LOW GENETIC DIVERSITY IN THE ANTARCTIC TOOTHFISH (*DISSOSTICHUS MAWSONI*) OBSERVED WITH MITOCHONDRIAL AND INTRON DNA MARKERS

P.J. Smith✉
National Institute of Water and
Atmospheric Research Ltd
Private Bag 14 901, Kilbirnie
Wellington, New Zealand
Email – p.smith@niwa.co.nz

P.M. Gaffney
College of Marine Studies
University of Delaware
Lewes, DE 19958, USA

Abstract

Two molecular methods, mitochondrial and intron DNA markers, were used to determine genetic relationships among Antarctic toothfish (*Dissostichus mawsoni*) samples from three CCAMLR areas: Subareas 48.1 and 88.1 and Division 58.4.2. *D. mawsoni* appeared to be characterised by low diversity; no genetic variation was detected with restriction enzyme digests of nine sub-regions of the mitochondrial genome. Polymorphisms were found in four of seven introns digested with the restriction enzymes, but there was no population differentiation among the three sea areas. Direct sequencing of cytochrome *b* (665 bp) and the left domain of the control region (639 bp) showed very little variation and no significant genetic differentiation among sea areas.

Résumé

Deux méthodes moléculaires, les marqueurs de l'ADN mitochondrial et des introns, ont permis de déterminer la relation génétique entre les échantillons de légine antarctique (*Dissostichus mawsoni*) provenant de trois secteurs de la CCAMLR : les sous-zones 48.1 et 88.1 et la division 58.4.2. *D. mawsoni* semble se caractériser par une faible diversité. En effet, aucune variation génétique n'est détectée à l'aide des digestions des enzymes de restriction de neuf sous-régions du génome mitochondrial. Des polymorphismes apparaissent dans quatre des sept introns digérés avec les enzymes de restriction, mais on ne relève aucune différenciation entre les populations des trois secteurs marins. Le séquençage direct du cytochrome *b* (665 bp) et la partie gauche de la région régulatrice (639 bp) n'indiquent qu'une très faible variation et pas de différenciation génétique importante entre les secteurs marins.

Резюме

Для определения генетической взаимосвязи между образцами антарктического клыкача (*Dissostichus mawsoni*) из трех районов АНТКОМа (подрайонов 48.1 и 88.1 и Участка 58.4.2) использовались два молекулярных метода: митохондриальные и интронные ДНК-маркеры. Представляется, что *D. mawsoni* характеризуется низким разнообразием; расщепление девяти подобластей митохондриального генома с помощью рестриктазы не выявило генетической изменчивости. В четырех из семи интронов, расщепленных с помощью рестриктазы, был обнаружен полиморфизм, но дифференциация популяции между этими тремя морскими районами отсутствовала. Прямое секвенирование цитохрома *b* (665 bp) и левого домена контрольной области (639 bp) выявило очень низкую изменчивость и отсутствие существенной генетической дифференциации между морскими районами.

Resumen

Se utilizaron dos métodos moleculares, marcadores del ADN mitocondrial y de intrones, para determinar la relación genética entre las muestras de austromerluza antártica (*Dissostichus mawsoni*) de tres áreas de la CCRVMA: Subáreas 48.1 y 88.1 y División 58.4.2. *D. mawsoni* aparentemente se caracteriza por una baja biodiversidad; no se detectó variación genética en la digestión de nueve subregiones del genoma mitocondrial con enzimas de restricción. Se encontraron polimorfismos en cuatro de los siete intrones digeridos con enzimas de restricción, pero no hubo una diferenciación de la población

entre estas tres áreas marinas. La secuenciación directa del citocromo *b* (665 bp) y del dominio izquierdo de la región de control (639 bp) mostró muy poca variación y ninguna diferenciación genética significativa entre las áreas marinas.

