LOW GENETIC DIVERSITY AND TEMPORAL STABILITY IN THE ANTARCTIC TOOTHFISH (*DISSOSTICHUS MAWSONI*) FROM NEAR-CONTINENTAL SEAS OF ANTARCTICA

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Abstract

This study presents data on the DNA analysis of recent (2011–2013) samplings of Antarctic toothfish (*Dissostichus mawsoni*) from CCAMLR Subarea 48.5 (2013) and SSRUs 486G (2011), 5841E and 5841FG (2011), 5842E (2011), 881C (2011), 882A (2011, 2012) and 883C (2012). No significant genetic differences between geographic locations, or between samples collected in consecutive years, were observed. These results, based on analysis of the most informative subset of nuclear genetic markers of SNP variation presented in Kuhn and Gaffney (2008), do not support genetic differentiation of samples from the Ross Sea. Discrepancies in the allelic frequencies for several loci compared to previously published data highlight the need for further study of the genetics of Antarctic toothfish.

Introduction

Antarctic toothfish (*Dissostichus mawsoni*) is an apex notothenoid predator inhabiting Antarctic waters. It has a circumpolar distribution south from the Antarctic Convergence (Gon and Heemstra, 1990) and is restricted to the Convention Area managed by CCAMLR. The fishery for *D. mawsoni* started in the late 1990s and the fleet use mainly bottom-set longlines at depths of 1 200–1 800 m. Defining the population structure of *D. mawsoni* is crucial for improved management of the toothfish fishery, but the number of populations or stock units is unknown and is the subject of scientific debate. There may be one genetic stock with gene flow via the Antarctic Circumpolar Current or two or more stocks contained within the major gyres in the Weddell and Ross Seas (Orsi et al., 1995). Alternatively, genetic differentiation could take place among different sectors of Antarctica – most particularly between the Ross Sea and Weddell Sea – that cut deep inland and have their own systems of circular currents and a vast shelf area.

The life history of *D. mawsoni* has been extensively studied by Soviet (Andryashev, 1979, 1986; Yukhov, 1982) and, more recently, by Russian ichthyologists (Shust, 1998; Prutko, 2004; Shust et al., 2005; Piyanova et al., 2008; Petrov and Istomin, 2008), who proposed that *D. mawsoni* can be divided into three stages, with each stage being restricted to a particular depth. In the first (shelf) stage, young fish (age 0+ and 1+), with total length between 41 and 117 mm, are found during spring and summer in surface waters near the continental shoreline (Andryashev, 1979, 1986; Yukhov, 1982); 21 months after hatching, immature toothfish about 15 cm (alternative estimate – between 18.5 and 54.1 cm) move to the bottom, become benthic and spend a few years on the continental shelf at depths of 50–300 m (Roshchin, 1997; Yukhov, 1982; Near et al., 2003; La Mesa, 2007; Hanchet et al., 2008). The second (slope) stage begins as fish reach lengths of 50–95 cm and move to the upper continental slope area and nearby islands at a depth between 500 and 850 m (Shust et al., 2005). The third (deep-water) stage occurs when fish reach 100 cm in length and migrate to deep waters and stay at a depth of 1 000–2 000 m (Petrov and Istomin, 2008).

Results of tagging studies indicate that *D. mawsoni* is generally not a migratory fish and most fish are caught within 50 km of the release site.

Table 1: Details of genetic samples collected during longline fishing for toothfish.

Loaction number	Collector	Region/SSRU	Collection date	RNCRGM*	Sample size (N)
1	I.I. Gordeev	D'Urville Sea, SSRU 5841G	02.01.2011	DIS810-841	32
2	I.I. Gordeev	Mawson Sea, SSRU 5841E	10.02.2011	DIS896-924	29
3	I.I. Gordeev	Collaboration Sea, SSRU 5842E	18.02.2011	DIS925-959	35
4	I.I. Gordeev	Bouvet Island area, SSRU 486G	09.03.2011	DIS960-977	18
5	I.I. Gordeev	Ross Sea, SSRU 881C 64°56'S 179°45'W	04.12.2011	DIS978-1016	39
6	E.F. Kulish	Bellingshausen Sea, SSRU 883C 69°29.2'S 82°21.3'W	25.02.2012	DIS1191-1229	39
7	E.F. Kulish	Ross Sea, SSRU 882A	18.01.2011	DIS0678-0735	48
8	S.E. Akimov	Ross Sea, SSRU 882A 75°56'S 169°40'W	05.02.2012	DIS1259-1306	48
9	I.I. Gordeev	Weddell Sea, Subarea 48.5 74°S 27°W	25.02.2013	DIS1437-1484	48

* RNCRGM – Russian National Collection of Reference Genetic Material, VNIRO

However, one fish was recorded 2 300 km from the release site (Dunn et al., 2005a, 2007; Hanchet et al., 2008).

