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Genetic diversity, growth, maturity and migration of the Atlantic wolffish (*Anarhichas lupus* L.)

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Erfðabreytileiki, vöxtur, kynþroski og far hjá steinbít (*Anarhichas lupus* L.)

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Ágrip á íslensku:	Í þessari rannsókn var arfgerð, kynþroski og vöxtur steinbíts frá Vestfjörðum og Austfjörðum rannsökuð. Við arfgerðarrannsóknina voru notuð 16 erfðamörk og genið Rhodopsin. Þrátt fyrir að steinbítur sé staðbundin fiskur og lítið rek sé á eggjum hans og lirfum fannst ekki munur á arfgerð hans milli svæða eða ára með þeim erfðamörkum sem rannsökuð voru. Erfðarannsóknirnar sýndu minnkun stofnstærðar steinbíts við Ísland. Í hlýja sjónum út af Vestjörðum vex steinbítur hraðar og verður kynþroska yngri og minni en steinbítur í kaldari sjónum út af Austfjörðum. Niðurstöðurnar voru skoðaðar út frá líffræði steinbíts, tímalengd sem steinbítur hefur verið aðgreindur milli svæða við Ísland og gildi rannsóknarinnar fyrir sjálfbærar veiðar.		
Lykilorð á íslensku:	Arfgerð, vöxtur, kynþroski, far, steinbítur, *Anarhichas lupus*		
Summary in English:	The stock structure of the Atlantic wolffish was investigated at Icelandic fishing grounds from two areas with different temperature regimes, using 16 microsatellite loci and the Rhodopsin gene. Growth and maturity was also examined. Despite the potential of the Atlantic wolffish to exhibit genetic structure (lack of eggs/larval dispersal and adults are sedentary), the genetic tests applied in this study did not detected significant genetic differentiation among the samples analyzed. However, the results on genetic diversity revealed a significant decrease in population size (bottleneck effect). Atlantic wolffish grows faster and matures at a younger age and smaller size in the warmer sea west of Iceland than in the colder sea east of Iceland. These results are discussed in terms of biological characteristics of Atlantic wolffish, recent isolation of populations and their application to sustainable fisheries management issues.		
English keywords:	Genetic diversity, growth, maturity, migration, Atlantic wolffish, *Anarhichas lupus*		

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1. INTRODUCTION

The exploitation of many marine fishery resources exceeds the limits of sustainable harvesting, and several stocks have declined drastically during the past 50 years (Christensen et al., 2003; Morato et al., 2006; FAO 2011). The depletion and the collapse of commercial fish stocks have been widely interpreted as a failure of sustainable fisheries management due to the mismatch between management and actual biological units (Reiss et al., 2009). Fish stocks are often managed under the panmixia hypothesis in spite of life history, behavioral, or genetic differences among components of these management units. Today, there is a growing body of evidence that marine fish populations do not conform to the classical panmictic population view, but are rather characterized by population structure on a much finer scale than expected from their dispersal and migratory abilities (see Hauser and Carvalho, 2008 for a review). Information on population structure of commercial species becomes therefore crucial for conservation and sustainable management of stocks (Hilborn et al., 2003). In recent years, genetic markers such as mtDNA (Carr and Marshall, 1991; Carr et al., 1995) and microsatellite loci (Beacham et al., 2002; Carlsson et al., 2006; Hoarau et al., 2002; Pampoulie et al., 2006; Pampoulie et al., 2008c) have consequently been used increasingly to improve stock identification and to aid fisheries management, with some success (Gharrett et al., 2007; Hyde et al., 2008; Pampoulie et al., 2008b; Pampoulie et al., 2008c; Pampoulie et al., 2011b; Rico et al., 1997; Ruzzante et al., 1997; Westgaard and Fevolden, 2007).

The Atlantic wolffish (*Anarhichas lupus*) is widely distributed around the North Atlantic, and has considerable economic importance. Its abundance has decreased over the last years, at least in the Northwest Atlantic. Atlantic wolffish is currently listed by the Canadian Species at Risk Act (SARA) as species of 'special concern' (McCusker et al., 2008). Despite its status of endangered species, very few biological studies have been performed on Atlantic wolffish (Gunnarsson et al., 2006). Indeed most of the available studies have been carried out in the

context of aquaculture development. While several important commercial fish species have been intensively studied genetically (cod and herring for example) following their abundance decline and in response to the application of efficient stock discrimination methods, there is an obvious lack of studies on the genetic structure and on the biology of the Atlantic wolffish. So far, genetic markers have mostly been used for species identification (Johnstone et al., 2007; McCusker et al., 2008) but have rarely found any application to stock structure and fisheries management (but see McCusker et al., 2010). In addition, only one biological study has been performed on wild individuals of this species, where both growth and maturity were examined (Gunnarsson et al., 2006). It showed that Atlantic wolffish from two areas in Icelandic waters, one characterized by relative warm sea temperature and the other by cold sea temperature, had different growth maturity reaction norm. It is a question if this reaction norm is reasoned by genetic origin or not. By using neutral genetic markers it can be investigated whether heterozygosity-fitness correlations (HFCs) can be detected. The HFCs, the correlation between heterozygosity observed at marker loci and fitness related traits such as growth, survival, fecundity, or developmental stability, have been under study for decades in populations of many species. Although the correlations between genetic variability and fitness components as reflected by molecular marker heterozygosity in natural populations usually accounts for a small percentage (1–5%) of the observed phenotypic variance (David, 1998), there are good indicators for a genetic basis of growth or maturity variation among populations. For example, Pogson and Fevolden (1998) examined the relationships between growth and the degree of individual heterozygosity at 10 nuclear RFLP loci in two natural populations of Atlantic cod (*Gadus morhua*), using a rough measure of growth (size at age). A significant positive correlation was found in one population, supporting the hypothesis that neutral DNA markers can detect HFCs.

Figure 1. Atlantic wolffish in a hole or a nest on Látragrunn, Iceland, the main spawning area of Atlantic wolffish in Icelandic waters. The picture was taken during a research survey in 2012 performed by the Marine Research Institute in Reykjavík (MRI) targeting Atlantic wolffish at Látragrunn.

Prior to this project, Atlantic wolffish had never been tagged with Data Storage Tags (DSTs). Several studies have been done on its migration pattern based on release and recapture with anchor- and alcathene tags. From 1966-1975, about 13 thousand Atlantic wolffish were tagged off Iceland. The average recovery rate was 5.5% (Jónsson, 1982). Further, that research showed that there are two main migrations of Atlantic wolffish off Iceland, a spawning and a feeding migration. In autumn, mature Atlantic wolffish migrate from shallow waters into deeper waters for spawning. Soon after the spawning season, mainly from January to March, Atlantic wolffish migrate again to the shallow waters for feeding. There seems to be a similar migration pattern for Atlantic wolffish off Canada (Nelson and Ross, 1992). Atlantic wolffish seems to be a rather stationary fish, while occasionally taking longer migrations. In the Icelandic tagging study, some Atlantic wolffish were recaptured about 300 miles from the tagging place (Jónsson, 1982). As in most tagging studies, most of the recapture of Atlantic wolffish was within a year of tagging, but there were several examples

of Atlantic wolffish being recaptured a few miles from the tagging site, 2-7 years after being tagged (Templeman, 1984; Riget, 1988). However, none of the Atlantic wolffish were recaptured in waters of other countries than where the tagging was done. These studies indicate that some Atlantic wolffish take a long migration, while other seem to be rather sedentary.

Other species such as cod (Pálsson and Thorsteinsson, 2003), showed that individuals exhibited different migration mode such as shallow or deep water feeding migration. In recent years, Pampoulie et al. (2008a) have shown that this feeding migration behavior of cod could be related to different *Pan* I locus genotypes. They have defined two genetically distinct behavioral types for cod in Icelandic waters, which have so far, not been investigated fully. Preliminary results suggest that within a spawning ground, individuals carrying the *Pan* I^{AA} genotype are likely to display shallow water feeding migrations, while individuals carrying the *Pan* I^{BB} genotype prefer deeper waters and forage near thermal fronts. The heterozygote exhibits either a shallow or deep water feeding migration. One of the main questions remaining to be solved concerning these behavioral types of cod, is to assess whether or not they belong to the same population or represent locally adapted behavioral units (evolutionary significant units which could be considered as management units).

It has recently been recognized that behavioral ecology should meet molecular ecology as well as evolutionary ecology to fully fathom the dynamic of a commercial stock and/or of a species (Keogh, 2009). The use of DTSs information combined to a genetic study involving neutral as well as a HFCs approach might be promising to better understand the evolutionary trajectory of the targeted species, and to better understand the biodynamic processes responsible for stock structure. This project adds a new dimension in growth and maturity researches of Atlantic wolffish by attempting to assess the relationship among genetic diversity and life-history traits variation (HFCs). The present project will apply an integrated tagging-genetic approach combining genetic markers (involving neutral microsatellite loci) to Data Storage Tags (DSTs) as soon as the results of DSTs will be established. Furthermore, it will add information to life-history traits variation in order to

study the potential restricted gene flow among the Atlantic wolffish from the two areas and their potential adaptation to local environment.

2. MATERIALS AND METHODS

A more detailed description of the work can be found in a peer-reviewed paper entitled: "Genetic structure of the Atlantic wolffish (*Anarhichas lupus* L.) at Icelandic fishing grounds: another evidence of panmixia in Iceland" by C. Pampoulie, S. Skirnisdottir, A. Daníelsdottir, A. Gunnarsson (Published in ICES Journal of Marine Science; see Appendix 1).

2.1 Sampling

The main sampling period for this study was in autumn 2010. In addition, sampling was done in 2011 and 2012. Archived samples from spring 2002 and 2004 were also analyzed. All sampling was done in research surveys performed by the Marine Institute in Reykjavík (MRI). Muscle tissue was taken for genetic analysis from each fish. Total length, whole body weight and gutted weight were measured for each fish, as well as liver and gonad weight. Sex and maturity stage were assessed for each individual and sagittal otoliths were extracted for age determination.

