Population genetic structure of the glacier lanternfish, *Benthosema glaciale* (Myctophidae) in Norwegian waters

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It is hypothesised that the fjords with restricted water circulation may partially isolate fish populations living there. The pelagic life history stages of the mesopelagic lanternfish may potentially subject to dispersal over vast areas, preventing population subdivision. In the present paper, we test the hypothesis of no geographic population genetic structure of *B. glaciale* in Norwegian waters. Fish samples from six different locations, including five western fjords were analysed using allozyme electrophoresis. Among the 17 loci analysed, nine loci were polymorphic by 0.99 criterion. Deviation from the global (pooled sample) HW equilibrium was detected at two loci, *AAT-2** and *PGM*,* in terms of heterozygote deficiency, indicating a possible population subdivision in the area studied. Supporting such postulate, the allele frequencies at several loci were found significantly different, consequently rejecting the null hypothesis. The individual fjord samples were different from the offshore sample, and some between-fjord heterogeneity in allele frequencies was also found. However, Wright's F_{ST} value was apparently low, but significantly different from zero, indicating a low level of population differentiation in the area studied. Partial isolation of fjord units of *B. glaciale* as a possible mechanism for genetic differentiation is discussed.

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INTRODUCTION

It is common knowledge that marine fishes with high dispersal abilities often tend to show little genetic differentiation over large geographic scales (Gyllensten 1985). However, partial isolation of populations and subsequent genetic differentiation may occur on a geographic scale that is much less than that allowed by the dispersal potential of the species (Palumbi 1994). Fjords provide peculiar habitats with unique environmental conditions created by both their hydrological and topographic characteristics (Farmer & Freeland 1983). It can be hypothesised that the fjord habitats with restricted water circulation may act to partially isolate fish populations, causing restriction to gene flow across adjacent waters. We investigated this hypothesis in relation to a mesopelagic fish, *Benthosema glaciale* (Reinhardt), by elucidating the population genetic structure of the species.

The glacier lanternfish, *B. glaciale* (Myctophidae), is among the highly abundant mesopelagic fishes in the Norwegian fjords and adjacent areas (Gjøsæter 1973, 1981). It is a zooplanktivore and an important prey for a wide range of fish species, such as saithe *Pollachius virens* L., blue whiting *Micromesistius poutassou* (Giske & al. 1990) and mackerel *Scomber scombrus* (Walker & Nicholas 1993), and thus plays an important role in the marine ecosystem. Distribution, ecology and some aspects of life history of the species have been investigated (Halliday 1970; Gjøsæter 1973, 1981; Lopes 1979; Kawaguchi & Mauchline 1982; Salvanes & Kristoffersen, in press). Reproductive biology of the species is partly known (Gjøsæter 1981). It is a gonochoristic species with clear sexual dimorphism, and matures at an age of 2-3 years (Gjøsæter 1981). Sparse information indicates a mode of repeated rather than a single event of spawning that occurs mainly through summer in Norwegian waters (op. cit.). Existing literature on *B. glaciale* do not indicate any spawning migration or other types of horizontal migrations.

Pelagic early life stages of *B. glaciale* predict great dispersal abilities over vast areas, and hence population differentiation at micro-geographic scale is less likely. However, some aspects of the life history may be indicative of adaptations that can cause restrictions to gene flow among adjacent areas. *B. glaciale* under-

