

# Fine-scaled geographical population structuring in a highly mobile marine species: the Atlantic cod

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## Abstract

Compared with many terrestrial and freshwater environments, dispersal and interbreeding is generally much less restricted in the marine environment. We studied the tendency for a marine species, the Atlantic cod, to be sub-structured into genetically differentiated populations on a fine geographical scale. We selected a coastal area free of any obvious physical barriers and restricted sampling to a 300-km region, well within the dispersal ability of this species. Screening 10 polymorphic microsatellite loci in 6 samples we detected a weak, but consistent, differentiation at all 10 loci. The average  $F_{ST}$  over loci was small (0.0023) but highly significant statistically, demonstrating that genetically differentiated populations can arise and persist in the absence of physical barriers or great distance. We found no geographical pattern in the genetic differentiation and there was no apparent trend of isolation by distance along the coastline. These findings lend support to the notion that low levels of differentiation are due to passive transport of eggs or larvae by the ocean currents rather than to adult dispersal, the latter being strongly dependent on distance.

*Keywords:* *Gadus morhua*, gene flow, genetic differentiation, local populations, microsatellites

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## Introduction

Populations constitute interbreeding units with more or less autonomous dynamics and recruitment. In terrestrial and freshwater environments, populations are often well defined and distinct from each other, often separated physically by barriers to mixing and interbreeding (see, e.g. Avise 2000 for an extensive review). In the marine environment, in contrast, physical barriers are often absent and the continuous water instead represents a potential means for dispersal, favouring intermixing of individuals over the species range. Tides and ocean currents may further act to mix passively drifting organisms, primarily eggs and larvae, over appreciable distances (see Palumbi 2001). For these reasons, distinct populations are more difficult to detect in the marine environment and for many marine organisms it is unclear to what degree distinct populations exist at all, or whether they are organized into larger panmictic units (McQuinn 1997). The distinction is crucial, in particular for heavily exploited

organisms such as many marine fish, because recruitment and sustainability are properties of the population. Failure to identify the population could lead to local over-exploitation and subsequent decline, as has become abundantly clear in recent decades (e.g. Atlantic cod in Alaska, Hutchings 2000, and along the Swedish west coast, Svedäng *et al.* 2001).

Genetic markers are valuable tools for analysing population structure (see, e.g. Utter 1991 for a historical review of the application of genetic markers to fish populations). By characterizing the geographical distribution of allele or haplotype frequencies, population sub-structuring can be detected and local populations identified. Such applications of genetic markers have been very successful in uncovering cryptic population structure in freshwater (Allendorf *et al.* 1976; Jorde & Ryman 1996; Carlsson *et al.* 1999) and marine fish (Nesbø *et al.* 2000; Ruzzante *et al.* 2000; Hutchinson *et al.* 2001). The use of genetic markers for population delineation requires a detectable level of genetic differentiation, however, and this has presented problems in studies of many marine organisms (Ward *et al.* 1994). In the marine environment many studies have failed to detect statistically significant population structuring

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because of low differentiation, especially over small geographical distances (e.g. in cod see, Árnason *et al.* 1992; Gjørseter *et al.* 1992). Low levels of differentiation in marine organisms are most likely due to extensive gene flow (Waples 1998; Avise 2000) and do not necessarily imply that structuring does not exist, but that more powerful means are required to detect them (see Waples 1998 for a general discussion). Marine organisms, even if weakly differentiated on a small geographical scale, often show evidence of differentiation over larger distances, probably because the long distance acts as an isolation mechanism.

Here, we address the question whether marine species tend to form distinct populations when such structuring is not forced upon the organism in the form of physical barriers or great geographical distances. We chose the Atlantic cod as a model organism and focus on a geographical scale well within the dispersal ability of the species (Løversen 1946; Danielssen & Gjørseter 1994). Any differentiation at such a restricted geographical scale may be interpreted as an innate tendency for population sub-structuring and not just a consequence of external forces. We use microsatellite DNA, which has proven effective in uncovering population sub-structuring over larger areas in other parts of the species' range (Bentzen *et al.* 1996; Ruzzante *et al.* 1996, 2000, 2001; Hutchinson *et al.* 2001; Nielsen *et al.* 2001).