The first genetic study of Antarctic toothfish was based on random amplified polymorphic DNA (RAPD) markers and concluded that specimens from the Ross and Bellingshausen Seas were from two genetically distinct groups (Parker et al., 2002). Investigation of mitochondrial DNA (mtDNA) sequences and seven nuclear intronic single-nucleotide polymorphism (SNP) markers did not reveal any substantial genetic differentiation among samples taken from three CCAMLR areas: Subareas 48.1 and 88.1 and Division 58.4.2, though haplotype frequencies were slightly different (Smith and Gaffney, 2005). A later study used an extended set of 13 nuclear markers and detected pairwise genetic differentiation in five out of 28 sample pairs, however, the pattern of genetic differentiation was not consistent with the spatial or temporal distribution of the samples studied (Kuhn and Gaffney, 2008).

The existence of a stable and genetically isolated population of *D. mawsoni* seems very unlikely given the long (almost two years) pelagic stage before fish became benthic, the strong homogenising effect of the circum-Antarctic currents and the documented ability of mature fish to migrate for thousands of kilometres. This type of life history should prevent any stable genetic differentiation among major toothfish aggregations in Antarctic waters. The aim of this investigation was to evaluate the population structure of *D. mawsoni* in the Antarctic by assessing a subset of the most informative SNP loci from Kuhn and Gaffney (2008), using material from geographically and temporally extensive samplings.

Material and methods

Samples used in the investigation of the population-genetic structure of Antarctic toothfish were collected from 2011 to 2013 during longline fishing by VNIRO staff working as observers on commercial fishing vessels. Fin-clip samples for genetic analysis were fixed in 96% ethanol on board the fishery vessels. Details of the samples are presented in Table 1 and locations are shown in Figure 1.

DNA extraction was carried out using the Wizard SV 96 Genomic DNA Purification System ('Promega', USA) following the manufacturer's protocol.

Five of the most polymorphic nuclear intronic regions (one contains three SNPs), previously analysed by Kuhn and Gaffney (2008), were chosen for this analysis. These loci showed the highest genetic variability in the populations examined to date and therefore were presumably the most informative loci to reveal genetic differentiation. Loci and primers are given in Table 2.

Amplifications were performed in a final reactions volume of 15 µl (70 mM Tris-HCL (pH 8.3);

Table 2: *Dissostichus mawsoni* PCR primers.

Location number	Gene	Locus: SNP position	SNP	Primer	Primer sequence	Reference
1	Ribosomal protein S7 (RPS7)	RPS7:374	G/T	Rps7_F Rps7_R	AACTCGTCTGGCTTTTCGCC TGGCCTCTTCCTTGGCCGTC	Chow and Hazama, 1998
2	Triose phosphate isomerase (TPI)	TPI:127	A/G	TPI_F	GCATYGGGGAGAAGCTRGAT	Quattro et al., 2001
3		TPI:135	G/T	TPI_R	AGAACCACYTTRCTCCAGTC	
4		TPI:170	G/T			
5	Mixed lineage leukaemia-like protein (MLL)	MLL:95	A/G	MLL_F MLL_R	GACGAAGAACAGCAACAGCAA CTGGCCGATATGCTGCTT	Smith and Gaffney, 2005
6	Creatine kinase intron 7 (CK7)	CK7:417	C/G	CK7_F CK7_R	CCCAAGTTYGAGGAGATCCTGAC CAGTCGGTCRGCRTTGGAGATGTC	Quattro and Jones, 1999
7	Calmodulin (CaM)	CaM:203	A/C	CaM_F CaM_R	CTGACCATGATGGCCAGAAA CTGACCATGATGGCCAGAAA	Chow, 1998

16.6 mM $(NH_4)_2SO_4$; 1.8 mM $MgCl_2$; 200 μM of each dNTP; 3 pmol of each primer; 100 pg DNA-matrix; and 0.4 units of *Taq*-polymerase (Sileks, Moscow)) with the following cycle protocol: a preliminary denaturation of DNA at 94°C for 1 min was followed by syntheses of DNA products: melting at 92°C for 20 sec, annealing of primers at 55°C for 40 sec, DNA syntheses at 72°C for 40 sec. A final extension cycle of 10 minutes at 70°C followed.

