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Original Article

Large and fine scale population structure in European hake (*Merluccius merluccius*) in the Northeast Atlantic

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Recently, there have been reports of increased abundance and landings of European hake in the northern part of the species range. Biological studies are however scarce and information about finer scale population structure important for stock assessments and fishery management is largely lacking. Here, we report on a population genetic study using neutral and outlier SNP loci assessing population structure in hake in the north-eastern parts of its range in the Atlantic. Hake samples from localities along the west coast of Norway, the Kattegat, the northern North Sea, and one locality in the Bay of Biscay were analysed using 53 SNPs, six of which were outliers potentially influenced by natural selection. We detected small-scale structure among northern samples, all of which were also distinct from Bay of Biscay hake, with the exception of a few individuals from the North Sea and the coast of Norway who clustered genetically together with Bay of Biscay hake. Our findings suggest that the present management unit of a single northern stock of hake is not biologically correct, and that there is more detail in the fine-scale population structure indicating that independent population dynamics could be expected in response to fishing patterns or changing environmental conditions.

Keywords: demersal, genetic variability, Kattegat, North sea, Norwegian sea, single nucleotide polymorphism, stock structure.

Introduction

Identification of population subdivision in harvested marine fish is of vital importance since undetected population structure may lead to overfishing of local populations, their subsequent decline in biomass, and in the worst-case scenario, extirpation of subpopulations (Kell *et al.*, 2009), or a collapse of the resource (Hutchings, 2000; Svedäng, 2003; Svedäng and Bardon, 2003).

The European hake is a commercially important fish that is common in the Northeast Atlantic and the Mediterranean (Murua, 2010). Its distribution in the Northeast Atlantic stretches

from the coast off Northwest Africa into Portuguese and Spanish waters, to the west of France, Ireland and Scotland, into the North Sea and Kattegat, and along the north-western Norwegian coast (Hickling, 1927; Bergstad, 1991; Casey and Pereiro, 1995). European hake has been heavily fished for centuries in the Mediterranean and around the Iberian Peninsula and the Bay of Biscay (e.g. Fortibuoni *et al.*, 2010). The biology, ecology, distribution, and population structure are relatively well studied in this southern part of the range. Hake is also fished commercially in the Celtic Sea, west of Scotland, and in the North Sea, but it is not as highly valued in the northern part of the range (Statistics Norway; FAO). Consequently, there is less information available on the biology and status of this species in areas above 56°N. Despite the lack of supporting evidence, hake in the Northeast Atlantic is arbitrarily divided into two stocks for management purposes–northern and southern stocks (Roldan *et al.*, 1998). The division between the northern and southern stocks lies along the Cap Breton canyon, which extends west from the Bay of Biscay, and is assumed to hinder movement of fish between the Iberian Peninsula to the south and the northern Bay of Biscay and Celtic Sea to the north (Roldan *et al.*, 1998). Several studies have shown that this division into two management stocks ignores the biological evidence for population structure. Hake caught in the Celtic Sea and the Bay of Biscay are similar genetically and distinct from fish farther south along the Iberian coast (Lundy *et al.*, 1999; Castillo *et al.*, 2004; Milano *et al.*, 2011; Pita *et al.*, 2011).

Fisheries landings and scientific survey data from north of Ireland have indicated strong increases in abundance, especially in the northern North sea with larger commercial catches since 2005 (Cormon *et al.*, 2014; Baudron and Fernandes, 2015). Records describing European hake in Norwegian and adjacent waters date to the turn of the 19th century (Brunchorst, 1898; Schmidt, 1909). Official catch records for the Norwegian fishery show that hake have been caught in the North Sea and along the entire Norwegian coast, from the eastern Skagerrak in the south-east and as far north as the Lofoten Islands, since 1935 (Norges_Fiskerier, 1935). Historically landings were low (less than 1000 tonnes), but the last decade has seen a steep increase in spawning stock biomass in the North Sea (Baudron and Fernandes, 2015), and concurrently Norwegian landings increased sixfold during the same period (Bakketeig *et al.*, 2015). One of the major questions arising from such a change is whether the increase represents an increase in population abundance or a change in population distribution patterns, or both.

In the Skagerrak and Kattegat, European hake is a frequent demersal fish species (DATRAS database at ICES, www.ices.dk) where it has been targeted for centuries (Smitt, 1892). It spawns during summer in shallow areas; ripe hake are found, for instance, near Kummelbank ("Hake bank" in Swedish) at depths between 30–70 m. Information drawn from commercial fisheries shows that hake are mostly caught between June and September on the western side of the Norwegian Trench off the Jutland coast, i.e. close to the alleged spawning grounds (Smitt, 1892). Hake are often taken as by-catch and the species is of minor importance for the Swedish fishery, amounting to 50–100 tonnes annually.

The processes behind the apparent increase in hake abundance at more northern latitudes are difficult to evaluate because the population structure of hake is poorly known in this part of its distribution range. Indeed, few studies have investigated more than one sample of the "northern stock" outside the Celtic Sea. Lundy *et al.* (1999) analysed a large sample of fish from the west coast of Norway (Trondheimsfjord) and found that fish from Norway were genetically distinct from those sampled in the Celtic Sea and the Bay of Biscay. Significant differences in otolith composition were also found between hake from the west coast of Norway (Romsdalsfjord), Reykjanes Ridge, and the Portuguese coast (Swan *et al.*, 2006). Tanner *et al.* (2014), on the other hand, found some evidence of exchange between hake sampled off Galicia and the Celtic sea to the north and Portugal to the south, based on the otolith elemental composition. In a SNP marker-based study, Milano *et al.* (2014) included three sampling locations in the northern range: Celtic Sea, west coast of Scotland, and northern North Sea. They found no genetic differentiation among these three samples with neutral loci, but a clear divergence using "outlier" loci, potentially affected by natural selection. These results underline the utility of considering not only neutral genetic markers but also gene-associated markers for stock identification (Nielsen *et al.*, 2012; Berg *et al.*, 2015; Gagnaire *et al.*, 2015).