2.2 Growth and maturity

A total of 763 Atlantic wolffish, which were collected during the year 2010 were aged during this study, 397 from the west and 366 from the east areas. The age determination was done at MRI by counting the winter zone in sagittal otoliths under transmitted light, performed by a highly experienced person in age determination of Atlantic wolffish. The beginning of the first year was determined according to Gunnarsson *et al*. (2006).

The analyses of growth of Atlantic wolffish were based on length-at-age data. The Schnute and Fournier (1980) growth model, which is derived from the Von Bertalanffy growth model and revised by Cook et al. (1999), was used to estimate growth. The t-test was performed to assess the difference in growth between the two locations (west and east) (Zar, 1999).

During the estimation of maturity, only female Atlantic wolffish were used because the estimation of maturity stages for male wolffish is much more complicated than of the female ones. Maturity stages were determined according to the maturity scale of Barsukov (1959), revised by Mazhirina (1988) (see also description in Gunnarsson et al., 2006). The estimation of maturity ogives was performed using a logistic regression analysis (Crawley, 2002), and age (A_{50}) and length (L_{50}) at 50% maturity were then calculated. Chi square test was used to compare maturity ogives between areas (Crawley, 2002) and t-test to assess if A_{50} and L_{50} were different between areas. The measure of model fit was based on a pseudo-coefficient of determination (Swartzman et al., 1995). The biological data sampled was combined with the genetic markers to analyse HFCs (section 2.6), i.e. relationship between biology and heterozygosity indices of the genetic markers.

2.3 Microsatellite genotyping

Muscle samples were collected from individual fish and conserved in 99% ethanol. Two DNA extraction methods were tested and their quality evaluated. These two methods were Agowa mag Midi DNA Isolation Kit (Agowa GmbH) and Chelex 100 (Walsh et al., 1991). Samples were genotyped with 16 published microsatellite loci: Alu7, Alu9, Alu10, Alu11, Alu14, Alu21, Alu22, Alu23, Alu24, Alu25, Alu26, Alu27, Alu28, Alu29, Alu30 and Alu31 (McCusker et al., 2008). The forward primers of each microsatellite pair was labeled with one fluorescent dye (6-FAM, VIC, NED or PET) and the reverse primers tagged on the 5´-end with a GTTTCTT PIG-tail (Brownstein et al., 1996). Polymerase Chain Reactions (PCR) were performed in a 10 µL volume containing 2 µL of Agowa DNA, 200 µM of each dNTP, 1X Teg buffer (100 mM Tris-HCl, pH 8.8; 500mM KCl; 15mM $MgCl_2$; 1% Triton X-100), 0.9 U Teg polymerase (Olafsson et al., 2010), 0.0075-0.10 µL (100 µM) of the labeled forward primers and the same amount of reverse primers. PCR reactions were performed on a Tetrad2 Peltier (Bio-Rad) thermal cycler as follows: an initial denaturation step of 3 min at 94 °C followed by 30 cycles of 30 s at 94°C, 50 s at 58°C, 50 s at 72 °C, and a final elongation step of 7 min at 72 °C. The 16 microsatellite loci were run in four multiplex systems where Multiplex 1 contained loci Alu21, Alu24, Alu25, Alu26 and Alu29; Multiplex 2 contained loci Alu7, Alu10 and Alu11 (Alu7 PCRs were performed separately); Multiplex 3 contained loci Alu22, Alu27,

Alu28, Alu30 and Alu31; and Multiplex 4 contained loci Alu9, Alu14 and Alu23 (Alu9 PCRs were performed separately) but developmental work was needed for optimization i.e. by varying primer concentration and primers. In addition to the multiplex PCRs, all the microsatellite loci were amplified in separated PCRs in order to compare single locus genotyping results to the multiplex systems. Samples were analyzed on an ABI PRISM 3730 sequencer using the GeneScan-500 LIZ size standard and genotyping scorings were done by using GeneMapper v4.0 and v4.1 (Applied Biosystems).

2.4 Microsatellite statistical analysis

The quality of the microsatellite loci was investigated using different software such as LOSITAN (Antao et al., 2008) to assess their neutrality, and GENEPOP'007 (Rousset, 2008) to assess their diversity. Genetic diversity of each sample (archived and contemporary) was evaluated using allele frequencies, observed (H_o) and unbiased expected heterozygosity (H_e) calculated in GENEPOP'007 (Rousset, 2008). Deviations from Hardy–Weinberg Expectation (HWE) were tested using the inbreeding coefficient F_{IS} (Weir and Cockerham, 1984) implemented in GENEPOP and significance assessed with exact tests. Genetic differentiation was estimated using theta estimates (θ) (Weir and Cockerham, 1984) implemented in GENEPOP, and significance was assessed using allelic and genotypic frequency homogeneity tests (5000 permutations). The significance levels were adjusted by a simple Bonferroni correction (Rice, 1989) when multiple tests were applied.

STRUCTURE 2.3.2 (Pritchard et al., 2000) was used to assess the potential number of populations within the contemporary samples. Due to the very low genetic differentiation level detected (see Results), the admixture model with the LOCPRIOR setting was used, which considers location information. This recently developed method (Hubisz et al., 2009) has been suggested to perform better than the traditional STRUCTURE methods when the genetic structure is weak or when the number of loci is low (< 20). The model was run with a "burn-in" period of 300 000 iterations and 600 000 MCMC iterations. The potential number of populations (*K*) varied from 1 to 8, and was tested with 5 independent analyses for each *K*. Then the archived samples were incorporated in the analysis.

As previously published, biological information have indicated differences between the eastern (STA) and western (STV) populations in Iceland (Gunnarsson et al., 2006), additional analyses were performed based on the possible existence of these two genetic groups, only using contemporary samples. First, the program FSTAT (Goudet, 1995) was used to assess potential differences of genetic diversity indices such as allele richness and observed and expected heterozygosity. Then a locus-by-locus hierarchical analysis of molecular (AMOVA) variance was performed using the program Arlequin (Excoffier et al., 2005). Because the previous biological investigation suggested a drastic reduction in population size, the effective population size (N_e) of Atlantic wolffish population using the linkage disequilibrium (Hill, 1981) and the temporal methods of Waples (1989) implemented in NEESTIMATOR (Peel et al., 2004) was estimated. For the temporal approach, all the archived samples from all regions were combined and they used as a reference point, both for the samples collected at the western and eastern regions. N_e estimates were also calculated for one single panmictic population of Atlantic wolffish. The archived samples were then only used for the temporal methods (Waples, 1989).

2.5 Genes investigation

An 850 bp fragment of the Rhodopsin gene was amplified for 160 selected samples in a 25 µL reaction containing forward primer Rh193-FM3 5´ATGANTAYCCNCAGTACTACC´3 (Matís redesign from Chen et al. 2003) and the reverse primer Rh1039r 5´TGCTTGTTCATGCAGATGTAGA´3 (Chen et al., 2003). The 25 µL PCR reactions contained 3 µL Agowa DNA, 0.75 U of Taq polymerase (NEB), 1X Standard buffer (NEB), 50 µM each dNTP, and 0.16 µM of the forward and the reverse primers. The thermal cycling was performed in a Tetrad2 Peltier (Bio-Rad) but the PCR amplification cycle consisted of a 4 min denaturation at 94°C, followed by 35 cycles of 94 °C denaturing for 30 s, 58°C annealing for 30 s and 68°C extension for 90 s. Cycling was concluded with a 7 min extension at 68°C. For PCR clean-up, 2.5 µL of the PCR products were mixed with 0.25 µL of ExoSAP-IT reagent and cycled according to the producer (Affymetrix). The sequencing of the fragment was performed by using the forward and reverse PCR primers and the BigDye Terminator v3.1 Cycle Sequencing Kit according to the manufacturer's instructions (Applied Biosystems). The

sequences were aligned and SNPs detection was done by using the software Sequencher 4.8 (Gene Codes Coperation). PCR and sequencing of hemoglobin β and Hsc70 for the same selected samples were also tested by using published and Matís designed primers.

2.6 Heterozygosity-Fitness relationships (HFCs)

Although several genetic indices can be used for HFCs investigation, a general consensus emerges when it comes to natural wild populations. For microsatellite loci, the Multi-locus heterozygosity (MLH_{ms}), e.g. the number of heterozygous loci divided by the number of loci types, and the mean d^2 distance, e.g. the mean square difference in microsatellite allele length of the two alleles, have been highly recommended for such investigations (Forstmeier et al., 2012; Coulson et al., 1998). Here, by using neutral genetic markers, it was investigated whether heterozygosity-fitness correlations (HFCs) could be detected. Namely, to investigate possible relationships among these two genetic parameters and growth rate, a measure of survival, in the Atlantic wolffish. To do so, MLH_{ms} and d^2 distances for four different groups were calculated (the fast growing fish from the West and East and the slow growing fish from the West and East of Iceland). Furthermore, analyses for two groups, e.g. fast and slow growing fish (combined from each area) were also done.

2.7 Data storage tags

Before going further it should be mentioned that the tagging was scheduled to be in the first year of this project or 2010, however, as the project was not granted from Tækjasjóður Rannís (The Rannis Equipment Fund) for pursuing the DSTs tags until the year 2012, the tagging was delayed. The DSTs (see technical description in Appendix 2) was located on the right side of the abdomen of the fish and the T-bar on the left side just below the dorsal fin, to increase the probability that the tags will be noticed by fishermen i.e. the yellow tube that is attached to the DSTs tag was visible on the right side of the fish and the T-bar on the left side. The tagging was done in late 2012 and the fish was caught either by long line or bottom trawl. Each fish was weighted to the nearest grams (g) and measured to nearest centimeters (total length). Fin clip was collected from the tagged fish for future genetic projects.

3. RESULTS

3.1 Sampling

About 1800 genetic samples were collected during the project time (see Figure 2). A total of 400 genetic samples were collected from the west of Iceland and 370 samples from the east of Iceland or a total of 770 samples from eight locations (Figure 2) in 2010. From each location, 100 samples were taken, except for one location where only 70 Atlantic wolffish were available.

