Short Communication

A "seascape genetic" snapshot of *Sebastes marinus* calls for further investigation across the North Atlantic

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A collection of 376 golden redfish (*Sebastes marinus*) from several fishing grounds in the North Atlantic in late 2001 was genotyped at nine microsatellite loci to provide preliminary information on the possible genetic structure in this species. Landscape genetic analysis revealed the presence of two distinct genetic pools within the North Atlantic, suggesting that *S. marinus* might be structured within the North Atlantic and should be the subject of more investigation.

Keywords: landscape genetics, microsatellite loci, migration, North Atlantic, redfish, *Sebastes marinus*.

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Introduction

Species of the genus *Sebastes* exhibit complex patterns of genetic structure and connectivity among their populations in both the North Pacific and North Atlantic and have historically been commercially important. Information on the population structure of commercially exploited fish stocks has now been widely acknowledged as crucial for the conservation and sustainable management of marine fish stocks (Hilborn *et al.*, 2003). Consequently, genetic markers have been used to improve stock delineation and to aid fisheries management of several species including those in the genus *Sebastes* (Gharrett *et al.*, 2007; Daníelsdóttir *et al.*, 2008; Hyde *et al.*, 2008; Stefánsson *et al.*, 2009a, b). For *Sebastes*, these studies often revealed that cryptic species (species distinguished only by genetic markers) often have overlapping distributions, but are segregated in habitat use because depth was the main driver for the genetic structure observed. Still, these species have been exploited and managed for decades as a single unit in the North Pacific and the North Atlantic (Daníelsdóttir *et al.*, 2008; Hyde *et al.*, 2008).

In the North Atlantic, the genus *Sebastes* is represented by four species: the deep-sea redfish (*Sebastes mentella* Travin 1951), the golden redfish (*Sebastes marinus* Linnaeus 1758), the Acadian redfish (*Sebastes fasciatus* Storer 1854), and the Norwegian redfish (*Sebastes viviparous* Krøyer 1845). These four have overlapping distributions and have been suggested to contribute to mixed fisheries (Roques *et al.*, 1999). The main fisheries activity is concentrated on the deep-sea redfish, which has been studied extensively in terms of genetic structure to assist fisheries management (Johansen *et al.*, 2000; Roques *et al.*, 2002; Daníelsdóttir *et al.*, 2008; Stefánsson *et al.*, 2009a, b). As a consequence, the other *Sebastes* species have received little attention, and limited information exists on their potential genetic structure within their distributional range.

The aim of the present study is, therefore, to provide preliminary information on the possible genetic structure of *S. marinus* within the North Atlantic using samples collected from several fishing grounds in 2001 and genotyped at nine microsatellite loci. Landscape genetic analysis of these samples revealed the presence of two distinct genetic pools within the area studied, suggesting further investigation to fully assess the connectivity of *S. marinus* populations.

Material and methods

In all, 376 *S. marinus* were collected from several geographic regions during a survey carried out in 2001 (Figure 1a). DNA extraction, polymerase chain reactions (PCR), and genotyping of nine microsatellite loci were performed as described in Stefánsson *et al.* (2009b). Details of PCR protocols are available on request.

Genetic diversity was evaluated using allele frequencies, observed (H_o), and unbiased expected heterozygosity (H_e) calculated with the GENEPOP version 4 (Rousset, 2008). Deviations from the Hardy–Weinberg expectation (HWE) were tested using the inbreeding coefficient F_{IS} (Weir and Cockerham, 1984) implemented with GENEPOP; significance was assessed with exact tests. Genetic differentiation was estimated using theta estimates (θ; Weir and Cockerham, 1984) implemented in FSTAT version 2.9.3.2 (Goudet, 1995). Significance of multilocus F_{ST} was assessed with a permutation test (5000). The significance levels were adjusted by sequential Bonferroni corrections (Rice, 1989) when multiple tests were applied.