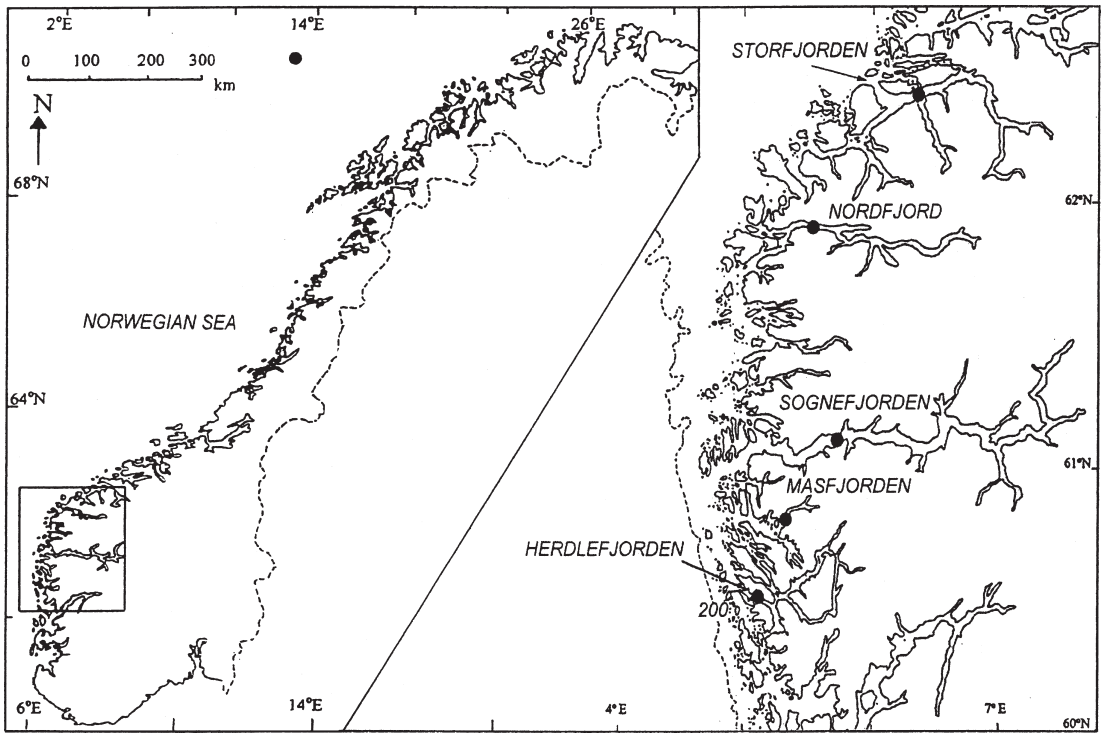

Fig. 1. Collection sites of *Benthosema glaciale* samples (marked with filled circles) in Norway and adjacent ocean.

take diurnal vertical migrations to the shallow waters, and the daytime distribution of adult *B. glaciale* in Norwegian fjords is mainly restricted to the deep water column below sill depth (Kaartvedt & al. 1988; Giske & al. 1990). At this depth, the wind-generated dynamic water circulation is expected to have little impact on the distribution of the fishes (Aksnes & al. 1989; Asplin & al. 1999). Deep distribution of eggs and late-larval stage (Kawaguchi & Mauchline 1982) may also be an adaptation to reduce advection out of the native area (Asplin & al. 1999). However, the early larval stage which concentrates mainly in the upper or intermediate layers (Kawaguchi & Mauchline 1982), and adults and juveniles feeding in the shallow advective layers are likely to be subjected to passive drift. The drifting life stages may either be retained within inner fjords or transported out of the fjord, depending on the direction of the coastal winds (Asplin & al. 1999). If a restricted level of gene flow is somehow maintained among adjacent localities, this may subsequently lead to subdivision of the *B. glaciale* population.

Spatial variations in meristic, growth and life history parameters of *B. glaciale* have been observed (Gjøsæter 1973, 1981; Badcock 1981; Kawaguchi & Mauchline 1982). The size at sexual maturity and maximum size of mesopelagic fishes in partially enclosed seas, such as the Gulf of Mexico and the Mediterranean Sea have been found to be smaller than those of their Atlantic counterparts (cited in Gartner 1993). Gjøsæter (1981) reported indications of differences between oceanic and fjord samples of *B. glaciale* based on otolith characteristics, length-weight relationship and growth parameters. Badcock (1981) reported marked differences in meristic characters in *B. glaciale* between the Mediterranean Sea and the Atlantic populations. Although the mechanisms responsible for such differences are not apparent, it has been suggested that the hydrography of partially enclosed seas may provide sufficient barriers to gene flow such that populations within seas may be diverging from those outside the seas (Gartner 1993). Investigation of these differences at population genetic level seems to be a necessity as it will reveal whether such variability in phenotypes has a genetic basis.

In this paper, the null hypothesis of no geographic population genetic structure in *B. glaciale* from Norwegian fjords and oceanic area is tested. Samples of *B. glaciale* from five west Norwegian fjords and from the Norwegian Sea were utilised for the analysis of allozymes using starch gel electrophoresis.

