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Lack of genetic divergence found with microsatellite DNA markers in the tarakihi *Nemadactylus macropterus*

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Abstract Three classes of molecular markers are commonly employed during population genetic studies of marine taxa: allozymes, mitochondrial DNA (mtDNA), and microsatellite DNA. These markers differ in their levels of polymorphism, and the ease and cost of their application. *Nemadactylus macropterus* is a commercially important marine fish from New Zealand and southern Australia that has been the subject of genetic (allozyme, mtDNA) and non-genetic (otolith microchemistry, larval advection) studies of stock structure. We collected microsatellite DNA data from this species to compare the utility of these molecular markers with those genetic methods previously applied to *N. macropterus*. Microsatellites did not indicate significant divergence among Australian samples, or between Australian and New Zealand samples. The latter is incongruent with the allozyme and mtDNA studies, and it is suggested that allelic homoplasy has hindered the resolution of population structure when using microsatellites.

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INTRODUCTION

Populations in different regions are often demographically and genetically independent, even in continuously distributed species, and the term "stock" has been used to describe such populations in a fisheries context (Carvalho & Hauser 1995). Separate management of stocks is desirable for maintenance of genetic variation and avoidance of regional over-exploitation, and central to this objective is knowledge of stock boundaries (Carvalho & Hauser 1995; Hauser & Ward 1998). A variety of approaches have been employed in an effort to identify stock boundaries, examining molecular, morphological, or ecological characters.

Two classes of molecular genetic characters have been commonly employed during studies of population divergence and fisheries management: allozyme electromorphs and mitochondrial DNA haplotypes (mtDNA). Studies of marine taxa employing these characters generally observed only limited population divergence due to the paucity of barriers to long-distance dispersal in the marine environment (Gyllensten 1985; Ward et al. 1994). Microsatellites are a more recently developed class of molecular characters, comprising regions of tandemly repeated DNA where the length of the repeated sequence is 1–5 base pairs (Weber & May 1989; Jarne & Lagorda 1996). These characters typically exhibit more variation than either allozymes or mtDNA (O'Connell & Wright 1997), and have often identified population divergence within marine taxa not evident from other molecular characters (Bentzen et al. 1996; O'Connell et al. 1998; Shaw et al. 1999a,b; Ball et al. 2000; Smith & McVeagh 2000; Wirth & Bernatchez 2001). However, microsatellites are technically more demanding and expensive to analyse compared with allozymes and mtDNA (Carvalho & Hauser 1995).

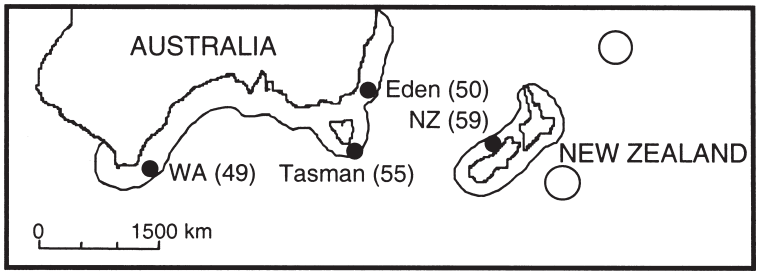

Fig. 1 *Nemadactylus macropterus* range (encircled), sample sites (•), sizes and names (WA = Western Australia, NZ = New Zealand).

The tarakihi *Nemadactylus macropterus* Bloch & Schneider 1801 is an abundant and commercially important marine fish of New Zealand and southern Australia (Fig. 1). This species occurs demersally in near shore and continental shelf waters at depths of 10–200 m (Annala 1987). Maximum ages exceed 35 years, yet maturity is attained within 3–6 years (Annala 1987). Fecundity is high, and spawning occurs serially during late summer and autumn (Annala 1987; Jordan 1997). The species has potentially high dispersal capability, indicated by an offshore pelagic larval stage of 8–12 months in duration (Annala 1987). The movement of larvae appears linked to mesoscale oceanographic processes, with records up to 250 km offshore (Bruce et al. 2001). Adult movement of 300 km within a year has also been observed (Annala 1987, 1993; Smith 1989).

