Challenges to Marine Ecosystems

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Challenges to Marine Ecosystems

Proceedings of the 41st European Marine Biology Symposium

Edited by

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Foreword

This volume presents a representative sample of contributions to the 41st European Marine Biology Symposium held on 4–8 September 2005 in Cork, Ireland. The theme of the symposium was 'Challenges to Marine Ecosystems', and this was divided into four sub-themes: Genetics, Marine Protected Areas, Global Climate Change and Marine Ecosystems, Sustainable Fisheries and Agriculture. The symposium attracted 308 participants from 33 countries and, over the 5 days, 92 oral presentations were given as well as 175 posters presented.

The world's marine ecosystems face multiple challenges, some natural, but many resulting from humankind's activities. Global climate change, driven by influences of energy usage and industrial practices, is a reality now accepted by most of the world's scientists, media and political establishments. Warming seas and rising sea levels are regarded as threats, while visionaries consider deep ocean carbon disposal as a technological opportunity. Exploitation of the seas continues apace, with repeated concerns over the impact of over-fishing, plus reservations about the environmental effects of marine aquaculture. We need to understand how resilient organisms and ecosystems are to these challenges, while responding by protecting biologically meaningful areas of the oceans. The sub-themes of the 41st European Marine Biology Symposium address all these matters.

The symposium was hosted by the Department of Zoology, Ecology and Plant Science, University College Cork, and we would like to thank all members of the Department who assisted in the running of the event. We would also like to thank all our sponsors for their vital financial support.

Thanks are also due to all the referees for their efforts in evaluating and improving the manuscripts that were submitted for publication in this volume.

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Mixed stock analysis and the power of different classes of molecular markers in discriminating coastal and oceanic Atlantic cod (*Gadus morhua* L.) on the Lofoten spawning grounds, Northern Norway

Vidar Wennevik · Knut Eirik Jørstad · Geir Dahle · Svein-Erik Fevolden

Abstract Atlantic cod (*Gadus morhua*) encompasses many different populations or stocks, which in part are managed separately. In the northeast Atlantic cod is divided into two main management units; northeast Arctic cod and coastal cod. These two groups have traditionally been separated by otolith classification. In this study, the power of different classes of genetic markers in separating the two cod groups was investigated. The variation in thirteen genetic markers, including allozymes, haemoglobin, the scDNA locus Pantophysin (*Pan* I) and a number of microsatellites was investigated, and mixed stock analysis and individual assignment tests were performed on samples comprising a mixture of individuals of putative coastal and oceanic type cod. The genetic analyses showed a large genetic differentiation between outer stations and stations located closer to the mainland shore. Mixed stock analysis and individual assignment tests used for estimation of stock proportions gave results similar to those obtained by otolith classification.

Keywords Atlantic cod · Mixed stock analysis · *Pan* I

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Introduction

The Atlantic cod (*Gadus morhua* L.) is one of the most important commercial fish species in the North Atlantic, and a key species in the Barents Sea ecosystem. Much effort has been directed towards achieving optimal management regimes for Atlantic cod. In northern Norway, the cod is divided into two separate management units - Northeast Arctic Cod (NEAC) and Norwegian coastal cod (NCC). The NCC, although managed as a single entity, probably consists of a number of different populations spawning in different fjords along the Norwegian coast. During the last years the total annual harvest of NEAC has been in the range of 400,000–500,000 tonnes, whereas landings of NCC has been around 30,000–40,000 tonnes. Both research surveys and harvest data have indicated that the NCC stock complex is in a depleted condition, and managers aim to reduce the fishing pressure. ICES has suggested that no quotas should be allocated for NCC in 2006.

The distinction of northeast Atlantic cod into separate groups or stocks dates back to early studies

of otolith structure and life history characteristics that revealed differences between oceanic cod and cod inhabiting coastal areas (Rollefsen, 1933). In the 1960s genetic analysis based on blood groups (Møller, 1966) and blood proteins (Frydenberg et al., 1965; Møller, 1968) supported the view that cod could be divided into migratory Arctic cod, and more stationary coastal cod. However, Frydenberg et al. (1969) argued that the variation in frequency of one of the blood proteins (haemoglobin—Hb I) along the Norwegian coast could also be explained by selection from different environmental conditions. Studies employing allozyme markers have shown relatively limited variation among cod populations along the Norwegian coast, and also between NCC and NEAC (Jørstad, 1984; Mork et al., 1985; Jørstad & Nævdal, 1989; Mork & Giæver, 1999). More recent studies employing various DNA markers have yielded results ranging from panmixia or high gene flow (mtDNA) across much of the Atlantic (Smith et al., 1989; Dahle, 1991; Arnason et al., 1992, 2000) to the presence of significant population structuring (microsatellite DNA, scnDNA) on small to medium spatial scales (Fevolden & Pogson, 1997; Hutchinson et al., 2001; Jonsdottir et al., 2001; Knutsen et al., 2003; Nielsen et al., 2003; Pogson & Fevolden, 2003; Sarvas & Fevolden, 2005; Pampoulie et al., 2006). Similar results have been reported from the western Atlantic, where temporally stable differences between inshore and offshore cod off Newfoundland have been demonstrated (Ruzzante et al., 1996, 1997, 1999). The gene marker *Pan* I (identical to GM798 in Pogson et al. (1995) and *Syp*I in Fevolden & Pogson (1997)) exhibits particularly large differences in allele frequencies between samples collected in the Barents Sea and in coastal areas of Norway. While samples of NEAC are almost fixed for the *Pan* I^B allele, samples of NCC exhibit high frequencies of the *Pan* I^A allele (Fevolden & Pogson, 1997; Sarvas & Fevolden, 2005).

