New Zealand Journal of Marine and Freshwater Research, 2002, Vol. 36: 737–750
0028–8330/02/3604–0737 $7.00

Genetic and meristic variation in black and smooth oreos in the New Zealand Exclusive Economic Zone

P. J. SMITH
P. J. McMILLAN
B. BULL
S. M. McVEAGH
National Institute of Water and Atmospheric Research Limited
P.O. Box 14 902
Wellington, New Zealand
email: p.smith@niwa.cri.nz

P. M. GAFFNEY
College of Marine Studies
University of Delaware
Lewes
DE 19958, United States

S. CHOW
Division of Pelagic Fish Resources
National Research Institute of Far Seas Fisheries
Orido 5-7-1, Smimizu
Shizuoka 424, Japan

Abstract Meristic and genetic methods were used to determine the stock relationships of black *Allocytus niger* (James, Inada & Nakamura, 1988) and smooth oreo *Pseudocyttus maculatus* (Gilchrist, 1906) in the New Zealand Exclusive Economic Zone (EEZ). Samples were collected from four management areas—OEO 1 (south-west), OEO 3A (Chatham Rise west), OEO 4 (Chatham Rise east), and OEO 6 (subantarctic) during the 1998 October–December spawning period. Lateral line scale counts and pyloric caeca counts revealed differences between black oreo samples from OEO 6 and the other three OEO management areas. Lateral line scale counts in smooth oreo showed no significant differences between areas. Genetic analyses of four non-coding regions of nuclear DNA and mitochondrial (mt) DNA haplotypes in black oreo showed no significant differentiation among the four management areas. Likewise for smooth oreo, genetic analyses of five non-coding regions of nuclear DNA and mtDNA haplotypes showed no overall regional differentiation, although there was weak evidence at one locus for a difference between OEO 3A and OEO 6. The data do not reject the null hypothesis of a single genetic stock in New Zealand waters, and are typical of marine species with long pelagic juvenile stages.

Keywords oreo; *Allocytus niger*; *Pseudocyttus maculatus*; DNA introns; mitochondrial DNA; meristics; stock discrimination

INTRODUCTION

Two species of oreo (family Oreosomatidae), the black oreo *Allocytus niger* (James, Inada & Nakamura, 1988) and the smooth oreo *Pseudocyttus maculatus* (Gilchrist, 1906), support major fisheries in the New Zealand Exclusive Economic Zone (EEZ), and smaller fisheries in the Australian Fishing Zone. In New Zealand, black and smooth oreo, together with the less abundant spiky oreo *Neocyttus rhomboidalis*, have been managed together under a combined quota. The fisheries have been divided into five management areas (Fig 1): the North Island east and west coasts and South Island west and south coasts, including the Puysegur-Snares-Macquarie Ridge, (Management area OEO 1); the South Island east coast and Chatham Rise west (OEO 3A); the Chatham Rise east (OEO 4); the subantarctic, including the Campbell and Bounty Plateaux (OEO 6); and the Kermadec area (OEO 10).

The main oreo fisheries are on the Chatham Rise (OEO 3A and OEO 4) and were developed by Soviet vessels in the early 1970s, with catches reaching a peak of c. 27 000 t in 1981–82 (Annala et al. 1998).

M02028; Published 14 November
Received 19 April 2002; accepted 7 August 2002

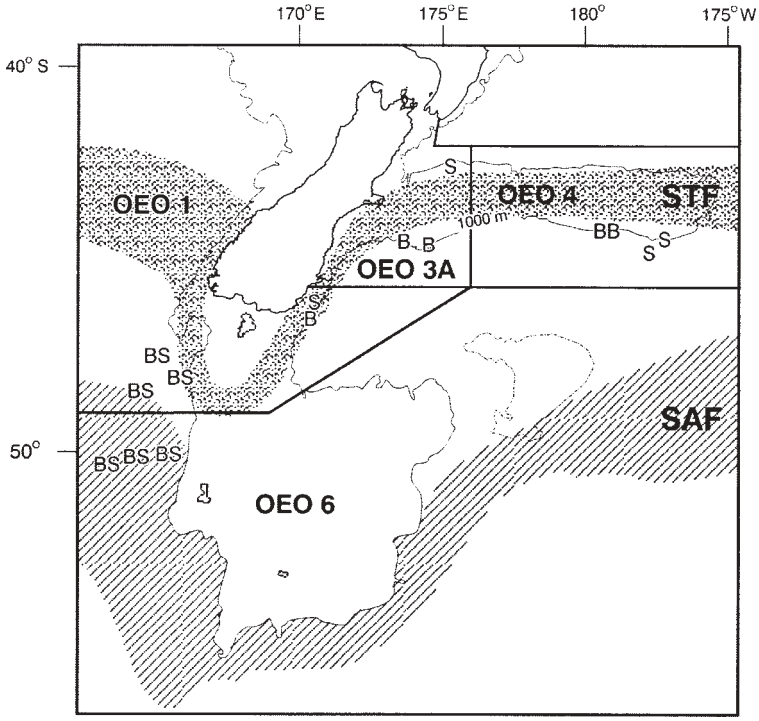

Fig. 1 Location of black *Allocytus niger* (B) and smooth *Pseudocyttus maculatus* (S) oreo sampling sites and oreo fishery management areas (OEO) around New Zealand. (STF = Subtropical Front; SAF = Subantarctic Front.)

The two fisheries are separated by c. 100 n miles (Doonan et al. 1995). The western fishery (OEO 3A) is a target fishery for black and smooth oreo which are caught in approximately equal quantities, with a minor by-catch of orange roughy *Hoplostethus atlanticus*. The eastern fishery (OEO 4) is a target fishery for orange roughy with a bycatch of black and smooth oreo, the former in much smaller quantities. Subsequently, other oreo fisheries have developed on the Puysegur-Snares-Macquarie Ridge (OEO 1) and on the Pukaki and Bounty slopes (OEO 6).

Black and smooth oreo are both slow growing and long lived, with maximum ages of 150 and 86 years respectively, but have different growth rates, natural mortalities, depth, and geographical distributions (Annala et al. 1998), and probably different population sizes, and therefore could be managed as separate species. If black and smooth oreos are managed as separate species then it is appropriate to base the management units on biologically meaningful stocks.

There have been few stock discrimination studies on black and smooth oreos compared with other deep water marine species such as orange roughy (Elliot & Ward 1992; Smolenski et al. 1993; Smith et al. 1997). Genetic studies on oreos have focused on phylogeny; allozyme markers have shown relatively high levels of genetic variation (Lowry et al. 1996), but no evidence for discrete stocks (Ward et al. 1996). A mitochondrial (mt) DNA haplotype found in small numbers of black oreo (6/96) from Tasmania, was absent in a sample (0/76) from the east of New Zealand, providing weak evidence for trans-Tasman differentiation (Ward et al. 1998). Allozyme and mtDNA haplotype frequencies among samples of smooth oreo from eastern New Zealand and Australia showed no evidence of genetic differentiation (Ward et al. 1998).

