

Population structure of the Patagonian toothfish around Heard, McDonald and Macquarie Islands

SHARON A. APPELYARD^{1*}, ROBERT D. WARD¹ and RICHARD WILLIAMS²

¹CSIRO Marine Research, GPO Box 1538, Hobart, TAS 7001, Australia

²Australian Antarctic Division, Channel Highway, Kingston, TAS 7050, Australia

*Corresponding author: Sharon.Appleyard@csiro.au

Abstract: Two mitochondrial DNA regions and seven microsatellite loci were examined in Patagonian toothfish from three locations in the Southern Ocean (Macquarie Island, five collections; Heard and McDonald Islands, four collections; Shag Rocks/South Georgia area, one collection). Striking mtDNA heterogeneity was detected between the three fishing locations ($F_{ST} = 0.445$, $P < 0.001$), but spatial and temporal collections within the same location were not significantly different. No significant overall microsatellite differentiation between the three locations was apparent ($F_{ST} = -0.009$, $P = 0.785$). However, some individual loci showed small but significant differentiation, which in each case was attributable to between rather than within-location differentiation. Greater differentiation of mtDNA can, in principle, be explained either by female philopatry and male dispersal, or by its greater sensitivity to changes in effective population size. The latter seems more likely as tagging indicates that toothfish is generally a sedentary species. The genetic heterogeneity between the three locations indicates restricted gene flow, with the fish at each location comprising independent units. Depletion in one location is therefore unlikely to be quickly replaced by immigration from another.

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Introduction

The Patagonian toothfish (*Dissostichus eleginoides* Smitt, 1897) is a benthopelagic shelf species of the sub-Antarctic, and is the largest member of the family Nototheniidae (the 'Notothen's') (Evseenko *et al.* 1995). The species is widely distributed from the slope waters off Chile and Argentina south of 30–35°S to the islands and shelf areas in sub-Antarctic waters of the Atlantic, Indian and Pacific Ocean sectors of the Southern Ocean (Kock *et al.* 1985, DeWitt *et al.* 1990, Kock 1992).

The Southern Ocean toothfish fishery has considerable economic value. Commercial exploitation has become significant only over the past 17 years at several fishing locations within CCAMLR (Convention on the Conservation of Antarctic Marine Living Resources) area. The total catch reported for 1999 in the CCAMLR area was 41045 t (FAO 1999). There is also unsustainable fishing pressure in some locations due to illegal, unreported and unregulated (IUU) fishing (CCAMLR 1998, Constable *et al.* 2000, Lack & Sant 2001).

Little is known about the stock structure or degree of stock separation of toothfish (CCAMLR 1995, Evseenko *et al.* 1995). Initial studies have indicated the possibility of separate toothfish stocks in the Southern Ocean with morphological evidence showing that populations around the Falkland Islands and South Georgia are distinct from each other (Kock *et al.* 1985). Chemical data from otolith cores collected from fisheries off Chile, the Falkland

Islands, Heard and McDonald Islands, Îles Kerguelen, Macquarie Island, Prince Edward Island and South Georgia suggest that there were at least four stocks over the greater Southern Ocean (Kalish & Timmis 2000). Toothfish collections from Îles Crozet and Kerguelen appear to show slight differences in an enzyme parameter (K_m) for acid phosphatase, with differences being attributed to sensitive adaptation to different local conditions (Diano 1989).

Toothfish are largely demersal, and clearly not highly migratory (DeWitt *et al.* 1990). There have been quite extensive toothfish tagging programs carried out in Australian waters at Macquarie Island and Heard and McDonald Islands for sub-adult and adult fish (Williams & Lamb 2001, Williams *et al.* 2002). Available data indicate that approximately 99.5% of toothfish recaptures ($n = 1250$) derive from the fishing ground of release, with fish having swum 15 miles or less from the point of release. Very few toothfish appear to move long distances, although three fish released at Heard Island were recaptured two to three years later at Îles Crozet, 1000 nautical miles (n.m.) away (Williams *et al.* 2002). Nonetheless, the overriding conclusion from tagging studies is that adult toothfish are essentially sedentary. Geographically separated toothfish populations are therefore expected to be quite genetically isolated from one another, at least as a function of the movement of adult fish.

Direct genetic evidence for sub-structuring of toothfish populations has come from nuclear DNA analysis. Smith &

McVeagh (2000) examined eight polymorphic microsatellite loci and seven polymorphic allozyme loci in toothfish populations from the Atlantic, Indian and Pacific Ocean sectors of the Southern Ocean. Overall, the microsatellite loci showed small but significant population differentiation while the allozyme loci showed no significant differentiation. Such microsatellite heterogeneity led Smith & McVeagh (2000) to conclude that different fishing grounds may support independent stocks. Preliminary microsatellite data led Reilly & Ward (1999) to suggest that two samples of toothfish from sites 40 n.m. apart off Macquarie Island may not be genetically homogeneous, but the sample sizes used were very small (< 20).

A combination of both mitochondrial DNA (mtDNA) and nuclear DNA analyses is more powerful than either single approach, as these genomes have unique characteristics and different modes of inheritance (mtDNA is maternally inherited while microsatellites are bi-parentally inherited) (Nei & Tajima 1981, Wilson *et al.* 1985, Avise *et al.* 1987, Moritz *et al.* 1987, Tautz 1989, Ovenden 1990, Billington & Hebert 1991, O'Connell & Wright 1997, DeWoody & Avise 2000). The study reported here employed both techniques (with microsatellite loci being used to assess nuclear DNA variation) in temporally and spatially separate collections around Macquarie Island and Heard and McDonald Islands (CCAMLR SubArea 58.5.2) in order to investigate the stock structure of toothfish within the Australian Fishing Zone (AFZ). For a more global perspective, a small collection of toothfish from Shag Rocks and South Georgia

(CCAMLR Area 48.3) was compared to the AFZ collections.

Methods

Sampling

Individual toothfish samples from within the AFZ were collected at two fishing locations: around Heard and McDonald Islands (HIMI) (south central Indian Ocean) and Macquarie Island (Macquarie) (south-west Pacific Ocean) from 1997 to 1999. Fish from the southwest Atlantic Ocean sector (Shag Rocks and South Georgia) were also examined (Table I). A hierarchical sampling design was undertaken for the analysis of toothfish stock structure within this study.

Early results from tagging experiments had shown at the time of sampling for this study, that there was little movement between fishing grounds separated by as little as 40 n.m. at the respective fishing locations. The first level of sampling was thus to collect material from each known fishing ground at each of the fishing locations. Commercial confidentiality agreements prevent us from detailing exact positions of sampling sites, but the Macquarie fishery is centred on 54°35'S, 158°50'E and the HIMI fishery is centred on 53°05'S, 73°30'E. The Macquarie fishing location is thus *c.* 3000 n.m. east of the HIMI fishing location and the intervening ocean is mostly over 3000 m deep.