A factorial correspondence analysis (FCA) was computed with GENETIX (Belkhir *et al.*, 2004) to plot multilocus genotypes of the different samples on a two-dimensional plan and to visualize relationships among samples. We performed a Mantel test

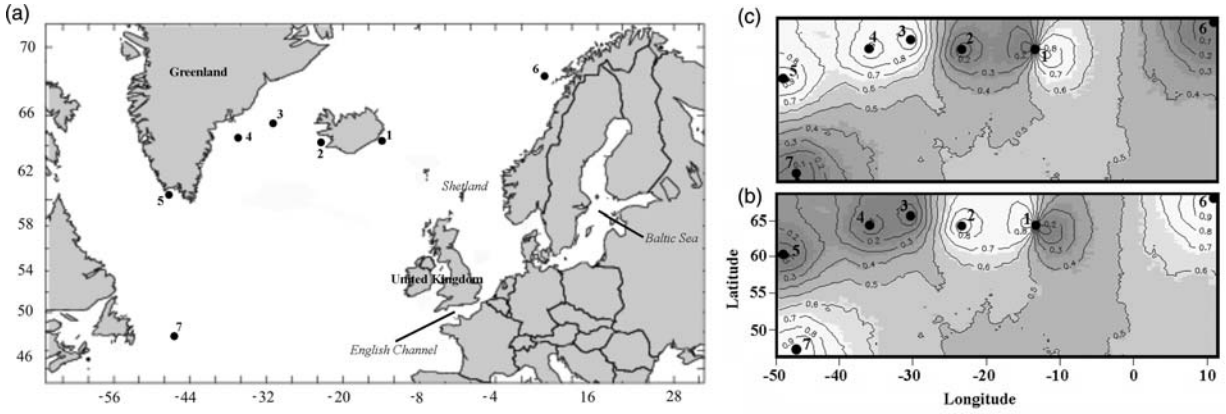

Figure 1. (a) Locations of collection of the seven samples of *S. marinus* from the North Atlantic in 2001. (b) Map of posterior probability of belonging to subpopulation 1 detected in the GENELAND analysis. Subpopulation 1 consists of samples from southeast ($n = 50$) and southwest Iceland ($n = 29$), the Flemish Cap ($n = 55$), and Norway ($n = 79$). (c) Map of posterior probability of belonging to subpopulation 2 detected in the GENELAND analysis. Subpopulation 2 consists of samples from West ($n = 50$) and East ($n3 = 50$; $n4 = 55$) Greenland. Note that eight fish from southeast Iceland were assigned to that group.

(Mantel, 1967) for correlation between geographic and genetic distance between samples (isolation-by-distance), as implemented with GENETIX (after 1000 permutations).

To infer the number of subpopulations (K) in our sample sets, we used the Bayesian approach implemented in GENELAND (version 0.3; Guillot *et al.*, 2005a), which incorporates geographic coordinates in the model development to detect discontinuities among populations with possible uncertainty in spatial coordinates (Guillot *et al.*, 2005b). We first estimated the number of subpopulations from 1 to 7 (number of samples collected), using 100 000 MCMC iterations and 200 thinning. Allele frequencies were drawn from independent Dirichlet distributions (Pritchard *et al.*, 2000), which perform better than the alternative model (*F*-model; Guillot *et al.*, 2005b). Five runs with fixed K were performed for the spatially explicit model (uncertainty 50 km), and for each run, the posterior probability of subpopulation membership was computed for each pixel of the spatial domain (150 × 150 pixels) using a burn-in of 200 iterations. For each of the inferred subpopulations (based on GENELAND analysis), deviations from the HWE and significance were assessed in GENEPOP. Tests for significant differences in H_o, H_e, and A_R (allelic richness) were performed with the software FSTAT. Genetic differentiation and significance were estimated in FSTAT.

Results

Genetic variability, assessed as the number of alleles per locus, was high, ranging from 10 (Smen10) to 49 (SEB33). Observed heterozygosity per sample averaged over all loci ranged from 0.7468 (Sample 3) to 0.9190 (Sample 1), and the expected heterozygosity per sample ranged from 0.7939 (Sample 2) to 0.8592 (Sample 1). Genotypic proportions in samples were out of HWE in just 3 of 63 exact tests after Bonferroni correction for multiple tests and were not attributable to any specific loci or samples. This is less than that by chance alone. Only Samples 3 and 5 deviated from HWE (Table 1).