Meristic differences in lateral line scale counts and number of pyloric caeca have been reported between samples of black oreo from Australia and New Zealand (Ward et al. 1996), although sample sizes were small. Lateral line scale counts in smooth oreo showed significant differentiation among Australian samples (Ward et al. 1996) but have not been tested in New Zealand specimens.

Here we use two stock discrimination tools, genetics and meristics, on black and smooth oreo in the New Zealand EEZ.

MATERIALS AND METHODS

Samples

Samples of black and smooth oreo were collected from the major fisheries in four oreo management areas in the New Zealand EEZ: OEO 1, OEO 3A, OEO 4, and OEO 6 (Fig. 1). Samples were collected through the Ministry of Fisheries Observer programme and NIWA research voyages during the 1998 spawning season (October–December). In each area muscle tissue samples were collected from up to 100 black oreo and 100 smooth oreo; based on two sets of 25 fish from four tows. For each fish c. 5 g of muscle tissue was removed from the abdomen and frozen in individual plastic bags. A further 100 black oreo and 100 smooth oreo, based on two sets of 25 fish from four tows, were collected whole and undamaged from each area for meristic counts made in the laboratory.

Genetics

DNA was extracted from frozen black and smooth oreo muscle tissue samples using proteinase-K digestion and phenol-chloroform extraction, modified from Bruford et al. (1992). DNA was then washed with 70% ethanol, precipitated by centrifugation, air dried, and re-suspended in 40 µl of sterile water and stored at –20°C. DNA concentrations were estimated with a Hoefer DyNA Quant fluorometer and a subset of dilutions made to provide 50 ng DNA/µl. A suite of primers was used to amplify different regions of the mitochondrial and nuclear genomes (Appendices 1 and 2). Amplification reactions were performed in 50 µl volumes overlaid with mineral oil in a Perkin Elmer Cetus DNA thermocycler or in 50 µl volumes in a Perkin Elmer 9600 thermocycler. The presence of a single band in the amplified product was taken to indicate optimum amplification conditions. Smooth oreo analyses were undertaken in 1998 and black oreo analyses in 1999.

Nine regions of mtDNA were tested in both black and smooth oreo (Appendix 1), but only those regions with a single amplified product smaller than c. 500 bp were screened for polymorphisms with 5- and 4-base restriction enzymes. In black oreo the ND1, ND2, and COII regions were digested independently with 19 restriction enzymes (*Alu* I, *Ava* II, *Bst* NI, *Bst* UI, *Cfo* I, *Dde* I, *Dpn* I, *Hae* III, *Hin*f I, *Msp* I, *Nci* I, *Nde* II, *Nla* III, *Sau* 3A, *Sau* 96 I, *ScrF* I, *Rsa* I, *Taq* I, and *Tsp 509* I), and in smooth oreo the control regions 1 and 2 were digested independently with 10 restriction enzymes (*Alu* I, *BSt* UI, *Cfo* I, *Hae* III, *Hin*f I, *MSp* I, *Nla* III, *RSa* I, *Sal* I, and *Taq* I). Twelve fish of each species (3 fish × 4 management areas) were used in the initial screening. Enzyme digestions were performed in 20 µl volumes for a minimum of 4 h, following the manufacturer's recommendations (New England BioLabs, Beverly, MA, United States). 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, which had been incorporated into the gel, and viewed under UV light.

Introns, non-coding regions of DNA, are more variable than protein-coding exon sequences (Chow & Takeyama 2000). Introns producing small amplified (<500 bp) regions were screened for size polymorphisms in acrylamide gels of varying percentage (5, 10, 15%) and agarose gels. Regions of the genome revealing polymorphism were tested in larger samples (28–92 fish) from each of the four oreo management areas.

Heterogeneity in mtDNA haplotype frequencies in the total data was tested by the χ^2 randomisation test described by Roff & Bentzen (1989) using the REAP package (McElroy et al. 1992). This method overcomes the problem of a large number of observed haplotypes at low frequency by comparing χ^2 values in 1000 random rearrangements of the data. Probabilities were estimated from the number of randomisations that were equal to or greater than the observed χ^2 value. Analyses were carried out on individual regions of mtDNA/restriction enzymes and on composite haplotypes. The proportion of haplotype variation due to differentiation between population samples was estimated with Nei's gene-diversity statistic, G_{ST} (Nei 1973). Sampling error will produce differences in haplotype frequencies, even when samples are drawn from the same population, therefore a randomisation test was used to assess if differences were the result of sampling error (Elliott & Ward 1992). One thousand randomisations were used and the probability was estimated from the number of randomisations that were equal to or greater than the observed G_{ST}.

Genotype frequencies at intron loci were tested for Hardy-Weinberg (HW) equilibrium with χ^2 tests carried out on observed and expected numbers of genotypes. A Monte Carlo χ^2 test was used when there were several cells with less than five fish (Zaykin & Pudovkin 1993). Allele frequencies at each locus were tested for heterogeneity among areas with contingency χ^2 tests using the GENEPOP

program (Raymond & Rousset 1995); probability levels were modified by the Bonferroni procedure for multiple tests after Rice (1989). The proportion of intron variation because of differentiation among populations was estimated with G_{ST} (Nei 1973), as described for mtDNA.

Meristics

Meristic counts were restricted to those characters that have shown regional differentiation: lateral line scale counts and number of pyloric caeca in black oreo between Australia and New Zealand (Ward et al. 1996), and lateral line scale counts in smooth oreo among Australian samples (Ward et al. 1996).

Lateral line scales were counted in black and smooth oreos from the four fishery management areas: OEO 1, OEO 3A, OEO 4, and OEO 6. Scales were counted in undamaged whole specimens, along the entire lateral line, on the left side. The length and sex of each individual were recorded and the otoliths removed for settlement zone analyses. The black oreos were dissected and the pyloric caeca counted.

Differences in scale counts and pyloric caeca counts between sampling areas were tested by linear regression, controlling for fish length and sex.