The importance of understanding the population structure of exploited species has long been acknowledged. Failure to recognize sub-structuring of a population subjected to mixed-stock fisheries may lead to over-exploitation of components of the population and erosion of genetic diversity (Ruzzante et al., 2000; Ward, 2000; Nielsen & Kenchington, 2001). Management units and biological entities as populations do not necessarily overlap completely. Management units, usually termed stocks, are sometimes defined not only on the basis of biological knowledge of the species, but may often be constructs that reflect management goals and policy rather than natural biological divisions (Carvalho & Hauser, 1994).

The development of statistical methods for determining the different components of mixed-stock fisheries, Mixed Stock Analysis (MSA), expanded the potential of genetic methods in fishery management (see Cadrin et al., 2005 and references therein). These methods allow the estimation of the relative exploitation, mortality and harvest rates of the different components of mixed-stock fisheries. The most common method applied with genetic data in fisheries management has been the maximum likelihood method (Fournier et al., 1984; Millar, 1987; Pella & Milner, 1987). A weakness of the maximum likelihood methods is that when the contribution of different stock components in the sample is uneven, the contribution of the less common components in the mixture tends to be underestimated (Pella & Milner, 1987; Xu et al., 1994). Also, the issue of "sampling zeroes", the absence of alleles found in the mixed sample in any of the baseline populations, have led to alternative Bayesian estimators of allele frequencies (Rannala and Mountain, 1997; Pella & Masuda, 2001).

Related to MSA are the so-called individual assignment tests. In these tests, the information derived from the multilocus genotypes of individuals in a mixed sample are used to assign the individual to one of a set of possible baseline populations (see reviews by Waser & Strobeck, 1998 and Hansen et al., 2001). The original assignment test was developed by Paetkau et al. (1995), but numerous variations and improvements have since been introduced (e.g. Rannala & Mountain, 1997; Cornuet et al., 1999; Banks & Eichert, 2000; Pritchard et al., 2000; Dawson & Belkhir, 2001; Pella & Masuda, 2001; Piry et al., 2004).

Concerning the two main groups of cod in the northeast Atlantics (NEAC and NCC), there is some overlap in spawning areas since major spawning for both occur around the Lofoten Islands. Normally, the NEAC stock is dominating the offshore banks west of the islands, while the NCC stock is found mainly near the coastline and inside the Vestfjord, and other

fjords of northern Norway (Hylen, 1964; Møller, 1968; Dahle & Jørstad, 1993; Nordeide, 1998). Depending on environmental conditions, especially temperature, some of the NEAC will spawn in the inner parts of Vestfjorden, on the same spawning sites as the NCC. The detailed mechanisms that limit or prevent interbreeding between the two main groups are unknown, but recent studies on depth distribution (Nordeide, 1998), spawning cod sound (Nordeide & Kjellsby, 1999), modelling of egg and larvae dispersal (Vikebø et al., 2005) and population specific egg buoyancy (Stenevik & Sundby, 2005), have suggested several factors that possibly are involved. Thus, in such intermingling areas the harvest is a typical mixed stock fishery. Recently, the general decline and weakness of the NCC group have put focus on the potential negative effects of the present management regime, and in particular, has accentuated the need for proper estimates of the proportions of the two groups in the commercial catches in major spawning areas. In this aspect, methods such as individual assignment and mixed stock analysis (based on genetic information) offer a potential opportunity to discriminate between the two groups and estimate the stock proportions in catches of various geographic areas.

In this article we investigate whether the previously observed structuring of cod at the Lofoten spawning grounds into two main groups, NCC and NEAC, based morphological differences in otoliths, is supported by information from genetic markers, and whether otolith classification can be regarded as a reliable tool for discrimination of cod in this area into different groups. We apply both mixed stock analysis and individual assignment to samples collected both near-shore and more distant from the coast at the Lofoten spawning grounds.

Material and methods

Collection of samples

Samples were collected during the annual cod survey carried out in the area from shelf banks outside Malangen and south to the Lofoten areas, including Vestfjorden. These surveys were conducted during the spawning season for cod in the area, usually from the middle of March to about April 10. In 2002 the R/V "G.O. Sars" were used, while R/V "Sarsen" conducted the survey in 2003. The main approaches used in the surveys were acoustic estimates of cod abundance, supplemented by pelagic and bottom trawling to collect samples for biological characterization. For all trawl catches, the cod were routinely characterised with regards to biological parameters (length, weight, sex, maturation etc). Otoliths were collected for routine classification to otolith type according to the procedures in the manual for sampling of fish and crustaceans at the Institute of Marine Research (IMR) (Borge et al., 2002).