As there is a limited amount of data regarding the genetic population structure in the northern part of the distribution range of European hake, we aimed at resolving this question. By including two fjord samples from the Norwegian coast, one offshore sample from the North Sea and two samples from Kattegat, we were able to cover major parts of the species' northern range. One sample from the Bay of Biscay was included as an outgroup. An additional objective was to correlate genetic structure to environmental factors, such as temperature and salinity, as such correlations have previously been indicated (e. g. Milano *et al.*, 2014).

Material and methods

Samples

Fish were collected between 2004 and 2012 at six locations (Table 1, Figure 1). Hake from the Norwegian Sea off central Norway (TRØ) were sampled at three locations (two inside fjord systems and one outside) toward the end of the spawning season in October and consisted mainly of mature but spent fish. Hake from Fanafjord (FAN) on the Norwegian west coast were collected from August to October and consisted mainly of immature fish and only a few non-spawning adults (> 35 cm). The North Sea fish (NS) were caught at two locations in July and consisted of both immature (< 40 cm) and maturing/spawning fish (Figure 2). The Kattegat hake sample consisted of fish from two locations: KAN representing large spawning hake at Kummelbank (Hake bank in Swedish) during summer, and KAS consisting of juveniles and smaller sized adults during the non-spawning season from a large part of the Kattegat (Figure 2). The Bay of Biscay (BB) sample was collected outside the spawning season and was composed of both immature (<~30 cm) and adult (>30 cm) hake (Figure 2).

Muscle tissue was the source of DNA for TRØ, KAN, KAS, and BB hake, and otoliths for FAN and NS hake were used to extract DNA due to the lack of tissue samples. Here after, the term "sample" is defined as a collection of fish from a specific location.

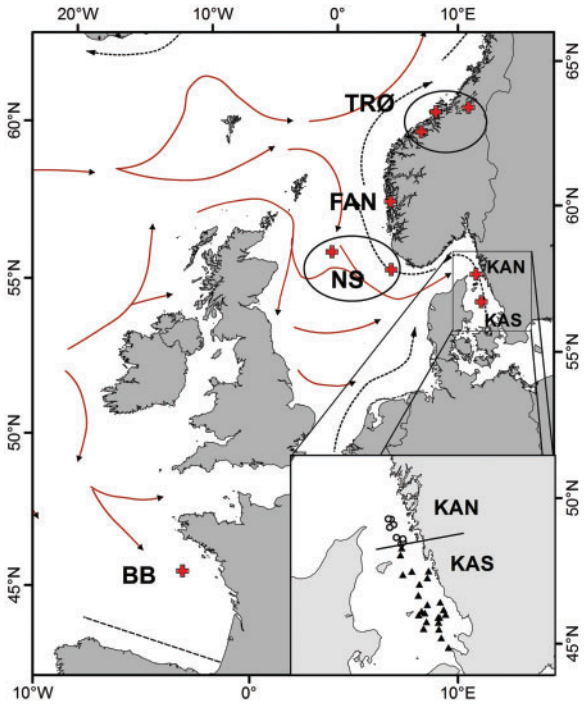

Figure 1. Map showing sampling locations (crosses) and surface currents (solid lines indicate Atlantic water, and dotted lines coastal water). The division between "southern" and "northern" European hake stocks is shown as a dashed line. Stations sampled, respectively, in the North Sea (NS) and Trøndelag (TRØ) are shown in circles. The highlighted area of the Kattegat shows locations of samples from the northern Kattegat (KAN) and southern Kattegat (KAS). Abbreviations of other sampling locations: Fanafjorden (FAN) an Bay of Biscay (BB).

Genetic analysis

DNA from otoliths was isolated using the QIAamp DNA Micro Kit (Qiagen N. V.) with two modifications: (i) the volume of the lysate was increased by 100% to cover as much of the otolith as possible, and (ii) samples were incubated in a thermo mixer at 56°C and 750 rpm for 24 h. The elution volume was set to 30 µl. DNA from muscle tissue was isolated using the E.Z.N.A 96 kit (Omega Bio-Tek) according to the manufacturer's protocol.

SNP loci previously characterized as neutral and outliers, possibly affected by selection within the Atlantic, were selected based on the work of Milano *et al.* (2011). We selected a subset of the 381 SNP markers used by Milano *et al.* (2014) based on the following criteria: (i) a set of SNPs detected as outliers within the Atlantic (22) and (ii) a random selection of "neutral" SNPs (61). This resulted in a total of 83 SNP markers arranged in three multiplexes that were genotyped using matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF MS) assays. Primers used for genotyping are listed in Supplementary data Table S1. Genotyping was performed using the IPLEX protocol following the manufacturer's instructions (Agena Bioscience Inc., Hamburg, Germany). The MassARRAY Typer software was used for automated genotype calling. Loci with more than 10% missing data per sample and a minor allele frequency below 5% were discarded. This resulted in the removal of 24 loci. Thus, the final dataset consisted of 59 SNP loci.

Genetic variation

Genetic variability at SNP markers was evaluated through basic population genetic parameters such as observed and expected heterozygosities, gametic disequilibrium and conformity to gametic and Hardy-Weinberg expectations, calculated using the software Arlequin 3.5 (Excoffier and Lischer, 2010). In cases of multiple testing, the Type I error rates were corrected using the False Discovery Rate (FDR) approach (Benjamini and Hochberg, 1995). Sampling stations were pooled if they were in close proximity and after having been tested for population differentiation among stations from the same location (see Figure 1). This increased the sample size and thereby statistical power, especially for TRØ and NS.