RESULTS

Black oreo mtDNA

The initial screening found two regions of mtDNA that revealed polymorphism when cut independently with six restriction enzymes (ND1: *Hae* III, *Msp* I, *Sau* 96 I, and *ScrF* I; and ND2: *Alu* I, and *Hin*f I). These two regions, cut with the six restriction enzymes, were tested in larger samples from each of the four management areas. Independent χ^2 tests found only one region/restriction enzyme with a marginal significant heterogeneity (ND1: *ScRF* I, $\chi^2 = 10.06$, $P = 0.049$) among the four management areas, which was non-significant after applying a Bonferroni modified P for multiple tests. The composite haplotypes (based on ND1: *Hae* III, *Msp* I, *Sau* 96 I, and *ScrF* I; and ND2: *Alu* I, and *Hin*f I), showed three common haplotypes and a large number of rare haplotypes (Table 1). There was no significant heterogeneity among the four management areas ($\chi^2 = 101.8$, $P = 0.099$); G_{ST} was estimated to be 0.035, which was not significantly different from G_{ST} because of sampling error ($P = 0.208$).

Table 1 Black oreo (*Allocytus niger*) composite mtDNA haplotypes (based on ND1: *Hae* III, *Msp* I, *Sau* 96 I, and *ScrF* I; and ND2: *Alu* I and *Hin*f I) observed in four management areas.

Composite haplotype	No. black oreo/management area			
	OEO 1	OEO 3A	OEO 4	OEO 6
AAAAAA	3	1	3	2
AAAAAB	6	8	4	13
AAAABA	1	2	3	4
AAAADA	1			
AACABA	2			1
AACABB	1			
AABBCB	1			
AAAACA	4		2	1
BAADBA	1		1	
BACDAB	1			
AACADA	2			1
CAAAAB	1			
ACAAAA		1		
AAAACB		1		
AACAAA		1		1
AAAADB		1	1	2
AAABCA		1	1	3
AACADB		1	1	
AACADA			1	
CBAAAB		1		
CAAAAB		1		
AAAADA			2	
AABDCA				
AAAABB			2	
AAABAA			1	
ACADBB			1	
CBABAB				1
AABBCA			1	3
AABBCB			1	
AACABA			1	
BBADBA		1		
AAABCC				1

Table 2 Smooth oreo (*Pseudocyttus maculatus*) composite mtDNA haplotypes (based on control region 1 cut with the restriction enzymes *Alu* I and *Rsa* I) from four management areas.

Composite haplotype	No. black oreo/management area			
	OEO 1	OEO 3A	OEO 4	OEO 6
CA	1	3	4	6
BC	0	0	4	3
CB	3	4	4	3
CC	18	30	66	19
CD	3	0	4	1
DC	0	0	4	1

Smooth oreo mtDNA

The initial screening found polymorphisms only in control region 1 cut with two restriction enzymes, *Alu* I and *Rsa* I, which were tested in larger samples from each management area. Composite haplotypes based on *Alu* I and *Rsa* I, showed one haplotype was common, occurring in 58–81% of individuals over the four management areas (Table 2). There was no significant heterogeneity in the distribution of haplotypes in the total data ($\chi^2 = 16.11$, $P = 0.184$), and no significant heterogeneity in all possible area pairwise comparisons. G_{ST} was estimated to be 0.035, which was not significantly different from G_{ST} because of sampling error ($P = 0.15$).

Black oreo introns

Most introns that were successfully amplified in black oreo produced a single amplification product. Two introns produced two or more amplified products that appeared to be the result of a direct allele size polymorphism. The ribosomal protein 1 (RP-1) intron showed several different genotypes: a common one-band genotype and several two-band genotypes, including the common band; and were interpreted as the products of a single nuclear genome locus. This locus was weakly polymorphic, with common allele frequencies shown in Table 3. The LDHA6 intron appeared as a single locus with one- and two-band genotypes (Fig. 2) that were in Hardy-Weinberg equilibrium (Table 3).

The calmodulin (CaM-1) intron, digested with eight restriction enzymes, also revealed polymorphism with two restriction enzymes. The observed genotypes at the CaM-1 intron revealed with the restriction enzyme *Hin*f I were in Hardy-Weinberg equilibrium (Table 3); a weak polymorphism was revealed with the restriction enzyme *Alu* I (Table 3).

There was no significant heterogeneity in the data for LDHA6, RP-1, CaM-1 *Hin*f I, and CaM-1 *Alu* I with individual locus χ^2 heterogeneity tests (LDHA6 $\chi^2 = 1.75$; RP-1 $\chi^2 = 9.44$; CaM-1 *Hin*f I $\chi^2 = 0.74$; and CaM-1 *Alu* I $\chi^2 = 1.66$) and G_{ST} (LDHA6 $G_{ST} = 0.007$; RP-1 $G_{ST} = 0.012$; CaM-1 *Hin*f I $G_{ST} = 0.002$; and CaM-1 *Alu* I $G_{ST} = 0.019$). Overall heterogeneity was not significant ($\chi^2 = 13.59$, $P = 0.886$; $G_{ST} = 0.0012$, $P = 0.909$) indicating that the management area samples had been taken from populations with similar allele frequencies.

No variation was found at the RP-2, glutamate synthetase, and CaM-2 introns when cut with 10 restriction enzymes. Amplification of other intron

Table 3 Black oreo (*Allocytus niger*) intron allele frequencies at four loci and agreement with Hardy-Weinberg (HW) equilibrium (χ^2) in samples from four management areas.

Intron common alleles (no. alleles)	Allele frequencies in four management areas			
	OEO 1	OEO 3A	OEO 4	OEO 6
RP-1 (5)				
1	0.97	0.98	0.97	0.98
No. fish	20	24	15	22
HW χ^2 *P*	0.91	0.92	0.89	0.99
LDHA6 (3)				
1	0.79	0.74	0.84	0.80
2	0.21	0.26	0.16	0.20
No. fish	33	31	28	28
HW χ^2 *P*	0.12	0.95	0.70	0.27
CaM-1 *Hin*f 1 (2)				
1	0.37	0.33	0.41	0.38
2	0.63	0.67	0.59	0.62
No. fish	31	33	29	42
HW χ^2 *P*	0.57	0.79	0.70	0.95
CaM-1 *Alu* 1 (3)				
1	1.00	1.00	0.96	0.97
No. fish	10	12	12	17
HW χ^2 *P*	–	–	0.89	0.90

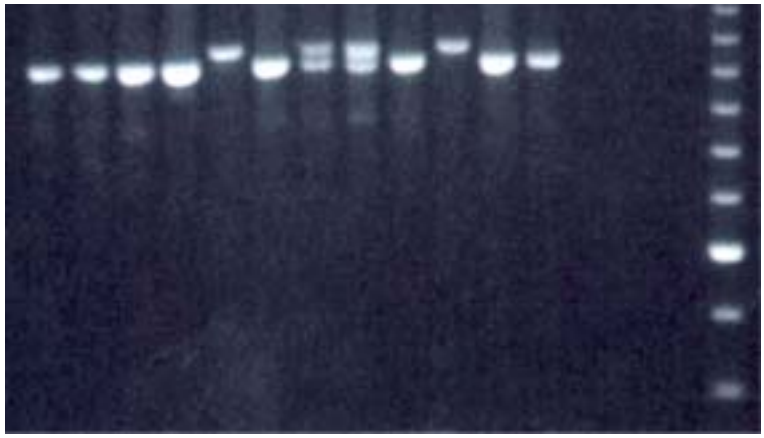

Fig. 2 LDH intron polymorphism in black oreo (*Allocytus niger*); amplified products separated in an agarose gel. Right hand column is a DNA size ladder.