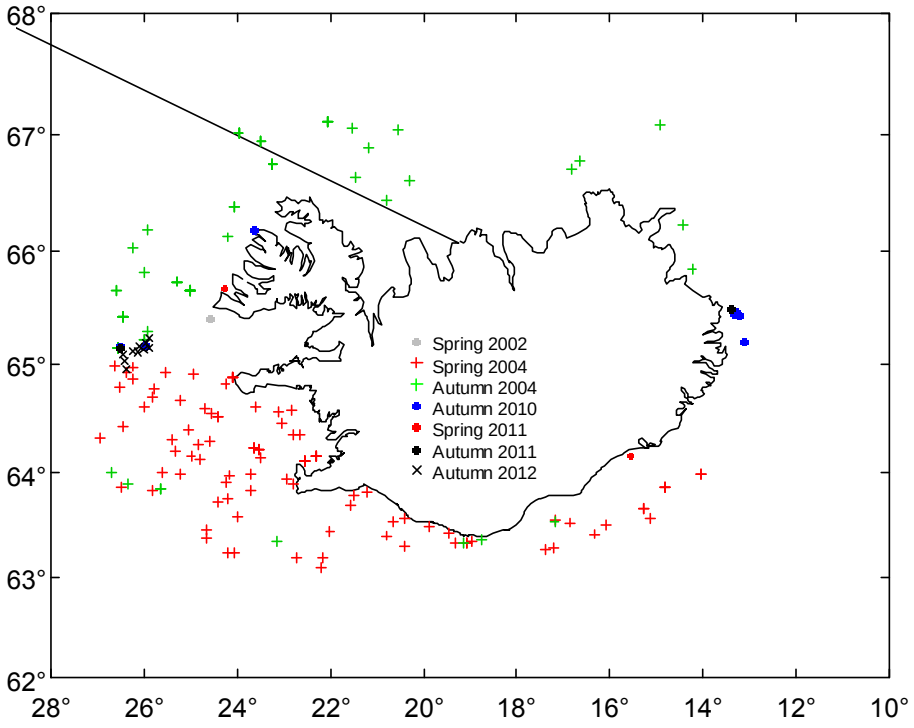

Figure 2. Sampling locations of Atlantic wolffish in Icelandic waters. Samples collected for the purpose of this project were collected in 2010, 2011 and 2012. Additional archived samples collected in 2002 and 2004 were analyzed to assess temporal variation of genetic diversity.

In spring 2002, a total of 100 samples were collected from west of Iceland from one location (Figure 2). In the year 2004 a total of 173 samples were taken; 100 from 79 locations in the annual ground fish survey in spring and 73 samples from 34 locations in annual ground fish survey in autumn (Figure 2). Both surveys are performed annually by MRI. In the year 2011, a total of 400 samples were collected. In spring 2011, 100 samples were collected from the south of Iceland and 100 from the west and in the autumn, 100 samples were taken from

the west of Iceland and 100 from the east (Figure 2). In autumn 2012, a total of 100 samples were collected in a research survey performed by MRI on the main spawning ground of Atlantic wolffish west of Iceland (Figure 2). In addition, 254 genetic samples were collected from tagged Atlantic wolffish (fin clip).

3.2 Growth and maturity

The growth and maturity study showed that Atlantic wolffish west of Iceland grows faster, and matures younger and at a smaller size than those east of Iceland. In addition, the growth model indicates that the growth of Atlantic wolffish decreases with age. In the west area, 6 years old fish grow 5 cm per year, while 17 years old fish grow 0.5 cm (Figure 3). In the east area, 7 years old fish grow 4 cm per year, while 17 years old fish grow 0.5 cm. The growth difference between same age classes between areas, decrease with higher age, it is about 0.6 cm for 7 years old fish and 0.05 cm for 17 years old fish (Figure 3). The cohort specific effect (gc), which is a growth parameter indicating how fast the fish grows, was larger for fish in the west than in the east area (t-test, n = 761, $P < 0.001$; Figure 4).

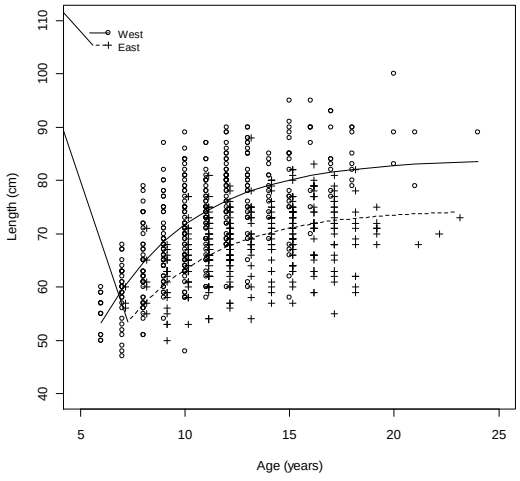

Figure 3. Growth of Atlantic wolffish west and east areas of Iceland. The symbol represents observed values and the lines fitted values from the growth model.

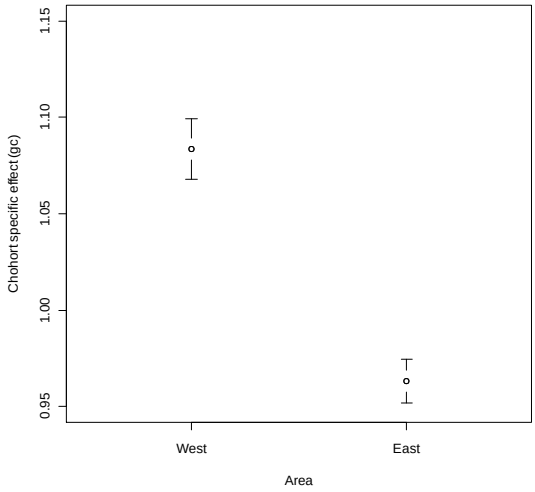

Figure 4. Cohort specific effect (gc) in Atlantic wolffish west and east areas of Iceland. The error bars represents 95 % confidential intervals.

The maturity ogives were different between areas for length (χ^2, df = *1; 244*, P < 0.001) and age (χ^2, df = *1; 244*, P < 0.001; Figure 5).

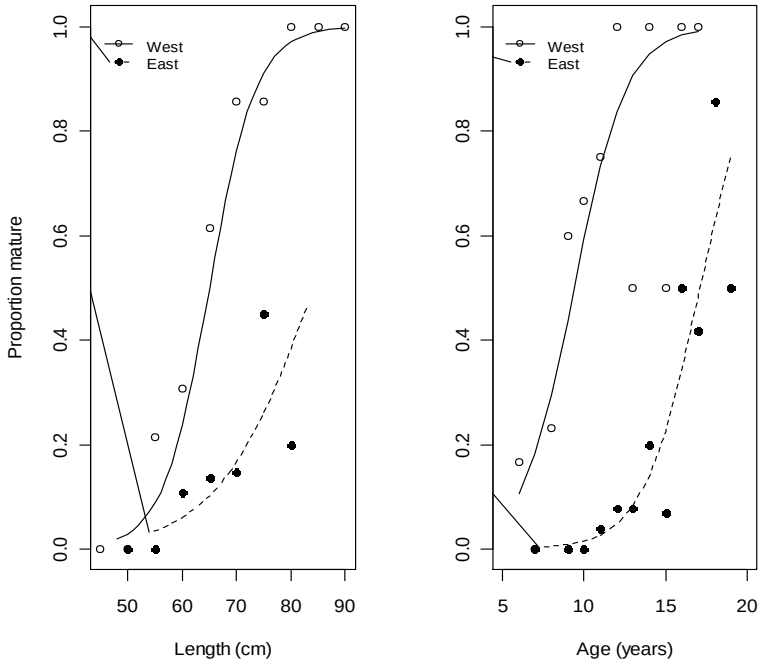

Figure 5. Maturity ogives for length and age for Atlantic wolffish west and east areas of Iceland. The symbols represent observed values and the lines are based on predictive values from logistic regression.

However, the parameter L_{50} or the length when 50 % of the fish are mature was not different between areas (t-test, df = 245, P > 0.05, Table 1). This result can be explained by the large standard error of the L_{50} in the east area, resulting in an inaccurate estimate (Table 1). The parameter $A_{50,}$ or the age where 50 % of the fish are mature, was however different between areas (t-test, df = 243, P < 0.001, Table 1).

Table 1. Estimates ± SE of total length (L_{50}) and age (A_{50}) at 50 % maturity by years. A pseudo-r^2 value is also given.

Area	L_{50}	r^2	*n*	A_{50}	r^2	*n*
West	65.00 ± 2.01	0.39	75	9.40 ± 0.18	0.25	75
East	84.17 ± 23.09	0.06	172	17.10 ± 0.31	0.23	170

3.3 Microsatellite genotyping

Both DNA extraction methods (Agowa and Chelex) were successful and gave good quality DNA. However, the Agowa DNA isolation method was preferred for all samples. DNA was extracted from 770 samples sampled in 2010 and 265 archived samples sampled in 2002 and 2004. In total 35 multiplex mixes were tested before the final four multiplexes were obtained. A total of 16 loci were genotyped with high efficiency for the 770 samples, which were sampled in autumn 2010 and 252 archived samples that were sampled in 2002 and 2004. Comparisons of genotyping results from multiplex systems versus PCRs with single locus gave the same results.

3.4 Population structure of Atlantic wolffish in Icelandic waters

A total of 770 samples collected in autumn 2010 were genotyped (400 samples from the west of Iceland and 370 samples from the east) (Figure 2). All 16 microsatellite loci were highly polymorphic. Genetic diversity assessed as the number of alleles per locus was high, ranging from 6 (Alu7, Alu24 and Alu30) to 27 (Alu11) (data not shown). The expected heterozygosity (H_e) per sample ranged from 0.682 (archived sample of 2004) to 0.696 (west sample collected in 2010) (see Appendix 1 Table 2). Genotypic proportions were not in HWE in 14 out of 176 exact tests before correction for multiple tests (none of them were

significant after correction) and were not attributable to any specific loci or samples. None of the samples collected deviated from HWE (see Appendix 1 Table 2) significantly. Simulations for selection suggested that none of the observed variation detected at the studied microsatellite loci departed significantly from neutral expectations (data not shown).