Keywords: *Dissostichus mawsoni*, mitochondrial DNA, introns, stock structure, CCAMLR

Introduction

Toothfish are large notothenioids living in Antarctic and sub-Antarctic waters. Two species are harvested, the Antarctic toothfish (*Dissostichus mawsoni*) and the Patagonian toothfish (*D. eleginoides*); both are circumpolar in distribution. *D. mawsoni* is found at high latitudes south of the Antarctic Convergence around 60°S, while *D. eleginoides* ranges from about 50°S to 75°S (Gon and Heemstra, 1990). *D. mawsoni* appears to be restricted to waters managed solely by CCAMLR, and the main fishery for this species has taken place in Subareas 88.1 and to a lesser extent 88.2, in the region of the Ross Sea, since 1997; small catches have also been made in Division 58.4.1 of the Australian Antarctic Territory (AAT). While the physiological adaptations of *D. mawsoni* are well known (Chen et al., 1997), and life history parameters are being studied (Horn, 2002), the number of populations or stock units of *D. mawsoni* is unknown. There may be one genetic stock with gene flow via the Antarctic Circumpolar Current or two, or more, stocks contained within major gyres in the Weddell and Ross Seas (Orsi et al., 1995).

There have been relatively few genetic studies of Antarctic circumpolar marine species. Tests of population structure in krill (*Euphausia superba*), using allozyme markers, produced conflicting results (Fevolden and Ayala, 1981; Schneppenheim and MacDonald, 1984; Williams et al., 1994), but DNA analyses showed evidence for two genetic units, one around South Georgia and the other in the Weddell Sea (Zane et al., 1998). Several molecular studies of *D. eleginoides* have been carried out. Allozyme markers showed no significant regional differentiation among samples from the Southern Ocean, but microsatellite DNA markers showed significant heterogeneity, allowing rejection of the null hypothesis of one unit stock (Smith and McVeagh, 2000). Mitochondrial DNA (mtDNA) markers revealed marked genetic differentiation among regions; samples from the South American Plateau were fixed, or almost fixed, for a haplotype that was absent in samples from the Atlantic and Indian Ocean sectors of the Southern Ocean, while a third genetic group occurred in the Pacific Sector (Smith and Gaffney, 2000a). Subsequently, Appleyard et al. (2002) reported significant mtDNA heterogeneity among samples from Macquarie Island and Heard and MacDonald Islands, while samples from the Indian Ocean were homogeneous for both mtDNA and microsatellite markers (Appleyard et al., 2004). A major genetic break was reported north and south of the convergence zone in the Atlantic (Shaw et al., 2004; Smith and Gaffney, 2000a; Smith and Gaffney, 2000b).

Recently Parker et al. (2002) examined random amplified polymorphic DNA (RAPD) markers in *D. mawsoni* and concluded that samples taken from McMurdo Sound (Ross Sea) and the Bellingshausen Sea were from two different genetic groups. Gaffney (2000) recommended using a range of molecular methods to increase the probability of finding informative markers in Antarctic species. In general, greater genetic differentiation has been detected with mtDNA than with nuclear DNA markers in *D. eleginoides* (Appleyard et al., 2002, 2004; Smith and Gaffney, 2000a; Smith and McVeagh, 2000). This study evaluates sub-regions of the mitochondrial genome and seven introns in the nuclear genome for detecting genetic variation in *D. mawsoni*, and for determining stock relationships.

Methods and materials

Sampling of *D. mawsoni*

During summer 2001, tissue samples were collected from three CCAMLR areas: the South Shetland Islands (SS), Subarea 48.1 in the Atlantic sector; the Ross Sea (Ross), Subarea 88.1 in the Pacific Ocean sector; and Division 58.4.2 in the Indian Ocean sector (AAT). Muscle tissue samples were removed from individual fish sampled for routine biological analyses and fixed in 90% ethanol.

DNA extraction

Total genomic DNA was extracted from 200–500 mg of muscle tissue by homogenisation and digestion with proteinase-K at 55°C for 4 h. After digestion, DNA was extracted with phenol:chloroform, followed by chloroform:isoamyl alcohol, and precipitated with 70% ethanol at –20°C (adapted from Taggart et al., 1992). The DNA pellets were air dried and resuspended in 40 µl sterile water and stored at –20°C. Two classes of markers, mtDNA and nuclear introns, were

screened in subsamples of *D. mawsoni* specimens from the three CCAMLR areas (Subareas 48.1 and 88.1 and Division 58.4.2).