Table 4 Smooth oreo (*Pseudocyttus maculatus*) intron allele frequencies at five loci and agreement with Hardy-Weinberg (HW) equilibrium (χ^2) in samples from four management areas. Monte Carlo χ^2 test (Zaykin & Pudovkin 1993) was used to estimate agreement with HW equilibrium for ribosomal protein 1.

Intron common alleles (no. alleles)	Allele frequencies in four management areas			
	OEO 1	OEO 3A	OEO 4	OEO 6
RP-1 (14)				
80	0.44	0.52	0.42	0.46
85	0.16	0.16	0.19	0.16
90	0.13	0.06	0.09	0.11
100	0.15	0.09	0.12	0.10
No. fish	76	77	79	78
HW χ^2 *P*	<0.001*	<0.001*	0.001*	0.07
CaM-1 (2)				
1	0.83	0.80	0.81	0.81
No. fish	84	84	83	92
HW χ^2 *P*	0.228	0.448	0.001*	0.999
Gsyn (2)				
2	0.85	0.94	0.85	0.79
No. fish	56	49	49	48
HW χ^2 *P*	0.196	0.678	0.224	0.376
CaM-2 (2)				
2	0.82	0.81	0.89	0.80
No. fish	38	43	44	44
HW χ^2 *P*	0.182	0.673	0.864	0.476
GH-11 (2)				
1	0.97	1.00	0.97	0.96
No. fish	16	14	38	12

*Significant at the 5% level with a Bonferroni modified *P* for multiple tests.

regions (creatine kinase, ependymin 2, growth hormone 2) produced weak or multiple fragments, perhaps indicating paralogous or unrelated loci (Chow 1998).

Smooth oreo introns

In smooth oreo, four introns, with amplified fragments less than c. 500 bp, were cut with 11 restriction enzymes (*Alu* I, *Bam* HI, *Bfa* I, *BSA* HI, *BSt* UI, *Cf*0 I, *Hae* III, *Hin*f I, *Nla* III, *RSA* I, *Sty* I, and *Taq* I) to detect polymorphisms. Two introns revealed restriction fragment polymorphisms in 12 fish: CaM-1 cut with *Taq* I, produced one- and two-band genotypes. The glutamate synthetase intron Gsyn cut with *Hae* III produced one- and two-band genotypes. The RP-2 intron revealed no genetic variation when cut with 11 restriction enzymes.

Three intron loci produced direct polymorphisms with one- and two-band amplified products. Amplification of the CaM-2 intron produced one- and two-band phenotypes that appeared to fit a simple genetic model with two alleles with homozygous and heterozygous phenotypes. Amplification of the GH-11 intron also produced one- and two-band products, but with a low level of polymorphism (common allele >0.95) and this locus was excluded from statistical analyses (Table 4).

The RP-1 intron showed several different one- and two-band genotypes (Fig. 3). There were 14 alleles, with a similar distribution of alleles in all areas (Table 4). Heterogeneity χ^2 tests showed no significant heterogeneity among the four area samples at three loci (RP-1 $\chi^2 = 30.9$, $P = 0.28$; CaM-1 $\chi^2 = 0.761$, $P = 0.86$; CaM-2 $\chi^2 = 2.96$, $P = 0.40$). The Gsyn intron showed a marginally significant heterogeneity ($\chi^2 = 8.81$, $P = 0.03$), although this was not significant with a Bonferroni correction for multiple tests. Likewise the G_{ST} analyses showed heterogeneity only for Gsyn (RP-1 $G_{ST} = 0.006$, $P = 0.17$; CaM-1 $G_{ST} = 0.0008$ $P = 0.90$; CaM-2 $G_{ST} = 0.008$ $P = 0.42$; Gsyn $G_{ST} = 0.023$, $P = 0.02$), but this was not significant with a Bonferroni correction for multiple tests.

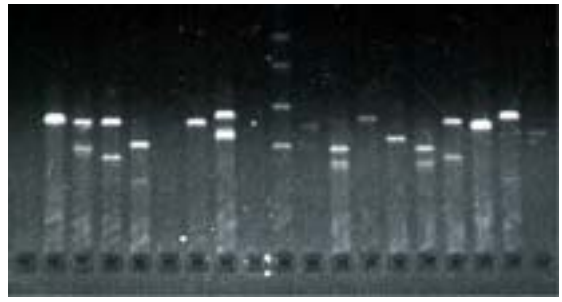

Fig. 3 Smooth oreo (*Pseudocyttus maculatus*) RP-1 intron amplified fragments separated in an agarose gel. Ninth column from the left is a DNA size ladder.

Pairwise comparisons of allele frequencies for the four loci among all areas revealed only one test pair that was significant ($P = 0.003$) with a Bonferroni modified P; Gsyn OEO 3A/OEO 6. The lack of heterogeneity in the total data was confirmed by the G_{ST} analysis which showed no overall heterogeneity ($G_{ST} = 0.008$, $P = 0.14$). Amplification of other intron regions (CK, EPD-2, IgH, Ras-I, and TP-I) produced weak or multiple fragments and may indicate paralogous or unrelated loci (Chow 1998).

Black oreo meristics

The mean lateral line scale counts for areas OEO 1, OEO 3A, and OEO 4 were similar with a higher count in OEO 6. The mean pyloric caeca counts for OEO 1, OEO 3A, and OEO 4 were similar, but OEO 6 was lower (Table 5). Mean lengths in the four black oreo samples differed widely, but the OEO 6 mean length was intermediate (Table 5). Counts of scales and pyloric caeca in black oreo were not closely associated with length (correlations with length were: scales, males 0.13; scales, females 0.13; scales, total 0.14; caeca, males –0.05; caeca, females –0.04; caeca, total –0.03).

Table 5 Black oreo (*Allocytus niger*) lateral line scale counts and pyloric caeca counts for four management areas in the New Zealand Exclusive Economic Zone.