The overall genetic estimates based on combined archived and contemporary samples did not reveal significant level of differentiation (F_{ST} = -0.00006, p > 0.05, 95% CI: -0.0005–0.0004) and of inbreeding (F_{IS} = -0.00006, p > 0.05, 95% CI: -0.0086–0.0066). This genetic pattern was reflected in the pairwise F_{ST} comparisons of samples as none of the comparisons remained significant after Bonferroni correction (see Appendix 1 Table 3). The pairwise F_{ST} comparisons among weekly temporal samples (sample 5 *vs*. 6; samples 1, 2 and 4) did not reveal any significant temporal variation within the two weeks interval considered. In addition, none of the archived samples were genetically different from the contemporary ones (see Appendix 1 Table 3).

The Bayesian cluster analysis performed on contemporary samples (using location information) confirmed the observed pattern with the pairwise F_{ST} comparisons and showed that the most likely number of K (populations) was 1 (mean Ln $P(D)$±S.D.: K=1, −32692±85; K=2, −39459±709; K=3, −40107±2213 K=4, −42522±7106; K=5, −46172±14426; K=6, −50813±23676; K=7, −42075±5874; K=8, −40390±2483). The inclusion of archived samples did not affect the results (data not shown).

The locus-by-locus AMOVA also confirmed this pattern as the overall among-groups differentiation was not significant between contemporary samples collected in waters east and west of Iceland (see Appendix 1 Table 4), even if two microsatellite loci revealed weak significant differences between groups.

Finally, the genetic diversity indices comparison among regions (eastern vs. western) using permutation tests implemented in FSTAT did not reveal any significant pattern (Eastern: H_O = 0.689, H_E = 0.691, A_R = 9.563; Western: H_O = 0.697, H_E = 0.695, A_R = 9.679, p > 0.05). Genetic differentiation among eastern and western regions was also not significant (F_{ST} = 0.0002, p > 0.05, 95%). In addition, comparison between each region and archived samples using the

same test did not reveal any loss of genetic diversity within the time period investigated (Archived: H_O = 0.690, H_E = 0.690, A_R = 9.608; P > 0.05).

Estimates of effective population size for each region using several methods are listed in Appendix 1 Table 5. The linkage disequilibrium methods (Hill, 1981) led to large 95% confidence intervals for both regions and suggested that N_e was usually higher in the western region than in the eastern one (see Appendix 1 Table 5). The temporal approach (Waples, 1989) also led to very large estimates of N_e for both regions (see Appendix 1 Table 5).

3.5 Genes investigation

The sequencing of the Rhodopsin gene gave 744 bp long sequences for 156 samples. Three SNPs were detected in the Rhodopsin gene sequence and none of the mutations observed (SNPs) induced a change in the amino-acid sequence coded by the gene (synonymous mutation). In addition, these SNPs were observed in a very limited number of individuals, suggesting that the Rhodopsin gene is relatively conserved in Icelandic Atlantic wolffish and cannot be used to asses genetic structure. Only eight individuals out of 156 (5 from the east and 3 from the west) exhibited changes (SNPs) in their sequences compared to the reference sequence. No sequencing results were obtained for the two other genes investigated, e.g. the hemoglobin β and Hsc70 genes. Therefore no other statistical analyses than descriptive approach were performed for these three genes.

3.6 Heterozygosity-Fitness relationships (HFCs)

By using neutral genetic markers, it was investigated whether heterozygosity-fitness correlations (HFCs) could be detected. By using the following groups: fast growing fish from the east, fast growing fish from the west, slow growing fish from the east, slow growing fish from the west, no differences in MLH (ANOVA, $df_{[3,156]}$ F=0.255, P=0.858) or d^2 (ANOVA, $df_{[3,156]}$ F=0.504, P=0.680) were observed among the groups. Combining the fast growing and slow growing fish from the different areas resulted in the same pattern, no differences in the genetic indices could be found between the slow and fast growing fish (MLH: ANOVA, $df_{[1,158]}$ F=0.051, P=0.822; d^2: ANOVA, $df_{[1,158]}$ F=0.327, P=0.568). In addition, for this set-up, no

correlation could be found between the growth rate and the genetic indices (fast versus slow growing fish). Therefore HFCs could not be investigated further.

3.7 Data storage tags

In September 2012, a total of 40 Atlantic wolffish were tagged at Glettingarnes east of Iceland (a similar location to where most of the genetic samples were sampled east of Iceland in 2010) (Figure 2). Among them, 31 fish were tagged with DSTs and T-bar tags and 9 with only T-bar tags (Figure 6). The fish were caught by long line. Fin clip was collected from each fish for the genetic analysis. Then, in late November and in the beginning of December 2012, 394 Atlantic wolffish were tagged at Látragrunn west of Iceland (at same or similar locations where the genetic samples were collected in 2012) (Figure 2). The fish were caught by bottom trawl. Out of the 394, 191 were tagged with DSTs and T-bar tags and 203 with T-bar tags. Fin clip was collected from each fish tagged with DSTs and from 32 fish tagged only with T-bar tags for future genetic projects.

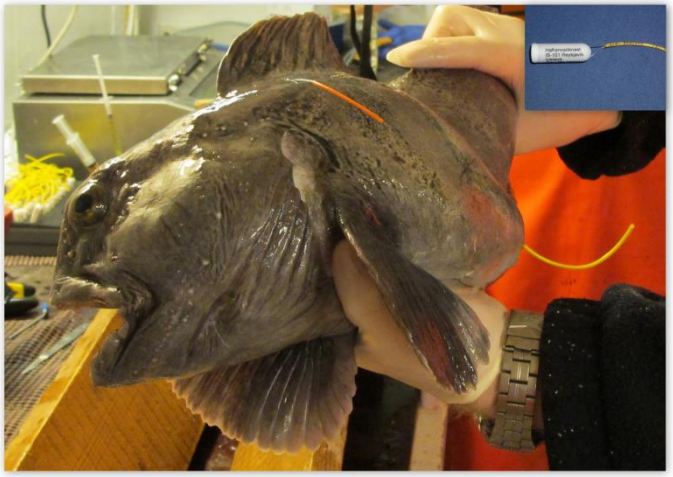

Figure 6. Tagged Atlantic wolffish from Glettingarnesgrunni east of Iceland. The yellow tube is connected to the DSTs tag (see picture in the upper right corner) and goes out of the abdomen of the fish.

4. DISCUSSION

The major aim of this project was to apply state of the art population genetics approaches to estimate the genetic variations within and among each geographically separated fishing area of Atlantic wolffish in Icelandic waters. In this study, SNP study of the Rhodopsin gene and 16 previously developed microsatellite loci were employed to indirectly assess gene flow, e.g. the exchange of genes, among populations of the targeted species located at the west and east of Iceland. The results of the present study revealed that this species is not genetically structured around Iceland. The results are supported by the lack of differences in the genetic diversity indices of the collected samples, a non-significant overall F_{ST}, the temporal stability of replicates both on a weekly and long-temporal scale (see F_{ST} results), and the absence of distinguishable genetic groups during the Bayesian cluster analysis. The lack of genetic structure for this species is discussed in terms of gene flow, recent isolation of populations and effective population size.

In the case of the Atlantic wolffish, one would expect to find reproductively isolated populations due to its peculiar life-cycle, e.g. genetic differences among the investigated geographical samples. Although the species undertakes feeding as well as breeding migration, it has been suggested that adults of Atlantic wolffish are sedentary and do not usually undertake extensive migration (Jónsson, 1982; Nelson and Ross, 1992), therefore limiting gene flow among populations. In addition, the reproductive tactics and the peculiarities of the eggs and larvae are also likely to prevent passive gene flow among populations through dispersal of young stages. The fertilization of the eggs is internal (Pavlov and Mokness, 1995) and eggs are deposited in a nest that the male guards until hatching (Pavlov and Novikov, 1993). The larvae will typically hatch at a size around 20 mm and almost exclusively stay around the nest area until the juveniles become bottom dwelling owing to their large size and negative buoyancy (Bigelow and Schroeder, 1953; Moksness and Pavlov, 1996). Therefore, even though there is an apparent lack of gene flow due to the biology of the species, the observed genetic pattern might be explained by other alternative hypotheses.

One of the most likely explanations for the absence of genetic structure despite the apparent lack of gene flow would be the recent isolation of the west and east wolffish populations in Icelandic waters. Given enough time, drift would typically have led to genetic differentiation of subpopulations in such a species, but marine populations around Iceland tend to be young and to originate from ice-free refugia during the Last Glacial Maximum (LGM) some 20-25 cal. Kyr BP (Coyer et al., 2003; Maggs et al., 2008; Pampoulie et al., 2011a; Pampoulie et al., 2008b). During LGM, most of the Icelandic waters were covered with a 1,500-2,000 m thick ice-cap, which reached the break of the shelf (Ingólfsson et al., 2010; see Fig. 4.1). The ice-cap rapidly collapsed from sea-waters and retreated onto present-day dry land between 14.9-13.9 cal. Kyr BP (Ingólfsson et al., 2010; see Fig. 4.2). The re-colonization of ice-free environment by marine organisms could therefore start at around 13 cal. Kyr BP in Iceland, a time, which might not have been sufficient enough to promote genetic differentiation at neutral markers. The post-glacial history of re-colonization of Icelandic waters has already been suggested to be at the origin of the present-day genetic pattern of commercial fish species such as the Atlantic cod (Pampoulie et al., 2008b; Pampoulie et al., 2008c; Pampoulie et al., 2011b), and has also been suggested to be at the origin of the genetic structure of Atlantic wolffish across the North Atlantic ocean (McCusker and Bentzen, 2010). The recent re-colonization of Icelandic waters by the Atlantic wolffish would have resulted in a typical lack of mutation-drift equilibrium due to recent population expansion, hence the lack of genetic differentiation, a pattern that has been consistent within Icelandic marine populations (Pampoulie et al., 2008b; Pampoulie et al., 2008c; Pampoulie et al., 2011b). Therefore, the present study is fully consistent with genetic studies performed on the genus *Anarhichas* in the North Atlantic suggesting a very limited genetic structure due to recent isolation of populations (McCusker and Bentzen, 2010; McCusker and Bentzen, 2011).