Outlier detection

Genetic loci were tested for diversifying or balancing selection using outlier detection tests. These tests assess the probability that the loci reflect neutral population genetic processes such as drift and gene flow among populations rather than local adaptation or selection (e. g. Narum and Hess, 2011; Gagnaire *et al.*, 2015). We used two approaches to identify such loci. First, a coalescent-based simulation approach was used to identify outlier loci potentially influenced by natural selection. This was done by comparing observed F_{ST} at each locus with expected values under neutrality. Then, loci displaying unusually high and low F_{ST} values were characterized as outliers (Beaumont and Nichols, 1996). This procedure is implemented in the Lositan Selection Workbench (Antao *et al.*, 2008). An initial run was performed with 50 000 simulations over all loci, using the mean neutral F_{ST} as a preliminary value. A more accurate estimate of the mean neutral F_{ST} was obtained by excluding all loci lying outside the 99% confidence interval following the first run, as their distribution could be a result of selection rather than neutral evolution. The refined estimate was used for a final set of 50 000 simulations over all loci, and this approach was used both on the global data and in pairwise tests between locations. We also employed a Bayesian simulation-based test implemented in BayeScan 2.1 (Foll and Gaggiotti, 2008). The locus-population F_{ST} coefficients were separated into a population specific component across all loci (β) and a locus-specific component across all populations (α). An α significantly different from 0 (negative or positive) indicates diversifying or balancing selection, respectively. The software uses a reversible-jump MCMC algorithm to estimate the posterior probability that a locus is showing signs of diversifying or balancing selection in relation to the global F_{ST}. We based our analysis on ten pilot runs each consisting of 5000 iteration, followed by 100 000 iterations with a burn-in of 50 000 iterations.

Population structure

An initial estimation of genetic structure was performed using pairwise F_{ST} values assessed according to Weir and Cockerham (1984) as implemented in Genepop 4.3 (Rousset, 2008). A Pearson's traditional χ^2 test in CHIFISH (Ryman, 2006) was used to assess whether the samples were significantly different from each other. The chi-squared test was chosen because it has been shown that it performs best when summing *p*–values across bi-allelic loci (Ryman and Jorde, 2001). The robustness of the observed structure and the direction of gene flow was assessed using the assignment test implemented in Arlequin 3.5 (Excoffier and Lischer, 2010). Here, the log likelihood that the genotype of

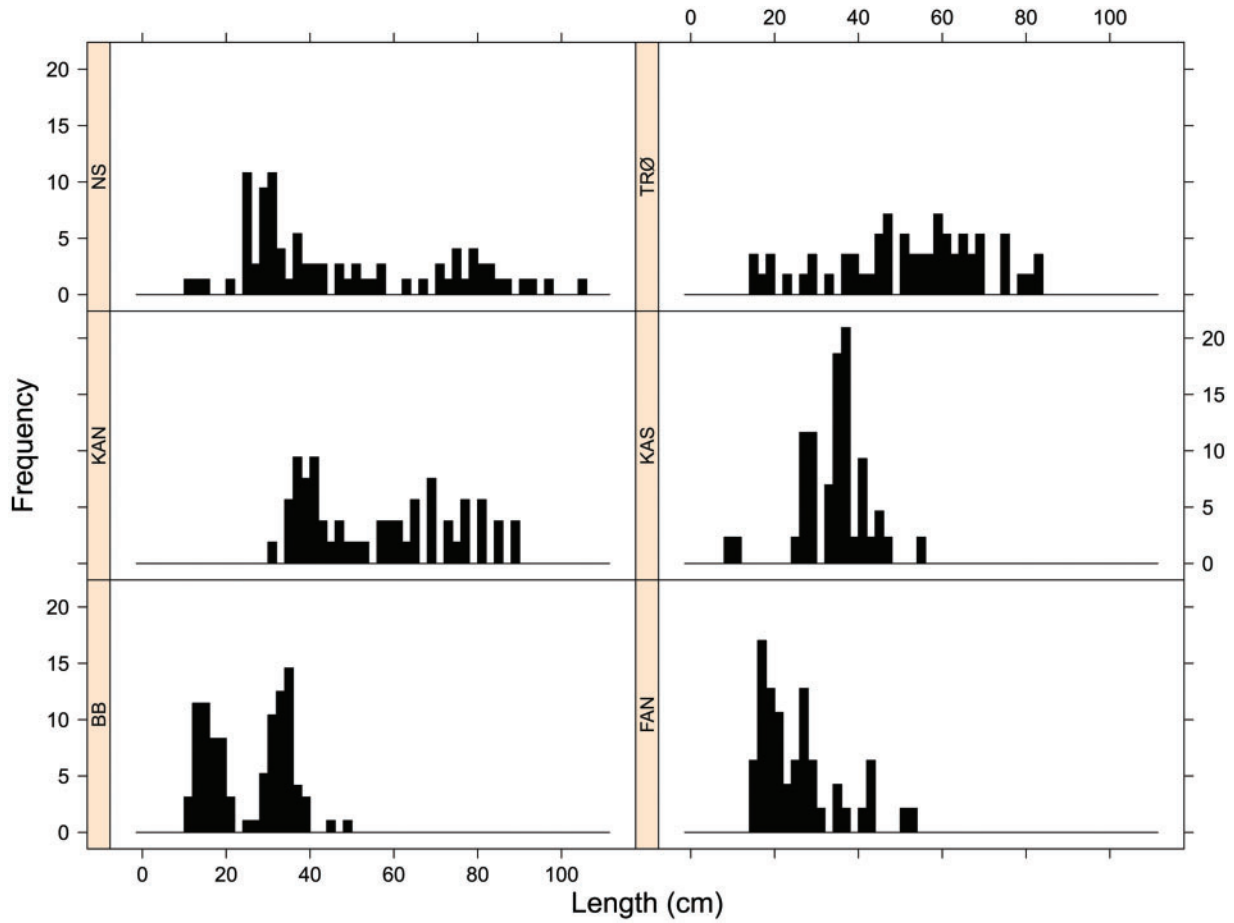

Figure 2. Length-frequency distribution of fish in samples included in this study. Locations indicated in the top bar.

Table 1. Overview of sample origin, abbreviations used in the text (material used for DNA extraction: M muscle tissue, O otolith), year samples were collected and sample size.

