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Genetic structure of the lumpfish *Cyclopterus lumpus* across the North Atlantic

Christophe Pampoulie[1]*, Sigurlaug Skirnisdottir[2], Guðbjorg Olafsdottir[2], Sarah J. Helyar[2], Vilhjálmur Thorsteinsson[1], Sigurður Þ. Jónsson[1], Alain Fréchet[3], Caroline M. F. Durif[4], Sally Sherman[5], Magdalena Lampart-Kałużniacka[6], Rasmus Hedeholm[7], Halldór Ólafsson[8], Anna K. Daníelsdóttir[2], and Jacob M. Kasper[1,8]

[1]*Marine Research Institute, Skúlagata 4, 101 Reykjavík, Iceland*
[2]*Matís ohf., Vínlandsleið 12, 113 Reykjavík, Iceland*
[3]*Maurice-Lamontagne Institute, Fisheries and Oceans Canada, Mont-Joli, QC, Canada G5H 3Z4*
[4]*Institute of Marine Research – Austevoll, 5392 Storebø, Norway*
[5]*Maine Department of Marine Resources, PO Box 8, 194 McKown Pt. Rd., W. Boothbay Hbr, ME 04575, USA*
[6]*Department of Environmental Biology, Technical University of Koszalin, 75-453 Koszalin, Sniadeckich, Poland*
[7]*Greenland Institute of Natural Resources, PO Box 570, 3900 Nuuk, Greenland*
[8]*BioPol, Einbúastíg 2, 545 Skagaströnd, Iceland*

**Corresponding author: tel: +354 575 2038; fax: +354 575 2001; e-mail: chrisp@hafro.is*

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Lumpfish, or lumpsucker, *Cyclopterus lumpus* (Linnaeus, 1758) is widely distributed in the North Atlantic Ocean. It has a considerable economic value and substantial fisheries occur in several North Atlantic regions owing to the use of its fully ripe internal egg masses in the ovaries as an alternative to sturgeon caviar. Despite being intensively fished in several locations, biological knowledge is limited and no genetic structure information is available. In this study, the stock structure of *C. lumpus* was investigated across the North Atlantic using ten microsatellite loci. Out of ten loci, two exhibited higher level of differentiation but their inclusion/exclusion from the analyses did not drastically change the observed genetic pattern. A total of three distinct genetic groups were detected: Maine–Canada–Greenland, Iceland–Norway and Baltic Sea. These results, discussed in terms of origin of differentiation, gene flow, and selection, showed that gene flow was rather limited among the detected groups, and also between Greenland and Maine–Canada.

Keywords: gene flow, lumpfish, microsatellite loci, migration, North Atlantic, selection.

Introduction

Lumpfish, or lumpsucker, *Cyclopterus lumpus* (Linnaeus, 1758) is a demersal and solitary species living in temperate and cold waters of the high latitudes. It is distributed on both sides of the North Atlantic Ocean, from Cape Cod (USA) to Canada on the western side, and from the south of Portugal to Iceland, Greenland, and Spitsbergen on the eastern side (Stein, 1986). This species has recently been recorded in Galicia, Spain (Bañón *et al.*, 2008) and in the Mediterranean Sea (Dulčić and Golani, 2006), and southward range extension has been identified along the coast of Portugal (Vasconcelos *et al.*, 2004). Lumpfish feeds in cold waters during winter and clusters in spawning aggregates in shallow and warm waters during the breeding season. It may undertake annual long-distance migrations from spawning to winter grounds and *vice versa* (Schopka, 1974). It has a considerable economic value and substantial fisheries occur in several North Atlantic regions owing to the use of its fully ripe internal egg masses in the ovaries as an alternative to sturgeon caviar. Lumpfish abundance has decreased in Canada (DFO, 2011) while the harvest has increased considerably in Greenland in the last ten years (Hedeholm *et al.*, 2014). In Iceland,

the landings fluctuate greatly, depending on the market price and reached a decadal maximum in 2010 (Hafrannsóknastofnun, 2013). Yet, despite its important economic value, no studies exist on stock connectivity and biocomplexity, which are crucial to sustainable fisheries management and conservation practices (Ruzzante *et al.*, 2006; Pampoulie *et al.*, 2012a).