Four population genetic studies have been conducted on *N. macropterus* in an effort to resolve its stock structure for management, using allozyme electrophoresis (Gauldie & Johnston 1980; Richardson 1982; Elliott & Ward 1994), and mitochondrial DNA restriction fragment length polymorphism (mtDNA RFLP; Grewe et al. 1994). Small but significant divergence was detected between Australian and New Zealand samples based on both techniques, but population structuring was absent within Australian waters (Richardson 1982; Elliott & Ward 1994; Grewe et al. 1994). The latter is in contrast with results from studies based on otolith microchemistry (Thresher et al. 1994) and larval advection (Bruce et al. 2001). Thresher et al. (1994) suggested four stocks within Australian waters, three of which were restricted to south-east Australia. Bruce et al. (2001) also suggested three stocks within south-east Australia, compatible with those of Thresher et al. (1994). In New Zealand waters, Gauldie & Johnston (1980) identified seven genetically defined stocks, although differences in allele frequencies appeared attributable to selection rather than genetic isolation, and only three stocks were suggested by analysis of otolith iron content (Gauldie & Nathan 1977).

The aims of this study were to assay microsatellite DNA markers for *N. macropterus*, to assess genetic divergence among Australian and New Zealand samples, and to compare these divergences with those observed from studies of allozymes and mtDNA. The same individuals from the investigations of Elliott & Ward (1994) and Grewe et al. (1994) were assayed, providing an absolute comparison of these different molecular approaches to resolving stock structure. As the lack of significant genetic divergence among Australian samples of *N. macropterus* based on allozymes and mtDNA could be explained by insufficient time for genetic drift, we also tested for deviation from mutation-drift equilibrium during our study.

MATERIALS AND METHODS

Nemadactylus macropterus DNA and frozen tissue samples were obtained from the studies of Elliott & Ward (1994) and Grewe et al. (1994). Samples were selected to cover the majority of the species' range (Fig. 1). Total genomic DNA was extracted from tissue samples as for *N. macropterus* library construction (Burridge & Smolenski 2000). Seven microsatellite loci developed for *N. macropterus* by Burridge & Smolenski (2000) were employed during this study. PCR amplification and electrophoresis conditions are described in the aforementioned paper and the repeat motifs of the loci appear in Table 1.

Measures of genetic variability and tests for Hardy-Weinberg equilibrium, genotypic disequilibrium, and sample divergence were calculated by Genepop 3.1c (Raymond & Rousset 1995). Fisher's exact test was used when there were less than five alleles per locus; otherwise an unbiased estimate of the exact test statistic was calculated using a Markov

chain procedure. Critical significance levels were adjusted for simultaneous tests, using the sequential Bonferroni procedure (Rice 1989). Sample divergence was quantified by the calculation of the fixation indices F_{ST} and R_{ST}, using the programs FSTAT 1.2 (Goudet 1995) and R_{ST}-calc (Goodman 1997) respectively. These measures differ in their assumptions of allele mutation, with F_{ST} assuming the infinite allele model (IAM), while R_{ST} assumes a stepwise mutation model (SMM). Multilocus estimates of both measures were obtained by averaging the variance components across loci (Weir & Cockerham 1984; Slatkin 1995). One thousand replicates of allele permutation among samples were used to determine whether divergence was significantly greater than zero.

Deviation from mutation-drift equilibrium was tested using the heterozygosity method of Cornuet & Luikart (1996). The program BOTTLENECK 1.1 (Piry et al. 1999) was used to calculate the difference between the Hardy-Weinberg expected heterozygosity (H_e, gene diversity) and the expected heterozygosity based on the number of alleles and sample size assuming mutation-drift equilibrium (H_{eq}). Three models of allele mutation were employed when calculating heterozygosity: IAM, SMM, and a two-phase model (TPM) incorporating 95% single-step mutations and a 12% variance of multiple-step mutations. Significant heterozygosity excess ($H_e > H_{eq}$) or deficiency ($H_e < H_{eq}$) across loci was determined from the Wilcoxon signed-ranks test.

RESULTS

Allele frequencies are available from the authors. Microsatellite diversity, measured in terms of expected heterozygosity (H_e) and allelic diversity (A) are listed in Table 1. Significant deviation from Hardy-Weinberg expected genotype frequencies were observed for three loci after applying sequential Bonferroni corrections, involving heterozygote deficiency at *Nma 106*, *Nma 305*, and *Nma 311* in several individual samples and when all samples were pooled (Table 1). Significant genotypic disequilibrium after Bonferroni correction was not observed.

