†	56	87–115	8	0.0018	0.604	0.611
Gmo35†	56	119–155	10	0.0035	0.836	0.769
Gmo36†	56	178–190	4	0.0189	0.523	0.083
Gmo37†	56	198–314	26	0.0002	0.867	0.806
*Gmo132**	56	100–186	30	0.0535	0.819	0.824
Tch11‡	56	124–212	22	0.0050	0.929	0.845

*Brooker *et al.*, 1994.
†Miller *et al.*, 2000.
‡O'Reilly *et al.*, 2000.

and Pogson (1997). This produces only two different alleles, A and B, identified by simple agarose electrophoresis.

All microsatellite data were analysed using MicroChecker, version 2.2.1 (Shipley, 2003), and the estimated null allele frequency for each locus was compared with the null allele frequencies obtained using a method by Brookfield (1996), after omitting non-amplified samples.

Samples were tested for conformation to Hardy–Weinberg equilibrium (HWE) expectations by the Markov chain method (Guo and Thomson, 1992), by resampling 2000 iterations per batch for 200 batches with GENEPOP (Raymond and Rousset, 1995), version 3.4, June 2003. The tests for genetic differentiation included a global test for each locus as well as tests across all loci analysed. In addition, genetic differentiation was tested between all population pairs, against each locus separately, and across all loci. *F*-statistics were calculated according to Weir and Cockerham (1984) as implemented in GENEPOP, including values estimated for each locus, and pairwise comparisons made between the four samples of broodstock. Standard deviation of F_{st} was calculated using FSTAT (v2.9.3), jackknifing over loci.

Results

The selected broodstock, representing coastal cod from four geographic regions, were analysed for four different types of genetic markers. These data, together with previous data from the same regions on *HbI* variation (Møller, 1966, 1968; Jørstad, 1984; Dahle and Jørstad, 1993), allozyme loci (Jørstad, 1984; Mork *et al.*, 1985; Mork and Giæver, 1999), and the *Pan*I locus (Fevolden and Pogson, 1997), provide a comprehensive database for revealing genetic differentiation within the coastal cod group in Norway. Data for the microsatellite loci used in the present study are summarized in Table 1, including information about annealing temperature as well as size range. The number of alleles in the sample collection varied from 4 (*Gmo36*) to 33 (*Gmo19*). The F_{st} for each locus is also shown in Table 1, demonstrating the highest values obtained for *Gmo132* (0.0535) and *Gmo36* (0.0189).

When the samples were tested for HWE, all samples deviated in the same four loci: *Gmo2*, *Gmo8*, *Gmo19*, and *Gmo36*. In all cases, the deviation was caused by an excess of homozygotes, and interestingly, deviation caused by an excess of homozygotes of *Gmo8* and *Gmo19* has been shown in other studies (Lage *et al.*, 2004). Possible explanations of this observed deviation from HWE might include null alleles, genetic drift, selection, admixture, or insufficient sampling.

Table 2 presents the allele frequencies of the most common allele for each of the loci analysed, including one haemoglobin, five allozymes, ten microsatellites, and pantophysin. As shown, the overall test for genetic differentiation at each locus and the corresponding F_{st} values revealed significant ($p < 0.001$) genetic differences among the coastal cod broodstock at two loci, *HbI** and *GPI-1**. The largest variation was found for the *Hb*I locus, where the frequency of *HbI*1* varied from 0.628 in the Øygarden sample to 0.232 in the cod collected from Porsangerfjord, giving an F_{st} value for that locus of 0.101.

As expected, significant variation was found in the *Pan*I locus, where the Øygarden sample was fixed for *Pan*I**AA* compared with an estimated frequency 0.75 in the Porsangerfjord samples. A high F_{st} value (0.116) was observed for this locus. For the ten microsatellites, only three loci (*Gmo34*, *Gmo132*, and *Tch11*) indicated significant differentiation in overall testing. The frequencies of the most common allele clearly show that the most informative locus revealing population structuring was *Gmo132*, where the frequency varied from 0.125 in the sample from Øygarden to 0.589 in the sample from Porsangerfjord (Table 2, Figure 2). The estimate of F_{st} for that locus was the highest (0.0526) observed among the microsatellite loci tested. Considering all loci investigated in this study, the largest values of F_{st} (Weir and Cockerham, 1984) were found for the *Pan*I, *Hb1*, and *Gmo132* loci (Table 2). The overall estimate of F_{st}, across all 17 loci analysed, was 0.015 (s.d. ± 0.007).