In 2002, samples for genetic analyses were collected from six of the trawl stations, mainly covering the spawning areas around the Lofoten, including the outer shelf areas (Moskenes bank and Røst bank) as well as in Vestfjorden (see Fig. 1 for details). A similar approach was used during the survey in 2003, and six samples were collected in essentially the same geographic areas. The number of cod sampled at each station varied according to catch size, and in large catches 96 specimens were sampled (Table 1).

The genetic sampling was coordinated with the individual recording of biological characterization and included blood for haemoglobin analyses, white muscle for allozyme analyses and fin clips for DNA investigations. Heparin was added to the blood samples, which were kept cold, at least overnight, until analysis. White muscle samples were frozen as soon as possible on the research vessel and stored frozen (−80 C) until later analysis in the laboratory in Bergen. The fin clips (dorsal fin) were stored in 96% ethanol for later extraction of DNA, and microsatellite and *Pan* I analyses.

Biological characterization

All biological measurement were carried out on the research vessels during the surveys and later loaded into the IMR databases. This also included otolith analyses, which were performed during the surveys by experienced otolith readers. The classification is based on the established procedures (Borge et al., 2002) based on the classification criteria first described by Rollefsen (1933). Based on otolith classification, each fish was grouped into one of five otolith categories or types, where otolith type 1 and 2 correspond to the NCC group (certain NCC and

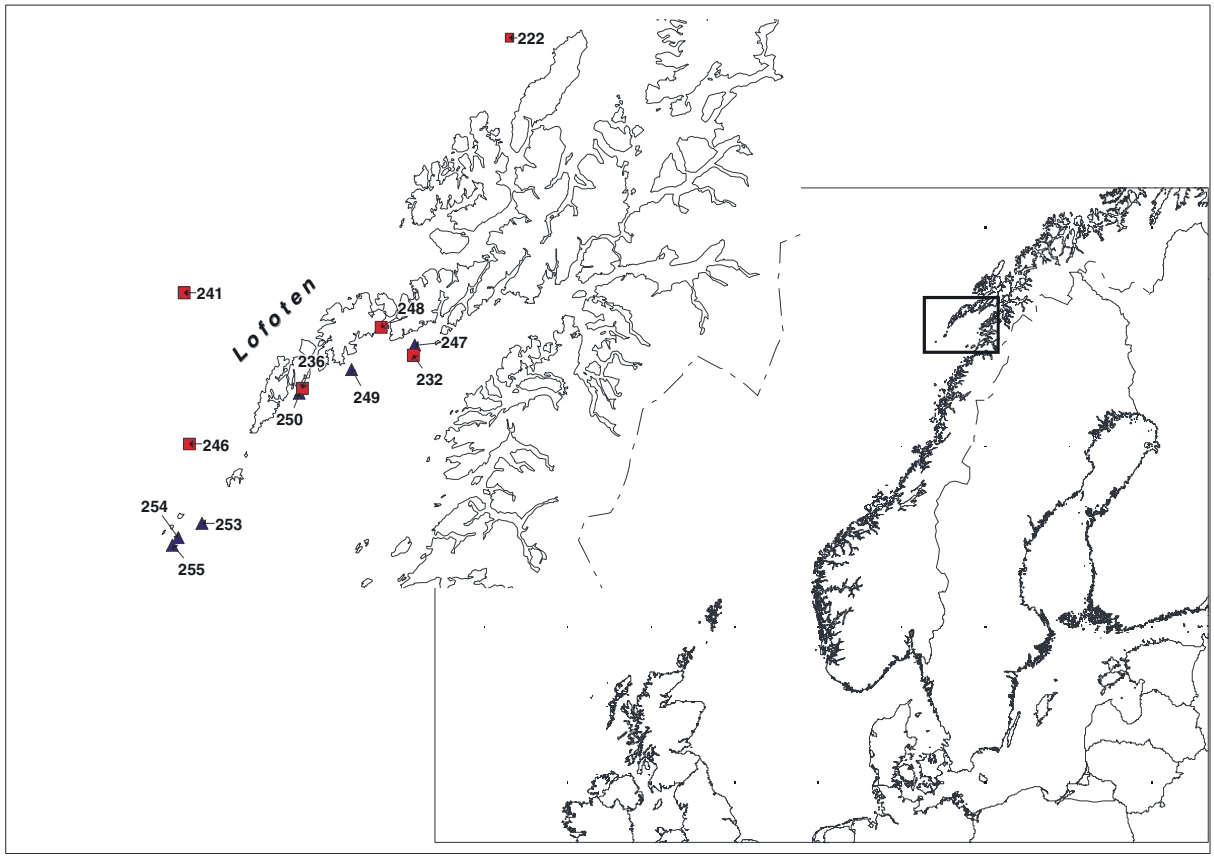

Fig. 1 Sampling stations in 2002 (black triangles) and 2003 (red squares)

Table 1 Location, date collected, number of individuals in samples, otolith classification of individuals and average age at sampling locations. Latitudes are given in degrees N, and longitudes are degrees E