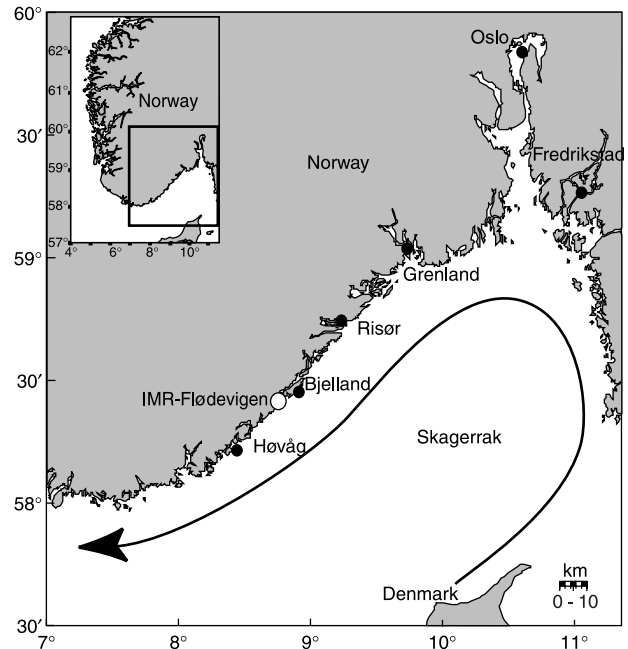
## Materials and methods

### The study species

The Atlantic cod (*Gadus morhua*) is one of the commercially most important marine fish in the world (FAO 2000). It has a wide geographical distribution in continental shelf waters in the North Atlantic, extending northward to Spitsbergen, Disco Bay and Labrador, and southwards to Cape Hatteras and the Bay of Biscay. To the east, cod also enters the Baltic Sea.

Atlantic cod display a wide range of mobility behaviours with regards to breeding, from long-distance spawning migration of oceanic cod to stationary coastal cod. While largely stationary, coastal cod may migrate appreciable distances (several hundred kilometres: Danielssen & Gjørseter 1994). Spawning along the coast usually takes place from January to April, depending on seawater temperature. A female cod can produce in excess of one million eggs, distributed over multiple spawning events. The eggs hatch after two or three weeks and the larvae stay in the water column where they feed on zooplankton. Around May–June the larvae metamorphose into small fish (juveniles), which settle on the bottom of the nursery grounds when they are  $\approx 3$ –5 cm long and are then referred to as the 0-group.

Maturation starts around 2 years of age when the cod is  $\approx 35$  cm and the 2-year-old fish constitute  $> 50\%$  of the



**Fig. 1** Map of the sampled region along the Skagerrak coast. The positions of the six sampling sites are identified by solid circles and the location of the Institute of Marine Research, Flødevigen Research Station by an open circle. The curved arrow shows the predominant ocean current, and indicates a possible route for the passive drift of eggs and larvae.

spawning stock (Gjørseter *et al.* 1996). Annual survival is  $\approx 0.5$ , and  $< 2\%$  of 1-year-old cod survive to reach 6 years or more. The generation length is  $\approx 3$  years.

### The study area

We chose to study cod along the Norwegian Skagerrak coast (Fig. 1). The choice was motivated, in part, to aid the statistical analysis of a seine haul data set that have been collected in this area for a long time (below) (Chan *et al.* 2003). The Skagerrak is an ocean basin extending from the North Sea and is delineated by the landmasses of Denmark, Sweden and Norway (Fig. 1). Within the basin, the ocean current flows in a loop along the coastline (Danielssen *et al.* 1997). The Norwegian Skagerrak coast is characterized by numerous small islands and skerries and with fjords typically extending a few kilometres inland. Cod is common along the entire coast, and we selected six roughly equidistant ( $\approx 60$  km apart) sites covering some 300 km of coastline (Fig. 1). Samples of  $\approx 100$  adult Atlantic cod were collected from each site (from west): Høvåg, Bjelland, Risør, Grenland, Oslo and Fredrikstad. Sampling with gill nets was carried out during the spawning season (January–March 2000), near presumed spawning sites, with the help of local fishermen. All sampled cod were aged, sexed, weighed, measured and assigned to a sexual

maturity index (according to Fotland *et al.* 2000). White skeletal muscle was taken from each individual and stored in 96% ethanol until genetic analysis.

A cod hatch-and-release programme had previously been in operation in the general study area in an attempt (largely unsuccessful as it turned out: Chan *et al.* 2003) to increase the coastal cod stock. In total, the programme lasted almost 90 years (from the 1880s to 1971) and included the release of 7 billion yolk sac larvae. As broodstock for the released fish, several hundred adult cod were captured annually in the vicinity of the Flødevigen Research Station (cf. Fig. 1) and put together in an enclosed salt-water basin where spawning occurred at random. Hatching larvae from the basin were thereafter released along the Norwegian Skagerrak coastline (Solemdal *et al.* 1984; Smith *et al.* 2002). An ongoing monitoring programme using systematic beach seine hauls was initiated early in the 20th century (annually since 1919) in order to study the effects, if any, of fry releases on the natural cod stock (Gjøsæter *et al.* 2002).