Within the two fishing locations, Ground A at Macquarie is 40 n.m. from Ground B. At HIMI, Ground A is greater than 165 n.m. from Ground B and Ground B is 123 n.m. from Ground C, which is greater than 240 n.m. from Ground A. Some grounds (e.g. Macquarie Ground A and HIMI Ground B) are further divisible into 'suburbs' – usually particular trawl tracks or groups of tracks separated by not more than 5 n.m. from each other. While such subdivisions were not stratified *a priori* into suburbs, the particular suburb where samples happened to be taken was noted and such samples were initially treated separately until homogeneity tests were undertaken on each fishing ground. In all cases, fish were sampled randomly from the hauls.

A second level of sampling was designed to investigate temporal differences. Thus Macquarie Grounds A and B were sampled in November 1997 to January 1998 and January 1999, and January 1998 and January 1999 respectively. HIMI Ground B was sampled in July 1998 and April 1999 (Table I). Again, these samples were treated separately until homogeneity tests were undertaken.

All samples in the study were collected from commercial fishing boats, fishing legally for toothfish, by scientific observers. Samples comprised pieces of white muscle tissue dissected from whole fish and stored frozen in alcohol preserving solutions at -80°C until DNA extraction.

Table I. *Dissostichus eleginoides* samples, lengths and sampling locations. Suburbs were considered different locations within fishing grounds and are shown in italics

Fishing location	Ground	Suburb	Date of sampling	Sample size	Average length cm (s.e)
Macquarie	A		11.97	11	479.09 (14.84)
Macquarie	B		1.98	60	686.78 (11.41)
Macquarie	A		1.98	56	746.73 (17.68)
HIMI	B		7.98	126	656.97 (7.12)
		<i>SA</i>	7.98	25	
		<i>ES</i>	7.98	76	
		<i>HE</i>	7.98	16	
		<i>FH</i>	7.98	9	
HIMI	A		7.98	58	874.62 (35.92)
HIMI	C		9.98	73	634.30 (15.34)
Macquarie	B		1.99	78	581.19 (15.51)
		<i>CV</i>	1.99	16	
		<i>BG</i>	1.99	62	
Macquarie	A		1.99	60	660.27 (26.52)
		<i>AT</i>	1.99	40	
		<i>YL</i>	1.99	20	
HIMI	B		4.99	56	619.30 (18.58)
		<i>HG</i>	4.99	25	
		<i>FH</i>	4.99	31	
Shag Rocks	-		6.99	24	655.50 (1.44)
South Georgia	-		6.99	24	679.50 (1.43)

DNA extraction

For both mtDNA and nuclear DNA, total genomic DNA was extracted from *c.* 50 mg of tissue from individual fish using a modified CTAB (hexadecyltrimethylammonium bromide) extraction protocol (Doyle & Doyle 1987, modified as in Appleyard 1998). After precipitation with isopropanol and ethanol, genomic DNA pellets were resuspended in 150 μ l of deionised water and stored at 4°C. Genomic DNA was diluted as in Appleyard *et al.* (2001).

MtDNA PCR amplification and digestion

Restriction fragment length polymorphism (RFLP) analysis of mtDNA was examined through restriction digestion of two regions (ND2 and BCL). The ND2 fragment contains the NADH dehydrogenase subunit 2 gene and was amplified using the forward primer t-Met of Park *et al.* (1993) and the reverse primer (Mt-76) of Smith *et al.* (2001), which target the tRNA^{Trp} gene. Amplified fragments were approximately 1.1 kbp in size, as in Smith *et al.* (2001). The more variable BCL fragment contains the control region or D-loop of the mtDNA and is flanked by the transfer RNA proline gene and the 12S rRNA gene. This fragment was amplified using primers 12SAR-H (Palumbi *et al.* 1991) and L16498 (following Smith *et al.* 2001, without the GC clamp). Amplified fragments were *c.* 1.3 kbp (as in Smith *et al.* 2001).

PCR amplifications were performed in a PE-Applied Biosystems 9600 thermocycler in a total volume of 50 μ l as in Appleyard *et al.* (2001). After an initial denaturing cycle of 93°C \times 10 min, samples were subjected to 93°C \times 30 sec, 50°C \times 1 min (BCL)/55°C \times 1 min (ND2) and 72°C \times 2 min for 40 cycles with a final extension step of 72°C \times 10 min.

Based on prior investigations on toothfish mtDNA (P. Gaffney, personal communication 2000), PCR products for ND2 and BCL were digested with the enzymes *NlaIII* (New England Biolabs, 10 000 U ml⁻¹) and *BstNI* (New England Biolabs, 10 000 U ml⁻¹) respectively. The products from each restriction digest were run separately on a 2.5% agarose, 1X TBE gel (containing 0.5 μ g.ml⁻¹ ethidium bromide) at 140 volts for 40–45 min. A 100 base pair size standard (Promega) was loaded on each gel to enable sizing of various fragments. Resulting fragments were visualized under UV light and photographed using a DC120 digital camera (Kodak).

DNA microsatellite markers

Seven microsatellite loci were used (*cmrDe2*, *cmrDe9*, *cmrDe30*, *cmrDe4* and *cmrDe13* following Reilly & Ward 1999, *To2* and *To5* following Smith & Moon, unpublished). Oligonucleotides were synthesized by GeneWorks (Adelaide, South Australia) with one of the primer pairs being end-labelled with a fluorescent tag - FAM, TET or

HEX. Four loci (*cmrDe2*, *cmrDe30*, *To5* and *To2*) were optimized for use in one multiplex reaction and the remaining loci (*cmrDe9*, *cmrDe4*, *cmrDe13*) were optimized for use in another. PCR amplifications were performed in a PE-Applied Biosystems 9600 thermocycler in a total volume of 25 μ l as per Appleyard *et al.* (2001) with between 0.12–0.32 μ M for forward and reverse primers (varies according to primer). After an initial denaturing cycle of 93°C \times 10 min, samples were subjected to 93°C \times 30 sec, 54°C \times 1 min and 72°C \times 2 min for 40 cycles with a final extension step of 72°C \times 10 min.