Location	Abbreviation	Year	*N*	H_E	H_O	F_{IS}
Trøndelag	TRØ (M)	Oct/Nov 2012	56	0.283	0.316	−0.117
Fanafjord	FAN (O)	Sept/Oct 2004	47	0.244	0.272	0.103
North sea	NS (O)	July 2012	74	0.302	0.303	−0.003
Kummelbank	KAN (M)	July 2010	53	0.318	0.282	−0.128
Kattegat	KAS (M)	Nov/Dec 2010	38	0.285	0.299	0.049
Bay of Biscay	BB (M)	Nov/Dec 2012	94	0.271	0.288	0.058

Also included is observed and expected heterozygosity, H_O and H_E, respectively, and the inbreeding coefficient (F_{IS}).

each individual belongs to each population sample is computed as if it was drawn from a population having equal allele frequencies to those estimated for each sample.

Since our data comprise both samples from different years (temporal samples) and a mix of juveniles and adults, we performed an AMOVA analysis (Excoffier *et al.*, 1992) implemented in the Arlequin 3.5 (Excoffier and Lischer, 2010) to assess the influence of these factors on the genetic structure. To account for temporal samples, we grouped the data according to the year sampled. If temporal sampling contributed more than spatial sampling, then the variation among groups (year; F_{CT}) is expected to be larger than the variation within groups (spatial location; F_{SC}). Assessing if the genetic structure depended on whether juveniles or adults were analysed, we used a similar approach as described above. Here each sample was split into two size groups, above and below 30 cm total length (adults and juveniles, respectively). Total length was used as it was available for all individuals and was considered as a proxy for age. Differences in the genetic structure between adults and juveniles should then reflect variation among age groups (adults/juveniles; F_{CT}) and may be larger than variation within groups (spatial location; F_{SC}). This approach was, however, hampered by small sample sizes in some cases, especially for the TRØ and KAT samples ($n = 9$ and 14, respectively), and for the KAN sample, which included no individuals with a total length below 30 cm.

An individual-based analysis of population structure was conducted by estimating the number of clusters (K) in the data using the Bayesian cluster analysis implemented in BAPS 6.0 (Corander *et al.*, 2006). BAPS treats K as an unknown parameter and uses a stochastic optimization algorithm to estimate the posterior mode of K by modelling the underlying population allele frequencies assuming Hardy-Weinberg equilibrium and non-linkage of markers (Corander *et al.*, 2003). The analysis in BAPS was made with two models incorporated in the software. First we used the "clustering of individuals" model, where only the genetic data were considered. The second model, "spatial clustering of individuals", incorporates spatial data as a prior as described in

Corander *et al.* (2008). A predefined number of clusters (K), here 1–10, were explored over 10 different runs to ensure proper replication for the given number of clusters. This approach was pursued for both steps.

Genotype-environment association

The correlation between the genetic data and the environmental variables temperature and salinity was explored with multiple regression on distance matrices (MRDM) using the "ecodist" package in R (Goslee and Urban, 2007). This analysis was based on a single dependant matrix, here the F_{ST} matrix based on all 53 loci, expressed as a function of several independent matrices, which were temperature and salinity at the surface and 25 meters depth. The analysis was conducted both with and without the Bay of Biscay sample. Annual mean values of seawater temperature and salinity at the different depths were retrieved from the National Oceanographic Data Center (NODC) database (Boyer *et al.*, 2013), using the closest geographic coordinates to the actual sampling locations. In addition, the relationship between outlier SNP loci and environmental variables was tested with a simple linear model, where allele frequencies at each of the six loci were fitted against the environmental variables temperature and salinity at different depths.

Results

Genetic variation

The observed and expected heterozygosity within loci (H_O) ranged from 0 to 0.723 and 0.018 to 0.527, respectively (Supplementary data Table S1) and was similar for samples from which the DNA originated from otoliths or muscle (Supplementary data Table S1). Conformity to Hardy-Weinberg expectations was violated in 6 of 318 tests. Of these six significant tests, three were at the locus X2592_fpt, and the remaining five were scattered across the remaining loci (Supplementary data Table S1). Evidence of gametic disequilibrium was discovered in 10 locus-pair combinations in a majority of the samples. The most informative SNPs in these combinations were used in the subsequent analysis. A total of six loci were discarded. Details are found in Supplementary data Table S3.

Outlier detection

The global test for outlier loci returned 6 of 53 loci likely to be influenced by positive directional selection as suggested by the results from Lositan. The BayeScan analysis suggested four outlier loci, which were also found by Lositan. Thus, combining the results from the two software we found four outlier loci in common (Figure 3, Supplementary data Table S2). In the study of Milano *et al.* (2014), three of these loci were also reported as outliers. The one outlier unique to the present study was x890_fpt. The analysis for the northern samples, without the Bay of Biscay sample, returned two outlier loci in common for the two software (results not shown), where one (X778ms) was in common with the global dataset including all six samples. The second outlier locus (X891_fpt) was detected only in the dataset without the Bay of Biscay sample. Only the four outlier loci detected by both software were considered true outliers and treated accordingly in the subsequent analysis. This applies both to the global dataset and the dataset with only the five northern samples.

Population structure

Pairwise estimates of genetic differentiation, $F_{ST,}$ showed a high degree of structure (Table 2), reflected by the number of significant tests, both with (15/15) and without (14/15) outlier loci (Table 2). The pattern of genetic differentiation among population samples for all 53 SNPs was congruent with their geographical distribution. The Bay of Biscay sample was most differentiated, with F_{ST} values ranging from 0.027 to 0.056, while the genetic structure was much lower among northern samples, with F_{ST} between 0.002 and 0.017 (Table 2). Excluding the four outlier loci resulted in markedly reduced, but still significant, differentiation between Bay of Biscay and the northern samples. Among the northern samples, only minor changes in the levels of differentiation were observed with this approach. That is, the outliers' influence of the genetic structure was most profound in differentiating the Bay of Biscay and Scandinavia.