MATERIAL AND METHODS

Samples

One sample (n = 120-130) of adult *B. glaciale* from each of five west Norwegian fjords, namely, Herdlefjorden, Masfjorden, Sognefjorden, Nordfjord and Storfjorden was collected by a small meshed pelagic trawl on board the RV *Håkon Mosby* in Feb-March 1998 (Fig. 1, Table 1). One sample (n = 120) from the Norwegian Sea off northern Norway collected on board the RV *G.O. SARS* in August 1997 was also included in the study.

Electrophoresis

Horizontal starch (11 %) gel electrophoresis was performed following the techniques described in Murphy & al. (1996), using aqueous homogenates of a piece of muscle from individual fish. Two continuous buffer systems, CAME or Amine-Citrate buffer (Morpholine, pH 6.8) (modified from Clayton & Tretiak 1972, with 1mM EDTA added) and Tris-Citrate II (pH 8.0, Selander & al. 1971) were selected. After electrophoresis, the activities of specific enzymes were visualised using histochemical staining procedures described in Murphy & al. (1996). Of the 36 enzymes analysed, only the following enzymes were adequately resolved; Aspartate aminotransferase (AAT, EC 2.6.1.1), Creatine kinase (CK, EC 2.7.3.2), Fumarate hydratase (FUMH, EC 4.2.1.2), Glycerol-3-phosphate dehydrogenase (GPD, EC 1.1.1.8), Isocitrate dehydrogenase (IDH, EC 1.1.1.42), L-Lactate dehydrogenase (LDH, EC 1.1.1.27), Malate dehydrogenase (MDH, EC 1.1.1.37), Malic enzyme (ME or MDHP, EC 1.1.1.40), Phosphogluconate dehydrogenase (PGDH, EC 1.1.1.44), Glucose-6-phosphate isomerase (GPI or PGI, EC 5.3.1.9) and Phosphoglucomutase (PGM, EC 5.4.2.2). CK, FUMH, GPD, LDH and ME enzymes were resolved using Tris-citrate II buffer system while the rest were resolved by the CAME buffer system. Although both AAT loci could be resolved by CAME buffer system, Tris-Citrate buffer was needed to identify the genotypes at the *AAT-2** locus. Locus nomenclature followed Shaklee & al. (1990). Multiple alleles were designated as the extent of migration relative to the most common allele (designated as 100).

Statistical analysis

Data and statistical analyses were performed using the software POPGENE V.1.31 (Yeh & al. 1997), FSTAT V.1.2 (Goudet 1996), and CHIHW and CHIRXC (Zaykin & Pudovkin 1993). Polymorphic loci were selected according to 0.99 criterion (if the frequency of the most common allele is < 0.99 in at least one sample) for population analysis. The selected loci were tested for linkage disequilibrium (by POPGENE).

Deviation from Hardy-Weinberg (HW) equilibrium within the individual samples, and within the pooled sample was tested by the pseudo-probability (χ^2) test at each locus using Monte Carlo simulation in CHIHW program. Per-locus probability level was adjusted by sequential Bonferroni method (Rice 1989). Homogeneity of allele frequencies across all samples and pairs of samples was tested by the contingency χ^2 test (by CHIRXC), and the combined probability for total chi-square value was reported. Pair-wise Rogers' modified genetic distance (Wright 1978) was calculated on all loci, and UPGMA dendrogram was constructed to depict the hypothetical genetic relationship among the samples. The robustness of the branches was evaluated by bootstrapping the distance matrix (100 replications), and finding the consensus tree, by the programs NEIGHBOR and CONSENSE in PHYLIP 3.57c software package (Felsenstein 1997). Single-locus *F*-statistics (Wright 1978) were computed on all polymorphic loci, and a multi-locus, weighted estimate was calculated according to Weir & Cockerham (1984). The standard deviations over loci for the F_{IT}, F_{IS} and F_{ST} were estimated using bootstrapping and jack-knife procedure (Weir 1990), and the statistical probability of $F \leq 0$ was obtained. An approximate estimate of gene flow (M) was obtained by private allele method of Slatkin (1985). Mantel tests for correlation between pair-wise estimate of F_{ST} or gene flow and geographic distances were used to test the isolation by distance (Slatkin 1993).

Table 1. Sampling locations and relevant data for the six *Benthosema glaciale* samples.