Genotyping of SNP loci was done either by direct sequencing of polymerase chain reaction (PCR) product or by restriction fragment length polymorphism (PCR-RFLP).

SNP loci Rsp7:374, CaM:203, TPI:127, TPI:135 and TPI:170 were genotyped by direct sequencing of purified PCR product. Homozygous positions could be identified by the presence of single peaks in the chromatogram, and the same position was considered as heterozygous if two peaks were present and the height of the lower peak was not less than 40% of the height of the alternative peak.

Two loci (MLL:95 and RPS7:374) were genotyped by restriction of the PCR fragment as described by Kuhn and Gaffney (2008). Restriction with the enzyme MboI was used for genotyping of the locus MLL (^GATC recognition site). To assess locus CK7, the PCR product was digested with Csp6I, (G^TAC recognition site).

Statistical analysis was performed using add-ons to Microsoft Excel: *Microsatellite Toolkit* (Park, 2001) and GenAlEx6 (Peakall and Smouse, 2012). Evaluations of the level of genetic (expected) (He) and observed (Ho) heterozygocities and allelic diversity and conformity of genotypic proportions to Hardy-Weinberg equilibrium (inbreeding coefficient (Fis) and the significance level of probability test) were performed in GenAlEx6.

Results

Data in this study suggested a high level of polymorphism in the loci examined in all samples. The frequencies of alleles recorded for these markers are given in Table 3.

The frequency distribution of the investigated loci indicates the lack of a clear population structure among the regions sampled (Figure 2). High

Table 3: SNP frequencies for the loci studied (numbers in the top row refer to sampling locations described in Table 1).

Loci	Allele	1	2	3	4	5	6	7	8	9
RPS7:374	*N*	25	12	25	9	28	26	44	46	45
	G	0.640	0.583	0.700	0.667	0.750	0.692	0.750	0.685	0.700
	T	0.360	0.417	0.300	0.333	0.250	0.308	0.250	0.315	0.300
TPI:127	*N*	18	17	22	10	18	21	24	21	20
	A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
TPI:135	*N*	18	17	22	10	18	21	24	21	20
	G	0.861	0.941	0.977	0.950	0.833	0.952	0.917	0.952	0.900
	T	0.139	0.059	0.023	0.050	0.167	0.048	0.083	0.048	0.100
TPI:170	*N*	18	17	22	10	18	21	24	21	20
	G	0.861	0.941	0.886	0.950	0.833	0.952	0.900	1.000	0.932
	T	0.139	0.059	0.114	0.050	0.167	0.048	0.100	0.000	0.068
MLL:95	*N*	32	25	35	16	37	38	39	46	48
	A	0.375	0.360	0.343	0.313	0.405	0.316	0.487	0.370	0.354
	G	0.625	0.640	0.657	0.688	0.595	0.684	0.513	0.630	0.646
CK7:136	*N*	9	8	22	9	33	38	46	48	46
	C	0.389	0.188	0.045	0.000	0.091	0.158	0.120	0.167	0.163
	G	0.611	0.813	0.955	1.000	0.909	0.842	0.880	0.833	0.837
CaM:203	*N*	11	16	13	16	13	14	15	16	13
	C	0.955	0.875	0.846	0.813	0.885	0.929	0.767	0.875	0.846
	A	0.045	0.125	0.154	0.188	0.115	0.071	0.233	0.125	0.154

Table 4: Distribution of pairwise values of the fixation index (Fst) in the studied populations. (Numbers in the top row and left-hand column refer to sampling locations described in Table 1).

Location number	1	2	3	4	5	6	7	8
2	0.018	0.000						
3	0.043	0.014	0.000					
4	0.057	0.020	0.008	0.000				
5	0.026	0.019	0.014	0.024	0.000			
6	0.021	0.004	0.012	0.019	0.017	0.000		
7	0.035	0.014	0.012	0.020	0.010	0.017	0.000	
8	0.031	0.007	0.018	0.021	0.025	0.006	0.017	0.000
9	0.020	0.004	0.012	0.017	0.009	0.005	0.007	0.008

levels of gene flow, assisted by the circumpolar currents, have probably led to homogenisation of allele frequencies among populations that inhabit near-continental seas of Antarctica. Pairwise genetic distances between the samples in this study do not exceed 0.027 and this indicates that there is no grouping of populations in this region (Table 4).