Despite smaller F_{ST} values, significant genetic structure was present among the northern population samples, for both sets of SNPs. In general, Trøndelag was the most differentiated sample among the northern samples (without Bay of Biscay) (Table 2). Interestingly, adults (Kummelbank) and juveniles (Kattegat) were significantly differentiated from each other within the Kattegat ($F_{ST} = 0.012$). However, the North Sea sample was weakly differentiated from the Kattegat (Kummelbank and Kattegat) and southern Norwegian (Fanafjord) samples ($F_{ST} < 0.01$). North Sea and Kattegat were not significantly differentiated when considering only the 49 "neutral" SNPs ($F_{ST} = 0.001$, Table 2). The robustness of the population structure was shown in the assignment test results, which confirmed the pattern of the F_{ST} estimates. In general, the majority of the individuals from Kattegat, Fanafjord and Bay of Biscay were reassigned to the sample of origin (Table 3a). Fish from the spawning aggregation at Kummelbank were less often reassigned to the sample of origin, as were hake collected at Trøndelag and North Sea. The assignment without outlier loci showed a similar pattern, but the self-assignment rate was lower for the Bay of Biscay population (Table 3b). The AMOVA analysis showed that sampling year did not contribute to the genetic variation (Table 4). Also, a comparison of juveniles vs. adults did not show any difference in structure between life stages (Table 4).

The most likely number of genetic clusters was $K = 4$, when using all 53 loci and $K = 3$, using the 49 "neutral" SNPs. The individual-based BAPS analysis corroborated much of the findings from the pairwise F_{ST} estimates in that most of the individuals from the Bay of Biscay were placed in two clusters considering all 53 loci (shown in red and yellow; Figure 4a) and a shallower structure among the northern samples, with a majority of the individuals placed within the same cluster (blue; Figure 4a). However, a higher proportion of the individuals from the samples Trøndelag, North Sea and Fanafjord were placed in the Bay of Biscay group, which was most pronounced for the North Sea sample (Figure 4a). This feature was also found in the F_{ST} estimates (Table 2). Considering the 49 "neutral" loci ($K = 3$), we found a more erratic pattern with the Bay of Biscay sample no longer as differentiated from the northern samples. Thus the influence of the four outlier loci on the genetic structure is clearly demonstrated (Figure 4b).

Genotype-environment association

Correlations of the F_{ST}-based differences from the four outlier loci only (all samples) with the environmental variables showed a