Pairwise tests were carried out for all individual loci and sample pairs, and significant differences were demonstrated. In the overall tests (across all 17 loci), the differences between Porsangerfjord and Øygarden, as well as from the samples from Tysfjord and Helgeland, were highly significant ($p < 0.001$). The pairwise test between Helgeland and Tysfjord across all loci analysed was not significant ($p = 0.117$). A closer look at each of the individual loci, however, revealed three loci with significant values, *Pan*I ($p = 0.050$), *Tch11* ($p = 0.037$), and *GPI-1* ($p = 0.002$), also suggesting minor but significant genetic differences between these two adjacent samples (Figure 1).

Table 2. Frequencies of the most common allele estimated for six protein loci, ten microsatellite loci, and *Pan*I in samples of broodstock collected from four coastal cod locations. The F_{st} values estimated for each locus and *p*-values obtained in global tests for genetic differentiation are also given. Statistically significant values are given in bold.

		Haemoglobin	Allozymes					Microsatellites										Pantophysin
		HbI	*LDH-3*	*GPD*	*IDH-2*	*PGM*	*PGI-1*	*Gmo2*	*Gmo3*	*Gmo8*	*Gmo19*	*Gmo34*	*Gmo35*	*Gmo36**	*Gmo37*	*Gmo132*	*Tch11*	*Pan*I
Site	*n*	1	100	100	100	100	100	104	191	130	144	99	128	190	290	106	171	A
Porsangerfjord	112	0.232	0.563	0.968	0.972	0.955	0.737	0.250	0.911	0.203	0.113	0.647	0.210	0.657	0.205	0.589	0.119	0.755
Herøy/Helgeland	44	0.430	0.659	0.943	0.989	0.977	0.693	0.307	0.920	0.261	0.143	0.614	0.273	0.477	0.227	0.341	0.114	0.885
Tysfjord	74	0.363	0.655	0.980	0.993	0.986	0.709	0.223	0.896	0.181	0.136	0.593	0.247	0.493	0.203	0.349	0.096	0.958
Øygarden	40	0.628	0.513	0.963	0.975	0.963	0.663	0.238	0.863	0.167	0.163	0.525	0.283	0.588	0.205	0.125	0.100	1.000
F_{st}		**0.101**	0.01	0.0013	0.0002	0.0008	0.0008	0.0005	0.0000	0.0008	−0.0005	−0.0034	0.0001	0.0330	0.000	**0.0526**	0.0060	**0.116**
p-Value genetic differentiation		<0.001	0.139	0.232	0.315	0.366	0.001	0.057	0.341	0.078	0.127	0.212	0.387	0.003	0.262	<0.001	0.001	<0.001

A is the most common allele in the *Pan*I system.

Several loci showed evidence of null alleles in all populations (*Gmo2*, *Gmo8*, *Gmo19*, *Gmo36*), the null allele frequency estimates varying between 0.033 and 0.303. In addition, the *Tch11* locus in the Porsanger sample showed evidence of null alleles, the null allele frequency estimated at 0.032.

The F_{st} values obtained in sample pair comparisons confirmed the large differences found in the three loci *HbI*, *Pan*I, and *Gmo132* (Table 3). The largest difference was found between Porsangerfjord and Øygarden at the *HbI* locus ($F_{st} = 0.283$), but large values were obtained at the *Pan*I locus ($F_{st} = 0.1722$) and *Gmo132* ($F_{st} = 0.1500$) as well. The F_{st} estimate based on all 17 loci varied between −0.0021 and 0.0372 (Table 3), the largest F_{st} observed between Øygarden and Porsanger.