Genetic markers such as microsatellite loci have increasingly been used to assess stock/population connectivity in the two last decades (see Hauser and Carvalho, 2008 for a review) and proved useful in the context of fisheries management (see Reiss *et al.*, 2009). The analysis of genetic markers has revealed that the decline of most exploited marine fish in the last century might potentially be due to a mismatch between biological and fisheries management units (Reiss *et al.*, 2009). Although interpretation of genetic results crucially depends on the nature of the marker used, microsatellite loci that are neutral or putatively under selection (see Nielsen *et al.*, 2006) remain useful to investigate reproductive isolation of populations and delineate stock boundaries. In addition, the combination of neutral vs. putatively non-neutral loci represents a robust approach to investigate the forces shaping the connectivity of the populations under study, e.g. the respective effects of drift vs. selection, and potentially adds an ecological time-scale approach to stock delineation and management.

The aim of the present study is to provide information on the genetic structure of lumpfish *C. lumpus* using microsatellite loci on samples collected across a wide range of habitats within the North Atlantic Ocean (spanning from Gulf of Maine to the Baltic Sea). To our knowledge, this is the first genetic study of *C. lumpus* within its distribution range, and the presented results are intended to assist fisheries management.

Material and methods

Sampling areas and protocols

A total of 1116 fish were collected at several fishing grounds in the North Atlantic (Figure 1; Table 1) from 2010 to 2012, with an additional Canadian sample collected in 2008. As genetic variation was expected to be large within Icelandic waters, the sampling strategy was intensive in these waters and included several fjord samples (Figure 1). DNA was isolated either from muscle or fin clips preserved in 96% ethanol using AGOWA mag Midi DNA isolation kit (AGOWA Gmbh).

A total of ten new microsatellite loci were genotyped, namely *Clu*12, *Clu*26, *Clu*29, *Clu*33, *Clu*34, *Clu*36, *Clu*37, *Clu*40, *Clu*44, and *Clu*45 (Skirnisdottir *et al.*, 2013). PCR were carried out on a Tetrad2 Peltier thermal cycler (BioRad) in a 10 μl volume containing 10–50 ng DNA, 200 μM of each dNTP, 1 U Teg polymerase (Matís Ltd.) (Ólafsson *et al.*, 2010), 1× buffer (Matís Ltd.), 0.3–2.5 μM of a 50:50 ratio of labelled forward and reverse primer tagged on the 5′-end with a GTTTCTT PIG tail (Brownstein *et al.*, 1996), adding 500 mM betaine to enhance PCR quality when needed. Details of PCR conditions are fully described in Skirnisdottir *et al.* (2013). Samples were analysed on an ABI PRISM 3730 sequencer using the GeneScan-500 LIZ size standard and genotyping performed with GeneMapper v4.1 (Applied Biosystems).