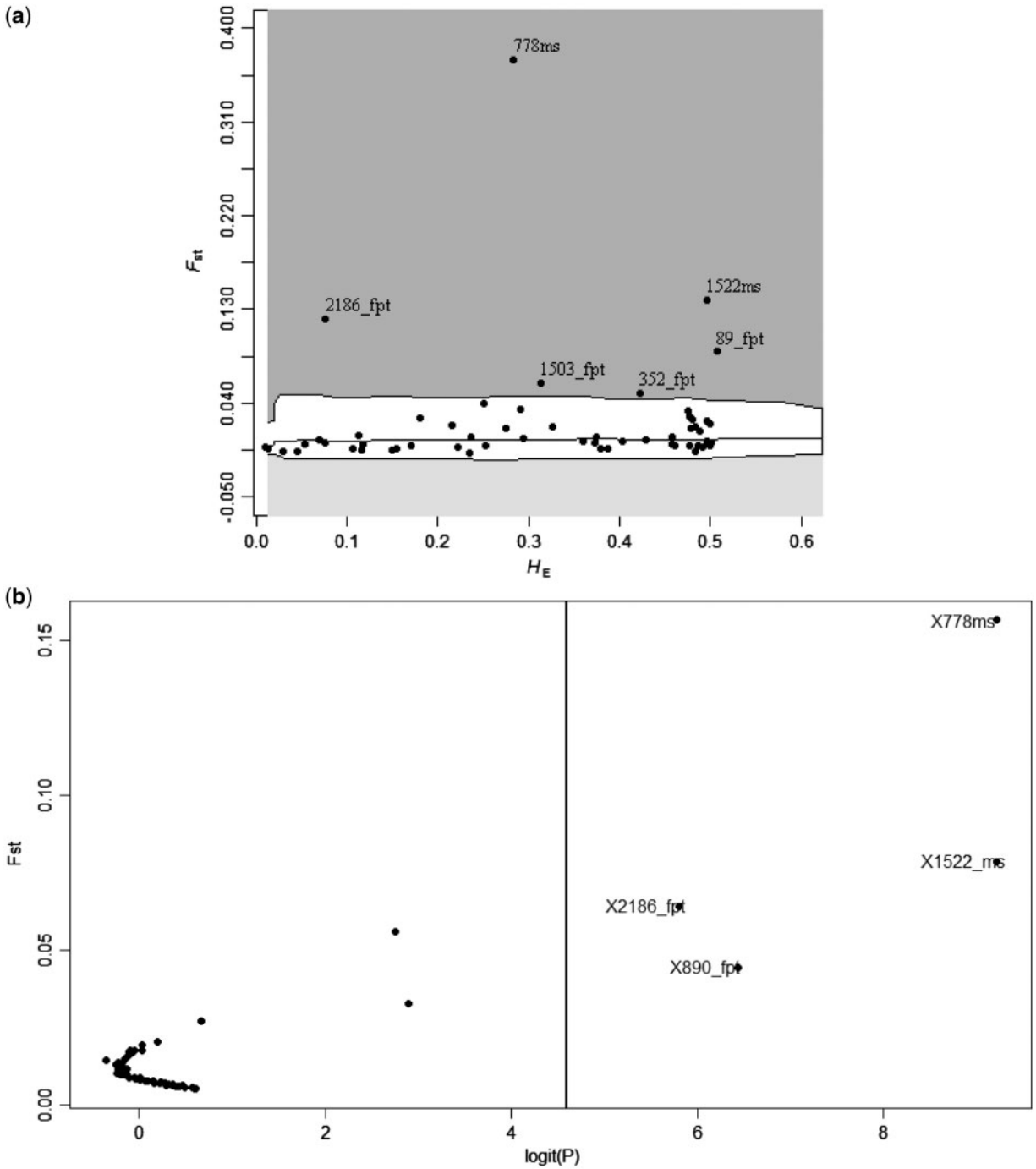

Figure 3. Outlier detection among 53 SNP loci using (a) F_{ST} vs. heterozygosity for the 53 SNP loci from the software Lositan (Antao *et al.*, 2008), based on the Fdist approach (Beaumont and Nichols, 1996). The dark grey area indicates the upper 99% confidence interval, while the light grey area indicates the lower 99% confidence interval. The black line in the middle of the plot shows mean F_{ST}. (b) Results from the software BayeScan (Foll and Gaggiotti, 2008), showing the logarithmic Bayes factor on the X-axis and F_{ST} values on the Y-axis. The vertical line indicates the threshold for significant outliers, which was set to "decisive", corresponding to a posterior probability of 0.99.

significant association between genetic distance and temperature at both depths (Table 5). A weaker, but significant correlation was also found between F_{ST} and salinity at 25 m depth. Removing the Bay of Biscay sample led to no significant association between the genetic and environmental data (Table 5), suggesting that the environmental heterogeneity among the northern localities was not great enough to act as a selective agent. At the individual locus level, all four outliers were correlated to at least one of the environmental variables, temperature and salinity (Table 6). This was most pronounced regarding temperature.

Discussion

This study detected an overall population structure in European hake in the Northeast Atlantic, with Bay of Biscay hake clearly

Table 2. Population-pairwise genetic differentiation (F_{ST}).

	TRØ	FAN	NS	KAN	KAS	BB
TRØ	*	**0.010**	**0.008**	**0.009**	**0.015**	**0.014**
FAN	**0.009**	*	**0.002**	**0.015**	**0.007**	**0.006**
NS	**0.008**	**0.005**	*	0.004	0.001	**0.011**
KAN	**0.006**	**0.017**	**0.009**	*	**0.012**	**0.016**
KAS	**0.013**	**0.010**	**0.008**	**0.012**	*	**0.011**
BB	**0.054**	**0.046**	**0.032**	**0.069**	**0.062**	*

All 53 SNPs, including outlier loci below diagonal and 49 neutral SNPs above diagonal. Significant values based on Pearson's traditional χ^2 test are indicated in bold.

Table 3. Genetic assignment showing the number of individuals from each sample (rows) assigned to each of the samples (column).

(a)

	TRØ12	FAN04	NS12	KAN10	KAS10	BB12
TRØ12	**31**	0	3	2	19	1
FAN04	2	**38**	1	1	3	2
NS12	4	6	**26**	2	24	12
KAN10	11	4	3	**24**	11	0
KAS10	1	0	1	0	**36**	0
BB12	1	4	4	0	6	**79**

(b)

	TRØ12	FAN04	NS12	KAN10	KAS10	BB12
TRØ12	**28**	0	5	5	15	3
FAN04	1	**38**	2	0	2	4
NS12	2	4	**25**	5	27	11
KAN10	8	4	5	**22**	10	4
KAS10	1	0	2	1	**32**	2
BB12	3	4	3	2	29	**54**

Highlighted in bold is the number of individuals assigned to the sample of origin (diagonal). The individual assigned was not included in the reference population (the leave one out procedure). (a) With all 53 loci, and (b) 49 neutral loci only. The numbers behind sample abbreviations indicate sampling year.

Table 4. Results from the AMOVA test (Excoffier et al., 1992), assessing (i) the impact of sampling year on the genetic structure and (ii) differences in genetic structure between juveniles and adults.

Source of variation	Percentage variation	*p*
The effect of sampling year		
Among sampling years	−0.12	0.55
Among population within years	3.49	0.00
Within populations	96.63	
Juveniles vs. Adults		
Among life stages	−0.14	0.43
Among populations within life stages	3.21	0.00
Within populations	96.94	

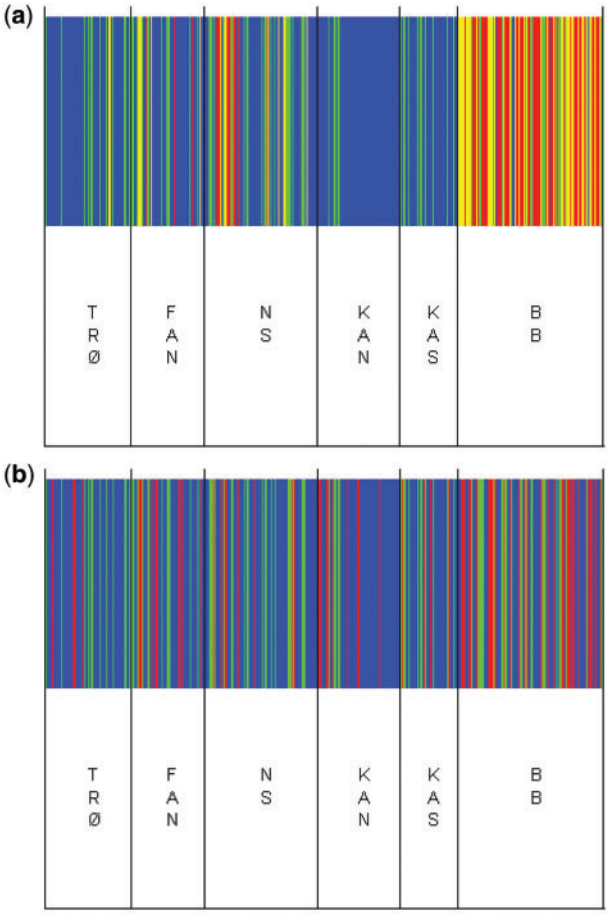

Figure 4. Individual genetic clustering using BAPS (Corander *et al.*, 2006). Bar plot with $K = 4$ clusters, using all 53 loci including four outlier loci (a), where the Bay of Biscay cluster is dominated by red and yellow, and $K = 3$ using 49 neutral loci (b), demonstrating the effect of the outlier loci in which the Bay of Biscay cluster is no longer well defined. Each bar represents one individual and the different colours represent the cluster to which the individual belongs.