#### Genetic analysis

DNA from individual cod was extracted from muscle tissue using the DNEASY kit (Qiagen). Polymerase chain reaction (PCR) amplification of 10 microsatellite loci was carried out using a PCT-100 machine (MJ-Research), following published descriptions with only slight modifications. The following loci were screened: *Gmo2* and *Gmo132* (Brooker *et al.* 1994); *Gmo3*, *Gmo19*, *Gmo34*, *Gmo35*, *Gmo36* and *Gmo37* (Miller *et al.* 2000); and *Tch12* and *Tch13* (O'Reilly *et al.* 2000). Microsatellite DNA fragments were separated on an ALFexpress II automatic sequencer (Amersham Pharmacia Biotech). Genotypes were scored independently by two people. Any deviating scores were reanalysed, using new PCR products, and dismissed if a consistent scoring could not be obtained after three or four attempts. Alleles were designated according to size (number of nucleotides), determined by comparison with standard fragments of various sizes (Amersham Pharmacia Biotech), and corrected for bias as described by Jorde *et al.* (unpublished).

#### Statistical analysis

Allele and genotype frequencies were estimated from samples by gene counting. Deviations from Hardy-Weinberg genotype proportions were estimated as  $F_{IS}$  (Nei & Chesser 1983) and the null-hypothesis of  $F_{IS} = 0$  was tested at each locus with an exact test (Guo & Thompson 1992). We further investigated heterozygosity excesses and deficiencies separately (with one-sided tests), using the score test in GENEPOP (Version 3.1d; Rousset & Raymond 1995b).

We tested the null-hypothesis of one single population occupying the Skagerrak coast by testing for allele frequency heterogeneity among the six samples. Because of potential statistical problems associated with the large number of alleles at most microsatellite loci, many occurring at low frequencies, we applied several standard statistical tests. These include the contingency  $\chi^2$  test, the log-likelihood test (*G*-test; Sokal & Rohlf 1981), and an exact test for allele frequency differences (Raymond & Rousset 1995a). When testing the joint hypothesis of no genetic differentiation at any locus we combined the single-locus statistics by summarizing the  $\chi^2$  and *G*-values as recommended by Ryman & Jorde (2001) and by summarizing twice the negative logarithms of the exact test *P*-values (Fisher 1950). The joint hypothesis was also tested using  $F_{ST}$  (below). For each individual we also calculated the likelihood of it belonging to each of the sample sites on the basis of its multilocus genotype, using the jackknifed 'leave one out procedure' in the WHICHRUN 4.1 software by Banks & Eichert (2000). We compared the proportion of individuals assigned to the site of capture with the proportions assigned to different sites.

Amounts of genetic variation were estimated both as the observed number of alleles at each locus and as average gene diversities within ( $H_S$ ) and among ( $H_T$ ) samples (Nei & Chesser 1983). The level of genetic differentiation among samples was characterized using Wright's  $F_{ST}$ , with Weir & Cockerham's (1984) estimator  $\theta$  as calculated using the GENEPOP software. We tested the null-hypothesis of no spatial structure (i.e. average  $F_{ST} = 0$ ) by a permutation test, whereby all individuals were reassigned to a sample at random, and  $\theta$  recalculated for the rearranged data set. Permutations were carried out for each locus separately, and for all loci simultaneously (i.e. by permutating the arrays of 10 genotypes representing individuals). Permutations were repeated 10 000 times, and the probability of the null-hypothesis was taken as the proportions of replicates that yielded an estimate ( $\theta$ ) of  $F_{ST}$  that is as high, or higher than, the observed value. To check that the observed  $F_{ST}$  reflects an actual spatial differentiation, and is not confounded by temporal heterogeneity, we estimated the temporal component ( $F_{MS}$ ) between immature (sexual maturity index 1) and mature cod (sexual maturity index 2 or larger) within sites, again using Weir & Cockerham's (1984) estimator  $\theta$ . The null-hypothesis of no temporal heterogeneity ( $F_{MS} = 0$ ) was tested with GENEPOP using the exact test for allele frequency heterogeneity within each site.

A possible geographical pattern in the distribution of genetic variability was analysed using two different approaches. First, we estimated  $F_{ST}$  (Weir & Cockerham 1984) between each pair of samples (15 pairs) and used these pairwise estimates to calculate the relationship between genetic differentiation and geographical distance,

**Table 1** The sampled localities and summary statistics for genetic variability within sites.  $H_S$  is the estimate of gene diversity,  $a$  is the average number of alleles per locus;  $F_{IS}$  measures deviation from Hardy–Weinberg genotype proportions ( $P$ -values for two-sided tests). Also indicated are the loci that appear to have excesses and deficiencies of heterozygotes, representing significant single locus tests in the corresponding direction (one sided tests: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ )

Sample site	Sample size	Average $H_S$	Average $a$	Deviations from Hardy–Weinberg proportions			
				Average $F_{IS}$	$P$ -value	Excess of heterozygotes	Deficiency of heterozygotes
Høvåg	100	0.687	12.7	–0.0019	0.183	6	4Gmo34*
Bjelland	92	0.692	13.3	0.0163	0.289	5	5Gmo132*,Gmo19**
Risør	101	0.691	13.1	–0.0038	0.390	8	2Gmo19*
Grenland	100	0.668	12.8	0.0085	0.482	5	5Gmo34*
Oslo	109	0.679	13.5	–0.0033	0.225	7Gmo132*	3
Fredrikstad	109	0.684	13.3	0.0313	0.076	1	9Gmo19***
Average		0.684	13.1	0.0078	0.149	5.3	4.7

by regressing  $F_{ST}/(1 - F_{ST})$  against the logarithm of geographical distance (Rousset 1997). Second, we calculated genetic distances ( $D_A$ : Nei *et al.* 1983) among sample pairs (see Takezaki & Nei 1996 for a discussion on alternative distance measures), and used these genetic distances to construct a phylogenetic tree among samples, using the UPGMA method. The topology of the resulting tree was tested for robustness by bootstrapping over loci (5000 replicates), using POPTREE software (N. Takezaki, unpublished; ftp://ftp.nig.ac.jp/pub/Bio/njbafd/dos/).

## Results

In total, 611 individual cod from 6 localities were genotyped at 10 microsatellite loci. The amount of genetic variability within sites, as judged by average gene diversities ( $H_S$ ) and average number of alleles per locus ( $a$ ), was very similar among sites (Table 1). There were large differences among loci, however, and for the total material,  $H_T$  ranged from 0.196 at locus *Gmo3* to 0.914 at loci *Gmo19* and *Tch13*, and the total number of alleles varied from 4 (*Gmo36*) to 38 (*Tch13*) (Table 2). These values are close to those found in other samples of cod at the same loci (cf. Bentzen *et al.* 1996; Miller *et al.* 2000; Ruzzante *et al.* 2000; Beacham *et al.* 2001; Bekkevold *et al.* 2002).

There was a slight overall tendency for deficiencies of heterozygotes within sites compared with Hardy–Weinberg genotype proportions (Table 1). Of the total 60 estimates of  $F_{IS}$  (i.e. at 10 loci from each of 6 sites), 32 were negative (representing heterozygote excesses) and 28 were positive (heterozygote deficiencies). The positive  $F_{IS}$  values were, on average, slightly higher than the absolute of the negative values, resulting in a positive (but nonsignificant) average  $F_{IS} = 0.0078$  over all sites and loci. Seven of the 60 single-locus tests for Hardy–Weinberg genotype proportions were statistically significant at the  $\alpha = 0.05$  level (one-sided tests), representing six deficiencies and one excess of

**Table 2** Amount of gene diversity within ( $H_T$ ,  $a$ ) and among ( $F_{ST}$ ) sample sites. The  $P$ -values refer to permutation tests for the null-hypotheses of  $F_{ST} = 0$  and represent the percentage of 10 000 random replicates that yield a value that is as high or higher than the observed one. Significant values in italics

Locus	Total $a$	$H_T$	Among sites	
			$F_{ST}$	$P$ -value
<i>Gmo2</i>	19	0.855	0.0004	0.326
<i>Gmo3</i>	8	0.196	0.0045	0.040
<i>Gmo19</i>	25	0.914	0.0022	0.026
<i>Gmo34</i>	11	0.626	0.0009	0.269
<i>Gmo35</i>	10	0.825	0.0020	0.096
<i>Gmo36</i>	4	0.493	0.0051	0.062
<i>Gmo37</i>	20	0.843	0.0036	0.009
<i>Gmo132</i>	29	0.896	0.0047	< 0.001
<i>Tch12</i>	18	0.290	0.0002	0.385
<i>Tch13</i>	38	0.914	0.0006	0.248
Average	18.2	0.685	0.0023	< < 0.0001
SE	3.3	0.086	0.0006	

heterozygotes. The deficiencies refer to two loci in the sample from Bjelland and one locus in each of Høvåg, Risør, Grenland and Fredrikstad, whereas a single excess of heterozygotes was observed in the sample from Oslo (Table 1). All significant deviations, in either direction, occurred at three loci only (*Gmo19*, *Gmo34* and *Gmo132*). Testing jointly over all 10 loci, however, no sample deviated significantly from Hardy–Weinberg proportions (two-sided tests), although the sample from Fredrikstad was nearly significant ( $P = 0.076$ ). At this site, 9 of the 10 loci showed heterozygote deficits, but this was significant only at locus *Gmo19* (Table 1). Pursuing this further, we find that deviations from Hardy–Weinberg genotype proportions at this site are most pronounced in adult cod aged 3 years and older (35 individuals), possibly indicating

admixture of cod (Wahlund effect) from different populations at the Fredrikstad site.