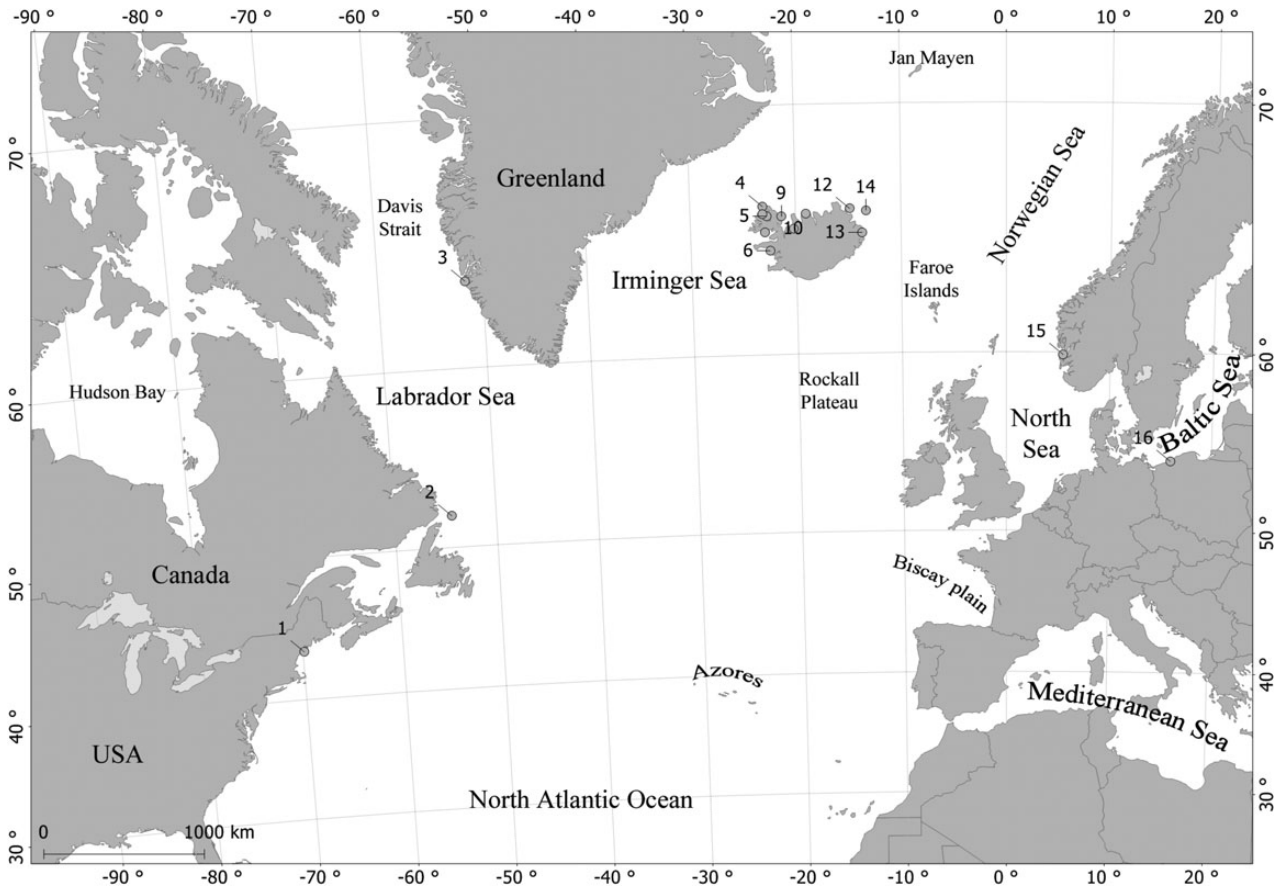

Figure 1. Sampling location of lumpfish *C. lumpus* across the North Atlantic. Numbers refer to sample number in Table 1.

Table 1. Sampling areas and information for 16 samples of lumpfish *C. lumpus*.

Sampling area	Maine	Canada	Greenland	Iceland Southwest	Iceland Sandgerði	Iceland Akranes	Iceland Breiðafjörður	Iceland Bolungarvík	Iceland Hunaflói	Iceland Skagaströnd	Iceland Siglufjörður	Iceland Bakkafjörður	Iceland Seyðisfjörður	Iceland Southeast	Norway	Baltic Sea
Sample number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Date (day.month.year)	31.05.2012	08.06.2008	11.05.2011	15.08.2011	09.05.2011	17.05.2011	24.06.2011	13.04.2011	12.05.2011	28.02.2012	03.12.2012	29.04.2011	15.05.2011	15.08.2011	15.05.2012	27.02.2012
Sample size	18	57	93	12	101	119	101	108	117	50	50	95	50	17	21	107

Statistical analyses

Genetic diversity indices such as the number of alleles (n_a), the expected (H_e) and observed (H_o) heterozygosities, and deviations from Hardy–Weinberg equilibrium (HWE) were calculated in GENEPOP 4.2 (Rousset, 2008).

As the neutrality assumption of genetic markers is crucial to the conclusion drawn from genetic data, coalescent-based simulation methods (Beaumont and Nichols, 1996) for the detection of potential outlier loci (loci under selection) were applied. Coalescent simulations were performed with the software LOSITAN (Antao *et al.*, 2008), with samples of the same size as the observed samples assuming an island model with 100 islands. A total of 100 000 independent loci were generated with the infinite allele mutation model. Simulated distribution of F_{ST} values conditional to heterozygosity under a neutral model were obtained and thus compared with observed F_{ST} values to identify potential outlier loci. Simulations were also performed using the hierarchical-island model implemented in ARLEQUIN (Excoffier *et al.*, 2005), taking into account the detected hierarchical population structure. A total of 10 000 simulations were performed considering standard recommended approach, e.g. considering 10 groups and 100 demes within each of them, then using only three groups and eight demes within each of them. The observed results were similar and only those obtained with the standard recommended approach is shown.

Both overall and pairwise population differentiation was estimated using the unbiased F_{ST} estimator (θ of Weir and Cockerham, 1984). Statistical significance was assessed using permutation tests implemented in GENEPOP.