The apparent lack of the genetic structure, and the long-term stability were also reflected in the estimates of population sizes (see N_e in Table 5 in Appendix 1). Both the linkage disequilibrium and the temporal methods revealed that the lower estimated N_e was around or higher than 2000 for the Eastern and Western populations, even when one panmictic

population was considered. Only the archived samples exhibited lower estimates ($642 < N_e < 1253$). However, such high estimates of N_e are commonly interpreted as evidence of conserved evolutionary potential of exploited populations (Franklin, 1980; Poulsen et al., 2006). In addition, the comparison of allele frequencies and the comparison of genetic diversity indices among contemporary and archived samples, revealed a lack of genetic differences and did not bring evidence for any loss of genetic diversity despite the previously mentioned decline of population size in Icelandic populations of Atlantic wolffish (Anon, 2010). The present study is therefore in line with several studies performed on neutral genetic diversity and showing a stable temporal pattern despite drastic stocks' overexploitation (Cuveliers et al., 2011; Hauser et al. 2002; Pujolar et al., 2011; Ruzzante et al., 2001). However, detecting a loss of genetic diversity at neutral genetic markers might not be suitable to assess the potential loss of adaptive genetic variation due to fisheries as mentioned in the case of the Icelandic cod (Jakobsdóttir et al., 2011).

In this study Atlantic wolffish grew faster and matured younger and at a smaller size in the warm water west of Iceland than in the colder one east of Iceland. Atlantic wolffish has been previously reported to grow faster in warmer than in colder waters (Barsukov, 1959; Gjøsæter et al., 1990; Gunnarsson et al., 2006; Hansen, 1992; Liao and Lucas, 2000; Nelson and Ross, 1992; Pavlov and Novikov, 1993). However, the present results showed that fast growing female Atlantic wolffish mature younger and at a smaller size than the slow growing ones (Figure 4-6), which is in accordance with Gunnarsson *et al.* (2006) and life-history theory, which predicts that the relationship between growth and maturity involved either fast growing and early maturation, or slow growing and delayed maturation (Lambert et al., 2003). No differences in the genetic indices could be found between the slow and fast growing fish. In addition, no correlation could be found between the growth rate and the genetic indices (fast versus slow growing fish).

The Atlantic wolffish is currently managed as a single fishing unit in Icelandic waters, and although the genetic approach developed during this study does not suggest the presence of reproductively isolated populations, we recommend that biological parameters, such as variability in mean size, age at maturity and growth pattern, which vary between Western

and Eastern populations, should be taken into account for future management advice as already stated (Gunnarsson et al., 2006).

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APPENDIX 1

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Genetic structure of the Atlantic wolffish (*Anarhichas lupus* L.) at Icelandic fishing grounds: another evidence of panmixia in Iceland?

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The stock structure of the Atlantic wolffish was investigated at Icelandic fishing grounds, using 16 microsatellite loci. Despite the potential of the Atlantic wolffish to exhibit genetic structure (lack of eggs/larval dispersal and adults are sedentary), none of the genetic tests applied in this study detected significant genetic differentiation among the contemporary samples as well as among the contemporary and archived samples. The results of this study therefore suggested a lack of genetic structure among the populations of Atlantic wolffish in Icelandic waters and temporal stability over a period of ~10 years. These results are discussed in terms of biological characteristics of Atlantic wolffish, recent isolation of populations, and their application to sustainable fisheries management issues.

Keywords: Atlantic wolffish, gene flow, last glacial maximum, microsatellite loci, temporal stability.

Introduction

The Atlantic wolffish *Anarhichas lupus* (Linnaeus, 1758) is widely distributed across the North Atlantic and is an important commercial fish species. Like many other marine fishery resources, the abundance of the Atlantic wolffish has drastically declined over the last decades, especially in the Northwest Atlantic where it has been listed by the Canadian Species at Risk Act (SARA) as a species of "special concern" (McCusker *et al.*, 2008). Habitat destruction by bottom trawl and bycatch and recreational fishing activities have been mentioned as possible reasons for the decline in the species (Collie *et al.*, 2000). However, despite its status of endangered species, very few biological studies exist for the Atlantic wolffish, although length and age at maturity as well as growth and fecundity have been studied in various environments (Barsukov, 1959; Jónsson, 1982; Templeman, 1986; Nelson and Ross, 1992; Pavlov and Novikov, 1993; Liao and Lucas, 2000).

Available biological data suggest life-history trait (LHT) differences between Atlantic wolffish collected at the east and the west of Iceland (Gunnarsson *et al.*, 2006). Fish collected in the west in warmer water tend to grow faster and to mature at an earlier age and at a smaller size, than fish collected in the cold water in the east (Gunnarsson *et al.*, 2006). In addition, tagging experiments using anchor and alcathene tags have been performed from 1966 to 1975 to investigate the migration pattern of the species and showed that the Atlantic wolffish exhibits spawning-site fidelity and migration to feeding grounds of <100 miles in Icelandic waters (Jónsson, 1982).

Data from the fisheries of Atlantic wolffish in the North Atlantic date back to the 1950s with landings averaging 20 000 to 40 000 t. In the year 2000, the catches averaged 40 000 t within the whole North Atlantic, but since then it has been constantly decreasing, with catches dropping to 23 000 t in 2008 (FAO, 2011). In Icelandic waters, annual landings averaged 19 000 t from the 1950s to the 1960s, and subsequently declined to 11 000 to 12 000 t in the 1970s–1980s (Figure 1). In 1977, foreign ships stopped fishing in Icelandic grounds, which resulted in a slight decline in catches until 1985 (~10 000 t). From 1986 to 1992, the long-line effort increased again and the catches averaged 15 000 t year^{-1}. Since 1999, the catches have been steady and were on average ~15 500 t (Anon., 2010). However, during this period, increased catch efforts using bottom trawls at one of the main spawning locations (west of Iceland) have raised concern about the recruitment of Atlantic wolffish in Icelandic waters. In 2010, the area of this location, which was closed during spawning time (from 15 September to the end of April), was therefore increased from 500 to 1000 km^2.

Figure 1. Annual landings of *A. lupus* (solid line) and fishable stock size (dashed line) in Iceland. The fishable stock size is based on the Bormicon model. Recruitment index (dotted line) represents the number of Atlantic wolffish at the length between 19 and 41 cm (in millions) and is based on data collected from the annual groundfish survey performed in March by the Marine Research Institute of Iceland.

Although genetic structure has been intensively studied in several important commercial species, e.g. because of the drastic decline in their stock, there is an obvious lack of genetic structure studies in the Atlantic wolffish. A recent study based on microsatellite loci revealed a weak genetic structure of the Atlantic wolffish from Western Greenland and Iceland to the Barents Sea (McCusker and Bentzen, 2010a). The apparent lack of genetic structure within these geographical locations was explained by a possible recent recolonization of these waters after the last glacial maximum from a refugia area located in the Rockall Bank (McCusker and Bentzen, 2010a).

For decades, marine species have been thought to be genetically homogenous due to their extensive eggs/larval dispersal and the obvious lack of barriers in the marine environment; thoughts that have been largely challenged in recent years (Ruzzante *et al.*, 1996; Lage *et al.*, 2004; Bekkevold *et al.*, 2005; Jørgensen *et al.*, 2005; Pampoulie *et al.*, 2008b). For the Atlantic wolffish, one would expect to find reproductively isolated populations due to its peculiar life cycle and to the absence of long-distance migrations of adults. In addition, the reproductive tactics and the peculiarities of the eggs and larvae are likely to prevent passive gene flow among populations through dispersal of young stages. The fertilization of the eggs is internal (Pavlov and Moksness, 1995) and eggs are deposited in a nest that the male guards until hatching (Pavlov and Novikov, 1993). The larvae will typically hatch at a size around 20 mm and almost exclusively stay around the nest area until the juveniles become bottom dwelling owing to their large size and negative buoyancy (Bigelow and Schroeder, 1953; Moksness and Pavlov, 1996).

In Icelandic waters, the strong variability of LHTs previously observed between samples collected at the east and the west Icelandic regions (Gunnarsson *et al.*, 2006), as well as the biological characteristics of the species described above fuelled our interest in testing whether or not population genetic approaches would reveal:

(i) reproductive isolation of these potentially separated fishing units (east vs. west) and

(ii) a loss of genetic variability due to the decline in the population and recruitment.

To achieve our goals, contemporary as well as archived genetic samples were genotyped for 16 microsatellite loci, shedding light into contemporary genetic structure as well as possible temporal variation.

Material and methods

Sampling areas and protocol

In all, 770 individuals were collected at two spawning grounds of the Atlantic wolffish, located the east and the west of Iceland, during fisheries (commercial catches) in autumn of 2010 (Figure 2; Table 1). Three of eight samples were collected at the main spawning ground (west of Iceland, samples 5, 6, and 8), which is usually closed for fisheries during spawning time (from 15 September to the end of April), except in 2010 (the area was closed from 8 October to the end of April because of the extension of the area). Since biological data suggest that the arrival of mature Atlantic wolffish at spawning grounds occurred in successive waves, a temporal sampling scheme was developed at the two spawning grounds, e.g. samples were taken in August and later in September 2010 (Table 1; Figure 2). In addition, three archived samples collected in 2002 and 2004 were also genotyped for temporal stability analyses (Table 1, archived samples A1, A2, and A3; $n = 240$). Two of these samples (A2 and A3) were already used in a previous large-scale analysis of Atlantic wolffish genetic structure across the North Atlantic (McCusker and Bentzen, 2010a).