MtDNA restriction fragment length polymorphism (RFLP)

Nine sub-units of mtDNA were amplified in *D. mawsoni* (Table 1), and the single amplified products were screened for polymorphisms with 5- and 4-base restriction enzymes following methods successfully used for *D. eleginoides* (Smith and Gaffney, 2000a). Twenty-four DNA templates of *D. mawsoni* (8 fish x 3 areas) were amplified for ATPase, ND1, ND2, ND3/4, COI, COII, cytochrome *b*, and two portions of the control region, and the amplified products digested independently with restriction enzymes. Restriction enzyme digestions were performed in 20 µl volumes for a minimum of 4 h, following the manufacturer's recommendations (New England BioLabs, USA). The digested products were separated in agarose gels (from 1.2 to 2%) and run at 60 v for 1.5–4 h. A DNA size ladder was included to estimate size of the amplified fragments. The amplified products were detected with ethidium bromide, incorporated into the gel, and viewed under ultraviolet light.

A ~700 bp region of the ATPase 6 sub-unit was digested with 18 restriction enzymes (*Ava* II, *BamH* I, *Bfa* I, *BseD* I, *BsiHKA* I, *BsmA* I, *BstY* I, *Cfr*131, *Dpn* II, *Hinf* I, *Hpa* II, *Hpy*188 I, *Hpy*188 III, *Pvu* II, *Rsa* I, *ScrF* I, *Taq* I and *Tsp*509 I); a ~1.4 kb fragment of NADH dehydrogenase sub-unit 1 was digested with 27 enzymes (*Acc* I, *Alu* I, *Apa* I, Ase I, *Ava* II, *BamH* I, *Ban* II, *Bsa*J I, *Bst*N I, *Bst*U I, *Bst*Y I, *Cfr* 131, *Dde* I, *Dpn* II, *Dra* I, *Eco*R I, *Hae* III, *Hinf* I, *Hpy*188 I, *Hpy*188 III, *Hpy*CH4 IV, *Rsa* I, *Sac* I, *Scr*F I, *Stu* I, *Sty* I and *Xba* I); a ~1 300 bp fragment of the NADH dehydrogeanse sub-unit 2 (ND2) was digested with 10 restriction enzymes (*Alu* I, *Ava* II, *Bst*U I, *Cfo* I, *Dpn* I, *Hae* III, *Hinf* I, *Hpa* II, *Rsa* I and *Taq* I); a ~2.2 kb region encompassing NADH dehydrogenase sub-units 3/4L/4 was digested with 16 enzymes (*Acc* I, *Alu* I, *Apa* I, *Ava* II, *BamH* I, *Ban* II, *Bsa*J I, *Bst*N I, *Bst*U I, *Bst*Y I, *Cfr*131, *Dra* I, *Eco*R I, *Hae* III, *Hinf* I and *Hpy*188 I); a ~650 bp fragment of the cytochrome oxidase II (COII) sub-unit was digested with 10 restriction enzymes (*Alu* I, *Ava* II, *Cfo* I, *Dpn* I, *Kpn* I, *Hpa* II, *Nci* I, *Nla* III, *Rsa* I and *Taq* I); a ~700 bp fragment of the cytochrome oxidase I (COI) sub-unit was digested with three restriction enzymes (*Cfo* I, *Kpn* I and *Rsa* I); and a 709 bp fragment of cytochrome *b* was digested with 16 restriction enzymes (*Acc* I, *Afl* III, *Ava* II, *BsiHKA* I, *Bsr* I, *BstN* I, *Hae* III, *Hha* I, *Hinf* I, *Hpy*188 I, *Hpy*CH4 IV, *Msp* I, *Rsa* I, *Sac* I, *Sau*96 I and *Sty* I).