A Bayesian cluster analysis was performed in STRUCTURE (Pritchard *et al.*, 2000) to assess the potential number of populations present in the samples. STRUCTURE is a model-based Bayesian, Markov-chain Monte Carlo (MCMC) approach that clusters individuals to minimize Hardy–Weinberg disequilibrium and gametic phase disequilibrium between loci within groups, and constructs a specified number of groups (K) based on these parameters. The posterior distribution of individual admixture proportions (q) of individuals was estimated by assuming an admixture without any foregone assumptions on the number of potential K populations contained in the data. A burn-in period of 400 000 steps and 600 000 MCMC simulations was used. The number of potential genetic distinct groups represented in the samples was estimated from $K = 1–10$ without information on the origin of the samples (without LOC PRIOR). Several runs were carried out to estimate convergence of both K and the individual admixture proportions (q). The runs were performed with all loci, with neutral loci only and with outliers only. The results were scrutinized in STRUCTURE HARVESTER (Earl and vonHoldt, 2012) to estimate the optimal number of K using the Evanno's method (Evanno *et al.*, 2005). DISTRUCT was then used to visualize the data (Rosenberg, 2004). As STRUCTURE is likely to detect the highest level of differentiation among the samples, a hierarchical analysis by performing similar STRUCTURE runs on detected populations (K) containing several samples were conducted.

An overall hierarchical analysis of variance (AMOVA) as well as a locus-by-locus AMOVA was performed in ARLEQUIN (Excoffier *et al.*, 2005) among post-hoc defined regions identified by the STRUCTURE analysis.

Results

All studied microsatellite loci were highly polymorphic. The number of alleles per locus was moderate, ranging from five (*Clu*12) to 18

(*Clu*34) (Supplementary Table 1). The unbiased expected heterozygosity per sample ranged from 0.567 (Baltic Sea) to 0.712 (Iceland Southwest), while the observed heterozygosity ranged from 0.563 (Baltic Sea) to 0.708 (Iceland Southwest). Genotypic proportions were out of HWE in four of the 160 exact tests, of which none remained significant after the Bonferroni correction for multiple tests (Supplementary Table 1).

The coalescent-based simulation methods (Beaumont and Nichols, 1996) revealed that two out of ten loci were potential outliers (loci under selection): *Clu*29 ($p < 0.001$) and *Clu*34 ($p < 0.001$). Both microsatellite loci were suggested to be under positive selection (Table 2). However, none of the microsatellite loci were suggested to be under selection using the hierarchical-island model implemented in ARLEQUIN and the genetic structure detected (see below and Table 2). Therefore, analyses were performed with and without the outlier loci (see below).

Using all loci, the overall genetic estimates revealed a highly significant F_{ST} ($F_{ST} = 0.041, p < 0.00001$, 95% CI: 0.032–0.052) and a non-significant overall F_{IS} ($F_{IS} = 0.012$, $p > 0.05$, 95% CI: 0.000–0.025). Of 120 pairwise F_{ST} comparisons, 65 were significantly different from zero, and 54 remained significant after Bonferroni correction (Table 3). Removing loci under selection (*Clu*29 and *Clu*34) resulted in a similar genetic pattern, e.g. a highly significant F_{ST} ($F_{ST} = 0.035$, $p < 0.00001$, 95% CI: 0.029–0.041) and a non-significant F_{IS} ($F_{IS} = 0.016$, $p > 0.05$, 95% CI: 0.003–0.031). Of 120 pairwise F_{ST} comparisons, 65 were significantly different from zero, and 53 remained significant after Bonferroni correction (Table 3). Pairwise F_{ST} values were similar to those observed using all loci (Table 3) and the observed slight decrease in F_{ST} values might be due to a loss of power when eight out of ten microsatellite loci were used. The only difference observed between the two datasets was the non-significance of the Maine–Canada comparison for the neutral loci.

The Bayesian cluster analysis (STRUCTURE) revealed that the most likely number of populations contained in our samples was for $K = 3$, both when using all loci (Figure 2a; Supplementary Figure 1) and neutral loci only (Figure 2b; Supplementary Figure 2). Additional hierarchical analysis of the Northwest Atlantic and Iceland–Norway clusters did not reveal any further structuring (Supplementary Table 2 and 3).

Table 2. Results of the outlier analysis using LOSITAN (Antao *et al.*, 2008) and the hierarchical-island model implemented in ARLEQUIN (Excoffier *et al.*, 2005).