Station no	Date	Latitude	Longitude	No fish	Otolith classification					# classified	Av. age (years)
					1	2	3	4	5		
247	27.03.2002	68.158	14.457	80	33	4	0	6	16	59	5.7
249	29.03.2002	68.067	13.815	51	26	4	0	3	18	51	5.6
250	30.03.2002	67.978	13.289	51	24	3	0	5	19	51	6.2
253	30.03.2002	67.495	12.31	60	6	2	0	10	29	47	6.9
254	31.03.2002	67.443	12.078	58	6	3	1	6	42	57	6.8
255	31.03.2002	67.413	12.017	36	0	3	0	5	28	36	6.8
222	24.03.2003	69.3	15.4	95	2	1	0	9	24	36	6.3
232	29.03.2003	68.12	14.44	96	45	14	0	11	11	81	6.4
236	30.03.2003	67.998	13.328	96	15	10	0	18	53	96	6.4
241	02.04.2003	68.352	12.137	96	3	1	0	10	76	90	6.8
246	03.04.2003	67.792	12.19	95	1	3	0	14	74	92	6.8
248	05.04.2003	68.223	14.115	96	72	14	0	6	3	95	5.6

uncertain NCC, respectively), while otolith type 4 and 5 correspond to the NEAC group (uncertain NEAC and certain NEAC, respectively). Otoliths not attributable to NCC of NEAC are typed as 3. This classification approach has been used for decades in cod stock management in the Northeast Atlantic.

Genetic analyses

Due to lack of stability, the blood samples had to be analysed within 3 to 4 days. After sedimentation of the blood cells, distilled water was added and the cells were disrupted. The samples were then analysed using the agar gel electrophoresis approach, first described by Sick (1961) and later modified by Jørstad (1984). The different banding patterns corresponding to the most common genotypes have earlier been described by Sick (1961). More detailed descriptions are also given in Dahle & Jørstad (1993) and Husebø et al. (2004). All blood sample analyses were conducted on fresh samples on the research vessel during the ongoing survey.

The samples of white muscle tissue were stored at low temperature (−80 °C) at the molecular biology laboratory of IMR in Bergen until analyses. The samples were analysed for 3 to 5 months after collection at sea, using standard starch gel electrophoresis with modifications as described by Jørstad (1984). Five different tissue enzymes were analysed, including lactate dehydrogenase (LDH), isocitrate dehydrogenase (IDH), glycerophosphate dehydrogenase (GPD), phosphoglucomutase (PGM) and phosphoglucose isomerase (GPI). These allozymes have earlier been described as polymorphic in cod (Mork et al., 1985; Jørstad & Nævdal, 1989).

Fin clips were stored (4°C) in the laboratory in Bergen until DNA extraction, which was carried out by using Qiagen DNeasy 96 Tissue kit (Qiagen). DNA quality was controlled by running the extractions on ready-to-run 1.2% agarose gels (Amersham Biosciences). The analyses of the *Pan* I locus followed the procedures described by Fevolden & Pogson (1997). The samples obtained from the 2002 survey were analysed in the laboratory at the Norwegian College of Fishery Science, University of Tromsø, while the 2003 collections were analysed in the laboratory in Bergen. The samples were analysed for six microsatellite loci (Gmo2; Gmo3; Gmo34; Gmo35; Gmo132; Tch11), all well established in cod studies and used in a number of other population studies in cod (Brooker et al., 1994; Miller et al., 2000; O'Reilly et al., 2000). Primer labels were according to ABI 3100 requirements, and the PCR reactions were mainly run separately for each primer-set to ensure sufficient amplification. The PCR products were mixed in appropriate groups according to primer labelling and fragment size and co-loaded on an ABI 3100 genetic analyser. Size standard GeneScan 500 LIZ (Applied Biosystems) was added to each sample. Fragment sizing was conducted by using the ABI Genotyper computer program package, with manual control of the automatically scored peaks.

Statistical analysis

Allele frequencies, F_{ST} values and exact tests of population difference between population pairs, tests for linkage disequilibrium and deviations from Hardy-Weinberg equilibrium were calculated in the program GENEPOP 3.4 (Raymond & Rousset, 1995). Nei's (1978) genetic distance and heterozygosities were computed in TFPGA (Miller, 1997). This program was also used to compute the UPGM dendrogram, and the % of bootstraps supporting the nodes based upon Nei's (1978) genetic distance. Allelic richness was calculated in FSTAT 2.9.3.2 (Goudet, 2001).

For the assignment and mixed-stock analysis of the samples collected in 2002 & 2003, two different approaches were used. First, base populations for presumed NCC and NEAC were constructed by pooling all individuals from stations 232 to 248 as a baseline for NCC and all individuals from stations 222, 241 and 246 as the NEAC baseline. Based on otolith classification these two groups of stations appeared to be dominated by NCC and NEAC, respectively, although individuals with otoliths of NEAC type were present in the NCC baseline and individuals with CC type otoliths were present in the NEAC baseline. The mixed samples consisted of stations 236, 247, 249, 250, 253, 254 and 255. Nineteen individuals in the mixed samples had alleles not present in the two baseline samples, and blanks were substituted for alleles at affected loci for these individuals before conducting the analysis (Gmo 2; 1 ind., Gmo35; 2 ind., Gmo132; 5 ind., Tch11; 5 ind. GPI-1; 3 ind., and GPD; 3 ind.). No individual in the mixed sample had more than one allele not represented in the baseline.