Location	Sill depth (m)	Position	Sampling depth (m)	Date	Sample size
Herdlefjorden	130	60°31'N, 05°08'E	96	3 Mar 1998	120
Masfjorden	75	60°52'N, 05°27'E	190	3 Mar 1998	130
Sognefjorden	240	61°07'N, 05°41'E	300	28 Feb 1998	120
Nordfjord	130	61°54'N, 05°35'E	260	1 Mar 1998	125
Storfjorden	190	62°24'N, 06°16'E	130	1 Mar 1998	120
Norwegian Sea	No sill	70°25'N, 13°29'E	300 - 400	16 Aug 1997	120

RESULTS

Patterns of allozyme differentiation

The analysis resulted in 17 presumptive loci of which nine were polymorphic by 0.99 criterion (Table 2). As no significant linkage disequilibrium was found between the selected loci (χ^2 test, $p > 0.05$), random association of alleles between the loci was assumed. The *PGM** locus was the most variable with six different alleles and more than 16 genotypes. Genetic variability esti-

Table 2. Allele frequencies at all variable loci in the six samples of *Benthosema glaciale*. Number of individuals analysed at each locus is equal to N in all samples. *CK-1**, *CK-2**, *LDH** and *MDH-3** loci were monomorphic.

		Nordfjord	Masfjorden	Herdlefjorden	Sognefjorden	Storfjorden	Norwegian Sea
	(N)	125	130	120	120	120	120
Locus	Allele						
*AAT-1**	70	–	–	–	–	0.004	–
	100	1.00	0.989	0.992	0.979	0.996	0.988
	130	–	0.004	–	0.021	–	0.008
	150	–	0.008	0.008	–	–	0.004
*AAT-2**	80	0.068	0.054	0.079	0.142	0.113	0.058
	100	0.884	0.919	0.817	0.813	0.838	0.854
	110	0.020	0.015	0.075	0.029	0.033	0.033
	120	0.028	0.012	0.029	0.017	0.017	0.054
*FUMH**	80	0.012	0.015	0.004	–	0.008	–
	100	0.988	0.981	0.996	1.000	0.988	0.988
	120	–	0.004	–	–	0.004	0.013
*GPD-1**	70	0.024	0.015	0.029	0.029	0.004	0.008
	100	0.968	0.981	0.971	0.971	0.996	0.992
	130	0.008	0.004	–	–	–	–
*GPD-2**	50	0.016	0.008	0.011	0.008	0.021	0.004
	100	0.964	0.977	0.967	0.988	0.954	0.992
	150	0.020	0.015	0.021	0.004	0.025	0.004
*IDH**	70	0.004	–	–	0.008	–	–
	100	0.948	0.977	0.933	0.933	0.963	0.996
	120	0.048	0.023	0.067	0.058	0.038	0.004
*MDH-1**	25	–	–	0.004	–	–	0.038
	100	0.988	1.000	0.996	1.000	0.967	0.958
	175	0.012	–	–	–	0.033	0.004
*MDH-2**	70	0.024	0.012	–	0.013	–	–
	100	0.976	0.988	1.000	0.987	1.000	1.000
*ME**	90	0.016	0.027	0.050	0.033	0.054	0.029
	100	0.964	0.942	0.921	0.950	0.946	0.942
	110	0.020	0.031	0.029	0.017	–	0.029
*PGDH**	50	0.012	–	–	–	0.008	0.004
	100	0.984	1.000	0.988	1.000	0.988	0.979
	150	0.004	–	0.013	–	0.004	0.017
*PGI-1**	70	0.004	0.008	0.017	0.008	0.021	0.017
	100	0.996	0.992	0.975	0.992	0.979	0.983
	130	–	–	0.008	–	–	–
*PGI-2**	–100	0.996	0.988	0.996	0.987	1.000	0.996
	5	0.004	0.012	0.004	0.013	–	0.004
*PGM**	70	0.064	0.081	0.033	0.054	0.071	0.100
	80	0.172	0.154	0.142	0.183	0.167	0.221
	90	0.036	0.027	0.013	0.013	0.017	0.013
	100	0.468	0.500	0.558	0.567	0.563	0.558
	110	0.032	0.042	0.063	0.050	0.029	0.096
	120	0.228	0.196	0.192	0.133	0.154	0.013

Table 3. Summary of genetic diversity measures in *Benthosema glaciale* calculated on 17 loci (standard error in parentheses).