	LOSITAN			ARLEQUIN		
Locus	H_o	F_{ST}	p	H_o	F_{ST}	p
*Clu*12	0.548	0.040	0.187	0.555	0.099	0.297
*Clu*26	0.704	0.052	0.027	0.707	0.072	0.489
***Clu*29**	**0.740**	**0.095**	**0.000**	0.803	0.148	0.084
*Clu*33	0.709	0.025	0.522	0.699	0.080	0.423
***Clu*34**	**0.591**	**0.087**	**0.000**	0.601	0.115	0.202
*Clu*36	0.676	0.011	0.933	0.718	0.057	0.368
*Clu*37	0.600	0.053	0.032	0.632	0.095	0.277
*Clu*40	0.808	0.020	0.792	0.827	0.042	0.154
*Clu*44	0.583	0.020	0.638	0.594	0.062	0.154
*Clu*45	0.830	0.020	0.800	0.862	0.059	0.290

Significant loci are indicated in bold. Both outlier loci were only suggested to be under positive selection with LOSITAN. H_o indicates observed heterozygosity, p indicates probability values.

Table 3. Pairwise F_{ST} for all loci (above diagonal) and for neutral loci only (below diagonal) among 16 samples of Atlantic lumpfish *C. lumpus* based on allelic frequencies.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0	**0.025***	**0.029***	**0.065***	**0.099***	**0.099***	**0.095***	**0.099***	**0.096***	**0.089***	**0.106***	**0.100***	**0.102***	**0.141***	**0.096***	**0.136***
2	0.027*	0	**0.022***	**0.109***	**0.134***	**0.131***	**0.128***	**0.132***	**0.128***	**0.120***	**0.135***	**0.136***	**0.129***	**0.175***	**0.135***	**0.188***
3	**0.030***	**0.023***	0	**0.061***	**0.080***	**0.081***	**0.077***	**0.081***	**0.079***	**0.069***	**0.079***	**0.084***	**0.077***	**0.117***	**0.082***	**0.140***
4	**0.041***	**0.101***	**0.054***	0	0.014*	0.014	0.006	0.009*	0.010*	0.006	0.008	0.012*	0.007*	0.028	0.004*	**0.065***
5	**0.055***	**0.113***	**0.058***	0.013*	0	0.000	0.001	−0.001*	−0.001	0.001	0.001	0.000	−0.002	0.008	0.004*	**0.049***
6	**0.059***	**0.113***	**0.062***	0.013	0.001	0	0.002	0.001	−0.001	0.001	0.004	0.001	0.004*	0.007	0.004	**0.041***
7	**0.050***	**0.104***	**0.053***	0.005	0.001	0.003	0	−0.001	−0.001	−0.001	0.001	0.001	0.001	0.009	−0.002	**0.056***
8	**0.059***	**0.114***	**0.062***	0.006*	0.001*	0.001	−0.001	0	−0.001	−0.001	−0.001	−0.002	−0.001	0.007	−0.003	**0.055***
9	**0.049***	**0.103***	**0.054***	0.007*	−0.001	0.001	0.001	−0.001	0	−0.003	0.001	−0.001	−0.002	0.003	−0.002	**0.053***
10	**0.046***	**0.099***	**0.047***	0.004	0.001	−0.001	0.001	0.001	−0.002	0	0.001	0.001	−0.001	0.003	−0.001	**0.053***
11	**0.064***	**0.115***	**0.058***	0.007	0.001	0.004	−0.001	−0.001	−0.001	0.001	0	0.001	0.001	0.014	0.003*	**0.070***
12	**0.057***	**0.117***	**0.063***	0.009*	0.001	0.002	0.001	−0.001	−0.001	0.001	−0.001	0	0.001	0.008*	−0.002	**0.059***
13	**0.053***	**0.104***	**0.052***	0.001	0.002	0.004*	0.001	−0.001	−0.001	−0.002	−0.002	0.001	0	0.006	−0.001	**0.070***
14	**0.069***	**0.134***	**0.076***	0.017	0.004	0.001	0.005	0.002	0.002	−0.002	0.008	0.004*	0.005	0	0.002	**0.049***
15	**0.058***	**0.119***	**0.065***	0.006	0.006*	0.005	−0.001	−0.003	−0.001	0.001	0.003*	−0.002	−0.001	−0.002	0	**0.060***
16	**0.104***	**0.179***	**0.133***	**0.079***	**0.057***	**0.047***	**0.065***	**0.062***	**0.060***	**0.061***	**0.083***	**0.068***	**0.079***	**0.048***	**0.074***	0

Significance was assessed using exact test. See Table 1 and Figure 1 for sample codes.
Emboldened values remained significant after Bonferroni correction ($\alpha = 0.05/120 = 0.00042$).
*Values significantly different from zero (Fisher's exact test, $p < 0.05$).