Muscle samples were collected from each individual and conserved in 99% ethanol. Samples were genotyped at 16 microsatellite loci, namely *Alu7*, *Alu9*, *Alu10*, *Alu11*, *Alu14*, *Alu21*, *Alu22*, *Alu23*, *Alu24*, *Alu25*, *Alu26*, *Alu27*, *Alu28*, *Alu29*, *Alu30*, and *Alu31* (McCusker *et al.*, 2008). DNA was isolated using Agowa mag Midi DNA Isolation Kit (Agowa GmbH). Polymerase chain reactions (PCRs) were performed in a 10-µl volume containing 2 µl of DNA, 200 µM of each dNTP, 1× Teg buffer (100 mM Tris–HCl, pH 8.8; 500 mM KCl; 15 mM $MgCl_2$; 1% Triton X-100), 0.9 U Teg polymerase (Matis, Taq comparable; see Ólafsson *et al.*, 2010), 0.0075–0.10 µl (100 µM) of the labelled forward primers, and the same amount of reverse primers fitted with a GTTTCTT PIG tail (Brownstein *et al.*, 1996). The 16 microsatellite loci were run in four multiplex systems: multiplex 1 (ML1): *Alu21*, *Alu24*, *Alu25*, *Alu26*, and *Alu29*; ML2: *Alu7*, *Alu10*, and *Alu11* (*Alu7* PCRs were performed separately); ML3: *Alu22*, *Alu27*, *Alu28*, *Alu30*, and *Alu31*; ML4: *Alu9*, *Alu14*, and *Alu23* (*Alu9* PCRs were performed separately).

PCRs were performed on a Tetrad2 Peltier (Bio-Rad) thermal cycler as follows: an initial denaturation step of 3 min at 94°C followed by 30 cycles of 30 s at 94°C, 50 s at 58°C, 50 s at 72°C, and a final elongation step of 7 min at 72°C. PCR products were analysed on an ABI PRISM 3730 sequencer using the GeneScan-500 LIZ size standard and genotyped with GeneMapper 4.0 (Applied Biosystems).

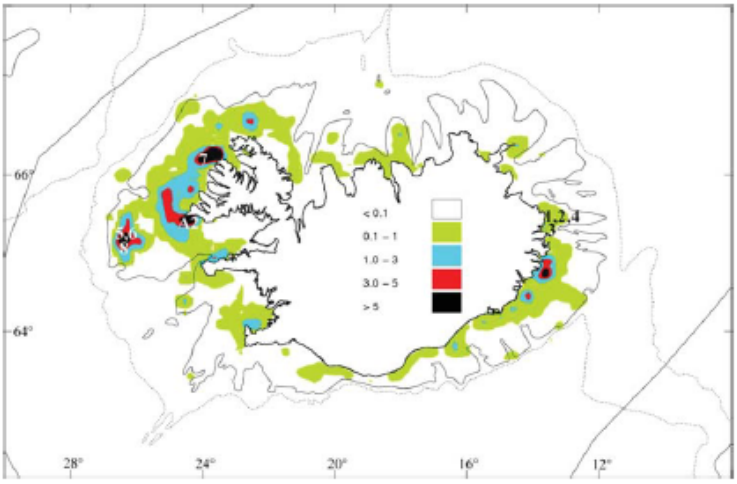

Figure 2. Sampling location of *A. lupus*, in Icelandic waters. Numbers refer to the samples depicted in Table 1 and fishing areas are indicated by coloured areas. The scale indicates the density of catches in 2009 (all gears combined, t nm^{-2}). The archived samples collected during autumn (A1 and A3) were not presented in the figure as they were collected during feeding time and not at a single geographical location.

Genetic analyses

As the conclusion drawn from microsatellite loci strongly depends on their neutrality, the coalescent-based simulation methods of Beaumont and Nichols (1996) implemented in the software LOSITAN were applied (Antao *et al.*, 2008). The software calculated F_{ST} values and heterozygosity for each locus according to Weir and Cockerham (1984) and expected F_{ST} values for each locus weighted by their heterozygosity. Coalescent simulations were then performed using samples of the same size as the observed samples and assuming an island model of 100 islands. One hundred thousand independent loci were generated using the infinite allele and stepwise mutation models, respectively. Simulated distribution of F_{ST} values conditional on heterozygosity under a neutral model are thus obtained and compared with the observed F_{ST} values to identify potential outlier loci.

Genetic diversity of each sample (archived and contemporary) was evaluated using allele frequencies, observed (H_o) and unbiased expected heterozygosity (H_e) calculated in the GENEPOP'007 (Rousset, 2008). Deviations from Hardy–Weinberg expectation (HWE) were tested using the inbreeding coefficient F_{IS} (Weir and Cockerham, 1984) implemented in GENEPOP and significance assessed with exact tests. Genetic differentiation was estimated using theta estimates (θ; Weir and Cockerham, 1984) implemented in GENEPOP, and significance was assessed using allelic and genotypic frequency homogeneity tests (5000 permutations). The significance levels were adjusted by a simple Bonferroni correction (Rice, 1989) when multiple tests were applied.

STRUCTURE 2.3.2 (Pritchard *et al.*, 2000) was used to assess the potential number of populations within our contemporary samples. Due to the very low genetic differentiation level detected (see the "Results" section), the admixture model with the LOCPRIOR setting was used, which considers location information. This recently developed method (Hubisz *et al.*, 2009) has been suggested to perform better than the traditional STRUCTURE methods when the genetic structure is weak or when the number of loci is low (<20). The model was run with a "burn-in" period of 300 000 iterations and 600 000 Markov chain Monte Carlo iterations. The potential number of populations (K) varied from 1 to 8 and was tested with five independent analyses for each K. Then, the archived samples were incorporated in the analysis.

As previously published biological information suggested differences between the eastern and western populations in Iceland (Gunnarsson *et al.*, 2006), additional analyses based on the possible existence of these two genetic groups were performed, by only using the contemporary samples. First, the program FSTAT (Goudet, 1995) was used to assess potential differences of genetic diversity indices such as allele richness and observed and expected heterozygosity. Then, a locus-by-locus hierarchical analysis of molecular (AMOVA) variance using the program Arlequin was performed (Excoffier *et al.*, 2005). Because previous biological investigation suggested a drastic reduction in population size (Anon., 2010; Figure 1), the estimation of the effective population size (N_e) of Atlantic wolffish population was done by using the linkage disequilibrium (Hill, 1981) and the temporal methods of Waples (1989) implemented in NEESTIMATOR (Peel *et al.*, 2004). For the temporal approach, all the archived samples from all regions were combined and used as a reference point, both for the samples collected at the western and eastern regions. N_e estimates were also calculated for one single panmictic population of Atlantic wolffish. The archived samples were then only used for the temporal methods (Waples, 1989).

Results

Biological and geographical information of the collected samples are presented in Table 1.

Table 1. Sampling area and information (coordinates, depth range, sampling dates), sample size, and size distribution (mean standard length in mm, standard deviation s.d., and range) for 11 samples of *A. lupus*.

Sampling identifier	1	2	3	4	5	6	7	8	A1	A2	A3
Coordinates	65°28.00′N 13°19.00′W	65°27.00′N 13°21.00′W	65°12.03′N 13°21.00′W	65°26.50′N 13°14.00′W	65°10.00′N 26°00.00′W	65°10.00′N 26°30.00′W	66°11.00′N 23°28.00′W	65°11.00′N 26°00.00′W	n.a.	65°25.00′N 24°33.00′W	n.a.
Depth (m)	75	66	82	82	156	156	46	156	n.a.	52	n.a.
Sampling dates	30 August 2010	31 August 2010	13 September 2010	13 September 2010	18 August 2010	25 August 2010	13 September 2010	21 September 2010	18 October 2004	19 April 2002	11 March 2004
Sample size	100	100	70	100	100	100	100	100	67	95	78[a]
Length (mm)											
Mean	68.72	69.97	66.39	67.98	76.78	74.84	61.33	74.46	44.86	66.91	50.05
s.d.	5.78	6.38	6.42	6.46	8.95	8.81	7.55	8.30	18.71	9.12	19.61
Range	53–82	53–83	50–88	53–82	48–94	57–95	47–87	58–100	16–85	46–91	11–110

n.a. depicts information that could not be collected from the archived samples. Dates are depicted in day/month/year.
[a]In all, 96 individuals were genotyped and only 78 were successfully screened for more than three-fourths of the loci investigated.

All studied microsatellite loci were highly polymorphic. Genetic diversity assessed as the number of alleles per locus was high, ranging from 6 (*Alu*7, *Alu*24, and *Alu*30) to 27 (*Alu*11; data not shown). The expected heterozygosity (H_e) per sample ranged from 0.682 (sample 11) to 0.696 (sample 8; Table 2). Genotypic proportions were out of HWE in 14 of 176 exact tests before correction for multiple tests (none of them were significant after correction) and were not attributable to any specific loci or samples. None of the samples collected deviated from HWE (Table 2) significantly. Simulations for selection suggested that none of the observed variation detected at the studied microsatellite loci departed significantly from neutral expectations (data not shown).

The overall genetic estimates based on combined archived and contemporary samples did not reveal significant F_{ST} ($F_{ST} = -0.00006$, $p > 0.05$, 95% CI: −0.0005 to 0.0004) and F_{IS} values ($F_{IS} = -0.00006$, $p > 0.05$, 95% CI: −0.0086 to 0.0066). This genetic pattern was reflected in the pairwise F_{ST} comparisons of samples as none of the comparisons remained significant after Bonferroni correction (Table 3). The pairwise F_{ST} comparisons among samples collected in August and September within a spawning ground were therefore not different which suggests the absence of temporal variation for the contemporary timeframe investigated. In addition, none of the archived samples were genetically different from the contemporary ones (Table 3).

The Bayesian cluster analysis performed on contemporary samples (using location information) confirmed the observed pattern with the pairwise F_{ST} comparisons and showed that the most likely number of K was 1 (mean Ln $P(D) \pm$ s.d.: $K=1$, $-32\,692 \pm 85$; $K=2$, $-39\,459 \pm 709$; $K=3$, $-40\,107 \pm 2213$ $K=4$, $-42\,522 \pm 7106$; $K=5$, $-46\,172 \pm 14\,426$; $K=6$, $-50\,813 \pm 23\,676$; $K=7$, $-42\,075 \pm 5874$; $K=8$, -40390 ± 2483). The inclusion of archived samples did not affect the results (data not shown).

The locus-by-locus AMOVA also confirmed this pattern as the overall among-groups differentiation was not significant between contemporary samples collected at the east and the west of Icelandic waters (Table 4), even if two microsatellite loci revealed weak significant differences between groups.