The program GENECLASS2 (Piry et al., 2004) was used for individual assignment analyses, and mixed stock analysis (MSA) was conducted in the program BAYES (Pella & Masuda, 2001). MSA and individual assignment analysis was conducted on all samples from 2002, and on the sample from station 236 from

2003, using various combinations of loci. The assignment power of the loci was evaluated using the program WHICHLOCI (Banks et al., 2003). This program uses successive assignment trials from one locus at a time, on a test data set generated by random sampling of the alleles according to their frequency in the populations in the given data set, to generate a ranking of the loci's efficiency for correct population assignment. In the present study, the population size parameter for the randomly generated populations was set to 500. The program GENETIX (Belkhir et al., 2004) was used to conduct a factorial analysis of the baseline populations constructed for the mixed stock analysis.

In order to verify the validity of this approach, with defining baseline populations from geography, a second analysis of the data was conducted in the program STRUCTURE (Pritchard et al., 2000). In this analysis, no prior information of morphology (otoliths) or geography (sampling locality) was used, and individuals were partitioned into putative groups based on genetic information alone. In the runs, the burn in length was set to 30,000, and the run length to 500,000. Possible values of K from 1 to 4 were evaluated, with three parallel runs at each value of K. The samples from 2002 and 2003 were run in separate tests. For all analyses the admixture model was used.

Results

Otolith types and age structure at the sampled stations

According to otolith classification, all stations in both years had individuals of NCC and NEAC type, but in different proportions (Table 1). The trawl stations in 2002 were distributed in increasing distance from the mainland in a southwesterly transect along the Lofoten Islands, with rising station numbers (see Fig. 1). The distribution of otolith types show a decreasing trend in the frequencies of NCC (types 1 and 2) and a corresponding increase in NEAC (type 4 and 5) along this transect. At the innermost trawl station (247), the fraction of NCC according to otolith classification was 62%, while it was reduced to only 8% at station 255. Ages from 1 to 15 years were represented in the samples, with age-classes from 4 to 8 being most abundant. There was also a slight increase in average age from the inner to the outer stations (Table 1).

The trawl stations sampled in 2003 were distributed somewhat differently. Three stations, 222, 241 and 246, were located offshore from the mainland, on the shelf banks west of the Lofoten islands, while the other three stations were situated closer to shore (Fig. 1) within the Vestfjorden area. The distribution of otolith types shows that the three outer stations were almost totally dominated by NEAC (92–96%), while the two innermost stations appear to be dominated by NCC (73% and 91%). One station, 236, appears to consist of a more even mix of individuals of both types. Age-classes from three to ten were represented, with ages from five to eight being most abundant. The average age of individuals was quite uniform among the stations, except for station 248 where the average age was lower (Table 1).

Allelic variation among loci and samples

The number of alleles across samples ranged from two at the *Pan* I locus to 33 at the Gmo132 locus, and mean heterozygosity among loci varied from 0.017 to 0.932 (Table 2). Allelic richness among loci across samples varied from 1.0 at the IDH locus at several stations to 16.8 at the Gmo132 locus at station 232 in 2003 (Table 2).

In general, heterozygosity and allelic richness were much higher at the microsatellite loci than at the *Pan* I, haemoglobin and allozyme loci. Allele numbers across samples at microsatellite loci ranged from 8 to 33. Lower numbers of alleles at the microsatellite loci Gmo34 and Gmo132 were observed in the samples from the outer stations. This difference between inner and outer stations in allelic richness at these two loci was apparent in both years, with a declining trend in number of alleles at the Gmo34 locus with increasing distance from the mainland at the stations sampled in 2002. Average heterozygosity at individual loci across samples varied from 0.176 at the Gmo3 locus to 0.932 at the Tch11 locus. The allelic distribution at four of the loci was similar across samples, but the loci Gmo34 and Gmo132 displayed a clear difference between samples dominated by individuals with otoliths of NEAC and NCC type.

Table 2 Summary statistics for the 13 loci in the 12 samples.Abbreviations are as follows: number of individuals (*N*), scoring percentage achieved at locus (*S*), number of alleles at locus (N_A), allelic richness (AR) F_{ST} in individual loci across samples (F_{ST}), and Nei's unbiased heterozygosity (*H*)