Although a larger fraction of the genetic variability resides within sample sites, a small but significant part ( $F_{ST} = 0.0023$  on the average: cf. Table 2) is ascribed to genetic differences among sites. The joint null-hypothesis of no genetic differentiation among sites at any locus was rejected with high probability by all tests ( $P$ -values ranging from  $<< 0.0001$  for the permutation test on  $F_{ST}$  to 0.0007 for the  $G$ -test on allele frequencies). Furthermore, all 10 single-locus estimates of genetic differentiation were positive and fairly similar, ranging from  $F_{ST} = 0.0002$  (at locus *Tch12*) to 0.0051 (*Gmo36*). Four of these single-locus values were statistically different from 0, as judged both by the permutation tests on  $F_{ST}$  (Table 2) and also by the various heterogeneity tests on allele frequencies (i.e. the  $\chi^2$  test, the  $G$ -test, and the exact test: values not shown). There is, therefore, strong evidence that the sample sites together represent several genetically distinct populations. This conclusion is consistent with the finding that a much higher fraction (47–62%) of individual cod was assigned to the site of sampling than to other sites (between 3 and 15% were assigned to each of the other localities: Fig. 2).

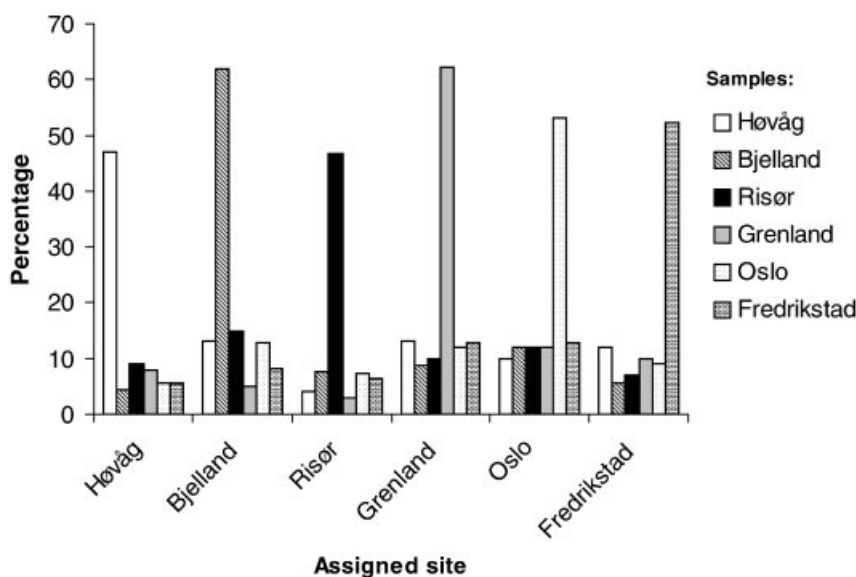
As a rather large fraction of the sampled cod (between 19% in Høvåg and 61% in Fredrikstad) turned out to be sexually immature, despite being caught near the presumed spawning sites during the breeding season, we estimated the level of genetic differentiation ( $F_{MS}$ ) between immature and mature cod from each sites (Table 3). For most sites the estimate is negative, implying that the observed differences between immature and mature cod were very small and can be explained by sampling errors alone. For Høvåg, the difference was somewhat larger ( $F_{MS} = 0.0016$ ) and may indicate a possible influence of more than one popu-

**Table 3** Proportion of mature individuals within sites and the distribution of genetic variation ( $F_{MS}$ ) between sexual mature and immature individuals within sites, and overall sites ( $F_{MT}$ ).  $P$ -values refer to exact tests for allele frequency heterogeneity between maturity classes within sites

Locality	% mature	$F_{MS}$	$P$ -value
Høvåg	81.1	0.0016	0.115
Bjelland	46.7	-0.0018	0.239
Risør	57.5	-0.0011	0.835
Grenland	64.0	0.0004	0.358
Oslo	68.8	-0.0012	0.254
Fredrikstad	38.5	-0.0012	0.779
All	63.8	$F_{MT} = 0.0006$	0.975

lation at this site. Nevertheless, the difference was not statistically significant ( $P = 0.115$ ), and we therefore chose to retain the immature fish in the further analysis of geographical population structure.

Apart from the finding that most samples were genetic differentiates of each other (cf. Table 4), we found no clear geographical pattern to this differentiation. First, the regression of pairwise  $F_{ST}/(1 - F_{ST})$  values against log-distance was indistinguishable from 0, the point estimate being slightly negative ( $b = -0.0015$ ). This showed that there was no tendency for increased genetic differentiation within the distance range investigated (50–300 km). Second, although most cod were assigned to the site of capture, assignment to other sites was rather uniform (cf. Figure 2), showing that cod resemble cod from the same site and are approximately equally different from cod from other sites. Third, the UPGMA dendrogram (Fig. 3) revealed an almost star-shaped phylogeny with little support for



**Fig. 2** Distribution of statistical assignment of sampled individuals based on genotypes at 10 microsatellite loci. For each sample, the six bars represent the percentage ( $y$ -axis) of individuals assigned to each sample site ( $x$ -axis). Note that for all samples a much higher fraction of individuals (46.5–62%) was assigned to the site of capture than to any other site (3–15%).