Finally, the genetic diversity indices comparison among regions (eastern vs. western) using permutation tests implemented in FSTAT did not reveal any significant pattern (Eastern: $H_O = 0.689$, $H_E = 0.691$, $A_R = 9.563$; Western: $H_O = 0.697$, $H_E = 0.695$, $A_R = 9.679$, $p > 0.05$). Genetic differentiation among eastern and western regions was also not significant ($F_{ST} = 0.0002$, $p > 0.05$, 95%). In addition, comparison between each region and archived samples using the same test did not reveal any loss of genetic diversity within the period investigated (Archived: $H_O = 0.690$, $H_E = 0.690$, $A_R = 9.608$; $P > 0.05$).

Estimates of effective population size for each region using several methods are listed in Table 5. The linkage disequilibrium methods (Hill, 1981) led to large 95% CIs for both regions and suggested that N_e was usually higher in the western region than in the eastern one (Table 5). The temporal approach (Waples, 1989) also led to very large estimates of N_e (Table 5).

Discussion

The present study aimed to assess the genetic structure of the Atlantic wolffish, *A. lupus*, in Icelandic waters using microsatellite loci. The obtained results revealed that this species is not genetically structured around Iceland at neutral markers. The results are supported by the lack of differences in the genetic diversity indices

Table 2. Expected (H_e) and observed (H_o) heterozygosities, number of alleles (N_a) for 16 microsatellite loci in 11 samples of Atlantic *A. lupus*.

	1			2			3			4			5			6		
Locus	H_e	H_o	N_a	H_e	H_o	N_a	H_e	H_o	N_a	H_e	H_o	N_a	H_e	H_o	N_a	H_e	H_o	N_a
Alu7	0.686	0.710	6	0.655	**0.667**	6	0.655	0.686	5	0.702	0.750	6	0.679	**0.650**	6	0.631	**0.620**	6
Alu9	0.703	0.729	7	0.721	0.748	7	0.754	0.750	7	0.700	0.7368	7	0.770	0.802	8	0.722	0.745	7
Alu10	0.711	0.640	7	0.673	0.670	7	0.711	0.714	8	0.720	0.750	7	0.718	**0.730**	8	0.684	**0.640**	7
Alu11	0.829	**0.810**	21	0.853	0.880	20	0.838	0.843	17	0.838	0.810	14	0.871	0.850	20	0.850	0.891	19
Alu14	0.765	0.707	14	0.823	0.850	14	0.779	0.800	13	0.769	0.820	13	0.807	0.800	14	0.765	0.790	15
Alu21	0.700	0.780	9	0.733	0.760	20	0.710	0.771	16	0.711	0.640	10	0.730	0.660	10	0.727	0.810	9
Alu22	0.429	0.420	6	0.428	0.412	9	0.489	0.471	7	0.414	0.380	7	0.427	0.410	6	0.424	0.465	5
Alu23	0.582	0.630	7	0.583	**0.560**	8	0.594	0.529	6	0.586	0.580	6	0.615	0.650	6	0.586	0.680	6
Alu24	0.661	0.750	5	0.628	0.620	4	0.648	0.620	4	0.663	0.604	4	0.670	0.680	5	0.669	0.744	5
Alu25	0.784	0.790	12	0.780	0.790	10	0.760	0.757	10	0.762	0.740	8	0.769	0.760	9	0.791	0.810	11
Alu26	0.841	0.810	15	0.859	0.887	16	0.846	0.814	17	0.834	0.761	14	0.854	0.930	16	0.863	0.895	20
Alu27	0.822	0.818	20	0.844	0.810	18	0.795	0.771	17	0.868	0.887	20	0.814	0.810	18	0.854	0.850	20
Alu28	0.900	0.890	16	0.904	0.920	16	0.898	0.871	15	0.913	0.920	15	0.912	0.910	17	0.880	0.850	17
Alu29	0.600	0.556	6	0.506	0.536	7	0.540	0.544	5	0.554	0.568	6	0.524	0.494	8	0.532	0.604	7
Alu30	0.174	0.170	3	0.124	0.130	5	0.134	0.143	4	0.141	0.140	4	0.114	0.120	4	0.164	0.180	2
Alu31	0.830	0.889	12	0.841	0.860	13	0.842	0.886	11	0.841	0.840	13	0.839	0.760	11	0.831	0.849	13
Overall	0.689	0.694	9.78	0.685	0.694	9.83	0.687	0.685	9.46	0.689	0.683	9.19	0.695	0.689	9.73	0.687	0.714	9.77

	7			8			A1			A2			A3		
	H_e	H_o	N_a	H_e	H_o	N_a	H_e	H_o	N_a	H_e	H_o	N_a	H_e	H_o	N_a
Alu7	0.670	0.590	6	0.714	0.700	6	0.661	0.667	6	0.643	0.653	6	0.680	0.597	6
Alu9	0.743	0.806	7	0.733	0.750	7	0.722	0.723	7	0.762	0.731	8	0.751	0.776	8
Alu10	0.692	0.760	8	0.711	0.720	8	0.739	0.671	8	0.716	**0.842**	8	0.718	0.667	7
Alu11	0.857	0.790	17	0.857	0.904	18	0.839	0.879	19	0.817	**0.800**	18	0.809	0.805	16
Alu14	0.793	0.780	13	0.816	0.840	16	0.802	0.821	13	0.774	0.800	12	0.765	0.769	14
Alu21	0.697	0.690	7	0.719	0.770	8	0.751	0.758	11	0.687	0.670	7	0.721	0.718	8
Alu22	0.365	0.340	7	0.413	**0.360**	8	0.407	0.418	7	0.440	0.451	7	0.361	**0.338**	7
Alu23	0.616	0.590	8	0.606	0.570	7	0.599	0.537	6	0.603	0.632	5	0.579	0.705	4
Alu24	0.651	0.700	5	0.674	0.670	5	0.646	0.692	5	0.655	0.733	4	0.649	0.653	4
Alu25	0.762	0.750	11	0.776	**0.800**	11	0.771	0.716	10	0.764	0.716	10	0.749	0.692	10
Alu26	0.843	0.880	17	0.843	0.897	15	0.842	**0.862**	16	0.851	0.843	17	0.836	0.878	15
Alu27	0.831	0.860	19	0.836	0.810	20	0.831	0.773	19	0.826	0.898	20	0.819	0.816	19
Alu28	0.900	0.890	16	0.909	**0.880**	16	0.895	0.925	14	0.904	**0.913**	15	0.912	0.936	16
Alu29	0.550	0.540	6	0.537	0.573	8	0.567	0.578	6	0.536	0.578	5	0.583	0.600	6
Alu30	0.165	0.180	4	0.141	0.130	4	0.180	0.197	3	0.102	0.106	4	0.147	0.158	3
Alu31	0.852	0.870	11	0.853	0.810	12	0.844	**0.806**	12	0.848	0.842	14	0.827	0.829	11
Overall	0.687	0.689	9.39	0.696	0.699	9.83	0.694	0.689	10.13	0.683	0.696	10.00	0.682	0.684	9.63

Emboldened values indicate samples that were not in HWE (exact test, $p < 0.05$), none remained significant after correction for multiple tests ($\alpha = 0.05/176 = 0.00025$).

Table 3. Pairwise F_{ST} (above diagonal) and values of p (below diagonal) among the 11 samples of *A. lupus* based on allele frequencies.

	1	2	3	4	5	6	7	8	A1	A2	A3
1	0	0.0024	0.0001	−0.0002	0.0012	0.0004	0.0004	−0.0006	−0.0016	0.0010	−0.0006
2	0.367	0	0.001	0.0035	0.0026	0.0022	0.0023	0.0005	0.0025	0.0015	0.0012
3	0.630	0.1189	0	0.0011	−0.0006	0.0003	0.0004	−0.0008	−0.0015	−0.0016	−0.0004
4	0.494	**0.001**	**0.031**	0	−0.0016	−0.0001	−0.0005	−0.0013	−0.0009	−0.0002	−0.0008
5	0.113	0.069	0.380	0.286	0	−0.0005	−0.0011	−0.0018	−0.0005	−0.0008	−0.0011
6	0.338	**0.029**	0.536	0.228	0.725	0	−0.0009	−0.0001	−0.0004	−0.0004	0.0003
7	0.250	**0.024**	0.368	0.358	0.609	0.736	0	−0.0017	−0.0014	−0.0014	−0.0005
8	0.836	0.289	0.317	0.206	0.713	0.247	0.891	0	−0.0017	−0.0011	−0.0012
9	0.838	0.104	0.752	0.537	0.363	0.544	0.926	0.738	0	−0.0005	−0.0001
10	0.453	0.326	0.656	0.666	0.304	0.304	0.983	0.749	0.737	0	−0.0010
11	0.772	0.657	0.225	0.789	0.280	0.393	0.295	0.463	0.402	0.762	0

Emboldened values differ significantly from zero (Fisher's exact test, $p < 0.05$). None of the comparisons remained significant after Bonferroni correction ($\alpha = 0.05/55 = 0.0009$).

of the collected samples, a non-significant overall F_{ST}, the temporal stability of the genetic pattern detected (see F_{ST} results), and the absence of distinguishable genetic groups during the Bayesian cluster analysis. The lack of genetic structure for this species is discussed in terms of gene flow, recent isolation of populations, and effective population size.

Table 4. Locus-by-locus AMOVA variance among the east and the west group of *A. lupus* (AEW, F_{CT}), among samples within group (ASWG, F_{SC}), and within samples (WS, F_{ST}).