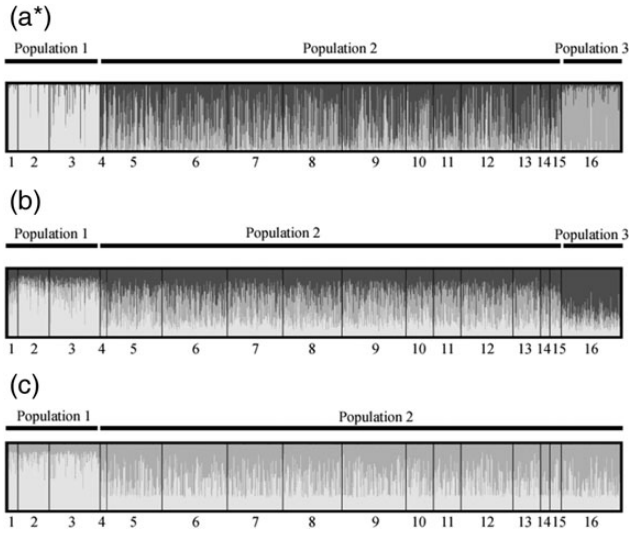

Figure 2. Bayesian cluster analyses performed in STRUCTURE (Pritchard *et al.*, 2000). (a) All loci, (b) neutral loci only, and (c) outlier loci only for $K = 2$. Within each plot, each vertical bar represents an individual while colours indicate the different clusters detected. The *y*-axis indicates the proportion of an individual to belong to each cluster. All runs were performed using an admixture model with correlated allele frequencies. Plots marked with * indicate a lack of convergence of ΔK analysis and the value of K was therefore chosen based on log-likelihood values. Additional hierarchical analysis of the detected clusters did not reveal any further structure (see Supplementary material). Numbers refer to sample number in Table 1 and Figure 1.

Using the outliers loci only (*Clu*29 and *Clu*34), the most likely number of cluster detected with STRUCTURE was for $K = 1$ (Supplementary Figure 3). However, for $K = 2$ (Figure 2c), a clear distinction among Northwest samples (Maine, Canada, and Greenland) and other samples was observed. Further hierarchical analysis did not reveal any more clustering.

AMOVA among post-hoc defined regions using the detected groups in the STRUCTURE analysis (Maine–Canada–Greenland, Iceland–Norway, and Baltic Sea) confirmed that a significant portion of the variation was due to among groups component (Table 4). The locus-by-locus revealed that all microsatellite loci contributed significantly to the observed genetic pattern (although loci under selection exhibited a twofold higher F_{ST} value) and that F_{CT} exhibited a twofold higher value than F_{SC} (Table 5).

Discussion

The main objective of the present study was to investigate the genetic structure of *C. lumpus* across the North Atlantic. The results show that this species is genetically structured on a large geographical scale but not at a relatively small geographical scale, at least for Iceland. The results, which are fully discussed below, mainly suggested the presence of three genetically distinct populations of lumpfish in North Atlantic, Maine–Canada–Greenland (Northwest), Iceland–Norway (Northeast), and the Baltic Sea.

The *F*-statistics revealed that, with or without outlier loci, the main differentiation occurred among samples from the Northwest Atlantic, the Northeast Atlantic, and the Baltic Sea. These results were corroborated further by Bayesian (STRUCTURE) and hierarchical analysis of variance (AMOVA) approaches.