	Nordfjord	Masfjorden	Herdlefjorden	Sognefjorden	Storfjorden	Norwegian Sea
% polymorphic loci [a]	58.8	58.8	47.1	52.9	52.9	47.1
Mean number of alleles per locus	2.41 (0.3)	2.29 (0.3)	2.24 (0.3)	2.12 (0.3)	2.18 (0.3)	2.41 (0.3)
Mean observed heterozygosity	0.082 (0.04)	0.072 (0.04)	0.078 (0.04)	0.077 (0.04)	0.076 (0.04)	0.072 (0.04)
Expected heterozygosity [b]	0.080 (0.04)	0.070 (0.04)	0.086 (0.04)	0.080 (0.04)	0.079 (0.04)	0.074 (0.04)
Fixation index [c] F_{IS} (f)	–0.028	–0.010	0.100	0.035	0.044	0.029

[a] 0.99 criterion
[b] mean (across loci) unbiased heterozygosity (Nei 1978)
[c] multilocus estimate per sample population (Weir & Cockerham 1984)

mates at 17 loci within the population samples revealed that the level of polymorphism was in the range 47-59 %, while the mean (across loci) observed heterozygosity varied between 0.072 and 0.082 with an average of 0.076 (Table 3). No deviation from the Hardy-Weinberg equilibrium within samples could be detected at any loci ($p > 0.05$, χ^2 test, after Bonferroni adjustment). However, the pooled sample displayed significant deviation ($p < 0.05$, χ^2 test) at two loci, *AAT-2** and *PGM**, and the corresponding F_{IS} values were 0.055 and 0.040 respectively (both significantly different from zero at $p < 0.05$), indicating a significant heterozygote deficiency (Table 4). There was no H-W disequilibrium, when the pooled fjord sample was tested ($p > 0.05$, χ^2 test, after Bonferroni adjustment).

The hypothesis of no heterogeneity of allele frequencies across samples was rejected (combined $p < 0.001$, χ^2 test), as significant differences were detected at six variable loci (Table 4). The most genetically divergent was the Norwegian Sea sample (hereafter called the offshore sample). Among the allelic differences, a markedly low frequency of the *PGM**120 allele and a higher frequency of the *PGM**110 allele, and the unique occurrence of the *MDH-1**25 allele with a moderate frequency were observed in the offshore sample. Moreover, even when tested after excluding the offshore sam-

Table 4. Summary results of the test for conformity to Hardy-Weinberg equilibrium (for the pooled sample), test for homogeneity (across all samples), and F_{ST} estimates with the corresponding probabilities, for 13 variable loci in *Benthosema glaciale*.

Locus	H-W Equilibrium (χ^2 test)	Homogeneity of Allele frequency (χ^2 test)		Wright's F_{ST}	
	P	χ^2 (df)	P	F_{ST}	P ($F_{ST} \leq 0$) (by permuting)
*AAT-1**	1.000	25.75 (15)	0.017 †	0.003	0.040 †
*AAT-2**	0.0005 † ‡	46.96 (15)	0.0001 † ‡	0.010	0.005 †
*FUMH**	0.995	15.84 (10)	0.086	0.001	0.175
*GPD-1**	0.958	14.45 (10)	0.123	0.003	0.150
*GPD-2**	0.912	10.51 (10)	0.397	0.002	0.125
*IDH**	0.322	24.42 (10)	0.004 †	0.010	0.005 †
*MDH-1**	0.984	66.21 (10)	< 0.0001 † ‡	0.020	0.005 †
*MDH-2**	0.831	14.59 (5)	0.012 †	0.008	0.005 †
*ME**	0.574	15.63 (10)	0.109	0.001	0.240
*PGDH**	0.995	17.36 (10)	0.054	0.003	0.095
*PGI-1**	0.988	14.68 (10)	0.144	0.001	0.315
*PGI-2**	0.874	4.82 (5)	0.437	0.000	0.515
*PGM**	0.0003 † ‡	84.97 (25)	< 0.0001 † ‡	0.010	0.005 †
All loci		356.20 (145)	< 0.0001***	0.008	0.005**

† significant at 0.05 level without sequential Bonferroni adjustment (Rice 1989)
‡ significant at 0.05 level after sequential Bonferroni adjustment
** multilocus probability, significant at < 0.01, *** combined probability, significant at < 0.001 level.

ple, the null hypothesis of homogeneity of allele frequencies across the fjord samples was rejected (combined $p < 0.001$, χ^2 test). Allele frequency differences at two loci, *AAT-2** and *MDH-1** was responsible for this heterogeneity. Differences at these loci were found between the sample from Masfjorden and other fjords except Nordfjord ($p < 0.05$, χ^2 test) (Table 5).