The overall genetic estimates revealed highly significant F_{ST} and F_{IS} values of 0.0296 ($p < 0.001$, 95% *CI*: 0.0183–0.0446) and 0.0609 ($p < 0.001$, 95% *CI*: 0.0411–0.0819), respectively. This genetic pattern was reflected in the values of F_{ST} in pairwise comparisons of samples, because 18 of 21 comparisons were significant after the Bonferroni correction. Pairwise F_{ST} varied from 0.0003 (Samples 2 and 6) to 0.0776 (Samples 2 and 4). The non-significant pairwise comparisons were observed among samples from West Greenland and southeast Iceland (Samples 1 and 5, $F_{ST} = 0.0074$), southeast Iceland and Norway (Samples 2 and 6, $F_{ST} = 0.0003$), and East Greenland and West Greenland (Samples 3 and 5, $F_{ST} = 0.009$). A graphic representation of the pairwise F_{ST} comparisons on a two-dimensional plot using an FCA analysis revealed the presence of two clusters along the first two axes, explaining 65% of the total variation (Figure 2). Cluster 1 comprised Samples 2, 6, and 7, and Cluster 2 comprised Samples 1, 3, 4, and 5. A Mantel test failed to show any correlation

Table 1. Genetic variability of *S. marinus* samples collected within the North Atlantic.

Parameter	1	2	3	4	5	6	7
n	58	29	50	55	50	79	55
N_A	17.22	11.67	15.33	16.56	14.89	15.11	14.44
H_e	0.859	0.740	0.826	0.834	0.847	0.807	0.833
H_o	0.820	0.781	0.747	0.785	0.761	0.794	0.808
F_{IS}	0.055	0.034	0.106*	0.064	0.111*	0.023	0.040

n, number of individuals genotypes; N_A, mean number of alleles; H_e, expected heterozygosity; H_o, observed heterozygosity; F_{IS}, inbreeding coefficient.
*Significant values after correction for multiple tests.

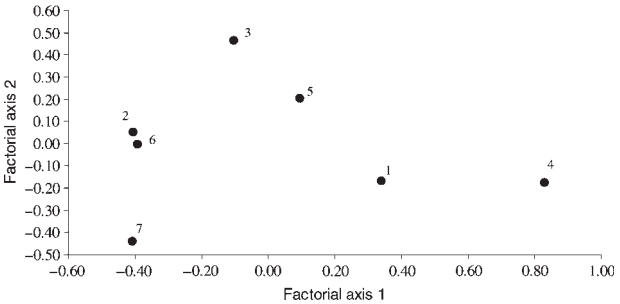

Figure 2. FCA performed on allele frequencies of *S. marinus* samples collected in North Atlantic waters using nine microsatellite loci.

Table 2. Statistical comparison of genetic diversity indices among subpopulations detected with GENELAND.

	Group 1	Group 2	
Parameter	**Samples 1, 2, 6, and 7**	**Samples 3, 4, and 5**	***p*-values**
A_R	16.913	17.484	0.999
H_o	0.803	0.766	0.329
H_e	0.841	0.864	0.329
F_{IS}	0.045	0.113*	0.329
F_{ST}	0.016	0.002	0.999
Relatedness	0.029	0.004	0.999
Corrected relatedness	−0.095	−0.255	0.999

*Significant F_{IS} value detected within the subpopulation.

between genetic (F_{ST}) and geographic distances ($Z = 45.087$, $r = -0.213$, $p = 0.6838$).