Locus	%AEW	%ASWG	%WS	F_{CT}	F_{SC}	F_{ST}
Alu7	−0.0795	0.3392	100.0964	−0.0002	−0.0010	−0.0008
Alu9	0.3841	0.3675	99.7652	−0.0015	0.0024	0.0038*
Alu10	0.1169	0.3529	99.8853	0.0000	0.0012	0.0012
Alu11	0.0623	0.4269	100.0156	−0.0008	−0.0002	0.0006
Alu14	0.0269	0.3971	99.6924	0.0028*	0.0031*	0.0003
Alu21	−0.0728	0.3600	100.1300	−0.0006	−0.0013	−0.0007
Alu22	0.0005	0.2116	100.1475	−0.0015	−0.0015	0.0000
Alu23	0.0149	0.2996	100.2020	−0.0022	−0.0020	0.0002
Alu24	−0.0060	0.3309	100.0192	−0.0001	−0.0002	−0.0001
Alu25	−0.0074	0.3888	99.9614	0.0005	0.0004	−0.0001
Alu26	0.0010	0.4263	100.0084	−0.0001	−0.0001	0.0000
Alu27	−0.0429	0.4193	99.9489	0.0009	0.0005	−0.0004
Alu28	0.0145	0.4545	99.9026	0.0008	0.0010	0.0001
Alu29	−0.0214	0.2729	99.9771	0.0004	0.0002	−0.0002
Alu30	−0.0262	0.0730	100.1485	−0.0012	−0.0015	−0.0003
Alu31	−0.1529	0.4228	99.9284	0.0022*	0.0007	−0.0015
Total	0.0133	0.3465	99.9893	0.0002	0.0002	0.0003

This analysis was performed on contemporary samples only. The loci that showed significant group comparison are indicated emboldened.
*$p < 0.05$.

Table 5. Estimates of effective population sizes (N_e) using linkage disequilibrium (Hill, 1981) and temporal methods (Waples, 1989).

Population	Method applied	Median	95% confidence interval
East	Hill (1981)	1 938	1 421–2 933
	Waples (1989)	∞	469–∞
West	Hill (1981)	3 158	2 109–6 110
	Waples (1989)	∞	1 363–∞
Archived East	Hill (1981)	642	479–955
Archived West	Hill (1981)	1 253	589–∞
One population	Hill (1981)	1 817	1 574–2 132
	Waples (1989)	∞	1 420–∞

Indeed, despite the apparent lack of gene flow due to the biology of the species, the observed genetic pattern might be explained by at least two alternative hypotheses detailed below.

(i) One of the most likely explanations for the absence of genetic structure despite the apparent lack of gene flow would be the recent isolation of the west and east Atlantic wolffish populations in Iceland. Given enough time, drift would typically have led to genetic differentiation of subpopulations in such a species, but marine populations around Iceland tend to be young and to originate from ice-free refugia during the last glacial maximum (LGM) some 20–25 Kyr BP (Coyer *et al.*, 2003; Maggs *et al.*, 2008; Pampoulie *et al.*, 2008a, 2011). During LGM, most of the Icelandic waters were covered with an ice-cap, which reached the break of the shelf and was ca. 1500–2000 m thick (Ingólfsson *et al.*, 2009; see Figure 4.1). The ice-cap rapidly collapsed from the seawater and retreated onto present-day dry land between 14.9 and 13.9 Kyr BP (Ingólfsson *et al.*, 2009; see Figure 4.2). The recolonization of ice-free environment by marine organisms could therefore start at around 13 Kyr BP in Iceland, a time, which might not have been sufficient to promote genetic differentiation at neutral markers (Slatkin, 1987; Cavalli-Sforza, 1998). In fact, for marine species such as the Atlantic wolffish with large effective population size and long generation time, drift might not have promoted genetic differentiation among isolated populations since the last glacial event (see Slatkin, 1987 and Pampoulie *et al.*, 2008a, b, 2011, for Icelandic cases). The post-glacial history of recolonization of Icelandic waters has already been suggested to be responsible for the weak genetic differentiation among populations of several commercial fish species in Iceland (Pampoulie *et al.*, 2008a, b, 2011) and for the weak genetic structure of Atlantic wolffish across the North Atlantic ocean (McCusker and Bentzen, 2010a). The recent recolonization of Icelandic waters by the Atlantic wolffish would have resulted in a typical lack of mutation-drift equilibrium due to recent population expansion, hence the lack of genetic differentiation. Therefore, the present study is fully consistent with genetic studies performed on the genus *Anarhichas* in the North Atlantic, suggesting a very limited genetic structure due to recent isolation of populations (McCusker and Bentzen, 2010a, b, 2011).

(ii) A second possible explanation for the genetic pattern detected might be the observed large effective population size (N_e) of the studied species. Indeed, the apparent lack of the genetic structure and the long-term stability were also reflected in the estimates of population sizes (see N_e in Table 5). Both the linkage disequilibrium and the temporal methods revealed that the lower estimated N_e was around or higher than 2000 for the Eastern and Western populations, even when one panmictic population was considered. Only the archived samples exhibited lower estimates ($642 < N_e < 1253$). As the magnitude of genetic drift crucially depends on N_e, such large effective population sizes for the Atlantic wolffish might not have favoured genetic differentiation in Icelandic waters (Slatkin, 1987).

Such high estimates of N_e are also commonly interpreted as evidence of conserved evolutionary potential of exploited populations (Franklin, 1980; Poulsen *et al.*, 2006). In addition, the comparison of allele frequencies and the comparison of genetic diversity indices among contemporary and archived samples revealed a lack of genetic differences and did not bring evidence for any loss of genetic diversity despite the previously mentioned decline in population size in Icelandic populations of Atlantic wolffish (Anon., 2010). The present study is therefore in line with several studies performed on neutral genetic diversity and showing a stable temporal pattern despite drastic stocks' overexploitation (Ruzzante *et al.*, 2001; Hauser *et al.*, 2002; Cuveliers *et al.*, 2011; Pujolar *et al.*, 2011). However, detecting a loss of genetic diversity at neutral genetic markers might not be appropriate to assess the potential loss of adaptive genetic variation due to fisheries as mentioned for the Icelandic cod (Jakobsdóttir *et al.*, 2011).

The Atlantic wolffish is currently managed as a single fishing unit in Icelandic waters, and although this study does not suggest the presence of reproductively isolated populations, we recommend that biological parameters, such as variability in mean size, age at maturity, and growth pattern, which vary between Western and Eastern populations, should be taken into account for future management advice as already stated (Gunnarsson *et al.*, 2006), as well as the decrease in recruitment.

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APPENDIX 2

Data storage tags characteristics

DST milli - L

Data Storage Tag milli L, low cost TD logger

Overview

Dimensions (diameter x length): 12.5mm x 38.4mm
Sensors: Temperature, pressure (depth)
Standard temperature range: -1°C to +40°C
Temperature accuracy: +/-0.1°C
Depth ranges: 10cm to 20m, 10 cm to 50m, 10cm to 100 m, 1 m to 250m, 5m to 500m or 5m to 800m
Depth accuracy: +/-0.8% of selected range
Memory size: 87,000 measurements in total
Battery life: 3 years
Housing material: Alumina (implantable, biocompatible ceramic material)

Description

The DST milli-L is a miniature data logger that records temperature and pressure (depth). Recorded data is stored in the logger‘s internal memory with a real-time reference for each measurement.

The DST milli-L is supported by the SeaStar software and the Communication Box which serves as an interface between the logger and a PC. Communication between the logger and the Communication Box is wireless.

In SeaStar, the user sets the start time, start date and sampling interval before starting the recorder. Up to seven different intervals can be set for the same measurement sequence. This is especially useful when more frequent measurements are needed at a certain time period.

With default programming temperature and pressure are recorded as pairs. Optionally, they can be set as primary and secondary parameters with different sampling frequency. That way memory partitioning can be customized according to individual preferences.

After recovering the DST recorded data is uploaded to SeaStar where results are displayed both in graphic and tabular form. After retrieving the data, the DST can be re-programmed and reused as long as the battery lasts.
A set of Communication Box and SeaStar software needs to be purchased with the first order.

Star-Oddi also offers protective logger housings for use at fixed locations or gear.

For more information about accessories please click here.

Features on Request

Extended memory
All DST milli products are available with an extended memory capacity of 682,000 measurements in total (FLASH memory, DST milli-F). Total memory size is divided between the two sensors temperature and depth. Please note that the FLASH memory consumes a little more energy from the batteries.

Temperature calibration outside standard range
Temperature calibration outside of standard ranges or for smaller ranges is available for all products on request.

Examples of Application

DST milli-L is an ideal low cost temperature depth logger for fish or marine animal tagging projects, where large quantities of archival tags are needed at reasonable cost. For recapture purposes Star-Oddi marks each tag with a custom defined text containing information on how to return tags and reward. The DST milli-L can also be deployed subsea at mooringsr, fastened to fishing gear or other underwater equipment, collecting valuable data.

The DST milli-L is suitable for studies within:

- Fish and marine animal tagging (internally or externally)
- Oceanography
- Marine biology
- Agricultural and cattle
- Hydrology
- Ecosystems research
- Temperature and pressure measurement in liquids, vials etc.
- Pharmaceutical production (quality control)
- Any other field where temperature and pressure recordings are required

You can read more about research studies and scientific papers here.

Technical Specifications

Sensors	Temperature and pressure (depth)
Size (diameter x length)	12.5mm x 38.4mm
Housing material	Alumina (Ceramic)
Weight (in air/in water)	in air: 9.2g in water: 5g
Memory type	Non-volatile EEPROM
Memory capacity	87,000 measurements in total *
Memory capacity bytes	130,750 bytes / temperature 1.5 bytes, pressure 1.5 bytes *
Memory extension option	1,048,064 bytes (FLASH memory) *
Memory management	- Custom programming of intervals - Primary and secondary parameter
Data resolution	12 bits
Temperature resolution	0.032°C (0.058°F)
Temperature accuracy	+/-0.1°C (0.18°F)
Temperature range	-1°C to +40°C (30°F to 104°F)**
Temperature response time	Time constant (63%) reached in 12 sec.
Standard depth ranges	0.1m-20m, 0.1m-50m, 0.1m-100m, 1m-250m, 5m-500m, 5m-800m
Depth	0.03% of selected range

resolution	
Depth accuracy	+/- 0.8% of selected range
Depth response time	Immediate
Data retention	25 years
Clock	Real time clock Accuracy +/-1 min/month
Sampling interval	In second(s), minute(s) or hour(s)
Number of different sampling intervals	1 to 7
Communications	Communication Box, RS-232C 9 pin serial and USB
Attachment hole	0.9 mm (in diameter)
Battery life	3 years***

* Total memory is divided between the two sensors.
** Outside ranges available upon request.
*** For sampling interval of 10 minutes, temperature & depth recorded simultaneously.
Specifications may change without notice.