The observed genetic pattern is likely to reflect a combination of events, from colonization of recently available habitats after the last glacial maximum (LGM), ~25 000 years ago, to a consecutive lack of gene flow among the studied populations. Indeed, genetic markers such as microsatellite loci have often been shown to reflect historical events and recolonization processes after the LGM, even for marine commercial species such as Atlantic cod (Hardie *et al.*, 2006; Pampoulie *et al.*, 2008) and Atlantic wolfish (Pampoulie *et al.*, 2012b). For several species, it has been suggested that the contemporary genetic patterns at microsatellite loci originate from the isolation of populations in glacial refugia during the Pleistocene ice ages (Hardie *et al.*, 2006; Hoarau *et al.*, 2007; Pampoulie *et al.*, 2008; Stefánsson *et al.*, 2009). During the LGM, the northern part of the North Atlantic Ocean was covered with ice and the average reconstructed temperatures ranged from −4 to −2°C (Siegert and Dowdeswell, 2004), conditions which were likely to be too extreme for the lumpfish. Several refugia have been suggested for marine species, from the Ancylus lake (Baltic Sea, see Verspoor *et al.*, 1999) to the Rockall plateau (Jolly *et al.*, 2006; Hoarau *et al.*, 2007; Pampoulie *et al.*, 2008; McCusker and Bentzen, 2010) and/or the Irminger Sea (Cross and Payne, 1978; Hardie *et al.*, 2006). Although the Baltic Sea was formed millions of years ago, the present-day connection with the North Sea was only established when the saline Lake Ancylus became a sea again, some 8000–9000 years ago. In the last two decades, genetic studies have shown that a number of marine populations from the Baltic Sea are highly differentiated from North Sea and North Atlantic populations (Case *et al.*, 2005; Pampoulie *et al.*, 2008; Gaggiotti *et al.*, 2009), and that a hybrid zone exists around the Danish belt (Nielsen *et al.*, 2003). The uniqueness of the Baltic Sea environment and its recent colonization by the lumpfish most likely led to the establishment and evolution of a genetically distinct population of lumpfish in the area after the LGM, with limited connection to the North Atlantic populations. Lumpfish from the Baltic Sea present slightly different morphological characteristics than individuals from other areas. They display less skin tuberculation and reduced dermal lumps (Davenport, 1985); they are also smaller than other lumpfish groups (Lampart-Kałużniacka and Heese, 2000; Kudryavtseva and Karamuschko, 2002). In addition, the power associated with the STRUCTURE analysis with the two outlier loci was as expected quite low, but the removal of the two outliers from the analysis resulted in a less distinct Baltic Sea group (Figure 2b). These results tend to confirm that the colonization of the Baltic Sea by lumpfish is quite recent and that gene flow between this population and the Northeast Atlantic populations was quite limited after the colonization of the Baltic Sea.

There was a lack of divergence among Iceland samples, as well as a lack of differentiation among the Iceland and the Norway samples. Although evidence from surveys indicate that the distribution of lumpfish extends almost continuously from Iceland to Norway (Holst, 1993; Nøttestad *et al.*, 2012), biological (Bagge, 1967) and tagging information (Schopka, 1974; Mitamura *et al.*, 2007; DFO, 2011) tend to confirm that the species is quite sedentary, and exhibits spawning site fidelity. Therefore, the lack of genetic divergence among these two populations might be due to a recent recolonization of the habitats from the same ancestral population and/or to their large effective population size which prevents drift to favour genetic differentiation (Slatkin, 1987). Together, genetic and tagging results suggest that lumpfish exhibit repeat-homing patterns. In addition, larval dispersal might also be limited. Male lumpfish are known to attend and guard the egg masses released by females until hatching (Davenport, 1985). Yet, little is known

Table 4. Hierarchical analysis of molecular variance among samples of lumpfish *C. lumpus* grouped according to STRUCTURE analysis (number of iterations 5000).

Loci	Source of variation	Variance components	% variation	Fixation indices	*p*-values
Microsatellites	Among groups	0.287	8.203	CT = 0.082	<0.00001
	Among samples within groups	0.012	0.340	SC = 0.004	<0.00001
	Within samples	3.202	91.457	ST = 0.085	<0.00001
	Total	3.519	100		

Genetic partition was tested among the three following groups: Group 1: Maine, Canada, and Greenland samples; Group 2: Iceland and Norway samples; Group 3: Baltic Sea sample.

Table 5. Locus-by-locus analysis of molecular variance among samples of *C. lumpus* (AS, F_{CT}), among samples within group (ASWG, F_{SC}) and within samples (WS, F_{ST}).

Locus	%AS	%ASWG	%WS	F_{CT}	F_{SC}	F_{ST}
*Clu*12	0.027	0.001	0.250	0.099***	0.002	0.100***
*Clu*26	0.029	0.001	0.033	0.072**	0.007	0.078***
***Clu*29**	**0.060**	**0.001**	**0.341**	**0.148*****	**0.002**	**0.150*****
*Clu*33	0.028	0.003	0.319	0.080***	0.008***	0.088***
***Clu*34**	**0.037**	**0.000**	**0.265**	**0.115****	**0.005****	**0.119*****
*Clu*36	0.020	−0.001	0.339	0.057**	−0.001	0.056**
*Clu*37	0.033	−0.001	0.285	0.095**	0.004*	0.099***
*Clu*40	0.022	0.000	0.484	0.043***	0.005*	0.047***
*Clu*44	0.021	0.001	0.019	0.070**	0.001	0.070***
*Clu*45	0.025	−0.001	0.406	0.062***	0.005*	0.066***
Total	0.302	0.003	2.741	0.082***	0.004	0.085***