Amplified products were used undiluted and mixed with formamide loading dye containing ABI Prism GeneScan 500 Tamra internal lane size standards (PE Applied Biosystems) and blue dextran loading dye. This was then denatured at 94°C \times 2 min, and immediately placed on ice. PCR products were then stagger loaded into a 4.8% urea (6 M) denaturing polyacrylamide gels on an ABI Prism 377 DNA sequencer (PE Applied Biosystems) following Appleyard *et al.* (2001). PCR products for each locus were analysed as in Appleyard *et al.* (2001) using GENESCAN™ 3.1 collection software (PE Applied Biosystems). GENOTYPER™ 1.1.1 software (PE Applied Biosystems) enabled the formation of approximately two (for the five dinucleotide repeat loci) and three (for the two trinucleotide repeat loci) base pair bins for each locus. Bin widths thus generally corresponded to a repeat unit.

Statistical analysis

In all instances where data were available from different toothfish suburbs, these were tested within fishing grounds for genetic homogeneity. If these groupings were not significantly different, the data were tested by fishing ground and then fishing location. If temporal collections from the various fishing grounds were available, these were also tested for genetic homogeneity.

Composite ND2 and BCL haplotypes were used for mtDNA analysis as the mtDNA genome is considered a single genotype that does not undergo recombination (Wilson *et al.* 1985). Only those individuals scored for both ND2 and BCL haplotypes were used. Levels of mtDNA variation within each collection were estimated as unbiased haplotype diversity (*h*) (following Nei 1987) (using DA in program REAP, McElroy *et al.* 1992). Haplotype diversity potentially ranges from zero (all individuals share a common haplotype) to one (all individuals have different haplotypes). Both temporal and spatial variation in mtDNA composite haplotype frequencies was assessed using standard Monte-Carlo chi-square approaches (Roff & Bentzen 1989) in the program CHIRXC (Zaykin & Pudovkin 1993), with 10 000 randomisations of the data being used to estimate *P* values. Differentiation among collections was quantified by Analysis of Molecular Variance (AMOVA) in ARLEQUIN version 2.00

Table II. Estimated approximate sizes (base pairs) of major restriction fragments for the amplified ND2 and BCL fragments of toothfish mtDNA. Variant restriction patterns are designated by capital letters. Fragments smaller than 100 bp not shown.

ND2 <i>NlaIII</i>		BCL <i>BstNI</i>						
A	B	A	B	C	D	E	F	G
900							1300	
	500							950
	400		900					
200	200	600		600		600		
					500			
		340	340	340	340	340		340
		300			300			
				280				
						210		
					130			

(Schneider *et al.* 2000). ϕ_{ST} (an analogue of F_{ST}) was obtained as the estimated variance component resulting from differences among collections divided by the estimated total variance (as in Michalakis & Excoffier 1996). Standard RFLP coding was adopted (based on the presence/absence of restriction sites, Table II) so as to take into consideration any genealogical relationships among haplotypes. Significance values for AMOVA were calculated after 16 000 permutations. F_{ST} values for all pairwise comparisons of collections and exact tests of differentiation were also undertaken using ARLEQUIN version 2.00. Significance of values was based on 100 000 Markov chain lengths.

For microsatellite data, genetic diversity for each microsatellite locus per collection was estimated by the number of alleles per locus and by the observed (H_{obs}) and Hardy-Weinberg expected (H_{exp}) heterozygosity. H_{obs} , H_{exp} and tests for deviations from Hardy-Weinberg Equilibrium (HWE) within samples were estimated using ARLEQUIN version 2.00, with significance levels determined from 100 000 Markov chain lengths. GENEPOP version 3.2 (Raymond & Rousset 2000) was used to test for linkage disequilibrium between all possible pairs of microsatellite loci, and to test for allele frequency differences at each locus among collections. Significance levels were determined after 500 batches of 5000 iterations each of a Markov chain. GENEPOP was also used to estimate F_{ST} values at each locus among all collections. As in the mtDNA analysis, AMOVA (ϕ_{ST} calculations for partitioning of microsatellite genetic variance of toothfish collections), F_{ST} comparisons (for all pairwise comparisons of collections) and global exact tests (based on all seven microsatellite loci considered jointly) were undertaken in ARLEQUIN version 2.00 (Schneider *et al.* 2000). Significance levels were based on 16 000 permutations (AMOVA) and 100 000 steps of a Markov chain procedure (F_{ST} and exact tests). The program BOTTLENECK (Piry *et al.* 1999) was also used to test for historic changes in allele frequency distributions at the seven microsatellite loci. For

Table III. Homogeneity χ^2 analysis for comparisons of composite mtDNA haplotype frequencies from various suburbs, P values are probability of H_0 . Significant values (after sequential Bonferroni correction) are shown in bold.

Fishing ground	Suburb (n)	Suburb (n)	Suburb (n)	Suburb (n)	χ^2	P
Macquarie A 1999	AT (38)	YL (19)			8.400	0.052
Macquarie B 1999	CV (14)	BG (63)			5.409	0.473
HIMI B 1998	SA (25)	ES (71)	HE (15)	FH (9)	15.081	0.601
HIMI B 1999	HG (25)	FH (29)			8.337	0.314
SubArea 48.3 1999	SR (2)	SG (13)			0.578	1.000

SR = Shag Rocks, SG = South Georgia

each polymorphic locus, 1000 simulations were undertaken for two microsatellite mutation models assuming mutation-drift equilibrium.

In all cases with multiple tests, significance levels were adjusted using a sequential Bonferroni procedure (Rice 1987). P values had to be equal to or less than this adjusted value (0.05 divided by the rank of the P value in the multiple tests) to be deemed significant.

Results

Pooling small collections for mtDNA analysis

Restriction digestion of the ND2 and BCL regions produced two and seven haplotypes respectively (Table II). Those fishing grounds that had been divided into suburbs were first tested for genetic homogeneity based on composite haplotypes from ND2 and BCL (Table III). There was no significant mtDNA heterogeneity evident between the various suburbs at the fishing grounds at Macquarie or HIMI. The suburbs that formed the sampling events for HIMI Ground B 1998 were therefore pooled as the H-B98 collection, those for HIMI Ground B 1999 pooled as H-B99, and those from Macquarie formed M-B99 and M-A99. Individuals from the Shag Rocks and South Georgia collections, both of small sample size, were pooled after chi-square homogeneity tests also detected no significant difference between the collections in the distribution of the composite haplotypes (Table III). This combined collection was referred to as SRG-99. After this pooling, ten collections were formed, each being identified by the fishing location, fishing ground, and year collected (e.g. M-A97; Macquarie, area A, 1997).

Spatial and temporal mtDNA haplotype tests for genetic homogeneity

Eleven different composite haplotypes were identified among the 557 individuals examined (Table IV). The two main composite haplotypes, AA and FA, were clearly not distributed uniformly among collections. FA was common in the Macquarie collections but rare at HIMI and SRG collections. AA was common at HIMI and a third haplotype,

Table IV. Haplotype frequencies at combined BCL and ND2 genes, sample sizes (*n*) and haplotype diversity (*h*) for mtDNA in toothfish collections within the AFZ and CCAMLR SubArea 48.3.