Table 5. *p*-Values for the correlation between genetic differentiation (F_{ST}) and environmental variables.

	T_0	T_{25}	S_0	S_{25}
All six samples	0.0002	0.0010	0.4634	0.0410
Northern samples	0.8993	0.1445	0.2414	0.3907

F_{ST} was calculated for all six samples based on four outlier loci, or the five northern samples only based on two outlier loci. T_0 and T_{25} refer to temperature at the surface (0 meters) and 25 meters, respectively. *S* refers to salinity at the same depths.

distinct from the northern samples. Within the northern samples we found a significant population structure, but using only neutral loci, a connectivity between the North Sea and Kattegat was discovered. One mechanism contributing to this pattern may be the transport of eggs and larvae from the spawning grounds in the North Sea into Kattegat, as documented for e. g. Atlantic cod (André *et al.*, 2016). While some of the observed genetic heterogeneity was due to loci likely influenced by local selection pressures, a neutral population structure was also evident, indicating a restricted migration/gene flow between the populations.

Fjord and coastal waters

The low genetic differentiation between Fanafjord and North Sea samples (Table 2) implies a large degree of genetic exchange either via direct migration of juvenile or adult fish, or the dispersal

Table 6. Estimation of the fit between individual outlier loci and different environmental variables using all six samples.

		X1522ms	X2186_fpt	X778ms	X890_fpt
T_0	*p*	0.008**	0.001***	0.005**	0.011*
	Adjusted R^2	0.822	0.931	0.855	0.793
T_{25}	*p*	0.009**	0.004**	0.008**	0.013*
	Adjusted R^2	0.812	0.869	0.823	0.779
S_0	*p*	0.153	0.356	0.194	0.109
	Adjusted R^2	0.296	0.213	0.222	0.393
S_{25}	*p*	0.031*	0.123	0.048*	0.017*
	Adjusted R^2	0.660	0.487	0.580	0.743

T_0 and T_{25} refer to temperatures at the surface (0 meters) and 25 meters, respectively. *S* refers to salinity at the same depths.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

of eggs and larvae via the interaction of the Norwegian coastal current and Atlantic water flowing southwards.

Mixing of seasonal migrating hake and local populations was suggested by both Hickling (1930) and Hart (1948). The Fanafjord sample, a mixture of juveniles and adults, was also a mixture of a local fjord population and North Sea hake, as supported by both the pairwise F_{ST} estimates (Table 2) and the BAPS analysis (Figure 4). Hake at Trøndelag were also affected by North Sea fish, but to a lesser extent. The absence of physical barriers and short geographic distances do not necessarily reduce the potential for the establishment of genetically different populations (Hauser and Carvalho, 2008). Low, but statistically significant, genetic sub-structuring between inner-fjord and outer-fjord environments, as well as between coastal skerries has been shown for cod in the Skagerrak (Knutsen *et al.*, 2003; Jorde *et al.*, 2007; Knutsen *et al.*, 2011). The topographic characteristics of a fjord, including its sill depth, climatic variables and current regimes influence the volume and layer of water exchanged with the outer water masses (Aksnes *et al.*, 1989). These factors impact the amount of eggs and larvae transported into or out of the fjord, knowing that it is not the only prerequisite for genetic differentiation. Provided that the home range of hake is largely within fjord bounds and spawning products are retained, it is likely that genetic divergence of fjord populations can develop, as would be the case for the Trøndelag sample and perhaps to some extent for the Fanafjord sample.

Both published and anecdotal information indicates that hake in the northern North Sea and in some Norwegian fjords and coastal waters, as well as off the coast of Møre and Romsdal (Norwegian Sea), spawn primarily in the period July–October (Hickling, 1927; Kjesbu *et al.*, 2006; Groison *et al.*, 2010; Werner *et al.*, 2016). According to local fishermen adult hake are less available in late autumn (October–November) in coastal waters of southern Norway, implying a possible emigration of spawning fish. However, the certainty that adult fish may migrate between fjords and open ocean is obscured by observations of adult fish in some fjords in January–February (Staby unpublished data). The fjord sample included in this study (Fanafjord) shows that 81% of the individuals here are assigned back to the population of origin. A more comprehensive survey of fjords with wider temporal/seasonal coverage would be required to determine the extent of population connectivity for hake in different parts of the coastline.

An emerging pattern is that both Fanafjord and Trøndelag are affected by the North Sea hake, which again is affected by the Bay of Biscay hake (Table 2 and Figure 4a). Two scenarios could cause this observed pattern. One is that present-day hake populations share a common ancestry. If the populations diverged recently and have not yet reached genetic drift-migration equilibrium, this will result in no, or shallow, divergence in the neutral regions of the genome (Roderick and Navajas, 2003). Evolution in the parts of the genome subjected to selection is a faster process (Roesti *et al.*, 2012). Therefore, we find much larger divergences between the Bay of Biscay and Scandinavian samples when we include the outlier loci in our analysis.

The second scenario includes connectivity/gene flow between the North Sea and the Bay of Biscay, and that connectivity is more limited farther north toward TRØ. The loci detected as outliers, possibly influenced by selection, affect the genetic structure first and foremost by clearly differentiated the Bay of Biscay from Scandinavian samples (Table 3a), indicating that these outliers are temperature and salinity driven (see below).