All loci showed significant comparisons among the three clusters detected using STRUCTURE (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). Outlier loci are indicated in bold.

about the larval stage of this species, and two hypotheses have been suggested (see Davenport, 1985 for a full discussion). For over a century, it has been suggested that newly hatched lumpfish will attached themselves to their father to benefit from its protection; an idea that has been confirmed by scuba-divers (see Davenport, 1985). Recent studies in near natural conditions have nevertheless suggested that the rapid dispersal of the larvae was also possible (see Davenport, 1985). These two different larval behaviour will have drastic opposite consequences on the genetic pattern of the species, the first hypothesis limiting larval dispersal, and potential for gene flow among populations, the second favouring it. A third possibility is that the newly hatched larvae do not attach to the father and do not disperse rapidly, but instead attach themselves "immediatly" to some substratum in the intertidal zone, which might limit larval dispersal.

While mainly observed with the Bayesian approach (STRUCTURE), the grouping of Greenland samples with the Maine–Canada group was surprising. This pattern can, however, probably be explained by the location of the samples which were collected on the west coast of Greenland (Figure 1). No samples were available from the East Greenland coast, and no fishery takes place in this region (GINR, unpublished data). This would suggest that East Greenland might constitute a potential barrier for a gene flow between the east and west clusters, thereby explaining the observed divergence. Also, colonization of Greenland waters since LGM from North America seems the most likely explanation. Coastal East Greenland waters are highly influenced by southward flowing cold Polar Water, and during lumpfish spawning time (March–June) temperatures are low (Straneo *et al.*, 2010) and most likely outside the tolerated range for lumpfish spawning. Iceland and West Greenland coastal waters are heavily influenced by the warm Atlantic Irminger current making conditions favourable. In addition, some degree of spatial genetic structure might be maintained through a high degree of site fidelity (repeated homing). Nevertheless, sampling at a scale similar to that performed in Iceland should be repeated in Greenland for the lumpfish, especially including samples from the geographical extremes. The observed pairwise F_{ST} for lumpfish suggested a certain degree of reproductive isolation between Greenland and Maine–Canada, even if the level of differentiation is two- to fourfolds lower than between Greenland and the Northeastern group. Therefore, the Bayesian approach results might reflect the common origin of Greenland, Maine, and Canada populations, and the Greenland samples might be part of a separate unit or belong to populations units which were not sampled during this study.

In general, selection will typically lead to faster genetic divergence than drift alone, and thus result in pronounced differentiation at certain microsatellite loci over a similar evolutionary time-scale (see Pampoulie *et al.*, 2006; Hemmer-Hansen *et al.*, 2007, 2013; Therkildsen *et al.*, 2013). For the lumpfish, the inclusion/exclusion of *Clu*29 and *Clu*34, both shown to be under positive selection in one test, did not result in a drastic change of the observed genetic pattern although F_{ST} values were higher for these two loci. This study however represents more evidence of the usefulness of combining neutral and non-neutral loci to fully fathom the genetic structure of marine organisms and potentially integrate the observed results into fisheries management. To our knowledge, this study is the first to investigate the population genetics of lumpfish across the North Atlantic, and is therefore relevant to fisheries management.

Overall, the present study revealed the presence of three distinct units of common ancestral origin, Maine–Canada–Greenland (western Atlantic), Iceland–Norway (Eastern Atlantic), and the Baltic Sea. Indeed, the Bayesian cluster analysis tends to confirm the common origin of the different groups while F_{ST} tend to support

differentiation among some of these samples (for example, Greenland and Canada–Maine). These results are therefore likely to reflect series of recolonization events after the LGM from different ancestral populations, associated with consecutive spatial isolation. At present, Greenland has no fisheries advice or Transferable Allowable Catch (TAC) despite a tenfold increase in harvesting since 2000 (Hedeholm *et al.*, 2014). In Iceland, the lumpfish fishery is managed by a proxy for trends in the fishing mortality rate (F_{proxy}), which is computed as the ratio of annual landings to the female biomass survey index assessed during a groundfish survey (Hafrannsóknastofnun, 2013). Both Canada and Iceland have also seen a decrease in spawning-stock biomass (Hafrannsóknastofnun, 2013) suggesting the immediate need for an improved management strategy.

Supplementary data

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

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