	Location		*Pan* I	Gmo2	Gmo3	Gmo34	Gmo35	Gmo132	Tch11	Hem	GPD	IDH	PGM	GPI-1	LDH-3	Average
2002	St. 247	*N*	77	77	78	77	77	78	71	59	59	59	59	59	59	68.38
		S	96	96	98	96	96	98	89	74	74	74	74	74	74	85.61
		N_A	2	11	4	8	8	22	18	2	2	2	3	3	2	6.69
		AR	2.000	9.288	3.138	6.205	7.257	14.289	15.199	2.000	1.931	1.407	2.287	2.931	2.000	5.37
		H	0.446	0.872	0.201	0.506	0.829	0.724	0.927	0.374	0.082	0.017	0.082	0.483	0.504	0.47
	St. 249	*N*	51	49	51	48	47	49	51	50	51	51	51	51	48	49.84
		S	100	96	100	94	92	96	100	98	100	100	100	100	94	97.69
		N_A	2	12	4	7	7	15	19	3	2	1	2	4	3	6.23
		AR	2.000	9.601	3.118	5.843	6.510	11.074	16.302	2.732	1.722	1.000	1.962	3.396	2.500	5.21
		H	0.502	0.797	0.131	0.352	0.829	0.659	0.934	0.408	0.039	0.000	0.094	0.405	0.503	0.43
	St. 250	*N*	51	51	51	49	47	51	51	50	51	51	51	51	50	50.38
		S	100	100	100	96	92	100	100	98	100	100	100	100	98	98.77
		N_A	2	13	5	7	8	18	20	3	2	2	2	3	2	6.69
		AR	2.000	10.867	3.644	6.200	7.708	13.749	17.019	2.732	1.722	1.471	1.856	2.722	2.000	5.67
		H	0.502	0.872	0.184	0.477	0.852	0.760	0.938	0.377	0.039	0.020	0.058	0.522	0.498	0.47
	St. 253	*N*	56	59	56	59	55	58	57	58	60	60	60	60	60	58.31
		S	93	98	93	98	92	97	95	97	100	100	100	100	100	97.15
		N_A	2	14	3	6	7	13	21	2	2	2	2	3	3	6.15
		AR	2.000	10.981	2.104	4.449	6.812	7.843	16.334	2.000	1.642	1.400	1.875	2.400	2.642	4.81
		H	0.446	0.874	0.053	0.329	0.812	0.497	0.927	0.265	0.033	0.017	0.065	0.459	0.497	0.41
	St. 254	*N*	54	49	56	51	51	58	50	58	58	58	58	58	58	55.15
		S	93	84	97	88	88	100	86	100	100	100	100	100	100	95.08
		N_A	2	10	4	5	7	10	17	3	3	2	3	4	3	5.62
		AR	2.000	8.561	2.674	4.039	6.924	7.062	14.980	2.658	1.828	1.414	2.216	3.216	2.802	4.64
		H	0.316	0.839	0.087	0.235	0.839	0.453	0.930	0.395	0.034	0.017	0.068	0.489	0.484	0.40
	St. 255	*N*	35	35	36	35	35	36	33	24	36	36	36	36	36	34.54
		S	97	97	100	97	97	100	92	67	100	100	100	100	100	95.92
		N_A	2	12	5	3	7	8	20	2	1	2	2	3	2	5.31
		AR	1.998	10.412	4.299	2.896	6.904	6.871	18.095	2.000	1.000	1.667	1.990	2.667	2.000	4.83
		H	0.135	0.858	0.277	0.162	0.846	0.468	0.943	0.284	0.000	0.028	0.106	0.504	0.468	0.39

Table 2 continued

	Location		*Pan* I	Gmo2	Gmo3	Gmo34	Gmo35	Gmo132	Tch11	Hem	GPD	IDH	PGM	GPI-1	LDH-3	Average
2003	St. 222	*N*	87	95	96	95	89	95	94	94	96	96	96	96	96	94.23
		S	91	99	100	99	93	99	98	98	100	100	100	100	100	98.23
		N_A	2	15	5	4	8	14	23	3	3	2	3	2	3	6.69
		AR	2.000	11.278	2.952	3.233	7.318	7.424	17.506	2.251	1.500	1.438	2.198	2.000	2.438	4.89
		H	0.239	0.860	0.120	0.141	0.825	0.489	0.939	0.207	0.021	0.021	0.109	0.469	0.487	0.38
	St. 232	*N*	71	81	81	80	81	81	81	81	81	81	81	81	81	80.15
		S	88	100	100	99	100	100	100	100	100	100	100	100	100	99.00
		N_A	2	15	5	8	8	23	19	3	2	2	3	3	2	7.31
		AR	2.000	11.606	3.810	6.107	7.228	15.009	16.804	2.506	1.296	1.654	2.450	2.832	2.000	5.79
		H	0.336	0.879	0.266	0.557	0.833	0.798	0.937	0.459	0.012	0.037	0.118	0.479	0.481	0.48
	St. 236	*N*	96	94	96	96	96	96	96	96	96	96	96	96	95	95.76
		S	100	98	100	100	100	100	100	100	100	100	100	100	99	99.76
		N_A	2	11	7	7	10	19	18	3	3	1	2	5	2	6.92
		AR	2.000	8.872	4.429	5.082	7.614	10.480	14.890	2.250	2.161	1.000	1.871	3.405	2.000	5.08
		H	0.476	0.827	0.265	0.348	0.834	0.589	0.928	0.308	0.061	0.000	0.071	0.446	0.483	0.43
	St. 241	*N*	84	89	90	88	89	90	90	90	90	90	90	90	90	89.23
		S	93	99	100	98	99	100	100	100	100	100	100	100	100	99.15
		N_A	2	16	7	5	8	11	18	3	2	2	4	4	3	6.53
		AR	1.993	11.515	3.860	3.403	7.187	6.354	14.946	2.264	1.792	1.463	2.817	2.981	2.468	4.85
		H	0.154	0.860	0.149	0.152	0.837	0.403	0.930	0.191	0.054	0.022	0.117	0.460	0.512	0.37
	St. 246	*N*	90	92	93	93	93	93	91	93	93	93	93	93	93	92.53
		S	97	99	100	100	100	100	98	100	100	100	100	100	100	99.53
		N_A	2	12	8	5	9	11	20	2	3	2	3	3	3	6.38
		AR	1.998	9.056	3.849	3.188	7.230	6.094	15.508	2.000	1.709	1.451	2.171	2.779	2.451	4.57
		H	0.190	0.826	0.154	0.124	0.814	0.479	0.923	0.250	0.032	0.021	0.093	0.471	0.512	0.38
	St. 248	*N*	94	96	95	94	95	94	96	96	96	96	96	96	96	95.38
		S	98	100	99	98	99	99	100	100	100	100	100	100	100	99.46
		N_A	2	17	7	7	9	25	20	2	2	1	4	4	3	7.92
		AR	2.000	11.356	4.230	5.927	7.465	15.588	15.987	2.000	1.438	1.000	2.375	3.198	2.250	5.75
		H	0.277	0.877	0.224	0.601	0.833	0.842	0.930	0.415	0.021	0.000	0.071	0.505	0.489	0.47
		$TotN_A$	2	21	10	8	12	33	28	3	4	2	5	6	3	
		AvH	0.335	0.853	0.176	0.332	0.832	0.597	0.932	0.328	0.036	0.017	0.088	0.474	0.493	
		AvAR	1.999	10.185	3.443	4.604	7.154	9.569	16.144	2.271	1.682	1.326	2.191	2.926	2.308	
		F_{ST}	0.330	0.008	0.007	0.050	0.000	0.035	−0.001	0.029	0.001	−0.003	−0.003	0.006	0.001	