Population genetic structure

The estimated *F* statistics were generally low. The overall F_{IS} over all polymorphic loci was 0.029. The overall F_{ST} value (across polymorphic loci), that estimates the variation due to population subdivision was 0.008, with the 95 % confidence interval from bootstrapping over loci of 0.004 to 0.010, which indicated significant difference from zero ($p < 0.05$). The per-locus F_{ST} values for all samples were generally low and in the range 0.001-0.020 (Table 4). The testing of the F_{ST} using permutation of alleles showed that F_{ST} was significantly different from zero ($p < 0.05$) at the *AAT-2**, *IDH**, *MDH-1**, *MDH-2** and *PGM** loci (Table 4). The overall F_{ST} value only for fjord samples was 0.004 with the 95 % confidence interval from bootstrapping of 0.002 to 0.009, indicating significant difference from zero ($p < 0.05$). The per-locus F_{ST} values for fjord samples ranged from 0.000 to 0.018, and the testing using permutation of alleles resulted in significant difference from zero ($p < 0.05$) at the *AAT-1**, *AAT-2**, *MDH-1**, *MDH-2** and *PGDH** loci.

Pair-wise F_{ST} values were generally low and in the range 0.0002-0.025. The Rogers' modified genetic distance (Wright 1978) values calculated on all loci ranged from 0.016 to 0.047 (Table 5). The UPGMA dendrogram revealed two branches, one representing all the fjord samples, separated from the offshore sample, and high bootstrap support (> 67 %) for all nodes were obtained (Fig. 2). An approximate gene flow of 17 individuals per generation was estimated. No significant correlation between the F_{ST} estimates or gene flow values and the pair-wise geographic distances was found with Mantel tests (Pearson's correlation $r = 0.023$, $p > 0.05$ from 500 replications), suggesting no indication of isolation by distance.

DISCUSSION

Moderate level of biochemical genetic variation in glacier lantern fish, both in terms of the degree of polymorphism and the observed heterozygosity (H_{ob}) was found. The mean H_{ob} value reported here (0.076) was above the average reported for many other fish species (0.055 for 106 species by Smith & Fujio 1982, 0.051 for 195 species by Ward & al. 1992). Comparably high H_{ob} values have been reported in some other myctophids as well (Afanas'yev & al. 1990). High H_{ob} values may indicate an unbroken population history without bottlenecks in marine fishes with large populations (Gyllensten 1985). According to Smith & Fujio's (1982) hypothesis in explaining the variation in hetrozygosities among fishes, mesopelagic species showing many adaptations to their environment are considered as habitat specialists and are expected to have high heterozygosities.

The deficiency in heterozygotes and the associated deviation of the total sampling population from the Hardy-Weinberg equilibrium could be attributed to the mixing of individuals originating from groups with different allele frequencies. Excessive homozygosity re-

Table 5. Results of the contingency chi-square test (Monte-Carlo simulation) for homogeneity of two samples, in terms of loci that showed significant heterogeneity (at $p < 0.05$ after sequential Bonferroni adjustment) and the combined probability (above diagonal), and pairwise estimates of Rogers' modified genetic distance (below diagonal).

	Nordfjord	Masfjorden	Herdlefjorden	Sognefjorden	Storfjorden	Norwegian Sea
Nordfjord		NS	NS	NS	NS	*PGM*, IDH*, MDH-1**, ($p < 0.001$)
Masfjorden	0.016		*AAT-2** ($p < 0.01$)	*AAT-2** ($p < 0.05$)	*MDH-1** ($p < 0.01$)	*PGM*, MDH-1** ($p < 0.001$)
Herdlefjorden	0.028	0.029		NS	NS	*IDH*, PGM** ($p < 0.001$)
Sognefjorden	0.032	0.032	0.022		NS	*PGM*, AAT-2*, IDH*, MDH-1**, ($p < 0.001$)
Storfjorden	0.028	0.026	0.021	0.019		*MDH-1*, PGM** ($p < 0.001$)
Norwegian Sea	0.047	0.041	0.043	0.036	0.035	

NS = no significant heterogeneity (combined probability $p > 0.05$)