	Høvåg	Bjelland	Risør	Grenland	Oslo	Fredrikstad
Høvåg	—					
Bjelland	0.0029*	—				
Risør	0.0028***	0.0019	—			
Grenland	0.0022**	0.0034*	0.0054***	—		
Oslo	0.0022**	0.0028	0.0014	0.0013*	—	
Fredrikstad	0.0015*	0.0003	0.0023	0.0031**	0.0014*	—

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

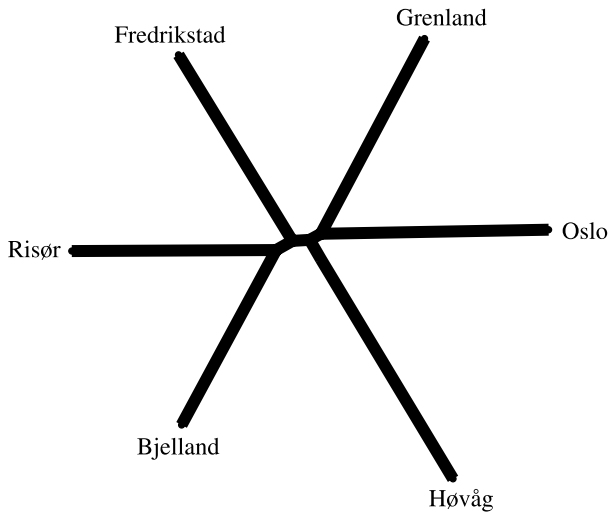


Fig. 3 UPGMA plot of Nei *et al.* (1983) genetic distances  $D_A$ , calculated for 10 microsatellite loci among six samples of coastal Atlantic cod.

any groupings of sites. The highest bootstrap support for any grouping was 39% and referred to Bjelland, Risør and Fredrikstad vs. the other three sites.

## Discussion

We detected and estimated statistically significant genetic differentiation among coastal Atlantic cod inhabiting a short shoreline. This differentiation was found despite ample opportunities for admixture and genetic homogenization in this species, due to passive drift of larvae, active dispersal by adult fish and the now terminated artificial release of larvae. As the distances covered by this study were intentionally short ( $\leq 300$  km), and well within the dispersal ability of the species, the observed structuring was unlikely to be caused by isolation due to geographical distance. This conclusion is supported by the apparent lack of any trend between distance and genetic differentiation, as such a trend is expected when dispersal among more distant sites is restricted in comparison with that among neighbouring sites.

The results indicate that marine species may actively group into local populations that remain partly isolated

Table 4 Genetic differentiation ( $F_{ST}$ ) between pairs of populations. Exact tests for differentiation ( $F_{ST} > 0$ ) are performed with the GENEPOP 3.3 software

from each other. Although on the basis of our data we cannot set a definite limit to the geographical extent of local populations, if indeed such limits exist, it appears that our six samples all represent different populations. This conclusion is based on the observation that there is no definite clustering of sites suggesting that two or more of the samples should represent the same population, either in the dendrogram (Fig. 3) or in the assignment percentages (Fig. 2). Although some pairwise  $F_{ST}$  estimates (Table 4) were not statistically significant, all point estimates were positive indicating greater differentiation than expected from sampling errors alone. Also, taking the slight tendency for sub-groupings among samples (above) at face value would produce geographically disparate 'populations', which is unlikely biologically.

Because the level of differentiation was quite low, it may at first be dismissed as not being 'biologically meaningful' (cf. Waples 1998; but see Wirth & Bernatchez 2001). In this context such a conclusion is unwarranted, however, because any statistically significant difference in allele frequency, no matter how small, indicates that the samples are from separate statistical populations. Although the issue of statistical vs. biological populations is complicated (e.g. samples from the same biological population taken at different times may be significantly different genetically; Jorde & Ryman 1996), in our case, separate statistical populations also imply separate biological populations. First, because all sites were sampled in the same season over a brief period, we reduced the possibility of spatial genetic differentiation being confounded by temporal genetic change. Accordingly, immature and mature individuals taken from the same sites were no more different genetically than could be accounted for by sampling errors (the average  $F_{MT}$  is very close to zero: Table 3). Hence, there is little temporal genetic change locally, indicating quite large effective population sizes (but see Palm *et al.* 2003 for a discussion on the probability of detecting drift in temporal samples). Second, by sampling adults, rather than younger individuals, we minimized the probability of nonrandom sampling within sites due to family aggregations (Allendorf & Phelps 1981). Third, great care was taken in scoring genotypes and uncertain individuals were reanalysed, sometimes several times. Hansen *et al.* (2001), using