The greatest differences in allele frequencies between samples were found at the two-allele *Pan* I locus, with frequencies of the *Pan* I^A allele ranging from 0.83 (station 248) to 0.07 (station 255) In general, the frequencies decreased with increasing distance from the mainland. The distribution of *Pan* I genotypes was also closely related to the distribution of otolith types, with the *Pan* I^A being most common in individuals with otolith type 1 and 2, and the *Pan* I^B allele in individuals with otolith types 4 and 5. The fraction of homozygotes for the *Pan* I^A allele decreases from over 70% in individuals with otolith type 1 (certain NCC) to around 5% in individuals with otolith type 5 (certain NEAC). The fraction of *Pan* I^{AB} heterozygotes is somewhat higher in individuals of otolith type 2 and 4 (uncertain NCC and uncertain NEAC, respectively), 29% and 45%, than in individuals of otolith type 1 and 5, which had heterozygote fractions of 24% and 17%, respectively.

The frequencies of haemoglobin variants varied between stations, and followed a similar pattern as the *Pan* I locus, with different frequency distributions in stations close to the mainland and further out. The frequency of the most common allele HbI^2, varied from around 0.7 at the stations dominated by individuals with otolith type 1 and 2 to around 0.85 to 0.90 at stations dominated by individuals with NEAC otolith types. Three alleles in all were found at this locus.

The number of alleles in allozymes varied from 2 (IDH) to 6 (GPI-1). In general, there was little variation between stations in both years, and no apparent trend in allele distributions at any locus. Some stations were monomorphic for IDH.

Hardy–Weinberg and linkage disequilibrium tests

The overall sample collection was tested according to Hardy–Weinberg equilibrium for each of the thirteen loci investigated. Five of the loci revealed significant departure from HW and all these were due to heterozygote deficiency. These loci included *Pan* I ($P < 0.0001$); Gmo132 ($P = 0.034$); Tech11 ($P < 0.0001$), Gmo3 ($P < 0.0001$) and HbI ($P = 0.0054$). The overall test including all loci and all samples demonstrated highly significant ($P < 0.0001$) departure from HW due to deficiency of heterozygotes.

The two-tailed test for Hardy–Weinberg equilibrium, considered for each population and with sequential Bonferroni correction for multiple tests (Rice, 1989), showed 11 significant deviations from equilibrium. Deficiency of heterozygotes was observed at locus *Pan* I at stations 222, 249 and 250, at haemoglobin at station 249, at locus Tch11 at stations 232, 236 and 246, at locus Gmo2 at stations 247, 254, and 246, and at locus Gmo3 at station 246. No instances of heterozygote excess were observed.

In the tests for linkage disequilibrium of the samples from 2002 to 2003 three significant deviations from equilibrium were found after Bonferroni correction for 78 tests; between *Pan* I and Gmo34, between *Pan* I and Gmo132 and between *Pan* I and Haemoglobin.

Genetic differentiation

The sample collection was tested for homogeneity at the various loci analysed and significant differentiation among samples following sequential Bonferroni correction was found at all loci except Gmo35, Tch11, GPD, IDH, PGM, GPI and LDH.