Discussion and conclusion

The first population genetic studies of Antarctic toothfish were conducted using samples from CCAMLR Subareas 48.1 and 88.1 and Division 58.4.2 (Smith and Gaffney, 2005), using two mitochondrial and intronic DNA molecular markers. Low polymorphism in the mitochondrial genome, as well as absence of differentiation of the four nuclear markers investigated, were shown by Smith and Gaffney (2005). In the follow-up study (Kuhn and Gaffney, 2008), population differentiation of fish from the Ross Sea was reported. This study, based on the same set of genetic markers as Kuhn and Gaffney (2008) but with extended modern sampling, does not show a significant difference of toothfish in the Ross Sea, thus confirming the findings of Smith and Gaffney (2005).

Significant differences were also found with the work of Kuhn and Gaffney (2008) in the assessment of polymorphism at a number of loci. For example, Kuhn and Gaffney (2008) reported loci TPI:127 to be highly polymorphic in all samples with allele frequencies from 0.368 in Division 58.4.1 to 1.000 in the Ross Sea. However, according to the data, obtained by direct sequencing, there is a total lack of polymorphism at this locus. Furthermore, at two other positions of the sequence (loci TPI:135 and TPI:170), Kuhn and Gaffney (2008) indicate the presence of unique SNP, which was found only once in all the investigated sequences. According to the data in this study, polymorphism of these loci has a regular character and was found in all populations (Table 3). The discrepancies between data in this study and Kuhn and Gaffney (2008) indicated the presence of methodological inconsistency in allelic scoring such that the consequential conclusions about genetic isolation of toothfish in the Ross Sea should be considered with caution.

Results of the toothfish tagging program carried out in Antarctica (Dunn et al., 2005b, 2007) for the last decade show that more than 80% of adult toothfish do not migrate far from the place where they were released (less than 50 km) and only exceptional individuals move a distance greater than 200 km (Petrov and Tatarnikov, 2010) or up to 2 300 km (Dunn et al., 2007; Hanchet et al., 2008). Based on generally low migration, the long lifespan of toothfish and highly overlapping generations, it is reasonable to assume that the population structure should be temporally stable and not fluctuate from one year to another. This assumption is supported by data from samples from the Ross Sea taken in 2011 and 2012 that indicated genetic similarity between the two years. This study, which included sampling from all major areas of the Antarctic, did not reveal genetic differentiation among any samples. Further investigation of genetic structure would be of benefit to improve fishery management of this valuable but understudied species, and development of new genetic markers is required in order to come to more robust conclusions.