Significant allele-frequency differences were absent during comparisons of samples at any locus based on exact tests followed by Bonferroni correction, including when pooled Australian samples were compared against the New Zealand sample ($P > 0.033$). When excluding loci deviating from Hardy-Weinberg expectations, F_{ST} and R_{ST} were –0.0010 and –0.0009 respectively, and neither differed significantly from zero ($P > 0.528$). Similarly, F_{ST} and R_{ST} for pair-wise comparisons of samples, including pooled Australia versus New Zealand samples, did not exceed 0.0094 or differ significantly from zero ($P > 0.099$). Values of F_{ST} and R_{ST} were also calculated based on all seven loci, to examine any effect of restricting analysis to those loci in agreement with Hardy-Weinberg expectations, but differences were minor and all values non-significant.

There was no evidence of deviation from mutation-drift equilibrium following Bonferroni correction for any of the samples, either when analysing all loci, or only those conforming to Hardy-Weinberg expectations ($P > 0.02$).

DISCUSSION

Deviation from Hardy-Weinberg expectations

Interpretations of sample divergences based on loci not conforming to Hardy-Weinberg expectations are potentially misleading, as differences observed may not be entirely the result of genetic drift. Consequently, the interpretation of results is based on those loci in agreement with Hardy-Weinberg expectations, although results from all loci were congruent. The presence of null alleles or scoring errors have been implicated for deviation from Hardy-Weinberg expectations at some microsatellite loci (e.g., García de León et al. 1997; Rico et al. 1997). Factors relating to species biology that could also contribute to heterozygote deficiency, such as Wahlund effect and inbreeding, are considered unlikely given the low values of F_{ST}, and the large population sizes and genetic variability for *N. macropterus*, respectively.

Comparisons with allozymes and mtDNA

Several microsatellite studies of marine species have identified small but significant population divergences that were not revealed from the analysis of allozyme or mtDNA data (Bentzen et al. 1996; O'Connell et al. 1998; Shaw et al. 1999a,b; Ball et al. 2000; Smith & McVeagh 2000; Wirth & Bernatchez 2001). In contrast, allozyme, mtDNA RFLP, and microsatellite studies each did not identify significant divergence among Australian samples of *N. macropterus* (Richardson 1982; Elliott & Ward 1994; Grewe et al. 1994; this study).

Table 1 Variation at seven microsatellite loci in samples of *Nemadactylus macropterus* (Fig. 1). Sample size (*n*), number of alleles (A), size range in base pairs, observed heterozygosity (H_o), Hardy-Weinberg expected heterozygosity (H_e, gene diversity), and the probability of deviation from Hardy-Weinberg equilibrium based on exact tests are given for each sample and a "pooled" *N. macropterus* sample.

	Western Australia	Eden	Tasman	New Zealand	Pooled
***Nma 106*, $(AC)_{16}$**					
n	47	49	53	59	207
A	25	30	32	27	40
Size range	91–147	93–185	91–185	91–177	91–185
H_o, H_e	0.61, 0.91	0.82, 0.94	0.65, 0.91	0.66, 0.95	0.69, 0.93
P	0.000*	0.093	0.001*	0.000*	0.004*
***Nma 118*, $(TCA)_9$**					
n	49	50	48	59	206
A	7	7	7	8	8
Size range	112–133	112–130	112–133	112–133	112–133
H_o, H_e	0.57, 0.61	0.50, 0.54	0.42, 0.50	0.56, 0.59	0.51, 0.56
P	0.657	0.449	0.206	0.615	0.360
***Nma 187*, $(CA)_{14}(GA)_4$**					
n	48	50	55	59	212
A	20	16	19	21	24
Size range	179–223	185–215	181–219	177–227	177–227
H_o, H_e	0.92, 0.90	0.92, 0.93	0.96, 0.93	0.92, 0.92	0.93, 0.92
P	0.623	0.964	0.899	0.287	0.627
***Nma 230*, $(GT)_{15}$**					
n	48	50	55	59	212
A	21	19	21	20	25
Size range	221–265	229–265	219–267	215–263	215–267
H_o, H_e	0.92, 0.91	0.88, 0.92	0.91, 0.91	0.95, 0.94	0.92, 0.92
P	0.638	0.094	0.124	0.515	0.537
***Nma 245*, $(GT)_{21}$**					
n	49	50	48	59	213
A	19	18	18	16	23
Size range	217–261	219–261	219–259	217–251	217–261
H_o, H_e	0.88, 0.92	0.84, 0.91	0.69, 0.80	0.83, 0.91	0.81, 0.89
P	0.417	0.805	0.105	0.606	0.019
***Nma 305*, $(GT)_7AT(GT)_9$**					
n	49	47	55	58	209
A	21	19	23	21	27
Size range	291–345	289–345	289–343	291–357	289–357
H_o, H_e	0.78, 0.94	0.74, 0.85	0.84, 0.95	0.81, 0.92	0.79, 0.92
P	0.000*	0.014*	0.006	0.078	0.000*
***Nma 311*, $(AT)_8$**					
n	49	50	55	58	211
A	7	6	8	10	11
Size range	297–313	297–309	297–327	297–327	297–327
H_o, H_e	0.37, 0.60	0.38, 0.55	0.45, 0.56	0.56, 0.63	0.45, 0.59
P	0.042	0.006*	0.008*	0.055	0.000*

*Significant after sequential Bonferroni correction for simultaneous tests.