All runs to infer the number of subpopulations (K) were performed in GENELAND and, using spatial data, indicated that the most likely number of K was 2. The five runs performed to assign individuals with K fixed to 2 were consistent across each of the ten runs. These geographically distinct subpopulations comprised samples from Norway (6), the Flemish Cap (7), southwest Iceland (2), and southeast Iceland (1) for the first group (Figure 1b) and from samples collected from East Greenland (3 and 4) and West Greenland (5) for the second group; eight fish from southeast Iceland were also included in this group (Figure 1c). The overall F_{ST} among the subpopulations detected with GENELAND ($F_{ST} = 0.012$, $p < 0.05$; 95% *CI*: 0.011–0.024) was significant, but slightly lower than the overall F_{ST} estimated among the samples collected (see above). The overall F_{IS} within subpopulations detected with GENELAND ($F_{IS} = 0.072$, $p < 0.01$, 95% *CI*: 0.065–0.113) was significant and similar to the observed F_{IS} within the samples collected (see above). Tests for significant differences in H_o, H_e, and A_R among subpopulations detected did not reveal a significant pattern (Table 2).

Discussion

Information on the population structure of commercially exploited fish stocks has now been widely acknowledged as crucial for the conservation and sustainable management of marine fish stocks (Hilborn *et al.*, 2003). Across the North Atlantic, redfish species have historically been commercially important. Interestingly, most of the genetic studies related to stock delineation in North Atlantic redfish have focused on the deep-sea redfish *S. mentella* (Roques *et al.*, 2002; Daníelsdóttir *et al.*, 2008; Stefánsson *et al.*, 2009a, b). The primary objective of this study was, therefore, to provide information on the potential genetic structure of *S. marinus* within its distributional range, using samples collected during a cruise in 2001.

The partitioning of genetic variance as estimated by *F*-statistics revealed significant differences in allele frequencies among the seven samples collected, but no explicit geographic pattern owing to the significance of almost all pairwise comparisons after Bonferroni correction. However, visualization of pairwise F_{ST} values on a two-dimensional plan (FCA, Figure 2) suggested the presence of two genetically distinguishable clusters. The inclusion of spatial information in the genetic analyses, e.g. utilization of the GENELAND spatial model, clearly confirmed the existence of genetic discontinuities in the dataset, which can be identified as barriers to gene flow between subpopulations. The runs were consistent and revealed the presence of two genetically distinct groups, one located on the east and west coasts of Greenland, and the other from the Flemish Cap to the south of Iceland and the coast of Norway. Only the clustering of Sample 1 was contradictory between the methods used in the present study.

The results confirmed previous findings on genetic discontinuity for: (i) microsatellite genotypes of *S. marinus* that revealed the presence of two genetically distinct clusters, one composed of Flemish Cap, and East and West Greenland samples, and the second of Icelandic, Faroese, and Norwegian samples (Schmidt, 2005); and (ii) isocitrate dehydrogenase (IDHP) genotypes of *S. marinus* that displayed a similar allelic distribution in samples collected in Icelandic waters and along the coast of Norway and the Faroe Islands, whereas samples collected in Greenlandic waters differed significantly (Nedreaas *et al.*, 1994). The comparable results of these studies using different genetic markers suggest, therefore, that the population of *S. marinus* from the Greenlandic regions is genetically different from the other populations in the North Atlantic. However, both studies suffer from a lack of samples and temporal approach. Indeed, the unbalanced sampling strategy developed for the current study did not allow a firm conclusion to be reached on the stock structure and its potential application to fisheries management. On the other hand, the results do demonstrate the need for further genetic investigation to understand better the pattern of *S. marinus* population connectivity across the North Atlantic.

The fisheries for *S. marinus* in ICES Subareas V and XIV (Icelandic and Greenlandic waters) are currently managed as a single unit. This management approach is likely to have a detrimental effect on the smaller or less productive of these stocks (Ward, 2000) if the genetic structure we observed is genuine. The total biomass index and total landings of *S. marinus* have been constantly decreasing since 1982, from 275 000 to 130 000 t and from 130 000 to 40 000 t, respectively (ICES, 2008). We believe that our results, along with the decrease in the biomass index for the species, demonstrate the need for further investigation of its stock structure for sustainable management.

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