A ~450 bp region of the left domain of the control region, amplified with the primer pair L15995 and H16498 (Meyer et al., 1990, 1994), was digested with 13 restriction enzymes (*Alu* I, *Ava* II, *BstU* I, *Cfo* I, *Dpn* I, *Hae* III, *Hinf* I, *Hpa* II, *Nci* I, *Nla* III, *Rsa* I, *Taq* I and *Tsp*509 I). The downstream fragment, approximately 950 bp, was amplified with the primers L16498 (Meyer et al., 1990) and 12SAR (Meyer et al., 1990; Palumbi et al., 1991), and digested with 10 restriction enzymes (*Alu* I, *Ava* II, *Cfo* I, *Dpn* I, *Hpa* II, *Kpn* I, *Nci* I, *Nla* III, *Rsa* I and *Taq* I).

MtDNA control region and cytochrome *b* sequencing

A ~0.7 kb fragment including the 3′ end of the cytochrome *b* gene, tRNA-Thr, tRNA-Pro, and a 5′ fragment of the control region, which is highly variable in some fishes (Ishikawa et al., 2001), and a 709 bp fragment of the cytochrome *b* gene were amplified and sequenced. PCRs were performed in 50 µl volumes in a Cetus DNA thermocycler (Perkin-Elmer Corporation, USA). The control region was amplified using the primer pair L-15774 and H-16498 (Meyer et al., 1990, 1994), and the cytochrome *b* fragment was amplified with the primers listed in Table 1. DNA samples were purified using the QIAquick gel extraction kit (Qiagen). Sequences were determined using the ABI Taq DyeDeoxy™ Terminator Cycle Sequencing Kit according to the manufacturer's directions (Applied Biosystems Inc., USA) and run on an ABI Prism autosequencer.

Sequences were edited in CHROMAS (Technelysium, Australia), and aligned in the BIOEDIT program (Hall, 1999). Tests of geographical differentiation were implemented by Arlequin 2.00 (Schneider et al., 2000).

Nuclear DNA introns

Seven introns were amplified (Table 1) and the products digested independently with restriction enzymes. The amplified digested products were separated in agarose gels, as for mtDNA RFLPs above. The S7 ribosomal protein sub-unit intron 1 (*RP*-1) and 2 (*RP*-2) amplicons (Chow and Hazama, 1998) were digested with 10 restriction enzymes (*Alu* I, Ava II, *BstU* I, *Cfo* I, *Dpn* I, *Hae* III, *Hinf* I, *Hpa* II, *Rsa* I and *Taq* I); intron 6 of the creatine kinase (*CK6*) gene (Chow and Takeyama, 1998) with

Table 1: Mitochondrial DNA and intron primers evaluated with *Dissostichus mawsoni*. PCR = polymerase chain reaction conditions (annealing and extension temperatures and Mg concentration).

Region	Primer sequences	PCR			Reference
		Anneal °C	Ext. °C	Mg^{++} mM	
mtDNA					
ATPase	TAAGCRNYAGCCTTTTAAG GGGGNCGRATRAANAGRCT	50	72	2	Quattro, unpubl.
Cytb	CGTTTTACCCTGGGGTCAAATGT GGGGAAGAAGATGAGGAAAAT	50	72	2	Gaffney, unpubl.
ND1	GCTATTAAGGGTTCGTTTGTTCA CCAAGAGCTTATTTAGCTGACTTTACT	50	72	2	Cronin et al., 1993
ND2	AAGCTATCGGGCCCATACCC CCGCTTAGYGCTTTGAAGGC	50	72	2	Park et al., 1993
ND3/4	AGTATAAGTGACTTCCAATCAC TTAGAATCACAATCTAATGTTTT	50	72	2	Cronin et al., 1993
COI	AGTATAAGCGTCTGGGTAGTC CCAGAGATTAGAGGGAATCAGTG	60	72	2	Palumbi et al., 1991
COII	AAAGGGAGGAATTGAACCC GCTCATGAGTGWAGGACRTCTT	50	72	2	Gaffney, unpubl.
control region	AACTCTCACCCCTAGCTCCCAAAG CCTGAAGTAGGAACCAGATG	49	72	2	Meyer et al., 1994 Meyer et al., 1990
control region	ATCTGGTTCCTACTTCAGG ATAGTGGGGTATCTAATCCCAGTT	51	72	2	Meyer et al., 1990 Palumbi et al., 1991
introns					
RP1	TGGCCTCTTCCTTTGGCCGTC AACTCGTCTGGCTTTTCGCC	60	72	2	Chow and Hazama, 1998
RP2	AGCGCCAAAATAGTGAAGCC GCCTTCAGGTCAGAGTTCAT	60	72	2	Chow and Hazama, 1998
CK6	GACCACCTCCGAGTCATCTC CAGGTGCTCGTTCCACATGA	55	72	2	Chow and Takeyama, 1998
CK7	CCCAAGTTYGAGGAGATCCTGAC CAGTCGGTCRGCRTTGGAGATGTC	50	72	1	Quattro and Jones, 1999
Gsyn	TCCAACAGCGACATGTACCT CTCCTGTTCCATTCCAAACC	50	75	2	Chow, unpubl.
Dyst6a	CAGCARACNAACAGTGAGAAGAT GCTGGAGAAGTTAACCACYTTGAC	50	72	1	Gaffney, unpubl.
MLL	GACGAAGAACAGCAACAGCAA CTGGCCGATATGCTGCTT	50	72	1	Gaffney, unpubl.