Haplotype	M-A97	M-B98	M-A98	M-B99	M-A99	H-B98	H-A98	H-C98	H-B99	SRG-99
AA	0.636	0.250	0.275	0.333	0.356	0.858	0.759	0.750	0.666	0.133
AB	-	0.036	0.025	0.013	0.036	0.058	0.190	0.125	0.129	-
BA	-	0.089	0.050	-	0.018	0.034	-	0.055	0.055	0.800
CA	-	-	-	-	-	0.008	-	-	-	-
DA	-	0.018	-	0.013	-	0.017	0.017	0.028	0.037	-
GA	-	-	0.025	0.013	0.036	0.017	0.017	0.028	0.019	-
GB	-	-	-	-	-	0.008	-	-	-	-
BB	-	-	-	-	-	-	-	-	0.019	-
EA	-	-	-	-	-	-	-	-	0.055	-
FA	0.364	0.571	0.575	0.600	0.554	-	0.017	0.014	-	0.067
FB	-	0.036	0.050	0.028	-	-	-	-	-	-
Parameter										
<i>n</i>	11	56	40	75	56	120	58	72	54	15
<i>h</i>	0.645	0.665	0.617	0.643	0.650	0.575	0.607	0.621	0.638	0.531

BA, was common at SRG. Within-population haplotype diversity ranged from 0.531 to 0.665, with an average of 0.619 ± 0.013 .

Pairwise tests for homogeneity of composite haplotype frequencies between collections (Table V) revealed no significant differentiation for any comparisons within fishing locations (among grounds, spatial comparisons) or between years within fishing locations (temporal comparisons). The lack of heterogeneity between collections within locations was also apparent from tests that compared multiple samples within locations simultaneously (irrespective of spatial or temporal collections, data not shown). In contrast, striking and significant differentiation between Macquarie and HIMI, between Macquarie and SRG, and between HIMI and SRG were observed (Table V). An overall global investigation of collection differentiation based on haplotype frequencies resulted in highly significant heterogeneity across HIMI, Macquarie and SRG collections ($P < 0.001$).

Pairwise F_{ST} comparisons among collections demonstrated very similar results to exact frequency tests (Table V). There were no significant F_{ST} values from pairwise comparisons of collections from the same fishing

location. In all cases however, except for the small M-A97 sample, HIMI collections were significantly different from Macquarie collections, and the SRG collection was significantly different to HIMI and Macquarie (Table V).

A hierarchical AMOVA analysis revealed a high overall ϕ_{ST} value of 0.445 ($P < 0.001$). This was almost entirely due to differences between the three localities, differences among collections within localities being trivial and non-significant. Clearly, the pronounced heterogeneity in mtDNA haplotype frequencies is attributable to spatial differences between the three fishing locations (HIMI, Macquarie and SRG).

Pooling small collections for microsatellite analysis

Following Bonferroni correction, no significant microsatellite heterogeneity was evident between any of the various suburbs within fishing grounds at the seven loci (P values ranging from 0.015 to 0.897). Samples (including those from Shag Rocks and South Georgia) were therefore pooled into the 10 collections as described in the mtDNA analyses.

Table V. Pairwise tests for homogeneity of mtDNA composite haplotype frequencies between toothfish collections sampled from the three fishing locations (temporal and spatial collections). χ^2 values below diagonal with probabilities estimated after 10 000 randomizations of the data. F_{ST} values above diagonal with probabilities estimated after 100 000 randomizations of the data. Significant values (after sequential Bonferroni correction) are shown in bold.

Collection	M-A97	M-B98	M-A98	M-B99	M-A99	H-B98	H-A98	H-C98	H-B99	SRG-99
M-A97	-	0.093	0.097	0.088	0.032	0.305	0.256	0.193	0.141	0.446
M-B98	7.082	-	-0.021	-0.009	-0.005	0.530	0.460	0.428	0.374	0.328
M-A98	5.570	3.401	-	-0.013	-0.007	0.560	0.482	0.448	0.387	0.355
M-B99	4.414	5.621	3.401	-	-0.007	0.525	0.470	0.436	0.388	0.387
M-A99	3.396	6.309	4.249	6.309	-	0.475	0.414	0.376	0.324	0.345
H-B98	45.786	98.702	91.661	89.332	83.332	-	0.036	0.003	0.022	0.676
H-A98	17.963	58.852	51.739	61.870	45.668	12.109	-	0.004	0.010	0.620
H-C98	22.519	61.375	55.801	65.148	50.844	7.757	4.900	-	-0.010	0.552
H-B99	17.625	49.392	45.079	55.063	42.785	17.213	8.891	6.291	-	0.476
SRG-99	16.349	33.498	44.075	53.673	48.776	84.761	58.219	49.608	40.818	-

Table VI. Summary of microsatellite variability data per locus in each toothfish collection.

Collection		Loci						
		<i>To5</i>	<i>To2</i>	<i>cmrDe30</i>	<i>cmrDe2</i>	<i>cmrDe13</i>	<i>cmrDe4</i>	<i>cmrDe9</i>
M-A97	N	11	11	11	11	10	11	7
	Nallele	1	8	6	15	5	8	8
	H _{obs}	-	0.727	0.636	1.000	0.700	0.818	0.571
	H _{exp}	-	0.861	0.684	0.965	0.716	0.879	0.967
M-B98	N	60	60	60	60	55	57	54
	Nallele	2	11	14	18	7	11	31
	H _{obs}	0.033	0.833	0.683	0.933	0.581	0.772	0.852
	H _{exp}	0.049	0.867	0.752	0.937	0.691	0.879	0.951
M-A98	N	56	56	56	56	54	56	51
	Nallele	2	12	15	19	8	12	30
	H _{obs}	0.036	0.788	0.750	0.893	0.593	0.875	0.941
	H _{exp}	0.053	0.813	0.827	0.946	0.691	0.886	0.955
M-B99	N	79	79	79	79	74	79	74
	Nallele	2	12	11	19	7	11	30
	H _{obs}	0.013	0.861	0.722	0.861	0.500*	0.759	0.824
	H _{exp}	0.025	0.854	0.757	0.934	0.660	0.876	0.958
M-A99	N	60	60	60	60	56	59	53
	Nallele	2	12	15	17	6	12	25
	H _{obs}	0.033	0.817	0.800	0.850	0.589*	0.763	0.850
	H _{exp}	0.082	0.824	0.761	0.941	0.670	0.864	0.951
H-B98	N	122	106	122	112	82	111	41
	Nallele	4	13	14	20	8	11	25
	H _{obs}	0.041	0.830	0.746	0.929	0.476	0.892	0.683*
	H _{exp}	0.049	0.837	0.678	0.927	0.531	0.845	0.944
H-A98	N	58	58	58	58	52	58	58
	Nallele	3	12	10	18	6	11	24
	H _{obs}	0.034	0.931	0.707	0.931	0.615	0.844	0.759*
	H _{exp}	0.085	0.859	0.752	0.936	0.683	0.879	0.964
H-C98	N	73	72	73	72	47	72	36
	Nallele	3	12	12	18	6	10	24
	H _{obs}	0.027	0.875	0.726	0.958	0.553	0.778	0.833
	H _{exp}	0.051	0.838	0.716	0.935	0.584	0.853	0.945
H-B99	N	56	55	56	54	46	53	40
	Nallele	2	11	12	19	7	11	28
	H _{obs}	0.018	0.855	0.714	0.963	0.500	0.887	0.825*
	H _{exp}	0.036	0.851	0.660	0.937	0.619	0.868	0.964
SRG-99	N	48	48	47	47	28	45	25
	Nallele	3	12	13	19	6	13	21
	H _{obs}	0.042	0.854	0.787	0.893	0.535	0.800	0.920
	H _{exp}	0.062	0.880	0.837	0.935	0.635	0.869	0.949
Mean ^b	N	62.3	60.5	69.3	60.9	50.4	60.1	43.9
	Nallele	2.4	11.5	12.2	18.2	6.6	11.0	24.6
	H _{obs}	0.028	0.837	0.727	0.921	0.564	0.819	0.806
	H _{exp}	0.049	0.848	0.748	0.939	0.648	0.870	0.955