Area	Mean scale count (range)	*N*	Mean caeca count (range)	*N*	Mean length
OEO 1	100.8 (88–117)	100	12.5 (9–17)	98	35.7
OEO 3A	100.9 (90–113)	100	12.4 (9–17)	100	31.2
OEO 4	100.1 (90–115)	99	12.7 (9–18)	96	28.5
OEO 6	103.0 (91–118)	82	11.6 (7–17)	79	33.6

Differences between areas were tested by two linear regressions, regressing scale and pyloric caeca counts on area, length, and sex. There was a statistically significant difference between areas for both traits after controlling for length and sex (scales $P = 0.015$, caeca $P = 0.0001$). For scale but not caeca counts, the length effect was significant (scale $P = 0.008$, caeca $P = 0.56$).

Both of the area effects were driven by the differences between OEO 6 and the other three OEO management areas. The OEO 1 samples were taken from two sublocations within the OEO 1 management area (Fig. 1): one sample from the Southland coast (Tow 5) and three samples from the Southern Plateau (Tows 10, 33, and 37), close to the boundary with OEO 6. The mean scale and pyloric caeca counts by tow for OEO 1 and OEO 6 are shown in Table 6. Plotting meristic counts against geographical location there was evidence for a north–south trend among the OEO 1 (excluding Tow 5) and OEO 6 samples in pyloric caeca counts (regression $R^2 = 0.825$, $P = 0.012$), but not with lateral line scale counts (regression $R^2 = 0.445$, $P = 0.148$).

Smooth oreo meristics

The mean scale counts for the smooth oreo from the four management areas were similar, although the mean lengths among the four management area samples differed widely (Table 7). Differences between areas were tested by linear regression, regressing scale counts on area, length, and sex. The difference between areas, after controlling for length and sex, was not statistically significant ($P = 0.51$). The length effect was significant ($P = 0.007$).

DISCUSSION

Meristic characters have a genetic basis but the environment may modify the expression of the character. The environmental component in meristic characters is determined during the early larval stages when variation in temperature, salinity, oxygen, pH, or food availability can modify the trait (Taning 1946; Barlow 1961; Lindsey 1988). Year class differences in meristics may occur within a single stock in long-lived species when environ-

Table 6 Black oreo (*Allocytus niger*) mean scale and pyloric caeca counts by tow (ranked north to south) for management areas OEO 1 and OEO 6.

Area/Tow	Mean scale count	*N*	Mean caeca count	*N*
OEO 1/5	98.7	25	12.6	25
OEO 1/10	101.4	30	12.5	29
OEO 1/33	100.6	26	12.7	26
OEO 1/37	102.8	19	11.9	19
OEO 6/49	103.4	25	11.9	25
OEO 6/56	102.2	28	11.7	26
OEO 6/81	103.3	29	11.3	28

Table 7 Smooth oreo (*Pseudocyttus maculatus*) lateral line scale counts in samples from four management areas in the New Zealand (NZ) Exclusive Economic Zone, Australia (TAS), and the Lord Howe Rise (LHR). (Australian and Lord Howe Rise data from Ward et al. (1996).)

Area	Mean scale count (range)	*N*	Mean length
OEO 1	113.6 (100–130)	100	37.2
OEO 3A	112.5 (102–126)	122	29.4
OEO 4	113.8 (104–123)	75	31.7
OEO 6	114.1 (105–123)	102	38.4
NZ sites pool	113.5 (100–130)	375	34.1
TAS	108.2 (98–129)	40	nd
LHR	107.9 (100–132)	14	nd
TAS/LHR pool	108.1 (98–132)	54	nd

mental conditions fluctuate between years (Schmidt 1921; Blouw et al. 1988).

The smooth oreo lateral line scale counts were similar among the four management areas, indicating that the fish had experienced similar environmental conditions during their early larval stages and might be derived from one common larval pool or from discrete pools of larvae experiencing similar conditions.

Regional differentiation of smooth oreo lateral line scale counts was reported in Australia (Ward et al. 1996): a sample from Western Australia (51 fish, mean scale count 117.2) had higher counts than a sample from southern Tasmania (40 fish, mean 108.2) and a small sample from Lord Howe Rise (14 fish, mean 107.9). The lateral line scale count was the only meristic character, out of six distinguishing the Australian samples, leading the authors to conclude that the differences might represent year class rather than stock differences (Ward et al. 1996).

The New Zealand lateral line scale counts for smooth oreo (range 100–130) fall within the range reported in Australia (98–133), but the mean scale count was consistently higher in the New Zealand smooth oreo (Table 7). The mean difference between the pooled New Zealand and the pooled Australian samples is 5.4 scales (Table 7), and is highly significant (*t*-test, $t = 6.24$, $P < 0.001$). The observed differences in lateral line scale counts between Australia and New Zealand would argue against one common larval pool of smooth oreo in the Southern Ocean, unless the samples were dominated by fish from different year classes with significantly different lateral line scale counts. These apparent trans-Tasman differences need to be confirmed by concurrent samples counted by the same readers.

The black oreo lateral line scale and pyloric caeca counts showed significant regional differentiation within the New Zealand EEZ, with OEO 6 different from all other area samples. The differences imply that the OEO 6 sample was taken from a subgroup of black oreo that had experienced different environmental conditions during their early larval stages.

Differences in lateral line scale and pyloric caeca counts in black oreo were reported by Ward et al. (1996), but were based on small sample sizes: southern Tasmania (55 fish), Western Australia (10 fish), and the Chatham Rise (9 fish). Lateral line scale counts for southern Tasmania fall within the range found in the New Zealand EEZ.

The observed differences in scale and pyloric caeca counts in our larger samples would argue against one common pool of black oreo larvae and juveniles in the New Zealand EEZ, unless the OEO 6 sample was dominated by fish from different year classes with significantly different counts. There is a significant effect for length with scale counts ($P = 0.008$), but not pyloric caeca counts ($P = 0.56$). However, the mean size of black oreo in the OEO 6 sample was intermediate between OEO 1 and OEO 3A and so the meristic differences were probably not because of fish length, and presumably age.

The major hydrological features of the Southern Plateau are the Subtropical and the Subantarctic Fronts (Fig. 1). It is possible that the samples from OEO 1 and OEO 6 were collected from different water masses, with the more northerly samples from OEO 1 influenced by the Subtropical Convergence Water and the more southerly samples from OEO 6 by Subantarctic Water. The water masses on the Southern Plateau act as barriers to species distributions of some southern species, such as southern blue whiting *Micromesistius australis* and Antarctic cods *Notothenia* spp. In black oreo there is evidence for a significant north–south decrease in pyloric caeca counts in the OEO 1 and OEO 6 samples from the Southern Plateau, but not in the lateral line scale counts. However, it is unlikely that this level of population subdivision is produced by subgroups of black oreo that remain as discrete units from larval to adult stages and the north–south differences in one of two meristic characters may represent sampling error.