15 restriction enzymes (*Alu* I, *Ava* II, *BstN* I, *BstU* I, *Cfo* I, *Dpn* I, *Hae* III, *Hinf* I, *Kpn* I, *Nci* I, *Nla* III, *ScrF* I, *Rsa* I, *Taq* I and *Tsp*509 I); the creatine kinase (*CK7*) intron 7 (Quattro and Jones, 1999) with six enzymes (*Dpn* II, *Hha* I, *Hpa* II, *Hpy*188 I, *HpyCH*4 IV and *Rsa* I); a glutamate synthetase (*Gsyn*) intron (Chow, unpubl.) with 14 restriction enzymes (*Alu* I, *Ava* II, *BamH* I, *BsiHKA* I, *BstU* I, *Cfo* I, *Dpn* I, *Hae* III, *Hinf* I, *Hpa* II, *Hpy*188 I, *Rsa* I, *ScrF* I and *Taq* I); a dystrophin intron (*Dyst6a*) with 11 enzymes (*Ban* II, *BsiHKA* I, *Bsr* I, *EcoR* I, *Hae* III, *Hind* III, *Hinf* I, *HpyCH4* IV, *Msp* I, *Nsi* I and *Sty* I); and an intron in the mixed lineage leukemia-like protein gene (*MLL*) with the restriction enzyme *BamH* I identified from the published intron sequence (GenBank Accession AF137249). The *Dyst6a* and *MLL* primer pairs were designed from published *D. mawsoni* intron sequences (Venkatesh et al., 1999).

Results

MtDNA RFLPs

No genetic variation was detected with the RFLP method for the nine regions of mtDNA in 24 *D. mawsoni*, which was surprising given that this method has revealed several polymorphsims in *D. eleginoides* (Appleyard et al., 2002; Smith and Gaffney, 2000a). The RFLP haplotype diversity in *D. eleginoides* was 0.143 (Smith and Gaffney, 2000a) and was 0 in *D. mawsoni*. Therefore two regions of mtDNA, the left domain of the control region and the cytochrome *b* gene, were sequenced.

Control region sequencing

Fifty-seven sequences (639 bp between primers) were obtained from the *D. mawsoni* control region amplicon by single-pass sequencing. Alignment of these sequences against available control region sequences from *D. eleginoides* (Derome et al., 2002, GenBank Accession No. AF490672; Kuhn and Gaffney, unpubl., 447 bp) provided a 433 bp overlap, which showed 89.9% sequence identity, with two indels (1 nt, 14 nt). The *D. mawsoni* amplicon contained two variable sites in the control region. One SS fish had a T > C transition at position 198, and two AAT individuals had a G > A transition at position 471.

The control region *per se* (385 bp) exhibited extremely low nucleotide diversity (0.00018) in the 57 available sequences. Analysis of molecular variance (AMOVA) showed no significant differentiation among the three samples (overall F_{ST} = 0.029). In contrast, the homologous region in *D. eleginoides* contained 12 segregrating sites and 25 haplotypes, with a ~30x greater nucleotide diversity (0.0052).