N = total number of fish, Nallele = number of alleles, H_{obs} = observed heterozygosity, H_{exp} = expected heterozygosity under Hardy-Weinberg expectations (Nei 1978), *significant deviation from Hardy-Weinberg equilibrium after sequential Bonferroni correction per locus

Spatial and temporal microsatellite genotype tests for genetic homogeneity

Genetic diversity statistics for the toothfish collections were estimated by the numbers of alleles per locus and by observed and Hardy-Weinberg expected heterozygosity per locus and per collection (Table VI). Total numbers of alleles per locus per collection ranged from 1 to 31 (M-A97 locus *To5* to M-B98 locus *cmrDe9*). Microsatellite allele

Table VII. F_{ST} statistics at seven microsatellite loci in ten toothfish collections. Significant values (after sequential Bonferroni correction and using exact tests of allelic differentiation) are shown in bold.

Locus	No. of fish	F _{ST} *	P
<i>To5</i>	623	-0.001	0.168
<i>To2</i>	605	0.005	< 0.001
<i>cmrDe30</i>	693	0.006	0.002
<i>cmrDe2</i>	609	0.000	0.015
<i>cmrDe13</i>	504	0.005	0.026
<i>cmrDe4</i>	601	0.005	< 0.001
<i>cmrDe9</i>	439	0.003	0.002

*F statistics are estimated as in Weir & Cockerham (1984)

frequencies at each of the seven loci are available from the authors upon request. Nei's (1978) unbiased estimate of expected heterozygosity per locus (under HWE) for each of the collections ranged from 0.000 (M-A97, *To5*) to 0.967 (M-A97, *cmrDe9*) (Table VI).

Genotype proportions in each collection for each locus were tested for goodness-of-fit to HWE (Table VI). After Bonferroni correction, *cmrDe13* (in M-B99 and M-A99) and *cmrDe9* (in H-A98, H-B98 and H-B99) demonstrated significant deviations from expectations, in each case with heterozygote deficiencies. As only five of the 69 tests were significant, in general it was considered that the collections accorded satisfactorily with Hardy-Weinberg expectations. Linkage disequilibrium tests demonstrated no significant linkage disequilibrium (after correction) in any collection except between *cmrDe2* and *cmrDe9* in M-A99. As this association was not observed in any other collection, and the level of observed linkage across all collections was only 0.51%, it was concluded that the seven loci were not linked and that they represented independent genetic markers.

The extent of microsatellite differentiation among the ten collections was very limited. F_{ST} values for each locus were all less than 1% (Table VII), but exact tests showed that five loci (after Bonferroni correction) did in fact show significant allelic differentiation (*To2*, *cmrDe30*, *cmrDe2*, *cmrDe4* and *cmrDe9*). Exact tests provided no evidence for temporal heterogeneity of allele frequencies at any locus among the collections (data not shown), but did reveal nine (out of 315) instances of significant (all P < 0.001) spatial differentiation of pairwise collection comparisons after Bonferroni correction (*To2*: H-B98 & M-B99; *cmrDe30*: H-B98 & M-A98, H-B99 & M-A98; *cmrDe2*: H-B98 & M-B99; *cmrDe13*: H-B98 & M-A99; *cmrDe4*: H-B98 & M-B98, H-B98 & M-A98, H-C98 & M-B98, H-C98 & M-A98). All these differences were between fishing localities; none were within localities.

Pairwise location comparisons based on F_{ST} values (when all loci were considered jointly) were not significant. Overall exact test of collection heterogeneity (again when loci were considered jointly), also showed no significant differentiation among all collections (P = 1.000). Similarly, the hierarchical AMOVA showed that φ_{ST} within the ten

Table VIII. Mutation-drift computations and probability distributions based on the IAM and SMM models used to test for heterozygosity excess at seven microsatellite loci in ten toothfish collections (as calculated in BOTTLECK, Piry *et al.* (1999))

Collection	Model ^a	Sign test ^b		P^c	P_{excess}^d	Mode shift ^e
		Deficiency	Excess			
M-A97	IAM	2	4	0.580	0.000	normal
	SMM	3	3	0.414	0.000	normal
M-B98	IAM	2	5	0.392	0.000	normal
	SMM	4	3	0.294	0.000	normal
M-A98	IAM	1	6	0.124	0.000	normal
	SMM	5	2	0.123	0.000	normal
M-B99	IAM	1	6	0.133	0.000	normal
	SMM	4	3	0.341	0.000	normal
M-A99	IAM	3	5	0.362	0.000	normal
	SMM	5	2	0.121	0.000	normal
H-B98	IAM	3	4	0.601	0.000	normal
	SMM	6	1	0.022	0.001	normal
H-A98	IAM	1	6	0.138	0.000	normal
	SMM	4	3	0.300	0.000	normal
H-C98	IAM	3	4	0.614	0.000	normal
	SMM	5	2	0.108	0.000	normal
H-B99	IAM	3	4	0.634	0.000	normal
	SMM	4	3	0.335	0.000	normal
SRG-99	IAM	1	6	0.147	0.000	normal
	SMM	5	2	0.102	0.000	normal

^aMutation models demonstrating the relationship between number of alleles and heterozygosity under IAM (Watterson 1984) and SMM (Chakraborty & Nei 1977).