Discussion

Among all four classes of genetic markers used in this study, significant genetic differentiation was detected between the broodstock collected from four geographic areas along the Norwegian coast. Three loci, including *HbI*, *Pan*I, and *Gmo132*, were of particular interest and showed large genetic differences between the broodstocks. The results obtained are consistent with earlier reported information on *HbI* variations (Møller, 1968; Jørstad, 1984) in coastal areas. This was also the situation for the *Pan*I locus, where the frequencies detected were comparable to those reported by Fevolden and Pogson (1997). The microsatellite locus *Gmo132* has also been reported as the most informative marker in other population studies (Knutsen *et al.*, 2003; Lage *et al.*, 2004). In contrast with earlier studies, we included four different gene marker systems in the same investigation. The different genetic marker systems indicated

Figure 2. Diagram of fragment size distributions (*y*-axis, fragment size in bp) for the *Gmo132* locus in samples of broodstock collected from four coastal cod locations along the Norwegian coast. Dark circle − the most frequent fragment. Samples: 1, Porsangerfjord; 2, Herøy/Helgeland; 3, Tysfjord; 4, Øygarden.

Table 3. F_{st} between the four populations estimated for all 17 loci as in Weir and Cockerham (1984) (data for each locus not shown).

Population	Porsanger	Helgeland	Tysfjord
Helgeland	0.0124		
Tysfjord	0.0129	−0.0021	
Øygarden	0.0372	0.0085	0.0120

substantial genetic differentiation among the coastal cod populations in question.

On the other hand, we do not know if the broodstock fish captured in this study actually reflect the wild cod stock in each of the areas. Both selection of fish from the actual catches and the losses incurred during transportation and during adaptation to the farming environment may have changed, at least in theory, the frequencies of some of the markers used in the broodstock. For this reason, we have also collected random samples from the same areas, and these are now being analysed for the same set of genetic markers.

The *HbI*(*1*) frequency found in the broodstock from Porsangerfjord was lower than that found in earlier studies (Møller, 1968; Jørstad, 1984). This could be explained by some intermingling with fish of northeast Arctic cod origin (Dahle and Jørstad, 1993). The value obtained for the *Pan*I locus gave some indication of a minor fraction of the migratory stock in our sample. These were identified as homozygotes for the *Pan*I B-allele described by Fevolden and Pogson (1997). However, these few fish had a negligible influence on the actual frequencies of the two loci (*HbI* and *Pan*I) in the sample from Porsangerfjord and thus did not change the results of genetic comparisons.

These broodstocks are now being used to produce family groups for performance studies under the same farmed conditions. This will address how the offspring of these broodstock perform among coastal cod stocks. In this case, it was important to identify and remove the fish that could belong to the northeast Arctic stock. This was done by removing all fish that were of genotype *Pan*I (*BB*) from the Porsangerfjord broodstock, although coastal cod with this genotype might exist in very low numbers. Based on the comprehensive screening of the broodstock, we know that our test groups are significantly different for several genetic systems. They are also collected from areas with very different environmental conditions, and their offspring are currently being tested under equal conditions at the Parisvatnet Field Station in Øygarden and at the Austevoll Aquaculture Station. So far, we have generated offspring (families) from two year classes (2003 and 2004) that are being evaluated.

In the present study, we have demonstrated significant genetic differences among broodstock groups collected from four geographic regions. With regard to the spawning season, clear differences in spawning time between the four broodstock groups have been observed over two years (Otterå *et al.*, 2006). The more important issue, however, is whether other key life history characteristics (e.g. growth, age at maturation) will be related to the different genetic groups. If parameters such as time of spawning are part of adaptation to local environmental conditions, non-adaptive escaped farmed cod from the industry could have serious consequences for local cod stocks. Introgression will possibly reduce the fitness of the local stock and reduce the overall viability and abundance of wild fish. Thus, information is urgently needed to evaluate the potential risks caused by large-scale farming of cod, in addition to developing a sustainable management strategy for the cod farming industry, including selective breeding programmes.

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