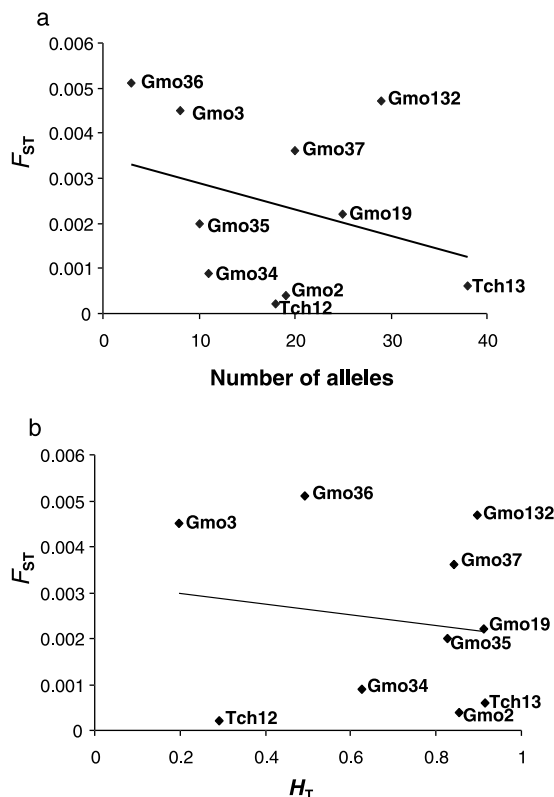


Fig. 4 Linear regression of  $F_{ST}$  against (a) the number of alleles and (b) gene diversity ( $H_T$ ) for 10 microsatellite loci. Both slopes are nonsignificant, but indicate a tendency of lower  $F_{ST}$  with increasing genetic variability.

computer simulations to assess the consequences of different types of scoring errors on estimates of  $F_{ST}$ , found that misclassifications of 4% of the genes could yield an apparent  $F_{ST}$  between 0.001 and 0.003, i.e. similar to our estimate, when the true value was 0. To check if our estimate could be inflated by scoring errors at particularly difficult loci, we compared single-locus  $F_{ST}$  values with various quantities that could covariate with scoring difficulty (Fig. 4). These quantities include number of alleles at each locus, gene diversity ( $H_T$ ), average number of replicate runs for each individual, number of individuals that was left unscored, and deviation from Hardy–Weinberg genotype proportions ( $F_{IS}$ ). The results are negative, and only numbers of alleles show any tendency to correlate with estimates of  $F_{ST}$  (Fig. 4a). This (nonsignificant) correlation should result in a downward bias in  $F_{ST}$ , if scoring errors were responsible, and thus cannot explain our positive estimate.

The relatively low level of differentiation that we observe among the sites ( $F_{ST} = 0.0023$ ; Table 2) is in accordance with earlier findings of microsatellite DNA in cod from other coastal regions (cf. Ruzzante *et al.* 2000,  $F_{ST} = 0.0039$ ; Hutchinson *et al.* 2001,  $F_{ST} = 0.0080$ , excluding the Canadian and Barents Sea samples). Although the

latter values are slightly higher than our estimate, they refer to studies covering larger geographic areas than ours and physical distance may play a role in (partly) isolating the populations and resulting in a larger  $F_{ST}$  among them.

Earlier studies of coastal cod in the Skagerrak region employed other techniques, including haemoglobin (Frydenberg *et al.* 1965) and allozyme polymorphisms (Gjøsæter *et al.* 1992). These studies failed to detect statistically significant differentiation among cod in this area. Re-analysing their data (both studies report genotype data), excluding samples from outside our study area (Frydenberg *et al.* 1965) and lumping multiple samples from the same locality (Gjøsæter *et al.* 1992), we estimate an average  $F_{ST}$  (Weir & Cockerham 1984) of  $-0.0004$  for the Gjøsæter *et al.* (1992) data and  $0.0004$  for the Frydenberg *et al.* (1965) data. None of these estimates is significantly different from zero, and because the studies differed markedly from ours with regards to sample size, number of loci and degree of polymorphism, we used computer simulations to check whether these results are consistent with our estimate. The simulations were carried out with the same number of alleles and the same allele frequencies as in the respective study. We simulated genetic drift until the required population differentiation ( $F_{ST} = 0.0023$ ) was achieved. Sampling of individuals for genetic analysis was simulated by random drawing (with replacement) of genes, using the harmonic mean sample sizes (see below) for each locus. The random drift and sampling steps were repeated four times, one for each sample (both studies included four sample sites). We estimated  $F_{ST}$  among samples, averaged over loci, and compared the mean estimate with the observed mean  $F_{ST}$  value for that study. The simulations were repeated 10 000 times, and the proportion of simulations that yielded a mean  $F_{ST}$ -value as low as or lower than the observed ones ( $-0.0004$  or  $0.0004$ , respectively, for the two studies) were taken as the probability of obtaining such low values under the null-hypothesis that our estimate is correct.

For the Frydenberg *et al.* (1965) study (one locus only, sample sizes from  $n = 77$  to 236, with a harmonic mean of 105) we found a probability of 0.47 of obtaining an  $F_{ST}$  estimate as low as or lower than the observed value (0.0004), given that our higher estimate is correct. For the Gjøsæter *et al.* (1992) study (six protein-coding loci from four samples, ranging in size from  $n = 43$  to 583 with a harmonic mean of 121), the corresponding probability is 0.17. These probabilities are both reasonably high and we conclude that the slightly lower earlier values probably deviate from ours by chance alone, reflecting the difficulty in estimating small amounts of differentiation from a limited number of genes.