Additionally, microsatellites also did not distinguish Australian and New Zealand samples of *N. macropterus*, yet significant divergence of these samples was evident from allozymes and mtDNA (Elliott & Ward 1994; Grewe et al. 1994).

It has been suggested that reduced levels of population divergence detected by microsatellites relative to allozymes and mtDNA might result from allelic homoplasy at highly polymorphic microsatellite loci (Bentzen et al. 1996; Hauser & Ward 1998; Shaw et al. 1999a,b). Allelic homoplasy describes the situation where alleles of the same size are not identical by descent (IBD); they may differ in nucleotide sequence or represent convergent mutation to the same allelic state (Angers et al. 2000). Where homoplasious alleles differ in nucleotide sequence, the detection and correction of such homoplasy by single strand conformation polymorphism (SSCP) and nucleotide sequencing has increased the inferred levels of population divergence (Garza & Freimer 1996; Viard et al. 1998; Taylor et al. 1999; van Oppen et al. 2000). The homogenising effects of allelic homoplasy may be particularly applicable to *N. macropterus*, given the greater influence of mutation relative to drift in species with large effective population sizes (Nauta & Weissing 1996; Shaw et al. 1999b; Estoup et al. 2002). However, the increased potential of sampling error when employing highly variable loci during the estimation of stock structure cannot be discounted for this study (Hedrick 1999).

Conclusions for *Nemadactylus macropterus*

The absence of genetically detectable stock structuring in *N. macropterus* of southern Australia has been previously ascribed to dispersal (Elliott & Ward 1994; Grewe et al. 1994; Thresher et al. 1994; Bruce et al. 2001). This species possesses an offshore pelagic larval phase of 8–12 months in duration, suggestive of high dispersal capability, and adult movements of up to 300 km within a year have been recorded (Annala 1987, 1993; Smith 1989). Molecular phylogenetic analysis of *Nemadactylus* also suggests high dispersal capability within this genus (Burridge 1999). Larval dispersal of *N. macropterus* appears linked with surface circulation patterns (Bruce et al. 2001), and the principal ocean currents operating within southern Australia, the Leeuwin and East Australian Currents, could facilitate movement throughout much of this species' range. Alternatively, multiple stocks of *N. macropterus* may exist within Australia, but they have not existed long enough for any detectable signal to develop from genetic drift, regardless of sample size and marker sensitivity. A recent origin of *N. macropterus* is inferred from the molecular phylogenetic analysis of *Nemadactylus* (Burridge 1999), and the large population sizes of this species may result in slow genetic drift. However, this was not supported by tests of deviation from mutation-drift equilibrium, which were all non-significant but admittedly based on few loci (Cornuet & Luikart 1996; Luikart & Cornuet 1998).

It is important to note that the equilibrium levels of gene flow required to homogenise populations genetically are somewhat lower than the levels of migration at which managers would consider stocks distinct (Carvalho & Hauser 1995; Waples 1998). Although this and previous genetic studies of *N. macropterus* may suggest high levels of gene flow from an evolutionary perspective, in management terms such levels of gene flow do not guarantee that recovery of populations after regional overexploitation will be rapid. Otolith microchemistry and larval advection studies of *N. macropterus* suggested three stocks within south-east Australia alone, and these techniques are less sensitive to the effects of dispersal (Thresher et al. 1994; Bruce et al. 2001). However, an appreciation of their sensitivity to fluctuations in environmental conditions is required as they measure differences at a single point in time, whereas genetic divergence represents a long period of reduced gene flow. Any influence of post-settlement migration on the detection of genetic stock structure could be removed by the analysis of individuals collected within their first year (e.g., Graves et al. 1992; Jones & Quattro 1999; Buonaccorsi et al. 2001). This study provides further support to the suggestion that a variety of characters should be employed when attempting to resolve the stock structure of marine taxa (Ward 1998).

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