The lack of population differentiation at the nuclear intron loci and mtDNA markers indicate little genetic differentiation among the black oreo samples. Ward et al. (1998) reported weak evidence for genetic differentiation between black oreo samples from the Chatham Rise and Tasmania with a mtDNA marker, but the difference was only weakly significant with a heterogeneity χ^2 analysis ($P = 0.034$), and not with a G_{ST} analysis ($P = 0.223$).

In smooth oreo the RP-1 intron showed a highly significant excess of homozygotes in each sample. An excess of homozygotes can be produced by several mechanisms, such as null alleles resulting from polymorphism in the priming site, weakly amplified alleles, inbreeding, population subdivision (the Wahlund effect) or selection (Futuyma 1998). Similar excess of homozygotes have been reported from several studies of microsatellite loci in fishes (e.g., Bentzen et al. 1996; Rico et al. 1997) and it is difficult to determine if these disequilibria are real biological effects, or an artefact of the DNA analysis and PCR techniques, in the absence of pedigree

studies. Null alleles are difficult to detect in population samples unless the null allele occurs at high frequency and is detected in the homozygous condition. Simple subpopulation mixing (the Wahlund effect) would not account for the homozygous excess at the RP-1 intron as the excess is equally spread across all samples, likewise for year class differences. It is possible that the agarose media gave insufficient resolution of the amplified alleles and some smooth oreo that were scored as homozygotes were heterozygous for two alleles that were close in size. If the homozygous excess were produced by mis-typing of genotypes then the error is consistent across all samples.

The even geographical distribution of the homozygous excess, coupled with lack of population differentiation at the other intron loci and with mtDNA data, indicate little genetic differentiation among the smooth oreo samples, and although pairwise tests at one locus showed significant differences between OEO 3A and OEO 6, this difference was not significant with a Bonferroni modified *P* for multiple tests. Ward et al. (1996) also reported a lack of genetic differentiation in smooth oreo samples from Western Australia, Tasmania, and the Chatham Rise with a different suite of allozyme and mtDNA markers.

Lack of genetic differentiation is typical of many marine species with a potentially long pelagic juvenile stage and opportunity for gene flow, even if post recruits show little movement among areas (e.g., the armorhead, *Pseudopentaceros wheeleri* (Martin et al. 1992), and spiny lobster, *Panulirus argus* (Silberman et al. 1994)). The genetic similarity could be the result of present gene flow, particularly by juvenile movement, or to historical gene flow between stocks which are currently isolated but have not evolved genetic differences. The overall genetic data collected in this study support, and do not reject the null hypothesis of single stocks of black and smooth oreo within the New Zealand EEZ.

In shallow water teleosts a negative correlation between genetic differentiation and dispersal ability has been reported (Waples 1987). A similar relationship probably applies to deepwater species. The lack of genetic differentiation in black and smooth oreo within the New Zealand EEZ contrasts with orange roughy which shows significant genetic differentiation among spatially isolated spawning groups from the Chatham Rise, east coast South Island, and Puysegur (Smith et al. 1997). Orange roughy eggs hatch near the bottom (Zeldis et al. 1994) and it is assumed that the early juveniles are demersal, reducing the potential for extensive gene flow. In hoki (*Macruronus novaezelandiae*) there is little genetic differentiation among spatially isolated spawning groups within the New Zealand EEZ (Smith et al. 1996), and hoki juveniles remain in the pelagic environment for several months (May & Blaber 1989), promoting gene flow.

Smooth oreo have an unusual juvenile distribution. Adults are generally found north of 52°S, but most of the few recorded juveniles have been found between 60 and 68°S ($n = 31$), with only seven specimens north of 52°S, one off Patagonia and six off New Zealand (James et al. 1988; McMillan pers. obs.). Only 23 black oreo juveniles have been recorded from the New Zealand EEZ; an additional six juveniles came from the stomach of a butterfly tuna (*Gasterochisma melampus*) caught between New Zealand and South America, 45°46′S, 113°32′W (McMillan pers. obs.). The juveniles of both species appear to be pelagic based on their coloration and relatively small eye size (James et al. 1988; McMillan pers. obs). A study of ageing in black and smooth oreos, applying radiocarbon data, found low levels of $^{14}\Delta C$ in adults—hatched both before and after bomb radiocarbon inputs—suggesting that as juveniles these fish must have lived well below the surface or at high latitudes (Morison et al. 1999). The pelagic features, coupled with $^{14}\Delta C$ levels, led Morison et al. (1999) to favour a high latitude origin for black and smooth oreo juveniles, although no black oreo juveniles have been reported from the Southern Ocean.

If the limited juvenile collection is indicative of smooth oreo juvenile distribution then it is possible that there is a Southern Ocean pool of juveniles in the Atlantic, Indian, and Pacific sectors that recruit northwards. Given the circulation in the Southern Ocean then the juveniles may recruit from a common gene pool, so that there is one genetic stock of smooth oreo, but there may also be ecological stocks with restricted movement post recruitment.

The ages at settlement differ in the two oreo species, with smooth oreo settling at c. 6 years and black oreo c. 4 years (Smith et al. 2000). A longer pelagic phase may promote greater juvenile advection in smooth oreo than black oreo, reducing the opportunity for genetic differentiation among regional smooth oreo stocks.

In the New Zealand EEZ, spawning of black and smooth oreo occurs from October to December and is widespread on the Chatham Rise, and probably occurs over the same months elsewhere. The long pelagic juvenile phase may promote gene flow and thus limit

the opportunity for genetic isolation; alternatively, larvae may be retained within gyres, isolating stocks. Discrete post settlement areas, coupled with an absence of extensive adult movements, may lead to the development of differences in characters that are acquired or determined in the late juvenile and adult stages.

CONCLUSIONS

In black oreo, lateral line scale counts and the pyloric caeca counts showed significant regional differentiation, with OEO 6 different from all other area samples. The differences imply that the OEO 6 sample was taken from a different subgroup of black oreo to all other areas, and/or that this group of fish had experienced different environmental conditions during their early larval stages. In smooth oreo there was no significant difference in lateral line scale counts between samples from the four management areas. There was no evidence for genetic subdivision within black and smooth oreo among the four management areas, based on intron loci and mtDNA. The data support, and do not reject the null hypothesis of a single genetic stock in New Zealand waters, and are typical of marine species with long pelagic juvenile stages.

ACKNOWLEDGMENTS

We are grateful to Peter Benson, Alan Hart, and Caoimhghin Ó Maolagáin (NIWA) for technical assistance; and to two anonymous referees and John Booth (NIWA) for constructive comments on a draft of the manuscript. The project was funded by the New Zealand Ministry of Fisheries project DEE9801. Patrick Gaffney was supported by the NIWA Visiting Scientist Programme.