^bNumbers of loci showing a heterozygosity deficiency or excess (Cornuet & Luikart 1996)

^c P = probability of number of loci with heterozygosity excess not significantly different to that expected under mutation-drift equilibrium (Cornuet & Luikart 1996)

^d P = probability of all loci with heterozygosity excess in an equilibrium population; if a bottleneck has occurred, heterozygote excess is observed more often (Cornuet & Luikart 1996)

^eLuikart *et al.* (1997) descriptor of allele frequency distribution, discriminates bottlenecked populations from stable populations where allele frequencies expected under a mutation-drift equilibrium display normal L-shaped distributions

collections (-0.021 , $P = 1.000$), among the three localities (-0.009 , $P = 0.785$) and among collections within localities was also non-significant (-0.012 , $P = 1.000$).

The three tests outlined in BOTTLENECK (Piry *et al.* 1999) showed no significant departure from allele frequencies expected under mutation-drift equilibrium at the seven microsatellite loci in any of the toothfish collections (Table VIII). The mode shift indicator (calculated under either the infinite alleles model, IAM (Watterson 1984) or the stepwise mutation model, SMM (Chakraborty & Nei 1977)) also demonstrated that the microsatellite allele frequencies in all ten toothfish collections were normal (i.e., that expected under mutation-drift equilibrium)(Table VIII).

Discussion

RFLP analyses of the ND2 and BCL fragment of the

mtDNA genome in toothfish from the Southern Ocean revealed moderate levels of variation with 11 composite haplotypes being detected. Striking and highly significant heterogeneity between HIMI, Macquarie and SRG fishing localities was detected, with about 40% of all variation being ascribable to differences between these locations. No significant temporal or spatial differences were observed within the same fishing location.

In comparison, high levels of variation were detected at seven microsatellite loci with between six (*To5*) and 36 (*cmrDe9*) alleles per locus. Evidence of small but significant allelic differentiation among collections was found for most loci when loci were considered separately. This reflected small differences between localities, although no significant differences were observed between grounds from the same fishing locality. This more extensive data analysis therefore does not support preliminary results on the five *cmrDe* microsatellite loci that suggested small differences between different fishing sites at Macquarie Island (Reilly & Ward 1999). Sample sizes were much larger in the current study and temporal data from the same fishing ground were also available. The observed small differences at individual loci between localities disappeared when all loci were considered jointly, resulting in no significant microsatellite heterogeneity between the HIMI, Macquarie and SRG fishing localities.

Smith & McVeagh (2000) used the same suite of microsatellite loci plus one more (*To3*) on 30 to 50 fish from each of the Ross Dependency, Macquarie Island, Heard Island, Prince Edward Island and the Falkland Islands. Five of the eight loci showed small but significant allelic differentiation, and the mean F_{ST} at 0.028, was likewise small but significant. Their data indicated genetic patchiness with regional differentiation rather than a relationship between genetic diversity and geographic separation of the samples. Our microsatellite data suggest rather less differentiation. While five of the seven loci did show small but significant allelic differentiation, our mean F_{ST} across the ten collections (estimated from the F_{ST} values of the seven individual loci considered individually) was only 0.003, and the overall ϕ_{ST} (estimated from a pooled analysis of all loci) was a negative -0.021 . However, we did observe some scattered instances of significant inter-locality differences for particular loci. We did not find that the Macquarie allele frequencies for *To5* were significantly different from those at other localities, including HIMI, as reported by Smith & McVeagh (2000). Our total sample sizes were somewhat larger (ranging from 439 to 623 individuals per locus) than those of Smith & McVeagh (2000) ($n = 196-230$). Allozyme data from eight polymorphic allozyme (nuclear) loci were also assessed by Smith & McVeagh (2000), but no significant sample heterogeneity was recorded.

Combining the various genetic data sets, the overall picture for toothfish populations within the Southern Ocean

appears to be one of genetic differentiation between well-separated fishing localities (best detected with mtDNA analysis) and minimal differentiation between grounds within specific localities.

Spawning grounds for toothfish within the Southern Ocean are unknown (Evseenko *et al.* 1995), but spawning is thought to be in deep water (*c.* 1500 m) over the various continental slopes and probably between March and August (K.-H. Kock personal communication 1993, Evseenko *et al.* 1995, Des Clers *et al.* 1996). The pelagic eggs may remain in the water column for up to three months (Kellerman 1990, Evseenko *et al.* 1995) but the duration of the pelagic phase of toothfish larvae is unknown. Larvae may be displaced by the ocean currents, but may be geographically restricted to specific grounds if trapped in local gyres (Orsi *et al.* 1995).

In the case of Heard and McDonald Islands, toothfish appear to settle on the shallow plateau (< 500 m deep) and move into the fishing grounds on the peripheral slopes when about four years old (A. Constable, R. Williams, T. Lamb & E. Van Wijk personal communication 2001). Tagging data shows that adult toothfish do not generally move more than about 15 miles from their tagging point, even after several years, and rarely move between grounds even within fishing localities (Williams & Lamb 2001, Williams *et al.* 2002) thus indicating that they are clearly not highly migratory (DeWitt *et al.* 1990). It has therefore been thought that deep water basins might prevent toothfish mixing between fishing locations, however four toothfish tagged at Heard and McDonald Islands have recently made long distance movements (Williams *et al.* 2002). One was recaptured at Îles Kerguelen (200 n.m. from HIMI) and three were recaptured at Îles Crozet (*c.* 1000 n.m. away). These fish had been at liberty for 1.8 to 3.1 years, and this demonstrates that under some circumstances toothfish are (albeit rarely) capable of wide movement across deep ocean waters.

How does this information on dispersal capabilities correlate with the genetic data? The genetic homogeneity among fishing grounds at particular fishing locations (such as those at Macquarie Islands or Heard and McDonald Islands) may be maintained by gene flow brought about by few adult fish that do move between grounds within localities, together with pelagic egg and larval drift over short distances (*c.* 40–200 n.m.). The genetic heterogeneity between the three fishing locations considered here is no doubt due to their isolation – tagging data have not indicated any adult movements between Macquarie Island and Heard and McDonald Islands. Geographical separation of the islands (*c.* 3000 n.m.) would also minimise the chance of successful egg or larval admixture via drift in oceanic currents. The Shag Rocks/South Georgia location is still further separated from the two AFZ locations.