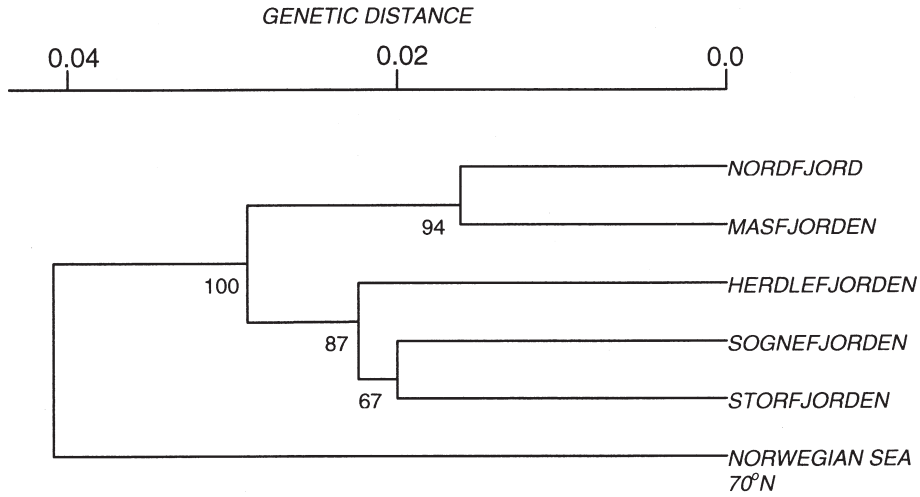

Fig. 2. UPGMA dendrogram based on pairwise modified Rogers' distance (Wright 1978) on all loci, summarising the genetic relationships among six samples of *Benthosema glaciale*. The bootstrap support values (from 100 replicated trees) are indicated at each node.

sulted from mixing of non-interbreeding populations is known as the Wahlund effect (cited in Nei 1987). Evidences for such an effect was observed, in terms of allele frequency heterogeneity between locations at several loci. However, other causes such as selection, inbreeding, assortative mating and the stochastic sampling error could also generate excess in homozygotes causing deviations from the H-W equilibrium (Nei 1987), even though the relative effects will be difficult to identify. Samples of same sub-populations in consecutive years will be beneficial in future studies to address the question of temporal stability of the observed genetic composition.

The results of the present study reveal clear genetic differences between the fish sampled from the fjords and those from the offshore, suggesting that these samples do not represent a single panmictic gene pool (samples are hereafter called sub-populations). The present data are insufficient to generalise this finding with regard to a single offshore sub-population. However, in a separate study of *B. glaciale* based on an analysis of four allozyme loci (unpublished data, JB Kristoffersen), no significant allelic heterogeneity was observed among five locations (62°N-70°N) in the Norwegian Sea (including the offshore location sampled in the present study), suggesting the existence of a single offshore sub-population. The hypothetical genetic structure depicted by the dendrogram further displayed the genetic divergence of fjord sub-populations from the offshore one. High bootstrap support values for the fjord and offshore sub-division (100 %) and also for inner nodes greatly supported the depicted genetic structure.

Population sub-division and subsequent complete reproductive isolation is generally common in species inhabiting environments boarded by physical barriers, such as fishes in different river drainages and separate lake-systems (Ward & al. 1994). On the contrary, in marine species that are characteristic of high dispersal potential, such a phenomenon may be regarded as less common since the marine environments present few absolute barriers to gene flow (Palumbi 1994). However, many opportunities exist for population subdivision of fish in the marine environment owing to partial isolation caused by mechanisms such as isolation by distance and invisible barriers (op. cit.). The reported F_{ST} values indicated a low, but significant level of differentiation across the total population of *B. glaciale*. Such low differentiation could be explained by a hypothesis of partial isolation of fjord units. Despite the fact that the geographic distance between the offshore and individual fjord locations (798-833 km) were several times greater than the between-fjord distances (27-216 km) apparently implying a correlation between the extent of divergence and the distance, there was no indication of isolation by distance. Apparently, the topographic barrier posed by the sill at the fjord mouth may restrict gene flow via adults to some extent. However, the possible advection of adults and juveniles that can occur during the time of feeding should be minimised by evolving behavioural adaptations in order to remain