The reason for the low level of genetic differentiation among coastal cod, as for many other marine organisms, is most likely a fair amount of gene flow among populations

(e.g. Ward *et al.* 1994; Waples 1998). Gene flow could occur at either the egg or larvae stage by passive drift along with the ocean current, by active migration of adult cod, or both. The apparent lack of a spatial pattern to the genetic differentiation observed here, especially the lack of a correlation between differentiation and geographical distance, indicates a mechanism of gene flow that is independent of distance. Passive transport of egg or larvae seems the most likely explanation because transport over long distances is possible in a short time (the current looping from Denmark to the southern part of Norway on average takes only 2–3 weeks: D. Danielssen, personal communication). Active dispersal of adult cod, however, appears to occur primarily over quite short distances as judged by capture–mark–recapture studies (Løversen 1946; Danielssen & Gjørseter 1994). Such a dispersal pattern is expected to result in neighbouring populations being more genetically similar and yielding a declining genetic correlation with distance (Wright 1943). Such a pattern was not found (the observed correlation coefficient was slightly negative), which implies that either there is no such trend at this geographical scale, or it is too weak to be detected by our sampling design. Other studies have reported an isolation-by-distance pattern, however, but primarily for much larger geographical distances than those involved in our study (e.g. Mork *et al.* 1985; Pogson *et al.* 1995, 2001). Even though passive transport of eggs or larvae is a plausible mechanism for gene flow that may explain the lack of a correlation with distance, such a pattern is also compatible with recent colonization (e.g. Slatkin 1993; Pogson *et al.* 2001). If the latter explanation is correct, it could imply a greater effect of the released larvae than had hitherto been thought.

The observed genetic differentiation was found in spite of the release of an enormous number of cod larvae over the years, raising the question of what genetic consequences, if any, larval release has had on the natural cod populations. As the released larvae originated from adult cod that were collected near the Flødevigen Marine Research Station the larvae should represent a single biological population. Furthermore, several hundred parents of each sex were used as broodstock each year and temporal differences among released larva should therefore be small. Hence, the larva releases should, if anything, have a homogenizing effect on the recipient populations. That we nevertheless observe genetic differences among extant populations may indicate that the larvae releases were largely unsuccessful and did not have any noticeable effect on the natural populations. Such a conclusion would be in accordance with findings from statistical analyses of demographic data collected through the beach seine hauls (Tveite 1971; Chan *et al.* 2003). An alternative explanation is that the larvae releases were successful and led more or less to the eradication of previous genetic differentiation in this area. Under this latter scenario the current differentiation is

recent, and caused by subsequent genetic differentiation after the releases ceased. As an example, the observed  $F_{ST} = 0.0023$  (Table 2) could be reached in the  $\approx 10$  generations that have passed since the last releases (in 1971) with random genetic drift, assuming that the local effective population sizes are no higher than  $N_e = 2000$  per generation (calculated from equation 7.3.17 in Crow & Kimura 1970). Such a limited effective size seems quite reasonable (cf. Ruzzante *et al.* 2001; Bekkevold *et al.* 2002), because it refers to the local population only and not to the entire coast. This scenario may also explain the star-like phylogeny among extant populations (cf. Fig. 3), as any historic pattern is eradicated under this scenario and all populations have diverged from each other in roughly the same time (30–40 years). We are not able to conclude with certainty what the genetic effect of the larvae releases have been, as the genetic data presented here are compatible with both scenarios. At any rate, the current genetic differentiation, whether old or more recent, does show that cod do not interbreed freely, but instead are aggregated into more or less local isolated populations, despite ample opportunity for mixing.

The finding that coastal cod are structured into local populations has important implications for coastal management. Because coastal cod populations depend on successful recruitment locally, both spawning and nursery areas should be preserved at the local scale. For exploited areas this may imply restrictions on human activities in the coastal zone, including restrictions on release of sewage from households and chemical pollutants from boats, harbours, factories, etc., and possibly restrictions on fishing at or near spawning grounds. For such management to be effective, local spawning and nursery grounds need to be identified (e.g. Knutsen *et al.* 2000). To date, no coastal management plan, to our knowledge, has taken recruitment to local marine fish populations into account, as it is for anadromous fish (e.g. the Atlantic salmon in Norway).

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This study is part of Halvor Knutsen's PhD thesis on genetic structure in Atlantic cod. Per Erik Jorde is a population geneticist, working on temporal genetic change and spatial genetic structure in various organisms. Carl André's research focuses on population biology and genetics of marine organisms. Professor Nils Christian Stenseth's research programmes focuses mainly on the population dynamics of terrestrial and marine species.

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