The mtDNA evidence strongly rejects the null hypothesis of a single panmictic toothfish population in the Southern

Ocean, yet the conclusion from the nuclear DNA is less clear. MtDNA is solely maternally inherited, while nuclear DNA is bi-parentally inherited. The greater extent of mtDNA differentiation could, in principle, reflect females returning to their place of origin for reproduction while males disperse. However, we are not aware of any data that suggest that female toothfish are more philopatric than males. Adult toothfish are relatively sedentary, and the sexes of the three exceptional toothfish that have swum about 1000 n.m. are unknown (Williams *et al.* 2002). Sex biased dispersal in marine mammals has been used to explain significant mtDNA heterogeneity observed between ocean basins (Palumbi & Baker 1994, Berube *et al.* 1998, Lyrholm *et al.* 1999, Rosel *et al.* 1999).

Another, and perhaps more likely explanation, is that mtDNA is more sensitive to genetic drift and population bottlenecks than nuclear DNA. MtDNA has an effective population size only one quarter that of nuclear DNA due to its haploid nature and maternal-only inheritance (Wilson *et al.* 1985). If the toothfish populations in the various fishing locations had ever been reduced to small numbers of individuals, or had been founded from small numbers of individuals, then genetic drift would be reflected in greater mtDNA than nuclear DNA differentiation. Evidence to differentiate the alternative scenarios could be gathered by mtDNA sequence data and clade analysis, but this could not be undertaken in the current study. A bottleneck analysis of the microsatellite allele frequency data (investigating historic frequency changes) was however carried out using the methods of Cornuet & Luikart (1996) and Piry *et al.* (1999). No evidence for population bottlenecks was observed at any of the microsatellite loci; these loci also showed no differentiation (exact tests, AMOVA) among toothfish collections. Therefore, mtDNA differentiation may reflect historic genetic drift or bottlenecks among the toothfish collections that are not detected with the nuclear microsatellite data.

Toothfish from Îles Kerguelen and Îles Crozet should now be examined in order to determine the extent of genetic differentiation between these sites and Heard and McDonald Islands, given the occasional migrant shown by tagging. Such genetic studies, especially of mtDNA, are planned and should help to resolve further gene flow issues among toothfish stocks within the western Indian Ocean sector. Further studies of South Atlantic and South American toothfish are also warranted.

Conclusion

In the current study, mtDNA was a more powerful indicator of toothfish population structure than microsatellite DNA. MtDNA analyses demonstrated striking genetic differentiation between the three fishing localities examined, indicating very restricted gene flow among the regions. These results will contribute to the more effective

management of commercial fisheries for Patagonian toothfish in Australian fishing waters and indeed more globally, as several genetically differentiated toothfish populations from fishing locations across the Southern Ocean have been identified. Geographic isolation by distance (due to deep water basins and a non-migratory species) and low levels of ocean drift of young pelagic stages over large distances are likely to be the factors that contribute to the restricted gene flow between fishing locations. Careful management of toothfish fisheries is therefore required, as depletion in one location is unlikely to be quickly replaced by immigration from another.

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References

- APPELYARD, S.A. 1998. *Application of genetic markers to Fijian Tilapia stock improvement*. PhD thesis, Queensland University of Technology, Brisbane, Australia, 181 pp. [Unpublished]
- APPELYARD, S.A., GREWE, P.M., INNES, B.H. & WARD, R.D. 2001. Population structure of yellowfin tuna (*Thunnus albacares*) in the western Pacific Ocean, inferred from microsatellite loci. *Marine Biology*, **139**, 383–393.
- AVISE, J.C., ARNOLD, J., BALL, R.M., BERMINGHAM, E., LAMB, T., NEIGEL, J.E., REEB, C.A. & SOUNDERS, N.C. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, **18**, 489–522.
- BERUBE, M., AGUILAR, A., DENDANTO, D., LARSEN, S.F., NOTARBARTOLO DI SCIARA, G., SEARS, R., SIGURJONSSON, J., URBAN, R.J. & PALSBO, P.J. 1998. Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus 1758): analysis of mitochondrial and nuclear loci. *Molecular Ecology*, **7**, 585–599.
- BILLINGTON, N. & HEBERT, P.D.N. 1991. Mitochondrial DNA diversity of fishes and its implications for introductions. *Canadian Journal of Fisheries and Aquatic Science*, **48** (Supplement 1), 80–94.
- CCAMLR. 1995. *Report of the Fourteenth Meeting of the Commission (CCAMLR–XIV)*. Hobart: CCAMLR, 153 pp.
- CCAMLR. 1998. *Report of the Seventeenth Meeting of the Commission (CCAMLR–XVII)*. Hobart: CCAMLR, 166 pp.
- CHAKRABORTY, R. & NEI, M. 1977. Bottleneck effects on average heterozygosity and genetic distance with the stepwise mutation model. *Evolution*, **31**, 347–356.
- CONSTABLE, A.J., DE LA MARE, W.K., AGNEW, D.J., EVERSON, I. & MILLER, D. 2000. Managing fisheries to conserve the Antarctic marine ecosystem: practical implementation of the Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR). *ICES Journal of Marine Science*, **57**, 778–791.
- CORNUET, J.M. & LUIKART, G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.
- DES CLERS, S., NOLAN, C.P., BARANOWSKI, R. & POMPERT, J. 1996. Preliminary stock assessment of the Patagonian toothfish longline fishery around the Falkland Islands. *Journal of Fish Biology*, **49** (Supplement A), 145–156.
- DEWITT, H., HEEMSTRA, P.C. & GON, O. 1990. Nototheniidae. In GON, O. & HEEMSTRA, P.C., eds. *Fishes of the Southern Ocean*. Grahamstown: J.L.B. Smith Institute of Ichthyology, 279–331.
- DEWOODY, J.A. & AVISE, J.C. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animal. *Journal of Fish Biology*, **56**, 461–473.
- DIANO, M. 1989. Enzymatic data as a criterion for characterising two populations of *Dissostichus eleginoides* (Nototheniidae). *Marine Biology*, **103**, 417–420.
- DOYLE, J.J. & DOYLE, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin*, **19**, 11–15.
- EVSEENKO, S.H., KOCK, K.-H. & NEVINSKY, M.M. 1995. Early life history of the Patagonian toothfish, *Dissostichus eleginoides* Smitt, 1897 in the Atlantic sector of the Southern Ocean. *Antarctic Science*, **7**, 221–226.
- FAO. 1999. *FAO Yearbook. Fishery statistics – capture production*. Rome: FAO Fisheries Series No. 57, FAO Statistics Series No. 154, 752 pp.
- KALISH, J.M. & TIMMISS, T. 2000. *Determination of Patagonian toothfish age, growth and population characteristics based on otoliths*. Report for the Fisheries Research and Development Corporation FRDC No. 97/123. Canberra: Australian National University.
- KELLERMANN, A. 1990. Catalogue of early life stages of Antarctic Notothenioid fishes. *Berichte zur Polarforschung*, **67**, 45–136.
- KOCK, K.-H. 1992. *Antarctic fish and fisheries*. Cambridge: Cambridge University Press, 359 pp.
- KOCK, K.-H., DUHAMEL, G. & HUREAU, J.-C. 1985. Biology and status of exploited Antarctic fish stocks: a review. *BIOMASS Scientific Series*, **6**, 1–143.
- LACK, M. & SANT, G. 2001. Patagonian toothfish – are conservation and trade measures working? *TRAFFIC Bulletin*, **19**, 1–18.
- LUIKART, G., ALLENDORF, F.W., CORNUET, J.M. & SHERWIN, W.B. 1997. Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity*, **89**, 238–247.
- LYRHOLM, T., LEIMAR, O., JOHANNSEON, B. & GYLLENSTEN, U. 1999. Sex-biased dispersal in sperm whales: contrasting mitochondrial and nuclear genetic structure of global populations. *Proceedings of the Royal Society of London*, **B266**, 347–354.
- MCELROY, D., MORAN, P., BERMINGHAM, E. & KORNFIELD, I. 1992. *REAP. The Restriction Enzyme Analysis Package, version 4.0*. Orono, ME: Department of Zoology, Migratory Fish Research Institute and Centre for Marine Studies, University of Maine.
- MICHALAKIS, Y. & EXCOFFIER, L. 1996. A generic estimation of population subdivision using distances between alleles with special reference to microsatellite loci. *Genetics*, **142**, 1061–1064.
- MORITZ, C., DOWLING, T.E. & BROWN, W.M. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annual Review of Ecology and Systematics*, **18**, 269–292.
- NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.
- NEI, M. 1987. *Molecular Evolutionary Genetics*. New York: Columbia University Press, 512 pp.
- NEI, M. & TAJIMA, F. 1981. DNA polymorphism detectable by restriction endonucleases. *Genetics*, **97**, 145–163.