within the fjords, such as active swimming against the currents and possible minor descents between in-flowing and out-flowing water masses. We speculate that, as an active swimmer, adult *B. glaciale* may be able to swim against small-scale currents. Such behaviour has been observed in some fish larvae too (e.g. sea bass *Dicentrarchus* sp., cited in Allegrucci & al. 1997). Minor night-time descents into deeper waters have been observed in a co-existing Sternoptychid fish, *Maurolicus muelleri* (Giske & al. 1990) but whether there is any relation to direction of water movement is unknown. Early studies have shown that particular larval stages of *B. glaciale* appear to be maintained within a very restricted temperature range irrespective of the time in the breeding season (cited in Badcock 1981). Accordingly, temperature seems to have a major effect on the development at early stages, and the drifted larvae may not survive once dislodged into offshore waters due to different temperature regimes and other environmental conditions. Annual natural mortality of more than 70 % (up to 83 % in Canadian waters) has been estimated for the species (Gjøsæter 1981). Evaluating the present results, the detected marked genetic heterogeneity could not be maintained if there was a substantial gene flow, suggesting the contribution from the passive drift of life history stages could not be substantial. Theory predicts, assuming no selection, that one successful migrant per generation is sufficient to prevent differentiation caused by genetic drift between populations (Nei 1987). Nevertheless, significant differentiation in natural populations can occur with up to 10 migrants per generation (Allendorf & Phelps 1981). In *B. glaciale*, the reported gene flow value (17 per generation) may still be regarded as very small when compared to very large population sizes of the species (adjusted estimate up to 12 million individuals in Masfjorden alone, Kaartvedt & al. 1988), and may be insufficient in preventing genetic differentiation.

Level of partial isolation seems to differ among the fjords studied. The Masfjorden sub-population was genetically distinct from all other fjords except Nordfjord, probably representing the greatest degree of isolation. This is in agreement with the topography of Masfjorden, which is a small inner branch bordering a much larger fjord system (Fensfjorden) (Kaartvedt & al. 1988), and has the shallowest sill depth among the fjords studied. Wind-driven coastal currents are likely to cause water exchange in the upper advective layer up to intermediate depths (Asplin & al. 1999) in adjacent fjords with deeper sills, causing passive drift of individuals, and subsequently, gene flow.

General lack of genetic structuring on a large geographic scale in marine fish species contrasts with the localised genetic heterogeneity among fjord stocks and oceanic stocks reported in some species (herring *Clupea harengus*: Jørstad & Nævdal 1983, Turan & al. 1998; cod *Gadus morhua*: Jørstad & Nævdal 1989, Dahle 1991; blue whiting *Micromesistius poutassou*: Giæver & Stien 1998). These studies also implicated a restricted gene flow between the fjords and the offshore waters. In addition to the physical barriers that can effectively play a role in limiting gene flow and creating genetic structure, important aspects of the life history can further facilitate the genetic divergence. It has been observed that the presence of distinct spawning aggregations in different areas such as those observed in coastal cod may facilitate intra-specific genetic heterogeneity in species with such reproductive behaviour (op. cit.). Since no extensive horizontal migrations have been found (Gjøsæter & Kawaguchi 1980), *B. glaciale* seem to spawn in the same water masses which they inhabit during the non-breeding period, and this presumably favours formation of locally adapted stocks in fjords.

The genetic differences revealed in the present study may also represent local adaptation to some extent. Environmental conditions, mainly the temperature and salinity regimes are different between the fjord habitats and the offshore waters, with colder deep layers with below zero temperatures in the Norwegian Sea compared to warmer deep waters (below sill depth) in the fjords (Kristoffersen & Salvanes 1998). As the eggs of *B. glaciale* are known to have a deep distribution, the eggs and early hatched larvae may have adapted to different temperature regimes in the two habitat types, thus the genetic differences between fjord and offshore populations are likely to reflect a degree of thermal adaptation. Local adaptation for ambient environmental conditions during more than 10 000 years and 5000 generations since the creation of fjords might be substantial. Mesopelagic fishes in general are known to respond rapidly to changes in the environment (see Gjøsæter & Kawaguchi 1980), and are therefore considered to be phenotypically plastic. The results of this study also support the hypothesis that reported spatial differences in growth and life history features of *B. glaciale* in the Norwegian waters may reflect a degree of reproductive isolation, and thus have a genetic origin. However, the relative importance of environmental and genetic causes is difficult to estimate (Allendorf & al. 1987), and conducting experimental studies of the heritability of life history traits of a deep living species like *B. glaciale* may be difficult.

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