REFERENCES

Annala, J. H.; Sullivan, K. J.; O'Brien, C. J.; Iball, S. D. 1998: Report from the Fishery Assessment Plenary, May 1998: stock assessments and yield estimates. Unpublished report held in NIWA library, Wellington. 409 p.

Barlow, G. G. 1961: Causes and significance of morphological variation in fishes. *Systematic Zoology 10*: 105–117.

Bentzen, P.; Taggart, C. T.; Ruzzante, D. E.; Cook, D. 1996: Microsatellite polymorphism and population structure of the Atlantic cod (*Gadus morhua*) in the northeast Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences 53*: 2706–2721.

Bielawski, J. P.; Gold, J. R. 1996: Unequal synonymous substitution rates within and between two protein-coding mitochondrial genes. *Molecular Biology and Evolution 13*: 889–892.

Blouw, D. M.; Saxon, S. D.; Chadwick, E. M. P. 1988: Temporal variation of meristic traits within an Atlantic salmon (*Salmo salar*) stock and implications for stock identification. *Canadian Journal of Fisheries and Aquatic Sciences 45*: 1330–1339.

Bruford, M. W.; Hanotte, O.; Brookfield, J. F. Y.; Burke, T. 1992: Mutli- and single locus DNA fingerprinting. *In*: Hoelzel, A. R. *ed*. Molecular analysis of populations: a practical approach. Oxford, IRL Oxford Press. Pp. 225–269.

Chow, S. 1998: Universal PCR primer for calmodulin gene intron in fish. *Fisheries Science 64*: 999–1000.

Chow, S.; Hazama K. 1998: Universal PCR primers for S7 ribosomal protein gene introns in fish. *Molecular Ecology 7*: 1247–1263.

Chow, S.; Inoue, S. 1993: Intra- and interspecific restriction fragment length polymorphism in mitochondrial genes of *Thunnus* tuna species. *Bulletin National Research Institute of Far Seas Fisheries 30*: 207–225.

Chow, S.; Takeyama, H. 1998: Intron length variation observed in the creatine kinase and ribosomal protein genes of the swordfish *Xiphias gladius*. *Fisheries Science 64*: 397–402.

Chow, S.; Takeyama, H. 2000: Nuclear and mitochondrial DNA analyses reveal four genetically separated breeding units of the swordfish. *Journal of Fish Biology 56*: 1087–1098.

Cronin, M. A.; Spearman, W. J.; Wilmot, R. L.; Patton, J. C.; Bickham, J. W. 1993: Mitochondrial DNA variation in chinook (*Oncorhynchus tshawytscha*) and chum (*O. keta*) detected by restriction enzyme analysis of polymerase chain reaction (PCR) products. *Canadian Journal of Fisheries and Aquatic Sciences 50*: 708–715.

Doonan, I. J.; McMillan, P. J.; Kalish, J. M.; Hart, A. C. 1995: Age estimates for black oreo and smooth oreo. *New Zealand Fisheries Assessment Research Document 95/14*. 26 p.

Elliott, N. G.; Ward, R. D. 1992: Enzyme variation in orange roughy (*Hoplostethus atlanticus*) samples from southern Australian and New Zealand waters. *Australian Journal of Marine and Freshwater Research 45*: 51–67.

Futuyma, D. 1998: Evolutionary biology. Sunderland, Massachussetts, Sinauer Associates.

James, G. D.; Inada, T.; Nakamura, I. 1988: Revision of the oreosomatid fishes (Family Oreosomatidae) from the southern oceans, with a description of a new species. *New Zealand Journal of Zoology 15*: 291–326.

Lindsey, C. C. 1988: Factors controlling meristic variation. *In*: Hoar, W. S.; Randall, D. J. *ed*. Fish physiology. Vol. X1B. New York, Academic Press. Pp. 179–274.

Lowry, P. S.; Elliott, N. G.; Yearsley, G. K.; Ward, R. D. 1996: Genetic variation and phylogenetic relationships of seven oreo species (Teleostei, Oreosomatidae) inferred from allozyme analysis. *Fishery Bulletin 94*: 692–706.

Martin, A. P.; Humphreys, R.; Palumbi, S. R. 1992: Population genetic structure of the armorhead *Pseudopentaceros wheeleri*, in the North Pacific Ocean: application of the polymerase chain reaction to fisheries problems. *Canadian Journal of Fisheries and Aquatic Sciences 49*: 2386–2391.

May, J. L.; Blaber, S. J. M. 1989: Benthic and pelagic fish biomass of the upper continental slope off eastern Tasmania. *Marine Biology 101*: 11–25.

McElroy, D.; Moran, P.; Bermingham, E.; Kornfield, I. 1992: REAP: an integrated environment for the manipulation and phylogenetic analysis of restriction data. *Journal of Heredity 83*: 157–158.

Meyer, A.; Morrissey, J. M.; Schartl, M. 1994: Recurrent origin of a sexually selected trait in *Xiphophorus* fishes inferred from a molecular phylogeny. *Nature 368*: 539–542.

Moran, P.; Dightman, D. A.; Waples, R. S.; Park, L. K. 1997: PCR-RFLP analysis reveals substantial population level variations in the introns of pacific salmon (*Onchorynchus* spp.). *Molecular Marine Biology and Biotechnology 6*: 315–327.

Morison, A. K.; Kalish, J. M.; Green, C. P.; Johnston, J. M. 1999: Estimation of orange roughy, black oreo and smooth oreo and natural mortality of black and smooth oreo. Final research report to the New Zealand Ministry of Fisheries, project DEE9702.

Nei, M. 1973: Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences USA 70*: 3321–3323.

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 keta*). *Molecular Marine Biology and Biotechnology 2*: 362–370.

Park, L. K.; Moran, P.; Dightman, D. A. 1995: A polymorphism in intron D of the chinook salmon growth hormone 2 gene. *Animal Genetics 26*: 277–285.

Quattro, J. M.; Jones, W. J. 1999: Amplification primers that target locus-specific introns in actinopterygian fishes. *Copeia 1999*: 191–196.

Raymond, M.; Rousset, F. 1995: GENEPOP (version 2.1): population genetic software for exact tests and ecumenicism. *Journal of Heredity 86*: 248–249.

Rice, W. R. 1989: Analyzing tables of statistical tests. *Evolution 43*: 223–225.

Rico, C.; Ibrahim, K. M.; Rico, I.; Hewitt, G. M. 1997: Stock composition in North Atlantic populations of whiting using microsatellite markers. *Journal of Fish Biology 51*: 462–475.

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.

Schmidt, J. 1921: Racial investigations. V11. Annual fluctuations of racial characters in *Zoarces viviparus*. *Comptes-Rendus des Travaux du Laboratoire Carlsberg 14*: 1–24.