- O'CONNELL, M. & WRIGHT, J.M. 1997. Microsatellite DNA in fishes. *Reviews in Fish Biology and Fisheries*, **7**, 331–363.
- ORSI, A.H., WHITWORTH, T. & NOWLIN, W.D. 1995. On the meridional extent and fronts of the Antarctic circumpolar current. *Deep-Sea Research*, **42**, 641–673.
- OVENDEN, J.R. 1990. Mitochondrial DNA and marine stock assessment: a review. *Australian Journal of Marine and Freshwater Research*, **41**, 835–853.
- PALUMBI, S.R. & BAKER, C.S. 1994. Contrasting population structure from nuclear intron sequences and mtDNA of humpback whales. *Molecular Biology and Evolution*, **11**, 426–435.
- PALUMBI, S.R., MARTIN, A., ROMANO, S., McMILLAN, W.O., STICE, L. & GRABOWSKI, G. 1991. *The simple fool's guide to PCR*. 2nd ed. Honolulu, HI: Department of Zoology, University of Hawaii, 47 pp.
- PARK, L.K., BRAINARD, M.A., DIGHTMAN, D.A. & WINANS, G.A. 1993. Low levels of intraspecific variation in the mitochondrial DNA of chum salmon (*Oncorhynchus ketu*). *Molecular Marine Biology and Biotechnology*, **2**, 362–370.
- PIRY, S., LUIKART, G. & CORNUET, J.M. 1999. BOTTLENECK: A program for detecting recent effective population size reductions from allele data frequencies. *Journal of Heredity*, **90**, 502–503.
- RAYMOND, M. & ROUSSET, F. 2000. Updated version of GENEPOP (v. 1.2) as described in: RAYMOND, M. AND ROUSSET, F. 1995. GENEPOP (v. 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- REILLY, A. & WARD, R.D. 1999. Microsatellite loci to determine stock structure of the Patagonian toothfish *Dissostichus eleginoides*. *Molecular Ecology*, **8**, 1753–1756.
- RICE, W.R. 1987. Analysing tables of statistical tests. *Evolution*, **43**, 223–225.
- ROFF, D.A. & BENTZEN, P. 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. *Molecular Biology and Evolution*, **6**, 539–545.
- ROSEL, P.E., FRANCE, S.C., WANG, J.Y. & KOCHER, T.D. 1999. Genetic structure of harbour porpoise *Phocoena phocoena* populations in the northwest Atlantic based on mitochondrial and nuclear markers. *Molecular Ecology*, **8**, S41–S54.
- SCHNEIDER, S., ROSSELLI, D. & EXCOFFIER, L. 2000. *ARLEQUIN ver 2.00: A software for population genetic data analysis*. Geneva: Genetics and Biometry Laboratory, University of Geneva.
- SMITH, P. & McVEAGH, M. 2000. Allozyme and microsatellite DNA markers of toothfish population structure in the Southern Ocean. *Journal of Fish Biology*, **57** (Supplement A), 72–83.
- SMITH, P.J., GAFFNEY, P.M. & PURVES, M. 2001. Genetic markers for identification of Patagonian toothfish and Antarctic toothfish. *Journal of Fish Biology*, **58**, 1190–1194.
- TAUTZ, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research*, **17**, 6463–6471.
- WATTERSON, G.A. 1984. Allele frequencies after a bottleneck. *Theoretical Population Biology*, **26**, 387–407.
- WEIR, B.S. & COCKERHAM, C.C. 1984. Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.
- WILLIAMS, R. & LAMB, T. 2001. History of the toothfish fishery. In HE, X. & FURLANI, D., eds. *Ecologically sustainable development of the fishery for Patagonian toothfish (Dissostichus eleginoides) around Macquarie Island*. Report for the Fisheries Research and Development Corporation FRDC No. 97/122. Hobart, TAS: CSIRO Marine Research.
- WILLIAMS, R., TUCK, G.N., CONSTABLE, A. & LAMB, T. 2002. Movement, growth and available abundance to the fishery of *Dissostichus eleginoides* Smitt, 1898 at Heard Island derived from tagging experiments. *CCAMLR Science*, **9**, 33–48.
- WILSON, A.C., CANN, R.L., CARR, S.M., GEORGE, M., GYLLENSTEN, U.B., HELM-BYCHOWSKI, K.M., GIGUCHI, R.G., PALUMBI, S.R., PRAGER, E.M., SAGE, R.D. & STONEKING, M. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society*, **26**, 375–400.
- ZAYKIN, D.V. & PUDOVKIN, A.I. 1993. Two programs to estimate significance of χ^2 values using pseudo-probability tests. *Journal of Heredity*, **84**, 152.