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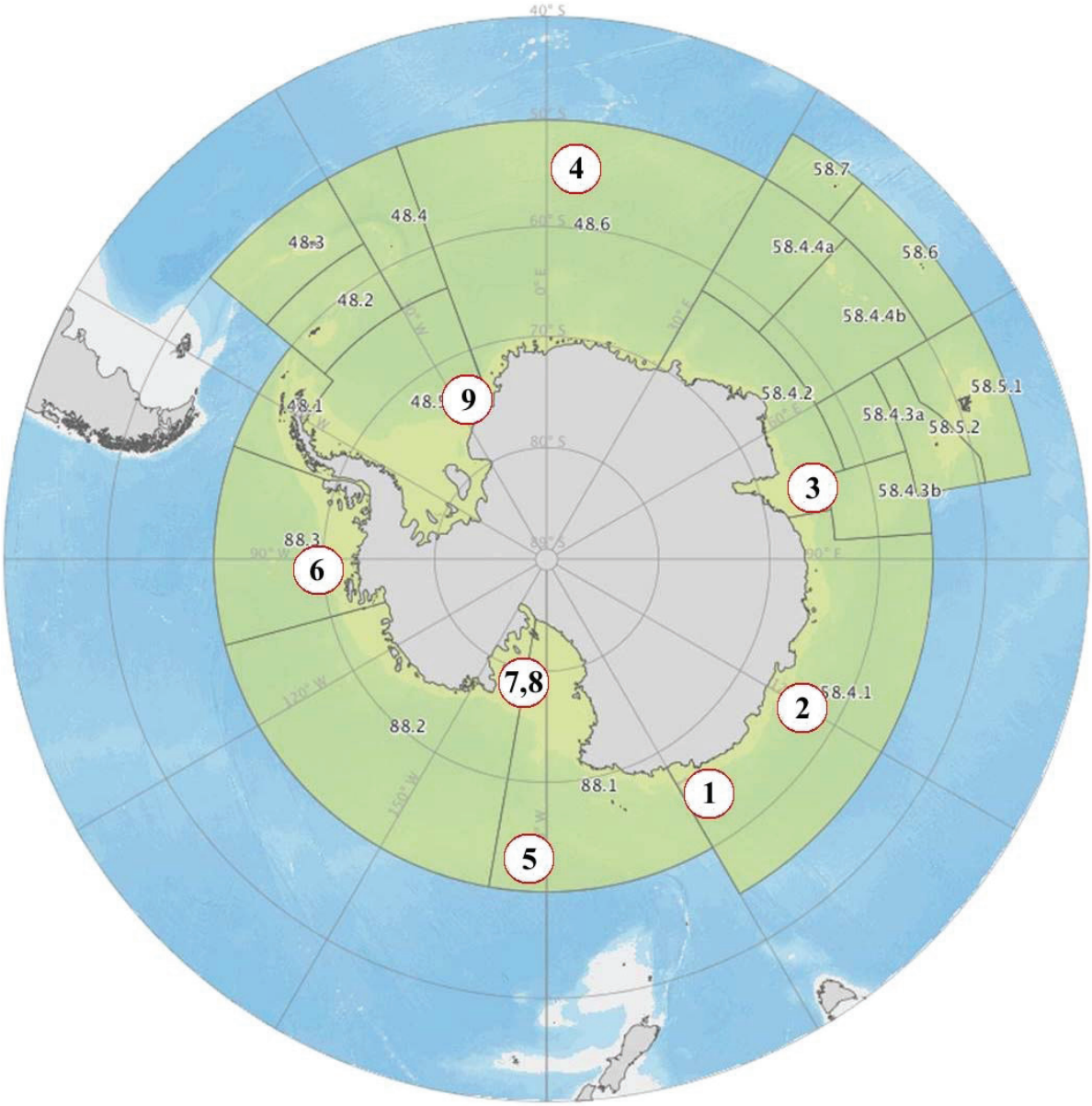

Figure 1: Map showing locations (numbers in circles refer to the number in Table 1) of the collection of genetic material from *Dissostichus mawsoni*. See Table 1 for details. The CAMLR Convention Area is shown in green with the CCAMLR subareas and divisions referred to in the text.

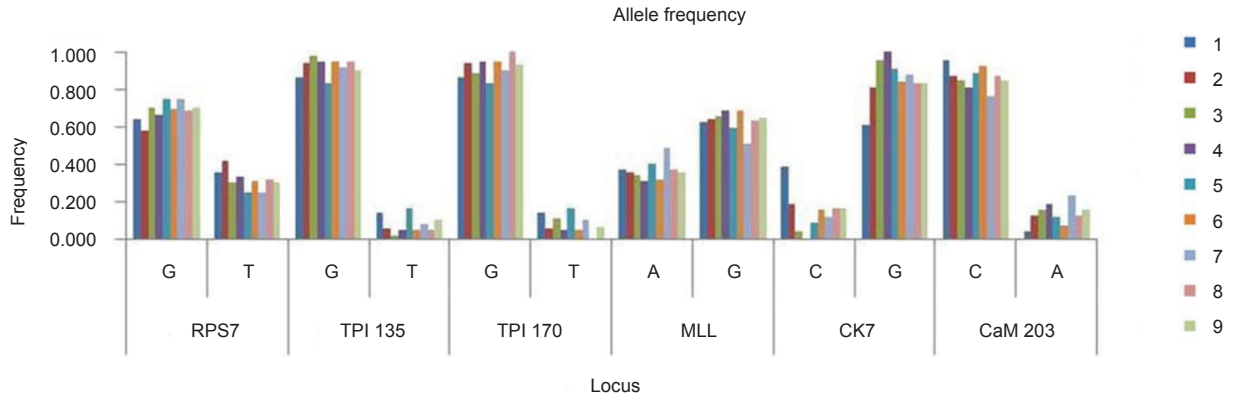

Figure 2: Frequency distribution of all loci in the investigated aggregations (numbers in the legend refer to sampling locations described in Table 1).