Silberman, J. D.; Sarver, S. K.; Walsh, P. J. 1994: Mitochondrial DNA variation and population structure in the spiny lobster *Panulirus argus*. *Marine Biology 120*: 601–608.

Smith, P. J.; McVeagh, S. M.; Ede, A. 1996: Genetically isolated stocks of orange roughy (*Hoplostethus atlanticus*) but not hoki (*Macruronus novaezelandiae*) in the Tasman Sea and Southwest Pacific Ocean around New Zealand. *Marine Biology 173*: 783–793.

Smith, P. J.; Benson, P. G.; McVeagh, S. M. 1997: A comparison of three genetic methods for stock discrimination of orange roughy, *Hoplostethus atlanticus*: allozymes, PCR amplified mitochondrial DNA and random amplified polymorphic DNA. *Fishery Bulletin 94*: 800–811.

Smith, P.; Proctor, C.; Robertson, S.; McMillan, P.; Bull, B.; Diggles, B. 2000: Final research report on stock relationships of black oreo in New Zealand waters. Report to New Zealand Ministry of Fisheries, November 2000. Unpublished report held in NIWA library, Wellington. 50 p.

Smolenski, A. J.; Ovenden, J. R.; White, R. W. G., 1993: Evidence of stock separation in southern hemisphere orange roughy (*Hoplostethus atlanticus*, Trachichthyidae) from restriction-enzyme analysis of mitochondrial DNA. *Marine Biology 116*: 219–230.

Taning, A. V. 1946: Stage of determination of vertebrae in teleostaen fishes. *Nature 157*: 594–595.

Waples, R. S. 1987: A multispecies approach to the analysis of gene flow in marine shore fishes. *Evolution 41*: 385–400.

Ward, R. D.; Elliott, N. G.; Yearsley, G. K.; Last, P. R. 1996: Species and stock delineation in Australian oreos (Oreosomatidae). Final report to Fisheries Research and Development Corporation, Australia.

Ward, R. D.; Elliott, N. G.; Grewe, P.; Last, P. R.; Lowry, P. S.; Innes, B. H.; Yearsley, G. K. 1998: Allozyme and mitochondrial DNA variation in three species of oreos (Teleostei: Oreosomatidae) from Australasian waters. *New Zealand Journal of Marine and Freshwater Research 32*: 233–245.

Zaykin, D. V.; Pudovkin, A. I. 1993: Two programs to estimate significance of χ^2 values using pseudo-probability tests. *Journal of Heredity 84*: 152–153.

Zeldis, J. R.; Grimes, P. J.; Ingerson, J. K. V. 1994: Ascent rates, vertical distribution, and a thermal history model of development of orange roughy *Hoplostethus atlanticus* eggs in the water column. *Fishery Bulletin 93*: 373–385.

Appendix 1 Mitochondrial DNA primers evaluated in black (*Allocytus niger*) and smooth oreo (*Pseudocyttus maculatus*). Polymerase chain reaction (PCR) conditions show the optimum conditions developed for pairs of primers.

Region	Primer sequences	PCR anneal (°C)	PCR extend (°C)	PCR Mg^{++} (m*M*)	Reference
ATPase 6	TAAGCRNYAGCCTTTTAAG GGGGNCGRATRAANAGRCT	52	72	2	Chow & Inoue (1993)
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)
ND4	CAAGACCCTTGATTTCGGCTCA CCAGAGTTTCAGGCTCCTAAGACCA	50	72	2	Bielawski & Gold (1996)
ND5/6	AATAGTTTATCCATTGGTCTTAGG TTACAACGATGGTTTTTCATGTCA	50	70	1.5	Cronin et al. (1993)
COIII	CTTCGACCAATTTATGAGCCC GCCATATCGTAGCCCTTTTT	52	70	2	Chow & Inoue (1993)
COII	AAAGGGAGGAATTGAACCC GCTCATGAGTGWAGGACRTCTT	50	72	2	Gaffney (unpubl. data)
Control region 1	CCTGAAGTAGGAACCAGATG AACTCTCACCCCTAGCTCCCAAAG	49	72	2	Meyer et al. (1994)
Control region 2	ATCTGGTTCCTACTTCAGG ATAGTGGGGTATCTAATCCCAGTT	51	72	2	Cronin et al. (1993)

Appendix 2 overleaf

Appendix 2 Nuclear DNA intron primers evaluated in black (*Allocytus niger*) and smooth oreo (*Pseudocyttus maculatus*). Polymerase chain reaction (PCR) conditions show the optimum conditions developed for smooth oreo.

Intron	Code	Primer sequence	PCR anneal (°C)	PCR extend (°C)	PCR Mg^{++} (m*M*)	Reference
Glutamate synthetase	Gsyn	TCCAACAGCGACATGTACCT CTCCTGTTCCATTCCAAACC	50	75	2	Chow (unpubl. data)
Intron 4 calmodulin 1	CaM-1	CTGACCATGATGGCCAGAAA GTTAGCTTCTCCCCCAGGTT	50	72	2	Chow (1998)
Calmodulin 2	CaM-2	GTTCCTGACCATGATGGCCA TGTCCGTCTCCGTCGATGTC	50	72	2	Chow (unpubl. data)
1st intron S7 ribosomal protein	RP-1	TGGCCTCTTCCTTTGGCCGTC AACTCGTCTGGCTTTTCGCC	50	72	2	Chow & Hazama (1998)
2nd intron S7 ribosomal protein	RP-2	AGCGCCAAAATAGTGAAGCC GCCTTCAGGTCAGAGTTCAT	50	72	2	Chow & Hazama (1998)
7th intron creatine kinase	CK	GACCACCTCCGAGTCATCTC CAGGTGCTCGTTCCACATGA	55	72	2	Chow & Takeyama (1998)
6th intron lactate dehydrogenase	LDHA6	TACACTTCCTGGGCSATYGGBATG GCGGAGAGCATCCTGAAGAACCTGC	58	70	2	Quattro & Jones (1999)
Ependymin 2	EPD-2	CCGTCGCTGCCCTCTCC GCGCCACTGTATCAGACAG	58	72	2	Moran et al. (1997)
Growth hormone 2, intron D	GH-2	CAGCCTAATGGTCAGAAACT CGTAGTTCCTCCTGACGTTG	52	68	3	Park et al. (1995)
Triose phopsphate isomerase	TP-I	GCATYGGGGAGAAGCTRGAT AGAACCACYTTRCTCCAGTC	55	72	2	Quattro (unpubl. data)
Ras-1 proto-oncogene	Raa-1	GAGCGCGCTCACCATCCAGCTC CATGTCTCCCCATCAATCAC	52	72	3	Moran et al. (1997)