Cytochrome *b* sequencing

The 665 bp cytochrome *b* sequence (709 bp amplicon between primers) obtained from *D. mawsoni* exhibited 90.1% identity to *D. eleginoides* (Kuhn and Gaffney, unpubl.) The 57 *D. mawsoni* individuals examined showed no sequence variation except for a single synonymous A > G transition in three Ross Sea fishes. In contrast, the cytochrome *b* region from *D. eleginoides* showed considerably more variation: aligned sequences of 562 bp from 51 individuals showed 27 haplotypes and 15 variable nucleotide positions (Kuhn and Gaffney, unpubl.). Although cytochrome *b* sequences in *D. mawsoni* were nearly monomorphic, it is possible that the single nucleotide polymorphism found in the Ross Sea sample may prove to be a private allele indicating some sub-population division.

Nuclear DNA introns

Four polymorphisms were found among the seven introns: the *CK6* intron digested with the *Cfo* I restriction enzyme, *CK7* digested with *Rsa* I, *Dyst6a* digested with *Hinf* I, and *MLL* digested with *BamH* I. For the *CK6* intron there was an excess of homozygotes in each area sample, and this was significant in the AAT and pooled samples, when tested with χ^2 tests on observed and expected numbers of genotypes (Table 2). Other loci were in Hardy–Weinberg equilibrium (Table 2). Allele frequencies were tested for heterogeneity among areas with a contingency χ^2 tests using the GENEPOP program (Raymond and Rousset, 1995). There was no significant heterogeneity at any of the loci (*CK6* χ^2 = 2.36, *P* = 0.31; *CK7* χ^2 = 4.06, *P* = 0.13; *MLL* χ^2 = 0.38, *P* = 0.83; *Dyst6a* χ^2 = 1.53, *P* = 0.46).

Discussion

Finding such low mtDNA diversity in *D. mawsoni* is surprising, given the high genetic diversity detected in *D. eleginoides* when using similar techniques. The two species of toothfish appear to have similar life histories (Horn, 2002), suggesting that the low mtDNA diversity seen in the control region and cytochrome *b* in *D. mawsoni* may reflect a historical population bottleneck in that species. MtDNA, with an effective population size one-quarter that of nuclear DNA, is more sensitive to genetic drift and population bottlenecks. Parker et al. (2002) also reported low genetic variation in *D. mawsoni* with RAPD markers, and suggested that low diversity may result from either a historical bottleneck, or low metabolic rate and severe environmental conditions leading to low rates of microevolutionary change in Antarctic fishes. However genetic variability in Antarctic notothenioid fishes measured with allozyme markers fell within the range for temperate water teleosts (Anderson, 1982; McDonald et al., 1992), although South Georgian fish appear to be more variable than those from McMurdo Sound (Anderson, 1982). Low allozyme diversity was reported in *Notothenia rossii* and the icefish *Champsocephalus gunnari* which occur south of the Antarctic Convergence (Duhamel et al., 1995), but average sequence diversities were reported in *C. gunnari* with mtDNA RFLP analyses (Williams et al., 1994).

Table 2: Genotype frequencies at the *CK6*, *CK7*, *Dyst*6a and *MLL* introns, Hardy-Weinberg χ^2 tests (HWχ^2) and probabilities (*P*) and common allele frequencies (f). Migration rate of the electrophoretic bands: ff – fast; fs – medium; ss – slow; SS 48 – South Shetland Islands (Subarea 48.1); AAT 58 – Australian Antarctic Territory (Division 58.4.2); Ross 88 – Ross Sea (Subarea 88.1).