The exact tests for pairwise genetic differentiation showed significant differences between most stations in both years (see Table 3), but there were also stations that were not significantly different. The largest differences in 2002 were found between the innermost and outermost stations. The inner (stations 232 and 248) and outer stations (stations 222, 242, 246) in 2003, consisting of mostly of individuals with coastal and oceanic type otoliths, respectively, formed two groups with small and not significant differences within the groups, and large differences between them (Table 3). F_{ST} values between stations ranged from zero to 0.12. A test of genetic differentiation between year classes 6, 7 and 8 of individuals pooled from outer stations 222, 241 and 246, with 85, 82 and 33 individuals, respectively, showed no significant differences across loci ($P = 0.55$), nor at any individual locus (*P*-values ranging from 0.11 to 0.86), demonstrating temporal stability and homogeneity within the samples from these stations.

The greatest genetic differentiation among the microsatellite loci was observed at loci Gmo34 and Gmo132, while the other four loci showed little or no differentiation measured as F_{ST} (see Table 4). Pairwise F_{ST} between stations calculated for microsatellites alone ranged from zero to 0.0387 across loci (data not shown, available from the authors upon request), with stations 241 and 248 being most

Table 3 Matrix of exact tests of genic differentiation at the 13 loci (above diagonal), and genetic differentiation estimated as F_{ST} (below diagonal). Bold indicates values that remain significant after sequential Bonferroni correction

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
St 222 (1)		**<0.0001**	**<0.0001**	0.4588	0.5349	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.0295	0.1524	0.5162
St 232 (2)	0.0861		**<0.0001**	**<0.0001**	**<0.0001**	0.5188	**0.0006**	**<0.0001**	0.0508	**<0.0001**	**<0.0001**	**<0.0001**
St 236 (3)	0.0144	0.0337		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0010**	0.2836	0.0126	**<0.0001**
St 241 (4)	0.0000	0.1014	0.0202		0.6405	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.0126	0.8710
St 246 (5)	0.0001	0.0942	0.0165	-0.0008		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.0200	0.4327
St 248 (6)	0.1015	-0.0005	0.0442	0.1174	0.1106		0.0169	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
St 247 (7)	0.0642	0.0064	0.0193	0.0748	0.0699	0.0065		0.0363	0.2406	**<0.0001**	**<0.0001**	**<0.0001**
St 249 (8)	0.0351	0.0300	0.0093	0.0415	0.0419	0.0354	0.0099		0.1399	**0.0011**	0.0080	**<0.0001**
St 250 (9)	0.0397	0.0109	0.0117	0.0532	0.0479	0.0165	0.0031	0.0111		0.00125	**<0.0001**	**<0.0001**
St 253 (10)	0.0073	0.0475	0.0009	0.0123	0.0130	0.0581	0.0260	0.0117	0.0149		0.4098	0.0098
St 254 (11)	0.0017	0.0689	0.0082	0.0072	0.0062	0.0828	0.0475	0.0166	0.0287	0.0026		0.3221
St 255 (12)	-0.0009	0.0945	0.0193	-0.0001	0.0024	0.1108	0.0715	0.0422	0.0438	0.0132	0.0018	

Table 4 Relative score of individual loci in individual assignment evaluated in WHICHLOCI

Rank	Locus	% (Relative score)
1	PAN	20.8
2	Gmo132	19.1
3	Gmo34	17.4
4	HEM	16.0
5	Tch11	9.8
6	Gmo2	8.5
7	Gmo3	2.9
8	Gmo35	2.3
9	PGM	1.5
10	PGI-1	1.0
11	GPD	0.4
12	LDH-3	0.2
13	IDH-2	0.2

differentiated. Pairwise F_{ST} values for the *Pan* I locus were high between inshore and offshore stations, with the highest observed value of 0.68 found between stations 232 and 241. The highest value for the haemoglobin locus of 0.14 was also found between these two stations. The pairwise F_{ST} values for the allozyme loci were in general low. A regression analysis of the correlation between F_{ST} values at the *Pan* I locus and the microsatellite loci was highly significant ($r^2 = 0.82$, $P < 0.0001$).

The three UPGMA dendrograms of the samples from 2003 presented in Fig. 2 show a very similar topology. In all three the stations 232 and 248 form a very tight cluster, as do the stations with dominance of NEAC otolith types; 222, 241 and 246. Station 236 takes an intermediate position. Excluding the *Pan* I and haemoglobin loci from the marker set did not have any influence on the tree topology.

Mixed stock analysis and individual assignment analysis

(a) Using baselines defined from geographical distribution of samples

The two baseline data sets constructed from outer (222, 241 and 246) and inner (232, 247) stations in 2003 were highly differentiated with an F_{ST} value of 0.11 across 13 loci. The largest differentiation was observed at the *Pan* I locus, which had an F_{ST} value of 0.68. Figure 3 shows the graphical distribution of individuals from the factorial analysis conducted in GENETIX. There is little overlap between the two groups, but the spread of individuals of NCC type is larger than for those of NEAC type. The self-assignment test conducted in GENECLASS2 resulted in correct assignment of 402 of the 456 individuals in the two baseline groups, corresponding to 88.2%. The analysis of the performance of the different loci in a self-assignment test on the two baselines conducted in WHICHLOCI, showed that the *Pan* I locus contributed most to correct assignment, followed by Gmo132, Gmo34 and Haemoglobin. The other loci contributed little or nothing (allozymes) to the assignment. The ranking and relative score of the loci are given in Table 4.