Cod and herring have complex population structures, with smaller local units and a larger migratory unit that covers long distances in north-south annual migrations for spawning or feeding (Neuenfeldt *et al.*, 2013). Hake are suspected of undertaking long migrations northward in summer from the Celtic Sea northward to the west of Scotland, entering the northern North Sea over the north of Scotland and the Shetland Islands. They continue their way southwards along the western slope of the Norwegian trench, possibly as far south as the Skagerrak (cf. Baudron and Fernandes, 2015). In addition, fish spawning in the North Sea, representing the "native" North Sea stock, may undertake shorter migrations along the Norwegian coasts. The presence of additional, local populations along the coast and in the fjords would complete the picture, with various amounts of contact between groups. What role the increased population abundance will play in replenishing, or replacing, these local populations is not known, but could be modelled (Kerr *et al.*, 2010).

Kattegat

The juvenile hake sampled in the Kattegat (Kattegat; KAS) were genetically differentiated from adult fish spawning in the northern Kattegat (Kummelbank; KAN). The genetic similarity between KAS and samples from the North Sea suggests high connectivity between the two areas. Hake progeny (eggs or larvae) might drift from spawning grounds in the North Sea into the Skagerrak and Kattegat, or hake with a "North Sea" genetic signal might have spawned in the Skagerrak/Kattegat, giving rise to progeny identified genetically as "KAS". This scenario may explain the asymmetry in gene flow as shown in the assignment test (Table 3), where a large part of the individuals from Trøndelag and the North Sea was assigned to Kattegat (KAN), whereas this is the case for only two individuals the other way around. It is possible that these individuals return to the North Sea as adults. Locally spawning hake in the Kattegat aggregate at the shallow grounds in the Kattegat, such as the Kummelbank, during the spawning period in summer from June to August (Fiskeriverket, 2011). This indicates that migrations may take place during the spawning season when hake presumably move in from the deeper parts of the Skagerrak or the Norwegian trench (Hickling, 1927). This enables a mixed spawning stock to occur, comprising local hake and migrating hake from the North Sea. The latter is "represented" as individuals from Trøndelag in the current data (Table 3). Such a mixture of local spawning stocks and incoming

juveniles from offshore spawning grounds has been found for Atlantic cod (*Gadus morhua*) in the Skagerrak-Kattegat area (Svedäng *et al.*, 2007; André *et al.*, 2016). What is missing from our picture is the fate of the progeny from the Kummelbank spawning grounds.

Adaptive vs. neutral population structure

Determining the selective agents for adaptive divergence is generally difficult, but the effect of environmental factors provides valuable insights. Here, the regression analyses showed a significant association between the global outlier data (four loci) and average annual water temperature, both at the surface and at 25 m. Also, a weaker association was found related to salinity at 25 meters depth. These findings are in concordance with the previous study by Milano *et al.* (2014) who also demonstrated a correlation with salinity and temperature for outlier loci. The relationship was no longer significant after removing the Bay of Biscay sample from the analysis, indicating that temperature, and to a degree salinity, may drive the divergence between Bay of Biscay and the more northern populations. However, Milano *et al.* (2014) detected three clusters of hake based on outlier loci in Atlantic samples: two distinct groups represented by the northern North Sea and northern Portugal, and a third cluster composed of fish from the west of Scotland, the Celtic Sea and the coast of Galicia. Therefore, the pattern of divergence observed here may actually represent a longer north-south continuum along the Atlantic shelf. The effect of environmental variables on the genetic structure of teleost fishes has previously been reported in several studies (e.g. Nanninga *et al.*, 2014; Sexton *et al.*, 2014; Henriques *et al.*, 2016).

Genetic variation and climate change

The North Sea has become warmer in recent decades (Perry *et al.*, 2005) as a result of global climate change. Changes in distribution are predicted as marine fish species shift or expand into new areas with optimal conditions (Beare *et al.*, 2004; Rijnsdorp *et al.*, 2009; Portner and Peck, 2010). Hake is often considered a southern species, but is rarely observed in waters warmer than 11–13° C. Alternatively, if the southern populations of European hake are adapted to higher temperatures, an increase in temperature could open the northern areas for colonization as suggested by an larger proportion of Bay of Biscay individuals in the most recent sample collected in 2012 in the North Sea (Table 3). Few studies have dealt with the effect of climate change on the genetic variation of commercially exploited fish species specifically (Pauls *et al.*, 2013; Crozier and Hutchings, 2014). But changes in the distribution of genetic variants as well as evolutionary responses in life history traits such as maturation schedules and migration timing have been documented (Crozier and Hutchings, 2014).

Implications for fisheries management

Hakes in the North Sea (ICES subarea IV) and Kattegat (ICES Division IIIa) are currently assessed as part of the "northern stock", even though the management documentation states that there is no biological basis for this designation (ICES, 2015). Our results clearly show that the "northern stock" is actually subdivided into several genetically differentiated units, of which Kattegat, Norwegian Coast, North Sea could be identified in this study. However, to fully understand the interconnectivities that seem to exist among the northern populations, further studies are needed to specifically resolve this issue. For now, we suggest that this structure should be included in any predictive modelling, especially population responses to climate change.

In recent years, the spawning stock biomass of the northern stock has reached record high levels (ICES, 2015), and annual TACs allocated to the North Sea and Kattegat have increased accordingly. The annually allocated TAC for these areas is based on a fixed percentage of the total "northern stock" TAC, i.e. 3% for the Kattegat and 3.5% for the North Sea. Neither the North Sea nor the Kattegat hake are assessed independently of the annual "northern stock" assessment, and the common management of the stock may obscure changes occurring on a smaller local scale, resulting in inappropriate exploitation patterns for the different components. Expanded reporting of catch data from coastal areas may allow for assessment in the North Sea and adjacent areas.

Conclusions

The present study provides evidence for both large scale and fine scale population structure in European hake in the Northeast Atlantic. The division between the Bay of Biscay and hake populations located in the North Sea and beyond is evident both from presumed neutral and outlier loci; the structure in the outlier loci correlates with water temperature most likely indicating adaptive differentiation. Moreover, significant population structuring was found at a regional scale in the North Sea and adjacent coastal areas. Our data have highlighted likely movement of hake between the Kattegat and North Sea, while indicating differentiation of hake found to the north of the North Sea (above 62°N).

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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