Area	ff	fs	ss	HWχ^2	*P*	f
	CK6 genotypes					
SS 48	19	29	24	2.81	0.09	0.47
AAT 58	31	34	23	4.50	0.03	0.54
Ross 88	16	19	12	1.81	0.18	0.54
Pooled	66	82	59	8.85	0.004	0.52
	CK7 genotypes					
SS 48	33	16	0	1.73	0.19	0.84
AAT 58	35	11	1	0.41	0.84	0.86
Ross 88	42	7	0	0.25	0.62	0.93
Pooled	110	34	1	0.89	0.48	0.88
	Dyst6a genotypes					
SS 48	23	21	1	2.11	0.15	0.74
AAT 58	28	15	3	0.34	0.56	0.77
Ross 88	33	11	3	2.36	0.12	0.82
Pooled	84	47	7	0.02	1.00	0.78
	MLL genotypes					
SS 48	18	27	3	2.69	0.10	0.66
AAT 58	19	24	3	1.44	0.23	0.67
Ross 88	24	19	5	0.25	0.62	0.70
Pooled	61	70	11	2.23	0.17	0.68

Because the mitochondrial and nuclear regions characterised in this study exhibited low genetic diversity in *D. mawsoni*, it is difficult to determine whether there are contemporary barriers to gene flow within the global range of the species. In *D. eleginoides*, major genetic differentiation was found between populations north and south of the Antarctic Convergence (Shaw et al., 2004; Smith and Gaffney, 2000a); and in krill (*Euphausia superba*) significant genetic differentiation was reported between samples from the Weddell Sea and South Georgia, although not among samples from the Ross, Bellingshausen and Weddell Seas (Zane et al., 1998). Finding regional genetic differentiation implies that oceanographic barriers are sufficient to restrict gene flow (Zane et al., 1998). Thus the two major sub-polar cyclonic circulations, the Weddell Sea gyre and the Ross Sea gyre (Orsi et al., 1995), may isolate populations of *D. mawsoni*.

Dispersal can occur during the pelagic larval and juvenile stages, and a lack of significant genetic differentiation would support wide dispersal across the range of the species. Small juvenile (4–15 cm long) *D. mawsoni* are believed to be mainly pelagic, after which they become benthopelagic (Roshchin, 1997). Juveniles have been caught in large numbers by Russian trawlers fishing for krill and Antarctic silverfish (*Pleuragramma antarcticum*) in surface waters (0–100 m) over bottom depths of 400–2 000 m; while larger immature fish (15–75 cm long) have been caught by bottom trawlers fishing for Wilson's mackerel icefish (*Chaenodraco wilsoni*) in the Indian Ocean (Roshchin, 1997).

Limited tag returns for *D. mawsoni* would suggest that the adults are not highly mobile, although long-distance movements may not be recognised because of the low level of fishing outside the Ross Sea area and the short history of the commercial fishery. Over 5 000 *D. mawsoni* have been tagged in McMurdo Sound (~77°S) since 1972 (DeVries and Eastman, 1998). Of the 13 recaptures, there was one long-distance movement, almost 800 km north (72°S) after 3.3 years (Hanchet et al., 2003). In the New Zealand exploratory fishery in the Ross Sea almost 2 000 *D. mawsoni* were tagged during the 2001, 2002 and 2003 seasons; the

30 recaptures have moved relatively short distances between 5 and 73 km over 3 to 695 days (Hanchet et al., 2001; Sullivan et al., 2003).

Conclusion

Populations of *D. mawsoni* are characterised by extremely low mitochondrial sequence diversity and homogeneous frequencies of both nuclear alleles and mitochondrial haplotypes among sea areas sampled. The preliminary results presented here, combined with the RAPD data (Parker et al., 2002), show little support for the notion that *D. mawsoni* is subdivided into regional populations that could form the basis of separate management units in this developing fishery. Further studies employing larger sample sizes are needed to determine whether the candidate private mitochondrial haplotypes observed in this study are indicative of restricted gene flow among sea areas.

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Tableau 2: Fréquences des génotypes pour les introns *CK6*, *CK7*, *Dyst*6a et *MLL*, les tests χ^2 d'Hardy-Weinberg (HWχ^2), les probabilités (*P*) et les fréquences des allèles communs (f). Taux de migration des bandes électrophorétiques : ff – rapide; fs – moyen; ss – lent; SS 48 – îles Shetland du Sud (sous-zone 48.1); AAT 58 – Territoire Antarctique australien (division 58.4.2); Ross 88 – mer de Ross (sous-zone 88.1).

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